



Human Dendritic Cells Segmentation Based on K-Means and Active Contour

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Abstract. Dendritic cells play a fundamental role in the immune system. The analysis of these cells in vitro is a new evaluation technique of the effects of food contaminants on the immune responses. This analysis that remains purely visual is a laborious and time-consuming process. An automatic analysis of dendritic cells is suggested to analyze their morphological features and behavior. It can serve as an assessment tool for dendritic cells image analysis to facilitate the evaluation of toxic impact. The suggested method will help biological experts to avoid subjective analysis and to save time. In this paper, we propose an automated approach for segmentation of dendritic cells that could assist pathologists in their evaluation. First, after a preprocessing step, we use k-means clustering and mathematical morphology to detect the location of cells in microscopic images. Second, a region-based Chan-Vese active contour model is applied to get boundaries of the detected cells. Finally, a post processing stage based on shape information is used to improve the results in case of over-segmentation or sub-segmentation in order to select only regions of interest. A segmentation accuracy of 99.44% on a real dataset demonstrates the effectiveness of the proposed approach and its suitability for automated identification of dendritic cells.

Keywords: Dendritic cells · Segmentation · K-means · Active contour

1 Introduction

Mycotoxins (food contaminants) generated by different types of moulds are the cause of severe food poisonings that affect humans. Indeed, mycotoxins are recognized for their hematotoxicity and immunotoxicity. The dendritic cells make the link among these two poisonousness. These cells are one of immune cells that are produced by stem cells, within the bone marrow. They appear as the initiator cell of the primary immune response, capable of stimulating the proliferation of naïve T lymphocytes. The highlighting of the key role of dendritic cells in the induction of immune responses of primary

types conducted to a multiplication of researches on these cells. Several works show that dendritic cells can be potentially a target of toxics and thus inhibit the primary immune response [1]. Taking into account the function provided by the dendritic cells, disturbances in the maturation of dendritic cells could be responsible for immunodepression due to a cytotoxic effect. Therefore, it seems essential to identify and assess adverse effects of mycotoxins on human dendritic cells. Recently, with computer technology advances and automation of diagnostic systems, image analysis techniques gained more attention as they are able to detect, characterize and classify cells in microscopic images. The segmentation is a primary and essential step in the process of image analysis. It is one of the most main tasks, but also one of the most difficult to achieve. Thus, the choice of segmentation operators in microscopic imaging remains a complex problem. On one hand, the observed biological structures are extremely variable (method of preparation, used magnification, natural variability, etc.). On the other hand, there is no universal segmentation method for all types of images. Currently, few works in image processing and analysis were conducted specifically on dendritic cells [2]. However, we can find a rich literature on the segmentation methods of cellular microscopic images in general. An assortment of methods is proposed in the literature for segmenting microscopic blood cell images. According to Díaz and Manzanera [3], segmentation techniques in this field can be roughly divided into two main categories: the region-based and the boundary-based methods. The first category comprises thresholding [4], region growing [5], watershed [6] and clustering based methods. Clustering algorithms are classified into two general classes: supervised approaches (e.g. support vector machine [7]) and unsupervised models (such as k-means [8], fuzzy c-means [9]). Boundary-based methods include active contours approaches [10] and edge detection techniques [11].

The remainder of this paper is planned as follows: in Sect. 2, we define our methodology describing the different segmentation steps. In Sect. 3, we present and discuss experimental results of the proposed approach on a collected cell image dataset. Finally, we conclude this work in Sect. 4.

2 Proposed Approach

The major difficulty in the images to be processed is related on the complexity of cell structures to be detected. Indeed, it remains sometimes difficult for the human expert to distinguish the dendritic cells (structures to be recognized) from nearby tissues that could not be separated from the culture medium (Fig. 1). Furthermore, unimodal grey-level histograms (Fig. 2) show the difficulty of separating the cells from the background. In this work, we propose an entirely automated image analysis approach for the segmentation of dendritic cells to be used for evaluating toxic impact on dendritic cells. The flow chart of our automatic dendritic cells segmentation is presented in Fig. 3. Our technique contains mainly three steps. The succeeding subsections provide the details of these steps.

