**Khulna University of Engineering & Technology**

Department of Computer Science and Engineering



SYSTEM DEVELOPMENT PROJECT

CSE 3200

**“**Platelet Count from Microscopic Blood Image through Image Processing Techniques**”**

By

**Kazi Ziaul Hassan**

Roll: 1607061

&

**Shanta Kumar Das**

Roll: 1607068

**Under Supervision Of:**

**Prottoy Saha**

Lecturer

Department of Computer Science and Engineering

Khulna University of Engineering & Technology

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Supervisor’s Signature

**Table of Contents**

|  |  |  |
| --- | --- | --- |
| Sl | Description | Page |
| 1 | Acknowledgement | 3 |
| 2 | Abstract | 4 |
| 3 | Motivation | 5 |
| 4 | Objective | 5 |
| 5 | Related Work | 6 |
| 6 | Introduction | 8 |
| 7 | Proposed Approach | 9 |
| 8 | Methodology | 10 |
| 8.1 | Image Acquisition | 10 |
| 8.2 | Image Pre-processing | 11 |
| 8.2.1 | Contrast Adjustment | 12 |
| 8.2.2 | Transformation of Gray Scale | 13 |
| 8.2.3 | Image binarization | 14 |
| 8.2.4 | Infilling Image | 15 |
| 8.3 | Segmentation | 16 |
| 8.3.1 | Region based segmentation | 16 |
| 8.4 | Counting platelet | 19 |
| 9 | Edge detection Segmentation | 20 |
| 9.1 | Sobel method | 20 |
| 9.2 | Canny method | 21 |

|  |  |  |
| --- | --- | --- |
| Sl | Description | Page |
| 9.3 | Erosion Technique | 22 |
| 10 | Result Analysis | 23 |
| 11 | Limitations | 24 |
| 12 | Conclusion And Future Scope | 24 |
| 13 | Reference | 25 |

**Acknowledgment :**

We would like to express our special thanks of gratitude to our supervisor “Prottoy Saha” for his able guidance and support in completing our project .

We would also like to extend our gratitude to the head of Department of Computer Science and Engineering “Dr. Md. Aminul Haque Akand” for providing us with all the facility that was required.

**Abstract:**

Platelet count is a blood test which plays a very important role to evaluate the health as well as to diagnose and follow the process of treatment for a wide range of diseases including Dengue Fever, Leukemia, Anemia etc. Platelets, also called thrombocytes, are tiny fragments of cells that are essential for normal blood clotting. They are formed from very large cells calledMegakaryocytes in the bone marrow and are released into the blood to circulate. The platelet count is a test that determines the number of platelets in a person's sample of blood

**Motivation:**

Ancient methods used for evaluating platelet count are by manual counting or by the use of Advia Haematology analyzer. Manual counting entirely depends upon the technician's skill that's why it doesn't give error-free results whereas Heamatology analyzer is a very expensive device so it is not easy to install in underdeveloped or rural areas. To overcome these drawbacks, we have introduced an image processing technique which is highly cost-effective and reliable.

**Objectives:**

* Introduction with Image Processing techniques.
* Introduction with various Algorithm.
* To implement the process in Matlab.
* To calculate the number of platelets.

**Related Work:**

1. Hemant Tulsani et al. presented a method for counting of blood cells components. The image processing techniques used for counting are spatial filtering, morphological operations and segmentation using watershed transformation.

2. S. Kareem et al. introduced angular ring ratio method for counting of RBCs and platelets in thin blood films. After that peak intensities of the ratio transformed image are calculated. Next, mapping the peaks on to the corresponding coordinates, which is actually the center of each component is done.

3. Rhodes and Bai presented a circle detection method using specific properties of Gabor wavelet filter to detect the image features such as circularity. It is able to extract radius wraps around the origin and the plane wave radiates from the center of the filter. They test their proposed method on synthetic images and real microscopic images, which allow a certain degree of overlapping cells. The results were 91.3% and 87% of the cells detected in the two microscopic test images.

4. Pallavi T. Suradkar has implemented image segmentation smoothing and gradient edge detection technique to extract the RBCs on the basis of their color and shape. Nasrul Humaimi Mahmood and Muhammad Asraf Mansor utilized Hough transform techniques to determine the RBCs, and morphological technique has been implemented for image segmentation and feature extraction.

