Contour-Based Algorithm for Tracking Cells and Cell-Material Analyses

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***Abstract* – Cellular tracking has been employed to investigate the complex cell-cell and cell-material interactions that play critical roles in tissue development and disease progression. Tracking is often performed manually, however limitations associated with manual tracking make it impractical for tracking dense populations of cells. To address these limitations, several automated tracking algorithms have been developed, but most of these algorithms are incapable of tracking cells after occlusion events or cell divisions. Here we have developed a custom algorithm in MATLAB that employs a contour-based segmentation approach to identify and track cell divisions and occlusion events. The algorithm further analyzes cell tracks during occlusion events using a cost analysis to detect and relabel mislabeled cells.**

Keywords: cell tracking, mechanobiology

I. Introduction

Tracking of cell migration has been widely used to study cell-cell interaction as well as cell-material interaction, including those interactions that play a critical role in tissue development and disease progression [1]. Traditionally, manual tracking of individual cells has been the most widespread method due to high accuracy. However several limitations of manual tracking, including potential user bias and being manually intensive, have made manual tracking infeasible for large cell populations. With recent advances in technology and computing power, several automated techniques for cell tracking have been developed. Automated techniques serve as a platform to minimize human bias while improving analytical efficiency [2]. Most automated methods, however, are incapable of accurately tracking cells that divide or undergo occlusion events (where one cell crosses over another in the field of view). To better understand complex cell behaviors that exist in dense cell populations, a method for tracking cells during occlusion events and divisions is needed. In this study we have developed a custom MATLAB algorithm capable of tracking cell divisions and identifying and correcting cell tracks following occlusion events. Individual cells atop wrinkled substrates as well as atop non-wrinkled substrates are tracked and migratory behavior compared.

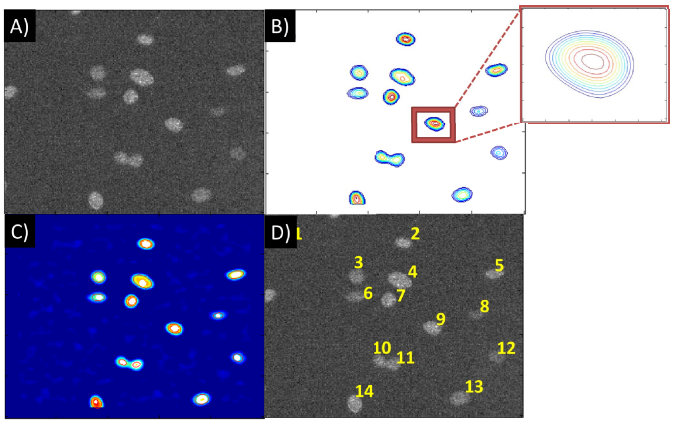

Figure 1. Segmentation and linking of cells is achieved using a contour-based approach. A) Cells are stained with Hoechst 33342 nuclear dye. B) Contour profiles are generated based on intensity variations after applying a bandpass filter (inset: zoomed cell 9 profile) C) An ellipse is fit to the cell half height and D) granted IDs for tracking over time. Cells 10 and 11 represent a sibling pair to be corrected.

II. Methods

We have developed an algorithm that achieves accurate cell tracking through four main processes: 1) cell and sibling identification, 2) cell tracking, 3) occlusion detection and correction, and 4) correlation analysis (Figure 1).

