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Counting of Blood Cells using DigitalImage Processing

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ABSTRACT

In healthcare, blood testing is observed to be as one of the most significant medical examination tests. In pathology labs, different types of blood cells are counted to diagnose the effected patient. By counting of RBCs (Red Blood Cells) and WBCs(White Blood Cells) in images of blood cells plays a key role in detection as well as to follow the treatment process of number of diseases such as anemia, leukemia etc. Counting and examination of blood cells manually by microscope is tedious, time intense andentails a lot of technical expertise. Hence, in this paper a method to digitally analyze the image of blood cells and find the RBC and WBC count values from the blood smear microscopic images. Plane Extraction of the microscopic images is done followed by edge detection and morphological filling operation. Circular Hough transform is performed for RBC count, whereas boundary is detected for WBC. The obtained resultsof the experiment are compared with lab reports and an accuracy of 91% is achieved for RBC while an accuracy of 85% is obtained for WBC.

Keywords Digital Image Processing, Cell Counting, Edge detection, Morphological Filling Operation, Circular Hough transform.

Introduction

A developing era in the field of Computer Science Engineering is Digital Image Processing(DIP) and it has its branches in all the fields. Oneof the growing fields in it is the Medicinal part.

DIP uses computer algorithms to perform image processing on digital images. The impact of digital images on modern society is so great, and image processing is a critical component in science. At present, the blood samples are taken to lab and processed with various substrates and the results are produced. Whereas this paper, a Biomedical – Computer Science basedinterdisciplinary work, applies the stain, makes the blood sample absorb the stain and then captures the image of it. Then it is digitally processed with software and the result is displayed immediately. The analysis of microscopy images is

extremely important in both the medical and the computer science fields. Many research problems are related to the analysis of microscopy images, such as complete blood count (CBC) tests [1] and the analysis of blood smears, which is considered the first step in detecting and diagnosing malaria, leukemia, and anemia. Additionally, during a complete physical exam a series of tests are performed. One of these tests is the CBC, which is used to evaluate the composition and concentration of all cellular blood components. The CBC determines red blood cell (RBC) counts, white blood cell (WBC) counts, platelet counts, hemoglobin (HB) measurements, and mean red blood cell volumes[2].

CBC tests and the analysis of blood smear images help to evaluate, diagnose, and monitor various health conditions, such as anemia, leukemia, infections, and allergic conditions[3]. For blood disorders, such as anemia, which is based on HB level, the production and destruction of red blood cells are evaluated. In red blood cell disorder such as anemia, other red cell indices such as (mean cell volume) MCV, mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC, and red blood cell distribution width (RDW or RCDW) are evaluated to narrow down on the causes of anemia. If the red cell indices are suggested of iron deficiency anemia (IDA), further tests to confirm the IDA will be done. In normal blood, red blood cell (RBC) counts range from 4.2 to 5.9 million cells per square centimeter. High RBC counts can be indicative of serious medical conditions, such as heart, lung, or kidney disease. Primary or secondary polycythemia in polycythemia HB is also raised; a bone marrow disorder also causes high RBC counts [2]. Normal WBC counts range from 4,500 to 10,000 WBCs per microliter of blood [4]. High WBC counts (above 30000 cells per microliter) indicate an infection, systemic illness, inflammation, allergy, leukemia, or burn induced tissue injury. If leukemia is suspected, analysis of blood smear is done to look for morphology of the leukemia cells and followed by bone marrow examinations [2–4]. For platelets, which are small blood cell fragments that assist in blood clotting, normal counts range from 150,000 to 450,000 platelets per microliter. In patients with low platelet count such as in patients with dengue infection, their platelet count is monitored closely and the value is within critical level, the patient might need platelet transfusion [2]. Generally, any abnormal blood smear reading indicates an infection or disease. Haemocytometer is the device, which is used in the labs to count the blood cells. A microscopic glass slide consists of a rectangular indentation, which creates a chamber. A perpendicular line grid is etched in this chamber. It is possible to count the chamber of cells in a specific volume of fluid, and calculate the concentration of cells in the fluid. Physician views the haemocytometer using a microscope and count the blood cells using the hand counter.[9]

Drawbacks of using manual method for blood count is listed below

- Counting the cells manually is time consuming and laborious.
- Overlapping cells is a great overhead.

