

RED BLOOD CELLS SEGMENTATION AND ESTIMATION

MUHAMMAD ASRAF BIN MANSOR

UNIVERSITI TEKNOLOGI MALAYSIA

RED BLOOD CELLS SEGMENTATION AND ESTIMATION

MUHAMMAD ASRAF BIN MANSOR

A project report submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Engineering (Electrical – Electronics and Telecommunications)

Faculty of Electrical Engineering
Universiti Teknologi Malaysia

JANUARY 2012

Specially dedicate to...

My beloved wife, son and family

ACKNOWLEDGEMENT

Alhamdulillah and praise to Allah, the Most Gracious and Most Merciful, Who has created the mankind with knowledge, wisdom and power. The Great Allah gives his continuous blessing and with His power, this works successfully achieved.

I would like extend my biggest gratitude and appreciation to everyone who has contributed directly or indirectly towards the success of this project entitled “Red Blood Cells Segmentation and Estimation”, particularly to my project supervisor, Dr. Nasrul Humaimi Bin Mahmood who has been very patient and understanding throughout the duration of this project. Without his continued support and guidance, this project would not have been completed in the first place.

Also, thanks to all my friends and colleagues for their support in covering and giving me their hands during the critical and ramp up period of my work in product development project.

Last but not least, I would like to express my love and gratitude to my beloved wife, son, parents, and family; for their understanding and endless love, through the duration of my studies.

ABSTRACT

The erythrocytes are the most numerous blood cells in human body and it also called red blood cells. The number of red blood cells contributes more to clinical diagnosis with respect to blood diseases. The aim of this research is to produce a computer vision system that can detect and estimate the number of red blood cells in blood sample image. The proposed system takes an input, color image of stained peripheral blood smear images. Since the object of interest is the red blood cells, the system is capability to detect or differentiate between the red blood cells with other blood cell based on size of object. In order to detect red blood cells, the segmentation and extraction step must come early before proceeded to the detection process. In addition this system also can provide the capability to estimate the number of red blood cells. This process is based on the circle detection process by considering that the red blood cells always in normal radius and circle shape of red blood cells. Thus, the result presented here is based on images with normal blood cells. The tested data consisting 20 samples produced the accurate estimation rate close to 96% from manual counting.

ABSTRAK

Sel-sel darah merah atau 'erythrocytes' merupakan antara kumpulan darah terbesar di dalam badan manusia. Jumlah bilangan sel-sel darah merah dijadikan penanda aras di dalam rawatan klinikal untuk menentukan berlakunya penyakit yang berkaitan dengan darah. Matlamat penyelidikan ini adalah untuk menghasilkan satu sistem berkomputer yang boleh mengesan dan mengira jumlah sel-sel darah merah di dalam sampel imej darah. Sistem ini menggunakan sampel imej dari kaca mikroskop. Sistem ini hanya fokus kepada sel-sel darah merah sahaja, maka sistem ini berkebolehan untuk kesan atau membandingkan sel-sel darah merah dengan sel-sel yang lain berdasarkan saiz sel-sel tersebut. Dalam proses untuk mengesan sel-sel darah merah, segmentasi dan pengekstrakan perlu dilakukan terlebih dahulu sebelum melalui proses pengesanan. Sistem ini juga berkebolehan untuk mengira jumlah sel-sel darah merah. Proses pengesanan ini berdasarkan mengenal pasti lingkaran objek dengan menjadikan normal jejari dan bentuk lingkaran sel-sel darah merah. Oleh sebab itu, keputusan yang diperolehi di dalam sistem ini adalah berdasarkan imej darah yang normal. Eksperimen ini menggunakan 10 sampel imej darah dan kejituan sistem ini hampir 96% daripada pengiraan secara manual.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	x
	LISTT OF FIGURES	xi
	LIST OF ABBREVIATIONS	xiv
	LIST OF APPENDICES	xv
1	INTRODUCTION	1
	1.1 Introduction	1
	1.2 Problem Statements	3
	1.3 Objective	3
	1.4 Scopes of the Work	3
	1.5 Significance of Study	4
	1.6 Thesis Overview	4
2	LITERATURE REVIEW	6
	2.1 Introduction	6
	2.2 Segmentation and Extraction	7

