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Scatter spectroscopic imaging distinguishes between breast pathologies in tissues relevant to surgical margin assessment

Ashley M. Laughney¹, Venkataramanan Krishnaswamy¹, Elizabeth J. Rizzo², Mary C. Schwab², Richard J. Barth³, Brian W. Pogue^{1,3}, Keith D. Paulsen^{1,4}, and Wendy A. Wells²

¹Thayer School of Engineering, Dartmouth College Hanover NH 03755

²Department of Pathology, Dartmouth Medical School, Lebanon NH 03756

³Department of Surgery, Dartmouth Medical School, Lebanon NH 03756

⁴Department of Diagnostic Radiology, Dartmouth Medical School, Lebanon NH 03756

Abstract

Purpose—A new approach to spectroscopic imaging was developed to detect and discriminate microscopic pathologies in resected breast tissues; diagnostic performance of the prototype system was tested in 27 tissues procured during breast conservative surgery.

Experimental Design—A custom-built, scanning *in situ* spectroscopy platform sampled broadband reflectance from a 150 μ m diameter spot over a 1 $\times$ 1cm² field using a dark field geometry and telecentric lens; the system was designed to balance sensitivity to cellular morphology and imaging the inherent diversity within tissue subtypes. Nearly 300,000 broadband spectra were parameterized using light scattering models and spatially dependent spectral signatures were interpreted using a co-occurrence matrix representation of image texture.

Results—Local scattering changes distinguished benign from malignant pathologies with 94% accuracy, 93% sensitivity, 95% specificity, and 93% positive & 95% negative predictive values using a threshold-based classifier. Texture and shape features were important to optimally discriminate benign from malignant tissues, including pixel-to-pixel correlation, contrast and homogeneity, and the shape features of fractal dimension and Euler number. Analysis of the region-based diagnostic performance showed that spectroscopic image features from 1 $\times$ 1mm² areas were diagnostically discriminant and enabled quantification of within-class tissue heterogeneities.

Conclusions—Localized scatter-imaging signatures detected by the scanning spectroscopy platform readily distinguished benign from malignant pathologies in surgical tissues and demonstrated new spectral-spatial signatures of clinical breast pathologies.

*Corresponding authors: Ashley.Laughney@dartmouth.edu or Brian.W.Pogue@dartmouth.edu Brian William Pogue, 14 Engineering Drive, Dartmouth College, Hanover NH 03755.

7 Competing interests

The authors declare they have no competing interests.

8 Author's contributions

AML participated in experiment design, data collection and interpretation, statistical analysis, and the writing of the manuscript. VK and BWP participated in optical system design and validation, experiment design, data interpretation, and the writing of the manuscript. EJR and MCW participated in tissue acquisition and histological processing. RJB participated in surgical tissue procurement. KDP and WAW participated in experiment design, data interpretation, and writing of the manuscript. All authors read and approved the final manuscript.

9 Author's information (Optional)

n/a

Keywords

Spectroscopy; imaging; light scattering; pathology; breast conserving surgery; texture; morphology

1 Introduction

A major limitation of breast conserving surgery is the inability to intra-operatively assess tumor margins; particularly, frozen section pathology has demonstrated a wide range of positive predictive values and is rarely used clinically due to freezing artifacts in adipose tissues[1, 2]. Instead, margin assessment is routinely performed post-operatively by standard histologic processing. Margins positive for residual disease are associated with an increased risk of local recurrence and decreased survival, so re-excision is the standard of care [3–5]. Intra-operative alternatives to paraffin histology are needed to reduce the secondary excision rate. The challenge in this is to optimize sensitivity to discriminating tissue ultra-structure, while sampling sufficient tissue to account for known patient heterogeneity. To achieve this goal, a scanning-beam platform for both spectroscopy and wide-field imaging was designed to rapidly image localized scattering spectra from intact breast surgical specimens[6, 7], and then to explore textural patterns for discrimination between pathologies *in situ*. The motivation for this approach was two-fold: (1) to exploit optical scattering, which is exquisitely sensitive to microscopic pathology, here used as the diagnostic gold standard, and (2) to capture spatial-spectral signatures because morphological patterns are otherwise difficult to interpret. Strong multiple scattering and absorption of light by tissue chromophores significantly confound direct measurement of the scattering response; here, signal localization was employed to minimize these effects on the detected spectrum and to simplify model-based spectral analysis. Studies using optical fiber-probe systems have indicated that localized spectroscopic measures can differentiate normal from diseased states, but parameters related to structure were heterogeneous [8] and likely under-sampled or underutilized. Indeed, the biological variation that has been observed with point measurements may actually reflect a spatial pattern that provides critically needed diagnostic accuracy, if the spectral and spatial features could be analyzed in concert. In this study, resected breast tissues were interpreted spatially to determine detectability levels for malignant versus benign pathologies.

