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REVIEW ARTICLE

Methodical Approaches of Image Analysis on Malarial Parasites Identification and Categorization in Thick Blood Smears-A Review

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ABSTRACT

Clinical trials have started focusing on other plasmodium species too, contrasting to the emphasis on Plasmodium falciparum so far. This review article lists out certain major techniques in detection and classification of malarial parasites. Although the world has succeeded in finding out the devices that detect and classify the malaria parasites well in thin blood films, technology still lacks sufficient innovation in identifying and classifying these infectious parasites in thick blood films. In order to fill the gap, it is aimed to provide a brief study of a collection of methods and systems especially via image processing in which these concepts are prioritized. Thick blood smear is primarily focused that would let in knowing the percentage of infected red blood cells by identifying the malarial parasites. Real time in vivo optical imaging of infected cells based on automated computer vision provides possible insights about the plasmodium species that could be applied to treat and prevent malaria.

Keywords: Malaria parasites, Detection, Classification, Image processing, Thick blood smears, Automated computer vision.

1. INTRODUCTION

1.1. Overview of malaria

Malaria is the deadliest tropical parasitic disease that takes away the lives of one to three million people per year (India had around 1 million deaths before independence and then gradually decreased by 1965, but then this low rate was not maintained). [1-3] Most of the research articles in malarial parasite detection and classification manifests the affected individuals and makes note of deaths in each year in their own respective regions. Even after the formulation of malaria eradication program, the situation became worse owing to the resistance development in these parasites. [4, 5] Its main victims are from endemic regions. Therefore effective methods in complete abolition of this disease should be developed. [6-9] But, several researches highlight the demand to control all phases of the parasites to prevent this disease. The infection begins when the sporozoites of infected mosquito enter into the human bloodstream.

Antibodies have role in sporozoites' transmission into the body from the extracellular environment [10-13]. 90% of the deaths caused by P.falciparum are due to the occurrence of acute complications. Crucial factors in malaria diagnosis include: ensuring the blood specimen is of malaria positive or negative, determining the species of the identified parasite and detecting its life cycle phases (trophozoite, schizont, and gametocyte). Only by identifying the correct

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parasitic species, apt treatment can be provided; while, identifying the life cycle phases is to appropriately evaluate parasitemia or to detect to which extent the cells are infected. Blood smear images may consist of disease causing several types of parasites apart from erythrocytes, leukocytes and platelets, thus making its contents complex. [14] So segmenting and defining its morphology is difficult. As manual cell counting system is not reliable, there is a need to switch to machine based advanced pattern recognition and computer vision programs.

Besides P.falciparum and P.vivax, other common plasmodium species that cause infection are ovale, malariae and knowlesi. But the worldwide distribution and mortality rate of these three species is less when compared to P.falciparum and P.vivax. Recently P.cynomologi has been identified that infects humans rarely but often affects other species [15-17].

1.2. Life cycle stages of parasites growth and membrane dynamics

Besides rising contribution in antimalarial drug advancement and control schemes, 50% of the world population is still exposed to this devastating tropical disease. [18, 19] Phospholipid hydrolyzing esterase has a great role in membrane dynamics in infection of the host cells. Mainly, studies of such related topics potentially target malarial therapy. [20] Such pathogens, when entering the body as infective sporozoites affects the liver, replicate in hepatocytes in turn to merozoites. On reaching the bloodstream, Red Blood Cells (RBCs) get infected, where the infected cycle lasts one to three days with respect to the plasmodium species.

