Voxel-level reproducibility assessment of modality independent elastography in a pre-clinical murine model

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ABSTRACT

Changes in tissue mechanical properties, measured non-invasively by elastography methods, have been shown to be an important diagnostic tool, particularly for cancer. Tissue elasticity information, tracked over the course of therapy, may be an important prognostic indicator of tumor response to treatment. While many elastography techniques exist, this work reports on the use of a novel form of elastography that uses image texture to reconstruct elastic property distributions in tissue (i.e., a modality independent elastography (MIE) method) within the context of a pre-clinical breast cancer system.[1,2] The elasticity results have previously shown good correlation with independent mechanical testing.[1] Furthermore, MIE has been successfully utilized to localize and characterize lesions in both phantom experiments and simulation experiments with clinical data.[2,3] However, the reproducibility of this method has not been characterized in previous work. The goal of this study is to evaluate voxel-level reproducibility of MIE in a pre-clinical model of breast cancer. Bland-Altman analysis of co-registered repeat MIE scans in this preliminary study showed a reproducibility index of 24.7% (scaled to a percent of maximum stiffness) at the voxel level. As opposed to many reports in the magnetic resonance elastography (MRE) literature that speak to reproducibility measures of the bulk organ, these results establish MIE reproducibility at the voxel level; i.e., the reproducibility of locally-defined mechanical property measurements throughout the tumor volume.

Keywords: elastography, reproducibility, computational modeling, MRI, breast cancer, pre-clinical, mechanical properties.

1. INTRODUCTION

Elastography utilizes non-invasive image-based methods in order to assess the mechanical properties of tissue.[4] Elastography has been applied towards the mechanical assessment of multiple types of tissues and has been implemented through the use of several imaging modalities, including magnetic resonance (MR)[5,6], ultrasound[7,8], and optical methods[9]. One important and emerging application of elastography that is of particular promise is in the assessment of mechanical properties to diagnose and assess cancer. Elastography has been shown to assess the mechanical properties of tumors and revealed high shear elasticity in breast tumors.[10] In a study of colorectal cancer by Li *et al*., MR elastography results after treatment with a vascular disrupting agent showed a significant change in stiffness, which was confirmed by central necrosis in histology reports, while in this particular study, other quantitative MR metrics did not yet reflect significant change.[11] Falou *et al.* showed that ultrasound elastography can be an early predictor of the response of breast cancer to neoadjuvant chemotherapy. Four weeks after treatment initiation, responding patients demonstrated a significant decrease in strain ratios and strain differences as compared to non-responding patients with 100% sensitivity and 100% specificity when comparing strain ratios with static regions of interest.[12] In previous work, elastography methods have been primarily focused on the evaluation of tumor stiffness at the region of interest level for diagnosis and staging of entire tumors as compared to healthy tissues. The goal of this study is to assess elastography reproducibility at the voxel level, using a pre-clinical murine model of breast cancer.

The elastography method that we employ in this work, modality independent elastography (MIE), has been

previously shown to generate estimates of mechanical properties that were validated with independent mechanical testing.[2] MIE is unique in that it is an image analysis elastography method that is somewhat independent of any particular modality in that it only needs image texture to operate; thus far, it has been used with MR, computed tomography (CT), and optical imaging data.[2, 3] Elastography as a general method is relatively underdeveloped in pre-clinical animal models of cancer, where studies that characterize initial therapeutic and mechanistic effects are frequently performed. One particularly advantageous feature of MIE is that it has cross-length scale translational applicability, due to the quasi-static excitation used in the method. This excitation, which is similar to traditional elastic testing of materials, is very similar in both the pre-clinical and clinical settings, allowing for direct comparisons.

While sparse within the literature, evidence of the reproducibility of MRE and other elastography methods has been previously documented at the clinical length scale. Lee *et al.* evaluated MR Elastography (MRE) reproducibility in ninety-four liver fibrosis patients. The Bland-Altman 95% limit of agreement was up to 35.35%.[13] In Bohte *et al*., the liver elasticity of thirty participants, both in healthy volunteers and patients with hepatic fibrosis, was evaluated with MRE. The threshold for declaring significant change in liver elasticity parameters over time was 22.2%.[14] These results are on the order of the results from other types of quantitative non-invasive imaging, however their validity is confined to the region of interest level. While these results are scientifically interesting, reproducibility assessment at the sub-region of interest level is necessary for proper determination of biological effect with therapeutic administration.

