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An Overview: Optimization of WBC and RBC from Blood Sample Based on Microscopic Images

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Abstract: At the moment, identification of blood disorders is through visual inspection of microscopic images of blood cells. From the identification of blood disorders, it can lead to classification of certain diseases related to blood. This paper describes a preliminary study of developing a detection and measurement of white blood cells (WBCs) and red blood cell (RBCs) using microscopic blood sample images. Analyzing through images is very important as from images, diseases can be detected and diagnosed at earlier stage. From there, further actions like controlling, monitoring and prevention of diseases can be done. Blood cell counting by laboratory task utilizes hemocytometer and microscope. The conventional task depends on physician skill. Images are used as they are cheap and do not require expensive testing and lab equipments. This paper will focus on white blood cells and red blood cell.

Keywords: White Blood Cells, Red Blood Cells, Microscopic Images, Reinforcement Learning.

I. INTRODUCTION

Blood is a connective tissue consisting of cells suspended in plasma. From the identification of blood disorders, it can lead to classification of certain diseases related to blood. This paper describes a preliminary study of developing a detection of leukemia types using microscopic blood sample images. Analyzing through images is very important as from images, diseases can be detected and diagnosed at earlier stage. From there, further actions like controlling, monitoring and prevention of diseases can be done.

Blood's major functions are to transport various agents such as oxygen, carbon dioxide, nutrients, wastes, and hormones. Blood cells are composed of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells, WBCs) and thrombocytes (platelets). The most abundant small reddish cells are erythrocytes and called red blood cell. An erythrocyte is a discoid cell with a thick rim and a thin sunken center [1]. RBCs' two principal functions are to move oxygen from lung to tissues elsewhere and transport carbon dioxide from tissues to the lung. Whereas, the Leukocytes or white blood cells are part of the immune system.

The conventional device used to count blood cells is the hemocytometer. It consists of a thick glass microscope slide with a rectangular indentation creating a chamber of certain dimensions. This chamber is etched with a grid of perpendicular lines. It is possible to count the chamber of cells in a specific volume of fluid, and calculate the concentration of cells in the fluid [2,3]. To count blood cell, physician must view hemocytometer through a microscope and count blood cells using hand tally counter. The overlapped blood cells on the top-side and right-side of hemocytometer are not counted. Normally, the counting task is time-consuming and laborious. Several attempts have been made to mimic the procedure of cell recognition from image. The process require human expert and prone to errors due to emotion disturbance and human physical capability that is of course have its own limit. Moreover, it is difficult to get consistent results from visual inspection [3]. Visual inspection also can only give qualitative results for further research [3]. Studies show that most of the recent techniques use all information about blood for e.g. number of red blood cells, hemoglobin level, hematocrit level, mean volume corpuscle and many more as the parameter for classifying diseases such as thalassaemia, cancer and etc. In order to know all information about blood, expensive testing and equipments of labs are required. Automatic image processing system is urgently needed and can overcome related constraints in visual inspection. The system to be developed will be based on microscopic images to measure number of WBCs and RBCs from blood samples.

II. BACKGROUND

Blood cells are produced in the bone marrow, a jellylike substance inside the bones that is composed of, among other things, fat, blood, and special cells that turn into the various kinds of blood cells. In children, the marrow of most of the bones produces blood. But in adults, only the marrow of certain bones -- the spine, ribs, pelvis, and some others -- continues to make blood. Bone marrow that actively

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produces blood cells is called red marrow, and bone marrow that no longer produces blood cells is called yellow marrow. Bone marrow tests allow doctors to look at the fluid (inner liquid part of the marrow also known as the spongy part of the bone) and tissue in the marrow to determine whether cancer or another disease is affecting blood cell production and/or the structure of the marrow. Marrow tests can help determine the type and extent of the disease. Certain changes to blood cells can be detected in marrow samples before they can be detected in blood samples.

