Applied Soft Computing 46 (2016) 629–638

Contents lists available at ScienceDirect

Applied Soft Computing

journal homepage: www.elsevier.com/locate/asoc

Blood smear analyzer for white blood cell counting: A hybrid microscopic image analyzing technique

Pramit Ghosh [a,*], Debotosh Bhattacharjee [b], Mita Nasipuri [b]

[a] Department of Computer Science and Engineering, RCC Institute of Information Technology, Kolkata 700015, India
[b] Department of Computer Science and Engineering, Jadavpur University, Kolkata 700032, India

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form
11 November 2015
Accepted 23 December 2015
Available online 28 January 2016

Keywords:
Boundary derivative
Euclidean distance
Fuzzy classification
HSI color model
Region growing
Texture

ABSTRACT

Total count and differential count of leukocytes or white blood cells (WBC) in blood samples are very important pathological factors for diagnosing a disease. There are not enough pathological infrastructures in the remote places of India and other developing countries. The objective of this work is to design a system, compatible with telemedicine, for automatic calculation of the total count and differential count of WBC from the blood smear slides. Hemocytometer based WBC counting provides more accurate result than manual counting, but hemocytometer preparation process needs expertise. As this device is targeted for remote places, blood smear technique is adopted to reduce the overhead of the operator. In the proposed system, microscopic images of blood smear sample are processed to highlight the WBC for segmentation. Region segmentation procedure involves background scaling and redundant region elimination from the region set. After segmentation, the more accurate region boundary is restored by using gradient based region growing with neighbourhood influence. Individual regions are separately classified on the basis of shape, size, color and texture features independently using different fuzzy and non-fuzzy techniques. A final decision is taken by combining these classification results, which is a kind of hybridization. A set of rules has been generated for making final classification decision based on outputs from various classifiers. The sensitivity and specificity of the system are found to be 96.4% and 79.6%, respectively on a database of 150 blood smear slides collected from different health centres of Kolkata Municipal Corporation, Kolkata, India.

1. Introduction

Human blood cells are mainly divided into three categories, namely Red blood cells (RBC) or Erythrocytes, White blood cells (WBC) or Leukocytes and Platelets or Thrombocytes. The main composition of RBC is haemoglobin, which primarily carries oxygen to living body cells and collect carbon dioxide from them. RBC have a lifetime of 120 days on an average. WBCs take care of the immune system that defends the body against both infectious diseases and foreign materials. Their life span is 3–4 days in the human body [1,2].

Platelets or Thrombocytes are tiny in size, 2–3 μm in diameter. They are irregular in shape and look like cell fragments. Platelets discharge thread-like fibbers to form clots that involved in haemostasis. The average lifetime of a platelet is normally just 5–9 days. Platelets release a multitude of growth factors which play significant roles in the repair and regeneration of connective tissues. If the number of platelets is too low, excessive bleeding may occur. However, if the number of platelets is too high, blood clots can form thrombosis that may obstruct blood vessels [3].

WBC or Leukocytes mainly protect our body against infectious diseases. They are created in the bone marrow and attack bacteria, viruses, and germs that enter into human body. There are five major types of WBC. These are Lymphocyte, Monocyte, Neutrophil, Eosinophil and Basophil. In a normal adult body, there are 4000–10,000 WBC per microliter of blood. Increase or decrease in the number of WBC in blood is an indication of an infection somewhere in the human body. The average percentage of each type of WBC in the blood are Neutrophil – 50–70%, Eosinophil – 1–4%, Basophil – 1%, Monocyte – 6% and Lymphocyte – 20–40% [4,5].

Based on the granularity of nucleus, Neutrophils, Eosinophils, and Basophils are grouped into a category, named granulocytes. These type of WBC contain digestive enzymes. The granularity of Basophils is the highest among them, Eosinophils have orange-red granules and Neutrophils have a faint blue-pink color.

* Corresponding author. Tel.: +91 9474575828.
E-mail addresses: pramitghosh2002@yahoo.co.in (P. Ghosh), debotoshb@hotmail.com (D. Bhattacharjee), mitanasipuri@gmail.com (M. Nasipuri).

http://dx.doi.org/10.1016/j.asoc.2015.12.038
1568-4946/

Neutrophils are one of the body's main defences against bacteria. They kill bacteria by ingesting them. Eosinophils also kill parasites and have a role in allergic actions in our body. Lymphocytes fight against viral and some bacterial infections by directly attacking the antibodies. Monocytes are the largest among the white blood cells. They clean our blood by eating foreign particles, bacteria, and dead Neutrophils, etc. Basophils release two chemicals, histamine, and heparin. Histamine reduces the allergic reactions, and heparin is an anticoagulant chemical, which prevents clotting of blood and helps bringing more blood to a damaged area in our body.

