COMPUTER AIDED SYSTEM FOR DENDRITIC CELLS DETECTION AND COUNTING

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A thesis submitted in
fulfillment of the requirement for the award of the
Degree of Master of Electrical Engineering

Faculty of Electrical and Electronic Engineering
Universiti Tun Hussein Onn Malaysia

May, 2017

DEDICATION

For my beloved mother and father,

My supervisor,

Lecturers,

Sisters,

Best friends,

Friends,

And everyone who involved in inspired me throughout my journey of completing this project.

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Wan Nurshazwani binti Wan Zakaria, for all the guidance and encouragement she provided me since the very beginning of my graduate program. Her invaluable suggestions and expertise in the field have helped me immensely in attaining the goals I set out to achieve with my research. I am also very grateful to my committee member, Dr. Mohd Razali bin Md Tomari for being so supportive and giving me important feedback over the course of this thesis. I am deeply indebted to Nicolas Jaccard and Marko Usaj from University College London (UCL) and University of Ljubljana, Slovenia respectively for taking time off their busy schedules to help with my research. Their inputs on the identification of various cells in the data set have helped in shaping a lot of my research work. I would also like to thank the members of the Cancer Research Malaysia (CRM) especially Dr Lim Kue Peng and Ms. San Jiun for providing the sample images of Dendritic Cells and clear explanation on the culturing process. Finally, I would like to thank my parents and my friends for all the smiles along the way that made the many months of my research a lot more pleasant and enjoyable.

This research was supported by the Fundamental Research Grant (FRGS) under Ministry of Education Malaysia (Vot 1583).

ABSTRACT

Immunotherapy is an entirely advanced class of cancer treatment which has been highly active and exciting field in clinical therapeutics. In numerous procedures, cancer immunotherapy demands a laborious practice to recognise and count Dendritic Cells (DCs) in the harnessing of immune system. Conventionally, the laser-based technology that provides a rapid analysis such as Flow Cytometry can affect the DCs viability as the staining procedure is involved. Another highly promising method which is Phase Contrast Microscopy (PCM) involves experienced pathologists to visually examine the respective microscopy images. In fact, PCM confronts complex issues regarding imaging artifacts which can deteriorate the recognition process. As DCs counting are crucial in most cancer treatment procedures, this research proposes a pioneering system called CasDC (Computer Aided System for Dendritic Cells identification) which implements an image processing algorithm to recognise and count DCs with a label-free method. Initially, the images undergo Grayscale Normalization, H-GLAT, and Halo Removal to remove the imaging artifacts. In segmentation, morphological operators and Canny edge detector are implemented to extract the cell contours. Following that, information from the contours are characterized through the use of One-Dimensional (1D) Fourier Descriptors (FDs) and classified using Template Matching (TM). The aim of developing this system is to establish a reliable and time saving-tool as a second reader in the clinical practice. The proposed system has an enormous potential towards helping Cancer Research Institute in improving the diagnosis of cancer. Through the experiments conducted on dataset provided by the Cancer Research Institute, performance measures of 83.8%, 94.2%, 99.5% and 88.7% have been recorded for precision, recall, accuracy and F1-score respectively .

