Clinical study of a noninvasive multimodal sono-contrast induced spectroscopy system for breast cancer diagnosis

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Purpose: To present a noninvasive multimodal sono-contrast induced spectroscopy (SCIS) system for breast cancer detection.

Methods: An IRB approved clinical study was carried out to evaluate its diagnostic power. A total of 66 subjects were enrolled with informed consent. The study data were grouped into healthy breast tissue (26), histologically proven cancer (14), and benign mass (26). The diffuse reflectance optical intensity and low intensity focused ultrasound (LIFU) signals, as well as ultrasound images, were collected during each study. The ratio of optical intensities at wavelengths 685 and 830 nm was analyzed using wavelet technique to compare the LIFU effects in cancer and noncancerous tissues. The ultrasound images were also processed to obtain tissue texture parameters, such as correlation, energy, contrast, homogeneity, etc. Backward stepwise regression method was performed to identify the statistically significant factors correlating to tissue types (cancer vs benign mass).

Results: Comparison of the optical signals showed that LIFU induced transitory fluctuation in noncancerous tissue, but not in malignant tissue, as quantified by the ratio of mean absolute deviation (RMAD) of the high frequency component. Statistical analysis revealed that the RMAD ratios were significantly different in tumor vs noncancerous masses ($p \ll 0.01$). For tissue texture parameters, energy and correlation were found to statistically correlate with the tissue types. A cancer characterization model was developed using the weighted factors to differentiate the tumor from the benign mass. Trade-off between sensitivity and specificity was obtained by varying the threshold value that estimated the upper-bound of the cancer output factor, from which the receiver-operating characteristic (ROC) curve was generated. The characterization model was optimized using ten modeling datasets and verified using another ten validation datasets randomly generated from the database. The optimization results show that an AUC of 0.93 can be achieved. With threshold 0.3, sensitivity of 96.0%, specificity of 84.1%, and negative predictive value (NPV) of 97.3% can be achieved.

Conclusions: The feasibility of the multimodal system in characterizing breast cancer vs benign mass is established. © 2012 American Association of Physicists in Medicine.

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Key words: low intensity focused ultrasound (LIFU), diffuse reflectance spectroscopy, blood flow, tissue oxygenation, optical imaging

I. BACKGROUND

Breast cancer is the most frequently diagnosed noncutaneous cancer and the second leading cause of cancer deaths after lung cancer among women in the United States.¹ About 275 000 women in the United States are diagnosed with breast cancer each year.² In 2011, there will be an estimated 232 620 new cases for breast cancer in the United States, among which there will be about 39 970 estimated deaths.³ Breast biopsy, in which samples of tissue are removed invasively for pathologic analysis, is currently considered the best way to evaluate a suspicious area in breast. Over 1×10^6 women go through breast biopsies each year in the United States, but only about 20–30% of these biopsies yield a diagnosis of breast cancer.⁴ Even larger numbers of

recommended short-term follow-ups and additional examinations are performed in routine clinical practice in the United States. This represents a large cost burden to the healthcare system. A highly specific, noninvasive screening technique based on mechanisms of tumor biology and physiology is needed to improve cancer detection and to reduce unnecessary invasive procedures.

Acoustic radiation force has been known to cause blood flow stasis.^{5–9} Studies in literature have mostly focused on the associated health risks, as well as methodologies to prevent stasis from occurring during ultrasound imaging.^{6–9} Previously, our group successfully demonstrated that the effects of low intensity focused ultrasound (LIFU) on banding of red blood cells can be used with optical spectroscopy to differentiate tumor from normal leg muscle tissue in

mice.¹⁰ The rationale behind this is that, under normal physiological conditions, oxygen dissociates from hemoglobin molecules in red blood cells to replenish the oxygen supply in tissues as blood flows through the vessels. When LIFU is used to slow or stop the blood flow, the oxyhemoglobin saturation (the amount of oxygen actually combined with hemoglobin, expressed as a percentage of the oxygen capacity of that hemoglobin) decreases as the available oxygen is depleted. It has been shown that oxy- and deoxyhemoglobin have signature absorption and scattering effects visible in diffuse reflectance optical spectroscopy.¹¹ Therefore, the change in oxyhemoglobin saturation during and after LIFU application can be measured noninvasively and without ionizing radiation. Interestingly, this LIFU sono-contrast (changes in spectroscopic parameters due to administration of ultrasound acoustic pressure) was strong in normal tissue vasculature but absent in those inside tumors. Therefore, LIFU can serve as an excellent discriminator of benign vs malignant vascular morphology reported by optical spectroscopy.

