Automatic Detection and Classification of Malarial Parasite

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Abstract

Recent advancement in genomic technologies has opened a new realm for early detection of diseases that shows potential to overcome the drawbacks of manual detection technologies. Computer based malarial parasite analysis and classification has opened a new area for the early malaria detection that showed potential to overcome the drawbacks of manual strategies. This paper presented a method for automatic detection of malarial infected cells. Blood cell segmentation and morphological analysis is a challenging due complexity of the blood cells. To improve the performance of malaria parasite segmentation and classification, we have used different set of features which are forward to the ANN for malaria classification. We have used Rao's method and bounding box for segmentation whereas we have used BPNN for classification on different set of texture and shape features.

Keywords: Malaria Detection, Segmentation, RBC Classification, Malaria Classification.

1. INTRODUCTION

Blood smear image consists of white blood cell, red blood cells, platelets as well as other possible contents such as malarial parasite, Aids virus etc. scattered across the background. The contents of blood cell images are very complex. The segmentation and morphological of blood cell is very difficult due to the complex nature of the blood cell images. Manual blood cell counting is not a reliable screening method when it is performed by non-expert due to lake of training expertise as it requires special training and considerable expertise [1-2].

Recent advancement in pattern recognition and computer vision algorithms made possible that the analysis of complex blood cells can be performed my machine. Automatic equipment for blood cell counting such as flow cytometry and automatic counting machines can examine cell quantity but not qualitatively, thus 21% of blood analysis is still need expert review [3]. Machine based visual analysis methods provides quantitative as well as qualitative blood cells analysis whereas this research area is not very much explored [4]. Automatic recognition and inspection of blood can assist hematologists in analyzing the blood sample and diagnosing diseases like Aids, malaria and blood cancer. Various research works are going on analyzing the microscopic blood cell images in the field of immunology, infectious diseases, transplantation, hematological malignancy and vaccine development in order to diagnose the infection existence in human blood. Thus, several image processing and pattern recognition base algorithms have been presented in literature for the automatic segmentation and classification of malarial infected blood cell.

Malaria is transmitted via saliva of female mosquito through bite which introduce the organisms form her saliva. There are five species of malarial plasmodium that can infect the human blood and even can be transmitted by human. The majority of malarial patient deaths are caused by falciparum and vivax plasmodium as shown in figure 1. Thus, in case of malarial infection, species identification is necessary for proper treatment. There are some rules for malarial species identification as discussed in table 3. However it is not necessary that every parasite will exhibit the morphological characteristics. Typically, malaria parasite diagnoses is a manual counting

process that use microscopic examination of Giemsa-stained thick and thin blood smears. Manual identification and counting of malarial parasite infected cells is very long, tedious process. Moreover it is prone to technician's ability to conduct the process correctly that requires training and skills, i.e. a trained expert takes about 15 min to evaluate and count 100 cells and blood sample of millions of patients is performed every year [7]. Early detection of malaria is vital in order to ensure prompt and effective treatment. People suffering from malaria should be diagnosed and given effective, affordable drug treatment as early as possible. Microscopy is a standard technique used for diagnosing, however in remote areas reagents are limited, equipment and electricity are unreliable, and a delay in obtaining results may lead to incorrect initial treatment.

Automatic analysis of microscopic blood cell is a powerful diagnostic tool that improves accuracy, saves time and reduces the required manpower as well as minimizes human errors. Automatic malarial diagnose has larger interest especially for clinics and laboratories; however, blood cell segmentation and morphological analysis is a challenging problem due to both the complex cell nature and uncertainty in microscopic videos. This paper presents a method for classifying malarial parasites. The organization of the rest of the paper is as follow: Section II briefly reviews the malarial parasite recognition approaches discussed earlier and Section III presents the proposed methodology whereas conclusion is drawn in section IV.

