Queensland University of Technology
Brisbane Australia

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Al-Dulaimi, Khamael Abbas Khudhair, Al-Sabaawi, Aiman Abdul-Razzak Fatehi, Daami Resen, Rajaa, Stephan, Jane, & Zwayen, Amani (2019)
Using adapted JSEG algorithm with Fuzzy C Mean for segmentation and counting of white blood cell and nucleus images. In *IEEE Asia-Pacific Conference on Computer Science and Data Engineering*, 2019-01-01.

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Using Adapted JSEG Algorithm with Fuzzy C Mean for Segmentation and Counting of White Blood Cell and Nucleus Images

Khamael AL-Dulaimi
[*]*Queensland University of Technology*
Brisbane, Australia
[**] *Department of Computer Science*
Al-Nahrain University
Baghdad, Iraq
khamaelabbaskhudhair.aldulaimi@hdr.qut.edu.au

Aiman Al-Sabaawi
[*]*Queensland University of Technology*
Brisbane, Australia
[**] *Department of Computer Science*
Al-Nahrain University
Baghdad, Iraq
a.alsabaawi@student.qut.edu.au

Rajaa Daami Resen
Information Technology
University of Information Technology and Communication
Baghdad, Iraq
rajaa.alnidway@gmail.uotic.edu.iq

Jane J. Stephan
Information Technology
University of Information Technology and Communication
Baghdad, Iraq
janejaleel@gmail.uotic.edu.iq

Amani Zwayen
Department of Computer Science
Al-Nahrain University
Baghdad, Iraq
amani.zwayen@gmail.com

***Abstract*—In this paper, an adapted unsupervised segmentation approach is proposed to fully automate the segmentation of white blood cells and their nuclei. Segmentation and counting of white blood cells from microscope images are challenging tasks, especially the segmentation of white blood cell nuclei from the cell wall and cytoplasm because of the need to consider intra-class variations arising from non-uniform illumination, stage of maturity, colour distribution, scale, and overlapped cells with other components of the blood. We propose the use of the JSEG algorithm based on colour-texture distribution, and adapted region growing using the Fuzzy C Mean to segment and count WBCs and their nuclei. First, colours in the image are quantized to represent differentiated regions in the image. Image pixel colours are then replaced by their corresponding colour class labels, thus forming a class-map of the image. A criterion for "good" segmentation using this spatial class-map is applied to local image windows resulting in J-images, which can be segmented using adapted region growing based on the Fuzzy C Mean algorithm. The Fuzzy C Mean is also employed for counting each white blood cell in images. Performance of the proposed method is evaluated on a combined dataset of 10 types of white blood cell with 200 digital images collected from 3 datasets. It achieves an average segmentation accuracy using four indices for WBC segmentation: jaccard distance, rand index, boundary detection error and F-value indices, 0.002, 0.93, 10.11, 0.93, respectively, while for WBC nuclei segmentation, it achieves indices values, 0.015, 0.88, 14.11, 0.90, respectively. The segmentation accuracy of the proposed method is also compared and benchmarked with the other existing techniques for segmentation of white blood cells over the same datasets and the results show that the proposed method is superior to other approaches.**

***Index Terms*—Segmentation, counting, white blood cells, JSEG, Fuzzy C Mean, region growing, and colour-texture.**

I. Introduction

Segmentation of white blood cells (WBCs) is an important process in the diagnosis of several blood disorders, such as leukaemia, some immunological disorders, and certain types of cancer. The traditional procedure needs a haematologist to manually segment and count the cells with the help of a microscope. The difficulties with manually segmenting and counting WBCs are that (a) there is a large amount of work for experts in relation to the output result, (b) it is a relatively expensive procedure in term of technological advances and automation, and (c) it is time consuming. Furthermore, manual segmentation and counting of WBCs have medical and scientific disadvantages, including poor sensitivity, specificity and predictive value and inaccuracy due to sampling error detection. In addition, manual segmentation and counting becomes more difficult when the number of cells and sampling are huge, with different shapes and overlapping cells [1], [2]. In contrast, automated segmentation of WBCs can process larger numbers of cells and different shapes and sizes of cells. Different techniques are used to segment WBCs and their nuclei and cytoplasm, including image processing, signal processing, machine learning and deep learning, potentially resulting in better accuracy. The most useful shape information for cell segmentation comes from the nuclei and colour of the cells due to the use different staining processes which result in different colouration of the cells and their nuclei and cytoplasm, and the blood image background (plasma) [2]. Multiple WBCs can be found in one image, as shown in Fig-1. Accurate segmentation of the nuclei is therefore a critical step in the segmentation process, which affects the performance

results of WBC classification. WBCs include three main types (Granulocyte, Monocyte and Lymphocyte) and seven sub-types (Neutrophil, Basophil, Eosinophil, Macrophage, Dendritic, B-lymphocyte and T-lymphocyte). An overview of the different WBC types and sub-types and a more detailed explanation about morphological characteristics, staining process and colour distribution and functions can be found in [2], [3].

