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A System for Bi-level Cell Tracking

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Abstract. Measurement of the proliferative behaviors of in vitro cells is important to many biomedical applications ranging from basic biological research to advanced applications, such as drug synthesis, stem cell manufacturing, and tissue engineering. The detection of borders within an image constitutes a process of digitalization of the image. Once obtained the digitized image, the next step is the application of a specific process, consisting in applying algorithms that allow the obtention of raw data of the image. In this case, the applied algorithm to the digitized images was the Canny algorithm. This work presents a system to compute a vector representation for a selected cell of an image. The representation is in bi-level raster image.

Keywords: bi-level, cell tracking, raster image

1. Introduction

Computational image analysis tools for semi-automated tracking of single molecules or molecular compounds within living cells have been developed and reported since early 1980s. Image sequences are commonly used for biologists to study living cell dynamics (for instance the study of cell aging in [1]). In order to produce quantitative and statistically relevant results, large amounts of data are required (say, several sequences, each one containing several hundreds of images), and automatic image analysis algorithms become necessary. Typical experiments produce over 100 gigabytes (GB) of image data consisting of about 40000 frames, representing thousands of cells in each frame. This makes automated tracking and analysis of cells critical in efficiently studying the underlying biological mechanisms. However, the high processing demand, the varying density of the cell culture (with cells dividing/dying, leaving/reentering the field-of-view), and the complexity of the cellular topologies (shape deformation, close contact, and partial overlap) pose many challenges to existing tracking techniques. One frequent aim is to extract from the image sequence the complete description of cell positions, shape and motion across time, leading, in the case of dividing cells, to a space-time classification. These segmentation and dividing/tracking issues have to be solved in the most reliable manner, since human post-processing is the limiting factor of the rate of processed data.

Recently, many cell proliferation assays have been developed for high throughput cell imaging and analysis. Specially, phase contrast microscopy is a superior imaging modality since it enables continuous monitoring of live cells without requiring destructive methods of cell manipulation, such as cell lysis and staining. Consequently, the need for extended-time observation tools and the proliferation of high-throughput imaging have made automated image analysis mandatory. Some approaches [10, 11] build a model of the cell motion in order to improve the segmentation performances. Khan et al tackled the problem of object contact [12] by incorporating a Markov random field prior distribution into the particle filter [13] framework, for modelling object interactions and maintaining object identities. The same authors addressed the issue of split/merged measurements [14], but with the assumption that the number of objects is fixed, which is not

considered in our problem. Furthermore, there is still no work that considers the scenario in which the object can divide and replicate itself.

In many graphical information systems, input consists of a picture or scanned image. In order to be able to manipulate, for example transform or select, lines and other primitives in such raster images, the primitives must be extracted from the raster image and vectorized. In the past, many techniques for extraction and vectorization of primitives from raster images have already been developed. In this work, we will focus on the extraction and vectorization of individual lines that may have arbitrary width, rough contours, crossings and branches.

Tracking-based approaches rely on cell tracking to determine individual cell trajectories, and then identify mitosis based on the temporal progression of cells featured along their trajectories [2-4]

A simple method is described in [2] to convert one-pixel-thick lines to a vector representation. However, if lines become thicker than one pixel, the conversion process acquires a much more complicated nature. Other techniques are based on the Hough transform. This kind of methods is mainly used for the detection of straight lines [4] or curves, such as circles [5].

The detection of borders of an image constitutes a process of image digitalization. Once completed, the next step is the processing of the digitized image applying algorithms that allows obtaining data from it. In this case the applied algorithm to the digitized images was the Canny algorithm.

The Canny algorithm is used to detect the borders of an image; nevertheless, one of our objectives is only the detection of the borders previously indicated by the user with the mouse pointer of the computer.

2. Methodology

For the development of the proprietary algorithm, basic concepts of images and algorithms for edge detection were reviewed. We review as well a lot of documentation related to the Matlab software tool.

The edge following algorithm used in our method, is based on the algorithm described in [4].We will use and describe it in the process of detecting the entire outline of an arbitrary 8-shaped object in the counter clockwise direction.

The outline will be an 8-connected set of foreground pixels, each of them having one or more neighbors belonging to the background. The general scheme of the proposed method in this work is depicted in Figure 1.

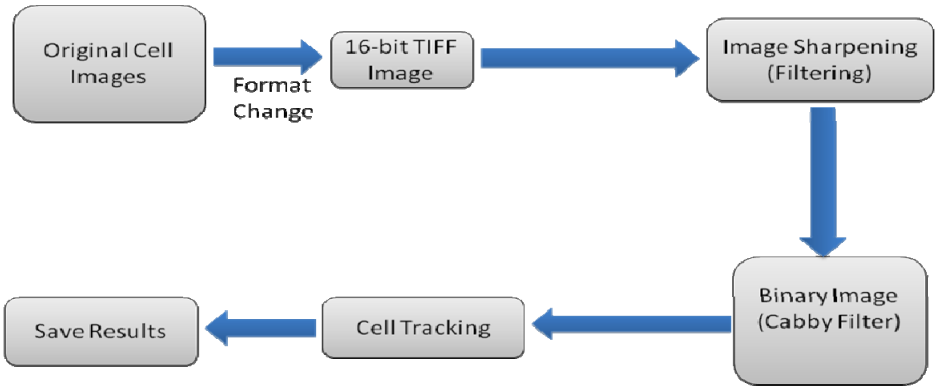

Fig. 1: General scheme of the proposed method

3. Test and results

Given an initial starting pixel (Si), the next outline pixel and next starting pixel are chosen as follows: search the neighbors in the counter clockwise direction, starting with Si, until a transition from background to foreground is found. The first foreground pixel thus found will be the next outline pixel and the last background pixel will be the next initial pixel, respectively. This process will continue with every transition found. Below there are some pictures of the developed application. The figure 2 depicts the original image, which consists of a set of cells. The pictures were provided by [7].

