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Automatic segmentation, counting, size determination and classification of white blood cells

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ABSTRACT

The counts, the so-called differential counts, and sizes of different types of white blood cells provide invaluable information to evaluate a wide range of important hematic pathologies from infections to leukemia. Today, the diagnosis of diseases can still be achieved mainly by manual techniques. However, this traditional method is very tedious and time-consuming. The accuracy of it depends on the operator's expertise. There are laser based cytometers used in laboratories. These advanced devices are costly and requires accurate hardware calibration. They also use actual blood samples. Thus there is always a need for a cost effective and robust automated system. The proposed system in this paper automatically counts the white blood cells, determine their sizes accurately and classifies them into five types such as basophil, lymphocyte, neutrophil, monocyte and eosinophil. The aim of the system is to help for diagnosing diseases. In our work, a new and completely automatic counting, segmentation and classification process is developed. The outputs of the system are the number of white blood cells, their sizes and types.

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1. Introduction

The main purpose of this paper is to describe the development of a blood smear image based process to help for diagnosis of diseases. The diseases can be diagnosed by the number and morphological changes of white blood cells. The diagnosis can still be performed mainly by manual techniques. However, the accuracy of it depends on the operator's expertise. The situation of the operator may highly affect the analysis. Another method is to use automated cell counter systems such as laser based cytometers [1]. In that paper, authors describe a device that allows carrying out optical excitation of separate cells in a flow cytometer using the radiation of YAG–Ni pulsed laser. There are a lot of cytometers on the market today. They may provide automated cell counting but they have lack of capabilities necessary for automated diagnosis of ALL disease. They do not have the capability to separate abnormal cells such as lymphoblasts from normal cells. They do not allow classifying white blood cells according to their morphologies. They are costly devices and require accurate hardware calibration and they have to use actual blood samples. After analysis, the blood sample is totally destroyed. In recent days, image based cell counting approaches attract the interest of researchers. Image based approaches can give rise to cost effective, automated and

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remote systems to be implemented. Although difficulties on image processing techniques to determine automatic threshold and segmentation still exist and intelligent classification has some problems, several good attempts are available in the literature on these approaches [2]. In [3], Otsu proposed in his famous paper a method for standardized and automatic threshold selection which is characterized by its nonparametric and unsupervised nature and has the desirable advantages such as it is very simple, straightforward extension to multi-threshold problems not based on the differentiation, but integration of the histogram, quite general covering a wide scope of unsupervised decision procedure. In the research in [4], an automatic threshold is used based on the Otsu's method. In that work, as is often done, the image mathematical morphology is used as a final step to smooth the region of interest giving a result of 92% accuracy. Edge detection methods were also used widely [5,6] but this method suffers from edges that are not sharp enough. Another method that joins two techniques, scale space filtering and watershed clustering for segmenting white blood cells is proposed in [7]. In that approach, nucleus and cytoplasm of white blood cells are extracted using different methods. K-mean clustering method and Fuzzy C-mean clustering method are used in segmenting white blood cells, respectively, in [8,9]. In the former, cropping the entire cell to get the real area of the cell is not clearly shown and in the latter, the computational time increases if the numbers of clusters are greater than 2. In [10], authors used MATLAB 7.1 toolkit to segment and localize the white blood cell nucleus. Our approach resembles their work in using MATLAB facilities but differs from it in such a way that we embed segmented cells in empty sub-matrices and apply them to the classifier for classifying five classes. We use a neural network (NN) structure as the classification purpose. In our work, a new and completely automatic counting, segmentation and classification process is developed. The overall process is given in Fig. 1. It consists of some important stages such as taking the image of blood smear in which the white blood cells were painted, passing it through a couple of image enhancement and segmentation processes, extracting individual images of white blood cells, counting the cells and determining the sizes of the cells, producing the percentage of malignant cells and applying individual images to a neural network based classifier. The target process is aimed to produce the following outputs: (1) the number of white blood cells within the image; (2) the sizes of individual white blood cells; (3) the percentage of malignant (grown) white blood cells called lymphoblasts; (4) important features by PCA for dimensionality reduction; (5) the classes of the white blood cells; and (6) the diagnosis of Acute Lymphocytic Leukemia (ALL) disease giving positive or negative answer. There are five classes of white blood cells such as basophil, lymphocyte, neutrophil, monocyte and eosinophil. In short, the cell types are called as {BP, LC, NP, MC, EP}, respectively. However, the diagnosis of ALL disease is out of the scope of this paper. The neural network classifier classifies the white blood cells in one of the above classes.

