Segmentation Based Approach to Detect Parasites and RBCs in Blood Cell Images

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ABSTRACT

This paper presents an approach to detect the parasites and RBCs in the blood cell images in view of malaria detection. We have used S and V component images of HSV color model. These images are segmented by using Zack's thresholding technique, sequential edge linking algorithm, Euclidian distance based clustering. Proposed approach is implemented with Pathology and CDC DPDx blood cell images.

Categories and Subject Descriptors

I.4 [**Image Processing and Computer Vision**]: Segmentation – *edge and feature detection.*

General Terms

Algorithms, Documentation, Performance, Design, Experimentation

Keywords

Image segmentation, image thresholding, clustering, parasites and RBC

1. INTRODUCTION

Most of the diseases are caused due to the blood. Malaria is one of those diseases. For the detection of the malaria, generally the blood samples are analyzed manually with the help of microscope. Many of the researchers have contributed in this area regarding the analysis of blood cell images by using the different image processing techniques. This paper presents the approach for the detection of the parasites and RBC count in the available blood cell image for the evaluation of malaria disease. Image segmentation technique is used for the analysis of the blood cell image.

This paper is organized as follows: Section 2 deals with the discussion on the available literature related to the blood cell image analysis of various diseases. Proposed approach is described in the section 3. Section 4 presents the experimental results. Conclusion and future scope is discussed in section 5 followed by the references in section 6.

2. RELATED WORK

In literature, different approaches are reported for the microscopic medical image analysis from disease detection point of view. Here, in this section, we are summarizing few of the approaches related to the medical image analysis. In general the images are the microscopic images.

Makkapati and Rao [1] explored the segmentation for HSV color space. A scheme presented in [1] is based on HSV color space that segments Red Blood Cells and parasites by detecting dominant hue range and by calculating optimal saturation thresholds. Methods that are less computation-intensive than existing approaches are presented to remove artifacts. The scheme is evaluated using images taken from Leishman-stained blood smears. Sensitivity of the scheme was found to be 83%. The method operates in HSV space and is dynamic in the sense that relevant thresholds are determined from the statistics of the given image rather than keeping them fixed for all images. Schemes determine optimal saturation thresholds to segment RBCs and chromatin dots that are robust with respect to the color variability encountered. The work in [1] illustrates the use of color image processing techniques.

Raviraja and et al. [2] introduces a blood image processing for detecting and classifying malarial parasites in images of Giemsa stained blood slides, in order to evaluate the parasitamia of the blood. To detect the red blood cells that are infected by malarial parasites, statistical based approach is used. To separate automatically the parasites (trophozoites, schizonts and gametocytes) from the rest of an infected blood image, color, shape and size information are used and later the image is compared with infected images after transformation of image by scaling, shaping to reconstruct the image. The images returned are statistically analyzed and compare to generate a mathematical base. Also the evaluation of the size and shape of the nuclei of the parasite is also considered.

Meyer and Beucher [3] uses marker constrained watershed algorithm for image segmentation which uses only the binary images and distance transform can lead to over segmentation. If the image objects are irregular in shape and the objects are overlapping and touching can lead to under segmentation. Only the binary images are suitable for this operation. To avoid problem of under segmentation and over segmentation, Rao and Dempster [4] suggests morphological area opening on distance transform for choosing markers to avoid over segmentation and under segmentation. This given technique is also suitable for grey image consideration if large variations in pixels within the image component. Rao and Dempster [5] give the improvement over the

calculating granulometry directly. They have introduced the technique for estimating the patches in the image component. The presented technique in [5] will compute granulometry function by two-stage process and helps to find out the size of patches and granulometry function for microscopic images of blood sample.

Ruberto et al. [6] uses the morphological method for detecting malaria parasites in Giemsa stained blood slide. The two facilities provided by [6] to detect parasites by selecting automatic thresholding based on morphological approach using Granulometries, evaluate the size of the RBC and the nuclei of parasite. The regional maxima mark the nuclei of the parasite. The presented method automatically identifies the parasite by using color and size information extracted by morphological approach. The filters component of saturation and hue are used after Granulometry analysis.

The architecture presented in [7] of a system of quantitative microscopy which yields an integrated platform for the morphological image analysis, pattern recognition, and visual content-based indexing of peripheral blood smear wide microscopic fields. This image analysis helps pathologists to diagnose lymphoproliferative disorders. A global image-based approach is used for the analysis and indexation of peripheral blood smear teleslide images which provides an objective description and classification of blood cells and which is designed to assist pathologist to diagnose haematological disorders in first phase. In second phase, erythrocyte morphology (shape and color) and erythrocyte inclusions yield important knowledge for detection of malaria status and seriousness.

Ruberto et al. [8] introduces morphological approach to cell image segmentation more accurate than the normal watershed based algorithm. The used non-flat disk-shape structuring element enhanced the roundness and compactness to improving the accuracy of normal watershed based algorithm whereas flat disk-shape structuring element to separate overlapping cells. These methods make use of knowledge of the RBC structure that is not used in existing watershed based algorithm.

The approach in [9] accounts for uncertain imaging conditions due to microscope settings as well as the quality of the blood smear preparation. In order to tackle in homogeneous backside illumination, compensation of imaging variability was carried out. The parasitaemia measure was carried out by partitioning the uninfected and infected cells using an unsupervised and in comparison a training-based technique. The obtained estimates were analyzed with respect to manually acquired results from professionals. Red blood cells detection by using a training based method, will improved the precision and recall rates. Based on pattern matching with parameter optimization and cross-validation against the expected biological characteristics, Red blood cells are determined. The selection of the infected cells out of the set of found RBCs was carried out using Variance based while the second one uses a color co-occurrence matrix.

The application of image processing technique is also reported in literature for the other type of medical images like cancer. Some of the related work is discussed here.

