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Implementation of Blood Cell Counting Algorithm using Digital Image Processing Techniques

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***Abstract*— Blood is a connective tissue with cellular components like RBC, WBC, and platelets. If the blood cells are below the standard range it leads to varies health diseases and hence proper blood cell counting technique plays importance in the pathology department. The most widely used method for counting blood cells is the microscopic technique which gives better results however, it takes more time to count the number of blood cells. Blood cell count using a digital image processing technique can be considered to automate the microscopic method and minimize the time taken by the conventional method. In this paper, a digital image processing technique is proposed to accurately count all the blood cells in a given slide with maximum accuracy. A method such as morphological operator, texture-based classification method and Circular Hough Transformation (CHT) used to count the RBC which gives accuracy around 90%. Similarly, Morphological Operator, Vegetation method, and Texture Object-Based classification are implemented to count the WBC and Platelet. However, Texture Object-Based classification method gave 100 % accuracy compared to Morphological Operator and Vegetation method. From the results discussed in this paper conclude that for RBC count using Circular Hough Transformation gives better result and WBC, platelets count using the Texture Object-Based classification method gives better results.**

Keywords*— *Digital Image processing, MATLAB, Circular Hough Transformation, Morphological Operation, Edge Base Segmentation, Texture based Classification

I. INTRODUCTION

CBC (Complete blood cell count) is a routine blood test that is done or recommended by a doctor to test his overall health condition. It is also used to identify and monitor disorders like anemia, leukemia, fatigue, fever, bleeding, thalassemia, polycythemia, and dengue, etc. The test performed for CBC will help the doctor to analyze factors and symptoms of the various disorders hence, CBC can be carried out by using a hematology analyze. The main aim of the Blood Cell Counting Algorithm is to automate blood count using a digital image processing technique. It forms accurate and easy procedures for determining and counting RBC, WBC, and platelets. The algorithm used for determining the blood cell count is based on CHT (Circular Hough Transformation) and Texture based algorithm where all the blood cells can be extracted and counted. RBC count can be determined by circular Hough transform and WBC and platelet count is determined by texture-based algorithms. Generally, the blood cell count is carried out by using digital image processing techniques like edge base methods, morphological operators, texture-based method and circular Hough transformation techniques, etc. Literature suggests that edge detection for cell counting simulation of human blood cells using MATLAB [1]. But the technique detects cell borders and edges are detected first and the holes get filled and the number of filling holes will be counted. One other method for determining RBC is by using the Counting and Segmentation method of the Blood Cell Image with Logical and morphological features of cells [2]. This method says a morphological operator which is used to detect RBC. Hough Transform is another method to detect RBC, circular Hough transformation is the best method to detect the RBC and the literature used is "Detection and Counting of Red Blood Cells in Blood Cell Images using Hough Transform"[3]. In this method, a cell is replaced by an accumulator concerning x-y co-coordinator and hence with the help of the cell border a circle is created, hence merged cells can be detected. For the detection and counting of WBC and platelets, the literature on "Segmentation using Morphological Watershed Transformation for Counting Blood Cells" is referred [4]. In this literature, it is said that the RGB image is converted to the YCbCr image. Here the luminous intensity of the RGB image is increased so that the WBC and platelets can be visualized very well. Hence, by using morphological operator WBC and platelet are extracted and counted. In the texture-based method, WBC and platelet count are detected based on the separation of RGB pixels so that the required pixels are extracted and counted [5]. In the texture-based method, WBC and platelet count are detected based on the separation of RGB pixels so that the required pixels are extracted and counted.

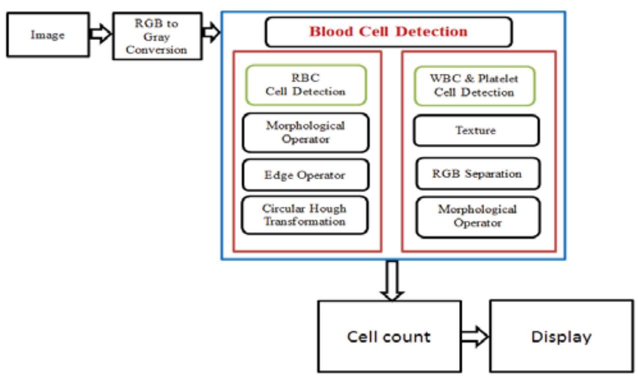

Fig. 1. Functional block diagram of blood cell detection

However major limitation of edge base technique is that the open edges cannot be filled and hence objects cannot be counted [1], however it can be read. The limitation of morphological operator is that cells can be extracted [2], but

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however merged cells cannot be counted separately. Circular Hough Transformation limitation is that, due to the non-uniform cells, the circles are created in unwanted locations [3].This paper mainly discusses the design and development a Blood Cell Counting Algorithm by using different image processing techniques in MATLAB tool is shown in Fig. 1. This developed system is validated by testing the system with different test cases.

