Medical Image Analysis 17 (2013) 337–347

Contents lists available at SciVerse ScienceDirect

Medical Image Analysis

journal homepage: www.elsevier.com/locate/media

A linear programming approach to reconstructing subcellular structures from confocal images for automated generation of representative 3D cellular models

Scott T. Wood [a], Brian C. Dean [b], Delphine Dean [a,*]

[a] Department of Bioengineering, Clemson University, 301 Rhodes Research Center, Clemson, SC 29634-0905, USA
[b] School of Computing, Clemson University, 100 McAdams Hall, Clemson, SC 29634, USA

ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 20 October 2012
Accepted 11 December 2012
Available online 9 January 2013

Keywords:
Automatic cell segmentation
Representative F-actin network
Confocal microscopy
Finite element model geometry

ABSTRACT

This paper presents a novel computer vision algorithm to analyze 3D stacks of confocal images of fluorescently stained single cells. The goal of the algorithm is to create representative *in silico* model structures that can be imported into finite element analysis software for mechanical characterization. Segmentation of cell and nucleus boundaries is accomplished via standard thresholding methods. Using novel linear programming methods, a representative actin stress fiber network is generated by computing a linear superposition of fibers having minimum discrepancy compared with an experimental 3D confocal image. Qualitative validation is performed through analysis of seven 3D confocal image stacks of adherent vascular smooth muscle cells (VSMCs) grown in 2D culture. The presented method is able to automatically generate 3D geometries of the cell's boundary, nucleus, and representative F-actin network based on standard cell microscopy data. These geometries can be used for direct importation and implementation in structural finite element models for analysis of the mechanics of a single cell to potentially speed discoveries in the fields of regenerative medicine, mechanobiology, and drug discovery.

1. Introduction

While cell mechanics has been recognized as an important area of study, current computational models to interpret experimental results tend to ignore individual cellular geometries. In particular, 3D computational models could help to improve the design of experiments to characterize cell mechanical properties and interactions. This could lead to reduced times for discovery of mechanobiology principles and to faster translation of those principles from benchtop to bedside in clinically relevant devices and medications. The goal of this study is to create a fully automated algorithm capable of reconstructing the geometries of the cell membrane, nucleus, and actin stress fiber network of single cells in 3D. We seek to accomplish this by processing fluorescent confocal microscopy images of each of those cellular components in such a way that the resulting geometries are optimized for structural analysis using finite element methods. If generated, such geometries could be utilized in various types of multiscale models to bridge the gap between the nano- and macro-scale models currently in use.

The traditional primary focus of modern medical research is the investigation of molecular biology and genetic factors in disease, which sometimes leads to a tendency to ignore changes in tissue structure and mechanics that can also lead to pain and morbidity (Ingber, 2003a). However, that lack of focus on the physical basis of disease has been changing in recent years with the growing emphasis on evidence-based medicine in US hospitals (Fielding et al., 2011; Kaufman, 2010) together with the substantial growth and maturation of the field of mechanobiology over the past decade (Butler and Wang, 2011). Indeed, there has been a great deal of effort to develop geometrically accurate 3D structural models at both the tissue and molecular levels (Biswas et al., 2009; Wu et al., 2010). However, there has been much less effort focused at the single-cell level and therefore comparatively little progress has been made toward generation of equally accurate 3D representations of the structural components of single cells.

The ability to predict the behavior of cells from their sub-micron and nanoscale structures could elucidate the mechanisms behind many tissue mechanical properties (Ingber, 2003b). For as long as there have been observations of the mechanical properties of cells, there have been models put forth to attempt to describe those observations. At the most basic level, there are two categories of these models: continuum and structure-based. Continuum

Abbreviations: AFM, Atomic Force Microscopy; DMEM, Dulbecco's Modified Eagle's Medium; F-actin, filamentous actin; FEA, Finite Element Analysis; FEM, Finite Element Model; FFT, fast Fourier transform; NA, numerical aperture; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; VSMC, vascular smooth muscle cell.

* Corresponding author. Tel.: +1 864 656 2611; fax: +1 864 656 4466.
E-mail addresses: stwood@clemson.edu (S.T. Wood), bcdean@clemson.edu (B.C. Dean), finou@clemson.edu (D. Dean).

1361-8415/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.media.2012.12.002

models, which lack internal structure, were the first type of model utilized to describe the mechanical behavior of cells and generally consider the cell to be equivalent to a simple "balloon full of molasses" (Ingber, 2003b; Li et al., 2007). These types of models therefore make predictions with minimal use of geometric variables (Cao and Chandra, 2010; Unnikrishnan et al., 2007). Despite the growing amount of evidence in support of the importance of structural elements within cells that has been published throughout the past several decades (Bathe et al., 2008; Bursac et al., 2005; Chaudhuri et al., 2007; Deng et al., 2006; Deshpande et al., 2008; Hardin and Walston, 2004; Hawkins et al., xxxx; Hemmer et al., 2009; Ingber, 2003a,b,c; Kasza et al., 2007; Li, 2008; Mizuno et al., 2007; Pollard, 2003; Pullarkat et al., 2007; Stamenović, 2005, 2008; Stamenović et al., 2009; Suresh, 2007; Tseng et al., 2005), these types of models remained popular with bioengineers due to their relative simplicity and ease of implementation.