Our approach resembles to the studies [11,12]. The difference from them is that the cells are cut through its edges and extracted one-by-one like a scissors. After extraction, each of the individual cells is put into empty sub-matrices whose dimensions are the same for each cell. In this way, a sub-matrix contains only the cell itself and no other disturbance. This is an innovative cell extraction process developed in this work. This type of extraction process can facilitate the training of the classifier and can help obtaining accurate results during operation [13]. Another difficulty is that a type of cell extracted and embedded into an empty matrix may have different size and orientation than the trained one. In literature, a couple of methods have been applied to overcome this difficulty. One of the methods may be to design a classifier that is invariant to

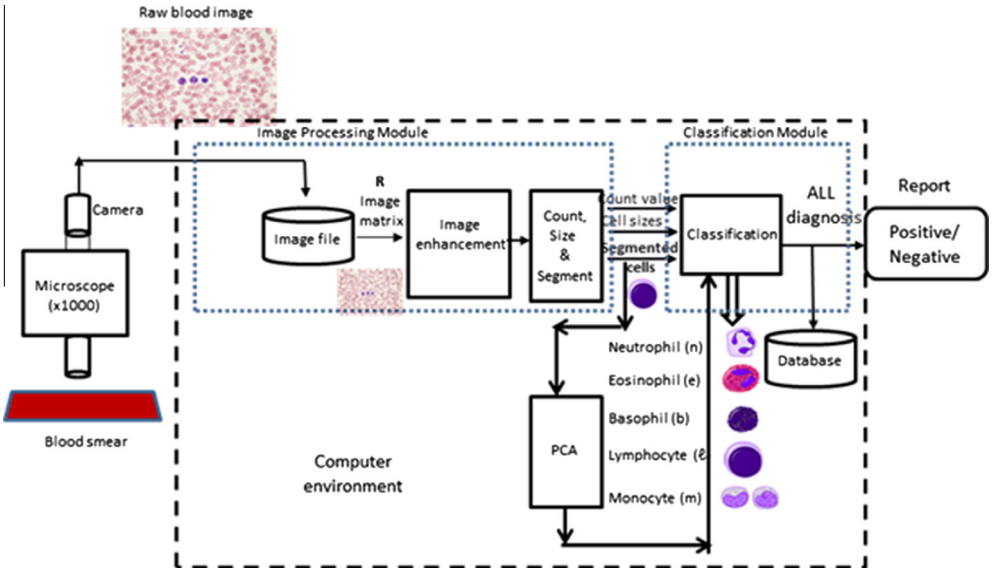

Fig. 1. Block diagram representation of overall process.

such transformations. Basically, there are at least three techniques for dealing with invariance [14]: invariance by structure, invariance by training and invariant feature space. For example, in [5] the capability of selected features in separating classes of cells has been qualitatively evaluated by plotting the classes with respect to three most relevant features as cell area, nucleus area and grey intensity of the cytoplasm. In our approach, we achieve invariance property somehow similar to invariance by training technique. We train the classifiers with different orientations of the same sample. We repeat it for each cell sample. In this way, the classifier can distinguish accurately the cells encountered after training. In recent papers [15–19], the authors apply similar methods to the process of segmentation and classification of white blood cells. However, the difference between our and their approaches is that we enforce the classification using NN by applying PCA to the complete original cells extracted from the smear after putting them into an empty matrix. In our case we do not need any expertise because of automatic threshold during segmentation by Otsu's method. The outputs of the image processing module are count value, cell sizes and segmented and extracted individual cells ready for classification. The microscope magnifies the blood smear by a magnification factor of $\times$1000 and the camera takes the image. The organization of the paper is as follows. In Section 2, the mathematical model of the system, cell counting, size determination and cell extraction are explained. In Section 3, the methods for the classification of white blood cells are given. In Section 4, discussion and conclusion are presented.

