Early-Stage Invasive Breast Cancers: Potential Role of Optical Tomography with US Localization in Assisting Diagnosis¹

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Purpose:

To investigate the potential role of optical tomography in the near-infrared (NIR) spectrum with ultrasonographic (US) localization as a means of differentiating early-stage cancers from benign lesions of the breast.

Materials and Methods:

The protocol was approved by the institutional review boards and was HIPAA compliant; all participants signed an informed consent. One hundred seventy-eight consecutive women (mean age, 52 years; range, 21–89 years) who underwent US-guided biopsy were imaged with a hand-held probe consisting of a coregistered US transducer and an NIR imager. The lesion location provided by coregistered US was used to guide optical imaging. Light absorption was measured at two optical wavelengths. From this measurement, tumor angiogenesis was assessed on the basis of calculated total hemoglobin concentration (tHb) and was correlated with core biopsy results. For patients diagnosed with carcinomas and followed up with subsequent excision, the tHb was correlated with pathologic parameters.

Results:

There were two in situ carcinomas (Tis), 35 T1 carcinomas, 24 T2–T4 carcinomas, and 114 benign lesions. The mean maximum and mean average tHb of the Tis–T1 group were $102.0 \mu\text{mol/L} \pm 28.5$ (standard deviation) and $71.9 \mu\text{mol/L} \pm 18.8$, and those of the T2–T4 group were $100.3 \mu\text{mol/L} \pm 26.4$ and $67.0 \mu\text{mol/L} \pm 18.3$, respectively. The mean maximum and mean average tHb of the benign group were $55.1 \mu\text{mol/L} \pm 22.7$ and $39.1 \mu\text{mol/L} \pm 14.9$, respectively. Both mean maximum and mean average tHb levels were significantly higher in the malignant groups than they were in the benign group ($P < .001$). The sensitivity, specificity, positive predictive value, and negative predictive value for Tis–T1 cancers were 92%, 93%, 81%, and 97%. The corresponding values for T2–T4 tumors were 75%, 93%, 69%, and 95%.

Conclusion:

The angiogenesis (tHb) contrast imaged by using the NIR technique with US holds promise as an adjunct to mammography and US for distinguishing early-stage invasive breast cancers from benign lesions.

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The increased use of breast cancer screening has resulted in more patients with small lesions being identified in mammographic screening programs (1–3). To further characterize solid lesions, ultrasonography (US) of the breast is more commonly being used as an adjunct to mammography (4,5). In addition, US may have a role in the screening of women with dense breasts (6,7). However, the correct diagnosis of a suspicious lesion at US may be difficult because of the overlap between features of malignant and benign breast lesions, especially when the lesions are small (8,9). Consequently, diagnostic confirmation often requires an image-guided biopsy procedure. In current clinical practice, 70%–80% of biopsies yield benign lesions (10).

Diffuse optical tomography in the near-infrared (NIR) region is an emerging modality with potential applications in radiology and oncology (11–28). Diffuse optical tomography provides functional quantification of tumor hemoglobin content, which is directly related to tumor angiogenesis (29) and tumor hypoxia (30). Thus, it is crucial to deter-

mine the optical properties of a lesion as accurately as possible to take advantage of these recognized attributes.

Most studies that are based on pure optical measurements have fundamental limitations owing to the intense light scattering in tissue. In general, localization or imaging that is based on tomographic inverse scattering approaches suffers from location uncertainty and imprecise target qualification. Many research groups have investigated the use of a priori lesion structure information provided by other imaging modalities, such as US (27,28), magnetic resonance (MR) imaging (24,25), and mammography (26) to improve the localization of optical tomography. A flexible light guide with the use of optical fibers makes optical imaging compatible with many other imaging modalities and allows for simultaneous imaging with identical geometric conditions. Furthermore, the lesion structure information provided by other modalities can be used to assist optical imaging reconstruction, therefore reducing the location uncertainty and improving the quantification accuracy of light.

Our initial clinical results obtained from US-guided optical tomography have shown that early-stage invasive cancers may be distinguished from benign lesions with an average of a twofold higher total hemoglobin concentration (tHb) contrast (hereafter referred to as *tHb contrast*) than that of benign lesions (27,28). However, breast cancer is a heterogeneous disease with different subtypes, grades, and metabolic rates that result in a wide range of functional differences. The purpose of our study was to investigate the potential role of optical tomography in the NIR spectrum with US localization as a means of differentiating early-stage cancers from benign lesions of the breast.

