Polarization Sensitive Multimodal Imaging for Detecting Breast Cancer

Rakesh Patel¹, Ashraf Khan², Robert Quinlan², and Anna N. Yaroslavsky*,¹

¹University of Massachusetts, Lowell, 1 University Ave., OL122, Lowell, MA, 01854
²University of Massachusetts Medical School and UMass Memorial Medical Center, Biotech 3, 1 Innovation Dr, Worcester, MA, 01605

Running Title: Multimodal Polarization Imaging for Detecting Breast Cancer

Keywords: Fluorescence, Polarization, Wide-Field Imaging, Optical Coherence Tomography, Optics

* Corresponding Author

Address: One University Avenue, Lowell, MA 01854

E-mail: Anna_Yaroslavsky@uml.edu

Tel.: 978 934 1350

Fax: 978 934 1305

The authors disclose no potential conflicts of interest
Abstract

Intra-operative delineation of breast cancer is a significant problem in surgical oncology. A reliable method for demarcation of malignant breast tissue during surgery would reduce the re-excision rate due to positive margins. We present a novel method of identifying breast cancer margins using combined dye-enhanced wide-field fluorescence polarization (FP) imaging for *en face* cancer margins and polarization sensitive (PS) optical coherence tomography (OCT) for cross-sectional evaluation. Tumor specimens were collected following breast surgery, stained with methylene blue, and imaged. Wide-field FP images were excited at 640 nm and registered between 660 and 750 nm. Standard and PS OCT images were acquired using a commercial 1310 nm swept-source system. The imaging results were validated against histopathology. Statistically significant higher fluorescence polarization of cancer as compared to both normal and fibrocystic tumor tissue was measured in all the samples. Fluorescence polarization delineated lateral breast cancer margins with contrast superior to that provided by OCT. However, OCT complemented fluorescence polarization imaging by facilitating cross-sectional inspection of tissue. PS OCT yielded higher contrast between cancer and connective tissue, as compared to standard OCT. Combined PS OCT and FP imaging shows promise for intra-operative delineation of breast cancer.
Introduction

Women have a 1 in 8 lifetime risk of having invasive breast cancer, which is the second leading cause of cancer death in women (1). Sixty to seventy percent of breast cancer patients undergo lumpectomy or breast conserving surgery (BCS) for surgical resection of the tumor and the rest have mastectomy (2,3). Alternative noninvasive treatments are also being developed such as MRI guided focused ultrasound surgery (4,5) but BCS presently remains the preferred method of treatment. Currently, breast cancer resection margins are defined several days post-operatively by pathologists analyzing the gross resection specimen macroscopically and representative histopathology sections of the tissue microscopically, thus rendering a final diagnosis. However, it is not practical to evaluate the entire tumor margin with this method, as thousands of histological tissue sections would have to be examined to fully evaluate the surface of a breast specimen measuring one centimeter in length (6). Therefore, pathologists render a diagnosis by analyzing the regions of interest selected from macroscopic evaluation, which may lead to sampling error. In addition, pathologic tissue processing takes days to complete and in 20% to 60% of cases additional tumor is found at the margins (7,8). Many patients require additional surgery, which results in higher treatment cost, greater morbidity, infection risk, delayed adjuvant therapy, poor cosmetic outcomes, and a patient’s loss of confidence in the adequacy of the treatment (6). Therefore, defining cancer margins intraoperatively is an important step for lowering the re-excision rate. Alternative pathology approaches such as Frozen Section (FS) pathology (9) and Touch Prep (TP) cytology (10) have been proposed. However, performance limitations (11) have prevented mainstream adoption of such methods. For example, TP cannot distinguish between in situ and invasive cancers (12). Similarly, FS processing is time consuming and may damage tissue compromising definitive permanent histopathology (13,14). Further, TP and FS are not widely available and not routinely used even at high volume centers.
due to the need for an onsite pathologist (8). Therefore, surgeons performing BCS have an urgent need for a method to observe the entire cancer margin intraoperatively in real time.

