Diagnosis of breast cancer biopsies using quantitative phase imaging

Hassaan Majeed[1], Mikhail E. Kandel[1], Kevin Han[1], Zelun Luo[1], Virgilia Macias[2], Krishnarao Tangella[3], Andre Balla[2], and Gabriel Popescu[*1]

[1]Quantitative Light Imaging Laboratory, Department of Electrical and Computer Engineering, Beckman Institute of Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [2]University of Illinois at Chicago, Department of Pathology, Chicago, Illinois 60612, USA; [3]Christie Clinic and University of Illinois at Urbana-Champaign, Department of Pathology 1400 West Park Street, Urbana, Illinois 61801, USA.

ABSTRACT

The standard practice in the histopathology of breast cancers is to examine a hematoxylin and eosin (H&E) stained tissue biopsy under a microscope. The pathologist looks at certain morphological features, visible under the stain, to diagnose whether a tumor is benign or malignant. This determination is made based on qualitative inspection making it subject to investigator bias. Furthermore, since this method requires a microscopic examination by the pathologist it suffers from low throughput. A quantitative, label-free and high throughput method for detection of these morphological features from images of tissue biopsies is, hence, highly desirable as it would assist the pathologist in making a quicker and more accurate diagnosis of cancers. We present here preliminary results showing the potential of using quantitative phase imaging for breast cancer screening and help with differential diagnosis. We generated optical path length maps of unstained breast tissue biopsies using Spatial Light Interference Microscopy (SLIM). As a first step towards diagnosis based on quantitative phase imaging, we carried out a qualitative evaluation of the imaging resolution and contrast of our label-free phase images. These images were shown to two pathologists who marked the tumors present in tissue as either benign or malignant. This diagnosis was then compared against the diagnosis of the two pathologists on H&E stained tissue images and the number of agreements were counted. In our experiment, the agreement between SLIM and H&E based diagnosis was measured to be 88%. Our preliminary results demonstrate the potential and promise of SLIM for a push in the future towards quantitative, label-free and high throughput diagnosis.

Keywords: breast cancer, cancer diagnosis, histopathology, quantitative phase imaging, microscopy, interferometry, label-free imaging, tissue microarray, SLIM

1. INTRODUCTION

Breast cancer is the second most common form of cancer diagnosed worldwide, accounting for 11.9% of all cancers diagnosed in 2012.[1] In spite of the high incidence and burden of disease the current histopathological analysis used for the diagnosis of breast cancers suffers from certain shortcomings. When an abnormality in the breast is discovered during a screening procedure such as mammography, a tissue biopsy is obtained by the pathologist and the section of tissue is stained using hematoxylin and eosin (H&E). This staining provides the necessary contrast needed for investigation of key morphological features by a trained, board certified pathologist using a conventional bright field microscope. Since this investigation is both qualitative and subjective it leads to both intra and inter observer discrepancy.[2,3] Furthermore, due to the qualitative nature of the histopathological information, the imaging modality does not lend itself to automation resulting in low throughput and in some cases, late disease diagnosis – a critical shortcoming given that early diagnosis significantly improves chances of survival.[4]

*gpopescu@illinois.edu; phone: 217-333-4840; light.ece.illinois.edu

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Quantitative phase imaging (QPI) refers to a sub-set of label-free microscopy techniques where contrast is generated by the variation of optical path length transerve to the direction of light propagation. The resulting image is a phase map $\phi(x,y)$ that is a quantitative measure of the product of the refractive index difference between the tissue $n_t(x,y)$ and its surrounding medium n_m and the thickness of the tissue $t(x,y)$ given by the equation

$$\phi(x,y) = \frac{2\pi}{\lambda}[n_t(x,y) - n_m]\, t(x,y) \quad (1)$$

where λ is the wavelength of light.[5-14] Since the information in the phase image $\phi(x,y)$ is a quantitative measure of the morphology of the tissue biopsy, diagnosis based on QPI provides the potential to eliminate inter and intra observer variation and lends itself to automation.

Spatial light interference microscopy (SLIM) provides access to this phase information by measuring four interferograms formed by the scattered and unscattered fields and solving for $\phi(x,y)$. It has been shown in previous publications from our group that SLIM provides diffraction limited resolution as well as low spatial and temporal noise leading to optical path length sensitivity of less than a nanometer.[15-17] The utility of refractive index of tissue (accessible through the phase images obtained by SLIM) as a marker for prostate tissue malignancy has also been shown in previous publications – motivating the investigation of SLIM as a histopathological analysis technique for detecting malignancy in breast tissue biopsies.[18]

In this work we present preliminary results that show the potential of a SLIM based technique for supporting diagnosis of breast cancers. Specifically, the resolution and contrast of SLIM phase images for diagnostic purposes were evaluated qualitatively by two board certified pathologists. As outlined in detail in the following sections, using the standard H&E staining based diagnosis protocol as a benchmark, the success of the pathologists in carrying out diagnosis on SLIM images was measured. Our results provide an indication of the signal to noise ratio available to us for subsequent quantitative analyses for carrying out diagnosis based on the relative phase values of various tissue components.