2.2.1	Conventional Segmentation	7
2.2.2	Otsu Thresholding	8
2.2.3	Morphology	9
2.2.3.1	Mass Center Calculation	10
2.2.3.2	Distance Calculation of Each Edge pixel from Mass Center	10
2.2.4	Active Contour Models	11
2.2.5	Zack's Method	12
2.2.6	Color and Texture Based Feature Extraction	13
2.2.7	Hough Transform	14
2.3	Classification and Counting	16
2.3.1	Support Vector Machine(SVM)	17
2.3.2	Counting Based on Neural Network	18
3	METHODOLOGY	21
3.1	Introduction	21
3.2	Project Overview	21
3.3	Data Acquisition	23
3.4	Image Enhancement	24
3.4.1	Hue-Saturation Value	24
3.4.2	Green Component Image	25
3.4.3	Median Filter	25
3.4.4	Piecewise Linear Contrasts Stretching	27
3.5	Red Blood Cells Segmentation	28
3.5.1	Thresholding	30
3.5.2	Morphological	31
3.5.2.1	Area Closing	31
3.5.2.2	Dilation	32
3.5.2.3	Logic Operation	33
3.5.2.4	Area Opening	33
3.5.3	Edge Detection	34
3.6	Red Blood Cells Extraction	35

3.7	Red Blood Cells Estimation	35
3.7.1	Morphological Erosion	36
3.7.2	Hough Transform	37
4	RESULTS AND DISCUSSION	39
4.1	Introduction	39
4.2	Experiment Setup	39
4.3	Read Blood Smear Image	40
4.4	Red Blood Cells Segmentation	41
4.4.1	Hue-Saturation Value Image	41
4.4.2	Green Component, G Image	42
4.4.3	Median Filter and Contrast Stretching	43
4.4.4	Threshold	44
4.4.5	Morphological Area Closing and Opening, Dilation and XOR Operation	44
4.4.6	Edge Detection and Overlay Technique	47
4.5	Red Blood Cells Extraction	48
4.6	Read Blood Cells Estimation	49
4.6.1	Morphological Erosion and Median Filter	49
4.6.2	Hough Transform	50
4.7	Graphical User Interface (GUI)	51
4.7.1	Browse Image Operation	51
4.7.2	Segmentation Operation	52
4.7.3	Estimation Operation	53
4.7.4	Calibration	54
4.8	Discussion	56
5	CONCLUSION	59
5.1	Conclusion	59
5.2	Further Works Issue	60
	REFERENCES	62
	Appendix A	65

LIST OF TABLE

TABLE NO.	TITLE	PAGE
4.1	Training Data	57

LIST OF FIGURE

FIGURE NO.	TITLE	PAGE
2.1	Isolated White Blood Cell	8
2.2	Comparison between the result of thresholding on direct Lapacian high pass filter product and high boost filter product	9
2.3	Mass center for three red blood cells	10
2.4	Segmentation step by using snakes active contour	12
2.5	Threshold for RBC segmentation in clear image	13
2.6	Hough transform of collinear points (a) 4 collinear points in the plane, and (b) 4 corresponding curves in parameter space	15
2.7	A circle and center determined by 3 not straight line points	16
2.8	Feed-forward neural network	18
3.1	Flow chart of red blood cells segmentation and estimation process	22
3.2	Input sample image of blood smear	23
3.3	Flow chart of image enhancement	24
3.4	Operation of median filter	27
3.5	Basic transformation of contras stretching	28
3.6	Flow chart of red blood cells segmentation	29
3.7	Multiple thresholding	30

