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RED BLOOD CELL COUNTING ANALYSIS BY CONSIDERING AN OVERLAPPING CONSTRAINT

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ABSTRACT

Red blood cells (RBCs) counting in blood smear image is very important to diagnose blood related diseases such as malaria and anemia before a proper treatment can be proposed. The conventional practice for such procedure is executed manually by pathologist under light microscope. However, manual visual inspection is laborious task and depends on subjective assessment which leads to variation in the RBC counting especially when there are many clumped RBC areas. In this paper a computer-aided systems is proposed to automate the process of counting the RBC from the blood smear image by considering an overlapping constraint. Initially RBCs region are extracted from the background by using global threshold method applied on green channel color image. Next, noise and holes in the RBCs are abolished by utilizing morphological filter and connected component labeling. Following that, information from the RBCs' area is extracted to determine single and overlapping RBC region. The former region can be counted directly while the latter need to be process further to estimate the number of individual cells. In this paper, two estimators which are Distance transform and Hough transform are utilized to count cells in the clumped regions. Eventually, the total RBCs is found by summing up information from the single cell number and from the estimator. The proposed method has been tested on blood cell images and it demonstrates that Hough transform is more reliable to predict number of total RBCs.

Keywords: red blood cell counting, overlapping, distance transforms, hough transform.

INTRODUCTION

Red blood cell (RBC) or erythrocyte is a one of blood type besides the other formed elements in the blood such as white blood cell (WBC), platelet and plasma. Its normal shape is round, biconcave and flattened, about 7 μm in diameter and 2.2 μm thick (Figure-1). The shape relates to its function that transporting oxygen and provides surface area for transmitting or diffusing the gasses (Fox, 2009).

In medical area, diagnosis of RBC contributes information about pathological diseases and condition. The shape of RBC and its deformability has connection to the relevant disease such as Huntington's disease, Myalgic Encephalomyelities (ME) and Multiple Sclerosis (MS) (Vromen, 2009, Wang, 2008, Wang, 2010) and because of that, the accurate diagnosis is very important in determining the correct treatment to the patient. Blood count, known as complete blood count (CBC) is a compilation test of blood components including RBC, and any abnormal finding will give a sign of disease such as decreased RBC indicates low of specific vitamin (Sharif, 2012, Zahir, 2006), anemia, reduction of hemoglobin (a protein that bind with oxygen molecule in RBC), and secondary effect of several other disorder (Webster, 2004). However some factors should be taken into consideration when perform the RBC counting, including level age of people (children and adult, younger and older) and strenuous physical activity (Webster, 2004).

Normally, the blood sample is being processed in laboratory by using chemical electronic device called hemocytometer or hematology analyzer. Such procedure is very dependent on the lab technologist's skill to count the cells by viewing the sample through microscope. The process of counting facing the major problem when the cells are overlapped and usually such finding is neglected. Despite its long clinical success, this method requires an expertise to manually classify the cells which is tedious, time-consuming and qualitative process (Venkatalakshmi, 2013). In addition, the existing method contributes to inaccuracy, inconsistency and poor reliability diagnosis that may lead to false diagnosis situation. In order to overcome the problem, an image processing technique is increasingly recognized as a very useful technique for the automated RBC count analysis. The counting technique of microscopic smear image requires three main steps 1) segmentation, 2) post processing and 3) clumped cell estimation (Habibzadeh, 2013, Mohammed, 2014).

The first segmentation step is very crucial because the accuracy of the next steps depend on the correct segmentation of the solitary red blood cells. It is also a difficult and challenging problem due to the complex nature of the cells and uncertainty in microscopic image (Kim, 2001). In most recent studies, various direct decision methods of segmentation technique have been developed. However, the main weaknesses of these methods lead to difficulties of correction process after a wrong decision has been made. As example in clustering process, the region between RBC, WBC and background is mixing together since both color components are very close to each other. In addition, a wrong clustering and scattering can lead to a similar color pixel between cell and plasma as a background. Consequently, contributing to unclear boundary between them (Rane, 2014, Chinwaraphat, 2008). Previous research has identified that the segmentation of blood cell is more exposed to errors in segmenting RBC from cytoplasm region of WBC due to a close color similarity between them in the complex nature of blood cells environment. Thus, it was considered to

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examine boundary point and conduct a pixel adjustment. However, errors were found to occur in the process of differentiating color pixel in RGB color spaces for color segmentation. A statistical model approach has been successfully demonstrated as the most practical method for segmentation of image that has special boundaries and texture distribution (Rongtai, 2012).