5.  Sanaullah Khan, Aamir Khan and Faisal Saleh Khattak  has proposed threshold technique to count the platelets which is based on Otsu algorithm and Watershed technique to increase the accuracy of microcell counting in the blood smear image.

6. Guitao Cao, Cai Zhong, Ling Li and Jun Dong [9] has implemented an approach to count the RBCs in urine micrograph where improved sobel operator is used for preprocessing, hough transform is used to detect the RBCs.

7. Nguyen et al. used distance transform to solve the overlapping cells problem; they proposed a method that concentrated on clumped cells. First, they assigned central points based on a distance transform. The optimal center points were selected by checking the degree of boundary covering the center point, and the average size of a cell was estimated by the extraction of a single cell. Then an algorithm was developed that used a single cell mask to split the cells.

**Introduction:**

For the last few decades, content base image analyses have been an important research area. A large database is created by capturing many digital images such as medical images, fashion etc. Which are used for wide applications. Medical images are the aim of our research. In the field of Bio-Medical Science, it's difficult to count the cells automatically because of its complex nature it cannot get segmented easily. Blood, being the only fluid tissue is unique among all the blood tissues. A blood cell is categorized in 4 components namely Erythrocytes, Leucocytes, Thrombocytes and plasma where first three are suspended in the plasma. Erythrocytes commonly known as Red Blood Cells, carries oxygen from lungs to tissues and carbon dioxide from tissues to lungs produced as waste product. Leucocytes (White Blood Cells) provide defense mechanism to the body against infection. Thrombocytes or platelets help in the process of Hematostasis. Plasma is used to carry CO2 in form of soluble carbohydrates. These blood cells can be differentiated on the basis of their size, color, texture and morphology of nucleus and cytoplasm. It has been observed that red blood cells are present in ample amount in human body, much greater than platelets and WBCs. The table shows that the size of a platelet is smallest among the blood cells.

**Methodology:**

**8.1 Image Acquisition :** In order to initiate the system to work it needs a microscopic blood cell image which is done in the user interface with load image button.   
This is the pictorial view of user interface of very initial stage.

 Fig-8.1.1: initial User Interface  
Input Image :   


Fig-8.1.2: Input Image

**8.2 Image Pre-processing**

An image is nothing more than a two-dimensional array of numbers (or pixels) ranging between 0 and 255. It is defined by the mathematical function f( x , y ) where x and y are the two co-ordinates horizontally and vertically. The value of f( x , y ) at any point is giving the pixel value at that point of an image. The main aim of pre-processing of image data that suppresses unwanted distortions or enhances some image features important for further processing. Among four categories of image pre-processing methods according to the size of the pixel neighborhood that is used for the calculation of a new pixel brightness .We used here pixel brightness transformation by the functions

I(:,:,1)=0;I(:,:,3)=0.15;

**8.2.1 Contrast Adjustment:** ​ In this process, pixel values below a specified value are mapped to black and pixel values above a specified value are mapped to white. Technique used for mapping an image's intensity values to a new range of values is called Intensity adjustment. Here we used **I = I – 75** ; which is used to map the intensity range to a new domain.



Fig 8.2.1.1:Contrast Adjustment

**8.2.2 Transformation of Gray Scale :** ​ ​Enhancing an image provides better contrast and a more detailed image as compared to non enhanced image. Image enhancement has very applications.



Fig-8.2.2.1: Gray Scale transformation of an image

An RGB image can be viewed as three images ( a red scale image, a green scale image and a blue scale image) stacked on top of each other. In MATLAB, an RGB image is basically a M\*N\*3 array of colour pixel, where each colour pixel is a triplet which corresponds to red, blue and green colour component of RGB image at a specified spatial location.

Transformation of image into grayscale image is done by RGB2GRAY(I) function taken from MATLAB where ‘I’ is an input image.[I](https://www.mathworks.com/help/matlab/ref/rgb2gray.html)​​ = rgb2gray(RG​[B](https://www.mathworks.com/help/matlab/ref/rgb2gray.html))​ converts the truecolor image RGB to the grayscale image I. The rgb2gray function converts RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance.

