

A Comprehensive Performance Comparison of Thresholding and the K-Means Clustering Algorithm in White Blood Cells Segmentation

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ARTICLE INFO	ABSTRACT
Article history: Received 24 March 2024 Received in revised form 25 September 2024 Accepted 8 October 2024 Available online 31 January 2025	In the medical field, the segmentation of white blood cells is an important process in machine learning techniques. The conventional process requires an expert, a hematopathologist, to manually analyse blood smear samples from patients. The sample is pre-processed on a slide using a staining procedure that provides the components of platelets, red blood cells and white blood cells. White blood cell images obtained by a microscope are important in guiding haematology imaging to diagnose blood cancers such as leukemia and lymphoma. However, there are some challenges while conducting manual segmentation of white blood cells, which requires expert labour to observe each blood sample individually to diagnose the patients. This process is also very iterative, time-consuming, and relatively expensive. Besides that, this procedure has medical and scientific drawbacks, including inaccurate output due to interobserver disagreement and inadequate sensitivity, specificity, and predictive value. Thus, new methodologies were proposed throughout the years to aid the white blood cell segmentation process. The objective of this study is to review thresholding and K-means clustering methods, which are two widely used segmentation approaches, to segment the microscopic image of white blood cells. The effectiveness of these approaches was then evaluated. The proposed methodology was tested on five types of white blood cells: neutrophils, eosinophils, basophils, monocytes, and lymphocytes, to compare their performances. The result shows that the K-means clustering technique's performance is more accurate than the thresholding, where the
<i>Keywords:</i> White blood cells; image segmentation; thresholding; K-means clustering	clustering technique's performance is more accurate than the thresholding, where the dice similarity is 93.88% and 93.23%, respectively. Contrarily, the thresholding technique runs much quicker than the K-means clustering algorithm, requiring 0.3238 seconds as compared to 6.4292 seconds.

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1. Introduction

In the medical field, the detection and segmentation of white blood cells (WBC) is an important process of haematology imaging that can aid in the diagnosis of several diseases such as blood cancer, leukemia and lymphoma. The conventional process used requires a hematopathologist, to manually check patient-provided bone marrow samples and peripheral blood stains. The sample is cleaned and prepared for microscopic viewing on a slide using a process of staining that presents the WBC component in multiple colorations, including red blood cells (RBC), platelets, cytoplasm and nucleus [1]. Both automatic and manual techniques of WBC detection, segmentation, and counting can be used in the analysis. Hematopathologists are the key figures in making therapeutic decisions. Figure 1 illustrates the manual diagnosis process from WBC's microscopic image.



Fig. 1. The manual diagnosis process from WBC microscopic image

However, there are some challenges while conducting manual segmentation and counting of WBC, which are that the procedure takes a lot of labour from experts concerning the output result, is iterative and time-consuming, and is a relatively expensive practice. In addition, there are medical and scientific drawbacks to this procedure, including inaccurate output due to interobserver disagreement and inadequate sensitivity and specificity [2]. Generally, medical image processing has four main procedures: pre-processing, segmentation, feature extraction, and classification. According to Adatrao and Mittal [3], the crucial process in image processing relates to image segmentation. This is because the resulting image from this process will directly impact the outcome of the following procedures, which are feature extraction and classification, thus increasing the accuracy of the final findings [4,5]. Hence, various automated cell segmentation methods have been developed.

This paper was divided into four sections. Section 2 discussed the background and related works of previous literature on the structure of WBC and WBC segmentation techniques. The details of the segmentation techniques used are shown in Section 3 which is the proposed methods section. Results and discussion of the WBC segmentation and performance evaluation were illustrated in Section 4, and lastly, Section 5 was the conclusion.

2. Background and Related Works

The microscopic image of blood is commonly comprised of RBC, WBC and platelet. WBCs are easier to identify since the nucleus of the cells is seemingly darker than the background image. The WBC is an important component in human blood that can be used in the diagnosis of several diseases, including leukaemia, infections, and blood-related diseases [6]. WBC's primary purpose is to fight against infectious diseases. Each of the types of WBC serves a particular role in protecting against infections such as bacterial, virus, fungal, and parasitic diseases. Neutrophils, eosinophils, lymphocytes, monocytes, and basophils are five distinct types of WBC and all are crucial for defending

the body from infection and foreign cells. Basophils, eosinophils, and neutrophils are called granulocytes, while lymphocytes and monocytes are called agranulocytes. There is a higher chance of disease if one type of cell is predominant over the other [7]. Different WBC types have different morphological features, including shape, size, nucleus and cytoplasm forms. These features will be employed in aiding the WBC counting, segmentation, and classification process. The five types of WBC structures are covered in Table 1.

	cture of WBC		
No.	Type of	Digital microscopic image	Structure of WBC [9]
	WBC	[8]	
1	Neutrophil		The nucleus consists of three to five lobes.
2	Eosinophil		The nucleus is bi-lobed and the cytoplasm is orange to red with larger granules.
3	Basophil		The granules are deep bluish, large, and abundant, and usually cover the nucleus.
4	Monocyte		The nucleus is usually kidney-shaped or bent (horseshoe-shaped).
5	Lymphocyte		The nucleus is dark-stained oval or round-shaped with Agranular cytoplasm.