ABSTRAK

Imunoterapi adalah satu kelas rawatan kanser maju di mana menjadi bidang yang sangat aktif dan menarik dalam terapi klinikal. Dalam pelbagai prosedur, kanser imunoterapi menuntut amalan yang membebankan untuk mengenal pasti dan mengira sel-sel dendrit (DC) dalam memanfaatkan sistem imun. Secara konvensional, teknologi berasaskan laser yang mampu menyediakan analisis yang pantas seperti sitometri aliran boleh menjejaskan daya ketahanan DC disebabkan oleh penglibatan prosedur pewarnaan. Satu lagi kaedah yang mendapat perhatian iaitu mikroskop kontras fasa (PCM) melibatkan ahli patologi yang berpengalaman untuk mengkaji imej mikroskop masing-masing. Malah, PCM menghadapi isu-isu yang kompleks berkenaan artifak pengimejan yang boleh menjejaskan proses pengenalpastian. Oleh kerana pengiraan DC ini mempunyai peranan yang besar dalam kebanyakan prosedur rawatan kanser, penyelidikan ini mencadangkan satu sistem perintis dipanggil CasDC (sistem berbantukan komputer untuk pengecaman sel-sel dendrit) yang dapat melaksanakan algoritma pemprosesan imej untuk mengenal pasti dan mengira DC dengan kaedah bebas pewarnaan. Pada peringkat awal, imej menjalani normalisasi skala kelabu, H-GLAT, dan penyingkiran halo untuk membuang artifak di dalam imej. Dalam segmentasi, operator morfologi dan pengesan *Canny* dilaksanakan untuk mendapatkan kontur sel. Berikutan itu, maklumat daripada kontur dicirikan melalui penggunaan satu dimensi (1D) deskriptor *Fourier* dan diklasifikasikan dengan menggunakan penyesuaian templat. Tujuan sistem ini dibangunkan adalah untuk menghasilkan alat yang berkebolehan dan menjimatkan masa sebagai bantuan kedua dalam amalan klinikal. Sistem yang dicadangkan ini mempunyai potensi yang besar ke arah membantu Institut Penyelidikan Kanser dalam meningkatkan diagnosis kanser. Melalui eksperimen yang berkaitan dengan set data yang disediakan oleh Institut Penyelidikan Kanser, ukuran prestasi telah direkodkan dengan masing-masing mencatat 83.8%, 94.2%, 99.5% dan 88.7% untuk kepersisan, kepekaan, kejituan dan skor-F1.

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LIST OF SYMBOLS AND ABBREVIATIONS

μ	-	Mean around each pixel
σ^2	-	Variance around each pixel
M	-	Number of rows of pixel in image
N	-	Number of columns of pixel in image
$\sum$	-	Sigma
η	-	N by M local neighborhood of each pixel in image
$\in$	-	Epsilon
Θ	-	Theta
$\bigoplus$	-	\bigoplus
A_{tc}	-	Area of T-cell
A_{db}	-	Area debris
A_{mc}	-	Area of Monocyte cell
A_{dc}	-	Area of Dendritic Cell
P_{tc}	-	Perimeter of T-cell
P_{db}	-	Perimeter of debris
P_{mc}	-	Perimeter of Monocyte cell
P_{dc}	-	Perimeter of Dendritic Cell
D_{tc}	-	Diameter of T-cell
D_{db}	-	Diameter of debris
D_{mc}	-	Diameter of Monocyte
D_{dc}	-	Diameter of Dendritic Cell

LIST OF APPENDICES

LIST OF PUBLICATIONS

1. **Anis Azwani Muhd Suberi;** Wan Nurshazwani Wan Zakaria; Razali Tomari and Mei Xia Lau. Dendritic cell recognition using template matching based on one-dimensional (1D) fourier descriptors (FD). *First International Workshop on Pattern Recognition*. Proc. SPIE. 2016. pp.100110K.

2. **Anis Azwani Muhd Suberi;** Wan Nurshazwani Wan Zakaria, Razali Tomari and Kue Peng Lim. Optimization of overlapping dendritic cell segmentation in phase contrast microscopy images. In 2016 *IEEE-EMBS Conference on Biomedical Engineering and Science.* IEEE. pp.246-250.

3. **Anis Azwani Muhd Suberi;** Wan Nurshazwani Wan Zakaria, Razali Tomari. Dendritic cell recognition in computer aided system for cancer immunotherapy. *Procedia Computer Science*. Elsevier. 105C: 177-182.

4. **Anis Azwani Muhd Suberi;** Wan Nurshazwani Wan Zakaria, Razali Tomari. Dendritic cells feature extraction using geometric features and 1D fourier descriptors. *In the 9th Int. Conference on Computer and Automation Engineering (ICCAE 2017)*. Accepted

5. **Anis Azwani Muhd Suberi;** Wan Nurshazwani Wan Zakaria, Razali Tomari. An Automated Detection and Quantitative Analysis of Dendritic Cells in Phase Contrast Microscopy Images. *Journal of the International Measurement Confederation (IMEKO)*. Elsevier. Under Review.

LIST OF AWARDS

1. Best Poster Award in the 1st FKEE Postgraduate Research Conference 2016, Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia.

2. Gold medal (Higher institution category) in the International Invention and Innovative Competition (INIIC-Series 2), Puteri Beach Resort, Port Dickson, Negeri Sembilan, 2016.

3. Bronze medal (Applied Research under staff category) in the Research and Innovation (R&I) Festival, Universiti Tun Hussein Onn Malaysia, 2016.

