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ORIGINAL ARTICLE

Automatic detection and classification of leukocytes using convolutional neural networks

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Abstract The detection and classification of white blood cells (WBCs, also known as Leukocytes) is a hot issue because of its important applications in disease diagnosis. Nowadays the morphological analysis of blood cells is operated manually by skilled operators, which results in some drawbacks such as slowness of the analysis, a non-standard accuracy, and the dependence on the operator's skills. Although there have been many papers studying the detection of WBCs or classification of WBCs independently, few papers consider them together. This paper proposes an automatic detection and classification system for WBCs from peripheral blood images. It firstly proposes an algorithm to detect WBCs from the microscope images based on the simple relation of colors R, B and morphological operation. Then a granularity feature (pairwise rotation invariant co-occurrence local binary pattern, PRICoLBP feature) and SVM are applied to classify eosinophil and basophil from other WBCs firstly. Lastly, convolution neural networks are used to extract features in high level from WBCs automatically, and a random forest is applied to these features to recognize the other three kinds of WBCs: neutrophil, monocyte and lymphocyte. Some detection experiments on Cellavison database and ALL-IDB database show that our proposed detection method has better effect almost than iterative threshold method with less cost time, and some classification experiments show that our proposed classification method has better accuracy almost than some other methods.

Keywords White blood cell · Detection · Classification · Convolutional neural networks · Random forest

1 Introduction

Nowadays, more and more computer technology and artificial intelligence take part in disease diagnosis [1–5]. Among them, the counting of white blood cells (WBCs, also known as Leukocytes) in peripheral blood is one of important issues because it can assist pathologists to diagnose diseases such as leukemia and other blood diseases. Generally, there are mainly five types of WBCs: eosinophils, basophils, neutrophils, monocytes and lymphocytes, as shown in Fig. 1. They can be counted with manual or automatic methods. Although the manual method can attain a recognition rate 100% when it is performed by very skilled operators, it results in some drawbacks such as slowness of the analysis, a non-standard accuracy, and the high dependence on the operator's skills. Hence, the automatic method for counting WBCs is more and more preferred in the computer-aided diagnosis system.

Generally speaking, an automatic WBC recognition system mainly consists of three key steps: (1) detecting WBCs from a peripheral blood image, (2) extracting effective features, (3) designing a classifier [4]. That is, it firstly detects WBCs from a peripheral blood image and then extracts effective features of WBCs for the classification.

To a certain extent, a good detection method for identifying WBCs from their background correctly is the first step to success. There have been a lot of methods for

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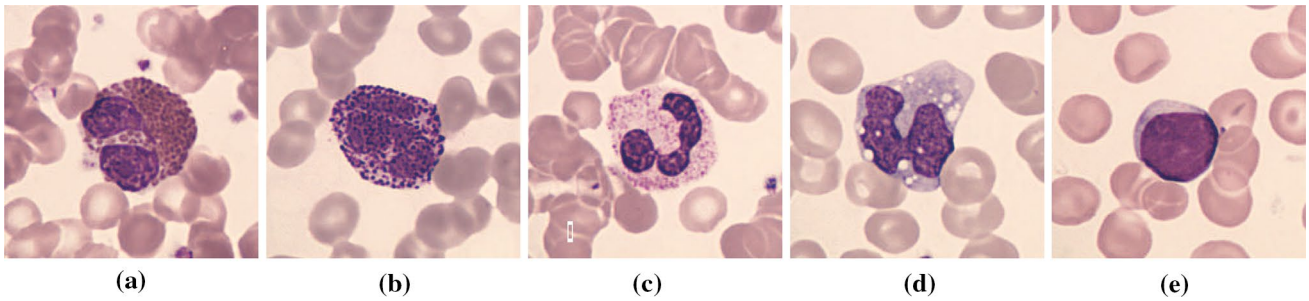

Fig. 1 Five types of white blood cells: eosinophils, basophils, neutrophils, monocytes and lymphocytes

detecting WBCs from the background, such like clustering [6], thresholding [7–14], morphological operator [15–17], Gram–Schmidt orthogonalization method [18], edge detection [19], region growing [20, 21], colors [14, 22, 23], optimization-based method [24, 25], fuzzy-based method [9, 26], and support vector machine (SVM) [27]. On the one hand, each method has its advantages and disadvantages. For example, the threshold method is simple but is not able to accurately segment WBCs from the background. Some methods (e.g., the SVM method and the region growing method) can produce reasonably accurate detection results, but they cost time and need high computational resources. While some color-based segmentation methods (e.g., [14]) were directly conducted on the RGB color space, some approaches (e.g., [22, 23]) adopted the hue-saturation-intensity (HSI) color space (especially on the *S* component). In general, the *S*-component-based methods outperformed the RGB-based methods. On the other hand, there are few automatic detection methods among them that detect WBCs from the microscope images. That is, many methods start the classification from the cropped WBCs images by some experts, which results in the inconvenience in real applications. For example, Tai et al. [31] begin the feature extraction such as roundness feature, color of cytoplasm, and nucleus-cytoplasm ratio. Therefore, some people begin to study the automatic WBCs detection methods for the applications. For examples, Guimaraes et al. [19] present a new automatic circular decomposition algorithm that proceeds the separation of connected circular particles to locate their center coordinates and estimate their radii. Sinha and Ramakrishnan [29] propose an automatic detection method using *k*-means clustering and EM-algorithm on the HSV-equivalent of the image. Nilufar et al. [12] propose an automatic detection method based on contour of blood cells. Rezatofighi and Soltanian-Zadeh [18] find the discriminating region of WBC on the hue-saturation-intensity (HSI) color space, segment WBC with a morphological process, extract some geometrical, color, texture features, and classify the obtained features with three kinds of neural networks: multilayer perceptron, SVM, and the hyper rectangular composite neural networks. Cuevas et al. [24, 25] propose some automatic detection methods based on the optimization theory, i.e., considering the automatic detection of WBCs as an ellipse or circle detection problem to improve the detection accuracy, robustness and stability.

