Wide-field quantitative micro-elastography of human breast tissue

WES M. ALLEN,1,2* KELSEY M. KENNEDY,1,2 QI FANG,1,2 LIXIN CHIN,1,2 ANDREA CURATOLO,1,2 LUCINDA WATTS,1,3 RENATE ZILKENS,1,3 SYNN LYNN CHIN,4 BENJAMIN F. DESSAUVAGIE,5,6 BRUCE LATHAM,5 CHRISTOBEL M. SAUNDERS,3,4,7 AND BRENDAN F. KENNEDY1,2

1BRITElab, Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands and Centre for Medical Research, The University of Western Australia, Perth, Western Australia, 6009, Australia
2Department of Electrical, Electronic & Computer Engineering, School of Engineering, The University of Western Australia, 35 Stirling Highway, Perth, Western Australia, 6009, Australia
3School of Surgery, The University of Western Australia, 35 Stirling Highway, Perth, Western Australia, 6009, Australia
4Breast Centre, Fiona Stanley Hospital, 11 Robin Warren Drive, Murdoch, Western Australia, 6150, Australia
5PathWest, Fiona Stanley Hospital, 11 Robin Warren Drive, Murdoch, Western Australia, 6150, Australia
6School of Pathology and Laboratory Medicine, The University of Western Australia, 35 Stirling Highway, Perth, Western Australia, 6009, Australia
7Breast Clinic, Royal Perth Hospital, 197 Wellington Street, Perth, Western Australia, 6000, Australia
*wes.allen@research.uwa.edu.au

Abstract: Currently, 20-30% of patients undergoing breast-conserving surgery require a second surgery due to insufficient surgical margins in the initial procedure. We have developed a wide-field quantitative micro-elastography system for the assessment of tumor margins. In this technique, we map tissue elasticity over a field-of-view of ~46 × 46 mm. We performed wide-field quantitative micro-elastography on thirteen specimens of freshly excised tissue acquired from patients undergoing mastectomy. We present wide-field optical coherence tomography (OCT) images, qualitative (strain) micro-elastograms and quantitative (elasticity) micro-elastograms, acquired in 10 minutes. We demonstrate that wide-field quantitative micro-elastography can extend the range of tumors visible using OCT-based elastography by providing contrast not present in either OCT or qualitative micro-elastography and, in addition, can reduce imaging artifacts caused by a lack of contact between tissue and the imaging window. Also, we describe how the combined evaluation of OCT, qualitative micro-elastograms and quantitative micro-elastograms can improve the visualization of tumor.

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1. Introduction

Breast cancer has the second highest mortality rate of cancers in females [1]. In the USA, breast-conserving surgery accounts for ~58% of surgeries in patients diagnosed with early stage breast cancer [2]. If post-operative histological assessment identifies tumor near the edge of the excised tissue (the surgical margin), the patient will often undergo a re-excision surgery to reduce the risk of recurrence. Currently, 20-30% of patients undergo re-excision due to tumor found within the surgical margin [3-6]. To reduce the number of re-excisions, there is a need for intraoperative margin assessment tools. A range of techniques have been proposed, including frozen section histology, imprint cytology and intraoperative specimen radiography, but these techniques have not significantly reduced re-excision rates [7]. More recently, a number of novel approaches for margin assessment have been proposed, including mass spectrometry [8]. The handheld pen-like device identifies tumor by its unique molecular profile. However, this technique is a surface measurement with the spatial resolution for each measurement limited by the smallest pen tip diameter: 1.5 mm [8].

A number of optical methods have also been proposed for tumor margin assessment in breast-conserving surgery [9-17]. Of these, optical coherence tomography (OCT) has shown great promise due to its unique combination of high spatial resolution, high imaging speed and an imaging depth, 1-2 mm, that is well-suited to margin assessment [11, 18]. In addition, OCT, in common with many optical methods, can readily be implemented in small form factor probes, making it well-suited for use in operating theaters [19]. A main drawback, in some instances, is that OCT has low contrast between tumor and uninvolved dense stromal tissue, making it potentially challenging to accurately assess margin status [12].
To increase contrast in dense tissues, we are developing OCT-based elastography techniques that utilize variations in mechanical properties between healthy and diseased tissue to improve the visualization of tumor [20-22]. One qualitative approach, optical coherence micro-elastography (OCME) maps the local axial strain, at each lateral (x, y) and axial (z) position, in response to a compressive load imparted to the tissue. A clinical feasibility study of 58 specimens demonstrated that images of local strain (qualitative micro-elastograms) can enhance contrast between uninvolved dense tissue and tumor, in comparison to OCT images alone [23]. We have recently described a wide-field imaging capability to extend the field-of-view of OCME, enabling an entire margin surface of a typical specimen excised during breast-conserving surgery to be viewed in one en face image [24]. Recent improvements to both the imaging system and the associated clinical scanning protocols, detailed in this study, allow us to acquire wide-field data volumes (46 × 46 × 3.5 mm) in 10 minutes.