Breast cancer continues to be a significant public health problem among women around the world. It has become the number one cause of cancer deaths amongst women. The key to improve the breast cancer prognosis is by early detection. The important sign for the breast cancer detection is the presence of Lesion such as

microcalcification clusters (MCCs). The mammogram- based approach is particularly suitable for detecting this type of lesion. To date, mammography remains the most effective diagnostic techniques for early breast cancer detection. While mammography is the best detection of breast cancer available today, however, not all breast cancer can be detected by mammograms [10]. For MCCs, the interpretations of their presence are very difficult because of its morphological features. Other segmentation, thresholding and classification techniques for MCCs identification are as follows:

Yu and Guan developed a CAD system that based on feature extraction and neural network classifier [11]. Firstly, the potential MCCs pixels are segmented out using wavelet features and grey level statistical features. Then, a multilayer feed forward neural network classifier was implemented to generate a likelihood map of potential MCCs. Secondly, an individual MCC is detected by 31 features that extracted from the potential individual MCCs objects. General regression neural network was implemented to analyze and select the most discriminatory features.

A research conducted by Lee and et al. [12] presented other automatic detection and classification of MCCs. A block region growing and k-means clustering-based thresholding is employed to extract the breast region. Then, a blanket method finds and locates the suspicious areas of possible MCCs clusters. The MCCs detection module is developed to automatically extract the MCCs from the ROIs. Among the image processing techniques that are involved in this module are gradient enhancement, contrast enhancement and Gaussian filters. The segmentation of MCCs from the background is done using entropy-based thresholding. Shape cognition which is based on neural network-like shape recognition systems is introduced as a classification technique of MCCs. The system in [12] achieved as high as 95% classification rate with 93% detection rate.

Due to its ability in enhancing the local contrast of MCCs, [13] focused on the wavelet multiresolution analysis for the enhancement of MCCs. The seed growing technique is applied for the segmentation purpose. Two techniques are being used to classify the MCCs i.e. geometrical (shape features) classification and cluster classification. The system gives good performance qualities by achieved 82.3% for geometrical classification and 73.7% for cluster classification.

Verma and Zakos [14] presented a system which is based on fuzzy-neural and feature extraction techniques for detecting and diagnosing microcalcifications patterns in digital mammograms. A combination of three features, such as entropy, standard deviation, and number of pixels, is the best combination to distinguish a benign microcalcification pattern from one that is malignant. A fuzzy technique in conjunction with three features was used to detect a microcalcification pattern and a neural network to classify it into benign/malignant.

Liu et al. [15] presented an adaptive automatic threshold segmentation based on the RGB and HSI color spaces to segment suspected cancer cells and nucleus from the complex backgrounds in the microscopic images. The nucleuses are segmented in RGB space whereas the cytolympths are segmented in HSI space. The feature values (perimeter, area, height, width, circularity, and rectangularity) of suspected cancer cells are extracted by using the technology of eight-chain code tracking.

The software system [16] that provides expert diagnosis of breast

cancer based on three step of cytological image analysis. The first step is based on segmentation using an active contour for cell tracking and isolating of the nucleus in the studied image. Then from this nucleus, some textural features using the wavelet transforms are extracted to characterize image using its texture, so that malign texture can be differentiated from benign on the assumption that tumoral texture is different from the texture of other kinds of tissues. Finally, the obtained features will be introduced as the input vector of a Multi-Layer Perceptron (MLP), to classify the images into malign and benign ones.

The analysis of review of the malaria microscopic image analysis related work is presented in Table 1. The analysis of review of the other related work is presented in Table 2.

Table 1: Summarization of Review of Malaria Related Work

Segmentation Technique	Image selection/ component	Color Space	Morpho-logical Processing	Feature extraction	Boundary / contour detection	Remark	Reference
Adoptive threshold (Otsu's method)	H, S	HSV	√	×	×	Used Hue to identify background	[1]
Statistical based approach to generate mathematical base	RGB color image	RGB	×	√ color, shape and size	×	Used moment theorem	[2]
Marker constrained watershed algorithm	Binary image	RGB	√	×	×	Performance depend upon selecting right marker	[3]
Watershed with morphological area opening to choose marker	Binary image and grey scale	RGB	√	×	×	Avoid over and under segmentation	[4]
×	S	HSV	√	×	×	Calculate size of each RBC	[5]
Reginal maxima	H, S	HSV	√	√	√ Reginal maxima	Color and size obtained by morphological processing	[6]
Auto thresholding Watershed ,region growing	Green component to binary	RGB	√	√	×	Color and size obtained by morphological processing	[7]
Auto Watershed with morphological area opening	Green component to binary	RGB	√	√	×	Opening with disk shape, flat and nonflat	[8]
×	RGB color image	RGB	×	√ Variance based and cooccurane matrix	×	Handled uncertain imaging condition and used unsupervised and supervise both	[9]

Table 2: Summarization of Review of Cancer Related Work

Segmentation Technique	Image selection / component	Color Space	Morpho-logical Processing	Feature extraction	Boundary /contour detection	Remark	Reference
Wavelet feature plus grey level statistical feature	Grey	×	×	√ 31 features used	√	Multilayer FFNN classifier	[11]
Block region growing & k-means clustering based thresholding plus entropy based thresholding	Grey	×	×	√ shape recognition technique	√	ROI blanket method for boundary	[12]
Seed growing	Grey	×	×	√ shape	√	Geometrical & cluster classi-fication	[13]
Fixed Threshold (0.5 & >0.5)	Grey	×	×	√ Entropy, std. deviation, no. of pixels	√	Based on fuzzy-Neural approach	[14]
Auto threshold	B component & HSI	RGB & HSI	√	√ Perimeter, area, height, width, circularity, rectangularity	√ Eight chain code	Used eight chain code to extract feature	[15]
Active contour GVF snake	Grey	RGB	×	√ texture	√ GVF snake	Use multilayer perceptron to classify the images	[16]

3. PROPOSED APPROACH

On the basis of review of above literature for the microscopic blood cell images, it is found that different image segmentation techniques are applied on color images, grey images and binary images. The different types of color models also used like RGB, HSV and in that a G, B and H, S components are used respectively for the identification of parasites and RBC by using image processing concept. But for the identification of correct number of parasite and RBC, proper segmentation technique is required. Most of the authors have applied combine approach of various segmentation schemes with morphological processing to improve the results.

