***In vivo* quantitative imaging of normal and cancerous breast tissue using broadband diffuse optical tomography**

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Purpose: A NIR tomography system that combines frequency domain (FD) and continuous wave (CW) measurements was used to image normal and malignant breast tissues.

Methods: FD acquisitions were confined to wavelengths less than 850 nm because of detector limitations, whereas light from longer wavelengths (up to 948 nm) was measured in CW mode with CCD-coupled spectrometer detection. The two data sets were combined and processed in a single spectrally constrained reconstruction to map concentrations of hemoglobin, water, and lipid, as well as scattering parameters in the breast.

Results: Chromophore concentrations were imaged in the breasts of nine asymptomatic volunteers to evaluate their intrasubject and intersubject variability. Normal subject data showed physiologically expected trends. Images from three cancer patients indicate that the added CW data is critical to recovering the expected increases in water and decreases in lipid content within malignancies. Contrasts of 1.5 to twofold in hemoglobin and water values were found in cancers.

Conclusions: *In vivo* breast imaging with instrumentation that combines FD and CW NIR data acquisition in a single spectral reconstruction produces more accurate hemoglobin, water, and lipid results relative to FD data alone. © 2010 American Association of Physicists in Medicine.

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I. INTRODUCTION

Near-infrared (NIR) spectroscopy and tomography have been studied in breast imaging for the past decade¹⁻³ and could play important supplementary roles to conventional imaging by providing quantitative functional information on tissue chromophores such as oxyhemoglobin (HbO₂), deoxyhemoglobin (Hb), water, and lipid. These chromophores act as absorbers of NIR light that is diffusively transmitted through scatter-dominated tissue. Based on the known molar absorption spectra of the chromophores, their concentrations can be estimated from NIR light measurements which, when col-

lected with an array of detectors, enable centimeter-scale spatial discrimination to be achieved at depth in tissue. One of the technical challenges associated with NIR tomography has been simultaneously satisfying the spatial and spectral sampling necessary to estimate chromophore concentrations with high accuracy using instrumentation that is moderate in cost.

NIR imaging systems typically have involved continuous wave (CW),⁴⁻⁶ frequency domain (FD),⁷⁻⁹ or time domain^{10,11} source-detector schemes. Among the three methods, the CW approach is relatively inexpensive and easy to implement, but the light measurements are incomplete, lack-

ing information about the optical scattering in tissue.¹² The latter two methods provide data related to both optical absorption and scattering, but the measurements need to be acquired at multiple wavelengths which cover the full spectrum of interest in order to attain accurate quantification of the molecular constituents in tissue. While a broadband FD system is conceptually the ideal choice, no single detector technology exists which offers both the sensitivity and spectral coverage (at an acceptable cost) that is required to achieve the best imaging performance. One practical compromise combines FD and CW methods to maximize spectral coverage with sufficient light detection performance and reasonable instrumentation costs to achieve quantitative molecular imaging of the relevant tissue chromophores in the breast. We recently described the implementation of such a system and demonstrated that the additional wavelengths (above 850 nm) recorded in CW mode improved the quantitative recovery and parameter decoupling of the optical absorbers in tissue-simulating phantoms, especially in terms of water content.¹³ Here, an extension of this work is shown to report initial experiences with this system when imaging the breast *in vivo*. Although the importance of broadband spectroscopy has already been elegantly demonstrated in the breast and the diagnostic significance of indexing oxyhemoglobin, deoxyhemoglobin, and water concentrations in breast abnormalities has been established,^{14–16} to the best of our knowledge, the data presented in this paper represent the first broadband tomographic images of the breast where quantitative water and lipid images in cancers are reported.