Implication for Patient Care

- The NIR technique with US can provide a safe, quick, noninvasive method that may aid US in accurate diagnosis of small indeterminate breast lesions.

Materials and Methods

Patients

The study was performed at the Department Radiology, University of Connecticut Health Center (Farmington, Conn), from September 1, 2004, to September 30, 2008, and at the Department of Radiology, Hartford Hospital (Hartford, Conn), from September 1, 2007, to September 30, 2008. The study protocol was approved by the institutional review boards of both institutions and was Health Insurance Portability and Accountability Act compliant. Written informed consent was obtained from all patients. The eligible patients were initially referred for US-guided biopsy with requisitions indicating solid masses, and their lesions were identified by using US at the time of the study. Individuals with lesions that were not identifiable by using US were not specifically examined; however, four such patients were used as control subjects. For these four patients, data were acquired at a different quadrant away from the questionable areas. Control data also included data from an additional 11 study patients

Advances in Knowledge

- With the use of optical tomography, a twofold higher total hemoglobin concentration (tHb) was found in malignant invasive breast cancer lesions (for Tis–T1 group, mean maximum was $102.0 \mu\text{mol/L} \pm 28.5$ [standard deviation] and mean average was $71.9 \mu\text{mol/L} \pm 18.8$; for T2–T4 group, mean maximum was $100.3 \mu\text{mol/L} \pm 26.4$ and mean average was $67.0 \mu\text{mol/L} \pm 18.3$) than in benign lesions (mean maximum, $55.1 \mu\text{mol/L} \pm 22.7$; mean average, $39.1 \mu\text{mol/L} \pm 14.9$), with $P < .001$.
- With the use of near-infrared (NIR) data with US location and a threshold level of tHb of $82 \mu\text{mol/L}$, the sensitivity, specificity, positive predictive value, and negative predictive value for Tis–T1 cancers were 92%, 93%, 81%, and 97%, respectively.

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Abbreviations:

CI = confidence interval
FWHM = full width at half maximum
NIR = near-infrared
ROI = region of interest
tHb = total hemoglobin concentration

Author contributions:

Guarantors of integrity of entire study, Q.Z., P.J.D.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, Q.Z., P.U.H., A.R., E.B.C.; clinical studies, Q.Z., P.U.H., M.K., E.B.C., Y.A., C.X., A.A., S.H.K., P.J.D., S.H.T.; experimental studies, Q.Z., Y.A., C.X., A.A., P.J.D.; statistical analysis, Q.Z.; and manuscript editing, Q.Z., P.U.H., A.R., M.K., E.B.C., S.H.K., P.J.D., S.H.T.

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whose data were acquired from quadrants away from reference lesions at presentation. These 11 study patients had large breasts and small lesions, and the control data were not affected in any way by the lesions. The final study group consisted of 178 consecutive women whose ages ranged from 21 to 89 years (mean age, 52 years). Of the 178 patients in the study, 16 were excluded from analysis. Data in four patients were not recorded appropriately owing to data acquisition problems. Four patients had small dense breasts with the chest wall about 1 cm deep to the skin surface. The poor probe-tissue contact and the heterogeneous chest wall caused substantial image artifacts. Data in six patients were from one breast each, and thus reference data from the contralateral breast was not available. Two patients had lesions in the dark nipple–areolar complex region that were not imaged correctly because of the high absorption of the dark nipple–areolar complex.

Pathologic Assessment

All core biopsy and surgical specimens were initially reviewed by an attending pathologist. Two breast pathologists (A.R., 25 years of experience; P.U.H., 8 years of experience), who were blinded to optical imaging results, retrospectively reviewed all the hematoxylin-eosin-stained slides to validate the information about primary tumor size with gross and/or microscopic measurements, tumor type, tumor grade (Nottingham score), as well as histologic evidence of carcinoma in situ, if any, for cases in patients from each corresponding hospital. For patients who underwent neoadjuvant chemotherapy ($n = 16$), the initial diagnostic core biopsy reports and the hematoxylin-eosin-stained slides, as well as the pretreatment clinical staging that was based on imaging results, were used to obtain tumor information.