Whilst wide-field OCME represents an important development, there are a number of obstacles that must be overcome before this technology can be translated to clinical use. For example, a primary mechanism by which OCME identifies regions of invasive tumor is by heterogeneous patterns in qualitative micro-elastograms. In these cases, strain heterogeneity often arises from localized changes in the mechanical properties between nests of tumor cells and surrounding immature fibrous connective tissue (desmoplastic stroma) [23]. However, this characteristic intermingling of tumor cells with desmoplastic stroma represents only one micro-architectural pattern in malignant tissue. For example, we have also identified cases in which invasive tumor exhibits homogeneous strain. Histopathological analysis of these cases has identified highly cellular tumors with little intervening stroma. To increase the clinical utility of wide-field OCT-based elastography, improved mechanical contrast is needed across a broader range of micro-architectural patterns characteristic of both invasive and in situ tumor. Another factor that can reduce contrast of tumor is the presence of artifacts in qualitative micro-elastograms. One such artifact arises when the specimen does not fully contact the imaging window used to impart deformation to the specimen. As described previously, micro- to milli-scale non-contact can cause tissue to deform in the opposite direction to the applied load [23]. This can result in strain heterogeneity with similar spatial frequency to invasive tumor. In a clinical setting, this heterogeneity could cause a reader to misinterpret a qualitative micro-elastogram, thereby potentially reducing diagnostic accuracy.

In this study, we propose wide-field quantitative micro-elastography (QME). We demonstrate improved visualization of malignant tissue by providing additional contrast, based on tissue elasticity, that complements the contrast provided by OCT and strain, and additionally, removes many of the artifacts present in OCME. The principle of QME has been described previously [25]. To perform QME, a translucent, compliant silicone layer with well-characterized mechanical properties is placed between the imaging window and the specimen, and OCT is used to estimate the axial stress at the layer-specimen interface [26]. Tissue elasticity is then estimated by dividing the axial stress (at each lateral position at the surface of the specimen) by the local axial strain (at each lateral and axial position in the OCT field-of-view) [25].

Here, we extend the field-of-view of QME to ~46 × 46 mm, allowing us to generate elasticity maps across the entire face of a typical specimen excised during breast-conserving surgery. We perform wide-field QME on thirteen freshly excised tissue specimens acquired from patients undergoing mastectomy. We demonstrate, through comparison with co-registered histology, the improved ability of QME to detect regions of highly cellular invasive tumor based on elevated elasticity. We highlight that by more readily conforming to the tissue surface, the compliant layer reduces heterogeneous strain arising from regions of micro- to milli-scale non-contact. In addition, by conforming to the surface, the layer increases overall contact area, allowing for more complete assessment of the margin. Together, these results demonstrate that quantitative micro-elastograms, used in conjunction with OCT images and
qualitative micro-elastograms, have the potential to improve the visualization of tumor in a broader range of breast cancers.

2. Methods

2.1 Wide-field imaging system

Our wide-field QME imaging system, shown in Figs. 1(a) and 1(b), comprises three main elements: the OCT system, the loading mechanism and the wide-field translation stages. The OCT system is based on a Telesto II spectral-domain OCT system (TEL220C1, Thorlabs). It uses a superluminescent diode light source with a central wavelength of 1300 nm and a bandwidth of 200 nm. The measured (full width half maximum) axial and lateral resolutions in air are 5.5 µm and 13 µm, respectively (Lens: LSM04, Thorlabs). The camera exposure time is 10 µs and the A-scan line period is 14 µs. The system is configured in common-path mode with the interface between the imaging window and the silicone layer acting as the reference reflector, Fig. 1(b). The measured displacement sensitivity of the OCT system is 1.44 nm at an OCT signal-to-noise (SNR) ratio of 40 dB, acquired under clinical testing conditions in the pathology laboratory (i.e., without a vibration isolation optical table).

