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Rapid Non-invasive Optical Imaging of Tissue Composition in Breast Tumor Margins

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Summary

Optical spectral imaging has the ability to identify differences between benign and malignant tissue in breast tumor margins. The use of this rapid, non-destructive technology could help reduce the need for second operations in women desiring breast conservation therapy (BCT).

Keywords

Breast Conservation Therapy; Optical Spectroscopy; Imaging; Margin Assessment

Background

Over the past 30 years, mammography has been the primary means for identifying breast malignancies and has proven effective in diagnosing cancer at earlier stages, leading to less aggressive surgical and adjuvant therapy.¹ A greater number of patients are being diagnosed with non-invasive neoplasms as well because of improved screening.² Multiple randomized clinical trials have previously shown that patients who undergo breast conservation therapy (BCT), for either invasive or non-invasive breast cancers, have equivalent long-term survival to those who undergo mastectomy.^{2–4} However, in a 15 year follow-up from The Early Breast Cancer Trialists meta-analysis, a survival benefit was identified for patients with BCT who did not have a local regional recurrence. For every 4 local regional recurrences avoided in patients treated with BCT, one breast cancer related death was averted.⁵ Thus for patients eligible for BCT, complete removal of the tumor or negative margins is desired to avoid a local recurrence. In a summary of 34 trials evaluating the risk of local recurrence based on margin status, clinically significant differences in local recurrence rates were identified when patients with positive or close versus negative margins were compared (16% vs. 6%). This adverse effect on local recurrence was seen to increase as time from diagnosis increased.⁶

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Breast cancer is a heterogeneous disease.⁷ The heterogeneity of this disease makes it challenging for surgeons to characterize tumors intra-operatively such that they can be completely removed at the initial surgery. Currently there is no widely available intra-operative tool to assure complete removal of a breast tumor during BCT. In experienced hands, frozen section and touch prep analysis of margins provide a good means of reducing the re-excision rate to lower than 20%.^{8,9} Because institutional resources may not permit pathologists to be readily available for BCT cases and because patients may undergo their procedures in off-site ambulatory locations, these specialized techniques have not become widely available.¹⁰ In order to reduce the local recurrence rate, patients are advised to have a re-excision lumpectomy if their margins are found to be positive or close by the pathologist with the definition of “close” varying from 0–3 mm. The quoted rates of second operations vary in the literature and range from 12% to as high as 60%.^{11–15} In order to more accurately characterize breast tumor margins intra-operatively and thereby reduce the re-excision and local recurrence rate, we have developed a device which utilizes optical spectral imaging to characterize differences in tissue composition of excised breast specimen margins. This device utilizes the primary light-tissue interactions of absorption and scattering in the visible part of the electromagnetic spectrum to characterize the underlying tissue composition. The sources of intrinsic optical contrast can be broadly classified as morphological (β -carotene, cell density) and physiological (deoxygenated and oxygenated hemoglobin and total hemoglobin content). It is fortuitous that a number of these biomarkers are hallmarks of carcinogenesis.^{16–17} We sought to determine whether these tissue compositional features could be exploited to rapidly identify malignant cells within the margin (0–2mm) of partial mastectomy specimens in women with invasive and non-invasive breast malignancies.

Materials and Methods

Patients

The study was approved by the Institutional Review Board (IRB) at Duke University in accordance with assurances filed with and approved by the Department of Health and Human Services. Informed consent was obtained from eligible participants (women > 18 years of age) undergoing primary BCT for an invasive or non-invasive breast malignancy. A sub-group recruited to this study had undergone neo-adjuvant endocrine or chemotherapy prior to their surgical procedure. Operations were performed by 5 breast surgical oncologists at the Duke University Ambulatory Surgery Center. Each surgeon performed the lumpectomy according to their standard practice. The tissue was assessed grossly and via specimen mammography. The surgeons removed additional breast tissue based on their assessment of the margins. The surgeons did not perform routine immediate re-excision of each of the 6 margins. Frozen section and touch prep analyses were not performed on these specimens. In this study, demographic data including patient age, tumor size and subtype, margin status and re-excision rate, receptor status, and presence of neoadjuvant endocrine or chemotherapy was recorded for each participant.