	Microscopy	RDTs
Requirement	Electricity	None,
	Special Training	Basic Training,
	Staining Chemical	None
Time	~60 Minutes	15-20 Minutes
Cost		
Specification		
Detection Threshold	50 pal/ul	~100par/ul
Detection of all species	Yes	Some bands
Quantification	Yes	None
Specie Identification	Yes	None
Life Stage Identification	Yes	None

TABLE 1: Comparison of Microscopy Diagnosis and Rapid Diagnosis Tests [34].
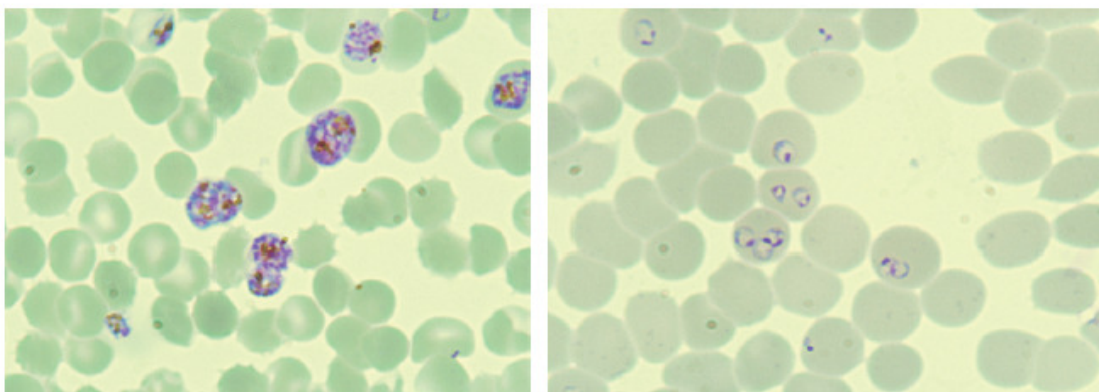

FIGURE 1: Malaria Effected Blood Images.

2. RELATED WORK
Microscopic Blood images consists of white blood cell, red blood cells, platelets as well as several other possible contents such as malarial parasite, Aids virus etc. that are scattered across blood

smear image. Due to the complexity of the images, cell identification and counting is time consuming job for biologist, e.g. expert requires 15 min to count and evaluate a slide. Moreover, it is painstaking and subjective job which also requires training and skills. Due to these complexities and hematologist restrictions, manual counting accuracy decreases and thus machine based blood classification system is essential for biologist in diagnosing disease. Machine based malarial diagnosis system can be designed by understanding the diagnostic expertise (hematologist knowledge) and representing it by specifically tailored image processing and pattern recognition algorithms. Several image processing based malarial diagnostic methods has been widely studied in order to provide early and accurate detection of malaria parasite. Table 2 summarized the recent development for malarial parasite identification.

Reference Article	Target Data	Preprocessing	Segmentation Method	Features	Classification Method	Accuracy (sensitivity /specificity)
Das et al. [10]	Malaria Parasite	Gray world assumption, geometric mean filter	Marker controlled watershed algorithm	80 textural & 16 Morphological features		Bayesian 98.10/68.91 SVM 96.62/88.51
Nasir et. al [23]	Malaria Parasite	Contrast enhancement	K-Mean		K-Mean Clustering	
Panchbhai et al. [24]	Malaria Parasite, RBC	Morphological Operation	Otsu Method			
Kumar et al. [21]	Malaria Parasite, RBC	Morphological Operation	Otsu Method		Threshold	
Savkare [29]	Malaria Parasite	Median Filter	Otsu Method	Geometrical, color and statistical chromatin size	SVM	92.26/99.09
Kaewkamnerd et al. [26]	Malaria Parasite	in-focus information merging	Histogram (HSV) and labeling			75% (pv), 90% (pf)
Ahirwar et al. [27]	Malaria Parasite	SUSAN	Threshold	Geometrical & Expert defined	Feed Forward Back propagation	
Yunda et al. [5]	Malaria Parasite	Morphological gradient method	AGNES and K-Median	Color Features and Textural	Multilayer Perceptron	77.19%
Savkare [20]	Malaria Parasite	Smoothing and Sharpening	Otsu Method	Geometrical, color and statistical	SVM	93.12%
Soni [29]	Malaria Parasite	Median and SUSAN	Region based (Watershed) Morphology	First Order Moment invariant	Tree	99
Tek et al. [7]	Malaria Parasite	Illumination correction, color correction and normalization	histogram-based thresholds and PDF	histogram & area granulometry features	KNN. FLD, BPNN	72:37/97:45
Diaz et al. [12]	Malaria Parasite	Low pass filter	Inclusion-Tree Structure and template based on EM	Color, Saturation level, Tamura texture and Sobel histograms	MLP SVM	94/98.7
Purwar et al. [30]	Malaria Parasite & RBC	Local Histogram equalization	energy minimization [54]	Pixel intensity	K-mean	100/88

