Unsupervised Segmentation of Leukocytes Images using Particle Swarm

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Abstract: Blood smear image analysis is an essential task for many health related issues. Among the many blood structures present in these images, leukocytes play an important role in the detection of many diseases (such as leukemias), which can be detected by the amount, or abnormal aspect, of the leukocytes. To address this problem, this paper presents an unsupervised segmentation method for the nuclear structures in leukocytes. Our method uses color deconvolution to separate the dyes in different channels and a PSO algorithm to estimate an optimal kernel filter to combine local features in different stain channels to emphasize the leukocytes structures so that simple thresholding techniques are able to perform image segmentation. We also used a postprocessing approach based on morphological operators to refine the border of detected structures, thus improving our performance. We performed a comparison with different approaches found in literature using 367 images containing leukocytes and other blood structures and results demonstrated the superiority of our approach in terms of Jaccard index.

1 INTRODUCTION

One of the main parts of the immune system are the White Blood Cells (WBCs), also called Leukocytes, which are produced in the bone marrow and lymphoid tissues. These cells are divided into five types Lymphocytes, Monocytes, Eosinophils Basophils, and Neutrophils. They are responsible for protecting the body against infections such as bacteria, viruses, and fungi.

Normally, a healthy human has four to eleven thousand leukocytes per cubic inch of blood (Banik et al., 2020), and the excess or lack of these cells can cause several diseases (Kutlu et al., 2020). The process for counting these cells usually involves the segmentation and classification process. Hematologists, with the aid of microscopes, have to manually segment the white cells to later classify them into their types. This process, in addition to requiring several hours from a trained professional, has its accuracy quite dependent on the agent who is doing the measurement (Banik et al., 2020).

Technological advances in the field of digital pathology have brought automatic procedures for the detection and classification of microscopic images of WBCs. This procedure consists of connecting digital cameras to microscopes to obtain high-resolution images to assist hematologists (Al-Dulaimi et al., 2020). As a consequence, the use of image processing systems for this task have grown every day. Typically, these systems have two main activities: segmentation and classification of blood cells.

Given the importance of the segmentation process for further classification, this work proposes an unsupervised segmentation method for the nuclear structures in leukocytes. This approach separates the RGB image into three channels and later uses the Particle Swarm Optimization(PSO) algorithm to estimate an optimal kernel filter to combine local features in different stain channels to emphasize the leukocyte's structures. After threshold segmentation, morphological operators are used to refine the edges of the detected structures.

The remainder of this paper is organized as follows: In Section 2, we present a review of the state of the art in leukocyte segmentation. In Section 3, we present the concepts used in this work. We present our approach in detail in Section 4. Section 5 presents the experiments and the results obtained. Finally, Section 6 concludes this paper.

2 RELATED WORKS

Due to its great scientific relevance, several works were proposed for the segmentation of WBCs

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(Rezatofighi et al., 2009) (Madhloom et al., 2010) (Mohamed and Far, 2012a) (Mohamed and Far, 2012b) (Mohamed et al., 2012) (Tosta et al., 2015) (Tareef et al., 2016) (Tareef et al., 2017). Below, we highlight some works that were used as a comparison with our approach.

The work of (Tosta et al., 2015) proposed an unsupervised approach to the segmentation of nuclear structures in leukocytes. The authors' method consists of four steps. Firstly, the deconvolution process is applied to the image to separate two components of Giemsa stained images, methylene blue and eosin, based on optical density, which is proportional to the concentration of each component in specific cellular structures. Later, in the second stage, a median filter is applied to remove noise and standardize nuclear regions. In the third stage, the Neighborhood Valley-emphasis method automatically determines a threshold value that separates regions of interest and background. In the last step, post-processing is performed with the morphological opening and closing operators to eliminate small holes. The work was evaluated using the Jaccard coefficient and Precision. The authors obtained a result of 89.89% in the Jaccard coefficient and 99.57% in Precision. In conclusion, the authors point out that the main limitation of this work is the low result in dealing with the edges of the structures.

