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A Method for Malaria Parasites Detection Systems

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Conflicts of Interest: Nil

Abstract:
Malaria disease is present in semi urban and non-urban areas especially in developing countries. It is always found that non-occurrence of the malaria disease in patients is more than occurrence of malaria disease in patients. It is necessary to develop a model based on decision support system with datasets. It is necessary for predicting the disease quite accurately using the model. This paper presents a model for malaria parasite identification and classification method. The proposed method detects falciparum and vivax plasmodium using the model.

Keywords: malaria; red blood cells segmentation; medical image analysis

Introduction

Malaria a vector borne disease, the data of which always creates a class imbalance problem because of the presence of samples of negative class (unaffected patients) dominating the samples of positive class (affected patients) [1-5]. Handling such a kind of imbalanced data, is a critical task because prediction of a patient with or without a disease becomes an important problem in the medical scenario [6-8]. Unbalanced data sets are include not only medical domain but also domains like credit card fraud detection[9], detection of problems in software's[9-10],detection of oil spills in satellite radar images[11-12], frauds in telecommunications[9], detection of frauds in financial sector[9] etc.

The disease, possibly, originated in Africa and spread to the Mediterranean region, South East Asia and India. It was very common in the marshy areas of Rome and the name originated from 'bad air' from the Italian language of 'mal-aria'. Over 500 million gets affected due to malaria infections annually, causing 1-2 million deaths, the majority of which are children in sub-Saharan Africa [5]. The occurrence of malaria cases is on an increasing trend due to a decrease of malaria control resulting in increased transmission of the disease.

Five species of plasmodium can infect human. The majority of deaths are caused by falciparum and vivax plasmodium. Every parasite will not exhibit the morphological characteristics. Malarial infection is diagnosed by microscopic examination of blood using blood films or with antigen based rapid diagnostic. Even after fifty years of malaria eradication program, it still continues to increase. The researchers are required to develop a rapid, accurate and affordable diagnostic method for early malaria parasite detection [1]. Detection of malaria is required early for effective treatment. The standard technique for blood cell analysis for diagnosing is based on Microscopic blood image analysis , however in remote area, a delay in obtaining results produce incorrect initial treatment due to unavailability of early diagnose system.

Malaria disease is one of the global health problem that occurred by a mosquito bite [1-2]. Malaria is a vector borne disease. Even though people maintain with healthy life style with good food habits and with neat surroundings, still due to the climate changes or for any other reason, many people are affected by malaria disease [1], [3]. According to 2016 report of World Health Organization (WHO), one million people are dying annually due to the vector borne diseases

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like malaria [4]. Overall, it is estimated that, the incessant presence of Malaria disease has decreased the world population by 60% [4-5].

The parasite and the vector are constantly evolving, so traditional methods are less effective in malaria control and new therapeutic methods are required [13-16]. The inherent problems associated with peripheral blood smear analysis with light microscope performed in laboratories have motivated scientists to develop methods to automate this process [33]. Modern diagnostic techniques are classified on the basis of cost and performance [17-20]. Polymerase Chain Reaction (PCR) techniques and Third Harmonic Generation (THG) imaging using infrared ultrafast pulsed laser excitation are some of the highly accurate and costly methods employed for parasite detection [34]. THG microscopes can detect parasite from unstained smear sample with high accuracy, but exorbitant costs of such system and maintenance issues prevent the usage of systems in developing countries [35]. Bio-chemical tests by identifying parasitic Lactate Dehydrogenase (LDH), the use of fluorescent microscopy and fluorometric analysis methods and using radio labelled precursors Hypoxanthine triturated, are “modern gold standard” in malaria detection [36]. Such tests are unavailable in under-developed countries due to the inherent high cost of system installation and maintenance [21-26]. Blood cell counters based on Coulter’s technique and flow cytometry based automated cell counters perform quantitative analysis, but experts are required for qualitative analysis which has been reported as 21% of the blood sample analysis by authors in ref [22]. Rapid Diagnostic Test (RDT) [37] and conventional microscopy [11,38] are low cost systems. RDT has an advantage over the latter with faster detection and can be performed by unskilled person but is less reliable method [38]. Moreover, only falciparum varieties are identified by such kits. The microscopic technique with thin blood smear stained with Giemsa remains the most reliable method in under-developed countries [27-30].

Literature Review

Researchers developed Malaria Diagnosis and Therapy by Web-Based Medical Assistant System [3]. Some researchers used machine learning technique where rough Set was used on training set for generation of classification model for malaria diagnosis. Malaria diagnosis was also developed using Fuzzy Expert System [7]. Health problem has a great threat to the existence of many communities and the complexities in medical practice make traditional quantitative approaches of analysis inappropriate.

The Application of Machine Learning Techniques for malaria diagnosis is needed to use computer technology to reduce the number of mortality and reduce the waiting time to see the specialist on malaria. Structured System and Design Methodology (SSADM) is also used. Clinical Protocol-Based Decision Support System for Malaria Treatment is developed[31-34].

The intervention of technology to assist pathologists and medical practitioners is vital for the fight against malaria and its early diagnosis to prevent mortality. The biologists and the chemists were busy discovering means to control the disease. Advancement in microscopy and computer technology has bolstered this effort. Several kinds of literature can be found in the research domain that has contributed to the research work. Several authors have tried to compare different methodology both in terms of detection technology and computational methods to establish better ways to identify and diagnose malaria. Some notable work is described in the following paragraphs in this section. [5] studied the different techniques for malaria detection. Apart from studying conventional microscopy for determination of malaria by an expert based on visual examination of peripheral smears, ‘gold standard for malaria’, the authors also studied other different laboratory methods for de-termination of malaria. The use of easy to implement mechanisms like using of RDTs was also studied to determine their efficacies in the real word scenario. Use of serological methods and determination of specific antigens was discussed by the authors. The authors surveyed different contemporary biotechnology related techniques like PCR, mass spectrophotometry and the use of automated cell counters. The study fundamentally compares different possibilities for the diagnosis of malaria on a medical perspective rather than on a technology based standpoint[37-41].

