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FIBROBLAST MECHANICS IN A MODEL EXTRACELLULAR MATRIX - AN APPLICATION OF OPTICAL SECTIONING MICROSCOPY

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INTRODUCTION: The cytoskeleton in a non-muscle cell is dynamic and versatile, and drives many essential processes ranging from cell division to wound healing. Through our work, we hope to understand how and where cells such as fibroblasts build and regulate the cytoskeletal machinery needed to carry out well-defined motile tasks. In essence, we aim to get, for non-muscle cells, the type of information routinely available to the muscle physiologist - detailed mechanics in addition to cytoskeletal kinematics. As a basis for this project, we need quantitative information on the spatial pattern and magnitude of the forces (tractions) applied by a cell to its surrounding matrix and neighbours, and a means for matching those patterns to identifiable cytoskeletal structures. The pioneering work of Harris [1] in growth of cells on transparent elastic substrata provides a means for estimating complex tractions through microscopy and digital image processing [2]. We have extended this approach to cells in collagen matrices, and we have automated the mechanics analysis.

METHODS: In our experiments, mouse 3T3 fibroblasts expressing GFP-alpha-actinin or YFP-actin are grown in a three-dimensional (3D) model extracellular matrix (ECM) composed of type I collagen hydrogel with specific isometric constraints and free-boundaries. Collagen is the primary constituent of connective tissue ECM and thus approximates a native environment for this cell type. The entire specimen is made small enough (5mm square x 0.3mm thickness) so that a large fraction of its volume is accessible to view with high-NA optics. By use of a perusable, thermostatted environmental chamber, the specimen can be maintained on the microscope for periods up to seven days. Our Automated Interactive Microscope (AIM) supports time-lapse, repetitive serial-focus operation, and switching between transmitted-light (Nomarski DIC) and fluorescence imaging modes. In the fluorescence illuminator light path, we utilize a grating imager [3] for optical sectioning. In a typical experiment, 10-60 fields are logged over which image sets are captured on a schedule. The AIM log and schedule can be complex, for example with differing frequency in DIC and fluorescence, and it can be modified interactively to eliminate or add fields as the specimen develops. In most cases, serial-focus data are acquired on each visit to each field. The AIM software creates a hierarchical data structure which can be tiled, or indexed by time, focus plane, or imaging mode for review and processing.

In DIC collagen fibrils are visible as a 3D network, so the model ECM can serve as an "in-situ strain gauge" readable through high-resolution time-lapse microscopy without extrinsic marker particles. The microscope is used in two ways: to image by fluorescence the distribution of key cytoskeletal proteins in individual cells, and by DIC the displacements in the ECM caused by the mechanical action of each cell. For most cells, which are embedded in the collagen matrix, the quasi-static displacement field, $\underline{U}(x,y,z,t)$, extends in all directions within the surrounding volume. Although our serial-focus DIC data in principle allows us to track displacements in 3D, we currently use a 2D plane-strain approximation to analyse image sets in which the cell major axis coincides with a plane of focus. The configuration of the model system produces a high percentage of cells that meet this condition. A series of 2D vector displacement fields, $\{Ux(x,y),Uy(x,y)\}$, is generated by use of our Deformation Quantification and Analysis software (DQA), which runs on a PC-based server. DQA utilizes windowed cross correlation to compare selected images from a time-lapse sequence. Using basic elasticity theory, DQA derives the 2D density increment, $d(x,y)$, and the plane-strain tensor field:

$$e_{ij}(x,y) = (\partial U_i/\partial x_j + \partial U_j/\partial x_i) / 2$$

Both d and [eij] are independent of material properties. Furthermore, from microrheometric measurements in cell-free gels [4], we know that the matrix is elastic under small deformations with a shear modulus averaging 50 Pa in the 2 mg/ml range. If a material constitutive relation is incorporated, the stress tensor field can be computed from the strain for estimating tractions. DQA software may be used on-line at dqa.web.cmu.edu.

RESULTS AND DISCUSSION: Over a period of several days in culture, 3T3 fibroblasts migrate long distances, proliferate, and contract the collagen gel to form a dense matrix resembling connective tissue. Deformations caused by the action of relatively isolated cells can produce anisotropy in the collagen which may affect other cells through contact guidance over 100-1000um distances. Sparse cells appear to organize into clusters or chains that

produce large-scale (non-elastic) condensation of the collagen. Single cells extend and retract long processes (pseudopods and filopods) and small lamellipods over 1- to 15-minute periods. In serum-deprived cultures, this activity produces little change in the model ECM. However, in growth medium (10% serum), cellular action leads to prominent elastic deformation of the collagen coupled to cell shape changes and locomotion. In fluorescence optical sections, GFP-alpha-actinin shows a highly non-uniform distribution throughout the cell (**Fig.1**), with major concentration in pseudopods and filopods. Deformation is quantified in corresponding