In the work of (Tareef et al., 2016), a three-stage approach to leukocyte segmentation is proposed. The first step is the segmentation of the nuclei, this segmentation consists of the transformation of the RGB color space to the CIE LAB, having both the RGB image and the CIE LAB, a grayscale image is generated by adding the red channel with the luminance and subtracting the color component A from the CIE LAB. With the grayscale image, the Poisson distribution based minimum error thresholding algorithm is applied to the obtained gray-scale image to get the nuclei candidates. In the second step, which consists of cytoplasm segmentation, the authors use discrete wavelet transform (DWT) and morphological filtering to eliminate small details and noise and to increase the contrast between the cytoplasm and the other structures. At the end of this stage, cytoplasm candidates are selected using the Otsu method. In the last step, the authors perform a refinement and filtering to obtain the final segmentation. First, the regularized level set is applied to refine the cytoplasm candidate contour. Subsequently, an opening is applied followed by an expansion to remove the excess of edges. In the end, a filtering process is carried out to remove false candidates for nuclei and cytoplasm, which consists of removing nuclei that are not surrounded by cytoplasm. The authors' work was evaluated using the similarity metric, obtaining a result of 85.10% for the BloodSeg dataset.

In the paper (Tareef et al., 2017), the authors proposed a framework based on four stages for the segmentation of leukocytes: clustering-based color enhancement and reduction, nuclei segmentation, cytoplasm segmentation, and post-processing. In the first stage, the authors created a technique that reduces the range of colors while preserving the contours of the cells. In this step, a median filter, followed by a contrast adjustment, is applied to the original image. Subsequently, they apply a clustering algorithm to the image to divide it into coherent regions. For each cluster found, they compute the median value for each color channel. Then, the authors use the Gram-Schmidt orthogonalization method to compute a vector of weights that is later used to highlight the region to be segmented. In the third stage, the method applies the watershed transform to segments the cytoplasm. In the end, the authors apply several morphological operations and filters to refine the results. The authors evaluated their results using the similarity metric, which obtained an average result of 88.2.

3 MATERIAL AND METHODS

3.1 Color Deconvolution

The main goal of color deconvolution is to separate immunohistochemical dye channels such as hematoxylin (H) and eosin (E). In this paper we used the method based on the orthonormal transformation of the RGB image in order to separate the dyes in different channels (Ruifrok et al., 2001; Wang et al., 2017). When a monochromatic radiation passes through an absorbing dye, that dye absorbs a fraction of the light according to the Bouguer-Lambert-Beer equation:

$$I = I_0.e^{-\delta.c} \tag{1}$$

where I is the intensity of the monochromatic radiation, I_0 is the intensity of the transmitted radiation, δ is the spectral molar optical density for a unified layer thickness and c is the dye concentration.

The optical density (OD) of a channel i is defined as

$$OD_i = -\log_{10}\frac{I_i}{I_0}, \tag{2}$$

and it has a linear relation with the concentration of absorbing material so that it is useful to estimate the contribution of each stain in a sample. The contribution of each stain is given by a matrix where each row represents a specific stain. After the orthonormal transformation and normalization, the contribution of

H, E and DAB stains in a RGB image is given by the following matrix, M:

$$\begin{bmatrix} 0.65 & 0.70 & 0.29 \\ 0.07 & 0.99 & 0.11 \\ 0.27 & 0.57 & 0.78 \end{bmatrix} \quad (3)$$

Given a RGB image, $y = CM$ describes the amount of each stain in a particular pixel, where y and C are 3×1 vector for, respectively, stains and RGB colors. Then, color deconvolution is defined as the matrix $D = M^{-1}y$. For H, E and DAB stains, D is defined as follows:

$$\begin{bmatrix} 1.88 & -0.70 & -0.29 \\ -0.07 & 1.13 & -0.11 \\ -0.55 & -0.13 & 1.57 \end{bmatrix} \quad (4)$$

3.2 Particle Swarm Optimization

Nature has been inspiring of human development in many subjects and it is not different in the computer sciences area. There exists many methods and techniques to solve computational problems that use models inspired in the biology, being one of them the Particle Swarm Optimization (PSO) algorithm. Proposed by (Kennedy and Eberhart, 1995), PSO is a swarm intelligent optimization algorithm. It is mostly inspired by the behavior of flocks of birds and schools of fishes, where these animals are capable to move synchronously while changing their direction, scattering and regrouping. By mimicking this swarm behavior, a system of particles is capable to search for global optimum by combining its own solution with the one provided by other members of the swarm.

