Cellular Tracking in Time-lapse Phase Contrast Images

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Abstract

The quantitative analysis of live cellular structures in time-lapse image sequences is a key issue in evaluating biological processes such as cellular motility and proliferation. The current clinical practice involves a manual tracking procedure, but with the arrival of modern image acquisition modalities, the amount of data required to be analysed by biologists is constantly increasing. As a result techniques that are able to process the data automatically are currently developed and evaluated. However, problems caused by cellular division, agglomeration, Brownian motion and topology changes are difficult issues that have to be accommodated by automatic tracking techniques. In this paper, we detail the development of a fully automated multi-target tracking system that is able to deal with Brownian motion and cellular division. During the tracking process our approach includes information such as the neighbourhood relationship and motion history to enforce the cellular tracking continuity in the spatial and temporal domain. The experimental results reported in this paper indicate that the proposed cellular tracking approach is able to accurately track cellular structures in time-lapse data.

1. Introduction

Biological processes such as proliferation and migration/motility of cellular structures are fundamental aspects that are studied to understand the multi-cellular development, wound healing, embryogenesis, inflammation, etc. [1-2]. In particular cellular motility analysis is important to understand these biological processes, as this opens the possibility to investigate various diseases including cancer, and to analyse the cellular response to different drug treatments [1], [12-13]. Cellular motility is evaluated in sequences of time-lapse data and the aim of this process is to assign the cell-cell association in consecutive images. Typically, the cellular tracking and analysis is performed manually or using semi-automated tracking techniques. Nonetheless with the development of image acquisition systems the amount of data to be analysed by biologists is constantly increasing and as a result the tracking process becomes a tedious and time consuming task. Thus, the development of image processing techniques that are able to achieve automatic cellular tracking is more necessary than ever before. The existing cellular tracking algorithms are based on feature matching, motion prediction, and model evaluation and they were developed to determine the self-propelled motility associated with live cellular structures. While these previous proposed algorithms are able to determine the cellular tracks when the cells' motility can be statistically evaluated they show poor performance when applied to data characterized by Brownian motion or cellular proliferation. In this scenario, feature-based approaches generate ambiguous tracking and the motion models are not able to adapt to the Brownian motion.

In this paper, a novel cellular tracking framework is detailed that is able to track multiple cells and accommodate cellular proliferation. To adapt to Brownian motion the neighbour information is utilised in a structural manner and during this process structures of cells are matched rather than individual cells. This method not only reduces the false associations caused by the Brownian motion, but also allows the tracking of cells that are generated by proliferation. In addition, failure in data association at a particular time does not affect the cellular tracking in the following images of the sequence. In our implementation we used a graph generation technique based on Delaunay triangulation that is employed to encode the spatial relationship [14-15] between the

cells contained in each image frame, where the cellular tracking process is performed by evaluating the changes in the graph structures in adjacent frames. The proposed tracking algorithm is generic and in this paper we have evaluated its performance when applied to different cellular data.

2. Previous methods

Cellular tracking has become an active area of research and a large number of approaches have been proposed to solve the cellular association in time-lapse multi-cell data. In general these techniques were developed in conjunction with well-defined applications where the cell association was carried out using features matching [3], motion prediction [7] and model evaluation [11] approaches.

The feature matching and motion prediction techniques involve the segmentation of cells in each frame and the association of the segmented data contained in consecutive images by the use of pattern recognition techniques that enforce continuity in the spatial and temporal domain. For instance, in [3] the feature matching process was carried out for user-selected cells by minimizing a criterion based on target location and feature similarity. The experiments demonstrated that this approach produces accurate results only when applied to sparse cellular datasets and is not able to handle the cell division and Brownian motion. A similar distance-based tracking approach is investigated in [4-5] and the experimental results further strengthened the conclusion that this solution alone is not suitable for robust cellular tracking.

To address the problems faced by feature-based tracking algorithms, motion prediction techniques such as those based on Kalman filtering and Particle filtering were developed [6-7]. These approaches proved to be robust only in situations when the cellular motion can be approximated by statistical models. However, the motion model-driven tracking techniques may fail when applied to dense cellular data that is characterised by Brownian motion. Their performance largely depends on the suitable selection of the noise covariance and elaborate simulation/training procedures have to be applied to determine the model parameters prior to the application of the tracking algorithms to real cellular data.

