A Contour-Based Approach Enables Individual Cell Identification for Cell-Material Analyses

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Statement of Purpose: Cellular tracking has been widely used to investigate critical cell-material interactions, including those that take place between cells and natural matrices during tissue development and disease [1] and those that affect the function and biocompatibility of implant and delivery materials. Rapid advances in the patterning of microenvironments for the study of morphogenesis and applications in tissue engineering have created a need for significantly improved methods of cell tracking. Manual tracking remains the gold standard in the field, but is often not feasible for analysis of dense cell populations in micropatterned environments. Automated techniques serve as a platform to maximize analytical efficiency and reduce human bias [2] but generally cannot track cells that divide or cross paths (occlude) over time. To advance current understanding of complex cell behaviors in patterned environments, it is necessary to accurately track cells during these events. Here we have developed a custom Matlab algorithm to resolve division and occlusion inaccuracies that occur during tracking of individual cell nuclei on biomaterial substrates and have assessed the approach when applied to cells on spatially defined substrates.

Methods: Our approach expands on an existing automated system [3,4] by employing a contour based segmentation method [5]. Cells are initially seeded on a material substrate and imaged using live-cell microscopy (Fig. 1A). Our developed algorithm analyzes the output based on four main components: 1) cell and sibling identification, 2) particle tracking, 3) occlusion detection and correction, and 4) correlation analysis. A contour map is created for each image based on intensity variations in cell nuclei. Segmentation is achieved after fitting ellipses to each cell's half-height contour profile. Cells exhibiting a shared profile are flagged as siblings and matched via their shared contour levels. Cell linking is achieved using the Kilfoil method [4]. Once each cell has been granted an identification tag (ID) over time, occlusion correction and division detection are performed. Cells with sibling events are isolated and classified as an occlusion or division event based on their existing profile. Occlusions are further examined with a cost function that corrects for inaccuracies associated with initial detection. Once ID adjustments have been made, the data is passed to a series of correlation functions, which allow for spatial and temporal classification of the microenvironment.

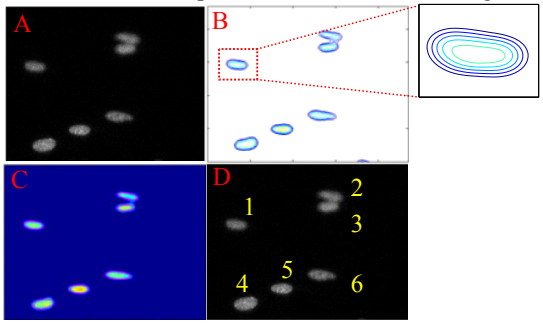

Figure 1: A) C3H10T1/2 cells are stained with Hoechst 33342 nuclear dye. B) Contour profiles are based on intensity variations (inset: zoomed cell 1 profile) C) Cells are fit at half height and D) granted IDs for tracking over time. Cells 2 and 3 represent a sibling pair to be corrected.

Results: Results demonstrate that the algorithm can segment cells based on their intensity profiles (Fig. 1A, B). The system can fit cells as ellipses (Fig. 1C) and utilize the Kilfoil [4] approach to track cells over time (Fig. 1D). The system can also modify cell IDs based on a cost function to ensure proper cell identification (Fig. 2A, B). Analysis of time-lapse data shows that the system can both track cells (Fig. 2C) and quantify their behavior (Fig. 2D) using correlation analyses. Assessment of dense cells on topographic substrates (Fig. 2C) revealed preferential movement not observed on flat controls (data not shown).

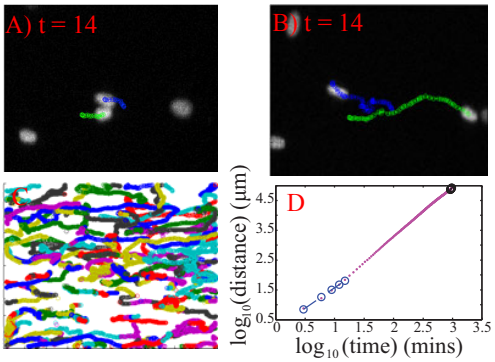

Figure 2: A) Sibling events are picked up by the system. B) With post-tracking analysis, occlusions are sorted by a cost function. C) Over time, cell tracks can be visualized and D) quantified by mean squared displacement.

Conclusions: Our work demonstrates the viability of applying a new contour-based approach for cell-material characterization. Results demonstrate the feasibility of utilizing post-collision correction to adjust for cell ID inaccuracies associated with occlusion events. The implementation of the sibling method allows for divisions to be identified, as they exhibit the same profile observed with occlusion events. This work is expected to enable more powerful, efficient, and accurate analysis of cell behaviors in micropatterned environments over extended periods of time. The modularity of the system offers flexibility for cell analysis and has the potential to accommodate dense cell population characterization in patterned microenvironments to drive morphogenesis studies and tissue engineering applications.

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