In PSO algorithm, given a system with N particles, we associate a position, $\{X_i^1(t), X_i^2(t), \ldots, X_i^D(t)\}$, and velocity, $\{V_i^1(t), V_i^2(t), \ldots, V_i^D(t)\}$, to a particle i, $i = 1, 2, \ldots, N$, where D represents the number of dimensions in the search space. For a current iteration t, $t = 1, 2, \ldots, T_{Max}$, where T_{Max} is the maximum number of iterations, we update the velocity and position of each particle by using the following set of equations:

$$V_i(t+1) = \omega(t)V_i(t) + c_1.r_1(Pbest_i(t) - X_i(t)) + c_2.r_2(Gbest(t) - X_i(t)) \quad (5)$$

$$X_i(t+1) = X_i(t) + V_i(t+1) \quad (6)$$

where c_1 and c_2 are the acceleration factors and r_1 and r_2 are randomly generated values ranging from $[0, 1]$. ω is the inertia weight and it is defined as a linear decreasing variable as follows:

$$\omega(t) = \omega_{max} - \frac{(\omega_{max} - \omega_{min})}{T_{Max}} \quad (7)$$

where $\omega_{max} = 0.9$ and $\omega_{min} = 0.4$. Personal and global best solutions, $Pbest_i$ and $Gbest$ respectively, are defined as follows:

$$Pbest_i(t) = \arg\min\{fit(X_i(1)), \ldots, fit(X_i(t))\} \quad (8)$$

$$Gbest(t) = \arg\min\{fit(Pbest_1(t)), \ldots, fit(Pbest_N(t))\} \quad (9)$$

3.3 Binarization

In many occasions it is necessary to reduce the number of gray-levels (or colors) of an image in order to better evaluate its content. When the resulting image is composed by only black and white pixels, this process is called as image binarization (Sezgin and Sankur, 2004).

Literature presents many and different approaches to convert an image into a binary one, being the simplest approach to apply a threshold value T to each pixel. Thus, a pixel whose value is greater than the threshold is classified as white or 1; otherwise, this pixel is classified as black or 0.

Although this threshold T can be manually defined, there is no guarantee that the chosen value is the best threshold for images under different acquisition conditions, such as illumination and contrast. This has motivated the development of algorithm that compute the best threshold value for each image based on the gray-levels distribution of the image, such as the Otsu (Otsu, 1979), Valley Emphasis (Ng, 2006), Modified Valley Emphasis (Fan and Lei, 2012) and Balanced Histogram (dos Anjos and Shahbazkia, 2008) methods.

It is also important to emphasize that due to factors such as non-uniform illumination, not all images can be converted to a binary one by using a single threshold value. For these images it is recommended the use of adaptive image binarization, where different threshold values are computed for different portions of the image.

4 PROPOSED FRAMEWORK

In this section we describe the proposed framework used to segment leukocytes from other blood structures in images. Given an input image, we compute its deconvolution to emphasize the color information of the leukocytes present in it. In the sequence, we use a $3 \times 3 \times 3$ kernel filter (computed using the PSO algorithm) to convert the image resulting from deconvolution into a grayscale image that highlights the leukocytes. Then we use a binarization algorithm to select

the leukocytes regions of the images. Since more objects are present in the image (e.g., other blood structures) and may be detected as leukocytes, we apply two a post processing steps to select actual leukocytes and to ensure the quality of the leukocytes detected. Figure 1 displays the framework of the method.