Some authors performed a critical review of different algorithms of computer vision, image analysis and pattern recognition for automated malaria screening using thin blood smears digitized images [39-45]. According to the authors, most of the research work surveyed by them are a partial solution and cannot be used as a diagnostic aid. The main objective of the review work undertaken by the authors was to identify the advance methods employed, to provide an overall structure for further research and to determine different aspects of the problem for effective solution. The review article discussed different image processing methods that can be applied for parasite detection. The digital image acquisition methods, the variation in colour image arising due to different camera parameters and light source used, illumination variation, different thresholding problems associated with the image and solutions thereof. The authors also surveyed methods that performed detection process based on colour, morphological features, scale and Granulometry, cell size estimation, segmentation, and different classification methods including cross validation performed to remove training bias. The authors provided a critical review of the different approaches found in the literature. They further discussed detection of per specimen results based upon the Parasitaemia level.

Other authors performed a detailed survey of methods in the machine learning domain that was utilized by different authors for parasite detection and classification and to determine the scope for further enhancement [33]. The authors discussed methods used under super-vised and unsupervised machine learning techniques. The feature extraction methods like the use of PCA and different type of features extracted by various research works was also listed by the authors. Similarly, the authors discussed the use of supervised and unsupervised ANN, SVM, k-Means clustering, Adaboost and Multiple Classifier Systems or MCS used at different levels of pattern classification, segmentation and pre-processing steps. Detailed descriptions of these methods were provided by the authors with the view that other co-research workers will be able to understand and implement such systems for the development of a CAD system.

Authors surveyed several types of research works related to CAD based systems for malaria parasite detection [46-50]. The authors dis-cussed several other modalities for obtaining malaria diagnosis. The use of digital microscopy as the best option and the use of ma-chine learning technique like ANN for the purpose of classification of lifecycle stage, parasite species and presence of parasite was concluded by the authors to be the best among all the methods de-scribed by them[51-53]. The authors, however, did not categorize the literature surveyed by them. This review citation provides a detailed description of the problem that is required to be solved. A detailed insight of contemporary technology for diagnosis of malaria dis-ease has been described. The citation has categorized contemporary research citation under two broad categories and has provided a detailed description of the proposed methods.

Proposed Method

The malaria parasites belong to the Phylum Apicomplexa, Class Sporozoea, Sub-Class Coccidia, Order Eucoccida and Sub- Order Haemosporina. Under this sub-order is Genus *Plasmodium* and this genus is characterized by the presence of two hosts in the lifecycle with Schizogony (asexual cycle) and Sporogony (sexual cycle). There are five different species affecting man. The more prevalent of them are *P. malaria* discovered by Laveran in 1881, *P. vivax* by Grassi and Feletti in 1890, *P. falciparum* by Welch in 1897 and *P. ovale* by Stephans in 1922. Similarly, the fifth species affecting only in South East Asia is known as P. knowlesi malaria and was discovered by Sinton and Mulliganin in 1932.Tropical regions of the world between 60° N and 40° S are naturally affected by the disease. The parasite resides in two hosts namely human for asexual lifecycle and female Anopheles mosquito for sexual lifecycle. Malaria protozoa have several forms within its lifecycle. They enter the human system as Sporozoite which is a minute thread-like protozoon with tapered ends on both sides. They are 9-12 μm ft in length as shown in Table 1.

Table 1: Plasmodium species during different stages of its lifecycle

Plasmodium	*P. vivax*	*P. falciparum*	*P. malaria*	*P. ovale*
	Benign Tertian Malaria	Malignant Tertian Malaria	Quartan malaria	Tertian Malaria
Trophozoite	Early Trophozoite (3µ dia size) have blue cytoplasmic ring, red nuclear mass& vacuole. Mature grows in size and takes amoeboid shape. RBC enlarges and irregular with Schaffner dots.	Early ring form (1.5 µ dia size) with fine and uniform cytoplasm ring with nucleus lying outside the ring often divided into two parts and at opposite pole. RBC remains normal with 6-12 Maurer's cleft.	They are similar to vivax but Mature Trophozoite assumes a band like shape and coarse brown to black pigment appears in cytoplasm. RBC is not enlarged and is undotted. But Ziemann's dot on prolonged staining.	Early ring form (2-.5µ dia size) and similar to malaria but without the band shape. Dark brown pigment in cytoplasm. RBC enlarged, irregular in shape with James dots.
Schizont	They almost fill the enlarged RBC (9-10µ dia size) the nucleus is large and lies on the periphery. After nuclear division on average 16 daughter individual form a roseate like cluster. RBC bursts at this stage.	They fill two third of RBC (5µ dia size). After nuclear division 8-32 daughter cell produced. RBC remains un-enlarged. They burst to release the cells.	The *plasmodium* is (6.5-7µ dia size) and fills the RBC. On nuclear division 6-12 daughter cells are arranged around a central mass. RBC remains un-enlarged and bursts at maturity of the daughter cells.	The *plasmodium* is (6.µ dia size) and fills three quarter of RBC. On nuclear division 6-12 daughter cells are arranged irregularly. RBC remains slightly enlarged before bursting.
Merozoite	12 - 24 oval cytoplasm containing mass (1.5-1.75µ length and 0.5µwidth)	18-24 circular cytoplasm mass (0.5-0.7 µ dia)	6-12 cytoplasm containing mass (2-2.5 µ dia)	6-12 cytoplasm containing mass with crescentic (2- 2.5 µ dia)
Gametocyte	Spherical in shape and slightly enlarged RBC containing granules.	Host RBC is filled, and the gametocyte is Crescent shaped.	Same size of the host RBC. They are round in shape.	Round in shape and host RBC is slightly enlarged.