II. METHODS AND MATERIALS

II.A. Theory

Under the assumption of scattering dominance over absorption, the propagation of NIR light can be described by the diffusion approximation to radiative transport in tissue

$$-\nabla \cdot D(r) \nabla \Phi(r, \omega) + \left[\mu_a(r) + \frac{i\omega}{c} \right] \Phi(r, \omega) = q_0(r, \omega), \quad (1)$$

where $\Phi(r, \omega)$ is the isotropic fluence at modulation frequency ω , c is the speed of light in tissue, and $q_0(r, \omega)$ is an isotropic light source at position r . The two unknown tissue property parameters in Eq. (1) are the absorption coefficient $\mu_a(r)$ and diffusion coefficient $D(r)$. Here, $D(r)$ can be written as

$$D(r) = \frac{1}{3[\mu_a(r) + \mu'_s(r)]}, \quad (2)$$

where the reduced scattering coefficient $\mu'_s(r) = \mu_s(r)[1 - g(r)]$, $\mu_s(r)$ is the scattering coefficient, and the anisotropy factor $g(r)$ is defined as the mean cosine of the scattering angular phase function. The measurement data at the tissue surface Φ includes two components: The amplitude and phase shift of transmitted light modulated at frequency ω . During CW measurements, only the intensity information is recorded [i.e., $\omega=0$ in Eq. (1)]. Estimating the distribution of

$\mu_a(r)$ and $D(r)$ is a nonlinear inverse problem, which has been described in previous publications.^{17,18} Briefly, a Newton-minimization approach is used to estimate $\mu_a(r)$ and $D(r)$ based on repetitive solution of Eq. (1) using the finite-element method (FEM). The updating equation for the tissue property parameters of interest can be written as a regularized inversion involving the computed sensitivity matrix J ,

$$\delta\mu = (J^T J + \lambda I)^{-1} J^T [\Phi_m - \Phi_c], \quad (3)$$

where Φ_m is the measurement data, Φ_c is the modeled results, and J is the Jacobian matrix which describes the sensitivity of the measurement data to variations in the optical properties. Since the Jacobian is typically not dimensionally square, the matrix to be inverted becomes $J^T J$, which is ill-conditioned and requires regularization. In the modified Tikhonov approach, regularization factor λ is added to the diagonal terms to $J^T J$ based on modification of the objective function to be minimized.¹⁸

Direct spectral reconstruction of data acquired from multiple wavelengths improves the quantification of chromophores and scattering properties,¹⁹ in which case the absorption coefficient is assumed to be a linear combination of the individual chromophore contributions, such that

$$\mu_a(\lambda) = \sum_i \varepsilon_i(\lambda) c_i, \quad (4)$$

where c_i is the concentration of the i th chromophore and $\varepsilon_i(\lambda)$ is its molar extinction coefficient. FD measurements can be obtained up to 850 nm—A limit imposed by the photocathode sensitivity of the PMTs used for detection. CW measurements can cover a wider range of wavelengths in the NIR spectrum if photodiodes or CCDs are used for signal detection.

Multispectral DOT reconstruction techniques have been described for CW (Ref. 20) and FD data¹⁹ where chromophore concentrations and scattering parameters are estimated directly from the multiwavelength measurements. Here, a combined FD and CW reconstruction process was developed to incorporate the two forms of data simultaneously. As defined in Eq. (5), the two data sets are combined to create a larger vector of measurements Φ , with the first half being FD and the second half being CW recordings. The Jacobian matrix J must be formulated accordingly as

$$\delta C = \begin{bmatrix} \delta C_1 \\ \delta C_2 \\ \vdots \\ \delta C_N \end{bmatrix}$$

FIG. 1. (a) NIR spectrum of four major chromophores in breast tissue and measurement wavelengths associated with FD and CW acquisitions in the NIR range. (b) An example of reduced scattering coefficient versus wavelength. The shaded area represents the wavelength range of frequency domain measurement, which provides both absorption and scattering information. The shaded area shows the range of CW measurement. (c) FD system using six intensity-modulated laser diodes. (d) Patient interface composed of arrays of 16 fibers in two planes. (e) Spectrometer-based detection system for CW tomography with 16 CCD-coupled imaging monochromators.