The goal of the present study is to assess the reproducibility of the MIE method at the voxel level, using a pre-clinical murine model of breast cancer. Characterization of reproducibility at this level is essential to study tumor heterogeneity for both pre-clinical treatment response studies and the translation to the clinical setting. Specifically, monitoring heterogeneous mechanical properties of tissue through elastography may help characterize cancer progression and predict the response of breast cancer to neoadjuvant chemotherapy.[15] To evaluate treatment response effectively, the analysis must be capable of revealing regional differences in response throughout the tumor. Tumors are widely known to exhibit significant heterogeneity in gene expression levels and responsiveness to therapy, particularly for targeted therapeutics. Therefore, we must be able to define a significant change in regional tumor stiffness at the voxel level in order to evaluate future treatment response studies in pre-clinical models. By assessing reproducibility at the voxel level, as opposed to the region of interest level, we will be able to identify specific tumor regional differential responses to treatment. Understanding of the spatial dependency of response will allow for a more robust measurement of longitudinal effects; therefore we must statistically characterize the reproducibility at voxel level.

2. METHODS

2.1 Animal model

This study was performed on a pre-clinical murine xenograft model of triple-negative breast cancer. Approximately 10^7 MDA-MB-231 cells in a 30% Matrigel suspension (Corning Life Sciences, Tewksbury, MA) were injected subcutaneously in the right flank of 4-6 week old female athymic nude mice. After the tumors grew to approximately 250 mm^3 in size, anatomical MR image volumes were acquired for four mice.

2.2 MR acquisition

Using a T_2-weighted fast spin echo sequence and a 7.0T MRI scanner (Agilent Technologies (formerly Varian), Palo Alto, CA) with a 38-mm quadrature RF coil (Doty Scientific, Columbia, SC), two anatomical images are acquired for each mouse. The two images differ in the amount of applied deformation (one image is considered undeformed and the other deformed). The mechanical deformation is applied externally *via* inflation of a 5 cc balloon catheter controlled by a syringe driver. The catheter and driver are placed within the MR imaging coil, with the balloon placed beside the tumor on the right flank of the mouse. Inhalation anesthesia in the form of 2% isoflurane in 98% oxygen is administered during imaging. The images are acquired consecutively, without removing the mouse, and classified as a single scan. The scan is then repeated on the same mouse on the same day. The mouse is removed and allowed to recover between scans.

2.3 Modality Independent Elastography

The MIE method utilizes two anatomical image volumes of a subject, each with a different level of mechanical compression, and produces a reconstruction of the elastic properties based on their analysis.[16] The method begins with the uncompressed image volume and builds a three-dimensional geometric model of the tissue. A K-means clustering algorithm followed by a Markov Random Field constraint is used to group tissue regions based on location and signal intensity. Utilizing both the uncompressed and compressed image volume, a non-rigid image registration is used to extract appropriate boundary condition information.[17] Using the geometric model and boundary condition data, a finite element model using linear elastic deformations is employed to simulate tissue compression. The deformation field is applied to the uncompressed image volume to generate a model-deformed compressed image volume. The acquired image volume with tissue compressed is compared to the model-deformed image volume with a similarity metric. Based on the similarity, the mechanical properties of the model are adjusted. This proceeds iteratively until better matching is not possible. For this work, in post-processing steps, elasticity maps were scaled such that the average elasticity value was 1.0 (current realization produces elastic property contrast between tissues). When analyzing the repeat measurement, elasticity maps were registered to a common reference to allow for voxel reproducibility (next section).

2.4 Post-processing for voxel-level comparisons

The independently reconstructed elasticity maps for each scan are post-processed to facilitate further voxel-level comparisons between scans of the same animal, in order to assess reproducibility. The first post-processing step is to scale the elasticity data maps produced for each scan in order to reflect stiffness relative to the average tumor stiffness. Next, scan 2 is non-rigidly registered to scan 1. As the animal is removed from the scanner and allowed to recover from anesthesia between scans, the two anatomical images are in different image spaces. Using a demons non-rigid registration algorithm[18], the two anatomical images are aligned. The resulting non-rigid deformation field from the registration process is then applied to the elasticity map from scan 2, transforming the image into scan 1 image space, to allow for comparison between analogous voxels in the different elasticity maps from the same animal. Variations in the placement of the animal and the deformation source between the two scans mimics the repositioning variation between scans that would be expected during a longitudinal treatment response study. Then, the elasticity maps are smoothed with a Gaussian filter. Finally, the elasticity maps are smoothed with a 4×4 kernel in-plane pixel averaging smoothing filter. The smoothing kernel limits the effect of local mis-registration. These steps facilitate voxel-level analysis between elasticity maps of the same animal.

2.5 Statistical analysis

Reproducibility was assessed using Bland-Altman analysis[19] to compare the elasticity maps created from the two sets of scans on a single animal at the voxel-level. Statistics were calculated for all animals, including the mean value, the mean difference, the root mean square deviation (rMSD), the within-subject standard deviation (wSD), and the coefficient of reproducibility (r).[20] The within-subject coefficient of variation (wCV) was also calculated as an indicator of precision. The 95% confidence interval (CI) was calculated to show the limits of the values considered a result of expected variability. A value outside of these limits would indicate significant change beyond that expected from measurement noise.

