**Anna University Chennai**

Research Support Scheme (Batch 2016)

**Format for submission of proposal for Student Innovative Projects**

(by Pre- final Year, odd semester, full time students)

***i. Title of the project:***

BLOOD CELL COUNT USING DIGITAL IMAGE PROCESSING

***ii. Name(s) of Students with roll numbers:***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Name*** | ***Department/***  ***Centre*** | ***Roll***  ***Number*** | ***Male/***  ***Female*** | ***e-mail Id and mobile Number*** |
| D.DYANESH VARUN | Department of Information Science and Technology | 2014115078 | Male | dyaneshvarun123[@gmail.com](mailto:gengashree@gmail.com)  7358618847 |
| S.LAKSHMI PRIYA | Department of Information Science and Technology | 2014115094 | Female | priyaceg1996[@gmail.com](mailto:himasethuraman@gmail.com)  9840912384 |
| K.REDDY VASAVI | Department of Information Science and Technology | 2014115087 | Female | kreddyvasavi[@gmail.com](mailto:himasethuraman@gmail.com)  9710699972 |

***iii. Degree programme (Pl.tick):*** B.E./B.Tech./B/Arch/M.E./M.Tech M.Arch./M.Sc/MBA/MCA

***iv. Year/ Semester of the degree programme:*** III year / VI sem

***v. Faculty member(s) associated with the project as mentors:***

|  |  |  |  |
| --- | --- | --- | --- |
| ***Name*** | ***Department/ centre*** | ***Designation*** | ***Phone/Mobile***  ***Number and email id*** |
| ***Dr.S.Abirami*** | Department of Information Science and Technology | Assistant Professor | [abirami@annauniv.edu](mailto:abirami@annauniv.edu) 9500082235 |

**1. Objective**

The basic aim of this project is to find the RBC and WBC count using Digital Image Processing.This project presents a method to digitally analyse the image of blood cells and find the required count values by using Thresholding technique .

**2. Introduction:**

Digital Image Processing is a developing era in the field of Computer Science and Engineering and has its branches in all the fields. One of the growing fields in it is the Medicine . DIP is the use of computer algorithms to perform image processing on digital images.The influence and impact of digital images on modern society is tremendous, and image processing is now a critical component in science and technology .At present, the blood samples are taken to lab and processed with various substrates and the results are produced. Whereas this project ,a Biomedical – Computer Science based inter-disciplinary project , applies the reagent , makes the blood sample floroscent and then captures it . Then it is digitally processed with software and the result is displayed immediately.

The conventional device used to count blood cells is the Haemocytometer. 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. To count blood cell, physician must view haemocytometer through a microscope and count blood cells using hand tally counter.

Drawbacks of the manual method are

* Manual counting task is time-consuming and laborious.
* Counting overlapping blood cells is a major problem.
* Difficult to get consistent results from visual inspection.

Complete blood count is performed by an automated analyser. The blood is well mixed (though not shaken) and placed on a rack in the analyser. This instrument has many different components to analyse different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review.

Drawbacks of automated method

* Automated analyser is Costly
* Cannot detect irregularities or variation in the shape and size of the cells.

**3. Literature Survey :**

Berge et al. presented an approach which is based on a morphological method and iterative threshold techniques. Segmentation was performed on red blood cells, which included clumped cells, and boundary curvatures were used to construct a Delaunay triangulation. They used real microscopy images prepared in the laboratory. Their method was not tolerated with a high degree of overlapping cells. Additionally, the iterative threshold method was unable to detect faded red cells.

Khan et al proposed a method to count WBCs, RBCs, and platelets. It requires several pre-processing steps before converting the image to binary. Segmentation and cell counting were performed based on the optimal threshold value, which was determined from a histogram. They achieved 95% accuracy with their proposed method compared to manual counting and a haematology analyzer. Drawback of this method is that, it is unable to detect overlapping cells. When using iterative thresholds, the probability of losing useful information from the image is high; this decreases the accuracy of segmentation.

Nguyen et al used distance transform to solve the overlapping cells problem; they proposed a method that concentrated on clumped cells.

Chiu et al presented a fast randomized Hough Transform method for detecting circles, to improve RHT which is less efficient in complex images due to its probability usage problem.

Mahmood and Mansor et al. examined 10 image samples of normal blood cells; image transformed to the HSV color space, and then Saturation or “S” channel was selected to proceed with image analysis. Morphological operators and thresholding method were used over S channel for cell segmentation. They used Circular Hough Transform to investigate the circularity feature of the red blood cells in order to perform detection and counting. Their proposed method achieved approximately 96% of accuracy rate in comparison to manual counting.

