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# Detection of Malarial Parasite from Blood Smear Image

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## Abstract

Members of the genus Plasmodium cause a febrile disease called malaria. The conventional method to diagnose malaria using microscopy consumes more time and difficult to reproduce the results. Diagnosis of malaria using microscope involves expert technician to examine the slide with intense visual and mental concentration and there is a high possibility of erroneous diagnosis even by experienced technicians. To overcome the limitations of the conventional method of manual counting, an automation of the evaluation process is presented in this paper. An automated system is presented to identify the presence of the malarial parasite in blood smear images using image processing techniques. The entire process of diagnosis can be done faster using image-based screening and is more advantageous than laboratory procedures. Pre-processing and segmentation techniques followed by detection of infected cells were employed to identify the presence of malarial parasites from stained blood smear images. A Graphical User Interface was created to provide a positive and negative diagnosis of malaria.



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## Introduction

Malaria is a harmful mosquito-borne blood disease, caused by the protozoan parasites of the genus Plasmodium. It is transmitted through the bite of an infected female Anopheles mosquito. The introduction of parasites into a person's blood is from the mosquito's saliva when the mosquito bites. The parasites mature and reproduce in the liver. During this process, the red blood cells (RBCs) are invaded by the parasites and are destroyed later. According to WHO, malarial infection is a life-threatening disease for approximately 40% of the world's population. It is estimated that nearly 500 million people are affected by malaria every year. Between 300 million and 500 million people in Africa, India, Southeast Asia, the Middle East, the South Pacific, Central and South America are diagnosed with this disease. In 2016, there were roughly 212 million malaria cases and an estimated 4, 29,000 malaria deaths. A rapid and accurate diagnosis which facilitates prompt treatment is an essential requirement to control malaria.

There are many new methods developed in recent years for the diagnosis of malaria, i.e., fluorescent microscopy, rapid antigen detection methods and polymerase chain reaction (PCR)-based techniques [8, 25]. Visual microscopically evaluation of Giemsa stained blood smears is the most widely used technique to detect the cells infected with parasite [13, 16, 17]. With the help of the staining process, RBCs, Plasmodium parasites, white blood cells (WBC), and platelets or artifacts are colored and highlighted. The detection of Plasmodium species requires detection of the stained objects. Further, the detailed analysis of stained objects is done to prevent false diagnosis. However, this manual method consisting of many steps is time-consuming and prone to human errors even by trained technicians or pathologists. The method to detect malaria is digitized may reduce the time taken for screening the disease leading to improvement in the consistency of malarial diagnosis. Digital image processing is a reliable method to detect the presence of the parasites in red blood digital image which optimizes the malaria diagnosis. A precise and an effective analysis of the expected image of microscopic RBC can be achieved by digital image processing [1].

Based on parasite type and stages in diagnosing malaria illness the microscopic digital image processing of malarial blood images incorporate several image processing steps. Different approaches of the algorithm used in each step yield different accuracy. Anand et al. [2] compared the shape profile of malaria-infected RBCs with that of the normal RBCs from the digital holographic interferometric microscopy for detecting the presence of malaria. They derived a correlation function to distinguish the infected RBCs. Bashir et al. [6], detected parasites based on the extraction of intensity and textural features. They created a database of cropped erythrocyte sub-images from both infected and non-infected image samples. The features detected from this database were evaluated using an Artificial Neural Network (ANN). Arco et al. [4] quantified the presence of malaria parasite from thick blood smear using adaptive threshold-based segmentation. Salamah et al. [20], presented image enhancement of low-quality thick blood smear images using contrast and edge correction techniques and also employed both global (Dark stretching) and local Contrast Limited Adaptive Histogram Equalization (CLAHE) correction. Unsharp Masking Filtering method was used for edge correction. From the pre-processing step to identification step Ary et al. [5] categorized different techniques of microscopic digital image processing and provided a comparison between them in terms of advantages and limitations and identification accuracy but the study did not include malaria identification methods for P.ovale, Knowlesi and Malariaea and its life cycle stages. Ghate et al. [11] extracted the RBCs from the Giemsa stained peripheral blood samples using the thresholding function and implemented a thinning algorithm for detecting edges of the cells.

Savkare et al. [21] separated the overlapped cells by applying the watershed transform. Shape-based features such as area, perimeter, the major axis and a minor axis, and texture-based features such as standard deviation, momentum, and kurtosis of RBCs were calculated. These features were fed to the SVM classifier for identification of the parasites. Shet et al. [22] implemented adaptive processing techniques in order to overcome the variability in images. Morphological operations and connected component analysis were performed for enumeration of parasites. Somasekar et al. [23] performed Gamma equalization for illumination maintenance and edge-based segmentation of infected erythrocytes. Extraction of infected erythrocytes was done using the FCM method, but the method was limited to classification of malarial parasite species. Neetu Ahirwal et al. [18] presented an advanced image analysis system for automatic detection and classification of the malarial parasite in blood images. Suryawanshi et al [24] compared the

efficiency of SVM and Euclidean distance classifier in detecting the presence of the malarial parasite.