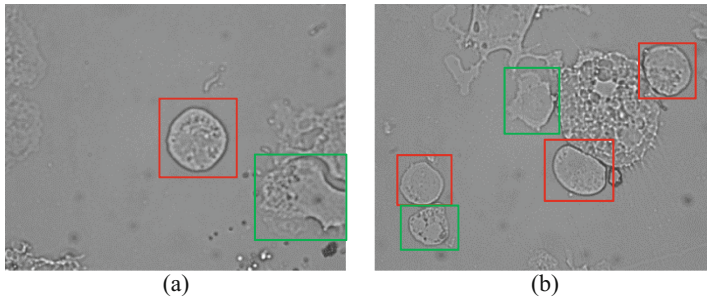


Fig. 1. Examples of microscopic dendritic cell images illustrating the difficulties to differentiate dendritic cell (surrounded by red box) from some parts of tissues (surrounded by green box). (Color figure online)

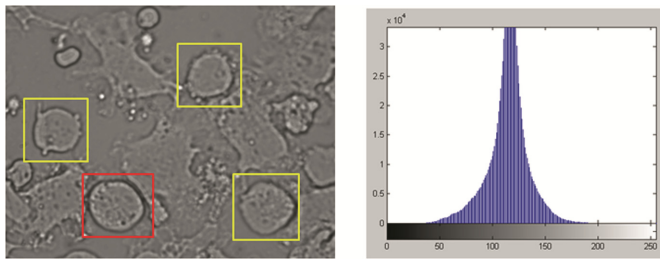


Fig. 2. Example of microscopic image with immature cells, some of which (surrounded by yellow) show some spontaneously developed dendrites, and the associated histogram. (Color figure online)

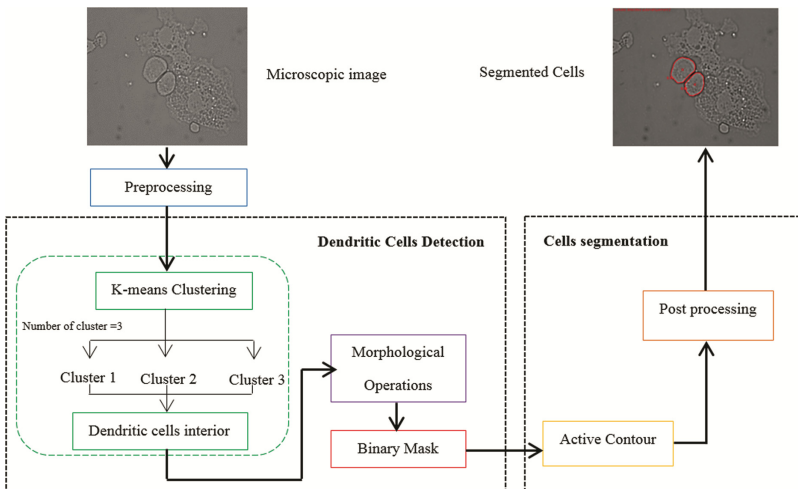


Fig. 3. Flow chart of the proposed segmentation procedure.

2.1 Image Preprocessing

The term of preprocessing is used for the processes that improve the acquired image to favor the subsequent analysis steps. Usually, this step consists to bring out the pertinent information and to reduce the noise. Numerous techniques are used in the preprocessing step such as illumination normalization, histogram equalization, contrast stretching and color normalization. Image smoothing is one of the most frequently procedures used for pretreatment. Indeed, different types of filters like average filter, median filter and Gaussian filter are used to preprocess the images. A non-linear anisotropic diffusion based on the formalism of the partial differential equations (PDEs) could be used to eliminate the noise while preserving the edge of cells. In this study, the Coherence Enhancing Diffusion (CED) model initially introduced by Weickert in [12] and subsequently developed in [13] was used to remove the noise without degrading the information about the dendritic cell edges. For more details on this method, we recommend to the reader to refer to the following reference [14].

