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Cell and tissue deformation measurements: Texture correlation with third-order approximation of displacement gradients

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ABSTRACT

Cells remarkably are capable of large deformations during motility and when subjected to mechanical force. Measurement of mechanical deformation (i.e. displacements, strain) is critical to understand functional changes in cells and biological tissues following disease, and to elucidate basic relationships between applied force and cellular biosynthesis. Microscopy-based imaging modalities provide the ability to noninvasively visualize small cell or tissue structures and track their motion over time, often using two-dimensional (2D) digital image (texture) correlation algorithms. For the measurement of complex and nonlinear motion in cells and tissues, implementation of texture correlation algorithms with high order approximations of displacement mapping terms are needed to minimize error. Here, we extend a texture correlation algorithm with up to third-order approximation of displacement mapping terms for the measurement of cell and tissue deformation. We additionally investigate relationships between measurement error and image texture, defined by subset entropy. Displacement measurement error is significantly reduced when the order of displacement mapping terms in the texture correlation algorithm matches or exceeds the order of the deformation observed. Displacement measurement error is also inversely proportional to subset entropy, with well-defined cell and tissue structures leading to high entropy and low error. For cell and tissue studies where complex or nonlinear displacements are expected, texture correlation algorithms with high order terms are required to best characterize the observed deformation.

1. Introduction

Cells and tissues in the body are capable of large deformation both *in vitro* and *in vivo*. In controlled studies by micropipette aspiration (Pravincumar et al., 2012) and optical tweezers (Henon et al., 1999), applied mechanical forces or pressure cause cells to undergo complex and viscoelastic changes in shape that relate to dynamics of the cytoskeleton. Flow and signal transduction pathways greatly influence cell polarization and motility with large changes to cellular shape (Maree et al., 2012). Red blood cells (Mills et al., 2004) and granulocytes (Evans and Kukan, 1984) undergo extreme deformations during flow. At the tissue level, excessive strain and strain rates have been linked to concussion in traumatic brain injury (Viano et al., 2005).

Because mechanical forces act upon our bodies, eliciting a diverse set of cellular responses, it is important to understand how our cells perceive and respond to force in the surrounding environment. One approach to address this question is to observe the cellular response to the mechanical forces and determine mechanistic relationships to the elicited chemical activity (Bershadsky et al., 2006; Janmey and McCulloch, 2007; Vogel and Sheetz, 2006; Zhu et al., 2000). To understand the influence of mechanical force on cells and tissues, unique methods are required to determine, in part, distributions of deformation (e.g. displacements and strain) throughout cellular subcomponents, including cytoskeleton, mitochondria, and the nucleus, as well as the surrounding tissue microenvironment (Gilchrist et al., 2004; Knight et al., 2006).

Texture correlation, a modified digital image correlation (DIC) procedure, utilizes the natural texture of biological tissues to measure displacement fields between two consecutive digital images (Bay, 1995). The texture correlation algorithm tracks the motion of a pixel within an image that is characterized by a unique intensity pattern defined by a subset of surrounding pixels. The displacement of the unique subset can be tracked by comparing images representing an object in a reference (i.e. initial) and deformed (i.e. current) configuration. Texture correlation has been applied to quantify bone, soft tissue, and intracellular deformations (Bay, 1995; Gilchrist et al., 2004,2007; Knight et al., 2006; Thompson et al., 2007; Wang et al., 2002; Zhang and Arola, 2004; Zhao and Simmons, 2012).

There are several unresolved concerns in the application of texture correlation algorithms to cell and tissue mechanics studies.

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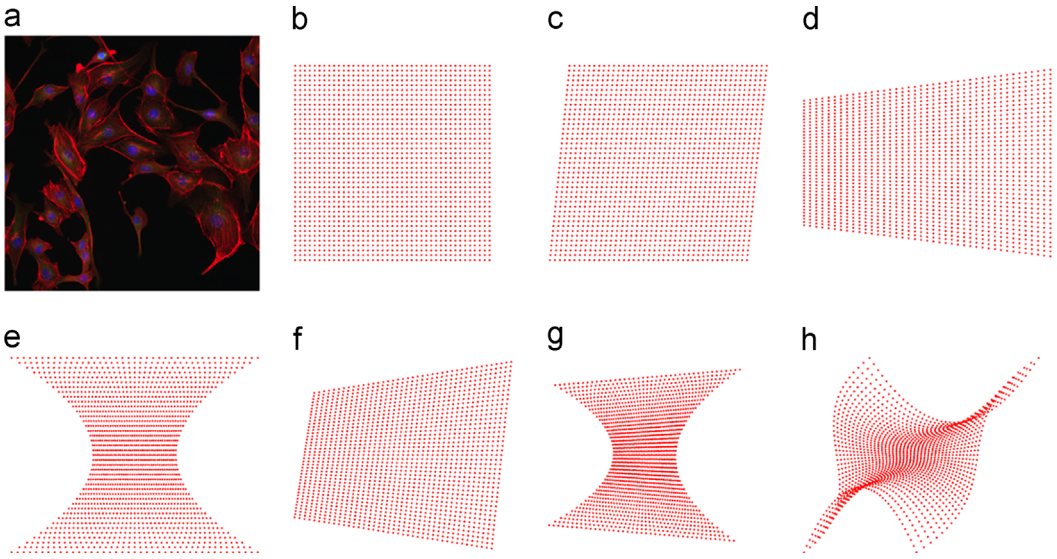

