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An Efficient Approach of Segmentation on White Blood Cells Using Morphological Operators and 2D-Discrete Wavelet Transform with Anfis Classification

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Abstract:

Segmentation of medical images gives an effective way of identifying the affected region of diseases in medical image processing. The main issue is to separate the homogenous regions and then assigning the objects to particular classes is called classification. Diagnosing the diseases address many constraints to limit the problem space and challenging with wide range of factors. The main objective of this work is to segment the nucleus and cytoplasm of WBC and classifying the object of the affected region. Isotropic Diffusion , a filtering process is applied in preprocessing stage and results in removal of noise. During segmentation, image is converted to binary image followed by a reverse morphological closing operation which erodes the boundaries of regions and foreground pixels and tend to extract the features, 2D-Discrete Wavelet Transform decomposes the binay image into sub bands and reduces the dimension, a Low-Pass(LL)-subband is used for classification. For the purpose of classification* Adaptive Neuro-fuzzy Inference System (*ANFIS*) *is used and compared with results obtained by Watershed transform and Level-set methods. Experimental analysis is done on Classification rate of different types of features and tabulated. Thus the proposed approach leads to promising segmentation and classification rate with 99.3% for varying cell appearance and image quality when compared to the existing system.

Keywords: Segmentation and classification, Homogenous regions, Nucleus and Cytoplasm of WBC, Isotropic Diffusion, Reverse morphological closing operation, Adaptive Neuro-fuzzy Inference System.

I. INTRODUCTION

With the increasing number of diseases today [1], there are many patients to look after and many more healthy people who require medical maintenance in order to maintain their good health. Therefore, a fast, durable, automated and intelligent system is required for doctors to carry out the necessary diagnoses [1,2,4]. Some of the main laboratories requirement are automated; and intelligent systems are used for bone marrow analysis and differential count of blood components (e.g. to count the number of red and white blood cells, platelets…etc). Any effort to help in reducing errors and speeding up the different types of blood diagnosis tools is valuable because it lessens the workload of the lab diagnosis experts [1-3,5]. White Blood Cells differential diagnosis is vital in today's medical industry because it helps in correctly analyzing the conditions of healthy and unhealthy patients. The normality and abnormality conditions of WBC provide hematologists with a great amount of useful data and knowledge about a patient's conditions [5].

Blood Cells (BC) are formed inside the bones in the "bone marrow" [4]. The "Stem Cell" is considered as the origin of all BC. The differentiation process occurs when stem cells get mature. At the beginning, immature cells are called blasts, which then develop into mature BC_s. As soon as BC is mature enough, it is released into the blood and circulated throughout the entire body to perform its individual functions. Also Cells can overlap each other, have large variation in shape and size, and be influenced by the external environment. Also, due to illumination inconsistencies, the contrast between the cell boundary and the background varies, and is different depending on the image capture conditions.

The need to automate this process helps reduce human errors in determining the cancerous cells or any other abnormalities of WBC. It will also help to speed up the process and provides more efficient diagnosis tool for many other blood diseases (e.g. blood cancer, anemia, AIDS or bone marrow cancer).

In this paper, the focus is the segmentation of white blood cells (WBC), also called leukocytes. WBC are cells of the immune system, and are found throughout the body, including bone marrow and blood. Since the number of WBC in the blood is often an indicator of some diseases, the count of different classes of WBC, named differential counting, plays a major role in the determination of the patient health in different stages: diagnosis, treatment and follow up [6]. WBC are classified according to their maturation levels, during what the cells can change shape, for example. Therefore, an accurate segmentation is essential for the success of subsequent high-level tasks, such as tracking and classification.

In this paper, an automated segmentation, identification and classification of Leukocytes (White Blood Cells) namely, lymphocyte, monocyte and neutrophil in light microscopic images based on isotropic Diffussion, thresholding and morphological algorithms is proposed . The experimental results are compared used the training and testing set, classification rate results are obtained with high accuracy than the existing system. The proposed method is more reliable and computationally less expensive.

The next section presents some related works, and Section 3 describes basic stages involved in the proposed work and its algorithm. Section 4 describes about the Experimental results. Finally, some conclusion in Section 5 and draw some conclusions and future work perspectives in Section 5.

II. RELATED WORK

Segmentation is one of the major problems in image analysis and considerable research has been performed in trying to solve this problem including methods of segmenting various types of cells or biological images. Aside from very simple techniques such as thresholding or basic edge detection, there are two types to segmentation — region based or edge based segmentation.

For region based methods, the prototypical approach is based on morphological operators and watersheds [7, 8]. Malpica et al. [8] describe an algorithm that segments clustered fluorescence microscopy based analytical cytology images using a watershed algorithm and simple morphological operators. Their method works quite well but the images are very different to the quality of the obtained results, and it is assumed that neighboring cells are adjacent rather than overlapping. Di Ruberto et al. [7] describe a method for analyzing and recognizing malarial red blood cell light microscopy images using morphological operators and a watershed algorithm. Of most relevance to problem is the method by which they process clustered or overlapping cells to detect individual cells.

Jiang and Yang [9] describes a method for finding elliptical cell boundaries using an evolutionary search. The search method is used to improve the localization of ellipses in an image and using Canny's edge detection algorithm detects the points for matching. Although their method works quite well, they did not include any images with overlapping or clustered cells (which would violate the elliptical condition). Liao [10] introduces the use of shape analysis into WBC segmentation, locating accurate WBC contour with a shape analysis step based on a roughly boundary obtained by thresholding.