TABLE 2: Summarization of Recent Development.

Most microscopes provide uniform or relatively uniform illumination images whereas several illumination and contrast enhancement technique have been applied in literature. One way to deal with uneven illumination is the predefined illumination correction but some time we don't have reference image [7]. Ruberto et al. used paraboloid modal for illumination correction [8]. Sriram et al. used diagonal modal for illumination modeling [9]. In diagonal modal, an image of unknown illumination is transformed to the known illuminant space by multiplying pixel values with a diagonal matrix. Das et al. performed gray world assumption for correcting illumination [10]. Diaz

used adaptive local low-pass filter to correct luminance differences on luminance channel [11-12]. Suradkar used the local histogram equalization for contrast enhancement of parasite and RBC [13] whereas Zou et al. and Sio et al. used adaptive histogram equalization [14] for image enhancement [15, 16]. The image is divided to several tiles and histogram shaping is applied to these tiles separately followed by bilinear interpolation to eliminate artificially induced boundaries. The filter was designed for a window size which contained the largest image feature, i.e. a typical erythrocyte size. Filter was selectively applied on higher luminance levels that represent the background pixels. Mehrjou used adaptive histogram shaping function for contrast enhancement [17]. Sabino et al. performed non-supervised nucleus region detection before nucleus color segmentation using the G channel from RGB color coordinates [18]. For colored images segmentation into ROIs, supervised classification method that is based on RGB color space is used.

In the identification of automatic malarial parasite classification procedures, the most important and complex phase is the segmentation of infected blood cells from other cells and background. There are several factors like cell shapes, light variation and noise that affect the accuracy of segmentation and make it complex task. Accurate segmentation allows fruitful result in subsequent levels. Blood cell segmentation can either be deductive or inductive. In deductive segmentation method, the microscopic image is first segmented into background and foreground images before segmenting the object whereas in inductive segmentation, the objects are located first by using intensity/RGB values followed by regions that contains stained. The recent has suggested several segmentation methods for blood cell summarized in table 2. Memeu et al. used both RGB and HIS for segmentation [19]. Green channel from RGB whereas hue and saturation channels are used for segmentation based on Otsu method and Zack algorithms for RBC and parasites respectively. The green channel gave good results for erythrocytes segmentation but it also added the parasite as part of the foreground. The hue component resulted to a binary image whose foreground had noisy boundaries whereas the saturation component failed to produce erythrocytes as the objects. Multilevel thresholding especially using Otsu method has been performed by many researchers for blood cell segmentation and it showed promising results. Savkare and Narote performed Global threshold and Otsu thresholdding on gray scale and green channel image [20]. Both images are added and median filter is applied to remove the unwanted points. Later on distance transform and watershed transform are applied to segment the cells. Kumar et al. used Otsu threshold on histogram of B component of RGB color space followed by the morphological operation [21]. Ahirwar et al. relied on two thresholds (one for erythrocytes, and one for parasites) for parasite segmentation [29]. The first threshold is selected to separate the erythrocytes from the background of the image. The second threshold is taking the first minimum after the principal mode of the histogram incorporating only the erythrocytes. Tek et al. modeled the stained and unstained pixel distributions with histograms and used the probability densities to determine whether a pixel on the input image is stained or not [12]. Diaz used inclusion-tree structure for segmentation. The background or foreground are label first using pixel classification. Different color spaces are used to build a pixel classification model because a particular color space may emphasize features that facilitate identification of searched objects [12].

