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Fast and Accurate Cell Tracking by a Novel Optical-Digital Hybrid Method

M. Torres-Cisneros · J. G. Aviña-Cervantes · E. Pérez-Careta · F. Ambriz-Colín · Verónica Tinoco · O. G. Ibarra-Manzano · H. Plascencia-Mora · E. Aguilera-Gómez · M. A. Ibarra-Manzano · R. Guzman-Cabrera · Olivier Debeir · J. J. Sánchez-Mondragón

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Abstract An innovative methodology to detect and track cells using microscope images enhanced by optical cross-correlation techniques is proposed in this paper. In order to increase the tracking sensibility, image pre-processing has been implemented as a morphological operator on the microscope image. Results show that the pre-processing process allows for additional frames of cell tracking, therefore increasing its robustness. The proposed methodology can be used in analyzing different problems such as mitosis, cell collisions, and cell overlapping, ultimately designed to identify and treat illnesses and malignancies.

Keywords Cell tracking · Morphological operators · Optical cross-correlation · Thermal and optical images

1 Introduction

Automatic and robotic image processing have introduced new demands and tolerances in processes that are presumed to be well known and controlled, creating a great deal of renewed interest and activity in the area. Such is the case of cell tracking. Due to

M. Torres-Cisneros (✉) · J. G. Aviña-Cervantes · E. Pérez-Careta · F. Ambriz-Colín · V. Tinoco · O. G. Ibarra-Manzano · H. Plascencia-Mora · E. Aguilera-Gómez · M. A. Ibarra-Manzano · R. Guzman-Cabrera
NanoBioPhotonics Group, DICIS, Universidad de Guanajuato, 36885 Salamanca, GTO, Mexico
e-mail: torres.cisneros@ugto.mx

O. Debeir
Information and Decision Systems, Université Libre de Bruxelles, Brussels, Belgium

J. J. Sánchez-Mondragón
Photonics and Optical Physics Laboratory, INAOE, Puebla, PUE, Mexico

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numerous potential applications, cell tracking is currently being extensively studied by a large number of research groups around the world. In medicine, for instance [1], cell motility could be used to indicate the effect of a certain drug over a cancerous tumor or other malignancies. This can become a routine lab test that can be performed efficiently and accurately on a large number of samples and on a limited time lapse.

It must be pointed out that cell tracking does not refer exclusively to the action of observing a cell or a group of cells in a specific context or scene. It consists of the identification of each cell in the context or scene and tracking each one along a complete sequence. Information regarding their instant position, velocity, acceleration, and even cell phenomena such as apoptosis, mitosis, collision, and overlapping, among others, can be recorded.

From one point of view, cell tracking issues can be considered as a traditional object recognition task; nevertheless, this conclusion is far from adequate. In object recognition, the image's quality ranges from medium to good in most of the cases and the object holds its shape along the sequence. In cell tracking variations of both, the texture and color in the whole sequence and scene can be observed. Figure 1 shows an optical microscope image of a group of cells, some detail can be observed (detail is required during the cell tracking process). The image has poor quality; the morphology of each cell is different, and there is practically no significative graylevel difference between the body of the cell and the background. There are certain objects in the scene that do not correspond to a cell and do not have the same size or profile. Furthermore, from the sequence of images that includes Fig. 1, it can be observed that the cell tracking is complex and difficult. For instance, the speed of each cell is different requiring that other phenomena such as mitosis, apoptosis, overlapping, collision, and migration be taken into account. Phenomena can be individual such as apoptosis, (the death of the cell, which can be observed when the cell does not present any voluntary movement), cell migration when the cell leaves the frame, or can be collective such as mitosis (the division of a cell into two different cells), and the collision or overlapping of cells (which occur when two or more cells collide or overlap).