Now an effective feature extraction method a good classifier is the second step to success for a WBC recognition system. Many papers extract some features of nucleus and cytoplasm, and then distinguish them with some neural networks classifiers. For example, Mohapatra et al. [28] use gray level co-occurrence matrix (GLCM) as well as some shape features of the leukocytes to classify five types. Sinha and Ramakrishnan [29] use shape, color and texture features to identify leukocyte. Kuse et al. [30] use GLCM texture to obtain 18 signatures to identify lymphocytes. Rezatofighi and Soltanian-Zadeh [18] extract the color and morphological features of cells, leaf area and texture feature (local binary pattern) to recognize leukocytes. Tai et al. [31] extract features such as roundness, color of cytoplasm, and nucleus-cytoplasm ratio. Su et al. [4] extract some geometrical, color, texture features. However, those features are designed by some experts with their experiences according to the characteristics of cells. These low-level features can be hand-crafted with great success for some specific data, but designing effective features for new data usually requires new domain knowledge because most hand-crafted features cannot be simply applied into new conditions. Therefore, people begin to find another way of learning features from the data of interest to remedy the limitation of hand-crafted features. A representative example of such methods is learning through deep neural networks, which attracts significant attention recently [32]. The idea of deep learning is to find higher-level features to provide more invariance to intra-class variability. One successful application of deep learning in image classification is the use of convolution neural network (CNN) [33, 34]

architecture. Human brain visual system can extract temporal information effortlessly from a cluttered scene in the external environment and analyze the target of interest or region formed on scene quickly and accurately from the aspect of understanding and awareness, while CNN just imitates this operation principle with its special network structure and learning rule. Therefore, CNN can extract high-level features from images.

Up to now, there have been many classifiers designed for pattern recognition, such as Bayes classifier [14], predictor of natural disordered regions (PONDR predictor) [40] for MobiDB database [41], feed-forward back propagation [35, 36], SVM [18], local linear map [37], fuzzy cellular neural network [38], extreme learning machine [39], random forest [46], and so on. Some of these classifiers are successfully applied in the process of classification for WBCs. As a random forest consists of a collection of tree-structured classifiers, it usually has a better classification effect than a single classifier.

In this paper, we address the issue of an automatic recognition system that can effectively detect and classify WBCs from peripheral blood images. We want to automatically detect WBCs from background with a simple and effective algorithm. Then we want to extract features in high level with CNN automatically. Finally, we consider applying an ensemble classifier: random forest to improve the classification. The main contributions of our study are highlighted as follows. (1) In the step of detecting WBCs from the microscope image, unlike some traditional methods that convert color image into other color spaces, we just apply the simple relationship of R and B components based on the special characteristics of WBC to separate WBCs from the background. Then we propose an algorithm that combines the lobes of nuclei using their own characteristics to detect WBCs automatically, which is timeless and accurate in experiments. (2) In the step of extracting features, unlike some traditional methods that design features by some experts with their experiences, we apply CNN to extract effective features of WBCs automatically. (3) In the step of choosing a classifier, unlike the traditional strategy that constructs a single classifier, we apply a random forest that consists of a collection of tree-structured classifiers to improve the accuracy for the features extracted by CNN.

2 Methods

In this section, we will propose a novel automatic WBC recognition system. The proposed system firstly detects WBCs from the microscope images based on the simple relationship of colors and the morphological operations. Then for each WBC, a granularity feature (pairwise rotation invariant co-occurrence local binary pattern (PRICoLBP) [42]) is extracted and SVM is applied on these granularity features to discern the eosinophil and basophil from other types of WBCs. For the remaining three types of WBCs, we apply CNN to extract their features in high level automatically. Then an effective classifier, random forest, is applied to recognize which kind of WBCs it belongs to: neutrophil, monocyte or lymphocyte? The flowchart of our proposed method is shown in Fig. 2.

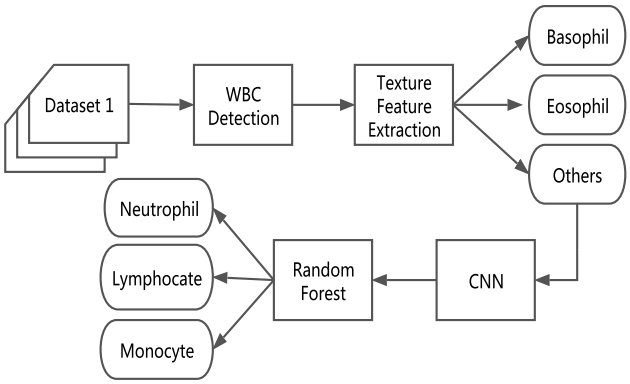

Fig. 2 A *block diagram* of our proposed automatic recognition system for WBCs: WBCs are detected from a microscope image first based on the simple relationship of colors and the morphological operations, then a granularity feature (PRICoLBP) is extracted for each WBC, and SVM is applied to discern the eosinophil and basophil from other types of WBCs. Last, CNN is applied to the left three types of WBCs to extract their features automatically, and a random forest is used to recognize them

2.1 Detection of WBCs

A peripheral blood image I_0 contains not only different kinds of WBCs, but also a lot of red blood cells. Therefore, the first step of our proposed method is to detect WBCs from the image I_0. Generally, after blood cells are dyed with some methods, e.g., the method of Wright's staining, the nuclei of WBCs will appear deep colored, which makes WBCs be easily distinguished from other cells. For example, see the sampled images (a) and (b) in Fig. 7. The classical methods for detecting the nucleus often convert the microscope image from the RGB space to some other color spaces such like HSI and HSV spaces, and then choose the significant components with an appropriate threshold value. Here, in our proposed method, we will adopt the special property of blood cell image to detect WBCs. That is, we first use the difference value $R - B$ of color R and B to get an $R - B$ image I_1, then give a binarization on the