The loading mechanism comprises a piezoelectric ring actuator, to which the imaging window is rigidly affixed, and a compression plate on which the specimen is placed. The piezoelectric ring actuator (Piezomechanik GmbH) has an aperture of 65 mm and a maximum stroke of 9.5 µm. The compression plate is mounted on three load cells (LSB200-FSH03874, Futek) arranged in a triangular configuration, which in turn are mounted on a motorized laboratory jack (MLJ050, Thorlabs). The load cells allow us to monitor the force applied to the specimen prior to testing. The motorized laboratory jack is used to bring the specimen into contact with the imaging window and to apply preload to the specimen to maximize contact area. The laboratory jack has 50 mm travel, which determines the maximum thickness of the specimen that can be scanned in this system. The motorized jack is controlled and the individual load cell readings are summed using a custom LabVIEW (National Instruments) program.

Wide-field datasets are generated by translating the loading mechanism, which includes the sample, silicone layer and imaging window, relative to the OCT scan head between sub-volume acquisitions, as described in a previous study [24]. The stages in this study have 100 mm travel and maximum velocity of 30 mm/s. Up to nine sub-volumes, each measuring 16 × 16 × 3.5 mm, are acquired with 1 mm overlap in the lateral plane. Sub-images in the en
The silicone layers used to measure axial stress at the tissue surface were formulated using Elastosil P7676 (Wacker Chemie AG), with a mixing ratio of 1:1 (Cross-linker:Catalyst) [27]. The diameter of the layers was ~55 mm. The thickness of each layer was measured before performing QME and recorded for subsequent processing of elasticity. The thickness of the layers ranged between 550 µm and 650 µm. Variation in layer thickness is due to the difficulty of pipetting exact amounts of the viscous silicone mixture into glass petri dishes for curing.

### 2.2 QME scanning

Prior to imaging, the specimen is placed on the compression plate and a preload, resulting in ~15-20% strain, is applied to ensure even contact between the imaging window, the silicone layer, and the specimen. The displacement imparted to the specimen by the actuator is synchronized with OCT B-scans pairs, such that alternate B-scans are acquired at different compression levels. Individual sub-volumes are acquired with 808 A-scans per B-scan and 2,424 B-scan pairs. During post-processing, phase difference is calculated between consecutive B-scans acquired in the same lateral position via the Kasai phase estimator and 3 phase-difference B-scans, acquired at different lateral locations, are block averaged together in the complex plane [28, 29]. The displacement at each lateral and axial location in a sub-volume is calculated from the phase difference [28, 29]. 3D qualitative micro-elastograms are generated by calculating the local axial strain at each lateral and axial location. Local axial strain is defined as the slope of the estimated axial displacement with respect to depth, and is calculated using weighted least squares linear regression, over a fitting range of 100 µm [28, 30].

The majority of strain measured in qualitative micro-elastograms is in the direction of loading (compression); however, strain in the direction opposite to loading can also occur due to variations in the compressibility of tissue. In incompressible tissue, due to the conservation of volume, axial compression results in lateral expansion, i.e., lateral tensile strain. This lateral expansion gives rise to reactive compressive forces along the lateral plane. In continuous and solid tissues, this expansion is similar across different sections of the tissue, with reactive forces cancelling and the axial strain being predominantly compressive (negative) strain, in qualitative micro-elastograms. However, compressible areas in tissue, such as hollow ducts and areas of poor contact, accommodate this expansion by sacrificing their volume. In material above such compressible areas, lateral compressive forces from nearby regions dominate, resulting in lateral compressive strain, which appears in qualitative micro-elastograms as axial tensile (positive) strain [23].

In processing of 3D quantitative micro-elastograms, the layer-specimen interface is detected in each B-scan using a detection algorithm based on the Canny edge detector [31]. The axial resolution of the system, ~3.9 µm (in silicone), determines the minimum measurable sensor thickness. The local axial strain at the layer-specimen interface, arising from compression introduced by the ring actuator, is then measured at each lateral position on the interface. Using this measured strain and the known stress-strain relationship of the layer, the axial stress at the layer-specimen interface is estimated. Under the assumption that the axial stress measured at the layer-specimen interface acts uniformly with depth, the 2D stress in the layer is divided by the 3D strain in the specimen, and the elasticity of the specimen is calculated at each lateral and axial position [25]. QME reports the elasticity as a tangent modulus at the preload strain. QME has previously been validated against standard compression tests, in each case the measured elasticity matched to within 8% of the expected elasticity and the inter-measurement variability was less than 12% [25]. During post-processing of the quantitative micro-elastogram, pixels in which the local strain is acting in the opposite direction to the applied stress are masked in the final quantitative micro-
elastograms, as these pixels give rise to negative elasticity, which is not physically meaningful.