Optical techniques such as wide-field polarization imaging (WFPI) (15–19), and optical coherence tomography (OCT) (20–22) can potentially perform rapid and accurate evaluation of the entire surgical margin intraoperatively. Previously our lab has shown promising results in delineating cancer in ex vivo samples of breast ductal carcinomas using a combination of WFPI and standard OCT (16). In the study, wide-field imaging was used to accurately evaluate lateral extent of the tumor of the tissue while standard OCT provided high-resolution information on the depth of the lesion. Standard OCT was capable of distinguishing adipose tissue and cancer, but it was difficult to discern tumor on the background of connective tissue. To remedy this deficiency, in this study we investigated the combination of wide-field fluorescence polarization and polarization sensitive OCT (PS OCT). PS OCT is capable of examining birefringence differences within the tissue sample (23). As the birefringence exhibited by fibrous tissue is higher than that of tumors, PS OCT can be expected to enhance contrast of cancer on the fibrous background (16), thus improving the results of tumor delineation as compared to standard OCT (24–26). In this paper we present en face fluorescence polarization and cross-sectional polarization sensitive OCT images of breast tumors and compare optical images to histopathology. In addition, we evaluated the values of fluorescence polarization in invasive ductal carcinoma (IDC), benign fibrocystic breast disease (FCC), and normal breast tissue.

**Materials and Methods**

*Contrast Agent*

Methylene blue (MB) was used as a fluorescent contrast agent for WFPI. MB is currently used for detecting breast cancer in sentinel node (27–30). It is approved for human use and does not affect OCT imaging because the MB absorption peaks (618 nm and 668 nm) do not overlap with
the wavelength (1325 nm) used for OCT. Furthermore, this dye has been shown to have similar staining pattern to hematoxylin and eosin (H&E) in histopathology (31–33), which simplifies morphological analysis of the images by clinical personnel. Commercially available methylene blue (MB 1% injection, USP, American Regent Laboratories, Inc., Shirley, NY) was purchased for this study. For staining, MB was diluted to a concentration of 0.05 mg/ml with Dulbecco phosphate buffered saline solution (DPBS 1X, pH 7.4, Mediatech, Manassas, Virginia).

Sample Preparation and Handling

Excess tissue specimens for the study were obtained from University of Massachusetts Medical Center following breast conserving surgery under an IRB approved protocol. In total, we have investigated 16 specimens from 16 patients. Information on the specimens is summarized in the first five columns of Table 1. The specimens for the study were obtained by a pathologist before fixation in formalin and after performing gross examination. The tissues included some tumor and a rim of adjoining residual breast tissue, which could contain normal tissue. The specimens were placed in vials with saline solution (DPBS pH 7.4 from Mediatech Inc. Manassas, VA) and immediately transported to UMASS Lowell for imaging. Imaging team was not informed of the diagnosis of the specimens. Of the 16 specimens, 5 were obtained from mastectomy and 11 from needle localization lumpectomy specimens either for a preoperative diagnosis of carcinoma or in 4 cases for a diagnosis of fibrocystic change with atypical ductal hyperplasia. Of the 5 fibrocystic change samples 4 were from lumpectomies for benign condition. The 5th sample was pre-operatively diagnosed as ductal carcinoma in situ. However, after histopathological examination of the imaged tissue, it was confirmed to be benign fibrocystic change.

Upon receiving samples at the Advanced Biophotonics Laboratory, the samples were stained in a 0.05 mg/mL Methylene Blue (MB) solution for 2 minutes. The excess stain rinsed off and the tissues were imaged with the WFPI system and PS OCT system. Following imaging,
the specimens were fixed in formalin and histopathology sections were processed from the approximately the same _en face_ and vertical planes that were imaged.

*Optical Coherence Tomography Imaging and Data Processing*

A commercially available Thorlabs swept-source system (OCS1300SS, Newton, New Jersey) with a polarization sensitive add-on module (PSOCT-1300, Newton, New Jersey) was used for OCT imaging in this study. The system incorporated a 1325 nm high-speed, frequency swept laser to illuminate the sample. The two orthogonal polarization states were detected using a fiber polarization beam splitter. The interference signals were fed to two balanced detectors measuring the vertical and horizontal polarization states, which were used to calculate birefringence induced phase-retardation images along with standard OCT images. The OCT system provided a lateral resolution of 25 μm and an axial resolution of 9 μm - 12 μm with a maximum imaging depth of 1.5 – 2 mm in breast tissue and a scanning rate of 25 fps. The data acquisition and processing was handled using Thorlabs integrated software package. The software allowed for automatic acquisition of multiple cross-sectional images of a given volume, which could be processed into a 3D volume block.