$$J = \begin{bmatrix} J_{\lambda_1}^{FD}(C_1) & J_{\lambda_1}^{FD}(C_2) & \cdots & J_{\lambda_1}^{FD}(C_N) \\ J_{\lambda_2}^{FD}(C_1) & J_{\lambda_2}^{FD}(C_2) & \cdots & J_{\lambda_2}^{FD}(C_N) \\ \vdots & \vdots & \ddots & \vdots \\ J_{\lambda_f}^{FD}(C_1) & J_{\lambda_f}^{FD}(C_2) & \cdots & J_{\lambda_f}^{FD}(C_N) \\ J_{\lambda_1}^{CW}(C_1) & J_{\lambda_1}^{CW}(C_2) & \cdots & J_{\lambda_1}^{CW}(C_N) \\ \vdots & \vdots & \ddots & \vdots \\ J_{\lambda_c}^{CW}(C_1) & J_{\lambda_c}^{CW}(C_2) & \cdots & J_{\lambda_c}^{CW}(C_N) \end{bmatrix}$$

$$\Phi = \begin{bmatrix} I_{\lambda_1}^{FD} \\ I_{\lambda_2}^{FD} \\ \vdots \\ I_{\lambda_f}^{FD} \\ I_{\lambda_1}^{CW} \\ \vdots \\ I_{\lambda_c}^{CW} \end{bmatrix}.$$

In Eq. (5), C_i represented a row vector including four absorbers (HbO₂, Hb, water, and lipid) and two scattering param-

eters (a and b). Jacobian matrices associated with each wavelength are assembled to allow the multiwavelength spectral reconstruction to occur. In the mixed (FD+CW) reconstruction, the Jacobian matrix elements at each wavelength were calculated using 100 and 0 MHz for the FD and CW contributions, respectively. The dimensions of the spectral Jacobian matrix were $[(f+c) \cdot M] \times N$, where f and c are the number of wavelengths used during FD and CW detection, respectively. M is the number of measurements at each wavelength and N is the number of nodes in the mesh involved in the FEM computations. M and N are 240 and 1785 in this study.

II.B. Experimental methods

Figure 1(a) shows the NIR absorption spectrum of the four main chromophores in breast tissue (HbO₂, Hb, water, and lipid) along with the FD and CW acquisition wavelengths. During FD measurements, six wavelengths (661, 761, 785, 808, 826, and 849 nm) were used to sample the absorption band of HbO₂ and Hb [as indicated by the shaded area from 650 to 850 nm in Fig. 1(a)]. The FD instrumentation is shown in Fig. 1(c). Six laser diodes were driven by a

TABLE I. Parameters used in the simulation example shown in Fig. 2.

	HbT (μM)	StO ₂ (%)	HbO ₂ (μM)	Hb (μM)	Water (%)	Lipid (%)
Background	16	75	12	4	30	80
Target	60	48	75	32	16	15

DC current source and frequency domain signal generator. The light detection array consisted of 16 PMTs.²¹ The transmitted modulated light signal was recorded with heterodyne circuitry to measure the change in AC amplitude and phase, from which both absorption and scattering properties were estimated. Figure 1(b) shows the typical wavelength dependence of reduced scattering coefficient $\mu'_s(r)$ across the NIR spectrum. Two tissue-related parameters, scattering amplitude a and scattering power b , were extracted from a Mie scattering model used to describe this functional dependence.