In this study, a custom, thresholding-based algorithm is used to segment regions of adipose and milli-scale non-contact. Quantitative micro-elastograms are presented in false color and overlaid on the grayscale OCT image. Additionally, at pixels masked due to a negative value of elasticity, OCT SNR is shown. Qualitative micro-elastograms are presented using a separate false color scale, overlaid on the grayscale OCT image [23, 24]. En face OCT images (from a single pixel depth), qualitative micro-elastograms, quantitative micro-elastograms, and corresponding plots, from QME measurements, are presented 100 µm below the layer-specimen interface. To demonstrate the ability of wide-field QME to generate 3D datasets, stacks of magnified en face images are presented as videos, to a depth of 500 µm for each wide-field QME result presented. In some dense tissues, the OCT SNR has degraded considerably by 500 µm, as a result, the custom thresholding-based algorithm fails to identify dense tissue and does not overlay elasticity and strain in these cases.

2.3 OCME scanning

In Fig. 3, we compare QME measurements to OCME measurements. To perform OCME, the experimental setup is the same as that described in Section 2.2, except for, in OCME, the silicone layer is not included. As a result, the interface between the imaging window and the specimen acts as the reference reflector, and displacement is imparted from the imaging window directly to the specimen. The force applied to the specimen during OCME measurements matched that for QME measurements to minimize any variation due to non-linear mechanical properties of tissue. OCME sub-volumes were acquired with the same scanning parameters as QME sub-volumes, described in Section 2.2. In OCME, 3D qualitative micro-elastograms are generated by calculating the local axial strain in the same manner as QME. In Fig. 3 en face OCT images and qualitative micro-elastograms, from OCME measurements, are presented 100 µm below the window-specimen interface.

2.4 Clinical scanning

Tissue was transferred directly from the operating theater to the anatomical pathology laboratory at Fiona Stanley Hospital. Mastectomies were dissected following standard protocols. During dissection, for imaging, a pathologist extracted a specimen with similar dimensions to a specimen excised during breast-conserving surgery. Specimens were kept hydrated in saline, and imaging took place within 30 minutes of dissection to minimize cold ischaemic time and prevent degradation of the tissue. Following imaging, a pathologist applied ink to the perimeter of the specimen for later orientation of the digital micrographs. Due to their size, specimens were further dissected, placed in cassettes, fixed in 10% neutral-buffered formalin, and processed and embedded in paraffin according to standard practice. Paraffin blocks were sectioned in the plane matching en face images and stained with hematoxylin and eosin. Scanned digital micrographs of the histology slides were manually stitched for histopathological analysis. Informed consent was obtained from patients and the ethical aspects of this research project have been approved by the South Metropolitan Health Service, Western Australia Human Research Ethics Committee and the Fiona Stanley Hospital Human Research Ethics Committee (FSH-2015-032).

3. Results

In Figs. 2-5, we present OCT images, qualitative micro-elastograms and quantitative micro-elastograms of human breast tissue specimens, freshly excised from patients undergoing mastectomy.

In Fig. 2, we present images from a dissected tumor, excised from a 93-year-old patient undergoing mastectomy. A photograph of the specimen, measuring ~40 × 40 × 20 mm, is shown in Fig. 2(a). This patient previously underwent breast-conserving surgery, and the
specimen scanned here was extracted from the location of the previous surgery. A cavity (C) from the previous procedure, can be seen in the photograph and is the cause of non-contact in the wide-field OCT image, Fig. 2(d), and micro-elastograms, Figs. 2(e) and 2(f). The specimen was processed in two sections for histopathological analysis, Fig. 2(b). Analysis revealed invasive tumor around the periphery of the prior excision (T). Surrounding the tumor there is adipose tissue (A) and uninvolved stroma (S). In Fig. 2(c), a representative region of tumor is magnified to show in more detail the tumor cells distributed within desmoplastic stroma.

A preload of ~20% was applied to the specimen, resulting in a contact area of ~30 × 45 mm, and wide-field QME was performed. In Figs. 2(d)-2(f), we present a wide-field OCT image, qualitative micro-elastogram, and quantitative micro-elastogram, respectively. In Figs. 2(g)-2(j), we present magnified images from a region of dense tumor with similar micro-architecture to that visible in the magnified histology, Fig. 2(c). Tumor can be identified in this specimen by a combination of heterogeneous OCT SNR, Fig. 2(g), heterogeneous strain, arising from a mix of stroma and desmoplastic stroma, Fig. 2(i), and high elasticity, Fig. 2(j) [11, 23-25].

