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RESEARCH ARTICLE

Detection of Malarial and Babesiosis Parasite in RBC using Combination of Annular Ring Ratio and Marker Controlled Watershed Segmentation

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ABSTRACT

Malaria is a disease caused by a single celled protozoan parasite. It can be fatal if not treated at early stage. The main goal of this paper is to provide an efficient algorithm to detect the presence of malarial parasite even at early stages. This method makes use of a combination algorithm of Annular Ring Ratio (ARR) and marker controlled watershed algorithm to identify the Red Blood Cells (RBC) in the blood smear image. The platelets and artifacts are removed by using morphological operations before identifying the RBC. The presence of malarial parasite is detected by using thresholding based on colour as the Giemsa stained nucleus are always blue. Using the same method the presence of Babesiosis parasite in blood can also be identified.

Keywords: Malaria, RBC, ARR, Marker controlled watershed algorithm, Thresholding.

1. INTRODUCTION

Malaria is an infectious disease caused by single celled protozoan parasite. A person gets affected by this parasite when it enters our blood stream due to the bite of infected female anopheles mosquito as they are the main transmitter of this disease. Babesiosis is rare illness which is caused by the Babesia protozoan parasite. The parasite is most often transmitted by ticks but in infrequent cases has been passed by blood transfusion. Both could be fatal if not detected and treated at early stages. For this purpose various diagnostic methods like microscopic, antigen and molecular diagnosis are introduced. Among them microscopic diagnosis is proved to be effective but it requires experienced technician and also there are chances for human error. In order to overcome this problem another method called automatic diagnosis method is introduced which does not require any human intervention. But the automatic diagnosis method requires an effective algorithm to identify the presence of malarial and babesiosis parasite in RBC even at early stage. Various algorithms are introduced to efficiently identify the presence of parasite in RBC.

The method proposed in [21-28] edge detection is used to segment the foreground objects and adaptive thresholding is used to segment the RBC as well as to identify the infected cells. The technique in [18] uses SUSAN edge based algorithm to segment the RBCs and Probabilistic Neural Network (PNN) is used to classify the infected cells from normal cells. The technique in [10] uses histogram based thresholding to identify the infected cells. The scheme in [4-6] uses colour and morphology based algorithm to identify the infected cells. The scheme in [16, 17] uses Annular Ring Ratio (ARR) method to segment the RBCs and histogram based thresholding to identify the infected cells in the blood smear image. In [29, 30] the detection of presence of parasite in blood smear is based on histogram based thresholding. In [7-9] Otsu thresholding is used to segment the foreground objects and Bayes classifier is used to detect the presence of malarial parasite. In [32, 33] thresholding is used to segment the RBCs and the overlapping

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cells are separated by using watershed algorithm and Support Vector Machine (SVM) is used to detect the presence of malarial parasite in RBC. The method proposed in [1] uses a combined algorithm of thresholding and watershed algorithm to segment the RBCs from the blood smear image and K Nearest Neighbour (KNN) is used to classify between infected and non infected cells. The method proposed in [12, 13] uses intensity profiles to detect the presence of malarial parasite. The technique in [2] uses colour models and K-means of clustering to identify the infected cells. The scheme in [20] uses Adaptive Resonance Theory (ART), SVM, Back Propagation Feed Forward Neural Network (BPFF) and KNN for the classification of malarial parasites. The method proposed in [19] uses Back Propagation Neural Network (BPNN) and Bayes classifier to detect the presence of malarial parasite in RBCs. The technique in [31] uses Neural Network (NN) and SVM to classify the infected cells. The method proposed in [14] uses geometrical and statistical features. NN and SVM are used to identify the infected cells. The method proposed in [11] uses thresholding to segment the RBCs and overlapping cells are addressed by using area and elongation of the object. In [15] adaptive thresholding method is used to segment the RBCs and the presence of malarial parasite is identified by extracting blue colour as the stained nucleus appears to be blue in Giemsa stained image. In [3] morphological operation along with histogram thresholding is used to segment the RBCs and a two stage classifier BPFF NN is used to detect the presence of malaria parasite.