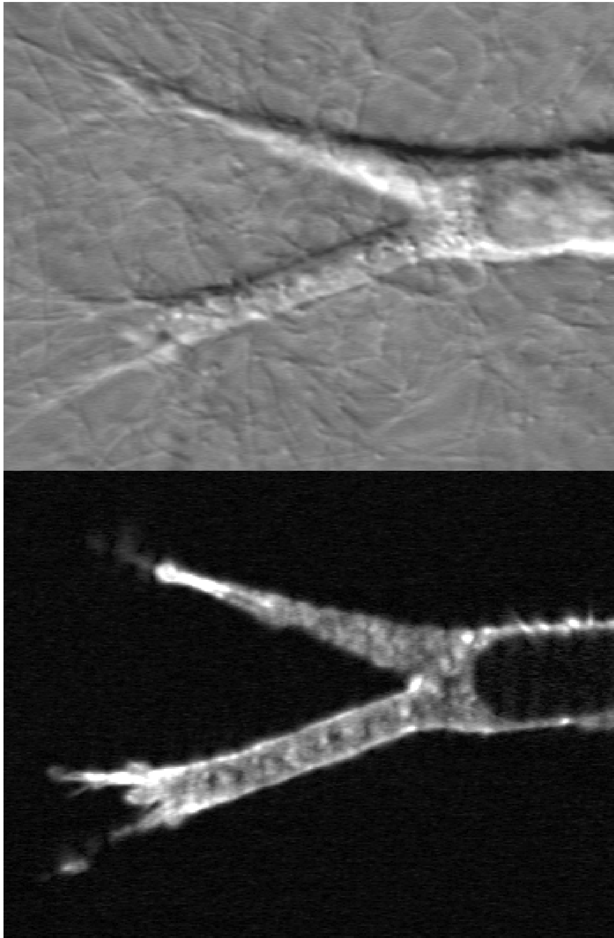

Figure 1. Images from time-lapse sequence. (upper panel) DIC image showing 3T3 fibroblast in collagen matrix. (lower panel) Corresponding fluorescence optical section showing GFP-alpha-Actinin. Field-of-view = 120um.

image maps of collagen density increment and of strain (**Fig.2**). Further data analysis will test the hypothesis that alpha-actinin is concentrated in the actin cytoskeleton at locations under the greatest tensile stress. In addition to the analysis of single-cell mechanics, it is possible to quantify large-scale deformation in tiled image sets. This opens the way to analysis of fibroblast behaviour in reshaping tissue in terms of single-cell effects, direct effects on neighbours, and collective action mediated by long-range effects on material properties.

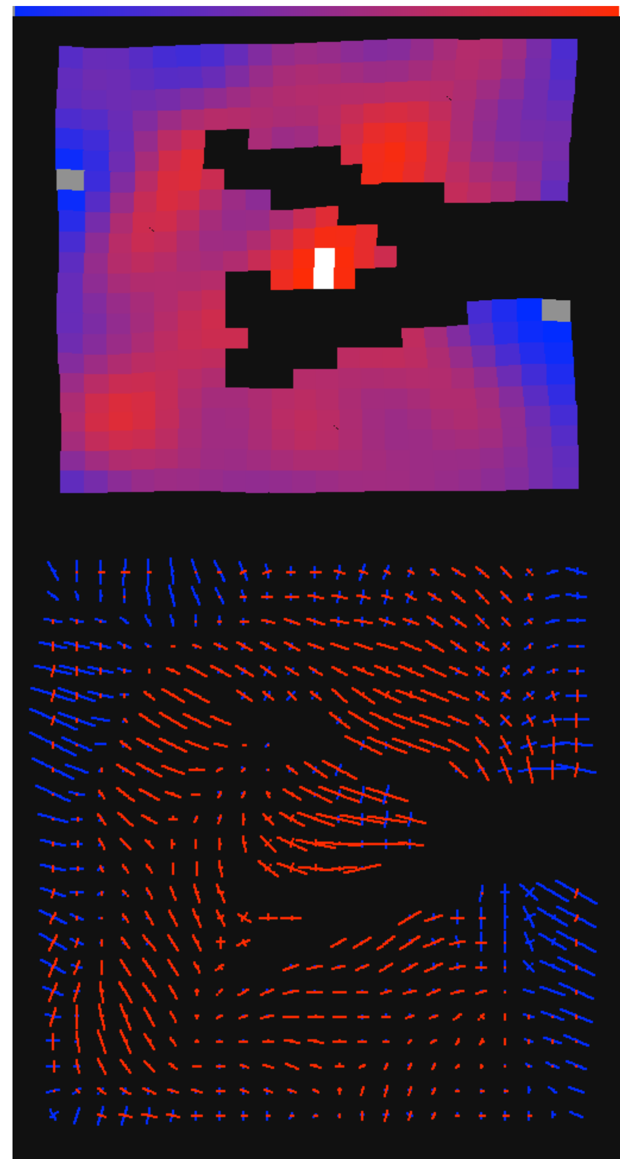

Figure 2. Mechanics analysis. (upper panel) 2D density increment (red = density increase, blue = density decrease, white = peak increase, gray = peak decrease). (lower panel) 2D principal strain field (red = material compression, blue = material extension).

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