The different life form of the parasite within the human host is shown in Figure 1. It shows the different life cycle form that is exhibited by the parasite within the RBC of human host. The development of gametocyte initiates the sexual life cycle of the parasite that takes place within the mosquito. Some infected cells instead of developing merozoites develop into gametocytes. They either form all male microgametes of size 9-10 µm or all female macrogamete of size 10-12 µm. They travel to the peripheral blood vessels of the host for transmission by female anopheles mosquito vector. The different species can be identified by changes in the shape of the infected cell, by the characteristic presence of some dots like Schaffner's dots, Maurer's clefts, Ziemann's Stippling, James' dot and the morphology of the parasite during different lifecycle stages [21]. At each of the life cycle stage, the parasite exhibit differences in its morphology, size, by the presence or absence of malarial pigment Haemozoin. Since the parasite exhibit rapid growth, often it becomes difficult to distinguish the transient

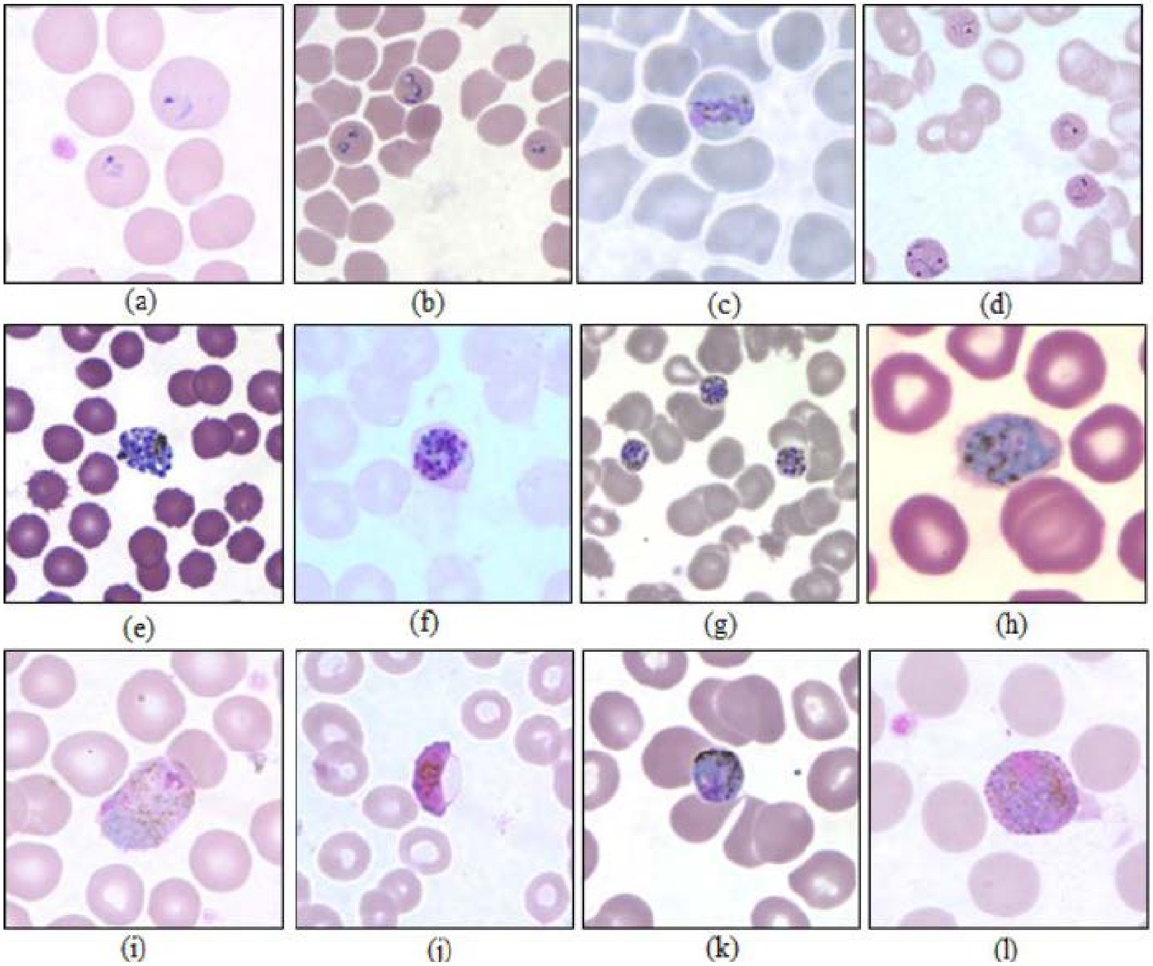

Figure 1: A comparison of the different asexual lifecycle forms of Plasmodium genus within the host Red Blood Cell. Figure (a)-(d) are Trophozoite of P. vivax, P. falciparum, P. malariae and P. ovale; Figure (e)- (h) Mature Schizont of P. vivax, P. falciparum, P. malariae and P. ovale; Figure (i)-(l) Gametocyte of P. vivax, P. falciparum, P. malariae and P. ovale.