1.1. The necessity of WBC counting

The WBC count indicates the total number of WBC and the percentage of each type of WBC in a person's blood. This WBC counting is used to determine a variety of illnesses. For example, Leukopenia, HIV, radiation therapy, liver and spleen diseases cause very low WBC count [6]. WBC count goes very high due to Leukocytosis, anaemia, stress, asthma, etc. [7,8]. The percentage of eosinophils increases when patient suffers from allergies and parasitic infections. Bacterial and fungi infection increases the density of Neutrophils, whereas sepsis and Aplastic anemia reduces Neutrophil count. Tuberculosis and other chronic infections increase the Monocytes-RBC ratio. So depending on the WBC count doctor will recommend a treatment plan for the patient. It is very difficult to arrange sufficient health infrastructure in the remote areas of a developing country. Telemedicine partially solves the lack of physician, but pathological infrastructures are not adequate in the remote areas. Sometimes it becomes very difficult to diagnose a disease properly due to the absence of a pathological report. A microscope based automatic WBC counting system compatible with telemedicine will be very helpful in those cases [9,10].

1.2. Review of existing works

Literature survey reveals that very few research works have been done in identification of WBC in blood smear images out of them some note worthy works are discussed here. Jiang et al. proposed a WBC segmentation technique based on histogram along with scale-space filtering and watershed clustering but they did not discuss anything about the procedure for distinguishing WBC from Protozoa (like Plasmodium) [11].

Bergen et al. described a Level set based technique to identify Leucocytes in a blood smear image [12]. However, they do not focus on the classification of different types of WBC. A WBC segmentation technique is proposed by Dorini et al. where the Selfdual Multiscale Morphological Toggle (SMMT) [13] approach is used. Their technique emphasizes to find WBC cell boundary (cytoplasm boundary) more accurately. The focus of their work is to detect only the Leucocytes, not on the classification of the different types of Leucocytes.

1.3. Motivation

A number of automatic WBC counting systems exist like Automatic blood analyser [14], haematology analyser [15], etc., but their works are based on some chemical processes. They also require a considerable amount of maintenances and recurring cost. On the other hand, the techniques discussed in the review section are developed in the academic interest and do not address the real life practical issues or attempt to assist the doctors with better diagnosis. For example, the method proposed by Kin Jiang, has not considered the artefact and Protozoa; because they use perfectly processed blood Smear slides. This is not possible in the real life situations. Dorini et al. emphasizes to find WBC cell boundary (cytoplasm boundary) more accurately, which has no additional benefits in the detection of WBC.

The objective of this work is to design a complete WBC counting system, which is based on analysis of microscopic images of blood smear slides and it can be implemented using the existing manual WBC counting system with minor modification. This system is aimed to be deployed in the remote areas of developing countries as a supporting aid to telemedicine system and any person with school education would be able to operate it.

The organization of the paper is as follows. Section 2 describes the detailed design methodology of the system. Section 3 reports the performance analysis of the system and comparison with other existing systems. The next section focuses on future scope of the work and concludes the paper.

2. The design principals and methods

The work focuses on the automation of counting of different types of white blood cells in a blood sample from the digital images of the blood smear slide. Olympus CX21i [16] microscope fitted with a CCD camera is used to capture microscopic images of blood smear slides. Leishman stain is used to stain the nucleuses of WBC, platelets and other parasites or bacteria (if they are present) in the blood samples. RBCs and platelets can be easily distinguished considering their shapes and sizes. RBC is concave, and it does not have any nucleus; Platelets are tiny in size [4]. A mechanical setup is used to change the slide position under the objective lenses of the microscope. According to WBC counting guidelines, images of more than 100 fields of a single blood smear slide need to be examined for better estimation of WBC count. For this reason, the automatic mechanical slide movement setup is required. This automatic slide movement is implemented by using two stepper motors [17]. NEMA 23 type stepper motors are used for the mechanical stage movement of the microscope. TB 6560 [18] stepper motor driver controller is used to control stepper motor more accurately. TB 6560 supports the micro-steps [19] to control the rotation of the shaft of the motor precisely by 0.1125° for each input pulse, and it is independent of the pulse width.

The proposed technique considers shape, size of the nucleus, color of the cytoplasm and texture features to eliminate cells other than WBC and classify different types of WBC. Each of these features are self-sufficient to classify each type of WBC. However, in this work, independent feature based WBC classification techniques are combined to reduce the false positive and false negative rates. Hence, it is a kind of hybrid approach. Prior to the classification of WBC, the WBC regions are detected. The WBC region detection process is common to all the independent feature based classification techniques. Fig. 1 shows the block diagram of the complete system. Pre-processing is common to all the WBC classification techniques that are discussed here. Initial detection of regions, containing WBC using color features is the first step of pre-processing. After initial region segmentation, wrongly detected regions are removed. Once the actual region has been detected then, the gradient based region detection technique is applied to obtain more accurate boundary points of the nucleus. Once the nucleus is detected, the next phase is to classify the WBC type. The shape feature, and size feature are independently used to classify all the WBC types except Neutrophil and Eosinophil. In those cases color feature of cytoplasm is used to categorize them. In parallel with these classifications, texture feature strongly classifies the artefact, malaria parasite, and Basophil. However, texture feature is also capable of classifying other WBC types.

2.1. Initial region segmentation

Leishman stain colors the nucleus materials of WBC with violet in the blood smear slide. In RGB color model, violet color is