Noninvasive *in vivo* tomographic optical imaging of cellular morphology in the breast: Possible convergence of microscopic pathology and macroscopic radiology

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This article presents a pilot study of multispectral diffuse optical tomography for noninvasively imaging volume fraction and mean size of cellular scattering components in the breast. Cellular morphology images for a total of 14 cases (four malignant breast and ten benign lesions) were obtained. Analyzing the images based on the pathological findings of the cases studied, we found that light scattering in the breast was contributed from both the nucleus and organelles such as mitochondria and nucleolus. Based on the image analysis of these 14 cases, we found that the differences in the mean size and volume fraction between the malignant and benign lesions are significant. The contrast ratio of the average mean size and volume fraction between malignant and benign lesions were calculated to be 3.38 and 2.63. These initial results suggest that cellular mean size and volume fraction may be two new criteria that could be used to differentiate malignant from benign lesions. © 2008 American Association of Physicists in Medicine.

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I. INTRODUCTION

The capability of imaging tumor cellular morphology noninvasively is significant because it not only leads to better understanding of tumor formation and growth in tumorigenesis studies, but also potentially generates a new imaging contrast mechanism for tumor diagnosis due to the fact that the cellular size and volume fraction of tumors are generally enlarged relative to that of the surrounding normal tissue.¹ In particular such a capability allows for possible correlation of microscopic pathologic findings with that from macroscopic radiology. Light scattering provides a way to achieve this capability since light is scattered differently when it propagates through different cellular components such as nucleus and mitochondria in tissue and the analysis of light spectroscopy allows the extraction of the cellular signatures.

Cellular analysis of light spectroscopy is, however, a challenging task because cells have complicated structures and the organelles inside a cell vary in size from a tenth of micron to 10 μm . The fact that all the organelles contribute to the scattering more or less complicates the cell scattering phenomena. Using fiber-optic polarized light scattering spec-

troscopy techniques, Mourant *et al.* estimated that the average scatterer radius in tissue was from 0.5 to 1.0 μm , which is much smaller than the nucleus.² In their study, the particle size distribution in mammal cells was also measured and the results suggested that small particles (possibly the mitochondria) contribute most to the scattering. However, other sub-cellular structures, such as the nucleoli and the nucleus, may also contribute significantly to the light scattering.³ Gurjar *et al.* demonstrated that polarized light spectroscopy was capable of providing the cell nuclei size distribution, which means the nucleus was also a major scattering contributor.⁴

Currently, two noninvasive optical imaging methods are being studied for measuring scattering particle size and density. One is the light scattering spectroscopy based technique,⁵ in which polarized light is delivered to the epithelial tissue and the single backscattering light that keeps the polarization is analyzed to extract the morphological features and the refractive index of the scatterers.⁶ It was reported that the initially polarized light lost its polarization after scattered propagation in turbid media such as biological tissues.⁷⁻⁹ In contrast, the single backscattered light kept its

polarization.¹⁰ After subtracting the unpolarized light, the polarized component of the backscattered light from the epithelial layer of the tissue was obtained. An alternative approach to differentiate the backscattering from background is to utilize a probe geometry that optimizes the detection of single scattered light.¹¹ It was demonstrated that the single optical fiber approach was highly sensitive to the light backscattered from layered superficial tissues. While highly sensitive, the polarization light scattering spectroscopy based methods are limited to superficial surface imaging.

The other one, which we are developing, is a tomographic imaging method based on multispectral diffuse optical tomography (DOT).^{12,13} In this method, the tomographic scattering images of tissue at multiple wavelengths are obtained using DOT and the scattering spectra were then used to extract the scatterer's size information with a Mie theory based reconstruction algorithm. We have obtained good results using phantom experiments when only one type of scattering particles was used in the target region.^{12,13} Furthermore, we have investigated how the size of scattering particles affects the extracted size using two types of scattering particles with different volume fractions in the target, and found that the reconstructed scattering particle size was larger than the actual size when two or more scattering particles of larger size were involved.¹⁴

It is noted that both polarization scattering spectroscopy and multispectral DOT based methods for particle/cellular sizing are developed under the classical Mie scattering theory where several assumptions have been made. For example, the particles are spherical and no interactions between particles are considered. In this paper, we demonstrate for the first time our multispectral DOT based method for obtaining *in vivo* cellular scatters' size and fraction in the breast. In the remainder of this article, we describe our method in Sec. II. We detail the clinical results in Sec. III. We present the discussion and conclusion remarks in Sec. IV.