3. RESULTS

MIE reproducibility was assessed in four mice. Figures 1 and 2 each show the anatomical MR images and the results of the MIE reconstruction for two representative animals. To facilitate comparison of the two scans, all elasticity maps were registered in the same orientation as the scan 1 anatomical image of that mouse, and each reconstructed elasticity map was scaled to unity for the average tumor elasticity value. The elastic property maps show the varying degrees of stiffness within the tumor. The two stiffness maps of each mouse show similar areas of high stiffness and low stiffness.

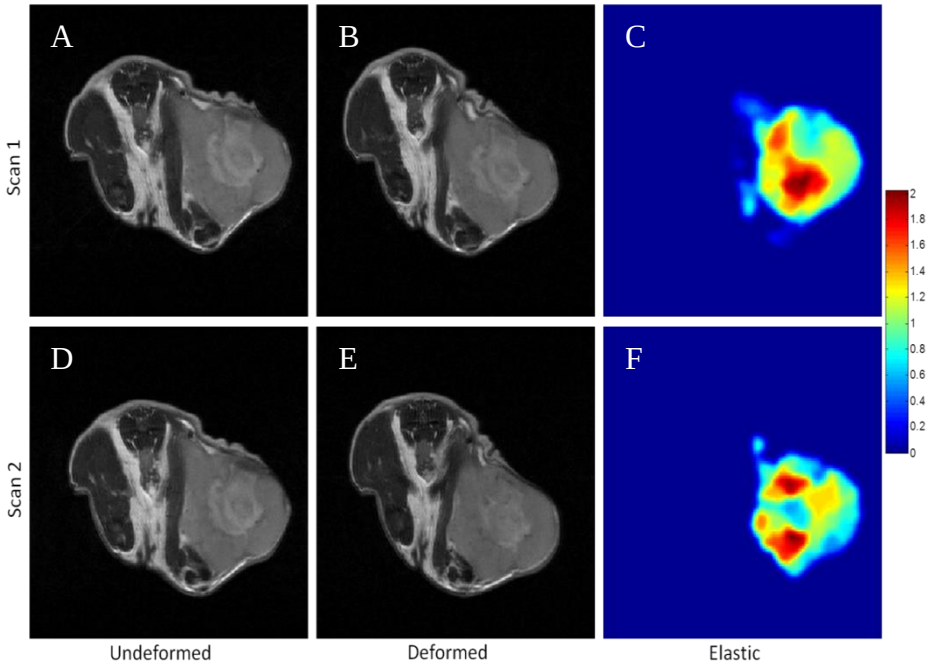

Figure 1. Undeformed, deformed, and MIE reconstructed elastic property map for scan 1 and scan 2 of mouse 1. Anatomical image A (undeformed) and anatomical image B (deformed) were processed with MIE to create the scan 1 elastic property map C. Anatomical image D (undeformed) and anatomical image E (deformed) were processed with MIE to create the scan 2 elastic property map F. The elasticity maps (C and F) show satisfactory agreement between ratios of high stiffness to average tumor stiffness in scan 1 and scan 2.