2.1. Types of WBCs and RBCs

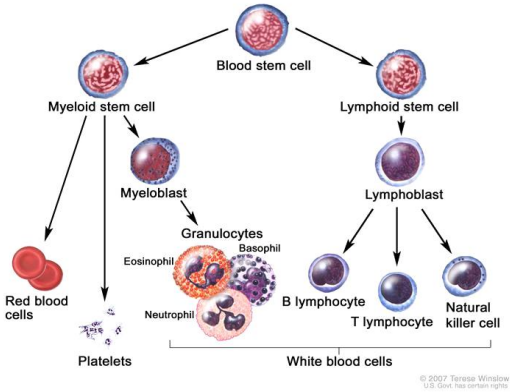

Fig.1. Production of Blood Stem Cell.

A. White Blood Cell (WBC)

White blood cells contain the immune cells that attack and remove viruses and bacteria in a person's body. Low WBC counts may indicate that a person is in danger of infection. High WBC counts might indicate an existing infection, tissue damage, or leukaemia. Typical levels are 4,000-10,800 cells per microliter of a person's blood. There are a number of different types of WBC's and their values differ; they are:

- Monocytes (2%-9% of all WBCs)
- Eosinophils (1%-4% of all WBCs)
- Basophils (0.5%-2% of all WBCs)
- Absolute Neutrophil Count (ANC)
- Neutrophils (50%-60% of all WBCs)
- Lymphocytes (20%-40% of all WBCs)

- *Neutrophils*

Neutrophils are a person's body's first line of defense against infection and disease. These cells assist with inflammation as a result of cuts or bacteria in the skin and are responsible for pus. A low level of neutrophils referred to as, 'neutropenia,' leaves a person susceptible to disease. Smoking and obesity increase a person's neutrophil count; for each pack of cigarettes a person smokes each day, their granulocyte count may increase. Mature neutrophils have a lobulated nucleus, but when demand is high immature cells with an unlobulated band nucleus may be released into circulation. They function as phagocytes and are important in infectious conditions and in inflammation. Increased neutrophil counts (neutrophilia) are caused by inflammation, bacterial infection, acute stress, steroid effects, and neoplasia of the granulocytic cell line (granulocytic leukemia can be difficult to differentiate from a simple neutrophilia without special stains or bone marrow biopsy). Decreased neutrophil counts (neutropenia) are caused by viral infections, toxin exposure (including foodborne toxins), certain drugs (eg, carbimazole and methimazole), autoimmune destruction of neutrophils, bone marrow neoplasia not involving the granulocytes, and bone marrow aplasia. Neutrophils are mainly present in human blood with a percentage ranging between 50 and 70%, have sizes around 10-12 microns and are distinguishable due to the number of lobes present in the nucleus, which can be up to a maximum of 5.

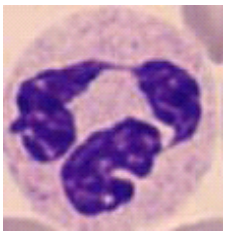

Fig.2. Neutrophils

- *Eosinophils*

Eosinophils are characterized by prominent pink-staining granules on a Romanowsky stain. They inactivate histamine and inhibit edema formation. Increased eosinophil counts (eosinophilia) are caused by allergic/hypersensitivity reactions, parasitism, tissue injury, mast cell tumors, estrus, and pregnancy or parturition in the bitch. Some large continental dog breeds (eg, German and Belgian Shepherds, Rottweilers) normally have a relatively high eosinophil count. Extremely high eosinophil counts (hypereosinophilic syndrome), possibly due to an out-of-control hypersensitivity reaction, and eosinophil leukaemia (a form of chronic myeloid leukaemia) are also described. Decreased eosinophil count (eosinopenia) is almost always caused by the action of glucocorticoids, either endogenous or therapeutic. Eosinophils are present for the 1-5% in human blood, have predominantly rounded shape with dimensions around 10-12 microns, and have a nucleus with more lobes, but not greater than 2. They differ from other white blood cells for the presence of granules, which include paracrystalline structures in the form of “coffee bean”.