4.1 Image Segmentation using PSO

Cell staining, such as H&E, is a procedure used to increase color contrast of different structures, thus allowing for a clearer view and improving the performance of segmentation algorithms. Therefore, instead of using color segmentation algorithms or other color space models, we propose to use a simple kernel filter to emphasize the leukocytes in images by combining local features in different stain channels.

We used a particle swarm algorithm to optimize the 27 floating point values that composes a $3 \times 3 \times 3$ kernel filter. After applying the color deconvolution algorithm on an input image, we applied this kernel filter to combine local characteristics present in each stain channel, thus producing a single gray scale image S. The main idea is that the kernel filter is able to generate an image S that highlights the main characteristics of leukocytes, so that it could be easily segmented using a simple automatic threshold approach (e.g., Otsu method), as shown in Figure 2. To measure how accurate the proposed segmentation is we used Jaccard index (Ghose et al., 2012), as described in Equation 10:

$$J(A,B) = \frac{|A \cap B|}{A \cup B} \tag{10}$$

where A and B are two binary images, respectively, our segmentation and the expert's segmentation. D, $0 \leq D \leq 1$, is the similarity level between the images, and the more the value D is close to 1, the more similar the images are.

4.2 Leukocytes Selection

After the image segmentation, it is necessary to verify whether each detected object is actually a leukocyte or not. To accomplish that, we proposed the following procedure. Initially we performed a morphological opening using a disk of radius $r = 5$ in order to separate near objects that are connected. We computed the area of all objects. This area is normalized by the area of the largest object. In the sequence, we removed all objects with a normalized area smaller than or equal to 0.2 from the image. We considered the remaining objects as leukocytes. This step was performed in order to remove objects that are too small in relation to the others. These objects represent small segmentation errors and noise present in the original image. Finally, we computed the image complement of the resulting segmentation containing the detected leukocytes and removed all objects with an area smaller than 100 pixels. The operation was carried out to eliminate small background regions present inside the leukocytes detected during the segmentation stage.

4.3 Border Refinement

When analyzing the leukocyte image it is possible to notice that there is a diffuse region separating the leukocyte from the image background. As a consequence, the segmentation process may not correctly detect the leukocyte border or to detect background regions adjacent to a leukocyte as part of it. Thus, we proposed a process of refining the leukocyte border detected in the segmentation step. This process begins with the dilation of the segmented image using a disk of radius $r = 5$. This is performed so that undetected regions of the leukocyte border are included in the refinement process. Then, we performed an erosion process guided by the color of the leukocyte. This process is executed for each leukocyte detected and it is defined as follows:

1. Given an binary object A, compute its morphological erosion using a disk of radius $r = 2$, B;
2. Compute the difference between objects A and B, $C = A - B$, where C is the region removed in the process of erosion;
3. Compute the average color of object B in the original image, $\bar{B}_{RGB}$;
4. For each pixel of C, compute the Euclidean distance between its color in the original image and the average color $\bar{B}_{RGB}$.
5. Remove from object A all point in C whose distance is greater than a threshold $T = 75$.
6. Repeat this process whenever more than 25% of the pixels of C are removed from object A.

Figure 3 shows an example of border refinement for a given leukocytes image.

5 RESULTS AND DISCUSSION

To evaluate our approach we used a dataset containing leukocytes images (Tosta et al., 2015). This dataset contains 367 color images of leukocytes stained with hematoxylin and eosin ($H\&E$). Each image has 640×480 pixels size and we applied color deconvolution over them before applying any other step of our

