Research Paper

Identification of Lymphocytes from Lymph node Images.

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Abstract

Manual counting of lymphocytes is a tedious job and also time consuming. Because of these drawbacks, an automated tool is essential for segmenting and counting the lymphocytes. The objective of this work is to develop an automated tool which segments and counts lymphocytes from lymph nodes. The dataset contains 25 stained lymphocyte images which are obtained from five different parts of the human body. These images are captured from scanning electron microscope (SEM) and camera fitted microscopes. These images are converted to gray scale images, and then edges are detected from canny edge detection method. Using morphological operators on the edge images we extract the contour boundary of the lymphocytes and count the number of lymphocytes. The proposed algorithm results with 76.00% of accuracy.

Keywords; White blood cells, lymphocytes, lympho node, segmentation, Lymphocytes count.

1. Introduction

Leukocyte cells composition reveals important diagnostic information to the doctors about the patients. Globally, blood is categorized into three different groups, erythrocytes, leukocytes and thrombocytes. Leukocytes are having the classification based on its Pathological (nucleus and cytoplasm) characteristics. They are granulocytes and agranulocytes, again the granulocytes contains three kinds, those are Neutrophil, Basophil and Eosinophil and agranulocytes contains LYMPHOCYTES and Monocytes as referred in figure 1. Here we are more concentrating on Lymphocytes. Lymphocytes are developed from the haemocytoblast (Stem cells) in the red bone marrow of the human body, and then spread in the blood to lymphoid tissue elsewhere in the body also. The lymphocytes are having large nuclei and cytoplasm. There are two functionally distinct types: T-Lymphocytes and B-Lymphocytes. These are present great in numbers in the lymphatic tissue. Out of 100 WBC cells lymphocytes are present in the range of 30 to 35 and it is the normal healthy person's lymphocytes range, else treated as cancer cell. These cancer lymphocyte cells combine to form a tissue called LYMPH NODES. Hence calculating the number of lymphocyte cells is an important task. Conventionally lymphocytes are counted by pathologist.

Pathologists make use of differential count (DC) and newbaur counting chamber (NCC) for manual counting of blood cells. In the literature of manual blood counting NCC stands at the first place and DC is at the second place. Till today DC is used for manual counting of blood cells. DC is a type of manual counting technique, where blood smears are viewed with the help of a microscope and cells are classified manually by the doctors. Doctors make use of five counters, which count the five different leukocytes cells. On the other hand in Newbaur Counting Chamber a grid structure is over lied on the image, which may be of the length and width about 1mm size. Then select any one grid area of an image, again divide it into number of subparts by putting the grid lines. Finally it ends with a microscopically one small region of an image. Count the number of cells present in that respective region of the microscopic image, then multiply it with that of one of gridded portion, again multiply it with the whole grids of an image. Finally, the result gives the number of cells present in an entire image respectively.

Both manual counting techniques have their own drawbacks. Each technique will consume more time and require more number of cytotechnicians. Sometimes these methods may not give required accuracy result. In some situations cells may be in confusion (illumination inconsistency) state. Because of this, ambiguity arises about the classification and counting of leukocyte cells. To overcome all these limitations we are proposing a novel automated algorithm for segmentation of lymphocytes and counting of lymphocytes in the image.

The rest of paper is organized as follows. In section 2 we provide a brief literature survey on segmentation of cells in the blood. In section 3 we discuss about the dataset. The new methodology proposed for segmentation of lymphocytes in the lymph node image with experimental results is provided in section 4. The paper is concluded in section 5.

2. Literature Survey

Many automatic segmentation methods have been proposed, most of them based on geometrical features and local image information such as shape, size, texture and histogram equalizations respectively.

