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Shape Characterization on Phase Microscopy Images using a Dispersion Indicator: Application to Amoeba Cells

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Abstract

Amoebiasis is due to mobility and shape changes of amoeba cells and can cause 70.000 deaths year. Amoeba cells can be observed on phase microscopy images, which are in general very poor in visual quality. Classical shape characterization methods do not perform well on such images. We present a new method of shape characterization of cells based on their skeleton extraction on grayscale images. The grayscale cells are segmented by computing a dispersion indicator. A signed sequential Euclidean distance map is elaborated and a method of extraction of the Euclidean skeleton is adapted to the grey level cells. The method is applied to biological cells in order to characterize shape changes of amoebas.

Keywords: grayscale images, skeleton extraction, shape characterization, adaptive segmentation, dispersion indicator.

Introduction

Amoebiasis is a disease due to mobility and sudden shape changes of amoeba cell membrane and can cause up to 70.000 deaths per year.

The notion of skeleton plays a major role in biological shape analysis[1]. It is introduced by Blum[2] and is the subject of an abundant literature[3,4]. Shape characterization using skeleton extraction is important for many applications as character recognition; data base analysis, or shape analysis in medical imaging, which leads to important decisions in diagnosis of some diseases. Segmentation is often applied before skeleton extraction. Zimmer and al. applied active contours segmentation to shape and motion analysis of amoeba. The method uses a binary image morphology combined with substitution of 3x3 pixel configurations, which represent an approximation of Level Sets Method. The approach allows automatic topological changes[5]. Another method has been applied to amoebas shape modification using a generalized diagram of Voronoï to extract their skeleton by computing their bisector network[6]. The method is an exact and fast one. However, it is know that exact algorithms are not able to preserve the shape of details of cells, as only some critical points are detected and connected, to define bisectors[7]. In fact, exact algorithms are used to compute an approximation of the bisector function. More than that, the above method uses binary images obtained by segmentation and by the way, after the loose of data about the shape. It is then important to study the shape changes of cells using directly grey level images obtain from phase contrast microscopy to extract quantitative data needed by biologists. Our method has been applied to amoeba cells (figure 1) as they can cause many diseases. For that, we present a method of amoebas shape deformation detection using direct skeleton extraction on grey level images for preserving contours data.

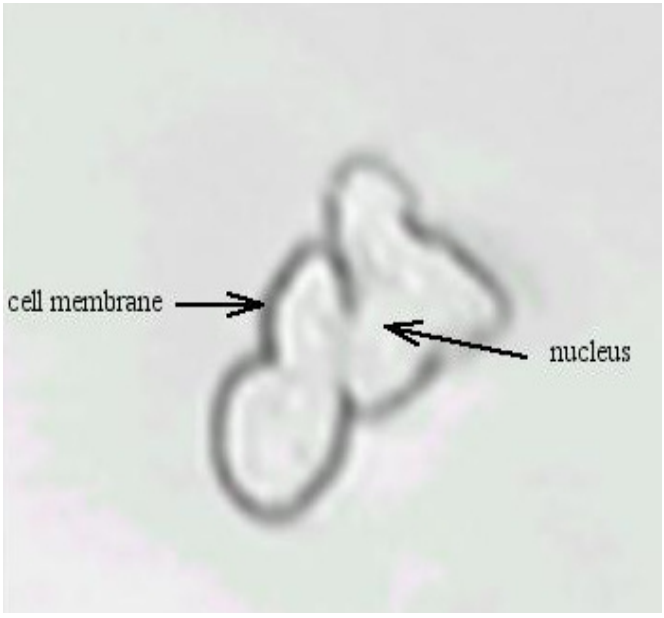

Figure-1
Structure of an amoeba

Section 2 presents the material and the method we have developed. The results of the method are presented in section 3. Sections 4 and 5 present discussions, conclusion and future work.