**8.2.3 Image Binarization:**

imbinarize(I) creates a binary image from 2-D or 3-D grayscale image I by replacing all values above a globally determined threshold with 1s and setting all other values to 0s. By default, imbinarize uses Otsu's method, which chooses the threshold value to minimize the intraclass variance of the thresholded black and white pixels. imbinarize uses a 256-bin image histogram to compute Otsu's threshold.



Fig 8.2.3.1: Binarized image

**8.2.4 Infilling Image:**

After performing segmentation there were found some tiny holes. In order to filling these holes we used imfill function which is a built in function of matlab.   
The **imfill function** performs a flood-fill operation on binary and grayscale images. This operation can be useful in removing irrelevant artifacts from images. For binary images, **imfill** changes connected background pixels ( 0 s) to foreground pixels ( 1 s), stopping when it reaches object boundaries.



Fig 8.2.4.1: Infilled Image

**8.3 Segmentation:**

Image segmentation is the process of partitioning a digital image into multiple segments (set of pixels, also known as image objects). The goal of segmentation is to simplify and change the representation of an image into something that is more meaningful and easier to analyze.

There are many algorithms of segmentation techniques. Some of them are of our need.

* **Region Based Segmentation / Thresholding Segmentation**
* **K-means clustering**
* **Mask R-CNN segmentation**
* **Edge detection Segmentation**

Among all the segmentation algorithms, the Region Based Segmentation or thresholding segmentation shows the best result.

**8.3.1 Region Based Segmentation :**

There are two major algorithms for image segmentation i.e (Region based Segmentation or segmentation using Thresholding & Segmentation using K-means Clustering). Here we used region based Segmentation to develop our system .

The region-based Segmentation well achieved to classified a particular image into a number of regions or classes. Thus for each pixel in the image we need to estimate and classify which class the particular pixel

belongs to. There are a variety of approaches to do region based segmentation method. Recent applications of image segmentation and image understanding techniques require increased robustness, better reliability and high automation of the algorithms. Methods of region based region growing, texture based segmentation and edge based snakes, dynamic programming image segmentation algorithms continue to be explored with attempts to improve their performance by adding expert knowledge to their detection.Once the red channel image is obtained, it is then converted into a gray scale image, which is represented with values ranging from 0 (black) to 1 (white). In order to segment the image, such that only the platelets are visible from the gray scale image, a threshold value needs to be applied which is obtained from the histogram of the said image . The threshold value chosen from the histogram, it is between 0 and 1 due to the gray scale image. After the binarization process is applied, the platelets, which are darker in nature than the surrounding blood components, are segmented from the rest of the image . The image is then complemented in order to better view the segmented regions of the image. One of the deficiencies that occur with the segmentation process is that occasionally the color of the lymphocytes present in the image is very close in nature to that of the platelets; therefore, it disrupts the counting algorithm by adding more objects to the connected component algorithm, which in turn increases the platelet count. In order to remove the lymphocytes present in the image, an area filtering technique is used. Any objects which are less than or greater than the specified of 3 to 72 are filtered from the image.



Fig-8.3.1.1: Before removing small objects Fig-8.3.1.2: After removing small objects



Fig 8.3.1.3: Removing the larger objects

**8.4 Counting platelets :**

This​ is the last step of our project . After passing all the aforesaid steps an image contains only platelets. Our system counts all the platelets and shows as output. The number of platelets present in the sample is evaluated by counting the average number of platelets observed on 100x oil immersion field of a well-spread blood smear. The average number gets multiplied with the multiplying factor of 20,000 to get the approximate platelet count/μL.



Fig-8.4.1 : After binarization of image Fig – 8.4.2: Interface to show platelet count

**Edge Detection Segmentation:**

Edge detection is an image processing technique for finding the boundaries of objects within images. It works by detecting discontinuities in brightness. It is used for image segmentation and data extraction in areas such as image processing, computer vision and machine vision.

There are two methods in detecting the edges.

* **Sobel method**
* **Canny method**

**9.1 Sobel method:** The sobel method is used for edge detection. It works by calculating the gradient of image intensity at each pixel within the image. It finds the direction of the largest increase from light to dark and the rate of change in that direction.