**4. Proposed work with methodology**

Significance:

\* The method of manual counting under a microscope yields inaccurate results and put an intolerable amount of stress to medical laboratory technicians. And also counting overlapping cells is a major problem. As a solution to this problem, this project proposes an image processing technique for counting the number of blood cells.

\* This counting of RBC and WBC help in diagnosing various diseases such as anaemia, leukaemia etc .

\* This project introduces a cost effective automatic blood cell counting method using image analysis technique and specifically aims at improving the results using thresholding and matching techniques.

**PHASE-1 [ BLOOD SAMPLE ACQUISITION AND IMAGE PRE-PROCESSING]**

Blood samples are collected , reagents are added and the cells are focused and captured in an IR Camera.The image so obtained is preprocessed to remove shadows and any other errors in the image and is ready to be processed for counting.

**PHASE-2 [ IMAGE PROCESSING]**

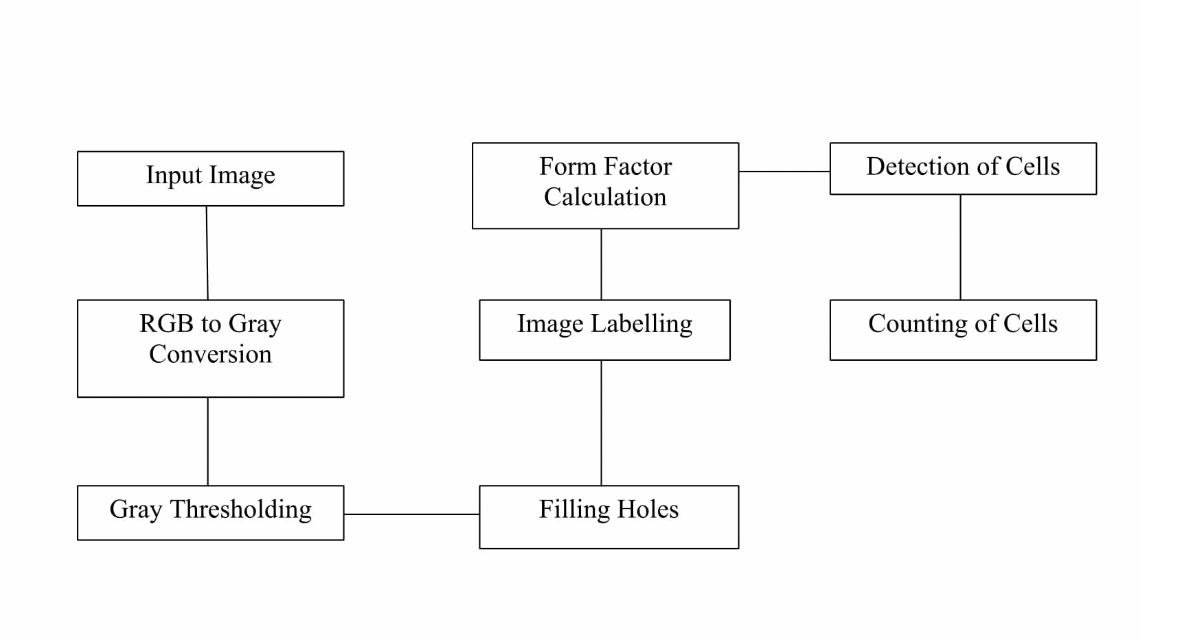
The gray scaled image of the blood sample obtained is subjected to gray thresholding technique. The gray thresholding function uses Otsu's method, which chooses the threshold to minimize the intra-class variance of the black and white pixels. Holes presented in the binary image are filled so that the blood cells are segmented

properly. This gives the count of the RBC and WBC of the blood sample collected. Using the count details obtained the state of the RBC and WBC is determined. (i.e.) the presence of anemia or leukemia is determined.

**PHASE-3 [ IMAGE POST-PROCESSING]**

The second half of phase 1 and phase 2 is repeated for the online users who provide te image of the blood sample which can be processed and the count report can be produced. As a part of phase 3 , all the details of the users and their report is stored in the database for future reference.

**PHASE-4 [ DOCUMENTATION , TESTING AND INTEGRATION]**

The idea, objectives ,requirements , components and procedure of pursuing with the project is completely documented in the form of a report with a clear and detailed explanation.

Flow chart representing the work flow.

**5. Implementation:**

**Image acquisition:**

The blood samples are collected in the cuvette followed by adding the below reagents which fluorescence the sample collected.

Reagent 1 is an isotonic electrolyte solution that dilutes whole blood samples, stabilizes cell membranes for accurate counting and sizing, conducts aperture current, rinses instrument components between analyses, and prevents duplicative cell counts by using the sweep-flow process.