Fig. 2. Wide-field QME of a freshly excised mastectomy specimen (see Visualization 1). (a) Specimen photograph. (b) Histology. (c) Magnified histology. (d) Wide-field en face OCT. (e) Wide-field en face qualitative micro-elastogram. (f) Wide-field en face quantitative micro-elastogram. (g) Magnified en face OCT. (h) OCT B-scan in the plane indicated by dashed line in (g). (i) Magnified en face qualitative micro-elastogram. (j) Magnified en face quantitative micro-elastogram. A, adipose; C, cavity; E, edge detection artifact; G, coherence gate curvature artifact; T, invasive tumor; R, specular reflection; S, uninvolved stroma.
The wide-field OCT image, Fig. 2(d), shows low OCT SNR in the corners of each of the 9 sub-images (G). As discussed previously, this arises from the coherence gate curvature of the lens used [24]. The binary segmentation algorithm used to generate elastogram overlays identifies regions of low OCT SNR and masks these pixels. As a result, no strain or elasticity data is presented in these areas, Figs. 2(e) and 2(f). Visible in Figs. 2(g)-2(j), is an artifact caused by the edge detection algorithm failing to identify the layer-specimen interface (E). In this case, the algorithm detected a horizontal specular reflection (R) visible in OCT B-scan, Fig. 2(b). The location of this B-scan is indicated by the white dashed line in Fig. 2(g). This strong reflection resulted in the layer thickness being measured incorrectly. This region was masked from elastograms using the segmentation algorithm, due to the low OCT SNR and, consequently, OCT SNR from the layer is displayed in en face images.

In Fig. 3, we present images acquired from a tumor freshly excised from a 66-year-old patient undergoing mastectomy. A photograph of the specimen, measuring ~45 × 45 × 15 mm, is shown in Fig. 3(a). Histopathological analysis of the digital micrographs, Fig. 3(b), reveals regions of invasive tumor (T) surrounded by uninvolved stroma (S) and normal adipose tissue (A). In the magnified histology, Fig. 3(c), we see nests of invasive tumor cells separated by regions of desmoplastic stroma (S). To compare OCME (without layer) and QME (with layer), we performed two scans on this specimen, OCME in Figs. 3(d)-3(g), and QME in Figs. 3(h)-3(m). A preload of ~20% was applied to the specimen in both cases, resulting in a contact area of ~40 × 40 mm. The contact region is indicated by the black square in Figs. 3(a) and 3(b).

The wide-field OCT image and qualitative micro-elastogram, generated by performing OCME, are shown in Figs. 3(d) and 3(e), respectively. Magnifying the OCT image in a region of dense invasive tumor, Fig. 3(f), we see heterogeneous OCT SNR indicative of malignancy. In the magnified qualitative micro-elastogram of the same region, Fig. 3(g), we see heterogeneous strain, corresponding to invasive tumor (T). The wide-field OCT image, qualitative micro-elastogram, and quantitative micro-elastogram, generated by performing QME, are shown in Figs. 3(h)-3(j), respectively. Magnifying the OCT image, Fig. 3(k), we see heterogeneous OCT SNR. In the magnified qualitative strain micro-elastogram, Fig. 3(l), we see that inclusion of the layer does not reduce strain heterogeneity in the invasive tumor (T). In the magnified quantitative micro-elastogram, we see a stiff region corresponding to invasive tumor, Fig. 3(m). Similar to Fig. 2, the tumor in this case presents as heterogeneous OCT and strain and as a region of elevated elasticity.

Comparison of OCME and QME images in Fig. 3 highlights advantages of including the layer. In wide-field OCME, the surface topology of this specimen causes regions of micro- and milli-scale non-contact (NC) between the rigid imaging window and the specimen in Fig. 3(f). Areas of micro-scale non-contact reduce the area of the specimen that is imaged. This could result in missing tumor in a specimen, if the malignant region is not in the scanned area. Milli-scale non-contact can also increase imaging artifacts; for example, strong reflections from air bubbles can saturate the detector. In QME incorporating the compliant layer, which more readily conforms to the surface topology we significantly reduce the regions of non-contact, as shown in Fig. 3(k).