The life cycle of malaria parasite inhabits within two distinct hosts. Within the human host, it undergoes asexual lifecycle or schizogony. Human beings are intermediary hosts while the sexual lifecycle or sporogony happens within female Anopheles mosquito, thus being the definitive host for the parasite. The development of gametes though takes place within the human host but the sexual process occurs within the mosquito. The asexual phase within the human is initiated by the injection of sporozoites through a bite by an infected mosquito. Within the human hosts, it undergoes three to four distinct cycles depending on the infected species. A Pre-Erythrocytic or Primary Exo-Erythrocytic Schizogony happens within the parenchymal cells of hepatic tissues of the liver. A single generation multiplication happens and merozoites are liberated called cryptozoites. The smaller micromerozoites enter the blood circulation to initiate the next cycle. Within the blood, the merozoites infect the RBC and the manifestation of the disease initiates after a definite number of days specific to each species. The Erythrocytic Schizogony cycle of development of Trophozoites, Schizonts, Merozoites and re-invasion of fresh RBC continues for several cycles at definite interval depending on the species. This is characterized by the recurrent febrile symptoms of the patient. This continues till the exhaustion of asexual life of the parasite. After achieving a certain number of asexual reproduction, the merozoites develop into gametocyte or the initiation of the sexual phase or gametogony. They generally develop within the smaller blood vessels of the internal organs like spleen and bone marrow. Mature gametes are found on the peripheral blood vessels ready for transmission by disease vector. A Latent Hepatic stage is observed in species like *P. vivax* and *P. ovale* where the merozoite enter a suspended state called Hypnozoite responsible for relapse of the disease.

Soon after a blood sucking event by female Anopheles mosquito from a malaria infected person the sexual cycle or Sporogony is initiated. A single blood meal requires more than 12 gametes/mm3 of which the female macrogametocytes should exceed the male microgametocytes. Each microgametocyte produces 4-8 threadlike microgametes whereas a macrogametocyte contains a single macrogamete. Through the process of flagellation and with the aid of chemotaxis a single microgamete comes in contact with the female macrogamete. By dissolving the wall, the nuclear material of the microgamete is infused inside the macorogamete. Fertilization occurs when the two pro-nuclei fuses to form a zygote. The zygote matures to form an ookinete. This process occurs within the mid-gut of mosquito. The ookinete further matures and form oocyte on the stomach wall. Within the oocyte several meiotic and mitotic division results in the development of thousands of sporozoites. The oocyst raptures and the released sporozoites travel all around the mosquito body through the body fluids. They have an affinity to be present within the salivary glands for easy transmission to the intermediary host via a bite on human tissue. A schematic diagram is summarizing the parasite lifecycle shown in Figure 2.

Early and effective treatment of malaria can prevent its rapid spread among the immediate geographic location. The malaria affecting areas being mostly backward, with inadequate medical resources, thus an efficient diagnostic plan can reduce the menace and burden to society . The diagnosis of malaria identifies the presence of malaria parasite cells, antigens and antibodies within the human blood. There are different malaria lifecycle stages and five types to identify from. Moreover, diagnosis further depends upon transmission of disease, Parasitaemia, immunity, drug resistance, penetration of the parasites in the deeper tissues and other factors. Malaria in humans are diagnosed by establishing presence/absence of malaria parasite in the blood stream in adequate number, establishing the species present and the lifecycle stage of the identified parasites . Early diagnosis can prevent mortality rates. Diagnosis of malaria also depends on the availability of proper diagnostic equipment and presence of adequate trained technicians. There are areas in Africa where malaria is present among a wide population but the symptoms do not manifest and remain asymptomatic. Economical backwardness often contributes to undertrained and underpaid technicians, while underequipped medical infrastructure hinders early diagnosis of malaria.

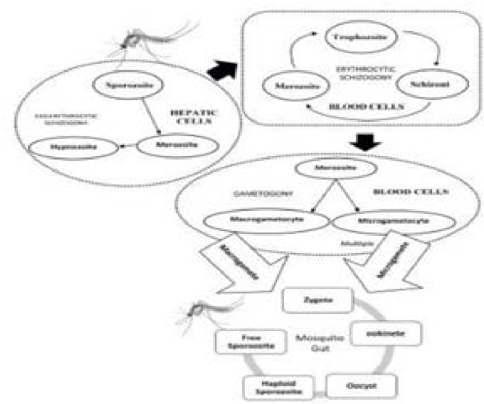

Figure 2: The life cycle of Plasmodium genus and its development in Human and Mosquito hosts

CAD based detection of Malaria Parasites consists of pre-processing, segmentation, feature extraction, classification and detection of infected red blood cells. It is actually parasitemia separation from infected RBC. Initially, the malaria images of three different stages, schizont, trophozoite, and gametocyte stages is captured from the blood smears. The malaria images taken from the thin blood smears of P. vivax samples. A database is created consisting of both types of samples parasitic and nonparasitic blood samples. Theses samples are taken from hospitals in west Bengal. CAD usually comprises four different image processing and analysis tasks, as follows:

1. Pre-processing.
2. Segmentation.
3. Feature extraction.
4. Classification

The proposed system is shown in figure 3.