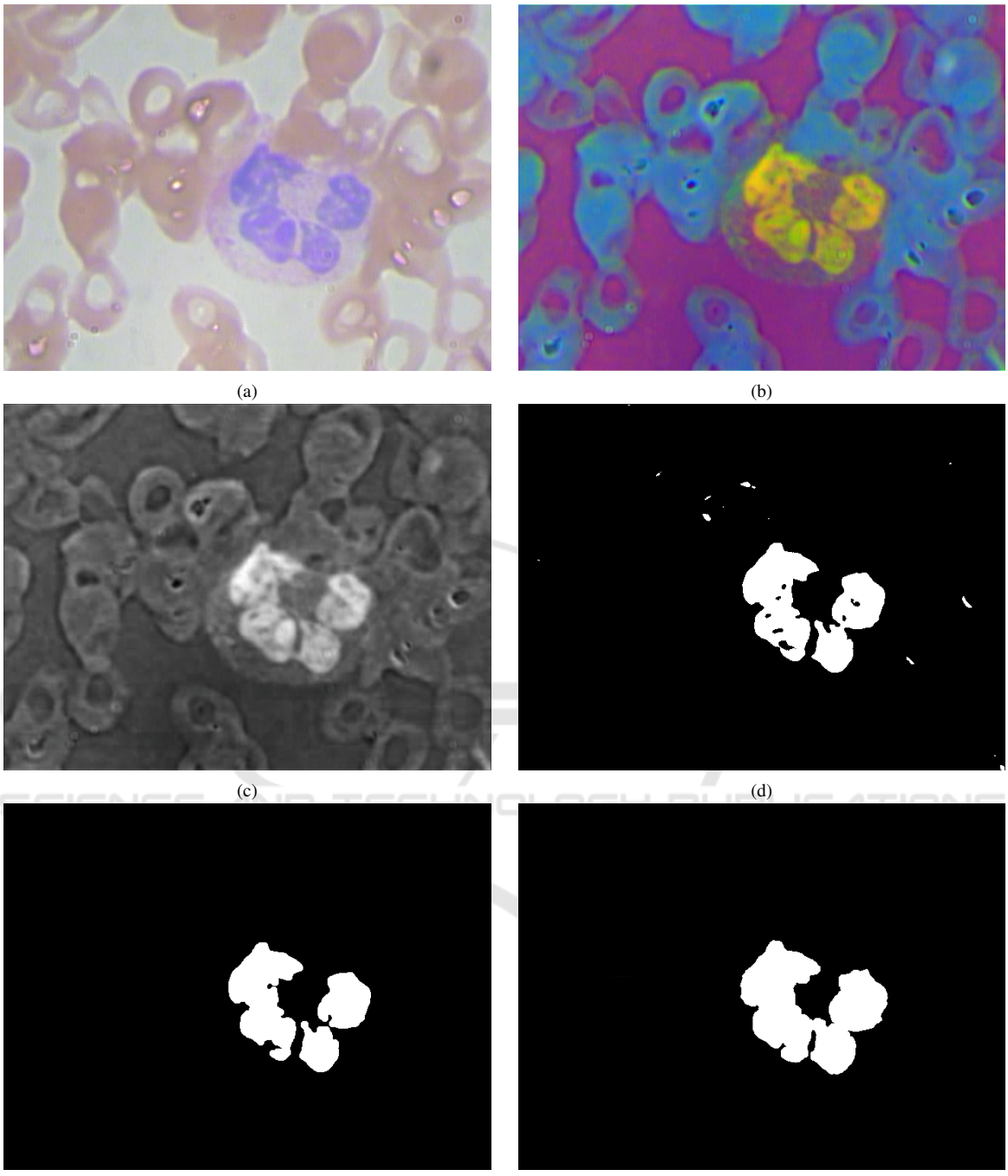
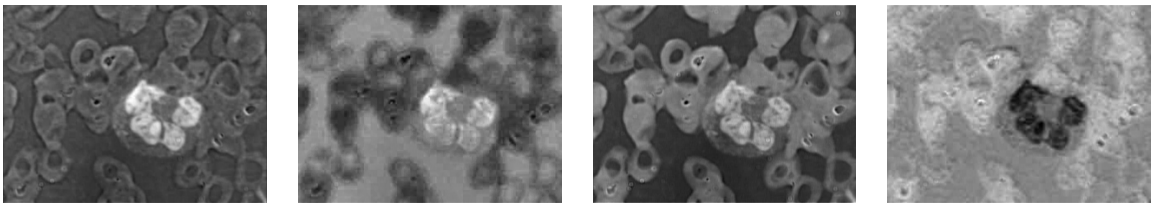