*Wide-Field Imaging and Data Processing*

The illumination was provided by a xenon arc lamp (Lambda LS, Sutter, Novanto, CA) combined with a 640 nm bandpass filter, with a full width at half maximum of 10 nm. The lamp was attached to a custom built ring light guide, which provided homogenous illumination of the sample. Images were acquired using a 0.5X Rodenstock lens coupled to a CCD camera (CoolSnap cf2, Roper Scientific, Tucson, AZ). Linear polarizing filters (Meadowlark Optics, Frederick, CO) were integrated into the ring light guide in the pathways of the light incident on the sample and light collected by the camera. The analyzing polarizer was calibrated for two positions, parallel or perpendicular to the polarization of the incident light, which allowed for
rapid sequential acquisition of co- and cross-polarized images. Data acquisition and processing was performed using Metamorph software (Molecular Devices, Inc., Sunnyvale, CA). Fluorescence polarization images were registered between 660 nm and 750 nm using a bandpass filter (660AELP, Omega Optical, Brattleboro, VT) placed in front of the CCD camera. The calibration factor (G=0.98) was measured to account for a bias in the detection of the two polarization states in the manner described by Lakowicz (34). The system provided a lateral resolution of 12 μm and a field of view of 2.2 cm x 1.6 cm. Fluorescence polarization images (fpli) were calculated using formula [1] (fpli: fluorescence polarization image; Ifco: fluorescence co-polarized image, Ifcross: fluorescence cross-polarized image)

\[
f_{\text{pli}} = \frac{I_{\text{fco}} - G \cdot I_{\text{fcr}}}{I_{\text{fco}} + G \cdot I_{\text{fcr}}} \]

[1]

**Histopathology**

Both en face and vertical histopathology sections were processed from each of the imaged tissue samples. After imaging, the fixed tissues were embedded horizontally in paraffin and several 5 μm thick slices were cut using a microtome. The sections were cut from the approximate planes that were imaged. Once cut, the tissue sections were transferred to glass slides and stained with hematoxylin and eosin (H&E). After the en face histopathology had been processed, the paraffin tissue blocks were melted, the tissue was reoriented and embedded for cross-sectional slices to be cut. These vertical sections were cut from the approximate locations of regions of interest defined from OCT and PS OCT images. The cross-sectional sections were then transferred to glass slides and stained as described for en face sections. All histopathology slides were digitized using a Zeiss Axioscope microscope (Zeiss, Germany, 5X objective lens, NA 0.13). The optical images were correlated with corresponding digitized histopathology.
Evaluation of Tumor Contrast in Optical Images

Contrast of tumor with respect to normal tissue was assessed quantitatively for wide-field fluorescence polarization, standard OCT, and PS OCT images. For correlating optical and histological images and for evaluating cancer contrast in the optical images, the tumor and normal areas were outlined in the optical images of each specimen, exactly as they present in histopathology. This procedure was described in detail elsewhere (15, 16, 35, 36). In short, cancerous and normal regions were outlined by a pathologist in digitized histopathology slides. Due to the preparation of paraffin embedded histopathology, sections may not preserve the shape and size of the imaged tissue block. To correct for this artifact, digitized histopathology slides were overlaid onto the optical images. Then affine, projective, or polynomial transformations were applied so that similar structures in the optical images coincided with corresponding structures in histopathology. After correction, the regions corresponding to cancer and normal breast tissue in histopathology were outlined in the optical images.

Mean fluorescence polarization values were determined for each specimen from mean pixel values of the entire tumor and normal areas in the fluorescence polarization images. Thus obtained mean fluorescence polarization values for cancer and normal tissue were averaged across all samples.