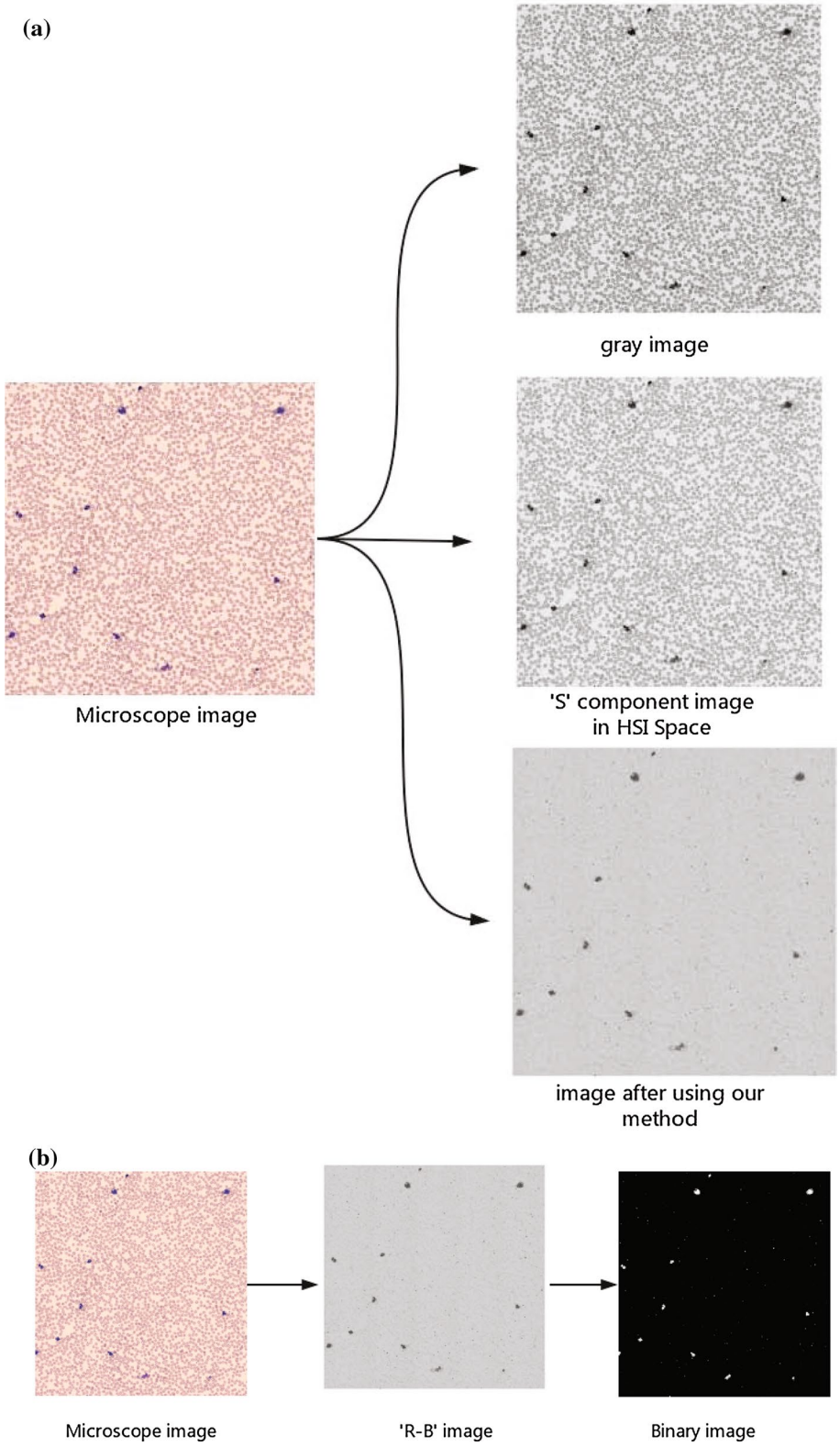

Fig. 3 Color transformation comparison of our method with some other methods for detecting WBCs initially. **a** Examples of converting color image into some other images. The *first row* shows the process of converting color microscope image into *gray image*. The *second row* shows the process of converting color image into "*S*" component image after HSI transformation. The *last row* shows the process of converting color image into $R - B$ image with our method. **b** The process of getting I_2 image with our proposed method. First, convert the color image into $R - B$ image, then get a binary image using a binarization on the $R - B$ image with a threshold value

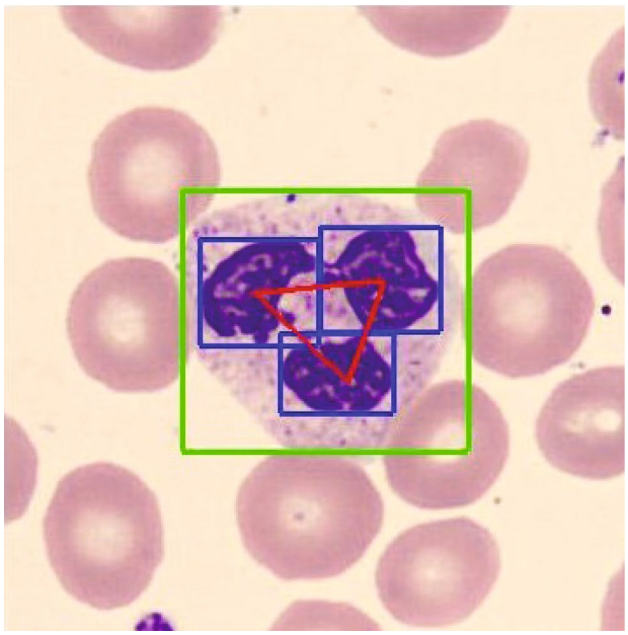

Fig. 4 One cropped WBC image illustrates the algorithm for mergence of lobe areas

$R - B$ image I_1 with a threshold value to get an image I_2 that makes the nuclei more obvious, as shown in Fig. 3.

We can see that the obtained image I_2 in Fig. 3 contains some small objects that are not nuclei of WBCs, and some nuclei may be not completed. In order to remove those small objects from image I_2 and complete the nuclei, we will apply some intersections of morphological operations [43], such like erosion and dilation operation on the image I_2. Erosion operation can eliminate small and insignificant objects. An erosion of B on X can be described as

$$X \ominus B = \left\{ z \in \mathbb{Z}^2 \mid (B)_z \subset X \right\},$$

where X is a binary image, B is a structuring element, and $(B)_z$ is the translation of B with respect to point z. As opposed to the erosion operation, dilation operation can fill in the holes of the objects by means of "growing" or "thickening" objects. An dilation of B on X can be described as

$$X \oplus B = \left\{ z \in \mathbb{Z}^2 \mid (\hat{B})_z \cap X \neq \varnothing \right\},$$

where $\hat{B}$ is the reflection of B. In our proposed method, we first use erosion operation once to get rid of small and insignificant objects, and dilation operation twice to grow the nucleus of WBCs. Its formula for getting a clear image I_3 from image I_2 can be described as follows:

$$I_3 = (I_2 \ominus B \oplus B \oplus B) \cap I_2,$$

where B is some chosen structuring element.