Reagent 2 is a lytic reagent that lyses red blood cells for white blood cell count and hemoglobin measurement. During this period, the image of fluorescent blood sample is captured using IR camera.

An infrared camera is a non-contact device that detects infrared energy (heat) and converts it into an electronic signal, which is then processed to produce a thermal image on a video monitor and perform temperature calculations. Heat sensed by an infrared camera can be very precisely quantified, or measured, allowing you to not only monitor thermal performance, but also identify and evaluate the relative severity of heat-related problems.

**Image pre-processing:**

During image acquisition and excessive staining, the images will be disturbed by noise. The noise may be due to illumination or shadows that make region of interest (ROI) appear as blurred image region. During this preprocess, contrast adjustment technique is applied which is capable of improving the medical image quality.This process adjusts intensity values of the image by performing histogram equalization. Contrast adjustment is done by manipulating the display range of the histogram by keeping data range same.

**Calculating the total blood cells count**

**Image processing:**

**Image segmentation:**

Image segmentation involves selecting only the region of interest in the image. Here only the blood cells are selected, because they are the areas of interest. In the next step, a circular Hough transform is applied which looks only for the circular objects in the image. Hence, not much of the image segmentation is needed.

**Detection and counting of cells:**

The circular Hough transform is then applied to the contrast adjusted image. This transform searches for the blood cells in the image and then detects them. The function “draw circle” draws circles around the detected cells. Even the overlapped circles are detected.

Counting the number of circles drawn gives the total number of Blood cells in the image.



**Calculating the White Blood Cells count**

**The image acquired and pre-processed is in RGB format. This image has to be converted into gray scale format for simpler processing.**

**RGB to Gray Conversion:**

[I](https://www.mathworks.com/help/matlab/ref/rgb2gray.html" \l "outputarg_I) = rgb2gray(RGB) function converts the RGB image to the grayscale intensity image I. The rgb2gray function converts RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance.

**Image Segmentation:**

**Image segmentation** is the process of partitioning a digital image into multiple segments. The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze. Image segmentation is typically used to locate objects and boundaries in images.

Here, Image segmentation is done by using Gray thresholding. This gray thresholding technique needs a threshold value .This threshold value is selected by developing a histogram. A Histogram is a distribution of pixel intensity values which can be constructed by splitting the range of the data into equal-sized bins.

**Gray thresholding technique:**

The gray thresholding function uses Otsu's method, which chooses the threshold to minimize the intra-class variance of the black and white pixels. Holes presented in the binary image are filled so that the blood cells are segmented properly.

level = graythresh (I) computes a global threshold (level) that can be used to convert an intensity image to a binary image with im2bw. level is a normalized intensity value that lies in the range [0, 1].

**Image Enhancement:**

**Image enhancement involves two steps. They are edge detection and image labelling.**

**Canny Edge Detection algorithm:**

Canny Edge Detection is used to detect edges that will result very thin and accurate edges. 'Canny Edge Detection' is known as edge detection algorithm that is the most accurate algorithm and resulting edges are very delicate and thin. This process takes 3 inputs: 'Low Threshold', 'High Threshold', 'Sigma'. All three inputs are used to obtain maximum accurate results. The algorithm runs in 5 separate steps:

1. **Smoothing:** Blurring of the image to remove noise

2. **Finding gradients:** The edges should be marked where the gradients of the image has large magnitudes.

3. **Non-maximum suppression:** Only local maxima should be marked as edges.

4. **Double thresholding:** Potential edges are determined by thresholding.

5. **Edge tracking by hysteresis:** Final edges are determined by suppressing all edges that are not connected to a very certain (strong) edges.

**Image Labelling:**

Connected components labeling scans an image and groups its pixels into components based on pixel connectivity, i.e. all pixels in a connected component share similar pixel intensity values and are in some way connected with each other. Once all groups have been determined, each pixel is labeled with a gray level or a color (color labeling) according to the component it was assigned to.

**6. Work Plan**

The table below gives the work plan , the key task and activities done by us.

|  |  |  |
| --- | --- | --- |
| **OBJECTIVE** | **LEAD PERSON** | **KEY TASK AND ACTIVITIES** |
| Requirement fetching and Integration | Dyanesh Varun | Cuvette. Reagents , IR Camera are collected. They are integrated in such a way that the IR camera captures photograph of the flourescent blood after the addition of reagents |
| Image conversion from RGB to Gray scale | Lakshmi Priya  Reddy Vasavi | The image captured from the camera are preprocessed in such a way that they are filtered and scaled  Key task – Coding |
| Detection and Counting | Dyanesh Varun  Lakshmi Priya  Reddy Vasavi | The algorithm is applied . Count of RBC and WBC are obtained after the application of the algorithm on the image.  Key task – Coding |
| Integration and Testing | Dyanesh Varun  Lakshmi Priya  Reddy Vasavi | The complete project is tested for fine working |