3.8	(a) Structuring element B “rolling” on the outer boundary of A . (b) Heavy line is the outer boundary of the closing. (c) Complete closing	31
3.9	(a) Before dilation. (b) After dilation	32
3.10	XOR operation between binary images	33
3.11	(a) Structuring element B “rolling” along the inner boundary of A (the dot indicates the origin of B). (c) The heavy line is the outer boundary of the opening. (d) Complete opening	34
3.12	Flow chart of red blood cells extraction	35
3.13	Flow chart of estimate the number of red blood cells	36
3.14	(a) Before erosion (b) After erosion	37
3.15	Calculated the center of circle by using Hough transform	38
4.1	Original image of blood smear	40
4.2	Saturation, S image of blood smear	42
4.3	Green component, G image of blood smear	42
4.4	(a) G image after pass through the 5×5 median filter. (b) Contrast Streching the (a) from low to high contrast image	43
4.5	(a) Image with high than 0.53 pixel value.(lower pixel). (b) Image with high than 0.96 pixel value(higher pixel)	44
4.6	Morphological area closing on lower pixel value image	45
4.7	Morphological dilation and area closing on higher pixel value image	45
4.8	Histogram of saturation, S image	46
4.9	Morphological XOR operation and area opening between higher pixel value image and lower pixel value image	47
4.10	Red blood cells segmentation	48
4.11	Result of morphological XOR operation	48
4.12	Red blood cells image after erosion and filter	49
4.13	Result of red blood cells estimation	50

4.14	GUI when perform loading image	51
4.15	GUI when perform segmentation task	52
4.16	GUI when perform estimation task	53
4.17	GUI show the calibrate button	54
4.18	How to determine radius of red blood cells	55
4.19	How to determine pixel value	56
4.20	Graph shows the accuracies of 10 image samples	58

LIST OF ABBREVIATIONS

WBC	-	White Blood Cell
RBC	-	Red Blood Cell
SMV	-	Support Vector Machine
MLP	-	Multilayer Perception
GUI	-	Graphical User Interface

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	Other results	65
B	MATLAB source codes	67

CHAPTER 1

INTRODUCTION

1.1 Introduction

Blood is a liquid tissue, which consists of three major cells in our blood, which are white blood cells (WBCs) or leukocytes, red blood cell (RBCs) or erythrocytes and platelets or thrombocytes. In an adult man, the blood is about 1/12th of the body weight, and this corresponds to 5-6 litres.

The erythrocytes are the most numerous blood cells in human body, and it also called red blood cells. The red blood is a blood that functioned as a carry oxygen throughout our body [1]. According to American Cancer Society (2009), the normal red blood cell in our body is divided into four categories of ages, which are newborn, children, women and men. The average amount of red blood cells each category is about 4.8-7.2 million per cubic millimeter, about 3.8–5.5 million per cubic millimeter, about 4.2-5.0 million of these cells per cubic millimeter and $4.6-6.0 \times 10^6$ per cubic millimeter respectively.

Red blood cells which are the ability to carry oxygen are measured by the amount of hemoglobin in our blood. If our level of hemoglobin is low, we are anemic and our body works much harder to supply oxygen to our tissues. This can make we feel fatigued and short of breath. In some cases fatigue becomes so severe that you must temporarily halt your treatment or reduce the dose you receive. Anemia can be relieved with a blood transfusion or with medication to increase your body's production of red blood cells [2].

The effect of having high red blood cells in our blood is it can be an indication of an undetected heart or lungs problems. When any of these organs is not functioning properly, then blood oxygen levels go down. In order to normalize oxygen supply, the body increases its production of red blood cells.

Counting of red blood cells in a blood sample can give the pathologists valuable information regarding various hematological disorders. Since the classical method for diagnosis of red blood examination in a blood sample is counted by manpower, it has are the following deficiencies such as poor reliability, low efficiency and strong subjectivity. The diagnosis is the process of finding out what kind of disease a certain patient has and this diagnosed must always be accurate. A wrong diagnosis may lead the situation and condition of a patient become worst and some case, patient die due to wrong dosage of drugs given [3].