Breast conserving therapy (BCT), which includes local tumor excision followed by moderate-dose radiation therapy, is the standard of care for patients with early invasive breast cancers (Stage I&II) and for patients with advanced disease (Stage II&III) whose tumor burden is successfully reduced with neo-adjuvant therapy; it has been demonstrated to be equally as safe and effective as mastectomy when surgical margins are clear of residual disease[9, 10]. During conservative surgery, the suspected lesion is removed with a targeted layer of grossly normal tissue (~1cm thick) and colored inks are superficially applied to the resected specimen to define six margins for postoperative assessment[11]. Multiple tissue sections are taken perpendicular to the margins and histologically processed overnight in order to quantify the nearest tumor-to-ink distance. At Dartmouth-Hitchcock Medical Center (DHMC), as well as most academic medical centers, re-excision of a margin is standard-of-care if invasive tumor is at and/or ductal carcinoma in situ (DCIS) is less than 1mm from the ink, but the patient is not informed of this requirement until at least 36 hours after the original surgery. Prospective, randomized trials have shown that over a fifteen year period, one breast cancer death is prevented for every four local recurrences avoided[12]; consequently 20–40% of patients undergo re-excision due to close or positive margins[13]. Detection of residual cancer at the time of primary surgery, rather than post-operatively by

histological processing, could reduce substantially the well-documented risks, costs and psychological effects caused by repeated surgery [14, 15].

Intra-operative alternatives to histology for margin assessment include gross tissue examination, frozen section analysis (FSA) and touch preparation cytology [16–18], but these are severely limited in their application to the breast. Gross tissue examination does not reflect microscopic margin status[17]; FSA gives histological artifacts in adipose tissues, samples only a minute area of the margin per frozen section, and minimizes viable tissue for post-operative assessment [18]; and reported correlations between touch preparation cytology and histologic margins are variable, with reported sensitivities ranging from 37.5% to 99.1% [19]. A study from Johns Hopkins evaluated the choice of some surgeons to take additional tissue from around the entire wall of the residual cavity immediately after the original lumpectomy had been removed. Additional cavity sampling significantly reduced the need for later re-excision [20], but such an increase in total tissue removed can diminish final cosmetic results. Most surgeons today prefer to identify each margin separately, with specific inks on the primary specimen, so they can perform directed re-excisions when necessary.

Optical spectroscopy has increasingly been explored as a powerful, intra-operative alternative to routine histological processing; specifically, localized spectroscopy has demonstrated an ability to distinguish microscopic pathologies *in situ*. Particular emphasis has been placed on diagnostic sensing during biopsy sampling [21–23] and improving resection completeness during breast conserving surgery[24–26]. To date, the wide-field extension of localized spectroscopy has been realized through multiplexed arrays of probes[25, 27] or raster-scanning techniques[28, 29], which mainly suffer from long data acquisition times, under-sampling, or larger probing volumes which may dilute intrinsic tumor signatures by volume averaging [23]. Ramanujam and colleagues pioneered a multi-channel probe array that samples diffuse broadband spectra at 5mm intervals over a 2×4cm area with manual translation of the sensor. Probing depths range from 0.5–2mm, depending on the tissue optical properties and source-to-detector distance in the array[27]. Initial efforts here and by others have employed stepper motors to raster scan the tissue sample across a more localized beam (100–200μm spot size), but significant limitations were imposed upon data acquisition time and field size [30, 31]. The Raman molecular fingerprint has also been used for point-based diagnostic sensing in the tumor resection cavity, first by Haka et al [26] and later by Keller et al [32], who demonstrated detection of breast cancers up to 2mm below a normal tissue layer with a spatially offset source-detector pair. Optical coherence tomography (OCT) is another high-resolution technique that has demonstrated success imaging morphology in the surgical setting [33]. The spectroscopy platform used here is different than these earlier methods because it is fundamentally a broadband imaging system (no sampling gaps) with specific control over the illumination-detection volume and consequently, microscopic sensitivity. As the first clinical demonstration of this prototype system, nearly 300,000 broadband spectra were parameterized in resected breast tissues and spatially dependent spectral signatures were interpreted using a co-occurrence matrix representation of image texture. The platform was designed to evaluate the diagnostic potential of localized scatter-imaging features and to quantify scattering heterogeneities observed within breast tissue types.