Recent research on feature extraction and selection of blood cell has shown the important of feature extraction phase for blood cell analysis. Researchers have used different features based on their target blood cells/ disease. The features which give predominant difference between normal cells and infected cells are identified as feature set. Textural [18,22] and color features are very important in order to differentiate from other cells and has been widely used for blood cell recognition, texture features. Color features play important role to differentiate similar shapes and overlapped cells. The blood cell images are composed of three color components: red, green and blue, for each of the pixels in the images. The color characteristics and other features are required to be calculated in each of the color components, i.e. each of the malaria parasites features a particular color tone (blue ring). Suradkar used color features for RBC extraction and extraction of infected cells by malarial parasite [13]. RBC are red whereas malaria infected RBC cells have blue ring. Based on red color, red blood cells are segmented and then blue color is

used in order to count malarial parasite. Yunda et al. used both color and textural characteristics [21]. They extracted 27 color characteristics for each three color, i.e. standard deviation, seven Hough moments for color and color range as features set. For textural characteristics, wavelets descriptors (energy, standard deviation, mean) and co-occurrence matrix in four different directions with descriptors (homogeneity, contrast, GLMSR, standard deviation, angular moment and correlation) are used. The texture features from co-occurrence matrix and wavelet transform are not used together. Thus, the total number of descriptors is 110 when the co-occurrence matrix is used and 92 when the wavelet transform is used. Das et al. computed set of 96 features textural and morphological features [10]. They extracted 80 textural (entropy, Haralick textural features, local binary pattern, fractal dimension, histogram based features, gray level run length matrix based texture) along with16 morphological features (shape features and Hu's moment) to discriminate six types of infected and non-infected erythrocytes.

Classification of malarial infected cell becomes the challenging task. Several classifiers have been reported for the computerized recognition of malarial parasites in the presence of other stained structures and artifacts form blood cells images. Bayes classifier and different types of Artificial Neural Networks (ANNs), local linear map, SVM, K-mean and fuzzy system has been extensively used as classifier in the literature for blood cell recognition. The summary of recently used classifier is discussed in table 2.

3. METHODLOGY
Due to complexity of the blood sample images, malarial parasite segmentation and morphological analysis is a challenging problem. Machine vision based malarial diagnostic methods has been widely studied in order to provide early and accurate diagnose of malaria parasite. An ideal diagnostic method would be accurate, non-invasive, and inexpensive. The key tasks for malarial parasite classification involve segmenting the malaria parasite infected cells from the complicated background. We presented an approach for classification of malarial infected cells using Rao's based segmentation and BPNN for classification. We have divided the proposed methodology in to four basic steps.

- Preprocessing
- ROI Segmentation
- Feature Extraction
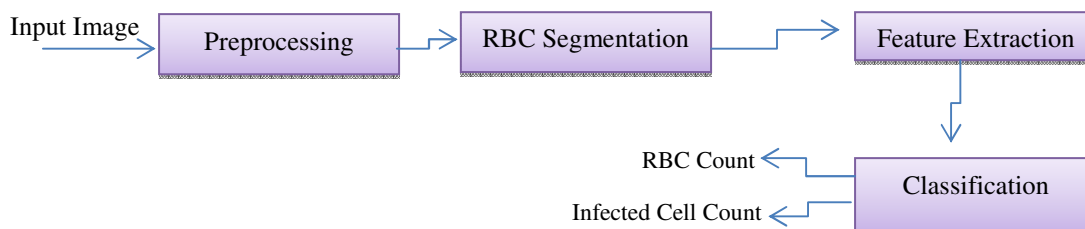- Classification of Infected Cells

FIGURE 2: System Diagram.

A. Preprocessing
Blood smear images might be affected by illumination and color distribution of blood images due to the camera calibration and staining variability. Most of the microscopes provide uniform or relatively uniform illumination images. The aim of preprocessing step is to obtain images with low noise, high contrast than original images for the further processing. This particular problem poses difficulties for classification of blood cells since it is hard to deal with proper segmentations of objects with quite similar colors. This process contains two operations image enhancement and noise reduction.