Techniques based on appearance and shape models were also applied in the development of cellular tracking algorithms. Using this approach, the cellular structure is initialised in the first frame and then propagated to subsequent images to identify the motility over the entire image sequence. For instance, techniques based on Active Contours, Level Sets and Mean-shift have been explored for multiple cellular tracking in [10], [11] and [13] respectively. However, the main restriction associated with these techniques is the fact that they require significant overlapping between the model and the target. Thus, if data shows frequent divisions, the initial model may overlap with multiple targets and the cellular association become ambiguous [11]. Recently, a combination of several techniques has been investigated for cellular tracking in [8] and [12]. The experiments indicated that their performance increased when compared to that offered by individual techniques but the number of parameters that have to be adjusted is very large.

Based on this brief overview of the cellular tracking algorithms we can conclude that most of the methods require user interaction for parameter estimation [11-13] and the tracking results are inaccurate when applied to dense cellular data or data characterised by frequent cellular division (proliferation). In this paper we propose a new tracking approach that is not hampered by problems caused by initialisation and is able to adapt to Brownian motion and cellular proliferation.

3. Proposed tracking framework

In our approach the tracking process evaluates the neighbourhood relationship between all cells in each frame and the cell association is performed by assessing the variation in cellular structures contained in consecutive frames of the image sequence (see Figure 1). Since tracking is carried out for cellular structures, the proposed solution does not require any initialisation procedure. In addition, our approach does not require user-defined constraints or the evaluation of feature similarity criteria in the process of cell association.

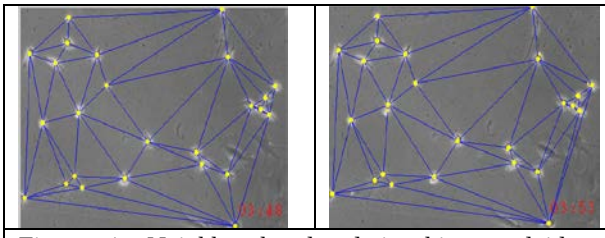


Figure 1. Neighbourhood relationship overlaid on NE4C cell data

The overview of the proposed fully automated tracking framework is illustrated in Figure 2.

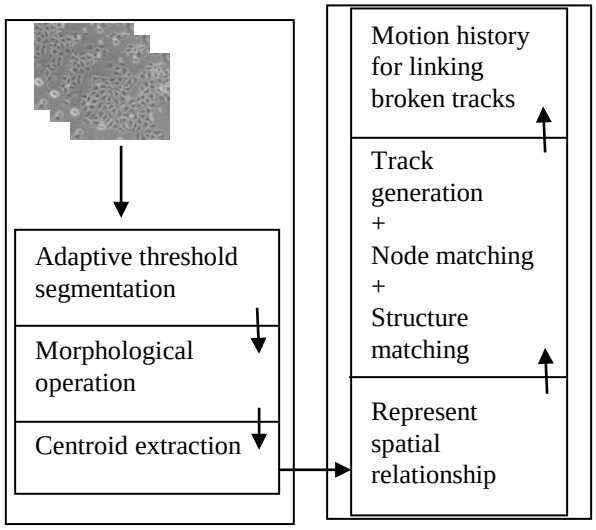


Figure 2. Flow diagram of the proposed tracking framework. Left – Centroid extraction module. Right – Tracking module

The cellular tracking framework has two major modules: a) *Centroid extraction module* (CEM) and b) *Tracking module* (TM). The centroid extraction module performs the segmentation and the extraction of the centroid points by the use of adaptive threshold and morphological operations. The generated output (centroid coordinates) will be passed to the tracking module.