Upon interpretation of the related work, it was inferred that preprocessing is necessary to enhance the image by removal of artifacts. Segmentation is performed on the preprocessed image to isolate individual RBCs and to identify parasite infected regions. Additionally, features may be extracted from the images to enable classification [19]. This paper presents the application of digital image processing techniques for detecting malarial parasites using microscopic color images. The rest of this paper is organized as follows. Section 2 describes the methodology to identify the infected cells, Section 3 presents results and related discussions, and finally, Section 4 concludes the paper.

## Materials and Methods

The method to detect the presence of malarial parasite using image processing techniques is shown in **Figure 1**. The acquired images are first pre-processed and segmentation techniques are employed to distinguish between normal and infected blood smear images. A Graphical User Interface (GUI) is created to provide a positive and negative diagnosis of malaria.

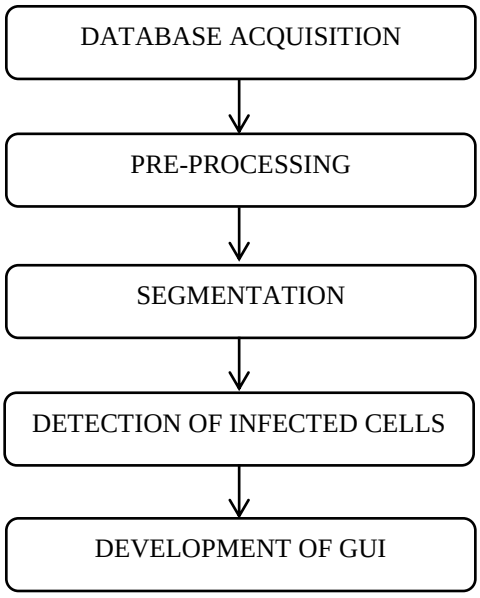


**Figure 1:** Methodology to detect the malarial parasite from blood smear image

***Database acquisition***

Giemsa stained peripheral blood smear images were obtained from SRM Hospital, Katangulathur. The database consisted of 25 malarial infected and 20 non-infected blood smear images of dimensions 4032 x 3024 pixels and resolution of 72 dots per inch which were viewed using a Leica DM750 microscope. Blood smear image for normal case and the malaria-infected case is shown in **Figure 2**.

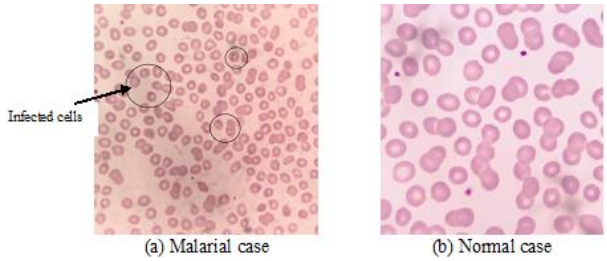


**Figure 2:** Blood smear image

***Pre-processing***

Pre-processing is done to improve the quality and remove noise from the image without losing the detail of the parasite and to process image segmentation into meaningful regions. Pre-processing techniques enhance the images by improving contrast thereby facilitating the segmentation process and also helps in better local and global feature detection [12]. This process overcomes issues such as non-uniform illumination, blurring, and presence of unwanted details. Histogram equalization, contrast stretching, Laplacian filter, arithmetic mean filter, geometric mean filter, median filter, adaptive median filter, minimum filter, maximum filter and wiener filter were the following techniques used in pre-processing. The efficiency of various pre-processing techniques can be quantitatively evaluated using performance metrics [9, 10, 14]. This will enable selection of the suitable pre-processing algorithm applicable for the acquired images. The various performance metrics used to evaluate the images, after processing, includes Mean Square Error (MSE), Contrast to Noise Ratio (CNR), Mean Absolute Error (MAE), Signal to Noise Ratio (SNR), Peak Signal to Noise Ratio (PSNR), Absolute Mean Brightness Error (AMBE), Universal Quality Index (UQI). Above listed performance metrics are calculated using equations (1) – (7).

$$\text{MSE(r,e)} = \frac{1}{RC}\sum_{i=0}^{R-1}\sum_{j=0}^{C-1}(I(i,j) - E(i,j))^2 \quad \text{.....(1)}$$

where, I and E denote input image and enhanced image respectively, and RC is the size of the image.

$$\text{PSNR} = 10log_{10}\left(\frac{(D-1)^2}{MSE(I,E)}\right) \quad \text{.....(2)}$$

where, D is the dynamic range of pixel values.

