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Rapid noninvasive optical imaging of tissue composition in breast tumor margins

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Abstract

BACKGROUND: In women undergoing breast conserving surgery (BCS), up to 60% can require re-excision. Our objective is to develop an optically based technology which can differentiate benign from malignant breast tissues intraoperatively through differences in tissue composition factors.

METHODS: A prospective study of optical imaging of BCS margins is being performed. Optical images are transformed into tissue composition maps with parameters of total hemoglobin concentration, b-carotene concentration and scattering. The predicted outcome is then compared to the margin-level pathology.

RESULTS: Fifty-five margins from 48 patients have undergone assessment. Within 34 specimens with pathologically confirmed positive margins, the ratio map of b-carotene/scattering showed the most significant difference reflecting a decrease in adipose and an increase in cell density within malignant margins ($p=.002$). These differences were notable in both in-situ and invasive disease.

CONCLUSIONS: We present a novel optical spectral imaging device that provides a rapid, non-destructive assay of the tissue composition of breast tumor margins.

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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 noninvasive 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 noninvasive breast cancers, have equivalent long-term survival to those who undergo mastectomy.^{2–4} However, in a 15-year follow-up evaluation 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, 1 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 re-

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currence 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

Breast cancer is a heterogeneous disease.⁷ The heterogeneity of this disease makes it challenging for surgeons to characterize tumors intraoperatively such that they can be removed completely at the initial surgery. Currently, there is no widely available intraoperative tool to ensure complete removal of a breast tumor during BCT. In experienced hands, frozen section and touch preparation cytology 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 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 to 3 mm. The quoted rates of second surgeries vary in the literature and range from 12% to as high as 60%.^{11–15} To characterize breast tumor margins more accurately intraoperatively and thereby reduce the re-excision and local recurrence rate, we have developed a device that uses optical spectral imaging to characterize differences in tissue composition of excised breast specimen margins. This device uses 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 classified broadly as morphologic (β -carotene, cell density) and physiologic (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 (0–2 mm) of partial mastectomy speci-

mens in women with invasive and noninvasive breast malignancies.

Materials and Methods

Patients

The study was approved by the institutional review board 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 y) undergoing primary BCT for an invasive or noninvasive breast malignancy. A subgroup recruited to this study had undergone neoadjuvant endocrine or chemotherapy before their surgical procedure. Surgeries 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 preparation cytology 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 hand-held optical spectral imaging probe interfaced to an adjustable tissue specimen box, an imaging spectrograph, and a charge-coupled device camera. A photograph of the device and the specimen box

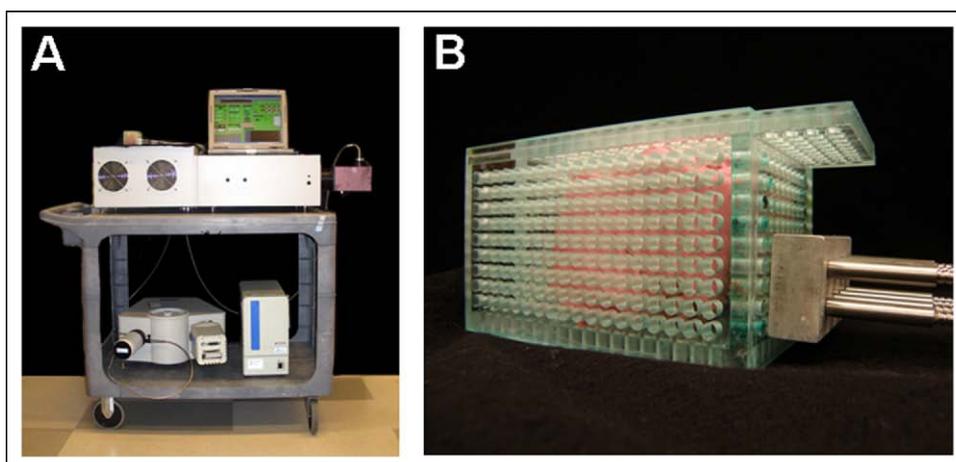


Figure 1 (A) Photograph of the portable optical spectral imaging device, and (B) photograph of a mock-up of a lumpectomy specimen being imaged by the hand-held imaging probe of the device.