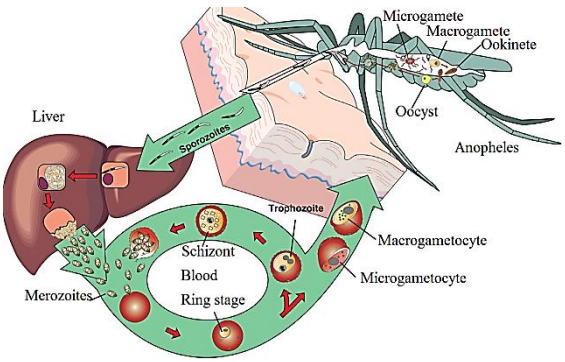

Figure 1.Life cycle of P.falciparum

The process continues in the generation of intra-erythrocytic gametocyte phases. It takes 10 days for the gametocytes of P.falciparum to get matured and forms gametes. They fuse to form ookinete which then become oocyst and sporozoites in mosquitoes. The life cycle of P. falciparum is shown in figure 1. Plasmodium remains mostly in human host cells and that can be hepatocytes or erythrocytes. This intracellular life process is followed by membrane biogenesis and growth of vacuolar system and sub-cells; thereby the structural and functional attributes of the host cells are substantially varied. Within the host cells, the plasmodium parasites are enclosed by parasitophorous vacuolar membrane, formed by invading the membranes of the blood cells. [21, 22] Parasites also form tubovesicular networks that permit molecule transfer between parasite cytosol and host cells. Meanwhile a large amount of lipids (phospholipids, neutral lipids and cholesterol) are required for parasites growth. Due to malarial infection, drastic changes occur in the quantity of these lipids.

Particularly, image processing and computer aided methods are employed to detect parasites in blood smears attained by microscopes. Upon availability of required data, the imaging algorithms could recognize plasmodium species. Further investigations are certainly required to classify them and identify their growth phases. Rather than using direct microscopic images, other mechanisms like fluorescence imaging or flow cytometry are also in practice. [23, 24] Utilizing image processing helps in remote diagnosis methods to improve the efficacy of experts and makes use of low cost microscopes with simple optical modules that adapts with portable and cellular units, and could include various tests with same images [25].

Numerous approaches for detection and classification of parasites have been proposed, and presently, image processing method is highly focused to localize cell movements and inspect in finding out the type of spatial dependence in respect of these movements. Image processing to detect malaria parasites require only low computational resources and so less complicated, which in turn results in proper detection and classification of plasmodium species. It is required to image the malarial

infection so as to explore the systems behind malarial pathophysiology and to enable perceptions in diagnosing this life threatening disease.

1.3. India's position on its challenges

In India, [26] the challenges of malaria diagnosis include resistance of common anti-malarial drugs. For the right drug administration, malarial detection must be specific. Estimation (figure 2) shows that only by 2030, malaria elimination would come to role. If that so, how one must be wise enough to build essential steps in contributing to the country's success.

At present, India concentrates on microscopic means mainly due to its cost effectiveness, though other better strategies based on spectrometry or chromatography are used by many other countries [27, 28]. As malarial transmission is complex due to its diverse geo-political and socio-economic parameters, still relying on conventional microscopic methods would not help in malaria eradication by the predicted time. In this perspective, computer aided method would go a long way fulfilling the country's target.

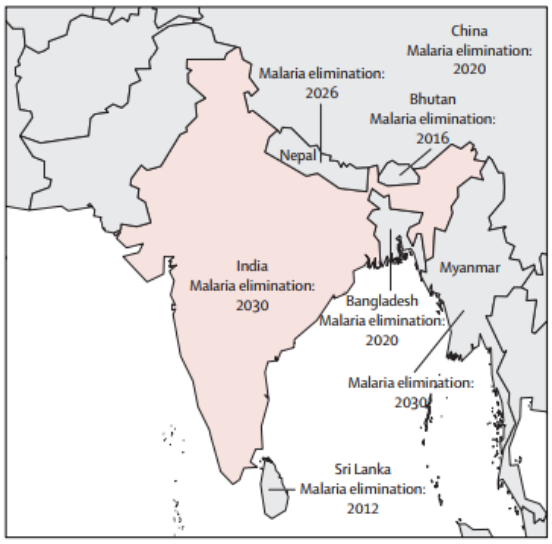

Figure 2.Malaria eradication targets for India and its neighborhood [26]

As a tool, a set of recent techniques that concentrates on the advancement in the imaging systems that is getting shifted from laboratory studies to biological fields is reviewed, thus targeting to the maximum extent possible to treat malaria so that technically effective computer aided pre-diagnosing process could also be facilitated in medically underserved regions.