Material and Methods

Material: The amoeba pathogenic phenotype is characterized by mobility and membrane shape deformation. Such mobility and deformation can cause many diseases such as amoebiasis, which is a gastrointestinal infection that may or may not be symptomatic and remain latent in an infected person for several years. This disease can cause up to 70.000 deaths year around the world from sudden amoeba shape change. Another disease is brain-eating amoeba which doesn't happen often. However, most summers, several people suffer sudden, tragic deaths from it. Other diseases are amoebic keratins, skin lesions and sinusitis caused by amoeba. As we are interested in sudden deformations of amoeba cells, we use images from phase contrast microscopy.

Phase contrast microscopy, is a technique utilized to produce images of transparent specimens. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes can be observed and recorded[8]. An example of images we have used is shown in figure 2. We first notice that the image is of very poor visual quality and by the way, classical segmentation techniques do not perform well on it.

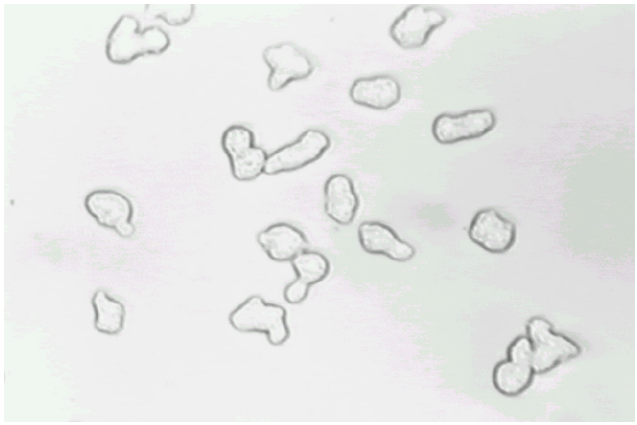

Figure-2
Phase microscopy image of amoeba cells

The method we have developed consists in adapting a signed Euclidean distance transform algorithm (SEDT)[9] to grey level images. The distance transform is defined as a mathematical operator which computes the distance map of a binary image[10]. Distance transform is an image *DT* obtained from the original image *I*, where each pixel value is the nearest distance from this to the object *F*.

$$DT(y) = \min_{x \in F} \left\{ d(x,y) \middle| y \in I \right\} \quad (1)$$

Numerous applications of the distance transform are known in computer vision, image analysis, pattern recognition, shape analysis, feature detection techniques. It can also be associated with the shortest-path algorithm, medial axes or skeleton extraction and other image segmentation techniques.

There are many ways to compute the distance transform (DT). The trivial method which is valid for all types of distances is called the brute force algorithm. This algorithm computes for each background pixel its distance to all the object pixels and out of these it selects only the minimum. Instead of the brute force algorithm a lot of scientists have tried to solve this algorithm in a faster way. Costa et al. in their survey[11] makes a classification and compare the most important algorithms. They classify the existent algorithms in three categories. The propagation algorithms proposed by Eggers[12] and Cuisenaire[13] compute the distance of the background pixels, starting from the boundary pixels of the object, from the closest to the farthest. The raster scanning algorithms[14], compute the distance transform algorithms supposing that a value of a pixel can be computed from the value of distance of its neighbors.

Given an object in an image, each point in the object is assigned a value equal to its distance to the nearest boundary of the object. This process of computing the DT produces a distance map whose ridges projected back onto the image plane generates skeleton structures. For a grayscale image, a DT cannot be computed directly, since the object boundary locations are not known. For some types of images, however, ridges of the intensity landscape tend to be at the center of anisotropic grayscale objects. Thus, ridges of a distance map or an intensity landscape are useful skeleton descriptors of a shape[15-17].

