Towards a Representative Phenotypic Representation of Structural Components of Vascular Smooth Muscle Cells

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Statement of Purpose: Vascular smooth muscle cells (VSMCs) are constantly under dynamic load due to arterial pressure in normal healthy conditions. In response to injurious mechanical loading, VSMCs can undergo significant cytoskeletal remodeling, leading to changes in mechanical properties that may eventually contribute to restenosis [1]. The ability to predict the behavior of cells from their nanoscale structures could elucidate the mechanisms behind many tissue mechanical properties [2]. (In this study we utilized novel image processing techniques to analyze confocal images of multiple VSMC phenotypes, leading to the creation of representative *in silico* model structures that can be imported into finite element analysis software for mechanical characterization.

Methods: Passage 5 – 8 Sprague-Dawley rat aortic VSMCs were cultured in subconfluent layer (~80%) on glass coverslips coated with type I collagen. A synthetic phenotype was induced by incubating the cells for 3 days in a solution of 95 parts DMEM, 5 parts fetal bovine serum, and 1 part antibiotic/antimycotic. A contractile phenotype was induced in by incubating the cells for 3 days in a solution of 99% DMEM, 1% anti/anti. The cells were then stained with AlexaFluor 488 (Invitrogen, Carlsbad, CA) phalloidin to label f-actin and DAPI to label nuclei, α- and β-tubulin antibodies to label microtubules, and β1 antibodies to label integrins. The cells were then imaged using laser scanning confocal microscopy and the images were subsequently imported into MATLAB where they were analyzed using custom scripts employing edge finding, 3D FFT, and statistical Hough transform algorithms to generate representative 3D *in silico* model structures for many of the structural components of each phenotype.

Results: Results presented in abstract will be limited to generation of a representative f-actin network due to limited space.

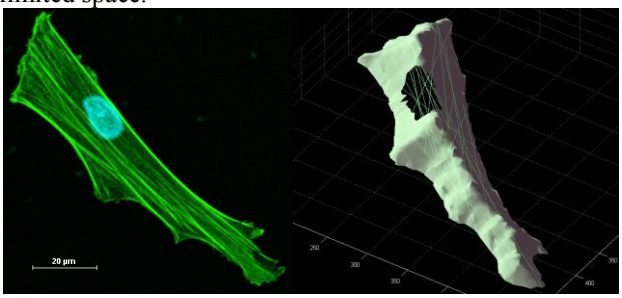

Figure 1. a) Confocal image of VSMC, with nucleus (blue) and f-actin (green) stained with phalloidin b) Representative 3D f-actin network obtained via use of entire stack of confocal images.

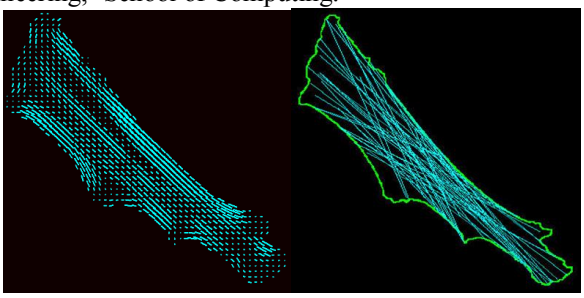

Figure 2. a) Vector field of f-actin network of one confocal image slice obtained via 2D FFT of Figure 1 b) Representative 2D f-actin network obtained via statistical analysis of Figure 2 around the cell boundary shown in green

As can be seen in Fig 2b, the calculated actin network for the cells is not identical to the structure seen in the original image (Fig. 1a). However, that is not the goal of this algorithm. The goal here is to allow for the easy and quick generation of a population of model structures that have structures similar to imaging data without having to image each cell individually. To move the models to 3D (Fig. 1b), the probability distribution calculated from the Hough transform (Fig. 2a&b) is generalized over the surface. The surface is calculated from a generation function estimated from confocal and AFM cell imaging data. To generalize the algorithm to nonlinear fiber structures, the first two steps are the same. However, instead of connecting the points using a line, the two sampled points on the cell surface are connected using a random walk where the steps are determined from the observed persistence length of the fiber in the images.

Conclusions: Our novel computer vision algorithms can quickly generate representative *in silico* cell structures based on standard imaging data. Besides using these structures for assessment of cell structure population from a set of images, these can be used to generate a population of structures with realistic geometries for modeling endeavors. In future studies, we will apply these techniques to a population of images of a given phenotype, thereby generating representative model structures of entire phenotypes which will be imported into finite element analysis software for mechanical characterization.

References: 1. Buerke M. BBA. 2007;1774:5-15.2. Ingber DE. J Cell Sci. 2003;116(7):1157-1173.

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