Benign lesions were similarly handled. They were initially reviewed by attending pathologists from the participating hospitals. The two pathologists mentioned above further retrospectively reviewed all the hematoxylin-eosin-stained slides

Figure 1

Figure 1: Combined US and NIR systems and a handheld probe with a centrally located US linear array and NIR source-detector fibers distributed at the periphery of the probe.

and classified benign lesions as proliferative with atypia, proliferative without atypia, and nonproliferative lesions. The proliferative lesions without atypia included adenosis variants, moderate to florid degrees of hyperplasia, sclerosing lesions, papilloma, radial scars or complex sclerosing lesions, and fibroadenoma with complex proliferative features (31). The nonproliferative lesions were fibrocystic changes, including mild hyperplasia, fibroadenoma without complex features, fat necrosis and/or other inflammatory changes, cysts, intramammary lymph nodes, and normal or physiologic breast tissue.

US and NIR Systems

US examinations were performed with a linear transducer (L12; Philips Medical Systems, Bothell, Wash) of the US unit (IU22; Philips Medical Systems) at University of Connecticut Health Center and a linear transducer of the US unit (15L8 Sequoia; Acuson, Mountain View, Calif) at Hartford Hospital. US lesions in patients who were referred for core biopsy were evaluated by two radiologists (M.K. and E.B.C., with 11 and 21 years of experience, respectively) and attending

radiologists from the participating hospitals. Two NIR systems with identical electronic and optical designs were used at the two hospitals (Fig 1, Appendix E1 [online]). The probe consists of a commercial US transducer located in the middle, with optical source and detector fibers distributed at the periphery (32). Two optical wavelengths, 780 and 830 nm, were used, and optical absorptions at these two wavelengths were used to calculate the tHb of the lesion, which was used to assess tumor angiogenesis. The entire NIR data acquisition interval was less than 5 seconds. For each patient, US images and optical measurements were acquired simultaneously before biopsy procedures at multiple locations, including the lesion region and a normal region of the contralateral breast in the same quadrant as that in which the lesion was located. The optical data acquired from the normal contralateral breast were used as reference for calculating the background optical absorption and reduced scattering coefficients that were used in image reconstruction of the lesions. Mammograms and US images obtained before the NIR image were reviewed by attending radiologists, and all

Table 1

Mean Values of Tis–T1, T2–T4, and Benign Groups

Group	Average Age*	No. of Lesions	Average Size Measured with US (cm) [†]	tHb (μmol/L) [‡]	
				Maximum	Average in FWHM
Tis–T1	59 (38–87)	37	$0.9 \pm 0.39 \times 0.7 \pm 0.23$	102.0 ± 28.5	71.9 ± 18.8
T2–T4	60 (37–89)	24	$2.9 \pm 1.07 \times 1.9 \pm 0.50$	100.3 ± 26.4	67.0 ± 18.3
Proliferative lesion	44 (23–72)	24	$1.2 \pm 0.46 \times 0.8 \pm 0.37$	65.5 ± 25.2	45.6 ± 17.3
Nonproliferative fibrocystic change	49 (21–76)	28	$1.3 \pm 0.99 \times 0.8 \pm 0.58$	55.4 ± 20.2	39.3 ± 12.2
Fibroadenoma	46 (29–84)	33	$1.2 \pm 0.83 \times 0.7 \pm 0.50$	51.5 ± 23.5	37.6 ± 16.0
Fat necrosis and/or inflammatory and reactive	51 (38–68)	8	$2.1 \pm 0.87 \times 1.3 \pm 0.45$	42.9 ± 25.7	29.4 ± 15.5
Complex cysts	55 (31–82)	16	$0.9 \pm 0.56 \times 0.61 \pm 0.29$	52.5 ± 19.0	37.8 ± 12.7
Breast tissue	53 (38–65)	3	$1.5 \pm 0.36 \times 0.9 \pm 0.37$	57.4 ± 17.3	36.7 ± 8.0
Lymph nodes	50 (42–57)	2	$0.7 \pm 0.23 \times 0.4 \pm 0.13$	51.4 ± 6.8	36.1 ± 3.8
Benign	48 (21–84)	114	...	55.1 ± 22.7	39.1 ± 14.9
Control	58 (30–84)	15	...	28.7 ± 12.5	21.8 ± 9.2

* Numbers in parentheses are ranges.

[†] Data are the mean $\pm$ standard deviation. Data are lateral $\times$ depth measurements.[‡] Data are the mean $\pm$ standard deviation.

contralateral measurements were taken at normal areas on the basis of the available information.