2 Materials and Methods

2.1 Scanning in situ spectroscopy platform

Surgical breast tissues, both lumpectomy and biopsy specimens, were imaged with a custom-built, scanning-beam spectroscopy platform. A schematic of the system is presented in Figure 1 and it is described elsewhere in the literature [7]. In brief, the imaging system

employs dark-field illumination and a telecentric, scanning lens to rapidly sample broadband spectra (450:700nm) at a 150 μ m lateral resolution over a 1 $\times$ 1cm² field-of-view (FOV). Each tissue sample, mounted on a glass plate above the optical assembly, was imaged in a non-contact and inverted geometry without mechanically translating the specimen or imaging system. Non-contact sampling avoided reflectance profile changes induced by probe contact pressure, a significant artifact quantified by Ti[34]. Measurement time per 1 $\times$ 1cm² FOV was approximately 12 minutes, although further improvements in data transfer rates are possible through hardware modifications not yet optimized in this prototype. Trace background reflection from the optical system, $R_{BG,meas}(x, y, \lambda)$, were acquired and subtracted from the measured spectra, $R_{TISSUE,meas}(x, y, \lambda)$, and data were normalized to the spectral response of the system, $R_{SPEC,meas}(x, y, \lambda)$, on a pixel-by-pixel basis using a 5% diffuse reflectance Spectralon standard (SRS-05-010 Labsphere, Inc., Northern Sutton, New Hampshire). Spectralon standards are highly stable, providing a daily calibration for direct comparison between tissue samples; this model number was chosen because it presented a similar reflectance level to the tissues imaged. Background, reference and sample measurements were acquired without removing the glass sample holder from the optical assembly, and produced a reflectance measure relative to the Spectralon standard according to

$$R_{TISSUE,ref}(x, y, \lambda) = \frac{R_{TISSUE,meas}(x, y, \lambda) - R_{BG,meas}(x, y, \lambda)}{R_{SPEC,meas}(x, y, \lambda) - R_{BG,meas}(x, y, \lambda)} \quad \text{Equation 1}$$

Dark field illumination efficiently rejected specular light from the detection path and the scan lens yielded normal illumination over the 1 $\times$ 1cm² FOV and illumination bandwidth. Signal localization limited detection to weakly scattered photons by obstructing multiply scattered and absorbed light from the detection path [35]. The advantage of this design is direct sampling of spectroscopic scattering, although the signal only originates from the tissue surface [6].

2.2 Simple, fast spectral parameterization using linear regression

Diagnostic classification was examined by sampling a comprehensive number of spectra and exploring their spatial relationships, rather than by increasing spectral model complexity. The localized illumination-detection geometry combined with typical tissue optical properties, limited detection to nearly single-event backscattering, so a power law dependence on wavelength could be used to describe the scattering spectrum[36]. Relative reflectance spectra, $R_{TISSUE,ref}(x, y, \lambda)$, acquired from clinically relevant breast pathologies are displayed in an inset of Figure 1. Their mostly linear spectral shape justifies application of this simple approximation

$$R_{TISSUE,ref}(x, y, \lambda) = A(x, y) \lambda^{-b(x, y)} \quad \text{Equation 2}$$

Here, parameters A and b are defined as the scattering amplitude and scattering power, respectively. These quantities reflect variations in the size and number density of scattering centers in the volume of tissue probed, which occur on sub-micron and even sub-nanometer length scales[37–39]. The data-model was log transformed and linear regression was employed to obtain estimates of the scattering amplitude and scattering power relative to Spectralon in a waveband that avoids hemoglobin absorption peaks (610:700nm) through direct matrix inversion. Additionally, a measure of average irradiance was calculated by integrating the reflectance spectrum over this waveband.