2. Mathematical model, cell counting, size determination and cell extraction process

The system performs the following processes: reading the row image to a file, eliminating noise, enhancing the image, counting the cells, segmenting the cells as sub-images in the form of sub-matrices, classifying the cells, storing the count value, size and type of cells. The cell counting process is the next step after filtering the image. The blood smear may contain hundreds of malicious white blood cells together with the other cells such as platelets and red cells. The white blood cells must be counted in a high accuracy and identified clearly. In the blood cell counting problem, five kinds of objects have to be identified based on their diameters, area, circularity and nucleus non-uniformities, etc., and also their magnitudes (whether they are abnormal or not) must be determined. Here, we added a new feature to the algorithm to solve the segmentation problems. The new feature added is extraction of individual cells as a compact body from its contours. This step is new since in the literature the attempts for segmentation are either to solve particular cluster of cells [2,20], to refine membrane segmentation [3], to detect incorrect segmentation [4] or to estimate the average diameter and perform segmentation with different techniques and combine the results in order to exploit all the available a-priori information achieving a robust identification of white blood cells. In contrast to these techniques, our method produces sub-images of single compact bodies of white blood cells. The new algorithm is explained as follows by means of MATLAB functions:

Step 0: The original image is taken.
Step 1: The image's intensity values are mapped to a new range by using *imadjust*.
Step 2: The RGB image is converted to the grayscale image by using *rgb2gray*.
Step 3: The complement of the image is computed by using *imcomplement*.
Step 4: Otsu's method is used to automatically convert the grayscale image to the binary image. The global threshold (level) is computed by using *graythresh*.
Step 5: The *imdilate* function dilates the binary image by using the flat, disk-shaped structuring element with radius 1. Thus areas of foreground pixels grow in size while holes within those regions become smaller.
Step 6: The holes of the binary image are filled by using *imfill*.
Step 7: The connected components in the image are found and label matrix from *bwconncomp* structure is created by using, respectively, *bwconncomp* and *labelmatrix*.
Step 8: The set of properties for each connected component in the image is measured by using *regionprops*. The measured properties are,

'*BoundingBox*' – the smallest rectangle containing the region.
'*Area*' – the actual number of pixels in the region.
'*MajorAxisLength*' – scalar specifying the length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.
'*MinorAxisLength*' – scalar specifying the length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.

Step 9: If the number of the connected components in the image is larger than one,

$$\text{Average axis length}_{\text{for connected component}} = \frac{\text{Major axis length} + \text{Minor axis length}}{2} \tag{1}$$

$$\text{Average axis length}_{\text{for image}} = \frac{\text{Average major axis length} + \text{Average minor axis length}}{2} \tag{2}$$

$$\text{Size of connected component}(\%) = 100 \times \frac{\text{Average axis length}_{\text{for connected component}}}{\text{Average axis length}_{\text{for image}}} \tag{3}$$

Step 10: If the number of the connected components in the image is larger than one, the connected components that have 30% fewer than average axis length for image are removed from the binary image by using *bwareaopen*.

Step 11: Using the structuring element defined in Step 5, the *imerode* function apply the erosion operation to the binary image.
Step 12: We produce an output image in which the pixel values of the eroded image are multiplied by the corresponding pixel values in the complement image.
Step 13: The steps from 7 to 9 are repeated until the last connected component.
Step 14: The connected components are labeled by using *bwlabel*.
Step 15: Each connected component obtained by using the smallest rectangle containing the region is located on the center of a black image with $(a \times b)$ pixel resolution.