Optical Spectral Imaging Procedure

The optical spectral imaging device consists of a Xenon lamp coupled to a monochromator, a handheld optical spectral imaging probe interfaced to an adjustable tissue specimen box, an imaging spectrograph, and a charge coupled device (CCD) camera. A photograph of the device and the specimen box are shown in (Fig. 1). The optical spectral images collected with this device are processed using a feature extraction algorithm based on a scalable inverse Monte Carlo model of reflectance to create maps of tissue composition for each margin that is imaged up to a sensing depth of 2 mm. A description of the underlying instrumentation and algorithm is described in previous publications.^{18–20}

Once the surgeon had completed his/her review of the margins, the specimen was placed into the box and interfaced with the imaging probe for assessment of the breast tumor margins (Fig. 1(b)). The engineering team was informed of the margins most likely to contain malignant cells based on the surgeon's assessment. Optical spectral images were obtained from 1–2 margins per specimen. Each placement of the probe covered an area of 3×1 cm and multiple placements were made if the margin was larger than the area covered by the probe. The spectral images recorded from each margin were entered into the feature extraction algorithm, which computes at each pixel (picture element), parameters related to light scattering (wavelength averaged reduced scattering coefficient) and light absorption (total hemoglobin, and β -carotene).¹⁸ These parameters were combined to create additional parameters, specifically, the ratios of total hemoglobin or β -carotene to the wavelength-averaged reduced scattering coefficient. In summary, two parameter maps were created per margin: a map of (1) the ratio of total hemoglobin and the wavelength-averaged reduced scattering coefficient and (2) the ratio of β -carotene and the wavelength averaged reduced scattering coefficient. Data acquisition and image processing took approximately 25 seconds per placement of the imaging probe. After imaging, specimens were marked with ink at each margin corner and with ink at each of 10 random optically measured sites, and were then fixed in formalin and sectioned according to standard protocols with subsequent routine pathologic review of margin-level pathology. The randomly inked sites were included in the margin sections. They were separately scored by the study pathologist (JG). The surgeons were blinded to the results of the optical data throughout the study.

Pathology

Two levels of optical-pathology concordance were performed. For the first, referred to as margin-level, the pathologic status of the entire imaged margin (positive, close, or negative) within the four inked corners as well as the type of residual carcinoma found at the margin, if any, was recorded and compared to summary measures derived from the two parameter maps of each imaged margin (as described below). A diagnosis of a margin as either positive (cancer extending to the edge of the inked margin) or close (cancer within 2 mm of the inked margin) were both considered as “positive,” since re-excision of the margin is usually performed for either case. The 2 mm distance of the tumor from the nearest margin represents the consensus definition of a “close” margin at the Duke University Medical Center and typically has the same therapeutic implication as a positive margin. Other medical centers have adopted the same definition of a close margin.²¹ If there was no residual carcinoma within 2 mm of the inked margin, the margin was classified as “negative.” For the second, referred to as site-level, the pathology from the randomly inked sites (diagnosed according to the same criteria described above) was used on a qualitative level to interpret the features in the parameter maps of the imaged margins.

Results

From December 2007 to September 2008, 54 patients were enrolled in this study. Optical spectral imaging data from 6 patients were not utilized in this analysis as the pathological outcomes could not be accurately co-registered with the specified margins. From the remaining 48 patients, 55 margins were evaluated with the optical spectral imaging device. Table I contains a summary of the patient and margin characteristics for the participants in this study. Twenty one patients had negative margins, and 15 had a positive margin on the main specimen which was analyzed with the optical probe with additional margin specimens taken by the surgeons at the original surgery to obtain negative margins. Of the 48 patients, 12 had to have a re-excision lumpectomy.