Being the reported work in literature is focused on the image segmentation, there is a scope to design some methodology or an approach for microscopic image segmentation.

3.1 Proposed Scheme

The presented scheme basically serves two purposes namely, parasite identification and RBC count. Hence, the presented scheme is divided into two phases. In first phase, chromatin dots are detected and in second phase, total numbers of RBC are counted. The presence of Howell Jolly bodies may also look like parasites when associated with blue stain deposits. So after first phase total numbers of blue stain deposited cells are counted including jolly bodies. The output of first phase and second phase will be useful to avoid the false counting of parasites by identifying pixel and object region. As presented scheme uses two phases, two different procedures are designed to achieve the desired results and these procedures are as follows:

Procedure to identify the parasites is as follows:

1) Collection of the microscopic blood cell images in RGB color space or model.

2) Conversion of the RGB color space image to HSV color space.

3) Extraction of S component image from HSV color space image.

4) Prepare the histogram of the S component image.

5) Use the Zack's method on histogram which gives the threshold value.

6) Calculated threshold value is not used directly to get the binary image, but some offset value is added to this threshold. This offset value is identified through some experimentation, and later it is found that the desirable value of this offset is 0.25 which is used in this procedure to get the binary image.

7) Do the hole filling using morphology and apply erosion with disk specification.

8) Calculate the Euclidean distance and form the clusters to get the contours boundary.

9) All contour shapes are filled with pseudo colors.

10) Finally count the total number of contour shapes with different pseudo colors which will provide the total count of the RBC cells.

Procedure to identify the RBC count is as follows:

1) Collection of the microscopic blood cell images in RGB color space or model.

2) Conversion of the RGB color space image to HSV color space.

3) Extraction of V component image from HSV color space image.

4) Prepare the histogram of the V component image.

5) Use the Zack's method on histogram (which gives the threshold value) to get the binary image.

6) Do the hole filling using morphology and apply erosion with disk specification.

7) Removal of small area contours using some particular threshold (it can be measured in terms of the total pixels covered by the contour shape), here the value of this threshold is 2000 and 100 for lab data and available data respectively.

8) Calculate the Euclidean distance and form the clusters to get the contours boundary.

9) Finally count the total number of contour shapes which will provide the total count of the RBC cells.

Block schematic of above two procedures is shown in Figure 1.
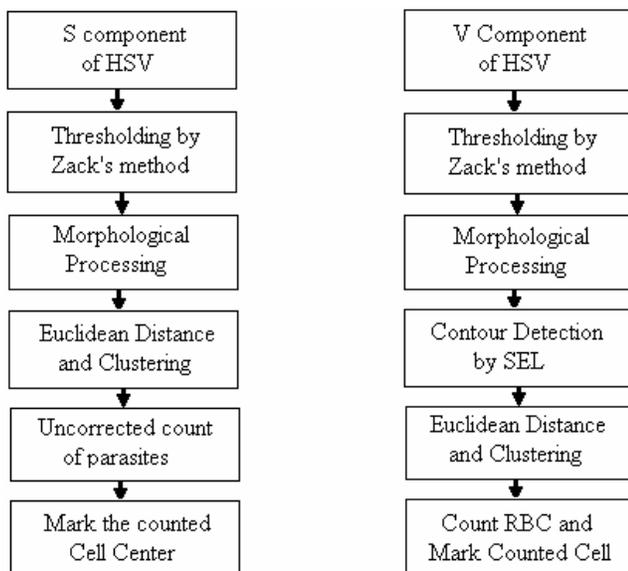
Figure 1: Block Schematic of Proposed Approach

3.2 Thresholding and Contour Detection by SEL

The technique due to Zack [17] is basically a graphical solution [18] and it gives effective result because some the object pixels produce a weak peak in the histogram. The found method also gives an optimal threshold for unimodal distributions of the histogram. A line is constructed between the maximum of the histogram at intensity b_{max} and the lowest value b_{min} in the image. The distance d between the line and the histogram h[b] is computed for all values of b from b = b_{min} to b = b_{max}. The brightness value b_o where the distance between h[b_o] and the line is maximal is the threshold value, i.e. t =b_o.

By the combination of thresholding and SEL (sequential edge linking) algorithm [19] accuracy of segmentation will increase. In SEL [19], edge decisions are made by using global information derived from past decisions on the existence of an edge pixel.

A path will be defined as a set of connected nodes with the following property: for any subset of three nodes r_1, r_2, r_3 in the ordered set, the directions of $r_1 \rightarrow r_2$ and $r_1 \rightarrow r_3$ differ by $\pi/4$ or

less. The path may be modeled as a kth-order Markov chain. SEL algorithm defines path metric to rank all the paths explored so far.

3.3 Euclidian Distance and Clustering

A cluster helps for the collection of objects which are "similar" between them. That is clustering refers to the classification of objects into groups according to certain properties of these objects. An important component of a clustering algorithm is the distance measure between data points.

If the components of the data instance vectors are all in the same physical units then it is possible that the simple Euclidean distance metric is sufficient to successfully group similar data instances. Cluster analysis is used to identified the parasites exist [20]. It will focus on detecting the white pixel with a max grey level value with four basic cluster analysis steps:

1. Collection of data.

2. Finding of a similar type of gray level value from image.

3. Used of Euclidean Distance to make a decision about number of clusters.

The Euclidean Distance is used to measure the mathematical distance between pixels and thus the pixels are classified to their nearest cluster. For the validation of cluster solution calculate the mean value for each data set-

a) Define the points of area with the same distance with the average value of data set.

b) All of the points defined determine the line of discrimination that will separate between clusters.