Skeleton extractions on binary images: Let F be the planar shape of a cell in an image I, C the contour of F and S (F) the skeleton of F. Let P be a point in F. If it exist a point Q belonging to C, outside of the maximal disk of center P at a distance D and called residual distance[18], then P belongs to S(F). In order to extract a Euclidean skeleton based on a connectivity criterion, an algorithm is presented in as follows[19]:

For a given I, compute the signed sequential Euclidean distance map (EDM).
For any P of I belonging to F:
Let x_i be a neighbor of P in the 3x3 kernel.
DO
Determine Q=SEDT (P) the closest point to P from EDM
Find Q_i the corresponding point of Xi such that
Q_i = SEDT (x_i), i=1,2,…,8
To obtain an exact connectivity, the following conditions must be verified:

$$D^2 = \left| Q_i - Q \right|^2 \geq \rho \quad (2)$$

$$\left\{ \left| Q_i \right|^2 - \left| Q \right|^2 \leq \max(X(Q_i - Q), Y(Q_i - Q)) \right. \quad (3)$$

In equation (2), D is the distance between two points of C closest to P and ρ is the minimum threshold of D^2.

In equation (3), X () and Y () are the coordinates of considerate expressions.

If any couple (Q_i, Q) satisfies criterions (2) and (3) then the corresponding P is considered as belonging to S (F).

Figure 3 is an illustration of the algorithm on binary images.

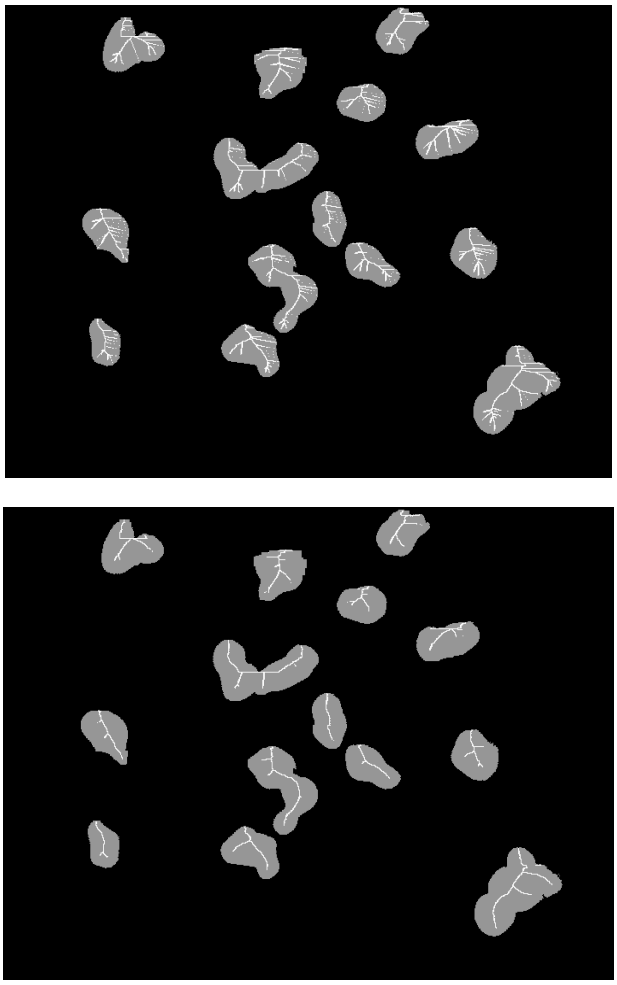

Figure-3
Skeleton extraction on binary images

Skeleton extractions on grey scale images: By observing figure 2, we can notice that pixels of the background of the image present a relative homogeneity compared to pixels of the cells. By this way, we define the dispersion measure. This measure is used for an adaptive segmentation as presented bellow. Let $\bar{x}$ be the central tendency in the observation window of size N and dI the dispersion indicator of the window. We compute dI as follows:

$$dI = \left[\frac{1}{N} * \sum_{i=1}^{N} \left(X_i + \bar{x} \right) \right]^{1/2} \quad (4)$$

X_i is the grey level of pixel i in the window of size N.