The use of thresholding techniques performs poorly in most part of the cases, since no spatial information is used during the selection of the segmentation thresholds. Sometimes, these techniques are combined with mathematical morphology operations [11].

An edge sensitive diffusion method is introduced in [12] for edge detection and segmentation. However, anisotropic diffusion is a nonlinear diffusion approach to spatial adaptive filtering, which demonstrates effective results of image segmentation in homogeneous regions while preserves edges effectively and significantly.

Accurate image segmentation is used in medical diagnosis since this technique is a noninvasive pre-processing step for biomedical treatment. In this work an efficient segmentation method for medical image analysis is presented. In particular, with this method blood cells can be segmented. For that, the wavelet transform is combined with morphological operations. Moreover, the wavelet thresholding technique is used to eliminate the noise and prepare the image for suitable segmentation. In wavelet denoising the best wavelet [13] is determined that shows a segmentation with the largest area in the cell. Different wavelet families and are studied it is concluded that the wavelet db1 is the best and it can serve for posterior works on blood pathologies. The proposed method generates goods results when it is applied on several images. Finally, the proposed algorithm made in MatLab environment is verified for a selected blood cells.

In [14] is shown the parallel edge-region-based segmentation algorithm targeted at reconfigurable MultiRing network. Finally, two techniques for WBC segmentation, scale-space filtering and watershed clustering, to extract nucleus and cytoplasm respectively.

Cell image segmentation is an important study direction and white blood cell composition reveals important diagnostic information about the patients. Manually counting of white blood cells is a tiresome, time consuming and susceptible to error procedure. Due to the tedious nature of this process, an automatic system is preferable. In this automatic process, segmentation of white blood cells is one of the most important stages. In order to solve problems of the traditional method for cell segmentation, a method of level-set 3D segmentation [15] for White blood cells was proposed using canny. The Level-set segmentation was based on geometric active contour models instead of parameter active contour models. The method overcame the obscurity of white blood cells' boundary by taking advantage of the structure of conforming anatomic arrange of threshold. Further, the initial segmented results preprocessed were applied using anisotropic diffusion and the real border of cell was detected using canny. Then level-set method is used for segmenting WBC. Thus, the segmentation of white blood cells could be done more accurately.

This paper presents a proposition for an algorithmic procedure to isolate and count the lymphocytes White Blood Cell (WBC) form microscopic images. The process involves segmentation of cells, scanning algorithm, feature extraction, and recognition of lymphocyte cells. The scanning algorithm returns the number and location of candidate area [16] in WBC images. For feature extraction, a combination of the shape feature moment invariants and the roundness are found to have an excellent recognition accuracy for identifying the lymphocyte cells from other WBC type.

Rezatofighi et al. [17] introduced another approach to WBC classification. It is based on Gram–Schmidt orthogonalization, and they used the snake algorithm [18] to segment nucleus and cytoplasm. Then, they extracted various features from the segmented region and selected the most discriminative features using a Sequential Forward Selection (SFS) algorithm. Next, they compared the performances of two classifiers: Artificial Neural Network (ANN) and SVM. Extracted features were composed of the morphological features, e.g., nucleus and cytoplasm areas, nucleus and whole cell perimeters, the number of separated parts of the nucleus, means and variances of nucleus and cytoplasm boundaries, and the ratio between cytoplasm and nucleus areas. Texture features such as co-occurrence matrix and local binary patterns were also used. The co-occurrence matrix included 14 features representing contrast, homogeneity, entropy and other texture quantities. Su et al. [19] proposed an idea to find the discriminating region of white blood cells

in the Hue-Saturation-Intensity (HSI) colour space. The colours of each pixel in the discriminating region were considered as nucleus and cytoplasm of WBC. Then a morphological process was used to segment WBC. They extracted geometrical, colour, and Local Derivative Pattern (LDP)-based texture features from the segmented result. These features were used to classify five types of WBCs using three kinds of neural networks: multilayer perceptron, the SVM and the hyper rectangular composite neural networks. Dhompongsa [20] showed that a nucleus alone could classify WBCs. They tested their algorithm with bone marrow images. The algorithm applies mathematic morphology to analyze WBC nucleus based features and uses Naïve Bayes classifiers and ANNs with five-fold-cross validation. The result showed that features from the nucleus alone led to a 77% classification rate on average.

Barbua et al [21] developed Anisotropic filter which follows an iterative smoothing procedure for denoising. Better denoising effect can be obtained but it tends to overblur the image leads to boundary sharpening with many texture details lost.It provides a smoothening effect to the images while preserving the edges. Buades et al [22] introduced Non-Local means filter which estimates each and every pixel intensity based on the presence of similar patterns and features in the image. Visual quality and objective quality gives poor results compared to other denoising methods. Pizurica and Philips [23] developed wavelet domain denoising in which information containing coefficients are thrown away by the process of Thresholding.

The proposed method concentrate on the denoising of high quality MRI image and Ultrasound image which combine three popular denoising techniques namely discrete wavelet transform, bilateral filter and wavelet thresholding.

Datasets

Blood smear microscopic images were collected from normal peripheral blood slides (dataset 1). The study's algorithm was tested on 555 images (a total of 601 WBC) under a light microscope with 100× magnification captured by a high-definition color camera head Nikon DS-Fi2. All images were recorded and saved in JPG format of 960 × 1,280 pixels. The calibration ruler scale from the manufacturer was 10 μm equal to 150 pixels. In addition, a database of white blood cells downloaded from the Cellavision Competency Software[24] (dataset 2) was tested for robustness. Dataset 2 has 477 images with a total of 477 WBCs. Each image was saved in JPG format of 360 × 363 pixels. The calibration scale was estimated from the size of RBCs to be 7 μm equal to 70 pixels. For comparison, all images were also manually segmented into nucleus and cell (or cytoplasm) areas and classified into normal leukocytes: basophil, eosinophil, lymphocyte, monocyte and neutrophil by a hematologist.