II. METHODS

II.A. Experimental system

The *in vivo* experiments were performed with our ten-wavelength DOT system. The system performance and calibration were described in details elsewhere.^{15,16} Briefly, there are ten laser modules with different wavelengths in the near-infrared region: 638, 673, 690, 733, 775, 808, 840, 915, 922, and 965 nm. Laser beam from one of the ten modules was delivered to the breast surface through one of the 64 source fiber bundles using a 10×64 optical switch. The diffused photon density was measured along the breast surface and used to reconstruct the absorption and scattering coefficient distributions inside the breast with our finite element based DOT algorithm. Although the experimental system was capable of three-dimensional data collection with 64×64 source/detector pairs at each wavelength, in this study, 16×16 source/detector pairs at each wavelength were used for two-dimensional (2D) DOT imaging.

FIG. 1. (a) Craniocaudal (CC) mammogram, (b) mediolateral oblique (MLO) mammogram for the right breast of a 52-year-old patient (patient ID G1). Tumor was indicated by the dotted curve.

II.B. Algorithms

Our tomographic method involves two separate steps for extracting the scattering particle size and volume fraction images in the breast. The first step is to obtain the scattering images at different wavelengths using our 2D DOT algorithm which has been described in detail elsewhere.^{17–19} Briefly, a regularized Newton's method is used to update an initial optical property distribution iteratively in order to minimize an object function composed of a weighted sum of the squared difference between computed and measured optical data at the surface of the breast. The computed optical data (i.e., photon density) are obtained by solving the photon diffusion equation with the finite element method.

The second step is to extract the scattering size and volume fraction images using our particle sizing algorithm in which the difference between measured and computed scattering spectra was iteratively minimized by adjusting the optimization parameters under Mie scattering theory.^{20–23} Once the reduced scattering spectra, $\mu'_s(\lambda)$, are recovered using DOT algorithm, the following relationship from Mie theory allows us to obtain the particle size distribution and concentration:^{20,22}

$$\mu'_s(\lambda) = \int_0^\infty \frac{3Q_{\text{scat}}(x, n, \lambda)[1 - g(x, n, \lambda)]}{2x} \phi f(x) dx, \quad (1)$$

where Q_{scat} is the scattering efficiency; g is the average cosine of scattering angles; x is the particle size; n is the refractive index of particles; ϕ is the particle concentration/volume fraction; $f(x)$ is the particle size distribution. Both Q_{scat} and g can be computed with Mie theory.²⁴ In order to solve for $f(x)$ and ϕ from measured scattering spectra, an inversion of Eq. (1) must be obtained. The Newton-type iterative inversion was used to minimize the squares of the objective functional:

FIG. 2. The reconstructed coronal scattering images at nine wavelengths from 638 to 922 nm (a to i) for patient G1. The axes (left and bottom) are the spatial scale (mm), whereas the color scale (right) is the reduced scattering coefficient (mm^{-1}). Fig. j indicated the corresponding orientations of the reconstructed optical images.

FIG. 3. The extracted coronal images of scattering particle MD (a) and VF (b) for patient G1. The axes (left and bottom) are the spatial scale (mm), whereas the color scale (right) is the value of MD (μm) or VF (%).

$$\chi^2 = \sum_{j=\lambda_1}^{\lambda_{10}} [(\mu'_j)^o - (\mu'_j)^c]^2, \quad (2)$$

where $(\mu'_j)^o$ and $(\mu'_j)^c$ are the observed and computed reduced scattering coefficients at ten wavelengths, $j = \lambda_1, \lambda_2, \dots, \lambda_{10}$ (more wavelengths can be used, depending on the number of wavelengths available with the experimental system). In the reconstruction, we have assumed a Gaussian particle size distribution in this study,

$$f(x) = \frac{1}{\sqrt{2\pi}b} e^{-\frac{(x-a)^2}{2b^2}}, \quad (3)$$

where a is the average diameter of particles and b is the standard deviation. Substituting Eq. (3) into Eq. (1), we obtain

$$\mu'_s(\lambda) = \int_0^\infty \frac{3Q_{\text{scat}}(x, n, \lambda)[1 - g(x, n, \lambda)]}{2x} \phi \frac{1}{\sqrt{2\pi}b} e^{-\frac{(x-a)^2}{2b^2}} dx. \quad (4)$$