- Visual inception is not consistent all thetime.

In case of an automated Blood Cell Counting, Complete blood count is performed by an automated analyzer. The bloodis well mixed without shaking and placed on ananalyzer rack. This instrument has many different components to analyze the different elements of the blood. The numbers and types of different cells within the blood are counted by the cell-counting component. The results areprinted out or sent to a computer for review.

- Drawbacks of the automated method would be,
- Automated analyzer is not cheap.
- Variations in the shape and irregularitiescannot be detected.

II. RELATED WORK

Morphological iterative threshold techniques were presented in the literature by Berge [1]. Segmentation has been performed on RBCs, along with clumped cells, and boundary curvatures were implemented to construct a Delaunay triangulation. Real microscopy imagesprepared in the laboratory were used in that experiment. This method could not tolerate a high degree of overlapping cells. This iterative threshold method could not detect faded red cells. In another work, a method to count the Red BloodCells, White Blood Cells and Platelets was proposed by Khan [2]. This method involvesseveral pre-processing steps for the conversion ofimage to binary (or) binary image. Optimal threshold value was calculated using histogram and it plays a major role in segmentation and cellcounting. They were able to achieve 95.23% accuracy in comparison with manual counting and a hematology analyzer but this cannot detect overlapping cells. Hence, iterative thresholds lead to a high probability of losing validinformation which is dangerous.

Distance transformation tosolve the overlapping cells problem was introduced by Nguyen [3]. Clumped cells wereconcentrated in this method in a higher degree. A randomized Hough Transformation method which detects circles and RHT is increased whichis lesser efficient in complex images due to itsprobability usage problem was proposed by Chiu[4] which was fast enough.

Mahmood and Mansor [5] analyzed ten image samples of normal blood cells. Here, image was converted to the HSV color space and Saturation or “S” channel was selected to pursue with the image analysis. Morphological operators and thresholding method were used instead of S channel for cell segmentation. They used Circular HoughTransformation to detect or check the presence ofcircularity feature which indicates the red blood cells in order to perform counting and detecting process. Their proposed method achieved approximately 96.54% accuracy rate when compared with manual counting process.

Miss. Madhuri G. Bhamare and Prof. D.S.Patil [6] presented a preliminary study of automatic blood cell counting using digital image processing. A few pre-processing and post-processing techniques have been implemented on blood cells image in order to obtain a much clearer and clearer image.

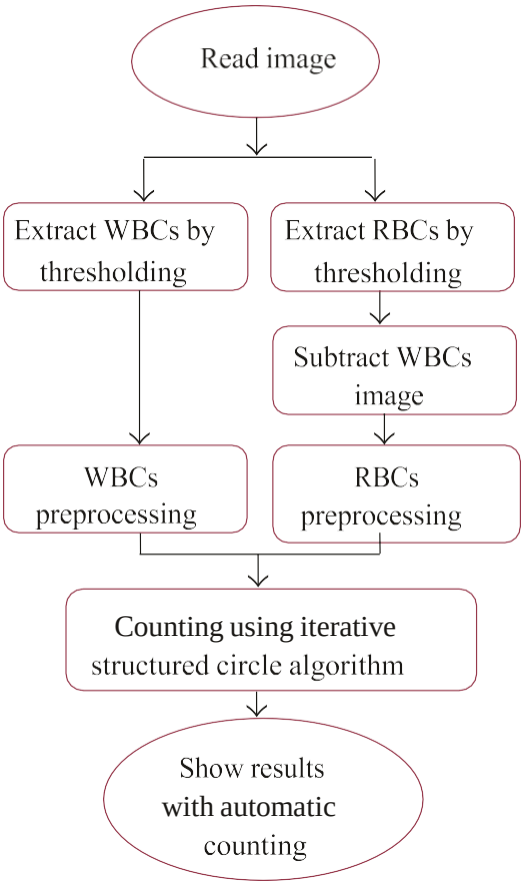

Figure 1: General Methodology for proposedmethod.