Of the various methods used for the segmentation of RBCs in blood smear image, Annular Ring Ratio method seems to be effective but the problem arises as it fails to separate the overlapping cells. In order to overcome this problem in our paper we propose a method which is a combination of ARR and marked watershed algorithm to segment the RBCs. Though the usage of classifiers seems to be effective to classify between the infected and non infected cells it is a complicated process. Algorithms like thresholding can also be used to detect the presence of malarial parasites. In order to make it more effective we propose a method called colour based thresholding that makes use of saturation component (S) in HSV colour space.

This paper consists of section 2.Methodology, section 3.Results and Discussion, section 4.Conclusion, section 5.Future work.

2. METHODOLODY

The main goal of this paper is to propose an effective and simple algorithm for the detection of presence of malarial parasite in blood smear.

2.1. Proposed methodology

The proposed method makes use of Annular Ring Ratio (ARR) and Marker Controlled Watershed (MCW) to segment the RBCs in the blood smear image. The presence of the parasite is identified by the use of saturation component in HSV colour space and thresholding.

The block diagram of our proposed system is given in figure 1. The input image is acquired from centre of disease control and prevention [21, 22] and Atlas database. The acquired image is first pre-processed by using grey scale conversion to easily differentiate the background and foreground objects. The contrast of the converted image is enhanced by using RMS contrast enhancement. The artifacts and the platelets are removed by using morphological dilation and erosion. The ARR transform is applied to the pre-processed image and the maximum peaks are detected by using peak detection algorithm. The marker controlled watershed algorithm is applied to the segmented image to separate the overlapping cells. The segmented image is converted to HSV colour space and the top hat transform is applied to the saturation component (S). Using thresholding the image is converted to binary image and the maximum peaks shows the presence of parasite.

2.2. Pre-processing

The pre-processing of our method involves the following steps

1. Grey scale conversion.
2. Contrast Enhancement.
3. Morphological operations in order to remove the platelets and artifacts in our image**.**

2.2.1. Grey scale conversion

The input image obtained from the database is converted to grey scale image to differentiate between lighter and darker

images. The conversion of RGB image to grey scale image involves conversion of 24 bit colour value to 8 bit grey value. Different methods are used for this process like simple averaging, weighted averaging, using function. The RGB image can be converted to grey scale image by using rgb2gray function. To obtain a more efficient grey scale image weighted averaging method is used, where from the RGB image, Red, Green and Blue component are obtained. Optimum weights are added to the Red, Green and Blue components. The RGB to grey scale conversion using optimum weights is given by equation (2.1).

$$\text{Grey} = W_R.R + W_G.G + W_B.B \quad (2.1)$$

where R – Red component, WR – optimum weight of R, G – Green component, WG – optimum weight of G, B – Blue component, WB – optimum weight of B, $W_R + W_G + W_B = 1$. Out of the 3 components, green component gives detailed morphology of the stained objects and hence by adding weight only to green component and keeping the rest of the components zero we get equation (2.2).

