DYNAMIK: A Software Environment for Cell DYNAMics, Motility, and Information TracKing, with an Application to Ras Pathways

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ABSTRACT
The emergence of new microscopy techniques in combination with the increasing resource of bioimaging data has given fresh impetus to utilizing image processing methods for studying biological processes. Cell tracking studies in particular, which are important for a wide range of biological processes such as embryonic development or the immune system, have recently become the focus of attention. These studies typically produce large volumes of data that are hard to investigate manually and therefore call for an automated approach. Due to the large variety of biological cells and the inhomogeneity of applications, however, there exists no widely accepted method or system for cell tracking until today. In this paper, we present our publicly available DYNAMIK software environment that allows users to compute a suit of cell features and plot the trajectory of multiple cells over a sequence of frames. Using chemotaxis and Ras pathways as an example, we show how users can employ our software to compute statistics about cell motility and other cell information, and how to evaluate their test series based on the data computed. We see that DYNAMIK’s segmentation and tracking compares favorably with the output produced by other software packages.

1 INTRODUCTION
Cell motility is an important factor in many biological processes, such as tissue repair, metastatic potential, chemotaxis, or analysis of drug performance (Nath et al., 2006). Cell migration is also crucial to the cells of the immune system, which migrate towards sites of inflammation to engage infectious agents, or the cells of the developing embryo that migrate to distant locations where they differentiate into different cell types (Zimmer et al., 2006). Cell tracking has therefore become one of the main applications for biological image processing, giving rise to an exploding number of publications over the last years. Two technical reasons also helped advance the field: First, the technological progress in microscopy led to an increasing stream of bioimages that makes it very often prohibitive for biologists to do manual labeling, and which let them search for automated alternatives. Second, the implementation of new image processing methods that allow biologists to process cell videos with lower contrast and under difficult lighting conditions. Despite these advances, automatic processing of cell videos is still a largely unexplored area with many obstacles yet to overcome.

Some of the inherent challenges in bioimaging are the following: Images with low contrast may display non-uniform intensity distributions with the result that parts of a cell can feature the same intensity as the background. This can let cells appear smaller than they are in reality, or it can lead to cells seemingly disintegrating into several components. One way to alleviate this problem is the use of fluorescent markers that color the cell membrane or diffuse throughout the cytoplasm and provide relatively uniform staining (Zimmer et al., 2006). Another problem is that cells can have very different shapes, ranging from simple circular blood cells or crescent-shaped malaria agents to almost arbitrary shaped animal cells. Moreover, many cell types change their shapes and size in time, extending and contracting their membrane in the process. In addition, cells do not necessarily move steadily across the section captured by a camera. Cells may display unstable behavior, depending on the experimental setup, camera settings (e.g. time-lapse photography), or biological processes in action. For instance, jumps, abrupt stops, and sharp direction changes are typical problems that a tracking method may have to cope with. Cells also very often interact with each other, resulting in merging and/or splitting cell objects in the cell video. The problem for a cell tracking method lies in keeping track of all cells before and after their contact. Naturally, the problem compounds with the number of cells aggregating. A related problem is the detection of cell division and cell fusion, which is a very common process in cell...
bioimaging systems for cell contrasted and spatially isolated cells, such as fluorescent processing can solve the tracking problem for well-intensive research. The current state of the art in image Due to these problems, bioimaging is still the subject of it. We have addressed these issues in another manuscript (Mosig et al., 2009).

Due to these problems, bioimaging is still the subject of intensive research. The current state of the art in image processing can solve the tracking problem for well- contrasted and spatially isolated cells, such as fluorescent bacteria. Unfortunately, while bioimaging systems for cell tracking have been reported before, there are currently few powerful open source solutions available to the research community (Davis et al., 2007), (Sacan et al., 2008). In the present paper, we present our DYNAMIK software, which offers cell biologists a publicly available tool for studying cell dynamics, and which is reasonably stable when facing the problems listed above.