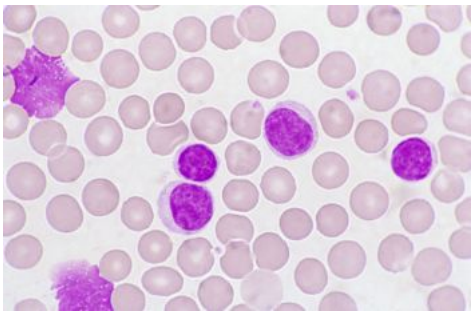


Fig. 1: Large lymphocyte cells in one image, RBCs and background from [4]

This paper is organized as follows: Section-II presents motivation and contribution. Section-III details a survey of background literature on WBC segmentation and counting problems. Section-IV outlines the proposed methodology. Experimental results are provided in Section-V. Finally, section-VI presents the conclusions derived from the work.

II. MOTIVATION AND CONTRIBUTION

Segmentation of the WBC, its nucleus and cytoplasm is still susceptible to errors because of several challenges mentioned in Section-III. The WBC classification accuracy is directly influenced by the quality of the segmentation and feature extraction steps. The aim of this work is to design an automated segmentation and counting procedure that can be robust to intra-class variations, such as colour-texture, shape, maturity stage, scale, illumination level and uneven contrast of cell nuclei images. The original contribution of this paper is that it is the first application of JSEG-based colour-texture distribution to the segmentation of WBCs, and to adapt JSEG algorithm in region growing with Fuzzy C Mean (FCM) to segment and count (three main types and seven sub-types) of WBC to a greater extent than previously. This paper provides the combination of JSEG and FCM in the region growing step, to extract the colour-texture of the WBC and its nucleus and to detect shape information regarding the nucleus. The shape information provides robustness to segmentation using colour transformation and indirectly to changes in illumination, colour-texture, shifting, scale, phase of maturity. This work considers multiple cells in one image. FCM is a fast procedure to count cells. The chosen dataset has all these variations. FCM has been successfully used in many pattern clustering applications and has performed well even with limited training data.

III. LITERATURE REVIEW

Image segmentation plays a key role in different areas, such as biometric detection [5], [6], [7], [8], steganography [9], [10], [11], facial recognition [12], [13], object detection [14] [15], satellite imaging [16], speaker recognition [17], and medical imaging [18], [19]. A major difficulty of medical image segmentation is the high variability in medical images due to the different modalities used to create those images, such as X-ray, ultrasound, CT, MRI, microscopy, PET, SPECT, Endoscopy, OCT, and many more. For decades, several methods have been proposed to detect and segment WBC images (cell, nucleus, cytoplasm or together). These methods included thresholding techniques [20], morphological operators and scale-space analysis [21], edge and boundary detection [22], multi-scale analysis [23], colour-space-based K-means clustering [24], fuzzy C-means (FCMs) [25], dual thresholding [26] and Otsu's thresholding [27]. Some more recent techniques include colour spaces with Otsu's threshold [28], K-means clustering method and Gram-Schmidt Orthogonal process [29], K-means clustering and a modified watershed algorithm [30], and optimal Thresholding and manifold-based Low-Rank Representation (HTLRR) [31]. A segmentation method has been proposed in [32] to detect WBCs using a colour band and thresholding procedure. This technique combined colour spaces with Otsu's thresholding as well as morphological filters. Deep Learning (DL) techniques have also been used to segment WBCs, such as a Self-Supervised Learning method (SSL) in [33] and a marker-controlled watershed based neural network algorithm in [34]. Those methods, including the colour space, threshold techniques, clustering techniques or edge detection rely on the difference of colour between the WBC and its nucleus, but these methods introduce difficulties due to using different staining during the preparation of WBC slides, shapes, illumination levels and maturity stages. WBC nuclei contain the most useful information required for segmentation, feature extraction and classification of cells, so accurate segmentation of WBC nuclei is essential. The recent research of DL in computer vision and image processing has opened possibilities to combine it with different techniques, and apply it to different fields. DL methods, especially Artificial Neural Network (ANN) [34], have been applied in the segmentation WBCs. At this point in time, DL approaches to this problem are still in the early stages of exploration and investigation, and are outside the scope of our work.