3. Classification of white blood cells and experimental results

The classification process is based on a neural network structure. The subimages contain the segmented individual white blood cells.

3.1. Image processing

An example for the overall process of cell segmenting, counting, size determination, cell extracting, cell labeling and placing into empty sub-matrices for further classification process is shown in Fig. 2. Each individual cell is extracted and put into an empty sub-matrix at the end of the image processing. The results of the image processing of blood smear cell segmentation are shown in Fig. 3. Notice that the individual cells are extracted, labeled and placed into sub-matrices one by one at the end of the image processing. They get ready for classification. Thresholds by using Otsu's method for Image 1, Image 2, Image 3 and Image 4 are 0.3549, 0.3922, 0.2863 and 0.4157 respectively. As an example, the properties of the cells in Image 4 are given in Table 1. According to Table 1, the sizes of Cell 4 and Cell 10 in Image 4 are, respectively, 156.11% and 132.74%. These cells are much greater than the others since the connected components labeled as Cell 4 and Cell 10 in Image 4 actually have two cells rather than one cell, as seen in Fig. 3. In such a situation, the count number will be erroneous. However, since we check the ratios as in Table 1, we can easily realize that the cells that have ratios greater than 100% are partly occluded by the others or they are so close that they touch together. In that case, although the algorithm counts them as a single cell, we correct the count number by increasing the counter by one if the ratio is in between 100% and 200%. We increment the counter by two if the ratio is greater than 200%. Normally this is enough in most of the applications. No manual intervention was needed for the experiments carried out in the above applications.

3.2. Classification by neural network

During the training and test phases of the neural networks, several white blood cells of each type obtained from