Fig. 1. Measurement error for a texture correlation algorithm with up to 3rd-order displacement mapping terms was determined using microscopy images and applied deformations. (a) Microscopy images of bovine pulmonary arterial endothelial (BPAE) cells revealed actin (Texas Red), microtubules (GFP), and nuclei (DAPI), which were merged for subsequent analysis in the study. Known deformation, defined by displacement mapping terms, was applied to subset points: (b) reference; (c) 1st-order deformation ($10\%\ \partial u/\partial y$); (d) 2nd-order deformation ($1\%\ \partial^2 v/\partial x\partial y$); (e) 3rd-order deformation ($1\%\ \partial^3 u/\partial x\partial^2 y$); (f) combination of 1st- and 2nd-order deformation ($10\%\ \partial u/\partial y + 1\%\ \partial^2 v/\partial x\partial y$); (g) combination of 1st-, 2nd-, and 3rd-order deformation ($10\%\ \partial u/\partial y + 0.5\%\ \partial^2 v/\partial x\partial y + 1\%\ \partial^3 u/\partial x\partial^2 y$), and (h) combination of 2nd- and 3rd-order deformation ($1\%\ \partial^2 v/\partial x\partial y + 1\%\ \partial^3 u/\partial^3 y + 1\%\ \partial^3 v/\partial^2 x\partial y$).

First, to reduce displacement measurement error for objects in complex motion, higher order (1st- and 2nd-order) displacement mapping terms were introduced to the algorithm (Chu et al., 1985; Lu and Cary, 2000; Vendroux and Knauss, 1998). However, for most studies of cell and tissue deformation, only 1st-order algorithms have been used to date (Gilchrist et al., 2004; Knight et al., 2006; Wang et al., 2002). High order approximations for displacement mapping terms may be required to best describe complex cell and tissue motion, especially when long image acquisition times limit the ability to visualize temporal changes, often occurring on millisecond time scales (e.g. (Pravincumar et al., 2012; Viano et al., 2005)). A related concern is that the distinct intensity patterns needed for texture correlation might not be found in small subset regions, especially in the extracellular matrix or the cytoplasm. A relatively large subset region (on the order of tens by tens of square pixels) may be necessary to provide a unique pattern that is tracked using the algorithm. In this case, however, linear deformation mapping may not be appropriate in the large subset region to characterize internal motion.

Second, relationships between the subset texture and measurement error of the algorithm have been largely unexplored. Two methods have typically been used to define subset texture: subset entropy and subset roughness (Gilchrist et al., 2004; Haralick et al., 1973; Sun and Pang, 2007). Subset entropy is a statistical measure of randomness of pixel values within a subset, in comparison to the entire image. Subset roughness depends on the standard deviation of the pixel values within a subset. Previous studies demonstrated an increase in subset entropy or roughness with the measurement accuracy. However, previous results were typically obtained using a single-loop simulation, i.e. only a pair of pre- and post-deformation images was used. Since texture correlation algorithms are sensitive to the image noise, it is not clear how the magnitude of error varies over multiple simulations representing arbitrary images acquired with superimposed random noise, a case more closely representing practical application of the algorithm.

In this study, we propose a texture correlation algorithm enabling higher order (up to 3rd-order Taylor series) approximation of the displacement gradients. We compare the utility of texture correlation with 0th-, 1st-, 2nd-, and 3rd-order displacement gradients for cell and tissue mechanics studies. Furthermore, we applied Monte-Carlo simulations to determine the relationship between subset texture, defined by entropy, and the error of displacement measurements.

2. Methods

2.1. Texture correlation algorithm

In this study, a texture correlation algorithm with up to 3rd-order displacement mapping terms was implemented. Texture correlation algorithms, that included 0th-, 1st-, and 2nd-order terms, were previously presented by Bay (Bay, 1995), Vendroux and Knauss (Vendroux and Knauss, 1998), and Lu and Cary (Lu and Cary, 2000), respectively. The 3rd-order algorithm is an expansion of the 2nd-order algorithm, considering deformation in two dimensions, with pixels of interest identified in image pairs that depict an object in reference (original) and deformed (current) configurations. For each point (e.g. pixel) of interest in the reference image, local texture centered about the point was defined using image intensity values in a square subset of pixels. The coordinates of each subset point in the reference image, (x, y), were mapped to their counterpart in the deformed image, $(\tilde{x}, \tilde{y})$, using

$$\tilde{x} = x_0 + U(x,y)$$
$$\tilde{y} = y_0 + V(x,y) \quad (1)$$

where U and V are the displacement components of each subset point. U and V can be approximated utilizing up to 3rd-order Taylor series expansion about a pixel of interest (x_0, y_0), as detailed in the Appendix. Briefly, the Taylor series expansion includes up to twenty displacement parameters, and allowed for the representation of complex deformations (Fig. 1). Bicubic spline interpolation was introduced to describe motion, in addition to a scaling parameter, w, to account for differences in reference and deformed image intensities. To find the mapping parameters, U and V, a least-squares correlation coefficient and Newton–Raphson optimization method was implemented. Importantly, a simple demonstration code for the algorithm, 'TextureCorrDemo.m', is available for download as a supplement online.

2.2. Cellular-scale images and simulated deformation

To compare the utility of a texture correlation algorithm with 0th-, 1st-, 2nd-, and 3rd-order displacement mapping terms, reference and deformed images of single cells were acquired and simulated, respectively. An image of bovine pulmonary artery endothelial (BPAE) cells (Invitrogen Inc., Carlsbad, CA), a model cell type with representative cytoskeletal and nuclear structures expected in a broad range of eukaryotic cells, was acquired by widefield fluorescent microscopy