Now the particle sizing task becomes to recover two parameters a and ϕ where b is typically set to be 1% of a (larger values of b , e.g., $b=10\%$ of a , were also tested which gave similar results as that for $b=1\%$ of a). As described in detail in Refs. 20 and 21, we have used a combined Marquardt–Tikhonov regularization scheme to stabilize the reconstruction procedure.

III.C. Patient examination

Our clinical study was approved and monitored by the Institutional Review Board. Each enrolled patient signed the consent form. The patients were examined with the fiber optic interface placed at the plane of the known breast lesions from mammography. Each optical exam took about 30 min. Optical imaging results were compared with the mammograms and the biopsy reports.

III. RESULTS

We have performed clinical exams on 14 women to test the idea of imaging cellular morphology *in vivo*. Here we first show typical cellular morphologic images for a malignant case and a benign case. We also summarize the results over the 14 cases (four malignant and ten benign lesions) where we attempt to correlate the tomographic images with

FIG. 4. (a) CC and (b) mediolateral (ML) mammogram of the right breast for patient S5. Tumor was indicated by the dotted curve.

pathologic findings for three of the four malignant cases.

III.A. Infiltrating ductal carcinoma

Optical exam was conducted for the right breast of a 52-year-old woman (patient ID G1). The right craniocaudal (CC) and mediolateral oblique (MLO) mammograms for the patient were shown in Figs. 1(a) and 1(b), respectively. An ill-defined spiculated mass was found in the center lateral portion of the breast, which lay under a marker for the palpable abnormality. BI-RADS category was 4. Sonograms also demonstrated an ill-defined hypoechoic mass with lobular margins measuring approximately $1.0 \times 1.6 \times 1.0$ cm in the position corresponding to the abnormality noted in the mammogram. After biopsy, mastectomy was performed and the surgery confirmed that the patient had an invasive ductal carcinoma in the right breast. Cut surfaces revealed a $1.2 \times 1.2 \times 1.3$ cm retracted and firm nodule of pink-tan tumor tissue in the lower outer quadrant subject to the biopsy site on the skin.

The DOT imaging was performed one week before the biopsy and the mastectomy surgery. The reconstructed scattering images at nine wavelengths from 638 to 922 nm of the examined breast were shown in Figs. 2(a) to 2(i), respectively. From these scattering images, we note that one target is detected at the location of 6 o'clock, while the mammograms indicate the tumor around 6–9 o'clock. A central artifact was noted in Fig. 2(a) due to the relatively lower signal-to-noise ratio at 638 nm. The artifact slightly degraded the subsequent reconstruction of scattering particle mean diameter and volume fraction in this case.

The scattering images at nine wavelengths were used to extract the scattering particle mean diameter (MD) image in μm [Fig. 3(a)] and the scattering particle volume fraction (VF) image in % [Fig. 3(b)]. From Fig. 3(a), the maximum MD in the tumor region was found to be $3.1 \mu\text{m}$ and the average MD in the tumor and its surrounding were found to be 2.18 and $0.45 \mu\text{m}$, respectively. This indicates a high tumor-to-tissue MD contrast ratio of 4.84. Similarly, from Fig. 3(b), the maximum VF in the tumor region was found to be 1.6% and the average VF in the tumor and its surrounding were 1.32% and 0.48%, suggesting a tumor-to-tissue VF contrast ratio of 2.75. In these calculations, the tumor area was estimated using the criterion of full width at half maximum of the parameter profiles. From both the MD and VF

FIG. 5. The reconstructed coronal scattering images at nine wavelengths from 638 to 922 nm (a to i) for patient S5. The axes (left and bottom) are the spatial scale (mm), whereas the color scale (right) is the reduced scattering coefficient (mm^{-1}).

images, the suspicious tumor was located in a relatively small region, which is consistent with the surgery report of $1.2 \times 1.2 \times 1.3$ cm tumor.

