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# Leukocyte Sub Type Identification using Spline Interpolation and Graphical Analysis

**Diana Baby [1,*], Sujitha Juliet Devaraj [2], Anishin Raj M. M. [3]**
[1,*] Part-time Research Scholar, Karunya Institute of Technology and Sciences, Coimbatore & Department of IT, Viswajyothi College of Engineering and Technology, Kerala
[1,*]dianaanishin@gmail.com(corresponding author)
[2]Department of CSE, Karunya Institute of Technology and Sciences,Coimbatore
sujitha@karunya.edu
[3] Department of CSE,Viswajyothi College of Engineering and Technology,Kerala
anishinraj@vjcet.org

## ABSTRACT

The automatic and computer assisted techniques for the categorisation of sub types of WBC's in the medical field is highly essential for the accurate and faster disease diagnosis. This work involves the segmentation of the nucleus of the leukocyte from the cytoplasm using K-Means clustering algorithm followed by morphological operations which is further interpolated using Cubic spline technique based on a heuristic on the change of edge values. Through interpolation the new data points within the range of a known data limit is estimated. There are different techniques for interpolation which varies in accuracy and computational performance. The variation of graphs based on computed values of the norm of difference of derivative of two vectors is computed in this work. This provides the information for the categorisation of sub types of WBCs such as Eosinophils, Lymphocytes, Monocytes and Neutrophils.

**Key words:** Leukocytes, Interpolation, Cubic Spline, Segmentation

## 1. INTRODUCTION

White blood cells (WBC) sub type identification is very important because it protects our body from various infections and it has a vital role in the immune system [14]. Various types of blood disorders like leukaemia and different types of blood cancers can be detected automatically by the analysis of WBC structure. Manual identification of leukocytes by pathologist is a tedious and time consuming process which has several hurdles. WBC's or leukocytes present in the blood are produced by bone marrow which consists of nucleus cytoplasm and cell wall. The nucleus of WBC's is lobbed and huge which makes it differ from other blood cells like red blood cells and platelets [1].

Nucleus of leukocytes is different in their shape, size and texture. The most prominent information about the leukocytes can be extracted from the nucleus. There are mainly three types of WBC's like Granulocytes, Monocytes and Lymphocytes. The four main sub types of WBC's are Eosinophils, Lymphocytes, Monocytes and Neutrophils [17]. The Eosinophil kills worms and parasites by releasing toxins from its cytoplasm. The nucleus of Eosinophil consists of two lobes and the diameter of nucleus ranges from 9 to 15(μm) which covers one to four percentage of entire WBCs [1].

Lymphocytes produce proteins and antibodies which contain large slightly oval or round nuclei which are single lobbed. The size of nucleus of Lymphocyte varies from 6 to 16 (μm). The monocytes stimulates osteoclasts which consists of vary large kidney shaped nucleus among all types of leukocytes whose diameter ranges from 10 to 13 (μm). The neutrophils fight against bacteria which contains multi lobbed nucleus whose diameter ranges from 10 to 18 (μm) and it covers 1 percentage to 3 percentage of peripheral WBCs [1].

This work concentrates on the identification of subtypes of leukocytes from the cropped part of segmented nucleus of White blood Cells (WBCs) which is again classified into five types Lymphocytes, Monocytes, Eosinophils, Basophils and Neutrophils. The image interpolation technique is used in this work to identify the class of leukocyte based on the analysis of interpolated graph.

## 2. RELATED WORK

Yung-Kuan Chan et.al proposed a method for segmentation of nucleus from Leukocytes and counting of nucleus lobes. In this work the segmentation of Leukocytes is performed in two stages such as object contour detection based on intensity values and object size followed by nucleus segmentation [2]. Oleg Ryabchykov et.al proposed a method for Leukocyte sub type classification using pseudo Zernike moments for feature extraction from Leukocytes which represents the cell morphology whereas the classification is performed using SVMs [3]. Nisha Ramesh et.al proposed a method for isolation and classification of normal White Blood cells from peripheral blood smears which comprises of two steps. Firstly partition the nucleus lobes by detecting maximum curvature points; secondly the feature extraction is performed using the technique Linear Discriminant Analysis (LDA) from the cytoplasm and nucleus [4]. Yunfeng Yang et.al performed image interpolation based on edge features. In this method an edge detection operator is used for the extraction of information from image edges. Then the bilinear interpolation is used in the non-edge areas whereas bicubic B-spline interpolation is used in near edge regions[5]. S.E.EI-Khamy et.al proposed a new approach for image interpolation problem based on non-iterative regularized inverse solution and this new method is compared with the basic bicubic and cubic spline interpolation technique and also with standard regularised interpolation scheme. The authors concluded that this method has much better edge prevention ability than other interpolation algorithms [6,16]. J.Antony Parker et.al performed a study and comparison of different interpolation methods for resampling. The researchers have concluded that high resolution cubic spline is better when compared to bilinear interpolation algorithm [7].An efficient segmentation technique and recognition of leukocytes from blood sample image was proposed by Syed H Shirazi et.al where the texture information is obtained using entropy filter from the image and the object boundaries are identified by textured image binarization and the masking operation. The morphological operation is applied to produce the segmented image and back propagation neural network is used for recognition of leukocytes [8]. The work by Narjes Ghane et.al enunciated a modified watershed algorithm for touching nuclei splitting and has employed K-Means clustering for the segmentation of nuclei from blood sample image [9].