A three-wavelength laser diode source (903, 912, and 948 nm) was assembled to extend the spectral range during CW acquisitions. The output of three laser diodes was directed into one fiber for simultaneous illumination of the breast. The spectrum of the received light was recorded with spectrometer-based detection.²² As shown in Fig. 1(e), the instrumentation consisted of 16 spectrometers integrated with CCD cameras (Pixis, Princeton Instruments, Inc., Acton, MA) cooled to -70°C . A 300 nm grating with center wavelength at 820 nm was set for each spectrometer and the source light was delivered sequentially to each fiber using a rotating stage. The exposure time for the cooled CCD cameras was determined by optimizing the signal-to-noise ratio at the first source position. When the longer wavelengths were used, the maximum exposure time for the furthest source-detector distance was 20 s. A complete acquisition of 15 measurements at 16 source positions required about 8 min. Calibration procedures were used to suppress background noise and to correct the measurement counts for their exposure time.²²

Separate sets of 16 bifurcated fiber bundles were used for source light delivery and transmitted light detection in the

FD and CW measurement modes, respectively. The fiber interfaces for the two acquisitions were located in vertically adjacent planes with a height difference of approximately 1 cm as shown in Fig. 1(d), but were considered to be in the same plane during image reconstruction.

III. RESULTS

III.A. Simulations

The combined FD+CW reconstruction approach was evaluated first in simulation studies of a two-region geometry. Typically, total hemoglobin ($\text{HbT}=\text{HbO}_2+\text{Hb}$) and oxygen saturation ($\text{StO}_2=\text{HbO}_2/\text{HbT}$) are reported instead of HbO_2 and Hb . The values of the four NIR imaging parameters comprising the background and inclusion are listed in Table I. A circular mesh with a diameter of 86 mm was used to simulate the coronal plane of the female breast. FD and CW data were calculated with FEM solutions of the diffusion approximation in Eq. (1). Normally distributed noise was added to the synthetic data sets. Simulated measurements were generated from nine wavelengths spanning 661–948 nm (the same as those available in the FD and CW imaging systems shown in Fig. 1) and were used for image reconstruction.

Figure 2 shows a comparison between the FD results with six wavelengths and the combined FD+CW approach using all nine wavelengths. Lipid was excluded from spectral reconstruction when only FD data were used because the absorption peak of lipid, which occurs around 920 nm, was not sampled by the FD wavelengths. The expected chromophore and scattering parameter images are shown in Fig. 2(a). Reconstructed images using only FD data appear in Fig. 2(b), where it is apparent that the water image is dominated by noise due to the lack of data acquired at wavelengths closer to the water absorption peak which occurs above 900 nm. Adding the longer wavelengths improves the quantification of water concentration and also allows the recovery of lipid values as shown in Fig. 2(c). The error in water content in the background decreased from over 100% in Fig. 2(b) to 1.2% when the combined FD+CW data sets were used. The false contrast observed in the StO_2 image in Fig. 2(b) also shows that recovery of hemoglobin becomes biased if the

FIG. 2. Reconstructed images of the four major chromophores in breast tissue. (a) Expected images and (b) results with only FD data (661, 761, 785, 808, 826, and 849 nm). (c) Results with combined FD and CW data adding CW measurements (903, 912, and 948 nm) to the FD acquisitions.

TABLE II. Normal subject demographic and radiologic information. HD =Heterogeneously dense; ED=Extremely dense.

Subject ID no.	Age (yr)	BMI (kg m^{-2})	Radiographic density
1	50	28.3	Scattered
2	58	26	Scattered
3	55	29.3	Scattered
4	67	25.8	Scattered
5	49	40.6	Scattered
6	37	27	ED
7	51	24	ED
8	52	26.5	HD
9	62	35.2	Fatty

FIG. 3. Reconstructed images of chromophore concentrations and scattering parameters for two breast types: (1) Scattered (subject 3) and (2) ED (subject 7). (Normal subject 3). The upper two rows show the results when only FD data below 850 nm is used. The two lower rows show the results of combined FD and CW data where lipid is quantified. Only the left breast was shown here for each.

water concentration is not well quantified.²³ This example illustrates that including longer wavelengths not only makes the quantification of lipid possible, but also improves the accuracy of the estimates of the major chromophores in breast tissue.