We structured our paper as follows: Following this introduction, Section 2 provides a brief overview of the existing approaches for cell tracking. In Section 3, we present the features offered by DYNAMIK and describe its built-in tracking method. Section 4 describes an example study on chemotaxis and Ras pathways for which reliable cell tracking is essential. Finally, in Section 5, we use our DYNAMIK software to identify and quantify meaningful differences between videos displaying the chemotaxis of various mutant cells.

2 EXISTING APPROACHES
Given the large number of publications, we confine ourselves to only a small subset of publications that we think are representative for the most important research directions. For a more extensive survey of automatic cell tracking methods, we refer readers to the references (Zimmer et al., 2006), (Miura, 2005). One criterion for classifying existing approaches is the distinction between segmentation-based approaches and approaches relying on deformable models.

2.1 Segmentation-based Approaches
The idea of segmentation-based approaches is to process the frames of a video sequence individually, followed by a matching in time of the cells segmented in each frame. A widespread technique for segmentation is thresholding, in combination with standard image operations, such as smoothing, and morphological operators for removing inclusions etc. Thresholding assumes that cells stand out against their background, and that pixels exceeding a certain brightness level; i.e. threshold, belong to a cell. By collecting all cell pixels and performing a connected component analysis, we can then identify individual cells. Thresholding relies on images with high contrast. However, in practice, this is very often not the case. For instance, parts of the same cell can appear brighter or darker due to uneven illumination; or photobleaching can result in a partly decay of fluorescence intensity. The actual tracking of cells can be done by nearest neighbor methods that link an object in frame i to the same object in frame i+1 based on the distance traveled (Tseng et al., 2002), (Apgar et al., 2000). Obviously, the time resolution of the video sequence and the number of moving objects are decisive factors for the tractability of this approach. A possible refinement of this approach is the inclusion of probability distributions for the anticipated positional changes (Schütz et al., 1997), (Anderson et al., 1992). Another possibility is to use graph-theoretical methods for resolving ties in case of multiple candidates that could equally likely be linked to the same object (Vallotton et al., 2003).

2.2 Deformable Models
Deformable models are typically closed curves in 2-D, or surfaces in 3-D, that evolve iteratively around the boundaries of objects (Zimmer et al., 2006). A cost function that is minimal for a curve coinciding with the object boundary is typically guiding the iterative process. This allows incorporation of domain specific knowledge, such as the length of the curve or the similarity to a given reference shape, which weighted energy terms can reflect in the cost function. Due to this flexibility of combining image characteristics with prior knowledge, deformable models have become very popular in medical imaging (McInerney and Terzopoulos, 2000). Zimmer et al. distinguish between two main categories of deformable models according to the mathematical representation of the contour or surface. In particular, they distinguish between explicit functions where the boundary is represented by parametric functions, such as (Zimmer et al., 2002) or (Kass et al., 1987) and implicit models, where the boundary is defined as the zero level set of a scalar function defined over the image domain, e.g., (Sethian, 1999), (Mukherjee et al., 2004).

