

LEUKOCYTES COUNTING AND SEGMENTATION USING NEURAL NETWORKS

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ABSTRACT: Human blood consists of three types of major cells: Red blood cells, White blood cells and platelets. WBC fights against infection. WBC is also called as leukocyte. White cell can be counted manually in Neubauer or with automated chambers. Later; it is done using core biopsy. The existing system performs segmentation, classification and counting based on size of white blood cells. Cell classifications were done on multilayer perception. But, this method lacks reliability. Since this method carries sub-images, the accuracy is very less. So, a system which offers better accuracy than the existing system is proposed. This paper deals images which are captured from peripheral blood areas images with varying intensities. To eliminate noise and produce enhanced information, preprocessing is used. This method uses gray scale imaging and thresholding for segmentation. This method even considers stained image conditions. The use of Alexnet algorithm in deep convolution neural networks offers a better accuracy than existing system. The use of both median and Weiner filtering offers a better picture of the clarity of the both images and their parameters.

KEYWORDS: AlexNet Algorithm, Classification, Detection, Leukocytes, Segmentation.

I. INTRODUCTION

A type of blood cell that is made in the bone marrow and found in the blood and lymph tissue. Leukocytes are a part of body's immune system. They help the body fight infections and other diseases. Types of leukocytes are granulocytes (neutrophils, eosinophils and basophils), monocytes and lymphocytes (T cells and B cells). Checking the number of leukocytes in the blood is usually part of a complete blood cell (CBC) test. It may be used to look for conditions such as infection inflammation, allergies and leukemia. Stem cells in the bone marrow are responsible for producing the WBC. The bone marrow then stores an estimated 80-90% of WBC. When an infection or inflammatory condition occurs, the body releases white blood cells to help to fight the infection. Basophils represent less than 1% of WBC in the body and result in increased number after an allergic reaction. Eosinophils

are responsible for responding to infections that parasite causes. They play a role in general immune system and as inflammatory response. Neutrophils represent the majority of WBC. They act as scavengers helping the surround and destroy bacteria and fungi. Lymphocytes include B cells, T cells and Natural killer cells(they are responsible for attacking and killing viral cells as well as cancer cells).Monocytes are WBC that make up around 2-8% of total WBC. They are present when body fights off chronic infections.

II. RELATED WORKS

Heng Li et al^[1], Visual percepts and electrode array are used in Low-resolution image and retinal identification to get clarity images. But this technique is difficult to complete more complex tasks like face /object detection. Therefore, it is essential to refer and use image processing techniques for reducing the visual perception. The use of saliency segmentation and image processing methods can certainly extricate and enhance objects, and substantially enhance object identification operation.

Razzak M et al^[2], a complete automatic method to identify and classify WBC using microscopic images has been proposed .The leukocytes are separated using division of watershed conversion. The second level of division relates to the detection of the nucleus and cytoplasm. The last step involves the feature extraction in which the pathologist can have the interpretation showing an accuracy of only 93%.

Syadia Nabilah Mohd Safuan et al^[3], Conventionally, WBC is counted manually which sometimes generate an erroneous output as the blood sample rises. Segmentation is one of the main steps in computer aided system. Any decline during segmentation will produce a fault in the upcoming process. This inspected at variance segmentation processes for counting WBC according to color band thresholding. Further, segmentation approach was done to extract the WBC

region from the background by relating the prediction of RGB, CMYK and HSV by Otsu thresholding. Eventually, Circle Hough Transform (CHT) is the effective method used to count the cells.

U.A.Nnolimin^[4], exposes an efficient color embellishment structure for logarithmic image processing (LIP)-based methods. This method does image processing by performing many mathematical functions and logarithms to find object in image but it does not modifies the intensity of the image of parameters. Thus this method is quite complicated than those other methods.

Malrey Lee et al in^[5], the idea is to produce an efficient procedure to recognize and count citrus by image detection approaches. Citrus detection and counting methods make use of the color characteristics to present a calculation of available citrus in the tree, and the analogous patterns are constructed to suggest a rapid estimation of the citrus yield. The citrus counting techniques consist various effective steps. This algorithm showed countless potentiality for initial fascination of the yield of a citrus tree.

Xuan Li et al^[6], Photo privacy protection has gathered more attention from the people. Visual information with various levels of privacy sensitivity can be filtered out using various image-

processing techniques. Objects in a photo usually contain visual information that can potentially reveal private information; this potential depends on the visual saliency of the objects and on the specific categories to which the objects belong.