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 has been 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. 1B). 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 to 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) the 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, 2 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 then were 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 scored separately by the study pathologist (J.G.). 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 4 inked corners, as well as the type of residual carcinoma found at the margin, if any, was recorded and compared with summary measures derived from the 2 parameter maps of each imaged margin (as described later). 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 because re-excision of the margin usually is 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

Table 1 Characteristics of the study population

Characteristics	
Average age, y (range)	57 (30–78)
Patient tumor histology	
Invasive ductal	8 (17%)
Ductal carcinoma in-situ	3 (6%)
Combined invasive ductal/ductal carcinoma in-situ	24 (50%)
Other	10 (21%)
No tumor present/negative	3 (6%) (postchemotherapy)
Estrogen positive	38 (79%)
HER-2/neu positive	6 (13%)
Node positive	13 (27%)
Surgical re-excision rate	12 (25%)
Neoadjuvant therapy	Chemotherapy, 6 (13%); endocrine 2 (4%)
Average lumpectomy volume (range)	513 cm ³ (93–2237 cm ³)
Margins assessed	Anterior, 14 (25%); Posterior, 15 (27%); Superior, 12 (22%); Inferior, 3 (5%); Medial, 7 (13%); Lateral, 4 (7%)

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 earlier) 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 used in this analysis because the pathologic outcomes could not be coregistered accurately with the specified margins. From the remaining 48 patients, 55 margins were evaluated with the optical spectral imaging device. Table 1 contains a summary of the patient and margin characteristics for the participants in this study. Twenty-one patients had negative margins, and 15 patients had a positive margin on the main specimen that 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.

Most patients (73%) enrolled in the study 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