With the information of nuclei in I_3 image, we begin to locate and crop the WBCs. Let (x_i, y_i) be the center coordinates of the minimum bounding rectangle A_i that contains the i-th WBC, $i = 1, 2, \ldots, N$. Sometimes, the selected rectangle may be not cover a whole cell, but just a lobe of a nucleus. For example, as shown in Fig. 4, for one WBC, there are three rectangles that contain one lobe, respectively. So we need to find a rectangle that contains the whole WBC. Here, we calculate the Euclidean distance of the centers of two rectangles. If the distance is less than the diameter of the nucleus and the number of pixels in two areas is less than the maximum value, we consider these two lobes are in the same nucleus and update the rectangle and its center coordinate with the following formula

$$A_i \leftarrow A_i \cup A_j \quad \text{and} \quad (x_i, y_i) \leftarrow \left(\frac{x_i + x_j}{2}, \frac{y_i + y_j}{2} \right)$$

until the condition above fails. Generally, the diameter of the nucleus and the maximum value is related to the magnification of the image. For example, in our sampled image, one nucleus contains around 3000 pixels. The detail procedure of merging lobes of one nucleus is shown in the following Algorithm 1.

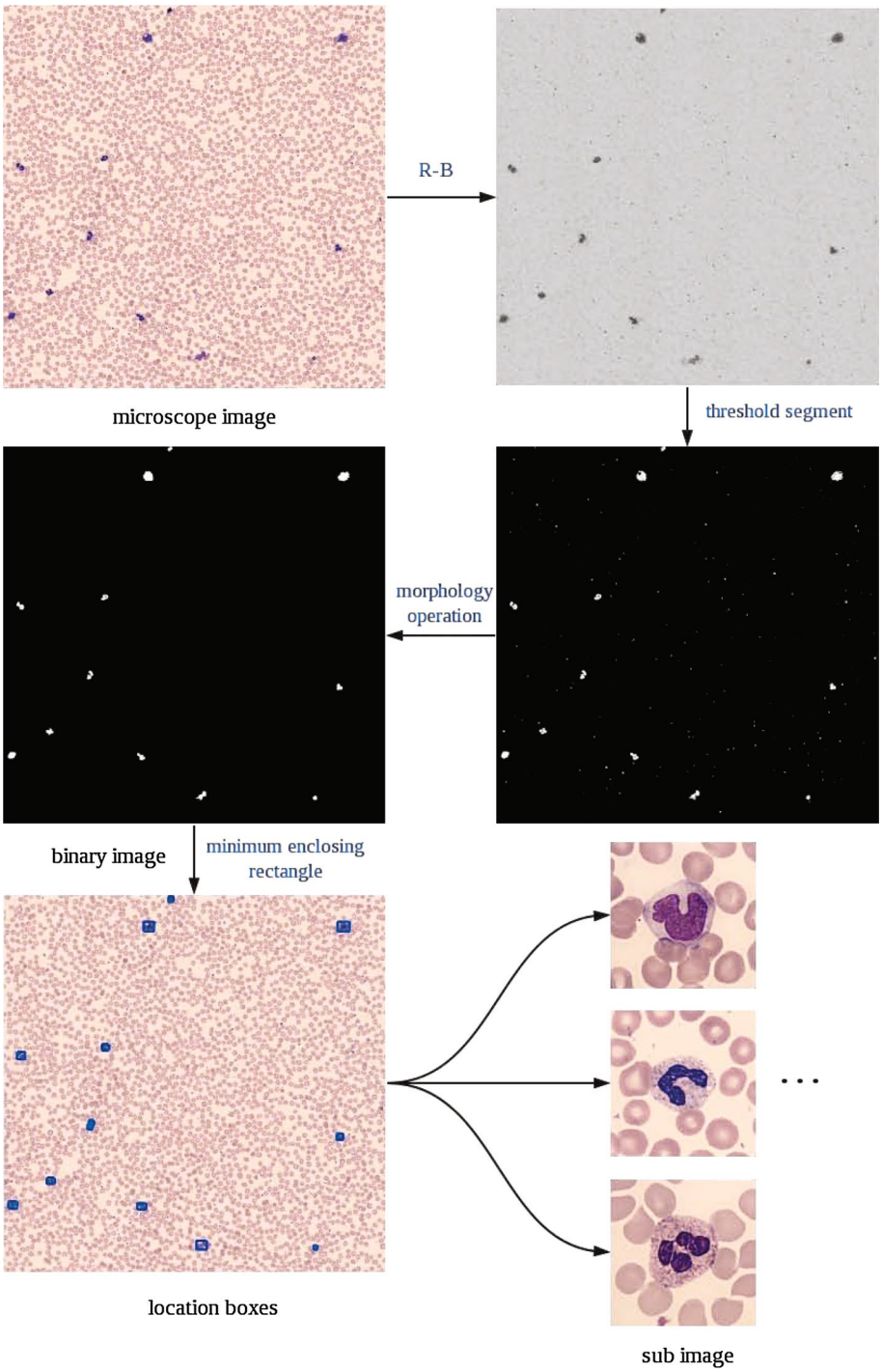

Fig. 5 The flowchart of our proposed method for detecting WBCs from a peripheral image. Convert color image into $R - B$ image, get a binary image using a binarization on the $R - B$ image with a threshold value, get rid of small objects and complete the nuclei with morphological operations, locate WBCs with our algorithm of merging lobes of one nucleus, and crop each WBC from the peripheral image

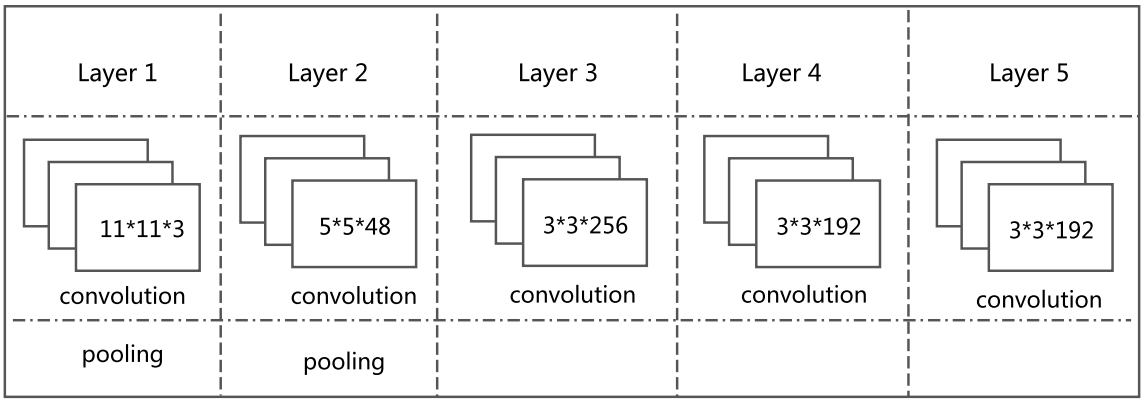

Fig. 6 The concrete architecture of CNN applied in our proposed method to extract features in high level. It consists of 5 convolutional layers and 2 pooling layers

Algorithm 1 The mergence of lobes of one nucleus.