To identify cells and sibling events, or events where cells occlude or divide, we employed a contour-based segmentation method [3] in which a contour map is generated for each image based on the intensity variations of cell nuclei. Cells are identified by fitting an ellipse to each set of contours at the half contour height. Cells that have a common contour are additionally tagged as siblings and undergo further analysis to determine if they are part of a cell division or occlusion event. Cell tracking is achieved by employing a widely used tracking method [4,5] where overall displacements of particles from frame-to-frame are minimized to link cells from frame-to-frame. Once cells have been linked and given an identification tag (ID) over time, occlusion correction and division detection are performed. Cells that were tagged as siblings are determined to be division events or occlusion events based on their existing profiles (whether both cells existed previously or not). Occlusions are further examined with a positional cost

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function and cell IDs previously mislabeled are corrected. After relabeling mislabeled IDs, particle information is then passed through a correlation function to analyze cell migratory behavior. Here we have looked at mean squared displacement to compare cell migration behavior atop different substrates. For this study, C3H10T1/2 mouse fibroblasts stained with Hoechst 33342 on wrinkled substrates [6] and non-wrinkled substrates were analyzed.

I. Results and Discussion

The algorithm is able to accurately segment cells based on their intensity profiles and fit them with a representative ellipse (Figure 1, A-B). The centroid of each ellipse is tracked using an established linking method [5], and post correction using the cost function can accurately relabel mislabeled tracks (Figure 2).

Analysis of cell behavior atop the wrinkled and non-wrinkled substrates revealed distinct differences in cell migration behavior. Cells atop the non-wrinkled substrate demonstrate no preferential direction of migration (Figure 3A) whereas cells atop the wrinkled substrate migrate in a preferential direction parallel to the wrinkle direction (Figure 3B). Mean squared displacement analysis revealed cells migrate in a ballistic behavior at small time scales regardless of topography, and at longer time scales cells atop wrinkled substrates continue to exhibit ballistic migratory behavior while cells atop flat substrates exhibit a more diffusive migratory behavior (data not shown).

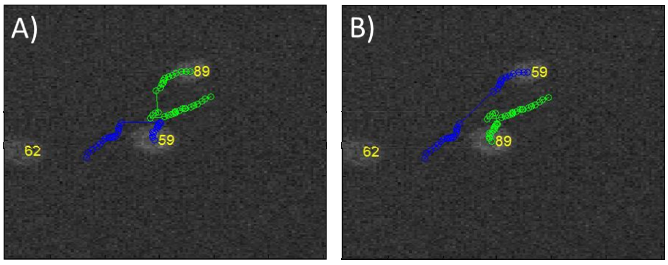

Figure 2. Cost function analysis relabels mislabeled cell IDs following an occlusion event. (A) Prior to performing cost function analysis,cell IDs during an occlusion event are mistakenly switched; (B) after performing cost function analyses, cell IDs are properly relabeled.

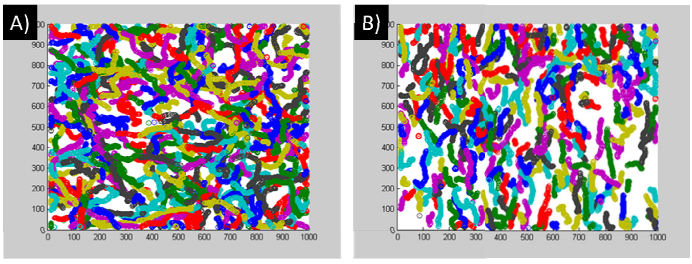

Figure 3. Cell migration differs atop ordered and non-ordered topographies. (A) cell tracks atop non-wrinkled substrates show no preferential direction of migration whereas (B) cells atop substrate with an ordered topography migrate parallel to wrinkle direction (vertical)

Conclusions

This work demonstrates the feasibility of employing a contour-based segmentation method to accurately track cells while accounting for division and occlusion events. Results reveal this technique can be used to identify occlusion events, analyze those events, and accurately relabel mislabeled cell IDs due to occlusions. This work is expected to enable more accurate tracking of cells in dense populations for investigating cell-cell and cell-material interactions and the effects they have on migratory behavior. The cell tracks generated by this algorithm can be used to investigate spatial and temporal correlations in individual and collective cell migratory behavior. Analyses such as these could further the understanding of how cell-cell and cell-material interactions drive biological processes during tissue development and disease progression.

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