Neelam et al [1] suggests a method for segmenting the blood cells using expectation maximization (EM) algorithm. Two part segmentation system enables to distinguish white blood cells into nucleus and cytoplasm from the color HSV images. Segmentation is done by 3D Gaussian distribution and clustering by K-mean clustering on the 3D feature vector. The respective scheme provide good results, as which is applied for 115 peripheral blood smears. Baldo et al [2] reported a novel method to segment nucleus and cytoplasm of white blood cells. WBC changes their shape in the level of maturation. Baldo et al use morphological operators and explore the scale-space properties of a toggle operator to improve the segmentation accuracy. The rate of segmentation mainly depends on selection of geometrical structure of the cell. Nipon Theera-Umpon [3] proposed a new method to segment single cell images of white blood cells in bone marrow into two regions, i.e., nucleus and non-nucleus. The method is based on fuzzy c-means clustering and mathematical morphology. WBC present in bone marrow is classified according their maturation. Though maturation is continuous, WBC are classified into discrete classes. The fuzzy clustering of pixels provides the over segmentation in which several patches are generated. These patches are then combined to form two segments of nucleus and non-nucleus regions depending upon their similarities. Farnoosh et al [4] projected WBC segmentation scheme, which is slightly different from other work, because the images are captured using L2 microscopic image for segmentation. All these schemes mainly contain edge and border detection, region growing, filtering, mathematical morphology, and watershed clustering. The result of the proposed framework is able to extract the nucleus and cytoplasm region in a WBC image sample. Ravi Kumar et al [5] proposed cell segmentation using Teager energy operator (TEO) method. TEO is used to differentiate the nucleus and cytoplasm present in the leucocytes. Nucleus is identified by high pass filtering property of TEO and cytoplasm is segmented by morphological operators.

Joost et al [6] proposed a method to segment the erythrocytes using the SEM images. Joost et al concentrate to segment the overlapped erythrocytes, i.e. only the upper most cells. Simple Greedy Contour technique is used for the segmentation. Duanggate et al [7] reported the scheme for automatic Pap smear screening process. This is developed over the manual count of nuclei and the cytoplasm of the cervical cancer smear. For the isolation of nuclei and cytoplasm using the dual wavelength method, as well for the segmentation process using the color model, genetic algorithm, fuzzy logics and Hough transformations are the techniques used sequentially for Pap smear screening. For classification purpose Duanggate et al used KNN classified and Tabu search methods.

Nilsson et al [8] proposed the method for leukocytes clustering. So many algorithms have been developed on the pair of normal leukocytes clustering. Aim of this work is to cluster the complex and abnormal leukocyte cells. The process is done on the base of morphological operators. Clustering is done using moving interface model based on combinatorial model optimization system. Hiremath et al [9] suggested a method for identification and classification of leukocyte cells. Many papers are developed over the same process based on the histogram analysis, measurement of distance among nuclei and segmentation based on Graham Schmidt orthogonalization process for amplifying color vectors. The method adopted is developed by using the histogram equalization, thresholding and edge detection methods and results with high accuracy, by the comparison of manual count done by pathologists. Animesh et al [10] suggested a method on circulating peripheral blood plasma cells as a prognostic indicator in patients with primary systemic amyloidosis (AL). Data set consists of two varieties of plasma cells i.e., peripheral blood plasma cells (PBPCs) and the bone marrow plasma cells (BMPCs). These two varieties of data set can be processed by the technique called sensitive slide-based immunofluorescence. By this process circulating peripheral blood plasma cells (PBPCs) as a prognostic indicator in patients' blood plasma, this technique gives the count of absolute circulating plasma cell count was determined. Domenico et al [11] proposed a method for correction of the motion blur alternation in the Human Lymphocyte Micro-Nucleus Image Based on Weiner's deconvolution. These corrections are always based on the Weiner's deconvolution, but evaluation of the point–spread function (PSF) of the image is performed by taking into account the motion angle. The image is altered by motion blur a proper index is defined. The theme of this paper is to reduce the number of rejected images for correct detection of Micro-Nucleus into human lymphocytes respectively. The proposed correction operates with the conjunction with spatial filters, pointed out to correct the bad exposure, the Gaussian out of focus and the Gaussian noise and the Weiner's devolution with PSF particular for the Gaussian out of focus alternation with high intensity. Kind of Morphological operators is used to develop this project. Miguel et al [12] designed the concept on the morphological Operators. This paper provides the relationship among the Medical image processing and the Mathematical Operators. The datasets are converted to polar logarithmic by morphological operators. To detect the Erythrocytes, Inclusions and Extrusions Extraction algorithm technique was used. Finally the fundamental idea here presented is that the conversion of image into other intuitive geometrical representation can be provided over the traditional Cartesian representation. The conversion of Polar-Logarithm coordinates as well as derived cyclic morphology appears this is the result of this paper.

3. Dataset

3.1. About the dataset :

Collection of data set is one of the challenging tasks. Here we have created our own image data set. We have collected lymph node images from five different parts of the human body, from each part of the body, randomly selected images are taken. They are head and neck, breast, respiratory, soft tissues and cardiovascular system.

The data set images are captured from SEM and camera fitted microscopes. SEM is nothing but scanning electron microscope. SEM can be controlled over a range of up to 6 orders of magnitude from about 10 to 5,00,000 times. These microscopic images are having high accuracy compared other microscopic images.

