FROM TIMELAPSE-DATA TO GENEALOGIC TREES: USING DIFFERENT CONTRAST MECHANISMS TO IMPROVE CELL TRACKING

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ABSTRACT

The analysis of time lapse data becomes a more and more important tool in (stem) cell biology, as this method enables a marker free analysis of cell populations over time and allows for the reconstruction of genealogical trees; these information can help to understand the mechanisms behind cell proliferation and differentiation. However, the crucial steps in a cell tracking algorithm (cell segmentation, cell tracking and mitosis detection) have to be performed with a high trustworthiness to find the correct lineage of each cell. Here, we present a novel cell tracking algorithm that combines microscopical images with different contrast mechanisms: first, oblique illumination is used to segment and track cells; second, phase contrast microscopy is used to detect the most critical events, the mitosis. The combination of both detection results in a more robust cell tracking. For evaluation, the free reference data CeTReS is used.

Index Terms— Cell Tracking, Time Lapse Microscopy, Image Segmentation

1. INTRODUCTION

In time lapse experiments, the complex and changing behavior of (stem) cell populations can be observed over a large time period of several days up to weeks. Such data can be used to analyze the behavior of a cell population over time as well as it enables the extraction of genealogical information [1, 2]. Furthermore, it is possible to use this kind of information to derive precise models of cell proliferation or differentiation [3]. In this work, we propose a new approach to enhance the reconstruction of spatio temporal tracking paths by combining two different contrast modalities, one superior for detecting and tracking cells, the other robust for detecting mitotic events. Cell detection and tracking is performed using the so-called Oblique Illumination (OI). The good contrast of theses images results in a good cell detection performance of about 95%; this high segmentation accuracy allows for a good reconstruction of spatio temporal cell paths, i.e. to follow each cell over time. Now, the most difficult task is to combine the resulting branch-free path segments to derive reliable genealogical information [4]. Here, one has to decide whether or not a mitotic event has occurred, i.e. a linear path segment can be linked to two subsequent paths. The intuitive approach is to use the tracking information to detect these events. Such a *tracking based* approach typically uses morphological changes (e.g. contraction of the cell) to detect mitosis [5, 6].

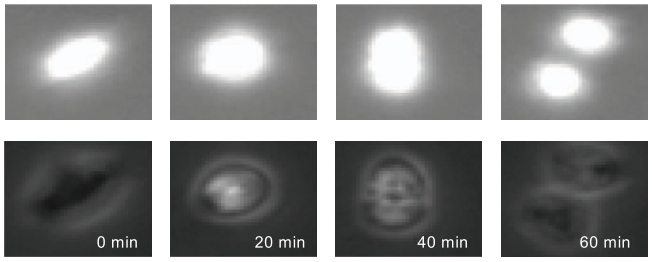


Fig. 1. Example of a cell division (mitosis) taken from the CeTReS data set. In oblique illumination (OI, upper row), the cell is easily detectable, while phase contrast (PC, lower row) is suitable to detect the physical changes within the cell during mitosis.

On the other hand, *tracking free* approaches make use of the characteristic optical signature caused by the physical appearance of a cell during mitosis. Such details can be e.g. observed in phase contrast microscopy (PC), which is a standard method to visualize cells and therefore widely used in the analysis of cell images [1, 2, 7]. In Figure 1, the dramatic differences between the two image modalities can be seen: obviously, the contour and position of the cell is easily extractable using the OI images (first row), while the structural changes within the cell during mitosis is much more visible on the PC image data (lower row).

In this work, we enhance an existing tracking system [6, 4] that is designed to work with OI images with a mitosis detection module operating on PC images. Furthermore, we introduce the concept of *critical point correction (CPC)*, where the detection results of both image types can be combined to extract genealogical nexus from time lapse data.

2. MATERIAL AND METHODS

The presented algorithms are based on the cell detection and tracking introduced in [4]. In the following, we give a short summary of the proposed cell detection algorithm and introduce our extension to the cell tracking algorithm, which uses different contrast mechanisms, the concept of *critical point correction (CPC)*.