The majority of patients (73%) enrolled had invasive and/or in-situ ductal carcinomas in the lumpectomy specimen. The remaining patients had a variety of histologic subtypes including lobular (invasive and in-situ), tubular, and papillary carcinoma. Three of the patients in this study had a complete pathologic response to their pre-operative chemotherapy. For each patient enrolled in this study, only 1–2 margins were assessed with the optical spectral imaging device. As such, the re-excision rate and device outputs could not be compared to determine what outcome could have occurred had the device results been revealed to the surgeons.

Figure 2 contains representative parameter maps of β -carotene concentration to wavelength-averaged reduced scattering coefficient (β -carotene:scattering) (A,C,E) and corresponding histograms which graphically represent the distribution of values within each image (B,D,F) for a margin negative for residual disease (A,B), a margin positive for ductal carcinoma in-situ (DCIS) (C,D) and a margin positive for invasive ductal carcinoma (IDC) (E,F). β -carotene is a dietary carotenoid known to be stored primarily in adipocytes, and is thus reflective of the amount of fat present in the sensing volume. The wavelength-averaged reduced scattering coefficient is a measure of the amount of light elastically scattered in the tissue, with higher scattering coefficients associated with more dense arrangements of cells and their subcellular scatterers such as organelles and membranes (scatter density) as well as with changes in the distribution of sizes of these scatterers (scatter size).²² Since malignant tissues are expected to have less fat (due to displacement of adipocytes by carcinoma cells) and higher scattering (due to increased cell density and changes in nuclear morphology), β -carotene:scattering is expected to be decreased in cancer tissue relative to normal breast tissue. Color maps in the images of Figure 2 are set such that lower values of β -carotene:scattering appear red, whereas higher values appear blue. As seen in the images, the negative margin (Figure 2A) is characterized by a higher proportion of blue pixels, whereas the positive margins (Figure 2C and 2E) are characterized by increased proportions of red pixels. Site-level histology of these margins indicated that generally, the blue areas were indicative of cancer-free regions of the margin, whereas the orange-red areas indicated regions of the margin containing residual disease.

In order to build predictors for margin-level assessment from the parameter maps, a threshold value for pixel intensity was determined (e.g., 6 $\mu\text{M}\cdot\text{cm}$ for β -carotene:scattering) and the percentage of pixels below that threshold was computed (as illustrated in Fig. 2(B,D,F)). A Wilcoxon Rank Sum test was carried out to determine if the percentage of pixels below that threshold was statistically different between positive and negative margins. The optimal threshold was determined by repeating the Wilcoxon tests across the full range of threshold values, the results of which showed that 6 $\mu\text{M}\cdot\text{cm}$ for β -carotene:scattering showed the greatest degree of association with pathology ($p < 0.002$, Fig. 3(A)). A similar process was applied to total hemoglobin:scattering, such that the percentage of pixels below a threshold value of 8 $\mu\text{M}\cdot\text{cm}$ resulted in the statistically most significant differences between positive and negative margins for that parameter ($p < 0.01$ Fig. 3(B)).

Next a multivariate predictive model was developed for classifying a margin as positive or negative based on the predictors shown in Fig. 3(A,B). A tree-based approach was taken to build the two-parameter model, such that a margin was classified as positive if the percentage for the β -carotene:scattering OR total hemoglobin:scattering parameters were above their respective thresholds; otherwise it was classified as negative. The percentages shown on the y-axes in Fig. 3(A) and 3(B) were each varied across the complete set of different threshold values (for example, 98% in Fig. 3(A) and 72% in Fig. 3(B)), and the sensitivity and specificity was then calculated against margin assessment by pathology. The optimal pair of threshold values was determined by a receiver operator characteristic analysis (ROC, Fig. 4) and the Youden index, in order to maximize the sensitivity and specificity in an additive manner. Then, a leave-one-out cross validation scheme was used to obtain an unbiased estimate of the operating characteristics of the predictive model using the same guiding principles as above,

and resulted in a sensitivity and specificity of 79% and 67%, for the two parameter decision tree.²³ The percentage pixel thresholds in the final model for β -carotene: scattering and total hemoglobin: scattering was $98 \pm 0.19\%$ and $72 \pm 1.0\%$, respectively.

