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A framework for automated cell tracking in phase contrast microscopic videos based on normal velocities

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ABSTRACT

This paper introduces a novel framework for the automated tracking of cells, with a particular focus on the challenging situation of phase contrast microscopic videos. Our framework is based on a topology preserving variational segmentation approach applied to normal velocity components obtained from optical flow computations, which appears to yield robust tracking and automated extraction of cell trajectories. In order to obtain improved trackings of local shape features we discuss an additional correction step based on active contours and the image Laplacian which we optimize for an example class of transformed renal epithelial (MDCK-F) cells. We also test the framework for human melanoma cells and murine neutrophil granulocytes that were seeded on different types of extracellular matrices. The results are validated with manual tracking results.

1. Introduction

Nearly all cells in the human body are moving at some point during their lifetime thereby contributing to the maintenance of the integrity of the human body and to medically important pathologies. Two prominent examples for the medical relevance of cell migration are the formation of tumor metastases and the immune response. Tumor cell migration is a critical step of the metastatic cascade that leads to the spread of tumor cells within the body (c.f., [14,16]). Thus, tumor cell migration is an important factor in determining the prognosis of cancer patients since patient outcome largely depends on the presence or absence of tumor metastases. Similarly, the ability of immune cells to migrate towards sites of infection is indispensable for clearing invading pathogens from the organism. On the other hand migration of immune cells towards foci of inflammation may contribute to the clinical symptoms of e.g., an autoimmune disease [39].

On a cellular level, cell migration represents the integration of a network of many different molecular mechanisms within the cell. Such mechanisms include among others the dynamic remodeling of the cytoskeleton [28], coordinated formation and release of cell-matrix contacts [31], and the activity of ion transporters and channels [34]. These molecular mechanisms are regulated by networks of intracellular and extracellular signaling molecules so that the migratory activity can be adapted to the respective requirements and migrating cells can respond to external cues. The contribution of individual components of the cellular migration machinery can be assessed by quantitatively evaluating the migratory behavior of individual cells, i.e., by determining speed, translocation and shape of single cells.

The "bottleneck" of such a detailed analysis is the extremely time-consuming segmentation of cells in image stacks of time-lapse videomicroscopic migration experiments. Thus, there is an urgent need for the development of automated sophisticated cell tracking software. Such a system should be able to detect not only the movement of the cell center but also to quantitatively assess changes of cell morphology during the process of cell migration. Large data sets obtained from automated cell tracking covering several time scales (from seconds to hours) could also be used for system biological modeling of cell migration. Finally, the automation of the image processing can facilitate the application of high-throughput screening regimes to single cell videomicroscopic migration experiments.

We propose a semi-automatic tracking method with minimal user interaction. The user only has to determine the rough positions of cells in the first frame. The method is based on a two step algorithm including a rough tracking using motion information and a contour refinement based on the gray level image. In this

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paper we will first summarize existing cell tracking techniques. We briefly describe the challenges in cell tracking (Section 3) as well as the data acquisition process for our cell migration experiments (Section 4) before we discuss how to tackle the problems and propose a tracking method in Section 5. Numerical results and an evaluation of our method in comparison to manual tracking will be given in Section 6 and we will draw conclusions in Section 7.

2. State of the art

There are many ways and methods for tracking movement. For a general summary of object tracking methods we refer to [41]. A good summary of the different techniques specifically developed for cell tracking can be found in [25]. Although the term 'cell tracking' is frequently used in literature, there is no general 'cell tracking' method, since the cell images can be very different depending on the cell type, the environment, and the image capture modality the experiment is recorded with. Furthermore, there are many relevant papers in the computer vision community addressing video tracking in general. However, we will try to summarize some approaches that usually have to be adapted to the data they are used for. We will structure the summary based on the techniques used for the tracking.

The simplest way besides manual segmentation is thresholding the data, which is not applicable in most cases since a big intensity difference between the objects of interest and the background as well as a noise free image are needed. Thresholding might be applicable to tracking of fluorescently labeled particles on a black background.

Many methods use templates to perform pattern matching. A template is moved over the image domain and compares the patch at the current position with the template e.g., via cross-correlation (e.g., [17]). Other methods try to fit Gaussian curves to their signal and use the peak position as the position of the object (e.g., [36,37]). Having segmented the cells in each frame the trajectory is recovered by searching for the nearest centroid position in the previous frame (e.g., [15]) or by using graph based methods (e.g., [38,25]). The approach of Debeir et al. [12] was to use a multikernel mean shift algorithm based on the assumption that the cell interior and the exterior close to the cell boundary have different gray levels.