In order to diminish noise as a result of segmentation, a morphological operation such as dilation and erosion were widely applied. Dilation adds pixels to the boundary of object in the image while erosion removes the pixels of boundary (Hamghalam, 2009, Angulo, 2003). Morphological operators also include a few steps, which are filling holes, area calculation, template calculation, opening, closing, and reconstruction. Mathematical morphological operators used to segment RBC by eliminating WBC appearance (Ruberto, 2000, Adagale, 2013). It is also used for extracting image components and useful information in representing or describing the region of shape such as boundary, skeleton and texture.

Clumped and overlapped cells in the blood images are very challenging issue in order to count RBC accurately. Several methods have been proposed to predicting the clumped cell regions. Some of them are based on Watershed and Distance transform (Huang, 2010, Sharif, 2012), morphological (Angulo, 2003), Appearance model (Rongtai, 2012), distance information (Hamghalam, 2009) and Hough transform (Venkatalakshmi, 2013).

In this paper we propose a computer aided system for automatically detect, and counting number of red blood cell (RBC) in blood smear image including the one in the clumped area. The construct system is difference in a sense that instead of running the clumped cell estimator in the whole image, we only run it in the clumped cell region. This procedure significantly reduces the processing time and also increase the system accuracy. The rest of the paper is organized as follows: Section 2 presents the architecture of the proposed system; Next, Section 3 shows experimental results with discussion; and finally, the conclusions and future research are presented in Section 4.

SYSTEM OVERVIEW

Introduction

The proposed method pipeline for automatically counting the number of RBC is outlined in Figure-1. It is operated by first acquired input image from a light microscope that attached with an eye piece static camera. Initially, the captured RGB image is converted into a single component color representation to make it convenient for the next processing. Next, the foreground is distinguished from the background by using adaptive global threshold method follow by low-level image post-processing methods in order to create a solid and noise-free foreground pixel map. Later, the connected regions of the foreground map are grouped together to identify overlapping and non-overlapping region. For the former region, further analysis is performed to predict the number of connected cells. In this paper two estimation algorithms which are Distance transform and Hough transform is investigate to determine its capability for separating the cells. Towards the end, the performances of the method are evaluated for accurately counting the number of the red blood cell.

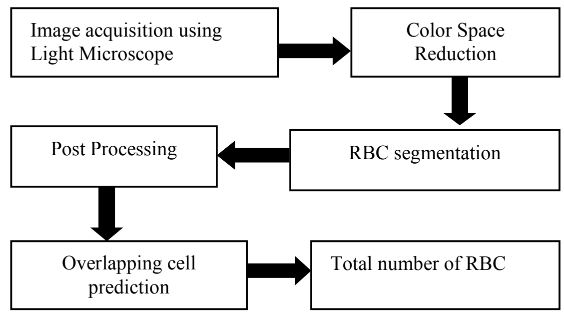

Figure-1. Method pipeline.

Image acquisition

The images are acquired from light microscope that equipped with DinoEye Eyepiece Camera as shown in Figure-2, and the process of capturing the image will involve blood smear process to the prepared sample. Blood smear is a process of preparation blood specimen on the slide that observed under microscope. The process for displaying the RBC image will involve digitization of image from the optical image with 40 times (40X) objective which equal to approximately 400 magnification.

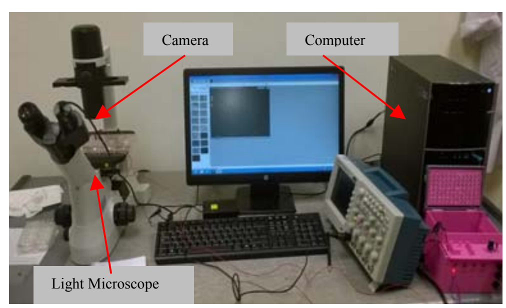

Figure-2. Image acquisition equipment.