III. PROPOSED SYSTEM

Automatic recognition of white blood Cell (WBC) light microscopic images usually consists of four major steps, including: preprocessing, image segmentation, feature extraction and classification. The pre-processing stage usually includes image enhancement that is removal of noise in acquired image and is essentially performed in order to prepare the image for the vital segmentation stage. Individual objects such as cytoplasm and nucleus of interest are separated from the background in the segmentation process.

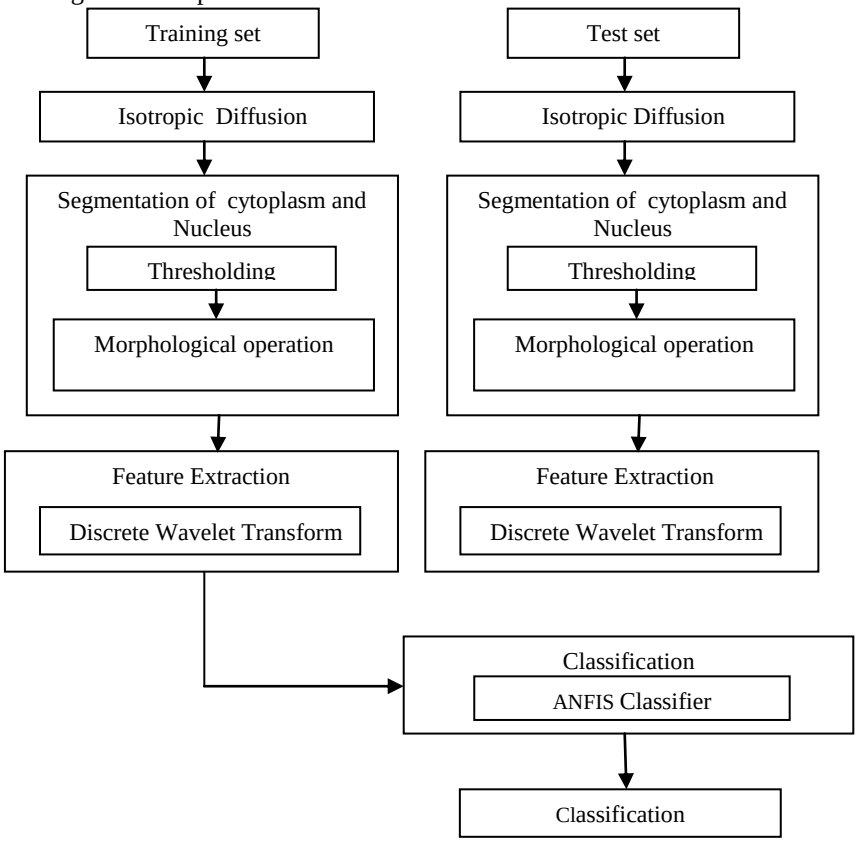


Fig 1: Overview of the Proposed System

These segmented information along with spatial information of the segmented objects are used for the subsequent feature extraction procedure. Feature extraction is done by applying the 2D-Dirichlet Wavelet Transformation to the de-noised image for decomposition which extracts the features from and retains the very low level frequency components in the LL-sub band of the decomposed image. An additional factor that pressures the outcome is that the maturation stages encompasses a constant variable, while the arrangement is discrete. In the experiments, six classes of WBC are taken for analysis. To differentiate the dissimilar cells, features connected to the geometrical shape of the nucleus and of the whole cell are extracted. In this work ANFIS is used for classification purpose. These geometrical features are used to identify and classify the leukocyte cells, namely, Band cells, Metamyelocyte, Myeloblas, lymphocyte, monocyte and neutrophil using Anfis Classifier. The proposed method for the segmentation and classification of blood cell (leukocytes) is given below:

Also an improved algorithm for identification and classification of white blood cells in digital microscopic images using image segmentation method. The ratio of areas of nucleus and cytoplasm of a cell as a prominent feature is presented. The experimental results are compared with the existing system. The performance of proposed algorithm is analyzed for four different feature sets.

Algorithm 1: Training phase:

Step 1:	Input the leukocyte colour cell image.
Step 2 :	Perform pre-processing by isoptropic Diffusion filter on cell image
Step 3 :	Convert the colour image into grayscale image by threshold operation.
Step 4 :	Perform segmentation by using morphological operations, namely, reverse closing method.
Step 5 :	Segment the image of Step 4 by threshold and obtain resulting binary image.
Step 6 :	Reduce the segmented image by applying 2D-DWT low level decomposition.
Step 7:	For each labeled segment, compute geometric shape features and store them. Let a_i be the value of ith parameter for kth class. The i=1,2,3 correspond to Area, Eccentricity, Perimeter, Image ratio respectively; and k=1,2,3,4,5,6 correspond to Metamyelocyte, Myeloblas, lymphocyte, monocyte and neutrophil respectively.
Step 8 :	Repeat Steps 1 to 7 for all the training images.
Step 9 :	Compute minimum and maximum values of features of leukocyte cells, denoted by a^k imin and a^kimax, for all i and k, and store them as knowledge base.