$$\text{Grey} = W_G.G \quad (2.2)$$

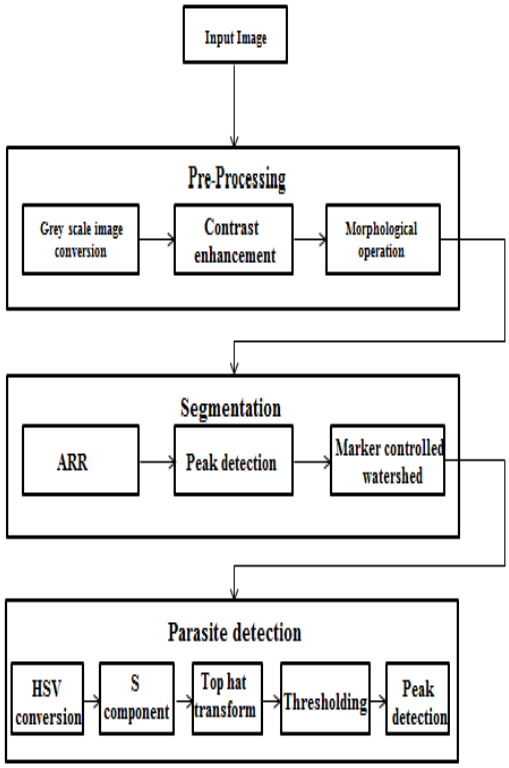


Figure 1.Block diagram of the proposed method

2.2.2. Contrast enhancement

The contrast of the converted grey scale image can be enhanced by using different methods like Michelson contrast, Weber contrast, RMS contrast etc. Michelson contrast is used for extreme dark and light patterns. It deals with images of high spatial frequency. It calculates the ratio of difference in maximum and minimum intensities to twice the average intensity. The Weber contrast is applicable to images having uniform background. As the blood smear images have non uniform background it's not applicable. In RMS contrast the contrast is measured by using root mean square value as given in equation (2.3) which does not depend on spatial frequency content of the image.

$$\text{RMS} = \frac{1}{\sqrt{M*N}}\sqrt{\sum_{i=0}^{N-1}\sum_{j=0}^{M-1}\left(I_{ij} - I_{avg}\right)^2} \quad (2.3)$$

where M*N – size of image, I_{ij} – normalized pixel intensity, I_{avg} – mean normalized pixel intensity.

2.2.3. Morphological operations

The morphological operation involves the following 2 steps to remove the platelets and artifacts

1. Morphological dilation.
2. Morphological erosion.

2.2.3.1. Morphological dilation

It is a procedure which replaces the original intensity value with the largest value in the neighbourhood. In an image that contains darker objects in lighter background, dilation reduces the size or fully eradicates any darker object smaller than structuring element. In this proposed methodology the structuring element chosen is of disc format and of size equal to the radius of RBC in pixels. In this case if dilation is applied more than 80% of the cells are eradicated as the size of RBC is not stable. For this purpose two structuring element of size 25% of radius of RBC and 75% of radius of RBC in pixels are considered. Dilation of the grey scale image I at any location (x, y) by structuring element (se) is calculated to be the maximum value of the neighbourhood outlined by se whose origin is at (x, y) is given [17] by equation (2.4).

$$I + se = \max(p,q)\varepsilon\, se\{f(x-p, y-q)\} \quad (2.4)$$

where se – structuring element, (x, y) – image pixels, (p, q) – Neighbourhood pixels, I – Grey scale image.

2.2.3.2. Morphological erosion

Erosion expands the size of the darker object in lighter background up to size of structuring element as it will pick the minimum value from the neighbourhood. The structuring element of disc format and size equal to 75% of radius of RBC in terms of pixels is used for erosion. The grey scale image I with a structuring element se for location (x, y) is given [17] by the equation (2.5).

$$I\theta se = \min(p,q)\varepsilon\, se\{f(x-p, y-q)\} \qquad (2.5)$$

2.3. Segmentation

The Segmentation of RBCs from the blood smear image involves the following steps

1. Annular Ring Ratio.
2. Peak detection.
3. Marker Controlled Watershed algorithm.

2.3.1. Annular Ring Ratio (ARR)

This method is based on local variation of pixel intensity values and information equal to the size of the cell obtained in terms of pixels. It calculates ratio of two regions for each pixels. The first is the annular ring whose outer radius is equal to 75% of radius of RBC in pixels and the inner radius is equal to 25% of radius of RBC in pixels. Let I_o be the average intensity with in the outer annular ring region and I_i be the average intensity with in the inner region [17]. The ARR is given by the equation (2.6).

$$ARR = \max\left[\left(\frac{I_o}{I_i} - 1\right), 0\right] \qquad (2.6)$$

ARR is computed for each pixel in the image, where the darker circular objects against the lighter background are transformed to lighter circular objects whose intensity increases towards the centre of the image against dark background. The result obtained is the transformed image in blob like structure [17].