NIR Imaging

The details of our dual-zone mesh optical imaging reconstruction algorithm have been described previously (28,33). Briefly, the reconstruction takes advantage of US localization of lesions and segments the imaging volume into the US-identified region of interest (ROI) and background nonlesion regions. Because the spatial resolution of diffused light is poorer than that of US, the ROI used for optical reconstruction is chosen to be at least two to three times larger than that seen by using US in the x-y dimensions. In addition, because the depth localization of diffused light is very poor, a tighter ROI in-depth dimension is mainly set by using coregistered US and, therefore, depends on margins seen by using US. Once the ROI is chosen, the optical imaging reconstruction is repeatable for each NIR data set and does not depend on operators. One author (Q.Z., 10 years of experience) performed optical imaging. In all optical images, a finer $0.25 \times 0.25 \times 0.5$ -cm imaging grid is used for the ROI, and a coarse $1.5 \times 1.5 \times 1.0$ -cm grid is

used for the background. This scheme effectively aids in reducing the total number of voxels with unknown optical absorption to the same order of total measurements. In addition, the total absorption is reconstructed, and the total is divided by the different voxel sizes of the lesion and background to obtain the absorption distribution. This scheme further conditions the inversion (28). As a result, the inversion is stable and converges quickly in three to four iterations. Furthermore, because the voxel size of the coarse grid is 72 times larger than that of the finer grid, the lesion-to-background tissue contrast is substantially improved. The tHb distribution is then computed from absorption maps (28,34). Maximum lesion tHb was measured, and the average was computed inside the lesion, with the tHb within 50% of the maximum value (ie, within the full width at half maximum [FWHM] region). To compute the sensitivity, specificity, and positive and negative predictive values that were based on all data obtained from this large patient pool at the end of the study, a tHb threshold level was retrospectively selected to separate most of the tumors from the benign lesions. False-negative cases were malignant tumors with tHb

content lower than the threshold level and false-positive cases were benign lesions with tHb content higher than the threshold level.

Because these quantitative measurements do not account for tHb pattern distribution differences as observed in large malignant cancers, we used three qualitative features previously described in other imaging techniques to assign a score to the large cancer tHb distribution at the time of imaging as heterogeneous peripheral enhancement, posterior shadowing, and uniform patterns. Peripheral enhancement and uniform patterns are frequently used in MR imaging to further differentiate benign from malignant lesions (35,36), whereas posterior acoustic shadowing is a finding used in US to suggest malignancy (5).

We acquired multiple coregistered US and NIR data sets at the lesion location and reconstructed the corresponding optical absorption maps, as well as the tHb distribution with coregistered US guidance. The NIR data sets with patient motion, as evaluated by using two coregistered US images acquired before and after each NIR data set, were excluded from averaging. The data for each patient were an average value that was obtained from three to four quality NIR images.

Figure 2

Figure 2: Maximum tHb in micromoles per liter in three patient groups. NPBL = nonproliferative benign lesions, PBL = proliferative benign lesions.

Figure 3

Figure 3: Scatterplot of reconstructed maximum tHb in micromoles per liter in 10 patient groups. The Tis-T1 group included ductal carcinoma in situ and lobular carcinoma in situ and stage I tumors. FA = fibroadenoma, FN-INF = fat necrosis and/or inflammatory and reactive changes, PBL = proliferative benign lesion, NPFCC = nonproliferative fibrocystic changes, ≥T2 = T2-T4 tumors.

Statistical Analysis

A two-sample *t* test was used to calculate significance for comparisons between groups and the 95% confidence interval (CI). A difference with a *P* value of .05 was considered to be significant. Binomial 95% confidence limits of sensitivity, specificity, positive predictive value, and negative predictive value were computed. Software (Minitab 15; Minitab, State College, Pa) was used for statistical calculation.

Results

The numbers of malignant lesions were as follows: Tis-T1a (*n* = 4), T1b (*n* = 10), T1c (*n* = 23), and T2-T4 (*n* = 24) tumors (Tables E1, E2 [online]; Appendix E1 [online]). Benign lesions were proliferative lesions with and without atypia (*n* = 24), nonproliferative fibrocystic changes (*n* = 28), fibroadenoma (*n* = 33), fat necrosis and/or inflammatory and reactive changes (*n* = 8), complex or simple cysts (*n* = 16), normal breast tissue (*n* = 3), lymph node (*n* = 2), and control cases (*n* = 15) (Table 1).