3 DYNAMIK
We developed DYNAMIK in response to the many requests of biologists for user-friendly cell tracking software allowing rapid prototyping, quick data evaluation, and proof of concept. To make a design decision between deformable models and segmentation-based techniques, we compared a
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DYNAMIK’s preprocessing is a mixture of filtering, edge detection, and morphological operations. It begins with a simple “salt and pepper” filter implemented through a 2-D median filter. Then, it applies a Sobel operator and thresholding to extract the cell outlines (Barber et al., 1996). Finally, it removes any remaining noise in the interior of the segmented cells and smoothes their outlines by means of eroding. DYNAMIK’s tracking method uses a nearest-neighbor technique that matches spatially close cells of neighboring frames to each other; under the assumption that cells are moving continuously over frames; i.e. a cell in frame i will have the shortest distance to its translocated cell among the cells in frame i+1. In particular, we use the symmetrical form of the Hausdorff distance to compute the distances between the convex hulls of cell outlines, which we compute by means of the Quickhull algorithm (Barber et al., 1996). The tracking problem then reduces to finding the closest pairs of cells in two neighboring frames, which is a classical matching problem. If the number of cells in frame i+1 is larger than the number of cells in frame i, which means that new cells have entered the field of vision, some cells of frame i+1 will remain unmatched and are thus identified as new. On the other hand, if the number of cells in frame i is larger than the number of cells in frame i+1, the unmatched cells in frame i have left the field of vision. For cases of cells leaving and entering the scene at the same time, we have introduced a threshold distance, which prevents DYNAMIK from matching unrelated cells that are too far from each other. Users can adjust this threshold to the specific requirements of their application. They can also filter objects according to their size, which allows them to filter out potential noise that could distort the feature statistics. In a related paper, we present a technique for dealing with merging and splitting cells, which we are about to integrate into DYNAMIK (Mosig et al., 2009).

3.3 Software Comparison
To evaluate the performance, we compared our DYNAMIK software with two other software packages, CellTrack (Scan et al., 2008) and a software based on flux and level sets, which we simply call Flux software in the following (Palaniappan et al., 2007), (Nath et al., 2006). Fig. 1 shows a comparison of the segmentation of all three software modules for Dictyostelium.
Fig. 1. Segmentation of Dictostelium: (A) DYNAMIK (B) Flux software, (C) CellTrack.

We can see that the segmentation of DYNAMIK (A) is slightly better than the segmentation of the Flux method (B) and CellTrack’s method (C) in that it undersegments slightly less than its competitors do. For instance, for Dictostelium cells in Figure 1, Arrow A points to a segmentation point correctly recognized by DYNAMIK, but not detected by Flux and CellTrack. On the other hand, Arrow B points to a segmentation point that goes undetected by all software packages.

In the following sections, we are going to trace the trajectories of these cells, and evaluate the results in a biological study on Ras pathways (Sasaki et al., 2004).

4 RAS PATHWAYS

This section introduces the biological background of our investigation of Ras, which is a family of genes that are involved in cellular signal transduction and that are responsible for regulating cell growth. Since dysregulated Ras signaling can lead to uncontrolled cell growth, understanding Ras pathways is very important for cancer research.

We investigate Ras pathways for Dictostelium. The single cellular Dictostelium belongs to the class of eukaryotic cells, which share common mechanisms for sensing of, and migrating to, chemoattractants (chemotaxis). The chemoattractant receptors and G-protein subunits distribute uniformly along its cell membrane. G plays an essential role to mediate the Ras activation, which then locally activates the PI3K (Class I phosphatidylinositol 3-kinases) preexisting on the membrane. PI(3,4,5)P3 (Phosphatidylinositol-(3,4,5)-trisphosphate) is the product of PI3K and its accumulation will result in the F-actin polymerization. F-actin is part of the cytoskeleton and its polymerization forms the pseudopod, which plays the role of a leading edge when the cell is moving. Accordingly, the wild-type Dictostelium cells in the chemoattractant gradient will be polarized and move to the chemoattractant source. This sense is very sensitive because the shallow extracellular signal difference is amplified to a steep intracellular gradient of the signal, in part, through a preferential activation of PI3K and localization of these PH domain-containing proteins. A positive feedback loop enhances further recruitment of PI3K from the cytosol to the leading edge. The translocation of PI3K, which depends on F-actin, reinforces the Ras pathway, resulting in a stabilization of the nascent leading edge. The direction of movement is dynamic, with the cells steadily sensing the extracellular signal and determining the directional change. A cell can form several lateral pseudopods. When a lateral pseudopod becomes stronger, it will replace the former main pseudopod and effectively lead the cell in a new direction. With the time the cell takes to polarize typically serving as a measure of sensitivity, the sensitivity of cells to a chemoattractant can vary. The speed of movement indicates the activation of F-actin polymerization, which measures the intracellular signal intensity.