3.4 Avoiding False Count

The second phase of proposed approach finds out the contour of the red blood cell after performing thresholding operation on image. The segmented images are often found red blood cells have holes inside. So we will try to conquer these problems to a certain level by using morphological processing.

With the help of morphology, clustering and Euclidean distance performed on the segmented RBCs, it is possible to count the red cells.

The segmented image of phase one counts the number of parasite by the clustering and Euclidean distance. We find out and mark the center of each parasites boundary and red cells, say (k, l) and (i, j) respectively. By using the structuring element, radius will be calculated. The addition of two different transform image of same resolution will perform and decide whether the mark points (center) is within the area of red cells by using the formula $D_E \leq r_{RBC} + r_{parasite}$, where D_E is Euclidean distance, r_{RBC} is radius of RBCs and $r_{parasite}$ is the radius of parasites.

4. EXPERIMENTAL RESULTS

4.1 Image Database and Experimental Setup

The two types of malaria generally found in India one is vivax and other is falciparum but mostly the vivax is common in most of the people. The scheme is evaluated using images taken from Leishman-stained blood smears. The images for the experiment collected from the Druv Pathalogy Lab, Laxminagar, Nagpur,

Maharashtra (India) by the Dr. Sheela Mundhada. The images having a size of 1280 × 960 resolution which will increase the computation time or elapsed time. The given images infected by the malaria parasite having a type of vivax and faiciparum respectively. In total, the database contains 6 red blood samples infected by malaria parasites and original images are shown in Figure 2. In Figure 2, image (a) is DSC09250; image (b) is DSC09261; image (c) is DSC09262; image (d) is DSC09263; image (e) is DSC09264; image (f) is DSC09265. The group contains mainly red blood cells infected by immature ring-form Schizont and Gametocyte of Plasmodium vivax, Plasmodium falciparum. The manual data with respect to total numbers of parasites, their types and total number of RBC count are observed in the laboratory. Some other original image samples [21] as shown in Figure 3 are also used in experimentation. In Figure 3, image (a) is Trophozite; image (a) is Gamatocyte; image (a) is Schizont. Implementation is carried out with Matlab 7.3 and 2 GB RAM. Implementation results are summarized in the Table 3. Figure 4 shows the implementation screenshots. Results for Lab data is shown in Figure 5 and results for blood cell images from [21] are shown in Figure 6.

Table 3: Summarization of Results

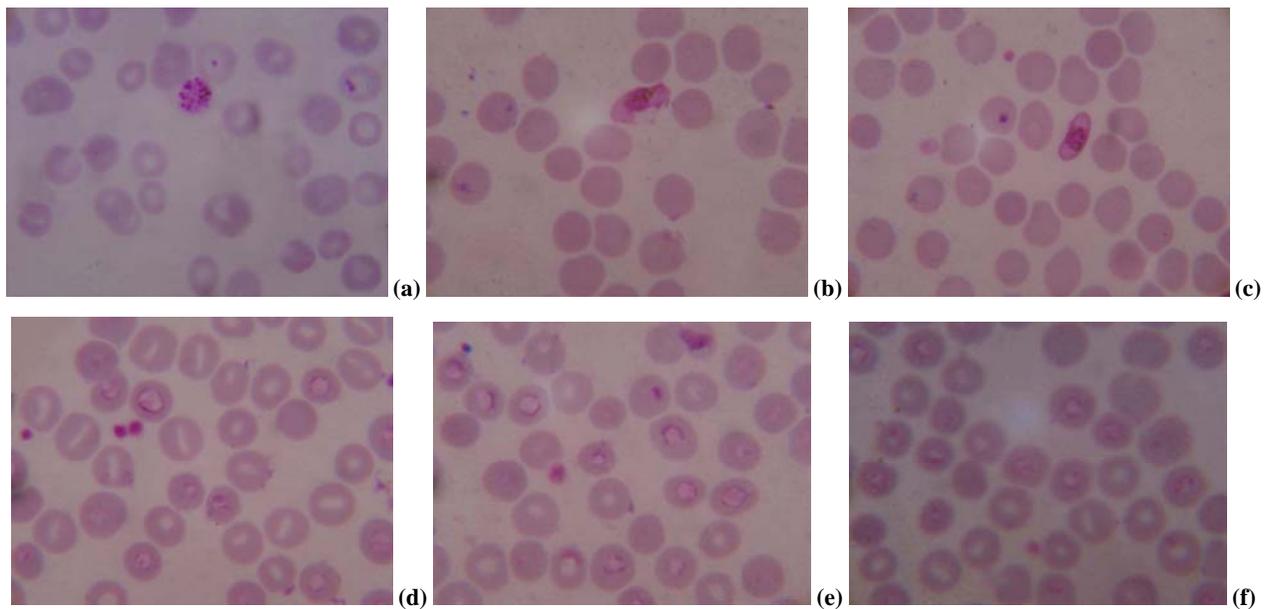| Image	Manual count of Parasites	Manual count of total RBC	Count of Parasites by proposed approach	Count of total RBC by proposed approach
DSC09250	3	34	4	19
DSC09261	5	29	6	28
DSC09262	5	43	5	35
DSC09263	16	42	16	38
DSC09264	17	42	15	35
DSC09265	22	40	23	26
Trophozite	NA	NA	2	10
Gamatocyte	NA	NA	2	11
Schizont	NA	NA	4	12