We assume that for a given value of dI, the central pixel of the window, belongs to the background of the image and for another value of dI, pixel belongs to a cell.

The use of dI for cells segmentation is a proof that we can obtain grey level image of cells for skeleton extraction as shown in figure 4

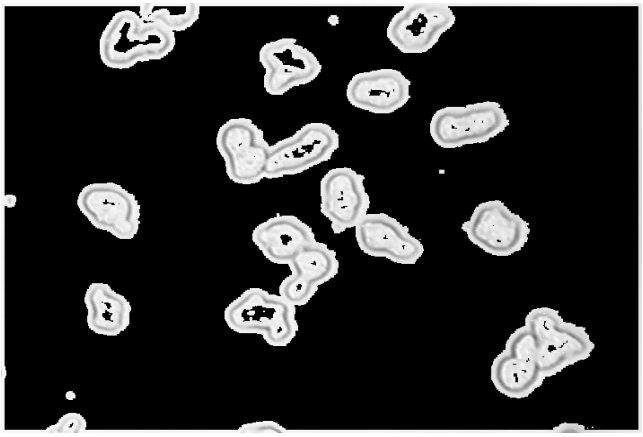

Figure 4
Cells segmentation on grey level images

We can notice that the segmentation is not entirely satisfactory because the pixels of cells are not discriminated by dI entirely. To compensate this drawback, we superimpose the original grey level image on the dI image to "close the gaps". Figure 5 is an illustration of the gap closure operation.

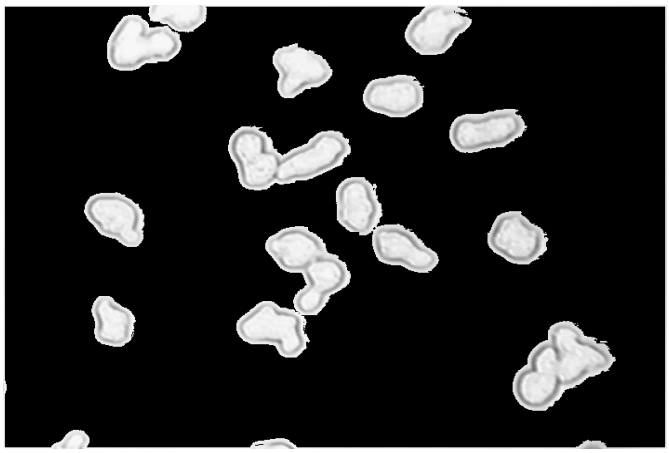

Figure 5
Enhanced cell segmentation

From images obtained by the grey level segmentation, we can extract the cells skeletons as if all pixels belonging to the background have an uniform value different from the values of cells. The new EDM is computed from images of dI and we apply equations (2) and (3) to get the grey level cells skeletons.