III. PROPOSED METHODOLOGY

The proposed method was developed to analyze microscopic images of blood smears by segmenting and counting both WBCs and RBCs. The segmentation is based on thresholding and morphological operations, and then counting is based on the circularity feature of the blood cells extracted using an iterative structured circle detection algorithm.

A new technique for binary images based on the fundamentals of RCD has been proposed and used for counting RBCs and WBCs. Therefore, the original image is separated into two images; the first image contains RBCs only and the second image contains WBCs; this step has been done using thresholding. We studythe histogram for 20 sample gray scale images, and we find out the best thresholding values to extract WBCs and RBCs; values were 64 and 140,respectively. After cells separation, each image is preprocessed using morphology operators to obtain the edge image using canny operator. Then, an iterative structured circle detection algorithm is used to count cells in each image.Figure 1 shows the

proposed method for WBC and RBC segmentation.

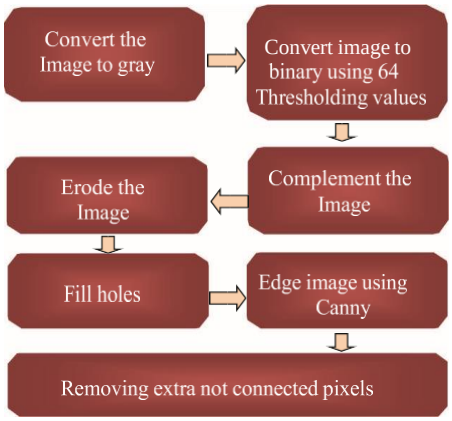

Figure 2: Preprocessing steps for WBCs.

Blood sample Acquisition

In this process, the slides are cleaned with ethanolusing cotton. The fingers are cleaned with ethanoland are pricked with the help of Lancets. The blood drop is dropped onto the slide, smeared with another clean glass slide with an angle of 45 degrees inclination. Now the slide is kept for drying for five minutes. Leishman's stain is applied over the smeared blood and kept aside fordrying. The stained slide is then washed with double distilled water to remove the excess stain

.Nikon E 100 is used for getting the microscopicimages. Adjustments are made to get the clear images. Nikon 21 Pixel Camera is used to obtain the images with the help of proper lighting and finer adjustments.

Image Processing

The image obtained from the preprocessing stepwill be in RGB scale. For counting the Red blood cells, the red plane of the image will be extracted and the counting process is done. [7] Similarly, for counting the White blood cells, theblue plane of the image is extracted and the counting process is applied. [8]

Noise Removal

This is a pre-processing step of an imagesequence before feeding into the Segmentation process.[7] The image is first converted to itscorresponding grey scale images. A pre-processing step is required to design a system thatmay be used under different conditions such as different blood staining techniques, types ofchemical materials used, microscope types,illumination conditions, human errors, etc., Certain noises, which are found in the images,include

- Salt and Pepper Noise: random occurrences of both black and white intensity values

Impulse Noise: Random occurrences ofwhite intensity values

- Gaussian Noise: Impulse noise

After observing various sample images it was found that the median filter would be the best noise removal filter.

Median Filter

Each pixel value is replaced with the median of the gray scale values in the region of the pixel (i,j). A 3 x 3 region centered around pixel (i,j) has been taken and sorted (in ascending order) according to the intensity values of the pixels in the region. The middle value among those values is selected as the intensity of the current pixel.This is the very effective in removing salt and pepper or impulsive noise while preserving the image detail.

Edge Detection

Edge preservation is an image processing technique to recover degraded and blurred images resulted while reducing the negative effect of noise in images. It can be a preliminary step toward better binarization and object segmentation.

In this paper, Canny edge detection algorithm is used over on the noise removed image to mark the edges of the cells. Canny edge detection algorithm detected the edges of the cells with maximal accuracy in case of sharp images whereas the accuracy of cell detection reduced in blurry images.[7] Since the cells are circular in shape, the edge detection wascircular and complete. But in the case of practicalimages, after applying edge detection certain edges were incomplete.