Table II contains the prediction accuracy resulting from the decision-making model described above. Of the 34 path-confirmed positive/close margins in the dataset, the predictive model correctly identified 27 of them as positive, yielding a sensitivity of 79.4%. In addition, of the 21 path-confirmed negative margins, the predictive model correctly identified 14 of them as negative, yielding a specificity of 66.7%. The performance of the model in predicting path-positive/close margins is also shown as a function of type of cancer found at the margin. For margins that were positive for IDC only, the predictive model correctly identified 11 out of 14, or 78.6% of positive margins. For margins that were positive for DCIS only, the predictive model correctly identified 8 out of 9, or 88.9% of positive margins. In the current dataset, in 6 of the positive margins the type of cancer tissue present at the margin was not specified, whereas a further 5 margins were positive for less common malignancies (lobular, mixed DCIS/IDC, and tubular). Of these “other” positive margins, the predictive model correctly identified 8 of 11, or 72.7% of positive margins. The margins were equally weighted with respect to the number of close margins ($n = 17$) and the number of positive margins ($n = 17$). The performance of the predictive model was also not biased significantly toward either positive or close margins, with positive margins being correctly predicted only slightly more frequently (14/17 or 82.4% sensitivity) than close margins (13/17 or 76.5% sensitivity).

Although only 7 of 34 positive margins (from 6 patients) were misclassified as falsely negative, it is important to understand why these margins may have been misclassified. Within this patient group were 2 patients who received neoadjuvant therapy; one with endocrine therapy and the other with chemotherapy. Figures 5A/B is imaged from the margin (posterior margin pathologically positive for IDC) of the patient who underwent neoadjuvant endocrine therapy. This patient had a significant decrease in proliferation rate between her pre- and post-therapy biopsies which may have decreased the scattering coefficient, which in turn could have resulted in a more flat histogram, which is typical of negative margins. In the second patient who had received neoadjuvant therapy, the device incorrectly called the posterior margin diagnosed with IDC at 2 mm as negative. The device likely called this margin negative because the residual disease was at a depth that was just at the 2 mm sensing depth of the device.

Of the remaining 5 patients, Figures 5C/D is imaged from a patient with IDC just beneath her nipple where there is greater ductal tissue and less fat. The histogram in this particular case looks like that of a positive margin but was just above the percentage pixel cutoff for margin positivity. This is an example of a near-miss. Another of the false negative cases had DCIS which was misclassified as negative by the optical spectral imaging device. The final pathology showed close margins anterior-inferiorly and anterior-laterally with additional shaved anterior, lateral and inferior margins negative for DCIS. This tumor specimen was large with a volume measuring greater than 1200 cm^3 . In this case, although the majority of the margin is reflective of normal tissue, there are some relatively small areas of very low β -carotene:scattering values, which are suggestive of the presence of residual disease. However, in the current method of automated image analysis, the contribution of these pixels to the overall pixel distribution was small, due to the very large size of these margins. This resulted in misclassification of the margin, since the percentage of pixels below the threshold was small. This is a weakness of the current paradigm for automated image analysis, in that error may be introduced when the area of suspicious pixels is small compared to the overall margin size.

Discussion

The majority of women with early stage breast cancer can undergo BCT. As the number of women who are treated neoadjuvantly with endocrine or chemotherapy increases, the eligible population for BCT will continue to grow. Recognizing, however, that anywhere from 16% to 60% of patients with BCT require a second surgery, a rapid, non-invasive, readily available technology is necessary to reduce this inability to detect tumor at the edge of a breast specimen in the operating room.^{11–15} This study highlights the potential for optical spectral imaging to evaluate the tissue composition of breast lumpectomy specimen margins intra-operatively. In this preliminary patient population, 79% of the pathologically positive margins were accurately identified by the device. This group of correctly identified margins included all the variant pathologies including one lobular cancer, several mixed in-situ and invasive carcinomas including lobular carcinoma in-situ, and a tubular cancer. Prior studies have shown that patients with lobular cancers have a higher likelihood of re-excision and/or mastectomy.^{12, 24–25} This study continues to accrue patients to improve the optical devices' sensitivity and specificity and will focus in future patient subsets on those with lobular cancers. This device showed an excellent ability to identify DCIS at the margin of a BCT specimen. DCIS has been identified as a tumor characteristic likely to increase the margin positivity and re-excision rate.² Future studies will also enrich for DCIS patients to ensure that the device can adequately identify DCIS at the margin of a BCT specimen.