2.3 Textural feature extraction

The scattering spectrum is relatively featureless compared to the visible absorption spectrum, limiting the unique information obtained per spectrum to the scattering power (or slope) and integrated intensity; but its spatial distribution is heterogeneous and region-based evaluation gave new signatures of diagnostic morphology. Direct sampling of larger tissue volumes may have otherwise masked this contrast because light transport becomes diffuse and absorption effects increase exponentially. The scattering slope was more localized than the integrated scattering intensity, so fundamental texture and shape features were computed based on the scattering power images [30, 40]. A 5×5 pixel neighborhood was chosen to compute texture features in a region that approximated the oxygen diffusion length in tissue (clinically observed to span 100–500µm[41]) because this was biologically relevant and showed outstanding discriminatory power. The gray-level co-occurrence matrix (GLCM) representation of texture features, first defined by Haralick[42], was used to mathematically represent intensity spatial dependencies in the images of scattering power using functions available in Matlab's image processing toolbox. Here, the texture features – contrast, correlation and homogeneity – were computed from the GLCM for a displacement vector of unit length and directionality symmetric about the angles, 0°, 45°, 90°, 135°. Reported values were averaged over the four angles because texture primitives were observed to be rotationally invariant. Contrast measures the amount of local variation present in the image, correlation provides an indication of the gray-tone linear-dependencies, and homogeneity represents the closeness of the distribution of elements in the GLCM to its diagonal (few dominant gray-tone transitions are expected in a highly homogeneous image). Additionally, a threshold to the sum of squared elements in the GLCM was applied to generate a binary map from which additional topological features related to scatter-image shape, the *Euler number* and *Fractal dimension*, were computed. The Euler number was computed from the binary map by summing the number of connected components (objects) in the image minus the number of holes in those objects[43]. Additionally, the fractal dimension of each binary image was computed to quantify intensity variations with scale using a box-counting method [44–46].

2.4 Imaging breast surgical specimens

In this HIPAA-compliant, prospective study, approved by the Institutional Review Board for the protection of human subjects, written informed consent was not required for participants, although an information sheet regarding the study was provided with an opt-out provision. Fresh tissue procured during breast conserving surgery or surgical biopsy was obtained directly from the Department of Pathology at DHMC from patients who did not decline this use of their tissue. Specimen imaging did not affect procedure time in the operating room or the content and verification of the final pathology report. Tissues were imaged within one hour of resection and returned to pathology for standard histological processing. An effort was made to image larger lumpectomy specimens, typical of the tissue volumes encountered during surgery. In the case of inked lumpectomy specimens, the three-dimensional tissue volume was loafed (standard pathology protocol) and one face of one slice of tissue was imaged in a region unaffected by ink. In some cases, tissues were cut from the larger specimen. Figure (2) illustrates the protocol developed for co-registration of the imaged field with histology from the large, fresh tissue sections: a thin, paraffin window bounding the image field was placed between the tissue surface and glass plate to locate the imaged field in an inverted geometry. When the paraffin windowed tissue was removed from the optical assembly, pins dipped in India ink were placed at the corners of the imaged field to secure the specimen to a piece of cork and to mark the imaged portion of the sample with black circles. The tissue-cork assembly was placed tissue-side down in 10% buffered formalin (Biochemical Sciences Inc., Swedesboro, NJ), dehydrated through graded alcohols, and paraffin embedded with the inked pins in place. After fixation, the pins were removed and

tissue sections (4 μ m) were coated with adhesive (sta-onTM, Surgipath Medical Industries Inc., Richmond, IL), mounted on glass slides, and stained with Hematoxylin and Eosin (H&E) for review. Circumscribed pin marks with inked borders were clearly evident on the H&E stained sections cut in the exact geometry imaged *in situ*, so that pathology correlates could be determined within areas bound by the pin markers. Figure (3) illustrates co-registration of scattering maps with pathology for the tissue types, *benign*, *DCIS* and *invasive cancer*. The 1 $\times$ 1cm²-imaged field was assigned one microscopic diagnosis between pin markers according to an experience pathologist (WAW). Imaging artifacts were automatically detected; pixels with low signal-to-background or detector saturation, which occurred when insufficient contact existed between tissue and the glass plate, were removed from the image. Specimens from a total of 32 patients were imaged with five excluded from the study – three because of insufficient contact between the tissue sample and the glass plate and two were confounded by chemotherapy treatment prior to surgery. Following automatic artifact removal, a total of 280,266 spectra were sampled from 32 FOVs in specimens acquired from 27 patients – demographics of sampled tissues are detailed in supplementary Table (1). The dataset represents a significantly larger number of broadband spectra than those reported in most probe-based classification studies[47].

2.5 Statistical analysis and performance metrics

Box plots of spectroscopic parameters and textural features were used for initial comparison of group medians – red bars indicate the median per diagnostic class, green dots indicate the mean value per patient, and boxes delineate the inter-quartile fractions with outliers represented by red crosses. Discrimination was assessed between benign and malignant pathologies and between the benign pathology sub-types, *normal*, *fibrocystic disease* and *fibroadenomas*, and the malignant pathology sub-types, *DCIS* and *invasive cancer*. DCIS was treated as a malignant pathology because clinically it is treated as a pre-invasive lesion. The mean and standard deviation of each parameter per 1 $\times$ 1cm² FOV were reported to quantify optical parameter heterogeneity within breast tissue types.