2. EXPERIMENTAL PROCEDURES

2.1 Sample

The samples comprised of an unstained tissue microarray (TMA) of 900 cores constructed from breast tissue biopsies of 400 different patients. The cores were mounted on three microscope slides and each core section was 4 μm thick. The sample was obtained from our collaborating pathologist at the University of Illinois at Chicago (UIC) Dr. Andre Balla. Corresponding to each unstained core, an H&E stained core from an adjacent parallel section of tissue was mounted onto separate slides for imaging under a bright field microscope, providing reference H&E images for comparison.

2.2 Slide scanning and stitching

The TMA was imaged using our SLIM imaging system, constructed as an add-on module to a commercial phase contrast microscope (Zeiss Axio Observer Z1). A 40x/0.75 NA phase contrast objective was used for the purpose. A slide scanning software, developed in-house in Visual C++, was used to obtain the raw images for the entire microscope slide (scanning area approx. 20 mm x 45 mm) at high throughput (approx. 2 hrs per slide). The phase maps were extracted using a MATLAB-based code. A Python-based stitching code was used for stitching the mosaic for the entire slide and segmenting out each individual core for subsequent processing and analysis. As shown in Figure 1, our processing allows the visualization of the entire TMA from the slide scale to the sub-cellular scale within each core. As illustrated in Figure 1 (c), our label-free SLIM images clearly delineate the epithelial stromal boundary allowing for assessment of tumor malignancy.

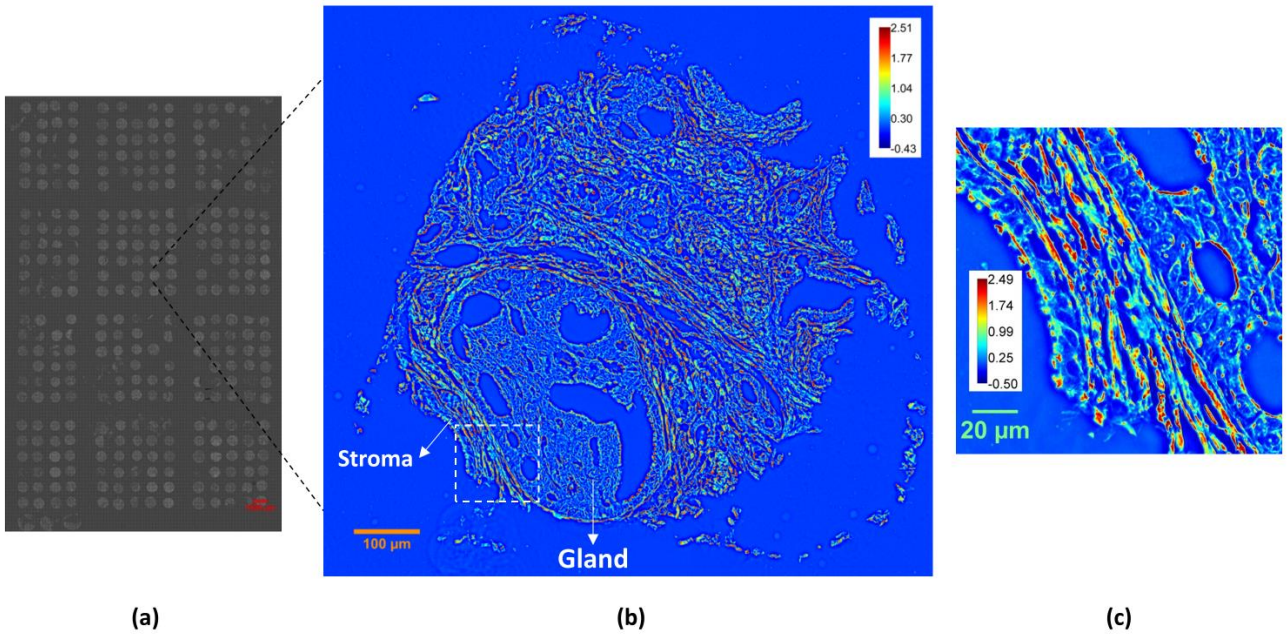

Figure 1. (a) The SLIM image of an entire TMA slide scanned and stitched using software developed in-house (b) Label-free SLIM image of a single TMA core delineating the boundary between the tumour and its extracellular environment (c) magnified image of region indicated in (b) clearly showing tumor cell nuclei and collagen fibers - typifying epithelial and stromal regions.