Step 1:	Input the leukocyte colour cell image.
Step 2:	Perform pre-processing by isoptropic Diffusion filter on cell image
Step 3:	Convert the colour image into grayscale image by threshold operation
Step 4:	Perform segmentation by using morphological operations, namely, reverse closing method.
Step 5:	Segment the image of Step 3 using global thresholding and obtain resulting binary image.
Step 6:	Reduce the segmented image by applying 2D-DWT low level decomposition.
Step 7:	For each labelled segment, compute geometric shape features a_i, i=1,2,3,4.
Step 8:	Apply rule for classification of the leukocyte cells; if a_i lies in the range [a^k imin, a^kimax], for i=1,2,3,4, then the cell (labelled segment) belongs to k^{th} class, where k=1,2,3,4,5 corresponds to Metamyelocyte, Myeloblas, lymphocyte, monocyte and neutrophil respectively.
Step 9:	Repeat the Steps 7 and 8 for all labeled segments and output the classification of identified leukocyte cells.

The input RGB image of leukocyte cell is converted into binary gray image and then only binary image is considered. Isotropic diffusion, a filtering process can be done preprocessing due to remove the noises from the image. It is also used to convert heterogeneous image in to homogeneous image. Isotropic diffusion filter is used in nucleus image preprocessing. After the diffusion the segmentation process can be applied to particular point from original image which also saves the processing time for further operations which has to be applied to the image. During segmentation using a threshold, in order to segment the image. In threshold function replace each colour pixel in a black pixel, also threshold converts gray level image into binary image. After thresholding morphological closing operation is applied on the threshold image to fill in the holes and small gaps in the image. Morphological closing is reverse method of morphological dilution and erosion method.

The dilation operator takes two pieces of data as inputs. The first is the image which is to be dilated. The second is a (usually small) set of coordinate points known as a structuring element (also known as a *kernel*). The structuring element that determines the precise effect of the dilation on the input image. The basic effect of the operator on a binary image is to erode away the boundaries of regions of foreground pixels (*i.e.* white pixels, typically). Thus areas of foreground pixels shrink in size, and holes within those areas become larger and strips away a layer of pixels from an object, shrinking it in the process.

The process of segmentation obtains the actual cell region after removing the spurious regions. Now, nucleus has to be extracted from the already extracted cell region. Here the saturation to extract the nucleus is also considered. Empirically, it is observed that, the nucleus has high saturation and it is above 0.45. Applying the above thresholding, it yields binarized images of nucleus and that of cell region. Finally, the cytoplasm region is obtained by subtracting binary image of nucleus from that of cell. For the experimentation, three feature sets, namely, f2, f3 and f4 for classification are used and compared with the results obtained for feature set f1 of the previous method: f2=(area, eccentricity) f3=(area, eccentricity, convex area of the nucleus and cytoplasm) f4=(area, eccentricity, perimeter, image ration of areas of nucleus and cytoplasm) The feature set f1=(area, majoraxislength/ minoraxislength, perimeter, circularity) is extended to f4 and f2 is extended to f3 by considering an extra feature, namely, the ratio of nucleus area and cytoplasm area. The experimental results demonstrate that the proposed color image segmentation method reverse method of closing morphological operation is efficient and effective in white blood cell segmentation, and provide better assistance to the pathologist in the process of classification of white blood cells.

A. Isotropic Diffusion

Prior to segmentation of the RBCs and the analysis of their motion and shape, the images are processed in order to improve their interpretation. Common procedures consist of filtering (de-noising) the images, followed by contrast enhancement to recapture smoothed features.

Red blood cells are clustered with white blood cells and the presence of noise, cell fragments and stains in the blood slides is significant. To overcome or reduce the effect of such factors, the images posteriori are standardized by means of applying Isotropic Difussion technique as the first pre-processing stage. Isotropic Diffusion based on spatial filtering methods ,also an edge sensitive diffusion method is for edge detection and segmentation. However, isotropic diffusion is a nonlinear diffusion approach to spatial adaptive filtering, which demonstrates effective results of restoration process involved image and the diffusion is oriented with respect to the structural characteristics of the image.

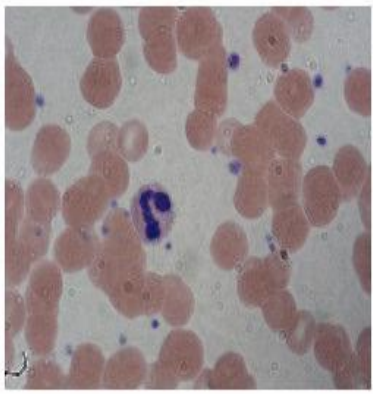


Fig. 1-a) Original Image

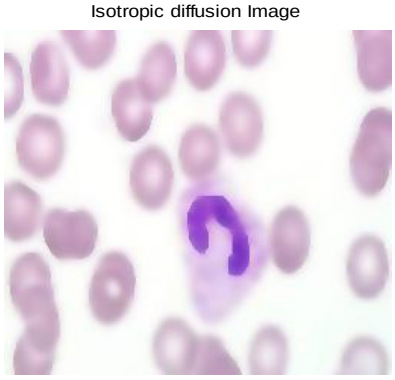


Fig. 1- b) Preprocessed Image

From Fig.2-a) the original image from the dataset, Fig.2-b) Filtered image. Thus removes the noises from the image and converted in to heterogeneous image in to homogeneous image also enhance image by smoothing noisy pixels without losing the important data like edges. Isotropic diffusion filter is used in nucleus image preprocessing on the current filtered image instead of the initial image incorporate adaption base. De-noising as a result of the pre-processing steps is necessary to obtain a reliable picture.