One-way analysis of variance was employed to assess whether parameters were drawn from a population with the same sample mean. This evaluation was followed by a paired, student t-test to determine which diagnostic groups were differentiable. The Behrens-Fisher null hypothesis tested whether parameters extracted from paired diagnostic groups were drawn from independent, normal distributions with equal means, but not necessarily equal variance. Variance was not assumed to be equal between diagnostic groups based on the group box plots. For all calculations, the null hypothesis was rejected with $\alpha=0.05$.

Receiver operator characteristic (ROC) analysis was used to evaluate the performance of a simple, threshold-based discrimination of benign from malignant pathologies according to the region-averaged scattering power as a function of region size (bin size is defined as the length of each square averaged region). Confidence intervals ($\alpha=0.05$) for the binomial sensitivity and specificity were computed according to the Yates χ^2 interval [48]. Area under the curve (AUC) as a function of bin size was used to characterize the scale of scattering variance observed within typical breast pathologies at this detection resolution and to identify the minimum sampling area that renders a robust diagnosis from scattering signatures. This approach was advantageous because spectral parameters were directly interpreted and diagnostic discrimination did not require training.

3 Results

3.1 Spectral parameters: variability and diagnostic relevance

Box plots of the region-averaged scattering slope and integrated irradiance as a function of diagnosis are illustrated in Figure 4(a–b); the mean and standard deviation per diagnosis are

listed in supplementary Table (1). The intra-patient scattering response was expectedly heterogeneous, but imaging-pathology correlates revealed that local variations reflected morphology like the organization of glandular structures, stroma and adipose compartments. Features averaged over a $1 \times 1 \text{ cm}^2$ FOV accounted for the known scattering heterogeneities at this sampling resolution and a natural separation between benign and malignant pathologies emerged. Box plot notches show scattering parameters did not independently discriminate pathology subtypes within the benign and malignant classes, except for fibroadenomas, which were distinguished from other benign pathologies by a lower integrated intensity. Higher scattering slopes were typical of benign, as compared to in situ and invasive pathologies. This finding is consistent with literature reports of an overall decrease in the reduced scattering coefficient at all wavelengths associated with benign relative to malignant tissues[8, 49, 50]. Histology revealed that the invasive cancer extreme with high scattering slope (indicated by the green star in Figure 4a) had dense stromal content, perhaps explaining its outlier behavior.

An understanding of within-class signal variance at the detection resolution is critical to the development of spectroscopic tools for diagnostic sensing; otherwise sampling artifacts can misinform a diagnosis. ROC analysis evaluated the ability of the region-averaged scattering slope to discriminate benign from malignant pathologies as a function of region size in order to characterize the length scale of scattering heterogeneity observed when typical breast pathologies at this sampling resolution, as shown is Figure 4(c–d). Region size spanned single spectra, a $100 \mu\text{m}$ detection spot size, to the $1 \times 1 \text{ cm}^2$ spectroscopic image. Performance curves suggest that localized spectra sampled over a $1 \times 1 \text{ mm}^2$ area are diagnostic and characterize the spatial extent of scattering variance observed within typical breast pathologies at this detection resolution. The $1 \times 1 \text{ cm}^2$ region-averaged scattering power separated benign from malignant pathologies with 94% accuracy, 93(77–100)% sensitivity, 95(79–100)% specificity, 93% positive predictive value and 95% negative predictive value for 32 regions (41% malignant). When a diagnosis was rendered on a per-spectrum basis, classification was achieved with 73% accuracy, 72(71.8–72.2)% sensitivity, 74(73.8–74.2)% specificity, 67% positive predictive value and 74% negative predictive value for 280,266 spectra (40% malignant); highlighting the importance of region-based assessment.

3.2 Spectrally-derived texture features and their diagnostic potential

Textural features were explored to better understand spatial patterns in the spectroscopic scattering images. Examples of texture maps and corresponding pathology for the tissue types, *benign*, *DCIS*, and *invasive cancer*, are presented in Figure 5. The first column shows a map of texture contrast for tissues with increasing diagnostic severity. Texture contrast is uniform and low for normal tissues, and tends to increase in intensity for in situ carcinomas, a pathology characterized by marked expansion of glandular units by neo-plastic cells, compressing (but not invading) the surrounding stromal environment. Invasive cancers have uniformly high texture contrast, likely because of their infiltrative epithelial component. Similar trends are observed in the maps of texture correlation (column 2) and inverse trends are evident in maps of texture homogeneity. Images suggest that epithelial regions have more local variance (contrast), mainly because the size of epithelium is nearer to the detection resolution. In contrast, predominantly stromal regions appear more homogeneous because collagen fibers are two orders of magnitude smaller than the detection spot size. Box plots summarizing texture parameters as a function of diagnosis for all patients, including the shape features, *Euler number* and *Fractal dimension*, are shown in supplementary data and highlight the unique information provided by these measures.