Zahra Khandan Khadem Alreza et al^[7], current retinal prostheses can only generate low resolution visual percepts constituted of limited phosphenes which are elicited by an electrode array and with uncontrollable color and restricted grayscale. Under this visual perception, prosthetic recipients can just complete some simple visual task, but more complex task like face identification/object recognition are extremely difficult.

Zunlei Feng et al ^[8], to obtain a color pigment that contains superior colors of the photo is done by Mining color themes. Here, they construct a color network to fabricate the intrinsic connection of color details. Using enriched linear iterative clustering (SLIC) algorithm, initial color themes are obtained. The sorted color result can be derived by learning color themes from human. This practically comes out with increased number of span, themes, and accuracy when compared to existing approaches.

III. METHOD

The existing system has reliability issues. Also the accuracy was not better. So, a system is proposed which produces a better accuracy than existing system. This paper uses techniques such as Green Plane Extraction, Arithmetic Operation, Linear Contrast Stretching, Histogram, Equalization, Global Thresholding and GLCM. It describes the results of fast and accurate blood cell segmentation of white blood cells. Here five types of WBC are identified. They are Neutrophils, Lymphocytes Monocytes, Basophil and Eosinophil. The images are captured from peripheral blood smear images. Preprocessing is used to eliminate noise and enhance their information.

Gray level intensities are used for segmentation because it requires less processing time and suitable for processing. The filters can be used for improving the segmentation result. This paper consists of two units Hardware and Software. The software part includes image acquisition gray scale conversion, median filtering and Weiner filtering, feature extraction using gray level co-occurrence matrix, adaptive histogram. The most important aspect of this project is the usage of neural networks. The proposed method uses Alexnet algorithm in deep convolution neural networks. The algorithm produces 98% accuracy in the identification of the cells.

Also the properties and volume information of the image is calculated. The hardware unit consists of power supply unit and arduino microcontroller. The power supply unit consists of step down transformer, bridge rectifier, and filter circuit and voltage regulator. Normally IC7805 and 7812 is used. The software unit is connected to Arduino microcontroller via UART cable. The software is implemented using MATLAB code. The hardware also has buzzer, LEDS, LCD display and a GSM module. The GSM is also connected to Arduino via UART cable.

Power supply unit offers power supply to the hardware circuit. Step down transformer is used to step down the power. A bridge rectifier is used to convert the AC input to DC. A full wave bridge rectifier is used. A power supply filter circuit is used to remove the remaining ac voltage. Normally, a low pass filter is used. In this paper, LM78XX series of three terminal regulators is used to protect from excessive current and overheating. Here,

Arduino UNO R3 is used. It is a microcontroller board based on the ATmega328. In hardware unit, a LCD display is used to display the results. A buzzer is used to alarm if there is any severity. GSM devices are used to indicate the user via messages and mail.

The software port starts with image acquisition. After the image has been obtained preprocessing has been done. The image is obtained as digital images. Digital imaging lends itself well to image analysis by software as well as to image editing. The acquired images are converted to gray scale images. Gray scale images are distinct from one bit black and white images. It is a kind of black and white or gray images, which is composed exclusively of shades of gray. After converting into gray scale image, feature extraction is done. Feature extraction is done using grey level co-occurrence matrix. This paper also measures correlation, contrast energy and homogeneity of the image. After feature extraction histogram equalization is done to increase the global contrast. The images parameters like mean squared error and peak signal-to-noise ratio, average difference, etc. Morphology includes image processing based on shapes. Dilation and erosion are two fundamental morphological operations. These objects are used for clarity in the boundary offering better results.

Proposed method uses deep convolution neural networks to provide 100% accuracy. Alexnet algorithm is used because this algorithm produces better results and less error compared to other algorithms. It uses a subnet of Image net with roughly 1000 images in each of 1000 categories. Alexnet consist of eight layers with weights. The first five layers are convoluted. The last three layers are fully connected. The neurons in the fully connected layers are connected to all neurons in kernel maps.

UV imaging is done to extract edges and analyze it. So after passing through neural network stage it is made to pass through segmentation. Segmentation is used to remove the affected parts from other parts. It also highlights the affected part. The type of WBC is identified using neural networks and the result is displayed. Now after performing all images processing it is interfaced with the UNO via UART cable. So the type of leukocyte identified is displayed on an LCD display. The intensity and severity of the image is obtained. The result is sent as an SMS to user mobile for this purpose and are port is generated and sent to user's mail id. This is done using GSM module. The existing system offers only 80% accuracy but this project offers 98% accuracy. This method uses both median and Wiener filtering to offer better image quality. The process flow is shown in Figure 1.