4. Silver medal in the 2017 Malaysia Technology Expo (MTE), Kuala Lumpur.

5. Gold medal in the 2017 Innovation Design Research International Symposium (IDRIS).

6. Gold medal in the 2017 Innovative Research, Invention and Application Expo (I-RIA).

CHAPTER 1

INTRODUCTION

Most of the established therapies such as surgery, chemotherapy and radiotherapy are widely used to treat cancer patients. Surgery is often considered as the first therapy option to remove the tumour (Sasada *et al.,* 2015). Nevertheless, there is a tendency of removing only parts of the tumour. Following that, a combination of surgery with radiotherapy or chemotherapy is typically used in patients to kill the cancer cells (Sasada *et al.*, 2015). However, both radiotherapy and chemotherapy can introduce non-favourable outcomes towards the patient, such as serious bleeding, lack of energy and experience depression (Mellman *et al.* 2011). Recently, Dendritic Cells (DCs) immunotherapy has been widely explored and introduced as an advanced approach to boost the immune system to fight cancer (Raïch-Regué *et al.*, 2014; Sasada *et al.*, 2015). DCs immunotherapy employs and activates the body's own immune cells to fight the cancer cells as shown in Figure 1.1.

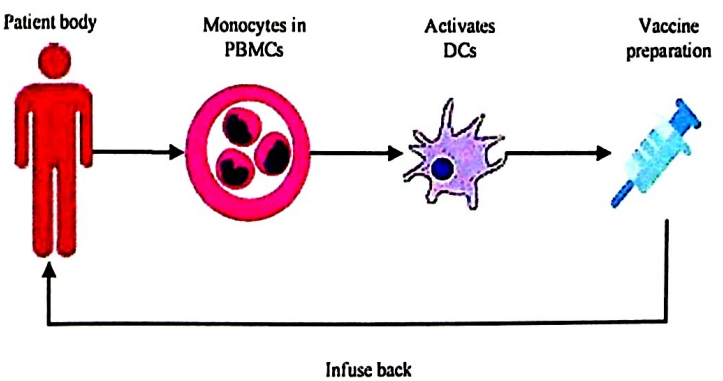

Figure 1.1: DCs immunotherapy procedure

In the cancer microenvironment, DCs have a key role for activation of T- and B-cell immunity as both represent as the Antigen-Presenting Cells (APCs) (Raïch-

Regué *et al.*, 2014; Tan *et al.*, 2010). DCs can be generated *in vitro* from Peripheral Blood Mononuclear Cells (PBMCs) (Tan *et al.*, 2010). On top of that, DCs have long and numerous tentacles as their main characteristic as shown in Figure 1.2. The identification of DCs in immunotherapy is important as the phenotype of DCs can determine the type of immune reaction in autoimmunity response. In brief, morphological cell analysis is a key issue for the preparation of cancer immunotherapy as the DCs are trained to recognise cancer antigens.

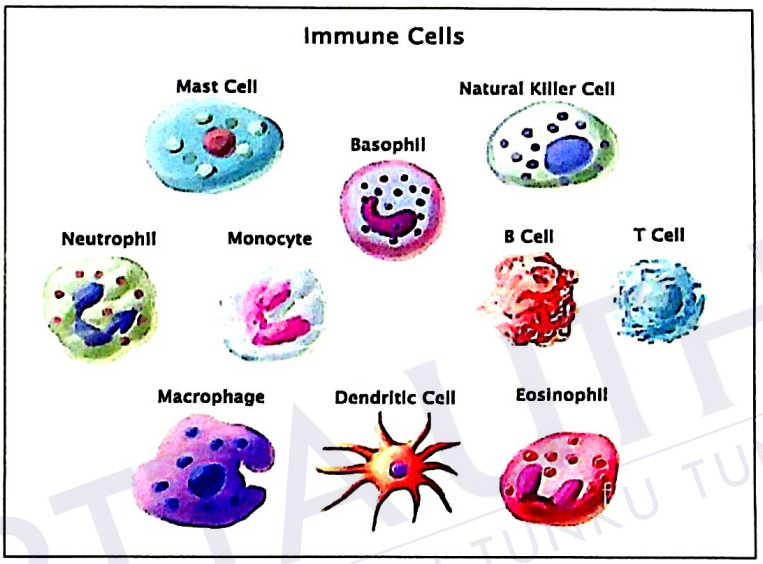

Figure 1.2: Component of immune cells in human blood system (IMDC, 2015)