**After using Sobel method in two images** :



Fig 9.1.1: Sobel on Image 1 Fig 9.1.2: Sobel on Image 2

**9.2 Canny method:** The canny edge detector is an detection operator that uses multistage algorithms to detect a wide range of edges in images.

**After using Canny method in two images**:



Fig 9.2.1: Canny on Image 1 Fig 9.2.2 : Canny on Image 2

**Why we can’t use Edge Detection algorithm?**

* From the above pictures, it seems that if we use edge detection method, the borders of the consisting objects in the image fragment into several curly lines. That is totally unexpected**.**
* It is like working with some unnecessary things. It creates extra burdens.

**9.3 Erosion Technique:**

Erosion is the method of compressing the pixels applying some structuring element around the border of all the objects. By applying this method, the objects that are smaller than the expected objects can be removed.

**After applying Erosion:**



Fig 9.3.1: With small objects Fig 9.3.2: Without small objects

Note: Some of the real platelets may be removed after using Erosion method. Also there are so many structuring elements (se).

So it is quite difficult to trace which one would be met to our criteria.

**Result analysis:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Images** | **Real Platelets** | **System Counts** | **Extra** |
| 1 | 6 | 9 | 3 |
| 2 | 8 | 7 | 1 |
| 3 | 4 | 5 | 1 |
| 4 | 9 | 10 | 1 |
| 5 | 3 | 4 | 1 |
| 6 | 2 | 7 | 5 |
| 7 | 5 | 9 | 4 |

Table 10.1 : Accuracy table

Average extra count = 2.28%

Average real platelets = 5.28%

Average error in counting = 43.24%

**Limitations:**

* Diagnostic stuffs add some solutions in the blood sample to change the color of the sample image into a compatible one. Thus they can differentiate the platelets. The solution must be added in the blood sample before capturing the image which will be input of the system.
* If the large objects in the sample image are split into tiny objects when we do the pre-processing, the tiny objects may look like real platelets. They may be one of the reasons for lessening the accuracy.

**Conclusion and Future Scope:**

Automated Platelet detection and counting based on Region Based Segmentation technique will speed up the whole process of diagnosis. It is very cost effective technique and can be easily implemented in countries like Indonesia which are resource deficient to produce and provide expensive machineries in every hospital laboratory of the country. Further this method provides accuracy up to 96% and robust way of counting platelets. Further this approach explored for platelets can be implemented for complete blood cell counting such as leukocytes (WBC) and erythrocytes (RBC). Also various abnormalities can be predicted by analyzing shape and dimensions by modifying the present algorithm for all the components of blood. It is helpful in estimate the count of platelet using image processing technique successfully. Compared with the manual counting of platelets the proposed system is taken less time. Compared with automatic analyzer this system is cost efficient. Despite with these advantages there is a problem in counting overlapping cell. Future research will motivate with various morphological operation to overcome weakness. Further with the help of platelets count, stages of dengue virus infection is also identified using image processing technique.