2. MICROSCOPIC BASED IMAGING SYSTEM

2.1. Conventional microscopy-Benefits and Disadvantages

Though advanced computerized techniques have emerged as a good platform to deal with malarial parasites detection and classification, microscopy based methods, have been the classical and important means to provide appropriate therapy in olden methods. All today's advancement has arisen from this classical approach.

[29] There are advantages like, simplicity, cost-effectiveness and the ability to detect the existence of parasites, and it has been the most accepted technique in clinical laboratories or field surveys, which also involves staining and direct visualization. Even in the forthcoming sections, merits and demerits of conventional microscopy methods (labor intensive, time consuming, less sensitive, prone to errors) are summarized.

[30] The interpretation mechanisms also involve several steps and require substantial expertise and training, especially in identification of plasmodium species at low parasitemia or combined infection. Additionally it is relatively low sensitive when the parasitic level is low. Several cases have led to underestimation of infection rates, which is also laborious, difficult in determining species and not suitable for high throughput applications. Certain drawbacks usually occur at low parasites density; still these challenging factors must be curbed.

2.1.1. Available methods for malaria diagnosis

The common tests are the thin/thick/peripheral smear and Quantitative Buffy Coat (QBC) that improve microscopic identification. [31, 32] There are also other immunological techniques such as Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Rapid Diagnostic Tests (RDTs). Polymerase Chain Reaction (PCR) comes under molecular classification.

- **Thin and thick blood smears:** Peripheral blood is collected, stained and examined

under microscope. Thin smears are fixed in methanol. Parasites identification is possible. They can also quantify and recognize parasites stages. They are not fit for large scale epidemiological researches when associated with low parasite density.

- **QBC:** [33] It is comprised of centrifuged and compressed erythrocytes with acridine orange staining and is subjected to UV source examination. [34] It is easier and more rapid than conventional peripheral film microscopy. On the other hand, it is not cost effective, and species recognition and enumeration are challenging.
- **IFAT:** [35] The infected antigen undergoes incubation process and then gets added with anti-human immunoglobulin and other solutions like fluorescin isothiocyanine, is examined using fluorescence microscopy. The need of this microscopy and high technical skills makes the process complicated.
- **ELISA:** [36] To assess whether the body has antibodies associated to malaria; can be supportive in malaria diagnosis.
- **RDT:** [37] It is low sensitive to detect asymptomatic individuals, especially at low parasitemia. Cross reactions, false negative/positive results are also bound to occur.
- **PCR:** [38, 39] It is tenfold more sensitive than microscopy (but too sensitive for clinical uses). Besides, it provides automation and quantitative detection and species classification. [40, 41] Some of its demerits are unsuitability for field purposes, time consuming, labor intensive and costlier. Again, it is unable to distinguish between viable and non-viable species.

Images acquired from stained films by classic microscopes include certain factors that adversely influence the cell colors because of attributes of color source/filters or intensity variations. [42] When comparing to recent technical advances, traditional microscopic examination for malarial diagnosis is too inappropriate to be used in laboratory, although inexpensive and reliable.

In this study, as thick blood smears are focused, a short description of them is given in the following sub-section.

2.2. Introduction to thick blood smears and its importance

Investigating thick blood films is more sensitive comparing to thin blood films. It is said that the so far implemented techniques (illumination correction, stained pixel identification) can be applied in the analysis of thick blood smears as well. The overall imaging methods available from in-vitro visualization of infected RBCs to in vivo imaging of plasmodium species in the liver have to be focused [43]. [44] recommends thick blood smears to detect plasmodium species as parasites would be missed in thin films due to low parasitaemia. Further, thin films also help in specifying the species. But negative in thick smears would not be positive in thin smears. Figure 3 shows the trophozoites in thick films. [45] Thick smear analysis should be precise enough since it is likely to predict artefacts and blood platelets as parasites.