2.2 Dendritic Cells Detection

In this stage, we first use K-means clustering method to associate every pixel of filtered image to one of three clusters in these microscopic images. These clusters correspond to cells interior, cells contour and halo artifacts. Then, we select the class assigned to the dendritic cell interior to support the following steps. Once the dendritic cells are located, some morphological operations such as opening and closing are used to obtain the binary masks that give an initial guess of the contours of the dendritic cells. Figure 4 shows steps of the initial contours identification on an example image. The contours will be used to initialize the active contour algorithm in the next stage.

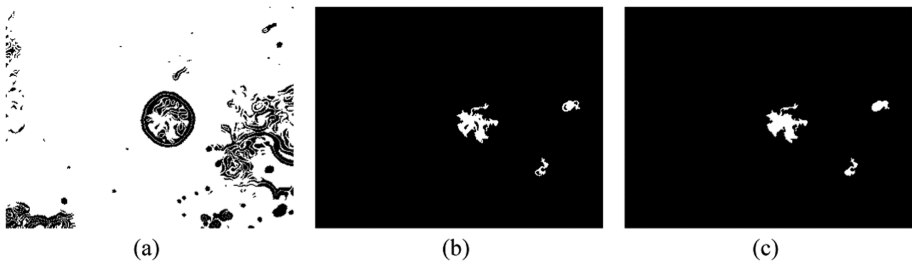


Fig. 4. Illustration on the image given in Fig. 1 (a) of the steps used to initialize the active contour algorithm: (a) initial segmentation by K-means, (b) clearing border and removing small objects, and (c) binary mask.

2.3 Cells Segmentation

We have obtained in the first step binary masks that give the regions of the dendritic cells. These masks define the position of the initial contours used for contour evolution to segment all cells in the microscopic images. We propose to use the Chan-Vese model

described in [15] as a region-based active contour model. We have specified 200 iterations to achieve desired segmentation results. Morphological operations are needed in order to refine results of active contour algorithm. Our microscopic images do not only contain dendritic cells; we can find also nearby tissues as shown in Fig. 1. Since these unwanted components have similar characteristics with dendritic cells, nearby tissues could be found in the binary mask obtained after the initialization step. However, from this illustrated culture sample, it is clear that the shape of dendritic cells is different from the other tissue components, which will allow to filter the segmented structures. More precisely, we exploit the circularity shape feature to remove the incorrectly segmented objects from the results. The circularity criterion is defined as:

$$\text{Circularity} = \frac{4\pi\text{Area}}{\text{Perimeter}^2} \quad (1)$$

Experimentally, we have selected the value 0.36 as a threshold to eliminate the unwanted segmented regions. Figure 5 illustrates some results achieved by the steps of the cells segmentation phase.

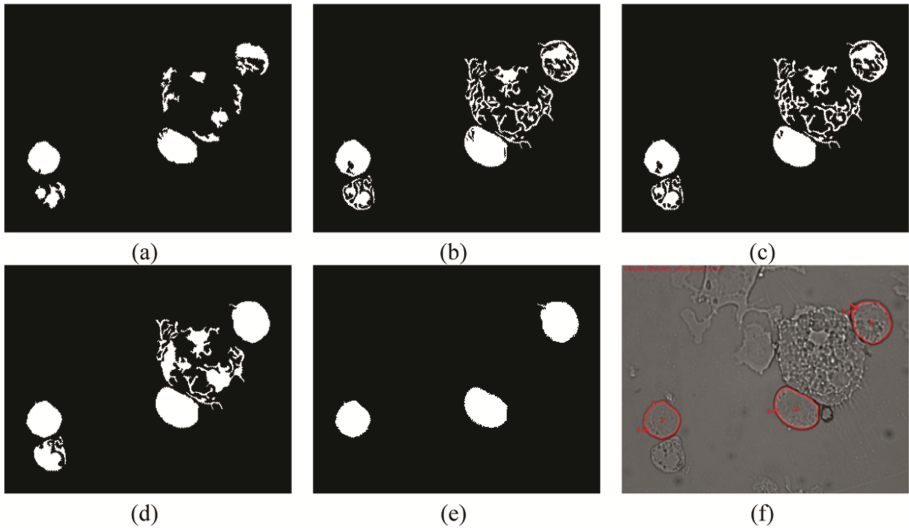


Fig. 5. Cells segmentation steps on the image given in Fig. 1 (b): (a) initial binary mask, (b) result of the region-based active contour algorithm, (c) closing, (d) filling holes, (e) selected dendritic cells, (f) segmented cells boundaries superimposed on the original image.