2.3.2. Peak detection

The image obtain from ARR transform are blob like structures whose centres are bright in the dark background. To identify the RBC and WBC in image, maximum pixel intensity in the image is found, as the centres of the cells have maximum pixel intensity. The identified pixel intensities are plotted on the original image which indicates the centres of RBC.

2.3.3. Marker controlled watershed

In watershed segmentation, over segmentation is one of the main problem. In order to overcome this problem we go for marked control watershed segmentation, where segmentation is based on given edges. The marker or the edges for segmentation is provided by imposed minima which is obtained from extended minima whose threshold is set as 75% of radius of RBC in pixels. It displays only the intensity values less than the threshold.

The catchment basins are initially constructed for watershed segmentation were the flooding process is performed on the imposed minima image. The set of pixels for which the flooding should start are chosen from the imposed image. The pixel with lowest priority is extracted from the queue, if the extracted pixel has the same label as that of the nearby pixels then the extracted pixel is labelled with their label. Non labelled markers are put in priority queue and this process is continued until the queue is empty. The non labelled markers are the watershed lines.

2.4. Parasite detection

The detection of presence of parasite involves the following steps

1. HSV conversion
2. Top hat transform
3. Thresholding

The segmented RGB image is converted to HSV colour space as it gives visible difference between the RBCs and the stained nucleus. The top hat transform is performed on the saturation component to see the difference between the foreground object (parasite) and the background object (RBC). The threshold is chosen based on the intensity values and the image is made a binary image. The parasites present in RBCs have maximum intensity values which can be plotted using peak detection algorithm.

3. RESULTS & DISCUSSIONS

3.1. Pre-processing

3.1.1. Grey scale conversion

The original image is obtained from CDC malaria prevention database [22]. The original image is converted to grey scale image by using the following methods

i. Standard grey scale function
ii. Using standard value as optimum weights
iii. By adding optimum weights to green component
iv. By adding optimum weights to red component
v. By using averaging method

The original image is converted to grey scale image for differentiation between the dark and lighter objects. It involves conversion of 24 bit colour value to 8 bit colour value, which represents luminance of pixel value ranging from 0 to 255. The standard grey scale function is obtained by using rgb2gray function. The conversion of RGB to grey using optimum weights involves separating the Red, Green and Blue component from the RGB image.

Standard weights are added to each component and are combined to convert the original scale image to grey image. WR=0.299, WG=0.587 and WB=0.114 are the weights used for red, green and blue component. The weights can be of any value but their total value must be 1. The values of the weights are changed to obtain a clear grey scale image; the green component gives clear morphology of the stained objects. So the weights of red and blue are set as 0 and the weight of green component is set as 1.

The outputs obtained by using various grey scale conversion techniques for malaria and babesiosis image is given in figure 2 where figure 2(a) is the original RGB image, figure 2(b) gives the grey image obtained by using grey function and figure 2(C) gives the grey image obtained by using standard weights. Figure 2(d) gives the grey image obtained by giving weights only to the green component Figure 2(e) gives the grey image obtained by adding weights only to the red component and figure 2(f) gives the grey scale image obtained by using averaging method.

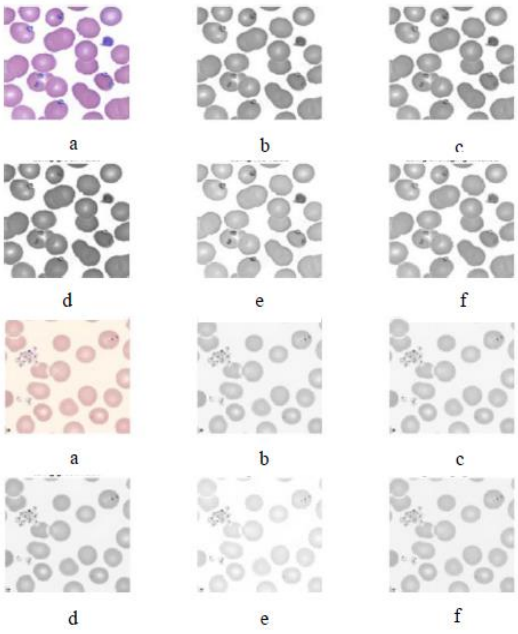


Figure 2.Converted grey scale image of malaria and Babesiosis blood smear using various methods