Based on that study, we proposed a novel technology, sono-contrast induced spectroscopy (SCIS), which uses LIFU as a “contrast agent” to improve the localization of diffuse reflectance spectroscopy. A multimodal system was developed incorporating this SCIS technique together with ultrasonography to improve breast cancer diagnosis noninvasively. A cancer characterization model combining the weighted inputs from both SCIS technique and ultrasonography was developed to characterize cancer from benign mass. An IRB-approved clinical trial has been carried out to evaluate its diagnostic power. This paper first gives a brief overview of this novel system and then presents the cancer detection methodology and the clinical study results in detail.

II. MATERIALS AND METHOD

II.A. System overview

There are three major modules designed into the SCIS system: LIFU, diffused reflectance spectroscopy (DRS), and ultrasonography. A scanhead system was developed to incorporate these modules.^{12–14} LIFU is generated by two 1 MHz focused transducers (Channel Industries, Santa Barbara, CA), aligned orthogonally inside the scanhead. The acoustic field was calibrated and maintained below FDA diagnostic ultrasound limits (0.72 W cm^{-2}).¹⁵

The DRS system is composed of 6 sources and 12 detectors mounted on the concave bottom plate of the scanhead. The sources are illuminated by two laser diodes with wavelengths 685 nm and 830 nm (LG-Laser Technologies, Germany). Laser power intensity was calibrated to be below 0.2 W cm^{-2} , according to ANSI Z136.1 (1993) Safe Use of Lasers. A multichannel optical switch (O/E land, Inc., Canada) is used to switch between the two laser inputs and the six sources based on a designed pattern.

A commercial ultrasound system (Philips iU22, Valhalla, NY) is used for tumor localization and image guidance during the procedure. The captured ultrasound images are used in tissue texture analysis (see Sec. II C 2 for details). This SCIS system has passed all the electrical safety tests (reverse

polarity test, neutral test, and leakage test) required for clinical study. A more detailed system description and patient safety related considerations can be found in Refs. 12–14.

II.B. Clinical study

An IRB approved clinical study was carried out in Cooper University Hospital, NJ. The intended patient populations are those present with a solitary mass in the breast that requires ultrasound-guided tissue biopsy to rule out malignancies. Figure 1 illustrates the clinical study design. The study was generally scheduled to be performed on the same day when the patient comes in for biopsy. Once the patient signed the consent form for participation, the SCIS scanning procedure was first performed, followed by the standard needle biopsy procedure. Tissue histology was then obtained from the standard pathology reports, based on which the study subjects were separated into different groups. This information also served as ground truth for developing the cancer characterization model and validating its effectiveness.

A total of 66 subjects were enrolled with informed consent during the course of study. The study data were grouped into “healthy breast tissue” (26), “histologically proven cancer” (14), and “benign mass” (26). A summary of cancer types, cancer stages, and Her2 statuses in the cancer group are listed in Table I. The healthy breast tissue group is composed of data from healthy volunteers or patients coming in just for routine or follow-up ultrasound examinations. These data were collected only in the very early stage of the clinical study to test system functionality by reproducing the signal contrast of healthy tissue and tumor. They are not included in the following cancer characterization model since they are not the intended study subjects.

During the SCIS scanning procedure, the patient sat in front of the scanhead. The scanhead was adjusted to a height such that the patient could comfortably place her breast under the bottom plate, in full contact with the sources and detectors, but without excessive compression. Ultrasound gel can be applied to the skin if necessary. The physician first used the commercial imaging ultrasound probe to locate the target of interest. Then, LIFU was adjusted using the knobs on the scanhead to position the focal spot within the target under inspection. Finally, data collection would be ready to start.