III.B. Normal breast imaging

Nine healthy women volunteered under informed consent to participate in NIR imaging studies according to a protocol approved by the Institutional Review Board at Dartmouth. Their age, body mass index (BMI) (weight/height squared), and breast radiographic density are listed in Table II. Subjects were assigned to high and low radiographic density groups based on their BI-RADS ratings, where fatty and scattered breasts were categorized as low density and heterogeneously dense (HD) and extremely dense (ED) breasts were considered as high density in order to investigate rela-

tionships with NIR image parameters. During NIR imaging, subjects were placed in a prone position on a padded examination platform with the breast to be imaged pendant within the circular fiber-optic array. The array was moved radially into firm but comfortable contact with the breast. All exams were performed on both breasts (except for subject 9 who was imaged only on the right breast). FD and CW data were acquired during each exam.

III.B.1. Improvement with longer wavelengths

Figure 3 shows reconstructed images of chromophore concentrations and scattering parameters in a single plane of two breast types: (1) Scattered (subject 3) and (2) ED (subject 7). To compare the effect of including CW data at the longer wavelengths above 850 nm, reconstructed results with only six wavelengths of FD data are also shown. When using only FD data, lipid was not included in the reconstruction. The images of HbT, StO₂, and water show generally homogeneous characteristics in all subjects. Low internal lipid content was observed in the images of some subjects such as in Fig. 3, which is consistent with the existence of central fibroglandular tissue surrounded by fat.

In healthy subjects, the comparison between FD and FD+CW imaging focused on the mean values of the reconstructed properties as presented in the Box-and-Whisker plots in Fig. 4. In general, the FD data with measurements below 850 nm produced higher HbT and StO₂ values and lower water concentrations compared to results obtained from the wider spectral sampling (up to 948 nm) available through the FD+CW approach. The difference of the three parameters between the two imaging approaches was all statistically significant ($p < 0.05$). These findings are consistent with the experiences reported in other spectroscopic studies of breast tissue.^{23,24} Previous simulation and phantom experiments have also shown that water concentration is underestimated when longer wavelengths are not included¹³ because some of the absorption effects of water are mistakenly attributed to hemoglobin. The cross-coupling between estimation

FIG. 4. Average values of (a) HbT, (b) StO₂, and (c) water of all normal subjects recovered using FD data from six wavelengths and FD+CW data from nine wavelengths.

FIG. 5. Top: Correlation plots for water and lipid concentrations versus scatter power. Bottom: Correlation plot for HbT versus water concentration.

parameters is particularly large in HbO₂ because of the similarity of its spectrum to that of water. Consequently, the overestimation of HbO₂ leads to errors in quantification of StO₂ as well. Addition of CW data (at wavelengths beyond 850 nm) serves to correct these misassignments of the tissue optical response into its chromophore constituents through the extended spectral sampling.

III.B.2. Intrasubject variability

The mean variance in HbT and StO₂ across the tomographic images of each subject was 10% and 6.7%, respectively, whereas the water and lipid estimates showed larger variation of 16%. The larger variation in these latter chromophores results from the variable breast tissue composition associated with the variety of parenchymal patterns that exist even in breasts having the same radiographic density classification. The low variability in StO₂ agrees with results reported by Shan *et al.*²⁵ and Svensson *et al.*³² However, the lipid variation observed here was larger than that found in Shah *et al.*,²⁵ likely because the Shah data resulted from a reflective measurement geometry that mostly samples the outer layer of breast tissue which is typically dominated by fat.

III.B.3. Intersubject variability

Mean HbT values within the breast ranged from 10 to 27 μM with an overall subject mean of 16 μM. Mean StO₂ within the breast varied from 49% to 72% with an overall subject mean value of 61%. Although the number of subjects is modest, these results agree with several other studies of asymptomatic breast tissue.^{26–30} Not surprisingly, the intersubject variation in water and lipid is larger because breast composition varies significantly between individuals.²³ The

TABLE III. Mean (standard error) of parameters for HD/ED versus scatter/fatty subject groups and *p*-values for differences between two groups.