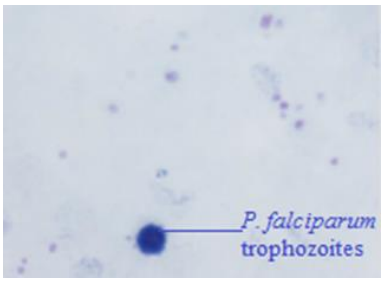

Figure 3.P.falciparum trophozoites in thick blood smear [44]

Standard operation procedures in microscopy examination on thick blood smear is detailed in [46]. [47] Quite a bulk amount of blood can be rapidly examined by thick blood smears and thus used in the identification of parasitemias. The normally used staining methods are Field's and slower Giemsa's techniques. The former one obviously requires expertise and extensive training while the result of other method is more predictable. In India, normally we use, Giemsa, Jaswant Singh Bhattacharji (JSB) and Leishman stains. For detection of parasites in dried thick smears, the hemoglobin is removed before or during staining where the parasites are stained from erythrocytes, in which the changes (infections) can be clearly noticed. Generally the sample is carefully made of venous blood without anticoagulants. To prepare thick smears, it is placed over slide and then dried in an incubator

by maintaining specific temperature and time, followed by air drying before lysis. By proper staining, and processing techniques [48], final malarial parasites identification and classification is performed. Blood samples with the proper staining aids in better detection, and by which the computer based identification systems are elaborated in section 3. Certain detection processes mainly rely upon Deoxyribonucleic Acid (DNA) staining, and Giemsa stain labels the parasites. It is tedious and time consuming.

[49] Thick smears have 20-40 times more sensitivity than thin blood films, though 40 times the blood quantity has to be analyzed; where the latter one also identifies and quantifies parasitemia. Sensitivity of thin films can be brought similar to thick films by examining for it about 30 minutes. Acridine orange stain is comparatively more effective than Giemsa stain and it can read thin and thick blood smears easily. In general, automated imaging based malarial diagnosing from blood smears includes image capture, feature extraction and categorization. The sensitiveness of such vision based automated therapy is more than RDTs [50], but could be an alternative to microscopy [51]. Its application is important in data collection when the results are maintained centrally, where spatial patterns of plasmodium parasites are obtained. More importantly, similar to malaria diagnosis, same principles could be followed in tests of other illness like tuberculosis.

These malarial diagnoses of thick blood films have begun right before several decades. [52] The differentiation of plasmodium parasites is more complicated than Giemsa stained smears. Experiments from various cases of acute malaria have revealed that thick blood smear morphology is consistent and fairly accurate. [53] It is said that reduction in damage while staining to WBCs and blood protozoa in thick films is possible by dilution of stain with phosphate buffer. Preservation of blood protozoa and white cells in thick smears are enabled when stained with methylene blue-saponin. Likewise many observations have pointed out staining in thick smears has resulted in appropriate detection of plasmodium species.

3. COMPUTER ASSISTED DETECTION

Most of the reviewed methods are based on thick blood smears; but a few focus on thin films too.

3.1. Advanced microscopy based method

3.1.1. Computer controlled microscopy

There are extensive studies related to advanced computer controlled microscopy. [54] deals with microscopic based image analyzing assessment of malarial parasites by which it could enhance the precision and reproducibility of pathogen quantitation. The limitations of conventional image processing in capturing considerable numbers of specimen images are identified and dealt using computer controlled motorized microscopic phases. The process is expensive and so digital counting system would be an answer to this.

An additional benefit is, faster processing of specimens. The proposed system has pplied open access Java based image processing software ignoring custom programs or special operators, and has resulted in precise digital counts, especially when the parasite density is high where conventional counting is complex. The steps included are simple:

- Capture images by field microscope and digital camera
- Segmentation of particles with respect to their thickness, area and degree of roundness by ImageJ software. Likewise segmentation by same methods could be used to detect in other fields as well [55, 56]
- Preliminary counting by standard ImageJ process.