During the cell culture, DCs can be characterised and recognised using Flow Cytometry (Rovati *et al.*, 2008; Tan *et al.*, 2010). However, this method involves a staining process which can affect the DC viabilities. Therefore, another promising microscopy technique called, Phase Contrast Microscopy (PCM) is used to visualise, recognise and count the amount of DCs manually before the sample is infused back into the patient body. Even though PCM is a label-free imaging modality, the identification purpose becomes challenging as the image is constituted with a variation of imaging artifacts such as halo region, low contrast and overlapping DCs (Jaccard *et al.*, 2014; Ra *et al.*, 2013; Usenik *et al.*, 2011). Such procedure is laborious, time-consuming and very dependent on the expert's skill to recognise and count DCs which can introduce human errors.

To address these circumstances, a pattern matching approach is developed to automatically recognise and count DCs in a large number of cells in PCM images. As

the initial condition, pre-processing schemes are applied to remove any imaging artifacts from the testing image. The complexity between DCs boundary and other cells can be considered distinctive to visually distinguish. Therefore, Fourier Descriptors (FDs), which are derived from One-Dimensional (1D) shape signatures is proposed in which the method has been applied in several studies particularly in shape-based image retrieval (Dalitz *et al.*, 2013; Sokic & Konjicija 2016; Yuan *et al.*, 2014). This proposed approach is capable to identify DCs with a label-free detection and low computational resources.

The overall process of DCs counting scheme of PCM image microscopy encompasses four primary stages namely, 1) Image Acquisition; 2) Pre-processing stage for both templates and testing images; 3) Computation of 1D Fourier Descriptors (FDs) to extract cell contour; and 4) DCs recognition, marking and counting process. Next, the sample images are compared to a set of template images to detect the most approximate target cell based on FDs. The cell templates have been already configured to match similar pattern of DCs to those of the templates.

During the features matching procedure, the cell shape signatures are obtained through the conversion of geometrical pixel information of shape descriptors to 1D FDs, which facilitate an efficient Template Matching (TM) to recognise, mark and count DCs in a pool of blood cells. The details on the DCs imaging classification schemes are described in Chapter 3, followed by the results and analysis in Chapter 4. Towards the end, the conclusion of the entire research with future works are discussed in Chapter 5.

1.1 Problem Statement

Identification of DCs especially in the cancer microenvironment is a unique disclosure since fighting tumor from the harnessing immune system has been a novel treatment under investigation. Besides that, pathologists figure this issue as a valuable case as the information about any diseases can be extracted from them. DCs can be defined through their key morphological feature which is tentacles (Wieder, 2003). Conventional Flow Cytometry provides an effective recognition of labelled DCs using fluorescent dyes that may cause phototoxic damage to the DCs tentacles (Tan *et al.*, 2010). Recent advances in cellular imaging have facilitated investigation of unstained living cell using PCM.

However, it involves tedious manual inspection and in certain cases, the DCs tentacles can hardly be identified due to the low image contrast and bright halo outlining the cells. The PCM turns the invisible phase shifts of the light waves emerging from the object into visible intensity by using interference with the 90° phase shifted illumination wave (Bensch & Ronneberger, 2015). Therefore, these would result in decreasing intensity which is proportional to the object thickness. This condition leads to halo region and shade-off artifacts to the image (Jaccard *et al.*, 2014; Bensch & Ronneberger, 2015). In the meantime, PCM has critical issues regarding clumping and overlapping cells which may deteriorate the recognition process (Tan *et al.*, 2010). Apart from imaging artifacts, the cell identification procedure can be very time consuming and qualitatively subjective. Such procedure is very dependent on the expert's skill to identify and count DCs. To address these circumstances, a Computer Aided System for DCs identification (CasDC) which involves pattern recognition is proposed to effectively identify and count DCs with a rapid and minimum processing time. The finding of this research would permit rapid, standardized and quantitative analysis for further cancer immunotherapy vaccine preparation.

1.2 Aim

This research aims to develop a Computer Aided System for Dendritic Cells identification (CasDC) for cancer immunotherapy vaccine preparation.