Color space reduction

For the red blood cell counting, appearance of the RBC with respect to its background plays an important role. The more significant the appearance, the better it will be and generally RGB color information only gives just small effect. Because of that, the RGB image is transformed into a single channel color representation for an efficient computation of a look-up table for detecting the RBC and consequently counting its corresponding number. In this project, the individual component of red, green and blue channel is investigated to determine an optimal colour channel that capable to distinguish between red blood cell and the background. Figure-2 shows sample of the results where (a) is the RGB image, (b),(c) and (d)

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indicate the corresponding component of red, green and blue in the RGB image and (e) illustrate the histogram of the corresponding color components . It can be seen that, the red colour component unable to precisely distinguish between the RBC and the background while the green and channel able to produce a better result. However, among the green and blue channel, qualitatively the green component gives the best contrast (wide histogram distribution) between the RBC and the background, and hence was selected for segmenting the RBC in the next section.

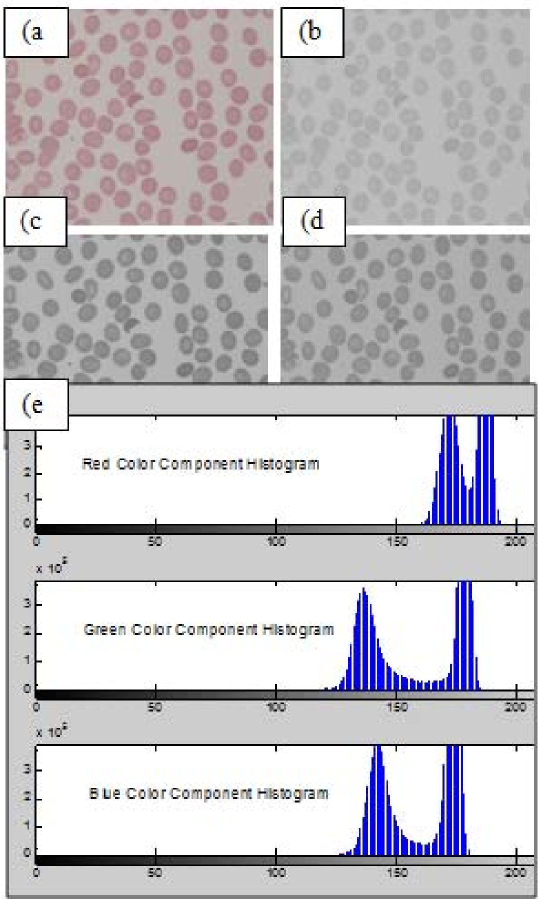

Figure-3. Color component selection. (a) RGB image (b) Red color component (c) Green color component (d) Blue color component (e) Color components histogram.

RBC segmentation and post processing

The image segmentation process is mainly implemented to partition an image into a region of homogenous representation corresponds to the object of interest in the image. Overall performance of an automated RBC classification system is considerably depends on its ability to segment the RBC region in the observed image accurately. A subsequent action, such as analyzing or identifying objects, requires an accurate extraction of the foreground objects, making the image segmentation a crucial part of the system. In this paper, an Otsu adaptive threshold strategy (Otsu, 1979) is applied in the green channel of the RBC image to separating between two classes of region. This method works by finding threshold value that minimizes the weighted within class variance. Sample of the produced binary image as a result of Otsu segmentation process of green channel image (Figure-4 (a)) is shown in Figure-4 (b). It can be seen that, even though the attained image capable to detect the RBC region, there are still some noise and holes exist. To overcome such problems, a series of post processing method is applied.