$$MAE(I,E) = \frac{1}{RC} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} |N(i,j)| \quad \text{.....(3)}$$

where, N(i.j)=I(i,j) - E(i,j)

$$SNR(I,E) = \frac{\sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (I(i,j))^2}{\sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (N(i,j))^2} \quad \text{.....(4)}$$

$$AMBE(I,E) = |\mu_I - \mu_E| \quad \text{.....(5)}$$

where , $\mu_I = \frac{1}{RC} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} I(i,j)$ $\mu_E = \frac{1}{RC} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} E(i,j)$

$$CNR(I,E) = \frac{(\mu_I - \mu_N)}{\sigma_N} \quad \text{.....(6)}$$

where, $\mu_N = \frac{1}{RC} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} N(i,j)$ $\sigma_N^2 = \frac{1}{RC-1} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (N(i,j) - \mu_N)^2$

$$UQI = \frac{4\mu_I \mu_E \sigma_{IE}}{(\mu_I^2 + \mu_E^2)(\sigma_E^{\ 2} + \sigma_I^{\ 2})} \quad \text{.....(7)}$$

where, $\sigma_{IE} = \frac{1}{RC-1} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (I(i,j) - \mu_R)(E(i,j) - \mu_E)$

$$\sigma_E^2 = \frac{1}{RC-1} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (E(i,j) - \mu_E)^2$$

$$\sigma_I^2 = \frac{1}{RC-1} \sum_{i=0}^{R-1} \sum_{j=0}^{C-1} (I(i,j) - \mu_I)^2$$

To identify a desired preprocessing method, the errors such as MSE, MAE, AMBE should be low and PSNR, SNR, CNR should be high. UQI should lie in the range of 0 to 1 and must be high.

***Segmentation and Detection of infected cells***

The objective of the segmentation algorithm is to separate the image into background and foreground, where the foreground consists of RBCs. These segmentation techniques are either region-based or edge-based. The former technique relies on common patterns in intensity values within a cluster of neighboring pixels and the edge-based techniques rely on discontinuities in the image. The algorithm should also isolate the parasite-infected cells. Adaptive k-means clustering [2, 7, 15], fuzzy c means, active contours, anisotropic diffusion, Otsu thresholding, Sobel edge detection, morphological operations and segmentation in HSV color space are the various segmentation techniques [3] were implemented and analyzed to identify the infected cells. The methodology of segmentation in HSV color space is shown in **Figure 3**. The acquired images are converted into HSV color space in which malaria-infected regions are highlighted. The hue and saturation component are separated from the image, histogram equalized, thresholded and combined to form the HS mask. This mask is used as seed points to run the active contour algorithm. This step outlines the infected cells and thus differentiates infected and non-infected RBCs.

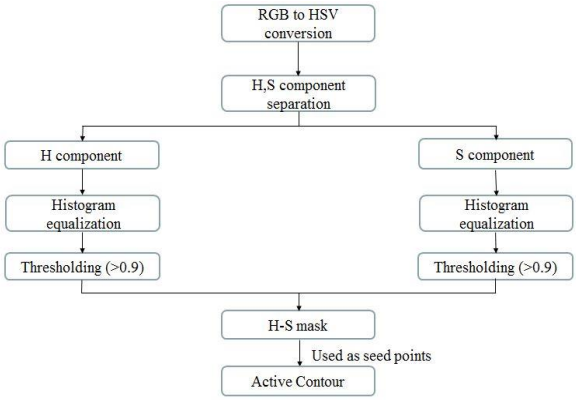


**Figure 3:** Segmentation in HSV color space

***Graphical User Interface (GUI)***

The Graphical User Interface (GUI) is used to facilitate the technicians or pathologists to automatically detect the malarial parasites from the given blood smear image. Its main aim is to improve the efficiency and feasibility of detection and diagnosis during the mass screening process. The user-friendly design ensures the good connectivity between the visual language and the tasks.

## Results and Discussion

The acquired images were pre-processed using different techniques such as Histogram Equalization, Contrast Stretching, and filters which include Laplacian, Arithmetic Mean, Geometric Mean, Median, Adaptive Median, Minimum, Maximum and Wiener filter and the simulated results of blood smear image for malaria case are shown in **Figure 4**. The performance metrics for every pre-processing method implemented was calculated and tabulated. The suitable method is chosen such that the errors (MSE, MAE, AMBE) are low, signal to noise ratio is high and the UQI is high within the range 0 to 1. From **Table 1 and 2**, it is observed that the Laplacian filter satisfied all of the criteria mentioned as the suitable pre-processing technique for the acquired images.

The pre-processed images are segmented using various techniques such as Adaptive k–means clustering, Fuzzy c means, Active contour, Anisotropic diffusion with Sobel edge detection, Sobel edge detection with Morphological operations, Sobel edge detection with Connected component analysis and segmentation in HSV color space and the simulated results are shown in **Figure 5**.