Table 1 Structure of WBC

The major challenge with the research topic is image segmentation, where accuracy and reliability are concerns with the research studies in the literature. According to Makem *et al.*, [10], the segmentation of WBCs is a crucial stage in the WBCs' classification and counting system based on digitized images of peripheral blood smears. The segmentation procedure for digital images is also included in the computerized detection of blood-related cancer. Many previous studies have been conducted to improve the image segmentation algorithms to build a study for cancer detection that is very effective, accurate, and has a low percentage of error. Mohamed, Far and Guaily [11] proposed using simple arithmetic operations to perform WBC segmentation to remove unnecessary components from the WBC area. The technique was based on filtering and grayscale contrast improvement. Compared with other published techniques performed on the same dataset, the efficiency was high.

Segmentation based on clustering to extract the precise interested area from the source image is one of the popular methods used. According to a comparative analysis carried out by Andrade *et al.,* [12], the most commonly used algorithm for WBC segmentation was K-means clustering because the technique produced the best results. When an appropriate input image is provided and the clustering

algorithms are capable of clearly segmenting WBC from blood smear sample images, they will produce great results.

In Amin *et al.*, [13], the WBC was segmented via K-means clustering the nuclei's features were extracted, and then the cells were classified as normal or cancerous using a support vector machine (SVM) classifier. Ferdosi *et al.*, [14] implied the use of a K-means clustering algorithm and morphological operators to segment WBC images with fluorescent conditions. Hafeez, Yan, and Guoliang [15] presented an automated WBC nuclei segmentation by combining several methods, which were contrast adjustment, K-means clustering algorithm, and thresholding (where the noise was removed). The results proved to have high accuracy for WBC nucleus segmentation. Besides, Sarrafzadeh *et al.*, [16] and Khamael *et al.*, [2] applied fuzzy C-means clustering in their studies to segment the WBC nuclei from the microscopic digital image.

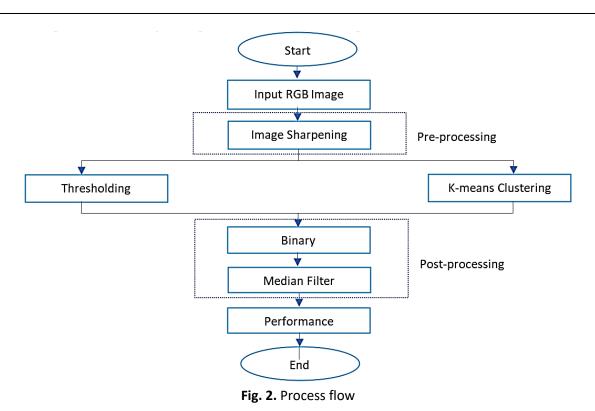
The K-means clustering method was widely utilized in different cases of medical imaging to aid in the segmentation process besides segmenting WBC blood smear sample images. Previous research from Abdullah and Ngadiman [17] used K-means clustering as unsupervised learning to find correct features of the breast cancer sub-type. Finding a pattern or structure in the data set and classifying it based on similarities is the aim of this learning process. Another study conducted by Nasir, Mashor and Mohamed [18] was conducted by implementing an enhanced K-means clustering algorithm, which is an advanced technique compared to conventional K-means clustering for malaria disease slide images. An improved version of the transferring procedure for clustered elements is employed to help allocate data to the appropriate centre during the clustering phase to produce a wellsegmented result image. The K-means clustering is a familiar method used in unsupervised learning for clustering medical imaging in various diseases.

Next, one of the most simple and easy-to-apply methods in the segmentation process is thresholding. The method has been frequently used independently or in conjunction with other methods to segment the WBC. As examples, Mohammed and Abdulla [5] focused on applying thresholding techniques to segment the WBC, and Mandyartha *et al.*, [19] applied global and adaptive thresholding approaches for WBC segmentation. Baihaqi, Widiawati, and Insani [20] used a K-means clustering approach based on Otsu thresholding to segment the WBC nuclei, and Deshpande *et al.*, [21] used multi-level thresholding with the Otsu and Kapur approaches to segment the WBC for leukemia diagnosis.