As described in Section 2.2, positive strain can arise from micro-scale non-contact, caused by localized peaks and troughs on the tissue surface, between the imaging window and the specimen in OCME [23]. In some cases, strain heterogeneity arising from such surface roughness can be similar to the heterogeneity arising from tumor. This could lead to misinterpretation of qualitative micro-elastograms. If we compare qualitative micro-elastograms from OCME, Fig. 3(g), and QME, Fig. 3(l), we see that an advantage of QME is the apparent reduction in positive strain caused by surface roughness while still maintaining strain contrast arising from tissue features.
Fig. 3. Wide-field OCME and QME of freshly excised mastectomy specimen containing invasive ductal carcinoma (see Visualization 2). (a) Photograph and (b) histology of specimen; black square indicates the scanned area. (c) Magnified histology. En face (d) OCT and (e) qualitative micro-elastogram from wide-field OCME. Magnified en face (f) OCT and (g) qualitative micro-elastogram from wide-field OCME. En face (h) OCT, (i) qualitative micro-elastogram, and (j) quantitative micro-elastogram from wide-field QME. Magnified en face (k) OCT, (l) qualitative micro-elastogram, and (m) quantitative micro-elastogram from wide-field QME. A, adipose; T, invasive tumor; NC, non-contact; S, uninvolved stroma.
In Fig. 4, we present QME images acquired from a tumor, excised from a 77-year-old patient undergoing mastectomy. A photograph of the specimen, which measured $\sim 70 \times 30 \times 15$ mm, is shown in Fig. 4(a). From the digital micrographs, Fig. 4(b), we see the specimen contains dilated ducts involved by ductal carcinoma in situ (DCIS) with necrosis (N) strands of stroma (S) and adipose tissue (A). Magnifying a region of the digital micrograph, Fig. 4(c), we see, in greater detail, ducts containing DCIS with necrosis (N) and ducts containing DCIS with necrosis and focal calcifications (FC). A preload of $\sim 20\%$ was applied to the specimen, resulting in a contact area of $\sim 25 \times 25$ mm. Wide-field images were generated by mosaicking four sub-images.

The wide-field OCT image, qualitative micro-elastogram and quantitative micro-elastogram are presented in Figs. 4(d)-4(f), respectively. In the magnified OCT image, Fig. 4(g), we see the outlines of the ducts; however, it is difficult to differentiate between DCIS and DCIS with focal calcifications from OCT SNR alone. Similarly, in the magnified qualitative micro-elastogram, Fig. 4(h), outlines of the ducts are highlighted by strain patterns. However, as in the OCT image, it is difficult to differentiate between DCIS and DCIS with focal calcifications. The magnified quantitative micro-elastogram, Fig. 4(i), on the other hand, uniquely provides differentiation between DCIS and DCIS with focal calcifications due to the lower elasticity of necrosis in comparison to calcifications. Viewing en face images at increasing depths, Visualization 3, allows the reader to follow the luminal cross-section of ducts through the specimen, which provides an appreciation of the 3D structure of the tissue.

In Fig. 5 we present wide-field QME images of a tumor, excised from a 59-year-old patient undergoing mastectomy. A photograph of the preloaded specimen, with surface area of $\sim 45 \times 45$ mm, is shown in Fig. 5(a). Analysis of the digital micrographs, Fig. 5(b), revealed the specimen contained regions of adipose tissue (A), ducts involved by DCIS (D), invasive tumor (T) and regions of uninvolved stroma (S). Magnified in Fig. 5(c) is a region of tissue containing a duct involved by DCIS. Surrounding the duct is a highly cellular invasive tumor (T) with little intervening desmoplastic stroma, and a region of paucicellular desmoplastic stroma (P), i.e., stroma containing few or sparse cells.