In addition to specimens with Lobular carcinoma or DCIS, an intraoperative margin device must also recognize the heterogeneous changes caused by exposure to neoadjuvant chemotherapy or endocrine therapy. Neoadjuvant therapies are increasing in prevalence for the treatment of breast cancer because of improvements in targeted agents. In a retrospective review of 478 breast cancer patients treated in Montreal, Canada, 76 had neoadjuvant chemotherapy. In this study there was no difference in re-excision rates (21 versus 18%) between the patients who received neoadjuvant chemotherapy and those treated adjuvantly.²⁶ In our study, of the 8 patients who received neoadjuvant therapy, 2 (25%) required surgical re-excision. However, only 50% of patients had their margins correctly assessed by the optical device (2 false negative, 2 false positive) The optical devices inability to correctly classify the margins in these cases may be due to the mosaic pattern of regression that can be found in some patients undergoing neoadjuvant therapy in comparison to a circumferential regression pattern. As the number of these cases increases in our clinical subset, it is likely that additional optical parameters will be added to account for the physiologic and metabolic changes encountered in specimens with complete responses and those that leave microscopic deposits within the tumor bed.

This study utilized the pathologically confirmed margin diagnosis to identify the tissue compositional parameters most likely to differentiate positive from malignant tissues. The ratio map of β -carotene:scattering showed the most significant difference reflecting a decrease in adipose content and an increase in cell density within malignant margins. Prior work by our group and others has shown that scattering, β -carotene content and total hemoglobin are good parameters with which to differentiate malignant from benign tissues.^{28–31} Results from the current study are consistent with earlier findings where optical measurements were made from tumor and normal biopsies.

It has been noted earlier that there are several reasons why a margin may be misclassified as false-negative including the fraction of the margin occupied by the tumor (DCIS example) and changes in proliferation rate (tumor treated with endocrine therapy). There are factors that could also contribute to a false positive, for example, the “pancake” phenomenon which has been previously described as a difficulty in margin assessment.³² The “flattening” of the immediately excised specimen for specimen radiograph could alter the tumor to margin ratio,

and/or affect the inking leading to a “false positive” pathology read on a margin that was truly negative for residual disease.³³ In the future we plan to use the device on the excised specimens prior to intra-operative specimen radiography to attempt to reduce the “pancake” phenomenon on the optical outputs and thereby potentially reduce the false positive readings.

During the past 3 years, over 50 journal articles have been written about the methodology of breast lumpectomy margin assessment or the patient characteristics most likely to result in a positive specimen margin.^{34–37} Three groups have shown a reduction in their re-excision rate to between 10–20% with the use of frozen section analysis.^{11,13,38} This reduction is important for those patients who benefited from the availability of these resources. However these intra-operative margin assessment tools of frozen section and touch prep analysis are not widely available and are not routinely utilized even in high volume centers. At Duke University we currently do not use either of these technologies for intra-operative margin assessment because of the need for an onsite pathologist or cytopathologist at an ambulatory surgery center which is not located within the main hospital. As this optical technology is refined, we hope to provide comparable and potentially improved results to frozen section and touch prep analyses for a broader patient population. Future clinical trials will be performed at medical centers that routinely perform intraoperative frozen sections or touch prep cytology for lumpectomy margin assessment, and the performance of our optical imaging device will be directly compared to intraoperative pathologic evaluation.