The tracking module receives the centroid coordinates for each cell in two successive images and neighbourhood relationship graphs are constructed for both frames by applying Delaunay triangulation. In this way, each node of the graph represents the cell position and edges define the spatial relationship between nearest cells. Using these cellular graph representations, the problem of node (cell) association can be formulated as a graph matching minimisation. The similarity between two cellular graph structures is evaluated in terms of triangle matching by using the Hausdorff distance. This process generates track segments that are connected by using global constraints such as motion history. The final output generates the tracks for each cell in the image. The main blocks of the proposed tracking algorithm will be detailed in the next sections of this paper.

3.1. Centroid extraction module

The goal of this module is to segment the images and extract the centroid points for all cells present in the image data. Segmentation of phase-contrast images is a challenging task as the cells' intensity values are not uniform and in general the cellular data is characterized by a high level of image noise. Thus, the simplistic thresholding operations are not able to achieve accurate cell segmentation. The main steps of the CEM are listed below.

(A) To reduce the level of noise, the image is filtered with a 3x3 median operator.

(B) The next step of CEM involves the application of Otsu thresholding to obtain the initial segmentation of the image data. This thresholding scheme determines the suitable threshold between foreground and background in an adaptive manner.

(C) Due to intensity variations, the cells are not completely segmented and morphological operations are applied to connect the incorrectly divided regions and fill the holes. In this process, the concave areas in the foreground data are connected using contour analysis and the small blobs are removed as they are generated by noise.

(D) Finally, the map resulting after the application of the distance transform is evaluated to determine the centroids for multiple cells that are agglomerated into a cluster. The distance transform map is calculated starting from the contour of the region that provides the peak in the clustered region and the local peaks were selected as the centroids of the individual cells. Figure 3 shows the results after the application of the centroid extraction module.

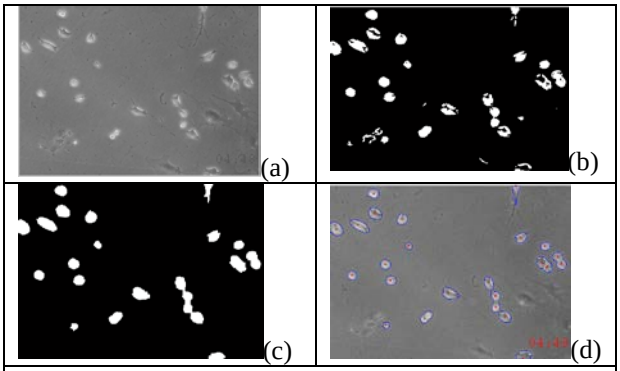


Figure 3. (a) Original image (NE4C cellular data). (b) Initial segmentation. (c) Final segmentation. (d) Centroid points.

3.2. Tracking module

In our approach cellular tracking is achieved by evaluating the neighbourhood relationship between Delaunay meshes calculated for two adjacent frames. Tracking module (TM) consists of a number of independent processes and they are shown in Figure 2.

As illustrated in Figure 2, the coordinates of the centroid points calculated by CEM are the input for this module whereas the output consists of the tracks resulting after cell association. The main components of the TM will be detailed below.

(A) To describe the neighbourhood relationship, we need to develop a technique that evaluates the spatial relationship between neighbouring cells using a graph representation. This is performed by applying Delaunay triangulation. This approach is able to generate the neighbourhood relationship between cells by partitioning the space covered by the controid points into a structure defined by a set of triangles.

(B) The next step of the tracking module attempts to identify the triangle structures that are common in Delaunay meshes that are calculated for each two adjacent frames in the image sequence. The triangle matching process should be flexible in order to accommodate small variations that are caused by cellular migration and reject the large variations that are usually caused by proliferation. To achieve this goal, in this implementation the Hausdorff distance was employed to perform triangle matching. Figure 4 illustrates the Delaunay meshes calculated for two consecutive frames where the structure marked in red returns the highest similarity.

Our approach outperforms the tracking techniques presented in [14] and [15] as these approaches are not able to handle situations caused by proliferation and appearance/disappearance of cells close to the image border. This is caused by the fact that these approaches evaluate the matching process by performing the correlation between independent triangles and as a result ambiguous matching is possible if the cellular structure is severely distorted by significant migration.