A twofold higher tHb was found in malignant groups (for the Tis-T1 group, the mean maximum was 102.0 μmol/L ± 28.5 [standard deviation] and the mean average was 71.9 μmol/L ± 18.8; for the T2-T4 group, the mean maximum was 100.3 μmol/L ± 26.4 and the mean average was 67.0 μmol/L ± 18.3) than in all benign groups (mean maximum, 55.1 μmol/L ± 22.7; mean average, 39.1 μmol/L ± 14.9 [*P* < .001]) (Fig 2). The mean differences of maximum and average were 47.2 μmol/L (95% CI: 38 μmol/L, 56 μmol/L) and 33.1 μmol/L (95% CI: 27 μmol/L, 39 μmol/L) between the Tis-T1 and benign groups, and the mean differences of maximum and average were 45.2 μmol/L (95% CI: 35 μmol/L, 56 μmol/L) and 27.9 μmol/L (95% CI: 21 μmol/L, 35 μmol/L) between the T2-T4 and benign groups. The proliferative lesion group showed a higher tHb content (mean maximum, 65.5 μmol/L ± 25.2; mean average, 45.6 μmol/L ± 17.3) than the other benign lesion groups (mean maximum, 52.3 μmol/L ± 21.3; mean average,

37.4 $\mu\text{mol/L} \pm 13.8$) ($P < .04$). Both maximum and average tHb levels of malignant groups, the proliferative lesion group, and the other benign lesion group were significantly higher than those in the control group (mean maximum, 28.7 $\mu\text{mol/L} \pm 12.5$; mean average, 21.8 $\mu\text{mol/L} \pm 9.2$) ($P < .001$).

Multiple NIR data sets were obtained at the lesion site, and average values of tHb were used to characterize each lesion. The mean standard deviations of maximum tHb of Tis-T1, T2-T4, and benign lesions were 6.2, 5.7, and 5.3 $\mu\text{mol/L}$, respectively; the mean standard deviations of average tHb were 4.2, 3.9, and 3.3 $\mu\text{mol/L}$, respectively, for the corresponding groups. Thus, on average, about a 5 $\mu\text{mol/L}$ change could be encountered in repeated imaging, and the change is independent of lesion type.

If 82 $\mu\text{mol/L}$ was chosen as a threshold level to distinguish between malignant and benign lesions, the sensitivity, specificity, positive predictive value, and negative predictive value for Tis-T1 tumors were 92% (34 of 37 lesions [95% CI: 78%, 98%]), 93% (106 of 114 lesions [95% CI: 87%, 97%]), 81% (34 of 42 lesions [95% CI: 66%, 91%]), and 97% (106 of 109 lesions [95% CI: 92%, 99%]) (Fig 3). The corresponding values for T2-T4 tumors were 75% (18 of 24 lesions [95% CI: 53%, 90%]), 93% (106 of 114 lesions [95% CI: 87%, 97%]), 69% (18 of 26 lesions [95% CI: 48%, 86%]), and 95% (106 of 112 lesions [95% CI: 89%, 98%]).

Typical examples of a benign lesion and T1 and T2-T4 malignant lesions are given in Figures 4-9.

Three false-negative cases were observed in the Tis-T1 group: a 0.11-cm microinvasive T1a ductal carcinoma, a 1.0-cm T1b lobular carcinoma, and a 1.3-cm T1c invasive ductal carcinoma (Table E1 [online], Appendix E1 [online]). The patient with T1c tumor had two lesions located 1.5 cm apart at depths of 1.3 and 2.4 cm. Tumor sizes were 1.3 and 2.0 cm measured from the pathologic specimen, respectively. The NIR image reconstructed at 2.4-cm depth showed one isolated tumor of a much higher tHb, whereas the NIR image

reconstructed at 1.3 cm showed two lesions of similar but lower tHb. Because NIR light does not have good depth resolution, signals from the deeper and larger 2-cm tumor produced some interference to the signals originating from the smaller tumor located at 1.3-cm depth. This resulted in the lower light quantification of the smaller tumor.

Thirty-eight percent (nine of 24) of the T2-T4 tumors showed heterogeneous peripheral enhancement, which was not observed in benign solid lesions. Interestingly, these tumors were all high grade. In addition, 33% (eight of 24) of the T2-T4 tumors showed posterior shadowing. This was caused by the substantial light absorption of a highly vascularized tumor, which causes the dramatic reduction of the reflected light from the deeper portion of the tumor. Four (17%) tumors in the T2-T4 group had a uniform tHb distribution with a lower tHb level, which made them indistinguishable from large benign lesions of a similar size (Table 2, Appendix E1 [online]).