Input: The number N of minimum bounding rectangles, and the center coordinate (x_i, y_i) of each bounding rectangle $A_i, i = 1, 2, \cdots, N$.

Output: The new number M and the coordinates of bounding rectangles $\{(x_i, y_i)\}_{i=1}^{M}$.

For $i = 1$ to N;

$k = 0$;

For $j = i + 1$ to N;

Calculate the distance of centers (x_i, y_i) and (x_j, y_j) of two bounding rectangles

$$d = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}.$$

If d is less than the diameter of the nucleus and the number of pixels in $A_i \cup A_j$ is less than the maximum value, update the rectangle and its center coordinate with the following formula

$$A_i \leftarrow A_i \cup A_j \quad \text{and} \quad (x_i, y_i) \leftarrow \left(\frac{x_i + x_j}{2}, \frac{y_i + y_j}{2} \right);$$

$k = k + 1$.

end if.

$N = N - k$.

end

end

Denote the final N as M, and collect all the new coordinates $\{(x_i, y_i)\}_{i=1}^{M}$.

Table 1 Databases

	Num	Resolution	Format	Usage
Cellavision database	14	2864 × 2909	JPG	Detection
	1080	300 × 300	JPG	Classification
ALL-IDB database	59	2592 × 1944	JPG	Detection
	130	1712 × 1368	JPG	Classification
Jiashan database	215	300 × 300	JPG	Classification

With the new coordinates $\{(x_i, y_i)\}_{i=1}^{M}$ of rectangles $A_{i=1}^{M}$, we can locate WBCs and crop them from image I_3. Up to now, we have detected WBCs from the peripheral image automatically. Its flowchart can be seen in Fig. 5 .

2.2 Classification of WBCs

From Sect. 2.1, we have detected all kinds of WBCs from the peripheral image automatically. The left work is to determine which type the WBC is: neutrophils, eosinophils, basophils, monocytes or lymphocytes? Generally speaking, traditional methods for classifying WBCs usually extract some features of nucleus or cytoplasm for the subsequent classifier, such like geometrical features, texture features or color features. Therefore, the classification results depend greatly on the dissimilarity of the designed features. That is to say, how to design an effective feature is very important for the WBC classification. In our proposed classification method, we want to seek a feature extraction of WBCs based on CNN [33, 34]. As CNN will exhibit better performance when it deals with larger set of images, while proportions of eosinophils and basophils in peripheral blood image are $1 \sim 5\%$ and less 1%, respectively, we want to distinguish the eosinophil and basophil from other types of WBCs firstly before applying CNN on WBCs.

Since eosinophil and basophil are full of some concentrate and big granules (as shown in Fig. 1a, b), we will apply PRICoLBP feature [42] to embody the granularity of eosinophil and basophil, which enhances the discriminative power of eosinophil and basophil from other types of WBCs. PRICoLBP is an variant of local binary pattern

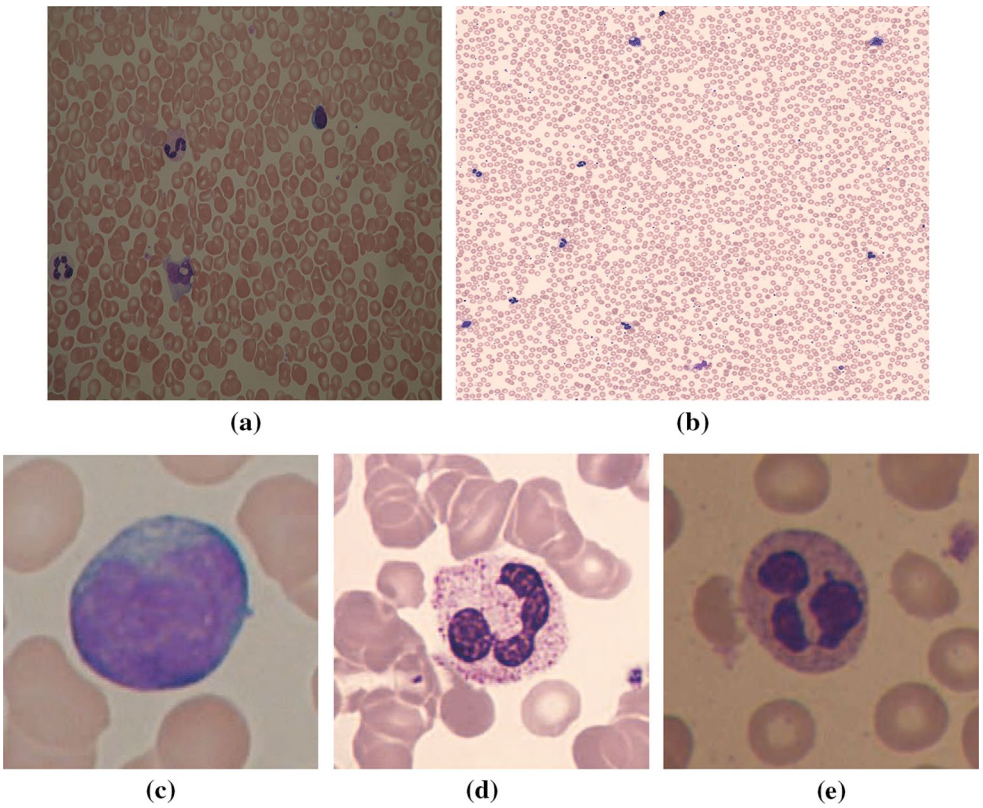

Fig. 7 Some images are sampled from ALL-IDB database, Cellavision database and Jiashan database. **a** One peripheral blood image sampled from ALL-IDB database. **b** One peripheral blood image sampled from Cellavision database. **c** One cropped image from ALL-IDB database. **d** One cropped image from Cellavision database. **e** One image from Jiashan database