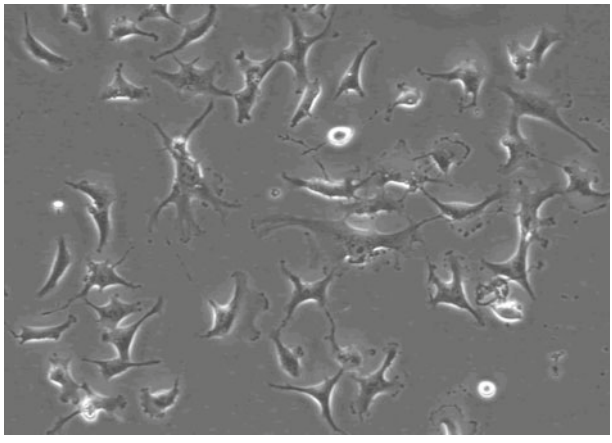

Fig. 1 Image obtained from a culture with phase-contrast video microscopy

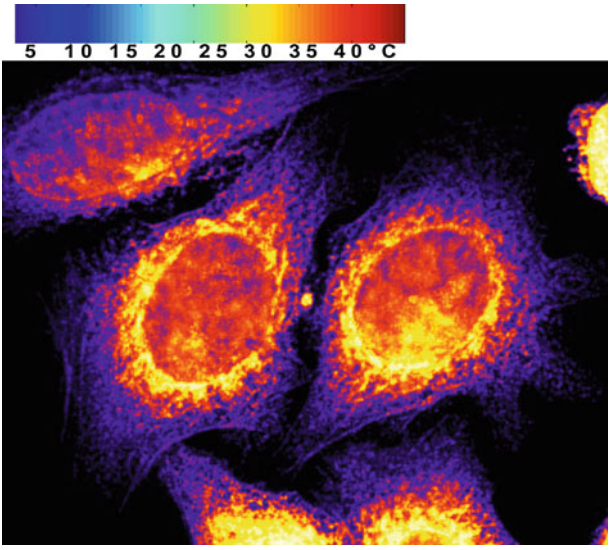

Fig. 2 Images of liver cells obtained from a thermal camera with a phase-contrast video microscope

Additional types of instrumentation beyond those included in the original setups need to be considered. The main goal of this work is to deal particularly with human bodies; therefore, the use of supplementary cameras is suggested, such as thermal imaging, for instance. As can be observed in Fig. 2, the characteristics of thermal imaging are not much better than those from optical cameras (see Fig. 1).

The complexity of cell tracking imaging and its demands in terms of speed and accuracy calls for better computer resources and capabilities (hardware, software, and time) in order to register every parameter, making it necessary to consider optical image processing. The key advantage of optics is the amazing speed in which it can perform image processing. One of the earlier works of optical imaging was the optical correlator, proposed by Vander Lugt [2].

Cross-correlation can be used in cell tracking in order to find a specific object (target) in a scene, based on the correlation peak size and location. In optics, the correlator have a simple experimental setup and it is able to perform image correlations in real time, with this being the essential difference when compared to computer correlation. A correlator cannot be used to solve cell tracking problems because of all the details that are present in a context or scene. These details will result in losing the cells after tracking them for just a few frames. However, this work proposes that if processing algorithms are applied beforehand on the cell images, *a priori* to the correlation operation, the detection sensitivity of the track cell process can be increased. Therefore, the aim is to increase the difference between correlation peaks of the positive target match as compared with the correlation peak of false positives on the scene. For that purpose, it has been decided to increase the border cells by applying processing algorithms on the images. The impact of several digital algorithms—such as K-media [3], binarization, dilatation, erosion [4], and other techniques such as nonlinear filters [5]—on the pre-processing methods have been successfully characterized on their own as well

as all of its possible combinations and sequences measuring the number of frames making it possible to track all cells in a context or scene.

In Sect. 2, the image features normalization, histogram equalization, image thresholding, and the morphological operations of dilation and erosion. In Sect. 3 some results are discussed and finally in Sect. 4, the conclusions are presented.