Benign pathologies tended towards a positive Euler number because predominantly stromal regions appear more homogeneous and invasive pathologies tended towards a negative Euler

number due to greater local spatial variance. The fractal dimension is expected to have a value between a line ($D=1$) and a plane ($D=2$) for a 2-dimensional image. A higher fractal dimension indicates greater pixel-to-pixel variance and was observed in malignant tissues. ROC curves in the supplementary figure compare the diagnostic performance of the spectral integrated intensity and slope, region-averaged scattering slope and textural parameters. The integrated intensity did not readily distinguish benign from malignant pathologies, but provided new information that contributed to pathology sub-type differentiation.

Pair-wise discrimination between normal and invasive pathologies, normal and DCIS pathologies, and invasive and DCIS pathologies, are evaluated according to region-averaged spectral and textural parameters; their significance values are presented in Table 1. Significance values within 95% confidence limits are underlined and show that spatial-spectral features differentiated the DCIS patients ($n=2$) from their benign and invasive counterparts. The spatial-spectral features, correlation and contrast, are the only parameters to successfully differentiate DCIS from both benign and malignant pathologies within the 95% confidence limits. Even though >18,000 spectra were sampled from the DCIS tissue type, this data was collected from just two patients due to limited availability and prospective determination of this diagnosis. While textural features may successfully decouple *in situ* pathologies from their normal and invasive counterparts, specimens from a larger patient population are needed to validate this hypothesis. Textural signatures of DCIS are rather intuitive because DCIS is composed of morphological features found in both normal tissue and invasive pathologies – the spatial relationship between these scattering components is responsible for uniquely identifying the pathology *in situ*.

4 Discussion

A scanning *in situ* spectroscopy platform was here used to investigate the diagnostic performance of highly localized scatter-imaging signatures in resected breast tissues. Its specifications were designed mainly for microscopic sensitivity to pathology, the diagnostic gold standard, and to avoid under-sampling in tissues relevant to surgical margin assessment. High-throughput imaging of light scattering, in contrast to probing discrete locations, better accounted for within-class spectroscopic variance and rapidly populated a training set for classification. The illumination-detection geometry was designed to explore direct scattering contrast and its relationship to diagnostic ultra-structure in breast tissues, so analysis focused on the spectral response of photons experiencing few scattering events and their value to diagnostic discrimination. The spot size and integrated phase function employed by the scanning spectroscopy platform detected a spectral slope that readily distinguished benign from malignant pathologies with 94% accuracy when evaluated per $1 \times 1 \text{ cm}^2$; the integrated irradiance further enhanced differentiation of pathologic subtypes. The texture features of *correlation*, *contrast* and *homogeneity*, and the shape features of *fractal dimension* and *Euler number*, significantly discriminated benign from malignant pathologies. Multi-parametric diagnostic classification was not presented here, but paired student t-tests suggest that the textural features may provide a unique measure for identifying DCIS.

A key advantage of the scanning *in situ* spectroscopy platform is that sampling and image feature quantification may be performed at multiple levels, each giving unique information tailored to the specific application. A higher sampling resolution could assess variance within intra-epithelial or extracellular compartments; perhaps identifying new markers of cancer or pre-cancer[51]; but translation of microscopic techniques to surgical problems like margin assessment will likely be flawed by sampling artifacts. Sampling larger volumes for increased spectral complexity (absorption probabilistic) and better coverage of the full tumor specimen is possible, the tradeoff, however, is loss of sensitivity to sub-cellular architecture

and the integrated phase function. The main advantage of the resolution and image size employed here was its relevance to standard clinical pathology; larger areas or sampling volumes could result in a mixed diagnosis and reduce sensitivity to microscopic residual disease. Current diagnostic performance is limited by our ability to co-register the spectrally-derived images with pathology. With improved co-registration, the system has the potential to differentiate light scattering from morphological features within tissue types which could lead to better understanding of tissue optical properties and determination of the minimum size of detectable, residual cancer at the present sampling resolution. Texture features were computed at length scales approximating the oxygen diffusion length in tissue ($500\mu\text{m}$) for increased sensitivity to DCIS, a powerful predictor of local disease recurrence and challenging pathology to detect intra-operatively[52–54]. In the absence of biological rationale, a feature-ranking algorithm could be used to optimize the neighborhood size employed for texture feature extraction [55].