Figure 4 shows the results in terms of Giemsa stained thick smears, which depicts the difference in staining color and intensity.

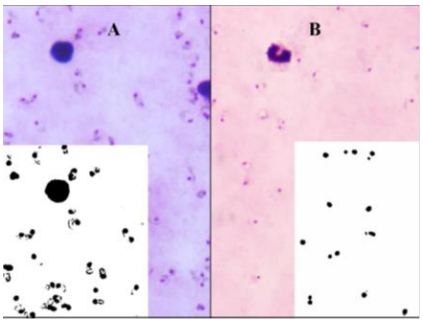

Figure 4.(A): noisy image of trophozoites (B): clear image of trophozoites

3.1.2. Fluorescence microscopy

[57] To enable fast and reliable diagnosis and to overcome the drawbacks of Light Microscopy (LM), RDTs are implemented. Even though it provides qualitative reports, it is not cost effective and is less durable. As a solution, Emission Diode Fluorescence Microscopy (LED FM) is developed and based on this, a systematic report is collected. Practically it (collects blood samples in EDTA) includes three steps viz. peripheral smear assessment using light and fluorescence microscopy and RDTs. [58] Both the thick and thin films are stained and analyzed. Leishman and acridine stained samples by LM and LED FM exhibiting the elements of P.vivax is given in figures 5 and 6 respectively. This is analyzed by means of 1000xoil immersion lens. The fluorescent color changed DNA and RNA stains in non-nucleated erythrocytes have confirmed the malarial infection.

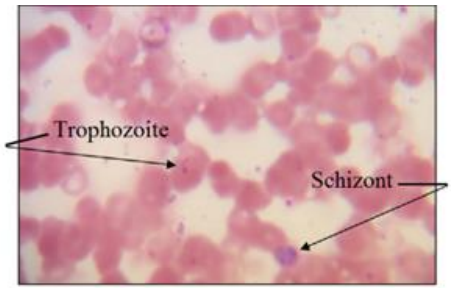

Figure 5.Leishman stained blood smear [57]

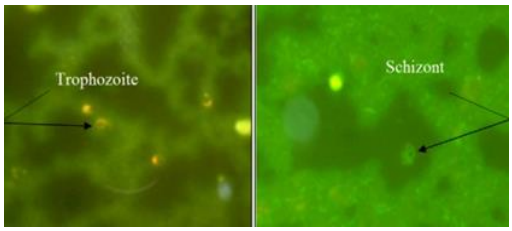

Figure 6.Acridine orange stained blood smear [57]

Parasitaemia is determined and rapid malarial antigen identification strategy is carried out in terms of monoclonal antibodies related to plasmodium species of falciparum and vivax. For comparing the values of several categorical variables, chi-square test is done. Comparatively, the fluorescence microscopy is effective. Still, training in staining and reading of images in this method needs further improvement. Here the specificity of LED FM is high but sensitivity tends to be low.

[59] On the contrary, RDTs are highly sensitive and specific. LED FM based detection is enhanced with increased parasitaemia whereas RDTs detection rate is minimal when parasitaemia is less than 1%. [60] Yet, RDTs include limited capability to stop low and unstable transmission.

3.1.3. Light microscopy

[61] also deals with machine learning system to characterize and classify malarial parasites using light microscopic blood film images. Investigations based on image retrieval, illumination modification, noise removal, RBC segmentation, features extraction and selection, and malaria life cycle phase categorization are performed. Marker controlled watershed conversion is used to segment RBCs. A total of 96 features of RBCs are retrieved in terms of infected and healthy cells, of which 94 are proved to have statistical significance in discrimination of six classes. Classification is accurate but not more than 84%, with the combined techniques of statistical learning methods.

