Adaptive Automatic Segmentation of Leishmaniasis Parasite in Indirect Immunofluorescence Images

F. Ouertani, H. Amiri, J.Bettaib, R.Yazidi and A.Ben Salah

Abstract—**This paper describes the first steps for the automation of the serum titration process. In fact, this process requires an Indirect Immunofluorescence (IIF) diagnosis automation. We deal with the initial phase that represents the fluorescence images segmentation. Our approach consists of three principle stages: (1) a color based segmentation which aims at extracting the fluorescent foreground based on k-means clustering, (2) the segmentation of the fluorescent clustered image, and (3) a region-based feature segmentation, intended to remove the fluorescent noisy regions and to locate fluorescent parasites. We evaluated the proposed method on 40 IIF images. Experimental results show that such a method provides reliable and robust automatic segmentation of fluorescent Promastigote parasite.**

I. Introduction

A titer is used to estimate the level of antibodies in a serum, it is expressed as the inverse of the highest dilution at which the fluorescence is still detectable. The manual measurement is a process obtained by logarithmic dilutions. In addition to being time consuming, it suffers from the usual problems in biomedical field, such as: (1) It requires a lot of manual work and (2) the results are subjective due to their dependence on the experience and on the biologist skill. Reliable automatic serum titration is therefore in great demand. The objective is to correlate the titer of a serum with the fluorescent parasites available in a fluorescent image. We are interested particularly in images derived from IIF technique applied on Promastigote parasites of Leishmania.

The automation of the titration task supposes in this case a beforehand IIF automation. This latter can be divided into three steps: (1)a fluorescent Promastigote form of the parasite detection, (2) a pattern classification and (3) computer aided diagnosis phases.

This paper deals with the first phase by presenting an efficient method for detecting and locating fluorescent Promastigote form in IIF images.

Several studies have been proposed for segmenting fluorescent images [4,7,9,10]. In [1], Du et al. developed and evaluated the performance of unsupervised data mining techniques in cell image segmentation. They compared k-means clustering, Expectation Maximization (EM), Otsu´s threshold method, and Global Minimization by Active Contour (GMAC). Huang et al. proposed in [2] two techniques for distinguishing between fluorescent cells and the background. The first one combines wavelets and bedirectional search, the second includes k-means clustering and Principal Component Analysis (PCA).

In [3], Bharati et al. presented a comparative study of k-means and Fuzzy C-Means clustering techniques for their performance in color images segmentation. Huang et al. [4] presented a classification based on connected regions for the segmentation of IIF images. Their method coupled Anisotropic diffusion with either Canny edge detection in case of sparse region cells images or Otsu´s thresholding in case of images with mass region cells.

From the above descriptions, it turns out that k-means and Fuzzy C-Means clustering can perform robust segmentation. Otsu's method cannot always guarantee a good segmentation result, mainly when performed on images with low contrast. EM performs weakly compared to the other techniques.

In our work, we have adapted an approach including distinctive, yet complementary, techniques from those previously mentioned for the fluorescent parasite detection.

The paper is organized as follows. Section II presents a brief description of the data acquisition. In Section III the proposed method for fluorescent parasites segmentation is introduced. Section IV presents the results and the evaluation of the proposed segmentation method. Conclusions and future works are presented in Section V.

II. Data acquisition

To be able to correlate the titer of a serum with the amount of the fluorescent parasites in an IIF image, we have to ensure automated form of the parasite recognition in IIF images taken from different dilutions of the serum. In this work, we are interested in the form of Promastigote parasites

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of Leishmania. We have performed IIF assay tests to detect anti-Leishmania antibodies in a serum of a naturally infected dog. We have considered the dilution of 1:800 as the cutoff point. For each dilution we have collected an IIF image with an optical fluorescence microscope Leica DFC 425 at 40-fold magnification. In each IIF image, we aim at detecting and locating the Promastigote parasite whose form is described in Fig. 1. This parasite has a streamlined body of 15 to 25 µm length and 1 to 4 µm width. It is extended by a flagellum.