We have applied median filter for noise reduction. The median filter replaces pixel value with the median of its neighboring value. To get the finest coefficients details of noise free image, a Forward Discrete Curvelet Transform is applied to the V channel as shown in Figure 3(b). It is a multi-dimensional transformation which can sense both the contours as well as curvy edges of the overlapping objects in the image. The FDCT has high directional sensitivity along with the capability to capture the singularities. Edge and singularity details are processed to extract the feature. After obtaining the highest detailed coefficients Inverse Discrete Curvelet Transform is applied to high frequency band to obtain the detailed image. This detailed image is now having the stronger edges than the original and would perform better in lending edge details to the segmentation step. The next step is the adaptive equalization operation to spread out the intensity values along the total ranges of values in order to achieve better contrast. Adaptive histogram equalization differ from ordinary histogram equalization in respect that it computes several histogram of each corresponding to distinct section and use these histogram to redistribute the lightness value. After applying the adaptive histogram equalization, the background pixels have higher intensities than the cells.
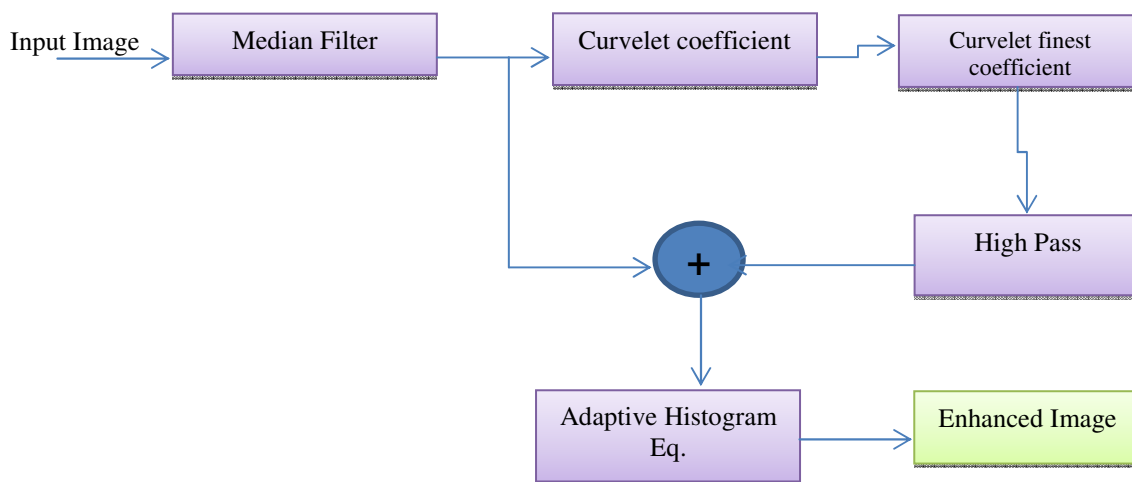

FIGURE 3: Preprocessing of Blood Cell Image.

B. ROI Segmentation

In the analysis of automatic classification of malarial parasite procedures, the most important and difficult part is segmentation of malaria parasite infected blood cells from the background and other cells because the blood cells are often overlaid with each other and is the basis of quantitative analysis of its deformability and hence its filterability[12]. Cell shapes, light variation and noise are the other factors that make segmentation a difficult task. Accurate segmentation allows fruitful result in sub-sequent levels. Malarial parasite lies in erythrocytes thus we need to segment the erythrocyte form the blood images. We have used Rao's method for background segmentation. Rao's method extracts a rough foreground image using morphological rea top-hats [26]. Two different threshold values are determined form these backgrounds and foreground that are used to produce the refined binary foreground mask.

At the end, a box counting algorithm is applied to the segmented image. Various algorithms are used for calculating the fractal dimensions, like the fractional (or fractal) Brownian motion and triangular-prism-surface area methods. The box counting algorithm counts the number of boxes having side length r needed to cover the surface of fractal objects and the number of boxes N, occupied by more than one pixel of the image. Two procedures are defined by two parameters in the box counting method. One is the selection of r and the other is the range of r. The blood cell image has finite set of points and the upper limit is the size of image while the lower is the pixel unit. Various researce propose using 2, 4, 8, 16, 2n pixels as box sizes to have a uniform spread of observation. The quadratic boxes cover the object, and the number of the boxes is recorded.