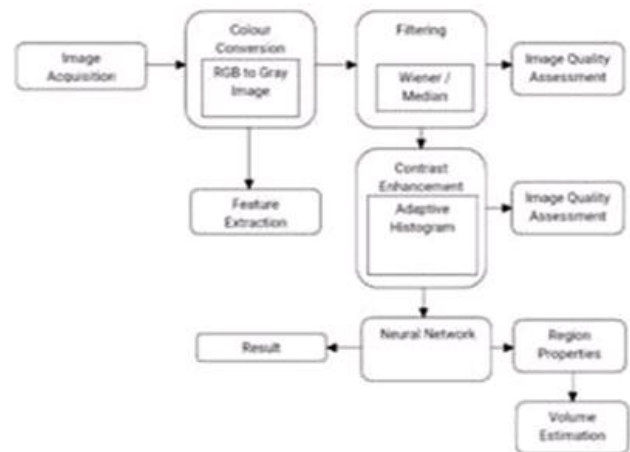


Figure 1 Block diagram of Software Unit

IV. PROPOSED SYSTEMS

- 1) Neural network require less formal statistical learning.
- 2) Ability to implicitly detect complex nonlinear relationship between dependent and independent variables.
- 3) AlexNet Algorithm is used to solve complex problems people face in real world situations.
- 4) Neural networks are trained using an optimization process that requires a loss function to calculate the model error.
- 5) AlexNet model is mainly employed due to its high performance.
- 6) AlexNet uses the non-saturating ReLU activation function which showed improved training performance over tanh and sigmoid.
- 7) A white blood cell count measures the number of WBC in the blood
- 8) A differential can also detect immature white blood cells and abnormalities both of which are signs of potential issues.
- 9) Learn how to identify the type of white blood cells normally present in a differential count.
- 10) Recognize abnormalities in WBC number and what they imply.

V. ALEXNET

AlexNet is the name of a convolutional neural network which has had a large impact on the field of machine learning, specifically in the application of deep learning

to machine vision. It famously won the 2012 ImageNet LSVRC-2012 competition by a large margin (15.3% VS 26.2% (second place) error rates). The network had a very similar architecture as LeNet by YannLe Cun et al but was deeper, with more filters per layer, and with stacked convolutional layers. It consisted of 11×11, 5×5, 3×3, convolutions, max pooling, dropout, data augmentation, ReLU activations, SGD with momentum. It attached ReLU activations after every convolutional and fully- connected layer. AlexNet was trained for 6 days simultaneously on two Nvidia Geforce GTX 580 GPUs which is the reason for why their network is split into two pipelines.

ImageNet is a dataset of over 15 million labeled high-resolution images belonging to roughly 22,000 categories. The images were collected from the web and labeled by human labelers using Amazon’s Mechanical Turk crowd-sourcing tool. Starting in 2010, as part of the Pascal Visual Object Challenge, an annual competition called the ImageNet consists of variable-resolution images. Therefore, the images have been down-sampled to a fixed resolution of 256×256. Given a rectangular image, the image is rescaled and cropped out the central 256×256 patch from the resulting image.

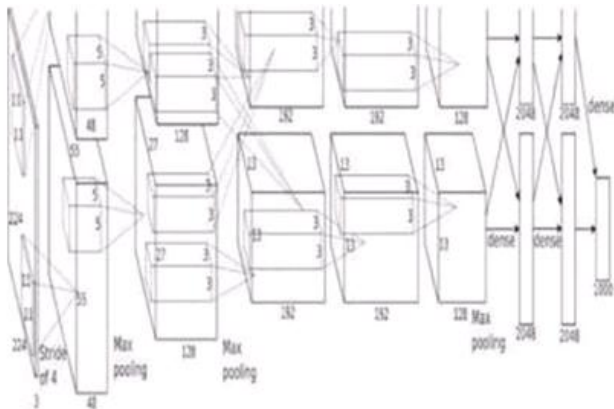


Figure 2 AlexNet: A typical D CNN Model

The architecture depicted in Figure 2, the AlexNet contains eight layers with weights; the first five are convolutional and the remaining three are fully connected. The output of the last fully- connected layer is fed to a 1000-way Softmax which produces a distribution over the 1000 class labels. The network maximizes the multinomial logistic regression objective, which is equivalent to maximizing the average across training cases of the log-probability of the correct label under the prediction distribution. The kernels of the second, fourth and fifth convolutional layers are connected only to those kernel maps in the previous layer which reside on the same GPU.