Parameters	Scatter/Fatty	ED/HD	<i>p</i> -value
HbT	13.1 (1.5)	21.4 (1.9)	0.01 ^a
StO ₂	59.2 (2.7)	62.4 (3.5)	0.49
Water	26.9 (5.4)	52.6 (6.9)	0.03 ^a
Lipid	76.2 (4.3)	61.4 (5.5)	0.08
Scatter amplitude	0.9 (0.1)	0.8 (0.1)	0.39
Scatter power	0.8 (0.2)	1.6 (0.2)	0.02 ^a

^aSignificant difference.

minimum water concentration was 20.0% and the maximum was 73%. The lipid fraction showed variations from 43% to 91%.

III.B.4. Correlation between physiological parameters

Figure 5 presents correlation plots of the average water and lipid concentrations versus scattering power. The solid lines represent linear fits and demonstrate a positive correlation between water content and scattering power, and a negative correlation between lipid content and scattering power. All correlations between parameters shown here were statistically significant ($p < 0.05$). Similar results have been reported by Cerussi *et al.*³¹ and Pifferi *et al.*²⁴ Water-abundant breasts are associated with a larger composition of collagen-rich glandular tissue, which usually has smaller scattering centers than fatty tissues resulting in the increased scattering effect. The correlation plot of HbT versus water content in Fig. 5 is also consistent with the scattering dependence because glandular tissue with larger water content is also characterized by well-vascularized lobular tissue.

III.B.5. Comparison of physiological parameters based on radiographic density

We applied a linear mixed model with random subject effects to analyze the outcomes. Covariates included breast density (fatty/scattered or heterogeneous/extreme) and side (left/right). SAS 9.1 (SAS Institute, Cary, NC) was used to conduct the statistical computations. Two-sided significance level was set to 0.05. The comparison results are shown in Table III. The high density group showed significantly higher values in HbT and water than the low density group. No significant differences were observed in StO₂ and scattering amplitude. The high density group also showed significantly higher scattering power than the low density group, which is consistent with earlier results reported by Pogue *et al.*²⁸ using frequency domain tomography. The statistically significant trend here suggests that a strong correlation exists between NIR scattering properties and radiographic density of breast tissues. Estimated mean of lipid for scatter/fatty (76.18) is higher than that for ED/HD (61.44) but it is insignificant (p -value is 0.08) due to the relatively high within group variance in lipid.

TABLE IV. Variations between left and right breasts (%).

Parameters	HbT	StO ₂	Water	Lipid	Scatter amplitude	Scatter power
$\Delta = \frac{ \text{Left-Right} }{\text{Average}}$	9.7	9.0	12.4	13.0	11.2	23.4

In addition to comparisons between the two density groups, differences in mean NIR imaging parameters between the two sides of the breast were also investigated. The relative deviation defined as $|\text{left-right}|/\text{average}$ was calculated for HbT, water, lipid, and scattering power and no statistically significant differences were observed between left and right breasts as summarized in Table IV. In general, the deviations found here are smaller than those reported by Shah *et al.*²⁵ and Svensson *et al.*,³² likely because we have used multiwavelength transmission data from 16 sources and 15 detectors to estimate breast tissue optical properties, which should be less sensitive to local near-surface variations in breast composition resulting from the reflectance measurements obtained by others.

III.C. Imaging patients with malignant breast tumors

Three female subjects with invasive ductal carcinoma were imaged. In order to place the imaging array in the region of interest, the tumor was localized by palpation guided by positional information contained in the radiology reports. Magnetic resonance (MR) images of the patients were also acquired with the subject in the prone position, either on a 1.5 T scanner (GE Signa, GE Healthcare, Waukesha, WI) for standard clinical indications or a 3 T (Philips Achieva, Philips Healthcare, Andover, MA) system for research studies. Dynamic contrast MR images were acquired with an injection of a bolus of contrast (Gadodiamide, Omniscan, GE Healthcare, Canada, Mississauga, ON).