II. Blood Cells

Blood cells are the ones that are formed in the bone marrow [6]. Blood cells are generated from the bone marrow of stem cells. Stem cells are always immortal, which means they never die. The stem cells give rise to erythrocytes, leukocytes, and platelets. Fig. 2 illustrates the different types of blood cells. It contains Leukocytes, also known as white blood cells, which are a group of related cell types that are involved in immune function. Leukocytes include neutrophils, eosinophils, basophils, lymphocytes, and monocytes.

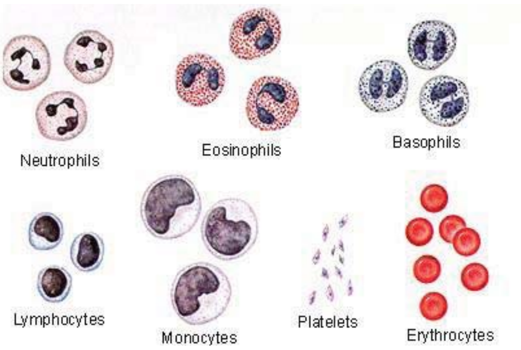

Fig. 2. Different types of blood cells

To detect and count the red blood cells various algorithms are implemented such as edge-based segmentation, morphological operator, texture region-based classification, Hough transformation with the morphological operator, Hough transformation with texture region-based classification, and Hough transformation with texture object-based classification. Fig. 3 show that microscopic input image; image involved 57 red blood cells different algorithms are implemented with image has input.

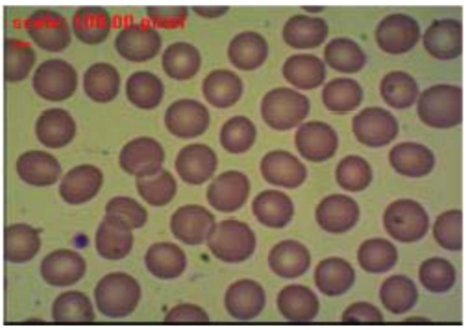

Fig. 3. Microscopic RBC blood cell input image

III. Blood Cell counting methods

There are different types of Blood cell counting methods such as Microscopic Method, Impedance Method, and Flow Cytometry. The microscopic method has few limitations such as The slide consists of several merged cells which is very difficult to count the individual cells. The number of cells is reduced in number which would lead to loss of information. Cells are occupied properly so that there is no information loss and hence it can be diagnosed properly. In impedance and flow, cytometry methods cell counters do not identify the cells which are considered here as particles, rather it counts and sizes them. This method doesn't count the cell whose size less than 100μm. The system should be calibrated once a month. During analysis, the overlapped cell will be ignored. Difficult to find mature & immature cells and this method requires more time to count the cells. To overcome these limitations automated blood cell counting algorithm technique is proposed. This technique uses different digital image processing algorithms to count the cells. Digital image processing techniques provide a new methodology to extract quantitative information from images of blood samples extracted through a microscope. In Electronics Microscope to prepare a slide, an EDTA solution which is an anticoagulant is mixed with a blood sample so that clotting of blood does not occur which is shown in Fig. 4. Using high electronic microscope cameras the image is captured and used to count the blood cell using different image processing algorithms.

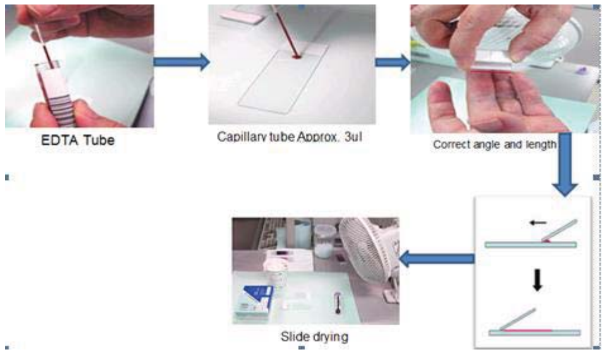

Fig. 4. Blood slide preparation for microscopic

IV. Digital Image Processing Techniques for Blood Cell Counting

A. RBC Extraction and Counting

To detect and count the red blood cell image processing algorithms such as edge-based segmentation, morphological operator, texture region based classification, and Hough transformation are implemented. Steps to implement the RBC count algorithm are explained below: -