In the following, we study the signal transportation pathway of Dictostelium by comparing the behavior of four types of mutated Dictostelium cells with the behavior of the wild type (control type). Using DYNAMIK software and five videos from a previous study of (Sasaki et al., 2004), we compute features that show significant differences between the five cell types. In our following analysis, we study in particular the direction, polarity, and speed of Dictostelium cells. The five videos, v5-v9, show the different types of Dictostelium cells, with frames taken every 6 s and with the micropipette containing the chemoattractant visible in the lower left corner. Video v5 displays the wild-type (WT, KAx-3) cells and Video v6 shows the rasG null cells with reduced polarity and directionality, and with more lateral pseudopodia. Video v7 shows the rasG null cells expressing RasG17N, which can block the activation of other Ras proteins that are regulated by RasG17N-sensitive GEFs (GDP/GTP exchange factor). Here, most of RasG17N/rasG null cells exhibit a severe loss of polarity and movement and produce random pseudopodia in response to chemoattractant stimulation. Video v8 shows aleA null cells, which carry a
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Disruption of a gene encoding the putative Ras GEF Aime- less. The cells exhibit a slightly reduced polarity but move even faster than wild-type cells. The aleA null cells still sense and move toward the chemoattractant although the directionality is reduced. The majority of cells eventually move to the chemoattractant source although many of them meander on their way toward the source and produce lateral pseudopodia. Some cells got lost for some period but then found the direction of the source. The video suggests Ras-mediated directional sensing may be independent from Ras-mediated cell polarization (Sasaki et al., 2004). Video v9 shows RasG$_{S17N}$/aleA null cells that appear to be composed of two different populations. One population exhibits a loss in cell polarity with cells not moving very far, similar to RasG$_{S17N}$/rasG null cells. The second population exhibits polarization and movement like those of aleA null cells, but the direction of movement is random.

Fig. 2. Two cells with low and high polarity.

Fig. 3. Cells without directional preference.

5 RESULTS

This section shows the results we computed for the Ras pathway videos v5-v9, using DYNAMIK.

Fig. 2 shows two Dictyostelium cells with different polarity. The circular cell is a cell with low polarity, while the cell with the elongated shape has high polarity and thus a high directional preference. In Fig.3 we see Dictyostelium cells with low polarity and low directional preferences. These cells exhibit none or only a small reaction when brought in contact with the chemical attractant, and will therefore stay local over the sequence of frames. This behavior is in stark contrast to the cells in Fig. 4, which shows Dictyostelium cells with high polarity together with their trajectories on which they approach the chemoattractant in the lower left corner.

Compared to CellTrack’s built-in tracking routines, the tracking of DYNAMIK is more stable, despite its seemingly less complex tracking method. This is because the implemented nearest-neighbor technique is very intuitive and robust against noise, while CellTrack’s dynamic flow and dynamic contour tracking can get confused when cells leave or enter the field of view.

DYNAMIK allows users to compute statistics about the directional preferences of cells. For instance, Fig. 6 is a direction compass showing the average directional preference for each cell, as indicated by a small red arrow. We can clearly see the preference of most cells for moving in the direction of the lower left corner; i.e. in the direction of the chemoattractant.

Fig. 4. Cell trajectories with directional preference.