3.1.2. Contrast enhancement

The contrast of the image is further enhanced by using following methods

i. Michelson contrast
ii. Weber contrast
iii. RMS contrast

Michelson contrast is commonly used for extremely light and dark patterns. It calculates the maximum and minimum intensities to twice the average intensity. It deals with images of high spatial frequency. Weber contrast is applicable only to images with uniform background but the blood smear images do not have a uniform background and hence no change occurs to the grey scale image. The RMS contrast uses the Root Mean Square (RMS) value to enhance the contrast. The contract enhancement when compared to the other methods is high for RMS contrast. The contrast enhancement outputs obtained for malaria and Babesiosis image using various contrast enhancements methods are given in figure 3 where 3(a) gives the contrast enhanced image obtained by applying Michelson contrast, 3(b) gives the contrast enhanced image obtained by applying Weber contrast and 3(c) gives the contrast enhanced image obtained by applying RMS contrast.

The contrast is enhanced for malaria by,

i. Michelson = 0.6190
ii. Weber = 0
iii. RMS = 10.518040

The contrast is enhanced for Babesiosis by,

i. Michelson = 0.5089
ii. Weber = 0
iii. RMS = 12.770633

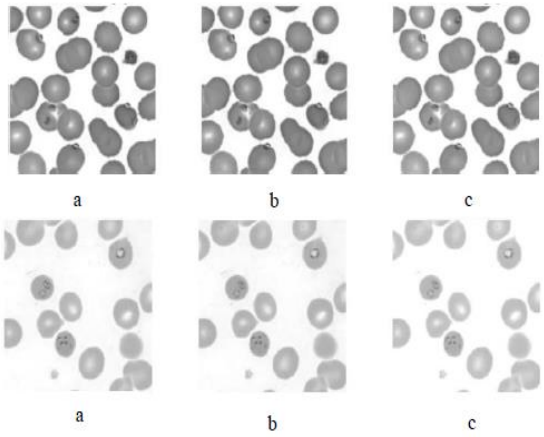


Figure 3.Contrast enhanced malaria and Babesiosis image

3.1.3. Morphological operations

The structural element for dilation and erosion is measured using the distance tool and is exported to work space. The size of structural element is 25% and 75% of radius of RBC in the blood smear image which is measured in terms of pixels. Figure 4 gives the measure of radius of RBC in terms of pixels.

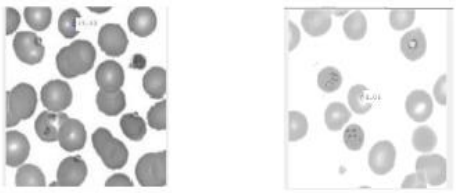


Figure 4.Radius of RBC

The values of structuring elements for malaria image are calculated as radius = [15.000000], 25 per of radius = [3.750000], 75 per of radius = [11.250000], 25 per of radius round off = [4.000000], 75 per of radius round off = [11.000000]. The values of structuring elements for Babesiosis image are calculated as Radius = [9.055385], 25 per of radius = [2.263846], 75 per of radius = [6.791539], 25 per of radius round off = [2.000000], 75 per of radius round off = [7.000000]. The contrast enhanced grey scale image is further pre-processed by using morphological filtering which involves morphological dilation and morphological erosion. The outputs obtained for morphologically dilated and eroded image is given below. The artifacts, platelets, parasites are removed by filtering process. The morphologically dilated and eroded images of malaria and babesiosis are given in figure 5(a) and figure 5(b).