2.1. Reconstruction of spatio temporal cell paths

As the OI image data is of a good quality, a pre-processing consisting of a background subtraction and a image normalization is sufficient. The image segmentation is performed using a global threshold (calculated using the method of Otsu [8]). The resulting binary image I_b is corrected using some morphological operators (opening and closing); after this, each cell shape is represented as a connected region C_t^j with $t = 1, \ldots, T, j = 1, \ldots, N_t$, where T is the total number of frames and N_t is the number of cells in image t. This segmentation results in a detection accuracy of 95%, see [4].

In the next step, the cells are followed over time (cell tracking). The aim of the first, initial cell tracking is not to reconstruct complete genealogical trees, but to extract spatio temporal cell paths with a high trustworthiness. Therefore, the intersection $\cap(C_t^i, C_{t+1}^j)$ of two cell shapes i, j in two consecutively images I_t, I_{t+1} is calculated. Starting in the first frame $t = 1$, a cell C_t^m only has a valid successor cell C_{t+1}^n if both shapes only intersect with each other. As long as this is true, the cell shapes are combined to a cell path $P_i = \left[C_t^j, C_{t+1}^{j+1} ..., C_{t+n}^{j+n}\right]$. If this is not true, i.e. if a cell overlaps with more than one possible successor cell, new paths are started at each of the intersecting cell shapes. The connections of these paths are stored in a graph representation as an adjacency matrix $\mathbf{A}$, where the connection of path P_m to P_n is stored with $\mathbf{A}(m, n) = \mathbf{A}(n, m) = 1$. The matrix $\mathbf{A}$ represents the basis for the critical point correction and the later reconstruction of genealogical trees.

2.2. Mitosis Detection

To connect the reconstructed cell paths P_i and to ultimately extract genealogical trees, it is necessary to find the mitotic events with a high accuracy. We tested two different mitosis detection algorithms based on the different contrast images. First, we give a brief introduction to the tracking based approach using the OI image data; for a detailed description see [6]. For the tracking free approach, a new algorithm was implemented using the PC images.

- **Morphology Based Detection (OI image data)**
  Using a tracking based detection, the typical spatio temporal pattern of a cell division is used to find a mitosis (a cell slows down and becomes smaller and rounder). This pattern can be detected using the changes of the parameters *compactness, area, brightness* and *length* $(\Delta C, \Delta A, \Delta B, \Delta L)$ of a cell path P_i using a maximum likelihood approach. To compensate for the changing morphology over time, these patterns can be adapted to different cell population parameters as cell density (confluency) or mean cell area [6].

- **SVM Classification (PC image data)**
  For the PC image data, a mitosis is detected using the unique representation of this event in phase contrast: the contraction of the cells results in a unique, bright pattern that can be easily searched for (see e.g. Fig. 1). To find this pattern, we use a Linear Support Vector Machine (LSVM) as a classifier for the given mitosis candidates (the endings of each path) in the frequency domain: we calculated the two-dimensional FFT (FFT2, MATLAB 7, Release 2011a) of the image patches and used the magnitude of the spectral data as feature input for the LSVM. For training, we used the labeled mitotic events in the reference data (image size 41×41 px).

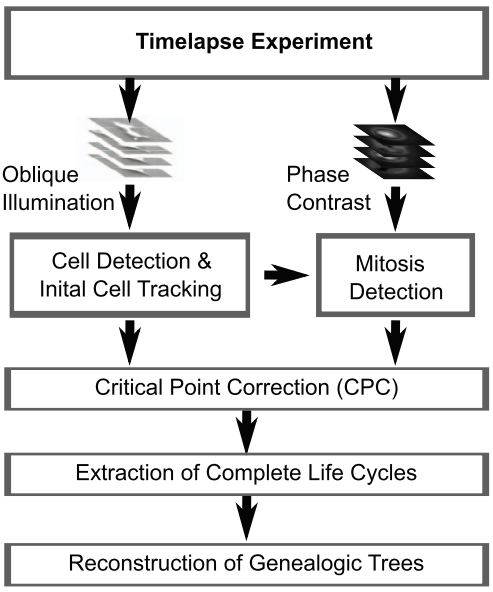


Fig. 2. Process chart for the complete cell tracking procedure. First, the cells are imaged using two different contrast mechanisms; one is used to track the cells, the other is used to find mitotic events. Both information are combined using the introduced Critical Point Correction (CPC) step; this results in a more robust cell tracking.