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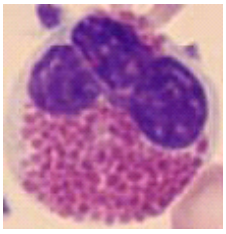

Fig.3. Eosinophils

- *Basophils*

Basophils are rare in most species and are characterized by blue-staining granules on a Romanowsky stain. They are closely related to mast cells and, like them, initiate the inflammatory response by releasing histamine. An increased basophil count (basophilia) accompanies eosinophilia in some species as part of the hypersensitivity reaction. Basophils instead represent only 0-1% of lymphocytes in human blood, have a diameter of about 10 microns and, generally, a nucleus with two lobes.

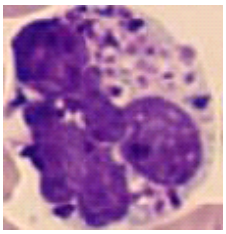

Fig.4. Basophils

- *Monocytes*

Monocytes are large cells with blue-gray cytoplasm, which may be vacuolated, and a kidney bean-shaped or lobulated nucleus. Their main function is phagocytosis, and they are essentially identical to tissue macrophages. An increased monocyte count (monocytosis) may occur in any chronic disease, especially chronic inflammation, and may be very marked in neoplasia. Monocytes also increase as part of the steroid response in dogs. Monocytes are the most voluminous white blood cells, with a diameter of 12-18 microns and representing 3-9% of circulating leukocytes.

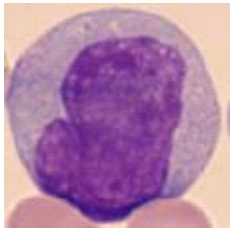

Fig.5. Monocytes

- *Lymphocytes*

Lymphocytes mainly develop outside the bone marrow in the lymph nodes, spleen, and gut-associated lymphoid tissue. They are the smallest of the white cells, with a round, evenly staining nucleus and sparse cytoplasm. Their primary function is immunologic, including both antibody production and cell-mediated immune responses. Some survive only a few days, but many are long-lived. The number in circulation is a balance between populations in the blood, lymph, lymph nodes, and splenic follicles and does not necessarily reflect changes in lymphopoiesis. An increased lymphocyte count (lymphocytosis) may occur for physiologic reasons, especially in cats, but significant increases usually indicate leukemia. Immature or bizarre cells may also be recognized. Decreased lymphocyte counts (lymphopenia) are usually due to an effect of corticosteroids, either endogenous (stress or Cushing's disease) or therapeutic, and may also accompany neutropenia in some viral infections, especially the parvoviruses. Lymphopenia may also be a feature of solid-organ lymphosarcomas, when leukemia is absent. In human blood is very common the presence of lymphocytes, with a percentage of 20-45% and a size of 7-15 microns, characterized by a rounded nucleus and a cytoplasm poor.

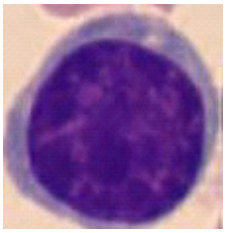

Fig.6. Lymphocytes

B. Red Blood Cells

Red blood cells carry oxygen from a person's lungs to the rest of their body. A depletion of red blood cells may lead to anemia. Anemia results in dizziness, fatigue, or even more serious symptoms if it is remains untreated. The red blood cell (RBC) count determines the total number of red cells (erythrocytes) in a sample of blood. The red cells, the most numerous of the cellular elements, carry oxygen from the lungs to the body's tissues. Hemoglobin (Hgb) is the protein-iron compound in the red blood cells that enables them to transport oxygen. Its concentration corresponds closely to the RBC count. Also closely tied to the RBC and hemoglobin values is the hematocrit (Hct), which measures the percentage of red blood cells in the total blood volume. The hematocrit (expressed as percentage points) is normally about three times the haemoglobin concentration (reported as grams per deciliter).

Red blood cell indices provide information about the size and haemoglobin content of the red cells. They are useful in differentiating types of anaemia. The indices include four measurements that are calculated using the RBC count, haemoglobin, and hematocrit results. Typical red blood cell count (RBC) levels are:

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- 4.2 to 5.4 million cells per micro liter for women
- 2.6 to 4.8 million cells per micro liter for children
- 4.5 to 6.2 million cells per micro liter of blood for men.