1.3 Objectives

To achieve the aim, the following objectives have been outlined:

1. To develop a pre-processing technique on the PCM images to reduce the image artifacts in preparation for segmentation of DCs.
2. To establish a feature extraction and classification algorithm for detection of DCs.
3. To develop an auxiliary Graphical User Interface (GUI) that supports efficient detection of DCs.
4. To evaluate the performance of developed system via a series of experimental programme (quantitative and qualitative analysis).

1.4 Scopes and Limitations

The restrictions and limitations during this research are:

1. This study focuses on the cell shape signatures in identifying the main characteristics of DCs for their classification.
2. MATLAB R2015a toolbox which utilizes GUI is developed to identify and count DCs.
3. The experimental studies are conducted and evaluated on selected samples of PBMCs images under PCM. All the selected samples are required to undergo culturing procedure to enhance the visibility of DCs from complex clumping region.
4. The image dataset is provided by Cancer Research Malaysia (CRM). All the ethical standards are conducted by CRM and the images are publicly available for research purposes.
5. The developed algorithm is tested on 1877 × 1408 and 2560 × 1920 size pixels of randomly selected from 135 PCM images to show the feasibility of the developed system.

1.5 Research Contributions

This research provides a framework of image processing approach in DCs classification and counting as shown in Figure 1.3. CasDC has been established in which this system offers a non-staining procedure to increase the viability of DCs and subsequently, beneficial to reduce error in DCs counting. This is the first time that Hybrid of Gaussian Kernel Filtering using low and high sigma parameter followed by Local Adaptive Threshold (H-GLAT) has been used to solve low contrast and overlapping cells in PCM images. Other than that, an automated DCs classification is provided to accelerate the clinical translation with rapid and minimum processing time through the use of Template Matching (TM) based on 1D FDs. Overall, a GUI has been developed to provide the end user with an interactive way to count DCs.