To compare contrast of the standard OCT and PS OCT images, the images were normalized by the maximum pixel value. Normalized mean pixel values for tumor and normal areas were calculated for each specimen. Then obtained mean pixel values for cancer and normal tissue were averaged across all specimens.

Tumor contrast was calculated using formula [2] (C: contrast; $pxl_T$: mean pixel value for tumor areas; $pxl_N$: mean pixel value for normal areas, including adipose and fibrous connective tissue). Contrast value of 1 indicates maximum contrast while 0 indicates no contrast.
\[ C = \frac{|pxl_T - pxl_N|}{pxl_T + pxl_N} \]  

**Statistical Analysis**

The differences in tumor and normal values were evaluated statistically for wide-field fluorescence polarization, standard OCT, and PS OCT images using a one-tailed student’s t-test for two independent populations. In all cases we tested the alternative hypothesis that the mean pixel value averaged over tumor regions in each sample was significantly different from the mean pixel value averaged over all normal regions in the samples. The p-value results were calculated for each modality and considered significant at p<0.005.

**Results/Discussion**

In total, eleven invasive ductal carcinomas and five benign fibrocystic breast tumors from 16 patients were imaged for this study. All samples came from female patients following breast conserving surgery. The patient ages ranged from 38 to 73. The sample sizes varied approximately between 6.1 mm – 12.9 mm in the lateral dimensions and 3 mm – 7 mm in depth. The respective data are summarized in Table 1. Histological analysis performed by the study pathologist after optical imaging established that all cancer specimens included tumor and normal regions. Fibrocystic tumor specimens did not contain normal tissue.

Example *en face* images of a representative invasive ductal carcinoma sample are presented in Fig. 1. Invasive ductal carcinoma shows architecturally abnormal proliferation of ducts, with ill-defined margins and variable amount of fibrocollagenous or adipose stroma. In this carcinoma sample the tumor presents on the background of fibrocollagenous connective tissue as seen in histopathology (Fig. 1A) and residual benign breast tissue seen in the bottom third of the tissue section is predominantly adipose (fat) tissue. Cancer affected area is bright in the fluorescence polarization image (Fig. 1B). Comparison of the tumor boundary in fluorescence polarization
image and in histopathology (Fig. 1A) demonstrates good correlation. In Fig. 1B the tumor is clearly distinguished from the surrounding normal tissue, as fluorescence polarization rapidly decreases upon transition into the normal tissue. The en face standard OCT and PS OCT images are presented in Figs. 1C and D, respectively. Tumor affected area, as well as connective tissue appear equally bright in the standard OCT image (Fig. 1C) and can hardly be distinguished. In contrast, in the PS OCT image (Fig. 1D) tumor is dark, whereas the fibrous tissue is bright, which confirms our hypothesis that birefringence imaging may help in delineating tumor and connective tissue. Comparison of the en-face optical images, including fluorescence polarization (Fig. 1B), standard OCT (Fig. 1C) and PS OCT (Fig. 1D) to histopathology (Fig. 1A) demonstrates that fluorescence polarization delineates the tumor margins more accurately as compared to standard OCT and PS OCT. In particular, neither of the OCT modalities highlights cancer in the upper left corner of the specimen. For this particular sample, the contrast for tumor to adipose tissue was measured to be 0.65 in the fluorescence polarization image (Fig. 1B), 0.14 and 0.03 in the standard OCT (Fig. 1C) and PS OCT (Fig. 1D) en face images, respectively. Moreover, it is hard to discern fibrous tissue surrounded by the tumor in Fig. 1C. However, the standard OCT image in Fig. 1C demonstrates remarkable morphological detail. In particular, honeycomb structure of adipose cells is very prominent. PS OCT (Fig. 1D) provides complimentary information by highlighting the morphology of connective tissue. The contrast for tumor to fibrous connective tissue was 0.34 in the fluorescence polarization image (Fig. 1A), 0.22 and 0.40 in the standard OCT (Fig. 1C) and PS OCT (Fig. 1D) en face images, respectively.