Despite previous work in this area, automatic shape segmentation of WBC nuclei is still facing challenges, particularly in the presence of non-uniform illumination, overlapped cells, different shapes of nuclei, and cell distortion. JSEG is based on colour quantisation and FCM can be used to address the WBC segmentation problem because of the ability of JSEG with FCM (region growing) to perform well in the presence of colour distribution between WBCs, background and RBCs, and overlapped cells. In WBC microscopic images,

it is difficult to segment image regions containing colour-texture patterns. The approach taken in this work takes account of the following:

1) Each region in the WBC image contains uniformly distributed colour-texture pattern.
2) The colour information in each WBC image region can be represented by a few quantized colours, which is true for most colour images of WBC.
3) The colours between two neighbouring regions are distinguishable, such as cell wall and nucleus.

A. Background of JSEG

Colour images with homogeneous regions are segmented to generate clusters in the colour space/class (classes are measured in the spectral distribution, with distinct intensity of visible electromagnetic radiation at many discrete wavelengths) [35], [36]. One way to segment images with textures is to consider the spatial arrangement of pixels using a region-growing technique whereby a homogeneous mode is defined with pixels grouped in the segmented region. This is known J measure based SEGmentation "JSEG" [37]. It is an unsupervised method used for segmentation and classification.

B. Application of JSEG

The JSEG algorithm has been used in different areas in term of segmentation and classification, for example, it used for segmentation images of natural scenes properly, without manual parameter adjustment for each image and simplifies texture and colour [38], [39]. It has also used in video detection [40], satellite imaging segmentation and classification [16], computer vision for agricultural mobile robot navigation [41], remote sensing [42] and more.

IV. PROPOSED METHOD

The proposed method of segmentation of WBC and its nucleus consists of different process:

A. Colour Quantization

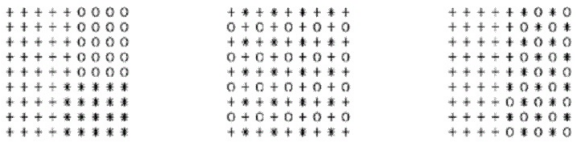
As used in this work, the main process in the JSEG algorithm is quantized colour to obtain edges that can be used for multi-scale differentiation in the colour image as in [16]. Let I is an input colour image, that contains N colours $c1, c2, ..., cN$, and each one has an occurrence frequency $f_i (i = 1, \dots N)$, where f_i is defined as the total number of pixels having the same colour vector c_i. Colour quantization is used to convert input image I into an indexed image I_Q^K in only K colours with least visual distortion, where $K \ll N$ and generally $K \leq 255$. K is always known "Quantization Level". Mean Squared Error (MSE) is employed to colour quantization distortion. Assuming that we have m rows and n columns in I, the colour quantization distortion can be written as:

$$D_Q = \frac{1}{m \times n} \sum_{x=0}^{m-1} \sum_{y=0}^{n-1} \| I(x,y) - I_Q^K(x,y) \|^2 \qquad (1)$$

where $I(x,y)$ and I_Q^K represent the colour vector of pixel (x,y) on the image plane before and after quantization respectively, $\| \bullet \|$ depicts the difference between these two colour vectors [43]. The JSEG algorithm using the colour quantization process not only analyses the colour space, but also analyses the colour space distribution [16].

B. Spatial Label Class

The characteristic of the J-images allows us to use labels " $+$ ","$*$" and " $\circ$ " points for calculating local J values, which form the same basic window. The quantized colours are assigned a colour class. A colour class is the set of image pixels quantized to the same colour. The image pixel colours are replaced by their corresponding colour class labels " $+$ ", "$*$" and "$\circ$". The newly constructed image of labels is called a "spatial class-map". In the class diagram, the value (x,y) for each point represents the position of the pixel within the image [44].



class-map 1 *J*=1.720 | class-map 2 *J*=0 | class-map 3 *J*=0.855

Fig. 2: The class diagram, the value (x,y) for each point represents the position of the pixel within the image after quantization colour [40].