Figure 2: Original Blood Sample Images from Druv Pathology Lab
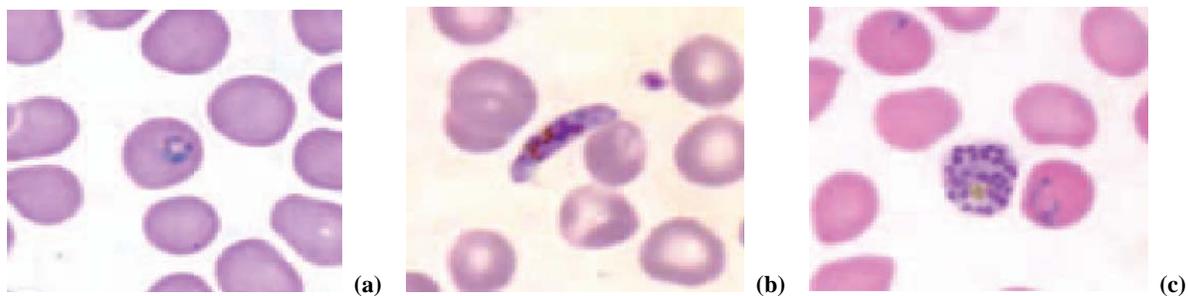
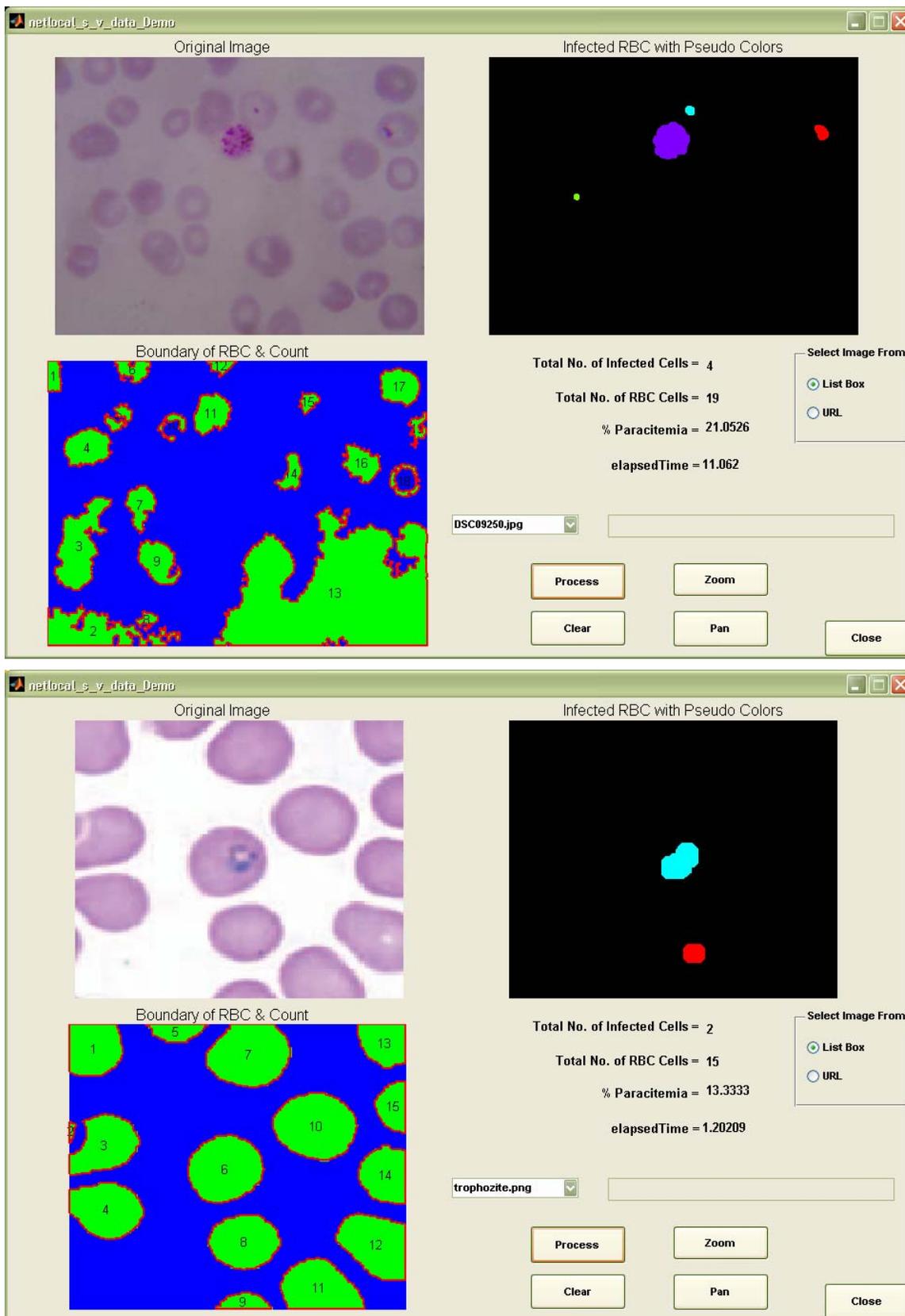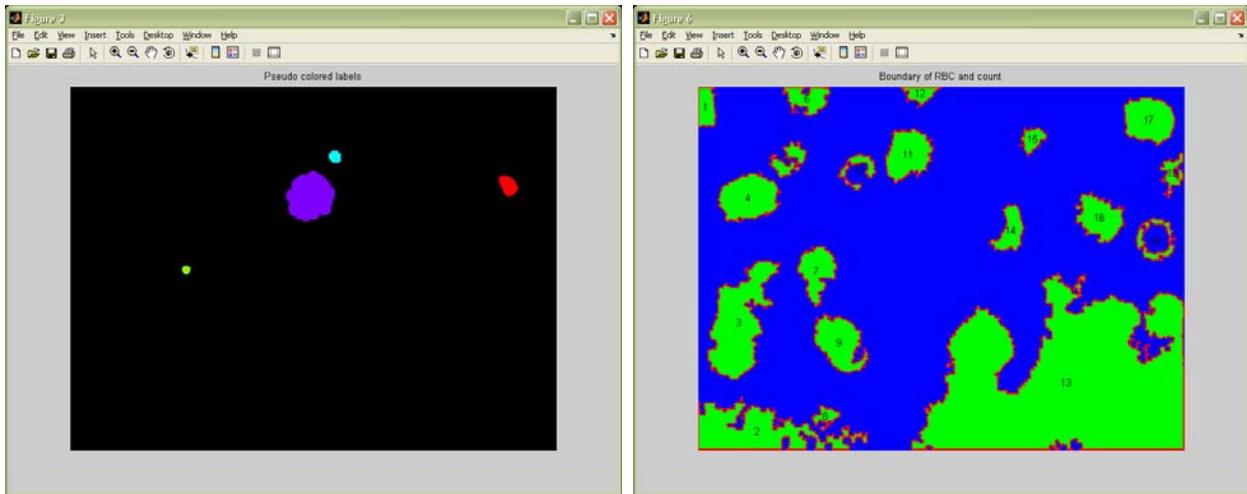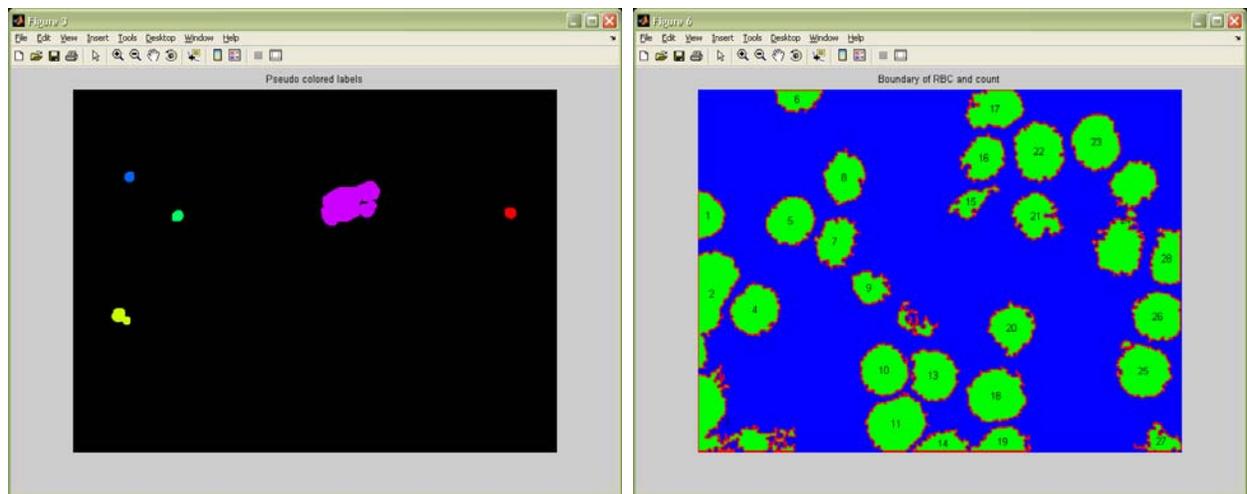**Figure 3: Original Image Samples [21]**