2.3. Critical Point Correction (CPC)

In the next step on the way to a completely reconstructed genealogical nexus we need to find complete cell paths, i.e. paths that trace a cell ideally from its initial *birth* mitosis to its *ending* one. But as we observe cells over a limited time in

a limited field of view, the following options exist for a cell to *appear/disappear*: it either can (1) exist in the first / last frame, (2) be born / dies in a mitosis or (3) appears / disappears in the field of view (image border). Now, we have to decide for each path whether it has such a valid begin/end or if the end of the cell path is caused by a detection or tracking error. Furthermore, we need (or at least should try) to correct as many non-valid paths as possible. For this task, we introduce the concept of *critical point correction (CPC)*.
Initially, all paths that do not appear / disappear over time (first/last frame) or at the image border are labeled as *critical*. Therefore, the adjacency matrix $\mathbf{A}$ is used. At each node, the incoming edges represent the end of a critical paths where the outgoing edges represent the begin. In the next step, a routine searches for known patterns that either can be used to connect two paths (one incoming edge, two outgoing edges, this pattern is denoted as *mitosis candidates*) or could be used for a following correction step. Now, a mitosis detection is used to decide for each mitosis candidate if the observed cell undergoes a mitosis or not. If a path end (node) is recognized as mitotic, this path P_i (and its corresponding node in $\mathbf{A}$) is labeled as *mitotic*.

Example Patterns: The most common patterns are shown in Figure 3. For example, a path can only be denoted as mitotic if two conditions are met: first, the cell needs to have a mitotic representation in the PC image data; second, two unique daughter cells need to exist, i.e. the node needs to have two outgoing edges, see Figure 3(A). An example for a correction step is given with Figure 3(C): a similarity measure can be used to connect the paths $1, 2$ to $4, 5$ (or $5, 4$); although useful, it is not necessary to correct for the wrong segmentation.

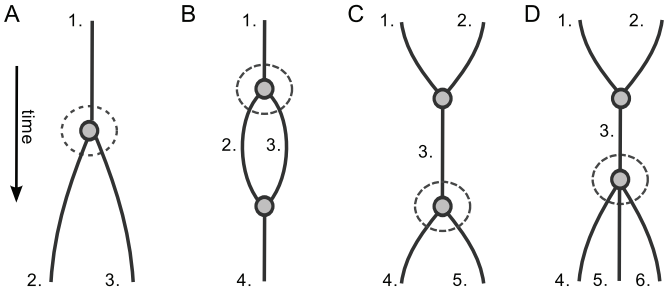


Fig. 3. Example of typical patterns for critical points in graph representation. Subfigure A shows the pattern corresponding to a mitosis, B shows an over segmentation (one cell is segmented as two), C shows a wrong segmentation (two cells are merged into one object). The last pattern D shows a more complex scenario: two cells merge before one undergoes a mitosis. The numbers enumerate different paths; all mitosis candidates are marked with a dotted circle.

2.4. Complete Paths and Genealogical Trees

The result of the CPC algorithm can be used to find and extract complete cell paths, i.e. cells that are followed for their complete life cycle. For this, the tracked cells (the paths P_i) and their connection (the adjacency matrix $\mathbf{A}$) are used. In this representation, a complete path correspondences to an edge connecting two mitotic-labeled nodes. With all detected mitotic events given, these paths / edges can be found easily. Furthermore, this data allows for a simple extraction of genealogical trees: after deleting all non-mitotic edges in $\mathbf{A}$, each tree is represented as a disjoint graph in $\mathbf{A}$.
An overview of the complete procedure from the time lapse experiment to the reconstruction of genealogical trees is shown in Figure 2.