Fig. 5. Trajectories.
For comparison, we show the corresponding values computed by Sasaki et al., using DIAS software, in brackets (Sasaki et al., 2004), (Soll and Wessels, 1998). The speed of a cell, or any other segmented biological entity, is the trajectory length of its centroid divided by the duration of the video, where we smooth the trajectory by removing the points of smaller deviations. Note that we compute the speed as pixel/frame, while Sasaki et al. know the scale and can thus compute it as micrometer/min. We compute directionality as the trajectory length divided by the distance between the start and end of the trajectory. For measuring both the distance between two points and the length of the trajectory, we use the standard Euclidean distance. As its name suggests, direction change describes the average directional change of the trajectory in degrees, which we measure in a straightforward way by computing the average angle between two neighboring vector segments of the trajectory. For the roundness feature, we use the following metric that is equal to one for ideal circles and equal to zero for the other extreme, a straight line:

\[ M = \frac{4 \cdot \pi \cdot \text{area}}{\text{perimeter}^2} \]

Accordingly, a large value of the roundness features indicates a low polarity, and vice versa. We multiply the above metric by hundred to express the roundness in percent. In addition to this roundness feature used in (Sasaki et al., 2004), we suggest measuring the polarity directly by using the eccentricity of a cell. The eccentricity describes the ratio of the distance between the foci of an ellipse and its major axis length, where the ellipse has the same second-moments as the cell region. Hence, the eccentricity value lies between zero, which indicates a perfect circle, and one, in which case the cell region degenerates into a straight line. Fig. 7 shows the average cell eccentricity of every frame for each video, where each cell type has a different color. The cyan curve shows that the WT cells are polarized on a high level throughout the video. Compared to the WT cells, the red curve shows that the rasG null cells have a reduced polarity, and they have a lower directionality according to Table 1. The blue curve displays the polarity of rasG null cells expressing RasG<sup>S17N</sup>, which is much lower than the polarity of WT or rasG null cells. According to Table 1, RasG<sup>S17N</sup>/rasG null cells also move much slower than WT or rasG null cells. The green curve for aleA null cells shows that aleA null cells have a reduced polarity, but that they can move much faster than WT cells, which is in accordance with the observation in (Sasaki et al., 2004). Since the population of RasG<sup>S17N</sup>/aleA null cells in Video 9 comprises two different populations, Table 1 only presents the average values for these two populations.

Fig. 8 shows the average migration distance for each video in a MatLab boxplot. The migration distance of a cell is the distance between its start position and end position; i.e. the distance between its position in the first and last frame. For each video, the boxplot shows a box with lines at the lower quartile, median, and upper quartile values. Whiskers extend from each end of the box to the adjacent values in the data, with the extreme values lying within 1.5 times the interquartile range from the ends of the box. The boxplot displays outliers beyond the ends of the whiskers with a red + sign.
Comparing the five videos, we see that the \textit{rasG} null cells (v6) and \textit{aleA} null cells (v8) migrate further away from their starting positions than cells in other videos.

![Cell Migration Distance](image)

Fig. 8. Cell migration distances for each video.

In summary, the data we computed with DYNAMIK is consistent with the DIAS data reported in (Sasaki et al., 2004), thus allowing us to draw the same biological conclusions. In addition to the analysis presented above, the accompanying online material provides further analysis and data computed by DYNAMIK, which again confirm the results of Sasaki et al.

6 SUMMARY

We presented our publicly available software DYNAMIK, which allows users to automatically segment and track cells in video sequences, plot their trajectories, and evaluate various cell parameters. The comparison with two other software packages showed that DYNAMIK compares favorably and that it, being a MATLAB implementation, offers many additional features, such as scalability and interactivity. For a practical Ras pathway application, in which we investigated the chemotaxis of several cell mutants, we showed the power of DYNAMIK. It allowed us to quickly visualize the different cell trajectories and compute the necessary statistics for our conclusions. Using well-established imaging methods, DYNAMIK provides results for a wide range of bioimaging data with sufficiently contrasted cells. We are continuously developing DYNAMIK and offer users the possibility to integrate their own features and segmentation functions. Our next step is to equip DYNAMIK with a more intuitive graphical user interface.

The latest DYNAMIK software, including the videos and results discussed in the present paper, is available under the web address: www.picb.ac.cn/sysbio/DYNAMIK.

REFERENCES