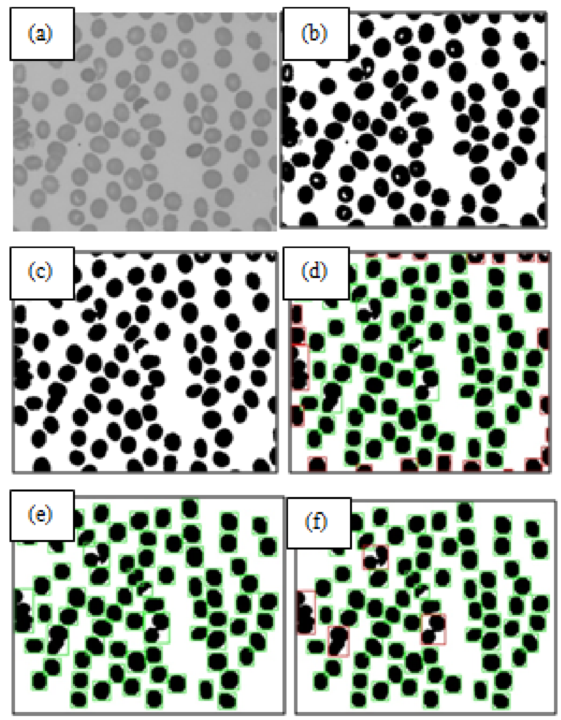

Figure-4. (a) Green channel image (b) Segmentation using Otsu Method (c) Morphological operation (erosion dilation and region filling) (d) Cell in border identification (e) Removing cell in border (f) Detecting overlapping cell.

The post processing aim is to remove noise and unwanted foreground cell from the segmented image. This process is crucial since the noise can significantly affect system ability to determine the RBC region accurately. In this paper, we use three methods which are morphological operation, connected component labeling (CCL) and bounding box filter to remove the unnecessary items.

Morphological operation works on binary image to change the size, shape, structure and connectivity of objects by using a structuring element and a set operator define by Erosion and Dilation. Erosion plays the role to ‘shrinks’ and ‘thins’ objects in image while dilation used to ‘grows’ and ‘thickens’ objects in image. The

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combination of both operators can be use to removes, break connection, clearing border and filling up holes. In this project, a sequence of two times of Erosion, two times of Dilation and contour filling algorithm is used to diminish the small noise and holes inside the cell. Sample output as a result of such process is shown in Figure-4(c). It can be seen that, solid cell's shape is attained and small noise was successfully eliminated. Once such cells are in hand, the object candidates are labeled via connected component labeling (CCL) as can seen in Figure-4(d). The bounding boxes indicate the minimum and maximum rectangular cell location in the image. Since cell object in the border does not provide valuable information, it was removed by detecting minimum and maximum *x* and *y* bounding box locations that touch the image boundary (Red rectangle in Figure-4(d)). Figure-4(e) shows result after performing the mentioned process where the cell in border was successfully removed. Finally, the single and clump cells are identified by calculating the cell area. The former cell can be count directly while the latter will be processed further to estimate the number of cells in the clumped area.

Cell estimation from clumped RBC area

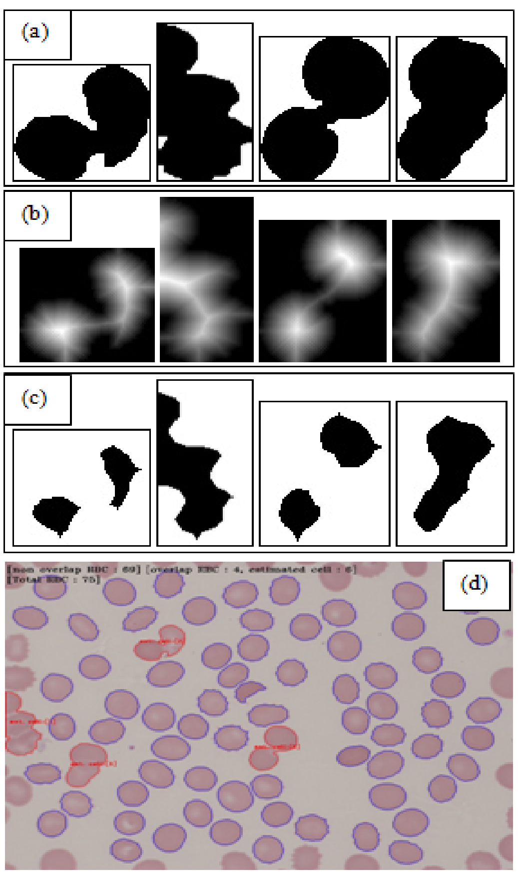

Figure-5. (a) Clumped cells area (b) Distance Transform result (c) Threshold distance transform region (d) Estimation number of cells in the clumped region.