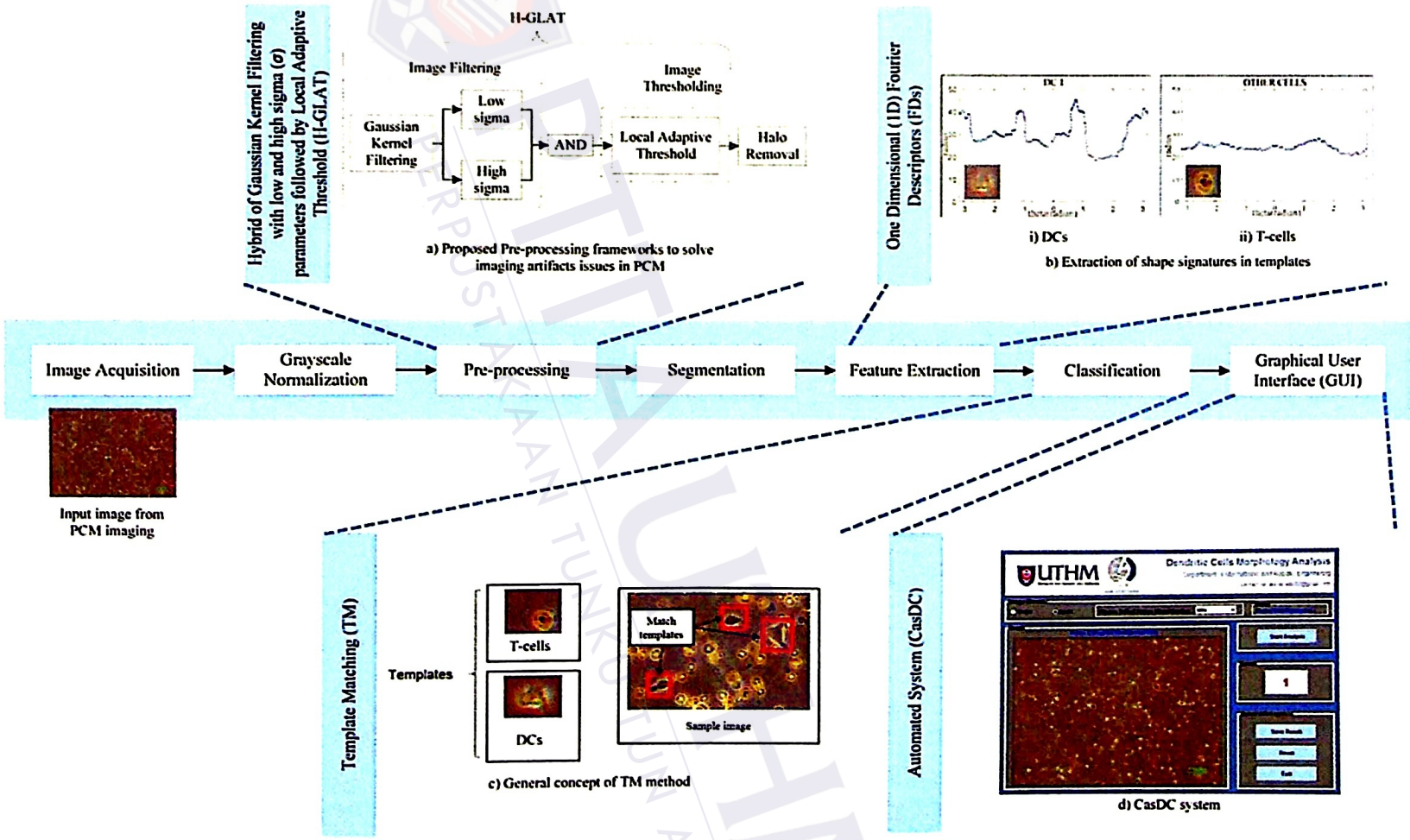

Figure 1.3: Overall research methods and contributions

1.6 Summary

This chapter briefly discusses the current issues in DCs identification for cancer immunotherapy purpose. In this procedure, DCs represent an appealing option in vaccine preparation as they have the ability to boost the immune system to fight cancer. DCs can be rapidly identified using a laser based tool called Flow Cytometry. However, this method affects the DCs viability as it acquires staining stage which might kill the tentacles. Therefore, manual counting has been practiced via a label free imaging modality which is PCM. The pathologists face a challenge to identify DCs based on the tentacles using this method due to a variation of imaging artifacts such as halo region, low contrast and clumping cells in the PCM image.

The manual counting of DCs is time consuming and highly prone to error as such procedure is laborious and depends on the expert's skill. In fact, it produces a high variance due to different levels of experience in DCs counting. Thus, this project aims to solve the problem by image processing approach to accelerate the clinical translation. Since the raw PCM images are constituted with imaging artifacts, applying image pre-processing is one of important step before classifying DCs. Towards the end, a system called CasDC is developed to provide a rapid identification of DCs in the PCM images. The next chapter describes in detail concerning DCs, PCM images and image processing techniques applied in the related studies.

CHAPTER 2

LITERATURE REVIEW

Many studies have discussed the motivation of carrying an in depth study on Dendritic Cells (DCs) in cancer immunotherapy by the clinical practitioners. In brief, this review focuses on four areas: 1) Introduction to Immune Cell Subsets; 2) Significance of Identifying DCs; 3) DCs Culture and Imaging; and 4) Current Technology in Identification for PCM Image.

2.1 Introduction to Immune Cell Subsets

White Blood Cells (WBCs) or known as Leukocytes involve in maintaining immune system by protecting the body against any diseases and sensing foreign organism. Besides that, they are essential in the diagnosis of cancer as the information about any diseases can be extracted from them (O'Neill *et al.*, 2015; Ma *et al.*, 2013). The production of WBCs is derived from multipotent cell in the bone marrow. There are five types of WBCs which are Monocyte, Lymphocyte, Neutrophil, Basophil, and Eosinophil as shown in Figure 2.1.

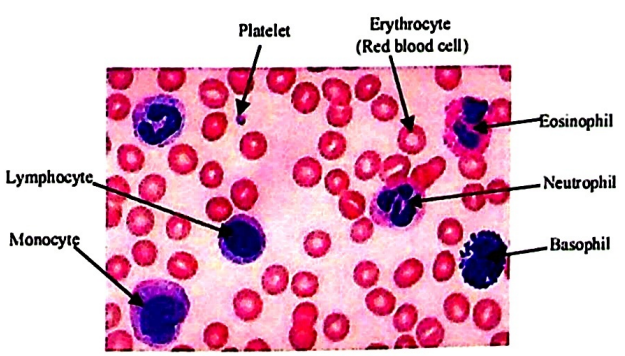

Figure 2.1: Types of blood smear under Light Microscope (Sarrafzadeh *et al.*, 2014)

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