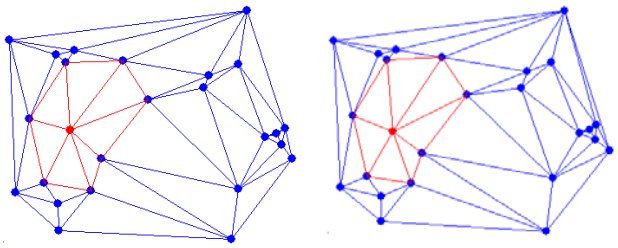
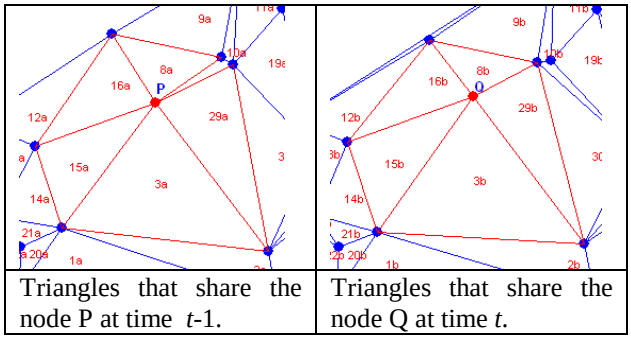


Figure 4. Neighbourhood relationship generated for adjacent images where each node represents the centroid of a cell.

The node (target) association in the proposed tracking scheme is formulated as follows. For each node *i* in the Delaunay graph generated for the frame captured at time *t*-1, a matrix T_{t-1}^{i} that represents pairs of triangles is constructed. In this matrix, the first column stores the triangles that share the node *i*, while the second column stores the corresponding matching triangles in the Delaunay graph generated for frame captured at time *t*. Similarly, T_t^j represents the neighbourhood for node *j* in the current graph at time *t*. In this matrix, the first column stores the triangles that share the node *j* at time *t* while the second column stores the matched triangles in frame at *t*-1.

The nodes *i* and *j* are associated if the pairs of triangles in the corresponding transition matrices (T_{t-1}^{i} and T_t^j) minimise the Hausdorf distance. For instance, in the graphs shown in Figure 5, the triangles corresponding to the nodes P and Q are labelled in both meshes.



Triangles that share the node P at time *t*-1. | Triangles that share the node Q at time *t*.

Figure 5. The triangle matching process.

The matrices constructed for nodes P and Q are illustrated in Figure 6. These nodes can be associated, because one-to-one triangle matching was possible and the fact that the matched triangles minimise the Hausdorff distance criterion.

Node P			Node Q	
8a	8b		8b	8a
29a	29b		29b	29a
3a	3b		3b	3a
15a	15b		15b	15a
16a	16b		16b	16a

Figure 6. Transition matrices constructed for the nodes P and Q shown in Figure 5.

This matching process was carried out for all nodes contained in the cellular mesh. This process generates pairs of associated nodes for every two successive images and also connects the tracks determined at each time *t* in the image sequence to generate full cellular tracks.

(C) The last stage of the tracking module performs the connection of broken cellular trajectories that are generated by image noise or by the appearance/disappearance of the cells that merge/split in continuous frames. In this process we use the assumption that a reliable cell estimate does not appear/disappear close to the centre of the image. If new cells appear close to the centre of the image, then these are generated either by noise or are the result of cellular division. To connect the broken tracks we employ motion history analysis that performs a validity check for all tracks in the image sequence. In this way, if a cell is tracked for a long sequence and it loses the track close to the centre of the image (at time *t*), then the algorithm searches for tracks that are newly generated after the frame t. The algorithm generates a list of nodes and it attempts to connect the broken tracks by evaluating the continuity in the spatial-temporal domain.

5. Experiments and Results

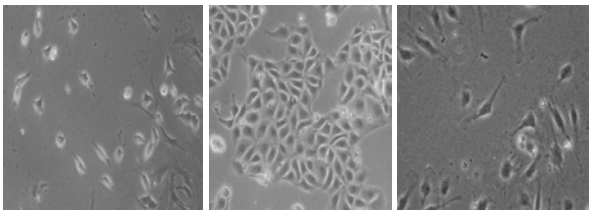
The proposed technique was evaluated on NE4C, MDCK and HUVEC data. The spatial resolutions of these image sequences are 560x400, 400x350, and 670x510 respectively. The temporal resolutions are 5 minutes for NE4C data and 10 minutes for others.