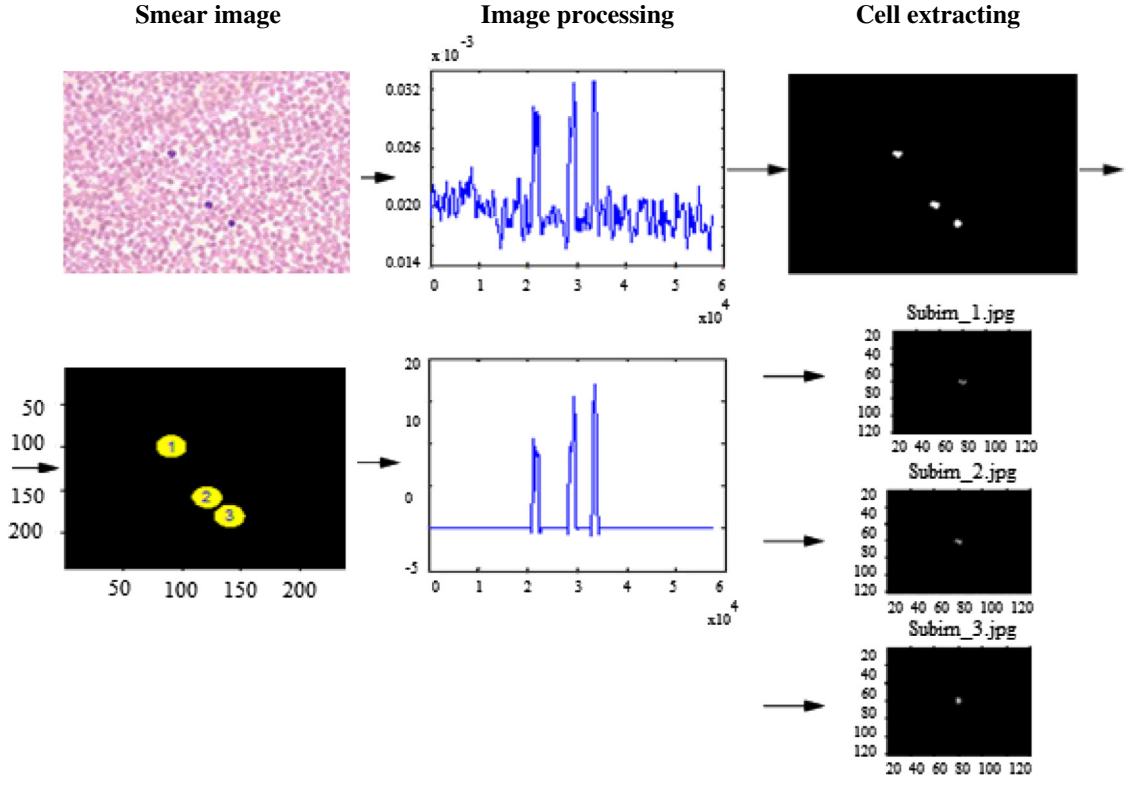

Fig. 2. Overall process of cell segmenting, counting, size determination, cell extracting, cell labeling and placing into an empty submatrix for further classification process.

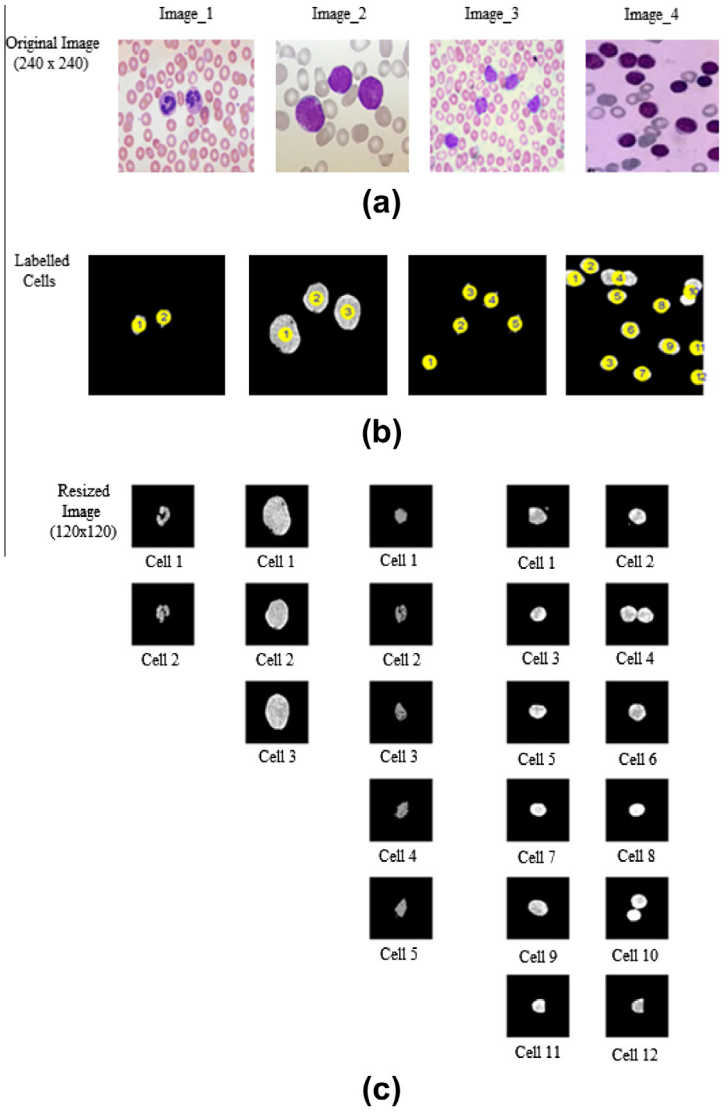

Fig. 3. Experimental results of the blood cell segmentation process; (a) original images; (b) labeled images and (c) cells extracted for each image.

Table 1
The properties of the cells in Image 4. average axis length for Image 4: 31.17.