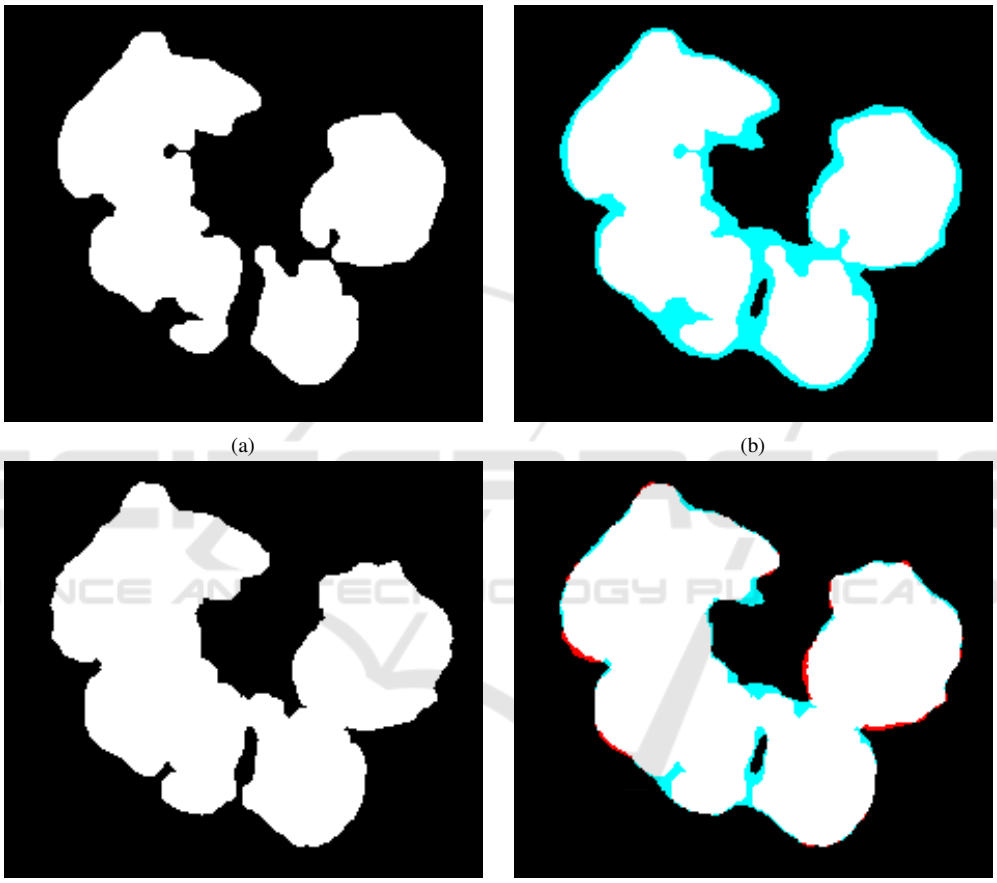
Figure 1: Proposed framework for leukocytes segmentation: (a) Input image; (b) Image deconvolution; (c) Filtered image; (d) Image after Binarization; (e) Leukocytes selection; (f) Border refinement.

proposed methodology. In order to segment the image we trained a PSO algorithm to optimize the 27 floating point values that composes a $3 \times 3 \times 3$ kernel filter. To accomplish that we randomly select 10% of the sample in the dataset for training. As for the fitness function, the PSO algorithm aimed to search for the kernel filter that maximized the average Jaccard index of the images. To execute the PSO algorithm we considered a population size of 100 individuals running for 5400 generations. In the sequence, we applied all steps of the proposed framework in all images of the dataset in order to report the average results obtained.