Distance transform (Pedro, 2012) is an operator mask applied to images to determine the distance map of objects in the image. The Euclidean distance metric is generally a preferred cost function for measuring the distance. Because of such operation, the result of the transformation is a gray level image that looks similar to the input image, except that the gray level intensities of points inside foreground regions are changed to show the distance to the closest boundary from each point to the nearest zero pixel. In this paper, distance transform is applied to the clumped cell region as in Figure-5(a), and the result of such process can be seen in Figure-5(b). It can be seen that, the intensity in the objects varied with brightest level in the center of object and slowly decreased as it reach the object border. Once the distance map is obtained, a threshold value is applied to separate the clumped cells. Empirically the value of 10 is selected to satisfy such requirement. Any pixel intensity in the object that is lower than 10 are assumed to be a background and the remaining is a foreground. Figure-5(c) shows the obtained image after the threshold operation and Figure-5(d) indicate the estimated number of cells in the clumped region.

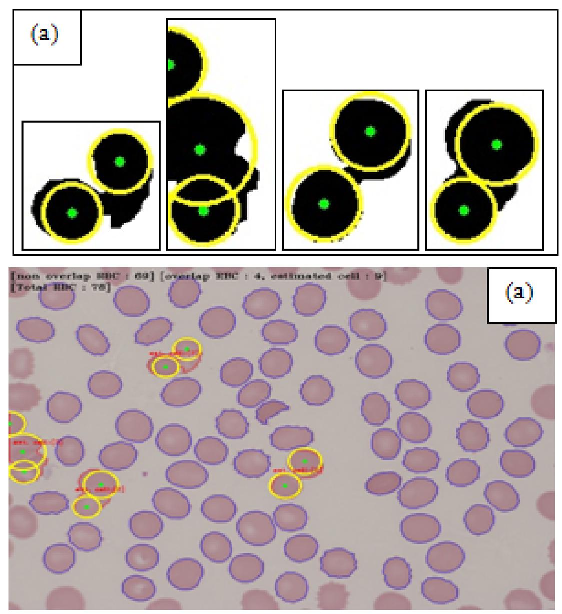

Figure-6. (a) Hough circle estimation of the clumped cells (b) Estimation number of cells in the clumped region.

The Hough transform (Duda, 1972) is a feature extraction technique used in image analysis, computer vision for finding lines, circles or other simple form in an image. It was initially suggested as a method for line detection in edge maps of images, then extended to detect general low-parametric objects such as circles .To estimate the RBC in the clumped region, only an estimation of circle is required, therefore this paper focusing on Hough Circle Transform (HCT). HCT works by initially converting the input image into edges via Canny edge detector (Canny, 1986). Following that, local gradient is

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calculated in every nonzero point in the edge maps by using Sobel filter. Using the gradient, every point along the line indicated by this slope, from a minimum to maximum is incremented in the accumulator. The candidate center is then selected from the accumulator points that provide the highest value. The clumped image cell as in Figure-5 (a) is fed to the HCT algorithm for cell estimation procedure. To determine optimal circle number, there are four parameters that need to be tune which are accumulator threshold value, minimum distance that must exist between two circles to consider them as distinct circle, minimum radius and maximum radius. In our work, such parameters are empirically determined to be 19, 12, 8 and 29 respectively. Sample of the estimated cell is shown in Figure-6(a) and the estimated number is depicted in Figure-6(b).

EXPERIMENTAL RESULT

In this section, the performance of RBC counting system is evaluated from smeared blood images obtained from the light microscope. The system was developed using Microsoft Visual Studio with OpenCV 2.4.7 and runs on 2.4 GHz i5-450M processor. We measured the performance on four sample images labeled im_1, im_2, im_3, and im_4 as shown in Figure-7 based on the ability to correctly estimate the number of cell including in the clumped region.

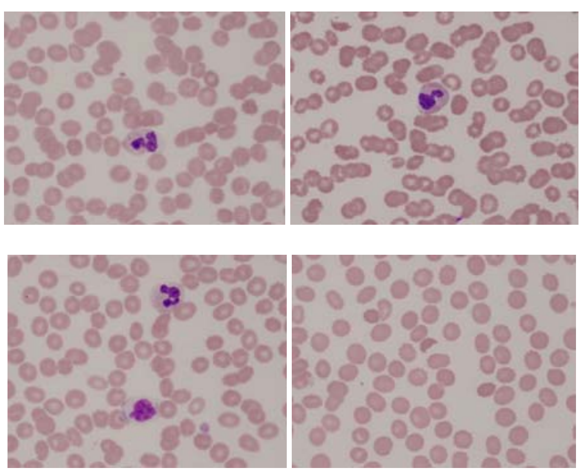

Figure-7. Images used for evaluating system performance. From top left to bottom right (im_1, im_2, im_3, im_4).