Cells in the Image 4	Major axis length	Minor axis length	Average axis length	Size (%)
Cell 1	36.07	28.07	32.07	102.88
Cell 2	33.62	25.95	29.79	95.55
Cell 3	31.07	24.00	27.53	88.32
Cell 4	**70.76**	**26.57**	**48.67**	**156.11**
Cell 5	33.49	23.62	28.55	91.60
Cell 6	33.11	28.50	30.80	98.81
Cell 7	31.53	22.53	27.03	86.71
Cell 8	30.48	22.17	26.32	84.44
Cell 9	38.42	26.26	32.34	103.73
Cell 10	**54.31**	**28.45**	**41.38**	**132.74**
Cell 11	26.19	24.05	25.12	80.58
Cell 12	25.38	23.59	24.48	78.53

www.kanbilim.com [21] have been used. The original white blood cells are shown in Fig. 4 and obtained cells after applying the steps mentioned in Section 2 are also in Fig. 4. The thresholds by using Otsu's method for the images are computed. To generate the training set for the classifiers, BP1, LC1, NP1, MC1 and EP1 are rotated by the steps of 30 degrees in a counterclockwise direction around their center points. The reason is that the blood smear may have cells with different orientations. In order to train the NN with cells having different orientations, we need to have a rich set of training samples. In addition, a Gaussian White Noise with zero mean and variances of 0.01 and 0.025 are added to each rotated one. The reason for adding Gaussian noise to the training samples is that we obtain an original cell extracted from the blood smear and it may be noisy originally. Since we do not try to

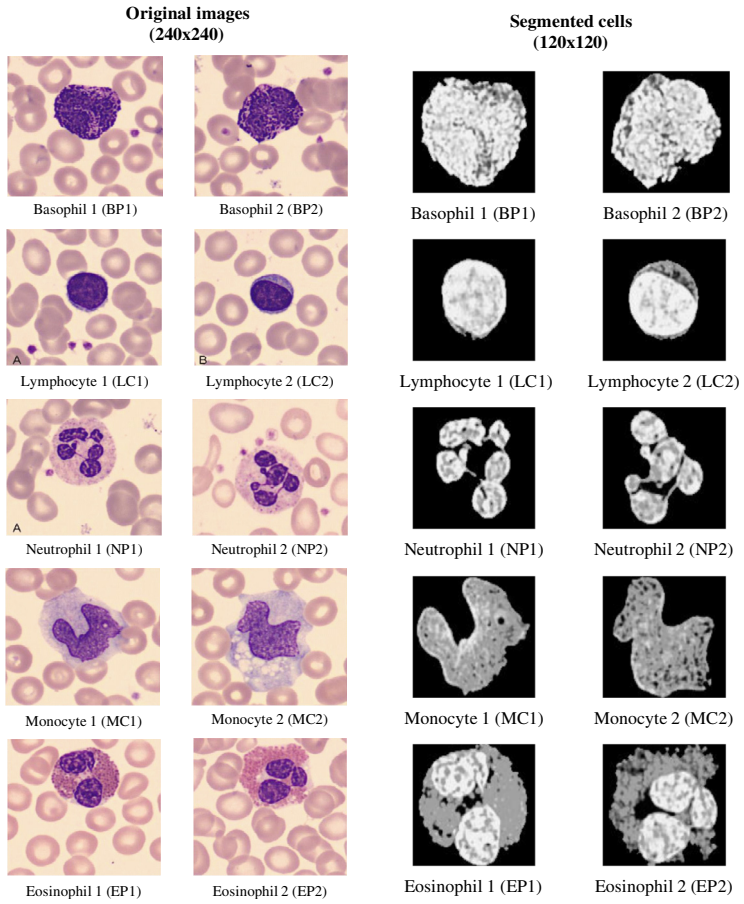

Fig. 4. Original and segmented white blood cells used for the classification.

eliminate noise from the cell itself during the image processing, we have to take into account this type of situation during classification. The overall training set has 180 images (36 images for BP1, 36 images for LC1, 36 images for NP1, 36 images for MC1, 36 images for EP1). During the test phases of the classifiers, the rotated images of BP2, LC2, NP2, MC2 and EP2 only have been used. Thus, the test set has 60 images (12 images for BP2, 12 images for LC2, 12 images for NP2, 12 images for MC2, 36 images for EP2). The trained NNs have been tested with the trained patterns and also untrained ones.