The kernels of the third convolutional layer are connected to all kernel maps in the second layer. The neurons in the

fully- connected layers are connected to all neurons in the previous layer. In short, AlexNet contains 5 convolutional layers and 3 fully connected layers. ReLU is applied after very convolutional and fully connected layer. Dropout is applied before the first and the second fully connected year. The network has 62.3 million parameters and needs 1.1 billion computation units in a forward pass. We can also see convolution layers, which accounts for 6% of all the parameters, consumes 95% of the computation.

VI. IMPLEMENTATION SEGMENTATION

Segmentation divides the image into its constituent regions or objects. The goal of segmentation is to simplify and / or change the representation of an image into something that is more meaningful and easier to analyze. Image segmentation is generally used to locate objects and boundaries in images. Here noise is eliminated to adjust the intensity of the image, perform a gray threshold and convert the image to a binary form.

MORPHOLOGY OPERATION

Morphological operations are image processing operations that process images based on shapes. Applies a structuring element of specific shape and size in the input image. The output image is created by comparing the value of each pixel with its neighbours. These operations are sensitive to the form of structuring.

CELL COUNTING

By comparing the superposition of the original image and the masked image and depending on the intensity profile, the differentiation between normal and infected cells is carried out. Finally, using mathematical operations, the count of the number of such cells is determined and displayed.

VII. EXPERIMENTAL RESULTS

The input image is the basic two-dimensional image is a monochrome (greyscale) image which has been digitized.

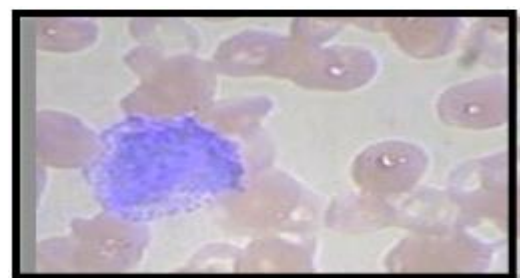


Figure 3 Input Image

The input taken from microscopic analysis of blood smear image is shown in Figure 3, which consists of affected leukocytes.

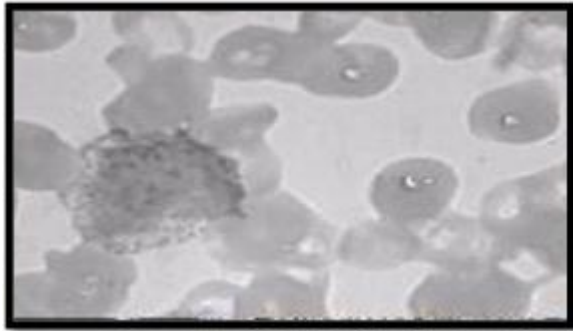


Figure 4 Gray Image

Grayscale images, a kind of black-and-white or gray monochrome, are composed exclusively of shades of gray. The conversion of input blood smear image to gray image is shown in Figure 4. The Median Filter is a nonlinear digital filtering technique, often used to remove noise from an image or signal which is shown in Figure 5. Such noise reduction is a typical pre-processing step to improve the results of later processing (for example, edge detection on an image).

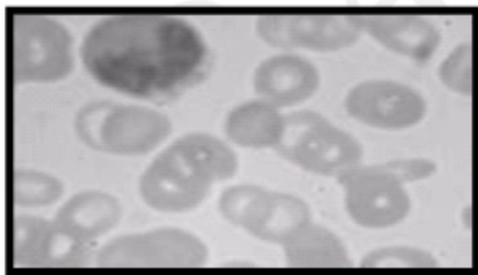


Figure 5 Median Filtering

Wiener filter is a filter used to produce an estimate of a desired or target random process by linear time-invariant (LTI) filtering of an observed noisy process, assuming known stationary signal and noise spectra, and additive noise. The Wiener filter minimizes the mean square error between the estimated random process and the desired process as shown in Figure 6.