The NIR data for tHb of four proliferative lesions (two each, with and without atypia), one hamartoma in the nonproliferative fibrocystic change category, one fibroadenoma, one fat necrosis with inflammation and reactive changes, and one complex cyst were above the 82 $\mu\text{mol/L}$ threshold level. These lesions were considered as false-positive cases (Table 3, Appendix E1 [online]).

Discussion

Optical tomography has tremendous potential to provide clinically useful functional information about tumor angiogenesis and tumor hypoxia (12). When targeted contrast agents are used, molecular information can also be revealed. Our previously published results obtained from 10 early-stage invasive cancers with size estimated from US and 90 benign lesions showed that malignant lesions presented a twofold higher tHb contrast compared with that in the benign group (27,28). This study, with 37 pathologically staged early-stage cancers and 114 benign lesions, further demonstrates the diagnostic potential of this

technique. The tHb threshold level of 82 $\mu\text{mol/L}$ used to distinguish most of the Tis-T1 tumors from benign lesions was based on the data generated by this large patient pool. In our earlier pilot studies (27,28), a tHb threshold level of 95 $\mu\text{mol/L}$ was used to distinguish between the 10 early-stage invasive cancers and the 90 benign lesions. Because the prior data included data from a limited number of malignant tumors, we believe that the current threshold level better represents the separation between diverse types of earlier-stage malignant cancers and benign lesions, and the data may be used as a training set for future large-scale prospective clinical trials.

Our technique is best suited to characterizing US-visible lesions, and it may prove to be valuable in investigating lesions that are small and considered indeterminate on the basis of mammographic and US results. When our technique is used as an adjunct to mammography and US in screening, the additional tumor angiogenesis information may yield a more accurate preoperative diagnosis. For large US-visible lesions, this technique can provide some tHb distribution features, such as heterogeneous peripheral enhancement and/or posterior shadowing, that can add diagnostic value to US. In addition, the tHb distribution can be monitored and used to assess response in patients who have locally advanced cancers and who are undergoing neoadjuvant chemotherapy (15,37-39).

In patients with benign disease who were examined, two proliferative lesions with atypia and two lesions with radial scar and fibroadenoma with complex features showed high tHb (Table 3). With regard to the breast cancer risk, there is clinicopathologic consensus that, among women who have had a benign breast biopsy, the subsequent breast cancer risk varies according to the histopathologic category of benign breast disease, with a moderately increased risk among women who have proliferative lesions without atypia and a substantially increased risk among women who have atypical hyperplasia (40-42). With

Figure 4

b.

Figure 4: (a) US image of a suspicious lesion (arrow) located at 3-o'clock position in the left breast in 37-year-old woman. The lesion was 7 mm by using US measurement. (b) tHb map shows low and diffused distribution of maximum of $35.3 \mu\text{mol/L}$ and average of $24.7 \mu\text{mol/L}$ within FWHM region at the corresponding location of section 4. Core biopsy revealed a fibroadenoma. In NIR image, the first section was 0.3 cm from the skin surface and the last section was 3.3 cm toward the chest wall. Each section is an x-y spatial image of $9 \times 9 \text{ cm}$, with x and y directions marked on the image. The spacing between the sections is 0.5 cm in depth. The vertical scale is the tHb concentration in micromoles per liter, ranging from 0 to $100 \mu\text{mol/L}$. In the following NIR images, the spacing between the sections is kept at 0.5 cm, and the vertical scale is kept from 0 to $100 \mu\text{mol/L}$. The spatial dimensions of each section were $9 \times 9 \text{ cm}$. *Slice* = section (also in Figs 5–9).

regard to the vascular transformation of proliferative lesions, Viacava et al (43) measured angiogenesis by using immunohistochemical techniques

in a spectrum of normal lobules and proliferative, atypical, and malignant breast lesions. Vascular proliferative parameters were higher in all breast

Figure 5

b.

