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Post Processing of Confocal Microscopy Images to Quantify Collagen Remodeling by Fibroblasts

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Abstract

The ways that fibroblast cells remodel their local microenvironment is critical to a number of pathologies. To quantify this in a 3D environment, I an existing MATLAB script was modified to process the qualitative, visual information from confocal microscopy images and generate quantitative graphs representing the density of collagen around a fibroblast as a function of distance from the cell. While the earlier script used each 2D image from a confocal image stack and dilated the shape of the recognized cell to extract to find the coordinates of the surrounding collagen. The work in this study builds on the analysis of the collagen density around an active fibroblast. Instead of setting a threshold and identifying the cell in each 2D image, the new method uses all the stack images collected and builds a 3D representation of the entire cell-collagen environment. This method contains the analysis capabilities of the previous method but can include collagen data from stacks directly above and below the cell in its analysis.

Introduction

Recognizing that cells may exhibit significantly different behavior depending on whether they are suspended on a 2D substratum or in a 3D substratum, scientists have studied the behavior of fibroblasts suspended in 3D extra cellular matrix (ECM) [1-5]. Quantifying cell behavior in a 3D environment is a major challenge, and there is a pressing need for data to fit the many models that have been proposed [6-12].

A critical question to determining how tissues and cells behave is the way that cells modulate their volume fraction and local microenvironment [13-18]. This is important in a range of composite materials applications [19-23]. In tissues, it determines the mechanical and viscoelastic functioning of structural tissues, and drives the electromechanical function of other tissues [24-30]. Our group hypothesizes that sites on fibroblasts, where they attach to the ECM, drive polymerization of ECM fibers and subsequently affect the fiber density in the immediate area around the cell [17,31-32]. As a result, the density of the collagen fibers around a fibroblast are of particular interest. Experimental evidence further supports a hypothesis that cellular regulation in response to their local mechanical environment is governed by the dynamics of the cell and the stiffness of the local ECM [33-40]. However, no existing tools are available for quantifying cellular dynamics of this character [41]. The methods used in this project measured the density of the collagen around a fibroblast, which may help scientists better understand how cells react to their immediate mechanical environment and respond to the mechanical signals given off by that environment.

Methods

Fibroblasts were suspended in ECMs of collagen in 8 well plates and placed in the confocal microscope and imaged over a series of timepoints. At the beginning of each experiment, individual cells were located manually on the microscope and their location recorded on the microscope software. Each 512 x 512-pixel image taken by the microscope is centered by the location of the cell. At each timepoint, images from 50 layers above to 50 layers below the cell are imaged, space allowing, yielding two stacks of images, one for the green-labeled collagen and the other for the red-labeled cell. For each timepoint, the script analyzes red stack images and uses a thresholding method to determine, pixel by pixel, whether the cell occupies it. The coordinates of the cell are fed into a function in MATLAB which dilates the volume data using a structuring element. In this case, a sphere of user-defined radius is effectively rolled around the outside of the original cell to simulate the space immediately around the cell. The coordinates of the dilated cell identify the coordinates of green light intensity values contained in the green images. The density of collagen and the intensity of green light imaged are directly related. By plotting the intensity values of green light on a color scale in the coordinates located by the dilation process, a color map of collagen local to the cell

was

produced.

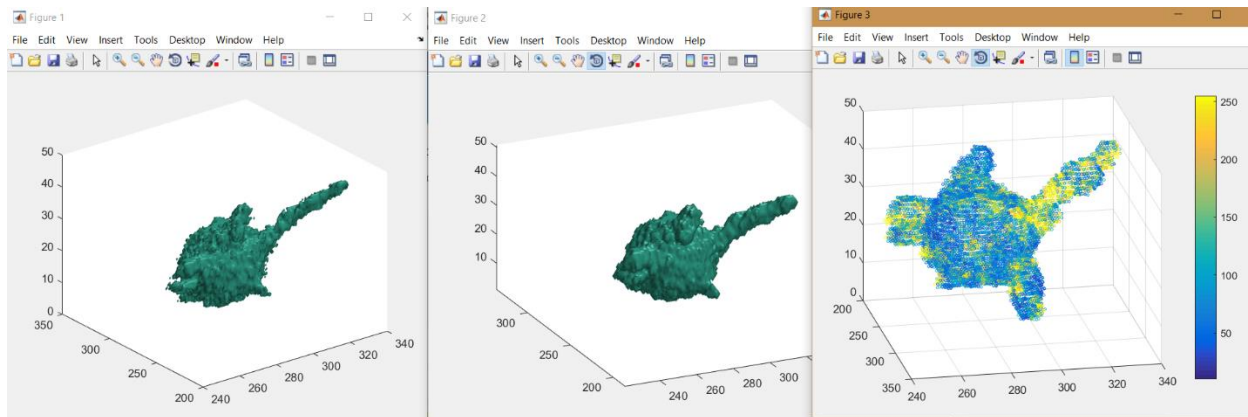


Fig. 1 Original cell (left), dilated cell (center), color map of local collagen intensity (right).

Another script produced movies by flipping through the images each time point at a single location, with the camera angle specified by user input.

By adding several layers of dilation to each cell, a range of data was analyzed, producing information on collagen relatively far from the cell surface (over 100um) as well as collagen only one or more micrometers away from the surface. This data was presented in the form of line graphs which showed the average intensity of collagen within each layer of dilation over each time point.