3. RESULTS

Reference data: To assess the quality of our proposed algorithms, the freely available reference data CeTReS[1] was used. In this data set, pancreatic stem cells were imaged with both contrast mechanisms (OI and PC) for $66.5h$ with an interval of 10 minutes, resulting in 2×400 images. An inverted Olympus ix-81 microscope with climatization control was used to image the cells. An integrated and motorized condenser allows to automatically image the cells using different contrasts mechanisms in a short period of time ($2 - 3$ seconds).
Cell detection and tracking: Our detection and tracking algorithm returns 4323 cell paths with 47 cells in the first and 611 in the last frame. From all 537 mitotic events in the reference data, 517 have a corresponding cell path P_i returned by our algorithm (the missing 20 mitosis were either not detected or to close to the image border to be imaged correctly), i.e. $> 96\%$ of all mitotic cells can be used to assess the quality of the different mitosis detectors.
Morphology mitosis detection: For the morphology based detection, different combinations of morphological features were tested. The performance of this algorithm is shown for the best feature combination ([area,brightness,length]) as Receiver Operator Characteristic (ROC) in Figure 4. The best six combinations were used to calculate the mean Area Under the Curve (AUC), see Figure 4. All AUC values are > 0.82, the best combination results in a performance of AUC $= 0.843$.
SVM based mitosis detection: To train the SVM classifier, 1157 images showing (labeled) mitotic cells and 2796 showing non mitotic cells were created using the CeTReS data (image size 41×41 px). To test the classification, the critical paths returned by the CPC were used (same paths as used to test the morphology based detection). For each of the given candidates, the given cell paths P_i were used to center and image the candidate cell for up to 5 frames before its end with a size of 41×41 px. One path was detected as mitotic if at

[1] `ftp://cetres.emb.fraunhofer.de`

least one corresponding image was classified as mitotic. This detection achieves an AUC $= 0.90$, the ROC and AUC are shown in Figure 4.

This detection accuracies shows, that the SVM based detection clearly outperforms the morphology based detection using OI images only. As a result, the combination of both images return the most accurate cell paths.

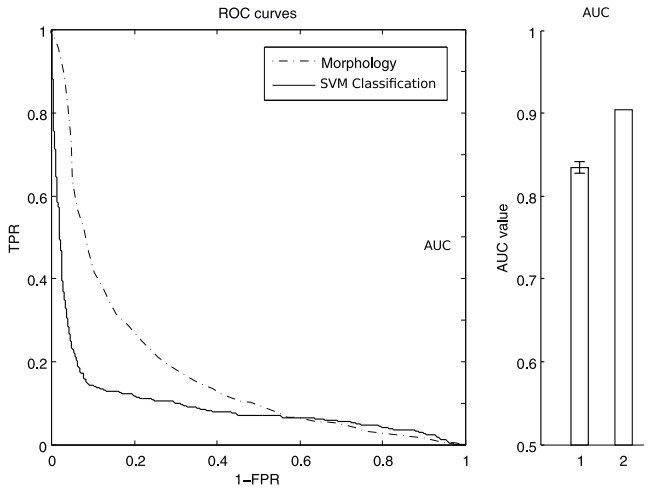


Fig. 4. **Detection performance** is shown for the morphology mitosis detection using OI image data and for the SVM based classification using PC images. The Area Under Curve is shown for the SVM (2) detection; the mean AUC for the morphology based detection (1) is calculated for the six best feature combinations.

4. DISCUSSION AND CONCLUSION

In current setups for time lapse microscopy motorized condenser (i.e. controllable by software) allow for the change of phase contrast modalities during a running experiment. For future tracking approaches, it can be expected that the combination of the information achieved by different image modalities will be used to reach the ultimate goal of highly reliable and marker-free cell tracking.

Due to the high error propagation, even when only roughly 15% of all mitotic events are missed, a complete reconstruction of the cells' genealogy up to the final frame is nearly impossible. Therefore, the presented gain in performance by the proposed framework that combines OI and PC images is promising.

In this work, we used a very basic approach to test for the characteristic features by using a simple linear classifier on the Fourier frequency magnitudes. We are optimistic to further improve the performance especially to detect dead cells and cropping effects at the image borders as these candidates are typically correctly classified by the weak mitosis detector on the OI images.

The fusion of these two image modalities will also allow for detection and correction of so far unsolvable problems in cell detection and tracking. Typically, tracking errors are additive as they highly relate on the correct detection of all predecessor cells. It would be tremendously helpful to have a reliable mitosis detection that performs widely independent from single cell detection.

5. REFERENCES

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