B. Segmentation

Precise and powerful segmentation methods are mandatory to separate the WBC's. Due to the fact that the elements of blood smears (WBC, RBC and Platelets) have lots of similar intensities, the problem of segmenting WBC from the digital image, realistically, is going to be a hard one. Also, each image has different segmentation's parameter values. In other words, the segmentation algorithm applied has to be returned to each image in order to be able to segment WBC.

Thresholding

Optimum segmentation, which will help automate WBC segmentation from blood smear sample image. Segmenting on an image also saves the processing time and to extract the desired objects using a threshold in order to segment the image. In threshold function replace each colour pixel in a black pixel, also threshold means it convert gray level image into binary image**.** Since the nucleus is already segmented, the aim is to separate cytoplasm from background and RBC. Based on the fact that in the image database the background pixels are always brighter than that of the foreground objects, the background is segmented using a thresholding procedure. The threshold value of 120 was chosen empirically and is the same for all images. Since RBC is generally smaller than WBC, the size distribution information of the blood smear images can be used to discard them.

Thresholding chooses the threshold to minimize the intraclass variance of the thresholded black and white pixels. The general mathematical model for the threshold technique algorithm is:

$P(x, y) \geq T \_ P(x, y) = 1$ And this pixel belongs to objects (3.1)

Else

$P(x,y) < T\_p(x,y=1) = 0$ and this pixel belongs to unwanted objects (3.2)

From equation (3.1) and (3.2), Threshold technique is effective when the intensity levels of the objects fall squarely outside the range of levels in the background and the unwanted object levels, because spatial information is ignored, however, blurred region boundaries can create confusion.

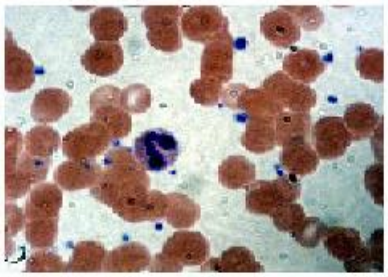


Fig 3-a) Original Image

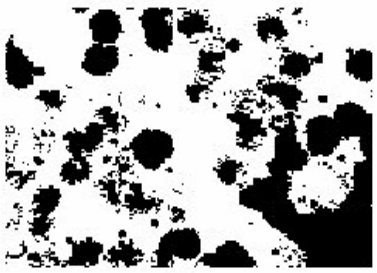


Fig 3-b) Binary Image

When adaptive thresholding algorithm has been applied on the preprocessed image, the WBC is emphasized notably and the thresholding method is effective for all the images existing in the database.

Reverse of Morphological closing operation

It is well-know that the morphological operations are used to image segmentation. The two most Mathematical morphology operations in mathematical morphology are erosion and dilation. It is a branch of nonlinear image processing and analysis. Morphological methods are used in many ways in image processing, for example, enhancement, segmentation, restoration, edge detection, texture analysis, shape analysis, etc It is also applied to several research areas, such as, medical imaging, remote sensing, military applications, etc.

- Dilation is one of the basic operations in mathematical morphology. Originally developed for binary images, it has been expanded first to grayscale images, and then to complete lattices. The dilation operation usually uses a structuring element for probing and expanding the shapes contained in the input image.
- The dilation operator takes two pieces of data as inputs. The first is the image which is to be dilated. The second is a (usually small) set of coordinate points known as a structuring element (also known as a kernel). It is this structuring element that determines the precise effect of the dilation on the input image Erosion is one of two fundamental operations (the other being dilation) in morphological image processing from which all other morphological operations are based. It was originally defined for binary images, later being extended to grayscale images, and subsequently to complete lattices.

After thresholding reverse morphological closing operation is applied on the thresholded image to fill in holes and small gaps in the image. Closing is opening performed in reverse. Closing is tends to smooth section of contour but, as opposite to closing, it generally fuses narrow breaks and long thin gulfs, eliminates small holes and fill small gaps in the contour.

Binary images can be considered as functions on two dimensional grids with values of 0 or 1 or, equivalently, as characteristic functions of subsets of the two-dimensional plane. The concept of structuring element is fundamental in morphology; it is the analogue of a convolution mask in linear image processing. The basic morphological operations involving an image S and a structuring element E are:

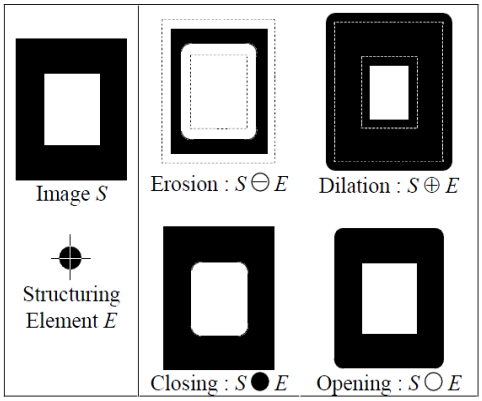


Fig. 2 Samples of an image S, structuring element E, and outputs of the erosion, dilation, closing and opening operators.