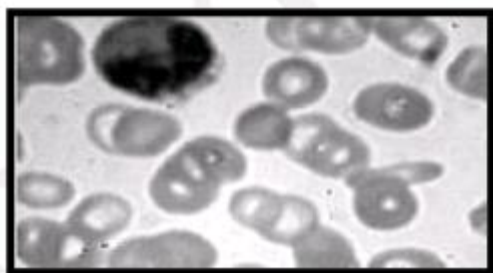


Figure 7 Histogram equalization

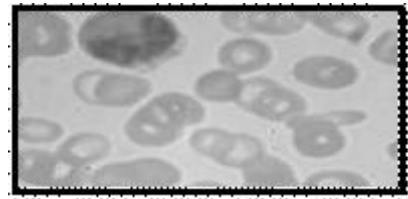


Figure 6 Weiner Filtration

The histogram equalization usually increases the global contrast of many images, especially when the usable data of the image is represented by close contrast values. Through this adjustment, the intensities can be better distributed on the histogram as shown in Figure 7.

After preprocessing and contrast enhancement, we obtain a noise-free image and clearly distinguishable components. Then, morphological operations are performed. The edges are detected by sliding a core over the image pixel by pixel. Figure 8 shows the processed image obtained using neural network. Thus the type of leukocyte is identified with great accuracy. The counting values by the proposed method differ slightly from the counting values obtained manually. Increasing the database by collecting images from various sources can make the algorithm used in the software more robust.



Figure 8 Segmented Image with the type of leukocyte identified

VIII. PROGRAM ANALYSIS

Table 1 provides the results of various quality assessment for identification of various leukocytes where AD indicates Average Difference, MD gives Maximum Difference, MSE gives Mean Squared Error, RMSE indicates Root Mean Squared Error, PSNR gives Peak Signal to Noise, NAE gives Normalized Absolute Error, NCC gives Normalized cross Correlation, SC gives Structural Content.

Table 1.Results of Quality Assessment

Para meters	Baso phil	lono cyte	Neutro phil	osino phil	Lympho cyte
AD	59.0	51.81	50.5	58.3	54
MD	144	134	136	145	135
MSE	7087.53	5705.53	5542.7	6520.77	5936.66
RMSE	84.18	75.535	74.449	80.75	77.04
PSNR	9.62	10.56	10.6	9.98	10.39
NAE	0.377	0.353	0.34	0.36	0.35
NCC	0.710	0.747	0.75	0.719	0.736
SC	1.604	1.491	1.47	1.59	1.539
Correlation	0.936	0.952	0.94	0.94	0.957
Contrast	0.051	0.057	0.06	0.056	0.059
Energy	0.405	0.338	0.32	0.37	0.333
Homogeneity	0.974	0.971	0.96	0.971	0.971
Area	288201	304340	301724	304625	306581
Centroid	317.363	321.69	322.94	321.86	320.881
Extend	0.93	0.990	0.98	0.99	0.99
Solidity	0.938	0.99	0.98	0.99	0.99
Orientation	1.095	0.84	-0.20	-0.019	-0.014
Eccentricity	0.68	0.66	0.65	0.658	0.660
Major Axis	758.93	740.67	741.65	739.35	739.08
Minor Axis	552.25	551.883	558.56	556.45	554.63

Various comparisons of Quality Assessment of Weiner and Median Filter are done in Table 2. Thus the success rate for identifying the leukocyte from the blood smear image has increased to a great accuracy.

Table 2.Comparison of Weiner and Median Filter

Parameters	Weiner	Median
AD	0.00239909	0.0107161
MD	10	203
MSE	0.66208	0.937643
RMSE	0.813683	0.96832
PSNR	49.9217	48.4104
NAE	0.00245411	0.00168129
NCC	0.999932	0.999912
SC	1.00012	1.00015

CONCLUSIONS

An effective method for WBC counting has been used in this method for the enhancement of accuracy. The white blood cells role in the accuracy of results. Despite all these facts sometimes the results will have no co-ordination with each other. Maybe for a same sample two are differentiated from noise and background resulting in segmentation. Thereby, the type of leukocyte is identified. The process of preprocessing helps in achieving image clarity. Further this helps in achieving efficient segmentation by employing neural network. Pathologists play an indispensable

pathologists can have difference of opinion. This may lack accuracy. In the proposed method we perform morphological operations which provide good efficiency as the cells are considered as boundary of image.

The deployment of AlexNet Algorithm through deep convolution neural network has enhanced the accuracy in the identification of leukocytes. The use of inbuilt functions makes the system faster and optimized. Since the biodegradable objects and diseases are variable in accordance to nature and time the research in this field is continuous and never ending process.

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