1. Select an input image
    a. Extract red pixel using multi threshold
    b. Convert extracted image into binary
2. Apply morphological operator for object segmentation
    a. Apply edge operator
    b. Find edges
    c. Hough begin
3. For each edge point. Draw a circle with center in the edge point with radius r and increment all the coordinates that the perimeter of the circle passes through in the accumulator.
    a. Find one or several maxima in the accumulator

b. Hough end and Map the found parameters (r,a,b) corresponding to the maxima black in the original image

- *Edge Base Segmentation*

The blood smear particles are determined by using an edge detection operator [1]. In edge base segmentation deformed RBC shapes such as teardrops, crescents, needles, etc., infected red blood cells, and additional overlapping cells which cannot be found with certain types of diseases. Edge operators such as the Sobel image with a median filter using a 3x3 mask function shown in Fig. 5(a), resulting in the image gives minimal information and also merged cell cannot be read. As shown in Fig. 5(b) edge line has been opened, holes could not be filled hence desired cells could not be segmented and counted. This leads to more error and less accuracy hence edge base segmentation is not recommended for RBC count.

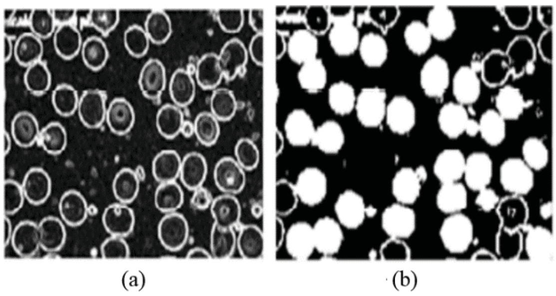

Fig. 5. Edge base segmentation implemented with I/P image, (a) Sobel edge output, (b) Segmentation output

- *Morphological Operator*

In this method blood cells are counted during a blood smear test. This method involves segmentation using morphological watershed transformation. The morphological operator is the one used for creating masks and marker-based watershed transform is used for cell segmentation. Counting of blood cells is carried out with the help of multiple image processing techniques that include spatial filtering, morphological operations, and segmentation based on watershed transformation. Morphological operators produce segmented white blood cell (WBC) nucleus as well as platelets when it is being used as a mask to remove WBC from the blood cell image and the process of counting is initiated. However, it is also concluded that this method is applicable only for handling small overlap in the image but not suitable for bigger overlaps of the image. Fig. 6 shows morphologically segmented object output. Since merged cells are counted as a single object, hence this leads to more error and less accuracy. A morphological operator is not recommended for RBC count.

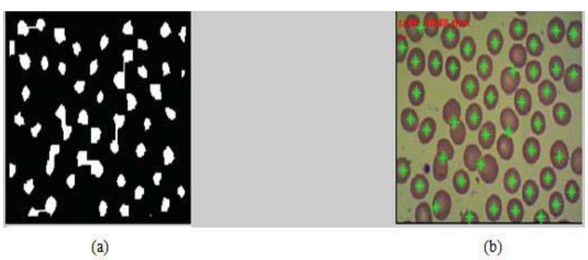

Fig. 6. Morphological operator implemented with I/P image, (a) Segmented object, (b) Centroid masked on RBC

- *Texture Region-Based Classification*

This method deals with pattern classification using texture features. The features such as standard deviation and entropy obtained after 3rd level decomposition using DWT and it is used for classification. For classification, distance similarity is used. To measure the distance between cells, Euclidean distance or Manhattan distance technique is used. The efficiency of classification is calculated for each distance and is compared [5]. Based on texture-based pattern classification, a blood cell group (region) can be extracted. An individual cell (i.e. RBC, WBC, and Platelets) cannot be extracted separately.