3 Experiments and Discussion

For the experiments, a database of 26 images was used to evaluate the proposed algorithm. We consider a dataset consisting of gray-scale images with resolution of 1344*1024 pixels. Some results obtained by our developed approach as well as the cells annotation established by a biological expert are given in Fig. 6. This figure illustrates

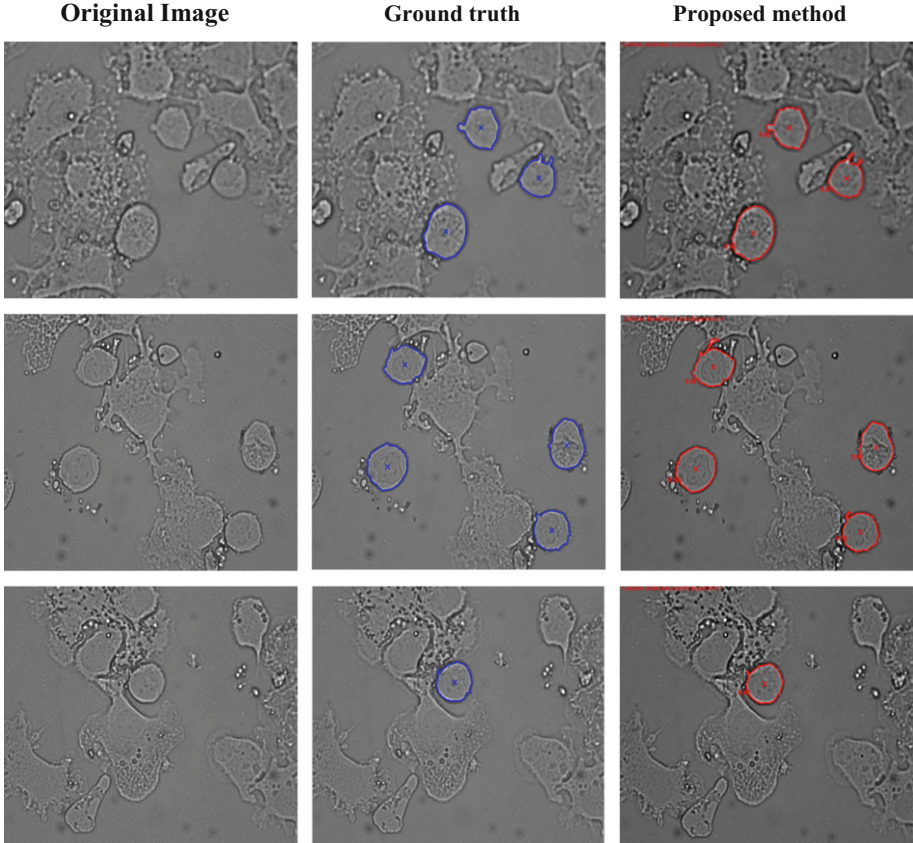


Fig. 6. Comparison of visual results of automatic and ground truth segmentation: original image (first column), ground truth (second column) and the proposed method (third column).

that our proposed method identifies both immature and mature dendritic cells correctly compared to the ground truth segmentation. We have already proposed a k-means based method to segment dendritic cells [16]. We will compare results obtained by the new proposed method to those obtained by only k-means to stress the benefit of the active contour step. In order to evaluate the accuracy of obtained segmentation, we compare the boundaries of all cells annotated by biological expert to the results obtained with the two automatic segmentation methods. For this purpose, we use four performance measures: accuracy (AC), specificity (SP) (also called true negative rate: TNR), sensitivity (SE) (also called recall: RC or true positive rate: TPR), precision (PR) (also called positive predictive value: PPV) and F-measure (FM). These measures are calculated according to the following equations:

$$\text{Sensitivity} = \text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \quad (2)$$

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{FP} + \text{FN} + \text{TN}} \quad (3)$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \quad (4)$$

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \quad (5)$$

$$\text{F - measure} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (6)$$

where TP (True Positive) denotes the number of pixels correctly identified as a dendritic cell, FP (False Positive) indicates the number of pixels incorrectly identified as a dendritic cell, TN (True Negative) represents the number of pixels correctly identified as a non-dendritic cell and FN (False Negative) denotes the number of pixels incorrectly identified as a non-dendritic cell.