2.3 Evaluation by pathologists

In order to assess the diagnostic capabilities of our SLIM imaging modality, we asked two board certified pathologists to evaluate our SLIM images. For practical reasons, 109 cores out of the total of 900 were chosen for evaluation, and stacks of SLIM and corresponding H&E images for these cores were assembled in ImageJ. Since pathologists are generally trained to recognize morphological features in H&E stained tissue, a training step was performed before the actual experiment was conducted. In this training step, both SLIM and H&E images for 10 benign and 10 malignant cores (determined as such by a third board certified pathologist a priori) were shown side by side to each pathologist. By comparing the SLIM and H&E images for each core the pathologists were able to learn how to interpret the tissue morphological details from SLIM phase maps. The total training time for each pathologist ranged from 10 – 15 minutes approximately. Figure 2 compares and contrasts how different tissue components are resolved in SLIM and H&E stained tissue images.

After the completion of the training step, each pathologist was first shown the stack of SLIM images for the 109 cores chosen. The pathologist classified each core as either benign or malignant. The process was repeated for the stack of H&E images for the same 109 cores. Using each pathologist's diagnosis on the H&E stained cores as the gold standard, the success of diagnosis using SLIM images was measured by counting the number of agreements between SLIM and H&E based diagnoses.

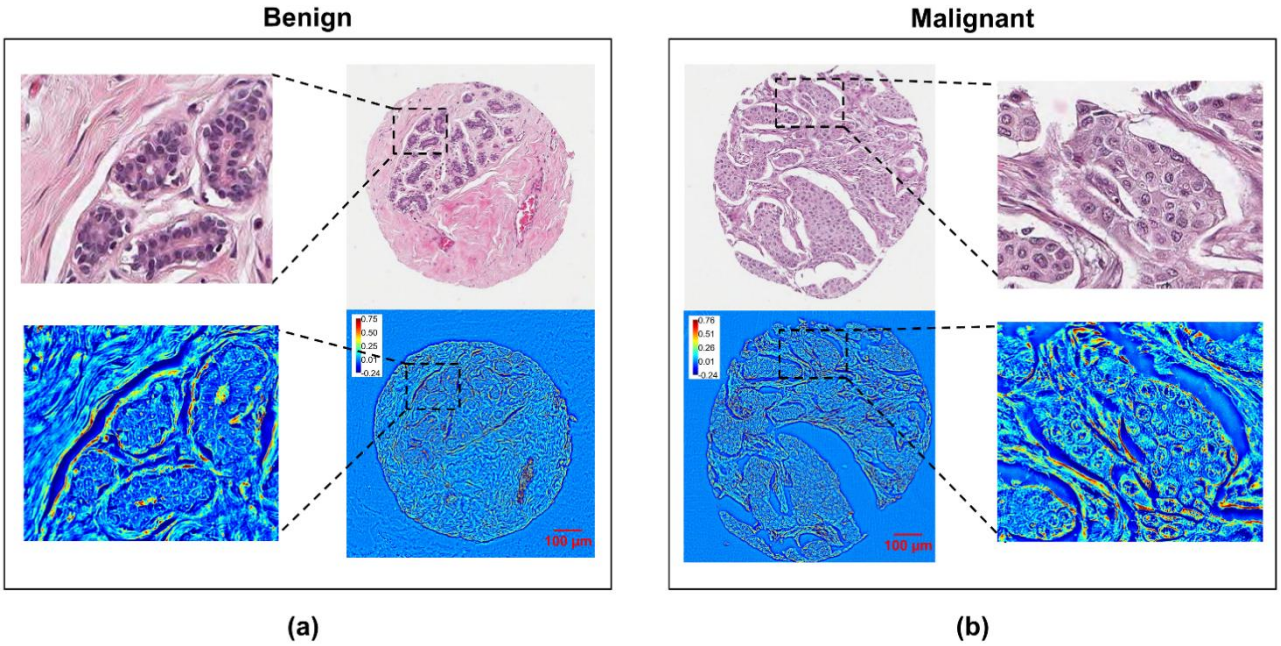

Figure 2. Comparison between SLIM (bottom row) and H&E stained bright field microscopy (top row) images in their respective abilities to resolve tissue morphology for (a) benign and (b) malignant cases. The H&E images were obtained from stained sections that were adjacent to the unstained sections used for SLIM imaging

3. RESULTS

The results of the classification of cores carried out by the two pathologists on both SLIM and H&E images are summarized in Figure 3. As shown in Figure 3 (c), the success rate of diagnosis on SLIM images (considering diagnosis on H&E as the gold standard) was 88%. As shown Figures 3 (a) and (b), the agreement between the two pathologists when rating SLIM images stood at 83% whereas the same for H&E images was much higher at 98%. The lower agreement between the two pathologists on SLIM images is not surprising when one takes into account the fact that, as part of their professional training, pathologists are trained to interpret images of H&E stained tissue for a number of years whereas, for this experiment, the training time for SLIM images was only a few minutes. We expect the agreement between the diagnoses of the two pathologists on SLIM images to increase significantly with longer training in interpreting SLIM images.