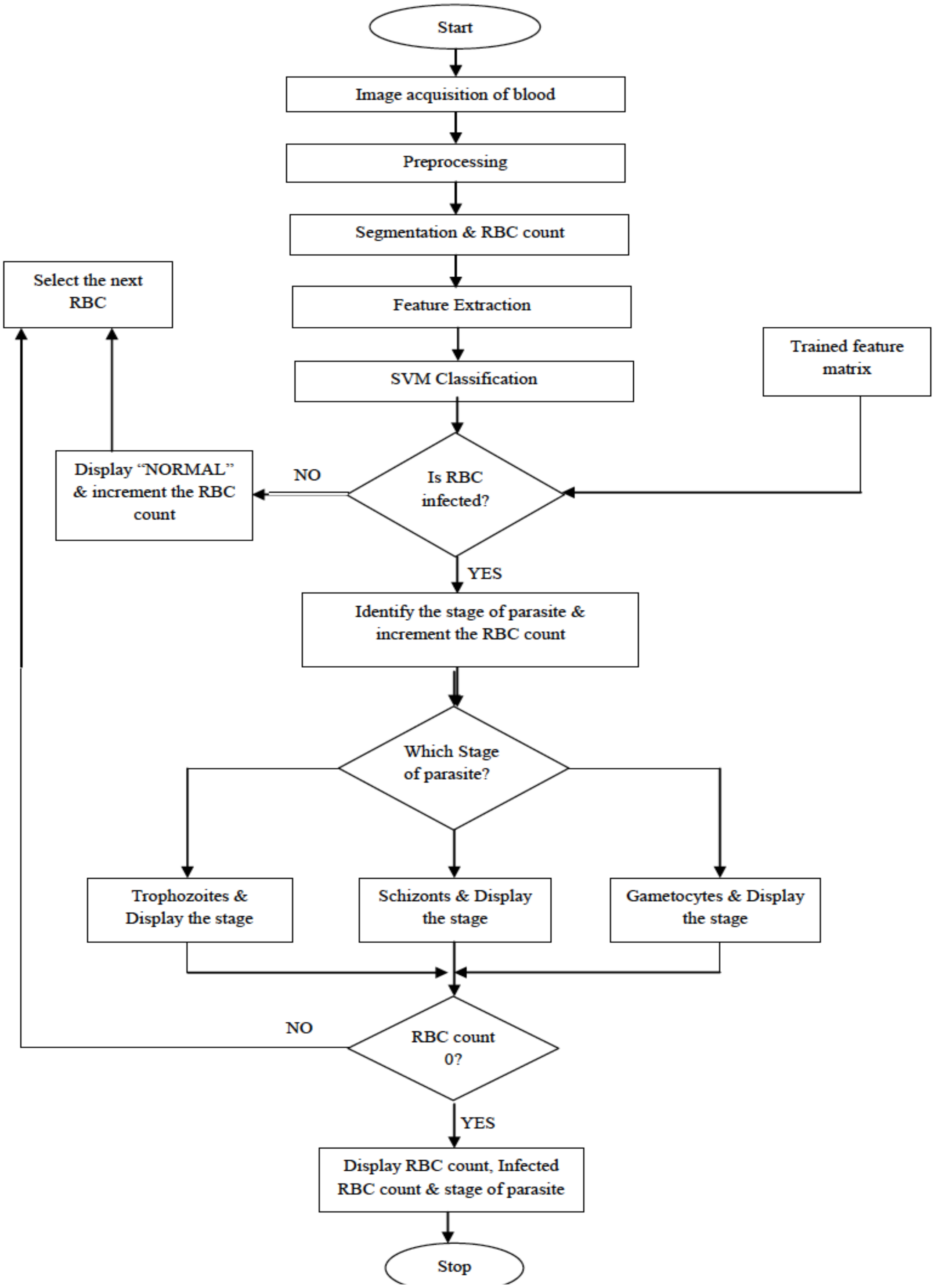

Figure 3: Proposed System

Results

For parasite detection RBC regions were investigated to determine edge presence using a binary mask and morphologi-cal erosion operation. The image processing was performed using MATLAB software. For testing, the authors compared the results of the software with manual counting by experts. Figure 4 shows raw image and detected parasite cells.

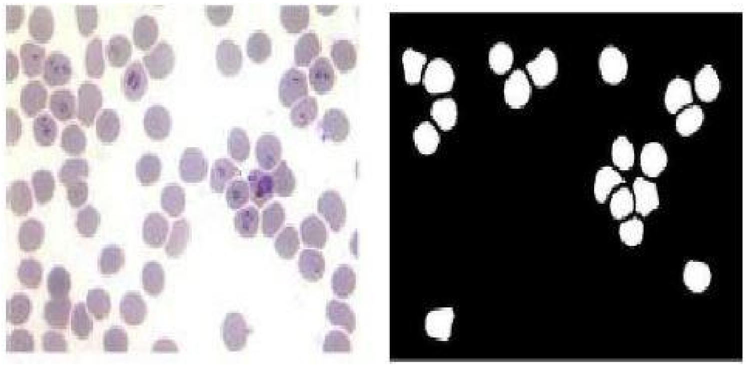

Figure 4: The raw image with parasites and the output mask of the parasite detected cells