(LBP) that is a gray-scale invariant texture descriptor. For a point A in an image, its LBP code is computed by

$$\text{LBP}\ (A) = \sum_{i=0}^{n-1} s(g_i - g_c)2^i,$$

where g_c is the pixel value of point A, g_i is the pixel value of point A's i-th neighbor, and $s(\cdot)$ is the signal function whose value is 0 or 1 [44]. Let $\mathcal{U}(\cdot)$ be the uniformity measure on LBPs defined as

$$\mathcal{U}(\text{LBP}\ (A)) = \sum_{i=1}^{n} |s(g_i - g_c) - s(g_{i-1} - g_c)|,$$

then $\text{LBP}^{\text{u}}(A)$, the uniform LBP of point A, is defined as those LBPs of point A with $\mathcal{U}(\text{LBP}\ (A)) \leq 2$, and $\text{LBP}^{\text{ru}}(A)$, rotation invariant uniform LBP of point A, is defined as [44]

$$\text{LBP}^{\text{ru}}(A) = \begin{cases} \sum_{i=0}^{n-1} s(g_i - g_c) & \mathcal{U}(\text{LBP}(A)) \leq 2; \\ n+1 & \text{otherwise} \end{cases}$$

Now the PRICoLBP of two points A and B can be described as

$$\text{PRICoLBP}\ (A, B) = [\ \text{LBP}^{\text{ru}}(A),\ \text{LBP}^{\text{u}}(B, i(A))]_{\text{co}},$$

where $\text{LBP}^{\text{u}}(B, i(A))$ is the uniform LBP of point B by using $i(A)$th index as the start point of the binary sequence [42]. PRICoLBP feature can not only capture the spatial context co-occurrence information effectively, but also possess rotation invariance.

With these obtained PRICoLBP features for five types of WBC, we use SVM [45] to classify them into three classes: eosinophil, basophil, and others.

Now the left work is to classify the remaining three kinds of WBCs: neutrophil, monocyte and lymphocyte. Here we want to apply CNN [34] to extract features of WBCs in high level, and take random forest [46] as a classifier to distinguish these features. The idea of CNN is to discover multiple levels of representation, with the assumption that higher-level features can represent more abstract semantics of the data. Those extracted features learned from a deep network are expected to provide more invariance to intra-class variability [32]. CNN is a special feed-forward neural network that consists of several convolutional layers and pooling layers. The convolution layer filters the input image with some small matrix of weights and applies some nonlinear function as an active function. For example, for an input image I, let $W \in \mathbb{R}^{k_1 \times k_2}$ be a filter of weights, then the operation in convolution layer is to take the $2D$ convolution $I \times W$, and the active function can be taken $f(x) = \max(0, x)$. The pooling layer does not contain weights and simply reduces the size of the preceding output with the max-pooling operation. In this paper, the applied CNN in our method consists of 5 convolutional layers and 2 pooling layers. Its concrete architecture is shown in Fig. 6.

The applied learning algorithm for training CNN is the stochastic gradient descent method. The update rule for weight w is

$$v_{i+1} := 0.9v_i - 0.0005\epsilon w_i - \epsilon \left\langle \frac{\partial L}{\partial w}|_{w_i} \right\rangle_{D_i},$$

$$w_{i+1} := w_i + v_{i+1},$$

where i is the iteration index, v is the momentum variable, ϵ is the learning rate, and $\langle \frac{\partial L}{\partial w}|_{w_i} \rangle_{D_i}$ is the average over the ith batch D_i of the derivative of the objective with respect to w, evaluated at w_i [34]. With the trained CNN, we can extract a feature vector of 4096-dimension from each WBC image.

Now a random forest [46] is used to classify those extracted features with CNN to determine the left three types of WBCs: neutrophil, monocyte and lymphocyte. A random forest is a classifier consisting of a collection of tree-structured classifiers, and each tree casts a unit vote for the most popular class at input [46]. The mechanism and structure of a random forest can be summarized as following two phases.

1. Training phase: Given a set of training set $X = \{x_i\}_{i=1}^N \subseteq \mathbb{R}^m$ and the corresponding class labels $Y = \{y_i\}_{i=1}^N \subseteq \{1, 2, \ldots, c\}$, where c is the number of classes, the number of trees L in the forests, and the i-th decision tree T_i in the random forests, $i = 1, 2, \ldots, L$.

Step 1. For each decision tree T_i, generate a training set with N bagging sampling, that is, sampling N times from training set X with replacement.

Step 2. At each non-leaf node t, the best split attribute is calculated by an approach of Gini impurity index defined by

$$\text{Gini}(t) = 1 - \sum_{j=1}^{c} [p(j|t)]^2,\ \ j = 1, \ldots, c$$

over the randomly chosen features, where $p(j|t)$ is the probability of class j in the node t.

Step 3. Go to Step 2 until T_i is fully grown without being pruned.2. Classification phase: For a given test sample x, it is pushed down each classifier in forests and every decision tree will give only one vote on the label of this sample. Then the predicted label of x is determined as the one which has the most votes in the forests.

Table 2 Sensitivity and precision comparison of our proposed method and the iterative threshold method in the paper [14] on the Cellavision database

No. of images	Our method					Iterative threshold method [14]				
	TP	FP	FN	TPR (%)	PPV (%)	TP	FP	FN	TPR (%)	PPV(%)
#1	8	2	0	100	80	10	0	9	52.6	100
#2	7	0	0	100	100	–	–	–	–	–
#3	7	0	0	100	100	6	1	5	54.5	85.7
#4	3	0	1	75	100	3	0	0	100	100
#5	0	0	0	100	100	0	0	3	0	100
#6	4	1	0	100	80	–	–	–	–	–
#7	6	0	0	100	100	6	0	0	100	100
#8	5	0	0	100	100	–	–	–	–	–
#9	5	0	0	100	100	5	0	8	38.5	100
#10	9	0	1	90	100	–	–	–	–	–
#11	12	1	2	85.7	92.3	13	0	4	76.5	100
#12	7	0	0	100	100	5	2	6	45.5	71.4
#13	1	0	0	100	100	–	–	–	–	–
#14	2	0	0	100	100	–	–	–	–	–

Table 3 Cost time (unit: s) comparison of our method and the iterative threshold method [14]

Database	Cost time of our method (s)	Cost time of iterative threshold method (s)
Cellavision	29.06	65.18
ALL-IDB	71.18	125.09

