Scatter Spectroscopic Imaging Distinguishes between Breast Pathologies in Tissues Relevant to Surgical Margin Assessment

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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-μm-diameter spot over a 1 × 1 cm2 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 cooccurrence matrix representation of image texture.

Results: Local scattering changes distinguished benign from malignant pathologies with 94% accuracy, 93% sensitivity, 95% specificity, and 93% positive and 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 × 1 mm2 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 showed new spectral-spatial signatures of clinical breast pathologies. Clin Cancer Res; 18(22); 6315–25. ©2012 AACR.

Introduction

A major limitation of breast conserving surgery is the inability to intraoperatively assess tumor margins; particularly, frozen section pathology has shown a wide range of positive predictive values and is rarely used clinically because of freezing artifacts in adipose tissues (1, 2). Instead, margin assessment is routinely conducted postoperatively by standard histologic processing. Margins positive for residual disease are associated with an increased risk of local recurrence and decreased survival, so reexcision is the standard of care (3–5). Intraoperative alternatives to paraffin histology are needed to reduce the secondary excision rate. The challenge in this is to optimize sensitivity to discriminating tissue ultrastructure, whereas 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 2-fold: (i) to exploit optical scattering, which is exquisitely sensitive to microscopic pathology, here used as the diagnostic gold standard, and (ii) 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 used 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 undersampled or underused. Indeed,
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 intraoperatively assess tumor margins; particularly, frozen section pathology has shown a wide range of reported positive predictive rates because of freezing artifacts in adipose tissues. Consequently, margin evaluation is routinely conducted postoperatively by standard histologic 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% to 40% reexcision rate. Here, scatter-imaging signatures were explored to detect and discriminate pathologies at the surface of surgical breast tissues 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 intraoperative pathology assessment.

Detection of residual cancer at the time of primary surgery, rather than postoperatively by histologic processing, could reduce substantially the well-documented risks, costs, and psychological effects caused by repeated surgery (14, 15).

Intraoperative alternatives to histology for margin assessment include gross tissue examination, frozen section analysis (FSA) and tough 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 histologic artifacts in adipose tissues, samples only a minute area of the margin per frozen section, and minimizes viable tissue for postoperative 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 reexcision (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 reexcisions when necessary.

Optical spectroscopy has increasingly been explored as a powerful, intraoperative alternative to routine histologic processing: specifically, localized spectroscopy has shown 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, undersampling, or larger probing volumes which may dilute intrinsic tumor signatures by volume averaging (23). Ramanujam and colleagues pioneered a multichannel probe array that samples diffuse broadband spectra at 5 mm intervals over a 2 × 4 cm area with manual translation of the sensor. Probing depths range from 0.5 to 2 mm, depending on the tissue optical properties and source-to-detector distance in the array (27). Initial efforts here and by others have used stepper motors to raster scan the tissue sample across a more localized beam (100–200-μm-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 and colleagues (26) and later by Keller and colleagues (32), who showed detection of breast cancers up to 2 mm below a normal tissue layer with a spatially offset source-detector pair. Optical coherence tomography is another high-resolution technique that has shown 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 tool.
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 cooccurrence 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.

Materials and Methods

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 Fig. 1 and it is described elsewhere in the literature (7). In brief, the imaging system uses dark-field illumination and a telecentric, scanning lens to rapidly sample broadband spectra (450-700 nm) at a 150 μm lateral resolution over a 1 cm² field of view (FOV). Each tissue sample, mounted on a glass plate above the optical assembly, was imaged in a noncontact and inverted geometry without mechanically translating the specimen or imaging system. Noncontact sampling avoided reflectance profile changes induced by probe contact pressure, a significant artifact quantified by Ti (34). Measurement time per 1 cm² 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,l), were acquired and subtracted from the measured spectra, R_{TISSUE}^{meas}(x,y,l), and data were normalized to the spectral response of the system, R_{SPEC}^{meas}(x,y,l), on a pixel-by-pixel basis using a 5% diffuse reflectance Spectralon standard (SRS-05-010 Labsphere, Inc.). 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

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 1 cm² FOV using a dark-field geometry. Light from a broadband, supercontinuum laser is collimated using a 200-μm 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 to 750 nm waveband. Specular light retraces the illumination path and light scattered from the sample is focused onto a 50-μm core-diameter fiber coupled to a charged couple device (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 50 μm fiber detects light scattered from a 100-μm-diameter spot size on the sample plane. Representative reflectance spectra and corresponding fits sampled from benign (green), in situ (blue), and invasive pathologies (red).
produced a reflectance measure relative to the Spectralon standard according to

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

Equation 1

Dark field illumination efficiently rejected specular light from the detection path and the scan lens yielded normal illumination over the 1 \times 1 \text{cm}^2 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).

**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, $R_{\text{TISSUE,off}}(x, y, \lambda)$, acquired from clinically relevant breast pathologies are displayed in an inset of Fig. 1. Their mostly linear spectral shape justifies application of this simple approximation

$$R_{\text{TISSUE,off}}(x, y, \lambda) = A(x, y) \lambda^{-b(x,y)}$$  

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 submicron and even subnanometer length scales (37–39). The data model was log transformed and linear regression was used to obtain estimates of the 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°, and 135°. Reported values were averaged over the 4 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). In addition, 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). In addition, the fractal dimension of each binary image was computed to quantify intensity variations with scale using a box-counting method (44–46).

**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 about 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 1 hour of resection and returned to pathology for standard histologic 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 3-dimensional tissue volume was loafed (standard pathology protocol) and 1 face of 1 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 coregistration 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 plate, 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.), dehydrated through graded alcohols, and paraffin embedded with the inked pins in place. After fixation, the pins were removed and tissue sections (4 μm) were coated with adhesive (sta-onTM, Surgipath Medical Industries Inc.), 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 marks indicated by the blue arrows.