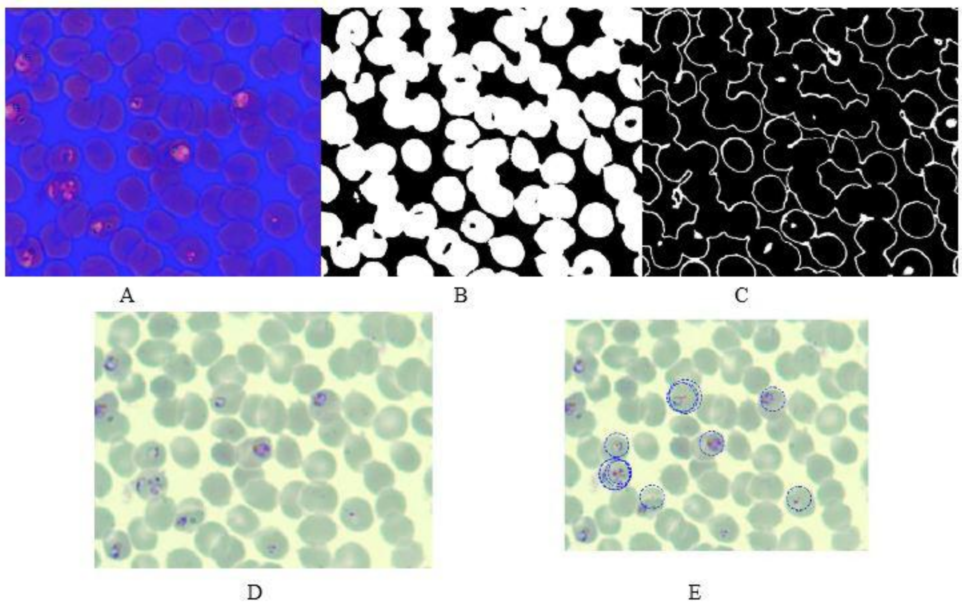

Figure 5: The original images (A) and the output image highlighting the infected RBC (B). The colour transformed image in HSV model (C), The RBC mask image (D) and the outline of the RBC image mask in (E)

To test the efficacy the algorithm was also tested on normal images taken from smear slides obtained from normal humans. The output of the system is shown in Figure 8. Detection and enumeration outcomes are shown in Figure.

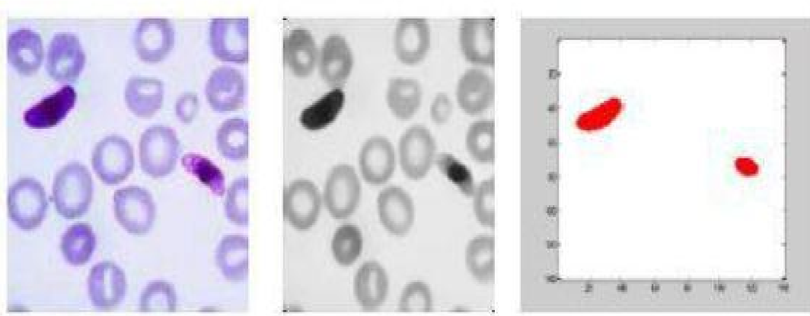

Figure 6: The original image, grayscale converted image and the output image colouring only the parasite detected by the system

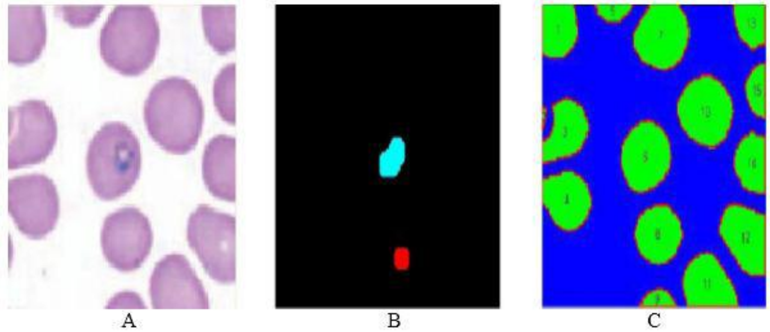

Figure 9: The output the proposed system where the original image containing a Tropozoite as in (A), the infected region mask in (B) and the enumeration of cells in (C).

The results of accuracy, sensitivity and specificity calculated in percentage are as shown in the table2.

Table 2: Accuracy, Sensitivity and Specificity calculated in %

Accuracy	Sensitivity	Specificity
97.7 %	97.4%	97.7%

Conclusions

The malaria parasite varies according to the growth and change in life cycle stages. Hence to detect its presence in blood images. In this paper CAD based malaria parasite detection is proposed.

References

1. Loddo, A.; Putzu, L.; Di Ruberto, C.; Fenu, G. A Computer-Aided System for Differential Count from Peripheral Blood Cell Images. In Proceedings of the 2016 12th International Conference on Signal-Image Technology Internet-Based Systems (SITIS), Naples, Italy, 28 November–1 December 2016; pp. 112–118.
2. Di Ruberto, C.; Loddo, A.; Putzu, L. A leucocytes count system from blood smear images: Segmentation and counting of white blood cells based on learning by sampling. Mach. Vis. Appl. 2016, 27, 1151–1160.
3. World Health Organization (WHO). Malaria Fact Sheet December 2016.
4. Somasekar, J. Computer vision for malaria parasite classification in erythrocytes. Int. J. Comput. Sci. Eng. 2011, 3, 2251–2256.
5. Soille, P. Morphological Image Analysis: Principles and Applications; Springer: Berlin, Germany, 2004; p. 392.
6. Giardina, C.; Dougherty, E. Morphological Methods in Image and Signal Processing; Prentice-Hall, Inc.: Upper Saddle River, NJ, USA, 1988.
7. Serra, J. Image Analysis and Mathematical Morphology; Academic Press: Cambridge, MA, USA, 1984.
8. Benavent, X.; Dura, E.; Vegara, F.; Domingo, J. Mathematical Morphology for Color Images: An Image-Dependent Approach. Math. Probl. Eng. 2012, 2012, 1–19.
9. Nachtegael, M.; Kerre, E. Classical and Fuzzy Approaches towards Mathematical Morphology. In Fuzzy Techniques in Image Processing; Studies in Fuzziness and Soft Computing; Kerre, E., Nachtegael, M., Eds.; Physica-Verlag: Heidelberg, Germany, 2000; Volume 52, Chapter 1, pp. 3–57.
10. Di Ruberto, C.; Dempster, A.; Khan, S.; Jarra, B. Analysis of infected blood cell images using morphological operators. Image Vis. Comput. 2002, 20, 133–146.
11. Rosado, L.; da Costa, J.M.C.; Elias, D.; Cardoso, J.S. Mobile-Based Analysis of