In the process of estimating red blood cells on blood sample images requires four steps. These steps are acquisition, segmentation, feature extraction and estimation. The acquisition step is done by taking the images that ready for analysis. Then the both segmentation and feature extraction is done by using a morphological technique in order to distinguish the red blood cells from background and other cells. The last step is estimating the number of red blood cells, and it has been done by using Hough Transform technique.

1.2 Problem Statement

The classical method of red blood examination in a blood sample is counted by manpower, which has the following deficiencies such as poor reliability, low efficiency and strong subjectivity. In order to overcome that weakness, some researchers have done some useful works [4-5]. The overlapping of clumped red blood cells each other, it also results in the problem in counting process. This project studies and develops the algorithm and creates user-friendly software to counting the red blood cell automatically in blood cells.

1.3 Objectives

The main objective of this project is to develop software or algorithm for the purpose of detecting the red blood cells in a blood sample. In order to detect the red blood cells of these blood samples, the software should have a capability to estimate the number of red blood cells in the image sample which will be taken by a microscope. In addition, this system should also have a capability to classify the red blood cells from other cells in blood samples before counting process starts. This is done by making sure that the algorithm can achieve high accuracy and high performance.

1.4 Scopes of the Work

In this study, there are scopes that need to consider in order to make this project can work accordingly to the objectives. The scopes are:

1. The program obtains image sequences (input) from the computer (offline).
2. Investigating the different between red blood cell and another cell in a blood sample.

3. Analyses the contour of red blood cell and segmented them.
4. Normal shape of single red blood cell will be considered.
5. The object of interest then should only be red blood cells and not any other cells.
6. Matlab is used to develop the software.

1.5 Significance of Study

Through the well study, it creates a path of idea on how to extract and counting the red blood cells inside the blood sample image. Besides that, it gave the user-friendly and practical approach to help a medical person in diagnosed the blood sample of a patient.

1.6 Thesis Overview

This thesis consists of five chapters. Each chapter has its own discussion on the aspects related to the project. The following are basically the aspects discussed in each chapter.

Chapter 1 discusses the introduction, objectives, scope and significance of the project. Moreover, it also describes briefly the general introduction on the environment and specifies the object of interest. Chapter 2 provides literature review of another research and published technical paper of the previous project that related to this project, and method approached for segmentation and estimation red blood cells.

Chapter 3 focused on the methodology, theory and approaches in building the project. This chapter reviews algorithm and design concepts, and its functionalities are explained. It also described design, work flow and methodology. The result and discussion are presented in Chapter 4. This chapter includes the basic usage guide on the system and comparison of manual results and system results. Chapter 5 deals with the summary and conclusions of the project. Some recommendation and suggestions for the future development of the project are also discussed.

REFERENCES

1. Michael R. Pinsky, Laurent Brochard and Jordi Mancebo. *Applied Physiology in Intensive Care Medicine*: Springer. 229-238; 2006.
2. Dondorp AM, Angus BJ, Chotivanich K, Silamut K, Ruangveerayuth R, Hardeman MR, Kager PA, Vreeken J, White NJ. Red cell deformability as a predictor of anemia in severe falciparum malaria. *Am J Trop Med Hyg* 60: 733–744; 1999.
3. Medicine Health;
http://www.emedicinehealth.com/leukemia/article_em.htm.
4. Yuzhang WEI. *The Research of Urinary Sediment Visual Component Analysis Based on Fuzzy Clustering*. Nanjing Information Engineering University, 2008:3-4,15-32.
5. Ran Ding. *Algorithm Research on Recognition and Classification of Microscopic Urinary Sediment Images*. Jilin University, 2006: 9-16, 20-31.
6. C.D. Ruberto, A.G. Dempster, S. Khan and B. Jarra. Segmentation of Blood Image using Morphological Operators. *Proceeding 15th International Conference on Pattern Recognition*. vol. 3, pp. 397-400, 2000.
7. Roy A. Dimayuga, Gerwin T. Ong, Rainier Carlo S. Perez, Gefferson O. Siy, Saman C. Sohrabi Langroudi and Miguel O.Gutierrez. *Leukemia Detection Using Digital Image Processing in Matlab*. ECE Student Forum, De La Salle University, Manila. March 26, 2010.
8. I. Cseke, A Fast Segmentation Scheme for White Blood Cell Images. *Proceedings. 11th IAPR International Conference Pattern Recognition, Conference C: Image, Speech and Signal Analysis*. Vol 3, pp. 530-533, 1992.
9. Ramin Soltanzadeh. Classification of Three Types of Red Blood Cells in Peripheral Blood Semear Based on Morphology. *Proceedings of ICSP*, 2010.

