博士論文の内容の要旨

論文題目　Cell Tracking Under Dense Cell Culture Conditions
for Cell Behavior Analysis
(細胞挙動解析のための密な細胞画像における細胞トラッキング)
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Background and motivation

Analysis of cell behaviors in populations is important for research and discovery in biology and medicine. For example, in biological research, Nikolic *et al.* manually tracked cell migration in wound healing assay to understand how multiple cells execute highly dynamic and coordinated movements during the healing process. In addition to individual cell trajectories, the analysis of cell lineage is important, in particular, to study cell differentiations. Ravin *et al.* developed an in vitro system that allows analysis of the fate transitions from central nervous system (CNS) stem cells to differentiated neurons and glia cells. In their system, the cells are manually annotated for identifying mother-daughter relationships and when and which stem cells are differentiated to other types of cells. To effectively obtain quantitative measurements of cell behaviors, many automatic cell-tracking methods have been developed. However, in biology and medicine, cells are often cultured under a variety of cell culture conditions, such as low-to-high density, as shown in Figure 1. For example, in regenerative medicine, the cells are cultured until they densely fill in the dish in order to mature them. Cell behavior metrics, such as migration speed and cell shape information, in high density are important to assess the quality of cells in non-invasive images before transplantation. Cell tracking under such high-density conditions still remains a non-trivial task. The main difficulty arises from the following aspects of this problem.

- Touching and partially overlapping: When multiple cells touch or partially overlap, they form a cell cluster with blurry intercellular boundaries. Such touching and/or partially overlapping cells present a performance bottleneck with most current cell-tracking methods; they may either lose track of one or more of the cells or confuse their identities.
- Cell division: The number of cells may change due to cell division and cells entering or leaving the field of view.
- Similar appearance: Neighboring cells often have similar appearances. This makes it difficult for appearance-based association methods to properly work.
- Large displacement: Cell movements between successive frames are often larger than the distances to the nearby cells when it takes time to obtain 3D volume data for a wide range of specimens. This makes it almost impossible to associate cells between frames based on their proximity.

In this thesis, I propose several cell-tracking methods for addressing these challenges. I quantitatively evaluated these methods by applying them to actual biological research and discuss their effectiveness.

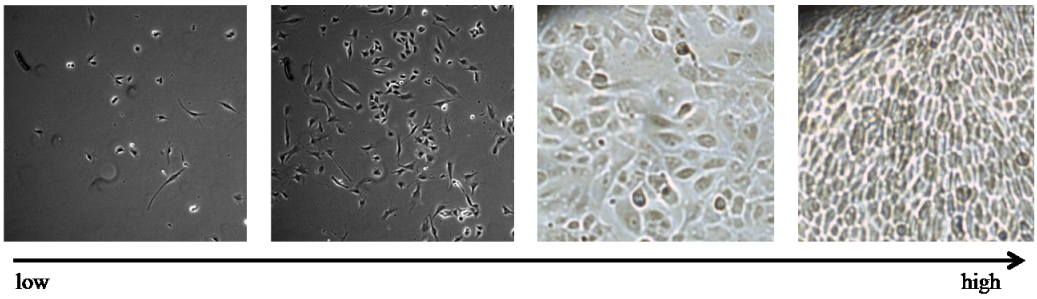

Figure 1: Example images in cell populations. Cell density increases from left to right.

Overview and contributions

The work presented in this thesis is based on the third group of cell-tracking methods , *i.e.*, detection and association, since these methods can easily handle various cell behaviors, including migration, mitosis, overlapping, entering, and exiting. Figure 2 shows the framework of all the proposed cell-tracking methods including the following five functions: detecting cell positions at each frame (cell detection); identifying cell division events in sequences (mitosis detection); associating detection results between successive frames to identify cell behaviors including migration, division, enter, exit, and overlapping (frame-by-frame association); segmenting cell cluster regions to their member cells (re-segmentation); and linking tracklets globally to obtain entire cell trajectories (global association). In particular, my methods contribute four functions, cell detection, frame-by-frame association, re-segmentation, and global association. I developed tracking methods step-by-step to address all the difficulties in tracking cells under dense conditions. Each proposed method and specific problems are presented in each chapter. I first present proposed cell-tracking methods for addressing difficulties, in which several cells touch and make a cluster under low-to-middle density conditions, in Chapters 2 and 3 before focusing on the difficulties under high-density conditions. Then, cell detection and tracking methods for addressing difficulties under high-density are presented in the remaining chapters. The contributions of each chapter are summarized as follows:

- Chapter 2: When multiple cells touch or overlap, they appear to form a cell cluster with blurry intercellular boundaries. In this case, it is often difficult to identify individual cells in the cluster from one image even though a human manually annotated. I propose a tracking method that identifies touching cell clusters from detection results by frame-by-frame data association then re-segments the clusters to their member cells by partial contour matching between cells and the cluster. This method makes it possible to robustly track two or three partially overlapping cells while maintaining the identity information of individual cells throughout the process from their initial contact to eventual separation.
- Chapter 3: When a false positive segmentation, such as tips of cells, appears near a mitotic cell, local temporal association methods may cause a mother-daughter relationship error. Global temporal information is important in solving this problem. If the cells are observed for several frames after the birth event, it can be easily determined that one of the children cells is a false positive since false positives usually quickly disappear. This allows to correct the relationship. Current global spatio-temporal data association methods for tracking non-dividing objects cannot be applied to cell tracking directly since they do not take into account cell division i.e., a

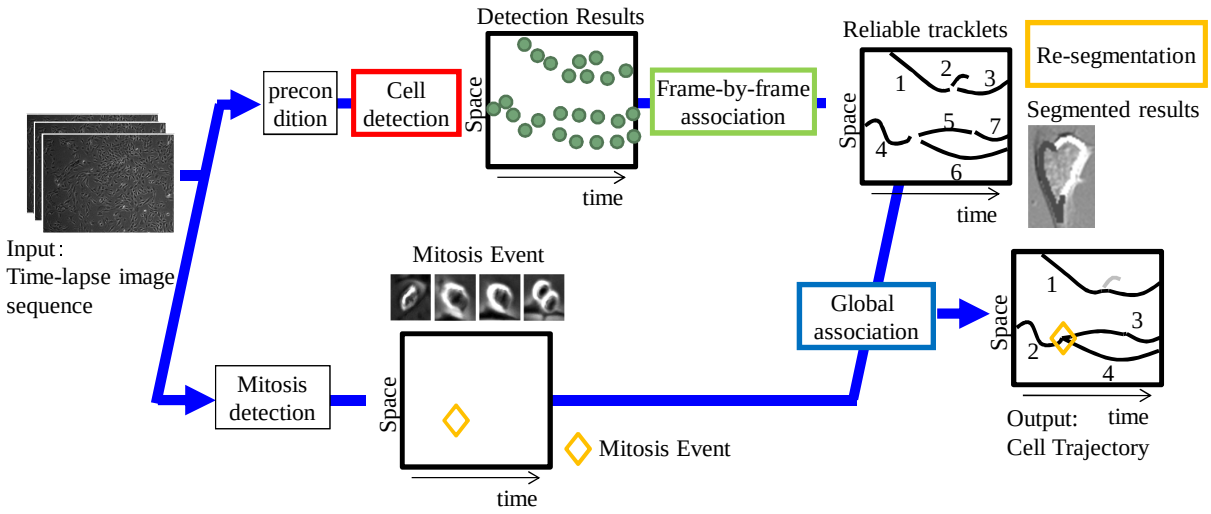

Figure 2: Overview of framework of proposed tracking methods. The proposed methods contribute to four functions: cell detection, frame-by-frame association, re-segmentation and global association.

mother cell divides into two daughter cells to form a tree structure in the trajectory. I propose a global spatio-temporal data association method for the tree structure to obtain cell trajectories and lineage trees. First, reliable tracklets (i.e., short trajectories) are generated by linking detection responses based on the frame-by-frame detection-and-association approach method in Chapter 2. Then, the global tracklet association for the tree structures is solved using linear programming. To the best of my knowledge, this is the first attempt at formulating tree structure global association to track dividing objects. By introducing global spatio-temporal information, we can easily determines the false positives of cell detection as cell or noise since false positives usually quickly disappear. This method was quantitatively evaluated on sequences with thousands of cells captured over several days.