III.B. Benign nodule

The second patient was a 69-year-old female volunteer (patient ID No. S5). Figure 4 presents the CC (a) and ML mammogram (b) of the right breast. In the mammograms, a stellate area of architectural distortion and asymmetric density was noted in the superior lateral quadrant, where the patient felt a palpable mass. But the sonography report indicated that no discrete mass was identified in the area of the known mammographic abnormality; there was some slight shadowing in this area. While biopsy was not performed for this patient, the stability of the lesion on the mammogram at 6 and 12 months following initial work-up served as an indicator that the lesion was most likely benign.

The reconstructed scattering images at nine wavelengths from 638 to 922 nm (a to i) were shown in Fig. 5, which were used to extract the scattering particle MD image [Fig. 6(a)] and VF image [Fig. 6(b)]. From Figs. 5 and 6, we see

that one target is identified at the position around 7–8 o'clock while the mammograms detect the abnormality around 9–12 o'clock.

As shown in Fig. 6, the scattering particle MD increased in the target region while the VF in the target region decreased. The maximum MD in the lesion region was found to be $0.65 \mu\text{m}$ and the average MD in the lesion and its surrounding were found to be 0.53 and $0.11 \mu\text{m}$, respectively. Again we see a high lesion-to-tissue MD contrast of 4.82 for this benign case. The minimum VF in the lesion region was found to be 0.2% while the average VF in the lesion and its surrounding were found to be 0.42% and 0.93% , giving a low lesion-to-tissue VF contrast of 0.45 .

III.C. Image analysis

The scattering particle MD and VF images for 14 cases (ten benign and four malignant) were reconstructed for image analysis. Figure 7(a) is the scatter plot (maximum MD vs. maximum VF in the lesion; averaged MD vs. averaged VF in normal tissue region) for all the 14 cases. We see that the malignant and benign lesions are clearly separable with the exception of a benign case that is embedded in the ma-

FIG. 6. The extracted coronal images of scattering particle MD (a) and VF (b) for patient S5. The axes (left and bottom) are the spatial scale (mm), whereas the color scale (right) is the value of MD (μm) or VF (%).

lignant group. In the exception benign case there was a strong central artifact dominating the reconstructed MD image (not shown here), resulting in its poor separation from the malignant lesions. The results shown in Fig. 7(a) indicate that the MD and VF of malignant lesions are different from that of the benign lesions and the normal tissue regions, while the MD and VF of benign lesions are similar to that of the normal tissue regions when all the benign cases are “statistically” considered. However, for each individual case, the benign lesions can be differentiated from the normal tissue regions, as shown in Fig. 6.

The average values and the standard deviations of the peak MD and peak VF in the malignant/benign lesions and their surroundings are shown in Fig. 7(b). We can immediately tell that the differences in the MD and VF between the malignant and benign lesions are significant. We note that the average MD for malignant lesions was calculated to be $4.4 \mu\text{m}$ with a standard deviation of $1.4 \mu\text{m}$, while it was $1.3 \mu\text{m}$ with a standard deviation of $1.4 \mu\text{m}$ for benign lesions. The ratio of average MD of malignant tumors to that of benign lesions was calculated to be 3.38. The average VF for malignant lesions was calculated to be 1.74% with a standard deviation of 0.65%, while it was 0.66% with a standard deviation of 0.51% for benign lesions. The ratio of average VF of malignant tumors to that of benign lesions was found to be 2.63. From Fig. 7(b), we also note that MD and VF of benign lesions are close to that of the normal tissue and MD and VF of the malignant tumors are three times larger than that of the normal tissue and benign lesions.