The procedure began with a 15-s background DRS signal collection followed by three cycles of LIFU bursts. Each cycle had a 5-s LIFU burst followed by 25-s relaxation period. Total study time was 1 min and 45 s. During the procedure, the optical switch swept through the two lasers and six sources. The 12 detectors continuously collected and transferred the DRS signals to the central database.

II.C. Cancer characterization technique

II.C.1. Optical signal analysis: Discrete wavelet analysis

Discrete wavelet analysis was used in this research to analyze the optical signals for features that characterize different study groups. The raw LIFU and DRS signals were first

FIG. 1. Clinical study design.

synchronized and segmented into 12 channels (corresponding to the 12 detectors). In this study, we only focus on analyzing signals from one channel located at the central proximal position (toward the patient side), due to the difficulty in setting up the breast to cover all the sources and detectors among all the patients. Only signals from the same channel were compared to avoid variations caused by factors other than LIFU effect.

The ratio of optical intensities at wavelengths 685 and 830 nm (I₆₈₅/I₈₃₀) was processed using two filters. The first filter was designed using the moving average technique to remove the noise and smooth the data; the second filter was designed using wavelet analysis. The output low frequency component (a) and high frequency component (d) were defined using Eqs. (1) and (2). Finally, the filtered signals were up-sampled by two using interpolation for comparison. Figure 2 demonstrates the data processing procedure.

$$a_i = \frac{1}{2} \sum_{j=1}^N c_{2i-j+1} f_j \quad i = 1, \dots, \frac{N}{2}, \quad (1)$$

TABLE I. Summary of the cancer types, stages, and Her2 statuses of the cancer group.

Pathology results	Summary
Cancer type	8 ductal carcinoma, 4 lobular carcinoma, 1 metastatic adenocarcinoma in lymph node, 1 invasive mammary carcinoma with ductal and lobular features
Cancer stage	5 stage 1A, 3 stage 2A, 1 stage 2B, 1 stage 3A, 2 stage 3B, 2 stage 4
Her2 status	4 negative/0, 6 negative/1+, 1 equivocal/2+, 2 positive/3+, 1 no staining

$$d_i = \frac{1}{2} \sum_{j=1}^N (-1)^{j+1} c_{j+2-2i} f_j \quad i = 1, \dots, \frac{N}{2}, \quad (2)$$

where N is the number of input data; f_j is the input data; a_i and d_i are the decomposed approximation and detail information, respectively; c_k is the coefficient. Daubechies-4 wavelet is used in this work to determine the coefficients.

II.C.2. Ultrasound tissue texture analysis and correlation study

Texture analysis of the B-scan image has been explored for classifying benign and malignant breast tumors in the literature.^{16–18} On breast ultrasound images, benign lesions

FIG. 2. Data processing flow chart.

typically manifest as homogeneous internal echoes, whereas malignant lesions appear as heterogeneous internal echoes. The texture-based analysis describes the similarity and correlation among the acquired image pixels, reflecting the variation in the structural echogenicity of a tumor.¹⁹ Although several texture-feature parameters have been studied, the most common ones are those extracted from the gray-level co-occurrence matrix (GLCM).^{20–23} In this research, ultrasound images of subjects in the cancer and benign mass groups were collected for image analysis. Four texture parameters (homogeneity, contrast, energy, and correlation) were analyzed based on the GLCM.

To determine the significance of these factors on the tissue types (cancer vs benign mass), backward stepwise regression was used. Initially, all of the factors were in the model. The least significant terms were then sequentially removed until all the remaining terms were statistically significant. The p -values, variance inflation factors (VIF), and Durbin–Watson number were used as criteria to evaluate the statistically significant factors. The p -values represent the significance of the factors. A p -value close to zero means that the parameters under testing are statistically significant and, thus, should be kept in the model. Here, we chose $p = 0.05$ as the cutoff value. VIF measures the multicollinearity in the independent variables. Both values 5 and 10, which correspond to R^2 (coefficient of determination) values of 0.8 and 0.9, have been used in the literature as a critical threshold as a rule of thumb. For this study, considering the small size of dataset, 5 was selected; that is, if VIF was larger than 5, then multicollinearity was high. Durbin–Watson number was examined to detect autocorrelation in the model residuals. A value around 2 generally indicates no autocorrelation. Considering the influence of the sample size and the number of statistically significant factors in the model, this statistic was compared with tabulated rejection values as shown in Durbin and Watson,²⁴ which were calculated based on those effects.