Another class of methods are motion estimation methods. They try to calculate the so-called optical flow and segment the video based on the object's movement. The most common methods are Horn–Schunck [18] and Lucas–Kanade [23]. A comparison of general optical flow algorithms can be found in [6]. An application of optical flow to cell tracking is presented in [32]. The optical flow equation can also be used for registration, which is done in [19] for tracking many cells in a phase-contrast video microscopy sequence. The tracking yields good results but only for about 50 frames. After that an accumulation of error causes large deviations from the actual cells centroid position.

Recently, active contour models or 'snakes' attracted much attention for general image segmentation. This approach minimizes an energy depending on the segmentation curve, where a low energy corresponds to a curve with the desired properties. Typically, these methods are driven by the data in some feature space and make a regularity assumption on the smoothness of the curve. They can also include a priori information about the shape, volume or position of the object of interest. Our method uses the latter approach with two different energies in two different steps. The most prominent representatives of these kind of models are Mumford–Shah, Chan–Vese, and Geodesic Active Contour models or Snakes. A very good summary of active contour cell tracking methods can be found in [44]. There are many examples for active contour methods in cell tracking. Ray et al. track leukocytes with active contours under size and shape constraints [33]. In [40] a statistical model is used to train the algorithm estimating the typical texture of cell boundaries and in [42] a local average filter is used to detect low contrast boundaries. Recent research on using level set functions for cell tracking in fluorescent microscopy can also be found in the work group of Meijering (see [11,10] and the references therein).

Other hybrid methods using active contours and motion information have been proposed. Paragios and Deriche [30] designed a method where the segmentation is driven by a statistical motion estimation force as well as a spatial gradient dependent force. Bascle et al. [4] present a tracking method using snakes with an additional motion constraint, whereas the motion is estimated in a multiscale approach using Gaussian pyramids. In [29] motion estimates are used to design parts of the force driving a level set function.

Due to the big differences in different cell images many data specific methods have to be developed and therefore address different problems. A performance comparison of single particle tracking methods in fluorescent microscopy can be found in [8]. A difficult problem in tracking multiple cells is the danger of wrongly merging adjacent cells, which was tackled in [43,26,44] by using multiple level set functions with a repulsion term. As we will describe in more detail in Section 3 the cells in phase contrast microscopy are often surrounded by bright spots, so-called halos, which are artifacts from the image acquisition process and make an accurate determination of the cell boundary difficult. For the case of a given rough segmentation, Ersoy et al. [13] addressed this problem and proposed a segmentation refinement based on geodesic active contours. The initial contour is assumed to be outside the cell of interest. It is then moved inwards until the gray value in normal direction changes from bright to dark. The second processing step of our approach will be closely related to this method.

3. Challenges in phase contrast microscopy videos

Phase contrast microscopy, which is applied in this study offers the advantages that – as opposed to fluorescence microscopy – it is essentially devoid of any phototoxicity and that cells can be observed in their native state without the necessity of dye-loading or transfection with fluorescent proteins. It thereby allows the monitoring of cell migration at high time resolution for extended time periods without any side effects on the cellular behavior. However, the downside of phase contrast microscopy is that it poses specific challenges to the tracking algorithms. Due to the specific illumination technique gray levels within a given cell or in its immediate vicinity may greatly vary during the course of an experiment. Thus, the gray level of the cell can be higher or lower than that of the background. Moreover, some parts of the cells will have gray levels that are almost indistinguishable from that of the background. Yet another difficulty in determining the cell contours in phase contrast images is that the rear part of migrating cells is frequently surrounded by a bright "halo". Fig. 1 shows a close up on three cells with different halos. As we can see the halo does not follow any regular model. It can be inside as well as outside the actual cell boundary. For these images even a human analyst can only guess the actual contour of each cell. This is a well known problem and causes different manual segmentations on the exact same dataset to yield relatively different results as we will show in the results section. Neither omitting all white spots nor including them nor looking for certain intensity changes in the halo yields the true contour.

Our proposed approach tries to take advantage of the halos by a two-step procedure. Since the halos lead to a large image contrast, motion of the halo is well visible. Therefore, our first step is