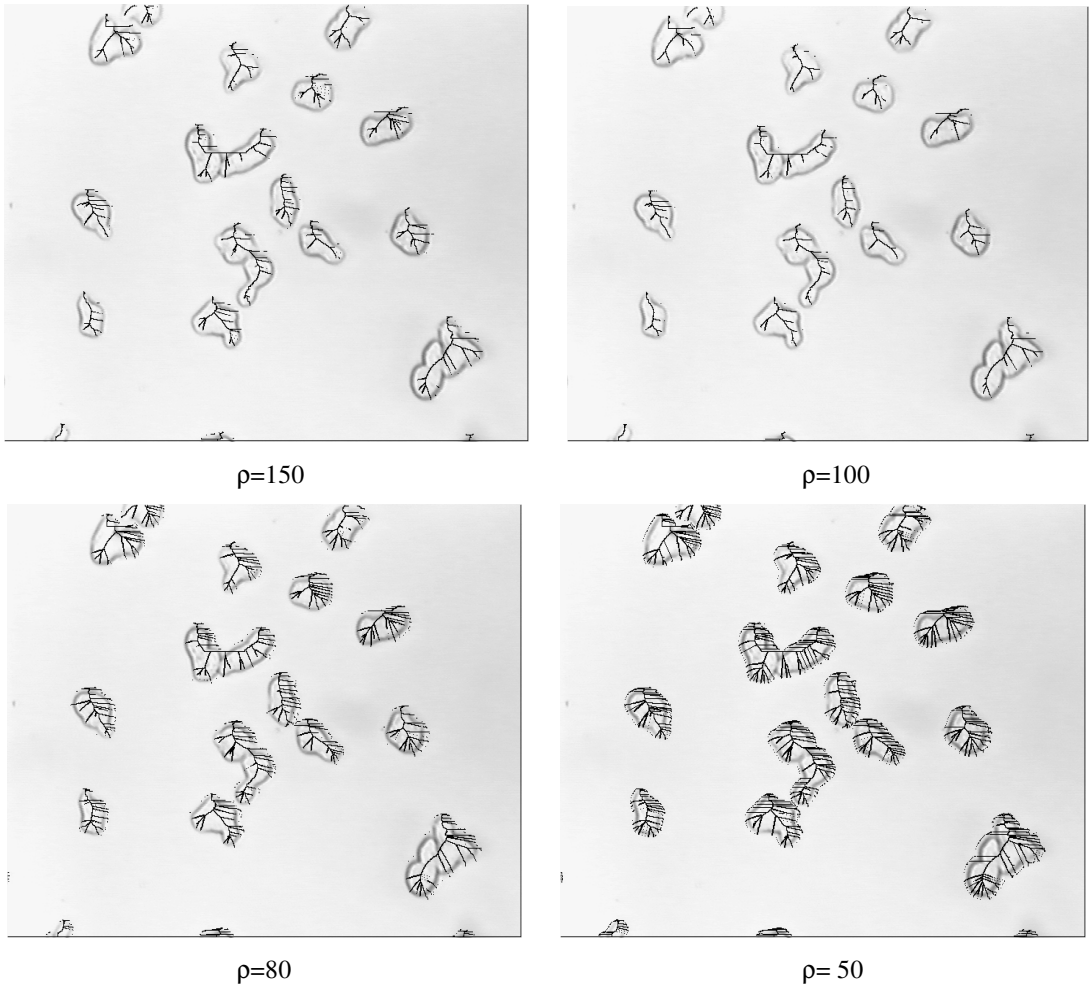

Figure-6
Grey level skeleton with various values of precision parameter ρ.

Results and Discussion

Results: The aim of this paper is to present a method to study shape changes of amoeba cells using grey level images obtained from phase contrast microscopy. However, one knows that exact skeleton extraction methods using binary images obtained after segmentation, are not able to preserve the shape of details of cells. We introduce a precision parameter ρ to overcome this drawback. The precision parameter is used to decide the level of details we would like to observe i.e. the user of the method decide how exact he wants the skeleton to be. From our empirical results, the lower the value of ρ is, the higher the skeleton details are. This is illustrated in figure 6.

In the figure, the value of ρ ranges from 150 to 50. Figure 6a shows results with ρ=150, figure 6b shows results with ρ=100, figure 6c shows results with ρ=80 figure 6d shows results with ρ= 50.

Discussions: Shape characterization using a dispersion indicator gives satisfactory results as shown above. By avoiding the loose of data due to classical segmentation methods, we can obtain a Euclidean skeleton based on a connectivity criterion on grey level cells.

The segmentation technique we have used, by superimposition of images need however to be improve.

Conclusion

We have shown that amoeba cells can be characterized using grey scale image obtained from a segmentation approach based on a dispersion indicator. The method uses a Euclidean skeleton algorithm based on a connectivity criterion.

Our further work will concern improving the segmentation method and pursuing the characterization approach in 3D.

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