Automated tracking results are compared against the manually tracked data and metrics such as the number of valid and invalid tracks and sensitivity are used to characterize the performance of the proposed automated tracking method. In this evaluation a track is defined as the cell trajectory from the time the cell is first detected until it leaves the areas imaged by the camera. Experimental results are depicted in Table 1.

Table 1. Sensitivity of tracking results.

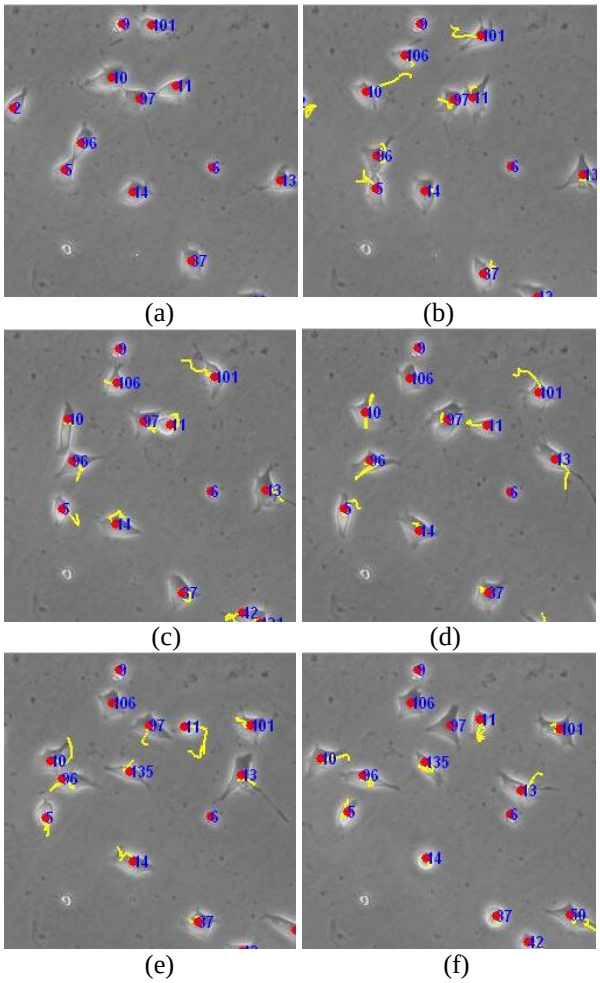
Cell sequence	Frames	Valid track	Invalid track	Sensitivity
NE4C	140	23	2	92%
MDCK	50	60	20	75%
HUVEC	90	30	8	73%

The results shown in Table 1 indicate that the proposed algorithm returns accurate tracking results when applied to NE4C data that is characterised by medium cell density but the performance of the tracking algorithm degrades when applied to MDCK and HUVEC cellular data that is characterised by high cellular density with a high frequency of cellular division (see Figure 7). Visual results that illustrate the performance of the proposed algorithm are shown in Figures 8 and 9. For visualization purposes, close-up images that illustrate the performance of the proposed tracking algorithm in the presence of cellular division and agglomeration are shown in Figures 10 and 11.



(a) (b) (c)

Figure 7. Cellular data evaluated in this paper. (a) NE4C. (b) MCDK. (c) HUVEC.



(a) (b) (c) (d) (e) (f)

Figure 8. Tracking results when applied to NE4C cellular data. (a) Frame 1. (b) Frame 10. (c) Frame 20. (d) Frame 30. (e) Frame 40. (f) Frame 50.