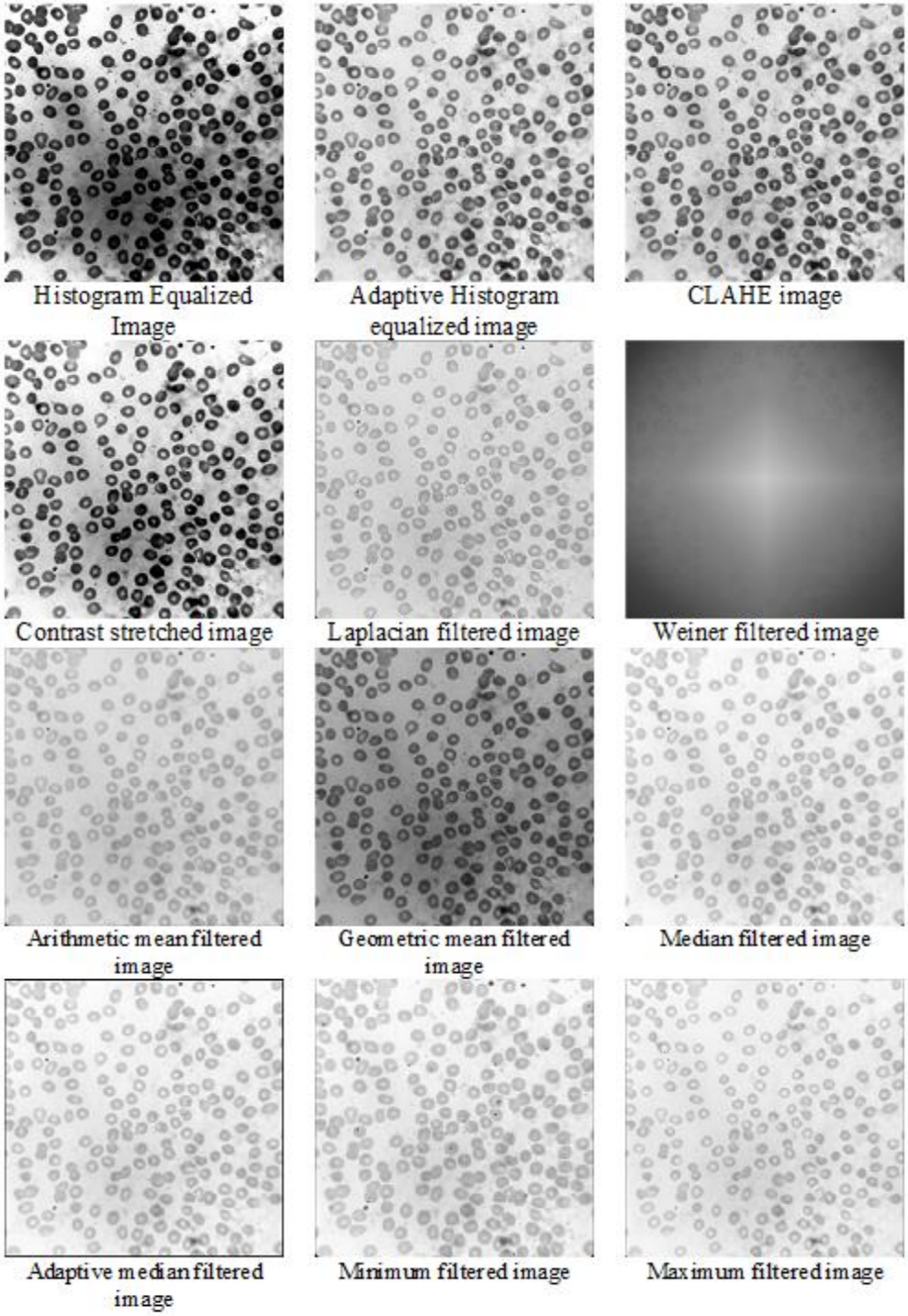


**Figure 4:** Simulated results of Pre-processing techniques

**Table 1:** Performance metrics of malarial cases

| TECHNIQUE | MSE | PSNR | MAE | SNR | AMBE | CNR | UQI |
|---|---|---|---|---|---|---|---|
| **Histogram Equalization** | 128.89 | 27.394 | 31.236 | 2.3966 | 30.189 | 12.782 | 0.9033 |
| **AHE** | 92.837 | 28.571 | 16.032 | 3.5730 | 10.456 | 15.712 | 0.9377 |
| **CLAHE** | 110.88 | 28.304 | 21.514 | 1.9072 | 17.737 | 14.211 | 0.9219 |
| **Contrast Stretching** | 4.8515 | 45.514 | 0.9564 | 20.516 | 20.484 | 218.83 | 0.9919 |
| **Median Filtering** | 7.3538 | 40.531 | 1.1023 | 36.768 | 0.0969 | 65.814 | 0.9992 |
| **Adaptive Median Filtering** | 461.94 | 20.516 | 4.7452 | 16.753 | 4.4452 | 6.0791 | 0.8848 |
| **Arithmetic Filtering** | 25903 | 3.8354 | 145.29 | 48.059 | 145.29 | 2.377 | 0.0001 |
| **Geometric Filtering** | 0.0767 | 59.478 | 0.2450 | 7.5844 | 0.3181 | 3.1740 | 0.7699 |
| **Minimum Filtering** | 0.0085 | 65.551 | 26.067 | 17.026 | 0.0390 | 7.0309 | 0.9376 |
| **Maximum Filtering** | 0.0082 | 69.052 | 0.0348 | 17.158 | 0.0267 | 6.9791 | 0.9347 |
| **Weiner Filter** | 0.1019 | 57.860 | 0.2995 | 5.9163 | 0.2125 | 1.6751 | 0.3471 |
| **Laplacian Filter** | 2.7258 | 43.988 | 0.8378 | 18.994 | 0.0312 | 127.81 | 0.9989 |

Note: MSE=Mean square error, PSNR= Peak signal to noise ratio, MAE: Mean absolute error, SNR: signal to noise ratio, AMBE: Absolute mean brightness error, CNR: Contrast to noise ratio, UQI: Universal quality index.