III.D. Pathological co-registration

The microscopic images of tissue for three malignant cases (patients G1, G2 and G8) were obtained, as shown in Fig. 8. From these microscopic images, we estimated the average MD and the approximate VF of the cancer cell nucleus and nucleolus. The MD of nucleolus was approximated as 0.2 times the corresponding nucleus. The estimated values were shown in Table I. The VF of nucleolus was calculated as $(0.2)^3 = 0.008$ times the corresponding nucleus VF. Average MD and VF calculated from the recovered optical images for the three cases are also given in Table I. We see that the recovered average scattering particle MD in the tumor for cases 1, 2, and 3 were, respectively, 55.3%, 6.5%, and 31.9% less than the average MD of nucleus and were several times larger than the average MD of nucleolus found

FIG. 7. (a) The peak value of the recovered MD versus the peak value of VF in the lesion and the average value of recovered MD versus the average value of VF in the normal regions for all the benign and malignant cases. (b) The average values of recovered peak MD and VF in the lesion region. And the average values of the averaged MD and VF in the normal region for the malignant and benign cases, respectively. The dark lines on bars indicate the standard deviations.

in the microscopy. Similarly, we found that the extracted average VFs were 77.9%, 60.3%, and 76.4% less than the nucleus VF and were ten times larger than the VF of nucleolus. These quantitative differences are largely due to the fact that the scattering in tissue is contributed by both nucleus and other smaller particles including nucleolus and mitochondria which is represented by a single modal scattering model in the reconstruction.

IV. DISCUSSION AND CONCLUSIONS

In this article, we have obtained the tomographic images of scattering particle MD and VF in the breast which are directly correlated with the subcellular structures. However, we note the clear quantitative discrepancy in the MD and VF values between the optically recovered and microscopy

FIG. 8. The pathological microscopic images of tumor cells for patient G1 (a), G2 (b), and G8 (c).

(Table I). In a prior study we found that the reconstructed MD value was always between the MD of small particles and the MD of large particles when bi-modal scattering particles were actually involved, but the single modal scattering model was used for reconstruction;¹⁴ the actual recovered MD was dependent on the VF ratio of the two kinds of

TABLE I. The MD and VF of the nucleus and nucleolus estimated from the microscopy and those of scattering particles calculated from the optical images reconstructed with one-particle scattering model.

Cases	Microscopy					
	Nucleus		Nucleolus		DOT	
	MD (μm)	VF (%)	MD (μm)	VF (%)	MD (μm)	VF (%)
G1	4.9 ± 0.48	5.9	1.0 ± 0.11	0.047	2.2 ± 0.25	1.3 ± 0.16
G2	4.4 ± 0.28	4.3	0.9 ± 0.06	0.034	4.1 ± 0.38	1.7 ± 0.10
G8	5.4 ± 0.44	6.8	1.1 ± 0.13	0.054	3.7 ± 0.09	1.6 ± 0.09

particles. Thus we believe that the reconstructed MD and VF presented in Table I were contributed by both large particles such as nucleus and small particles such as nucleolus and mitochondria. To illustrate this further, here we show recovered results using a bi-modal scattering model²⁵ where four parameters needed to be reconstructed based on the scattering spectra. We assumed that the total scattering at each pixel/node was the summation of the scattering from each kind of particle. In this model, there are four unknown parameters, a_1 and ϕ_1 for one kind of particles plus a_2 and ϕ_2 for another kind of particles. These four unknown parameters were then simultaneously reconstructed using an inversion algorithm similar to that described in Sec. II B. For a typical node in the tumor region, the bi-modal based results for cases G1, G2, and G8 are shown in Table II. The relative errors of the recovered MD/VF for the large scattering particle, compared with that for the nucleus found in the microscopy, have now become 8.6%/11.9%, 10.1%/47.4%, and 75.1%/54.6% for cases G1, G2 and G8, respectively. We see that the two-particle model gives better results than the one-particle model for the large particles such as nucleus.

For small particles, one can see from Table II that the reconstructed MD is close to that of nucleolus, but the recovered VF is two times larger than that of the nucleolus, which means that the scattering contribution from the small particles should include not only the nucleolus but also other small particles such as mitochondria. Figure 9 presents the scattering spectra at a typical node recovered using DOT as well as that fitted using one- or two-particle model. We see that the fitted spectra match much better with the recovered

TABLE II. The MD and VF of the nucleus and nucleolus estimated from the microscopy and those of scattering particles calculated from the optical images reconstructed with two-particle scattering model.