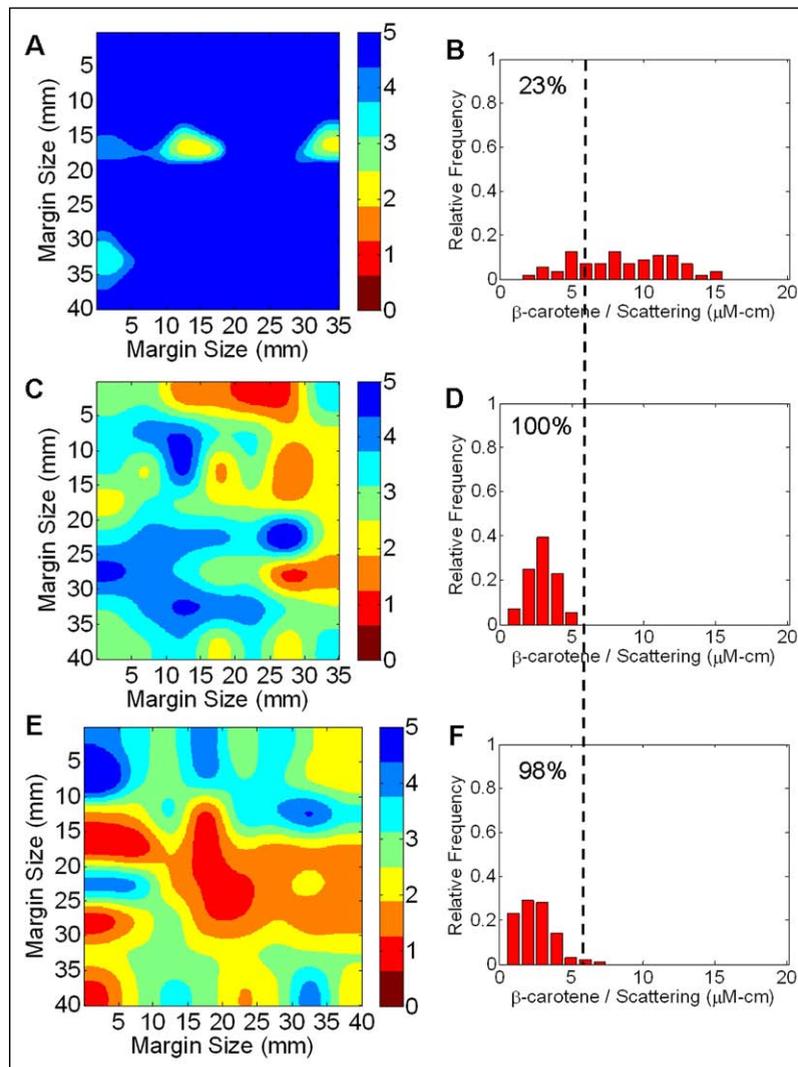


Figure 2 Maps of β -carotene:scattering coefficient for (A) negative margin, (C) margin positive for DCIS, and (E) margin positive for IDC. Site-level pathology for the margins indicated that the blue areas generally corresponded to cancer-free areas, whereas the red areas corresponded to regions with residual disease. Corresponding frequency histograms are given to the right of each map (B, D, and 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.

had a complete pathologic response to their preoperative chemotherapy. For each patient enrolled in this study, only 1 to 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.

Fig. 2 contains representative parameter maps of β -carotene concentration to wavelength-averaged reduced scattering coefficient (β -carotene:scattering) (Fig. 2A, C, and E) and corresponding histograms that graphically represent the distribution of values within each image (Fig. 2B, D, and F) for a margin negative for residual disease (Fig. 2A and B), a margin positive for ductal carcinoma in situ (DCIS) (Fig. 2C and D) and a margin positive for invasive ductal carcinoma (IDC) (Fig. 2E and F). β -carotene is a dietary carotenoid known to be stored primarily in adipocytes, and thus

is 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 Because malignant tissues are expected to have less fat (owing to displacement of adipocytes by carcinoma cells) and higher scattering (owing 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 Fig. 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

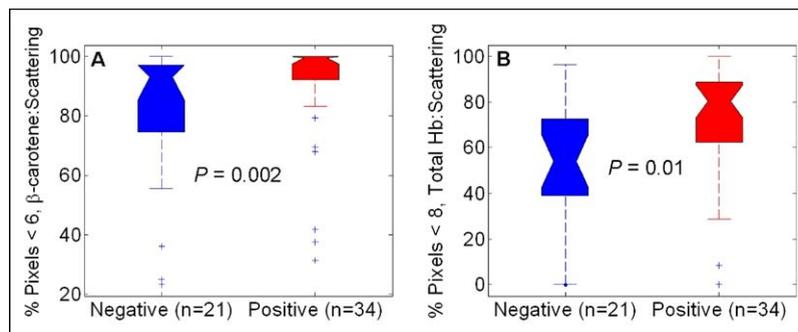


Figure 3 Box plots of (A) percentage of pixels less than 6 $\mu\text{M-cm}$ β -carotene:scattering, and (B) percentage of pixels less than 8 $\mu\text{M-cm}$ total hemoglobin:scattering, stratified by margin status (negative, blue; positive/close, red). *P* values from Wilcoxon rank-sum tests are indicated in the plots.

margin (Fig. 2A) is characterized by a higher proportion of blue pixels, whereas the positive margins (Fig. 2C and E) are characterized by increased proportions of red pixels. Site-level histology of these margins indicated that, generally, the blue areas indicated cancer-free regions of the margin, whereas the orange-red areas indicated regions of the margin containing residual disease.

To build predictors for margin-level assessment from the parameter maps, a threshold value for pixel intensity was determined (eg, 6 $\mu\text{M-cm}$ for β -carotene:scattering) and the percentage of pixels below that threshold was computed (as illustrated in Fig. 2B, D, and F). A Wilcoxon rank-sum test was performed 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 $\mu\text{M-cm}$ for β -carotene:scattering showed the greatest degree of association with pathology ($P < .002$; Fig. 3A). A similar process was applied to total hemoglobin:scattering, such that the percentage of pixels below a threshold value of 8 $\mu\text{M-cm}$ resulted in the most statistically significant differences between positive and negative margins for that parameter ($P < .01$; Fig. 3B).