II.C.3. Cancer characterization model optimization

A cancer characterization model was developed to identify the malignant tissue from the benign mass. It combines the weighted inputs from both the SCIS technique and ultrasonography, as shown below:

$$F_b = w_x \sum_{i=0}^N \alpha_i x_i + w_y \sum_{j=0}^M \beta_j y_j, \quad (3)$$

$$\sum_{i=0}^N \alpha_i = \sum_{j=0}^M \beta_j = 1, \quad (4)$$

$$w_x + w_y = 1. \quad (5)$$

x_i and y_j are the statistically significant factors correlating to tissue types (tumor vs benign mass), obtained from two independent and complimentary techniques: SCIS technique (group 1) and ultrasonography (group 2). N and M are the corresponding number of the factors in these two groups. α_i and β_j are the weights of these factors among their own groups, the summation of which equals to unity [Eq. (4)]. w_x and w_y

are the weighting factors of these two groups of inputs, the summation of which should also equal to unity [Eq. (5)].

The threshold model that estimated the upper-bound of the cancer output factor can be represented as

$$F_b \leq \Delta \quad (6)$$

where Δ , named “classifier,” represents the threshold factor separating the benign tissue from the cancerous tissue. Output factor F_b equal to or lower than the threshold will indicate the existence of cancerous tissue. Selecting different values of threshold has the effect of selecting different trade-offs between sensitivity and specificity. The area under the curve (AUC), which aggregates the performance across the range of trade-offs (sensitivity and specificity) and is independent of the chosen threshold, is of primary consideration for model optimization. Sensitivity and specificity are also included in the optimization objectives with assigned weighting factors. Sequential quadratic programming method was adopted for optimization. This method was previously described in Ref. 25 in details and will not be discussed in this paper.

III. RESULTS

III.A. LIFU effect in tumor, benign mass, and breast tissue

Analysis from the 66 subjects included in the study has not revealed any consistent trend or changes in the low frequency components that can be used to distinguish cancer from benign mass. However, in the high frequency components, there was a transitory fluctuation during LIFU actuation in the noncancerous tissues (breast tissue and benign mass), which was not observed in breast cancer. This fluctuation can be quantified in terms of the mean absolute deviation (MAD). The ratio of MAD (RMAD) during LIFU vs baseline in cancer, benign, and breast tissue are [mean (max/min/std)] 2.04 (3.27/1.41/0.43), 2.97 (4.29/1.86/0.62), and 3.06 (4.00/2.18/0.50), respectively. This suggests that the DRS fluctuation was amplified in noncancerous tissue during LIFU stimulation. Figure 3 shows the data distribution for the three groups. There are a few outliers due to poor patient setup or patient movement during procedure. Statistical analysis also reveals that these RMAD

FIG. 3. MAD distribution in tumor vs benign mass vs breast tissue.

values induced by LIFU are significantly different in tumor vs benign mass ($p \ll 0.01$). So far, it has not suggested significant difference in benign mass vs breast tissue ($p = 0.53$). Figures 4(a)–4(c) illustrate examples of DRS signals from breast tissue, benign mass, and tumor, respectively. s represents the raw DRS signal from a certain channel; a is the low frequency component; and d is the high frequency component. A total of three cycles were actuated during the procedure. As shown in

the figure, when LIFU was on, there were signal fluctuations in high frequency components in both benign mass and breast tissue; however, this did not occur in tumor.

III.B. Statistically significant texture factors

For each B-scan in the study, ROIs were manually selected both inside the index lesion and at the proximal

FIG. 4. Comparison of signals from (a) breast tissue (b) benign mass, and (c) tumor. The circles highlight the high frequency fluctuations in breast tissue and benign mass.