The wide-field OCT image, qualitative micro-elastogram and quantitative micro-elastogram are presented in Figs. 5(d)-5(f), respectively. Magnified in Fig. 5(g) is the OCT image corresponding to the region magnified in histology, Fig. 5(c). At this scale, OCT shows limited contrast between the duct, dense invasive tumor and stroma. A plot of OCT SNR through the duct, Fig. 5(h), confirms limited contrast between DCIS and invasive tumor. In the magnified qualitative micro-elastogram, Fig. 5(i), the duct is clearly delineated from the surrounding tissue as an oval-shaped feature, highlighted by positive and negative strain, as described previously [23]. The plot of strain through the duct, Fig. 5(j), highlights the transition from negative to positive strain found at the boundaries of the duct containing DCIS. However, in this case, the qualitative micro-elastogram shows homogeneous strain in both the tumor and paucicellular desmoplastic stroma. The magnified quantitative micro-elastogram, Fig. 5(k), delineates between the region of tumor, with high elasticity, and paucicellular desmoplastic stroma, with low elasticity. Within the duct, strain acts in the opposite direction to the applied stress. As a result, elasticity is not reported in these pixels and OCT is instead presented. The plot of elasticity through the duct, Fig. 5(l), highlights the pixels with masked elasticity and the elevated elasticity in this tumor. This result highlights the complementary contrast provided by qualitative and quantitative micro-elastograms. Horizontal dashed lines are plotted across Figs. 5(g)-5(l) to aid in locating the upper and lower boundaries of the duct in the corresponding plots.
Fig. 4. Wide-field QME of a freshly excised mastectomy specimen containing ductal carcinoma in situ (DCIS) (see Visualization 3). (a) Photograph and (b) histology of specimen; black square indicates the scanned area. (c) Magnified histology. (d) Wide-field \textit{en face} OCT image. (e) Wide-field \textit{en face} qualitative micro-elastogram. (f) Wide-field \textit{en face} quantitative micro-elastogram. (g) Magnified \textit{en face} OCT. (h) Magnified \textit{en face} qualitative micro-elastogram. (i) Magnified \textit{en face} quantitative micro-elastogram. A, adipose; FC, DCIS with focal calcification; N, DCIS with necrosis; S, stroma.
Fig. 5. Wide-field QME of freshly excised mastectomy specimen containing invasive ductal carcinoma and DCIS (See Visualization 4). (a) Photograph of preloaded specimen. (b) Histology. (c) Magnified histology. (d) Wide-field en face OCT. (e) Wide-field en face qualitative micro-elastogram. (f) Wide-field en face quantitative micro-elastogram. (g) Magnified en face OCT. (h) Plot of OCT SNR. (i) Magnified en face qualitative micro-elastogram. (j) Plot of strain. (k) Magnified en face quantitative micro-elastogram. (l) Plot of elasticity. A, Adipose; D, DCIS; P, Paucicellular desmoplastic stroma; T, Invasive ductal carcinoma; S, Uninvolved stroma. Plot location indicated by dashed lines in (g), (i) and (k). The upper and lower boundaries of the duct are indicated by the dashed black horizontal lines across (g)-(l).

4. Discussion

We demonstrate improved visualization of malignant tissue by providing additional contrast, based on tissue elasticity, that complements the contrast provided by OCT and strain, and additionally, removes many of the artifacts present in OCME. We have shown the ability of the technique to generate OCT images, qualitative micro-elastograms and quantitative micro-elastograms from a wide-field dataset, acquired in 10 minutes. We have shown that analysis of these three images can increase the ability of this technique to detect a wider range of tumors than can be achieved using any one of the individual images, as demonstrated in detail in Fig. 5.

Our results demonstrate that quantitative micro-elastography may be used to distinguish highly cellular tumors from surrounding tissue due to the tumor’s elevated elasticity, despite such tumors tending to exhibit homogeneous strain. However, it is unknown how accurate elasticity is in identifying tumor. To establish this accuracy, a larger clinical study is required
to generate a more extensive library of quantitative micro-elastograms across a range of
tumors of differing grades. In generating a library of tumor elasticity \textit{ex vivo}, it is important to
note the reported mechanical properties may differ from those generated \textit{in vivo} [32]. Another
consideration is the non-linear mechanical properties of soft tissue [33, 34]. To increase the
inter-specimen repeatability, the preload applied to the specimen should remain constant
across specimens, which was achieved in this study by monitoring the force applied prior to
testing.