Most of the existing literature focused on segmenting the WBC nuclei. However, cell segmentation is as important as nuclei segmentation in evaluating the disease and its severity. The preprocessing stage, WBC segmentation, and performance evaluation were all covered in the following proposed system. This paper proposed to analyse the comparative performance of two prominent segmentation techniques: thresholding and the K-means clustering algorithm in microscopic WBC images.

3. Proposed Methods

In this section, the process flow of this paper is explained. The segmentation methods used in this paper were thresholding and the technique for clustering with K-means, and the effectiveness of the two techniques were compared. The summary of the process flow is shown in Figure 2.



3.1 Pre-Processing

The first approach in this paper was pre-processing, which is an important procedure in the image segmentation process. This approach improved the quality of the original input image and enhanced the segmentation image result in the next process. In this process, the original image underwent a sharpening process where the colour of the pixel image became clearer.

3.2 Segmentation Techniques 3.2.1 Thresholding

The threshold technique was used in this paper as a comparison approach to the K-means clustering technique. The thresholding technique had previously been proposed using global and local approaches. While local thresholding techniques apply multiple threshold values to various areas of the image, global approaches apply a single threshold value to the entire image. Global thresholding is the simplest technique, and only one threshold parameter is applied to differentiate the component of the image from the background pixel, as the difference between the component and the background of the image is quite significant [22,23]. If g(x, y) is a threshold image version of the original f(x, y) image at some value of threshold T, the thresholding equation is shown as Eq. (1).

$$g(x,y) = \begin{cases} 1 & \text{if } f(x,y) \ge T \\ 0 & \text{otherwise} \end{cases}$$
(1)

3.2.2 K-means clustering

The K-means clustering approach in data exploration began with the initial set of randomgenerated centres, which served as starting points of every cluster group, as a way to learn about the information. The centroids' locations are then refined using repetitive or recurring calculations. When the centroids have stabilized and the values remain unchanged as a result of the clustering being effectively performed and the required number of repetitions has been accomplished, it stops creating and optimizing clusters. The most widely used clustering criterion is the sum of the squared Euclidean distances between each data point x_i and the centroid c_j of the cluster subset containing x_i [24]. The objective function, J of the K-means clustering algorithm was shown in Eq. (2) below.

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} \left\| x_i^{(j)} - c_j \right\|^2$$
(2)

where,

 $x_i^{(j)}$ indicates the i^{th} sample from j^{th} group of clusters k c_j indicates the center of the j^{th} region

The following stages are illustrations of the K-means clustering algorithm. Let $X = \{x_1, x_2, ..., x_n\}$ be a collection of data points, and $C = \{c_1, c_2, ..., c_i\}$ be the set of middle points.

Stage 1: Classify the data into k clusters, in which k is a predetermined number.

- Stage 2: Pick k randomly to serve as cluster middle points.
- Stage 3: Execute the Euclidean distance function to assign objects to their closest cluster middle points.
- Stage 4: Compute the middle point or mean of each cluster's components.
- Stage 5: Stage 2 is repeated until each cluster acquires the same number of points in subsequent rounds.

3.3 Post-processing

The post-processing described in this paper included the binarization and median filter processes. The resulting image from the segmentation process underwent the binary process to produce black and white pixels and a median filter was implemented on the binary image to remove the noise. These procedures were implemented on the segmented image results to aid in the performance evaluation process of the two segmentation techniques.

3.4 Performance Evaluation

The following equations were applied to compute the Dice score, sensitivity, and specificity for the performance evaluation of the indicated segmentation approaches.

Dice Score
$$= \frac{2 * |G T \cap S I|}{|G T| + |S I|}$$
 (3)

Sensitivity =
$$\frac{TP}{(TP+FN)}$$
 (4)

Specificity =
$$\frac{TN}{(TN + FP)}$$
 (5)

where the terms ground truth (GT) represented the number of cell pixels region of interest from the expert's segmentation, segmented image (SI) was the cell pixels of the segmented WBC image of the proposed techniques, true positive (TP) was the number of interested cell pixels accurately

classified as interested cell pixels, true negative (TN) was the number of inaccurate cell pixels classified as non-interested cell pixels, false positive (FP) was the number of non-interested cell pixels that are inaccurately classified as interested cell pixels and false negative (FN) represented the number of interested cell pixels that inaccurately classified as non-interested cell pixels.