Cross-sectional OCT images of the invasive ductal carcinoma specimen shown in Fig. 1 are presented in Fig. 2 B-C. The approximate location of the cross-sectional images is indicated in the en face histopathology image displayed in Fig. 1A. Cross-sectional histopathology (Fig. 2A)
shows an area of tumor and connective tissue surrounded by adipose tissue on either side. Similarly to the en face standard OCT image in Fig. 1, standard OCT shows good contrast of adipose tissue in the cross-section pictured (Fig. 2B). The standard OCT image correlates well with histopathology but does not differentiate tumor on the background of connective tissue. PS OCT (Fig. 2C) emphasizes an area of connective tissue that is not clearly delineated in the standard OCT image (Fig. 2B), although it does not clearly distinguish adipose tissue.

A representative benign fibrocystic breast disease case is shown in Figs. 3 & 4. Fibrocystic change (FCC) presents as a combination of features including stromal fibrosis, cystic dilatation of ducts, apocrine metaplasia and adenosis. En face histological and optical images are displayed in Fig. 3 with corresponding cross-sectional images shown in Fig. 4. In histopathology (Fig. 3A) the pink area seen above the fatty breast tissue shows FCC, which mainly includes stromal fibrosis and cystic dilatation of the ducts. Smaller foci include lobules that have some minimal degree of adenosis. Fluorescence polarization does not highlight any areas in the sample. Thus, the benign lesion does not exhibit significant fluorescence polarization.

As compared to the ductal carcinoma sample presented in Figures 1 and 2, the contrast in fluorescence polarization image of FCC is poor (Fig. 3A). FCC exhibited fluorescence polarization of 0.02. This value is significantly lower as compared to the average fluorescence polarization of the tumor area in the ductal carcinoma specimen presented above, which was measured as 0.09. Standard OCT (Fig. 3B) and PS OCT (Fig. 3C) also display low contrast except for small pockets of adipose tissue in between areas of dense fibrous tissue. Neither of the OCT images of FCC highlights the lesion.

Cross-sectional standard OCT (Fig. 4B) shows no distinction between fibrous tissue and benign ducts and lobules, as indicated in histopathology. There is no adipose tissue present in this
cross-section and as such the standard OCT image lacks contrast. In contrast, PS OCT (Fig. 4C) yields higher signal in areas corresponding to fibrous connective tissue while the benign ducts and lobules exhibit a lower signal. PS OCT highlights dense connective tissue within FCC sample. It also emphasizes the stromal fibrosis within the tissue except for the regions containing breast ducts and lobules with adenosis.

The pixel values of optical images for cancer, fibrocystic tumor, and normal areas averaged over all samples are summarized in Fig. 5. For fluorescence polarization images these averaged pixel values represent mean fluorescence polarization. The mean fluorescence polarization of normal, fibrocystic, and cancerous areas measured over all samples in this study was found to be 0.03 +/- 0.01, 0.02 +/- 0.01, and 0.06 +/- 0.02, respectively. The fluorescence polarization values found for normal and cancer tissue in this study are in good accordance with previously measured values from our earlier study (15). Higher fluorescence polarization of cancer with respect to benign tissue was measured in all the samples. There could be several explanations of these results. In our previous work we have ruled out the hypothesis that differences in elastic scattering from tumor and normal tissue lead to enhanced FP of breast cancer (13). Restricted rotational motion as well as reduced fluorescence lifetime of the dye molecules in cancer cells can result in higher fluorescence polarization (34). Restricted rotational motion of dye molecules could be caused by several factors such as binding of the dye molecules to large cell organelles and higher intracellular viscosity. The work is currently underway to investigate the origins of higher fluorescence polarization in breast cancer as compared to normal tissue and benign pathology.

Fluorescence polarization values averaged over the cancer and normal areas for each sample are presented in columns seven and eight of Table 1. Mean FP values for normal tissues varied
between 0.01 and 0.03, whereas mean FP of cancer was in the range between 0.04 and 0.09. Even though the highest mean FP, 0.03, of normal tissue appears to be close to the lowest mean FP exhibited by cancer, 0.04, it can be seen from the table that the samples with lower FP of normal tissue exhibit lower FP values of cancer and similarly samples with higher normal tissue FP exhibit higher cancer FP. These variations between the samples are most likely caused by the differences in the background optical properties of the specimens, and in particular, by differences in the scattering coefficient. It can also be appreciated that higher grade cancer tissue demonstrated overall higher fluorescence polarization. Further studies are required to investigate the apparent correlation between the stage of cancer and FP values exhibited by tissue.