C. JSEG Calculation

The JSEG value is used as a criterion for the region division of the JSEG algorithm. JSEG calculation is performed on the class diagram. The local JSEG value of each pixel is calculated by the circular template scanning class diagram centred on the pixel, and the composition JSEG (J-image) is defined as following:

z represents a set of N pixels in the class diagram, let $z = (x,y)$, $z \in Z$, the mean m can be calculated as:

$$m = \frac{1}{N} \sum_{z \in Z} z \qquad (2)$$

Then, S_T represents the sum of the variance values of the N data, N represents the number of pixels within the class, and S_W indicates the sum of the variance of each class, where S_T and S_W can be calculated as [16]:

$$S_T = \sum_{z \in Z} \|z - m\|^2 \qquad (3)$$

$$S_W = \sum_{i=1}^{c} S_T = \sum_{i=1}^{c} \sum_{z \in Z} \|z - m\|^2 \qquad (4)$$

The JSEG (J) value is:

$$J = \frac{S_T - S_W}{S_W} \qquad (5)$$

D. Region Growing Using Fuzzy C Mean (FCM)

Since the seed region is the basis for regional growth, it is necessary to determine the seed point. A threshold T_J is set in the algorithm to determine the local J value less than T_J in the region as the seed point, and T_J is defined as:

$$T_J = u_J + \sigma_J \tag{6}$$

To select more seed points in the area, the value of σ in the algorithm can be selected from $\{-0.6, -0.4, -0.2, 0, 0.2, 0.4\}$. u_J and σ_J represent the mean and variance of the local J values in each region of the image, and there are N pixels in the region i [16], [45]. This can be done by using Fuzzy C Mean (FCM), which is used to analyse data and construct models in order to find the structure in an unlabelled class by collecting it into n groups. Every pixel in region has a certain degree in the class belonging to every group. If a certain pixel lies close to the centres of a group, it will highly belong to that group [46]. Otherwise, it will have a low degree of membership to that group. The FCM can use a fuzzy partitioning term which a data point in region can be assigned a value between $[0, 1]$. The equation is written as in [47]:

$$J(X|U,V) = \sum_{i=1}^{n}\sum_{k=1}^{c} u_{ki}^{m} \parallel X_i - V_k \parallel^2 \rightarrow min, m \geqslant 1 \tag{7}$$

where

$$\sum_{k=1}^{c} u_{ki} = 1, \ \ i = 1...n \tag{8}$$

where n is number of data point and c is the number of region centres. $\parallel X_i - V_k \parallel^2$ is the Euclidean distance between i^{th} data point and the k^{th} region centre. u_{ki} is a calculation of the fuzzy membership. V_k represents the i^{th} region centre.

V. EXPERIMENTS AND RESULTS

A. Databases

Three databases used for the evaluation of the proposed methodology have all WBC type images and have been used in different works on WBC segmentation [2] and classification [46], [48]: Cellavision Database [49] Acute Lymphoblastic Leukaemia Image Database (ALL-IDB) [50] were collected by the Department of Information Technology Universit'a degli Studi di Milano and the Wadsworth Centre Database [4].

B. Proposed Method Implementation

The proposed segmentation method is tested using 200 digital images of multiple WBCs: 90 images from the Cellavision database, 50 images from ALL-IDB, and 60 images from the Wadsworth Centre database. The proposed method is implemented using 24 bmp images. The ground truth for all images and the actual number of cells was obtained by three pathologists. The number of WBC types (3 main types and 7 sub-types), from 200 images, is 300 cells and is shown in Table-I. The proposed method is implemented using MATLAB2017b. The code processes images of size $M \times N$. Pre-processing steps involved contrast stretching because some images in the databases suffered from poor contrast due to limited dynamic range in the image sensor during image acquisition. Initially, the main process of image segmentation is quantized colour of the image in order to obtain edges that can be used to differentiate between the WBC and its nucleus by using MSE measurement. Then, the image is divided into local windows of 3×3. Applying the criterion to local windows in the class-map results in the J-image, in which high and low values correspond to possible boundaries and interiors of colour texture regions. A region growing method is adapted using FCM to collect the region data into 10 groups. Every pixel in region has a certain value in the class belonging to every group. After region growing, an initial segmentation of the regions is obtained. If obtained regions have over-segmented regions, these regions are merged based on their colour similarity by calculating the distance between colour groups, as shown in Fig.3. After segmentation regions, FCM is also used to count WBCs from segmented regions.