Next, a multivariate predictive model was developed for classifying a margin as positive or negative based on the predictors shown in Fig. 3. A tree-based approach was taken to build the 2-parameter model, such that a margin was classified as positive if the percentage for the β -carotene:scattering or total hemoglobin:scattering parameters were greater than their respective thresholds; otherwise it was classified as negative. The percentages shown on the y-axes in Fig. 3 were each varied across the complete set of different threshold values (eg, 98% in Fig. 3A and 72% in Fig. 3B), and the sensitivity and specificity then were calculated against margin assessment by pathology. The optimal pair of threshold values was determined by a receiver operator characteristic analysis (Fig. 4) and the Youden index 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 described earlier, and resulted in a sensitivity and specificity of 79% and 67%, respectively, for the 2-parameter decision tree.²³ The percentage pixel thresholds in the final model for β -carotene:scattering and total hemoglobin:scattering were $98\% \pm .19\%$ and $72\% \pm 1.0\%$, respectively.

Table 2 contains the prediction accuracy resulting from the decision-making model described earlier. 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 also is 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 of 14 (78.6%) positive margins. For margins that were positive for DCIS only, the predictive model correctly identified 8 of 9 (88.9%) 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 (72.7%) positive margins. The margins were weighted equally with respect to the number of close margins ($n = 17$) and the number of positive margins ($n = 17$). The performance of the predictive model also was not biased significantly toward either positive or close margins, with positive margins

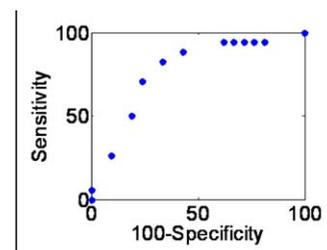


Figure 4 Receiver operator characteristic analysis of the 2 predictors from Figure 3.

Table 2 Prediction accuracy of cross-validated algorithm for margin classification on 55 margins from 48 patients

	All margins		Positive IDC	Positive DCIS	Positive other	All positive	All close
	Path positive	Path negative	Path positive	Path positive	Path positive	Path positive	Path close
Probe positive	27	7	11	8	8	14	13
Probe negative	7	14	3	1	3	3	4
Sensitivity	79.4%		78.6%	88.9%	72.7%	82.4%	76.5%
Specificity	66.7%						

Sensitivity is also given for path-positive margins separated by cancer subtype, as well as by positive versus close margin status.

being predicted correctly only slightly more frequently (14 of 17; 82.4% sensitivity) than close margins (13 of 17; 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; 1 with endocrine therapy and the other with chemotherapy. Fig. 5A and B are 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 pretherapy and posttherapy 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 classified the posterior margin diagnosed with IDC at 2 mm as

negative. The device likely classified this margin as 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, Fig. 5C and D are 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 similar to that of a positive margin but was just above the percentage pixel cut-off level for margin positivity. This is an example of a near-miss. Another of the false-negative patients 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 1,200 cm³. In this case, although most 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