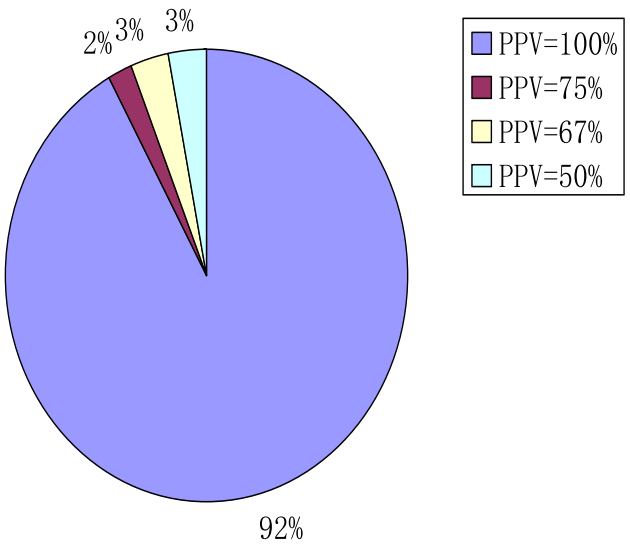

Fig. 8 Proportions of images with different PPV via our detection method for 59 images in ALL-IDB database. 92% of 59 peripheral blood images have PPV 100%; 2, 3, 3% of 59 peripheral blood images have PPV 75, 67, 50%, respectively

2.3 Databases and evaluation criteria

It is a phenomenon that there is no big and public database for WBC detection and classification. So many papers test their recognition system with only a few WBC images, or with their own databases that are not for public. In order to illustrate the high effect of our proposed method, we collect all the currently known datasets and some sample images from local hospital as possible as we can. Here Cellavision database [47] is an innovative and global medical technology company that develops and sells best-in-class systems for the routine analysis of blood and other body fluids. We download 14 microscope images of size 2864 × 2909 in JPG format with 24-bit color depth to test our detection method and 1080 cropped images of size 300 × 300 in JPG format with 24-bit color depth to test our classification method from the database in CellaVision Proficiency Software. ALL-IDB database [48] is a public image database consisting of the peripheral blood samples collected by some experts from some normal individuals and leukemia patients of childhood leukemia and hematological diseases. It contains two distinct versions. One includes 108 images in JPG format with 24-bit color depth, most of which were captured with an optical microscope in different magnifications ranging from 300 to 500 and a Canon PowerShot G5 camera with a resolution 2592 × 1944. The other images were captured with a microscope in a constant magnification and an Olympus C2500L camera with a resolution 1712 × 1368. In our experiments, the normal samples are chosen as our training and testing data. Jiashan database

Table 4 Accuracy comparison of our proposed classification method with Seyed method [18] and HSVM method [31] on the mixed database of Cellavision, ALL- IDB and Jiashan databases

Methods	Basophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	Classification accuracy (%)
Ours	100	70	74.8	85.3	97.1	92.8
Seyed [18]	53.0	63	85.0	39.0	50.8	76.8
HSVM [31]	43.8	0	66.8	0	7.5	76.3

is an image database consisting of the peripheral blood samples collected by some experts in Jiashan First People's Hospital from some patients. It includes 215 cropped images in JPG format with 24-bit color depth and a resolution 300 $\times$ 300 for testing the classification.

In all of these databases, each image for detection has an associated text file containing the coordinates of the centroid of each leukocyte. They are manually labelled by a skilled operator and can be used as a ground truth. The information of these databases is shown in Table 1, and some sampled images from these databases are shown in Fig. 7.

To evaluate the good performance of a method, we apply sensitivity (or true positive rate, TPR) and precision (or positive predictive value, PPV) as follows:

$$\mathrm{TPR} = \mathrm{TP}/(\mathrm{TP} + \mathrm{FN}), \quad \mathrm{PPV} = \mathrm{TP}/(\mathrm{TP} + \mathrm{FP}),$$

where TP is the number of WBC in a microscope image that has been detected to be a WBC correctly, FP is the number of WBC in a microscope image that has not been detected to be a WBC, and FN is the number of non-WBC in a microscope image that has been detected to be a WBC. Therefore, the higher the sensitivity and precision, the better the detected method.

All the programmes are carried out in MATLAB 8.4.0.150421 (R2014b) environment running on 8 processor with the speed of 2.40 GHz.

3 Results

3.1 Experiments on WBC detection

In this subsection, we compare the validity of our proposed detection method for WBCs with the iterative threshold method in the paper [14]. For the iterative threshold method [14], Wu et al. consider the WBC recognition from the global process. They start the image cropped by experts from microscope, find the discriminating region of WBC in the HSI color space, segment WBC with a morphological process, extract some geometrical, color, texture features, and classify the obtained features with three kinds of neural networks: multilayer perceptron, SVM, and the hyper rectangular composite neural networks. We take some microscope images of Cellavision database and ALL-IDB database as the testing data. According to our detection method described in Sect. 2.1, the threshold for separating nucleus from the background can be set from -5 to 0.

Table 2 shows sensitivity (TPR) and precision (PPV) of our proposed method with the iterative threshold method [14] on the Cellavison database. Furthermore, Table 3 exhibits their cost time on Cellavison database and ALL-IDB database.

As shown in Table 2, our proposed detection method has an outstanding performance than the iterative threshold method [14] for most sampled images. For example, method can not detect 6 microscope images that occupy 43% in the sampled images. Even for the images that method can detect, our proposed method almost has a higher sensitivity, and method easily detects other non-WBCs as WBCs, which will also bring the bad effect in diagnosis.

Table 3 exhibits the comparison of cost time of our proposed method with the iterative threshold method [14], where the cost time is taken from inputting the peripheral blood image to get the subimages of all kinds of WBCs. As observed from Table 3, the iterative threshold method takes twice times as our method does for detecting WBCs, which implies that our method is quick and the cost time is acceptable in medical diagnosis.

Table 2 illustrates the good performance of our detection method on Cellavision database. Also, we take the same experiments on the ALL-IDB database. Here we further show the entire effect of our detection method on 59 peripheral blood images in ALL-IDB database. Then the proportions of images with different PPV via our detection method are shown in Fig. 8.

Figure 8 shows that the images that have PPV 100% occupy 92% in 59 peripheral blood images; the left images that have 75, 67, 50% occupy 2, 3, 3 in 59 peripheral blood images, respectively, which implies that our detection method can detect almost WBCs from peripheral blood images.

3.2 Experiments on WBC classification

In this subsection, 1080 cropped images of Cellavision database, 20 cropped images of ALL-IDB database,

215 images of Jiashan database with normalized size of 227 × 227 are put together to test the effect of our classification method. Firstly, we extract the PRICoLBP feature for each image and apply SVM to distinguish the eosinophils and basophils from other three types of WBCs. Then for the left three kinds of WBCs: neutrophil, monocyte and lymphocyte, CNN is used to extract feature in high level with 4096 dimension. After normalizing these features, we use a random forest to classify them. Classification process is run 50 times, and the average result is taken as the final classification accuracy. The final classification comparison results of our proposed classification method with Seyed method [18] and hierarchical SVM (HSVM) method [31] for WBCs are shown in Table 4.