## 3.PROPOSED METHOD


**Figure 1:** Proposed Leukocyte identification method

The proposed leukocyte segmentation and identification method is shown in figure 1. In this method leukocyte cell image with cytoplasm and nucleus is given as input. This nucleus is further segmented based on K-means clustering algorithm [9][10][12][15] followed by morphological

operations [11][13]. Then crop the most varying part of nucleus and apply spline interpolation [7] based on the heuristics applied on the edge values. Finally the identification of the leukocyte is done by analysis of the graph.

## 3. METHODOLOGY

Step 1: Compute the edge point vectors of each categories of Leukocytes

Step 2: Choose the (x,y) pairs such that x varies at least by one in the vector of x, i.e neglect the (x,y) pair with same x values.

Step 3: Apply cubic interpolation on the vectors x and y

Step 4: Compute the norm of difference of derivative of two vectors.

Interpolation is the process of computing the new data points within the range of a known data limit. There are different techniques for interpolation which varies in accuracy, smoothness and computational complexity [7]. Linear interpolation and nearest neighbour interpolation are computationally more simple when compared to cubic spline and B spline techniques. Nearest neighbour interpolation assumes the next data point to be same as the sample which is just beside to that point. Cubic spline is basically a third order piece-wise continuous function.

The function should be symmetric about zero which can be expressed using the general cubic spline equation for the interval (0,1) and (1,2) are shown below [7]:

$$f(x) = a_{30}x^3 + a_{20}x^2 + a_{10}x + a_{00} \quad (1)$$

$$f(x) = a_{31}x^3 + a_{21}x^2 + a_{11}x + a_{01} \quad (2)$$

## 4.RESULTS AND DISCUSSION

The leukocyte segmentation, cropping and spline interpolation of the edges of the various categories of Leukocytes are shown in Figure 2 and Figure 3. The norm of difference of derivative is calculated to compare the two graphs. The results show that the value of norm of difference of derivative is 2.9614 for monocyte sub type which is the highest since the edges are tortuous and contains sudden changes than all other subtypes whereas the edge features of Eosinophil are smooth and the value of norm of difference of derivative is 0.4361 which is the lowest since the interpolation using cubic spline to have minimum variations form the actual edge points. The values obtained for Lymphocyte and Neutrophil are 0.6979 and 1.5146 respectively since Lymphocyte has a better smooth edge than Neutrophil.