TABLE I: Number of WBC in images used in the experiment.

Main class	Total cell	Sub-Class	Numbers of cell
Granulocyte	130	Neutrophil	60
		Basophil	35
		Eosinophil	35
Lymphocyte	90	B-cell	45
		T-cell	45
Monocyte	80	Macrophage	50
		Denditiric	30

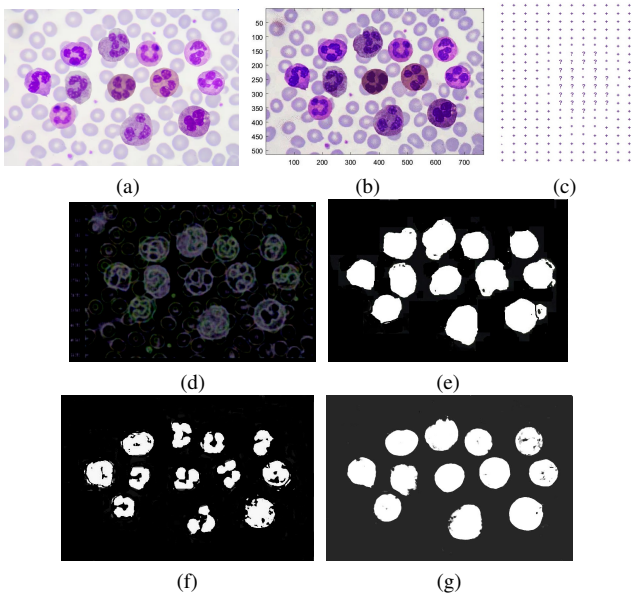


Fig. 3: Examples of WBCs and their nuclei segmentation. (a) WBCs image, (b) quantization colour, (c) class map for one WBC, (d) J-image, (e) WBC segmentation after region growing using FCM ,(f) nucleus segmentation, (g) ground truth of WBC.

C. Performance Evaluation Result

The proposed method is evaluated using four indices: Jaccard Distance error (JD), Rand Index (RI), Boundary Displacement Error (BDE), and F-Index [51]. These indices provide a measurement of the similarity between a segmented image and a ground truth. BDE provides the average displacement between the resulting boundaries of two segmentation regions; it ranges between $[0, \infty)$ in pixel units, where a lower value is better. JD calculates the accuracy of the segmentation; JD = 0 is best. RI measures the likelihood of a pair of pixels being grouped consistently in two segmentations, and ranges between $[0, 1]$, where higher is better [2], [19]. The F-Index is used to measure the accuracy of the segmentation and is computed based on precision P and recall R. P represents the number of correct positive results divided by the total number of positive results, and R represents the number of correct positive results divided by the number of positive results that should have been returned. Both P and R are calculated based on true positives *TP*, which represent the intersection between the segmentation result and the ground truth (pixels correctly segmented as foreground); false positives *FP*, which measure the result of the segmented area that is not in the ground truth (pixels falsely segmented as foreground); false negatives *FN*, which represent missed parts of the ground truth (pixels falsely detected as background); and true negatives *TN*, which represent the area that does not belong to the union of the segmentation result and the ground truth (pixels correctly detected as background). The best value for the F-Index is 1, which represents the best segmentation [52], [53].

The proposed method is implemented and the segmented cells result is compared with ground truth in the presence of WBCs and their nuclei. It obtains average segmentation accuracy results using four indices for WBC segmentation: JD, RD, BDE and F-index 0.002, 0.93, 10.11, 0.93, respectively, while for WBC nuclei segmentation, it achieves 0.015, 0.88, 14.11, 0.90, respectively. FCM is also used to count WBCs in images and the result is compared with actual number of cells in Table- I. The results show that the proposed method has been counted most cells comparing with actual number of cells, for example, the number of granulocyte cells counted is 125 comparing with the actual number, which is 130. Another example is that the number of lymphocyte cells counted is 90 which is equal to the actual number of cells, as shown in Table-II. However, few cells could not be counted because have low contrast and some cells overlapped with other components.

TABLE II: Number of WBC in images counted after segmentation process in the experiment.