2 Processing Method

The images presented in this work were acquired from the frames of a video sequence kindly provided by Debeir et al. [6]. These video sequences were obtained by phase-contrast microscopy, and each frame is 700 by 500 pixels. Images are presented in shades of gray, with 256 levels of intensity. The lowest intensity value is black; therefore, with white being the highest intensity value. Figure 1 shows a frame taken from such a sequence.

As has been mentioned before, some pre-processing on the images needs to be done prior to the correlation operation. For a better understanding of the procedure, some of the operations used along this process are described. This process includes image normalization, image thresholding, gray level morphological gradient and its two morphological operators, dilatation, and erosion.

A histogram is a representation of the repetition of the image intensity values for each pixel and can be represented by the discrete function [7]

$$P(r_k) = n_k/n, \tag{1}$$

where $P(r_k)$ is the probability of the appearance of the gray level (r_k), n_k is the number of pixels with the r_k level, and n is the total number of pixels in the image. Schalkoff [8] has pointed out that brightness and contrast in an image are given by its histogram. Image normalization is performed by stretching the histogram by distributing all the possibilities of appearance for each pixel in the image through the scale of intensities (256 values). Image normalization can be mathematically expressed by

$$N(m,n) = \frac{I(m,n) - \min(I)}{\max(I) - \min(I)} \times \max(I) \tag{2}$$

$N(m,n)$ is the normalized image between the values of $\max(I)$ and $\min(I)$, and $I(m,n)$ is the image during the normalization process.

To simplify the tracking of the objects, the image contrast has been improved by equalizing the image, which has been defined as a uniform distribution of the image histogram. The histogram equalization makes it possible to obtain the maximum value of intensity in the image, which in this case is represented in white, allowing one to threshold the image in two levels. The image equalization is performed by

$$E(m,n) = 255 \times AccuHis[I(m,n)] \tag{3}$$

$E(m,n)$ is the equalized image, m and n are the pixels in the image, and *AccuHis* is the accumulated histogram of image $I(m,n)$.

Image thresholding is a process that allows the transformation of the image in a gray scale to a binary image. The main objective of this process is to improve the detection of the cell bodies. Image thresholding produces a uniform background referred to the equalized image.

Gray level morphological gradient refers to the difference between the dilatation and the erosion of a given image in order to enhance existing cell gradients. Dilation and erosion are two morphological operators that allow a better contour definition of the tracked objects. The dilatation is an image process that adds pixels to the boundaries of the tracked objects in the image, namely,

$$G(m, n) = F(m, n) + H(m, n) \tag{4}$$

$F(m, n)$ for $1 \leq m,\ n \leq N$ is a binary-valued image and $H(m, n)$ for $1 \leq m,\ n \leq N$, where N is an odd integer, is a binary-valued array being called a structuring element. $H(m, n)$ is a square array.

The dilation process can be applied to a binary image. A different array window can be used to analyze it, for example, 3×3 depending on the size of the objects in the scene. In this image, it is possible to observe that the objects to be tracked seem to be better defined. Finally, erosion is an image process that removes pixels from the boundaries of the tracked object. Erosion can be expressed as

$$G(m, n) = F(m, n) - H(m, n) \tag{5}$$

$H(m, n)$ is an odd size $N \times N$ structuring element.

From the description of the last two morphological operations, it seems that by applying dilation to one picture and then applying erosion, the original image could be recovered. But, in fact, as can be observed in Fig. 3, the consecutive use of these operations makes it possible to enhance the object borders as desired. This is one of

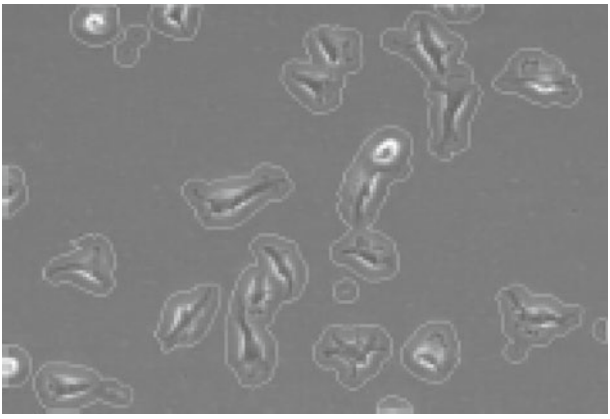

Fig. 3 Enhancement of border cells obtained after applying a sequence of the morphological operations (dilatation and erosion) over the image after some image preprocessing

the most visible results that can be obtained by doing image processing on the scenes, which are then ready to go into the optical correlator.

