AUTOMATED 5-D ANALYSIS OF CELL DYNAMICS FROM TIME-LAPSE SEQUENCES OF MULTI-CHANNEL IMAGES

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ABSTRACT

In this thesis, we aim to develop a broadly applicable framework of automated, robust, and scalable algorithms that accelerate the discovery of underlying mechanisms of complex and dynamic biological microenvironments from 5-dimensional (3-D spatial, temporal, spectral) images.

The automated framework starts with a 5-D movie that records dynamic phenomena of multiple object types in full spatial and temporal context. One of our contributions is a robust strategy that separates the image data into non-overlapping channels containing only one type of object. Our work has resulted in a simple yet effective thresholding method to separate 3 or fewer imaging channels without prior knowledge of spectral signatures. To handle M-channel (M>3) images, we adopt a Bayesian approach with Markov Random Field (MRF). This approach attempts to advance conventional solutions by integrating spatial context into spectrum separation. To evaluate algorithm performance, we design a voting strategy followed by an accordance ratio computation.

Each object class is extracted from spectrally separated images frame by frame. A mean-shift clustering algorithm is applied to delineate blob-like objects in crowded regions. It is adaptive to morphological complexity and heterogeneity, and requires the fewest adjustable parameters. After segmentation is accomplished, we extract morphological characteristics of objects in individual imaging channels, and determine temporal correspondences between successive frames using a multiple hypothesis tracking (MHT) algorithm. The strength of MHT is that it intrinsically accommodates all potential topological changes, which enables complex studies of cell behavior (e.g. migration, division, and death). Once objects are tracked, we compute a set of associative measurements that capture a dense network of spatial-temporal relationships among objects in the same or distinct imaging channels. Then, system-level understanding is developed for a specific application based on these quantitative results.

Validation of automated results in an efficient manner is a major focus of this study. Traditional methodologies are driven by multiple observers, and require manually generated ground truth. Such information is either unavailable or expensive in our case. With this in mind, we provide an edit-based framework, in which the human audit effort scales with the error rates of automated algorithms rather than data volume. Our work

has identified potential segmentation and tracking errors via conventional distribution-based outlier detection algorithm combined with graphical aides. For robustness, we adopt more advanced technique, distance-based data mining, in the process of outlier detection. User edits are maintained to evaluate algorithm performance, and suggest algorithm enhancement.

To validate the methodologies present in this study, diverse applications are considered. Experiments have demonstrated the effectiveness of the thresholding-based and MRF-based spectral separation methods on multi-channel images containing dendritic cells, thymocytes and blood vessels in the developing thymic cortex of mice. The 3-D segmentation and tracking algorithms were performed on both the thymic datasets and image data of dendritic cells and T-cells in the lymph node. We also tested the MHT tracking algorithm on images of developing embryos of *Caenorhabditis elegans* worms, and extracted cell lineage tree at early developing stage. In both thymic and lymph nodes datasets, automated quantitative measurements generally agreed with manual analysis. The experiment has confirmed biological hypotheses regarding the effects of genetic manipulations on immune system.