Overall the result of edge detection stage was an image in which there weretwo types of edges, one was the image where thecells were completely edge detected and another was the images where the cells edges were broken. To deal with these two different types of images and produce accurate results, a process called Morphological Filling was applied over the two sets of images which are explained below.

Morphological Filling

Morphological image processing is based on a strong mathematical concept which has been used to change the size, shape, structure and connectivity of objects in the image It involves binary erosion, dilation, opening, closing and reconstruction. [7] The erosion plays the role toshrinks and thins objects in image while dilation is used to grow and thicken the objects in image.Next, morphological opening is a combinational process of erosion and dilation while morphological closing is using the concept of dilation and continued by erosion. In other words, the functions of morphological opening are to remove, break and diminish the connection or objects which do not contain the structural elements. In contrary, morphological closing functions are used to join, fill and build connection and objects in the image. Using the closing image transformation with defined Structure Element (SE) unwanted edges,

typically generated, are removed. The input to this block is the edge detected image. Morphological operation of filling is applied in the edge detected image. The output of the morphological filling operation is an image where the objects which was detected by Canny is highlighted with lesser pixel and the background is completely dark which explicitly differentiates the objects that is cells - Red blood cells in case of red plane extracted image and White blood cells in case of blue plane extracted image.

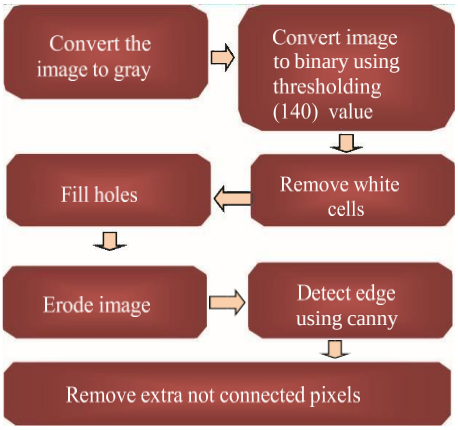

Figure 3: Preprocessing steps for RBCs.

Circular Hough Transform

The circular Hough transform is then applied on the Red plane extracted image to count the Red blood cells as red blood cells are spherical that is circular in shape. This transform searches for the blood cells in the image and then detects them.[7] Detection process for circular hough transform is easier as the input image is morphologically filled The function “draw circle” draws circles around the detected circular objects. Even the overlapped circles are detected. This method of counting cannot be used for white blood cells as they are not circular in shape.

Boundary Detection and counting

As the method of counting Red blood cells cannot be used for counting White blood cells, the method of Boundary detection and counting is used.[8] The input of this operation is morphologically filled Blue plane extracted image. A boundary around the filled objects is drawn and counting the number of cells drawn gives the total number of White blood cells in the image.

Blood Cell Calculation

The total number of Red Blood Cells in the body given the number of cells in the image with prescribed magnification and size is calculated using the formula with the unit - million cells per cubic millimeter of blood is mentioned in [Eq 1] for RBC’s and [Eq 2] for WBC’s blood count.

IV. PERFORMANCE ANALYSIS

The ABCCS method of counting blood cells wasexperimented from the initial stage of collecting blood samples for about 63 subjects in real-time. A drop of blood was collected from the subject and was placed in one corner of the glass slide cleaned with ethanol and was smeared using another clean glass slide. After the process of drying, the blood cells were stained using Leishman's stain. The slide was dried and cleaned with double distilled water to remove the excess stain. The stained blood cells were oil immersed using cedar wood oil and viewed under a compound microscope under 100x magnification. The images of the oil immersed blood cells were captured using a digital camera. A sample image is shown in the Fig 4.