The first case involved a 48-year-old woman with a $4.2 \times 2.6 \times 3.5 \text{ cm}^3$ ductal carcinoma *in situ* and invasive ductal

carcinoma in her left breast. Dynamic contrast MR images are shown in Figs. 6(a)–6(c). Spectral reconstruction images using FD data at six wavelengths [Fig. 6(d)] are compared to results obtained from FD+CW data at nine wavelengths [Fig. 6(e)]. The HbT image shows a localized increase concordant with the position of the tumor in MRI indicating an increase in vascularity presumably due to angiogenic activity.³³ No significant contrast was observed in the StO₂ distribution. The water image recovered from the FD data shows a homogeneous result; however, with the addition of the longer wavelengths from the CW acquisitions, contrast is found in both water and lipid concentrations in the spectral reconstruction. The increase in water and decrease in lipid content within the tumor is consistent with studies of breast cancers using spectroscopic reflectance measurements covering a similar wavelength range.^{15,34} The location of the water contrast in the NIR images is not completely congruent with the contrast in hemoglobin, which may be the result of our current imaging geometry where the data acquisition planes for the FD and CW recordings are not identical. Although FD and CW data from two adjoining planes were used together in a single reconstruction representing the same plane, the error caused by this approximation is likely not too large given the diffusive nature of NIR light in tissue and the size of the tumor in this subject.

Figure 7 shows images obtained from the breast of a 43-year-old female with inflammatory carcinoma. The tumor size was about 9 cm. Only T1-weighted MRI were available for this subject as shown in Figs. 7(a)–7(c). Due to the large tumor size, the HbT image presents diffusive enhancement in

FIG. 6. Dynamic contrast MRI images of a patient with an invasive tumor in the breast, showing: (a) Axial view, (b) coronal view, and (c) sagittal view. The coronal view (b) is the same view used in the NIR reconstructed images and the shape of the tumor region is outlined in a dotted line. Coronal NIR chromophore and scattering parameters: (d) With six wavelengths of FD data and (e) with nine wavelengths of FD+CW data.

FIG. 7. T1-weighted MRI images of a patient: (a) Axial view, (b) coronal view, and (c) sagittal view are shown, with the coronal view (b) being the one used for display of the NIR images. The tumor region is outlined with a dotted line on the coronal MR and the NIR images. Coronal NIR images of chromophore and scattering parameters: (d) With six wavelengths of FD data and (e) with nine wavelengths of FD+CW data.

the general region of disease. Interestingly, the Hb image reveals more focal contrast with more than 100% increase compared to surrounding breast tissue. The difference between the HbO₂ and Hb images could arise from the variable metabolism within the breast, which suggests it may be important to analyze these two parameters separately during diagnosis or therapy monitoring. The water image also shows high contrast in the tumor region, while the opposite contrast was found in the lipid image when the CW data was added to the image reconstruction. The average scattering power increased by 16% in the tumor region.

Image contrast in tumor relative to background tissues, defined as $\text{mean}(\text{tumor})/\text{mean}(\text{background})$, is summarized in Fig. 8 for the three patient cases. The improvement in water quantification is apparent when longer wavelengths above 850 nm were incorporated. The enhanced accuracy in the water results is also expected to decrease the error in hemoglobin concentration, which has been shown to occur in other spectroscopic²³ and time-domain²⁴ studies of the breast.

FIG. 8. Bar graph summarizing data from the three patients with breast cancer as a ratio of the tumor relative to background tissue values for chromophore concentrations and scattering parameters.