FIG. 4. (Continued)

edge as illustrated in Fig. 5. The internal ROI for the inside-lesion region was extracted from the inside-lesion region. The ROI around the proximal edge were extracted to include both breast tissue and malignant/benign tissue with the proximal boundary in the center. For consistent comparison, the size of ROI was chosen to be 40×40 pixels, due to several small lesions in the study group. The tissue texture parameters (energy, contrast, homogeneity, and correlation) were computed for both ROIs and included in the correlation study.

In the internal region, the average homogeneity values for tumor and benign mass were 0.966 and 0.971, respectively, indicating that benign tissues in the current cases were more homogeneous than tumors. Also, the average contrasts for benign masses and tumors were 0.062 and 0.069, respectively, also suggesting that benign masses have lower con-

trast. The energy and correlation for tumor/benign mass were found to be 0.379/0.467 and 0.933/0.899, respectively.

In the region around proximal edge, the differences between tumor and benign mass were less significant compared to the internal region, as shown in Table II. The energy, contrast, homogeneity, and correlation for tumor/benign mass were 0.255/0.234, 0.126/0.118, 0.964/0.965, and 0.955/0.966, respectively.

A total of six iterations were performed using data from the 40 subjects (tumor and benign mass groups) until a model with only significant factors was obtained. The final result reveals that energy ($p=0.046$) and correlation ($p=0.0002$) in the internal region have significant correlation with the tissue types. The p -value of the model was less than 0.0001, which suggests that the regression model is

FIG. 5. Illustration of ROI selection around proximal edge and internal region.

TABLE II. Tissue texture parameters.

		En_C	Ctr_C	Hm_C	Cor_C	En_prox	Ctr_prox	Hm_prox	Cor_prox
Mean	Tumor	0.379	0.069	0.966	0.933	0.255	0.126	0.964	0.955
	Benign mass	0.467	0.062	0.971	0.899	0.234	0.118	0.965	0.966
Variance	Tumor	0.009	0.0002	0.00003	0.00086	0.00259	0.011	0.00003	0.00043
	Benign mass	0.044	0.0006	0.0001	0.0046	0.002	0.012	0.00005	0.00038

Note: Parameters in internal region (En_C: energy, Ctr_C: contrast, Hm_C: homogeneity, Cor_C: correlation) and at proximal edge (En_prox: energy, Ctr_prox: contrast, Hm_prox: homogeneity, Cor_prox: correlation).

significant. Durbin–Watson value at 1.50, between 1.39 and 1.60, which are the cutoff values for two regressors and 40-sample size, does not reveal any autocorrelation. Correlation characterizes periodic texture patterns; energy is directly affected by edges and specular structures and, to some degree, by diffuse echoes and other highly disordered texture patterns.²⁶ These two factors will be included in the cancer characterization model together with input from SCIS technique (RMAD).

III.C. Cancer characterization model optimization

The optimization model was constructed using weighted inputs from SCIS technique (RMAD) and ultrasonography (correlation and energy). The optimization engine was implemented in MATLAB (MathWorks, Inc., MA). Ten modeling datasets and ten validation datasets were randomly generated from the 40 subjects' database. Each dataset has 20 subjects. Tissue histology results were used as ground truth to measure the correctness of the cancer detection method, from which sensitivity and specificity were computed. Optimization objectives include AUC, sensitivity, and specificity with the assigned weighting vector [5;5;1]. The optimization algorithm was run with the parameter vector initialized at [0.5;0.5;0.5;0.5], which corresponds to $[\beta_1; \beta_2; w_x; w_y]$. Note that α_1 equals to 1 since there is only one factor (RMAD) from SCIS technique.

The resulting optimal parameter vector was [0.28;0.72;0.27;0.73], with which the maximum AUC was achieved at 0.92. The model was then validated using another ten sets of validation datasets. By varying the classifier values in increments of 0.01, a receiver operating characteristics (ROC) curve was generated, as shown in Fig. 6. The AUC calculated using the independent sets of validation data was 0.93. With threshold 0.3, sensitivity of 96.0%, specificity of 84.1%, and negative predictive value (NPV) of 97.3% can be achieved.