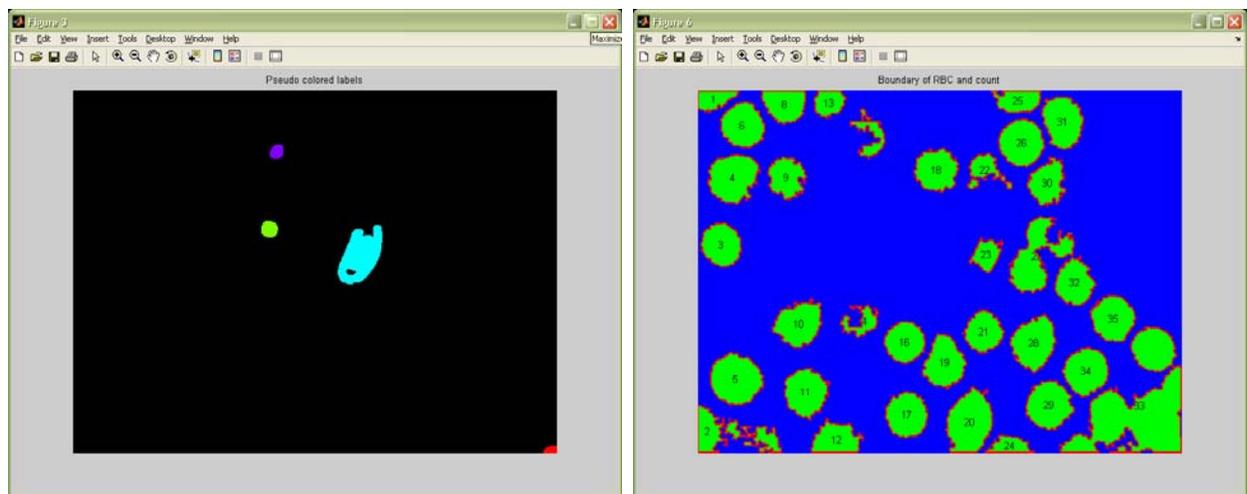**Figure 4: Main Screen of the Implementation with the Result of Image DSC09250 and Trophozite**
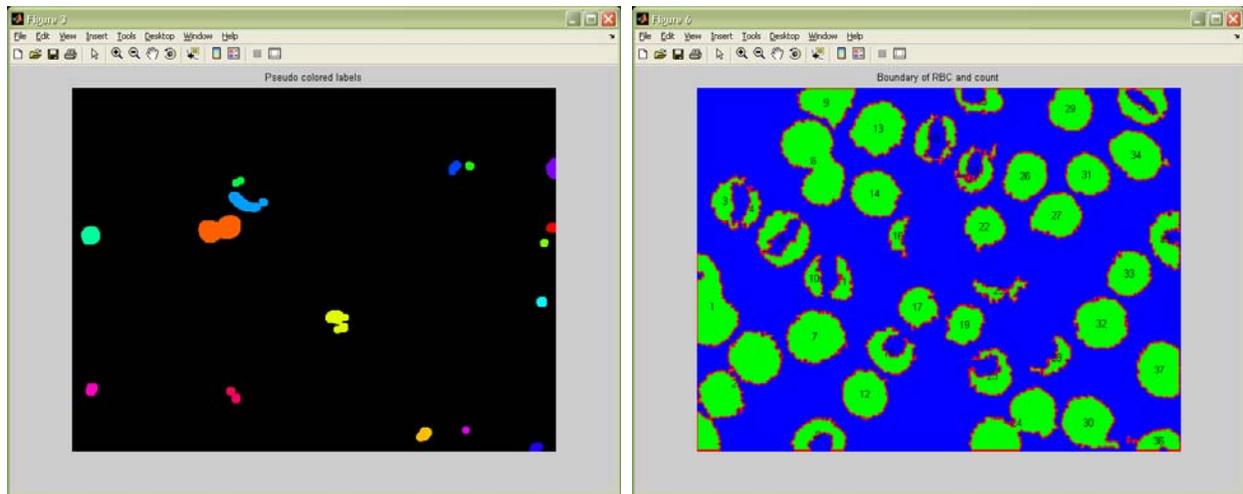
(a)