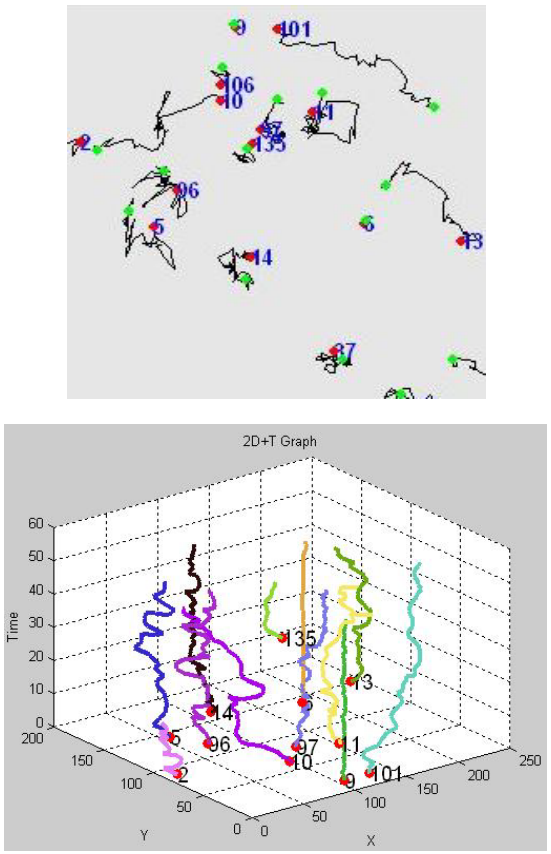
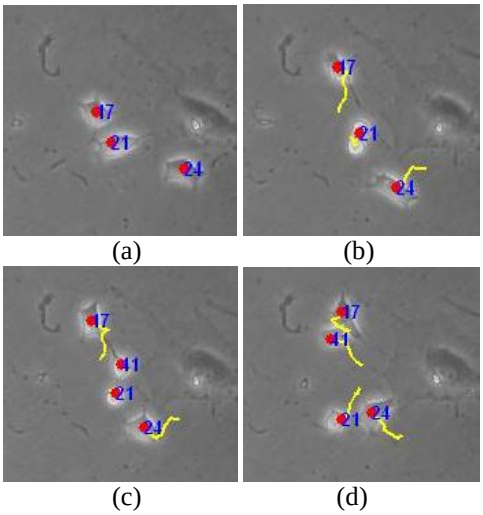
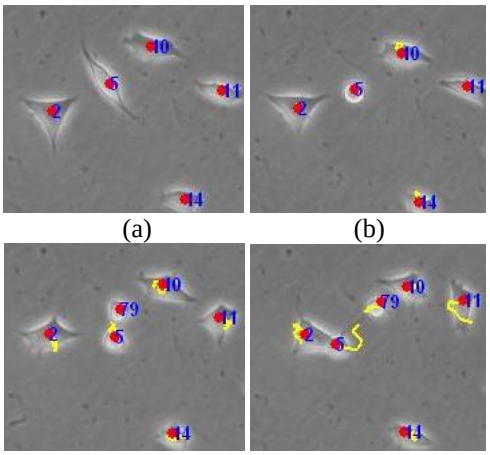


Figure 9. (Top) Tracking results obtained after the algorithm is applied for first 50 frames. (Bottom) Tracking results shown in a 2D+time diagram (time is represented on Z axis).



(a) (b) (c) (d)

Figure 10. Tracking results in the presence of cellular division. (a) Frame 1. (b) Frame 13. (c) Frame 15. (d) Frame 20.



(a) (b) (c) (d)

Figure 11. Tracking results in the presence of cellular agglomeration. (a) Frame 1. (b) Frame 10. (c) Frame 15. (d) Frame 20.

6. Conclusion

The aim of this paper was to introduce a novel and fully automated framework for cellular tracking. The proposed framework encodes the spatial distribution of the cells in the image using a graph-based representation and tracking is performed by evaluating the similarities in the mesh structures that are generated for consecutive frames. Full tracks for all detected cells in the image are generated using continuity constraints that are implemented based on motion history analysis. The developed tracking scheme is able to adapt to Brownian type motion, does not require initialisation procedures, is generic and it can handle difficult situations generated by cellular division and agglomeration.

This research is ongoing and future work will be focused on the inclusion of motion predictors and on the detailed analysis of the motion history to prevent the problems generated by over-segmentation in dense cellular data.

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