3.2.1. Classifier A

It is a MultiLayer Perceptron (MLP) with 14,400 (120×120) inputs and 5 outputs. It has 4 hidden layers. The number of neurons are 45, 50, 60, and 60 for these layers. The neurons have tangent sigmoid nonlinearities. Training period is 556 s (924 iterations). The mean square error (MSE) value at the end of the training period is 3.65e−30. The outputs are +1 and −1 for almost all degrees of rotations and cell types.

3.2.2. Classifier B

It is a MultiLayer Perceptron (MLP) with 242 (the number of principal components) inputs and 5 outputs. It has 3 hidden layers. The number of neurons are 35, 40 and 40 for these layers. The neurons have tangent sigmoid nonlinearities. Training period is 11.21 s (466 iterations). The mean square error, (MSE) value at the end of the training period is 8.22e−35. The outputs are +1 and −1 for almost all degrees of rotations and cell types. 65% accuracy for Classifier A and 95% accuracy for Classifier B have been obtained in the test phases. The accuracy is calculated as

$$\text{Accuracy} = 100 \times (\text{Number of correctly identified images}) / (\text{Number of images})$$

Moreover, the training period of Classifier B is much shorter than the training period of Classifier A.

3.2.3. Principal Component Analysis (PCA)

Principal Component Analysis is one of the oldest and most widely used data transformation techniques for multivariable analysis. The dimension of input dataset is reduced using this technique. PCA is mathematically defined as an orthogonal linear transformation that transforms the data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate and so on [22,23]. PCA is applied to the images in

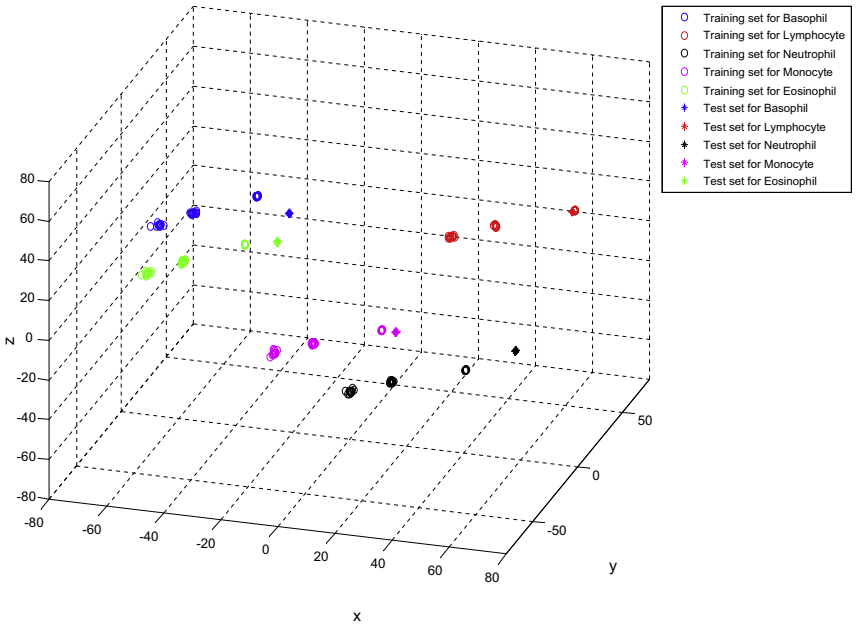

Fig. 5. The new data set derived by using $k = 3$.

the training and test sets. We choose $k = 242$ since $r(i) = 100\%$ is firstly achieved by 242nd eigenvector. Therefore, each image can be represented with 242 variables instead of $a \times b = 14{,}400$ (120×120). However, we can visualize the new data set derived by using $k = 3$, as shown in Fig.5.

