**Mechanisms of tissue deformation in OCME**

Under compression in OCME, tissue axial displacement is highest at the surface in contact with the mechanical loading device and, in most cases, decreases monotonically to zero at the side farthest from the device. This is illustrated in Supplementary Figure 1 for the case of a stiff, tumor-like feature embedded in a softer surrounding material. The axial displacement, d, versus depth, z, through the center of the material is illustrated in Supplementary Figure 1B. A change in the slope of displacement is observed in the stiff feature and is quantified by estimating the local strain, (1) (Supplementary Figure 1C). Lower local strain is present in the stiff feature, allowing it to be distinguished from the softer background (Supplementary Figure 1C). In this case, the slope of measured displacement versus depth is always negative (or zero). Furthermore, the negative local strain is inversely proportional to the stiffness of the tissue if it is assumed that stress is uniformly distributed throughout the tissue (1). Negative local strain is responsible for much of the contrast observed in Figures 2-7 of the manuscript.

Figure 1. Tissue deformation in OCME. A, Illustration of a tissue sample containing a stiff feature under compression in the OCME imaging system. B, tissue displacement, d, versus depth, z, at the location corresponding to the dashed line in A. C, corresponding local strain,**versus depth. The vertical dashed lines in B and C correspond to the upper and lower boundaries of the stiff feature in A.

Excised breast is, however, a structurally complex tissue that contains cavities, such as ducts, lobules and blood vessels. This structural heterogeneity causes more complex tissue deformation than that described above and can lead to positive local strain in OCME, as observed in Figures 2-7 in the manuscript. To demonstrate how positive local strain may occur, consider the upper boundary between a cavity (*e.g.*, a duct) and soft tissue, as shown in the illustration in Supplementary Figure 2. Under compression, as in Supplementary Figure 1, the displacement is highest close to the loading device and decreases with depth, corresponding to negative local strain. However, as there is no tissue in the cavity to restrict motion, the axial displacement of the tissue begins to increase at depths approaching the tissue-cavity boundary. As illustrated in Supplementary Figure 2B, this results in a gradient of displacement with depth that is opposite to that present in solid tissue and manifests as positive local strain in micro-elastograms (Supplementary Figure 2C). Positive local strain is also present below the cavity and arises because there is no axial force directly above the lower cavity-tissue boundary. This results in less displacement at the lower cavity-tissue boundary than in the tissue below the cavity and causes a positive gradient of displacement, corresponding to positive local strain (Supplementary Figure 2C).

Figure 2. Deformation in a tissue containing a cavity. A, Illustration of a soft tissue sample containing a cavity under compression in OCME imaging system. B, tissue displacement, d, versus depth, z, at the location corresponding to the dashed line in A. C, corresponding local strain, , versus depth. The vertical dashed lines in B and C correspond to the upper and lower boundaries of the cavity.

A number of other scenarios can also cause positive local strain in breast tissue. For example, excised breast tissue has surface roughness characterized by localized peaks and troughs caused by the tissue’s structure at the excision surface. This can result in small gaps between the tissue and the compression plate. Similarly to the case of the lower cavity-tissue boundary described above, this results in no axial force being applied directly above the troughs at the tissue surface. This causes a positive gradient of displacement as a result of the load applied to the tissue adjacent to the troughs, corresponding to positive local strain in the micro-elastograms. Positive local strain may also occur if the assumption that all constituent materials in breast tissue are incompressible is invalid (Incompressibility causes lateral expansion in response to an axially applied load and is quantified by Poisson’s ratio). Varying Poisson’s ratio between tissue types can allow the more incompressible tissue to expand laterally into the less incompressible tissue and can result in the incompressible tissue being forced upwards, causing a gradient of displacement with depth opposite to the direction of the applied load. A combination of these mechanisms cause the positive local strain observed in regions of invasive tumor visible in Figures 5, 6 and 7 of the manuscript, characterized by adjacent regions of positive and negative local strain.

To further illustrate the mechanism of compression in breast tissue and to demonstrate an example of positive local strain by the first mechanism described above, we performed mechanical simulations using the finite-element method (FEM), a numerical method commonly used to compute solutions to mechanical deformation problems by subdividing the problem into a mesh of discrete, homogeneous elements and solving the governing equilibrium equations in each element. The FEM simulations were constructed in the Abaqus simulation software package (Dassault Systèmes, Providence, USA, v6.12). Our group has previously used FEM simulations to analyze mechanical contrast in OCME (2) and to develop a multiphysics simulation of the imaging technique (3). In the simulations presented in Supplementary Figures 3 and 4, the simulated tissue has (*x*× *y*× *z*) dimensions 10 × 10 × 3 mm. To mechanically load the tissue, the tissue was first preloaded to 5%. The surface was then displaced a further 2 m to calculate local strain. These values are similar to those used in OCME imaging of excised breast tissue presented in the manuscript. Supplementary Figure 3 shows an FEM simulation of a tumor-like feature, 50 m in diameter and embedded 50 m below the tissue surface. The feature was ten times stiffer than the surrounding material in which it is embedded. The figure shows the local strain distributed throughout the sample in a two-dimensional image in which the vertical axis represents depth (*z*-direction) and the horizontal axis represents one lateral axis (*x*-direction). As observed, negative local strain is present at every location in the simulated micro-elastogram. The stiff feature is identified as a region with lower local strain than the surrounding soft material. This result is consistent with previous experimental and simulated OCME results (2,3).



Figure 3. FEM simulation of the tissue sample illustrated in Figure 1. Negative local strain is visible at each pixel in the image. Lower negative local strain in the stiff feature allows it to be distinguished from the softer background. Scale bar, 25 m.

To illustrate positive local strain using FEM, Supplementary Figure 4 shows simulations of a soft, incompressible material containing a cavity of similar dimension to the feature illustrated in Supplementary Figure 2. Distinct regions of positive local strain are visible above and below the cavity, as described above. There are also two distinct region of high negative local strain at the sides of the cavity. These are caused by the lateral expansion of the soft material into the cavity from either side, which allows the soft material adjacent to the cavity to undergo more negative local strain in these regions.



Figure 4. FEM simulation of the tissue sample, containing a cavity, illustrated in Figure 2. Both positive and negative local strain are prominent in the image. Fluctuations in local strain in the vicinity of the cavity provide contrast with surrounding tissue. The dashed circle represents the initial cavity diameter prior to mechanical loading. Scale bar, 25 m.

**References**

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