4. Results and Discussion

In this section, the segmented image results of thresholding and K-means clustering algorithm were provided for the five WBCs, namely neutrophil, eosinophil, basophil, monocyte, and lymphocyte. In this study, the sample images observed originated from a public source of peripheral blood cell databases in Kaggle [8]. The WBC microscopic images were all exported in JPG file format with 224×224 pixels. The segmentation evaluation using the Dice coefficient, sensitivity, and specificity was also provided, and the execution time for both techniques was analysed. Table 2 illustrates the segmented WBC image results of the five WBCs respectively.

The segmented WBC image results have been evaluated by comparing them with the ground truth image. According to the finding results, the K-means clustering algorithm evidently provided better segmentation as compared to thresholding techniques. The segmentation of the image of the monocyte highlighted a noticeable difference, with the K-means algorithm providing a more complete segmented image as opposed to thresholding. The execution of both techniques was then analysed through a comparison of both algorithms with the manual expert's segmentation by a hematopathologist. The similarity measures based on region overlapping Dice coefficient, sensitivity, and specificity were used and the results were provided in the next subsection.

Table 2

WBC segmentation image results

Type of Original WBC WBC Image		Pre-processing	Expert's Segmentation	Segmentation Techniques		Post-Processing			
		Sharpening	Ground Truth	Thresholding	K-means Clustering	Ground Truth	Thresholding	K-means Clustering	
Neutrophil									
Eosinophil									
Basophil			B *						
Monocyte									
Lymphocyte			, ,	•	·				

4.1 Segmentation Evaluation

Table 3 presented the image segmentation performance evaluation results of neutrophil, eosinophil, basophil, monocyte, and lymphocyte using thresholding and K-means clustering algorithm. Based on Table 3, the average results for the Dice coefficient can be seen to be slightly biased towards the K-means clustering algorithms, as well as the sensitivity and specificity results. Thus, it can be discovered that the K-means clustering algorithm outperformed thresholding in terms of WBC segmentation ability.

Table 3

Dice Coefficient			Sensitivity		Specificity	Specificity		
Type of WBC	Thresholding	K-means Clustering	Thresholding	K-means Clustering	Thresholding	K-means Clustering		
Neutrophil	0.9812	0.9771	0.9635	0.9553	0.9999	1.0000		
Eosinophil	0.9082	0.9063	0.8318	0.8291	1.0000	0.9999		
Basophil	0.9605	0.9424	0.9241	0.8910	1.0000	1.0000		
Monocyte	0.8711	0.9255	0.7864	0.8614	0.9969	1.0000		
Lymphocyte	0.9403	0.9427	0.8918	0.8925	0.9995	0.9999		
Average	0.9323	0.9388	0.8795	0.8859	0.9993	1.0000		

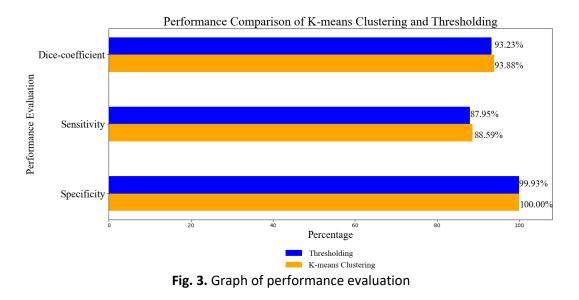
4.2 Segmentation Execution Time

Table 4 shows the time taken to execute the WBC segmentation using thresholding and the K-means clustering algorithm. Based on the findings, the average execution time for the thresholding technique is comparatively less than using the K-means clustering approach. The thresholding approach took less than a second for each execution, while the K-means clustering algorithm takes 6–8 seconds per execution.

Table 4

Execution time (sec)								
Execution time	Neutrophil	Eosinophil	Basophil	Monocyte	Lymphocyte	Average		
Thresholding	0.3512	0.3096	0.3130	0.3140	0.3310	0.3238		
K-means clustering	8.5866	6.0058	5.7620	5.7290	6.0626	6.4292		

Figure 3 visualizes the graph of the performance evaluation in percentage for each of the evaluation methods. There was a slight difference in performance percentage. However, the K-means clustering algorithm proved to be a better technique to be used in WBC image segmentation. In the next subsection, the execution time for both techniques will be discussed.



5. Conclusions

This paper compared the performance of two common techniques used in image segmentation, the thresholding technique and the K-means clustering approach, from the point of view of performance accuracy and execution time. Based on the observations, the performance accuracy of WBC image segmentation proved to be in favour of the K-means clustering algorithm. Meanwhile, by observing the execution time, the thresholding technique seems to be faster. However, the visual aspect of the WBC segmented image results is more important in the machine learning process to provide a good segmentation image for further process. Besides, the execution time variance was only approximately 6 seconds, which was not too much of a difference. Both techniques seem good to be implemented. However, the K-means clustering technique does give more accurate WBC image segmentation.

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