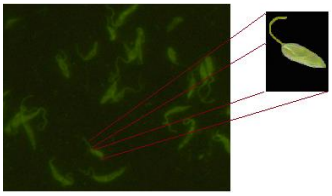

Figure 1. The Promastigote form

III. Overview of the Proposed Approach for Segmentation

The proposed method for the segmentation of fluorescent Promastigote parasites in the IIF images consists of three principle stages: (1) a color based segmentation whose aim is to extract the fluorescent foreground and separate it from the background. It makes use of the k-means clustering to classify the pixels, (2) the segmentation of the image representing the fluorescent cluster by means of the Otsu thresholding algorithm followed by some morphological operations; and (3) a region-based feature segmentation, which is intended to remove the fluorescent noisy regions and to locate fluorescent parasites.

In what follows, I_{rgb} denotes the color-scale input image for the proposed method of segmentation.

B and F refer, respectively, to the background and the foreground. A description of the procedure is shown in Fig. 2.

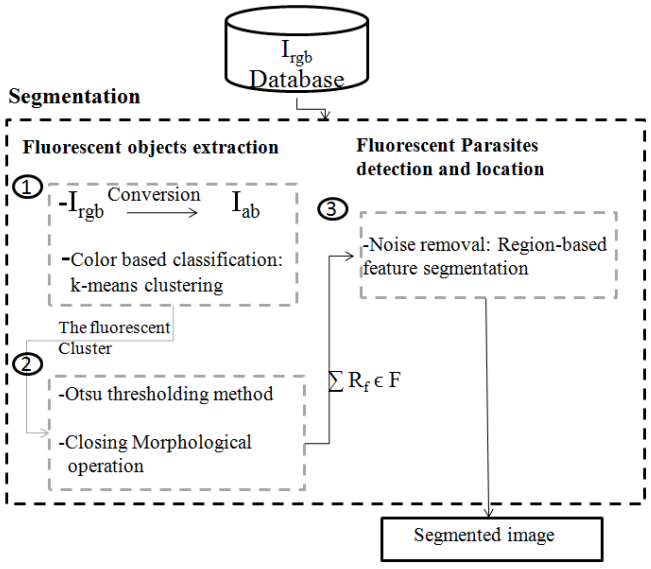

Figure 2. Overview of the proposed approach for segmenting fluorescent images

A. Color-based extraction

In IIF, a non optimal dilution of the primary antibody or a very strong concentration of the fluorochrome can cause problems of noise noted at the moment of microscopic observation either in the image background or as fluorescent particles. In this paragraph, the background noise is removed as we separate the foreground from the image. As for the foreground noise, we will present a solution for its removal in the next subsection.

Some proposed methods, as in [13] extract the green channel from the original RGB image (since they are stained by a green dye) and use it as input for the segmentation. In our approach, we have performed a color-based segmentation to extract the fluorescent objects. The advantage of this method, besides separating the foreground and the background, is that we get rid of the staining noise.

In what follows is a description of this step.

If we ignore variations in brightness, our fluorescent image as in Fig. 3 a), shows two main colors: the black one that constitutes B, and the green one that forms the fluorescent objects.

The light green represents noise in the background and the dark green depicts some artifacts in the foreground.

Thus, in order to separate the fluorescent objects without considering the luminosity factor, we first convert the image to L*a*b* color space. The three coordinates of L*a*b* represent the lightness L*of the color, its position between red and green a* and its position between yellow and blue b*.

As the whole color information is contained in the a*b* space, we denote I_{ab}, our I_{rgb} converted to L*a*b* and represented only in a*b* space, where the difference between two colors can be measured using the Euclidean distance metric.

$$I_{ab} = B \cup \sum R_f \qquad (1)$$

where R_f denotes a fluorescent region and $\sum R_f$ forms F. We consider p a pixel and (x,y) its coordinates in the a*b* space. We perform a classification of each pixel by employing the k-means clustering [5].

We have chosen k=3 number of clusters. A pixel p is classified either as $\{R_f\}$ or {B} or a noisy stained region.

The Euclidean distance is here the metric used to quantify how close two pixels to each other.

As a result, k-means provides the three clusters containing separately the background B, the foreground F and the noisy stained regions. Nevertheless, it does not precise which cluster includes F. A solution to this problem may be to experimentally check the value of each centroid (which contains the mean 'a*' and 'b*' value for each cluster).