4. Proposed Model

Automatic identification of lymphocytes from lymph node images consists of four steps, including pre-processing, histogram equalization, edge detection and morphological operations. The pre-processing stage includes image enhancement of the dataset images. Edges are detected from the image using canny edge detection. The post-processing steps involves morphological operations namely dilation and erode.

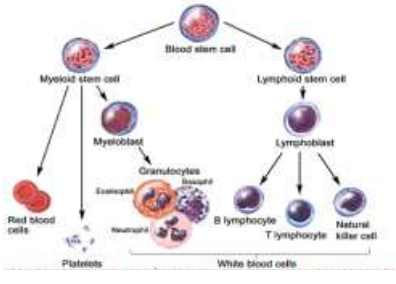

Figure 1 : Different types of WBC

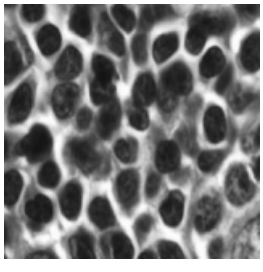

Figure 2: Sample Image from our dataset

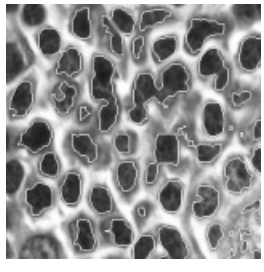

Figure 3
Single Cell Detection

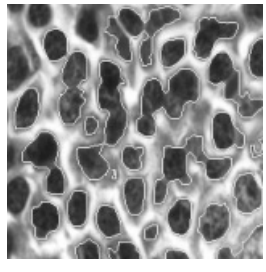

Figure 4. Combined Cell Detection.

The proposed method for lymphocyte segmentation is given below:

Algorithm: Lymphocytes Detection from Lymph node image.

Step 1: I ← Input color Image.

Step 2: Igray **w** Convert the color image to gray scale.

Step 3: Thersholding is applied separately all places ie red, green, and blue plain.

Step 4: Three plains are concatenated then once aging global Thersholding is applied.

Step 5: Edges are detected by Canny Method.

Step 6: Idilate Dilation is applied on Iedge_detected Image.

Step 7: Iimerode is applied in Idilate.

Step 8: Finally lymphocytes are detected from lymph node image.

For the purpose of experimentation five different parts of human body lymph node images are considered. Preprocessing is done on all images, then they are subjected to canny edge detection. Morphological operators namely dilation is applied on edge detected images. The simulation is done for different threshold values. Figure 2 shows sample image of our database.

Table 1: Accuracy Resulting Table.

Threshold Value	Single cell detection	Combined cell detection
0.2	60.08	34.40
0.25	53.92	32.12
0.3	63.04	35.12
0.35	19.56	22.48
0.40	12.48	18.28

The table 1. Represents the rate of detection of lymphocyte cells for different threshold values. Table 1 shows the rate of detection of lymphocytes cells for different threshold values. We have calculated two types accuracy i.e., single cell detection and combined cell detection. The former one gives accuracy of single cell detection whereas later one gives accuracy based on single and combined cell detection in which two or more cells are detected as a single cell. Figure 3 represents single cell detection and figure 4 represents combined cell detection.

Table 2: Experimental Results

Threshold Value	Average Result.
0.20	76.00%
0.25	75.78%
0.30	76.00%
0.35	36.00%
0.40	26.00%

Table 2. Represents the experimental result of the proposed method. The same average result is getting for two different threshold values i.e., 0.20 and 0.30. As like pathologist, we are also more concentrating on single cell detection. Single cells detection is high in threshold value 0.30 as compared with 0.20. The result of the 0.3 threshold value is considered as final result of the proposed algorithm.

5. Conclusion And Future Work

We have proposed a method for segmenting lymphocyte. The proposed method made to work on lymphocyte images which are taken from the five different parts of the human body; they are collected from head and neck, breast, respiratory system, soft tissues and cardiovascular system. In differential counting, pathologists are more concentrate on single cells. As like pathologist we are also concentrating on single cell segmentation, and then only we give preference to combined cell segmentation. The profiling is done on all five classes of images with 76.00% accuracy. From that, the result of 0.2 and 0.3 threshold values are same, but we conclude that 0.3 threshold value is the higher accuracy, because rate of single cell segmentation is high compared 0.2.

We have successfully segmented the lymphocytes from the lymphnodes. The same idea can be further extended to remaining all types of blood cells. This idea leads to a great thought, anyone can think of developing a device like multimedia card reader. As like card reader, it should read the blood smear and give the count of different types of blood cells automatically.

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