Fig 4 Samples of an image S,structuring element E and outputs of the erosion, dialation, closing and opening operation

erosion: $SE = \cap\{S - e : e \in E\}$ (3.3)

dilation : $S \oplus E = \cup\{E + s : s \in S\}$ (3.2)

Where ∩ and ∪ denote the set intersection and union, respectively. $A + x$ denotes the translation of a set A by a point x, i.e. $A + x = \{a + x: a \in A\}$. The closing and opening derived from the erosion and dilation, are defined by *closing:* $S\ E = (S \oplus (-E))\ (-E)$ *opening:* $S\ E = (S\ E) \oplus E$ where $-E = \{-e: e \in E\}$ denotes the 180° rotation of E about the origin

With the dilation related pixels can be joined; with the erosion small objects or irrelevant details can be eliminated. In a binary image, the background's value is 0 and the value of the object is 1. Dilation in this case makes that the background pixels that are neighbors to pixels of the object take the value 1, that is, dilation enlarges the size of the objects. On the other hand, erosion in this case makes that the object pixels that are neighbors to pixels of the background take the value 0, that is, erosion decreases the size of the objects.

Erosion can also be used to remove small spurious bright spots (‘salt noise’) in images. We can also use erosion for edge detection by taking the erosion of an image and then subtracting it away from the original image, thus highlighting just those pixels at the edges of objects that were removed by the erosion.

Fig-4a Binary Image

Fig-4b Erode and Dilate image

It is defined simply as dilation followed by erosion *using the same structuring element for both operations*. The closing operator therefore requires two inputs: an image to be closed and a structuring element. Gray level closing consists straightforwardly of a gray level dilation followed by gray level erosion. Closing is the dual of opening, closing the foreground pixels with a particular structuring element, is equivalent to closing the background with the same element. The segmentation process combined thresholding, morphological operation and ellipse curve fitting**[**26 **],** several features were extracted from the segmented nucleus and cytoplasm regions. Prominent features were then chosen by a greedy search algorithm called sequential forward selection. With a set of selected prominent features, both linear and naïve Bayes classifiers were applied for performance comparison. This system was tested on normal peripheral blood smear slide images from two datasets. the overall correction rate in the classification phase is about 98 and 94% for linear and naïve Bayes models, respectively.

C. Discrete Wavelet Transform

To classify WBC, features that represent their images have to be obtained. Those features have to be capable of distinguishing between WBC classes (to be discussed further shortly); and it is desired to have as few features as possible. Although the classification accuracy is dependant on the classifier, the performance of the classifier is totally dependent on having adequate features.

Feature extraction refers to finding common features in the Region of Interest (ROI) to classify it to classes. It is the objective of this work to find the minimum features required to classify four WBC classes. These classes are the main classes of WBC and they could change their formation based on their stage. The number of nucleus, color and WBC size play a major roles in determining the stage; and normality or abnormality of the WBC.

DWT also based on decomposition of a signal using an orthogonal family of basis function. The most important advantage of WT compared to FT is that FT is practical in analyzing periodic and stationary signals. DWT is a transformation from spatial domain to frequency domain that can be used to analyze the temporal and spectral properties of non-stationary signals.

DWT is seen as multi-resolution approximation expressions. Therefore, the signal analysis can be done at different frequency bands and different scales. Two channel banks low pass filter (G) and high pass filter (H) are needed to solve such multi resolution analysis problem.

$$H[k] = \sum_n x[k]h[2k - n] \qquad (3.5)$$

$$G[k]\sum_n x[n]g[2k - n] \qquad (3.6)$$

Consequently the resulting bank are down sampled by (1/2) of the previous frequency. This procedure is repeatable to obtain wavelets at any needed resolution level. In another words, DWT is developed to provide time-frequency information at various resolutions, which is a measure of the amount of detail information in the signal, levels of the image. When DWT is applied to an image, the image will be divided into four sub images in which each image represents one resolution level. DWT is a discrete function, the “detail” and “approximation” coefficients from all resolutions are generated by convolving the image.

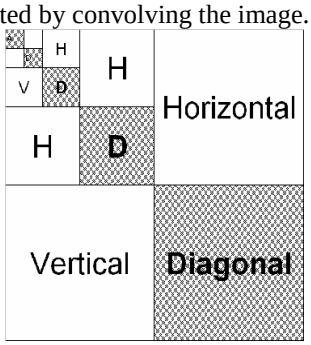


Fig- 5a Four sub Images from DWT

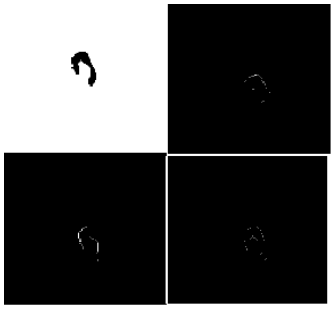


Fig- 5b Extracted Transformed Feature

Those sub images correspond to low frequency variation (approximation coefficients) of the original image (**a(m,n)**). The second, third and fourth resolutions level correspond to the high frequency (detailed coefficients) content on the vertical and horizontal and diagonal dimensions (**V(m,n), H(m,n) and D(m,n)**) respectively.

As Fig. 5a illustrates, the following represents the name of the four sub images transformed from the original image and Fig5b shows the segmented original image with its sub images such as vertical, horizontal and diagonal images. It is a db2 transformation using DWT, with lower frequency information. Sub-image "**LL**": Both horizontal and vertical directions have low-frequencies.

- Sub-image "**HL**": The horizontal direction has high-frequencies while the vertical has low-frequencies.
- Sub-image "**LH**": The horizontal direction has low-frequencies while the vertical has high-frequencies.
- Sub-image "**HH**": Both horizontal and vertical directions have high-frequencies.