The cluster containing the foreground is the one that has the highest centroid mean value.

Objects in each clusters are described in Fig. 3 b),c) and d).

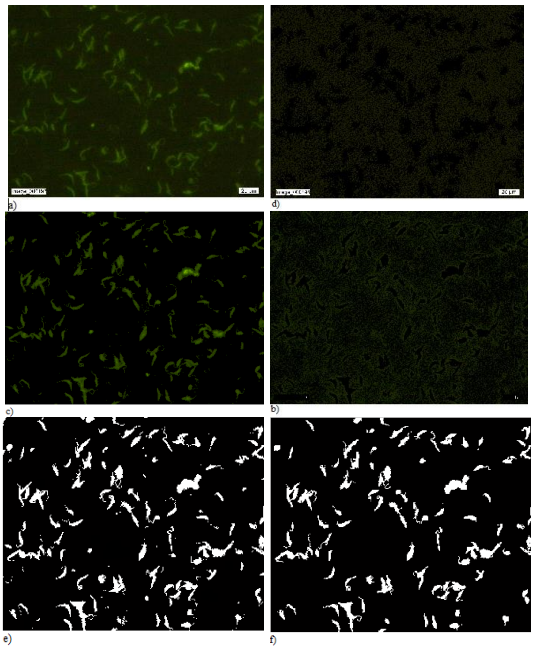

Figure 3. An IIF image processed with the proposed segmentation approach: a) original RGB image, b) Objects in cluster 1: noisy stained regions , c) Objects in cluster 2: fluorescent foreground, d) Objects in cluster 3: background, e) Image segmented by k-means clustering followed by Otsu thresholding and closing morphological operator, f) Final segmented image.

B. Otsu Segmentation

This step takes as input the image representing the fluorescent parasites cluster described in Fig. 3.c). The Otsu thresholding algorithm [12] was performed followed by the closing morphological operation to eliminate small regions and fill the holes in the foreground. The result is illustrated in Fig. 3 e).

C. Region-based feature detection

The foreground extracted and separated by k-means clustering from the background contains the fluorescent parasites that we want to detect, but it is also composed of noise due to artifacts when performing the IIF technique.

Knowing the characteristics of the fluorescent parasite that we want to extract, we opt for a removal of the fluorescent objects whose region characterization does not fit with the parasite one. These objects are of two types: the first one is the small fluorescent particles whose size is smaller than the one of the parasite. The second type is the fluorescent large objects, whose size exceeds the one of the parasite. We have removed only the first type and kept the second.

We will treat this second type of fluorescent objects separately in a future work, as it may be an overlapping or a superposition of parasites.

We have implemented a region characterization method using Matlab® functions including the *regionprops* function to compute geometrical features e.g. Area, Perimeter.

An example of the result image is shown in Fig. 3 f).

IV. Results and Evaluation of the Segmentation Method

This study experimented fluorescent objects with manual sketched outlines from 40 images to test the accuracy of the proposed method. The simulations were tested on a Core Duo CPU i7- 2.4 GHz personal computer with Microsoft Windows 8 operating system.

Fig. 4 shows the segmentation results of some IIF images corresponding to different dilutions of the serum. As shown in this figure, the proposed method performs well on almost all the images where the fluorescent parasites are scattered Fig. 4 a) and b); however, the performance on images of overlapped or superposed fluorescent objects is less satisfactory Fig. 4 c).

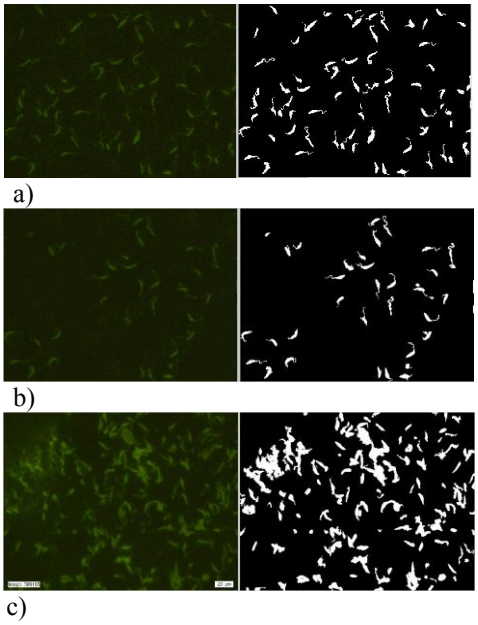

Figure 4. Example of some segmented images, on the left: the original images, on the right: the respective segmented ones

Table 1 compares the segmentation results derived from the proposed method with two other methods used in the same context in the literature: the Otsu automatic thresholding method with morphological operators and the Fuzzy C-Means clustering algorithm.