Main class	Total cell	Sub-Class	Numbers of cells
Granulocyte	125	Neutrophil	58
		Basophil	35
		Eosinophil	31
Lymphocyte	90	B-cell	45
		T-cell	45
Monocyte	70	Macrophage	42
		Denditiric	28

D. Comparison With Other Methods and Benchmarking

Table-III lists the performance values of proposed segmentation method using four indices where each value is an average computed over the performance of 200 digital images of multiple WBCs from three databases (Cellavision database, ALL-IDB, and Wadsworth Centre), and compared with 13 other segmentation methods: Morphological operators [21], edge and boundary detection [22], colour-space-based k-means clustering [24], region detection model and EM [54], FCM [25], self-supervised learning method [33], dual threshold [26], watershed-based neural network [34], K-Means clustering and watershed algorithm [30], Chan-Vese method [55] and Otsu's thresholding [27].

Table-III shows the proposed method outperformed than the other methods. The proposed method shows better ability to detect WBCs and their nuclei than the other 13 methods, which fail to produce good results when complex boundaries, overlapping cells, low contrast or colour distortion occur between the WBC and its nucleus. The proposed method achieves higher accuracy and performance using JD, RI, BDE and F-index, 0.002, 0.93, 10.11, 0.93, respectively, than the best accuracy of the recent methods in Table-III: K-means clustering and watershed algorithm [30], thresholding and manifold low-rank [31], colour-based k-means clustering [24] and Level set via GAC method [2] edge and boundary detection [22]. However, the performance of the level set method via GACs in [2] is better than the performance of the proposed method in the segmentation of WBC nuclei, based on segmentation shape boundary, splitting and merging of nuclei. The level set method via GACs yielded JD, RD, BDE and F-index values 0.003, 0.93, 12.50, 0.92, respectively, while the proposed method obtains 0.015, 0.88, 14.11, 0.92, respectively.

TABLE III: Performance comparison between the proposed segmentation method and other existing methods to segment a WBC and its nucleus using the same database (300 multiple cells in images from [4], [49], [50]), and using JD, RI, BDE and F-index measurement.

Method Name	**JD**	**RI**	**BDE**	**F-In**
Morphological operators [21]	0.31	0.75	15.12	0.76
Edge and boundary detection [22]	0.21	0.75	14.34	0.81
Colour-based k-means clustering [24]	0.31	0.75	15.43	0.82
Fuzzy c-means [25]	0.29	0.75	15.23	0.76
Region detection & EM [54]	0.12	0.74	16.45	0.79
Dual threshold [26]	0.19	0.67	N/A	0.74
Chan-vese method [55]	0.23	0.74	N/A	0.61
Otsu thresholding [27]	0.44	0.73	14.23	0.54
Thresholding & low-rank [31]	0.01	0.89	11.00	0.88
Clustering & mark Watershed [30]	0.02	0.86	12.42	0.90
Self-supervised learning [33]	0.11	0.83	10.30	0.78
Watershed Based Neural Network [34]	0.06	0.79	10.12	0.76
Level set via GACs [2]	0.002	0.92	10.46	0.92
Our proposed	**0.002**	**0.93**	**10.11**	**0.93**

VI. Conclusion

In this paper, an adapted JSEG Algorithm with Fuzzy C Mean has been proposed to segment WBCs and their nuclei

based on texture-colour. The proposed method can be used to segment the complex shape of WBC nuclei, low contrast images, distorted cells and overlapping cells because it can identify intricate details. Testing has been undertaken using 300 WBCs in images at different stages of maturity and with varying illumination, orientation and background, without any prior knowledge of the target shape. The results obtained are satisfactory despite such variations. The performance of the proposed method has been evaluated using different indices JD, RI, BDE and F-index measures. The performance of the proposed method has also been compared and benchmarked against 13 other WBC segmentation methods using the same database. For example, the proposed algorithm yields better segmentation results according to F-index, with a value 0.93, compared with the best results of the other methods: 0.90 for (K-Means clustering and watershed algorithm), and 0.82 for (colour-based k-means clustering), 0.81 for (edge and boundary detection). The proposed method has considered overlapped cells because it has been applied to images containing multiple cells in this work. However, the proposed method could not segment and count some WBCs and their nuclei because some images have very low contrast and the algorithm fail to process the merging and splitting during maturity stage of the WBC. In future, the results of cell segmentation using the proposed method will be considered in classification WBC into three main types and seven sub-types using the work in [46], [48]. In addition, the proposed method can be improved using DL techniques in [56], [57] and extending the database.

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