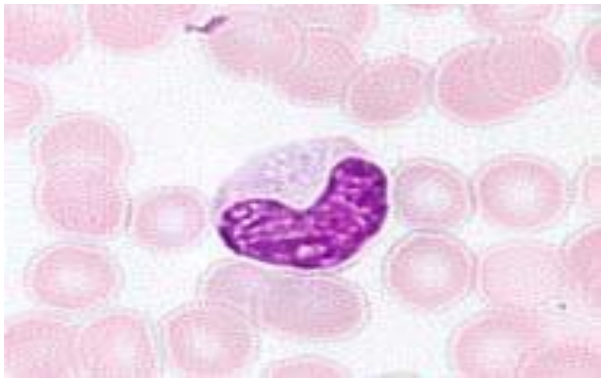

Figure 4: Sample Blood Smeared Imageobtained under 100x microscope

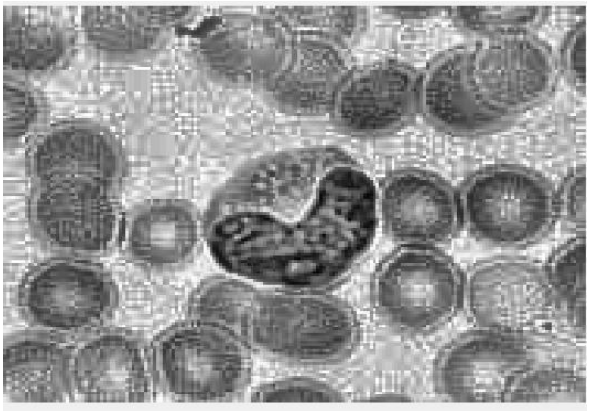

Figure 5: Gray Scale Image

The images obtained from the microscope under 100x magnification were subjected to Digital Image Processing. The unnecessary noises in the images were removed using Median Filter [8]. The Noise Removed Images were next subjected to the process of Plane extraction. Red plane was extracted in order to count the RBC as depicted in Figure 1 and Blue plane was extracted for the WBC counting as depicted in Figure 4. One sample image where the Blue plane was extractedfor WBC Counting process is shown in the Figure5.

Once the required plane is extracted, the basic DIP operation that is, Edge Detection [8] and Morphological Filling [8] is done to clearly distinguish the required plane objects in the image. A sample image after the application of the above two DIP operations is shown in the Figure 6