Although Seyed [18] and HSVM [31] methods are successful on their respective databases, Table 4 shows that they are not successful on our mixed database. The reason for this is that the database has a strong challenge and is larger. However, our method employed CNN that can extract multiple-levels features from WBCs effectively on the larger database. Therefore, it is almost superior to Seyed and HSVM methods from each kind of WBC or the total recognition rate on the challenge database.

4 Discussion

Since the counting of WBCs in peripheral blood image can assist pathologists to diagnose diseases, it is meaningful for the paper to discuss the automatic detection and classification for WBCs from microscope images. The proposed method firstly detects WBCs based on a simple relation of colors R and B and the morphological operation, then PRICoLBP feature is extracted, and SVM is applied to distinguish the eosinophils and basophils from other three types of WBCs. While for the left three kinds of WBCs: neutrophil, monocyte and lymphocyte, CNN is used to extract feature in high level and a random forest is applied to distinguish them. In a word, the design of our method is based on the essential information of the peripheral blood image and the structure of WBCs. And furthermore, some effective methods are introduced to improve the classification accuracy.

For the detection of our method, as Tables 2 and 3 show, our proposed method has an outstanding performance than the iterative threshold method [14] for most images. Because the iterative threshold method [14] uses the iterative Otsu's approach on HSI space based on circular histogram to detect WBCs, it sometimes cannot detect WBCs for some peripheral blood images. For example, as #2, #6, #8, #10, #13, #14 images shown in Table 2, no matter how the threshold is taken with iteration, WBCs cannot be segmented from the background. Even for the images that method [14] can detect, our proposed method has a higher sensitivity (TPR) and precision (PPV) mostly. Because our method makes the best of the simple relation of colors R, B and uses the morphological operation to delete the noises and complete the nucleus, it can possibly avoid recognizing non-WBCs as WBCs while iterative threshold method often regards non-WBCs as WBCs. At last, our detection method does not need the iteration, so it costs less times than the iterative threshold method does.

Of course, our detection method is not perfect. There is also some limitations. It cannot detect all the WBCs for some peripheral images, for example, as #1, #6, #11 images shown in Table 2. And it sometimes regards a few non-WBCs as WBCs, for example, as #4, #10, #11 images show. Hence, how to find a more effective detection method based on our method is the direction of our study in the future.

For the classification of our method, as shown in Table 4, the classification accuracy of our proposed method is almost superior to Seyed method [18] and HSVM method [31] do on the mixed database of Cellavision, ALL- IDB and Jiashan databases. Generally speaking, feature extraction and design of a classifier are two key steps in classification. Our proposed classification method considers these two factors in the process of design. For example, considering that eosinophil and basophil are full of some granules, we extract the PRICoLBP features to enhance the discriminative power of eosinophil and basophil from other types of WBCs. As expected, the accuracy of basophil and eosinophil with our method is 10 and 70%, respectively, which is higher far from the results for Seyed method and HSVM method. The reason is that we design a better features for eosinophil and basophil than Seyed method and HSVM method do. Furthermore, for the left three types of WBCs: lymphocyte, monocyte, and neutrophil, we apply CNN to discover features in high level to enforce the discrimination of these three WBCs. And an effective classifier: random forest is applied to classify the extracted features. Several studies have shown that combining multiple weak classifiers into one aggregated classifier can lead to better classification performance than any of the weak individuals [49], random forest shows an effective classification. As expected, the accuracy of monocyte and neutrophil with our method is 85.3 and 97.1%, respectively, which is higher far from the results for Seyed method and HSVM method do.

Of course, our classification method is not also perfect. The classification for lymphocyte is 74.7%, which is less than 85% of Seyed method. The reason is that the number of lymphocyte images for training CNN is less, while CNN does good jobs for large data. Therefore, how to improve the classification of lymphocyte images based on our method is another direction of our study in the future.

5 Conclusion

In this paper, we have proposed an automatic detection and classification system for WBCs from peripheral blood images. Our proposed method firstly uses the simple relation of color R, B to get $R - B$ image, applies morphological operation to delete the noises and complete nucleus, and then gives an algorithm of merging lobes of nucleus to help detecting WBCs from peripheral blood images. With the detected WBCs, we use PRICoLBP feature and SVM to distinguish the eosinophils and basophils from other three types of WBCs first. Then for the left three kinds of WBCs: neutrophil, monocyte and lymphocyte, CNN is used to extract feature in high level and a random forest is applied to distinguish them.

The main contributions of this paper are highlighted as follows:

1. In the step of detecting WBC from the peripheral blood image, unlike the traditional strategy that converts color image into other color space, we have applied the simple relation of R, B to replace the HSI space.
2. We have proposed an algorithm that combines the lobes of nuclei using their own characteristics, and then detect WBC using the location of nucleus of leukocyte, which is timeless and accurate in experiments.
3. Considering that eosinophil and basophil are full of some granules, we designed a plan to extract the granularity via PRICoLBP feature to classify the eosinophil and basophil from other types of WBCs.
4. For the left three types of WBCs: lymphocyte, monocyte, and neutrophil, we have applied CNN to extract the most effective features and a random forest to classify them accurately.
5. We have proposed an automatic recognition system for WBC, that is to say, a method for detecting WBCs from peripheral blood image directly and classifying them without manual operation.

Experiments on Cellavison database, ALL-IDB database and Jiashan database show that our proposed method has better detection and classification than some other methods with less cost time.

Of course, our proposed method is not 100% perfect. There is also some limitations. For example, it cannot detect all the WBCs for some peripheral images, and it sometimes regards a few non-WBCs as WBCs. What is more, the classification for lymphocyte needs to be improved. Hence, how to find a more effective detection method and how to improve the classification of lymphocyte images based on our method are the directions of our study in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This study did not involve human participants and animals.

Informed consent The all authors of this paper have consented the submission.

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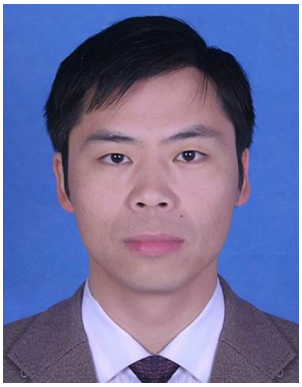

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