C. Hemoglobin

Hemoglobin is a molecule on a RBC that allows it to carry oxygen. Low hemoglobin counts may also result in fatigue and anemia. This is the amount of hemoglobin in a volume of blood. Hemoglobin is the protein molecule within red blood cells that carries oxygen and gives blood its red color. Typical levels are:

- 13-18 grams per deciliter in men (international units 8.1 to 11.2 millimoles/liter)
- 12-16 grams per deciliter in women (international units 7.4 to 9.9 for women)
- 11 to 13 grams per deciliter in children.

2.2 Existing methods

Some research has been done in automating the process of blood cell identification and next can diagnose the patient correctly. Some of them are, [1] they have detected malaria infected red blood cells by using digital holographic interferometric microscopy (DHIM) with numerical focusing. They have done the identification by comparing its shape profile with that of a healthy RBC. A correlation function is used to separate healthy and malaria-infected RBCs.

In [2], they have classified the WBCs and the count of white blood cells in microscopy images is done which allows the vivo assessment of a wide range of important hematic pathologies (i.e., from presence of infections to leukemia). Here they have used the morphological cell classification which is typically made by experienced operators.

While in [5], they have proposed a software base solution related health industry which will assist the medical laboratory technician (MLT) to detect and find a blood cell count and produce an accurate cell count report. This will be very helpful to a physician in identifying the cause of his patient's diseases. To count the blood cells in a clinical laboratory different two methods and techniques are used. One is the old conventional method of cell counting under the microscope and the other is to produce cell counting report by latest but very expensive hematology analyzer.

And in [4] they have presented a rapid, novel, minimally invasive approach for cancer detection based on Fourier transform infrared (FTIR) micro spectroscopic (MSP) analysis of peripheral blood plasma coupled with advanced computational methods. They have developed an automatic, computerized classification method that alerts for any signs of cancer presence, even in the early stages, regardless of the location of the solid tumor and without the need to search for a specific type of cancer.

In [14], they have proposed automatic Otsu's threshold blood cell segmentation method along with image enhancement and arithmetic for WBC segmentation. The K-nearest neighbor (KNN) classifier has been utilized to classify blast cells from normal lymphocyte cells. The system is applied for 108 images available in public image dataset for the study of leukemia. This method gives 93% accuracy.

[9] uses method curbs the human error while detecting the presence of malaria parasites in the blood sample by using image processing and automation. They have achieved this goal using Image Segmentation smoothing processing techniques, gradient edge detection technique to detect malaria parasites in images acquired from Giemsa stained peripheral blood samples. This system in a robust manner so that it is unaffected by the exceptional conditions and achieved high percentages of sensitivity, specificity, positive prediction and negative prediction values. And the extraction of red blood cells achieves a reliable performance and the actual classification of infected cells.

[11] they have proposed an image processing technique for counting the number of blood cells. The number of counted blood cells will then be used to calculate the ratio of blood cells for leukemia detection. For this purpose, few pre-processing and post-processing techniques have been implemented on blood cells image in order to provide a much clearer and cleaner image for blood cells ratio calculation. The results show that the ratio of blood cells which have been calculated using the proposed image processing techniques are able to differentiate between normal and abnormal blood cell image for leukemia detection. They have applied this method on 91 different images.

[10] They have proposed determining the convex hull of the cells which gives us an improved result in the context of cell count. Leukaemia can be broadly classified into acute and chronic. This method focuses on the detection of the Acute Lymphoblastic Leukaemia (ALL) which contributes to about 70% of most of the leukaemia cases every year. Segmentation of the white blood cells from red blood cells in the image of the blood smear is done. The Blood Cell Ratio is then computed to determine presence of increased white blood cells.

[7] This method shows the effectiveness of an automatic image processing method to detect normal red blood cells (RBCs) by peripheral blood smear microscope image. When single RBCs are extracted from sickle RBCs and white blood cells (WBCs) component, its images are analyzed and classified by neural network. Next RBCs are counted and displayed. They found that their method proposed system has sensitivity 0.86, specificity 0.76 and accuracy 0.74.