**Table 2:** Performance metrics of Normal cases

| TECHNIQUE | MSE | PSNR | MAE | SNR | AMBE | CNR | UQI |
|---|---|---|---|---|---|---|---|
| **Histogram Equalization** | 209.91 | 24.912 | 75.824 | 0.8331 | 74.060 | 11.555 | 0.9065 |
| **AHE** | 119.23 | 27.381 | 22.471 | 3.3043 | 17.682 | 19.496 | 0.9284 |
| **CLAHE** | 132.48 | 26.919 | 28.796 | 1.9287 | 25.925 | 17.977 | 0.9095 |
| **Contrast Stretching** | 131.39 | 25.599 | 34.162 | 4.5222 | 31.298 | 37.769 | 0.9817 |
| **Median Filtering** | 7.4638 | 39.513 | 0.8473 | 37.539 | 0.0284 | 75.360 | 0.9903 |
| **Adaptive Median Filtering** | 979.32 | 15.487 | 10.502 | 13.097 | 10.259 | 4.2849 | 0.4484 |
| **Arithmetic Filtering** | 40605 | 2.0553 | 200.09 | -48.049 | 199.91 | 8.3962 | 0.0001 |
| **Geometric Filtering** | 0.0801 | 59.146 | 0.2734 | 9.0413 | 0.2735 | 7.5561 | 0.7829 |
| **Minimum Filtering** | 0.0139 | 66.717 | 0.0500 | 16.611 | 0.0500 | 6.9624 | 0.5969 |
| **Maximum Filtering** | 0.0134 | 66.854 | 0.0469 | 16.750 | 0.0226 | 7.1704 | 0.4912 |
| **Weiner Filter** | 0.1283 | 57.048 | 0.3280 | 6.9492 | 0.3253 | 3.1214 | 0.0060 |
| **Laplacian Filter** | 1.6844 | 45.953 | 0.6926 | 21.876 | 0.0895 | 224.11 | 0.9984 |

**Note:** MSE=Mean square error, PSNR= Peak signal to noise ratio, MAE: Mean absolute error, SNR: signal to noise ratio, AMBE: Absolute mean brightness error, CNR: Contrast to noise ratio, UQI: Universal quality index.

In Adaptive K means clustering method the image is separated into background and foreground (RBCs). Parasites were not isolated since they belonged to the same cluster as the cells. No differentiation was observed between infected and non-infected cells. Thus, this method was not taken for further use. Fuzzy c means method yielded best results for 3 clusters and 15 iterations. Similar to adaptive K means, this method did not differentiate infected and non-infected cells.

Active contour method involves processing time for 10,000 iterations were around 30 minutes. Incomplete segmentation was observed. Better results may be obtained by increasing number of iterations thus, increasing processing time leading to limitation of this method. In the anisotropic diffusion algorithm number of iterations was set to 30. And this method was not considered for further processing as the edges of the cells detected were discontinuous. An alternate method of Sobel edge detection followed by implementing morphological operations such as dilation using line structural element, hole filling and erosion using diamond structural element was implemented.