The fractal dimension (FD) measures the dependence between the number of boxes N and the box side length r.

C. Feature Extraction

Recent researches on feature extraction and selection of red blood cell have shown the importance of feature extraction phase for red blood cell analysis. Researchers have used different features based on their target blood cells/disease. The features which give predominant difference between normal cells and infected cells are identified as feature set. Textural [35,21] and color features [1, 27, 35] are very important in order to differentiate form other cells and has been widely used for blood cell recognition whereas color features plays important role in order to differentiate similar shapes and overlapped cells. We have used all geometrical and intensity features along with GLCM based texture features. Rules for identification of malarial species are presented in table 3.

	P. Falciparum	P. Vivax	P. Ovale	P. Malariae
Size	Not enlarged	Enlarged	Enlarged	Enlarged
Shape	Round crescent gametocyte	Round or Oval	Round or Oval amoeboid	Round
Dots	Large red spots	Small red dots	Small red dots	Few tiny dots

TABLE 3: Some Rules for Species Identification.

Geometrical features

Geometrical features remain very important for complex shape recognition and lot of researchers used geometrical features for blood analysis. We have extracted geometrical features that are invariant under different condition and analogous to those used by hematologist. These features include nucleus area, relative area, nucleus parameter, nucleus relative parameter, nucleus roundness and nucleus relative roundness, nucleus mean, nucleus variance, cytoplasm area, cytoplasm parameter, cytoplasm mean, cytoplasm variance, cytoplasm ratio to nucleus and number of object of in nucleus. To reduce the scaling effect on the leukocyte recognition, we have used the relative based features, i.e. relative area, relative parameter, and relative roundness.

Relative Area

$$A_r = \frac{\sum_{x,y} I(x,y)}{\pi r^2}$$

Relative Parameter

$$P_r = \frac{\sum_{i=0}^{N-1} \sqrt{(x_i + x_{i+i})^2 + (y_i + y_{i+i})^2} + \sqrt{(x_{i_{max}} + x_0)^2 + (y_{i_{max}} + y_0)^2}}{2\pi r}$$

Circularity of the cell is defined as the ratio between the cell area and square of its parameter. The cell circularity features shows how close the shape of the cell to circle. More the roundness mean the cell is closer to normal cell.

$$R = \frac{4\pi A}{p^2}$$

Where A is the cell area and p is the cell perimeter

Relative Circularity

$$R_r = \frac{4\pi A_r}{p_r^2}$$

Where P_r is the relative cell perimeter and A_r is the relative area of the region

Medical axis ratio describes the property that shape of the cell is stretched. Medial axis ratio is calculated as

$$MAR = \frac{L_{minor}}{L_{major}}$$

Whereas L_{minor} represent the length of minor principle axis and L_{major} represent the length of major principle axis.

Texture Features
Due to the importance of textural feature for complex object classification, we have extracted several texture features, i.e. co-occurrence matrix and local binary pattern.

The co-occurrence feature matrix describes the second order probabilistic features relating to the gray level relationship in the pixel neighborhood. GLCM is statistical measure used to characterize the image texture by calculating how often pairs of pixel occurrence in special specified relationship. It is a symmetric matrix constructed on the basis of image gray levels with distance and angle. The disparate co-occurrence feature matrix is created by the divergence of angle and distance. As different type of nucleus represent different texture, thus GLCM based texture features are taken into account for classification. If an image M consists of N gray levels, the co-occurrence matrix dimension is NxN. Let I be the segmented region of the leukocyte nuclei, the GLCM is computed by summing all the texture information in image I including the average spatial relationship between neighboring gray tones.