(a) (b) (c) (d)

Figure 2: Comparison of the proposed filtering scheme with image deconvolution: (a) Resulting filtered image; (b) Hematoxylin; (c) Eosin; (d) DAB.



(a) (b)

(c) (d)

Figure 3: (a) Selected Leukocytes; (b) Comparison of (a) with the markings provided by experts; (c) Image (a) after border refinement; (d) Comparison of (c) with the markings provided by experts. Blue color indicates a region undetected by our method while red color indicates a region wrongly detected.

In our proposed framework, we use a binarization approach to convert the filtered image S into a binary one. During the training of our PSO optimized kernel filter we used Otsu method (Otsu, 1979) to compute the global threshold for the image binarization. However, Otsu may not be the best choice for this given problem so that we compared the results obtained by Otsu with a more recent method, Modified Valley Emphasis (MVE)(Fan and Lei, 2012). Figure 4 presents the average Jaccard index obtained for both Otsu and Modified Valley Emphasis methods at each stage of the proposed approach.

Results show that Modified Valley Emphasis method performs better than Otsu in all steps of our approach. However, the difference of performance is the highest at the first step (i.e., binarization), where the difference is of $\approx 2.00\%$. This indicates that Modified Valley Emphasis method is capable to achieve

a threshold value that is more suitable for the filtered image S and its content, even though the PSO was trained using Otsu. As we execute the other steps of our framework (leukocytes selection and border refinement), this difference of performance decrease until only 0.40%. This is expected as these are post-processing steps and they were proposed to compensate small segmentation problems that could arise from the initial step, such as the presence of noise or a very smooth or poorly defined leukocyte border.

To improve our analysis we compared our approach with the results obtained by other segmentation methods found in literature. Table 1 show that our approach is capable to surpass all compared approaches, independent of the binarization approach used. When we consider our best result (i.e., Modified Valley Emphasis), our framework achieves an average result 1.10% superior than the best compared approach, thus corroborating the effectiveness of our approach to segment leukocytes disregarding their size, shape and spatial distribution of the cells in the image.

Table 1: Jaccard index achieved by different approaches.

Method	Jaccard index
Paper (Rezatofighi et al., 2009)	83.20%
Paper (Madhloom et al., 2010)	55.90%
Paper (Mohamed and Far, 2012a)	85.40%
Paper (Mohamed and Far, 2012b)	80.60%
Paper (Mohamed et al., 2012)	79.70%
Paper (Tosta et al., 2015)	89.89%
Paper (Tareef et al., 2016)	85.10%
Paper (Tareef et al., 2017)	90.10%
Proposed approach (Otsu)	90.80%
Proposed approach (MVE)	91.20%

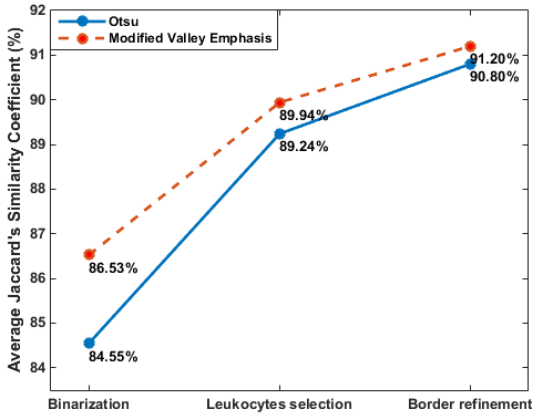


Figure 4: Average Jaccard index obtained for two different binarization methods at each stage of the proposed approach.

6 CONCLUSION

In this paper we presented a methodology to detect and segment nuclear structures in leukocytes. To accomplish that our methodology uses a PSO algorithm to estimate an optimal kernel filter, which is applied after the color deconvolution of the image, so that it is capable to explore and combine local features in different stain channels. Evaluation using a set of 367 images containing leukocytes and other blood structures showed that the estimated kernel filter highlights the structures composing the leukocyte so that simple thresholding techniques are able to perform image segmentation with high accuracy, surpassing the results of various compared approaches found in literature.

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