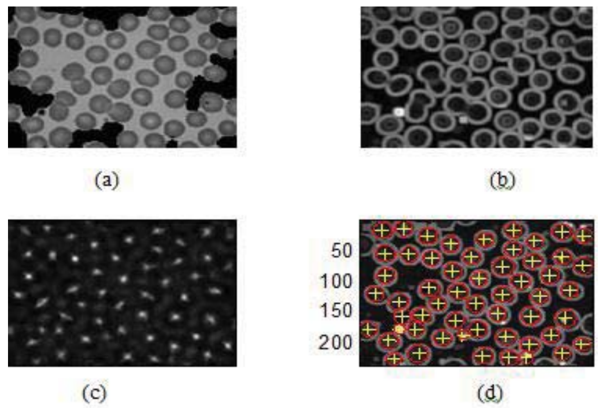

Fig. 7. CHT with texture classification, (a) Extracted cell boundary, (b) Edge detector,(c) Accumulation, (d) Circles plotted on cell object

Hence need to separate RGB from the segmented image. Circular Hough Transformation can be implemented with Texture based pattern classified image [3]. However, it causes to plot circles on unwanted cells. Hence morphological operator is recommended for segment required cells, before plotting CHT. Fig. 7 shows the Texture region-based algorithm extracted RBC and non-RBC cells within a region (a), Edge detector also created edges on non RBCs (b) Accumulator is acquired non-cell object area concerning XY coordinator and circles are plotted on RBC and non-RBC's hence this algorithm is not recommended for RBC count.

- *Circular Hough Transformation with Morphological Operator*

Hough transform is a transformation of a point X, Y plane in the parameter space; with the help of edge operator circle. This circle is drawn in the parameter space, such that the "x" axis is the 'a' - value and the "y" axis is the 'b' value while the "z" axis is known as radii. At XY coordinates which is related to the perimeter of the drawn circle the value in the accumulator matrix which has the same size as the parameter Space is incremented. The accumulator contains numbers corresponding to the number of circles passing through the individual coordinates. Thus the highest numbers of radius correspond to the center of the circles in the image.

The equation of the HCT is:

$$r = \sqrt{(x-a)^2 + (y-b)^2} \tag{1}$$

Representation of parametric of the circle is:

$$x = a + r * \cos(\theta) \tag{2}$$

$$y = b + r * sin\,(\theta) \tag{3}$$

Fig. 8 shows that CHT is implemented with a morphological operator. With the help of the Sobel edge operator segmented object border lines are detected. Later on, implemented 5 to 16 radiuses to the accumulator, and hence exactly individual circles are plotted on the merged object. This algorithm provides more accuracy and fewer errors, hence CHT with morphological operator recommended for RBC counting.

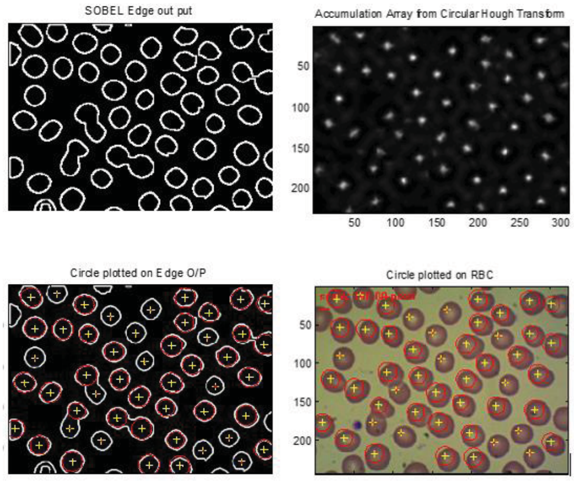

Fig. 8. Circular hough transformation with morphological operator

B. *WBC Extraction and Counting*

To detect and count WBCs algorithms such as Morphological operator and Texture object-based classification are implemented. Fig. 9 shows that the microscopic WBC input image, image involved 2 white blood cells which are counted manually. Steps to implement the WBC count algorithm are explained below: -

1. Select an input image
   a. Convert RGB to Gray Scale
   b. Convert gray into binary
2. Apply morphological operator for object segmentation
   a. Convert RGB to HSV for Color masking
   b. Separate RGB and extract blue pixels
3. Apply morphological operator for object segmentation
   a. Remove small object and
   b. Count the WBC

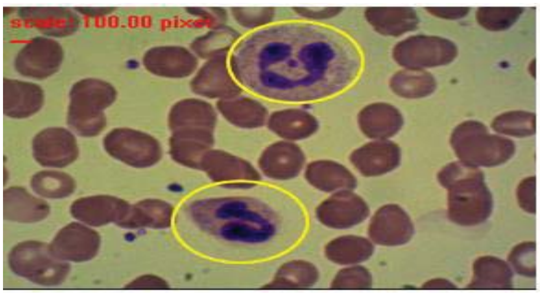

Fig. 9. Microscopic white blood cell input image

- *WBC Detection using Morphological*

The method involves segmentation using morphological watershed transformation. Morphological operations are the ones used for creating masks and marker-based watershed transform is used for cell segmentation. Counting of blood cells is carried out with the help of multiple image processing techniques that include spatial filtering, morphological operations, and segmentation based on watershed transformation. Fig. 10 shows WBC detection using a morphological operator. From the original microscopic blood image, the blue color is nothing but WBC hence blue pixels are extracted by using a morphological operator. A morphological operator extracted more number of non-WBC. Hence this algorithm leads to more error and less accuracy. A morphological operator is not recommended for WBC counting [8].