We have extracted 28 texture features from co-occurrence matrix to represent correlation, entropy, variance, difference entropy, sum entropy, dissimilarity and homogeneity. Co-occurrence feature matrix is computed as.

$$C_{\Delta x, \Delta y}(i,j) = \sum_{x=1}^{n} \sum_{y=1}^{m} \begin{cases} 1 & if\ I(x,y) = i\ \ I(x+\Delta x, y+\Delta y) = j \\ 0 & Otherwise \end{cases}$$

Entropy

$$E = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} P(i,j)\log\left(P(i,j)\right)$$

Energy

$$EN = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} P(i,j)^2$$

Correlation

$$C = \frac{\sum_{i=0}^{N-1} \sum_{j=0}^{N-1} (i,j) P(i,j) - \mu_x \mu_y}{\sigma_x \sigma_{xy}}$$

Sum entropy

$$E_s = \sum_{i=2}^{2(N-1)} P_{x+y}(i) \log P_{x+y}(i)$$

The gray level run length matrix describes the coarse structure analysis. For WBC segmented Image I(x,y), run length matrix R(I,j) specifies the number of length j in the given direction for a particular gray value i. We have computed 11 GLRLM based features (short run emphasis, high gray level emphasis, long run emphasis, low gray level emphasis, gray level non uniformity, run length non uniformity, short run low gray level run emphasis, short run high gray level run emphasis, long run high gray level runs emphasis, long run low gray level run emphasis and run percentage) for each 0, 45, 90, 135 direction angles.

Short run emphasis

$$SRE = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} r(i,j)/j^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$

High gray level emphasis

$$HGRE = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j) j^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$

Long run emphasis

$$LRE = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j) j^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$

Low gray level emphasis

$$LGRE = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} r(i,j)/i^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$

Gray level non uniformity

$$GLNU = \frac{\sum_{i=1}^{N_g} \left(\sum_{j=1}^{N_r} R(i,j) \right)^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$

Run length non uniformity

$$RLNU = \frac{\sum_{i=1}^{N_r} \left(\sum_{j=1}^{N_g} R(i,j) \right)^2}{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} R(i,j)}$$
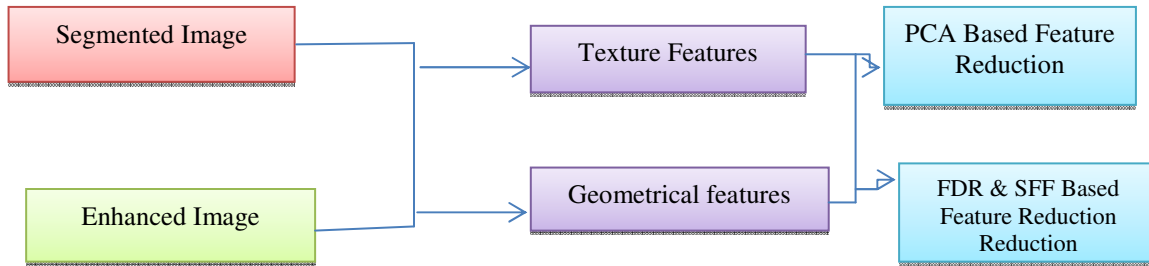
FIGURE 4: Feature Extraction and Selection.

4. CLASSIFICATION OF INFECTED CELLS

We have used Backpropogation neural networks (ANN) for classification of the malarial parasite. ANNs are the computational modals that simulated structure and function of biological neural networks. Training is an important task in utilizing the neural network. For this purpose, we have provided two types of input features as explained in feature selection section. There are some where size of cell can be abnormal shapes and size. Recognition results are summarized in table 4.

BPNN	P. Falciparum	P. Vivax	P. Ovale	P. Malariae
Feature Set-I	94.2	89.2	93.2	97.9
Feature Set-II	93.1	91	93.5	97.9
SFS Feature List	94.6	92.3	94.9	98.6

TABLE 4: Recognition Result.

5. CONCLUSION

The paper presented a method for automatic detection of falciparum and vivax plasmodium. Although, malaria cell segmentation and morphological analysis is a challenging problem due to both the complex cell nature uncertainty in microscopic videos. To improve the performance of malaria parasite segmentation and classification, we have used different set of features which are forward to the ANN for malaria classification. We have selected three feature set. Feature matrix III provided promising results that is computing after feature selection suing SFS and FDR.

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