Most of the variation occurred in diagonal and approximation coefficients, whereas the horizontal and vertical coefficients do not give adequate variation. Hence, the average of the diagonal detailed coefficient at the various resolution levels is taken as features for classification. The morphological features considered in this work to classify WBC and their analysis are presented below:

- ***Area***

Area (AR) of the object is scalar and is calculated by counting the actual number of pixels in the region of interest Area of objects in binary image, Total = bwarea (BW) estimates the area of the objects in binary image BW. Total is a scalar whose value corresponds roughly to the total number of on pixels in the image, but might not be exactly the same because different patterns of pixels are weighted differently. Returns a scalar that specifies the actual number of pixels in the region. (This value might differ slightly from the value returned by bwarea, which weights different patterns of pixels differently.)

- ***Eccentricity***

Returns a scalar that specifies the eccentricity of the ellipse that has the same second-moments as the region. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1. (0 and 1 are degenerate cases. An ellipse whose eccentricity is 0 is actually a circle, while an ellipse whose eccentricity is 1 is a line segment.)

- ***Convex area***

Returns a scalar that specifies the number of pixels in Convex Image. Then convex image means, Returns a binary image (logical) that specifies the convex hull, with all pixels within the hull filled in (set to on). The image is the size of the bounding box of the region. (For pixels that the boundary of the hull passes through, region props use the same logic as roipoly to determine whether the pixel is inside or outside the hull.)

- ***Perimeter***

Returns a scalar that specifies the distance around the boundary of the region. Region props compute the perimeter by calculating the distance between each adjoining pair of pixels around the border of the region. If the image contains discontinuous regions, region props returns unexpected results.

- ***Image ratio(aspect ratio)***

Aspect ratio is the term used to describe the dimensions of an image by comparing the width to the height and expressing it in ratio form. The aspect ratio of your images is primarily determined by the dimensions of your camera's sensor (or the film type plus camera design with film cameras). As these physical aspects are fixed, it is easy to take the aspect ratio of your images for granted, and to not consider the implications of the aspect ratio you are using in relation to composition.

After morphological process, feature extraction of Discrete Wavelet Transform is applied to the de-noised images. The image is separated into for different sub-bands low-low, high-high, high-low, low-high sub-bands. All the information of the image will be presented in the low-low sub-band. It is further used for the processing.

D. ANFIS Classification

Four features are extracted from each cell's nucleus. The features are used as the inputs to two types of classifiers, i.e., a ANFIS neural network classifier. A fivefold cross validation is applied to perform the training and testing on the data set. The classification results are evaluated in terms of the traditional classification rate and the class wise classification rate. In this section, we describe the nucleus feature extraction, the classification performance evaluation, and the experimental results and analysis. ANFIS concerns two methods in updating parameters. ANFIS is a fuzzy interference system (FIS) executed in the framework of an adaptive fuzzy neural network. It coalesce the overt knowledge representation of a FIS with the learning power of ANNs. The aim of ANFIS is to incorporate the best features of fuzzy systems and neural network.

In a classification problem, we generally evaluate a classifier's performance using the traditional classification rate, which is the ratio of the total correct classifications to the total number of samples classified. In addition to the traditional classification rate calculation, we consider another rate called the class wise classification rate. Basically, the class wise classification rate is the average of the classification rates of all classes, i.e.,

Class wise classification rate:=

$$\frac{1}{C}\sum_{i=1}^{c}\frac{number\ \ of\ correct\ \ classification\ \ \ in\ class\ i}{Total\ \ number\ \ of\ samples\ \ in\ class\ i} \qquad (3.8)$$

Where C is the number of classes. The basic idea of the class wise rate is to take out the effects of the number of samples in the training. While the traditional classification rate may be high if a large number of correct classifications occur in a class consisting of a large number of samples, the class wise rate is high only if all the classes have large numbers of correct classifications compared to their corresponding total number of samples. Therefore, we to have presented an ANFIS classifier that provides good classification performance in both the traditional and class wise senses.

IV. EXPERIMENTAL RESULTS

For the purpose of experimentation,100 light microscopic images of different types of leukocyte cells(non-overlapping) are considered which are taken from light microscopy. The implementation is done on a Intel core 2 duo processor @ 2.83 GHz machine using MATLAB 11b. The input of leukocyte cell image is converted into grayscale image and then we perform histogram equalization and the morphological operations are applied. The resulting image is global threshold to obtain segmented binary image. The segmented image is labeled and for each segmented region (known leukocyte cells), the geometric features are extracted.

All classifiers need training and testing phase, during the training phase, the classifier is offered a set of feature vectors with known class label. During the testing phase, class label are determined for each presented feature vector. The data is divided into two batches one for training the classifier while the other for testing them. The first batch consists of (808) segmented images of WBC and used for training the classifiers by ANFIS. The second batch which consists of (816) segmented images will be used for testing the classifiers.

Table-I: Accuracy Rate and Classification Rate using Isotropic diffusion segmentation with ANFIS classifier.