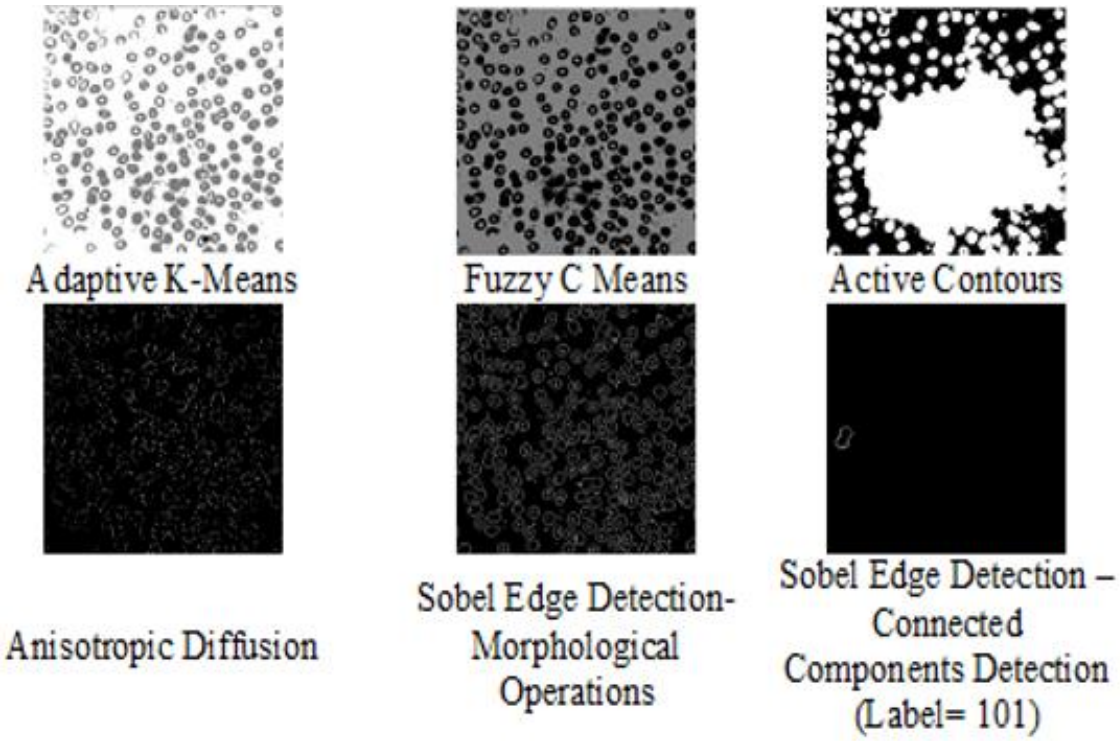


**Figure 5:** Results of Segmentation Techniques

It produced good segmentation results and can be used to distinguish infected and non-infected cells by extracting shape-based features. Similarly, the Sobel edge detection method with connected component resulted in discontinuous edges. While attempting to label the connected components, it was observed that most of the edges were line segments and only a few cells were detected completely. The desired segmentation results were obtained by performing the active contour method in HSV color space. The infected regions were identified by converting the image to HSV color space and the infected cells were highlighted using active contour. The simulated results of segmentation with detection of infected cells for the malarial and normal case at each stage have been displayed in **Figure 6 and 7**. A Graphical User Interface was created using MATLAB Guide to load the input image, display the output at different stages of segmentation and to identify the input as malaria-infected or non-infected blood smear image. A screenshot of the GUI is displayed in **Figure 8** for the malarial case and **Figure 9** for the normal case. The results of the work were validated with the diagnosis of the malarial images provided by the Department of Pathology, SRM Medical College and Research Centre, Kattankulathur.

## Conclusion

The method to detect the presence of malarial parasite from blood smear image using image processing techniques is presented. Various preprocessing methods were implemented, and the significant method is obtained by analyzing the performance metrics. From the analysis, it is observed that the Laplacian filter satisfied all of the criteria mentioned as the suitable pre-processing technique for the acquired images. The next step to preprocessing is segmentation and detection of infected cells. The blood smear images are classified as malarial infected or non-infected by converting the input image from RGB to HSV color space, performing histogram equalization and Otsu thresholding on H and S components individually and adding the H and S masks to obtain the seed points for active contour. The output of the active contour shows the infected cells, the presence of which is detected and used for determining if the blood smear image is infected or not. The work is combined with a Graphical User Interface which outputs the segmented results and shows if the input is malarial infected or non-infected. The work can be extended to identify the stage of the malarial parasite. The percentage of infected cells in the blood smear image can also be provided to know the extent of parasite infection.