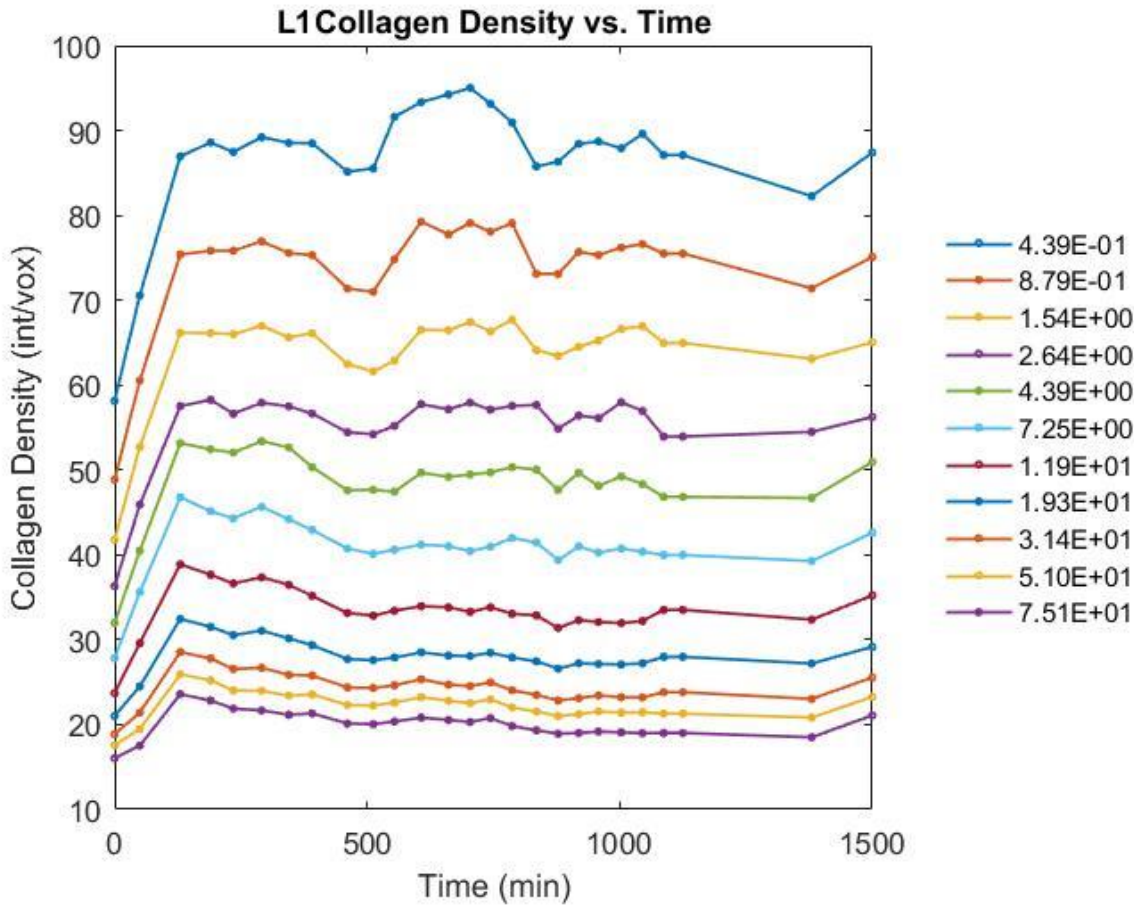


Fig 2. Data from an experiment conducted on 8-2-17 at a cell at location 1. Dilation distances in micrometers are listed on the right.

Discussion

Due to the heavy processing nature of this task, the script was eventually broken down into a few components. The most important component was a script that extracted the red and green image data from the microscopy image files and stored them in the form of 3D matrices. This allowed this data to be available for any sort of processing, rather than having to re-extract that data each time the script was run. Two other scripts were based off this data, and either

performed the dilations and generated dilation-based graphs or performed a single dilation and generated a time lapse movie.

There are a few limitations of this study that are important to consider when evaluating the quality of the data produced. One of the most significant is the resolution accuracy of the thresholding of the cell. It is unclear as to how well the red dye and imaging filter can resolve the boundaries of the cell. This is important since the interface of the cell and collagen fibers is of interest and needs to be resolved well to produce clear, compelling graphs and images. Preliminary analysis of dilations less than a few micrometers show potential in generating qualitative data on adhesion sites between the cell surface and collagen. These images appear to show bundles of collagen fibers gathered in adhesion sites along the length of fibroblast filopodia.

Some other considerations include the local fluctuations of collagen density in the ECM independent of the cell. For example, if a stack image is taken close to the surface of the incubation well, the collagen density is usually lower since gaps and tears of the ECM occur along the surface of the well. The optical effects of being close to the well surface also affect data there. While most cells in the study had adequate stacks above and below them, some were located close to the well surface or migrated over the course of the experiment. Another example is the presence of a second cell. Once again, most cells analyzed were found to be relatively isolated in the ECM. However, some cells were close to another cell, or had begun migrating closer to another cell. The current script is designed for one cell and cannot distinguish between two fibroblasts, so the presence of another cell affects the density and clouds the data produced. Cell-cell interactions are critical to a number of important

physiological and pathophysiological cell behaviors, and are an important topic for future investigation [6-7,]

Another consideration is the presence of small patches of cell material floating around the cell due to the process of blebbing. This material often passes the threshold and is considered part of the cell by the code. This artificially decreases the density of collagen data as it can treat collagen data farther from the surface as collagen close to the surface, depending on the location of the bleb material.

Further development of this project should begin with finding an answer to the question of cell boundary resolution and perhaps incorporation of smarter, automation features, such as automatic cell tracking on the microscope, to being able to distinguish between separate cells.

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