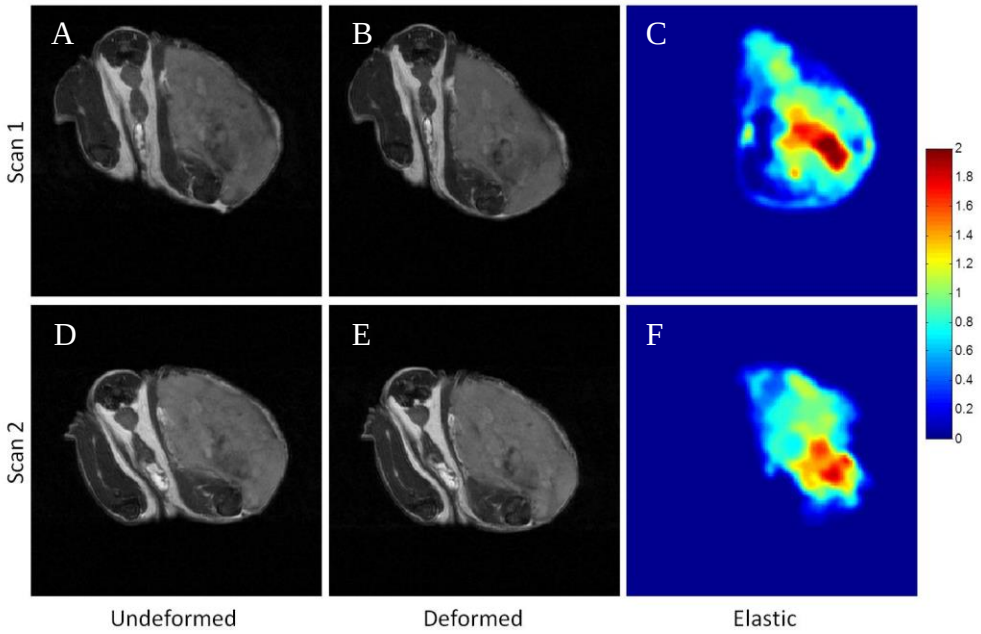

Figure 2. Undeformed, deformed, and MIE reconstructed elastic property map for scan 1 and scan 2 of mouse 2. Anatomical image A (undeformed) and anatomical image B (deformed) were processed with MIE to create the scan 1 elastic property map C. Anatomical image D (undeformed) and anatomical image E (deformed) were processed with MIE to create the scan 2 elastic property map F. The elasticity maps (C and F) show satisfactory agreement between ratios of high stiffness to average tumor stiffness in scan 1 and scan 2.

Metric	Value
Mean difference	0.0006
wSD	0.2785
wCV	32.02%
Reproducibility	0.7721
ICC	0.7110

Table 1. Statistics for voxel-level reproducibility analysis.

In Table 1, we show the results for several statistical metrics that are computed to assess MIE voxel-level reproducibility. All metrics are assessed by comparing normalized, registered, and smoothed elasticity maps from repeat MIE reconstructions of the same animal, from four animals. The mean difference indicates the average difference in stiffness results between analogous voxels in the elasticity maps produced from scan 1 and scan 2. The within-subject standard deviation (wSD) is a function of the differences between measurements and the total number of measurements.[20] The within-subject coefficient of variation (*wCV*) is the quotient of the wSD and the mean. The coefficient of reproducibility is the maximum difference expected in 95% of paired scans.[20] The Intra-class correlation coefficient (ICC) is a measure of reliability that compares the variance within individual subjects to overall variance.[21] These statistical tests are how we can discriminate between measurement error and real differences due to treatment. Bland-Altman analysis[19] of co-registered repeat MIE scans in this preliminary study (n = 4) showed a reproducibility index of 24.7% (scaled to a percent of maximum stiffness) at the voxel level; an observed difference greater than this value would indicate a significant difference at the 5% level.

4. DISCUSSION

For the last several years, MIE has been developed and assessed in our lab.[1-3, 15-17, 22-24] However, a study of reproducibility in a pre-clinical murine cancer model with voxel-level analysis had not previously been undertaken. The analysis in this work addresses the reproducibility of this method with regard to structural heterogeneity at the local voxel-level. Previous studies have investigated the reproducibility of elasticity imaging by comparing average tissue elasticity values and found the elastography measurement to be reproducible for analysis of overall tumor stiffness. However, assessment of reproducibility at only the region-of-interest level is blind to the potential spatial dependency of response expected during longitudinal therapeutic studies. Therefore, we sought to compare the separate MIE elasticity maps from test/re-test MIE analysis from voxels in the same position on each map.

In this study, we show the preliminary results for voxel-level reproducibility assessment of MIE in a pre-clinical model of breast cancer. We non-rigidly registered the elasticity maps produced from two separate elasticity assessments of the same animal using the MIE method and assessed statistical metrics of reproducibility at the individual voxel-level. We found wCV of 32.02%, ICC of 0.7110, and coefficient of reproducibility of 0.7721. These voxel-level results are found to be consistent with reproducibility results previously reported by other elastography methods at the region-of-interest level in the literature.[13, 14] The analysis in this work establishes the ability of the MIE method to assess heterogeneous tissue stiffness in a pre-clinical model. Additionally, this assessment will allow for detection of local changes in tumor stiffness over the course of treatment in a longitudinal study, even if the overall bulk stiffness does not change significantly. The metrics reported will allow for statistical discrimination between measurement noise in the MIE method and actual longitudinal changes in voxel-level stiffness. Combined with previous results characterizing the accuracy, the preliminary reproducibility results demonstrated in this work provide considerable promise for future pre-clinical treatment response studies and translation of MIE to the clinical setting. In future work, we intend to test reproducibility of MIE in human subjects and incorporate MIE into our existing image-based predictive oncology framework.[25]

ACKNOWLEDGEMENTS

This work was supported by the National Cancer Institute by R01CA138599, R25CA092043, U01CA142565, and U01CA174706. This work was also supported by the Vanderbilt initiative in Surgery and Engineering Pilot Award Program and the Vanderbilt University School of Engineering Summer Research Program. We thank the Kleberg Foundation for the generous support of our imaging program.

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