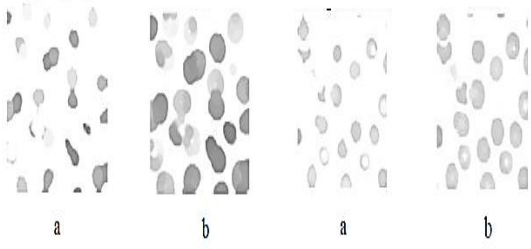


Figure 5.Morphologically filtered malaria and babesiosis image

3.2. Segmentation

3.2.1. Annular Ring Ratio (ARR)

The Euclidean distance is measured to check if the centre lies between the inner disc or annular disc and a binary mask is created. The binary mask created for malaria and Babesiosis image is shown in figure 6.

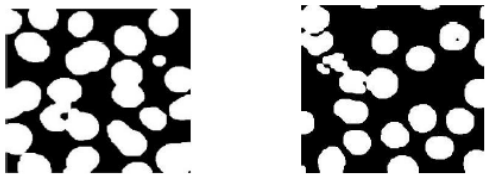


Figure 6.Binary mask created for malaria and Babesiosis image

The binary image is converted to ARR transformed image by calculating the ratio between the inner and outer intensities of the inner and annular disk. The ARR transformed image for malaria and babesiosis image is shown in figure 7.

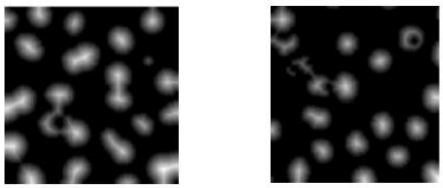


Figure 7.ARR transformed image for malaria and babesiosis image

3.2.2. Peak detection

The maximum intensity peaks are identified and are plotted on the original image to detect the RBCs as the RBCs sides are thicker in the end and thin on the insides. The

centres have maximum intensity of pixels. Figure 8 shows the RBCs segmented by using ARR transform for malaria and Babesiosis images are plotted by using peak detection algorithm.

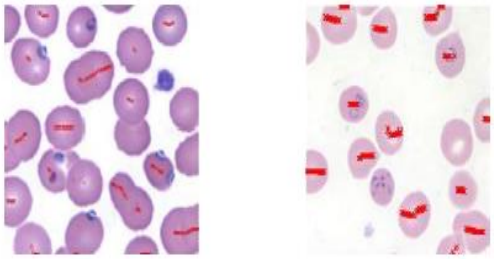
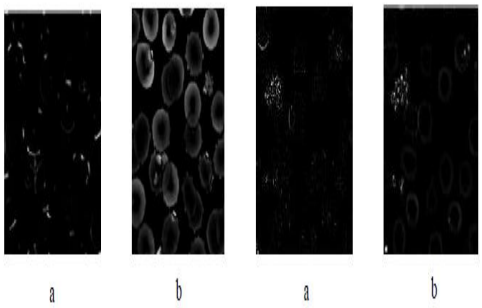


Figure 8.Peaks detected for malaria and babesiosis image

3.2.3. Marker controlled watershed

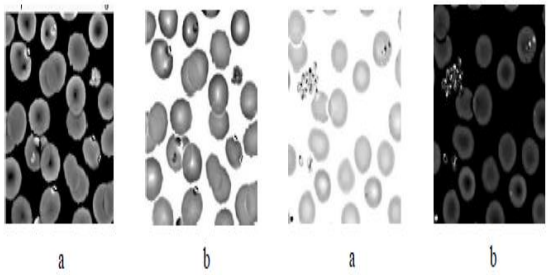
For marked controlled watershed segmentation the image contrast is enhanced by taking the top hat and bottom hat transform. The top hat transform contains peaks of objects that fit the structuring element. The output of this transform is the original image subtracted from the opening image. Similarly the bottom hat transform is used to increase the contrast between the objects and the gaps that separate them. The output of this transform is the original image subtracted from the closed image. Figure 9(a) and 9(b) shows the output obtained by using top hat and bottom hat transform for malaria and Babesiosis image.