Table-1 summarizes results of the counting performance. It can be seen that, Distance transform and HCT capable to correctly count the number of cell with 78% and 94 % average performance respectively. This means that HCT shows a good performance for estimating the number of cells in the clumped region with an acceptable false alarm error. HCT tend to fail only when the RBC shape looks like an ellipse and when cells overlapped too close with each other. As for the Distance transform, from our experiments it shows that it unable to estimate the clumped region accurately. The distance transform seems to fail when the dimension of the cells in the clumped region is inconsistent and when the degree of overlapping between cells is high, e.g., more than 50%.

Figure-8 and Figure-9 show the result of overall RBC counting by using Distance transform and Hough transform estimator respectively. The single cell is denoted by blue boundary while the clumped cell is mark by red boundary. Yellow circle in Figure-9 indicate the estimated circle using Hough transform. The result Hough transform shows a promising outcome over the Distance transform for completing its task to estimate the clumped region in the captured images.

Table-1. Result of total RBC counting by estimating the clumped regions using distance transform and Hough transform**.**

Image	Dist. transform	Hough transform	Manual count	Accuracy (%)	
				DT	HT
im_1	64	94	110	58	86
im_2	75	93	103	73	90
im_3	74	85	87	85	98
im_4	75	78	78	96	100

CONCLUSIONS

In this paper, a framework to automatically classify the RBC into overlap, normal and abnormal cluster is proposed. The system consists of combination of three main blocks which are segmentation and processing block, feature extraction block and classification block. Each of the algorithms in blocks gave a good performance during task completion with an acceptable error. Otsu segmentation method applied on green color channel image with a series of post processing filter (morphological and connected component labeling) is found to effectively prune out solid RBC shape from the background. For the feature clumped cells region, the Distance transform and Hough transform are able to separate the cell area with an average accuracy of 78% and 94% respectively. Apparently, it can be conclude that the Hough transform shows more promising result compared to the Distance transform. The bottleneck is only that Hough transform required many parameters to be tuned to determine an optimal output.

In future, the system can be improved by analyzing more overlapping cell estimator and evaluate the with more sample sets to determine the generality of the framework.