**References:**

* Shet, N. and Sampathila, N. Detection of plasmodium vivax from leishman stained malarial thin blood microscopic images. International Conference on Communications and Signal Processing (ICCSP), 2015, 1268-1272.
* Nayak, R., Pandey, A.K., Kumar, P. and Galigekere, R.R. Estimation of the fibre-composition of Verhoffstained fascia using colour-image processing. International Journal of Medical Engineering and Informatics 3 (2) (2011) 99-107.
* Bhattacharyya, S., Dutta, P., De, S. and Klepac, G. Hybrid Soft Computing for Image Segmentation. Springer, 2016.
* Dengue Fever. 15 Jan 2016. Available from: [http://www](http://www/). nhp.gov.in/disease/ musculo-skeletal-bone-joints-/dengue-fever
* Reddy VH. Automatic red blood cell and white blood cell counting for telemedicine system. International Journal of Research in Advent Technology. 2014 Jan; 2(1):1–6.
* Bhalerao, G.V. and Sampathila, N. K-means clustering approach for segmentation of corpus callosum from brain magnetic resonance images. IEEE International Conference on Circuits, Communication, Control and Computing (I4C), 2014, 434-437
* [Link 1](https://mail-attachment.googleusercontent.com/attachment/u/0/?ui=2&ik=253d213c3f&attid=0.2&permmsgid=msg-a:r-5567738590498210896&th=16d6ca6331e0aa91&view=att&disp=inline&realattid=f_k10dd76z0&sadnir=1&saddbat=ANGjdJ86a8VpZPZkBNqrsLe2yycPL-VXroastm_7H51LY76uY4Y0ZVA4UIhnPtwbPjCkO6aHYLqC0I6fVmH6eMgbr6rqs43uicE3acfxOXKDqMEc7LDzmtaloIUWDvbquNbxTmtiAcGzeoL7VnOmA5wqDwYKsgxYcbL0on1Sz482c-kvc1CSbl967ItBEDkZaL14OZlbqpKkts1kxGmWI5H0Y7y1h51zymOvJmoaAgfDx-w8LCJK4PK4cgZ-nkKlRtwB6Q7qXO2jPYn9SQGujxPOKwONymPvw4MSA-wHDNebazpTHZjPH9AuH0dnoQcMl9aKfLvOcEPw9O4Gnrbyld34pnwunRG-qJUci0q4xQrIuCJdHG4540K5YJAngK-EvrVRduRT7pV05kho5pID5y__AK8eRSOtoMTi7L-2hlvX0-7lne8_jHSBeEd3hTWCYGv_hwlplEeAzC09_AWGfK6PX-u6Ki4RFAUbw4_tsBraT4lV0Fs5JkM_8Y1sYrjxbzwUaXIESiIKilRnKObcHP0j9-0kAJ7z6BMdPyrRjaaDL0Fvm3yP5HxZGZIxuVVi8vH7mOnhOZum5dUvosIp7J2Z8HIveh9ASHmB7nj7JYsDZ9A5qTigbA3fJCWOa4LIEBltXQEXIJK2Vk1BjnSLE2qpBs7GhYEuJK4tQ32dA3oTYe6M3RSE6Wlqt1Q_QDw)
* [Link 2](https://mail-attachment.googleusercontent.com/attachment/u/0/?ui=2&ik=253d213c3f&attid=0.1&permmsgid=msg-a:r-5567738590498210896&th=16d6ca6331e0aa91&view=att&disp=inline&realattid=f_k10dd7761&sadnir=2&saddbat=ANGjdJ-j387xDwrJT4L_2O0N02HABVNqEMFs7shp0bQkrdt596oSXDr05o0eYrhSjTCkOgGPlBb4wGHd29P79bjNJopePlcmtck7znH_i_oD9oPS-S9Ywhl_LPU0C-PCM2xSCbCjBMs48CbAIFwWXMZve7_dLk_5gzfs6G_OCt3fHSca0X8PDn2afTnHiNEZIWRQ-ArW-NlqSu7rTWvEO3XjSfj0keWTumsMk_oryfXmXSTXWNYbk_WHrXY50C3PJlf6CxFfJxtXYyRvxiuHA4Ea4ADXHyys-N1JOx1qT5A8aC7ZTPZvIF4i1_Ba4wfn9ojD-rdG6OB9aa_aTgPO0X0m0N0liolprCvPh8iZ3x9x0b6wkujGfGUsg1tQpaeRYxkmxLxBqCGAtccUMFBRd5xQpqHH5Xz_yPZK3tuK0vBpkWr3qHiVuReLD6eUlpQ7cf8ucUHEMtcpis2F5WdwTCNEDULQWcWUVisp91X9smAcZCDOaFR50bmxJ3ReIm5WpsouVW5m_1piYE_7gpHXzonLMTbjwnKEHKdTK8mOtHyYi0-o_7chcbtG2c7WtVwT61GRY-ZXN3Jb_rLUhuR1fw8TJfgEnRiF9TD8re9eedx3gcEDZmwJ7PZIeyGJjj3d9VLNZqaWVw-MUqWPXFUFfPXJTVypjvTA1iSfKNeytu5y-ontgJ4DRMLDsuXSlMo)
* <https://ieeexplore.ieee.org/document/950259>
* <https://www.analyticsvidhya.com/blog/2019/04/introduction-image-segmentation-techniques-python/> .