a b a b

Figure 9.Top and bottom hat transform

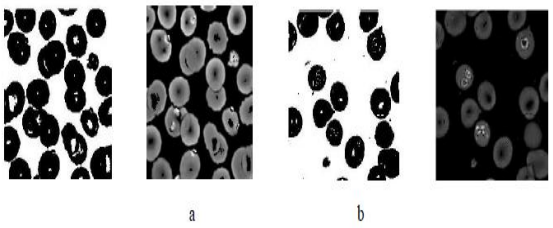
The enhanced image is obtained by adding the top hat image to the grey scale image and subtracting it with bottom hat image, the negative of this enhanced image is obtained by taking the complement to highlight the intensity values. The malaria and Babesiosis enhanced image and its complement is shown in figure 10(a) and figure 10(b).



a b a b

Figure 10.Enhanced image and its complement

To detect the intensity values deeper than 75% of radius of RBC the extended minima is used and the imposed minima converts the deeper intensity pixel values to 0, and all the edges of the imposed minima are detected by watershed transform. The malaria and Babesiosis extended minima and imposed minima outputs are shown in figure 11(a) and figure 11(b).



a b

Figure 11.Extended minima and minima of malaria and Babesiosis

The watershed transform of the edges obtained from the imposed minima is detected. The marker controlled watershed output of malaria and Babesiosis is shown in figure 12.

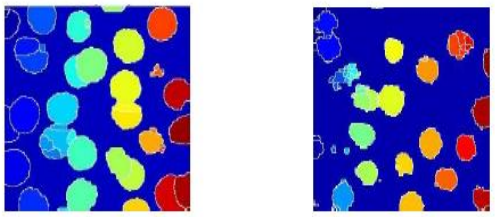


Figure 12.Marker controlled watershed transform of malaria and babesiosis

The watershed transformed output is super imposed on the peak detected image to obtain the combined segmented output of both ARR and watershed. The segmented output for malaria and babesiosis obtained by using combination of ARR and marker controlled watershed is shown in figure 13.

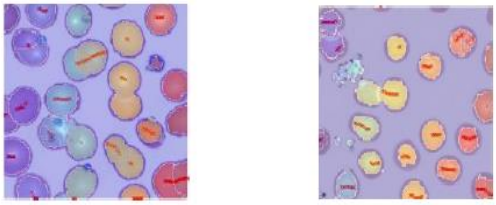


Figure 13.Segmented image using ARR and watershed of malaria and babesiosis

3.3. Parasite detection

The segmented RBCs are converted to HSV colour space and the hue (H), saturation (S) and value (V) component are separated. The figure 14(a) shows the HSV colour space of segmented RBS's. The separated H, S and V components of malaria and Babesiosis images are shown in figure 14(b, c, d).

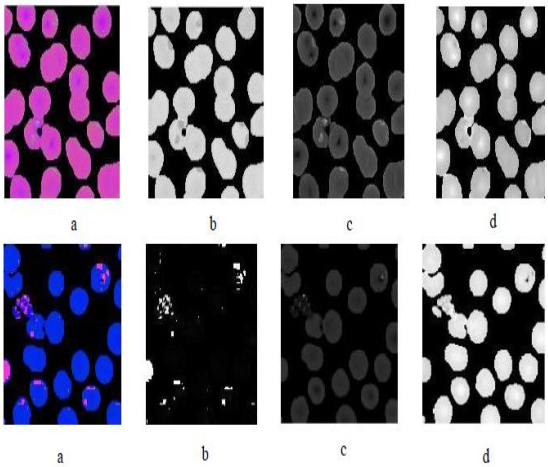


Figure 14.RBC converted to HSV colour space

The top hat of S component obtained for malaria and babesiosis is shown in figure 15 which shows the difference between the background objects (RBCs) and foreground objects (parasites).