4. CONCLUSION AND FUTURE WORK

Our preliminary results show the capability of our label-free imaging modality in resolving morphological features relevant for diagnosis of breast cancer. While this qualitative analysis shows the potential of quantitative phase imaging for diagnosing malignancy in breast cancer, the long term aim is to address the shortcomings in conventional histopathology with regards to inter and intra observer variability and low throughput by searching for quantitative parameters for classifying benign and malignant tumors. As has been reported in literature before, phase images of tissues can be used for extracting scattering parameters such as mean-scattering length and tissue anisotropy parameter that can be used to characterize the cellular scale organization of tissue.[19] Since malignancy is associated with changes in tissue organization and morphological state of cells, comparing scattering parameters between benign and malignant tumors has the potential to provide a quantitative basis for diagnosis. Hence, our future work is focused on leveraging the quantitative information regarding tissue morphology available to us in phase maps to come up with parameters for diagnosis of breast cancer biopsy images and build classifiers that can achieve this in an automated fashion.

In addition to discovering quantitative bases for diagnosis, we also hope to use our imaging modality for discovering new prognostic biomarkers. There is a search in the scientific community for new, readily accessible biomarkers to serve as prognostic indicators as the current set of biomarkers (histological grade, tumor size, hormonal receptor status etc.) are insufficient in predicting outcomes for some patients.[20] We hope to address this need by discovering new biomarkers, using our label free images, to serve as predictors of patient outcome.

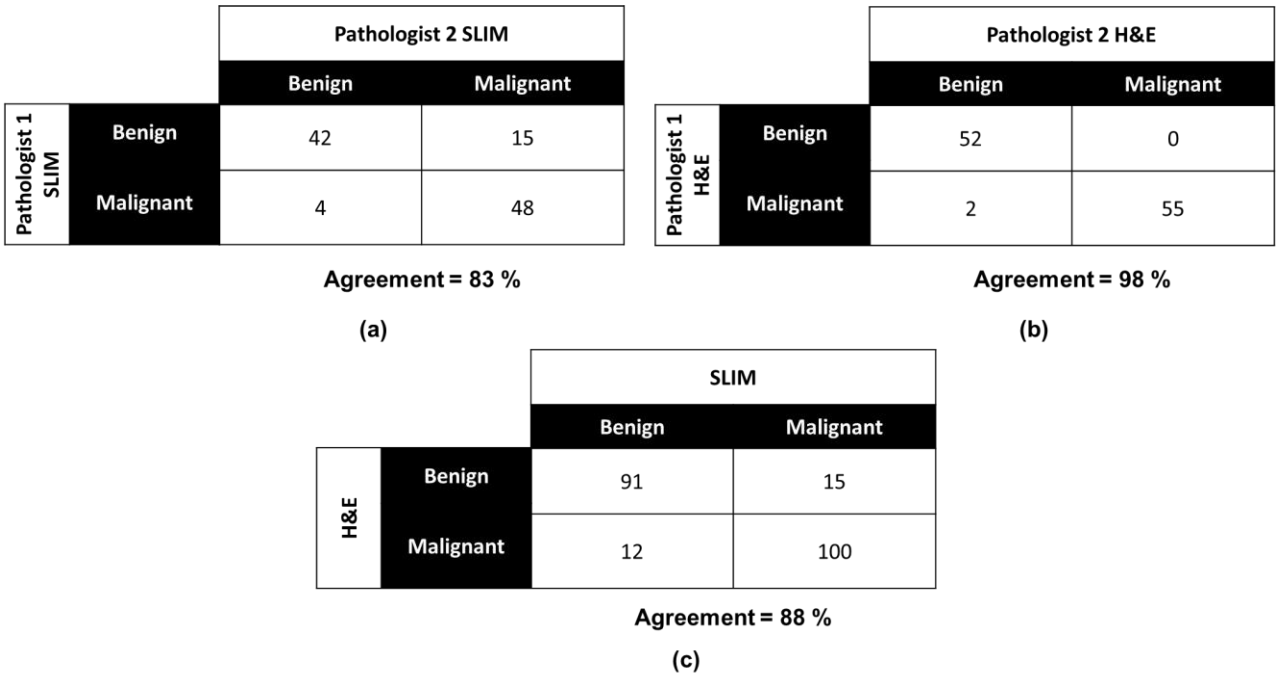

Figure 3. Confusion matrices showing results of qualitative diagnosis carried out by two pathologists on both SLIM and H&E stained tissue images for 109 cores (a) Pathologist agreement on SLIM images (b) Pathologist agreement on H&E images (c) Agreement between ratings on SLIM and H&E images

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