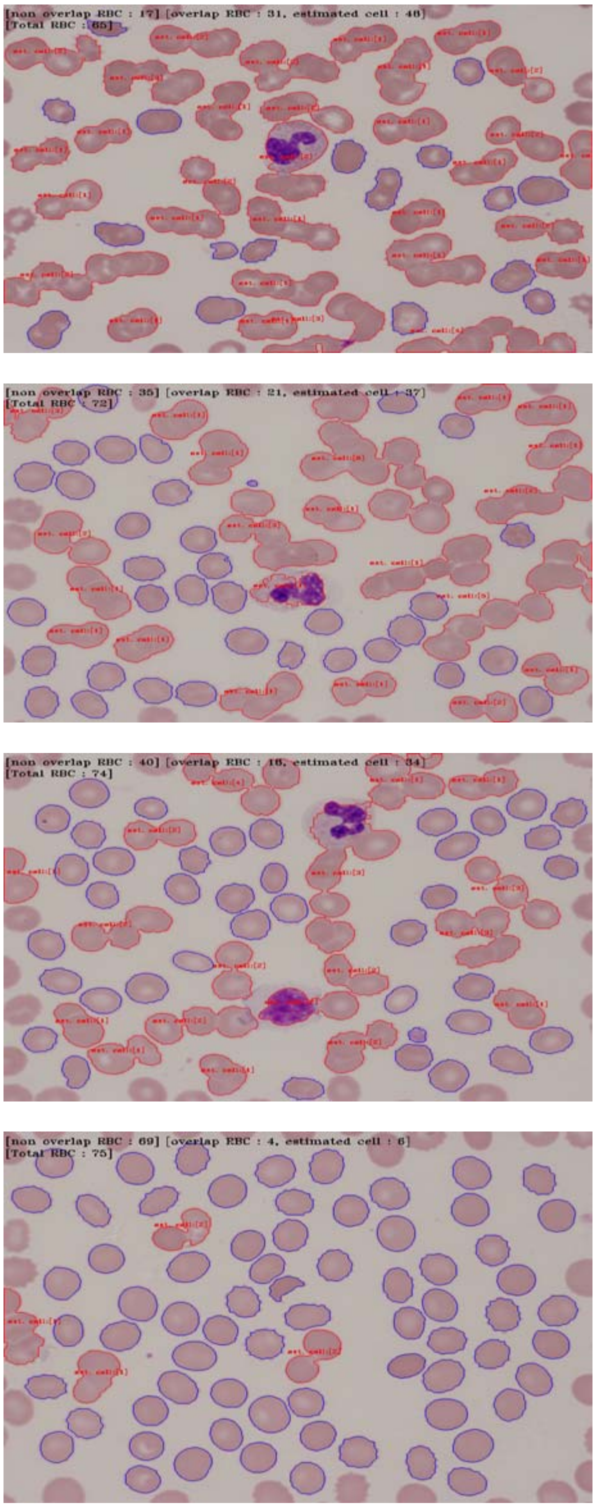

Figure-8. Result of cell counting by using distance transforms assisted clump cell estimation. From top to bottom (im_1,im_2,im_3, im_4).

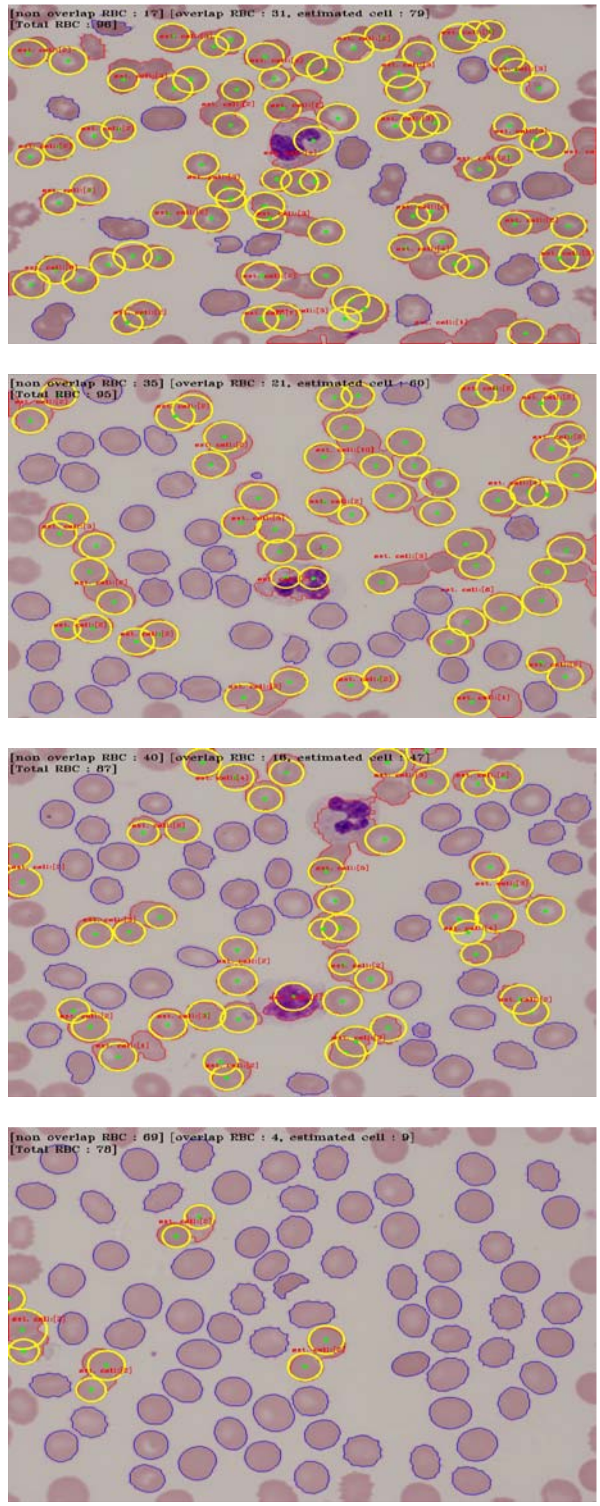

Figure-9. Result of cell counting by using Hough transforms assisted clump cell estimation. From top to bottom (im_1,im_2,im_3, im_4).

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