CHAPTER 1

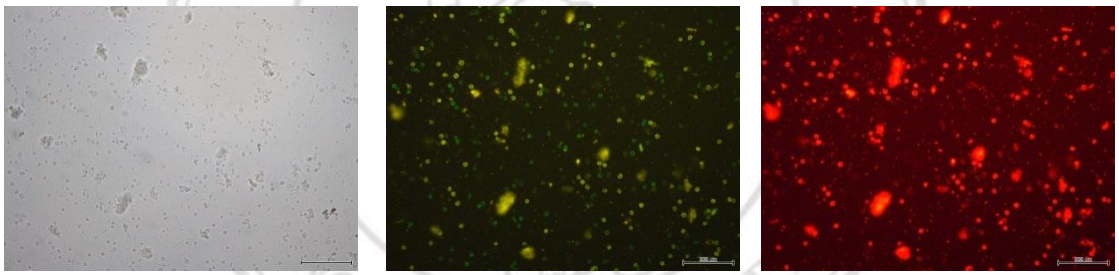
Introduction

1.1 Background

The CD or cluster of differentiation is a protein expressed on the surface of the cells in hematopoietic system. CD4 proteins are found on the surface of white blood cells such as T-helper cells, monocytes, macrophages and dendritic cells. T-helper cell, or technically called CD4+ lymphocyte, is a type of white blood cell whose surface contains CD3 and CD4 proteins. In the peripheral immune system, CD4+ lymphocyte occupies the crucial role in immune function regulation and it is the primary target of human immunodeficiency virus (HIV). HIV invades and replicates itself inside the cell and thus, the infected cell will finally be destroyed. The progressive loss of CD4+ lymphocytes eventually results in the loss of an ability to generate immune response to any pathogen, resulting in acquired immune deficiency syndrome (AIDS). So, the CD4+ lymphocyte circumstance in peripheral blood is a factor for monitoring the disease's progress and the effectiveness of anti-retroviral drug treatment.

The HIV infected person should be monitored for the quantity of CD4+ lymphocytes in blood every 6 or 12 months in order to evaluate the effectiveness of drug treatment and the condition of immune response. In laboratory testing, CD4+ lymphocytes are routinely counted using flow cytometer. However, flow cytometer is very expensive equipment, due to its inherent technology. Nowadays, automatic image-based CD4+ lymphocyte counting instrumentation named Alere Pima^{TM} CD4 [1], is available on the market. It consists of a portable bench-top, which allows a cheaper diagnostic equipment. CD4+ lymphocyte can be detected via bi-color fluorescence images captured under low magnification. However, counting cells using only fluorescence images has certain limitations due to no supporting evidence that color spots are truly cells. According to the fact that cells contain deoxyribonucleic acid

(DNA), 4',6-diamidino-2-phenylindole (DAPI), a strongly DNA binding fluorescent dye is occasionally used for this purpose but this technique causes an expansion of testing cost. Fortunately, as we have known that bright field imaging is the technique that is used for observing WBC morphology in clinical laboratory. Therefore, which color spots in fluorescence image are cells can be identified by using a corresponding bright field image where the diagnosis cost is much cheaper. Briefly, the color spots in green and red fluorescence image are counted as a CD4+ lymphocyte if both correspond to the cells which appear in bright field image. So, this approach acquires three types of images: bright field (Fig.1.1a), green fluorescence (Fig.1.1b) and red fluorescence (Fig.1.1c) images. Furthermore, we developed our approach on low resolution image which allows a large number of cells to be taken in order to provide the comparable outcome to flow cytometer.



(a) (b) (c)

Figure 1.1 Stained cells imaging of (a) bright field (b) green fluorescence (c) red fluorescence images.

1.2 Research Objective

To develop an image-based CD4+ lymphocyte detection and counting approach using three corresponding low resolution images, i.e., bright field, green fluorescence and red fluorescence images.

1.3 Research Scopes

1.3.1 Data input with immunofluorescence stained cells images are captured under the fluorescence microscope with 20x objective lens power by using the digital microscope camera (Olympus DP21).

1.3.2 Each microscopic image consists of three series from the microscope light source captured with the same scene; bright field, green fluorescence channel and red fluorescence image. The red fluorescence image is generated by using 490 nm of excitation light and emits the 670 nm wavelength. The green fluorescence image is generated by using 485 nm of excitation light and emits the 519 nm wavelength. The number of cells used in this experiment is at least 2500 cells.

1.3.3 The results from the algorithm are compared to the detection by visualization of at least three experts.

1.4 Educational Advantage

The implementation of cell counting on fluorescence image together with corresponding bright field image tends to increase the reliability of detection method. Besides, image-based method hopefully permits a cheaper and smaller technology than flow cytometer for cell counting.

1.5 Thesis Organization

This thesis is arranged into 5 chapters. A short overview of CD4+ lymphocyte counting method is stated in chapter 2. Also a review of previous studies on image-based cell detection and fundamental of image processing are described in this chapter. Chapter 3 is devoted to materials and methods involving sample preparation, image acquisition, algorithm development and performance evaluation. The results of the algorithm are then discussed in chapter 4. Finally, conclusions and possible further study are given in chapter 5.