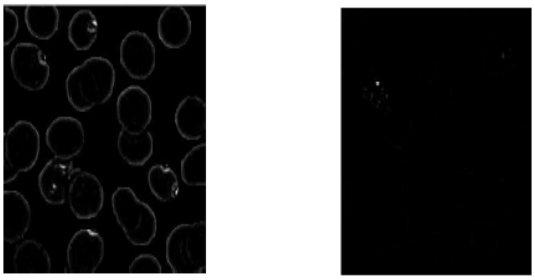


Figure 15.Top hat of S component for malaria and Babesiosis

The thresholding level is obtained from S component. The top hat of S component is converted to binary. If the pixel value lies within the thresholding level then the pixel value is set as 1 and if the pixel value is greater than the thresholding level then the pixel value is set as 0. Thus the binary image and its complement are shown in figure 16. The white regions in figure 16 are the parasites present in the RBCs.

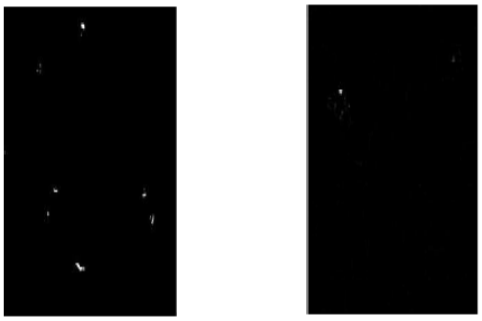


Figure 16.Binary image of malaria and Babesiosis highlighting parasites

The maximum intensity values from figure 16 are calculated and plotted on the original image using peak detection algorithm. The figure 17(a) shows the image of parasites segmented from the original image and the figure 17(b) shows the parasites plotted by using peak detection algorithm.

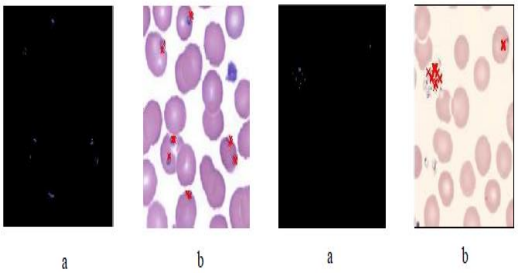


Figure 17.Presence of parasites detected in malaria and babesiosis

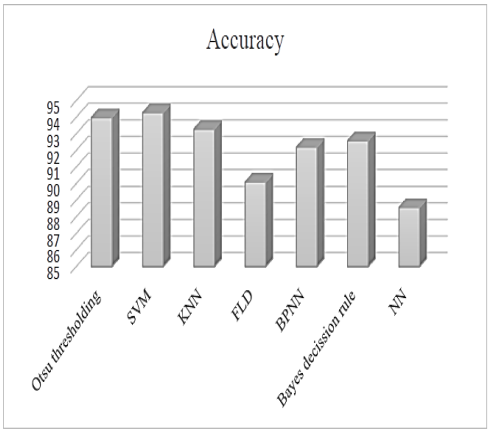


Figure 18.Performance measure

A total of 50 images, 25 normal and 25 infected images (25 malaria infected and 25 babesiosis infected) are considered to calculate the performance of this method. The overall accuracy obtained is of 94% which is similar to that of methods that used classification techniques for detection of parasites. The performance measure is highlighted in figure 18.

4. CONCLUSION

This paper proposes an effective algorithm to detect the presence of malarial and babesiosis parasites in RBCs. The image is pre-processed by grey scale conversion using optimum weights and then the contrast of the pre-processed image is enhanced by using RMS contrast. The morphological operations both morphological erosion and morphological dilation are used to remove the platelets and other artefacts. The ARR transform is applied on the mask created using Euclidean distance to highlight the centres of each RBCs. Once the centres are highlighted the peak detection algorithm is used to highlight the maximum intensities. The Marker controlled watershed algorithm is further applied on the segmented image to separate the overlapping cells. Thus the RBCs are successfully segmented by using combination of ARR and Marker controlled watershed algorithm. The presence of malarial parasite is detected by using colour based thresholding on the segmented image. This proposed method is used in the detection of both malaria and Babesia parasites in RBC's with an accuracy of 94%, sensitivity of 95.83% and specificity of 92.3%.

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