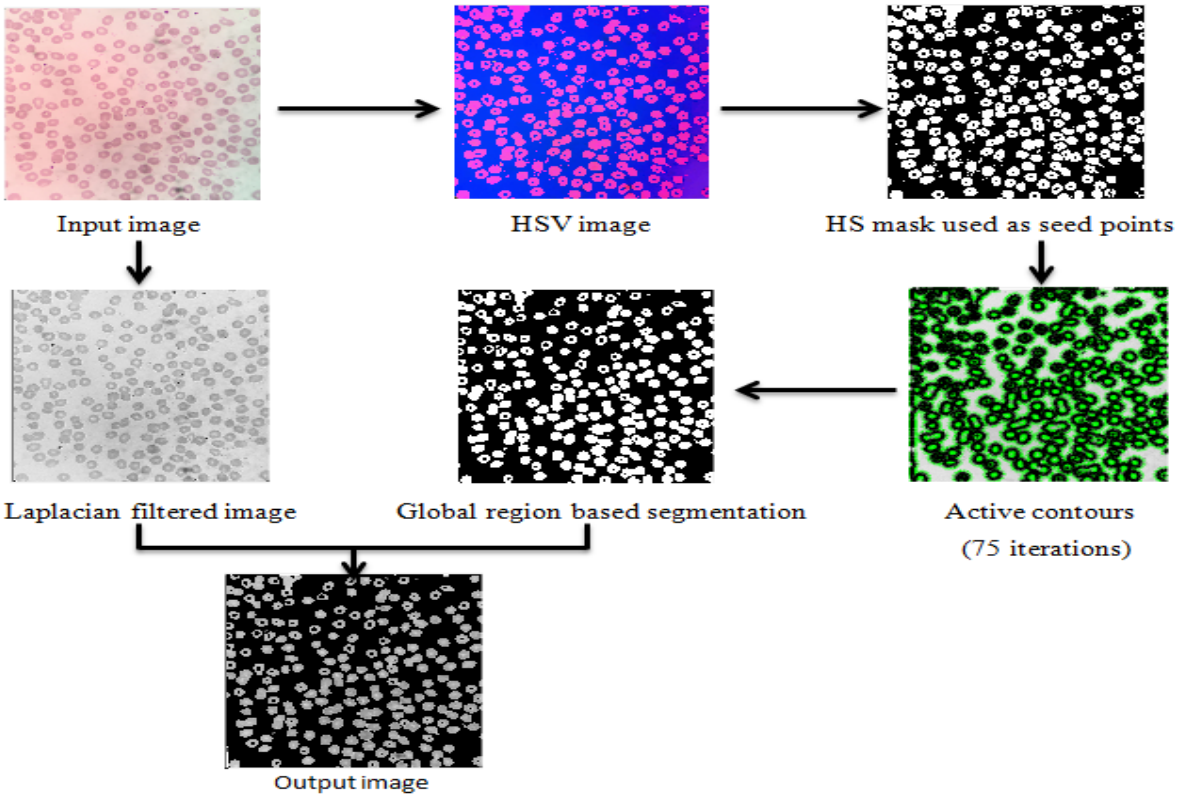


**Figure 6:** Segmentation and detection of infected cells Output for Malarial case

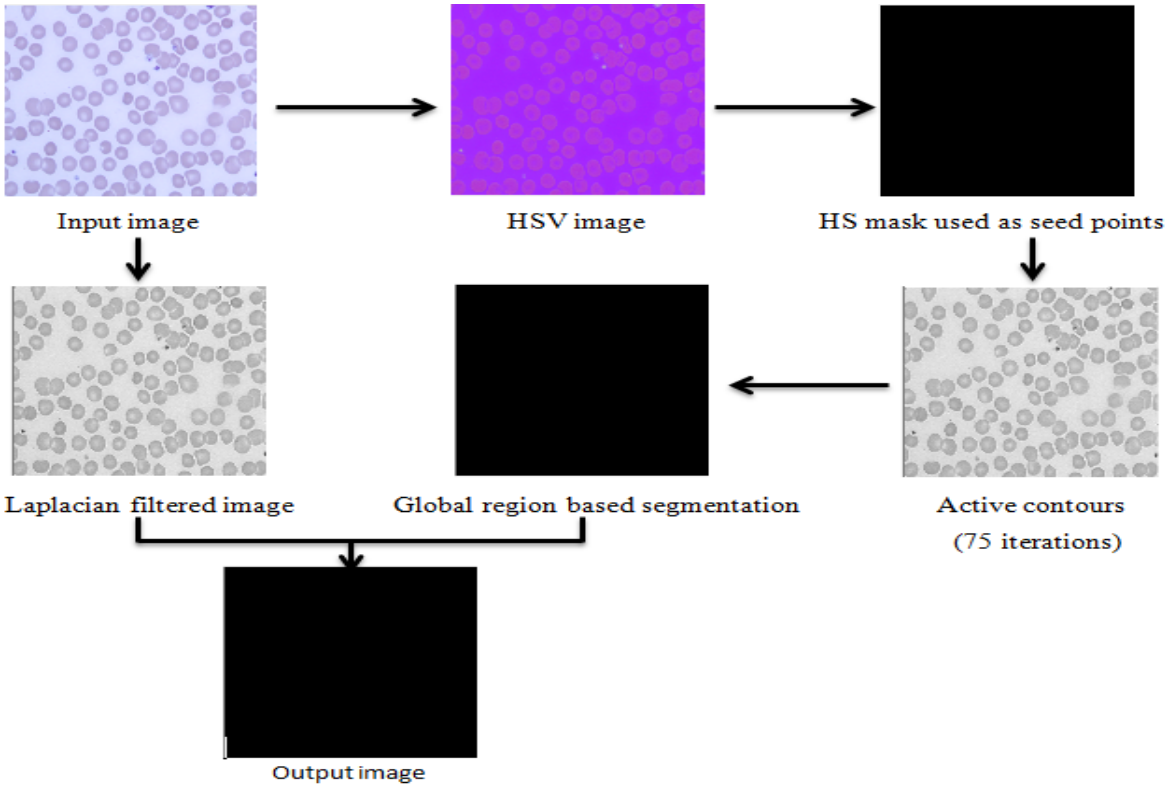


**Figure 7:** Segmentation and detection of infected cells Output for Normal case

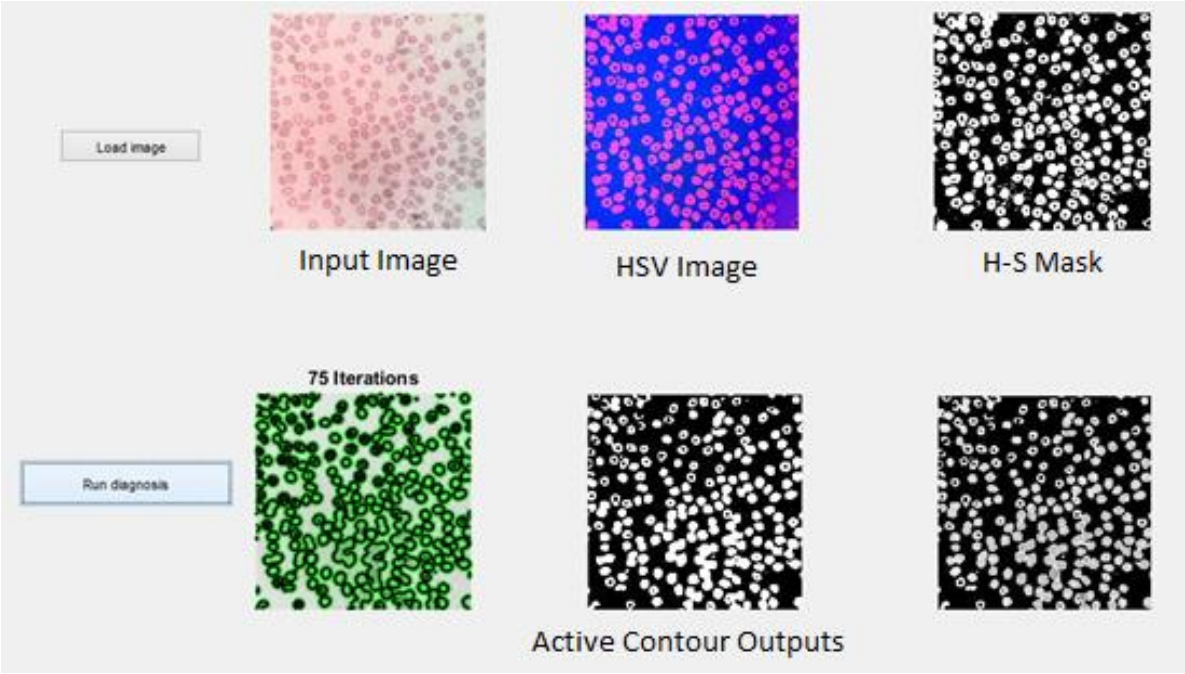