This comparison is based on the number of regions classified as True Positive TP, True Negative TN, False Positive FP or False Negative FN vis-a-vis the manual sketched parasites.

Let R be the region segmented by a segmentation method,

- TP: R is a parasite and it is correctly segmented.
- TN: R is a parasite but it is not segmented
- FP: R is not a parasite but it is segmented,
- FN: R is not a parasite and it is not segmented.

Comparison segmentation results (also illustrated in Fig. 5) show that the proposed method presents a small number of cases that might generate an undesired segmentation. We have found that, in the 40 tested images, 1231 out of 1702 parasites were correctly segmented. Only 347 were wrongly segmented. This is due to parasites overlapping as shown in

TABLE I. COMPARISON OF THE SEGMENTATION RESULTS BETWEEN THE PROPOSED METHOD, FCM CLUSTERING AND THE OTSU THRESHOLDING WITH MORPHOLOGICAL OPERATORS

Criteria of comparison	Segmentation Method		
	The proposed method	***FCM clustering***	***Otsu with morphological operators***
TP	1231	1089	1002
FP	209	3804	3786
FN	3398	11	25
TN	347	376	398
Mean Processing time (second/image)	37,16	113,72	8,60

Fig. 4 c) or to some artifacts caused by the IIF technique.
The high value of FN is explained by the fact that the proposed method has eliminated small regions that do not have the same characteristics as the parasite.
As it is shown in the Table, Fuzzy C-Means clustering is computationally expensive due to the fuzzy measures calculations involved in the algorithm.

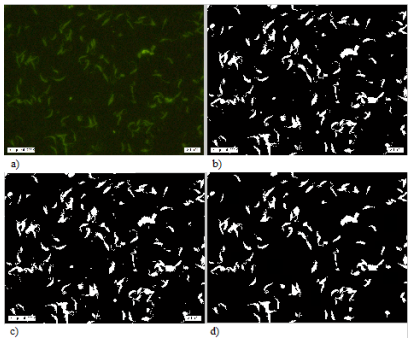

Figure 5. Segmentation results: a) Original IIF image, b)Otsu segmentation, c) FCM clustering, d) the proposed method

Although Otsu method and FCM clustering have considerable results when performed on some images, they cannot always guarantee a good segmentation result, mainly when performed on images with low contrast as it can be seen in Fig. 6. On the other hand, our method keeps providing reliable results.

V. CONCLUSION

In this paper, we have proposed an adaptive method for segmenting the Promastigote form of Leishmania parasite. This method enables easier and faster observations, it can save much of the time required to locate fluorescence parasites with high stability as it can serve as a second reader so that to reduce errors.

Although the k-means clustering perfectly separated the foreground from the fluorescent image and so we removed the background noise, applied alone it cannot give a satisfactory result in terms of segmentation. This led us to couple it to Otsu thresholding method and to a region-based feature detection which enabled to remove the small

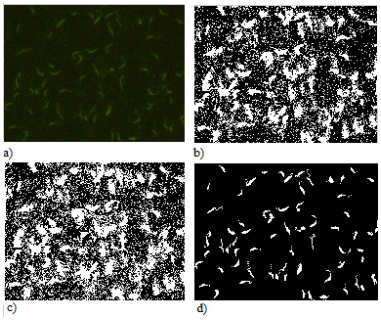

Figure 6. Segmentation results on a low-contrast image: a) Original IIF image, b)Otsu segmentation, c) FCM clustering, d) the proposed method

fluorescent regions. The large ones are kept to be treated separately in future work before we get started with the second step: the pattern recognition.

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