Malaria-Infected Thin Blood Smears: Automated Species and Life Cycle Stage Determination. Sensors 2017, 17, 2167.
12. Das, D.; Mukherjee, R.; Chakraborty, C. Computational microscopic imaging for malaria parasite detection: A systematic review. J. Microsc. 2015, 260, 1–19.
13. Tek, F.; Dempster, A.; Kale, I. Computer vision for microscopy diagnosis of malaria. Malar. J. 2009, 8, 1–14.
14. Oliveira, A.; Prats, C.; Espasa, M.; Serrat, F.Z.; Sales, C.M.; Silgado, A.; Codina, D.L.; Arruda, M.E.; i Prat, J.G.; Albuquerque, J. The Malaria System MicroApp: A New, Mobile Device-Based Tool for Malaria Diagnosis. JMIR Res. Protoc. 2017, 6, 1–11.
15. Dave, I.; Upla, K. Computer Aided Diagnosis of Malaria Disease for Thin and Thick Blood Smear Microscopic Images. In Proceedings of the 2017 4th International Conference on Signal Processing and Integrated Networks (SPIN), Noida, India, 2–3 February 2017; p. 561.
16. Devi, S.; Singha, J.; Sharma, M.; Laskar, R. Erythrocyte segmentation for quantification in microscopic images of thin blood smears. J. Intell. Fuzzy Syst. 2017, 32, 2847–2856.
17. Somasekar, J.; Reddy, B. A Novel Two-Stage Thresholding Method for Segmentation of Malaria Parasites in Microscopic Blood Images. J. Biomed. Eng. Med. Imaging 2017, 4, 31–42.
18. Sulistyawati, D.; Rahmanti, F.; Purnama, I.; Purnomo, M. Automatic Segmentation of Malaria Parasites on Thick Blood Film using Blob Analysis. In Proceedings of the 2015 International Seminar on Intelligent Technology and Its Applications, Surabaya, Indonesia, 20–21 May 2015; pp. 137–142.
19. Khan, M.; Acharya, B.; Singh, B.; Soni, J. Content based image retrieval approaches for detection of malarial parasite in blood images. Int. J. Biometr. Bioinform. (IJBB) 2011, 5, 97–110.
20. Savkare, S.; Narote, S. Automatic Classification of Normal and Infected Blood Cells for Parasitemia Detection. Int. J. Comput. Sci. Netw. Secur. (IJCSS) 2011, 11, 94–97.
21. Savkare, S.; Narote, S. Automatic detection of malaria parasites for estimating parasitemia. Int. J. Comput. Sci. Netw. Secur. (IJCSS) 2011, 5, 310–315.
22. Das, D.; Maiti, A.; Chakraborty, C. Textural pattern classification of microscopic images for malaria screening. In Advances in Therapeutic Engineering; Yu, W., Chattopadhyay, S., Lim, T., Acharya, U., Eds.; CRC Press: Boca Raton, FL, USA, 2014; Chapter 19, pp. 419–446.
23. Schulze, M. Biomedical Image Processing with Morphology-Based Nonlinear Filters. Ph.D. Thesis, University of Texas at Austin, Austin, TX, USA, 1994.
24. Špringl, V. Automatic Malaria Diagnosis Through Microscopy Imaging. Master's Thesis, Czech Technical University in Prague, Faculty of Elecrical Engineering, Prague, Czech Republic, 2009.
25. Khan, N.; Pervaz, H.; Latif, A.; Musharraf, A. Unsupervised identification of malaria parasites using computer vision. In Proceedings of the 2014 11th International Joint Conference on Computer Science and Software Engineering (JCSSE), Chonburi, Thailand, 14–16 May 2014; pp. 263–267.
26. Sathpathi, S.; Mohanty, A.; Satpathi, P.; Mishra, S.; Behera, P.; Patel, G.; Dondorp, A. Comparing Leishman and Giemsa staining for the assessment of peripheral blood smear preparations in a malaria-endemic region in India. Malar. J. 2014, 13, 512–516.
27. González-Betancourt, A.; Rodríguez-Ribalta, P.; Meneses-Marcel, A.; Sifontes-Rodríguez, S.; Lorenzo-Ginori, J.V.; Orozco-Morales, R. Automated marker identification using the Radon transform for watershed segmentation. IET Image Process. 2016, 11, 183–189.
28. Kareem, S.; Kale, I.; Morling, R. Automated Malaria Parasite Detection in Thin Blood Films:- A Hybrid Illumination and Color Constancy Insensitive, Morphological Approach. In Proceedings of the 2012 IEEE Asia Pacific Conference on Circuits and Systems (APCCAS), Kaohsiung, Taiwan, 2–5 December 2012; pp. 240–243.

29. Romero-Rondon, M.; Sanabria-Rosas, L.; Bautista-Rozo, L.; Mendoza-Castellanos, A. Algorithm for detection of overlapped red blood cells in microscopic images of blood smears. DYNA 2016, 83, 187–194.

30. Reni, S.K.; Kale, I.; Morling, R. Analysis of thin blood images for automated malaria diagnosis. In Proceedings of the 2015 E-Health and Bioengineering Conference (EHB), Iasi, Romania, 19–21 November 2015; pp. 1–4.