4. Discussion and conclusion

In this work, a new automatic system used to help the diagnosis of some important blood diseases is developed, tested and the results are presented. The image taken by a camera attached to a microscope is processed and then the results that are necessary for diagnosing the diseases such as the number of white blood cells, sizes of them and types of them are accurately produced. The mathematical model of the images and the process is established. The RGB image is converted to the grayscale image. Otsu's method is used to automatically convert the grayscale image to the binary image. The binary image is dilated by using the flat, disk-shaped structuring element with radius 1. The holes of the binary image are filled. The connected components in the image are found and label matrix created. The set of properties for each connected component in the image such as bounding box, area in pixels, major axis length and minor axis length is measured. If the number of the connected components in the image is larger than one, the average axis length for each connected component in the image is computed by using the major axis length and the minor axis length of the related connected component. Also, the average axis length for image is computed by using the average of the major axis lengths and the average of the minor axis lengths of all connected components in the image. The size of each connected component in the image is calculated. If the number of the connected components in the image is larger than one, the connected components that have 30% fewer than average axis length for image are removed from the binary image. We produce an output image in which the pixel values of the eroded image are multiplied by the corresponding pixel values in the complement image. The connected components are labeled by using *bwlabel*. Each connected component obtained by using the smallest rectangle containing the region is located on the center of a black image. There are some cells that have ratios greater than 100% are partly occluded by the others or they are so close that they touch together. In that case, although the algorithm counts them as a single cell, we correct the count number by incrementing the counter by one if the ratio is in between 100% and 200%. We increment the counter by two if the ratio is greater than 200%. Normally this is enough in most of the applications. No manual intervention was needed for the experiments carried out in the above applications. An image may contain two occluded or cells that stick which are about $2/14 = 0.143 \cong 14\%$ of all cells. They can easily be identified. If the cells that stick together exceed four or more, then a major difficulty arises for segmenting them. The white blood cells are extracted from their edges and original cells are put into empty sub-matrices. In the training phase of the classifier, the cells in the sub-matrices are applied to the classifier with different orientations and with additive Gaussian white noise. Any sub-image of a cell is rotated with 30-degree resolution. Two types of classifiers are tried in the system. Classifier A: it is a Multi-Layer Perceptron (MLP) with an input size of $a \times b$. For example, in our experiments we utilized $a \times b = 14{,}400$ (120×120) inputs and 5 outputs. Classifier B: it is a Multi-Layer Perceptron (MLP) with 242 (the number of principal components) inputs and 5 outputs. 65% accuracy for Classifier A and 95%

accuracy for Classifier B have been obtained in the test phases. PCA is applied to the images in the training and test sets. The percentage of the variance accounted for by the ith eigenvector is plotted. We choose $k = 242$ since $r(i) = 100.0000\%$ is firstly achieved by 242nd eigenvector. Therefore, each image can be represented with 242 variables instead of 14,400 (120×120). However, we can visualize the new data set derived by using $k = 3$. It is noted that the classification can be achieved clearly. The sizes of the cells have been determined and average cell size within an image has been calculated. This value facilitates the decision making on the blast cells that may be available in the blood smear. In order to automate the segmentation and classification, the Otsu's method that provides automatic determination of threshold is applied. Without PCA application, the classifier (NN) has worked with a success rate of 65% based on the rotated training set. The success rate has been increased to 95% with the PCA application to the training set, since the PCA extracts the most important features of the data vectors in reduced order. The variance percentage $r(i)$ becomes 100% that is firstly achieved at the 242nd eigenvector. Therefore, each image can be represented by 242 variables instead of $a \times b = 14{,}400$ (120×120). We believe that PCA application before classification by NN gives reasonable results.

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