3 Results

We characterize some of these algorithms by measuring the time and the image quality obtained from a HP Pavilion dv6220la Notebook PC with AMD Turion 64 X2 processor technology TL-50 1.61 GHz and 9 GB of RAM memory. Normalization was the first image processing algorithm implemented. It visibly improves the image's quality, and it takes an average time of 0.002 s to process each image (1080 total). Equalization was the second image processing algorithm used. Image equalization takes longer because it completes the cumulative histogram of the image. Image equalization of each frame takes between 0.005 s and 0.007 s, depending on the change frequency present in the image. The change in the image's frequency is directly dependent on the number of cells in the image. If there are numerous cells in the image, the frequency components are larger. After the equalization process, a much better defined cell on the scene or context can be obtained. The use of morphological operators helps to better define the contour of every object in the image, but the time requirements increase. Even morphological operators can be considered as area operators; the time frame of this image processing can take several minutes.

Image dilation was applied using a five-pixel element while the erosion process was applied to eliminate "noise" generated by the image binarization. "Noise" is used to refer to any region that contains a cell mixed with a background, where most of the regions that contain cells are well defined.

Once the pre-processing is applied to the pictures, optical correlation is used in an attempt to obtain a well-defined peak, in which its position denotes the geometrical center of the cell that is being detected. If it is not obtained, a distance algorithm to the center of the target is applied. It is based on the Euclidean distance algorithm, and when the target is located in the area defined as a cell, the cell position for the following frame is the same as the current one. If the position of the peak is located in the area corresponding to the halo or a boundary, the target is also established in the same position for the next frame; but if the Dirac delta function is in an area that does not belong to the cell or closer to it (5 pixels), the minimum distance to the closest pixel with maximum intensity is calculated. A pixel with the maximum intensity is a boundary or the halo of that cell.

After comparing the minimum distance, that distance is doubled in the same direction to establish the peak in the region defined as a cell. This process guarantees that the peak will always be inside the cell or, at least, contained within the region in which good quality tracking can be obtained.

To face the mitosis problem, the properties of the cell were analyzed. During the mitosis, the cell reduces its size considerably, and in most of the cases, it decreases in size and obtains a semi-circular shape, and the complete area becomes white. Therefore, using a circular window of radius equal to 15 pixels around the Dirac delta function, it is possible to identify when a mitosis process is about to take place. Once mitosis is recognized in one zone, a new rectangle with the same features is created.

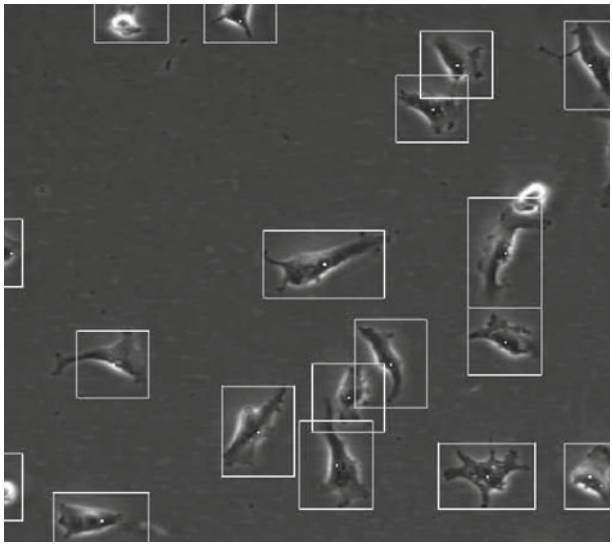

Fig. 4 Cell image after pre-processing. *White points* pinpoint the geometrical center of the cells, and the *rectangle* denotes the object that is classified as the cell that is to be tracked