Averaged over all samples fluorescence polarization exhibited by breast cancer and FCC are 0.06 +/- 0.02 and 0.02 +/- 0.01, respectively. The gradient of FP at the boundary between cancer and benign tissue is high in all the specimens investigated. This finding indicates that fluorescence polarization may be capable of differentiating between malignant and benign disease and accurately delineating cancer. It is known that dense breast tissue as a result of fibrocystic change can cause enhanced contrast on MR mammography leading to false-positives (37). Furthermore, with commonly used current breast cancer screening tests, conventional mammography and/or clinical breast examination, false positive rates over a 10 year period have been reported as high as 31.7% (38). Therefore if benign diseases could be reliably diagnosed preoperatively using “optical biopsy” it could prevent unnecessary resection of non-malignant tissue or surgery all together for these patients.

Normalized average pixel values of normal and cancerous areas, measured from OCT and PS OCT images, are summarized in Fig. 6. Averaged pixel values for normal and cancer tissue for standard OCT were 0.13 +/- 0.09 and, 0.29 +/- 0.07, respectively. For PS OCT the normalized
pixel value for normal areas was 0.26 +/- 0.08 and 0.29 +/- 0.08 for cancer. The contrast of cancer in the fluorescence polarization images was found to be 0.39 +/- 0.08 whereas in standard OCT and PS OCT images it was 0.39 +/- 0.15 and 0.24 +/- 0.09. Fluorescence polarization showed a significant difference between tumor and normal (p<0.00001). Standard OCT differences between tumor and normal were also significant (p<0.0001). Interestingly, even though PS OCT reliably delineated cancer on the background of birefringent connective tissue, it revealed no significant difference in contrast of cancer as compared to normal tissue (fibrous and adipose) averaged across all samples in this study. This could be explained by low birefringence exhibited by cancer and normal adipose tissue. It should also be noted that contrast of the tumor in the image is not the only important factor in evaluating the performance of the method. The ability of the technique to present morphology of tissue with adequate resolution should also be considered as equally important. In this respect, all the techniques investigated, including fluorescence polarization, standard OCT and PS OCT, are complimentary and demonstrated good performance in discerning the morphology of tumor, adipose and connective tissues, respectively.

A polarization sensitive combination of wide-field and OCT imaging would allow for more rapid and accurate evaluation of tumor margins, as compared to the OCT imaging alone. The OCT system is capable of simultaneously acquiring standard and polarization sensitive OCT cross-sectional images in approximately 40 ms. The en face imaging of 3 cm² sample surface will take more than 30 sec. A combination system could use wide-field fluorescence polarization to acquire en face images, rapidly acquired within 1 sec, and then use this image to guide cross-sectional OCT inspection of suspicious areas.
In summary, we have shown that fluorescence polarization wide-field imaging is suitable for delineating lateral extent of the breast cancer margins from *en face* images with contrast superior to that provided by OCT. However, OCT imaging can complement wide-field fluorescence polarization by enabling high-resolution cross-sectional imaging and thus facilitating inspection of the close margins. In our previous publication (16) we have reported that delineating tumor on the background of the fibrous tissue using standard OCT may be challenging and we proposed to use PS OCT to emphasize birefringence properties of the connective tissue and thus improve the contrast of the tumor margins. In this study we have confirmed that PS OCT is capable of differentiating cancer from fibrous tissue yielding the higher contrast of 0.40, as compared to 0.22 in the standard OCT. Fluorescence polarization has also demonstrated the capability to differentiate benign tissue from cancer. Thus, this technology may also prove to be useful as an optical biopsy tool to help avoid unnecessary surgical removal of benign tissue, such as FCC. The results of our pilot trial suggest that presented combined technology has potential to detect microscopic nests of ductal carcinoma *in situ*. The resolution, afforded by the device (∼12 μm), and high contrast of fluorescence polarization images point towards the feasibility of successful outcome. However, larger trials are required for conclusive proof of this hypothesis. Further clinical studies will focus on determining the sensitivity and specificity of this combined technology for delineating different types of breast cancer.