- Chapter 4: Cell detection in each frame is obviously important for improving tracking performance since detection errors usually cause association errors. When cell density is high, the performances of the above two methods proposed in Chapters 2 and 3 drastically decline because of the increase in cell-detection errors. Thus, I propose a cell-detection method for addressing all the difficulties in detecting dense cells simultaneously: multiple cells are mistakenly merged, a single cell is divided into multiple regions, and low-intensity cells are miss detected. The method first detects redundant candidate regions by allowing candidates to overlap to avoid miss detections. Then, to avoid over detections, I select an optimal set of cell regions from the redundant regions under non-overlapping constraints, in which a selected region looks like a single cell and does not overlap with other cells. This method improves the performance of cell detection and tracking. This idea of selecting an optimal set from redundant candidates is expanded to tracking in the following chapters.
- Chapter 5: Under dense cell-culture conditions, cells more often touch other cells with blurry intercellular boundaries. Such conditions cause difficulty in generating reliable tracklets with the global spatio-temporal data association method proposed in Chapter 3 since the frame-

by-frame detection-and-association tracking process heavily depends on the detection results. To mitigate this problem, I propose a tracking method for determining the detection results in the association step by using both image features in the current frame and the tracking results in the previous frame. This make it possible to make more reliable tracklets under high density conditions compared with typical detection-and-association methods. After generating reliable tracklets, the global data association method proposed in Chapter 3 is used to obtain all cell trajectories and lineage trees. This method was evaluated based on the challenging image sequences in which cells were cultured in high density and the boundaries of cells were blurring. The experimental results show that this method significantly improves the tracking performance comparing with the two other proposed methods introduced above.

- Chapter 6: The method proposed in Chapter 5 depends on initialization of cell detection since the joint problem of optimal region selection and association is solved at each frame independently. I propose a cell-tracking method that first generates redundant candidate tracklets, then solves the joint problem of optimal tracklets selection and association globally. This method generates redundant candidate tracklets, which include many false positives but in turn very few false negatives, by allowing tracklets to overlap. This is a similar idea with the detection step proposed in Chapter 5. Next, the problem of both selecting an optimal set of cell tracklets from the redundant tracklets and associating the tracklets over frames under non-overlapping constraints is solved simultaneously. This methods achieved the best performance on the comparison with the above tracking methods proposed in Chapters 2, 3 and 5.
- Chapter 7: I propose a cell-tracking method that enables to track cells with large movements. The increment in time-lapse imaging cannot be shortened to monitor a wide area, which results in the problem in which the movements of cells between successive frames are often larger than the distances to the nearby cells. This makes it almost impossible to associate cells between frames based on their proximity. To mitigate this problem, this method exploits the observation in which nearby cells under high-density conditions exhibit similar motion patterns. This is done by introducing global motion estimation and local pairwise spatial relationships. This method was evaluated on synthetic point-sets and compared against the existing methods. The proposed method was evaluated on synthetic point-set and compared against current methods.
- Chapter 8: I show how easily and effectively automated cell-tracking systems can provide detailed spatio-temporal cell behavior measurements for biological analysis. The spatio-temporal measurements of cell behaviors are important for critical analysis, because the cell culture conditions vary with time and space on the dish. For example, the effectiveness of a medicine may change with time and space since cell density can differ in the different space. I present an application of automatic cell-tracking for wound healing assay *in vitro* under three different culture conditions to demonstrate how easily and effectively automated cell-tracking systems can provide detailed spatio-temporal cell behavior measurements for biological research.

Finally, I present a conclusion and future directions in Chapter 9.