(b)

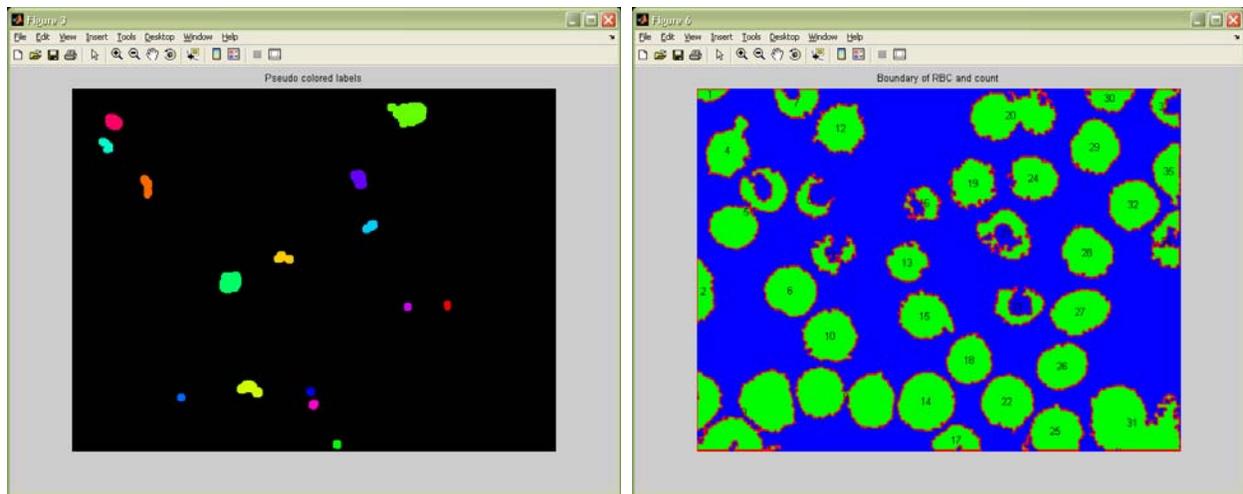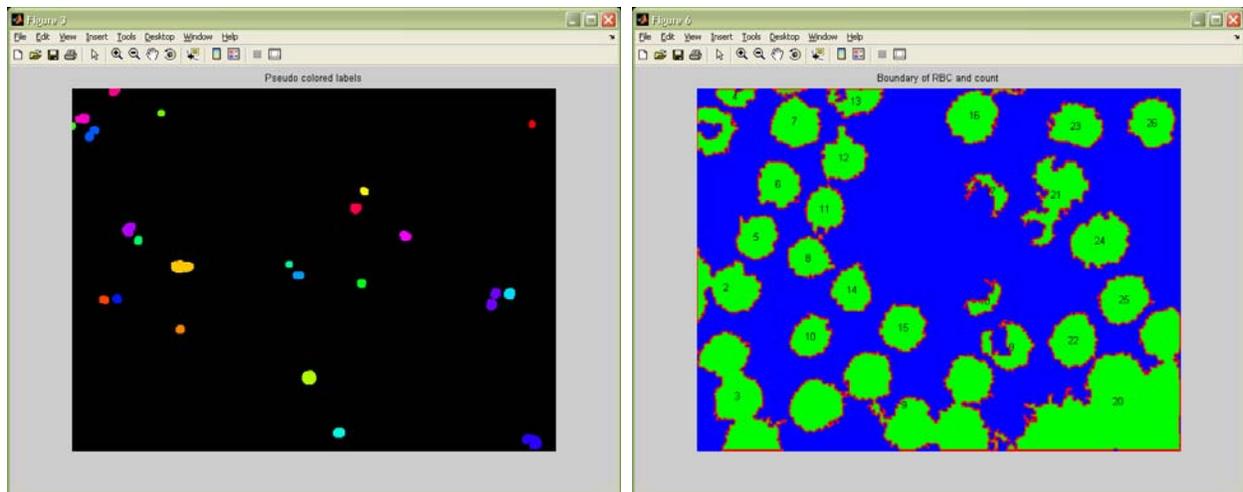**(c)**