Figure 5: (a) US image of a suspicious lesion (arrow) located at 8-o'clock position in the right breast in 65-year-old woman. The lesion was 9 mm by using US measurement. (b) tHb map showed an isolated, well-defined mass with high tHb of maximum of $112.3 \mu\text{mol/L}$ and average of $75.8 \mu\text{mol/L}$ at the corresponding location of section 3. The first section was 0.5 cm from the skin surface. The vertical scale is the same as on Figure 4. Biopsy revealed an invasive carcinoma with ductal and lobular features, and the pathologic stage was T1b (size, 1.0 cm).

lesions, as compared with normal lobules, and increased with lesion severity. This could account for the increasing tHb levels from benign nonproliferative lesions, to proliferative and atypical lesions, to malignant cancers.

The perturbations used to calculate absorption maps were the normalized

Figure 6

Figure 6: (a) US image of a suspicious tubular-like lesion (arrow) located at 12-o'clock position in the right breast in 71-year-old woman. The lesion was 7 mm by using US measurement. (b) thb map showed an isolated, well-defined mass with high thb of maximum of 97.8 $\mu\text{mol/L}$ and average of 65.7 $\mu\text{mol/L}$ at the corresponding location of section 3. The first section was 0.7 cm from the skin surface. The vertical scale is the same as that on Figure 4. Biopsy revealed an invasive ductal carcinoma, and pathologic tumor stage was T1c (size, 1.2 cm).

difference between the measurements obtained at the lesion region and the normal region of the contralateral breast in the same quadrant as that in which the lesion was identified. In this procedure, we used the optical properties of the patient's own background tissue as a reference or a control, and this background tissue is thus

insensitive to the patient's background optical property changes. However, this procedure is sensitive to contralateral site selection. We evaluated possible bilateral disease by reviewing mammograms and US images obtained before the NIR images, and we did not perform measurements in any sites near areas of potential disease. All

Figure 7

Figure 7: (a) US image of a suspicious lesion (arrow) located at 6-o'clock position in the left breast in 51-year-old woman. The lesion was 9 mm by using US measurement. (b) thb map showed an isolated and distinct mass with high thb of maximum of 108.4 $\mu\text{mol/L}$ and average of 77.3 $\mu\text{mol/L}$ at the corresponding location of section 3. The first section was 0.5 cm from the skin surface. The vertical scale is the same as that on Figure 4. Biopsy revealed an invasive carcinoma with ductal and lobular features, and the pathologic tumor stage was T1c (size, 1.5 cm).

contralateral measurements were taken at normal areas on the basis of the available information. Multiple NIR data sets were obtained at the lesion site, and average values of thb were used to characterize each lesion. On average, about a 5 $\mu\text{mol/L}$ variation could be encountered in repeated imaging by using the handheld probe. Further technology

Figure 8

Figure 8: (a) US image of a suspicious lesion (arrows) located at 11-o'clock position in the right breast in 63-year-old woman. The lesion was 3 cm by using US measurement. (b) tHb map showed heterogeneous distribution with a higher concentration located at the periphery. The first section was 0.2 cm from the skin surface. The vertical scale is the same as that on Figure 4. Biopsy revealed an invasive ductal carcinoma, and the pathologic tumor stage was T2 (size, 2.2 cm).

development on real-time NIR data acquisition could reduce this variation.

This technology has limitations. First, when the chest wall is present within a 1.0- to 1.5-cm depth, the chest wall underneath the breast tissue affects NIR measurements. In our clinical studies, we use real-time coregistered US to

position the handheld probe at the lesion site and the contralateral reference site of the same quadrant by matching the probe position and the chest wall depth. This effort minimizes the difference in chest wall positions at both sites and produces the best imaging results (44). Therefore, the operator's

Figure 9

Figure 9: (a) US image of a suspicious lesion (bottom arrow) located at 1-o'clock position in the left breast in 76-year-old woman. The patient had a prior cancer (top arrow) removed 10 years previously, and the new lesion grew at the prior surgical site. The exact size could not be measured because of scar tissue. (b) tHb map showed a much higher concentration at the top of the tumor (section 3) than that at the bottom (section 4). The first section was 0.5 cm from the skin surface. The vertical scale is the same as that on Figure 4. Core biopsy sampled from both scar and new lesion areas revealed a high-grade infiltrating lobular carcinoma. The patient was treated with neoadjuvant chemotherapy.

knowledge and skills are important, and guidelines have to be provided for future multi-institutional studies.

Second, the dual-zone mesh reconstruction method is based on a linear approximation to the photon density waves measured at the surface. This algorithm is robust and converges quickly.