Rapid, optical assessment of resection tissue margins at the time of primary surgery could have immediate clinical impact by reducing the high rate of secondary excision. The scanning-beam design used here efficiently imaged tissues relevant to surgical margin assessment and signal localization enabled linear spectral evaluation (computationally inexpensive). Furthermore, a simple threshold applied to the region-averaged scattering slope readily distinguished benign from malignant pathologies, rendering overall, a high degree of automation and near real-time diagnostic feedback. Data acquisition and analysis are currently rendered in less than 15minutes, but data transfer rates are not yet optimized in this prototype system. The system was designed to differentiate pathologies at the surface of resected tissues in the operating room at the time of primary surgery. Margin status could then be validated post-operatively by routine histology. Smaller tissue pieces were evaluated here for initial clinical testing, but the system is capable of imaging larger, uncut lumpectomy specimens. The demonstrated diagnostic performance supports further hardware optimization and a blinded clinical study to validate localized spectroscopic *imaging* for *in situ* characterization of breast tissue types. Other potential applications include pathology discrimination during prostatectomy or rectal carcinoma resection, where margin assessment is critical for curative therapy and frozen sections can be diagnostically unreliable and/or destroy all remaining tissue for routine histology [56–58]. Here, we have evaluated the diagnostic performance of near single-event light scattering in an imaging context and explored new spectral-spatial signatures of breast pathologies. These results appear to be the first demonstration of imaging highly localized scattering spectra in thick biological tissues using a scanning-beam approach that has shown significant diagnostic potential.

5 Conclusions

In this study, spectroscopic images of resected breast tissues were analyzed to characterize scatter-imaging signatures of clinically relevant breast pathologies. Images were acquired using a scanning *in situ* spectroscopy platform that selectively sampled the spectrum of near-single event light scattering over a 1cm^2 FOV, significantly decoupling the effects of absorption from scattering and allowing linear interpretation of the resulting spectra. Spatially, the intra-specimen scattering response was expectedly heterogeneous, but imaging accounted for this morphological diversity and improved region-based diagnosis. Spectra locally measured over a $1\times 1\text{mm}^2$ area sufficiently characterized the heterogeneity observed within breast tissue types at this sampling resolution and rendered a robust diagnosis according to scattering features. Local scattering images discerned benign from malignant pathologies with 94% accuracy using a simple, threshold-based classifier and revealed new spectral-spatial signatures of breast pathologies. The texture features of *correlation*, *contrast* and *homogeneity*, and the shape features of *fractal dimension* and *Euler number*, also

discriminated benign from malignant pathologies and suggested potential contrast mechanisms for DCIS, indicating that scattering variations encode key morphological patterns with diagnostic power. The system was designed to strike a balance between microscopic sensitivity and imaging diagnostically representative tissues for eventual use as an adjunct during surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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6 Abbreviations

DCIS	ductal carcinoma in situ
BCT	breast conserving therapy
DHMC	Dartmouth Hitchcock Medical Center
OCT	optical coherence tomography
FOV	field of view
GLCM	gray-level co-occurrence matrix
HIPAA	health insurance portability and accountability act
H&E	Hematoxylin and Eosin
ROC	receiver operator characteristic
AUC	area under the curve
NPV	negative predictive value
PPV	positive predictive value
L1	achromatic lens
GSM	galvanometer-abased scanning mirrors
CCD	charge-coupled device
L3	achromatic lens
CCD-SPEC	CCD-based spectrometer
Dx	diagnosis
b	scattering power
A	scattering amplitude
I_{avg}	integrated intensity
NOR	normal
FCD	fibrocystic disease

FA	fibroadenomas
INV	invasive cancer

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TRANSLATIONAL RELEVANCE

Breast conserving therapy (BCT), which includes local tumor excision followed by moderate-dose radiation therapy, is the standard of care for patients with early invasive breast cancers. A major limitation of BCT is the inability to intra-operatively assess tumor margins; particularly, frozen section pathology has demonstrated a wide range of reported positive predictive rates due to freezing artifacts in adipose tissues[1, 2]. Consequently, margin evaluation is routinely performed post-operatively by standard histological processing. Margin assessment is critical for local disease control because positive margins have been associated with an increased probability of local recurrence and mortality, resulting in a 20–40% re-excision rate. Here, scatter-imaging signatures were explored to detect and discriminate pathologies at the surface of surgical breast tissues in order to improve primary resection completeness. Direct scattering-imaging features readily distinguished benign from malignant pathologies, establishing the first clinical support of localized scatter imaging for intra-operative pathology assessment.