Figure 5: Parasites and RBCs Segmentation Result of Image DSC09250, DSC09261, DSC09262
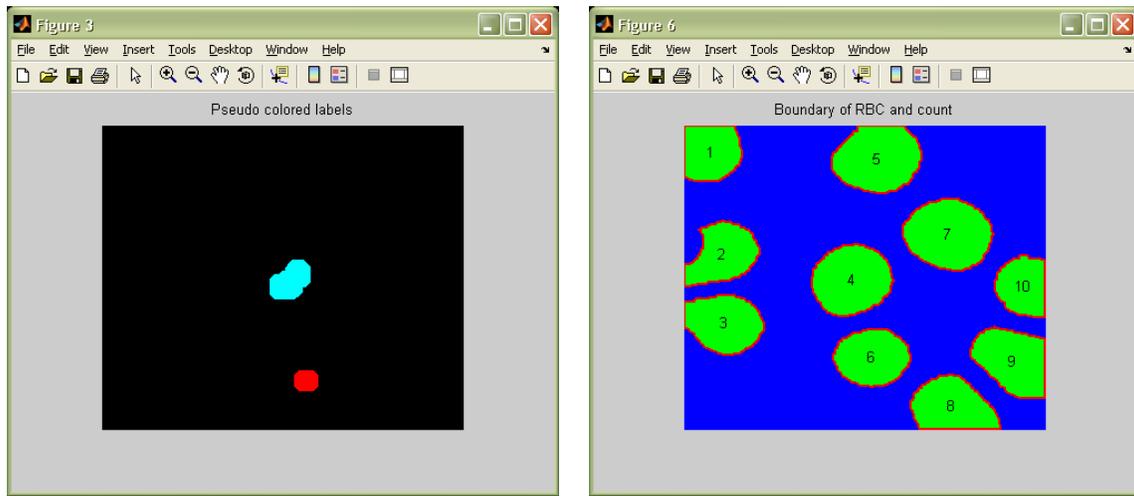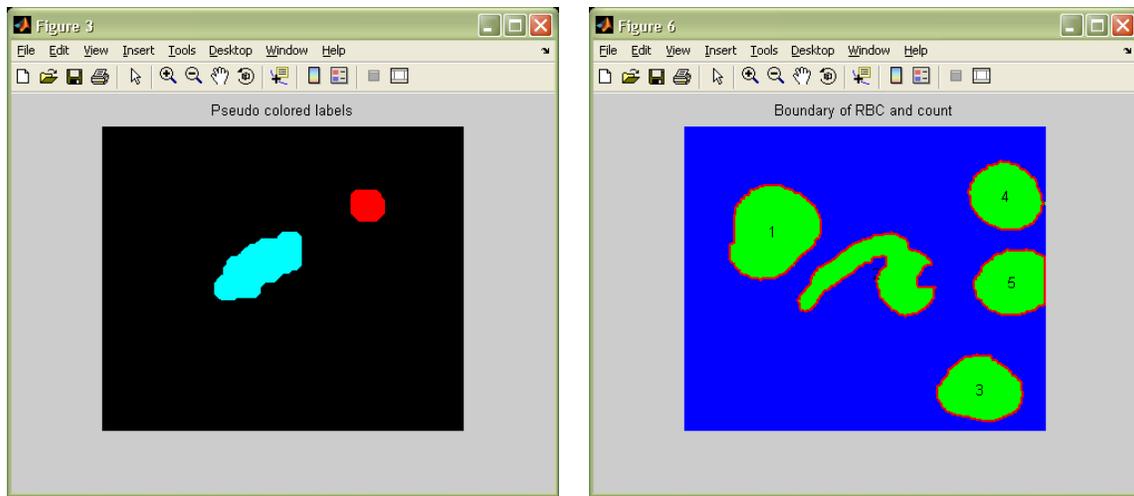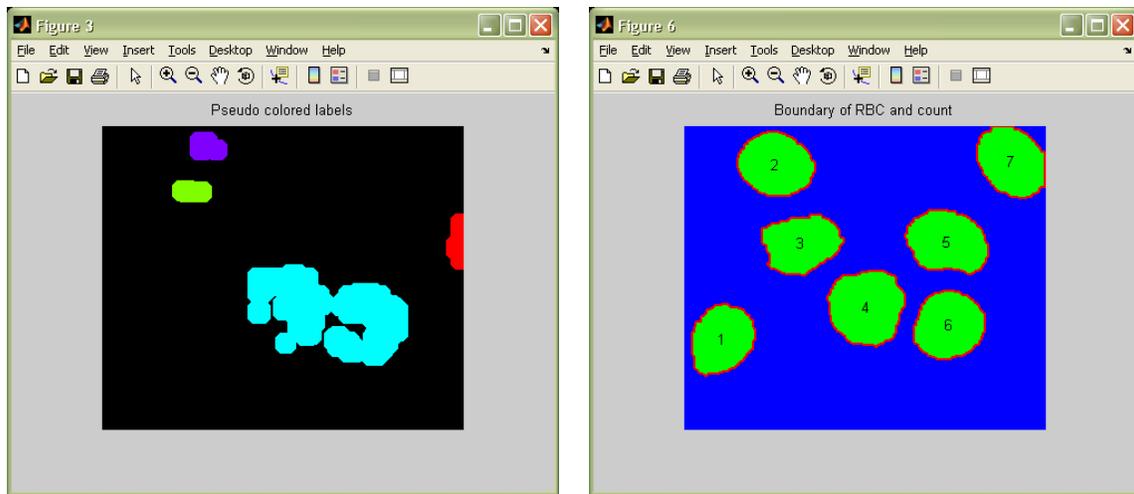
(d)

(e)

(f)

Figure 5: Parasites and RBCs Segmentation Result of Image DSC09263, DSC09264, DSC09265

(a)

(b)

(c)

Figure 6: Parasites and RBCs Segmentation Result of Image Trophozite, Gamatocyte and Schizont

5. CONCLUSION AND FUTURE SCOPE

After implementation of the proposed approach for the Lab sample images and for the available image database, it is found that the parasites count is near about matching with the manual count while in the RBCs count, some more difference is observed. Therefore, it become necessary to check the RBC count with the different plane image, for instance, S component only, in place of V component. From observation, it is clear that the proposed approach required less time in comparison with the manual evaluation. But for the available database image, the relevant data is not available so it becomes difficult for us to evaluate our approach. In future, we may go for the evaluation of proposed approach with S component only for the parasite and RBC count. Presently we have used the RGB color space images, in future; we would like to evaluate the proposed approach with different color spaces.

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