In conclusion, this study demonstrates the potential of combining wide-field fluorescence polarization imaging and OCT, standard and polarization sensitive, for rapid and reliable evaluation of breast cancer margins. The proposed approach can be used to image the excised tissue or the surgical wound *in situ*. In general, the two approaches should yield equivalent results and both can be implemented in clinical practice. For *in vivo* implementation it will be
required to rinse the surgical bed with aqueous solution of methylene blue, which should be safe, as currently MB is used clinically in much higher concentration for mapping sentinel nodes. Both methods, i.e., fluorescence polarization and multimodal OCT, are mature technologies that can be readily implemented in a single instrument. Further development of this combined technology may enable intraoperative evaluation of tissue and serve to lower positive margin and reoccurrence rates.

**Acknowledgments**

The authors would like to thank Anjul Davis, Ph.D. and Javier Jurado from Thorlabs for lending the PS OCT system and technical assistance.
References


Table 1

Study Subjects

IDC: invasive ductal carcinoma; FCC: benign fibrocystic change; * - with squamous differentiation; ** - with lobular growth; *** - preoperatively diagnosed as ductal carcinoma in situ

<table>
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<tr>
<th>Sample #</th>
<th>Gender</th>
<th>Age</th>
<th>Lateral Dimensions, mm X mm</th>
<th>Diagnosis, Grade</th>
<th>Surgical Treatment</th>
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<td></td>
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Figure Captions

**Figure 1**: *En face* images of a representative sample with invasive ductal carcinoma (IDC). Fig. 1A: histopathology; Fig. 1B: wide-field fluorescence polarization; Fig. 1C: standard optical coherence tomography (OCT); Fig. 1D: polarization-sensitive optical coherence tomography (PS OCT). Red line in Fig. 1A indicates approximate location of cross-sectional images shown in Fig. 2. (Bar: 1 mm)

**Figure 2**: Cross-sectional images of the invasive ductal carcinoma (IDC) sample presented in Fig. 1. The images were acquired from the approximate location indicated in Fig. 1A (red line). Fig. 2A illustrates the histopathology cross section of IDC sample outlining areas of tumor, adipose, and connective tissue. Corresponding standard optical coherence tomography (OCT) and polarization-sensitive optical coherence tomography (PS OCT) sections are shown in Figs. 2B and 2C respectively. (Bar: 1 mm)

**Figure 3**: *En face* images of a representative sample with benign fibrocystic breast disease (FCC). Fig. 3A: histopathology; Fig. 3B: wide-field fluorescence polarization; Fig. 3C: standard optical coherence tomography (OCT); Fig. 3D: polarization-sensitive optical coherence tomography (PS OCT). Red line in Fig. 3A indicates approximate location of cross-sectional images shown in Fig. 4. (Bar: 1 mm)

**Figure 4**: Cross-sectional images of the benign fibrocystic breast disease (FCC) sample presented in Fig. 3. The images were acquired from the approximate location
indicated in Fig. 3A (red line). Fig. 4A illustrates the histopathology cross section of FCC sample outlining areas of stromal fibrosis and benign ducts and lobules. Corresponding standard optical coherence tomography (OCT) and polarization-sensitive optical coherence tomography (PS OCT) sections are shown in Figs. 4B and 4C respectively. (Bar: 1 mm)

**Figure 5:** Averaged fluorescence polarization values for benign fibrocystic breast disease (FCC), normal, and malignant tissue. Bars are standard deviations above the mean.

**Figure 6:** Normalized standard optical coherence tomography (OCT) and polarization-sensitive optical coherence tomography (PS OCT) pixel values for normal and malignant tissue averaged over all samples. Bars are standard deviations above the mean.
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Rakesh Patel, Ashraf Khan, Robert Quinlan, et al.

Cancer Res  Published OnlineFirst June 23, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-13-2411

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