Table 2

False-Negative Cases with Low tHb and Uniform Distribution

Patient No./Age (y)	Tumor Type	Tumor Stage		US Measurements		tHb ($\mu\text{mol/L}$)	
		Size (cm)	Nottingham Score*	Size (cm) [†]	Depth	Maximum	Average in FWHM
1/50	Invasive ductal carcinoma	T2	9 (3)	2.7×1.6	1.5	78.5	49.6
2/49	Invasive ductal carcinoma [‡]	cT2	6 (1)	2.2×2.5	1.5	72.4	49.0
3/55	Invasive carcinoma with ductal and lobular features [§]	T2	6 (1)	2.6×1.5	2.0	80.3	60.3
4/68	Invasive ductal carcinoma	T2	9 (3)	2.2×1.9	1.3	63.7	39.7

* Numbers in parentheses are mitotic scores.

[†] Data are lateral $\times$ depth measurements.[‡] Mitotic counts were 0–1 per 10 high-power fields.[§] Mitotic counts were 1 per 10 high-power fields.

Table 3

False-Positive Cases

Patient No./Age (y)	Lesion Characteristics	US Measurements		tHb (μmol/L)	
		Size (cm)*	Depth	Maximum	Average in FWHM
PBL					
1/44	Proliferative FCC with atypia	1.9 × 1.5	2.3	104.1	71.3
2/44	Atypical apocrine adenosis	1.1 × 0.4	1.0	121.6	83.6
3/55	Focal ductal hyperplasia, fibrosis, and epitheliosis	1.3 × 0.8	1.0	88.2	63.8
4/48	Complex fibroadenoma	1.1 × 0.7	0.9	91.6	66.0
NPBL					
5/37	Mammary hamartoma	2.3 × 1.9	2.1	105.6	69.4
6/54	Extensive fat necrosis	3.2 × 1.7	1.3	96.6	57.7
7/30	Fibroadenoma	0.7 × 0.4	1.6	143.8	102.3
8/54	Complex cyst	0.8 × 0.5	2.0	83.3	57.8

Note.—FCC = fibrocystic changes, NPBL = nonproliferative benign lesions, PBL = proliferative benign lesions.

* Data are lateral $\times$ depth measurements.

For a higher optical contrast phantom target or tumor of 1 cm in size, we typically achieve about 80% and 70% accuracy when the maximum and the average values of a reconstructed absorption map are used, respectively (32,45). For a lower optical contrast phantom target or a benign lesion of the same size, about 100%–120% and 100%–140% accuracy can be achieved by using the same quantification criteria, respectively. However, for a larger phantom target of 3 cm in size, about 65%–70% and 55%–60% accuracy for the higher optical contrast one are achieved by using the same quantification criteria, respectively, and about

100%–130% accuracy for the lower optical contrast target (32,45). The results of the controlled phantom studies suggest that the in vivo malignant-benign lesion contrast for early-stage cancers versus benign lesions could be higher than the twofold higher contrast reported because a small cancer could be 20% underreconstructed and a small benign lesion could be 20% overreconstructed. Further technology developments by using nonlinear approximation to the measured diffusive waves and improving system dynamic range are warranted to advance this technology.

Third, because NIR light measures light absorption, a bruise or a hematoma

caused by injury or prior biopsy could appear as a false-positive case. In this study, coregistered NIR and US data for all patients were taken before their biopsies, and the potential issue related to the angiogenesis change after biopsy can be ruled out. In addition, a dark nipple–areolar complex could have higher absorption in the shallow depth near the skin surface. If the examined lesion was more than 1 cm deeper or 2 cm away from a dark nipple, the nipple–areolar complex did not appear to cause a problem. Lighter-colored nipple–areolar complex skin did not cause a problem. Another potential problem is the poor probe-tissue contact in the examination of small dense breasts. By developing probes of different sizes suitable for different breast sizes, good probe-tissue contact should be achieved.

A final limitation was that we used data from the current study population to retrospectively set our threshold level for tHb. This level likely leads to an overestimation of the values of sensitivity, specificity, and accuracy that would be obtained in a different patient population. A large prospective clinical trial is needed to further validate these results.

We showed that intrinsic tHb contrast holds promise as an adjunct to diagnostic mammography and US for distinguishing early-stage invasive breast cancers from benign lesions. The tHb contrast may also help to distinguish some high-risk proliferative lesions from nonproliferative lesions. For advanced cancers, some tHb distribution features,

such as heterogeneous peripheral enhancement and/or posterior shadowing, can add diagnostic value to US.

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