Since the introduction of our wide-field OCME technique [24], we have refined our
acquisition protocol to allow wide-field scans to be acquired in 10 minutes. However, incorporation
of the compliant layer requires additional time for positioning the specimen before scanning. The specimen is
positioned on a compression plate, and the layer is then placed on the specimen. The layers used in the study can be difficult to handle due to their
low tear strength and layer thickness of 550-650 µm. Positioning the layer can add up to two
minutes to each scan. A proposed solution is to bond the layer directly to the imaging
window. Initial testing proved promising, however, additional development is required before
this approach can be implemented in our clinical scanning protocol. Currently, the imaging
window-layer interface is lubricated to provide low friction. High friction at the imaging
window-layer interface restricts the lateral expansion of the layer and causes the stress in the
specimen to be underestimated [35, 36]. This results in an underestimation in the specimen
elasticity reported by QME. A computational method has been proposed to more accurately
estimate the stress at the surface of the layer using a finite element analysis approach [36].
Further work will be required to investigate the ability of computational methods to
compensate for very high friction caused by bonding the layer to the imaging window. Computational methods have also been proposed to more accurately characterize the stress field in mechanical heterogeneous tissue using an iterative solution of the inverse elasticity
problem [37]. This method would remove the assumption that stress at the layer-tissue
interface acts uniformly with depth. However, the computation time may render this solution
unfeasible for intraoperative margin assessment.

Wide-field QME can currently be performed on two margins within a clinically relevant
time frame of ~30 minutes, including time to position and load the specimen. Further
increases in speed may be required to compete with techniques such as intraoperative
specimen radiography. A recent study demonstrated the capability to simultaneously acquire
two 3D OCME datasets from opposite sides of a specimen [38]. The system uses two
interferometers with path-length differences that allow depth-ranging from each
interferometer to be performed simultaneously on a single spectrometer. Implementing this
depth-encoding technique in wide-field QME would effectively double the imaging speed,
allowing us to scan two opposite margins of a specimen in 10 minutes. It would further save
time by removing the need to reposition to specimen to scan the second margin.

The maximum A-scan frequency for the Telesto II OCT system used in this study is
76 kHz. Upgrading the OCT system would allow for further increases in acquisition speed.
Phase sensitive optical coherence elastography has been performed with A-scan frequencies
in the MHz range using a Fourier-domain mode-locked swept-source laser [39]. As the A-
scan frequency increases, for a set sampling density, so will the B-scan frequency and the
actuator loading frequency. To ensure tissue loading remains quasi-static at higher imaging
speeds, it may be necessary to calculate phase difference between volumes, as described in a
previous study [40].

In addition to reducing the acquisition time, processing time must be reduced to generate
micro-elastograms for rapid interpretation in a clinical setting. In this current feasibility study,
data was processed in the days following surgery. A graphics processing unit-accelerated
OCME system capable of generating qualitative micro-elastograms of soft tissue at near
video-rates has previously been described [41]. Future work will investigate the suitability of
this approach to generate en face wide-field OCT images and wide-field quantitative micro-elastograms to allow for rapid clinical interpretation.

In Fig. 2(g), we identified a region in which the edge detection algorithm failed to correctly detect the layer specimen interface. This was caused by a specular reflection from the layer interface. A method to overcome this artefact is to introduce a small amount of scatterers to the layer to reduce specular reflection. However, any increased scattering in the layer may reduce imaging depth in tissue. A more suitable option is to refine the algorithm used to detect the interface. A large number of edge detection methods have been developed for use in ophthalmology [42]. Future work will assess the suitability of these in detecting the layer-specimen interface in wide-field QME.

To translate wide-field QME to a clinical setting, the interpretation of images needs to be simplified. Wide-field QME generates three images (OCT, qualitative micro-elastograms, and quantitative micro-elastograms), which could prove difficult for rapid interpretation in a surgical setting. Here, and in previous studies, we have presented micro-elastograms as overlays on OCT images, but further work on visualization must be performed to simplify images. Previously, we have identified regions of tumor by mapping a heterogeneity index of qualitative micro-elastograms [43]. In ongoing work, we will further investigate image fusion to aid with clinical interpretation.

5. Conclusion

In conclusion, we have demonstrated that wide-field QME can be performed over fields-of-view of ~46 × 46 mm. We have demonstrated the ability of wide-field QME to provide improved mechanical contrast across a range of both invasive and in situ tumors in freshly excised human breast tissue. We have presented a comparison of OCME and QME images and have demonstrated preservation of OCT contrast. An advantage of QME over OCME is an apparent reduction in artifact caused from surface roughness, whilst maintaining contrast between tissue types. We have presented scans of mastectomy specimens demonstrating that OCT images, qualitative micro-elastograms, and quantitative micro-elastograms acquired from QME provide complementary contrast of malignant tissue, improving the visualization of both in situ and invasive tumors. Together, these are important advances toward clinical implementation of OCT-based elastography techniques for guidance of breast-conserving surgery. In future work, the diagnostic accuracy of wide-field QME to detect tumor in the surgical margin will be determined by performing a study on a large number of breast-conserving surgery specimens.

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