The routine removal of “shaved margins” has also been proposed to reduce the re-excision rate for patients undergoing breast conservation therapy. In two recent papers, the authors were able to reduce the number of patients going back to the operating room by 50%, yet 18–20% still required either a re-excision or mastectomy based on final pathological review.^{39–40} It should be noted that these extra excisions are likely at the expense of the patient’s cosmetic outcome due to the increase in volume excised. Currently the optical device has a specificity of 67% and thus could significantly reduce re-excision while preserving cosmesis.

Breast conservation therapy remains a mainstay in the treatment of patients with breast cancer. As new minimally invasive technologies such as cryosurgery, radiofrequency ablation and intra-operative radiation become more widely available, an intra-operative tool to assure complete resection of a breast malignancy is necessary. This preliminary subset of patients whose BCT specimens were assessed with optical spectral imaging device validates the early potential of this novel technology. As the number of patients and tumor types increases within this study, the accuracy and applicability of this non-invasive technique is expected to show significant promise for women with breast cancer who are candidates for breast conserving therapies.

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Figure 1.

A) Photograph of the portable optical spectral imaging device, and B) photograph of a mockup of a lumpectomy specimen being imaged by the handheld imaging probe of the device.

Figure 2.

Maps of β -carotene:scattering coefficient for A) negative margin, C) margin positive for DCIS, E) margin positive for IDC. Site-level pathology for the margins indicated that the blue areas generally corresponded to cancer-free areas, whereas the red areas corresponded to regions with residual disease. Corresponding frequency histograms are given to the right of each map (B, D, F, respectively), and depict the distribution of β -carotene:scattering values present in each image. The vertical dashed line indicates the threshold on β -carotene:scattering values used in the predictive model to separate positive from negative margins; the percentage of image pixels below the threshold is given in the histogram inset.

Figure 3. Boxplots of A) percentage of pixels < 6 μ M-cm β -carotene:scattering, and B) percentage of pixels < 8 μ M-cm total hemoglobin:scattering, stratified by margin status (negative: blue, positive/close: red). *P*-values from Wilcoxon rank-sum tests are indicated in the plots.

Figure 4.
ROC of the two predictors from Figure 3.

Figure 5.

Maps of β -carotene:scattering coefficient (A, C) for margins positive for IDC but falsely classified as negative by the predictive model from 2 different patients. Corresponding frequency histograms are given to the right of each map (B and D, respectively), and depict the distribution of β -carotene:scattering values present in each image; the percentage of image pixels below the threshold is given in the histogram inset.

Table I

Characteristics of the study population.

Characteristics of the Study Population	
Age (average)	57 (Range 30–78)
Patient Tumor Histology	
Invasive Ductal	8 (17%)
Ductal Carcinoma in Situ	3 (6%)
Combined Invasive Ductal/DCIS	24 (50%)
Other	10 (21%)
No Tumor Present/Negative	3 (6%) (post chemotherapy)
Estrogen Positive	38 (79%)
HER-2/neu Positive	6 (13%)
Node Positive	13 N1 (27%)
Surgical Re-excision Rate	12 (25%)
Neoadjuvant Therapy	Chemotherapy 6 (13%); Endocrine 2 (4%)
Lumpectomy Volume (average)	513 cm ³ (Range 93–2237 cm ³)
Margins Assessed	Anterior 14 (25%); Posterior 15 (27%); Superior 12 (22%); Inferior 3 (5%) Medial 7 (13%); Lateral 4 (7%)

Prediction accuracy of cross-validated algorithm for margin classification on 55 margins from 48 patients. Sensitivity is also given for path positive margins separated by cancer subtype, as well as by positive versus close margin status.

Table II

	All Margins		Positive, IDC		Positive, DCIS		Positive, Other		All Positive	
	Path Positive	Path Negative	Path Positive	Path Positive	Path Positive	Path Positive	Path Positive	Path Positive	Path Positive	Path Close
Probe Positive	27	7	11	8	3	14	13			
Probe Negative			3	1	3	3	4			
Sensitivity		79.4%	78.6%	88.9%		82.4%	76.5%			
Specificity		66.7%								