Quantitatively, the results show that the proposed algorithm is efficient in detecting dendritic cells from microscopic images with accuracy percentage that surpasses 99%. Table 1 shows the percentages of the accuracy, the specificity, the sensitivity, the precision and the F-measure calculated for the entire image database. Thus we conclude that the proposed technique offers very good results for the identification of the dendritic cells. The technique based on k-means clustering [16] structures the image into two classes but it still suffers from the difficulty to identify automatically the cells cluster. In this paper, we have used a three classes clustering to automatically detect the interior of the cells. The active contour step is then needed to detect more precisely the cells than using only k-means. Hence, the proposed method show improved performance in well defining the edges of cells that can be clearly seen in Fig. 7. In particular, this benefit of the new method is more relevant in the case of cells with dendrites (i.e. lot of details on the contour). Since our ultimate goal is to classify the detected cell as immature or mature according to the shape, the active contour is more efficient as it gives entire and precise cells edges leading to a better interpretation of the morphological modification of dendrites during maturation. From the Table 1, we remark some cells are not detected by the proposed method. These cells are not present in the mask obtained by the step of the initial contours identification because of the lack of contrast of these cells against nearby biological tissue. However, the detection of these cells can be achieved by readjusting some parameters we want to be generic for all cells in the database.

Table 1. Quantitative comparison results of segmentation performance: sensitivity (SE), specificity (SP), accuracy (AC), precision (PR), f-measure (FM).

Manual count by an expert	Auto count	SP (%)	SP (%)	AC (%)	PR (%)	FM (%)
53	49	90.71	99.90	99.44	98.40	94.00

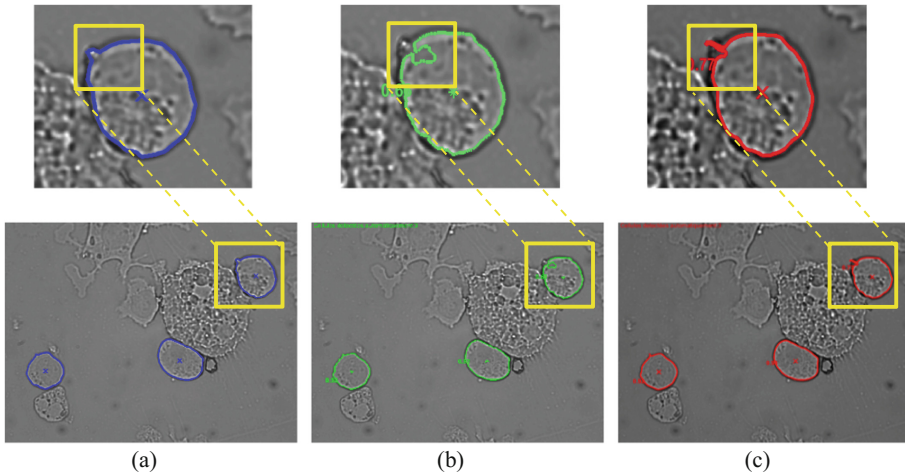


Fig. 7. Comparison results between the ground truth segmentation (a), k-means based approach (b) and proposed method (c). The cell edge is more precise by the proposed method (see the zoom in the square).

4 Conclusions

The main objective of this work was to develop an automatic technique of segmentation of dendritic cells microscopic images in order to facilitate the evaluation of toxic impact for biologists. In this paper, we have proposed an entirely automated image segmentation approach for evaluating toxic impact on dendritic cells. In our method, k-means clustering and Chan-Vese region-based model are combined to segment microscopic dendritic cells images. The experiment results show that the proposed technique offer an accurate results for the identification of the dendritic cells. In future work, we plan to address the classification issue of the dendritic cell as immature or mature by exploiting the deformation (expansion) of the cell contour.

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