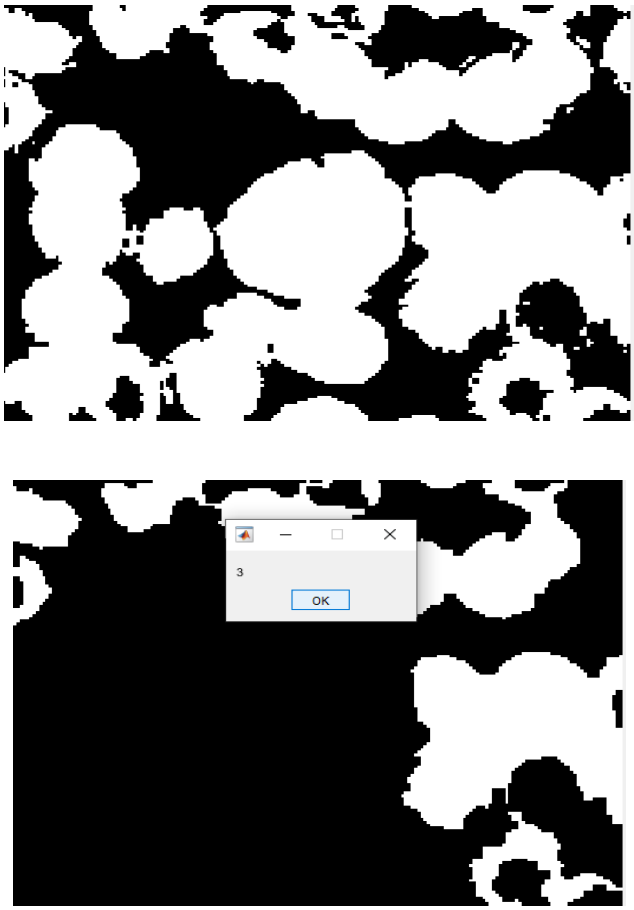

Figure 6: Thresholded Image

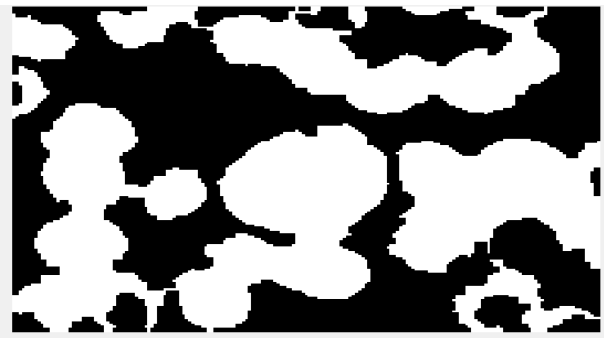

Figure 7 : Noise Removal Image

Figure 8 : Eroded Image

Now, two different methods are employed for counting RBC and WBC. RBCs arecircular in shape and hence, they can be detected and counted using Circular Hough Transform. An image under RBC counting process after the application of Circular Hough Transform is shown in the Figure 5. For WBC, Circular Hough transform cannot be used as WBC is shapeless and therefore, boundary is detected for any object in the blue plane extracted image and thereby the objects are counted. The boundary detected blue plane extracted image is shown in the Figure 6 which is under WBC Counting process.

Figure 10 : Image after application of Circular Hough Transform Operation for RBCFigure 11:

Displaying of RBC Count.

The ABCCS method was experimented for 63 subjects in real time and the results of them by ABCCS

method was validated or cross checked by the method of manual count using Haemocytometer. The RBC and WBC results given by the ABCCS method and by the Haemocytometer of a randomly selected ten subjects out of 63 are compared in the graph
which is depicted in the Figure 8 and Figure 9 respectively. The Accuracy Percentage of the RBC and WBC Count of the same ten subjects given by the ABCCS method was measured in comparison with the count given by the Haemocytometer and the same is depicted in theFigure 12.

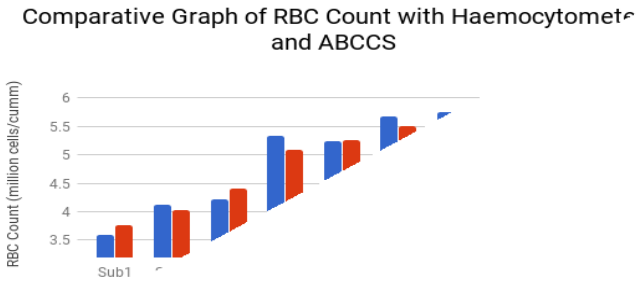

Figure 11: Comparative Graph of RBC Count with Haemocytometer and ABCCS

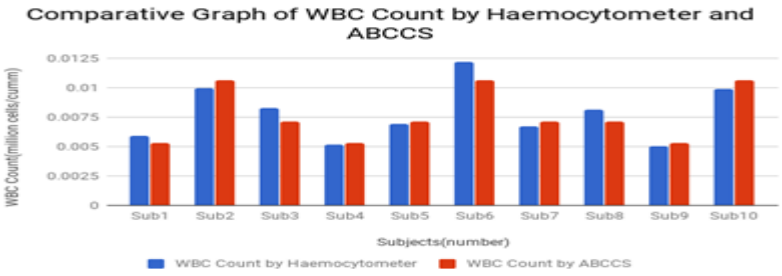

Figure 12: Comparative Graph of WBC Count ofHaemocytometer and ABCCS

V. CONCLUSIONS AND FUTURE WORK

The ABCCS method solves the problem of counting RBC with an overall accuracy of 91% and WBC with an accuracy of 85%.By using the proposed technique in image processing, analysis of blood cell image is more accurate as well as this method is efficient in terms of time and cost compared to existing techniques of blood cell analysis. The proposed method performed the segmentation and counting of RBCs and WBCs well when results were compared with the groundtruth (biosigdata.com), which was determined byexperts.

This system could further be extended for counting platelets and using complex functions to find the different kind of WBC present in the smeared image and hence improving the accuracyof the proposed method.

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