31. Sheikhhosseini, M.; Rabbani, H.; Zekri, M.; Talebi, A. Automatic diagnosis of malaria based on complete circle ellipse fitting search algorithm. J. Microsc. 2013, 252, 189–203.

32. Somasekar, J.; Reddy, B. Segmentation of erythrocytes infected with malaria parasites for the diagnosis using microscopy imaging. Comput. Electr. Eng. 2015, 45, 336–351.

33. Tek, F.; Dempster, A.; Kale, I. Parasite detection and identification for automated thin blood film malaria diagnosis. Comput. Vis. Image Underst. 2010, 114, 21–32.

34. Arco, J.; Gorriz, J.; Ramirez, J.; Alvarez, I.; Puntonet, C. Digital image analysis for automatic enumeration of malaria parasites using morphological operations. Expert Syst. Appl. 2014, 42, 3041–3047.

35. Anggraini, D.; Nugroho, A.; Pratama, C.; Rozi, I.; Iskandar, A.; Hartono, R. Automated status identification of microscopic images obtained from malaria thin blood smears. In Proceedings of the 2011 International Conference on Electrical Engineering and Informatics, Bandung, Indonesia, 17–19 July 2011.

36. Elter, M.; Haßlmeyer, E.; Zerfaß, T. Detection of malaria parasites in thick blood films. In Proceedings of the 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Boston, MA, USA, 30 August–3 September 2011; pp. 5140–5144.

37. Kareem, S.; Morling, R.; Kale, I. A novel method to count the red blood cells in thin blood films. In Proceedings of the 2011 IEEE International Symposium on Circuits and Systems (ISCAS), Rio de Janeiro, Brazil, 15–18 May 2011; pp. 1021–1024.

38. Mushabe, M.; Dendere, R.; Douglas, T. Automated detection of malaria in Giemsa-stained thin blood smears. In Proceedings of the 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Osaka, Japan, 3–7 July 2013; pp. 3698–3701.

39. Ross, N.; Pritchard, C.; Rubin, D.; Dusé, A. Automated image processing method for the diagnosis and classification of malaria on thin blood smears. Med. Biol. Eng. Comput. 2006, 44, 427–436.

40. Otsu, N. A threshold selection method from gray-level histograms. Automatica 1975, 11, 23–27.

41. Ahirwar, N.; Pattnaik, S.; Acharya, B. Advanced image analysis based system for automatic detection and classification of malarial parasite in blood images. Int. J. Inf. Technol. Knowl. Manag. 2012, 5, 59–64.

42. Soni, J.; Mishra, N.; Kamargaonkar, C. Automatic difference between RBC and Malaria parasites based on morphology with first order features using image processing. Int. J. Adv. Eng. Technol. 2011, 1, 290–297.

43. Yunda, L.; Alarcón, A.; Millán, J. Automated image analysis method for p-vivax malaria parasite detection in thick film blood images. Sistemas & Telemática 2012, 10, 9–25.

44. Das, D.; Ghosh, M.; Chakraborty, C.; Maiti, A.; Pal, M. Probabilistic prediction of malaria using morphological and textural information. In Proceedings of the 2011 International Conference on Image Information Processing (ICIIP), Shimla, India, 3–5 November 2011; pp. 1–6.

45. Das, D.; Ghosh, M.; Pal, M.; Maiti, A.; Chakraborty, C. Machine learning approach for automated screening of malaria parasite using light microscopic images. Micron 2013, 45, 97–106.

46. Dey, R.; Roy, K.; Bhattacharjee, D.; Nasipuri, M.; Ghosh, P. An Automated system for Segmenting platelets from Microscopic images of Blood Cells. In Proceedings of the 2015 International Symposium on Advanced Computing and Communication (ISACC), Silchar, India, 14–15 September 2015; pp. 230–237.

47. Diaz, G.; Gonzalez, F.; Romero, E. A semi-automatic method for quantification and classification of erythrocytes infected with malaria parasites in microscopic images. J. Biomed. Inform. 2009, 42, 296–307.

48. Savkare, S.; Narote, S. Blood Cell Segmentation from Microscopic Blood Images. In Proceedings of the 2015 International Conference on Information Processing (ICIP), Shimla, India, 21–24 December 2015; pp. 502–505.

49. Tek, F.; Dempster, A.; Kale, I. Malaria parasite detection in peripheral blood images. In Proceedings of the 2006 British Machine Vision Conference (BMVC), Edinburgh, UK, 4–7 September 2006; pp. 347–356.

50. Jiang, X. Feature extraction for image recognition and computer vision. In Proceedings of the 2009 2nd IEEE International Conference on Computer Science and Information Technology (ICCSIT), Beijing, China, 8–11 August 2009; pp. 1–15.

51. Malihi, L.; Ansari-Asl, K.; Behbahani, A. Malaria parasite detection in giemsa-stained blood cell images. In Proceedings of the 2013 8th Iranian Conference on Machine Vision and Image Processing (MVIP), Zanjan, Iran, 10–12 September 2013; pp. 360–365.

52. Chen, S.; Zhao, M.; Wu, G.; Yao, C.; Zhang, J. Recent Advances in Morphological Cell Image Analysis. Comput. Math. Methods Med. 2012, 2012, 1–10.

Rosado, L.; da Costa, J.M.C.; Elias, D.; Cardoso, J.S. A review of automatic malaria parasites detection and segmentation in microscopic images. Anti-Infect. Agents 2016, 14, 11–22.