**7. Expected outcome / Results**

|  |  |  |
| --- | --- | --- |
| ***Phase*** | ***Time Period(days)*** | ***Deliverables*** |
| 1. Blood sample Acquisition and Pre-processing of image:   a. Collection of blood samples.  b.Capturing images of the blood  samples using IR cameras. | 40 | Gray scale converted image of the blood samples. |
| 1. Image processing   a. Gray Thresholding of the  image.  b. Classification of cells | 60 | Count details of RBC and WBC of samples |
| 1. Image Post-processing   a. Repeat 1.a, 1.b, 2.a and 2.b  for online users.  b. Storing the details in the  database. | 60 | Count report of the blood sample images provided by online users |
| 1. Documentation, Testing and Integration | 20 | Submission of end product |

**8. Applications**

**\*** RBC and WBC count helps doctor check any symptoms, such as weakness or fatigue, or bruising.

\* It also helps to diagnose conditions, such as anemia, infection, and many other disorders.

\* The number of white blood cells is sometimes used to find an infection or to see how the body is dealing with cancer treatment.

\* When a person has a bacterial infection, the number of white cells rises very quickly.

\* Too many or too few of the different types of white blood cells can help find an infection, an allergic or toxic reaction to medicines or chemicals, and many conditions, such as leukemia.

\* If the RBC count is low (anemia), the body may not be getting the oxygen it needs. If the count is too high (a condition called polycythemia), there is a chance that the red blood cells will clump together and block tiny blood vessels (capillaries). This also makes it hard for your red blood cells to carry oxygen.

\***Hematocrit (Hct)**, the percent of RBCs in the blood. When the Hct values fall too low, it’s called [*anemia.*](http://www.cancer.org/ssLINK/anemia-in-people-with-cancer)

*\** The most important infection-fighting WBC is the *neutrophil* (NEW-truh-fil). The number doctors look at is called *absolute neutrophil count* (ANC). A healthy person has an ANC between 2,500 and 6,000.

The ANC is found by multiplying the WBC count by the percent of neutrophils in the blood. For instance, if the WBC count is 8,000 and 50% of the WBCs are neutrophils, the ANC is 4,000 (8,000 × 0.50 = 4,000).

When the ANC drops below 1,000 it is called *neutropenia* (new-truh-PEEN-e-uh). The risk of infection is much higher when the ANC is below 500.

|  |  |  |  |
| --- | --- | --- | --- |
| TEST | UNITS\* | NORMAL VALUES# | COMMENTS |
| WBC count | x 1000/mm3 | 5–10 | Number of infection-fighting cells |
| RBC count | x 1,000,000/mm3 | 4.2–6.1 | Measure of RBCs, which carry oxygen and carbon dioxide |

*\** The count information helps to diagnose Myelodysplastic Syndromes (MDS) which are a group of diverse bone marrow disorders in which the bone marrow does not produce enough healthy blood cells. MDS is often referred to as a “bone marrow failure disorder”. MDS is primarily a disease of the elderly (most patients are older than age 65) whose symptoms include anemia , leukemia and thrombocytopenia.

**9. Conclusion**

Proposed method of cell counting is fast, cost effective and produces accurate results. It can be easily implemented in medical facilities anywhere with minimal investment in infrastructure. This method can also recognise the overlapping cells and counts them separately. The average time required and the average accuracy of the proposed system is 20 seconds and 93.01% respectively.

**10. References**

**\*[http://www.webmd.com/a-to-z-guides/complete-blood-count-cbc#1](http://www.webmd.com/a-to-z-guides/complete-blood-count-cbc" \l "1)**

**\***[**http://www.healthline.com/health/blood-cell-disorders**](http://www.healthline.com/health/blood-cell-disorders)

**\***[**http://www.cancer.org/treatment/understandingyourdiagnosis/examsandtestdescriptions/understanding-your-lab-test-results**](http://www.cancer.org/treatment/understandingyourdiagnosis/examsandtestdescriptions/understanding-your-lab-test-results)

**\***[**www.mds-foundation.org/what-is-mds/**](http://www.mds-foundation.org/what-is-mds/)

**11. Financial Assistance**

***Estimated Cost of the project:*** Rs.25,000/-

|  |  |
| --- | --- |
| ***Item*** | ***Cost in Rupees*** |
| a) Material/ component/ fabrication | Rs. 10000/- |
| b) Contingencies | Rs. 10000/- |
| c) Conference | Rs. 5000/- |
| Total | RS. 25000/- |