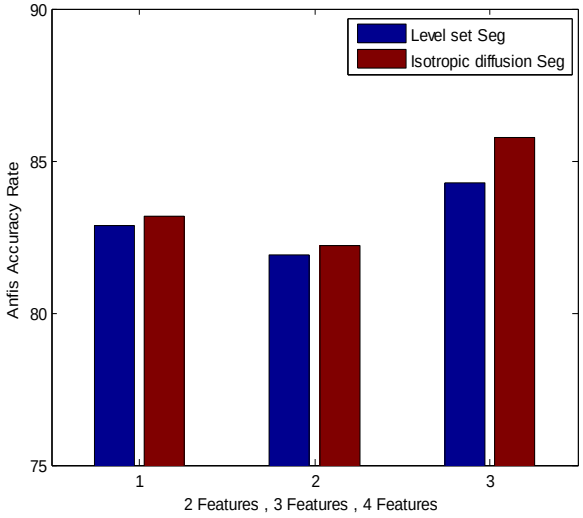
NO OF FEATURES	FEATURES TYPE	BD TYPE	ACCURACY RATE(%)	CLASSIFICATION RATE(%)
1	1.Area 2.Eccentricity	band cells	80	66.66
		Metamyelocyte	80	66.66
		Myeloblas	85.71	77.77
		N.myolocyte	80	66.66
		N.promyelocyte	80	66.66
		Neutrophil cells	93.33	87.5
2	1.Area 2.Eccentricity 3.ConvexArea	band cells	80	66.66
		Metamyelocyte	80	66.66
		Myeloblas	80	66.66
		N.myolocyte	80	66.66
		N.promyelocyte	80	66.66
		Neutrophil cells	93.33	87.5
3	1.Area 2.Eccentricity 3.Perimeter 4.Image Ratio	band cells	88.88	83.33
		Metamyelocyte	92.30	88.88
		Myeloblas	80	66.66
		N.myolocyte	80	66.66
		N.promyelocyte	80	66.66
		Neutrophil cells	93.3333	87.5

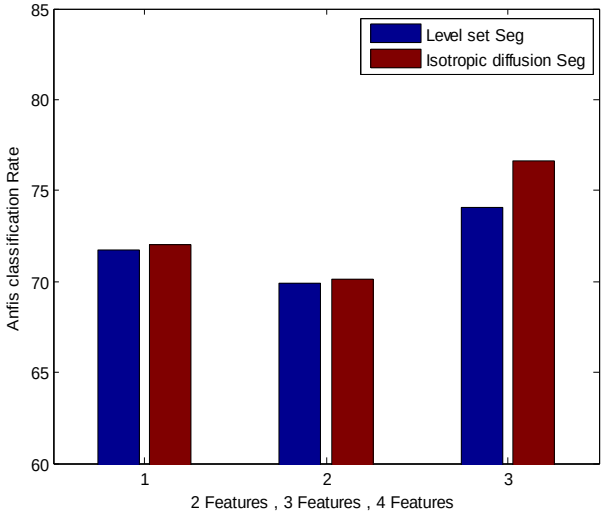
The Table 1 presents the geometric feature values computed for the segmented leukocyte cells and demonstrates the Accuracy rate and class wise Classification rate using ANFIS classifiers. The comparison is done separately for 2 features, 3 features and 4 features. Experiment is done separately for number of features is to analyze the accuracy. Also the classification is takes place for six cell types. Feature type f2 obtains the accuracy 83.17%, f3 has 82.22% and f4 has 85.7% which gives the highest accuracy and Classification rate for f4 is 76.6%, implies the more type of features achieves better result when compared to less number of features After de-noising, preserving edges and filtering, and deleting objects around the object desired, the resulting image can be applicable for training the ANFIS neural network and making comparisons based on the given instruction. Db2 transform has become the orthogonal foundation and transform with limited length and by the degree of removal moment. The atoms of a lot of consistency with the proposed classification of WBCs in two stages, that the standard DWT-filter bank have a faster implementation and lower computational complexity and this is a sign of their effectiveness.

Table II: Comparison of Accuracy and Classification rate of level set segmentation [25] and Proposed Isotropic diffusion segmentation

NO OF FEATURES	FEATURES TYPE	ISOTROPIC DIFFUSION ACCURACY RATE%	LEVEL SET ACCURACY RATE%	ISOTROPIC DIFFUSION CLASSIFICATION RATE%	LEVEL SET CLASSIFICATION RATE%
1	1.Area 2.Eccentricity	83.1746	82.8571	71.7593	71.9908
2	1.Area 2.Eccentricity 3.ConvexArea	82.2222	81.9048	69.9074	70.1389
3	1.Area 2.Eccentricity 3.Perimeter 4.Image ratio	85.7550	84.2857	76.6204	74.0741

From Table-2, it classifies the existing Level set segmentation based result and compared with Isotropic Diffusion method which shows the better accuracy than the existing Level set algorithm.





In the existing system, methodology followed for classification of WBC's are done using Multistage Morphological Toggle (SMMT) operator with scale space properties is used to segment the nucleus and to assure the accuracy of two well-known image segmentation Techniques. SMMT plays a major role in the segmentation part namely Watershed transform and Level-set methods and the two techniques are granulometric analysis and on morphological transformations. For the purpose of classification *Adaptive Neuro-fuzzy Inference System (ANFIS)* is used to demonstrate the classification rate and accuracy of existing and proposed system ANFIS classifiers. The comparison is done separately for 2 features, 3 features and 4 features. Experiment is done separately for number of features is to analyze the accuracy. And also the classification is takes place for various cell types. From the results it shows the feature (f4) gives best among the (f3) and (f2) with the Classification rate of 76.62% and Accuracy rate of 85.75% when compared to the Level set algorithm with the 84.28% and 74.07%. It is clearly analyzed that the proposed technique give good results.

V. CONCLUSION

The proposed system, based on normal White Blood Cell (WBC) morphology and its characteristics, was applied to Test and Train datasets. The results of the calibrated segmentation process on both datasets are fast, robust, efficient and coherent. Two sets of comparison were performed: Accuracy rate and classification rate. The automatically segmented results were compared to the ones obtained manually by a haematologist. It was found that the proposed method is consistent and coherent in both datasets, with dice similarity of 85.75% and 76.62% for average segmented nucleus and cell regions, respectively.

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