**Figure 8:** Graphical User Interface (GUI) interpretation for Malarial case

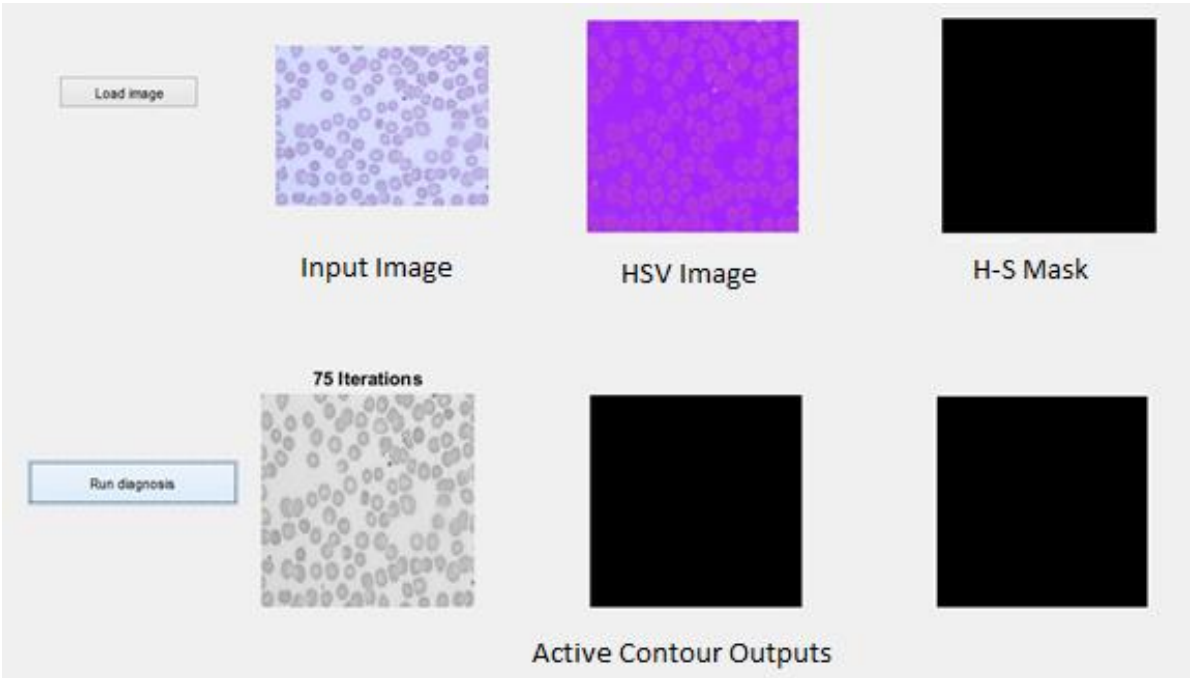


**Figure 9:** Graphical User Interface (GUI) interpretation for Normal case

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## Conflict of Interest

All authors have disclosed no conflicts of interest.

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