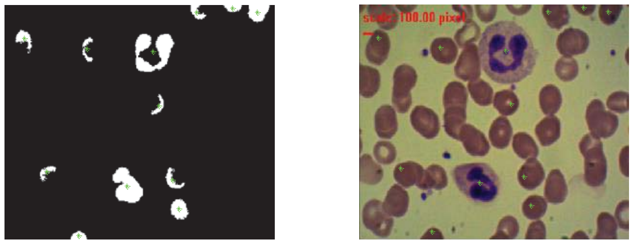

Fig. 10. WBC detection using morphological operator

- *WBC Detection using Texture Object Based Classification*

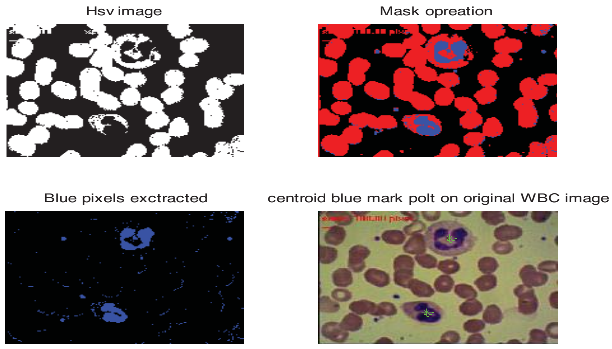

Fig. 11. WBC detection using texture object based classification

Fig. 11 shows that WBC detection using texture object based classification method. An original image involved blue object is nothing but white blood cell and Platelets. The original microscopic blood image is converted into HSV (Hue Saturation Value). Later on, mask operation is implemented to define RGB to respective input image pixels (RBC turns into a red object, WBC and Platelets are turns into blue color). Later on, Blue pixels are extracted by using a morphological operator; later on, the exact bigger object (WBC) is extracted and removed smaller objects using the logical function. Texture object-based classification gives better results with more accuracy and minimum errors.

C. *Platelet Extraction and Counting*

To detect and count Platelet cells were implemented Morphological Operator and Texture Object-Based classification are implemented. Fig. 12 shows that the microscopic input image; the image involved 6 platelet cells which are counted manually. Steps to implement the platelet count algorithm are explained below: -

1. Select an input image
   a. Convert RGB to Gray Scale
   b. Convert gray into binary
2. Apply morphological operator for object segmentation
   a. Convert RGB to HSV for Color masking
   b. Separate RGB

c. Extract blue pixels

3. Apply morphological operator for object segmentation

   a. Remove larger object and count platelets

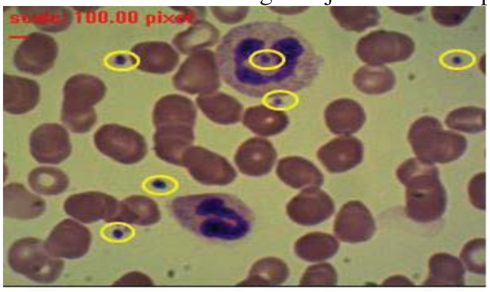

Fig. 12. Manually counted microscopic platelet input image

- *Platelet Detection using Morphological*

Platelet detection using a morphological operator is shown in Fig. 13. From the original microscopic blood image a blue color is nothing but platelet cells, hence blue pixels are extracted by using a morphological operator. A morphological operator extracted more number of non-platelets. Hence this algorithm leads to more error and less accuracy. A morphological operator is not recommended for Platelet counting [9].