Figure 2. Illustration of coregistration between optical images and pathology. Coregistration 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 and E, pathology correlates were determined within areas bound by the pin marks.

a significantly larger number of broadband spectra than those reported in most probe-based classification studies (47).

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 interquartile fractions with outliers represented by red crosses. Discrimination was assessed between benign and malignant pathologies and between the benign pathology subtypes, normal, fibrocystic disease, and fibroadenomas, and the malignant pathology subtypes, DCIS and invasive cancer. DCIS was treated as a malignant pathology because clinically it is treated as a preinvasive lesion. The mean and standard deviation of each parameter per 1 cm² FOV were reported to quantify optical parameter heterogeneity within breast tissue types.

One-way analysis of variance was used to assess whether parameters were drawn from a population with the same sample mean. This evaluation was followed by a paired, Student’s 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 $\chi^2$ interval (48). Area under the curve 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.

Results

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 Fig. 4A and B; the mean and standard deviation per diagnosis are listed in Supplementary Table S1. The intrapatient 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 $1 \times 1$ 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 with 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 Fig. 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 to characterize the length scale of scattering heterogeneity observed when typical breast pathologies at this sampling resolution, as shown in Fig. 4C and D. Region size spanned single spectra, a 100-µm detection spot size, to the $1 \times 1$ cm$^2$ spectroscopic image. Performance curves suggest that localized spectra sampled over a $1 \times 1$ mm$^2$ area are diagnostic and characterize the spatial extent of scattering variance observed within typical breast pathologies at
this detection resolution. The 1 × 1 cm² 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.

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 Fig. 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 2 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 toward a positive Euler number because predominantly stromal regions appear more homogeneous and invasive pathologies tended toward a negative Euler number because of 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 subtype 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 more than 18,000 spectra were sampled from the DCIS tissue type, this data was collected from just 2 patients because of limited availability and prospective determination of this diagnosis. While textural features may successfully decouple \textit{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 \textit{in situ}.

**Discussion**

A scanning \textit{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 undersampling in tissues relevant to surgical margin assessment. High-throughput imaging of light scattering, in

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**Table 1.** Pearson’s correlation coefficient for pair-wise comparisons between normal and invasive pathologies, normal and DCIS pathologies, and invasive and DCIS pathologies per parameter

<table>
<thead>
<tr>
<th>Paired diagnosis</th>
<th>(&lt;b&gt;)</th>
<th>(&lt;d_{avg}&gt;)</th>
<th>(&lt;\text{Correlation}&gt;)</th>
<th>(&lt;\text{Contrast}&gt;)</th>
<th>(&lt;\text{Homogeneity}&gt;)</th>
<th>Euler #</th>
<th>Fractal dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOR-INV</td>
<td>0.0006</td>
<td>0.0712</td>
<td>0.0150</td>
<td>0.0046</td>
<td>0.0013</td>
<td>0.0018</td>
<td>0.0016</td>
</tr>
<tr>
<td>NOR-DCIS</td>
<td>0.0300</td>
<td>0.0057</td>
<td>6.62E-10</td>
<td>0.0171</td>
<td>0.0134</td>
<td>0.1468</td>
<td>0.0007</td>
</tr>
<tr>
<td>INV-DCIS</td>
<td>0.4637</td>
<td>0.1553</td>
<td>0.0452</td>
<td>0.0145</td>
<td>0.3038</td>
<td>0.2673</td>
<td>0.1437</td>
</tr>
</tbody>
</table>

NOTE: Underlined values are significant within 95% confidence limits. Abbreviations: NOR, normal; INV, invasive.
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 ultrastructure 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 used 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’s $t$ 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 conducted at multiple levels, each giving unique information tailored to the specific application. A higher sampling resolution could assess variance within intrapelvic or extracellular compartments; perhaps identifying new markers of cancer or precancer (51); but translation of microscopic techniques to surgical problems such as 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 subcellular architecture and the integrated phase function. The main advantage of the resolution and image size used 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 coregister the spectrally derived images with pathology. With improved coregistration, 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 $\mu$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 biologic rationale, a feature-ranking algorithm could be used to optimize the neighborhood size used 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 15 minutes, 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 postoperatively 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 shown 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 biologic tissues using a scanning-beam approach that has shown significant diagnostic potential.

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 $1 \text{cm}^2$ FOV, significantly decoupling the effects of absorption from scattering and allowing linear interpretation of the resulting spectra. Spatially, the intraspecimen 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.
Disclosure of Potential Conflicts of Interest

K.D. Paulsen has ownership interests (including patents) in patents and pending patents. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: A.M. Laughney, V. Krishnaswamy, B.W. Pogue, K. D. Paulsen, W.A. Wells

Development of methodology: A.M. Laughney, V. Krishnaswamy, B.W. Pogue, K.D. Paulsen, W.A. Wells

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.M. Laughney, M.C. Schwab, R.J. Barth, K.D. Paulsen, W.A. Wells

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.M. Laughney, V. Krishnaswamy, B.W. Pogue, K.D. Paulsen, W.A. Wells, M.C. Schwab

Writing, review, and/or revision of the manuscript: A.M. Laughney, V. Krishnaswamy, B.W. Pogue, K.D. Paulsen, W.A. Wells, V. Krishnaswamy, R.J. Barth

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W.A. Wells

Study supervision: V. Krishnaswamy, B.W. Pogue, W.A. Wells

Tissue acquisition and histologic processing: E.J. Rizzo

Surgical tissue procurement: R.J. Barth

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