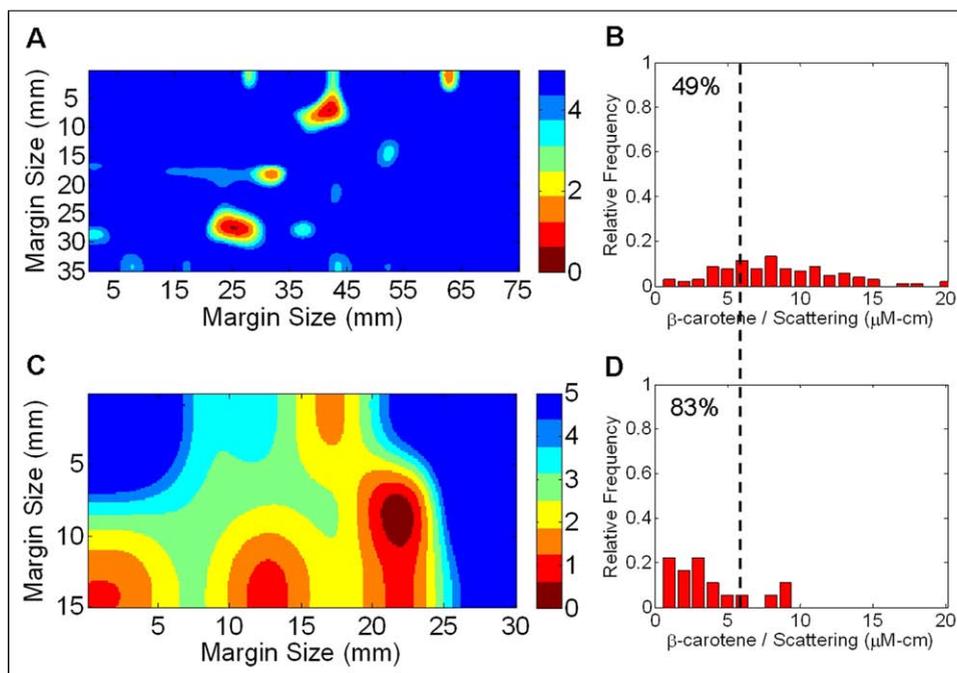


Figure 5 Maps of β -carotene:scattering coefficient (A and C) for margins positive for IDC but falsely classified as negative by the predictive model from 2 different patients. Corresponding frequency histograms are given to the right of each map (B and D, respectively), and depict the distribution of β -carotene:scattering values present in each image; the percentage of image pixels below the threshold is given in the histogram inset.

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 because of the very large size of these margins. This resulted in misclassification of the margin because 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 with the overall margin size.

Comments

Most 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, noninvasive, 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 intraoperatively. In this preliminary patient population, 79% of the pathologically positive margins were identified accurately by the device. This group of correctly identified margins included all the variant pathologies including 1 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 include additional patients with DCIS to ensure that the device adequately can identify DCIS at the margin of a BCT specimen.

In addition to specimens with lobular carcinoma or DCIS, an intraoperative margin device also must 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% vs 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 assessed correctly by the optical device (2 false negative, 2 false positive). The optical de-

vices inability to correctly classify the margins in these cases may be owing to the mosaic pattern of regression that can be found in some patients undergoing neoadjuvant therapy in comparison with 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 used 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.²⁷⁻³⁰ 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 also could contribute to a false-positive diagnosis, for example, the "pancake" phenomenon, which has been described previously as a difficulty in margin assessment.³¹ 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.³² In the future, we plan to use the device on the excised specimens before intraoperative 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, more than 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.³³⁻³⁶ Three groups have shown a reduction in their re-excision rate to between 10% and 20% using frozen section analysis.^{11,13,37} This reduction is important for those patients who benefited from the availability of these resources. However, these intraoperative margin assessment tools of frozen section and touch preparation cytology are not widely available and are not used routinely even in high-volume centers. At Duke University we currently do not use either of these technologies for intraoperative margin assessment because of the need for an on-site 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 preparation cytology for a broader patient population. Future clinical trials will be performed at

medical centers that routinely perform intraoperative frozen sections or touch preparation cytology for lumpectomy margin assessment, and the performance of our optical imaging device will be compared directly with intraoperative pathologic evaluation.

The routine removal of shaved margins also has been proposed to reduce the re-excision rate for patients undergoing breast conservation therapy. In 2 recent articles, the investigators were able to reduce the number of patients going back to the operating room by 50%, yet 18% to 20% still required either a re-excision or mastectomy based on final pathologic review.^{38,39} It should be noted that these extra excisions are likely at the expense of the patient's cosmetic outcome because of the increase in volume excised. Currently, the optical device has a specificity of 67% and thus significantly could reduce re-excision while preserving cosmesis.

BCT remains a mainstay in the treatment of patients with breast cancer. As new minimally invasive technologies such as cryosurgery, radiofrequency ablation, and intraoperative radiation become more widely available, an intraoperative tool to ensure 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 increase within this study, the accuracy and applicability of this noninvasive technique is expected to show significant promise for women with breast cancer who are candidates for breast conserving therapies.

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