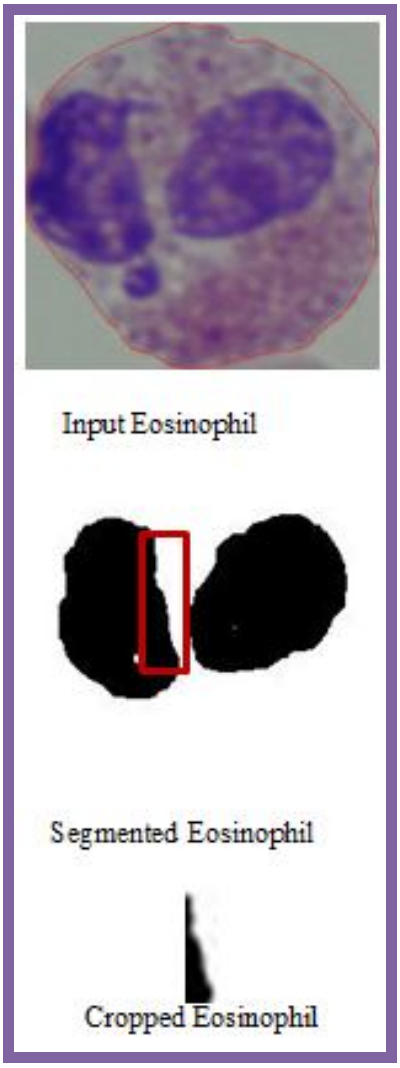


**Figure 2(a):** Eosinophil

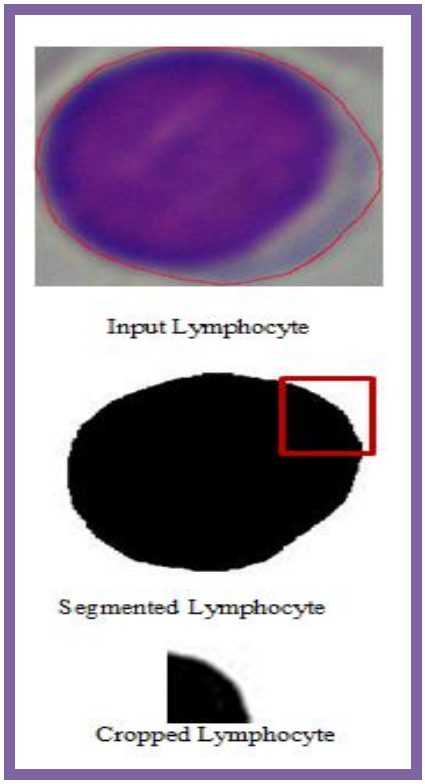


**Figure 2 (b)** :Lymphocyte

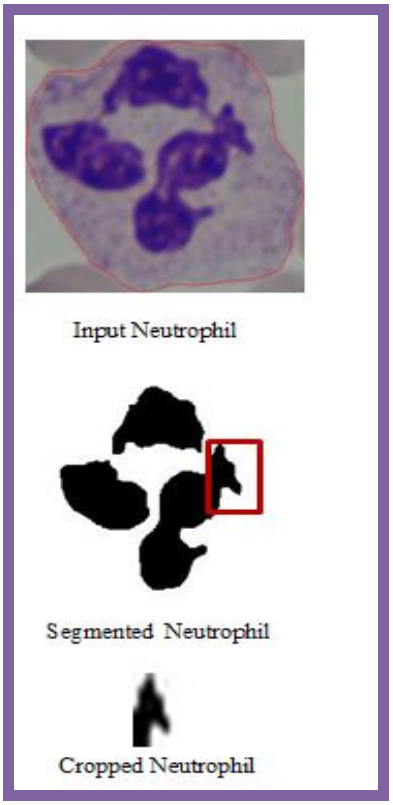


**Figure 2(d):** Neutrophil

**Figure 2(a, b, c & d):** Leukocyte segmentation & Cropping

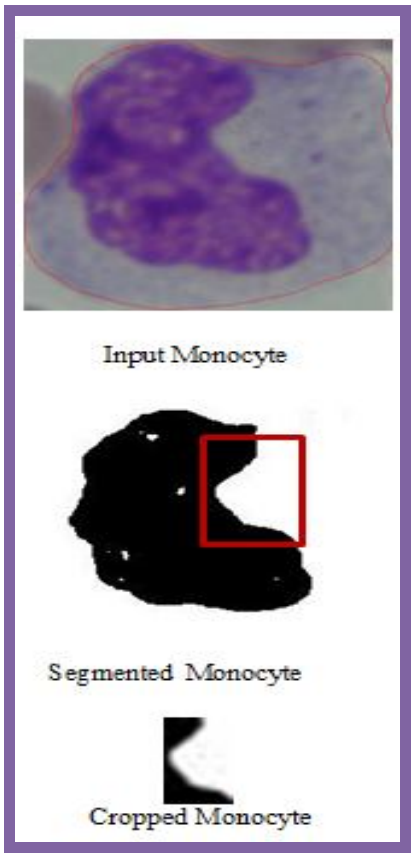


**Figure 2 (c):** Monocyte

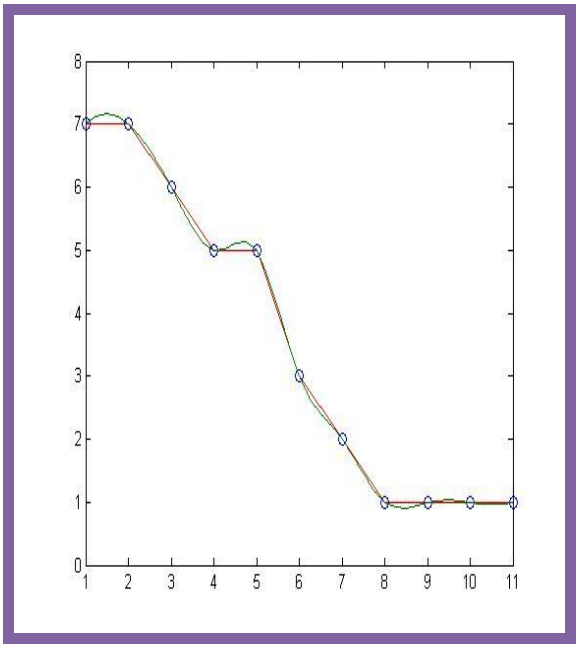


**Figure 3 (a):** Eosinophil

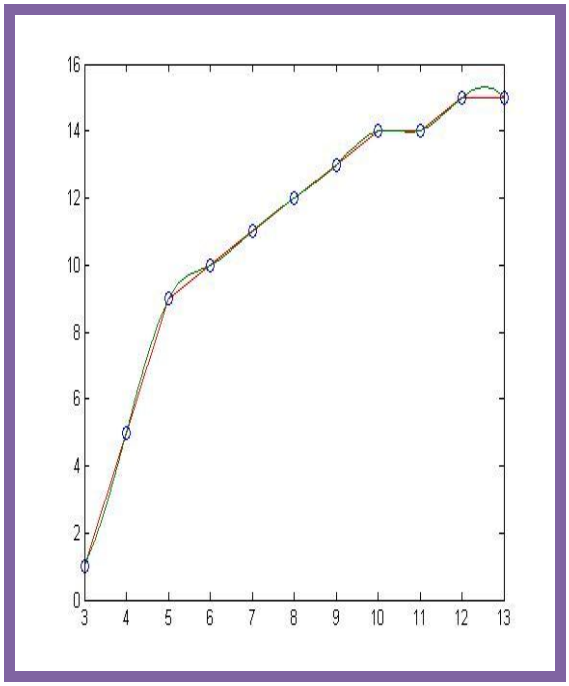


**Figure 3 (b):** Lymphocyte

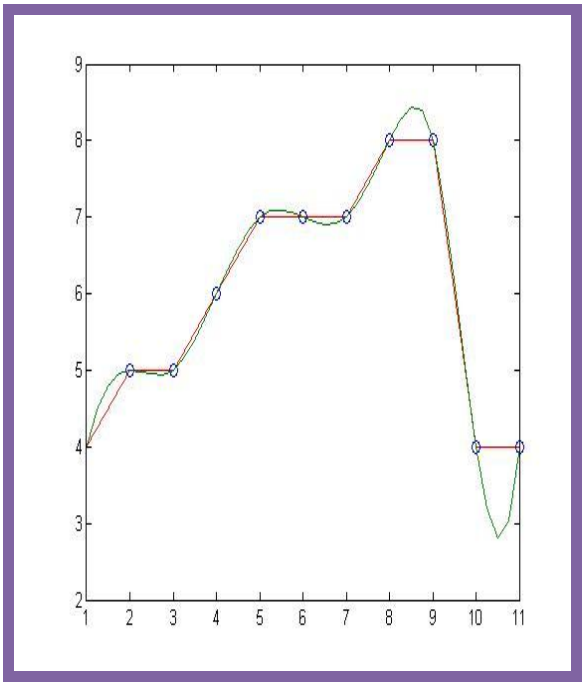


**Figure3 (d)**: Neutrophil

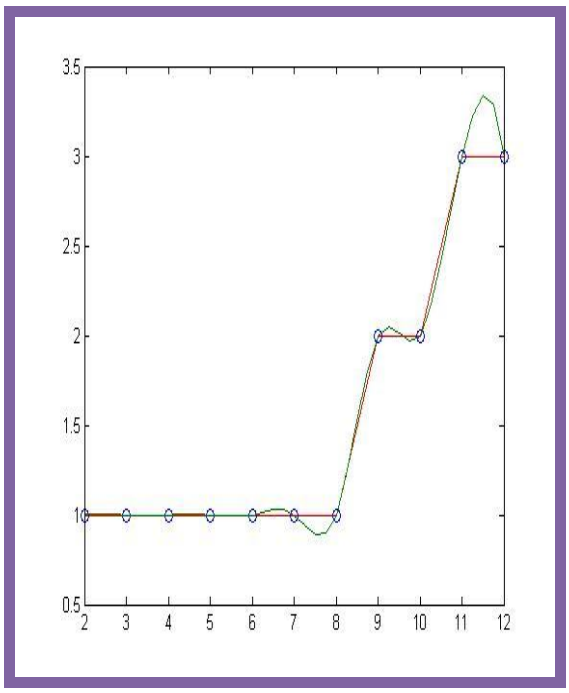


**Figure 3 (c)**: Monocyte

**Figure 3(a, b, c & d)**: Cubic spline interpolation

## 4. CONCLUSION

Leukocytes play a vital role in the immune system of human body which are basically classified into four major categories. This work proposes a novel technique for the categorization of subtypes of leukocytes such as Eosinophil, Lymphocyte, Neutrophil and Monocyte based on interpolation. The segmentation is performed using K-Means algorithm followed by morphological operations. The variation of graphs based on computed values of the norm of difference of derivative of two vectors is computed in this work which in turn provides the information for the categorisation of sub types of WBC's such as Eosinophils, Lymphocytes, Monocytes and Neutrophils.

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