Cases	Microscopy				DOT			
	Nucleus		Nucleolus		Large particles		Small particles	
	MD (μm)	VF (%)	MD (μm)	VF (%)	MD (μm)	VF (%)	MD (μm)	VF (%)
G1	4.9 ± 0.48	5.9	1.0 ± 0.11	0.047	5.3	3.1	0.7	0.10
G2	4.4 ± 0.28	4.3	0.9 ± 0.06	0.034	3.9	1.1	0.9	0.10
G8	5.4 ± 0.44	6.8	1.1 ± 0.13	0.054	5.9	3.1	0.9	0.10

FIG. 9. The scattering spectra at one pixel/node in the scattering image for a selected case (patient G1), where solid line with cross markers for total scattering, dashed line for large particle scattering, solid line for small particle scattering and scattered dots for reconstructed scattering coefficient. The fitting results were obtained using one-particle model (large or small particle) or two-particle model.

spectra, while we note that there are some spectral fluctuations at the shorter or longer wavelengths due to the relatively lower signal-to-noise ratio at these wavelengths.

In this work, the MD and VF images were obtained in two separated steps as described in Sec. II B. The first step was the finite element based DOT reconstruction for recovering scattering spectra at each wavelength. The second step was the extraction of MD and VF using the recovered scattering spectra at each nodal location without involving finite element method. In a tumor suspicious area found in a single scattering image [e.g., Figs. 2(a)–2(i)], each node has a different scattering spectrum, resulting in different MD and VF values at different nodal locations in the area, as shown in Fig. 3. We note that only a partial area of the tumor containing larger particle size/fraction is seen in Fig. 3, relative to that shown in Fig. 2.

In the first step, both absorption and reduced scattering images at each wavelength were reconstructed simultaneously, while one may note that the measurements at nine wavelengths were used to reconstruct the absorption and scattering images and the measurement at 965 nm was dropped off due to the poor signal-to-noise ratio at this wavelength. For brevity, the absorption images were not shown here as the study of scattering images was the focus of this work. The absorption spectral images could be used to extract the hemoglobin concentration and water content images in the tissue as described in Ref. 15. We have also recently implemented a method that allows direct reconstruction of hemoglobin concentration, water content, and volume fraction of different scattering particles.²⁶

In this study, mammograms, showing approximate positions of suspicious lesions, provided a method to validate the suspicious lesion locations detected by the optical methods. However, since the two imagings were performed very differently (compression of the breast for x-ray vs. noncompress-

sion for optical), exact anatomical correlation between the two methods is impossible. This also caused some discrepancies in the lesion locations detected by these two different modalities.

In this article, we have assumed that the scattering particles such as nucleus or mitochondria in the breast are spherical in order to use the Mie theory effectively (we note that in a recent study by Wang *et al.*²⁷ similar effective scatterer size and number density were estimated using an empirical power law that is an approximation to the Mie theory). In practice, however, these particles are nonspherical. Advanced scattering theories are needed to more accurately describe light scattering at cellular level. Recent progress in reduced-order expressions for the total scattering cross section spectra of nonspherical particles provides a possible solution to the problem.²⁸ Another method to study the scattering of complex structures is finite-difference time-domain modeling.^{29,30} Exact co-registration of optical imaging with other methods such as biopsy and surgery is important but difficult. We have compared our reconstructed MD and VF for three malignant cases with the pathological microscopy of tumor tissue samples. However, the exact spatial correlation between the samples and the reconstructed images are not available. A method to accurately realize the co-registration is expected to play an important role in tomographic imaging of cellular morphology.

In sum, in this work, we have studied 14 clinical cases including ten benign and four malignant using multispectral DOT for noninvasive cellular imaging. The image analysis has shown that the scattering particle MD and VF that can be obtained using multispectral DOT may provide new parameters for differentiating malignant from benign abnormalities in the breast. In addition, we have found that light scattering in breast tissue is contributed from both the nucleus and smaller particles such as nucleolus and mitochondria.

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