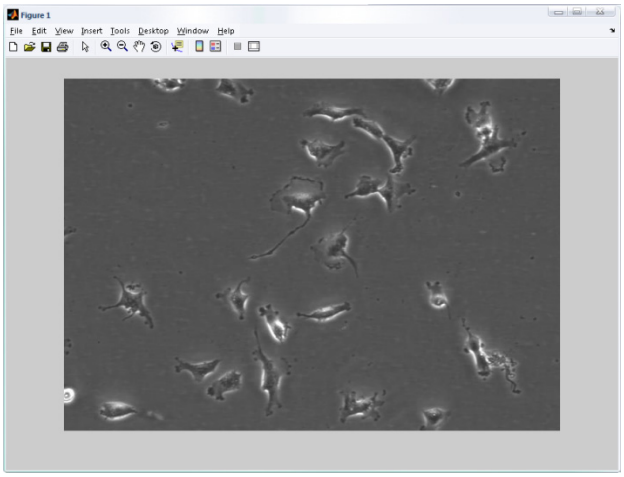

Fig. 2: Original image

The image read is converted to a binary file in order to apply the edge detection. To filter the image the Canny filter is used, which can be adjusted for reducing noise in the image. Figure 3 shows the filtered image, after this kind of filter is applied. The main objective is to sample the gray level at various places near the cells in the picture, and estimate the local gray-level thresholds that can be used to define seeds (low threshold) and extra-cell space (high threshold).

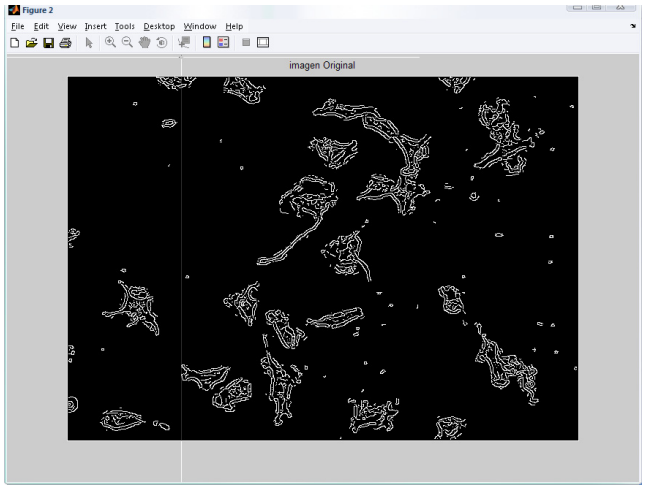

Fig. 3: Filtered image, is using a filter canny

The next step is to track the neighbouring selected pixel in the counter clockwise direction; this way, the pixel that matches the value of the pixel edge will be stored as part of the outline shape. Next, the searching process takes as its pivot the last pixel added to the edge and repeats the search using the next neighbouring pixel.

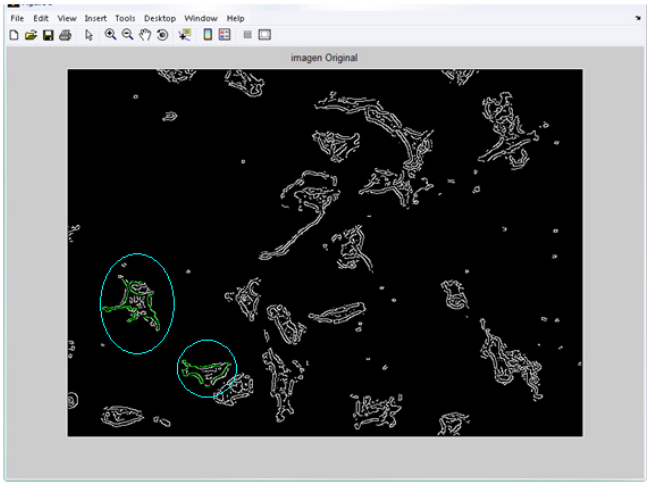

Fig. 4: Filtered image with edges marked

The figure 4 shows the filtered image with the edges marked. This is achieved by selecting the cell of interest. However, the basic concepts underlying the vast majority of published methods are virtually the same. The commonly used approach in motion tracking consists of at least the following steps: preprocessing the image data, detecting individual particles every time gap, linking particles detected at successive time points and analyzing the results.

The edge will be stored as ordered pair (x, y), representing the coordinates of pixels on the screen, having the upper left corner of the screen as the origin.

4. Conclusions.

Until recently, it is possible to manually identify incidents of mitosis because mitotic cells tend to retract, round up, and exhibit intensified surrounding halos under phase contrast illumination for short-period, small-scale studies. The proposed method does not depend on empirical parameters, ad hoc image processing, or cell tracking and consequently can be straightforwardly adapted to different cell types.

5. References

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