IV. DISCUSSIONS

The clinical study results demonstrated the potential of the SCIS system in characterizing breast cancer vs benign lesion. LIFU appears to exert more pronounced influence on blood flow in noncancerous tissue than in breast cancer. The possible mechanism could be that the acoustic force delivered by LIFU caused reversible compression of the blood vasculature and/or blood flow stasis, and therefore temporal changes in hemoglobin concentration, which were registered in the reflected optical signals. This lack of response to LIFU in breast cancer is possibly due to its abnormal blood vasculature.

In a previous animal (murine) study,¹⁰ the tumor was superficial on the hind leg, and the spectrometer probe was in direct contact with it. LIFU induced optical signal drop could be clearly observed. However, in this clinical study, the tumors were seated below layers of tissues. In addition, the detectors collected the reflectance lights indiscriminately from both the tumor and the surrounding tissues. Therefore, the LIFU induced effect was much more complex compared to that in the animal study. By using discrete wavelet analysis, the LIFU effect was revealed in the high frequency components.

Besides the SCIS technique, ultrasound image texture analysis was also performed to identify the statistically significant factors correlating to tissue types. Backward stepwise regression study reveals that energy and correlation in the internal region have significant correlation with the tissue types.

A cancer characterization model was then developed by combining the weighted inputs from these two complementary techniques. Optimization was performed to find the weighting factors that would give the optimal AUC as well as cancer detection sensitivity and specificity. In this study, we gave more weights to sensitivity than specificity since false negative cases would be of more concern. The weighting vector for the optimization objectives can be changed to address different emphasis/concerns on cancer detection. In addition, a classifier that would give higher sensitivity or specificity in the generated ROC curve should be selected as the threshold to further address the concern.

Twenty datasets were randomly generated from the 40 subjects' database, 10 for modeling and 10 for independent validation. The optimization results show that the resulting optimal parameter vector [0.28;0.72;0.27;0.73] can achieve

FIG. 6. ROC curve.

AUC 0.93. With threshold 0.3, sensitivity of 96.0%, specificity of 84.1%, and NPV of 97.3% can be achieved. For comparison, optimizations were performed with (1) only RMAD and (2) only ultrasound tissue texture factors (energy and correlation) in the cancer characterization model. Results revealed that the achieved optimal AUC is 0.89 for model with only RMAD and is 0.70 for model with only ultrasound tissue texture factors. This clearly suggests that, by combining the two techniques, the diagnostic power of the SCIS system is improved.

Currently, we used linear combination of the weighted inputs to construct the optimization model. In future, with more clinical data available, other methods, such as neural network or support vector machine, should also be explored.

The average power of the power spectral density (PSD) of I_{685}/I_{830} for each cancer subject was calculated. Statistical analysis was performed between the cancer subgroups and relating factors including PSD, RMAD, and the imaging tissue texture parameters. Below are some observations:

1. The p value for the average power of the PSD in ductal and lobular cancer subgroups is 0.0219 (<0.05), suggesting that it might be an indicator for differentiating these two groups.
2. The p value for ultrasound imaging tissue texture parameter (correlation_prox) in Her2 status negative/0 and negative/1 + groups is 0.044 (<0.05), also suggesting that ultrasound imaging tissue texture parameters may be used to characterize these two groups.

The current study is not powered for determining these relationships. More clinical data will be needed for concrete analysis.

V. CONCLUSIONS

The feasibility of the SCIS system in characterizing breast cancer from benign mass is established. LIFU appears to exert more pronounced influence on blood flow in noncancerous tissue than in tumor. A cancer characterization model was developed by combining the weighted inputs from SCIS technique and ultrasonography. The optimized model can achieve sensitivity of 96%, specificity of 84.1%, and NPV of 97.3% with a threshold of 0.3. The AUC was calculated to be 0.93. Combining this system with breast ultrasound has the potential to increase breast cancer detection and to reduce unnecessary invasive procedures.

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