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III. PROPOSED METHODOLOGY

From the literature, it is found that typical steps for the process of automating blood recognition are as in Figure 7. Proposed methodology that will be used in this research includes:

A. Image Pre-processing

The main image processing tasks consists of enhancing the image's qualities and deleting overlapped blood cells in the boundary area of the image. Both tasks can be subdivided into smaller tasks.

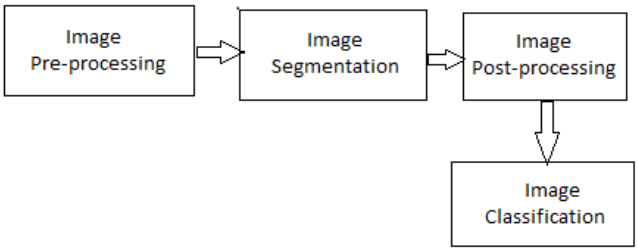

Fig.7. Steps for the process of blood recognition.

- Green Plane Extraction: The green plane is extracted from the imported bloodcell image. The other planes such as red and blue are not considered because they contain less information about the image.
- Histogram equalization: This process adjusts intensity values of the image by performing histogram equalization involving intensity transformation, so that the histogram of the output image approximately matches a predefined histogram.
- Contrast and brightness adjustment: To adjust brightness of an image, an histogram of the interested image is used to determine data and display ranges of the image. The data range is the range of intensity values actually used in the image. The display range is the black-to-white mapping used to display the image determined by the image class. Contrast adjustment is done by manipulating the display range of the histogram while the data range of the image remains constant.

B. Image Segmentation

This involves selecting only the area of interest in the image. Here only the blood cells are selected, because they are the areas of interest. When circular hough transform is applied, not much of the image segmentation is needed because the applied transform looks only for the circular objects in the image. A segmentation can be used for object recognition, boundary estimation within motion or stereo systems, image compression, image editing or image database look up. They are:

- Thresholding: techniques which make decisions based on pixel information are effective when the intensity levels of the objects fall squarely outside the range of levels in the background. Because spatial information is ignored however, blurred region boundaries can create havoc.
- Edge based methods center: around contour decision: their weakness in connecting together broken contour lines make them, too, prune to failure in the process of blurring.
- Segmentation by edge detection: The edge based methods make use of various edge operators to produce an "edginess" value at pixel. The values are then threshold to obtain the edges. The regions within connected edges can be considered as different segments because they lack continuity with adjacent regions.
- Region based methods: The image is partitioned into connected regions by grouping neighboring pixels of similar intensity levels. Adjacent regions are then merged under some criterion involving perhaps homogeneity or sharpness of region boundaries. Over stringent criteria create fragmentation, lenient ones overlook blurred boundaries and over merge.

C. Image Post-processing

The most important problem in generation of features of blood cells that characterize them in a way enabling the recognition of different blast types with the highest accuracy. The WBC appears rather darker than the background while red blood cell (RBC) appears in an intermediate intensity level. From an image we can indicate that white cells are the darker elements in images with RBC appear to be pale. Platelets are much smaller than white and red cells. All the features are extracted from the binary equivalent image of the nucleus with nonzero pixels representing the nucleus region:

- Area: The area was determined by counting the total number of none zero pixels within the image region.
- Perimeter: It was measured by calculating distance between successive boundary pixels.
- Circularity: This is a dimensionless parameter which changes with surface irregularities and is defined as,

Circularity = 4* Pi* Area/ Perimerer2

D. Image Classification

After the blood cell image has been implemented with image pre-processing techniques as mentioned above, the number of WBC and RBC from the samples will be counted.. First, the WBC number is determined and continued with the RBC number.

IV. CONCLUSION

This research involves detecting the types of WBCs and RBCs using microscopic blood sample images. The system will be built by using features in microscopic images by examining changes on texture, geometry, colors and

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statistical analysis as a classifier input. The system should be efficient, reliable, less processing time, smaller error, high accuracy, cheaper cost and must be robust towards varieties that exist in individual, sample collection protocols, time and etc. Information extracted from microscopic images of blood samples can benefit to people by predicting, solving and treating blood diseases immediately for a particular patient.

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