10. C.D. Ruberto, A.G. Dempster, S. Khan, and B. Jarra. Morphological Image Processing for Evaluating Malaria Disease. *Proceedings of IWVF*. pp.739~748, 2001.
11. Guclu Ongun, Ugur Halici, Kemal Leblebicioglu, Kemal Leblebicioglu, Meral Beksad and Sinan Beksad. Feature Extraction and Classification of Blood Cell for an Automated Different Blood Count System. *Proceedings of the 23rd Annual EMBS International Conference, Istanbul, Turkey*. October 25-28, 2001.
12. Guclu Ongun, Ugur Halici, Kemal Leblebicioglu, Kemal Leblebicioglu, Meral Beksad and Sinan Beksad. An Automated Differential Blood Count System. *Proceedings of the 23rd Annual EMBS International Conference, Istanbul, Turkey*. October 25-28, 2001.
13. Heidi Berge, Dale Taylor, Sriram Krishnan, and Tania S. Douglas. Improved Red Blood Cell Counting in thin Blood Smears. *Proceedings of ISBI*. 2011. pp.204~207.
14. Zack G.W., Rogers W.E. and Latt S.A. *Automatic-measurement of sister chromatid exchange frequency*. *Journal of Histochemistry & Cytochemistry* 25, 741--753 (1977).
15. Guitao Cao, Cai Zhong, Ling Li and Jun Dong. Detection of Red Blood Cell in Urine Micrograph. *The 3rd International Conference on Bioinformatics and Biomedical Engineering (ICBBE)*. 2009.
16. N. Theera-Umpon and P.D. Gader. Training Neural Networks to Count White Blood Cells via a Minimum Counting Error Objective Function. *Proceedings International Conference on Pattern Recognition*. 2000.
17. J.Poomcokrak and C. Neatpisarnvanit. Red Blood Cells Extraction and Counting. *The 3rd International Symposium on Biomedical Engineering (ISBME)*, 2008.
18. Centers for Disease Control and Prevention: Public Health Image Library (online). 2005. from;
<http://phil.cdc.gov/phil/home.asp>
19. <http://www.unomaha.edu/hpa/blood.html>.
20. <http://library.med.utah.edu/WebPath/HEMEHTML/HEME005.html>.
21. Rafael C. Gonzalez and Richard E. Woods. *Digital Images Processing*. 2nd Edition. New Jersey, USA: Prentice Hall Inc. 2002.

22. Fisher, R., Perkins, S., Walker, A. and Wolfart, E. *Morphology-Dilation*. Retrieved 2003, from;
<http://homepages.inf.ed.ac.uk/rbf/HIPR2/dilate.htm>
23. Fisher, R., Perkins, S., Walker, A. and Wolfart, E. *Morphology-Erosion*. Retrieved 2003, from;
<http://homepages.inf.ed.ac.uk/rbf/HIPR2/erosion.htm>
24. Kenneth.R.Castleman,Z. G. Zhu. *Digital Image Processing*. Publishing House of Electronics Industry, Beijing, 1999.
25. W. Meisel. *Computer-Oriented Approaches to Pattern Recognition*. Academic Press. New York, 1972.