Structure-based models, on the other hand, are comprised of one or more networks of discrete structural elements that work in harmony to determine the mechanical responses of cells. These models tend to utilize Finite Element Analysis (FEA) to allow for analysis of complicated cellular and subcellular geometries. Many single-cell Finite Element Models (FEMs) rely on idealized geometries (Karcher et al., 2003; Peeters et al., 2005; Unnikrishnan et al., 2007), however recent efforts have incorporated geometries obtained from image segmentation. The first efforts to generate accurate 3D representations of subcellular structural components using image segmentation techniques focused primarily on nuclei (Funnell and Maysinger, 2006; Gladilin et al., 2008), and the most advanced structure-based cellular mechanics models to date utilize stacks of confocal photomicrographs of a cell to generate 3D model structures. There have been a small number of these types of models proposed in the last several years (Dailey et al., 2009; Slomka and Gefen, 2010), each of which have been important advances towards the development of a fully representative 3D model of single cell mechanics. However, none of those models has been constructed with entirely non-idealized geometries for all mechanically relevant components of a cell.

Few 3D single cell models have included any form of cytoskeletal elements inside the cells (Slomka and Gefen, 2010); yet even though these models represent a significant step towards reality, they still rely on the manual addition of a limited number of cytoskeletal components. There has not yet been a system put forth in the literature that is either fully automated or capable of reconstructing any elements of the cytoskeletal networks of cells in a representative manner. The goal of this study is to present such a fully automated cellular geometric reconstruction system based on 3D confocal microscopy images of single subconfluent cells.

2. Methods

2.1. Data acquisition: Cell culture, staining, and imaging

Primary rat aortic vascular smooth muscle cells (VSMCs) obtained from female Sprague Dawley rats are used in this study. The cells are cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (HyClone Laboratories, Logan, UT, USA) with an antibiotic solution of penicillin and streptomycin (HyClone Laboratories) added to a concentration 0.5%, and an antimycotic solution of amphotericin B (HyClone Laboratories) added to a concentration 0.5%. Cells are cultured in T75 cell culture polystyrene flasks and maintained in an incubator at 37 °C and 5% CO_2 with fresh media being exchanged every other day. VSMCs are utilized between passages five and eight. Once the cells reach about 90% confluency, they are trypsinized with a solution of 0.25% trypsin and 0.02% ethyldiaminetetraacetic acid (EDTA) in 1X HBSS without sodium bicarbonate, calcium, or magnesium (Mediatech, Manassas, VA, USA) and seeded at 7000 cells/cm^2 on 25 mm diameter glass coverslips (VWR, Radnor, PA, USA) coated with 50 μg/mL type I rat tail collagen (BD Biosciences, Bedford, MA, USA) 24 h prior to seeding. The cells are then cultured for 3–5 days to reach about 25% confluency.

Upon reaching 25% confluency, cells are fixed with 4% paraformaldehyde (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C for 10 min. After fixation, cells are treated with 130 nM AlexaFluor 488 phalloidin (Invitrogen, Eugene, OR, USA) at room temperature for 15 min to visualize filamentous actin (F-actin), rinsed three times with phosphate buffered saline (PBS) (MP Biomedicals, Solon, OH, USA), and then mounted onto glass slides using SlowFade® Gold antifade reagent with DAPI (Invitrogen) to visualize the nucleus. The cells are then imaged using an Olympus PLAPON60XO 60× oil objective (NA = 1.42) on an Olympus IX81 inverted microscope equipped with a DSU spinning disc confocal unit and a Hamamatsu ImagEM CCD camera (Hamamatsu Photonics K.K., Hamamatsu City, Japan). Image stacks are taken using a Nyquist step size of 200 nm between image planes for maximum resolution in the *z*-direction as calculated by the microscope controller software (MetaMorph® for Olympus Basic, Version 7.7.1.0, Molecular Devices, Sunnyvale, CA, USA).

It should be noted that several types of microscopy were originally considered for this study. Atomic Force Microscopy (AFM) is capable of atomic-level resolution, but was eliminated from consideration due to its topographical nature and therefore inherent inability to image intracellular structures more than a few nanometers below the apical surface of a cell. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) also provide more-than-sufficient levels of resolution for imaging subcellular structures; however, each was ultimately eliminated from consideration due to limitations of the imaging environment and sample preparation. Electron microscopy usually requires samples to be imaged in an arid (i.e. non-aqueous) vacuum chamber and bombarded by an incident electron beam. Because electrons must pass through the specimen, TEM requires a very thin (40–90 nm thick) section which is difficult to accomplish with biological materials using traditional ultramicrotomy methods of sample preparation. In order to process the samples to make them electrically conductive for SEM, it is often necessary to coat them in harsh chemicals such as heavy metal salts and silver or osmium. While it is possible to image biological samples using electron microscopy techniques, the sample preparation and imaging environment (in particular the non-aqueous nature) are capable of producing artifacts (i.e. altering their structure) (Echlin, 2009) that could cause 3D reconstruction of those images to be inaccurate using the image processing techniques utilized in this study. Confocal microscopy does not provide the same level of resolution as any of the aforementioned techniques; however, with a maximum lateral resolution of approximately 180 nm and maximum axial resolution of roughly 500 nm (Spring et al., 2004; David, 2007) it is still sufficiently capable of imaging the structurally relevant subcellular components at the whole-cell level. Ultimately, confocal microscopy was chosen for this study due to its ability to image cells in their native aqueous environment, its non-destructive nature, its relative low-cost compared to electron microscopy, and the fact that it is generally considered to be the standard modality for cytoskeletal imaging. An additional benefit of this imaging technique is that it may be utilized to image live cells. This allows for imaging a cell for which mechanical characterization is also obtained, thus enabling direct validation of eventual models.

2.2. Image pre-processing

All images are saved and analyzed as 8-bit grayscale images in TIF format, at a size of 256 × 256 pixels. For each image, the F-actin