Figure 1. Schematic of the scanning *in situ* spectroscopy platform

The scanning *in situ* spectroscopy platform samples the local scattering spectrum from specimens over a 1cm² FOV using a dark-field geometry. Light from a broadband, supercontinuum laser is collimated using a 200μm core diameter multimode fiber and achromatic lens (L1). L1 is bonded to a 45° micro-rod mirror at its center; an aperture stop with this assembly is used to produce the dark-field. The cylindrical beam of light is steered with galvanometer-based scanning mirrors (GSM) through a custom broadband, telecentric, f-theta scan lens. The lens permits normal illumination of the sample in the FOV for the full 400–750nm waveband. Specular light retraces the illumination path and light scattered from the sample is focused onto a 50μm core-diameter fiber coupled to a CCD-based spectrometer (CCD-SPEC) by the micro-rod mirror and an additional achromatic lens (L3). The focal length of L3 was chosen to have a lateral magnification of 0.5, so that the 50μm fiber detects light scattered from a 100μm diameter spot size on the sample plane. Representative reflectance spectra and corresponding fits sampled from benign (green), *in situ* (blue) and invasive pathologies (red).

Figure 2. Illustration of co-registration between optical images and pathology

Co-registration of spectroscopic images with pathology in large, fresh tissue sections. (a) Slice of a fresh lumpectomy section inked on its margins. A thin paraffin window is placed between the tissue surface and glass plate during imaging to locate the imaged field in an inverted geometry. When the tissue is removed from the plate, inked pins are placed at the corners of the imaged field. (b) The imaged region is marked with inked pins and the tissue is fixed in formalin with pins in place. (c) Fixed tissue was paraffin embedded and processed for histology. (d–e) Pathology correlates were determined within areas bound by the pin marks indicated by the blue arrows.

Figure 3. Example of co-registration between diagnostic pathology and spectral feature maps
 Column (a) shows digital photographs of the fresh tissue specimen with a box bounding the imaged field; Column (b) contains the corresponding pathology with the bounding box highlighting the imaged FOV, blue arrows indicate pin markers; Columns (c–d) present the co-registered images of scattering power and integrated irradiance for normal tissue (row1), DCIS (row2) and invasive cancer (row3).

Figure 4. Diagnostic performance of spectral parameters

Box plots of fitted scattering parameters as a function of diagnosis: (a) mean scattering slope per $1 \times 1 \text{ cm}^2$ FOV, (b) mean integrated irradiance per $1 \times 1 \text{ cm}^2$ FOV. The green star in (a) is an indicator of the invasive cancer extreme (see text for further details). (c) ROC analysis of threshold-based diagnosis of benign and malignant pathologies according to region averaged scattering slope as a function of region size (bin length). (d) Area under the curve (AUC) as a function of region size revealed that 10×10 spectra, or spectra sampled within a $1 \times 1 \text{ mm}^2$ area, sufficiently accounted for biological variance and produced a robust diagnosis.

Figure 5. Images of spectrally derived texture features and corresponding pathology
 Example of textural images associated with normal (row 1), DCIS (row 3) and invasive cancer (row 5) pathologies: maps of spectroscopic textural contrast (column 1), textural correlation (column 2), and textural homogeneity (column 3) are shown. Column 4 includes the corresponding histology for the image sets with pin marks indicated by blue arrows.

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Table 1

Pearson’s correlation coefficient for pair-wise comparisons between normal (NOR) and invasive (INV) pathologies, normal (NOR) and DCIS pathologies, and invasive (INV) and DCIS pathologies per parameter. Underlined values are significant within 95% confidence limits. Pair-wise performance of region-based diagnosis using spectrally derived texture features

Paired Diagnosis		<I _{avg} >	<Correlation>	<Contrast>	<Homogeneity>	Euler #	Fractal Dimension
NOR-INV	<u>0.0006</u>	0.0712	<u>0.0150</u>	<u>0.0046</u>	<u>0.0013</u>	<u>0.0018</u>	<u>0.0016</u>
NOR-DCIS	0.0300	0.0057	6.62E-10	0.0171	0.0134	0.1468	0.0007
INV-DCIS	0.4637	0.1553	<u>0.0452</u>	<u>0.0145</u>	0.3038	0.2673	0.1437