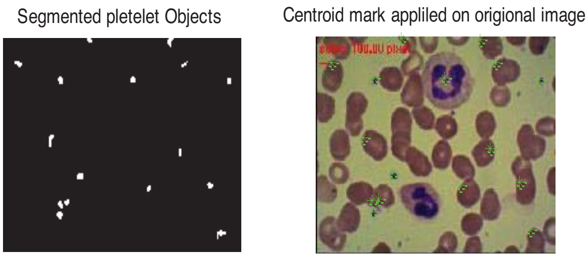

Fig. 13. Platelet detection using morphological

- *Platelet Detection using Texture Object-Based Algorithm*

Platelet detection using texture object-based classification method is shown in Fig. 14. An original image involved blue object is nothing but white blood cell and Platelets. The original microscopic blood image is converted into HSV (Hue Saturation Value). Later on, mask operation is implemented to define RGB to respective input image pixels (RBC turns into a red object, WBC and Platelets are turns into blue color). Later on, Blue pixels are extracted by using a morphological operator; later on, the exact smaller object (platelet) is extracted and removed larger objects using the logical function. Texture object-based classification gives better results with more accuracy and minimum errors [10].

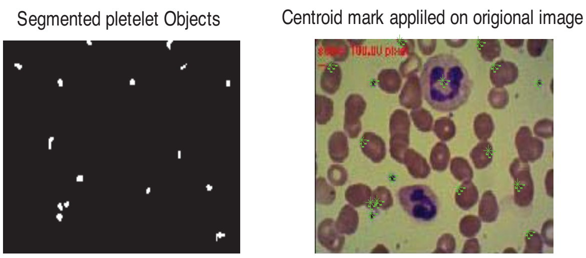

Fig. 14. Platelet detection using texture object based algorithm

V. RESULTS AND DISCUSSION

The developed digital image processing algorithms are tested different blood samples of different subjects. The images obtained from an electron microscope are used to count the blood cells by using different image processing algorithms. Several algorithms for RBC, WBC, and Platelets are tested out of this CHT with the morphological operator for RBC cell count gives 96% of accuracy is tabulated as in Table-I. Texture region-based classification for WBC and platelet cell count gives 100% accuracy is tabulated as in Table-II and Table-III.

TABLE I. PERFORMANCE RESULT OF IMAGE PROCESSING ALGORITHMS FOR RBC EXTRACTION AND COUNTING

	Manual Count	**Algorithm Count**	**Non-RBC**	**Accuracy**	**Error**
Edge Base Segmentation	57	76	19	75%	25%
Morphological Operator	57	53	--	92%	8%
Texture Region Based Classification	57	52	--	91%	9%
CHT with Morphological Operator	57	55	4	96%	4%

TABLE II. PERFORMANCE RESULT OF IMAGE PROCESSING ALGORITHMS FOR WBC EXTRACTION AND COUNTING

	Manual Count	**Algorithm Count**	**Accuracy**	**Error**
Morphological Operator	2	12	60%	40%
Texture Region Based Classification	2	2	100%	0%

TABLE III. PERFORMANCE RESULT OF IMAGE PROCESSING ALGORITHMS FOR PLATELETS EXTRACTION AND COUNTING

	Manual Count	**Algorithm Count**	**Non-Platelets**	**Accuracy**	**Error**
Morphological Operator	6	16	10	26%	74%
Texture Region Based Classification	6	6	--	100%	0%

VI. FUTURE WORK

- Image Stitching Algorithm to be implemented
- Differential Cells Eosinophil's, Basophils, Monocytes, Lymphocytes, and Neutrophils can be extracted
- Also in the future developed algorithms can be implemented with a microscopic urine sample to detect and epithelial cells, red blood cells, cast, crystal, and budding east, etc.

VII. CONCLUSION

Blood is the connective tissue with cellular components like RBC, WBC, and platelets. As per the TABLE I, II & III if blood cells are below the normal range it leads to health problems and hence blood cell counting is of great importance in pathology. The manual microscopic method gives the better result to detect and count blood cells but it takes a lengthy procedure, hence less time taking electronics microscopic blood counting along with digital image processing algorithms are implemented. RBC count such as edge base segmentation gives a result with 66 % accuracy and leading to counting error. However, the Morphological operator gives 92% accuracy, Texture based classification method gives 89% accuracy and CHT with a morphological operator gives better results with 91 % accuracy. Similarly, for WBC and Platelet count, two different algorithms such as the Morphological Operator and Texture Object-Based classification were implemented. It was found that the morphological operator gave only 60 % accuracy and the Texture Object-Based classification method gave 100 % accuracy. For platelets count Texture Object-Based classification method gave 100 % accuracy. Hence, this paper concludes that for RBC count using Circular Hough Transformation gives better results and WBC, platelets count using Texture Object-Based classification method gives a better result.

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