IV. DISCUSSION

NIR images based on the combination of FD and CW data have been used to quantify the chromophore and scattering parameters in breast tissue. The most significant improvement occurs in the accuracy of water content in both normal and cancer regions. The quantification of water is critical in the study of the breast because of the highly variable distributions of adipose and fibroglandular tissues between subjects. Srinivasan *et al.*²⁷ has shown water concentration in breast tissue has a large variation from 10% to 70% between different subjects, which has been largely attributed to changes in their adipose/fibroglandular distribution.³⁵ Cerussi *et al.*²³ and Srinivasan *et al.*²⁷ also showed a significant variation in water concentration in normal breast tissues with age. Spectroscopy of the breast with a handheld probe^{1,36} has benefited significantly from the addition of broadband CW data, and this conceptual approach has been extended to full tomography mode here in order to study the water fractions in breast tissue. With longer wavelengths added into the spectral reconstruction, tumor regions presented with contrasts up to 2 relative to surrounding normal tissues in all three cancer cases.

The contrast in HbT indicates an increase in vascular density in the tumor region and is related to angiogenesis in response to fibroblastic and vascular growth factors expressed by the cancer cells.³⁷ However, no consistent decrease in tumor StO₂ was observed in the current study. The weaker association of StO₂ changes with malignancy was also found in other DOS studies.^{15,38} Infiltrating breast ductal carcinomas are usually surrounded by dense peripheral infiltrates of inflammatory cells which are metabolically active. The intensity and distribution of the cellular infiltrates varies considerably within a given breast carcinoma and among different patients, so oxygen saturation changes caused by inflammatory cells is likely to be quite variable.³⁷ The results

presented here suggest that recovering HbO_2 and Hb separately is also important to the analysis the tumor properties. HbO_2 is related more directly to global vascular structures, while Hb is more sensitive to cellular oxygen consumption and local metabolism. The differences in these two chromophores are reflected in the results from patient 3 shown in Fig. 7, where Hb is more localized than HbO_2 .

The FD+CW acquisitions also allow quantification of lipid content. Several studies in normal breast tissues have shown that lipid properties in the breast are strongly affected by age and menopausal status.^{25,32} The quantification of lipid may be helpful in detecting breast lesions. Water and lipid have been shown to be related to the response of breast cancer patients in neoadjuvant chemotherapy monitoring studies with DOS.^{14,34} The imaging approach demonstrated here can provide a complete distribution of chemotherapy-induced changes in these physiological properties of tumors.

Scattering properties are another potentially important tissue parameter for diagnosing and/or characterizing breast malignancy. Cerussi et al.²³ showed that collagen-rich glandular tissue has higher scattering power than adipose tissue. Pogue et al.²⁸ have shown that scattering amplitude and scattering power correlate with radiographic density and can provide useful information for assessing breast physiology and potential risks of disease. FD data have the advantage of providing path-length information on the scattered light in tissue. Scattering amplitude is sensitive to the number density of scatterers, while scattering power is related to their size. The contrast shown in scattering power in Fig. 8 indicates a decrease in the size of the scattering centers, which is likely related to fibrosis occurring in the malignancies. Our findings agree with the results of malignant breast tumors reported by Cerussi et al.¹⁵ Wang et al.³⁹ also found that the effective scatter size in breast tumors is smaller than background tissues based on a Mie scattering model. Microscopic study of the scattering properties of tumors requires further investigation to fully appreciate the subcellular origins of this signal.

V. CONCLUSION

NIR imaging that combines FD and CW data can quantify the chromophore and scattering parameters more accurately in breast tissues. The major chromophores in breast tissue include HbO_2 , Hb, water, and lipid, and are estimated using spectral reconstruction with combined data sets. Based on the enhanced spectral information acquired, the *in vivo* studies on healthy subjects and in women with breast cancer showed promising improvements in imaging accuracy. The value of this type of imaging to clinical breast cancer management is still speculative, yet these developments are worth further clinical investigation in an ongoing study of women with breast abnormalities.

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