The new rectangle is set 15 pixels above and 15 pixels below the original position to make sure that each one is going to follow different targets (and not the same one). To identify the target in the scene, both images are correlated (image and target). The result of this correlation is obtained in 2.547 s. The correlation time depends directly on the size of the image. The average time for an image autocorrelation of 256×256 pixel size is 10 min. In this case, the initial correlation of two images (the 512×512 px scene and the 80×60 px target) was done in just 3.2 s.

After finishing the pre-characterization stage, interesting results were obtained. In Fig. 4 the initial frame, where targets were identified and were ready to be tracked, is presented. The targets were automatically detected after the application of pre-processing algorithms and the execution of logic operations between the original image and some cell pictures randomly taken from other frames. In order to start the task in an automated way, a logic operation "AND" between scene and one target was carried out, and then the logic operation "OR" was performed, in order to clear the noise produced as a result of the first logic operation. Results show that 15 from the total of 18 cells in the scene were detected. Subsequently, the detection of all cells in the scene was carried out by another logic operation "AND" between the previous result and a cell target. To reduce the regions of interest, a median filter was implemented to "smooth" the image. All cells were detected and, later, it was possible to track every cell in the scene with no loss.

4 Conclusions

The functional and reliable operation of a cell tracking optical digital method that shows an attractive perspective for a setup beyond the lab conditions has been

demonstrated. It has been found that image normalization improves the definition of the cells, but image equalization increases the contrast between the cell soma and the background, making the cell easier to detect. This reduces the localization of false targets in the correlation. Binarization was the fastest method to discard the background considering only the cell. The morphological gradient worked as the faster border detection method, furthermore, it was the best algorithm to eliminate the "garbage" or small objects presented in the image background. Dilation does not produce good results by itself because it increased the background, making the cell localization more difficult. The use of one of the morphological gradient operators alone should be discouraged. However, the importance of the pre-processing should be reinforced because, without it, the tracking algorithm lost its target on an average of 120 frames. By using normalization exclusively, the target was lost in about an average of 170 frames. Results have shown that the detection of the cell is not directly dependent on the position of the correlation peak that was obtained, but on the definition of the border of the cell and that of the halo. It can be therefore concluded that the main goal of the image processing task (previous to actual cell tracking) must focus on the enhancement of the cell borders.

A numerical cross-correlation was implemented in order to carry out the cell tracking task. Even if it can be considered to be time consuming, it was the best option since the time requirements were solved by implementing an optical correlator setup. Although the correlation tasks were performed in numerical or optical versions, there was concern about improving the robustness of the method and to reduce the number of false positives. In order to do that, a digital nonlinear filter [5] to be used in the correlation operation was implemented. It ponders the (frequency) information of the objects to be correlated in a different manner. It was found that the nonlinear filters result in faster performance because they are smaller in size than the linear filters. They also increase the sensitivity of the method because most of the time the correlation peak is located in the cell body. Specifically, the kth law for $k = 0.4$ presented the best performance of the filter in the correlation and $k = 0.1$ improves the detection of the cell with a finer peak. Nonlinear filtering helps in the resolution of some targets during the process. As the value of k in the kth law is reduced, the distance between true peaks and false peaks is increased. Because of the possibility of storing previous information, nonlinear filtering also helps detect cells under different positions and it is invariant to the rotation and size.

Even though this work was done experimentally using human umbilical vein endothelial cells (HUVEC), the method ensures a robust tracking method for frame cells based exclusively on the correct detection of the first cell, without considering its features. The best results for the proposed cell tracking method, considering time and cell localization, were obtained through the implementation of nonlinear filtering.

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