3.2. Voxel and biosensor based image processing

[62] has dealt with an image processing algorithm in terms of temporal changes of pixels. This variation of pixels is considered as independent set (voxels). Any change in dark pixel indicates intercellular actions, thereby stating the presence of plasmodium species. The parasite's existence, growth and motion in the affected erythrocyte are speculated through this method. As described earlier, the infection begins once the parasites break the membrane of RBC and target a new cell. This specific phase is said to be ring which develops by destructing hemoglobin, thus forming trophozoite and later divides to become schizont.

[63] These alter the dynamics of the cell motion based on unaffected cell. The proposed technique minimizes the temporal set of images to a single one that includes all the required data to categorize the cell as affected or not. Figure 7 is based on voxel based image processing technique, depicting uninfected and infected cells. The last block in the figure relates the failed classification.

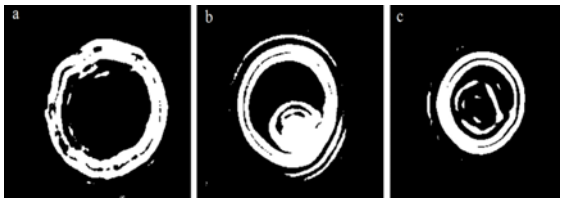

Figure 7.a-Healthy cells; b-Trophozoite; c-Classification unsuccessful [62]

This Matlab enabled program with the real time processing reduces the operational time as well. This method is supposed to overcome the limitations that occur in clustering based image thresholding. Therefore the used Otsu's method can be replaced by some other advanced one in the above process or in the conversion of gray scale to binary image. Still the accuracy in detecting healthy cells is less precise 5%. The pixel or the voxel based method is able to differentiate malarial phases and thus the process can be further enhanced with improved thresholding algorithms.

To digitally analyze the changes by P.vivax in red cells, [64] has divided the blood infected samples into low and high parasitemia to indicate the enhancing levels of disease severity. Both thin and thick blood smears to analyze RBC morphology and to quantify parasite density are prepared and air-dried. JSB stained thick smears are processed to determine density characteristics of pathogens.

The dried slides are observed by video-microscope and digital camera and the resultant images are preserved in the computer, which are then processed using Matlab. To calculate the shape factors, background extracted blood cell images are obtained as in figure 8.

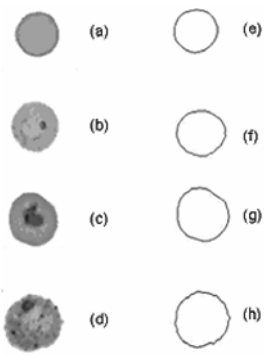

Figure 8.a) Normal sample, samples of parasitemia b) Low, c) Medium, d) High, e-h) Respective retrieved counters [64]

Similarly form factor is determined on the basis of perimeter and area. Such shape descriptors play a significant role in identifying deformability of RBCs. But, determining the growth pattern of parasites takes more time, and further improvement in automatic identification of variations caused by pathogens has to be formulated. [65-67] have made a note of biomarkers to detect plasmodium species. Though they do not have any relevance with thick smears, they are described just to validate the role of biomarkers in malaria diagnosis. [65] presents an aptamer based electrochemical biosensor method that targets P.falciparum lactate dehydrogenase.

It can be regenerated for several detections without compromising its sensitiveness, just by rinsing with denaturant substance (urea). Besides its effectiveness to offer better sensitivity and re-usability, it can be also good at other parameters like specificity, accuracy and robustness, and inexpensive too. Likewise [66] states that biomarker, haemozoin shows less accuracy in identification of younger parasites. But [67] suggests that rather than focusing single bio-marker, multiple biomarkers will be beneficial. [68] reveals that though primary investigation show negative reports of pathogens, there is a probability of repetitive tests to confirm the pathogenic infection.

According to a 14-year study conducted among individuals who had malarial diagnosis, 66% of the patients are affected by P.falciparum and that is diagnosed at the first blood smear itself. But 7% of the individuals, who had negative blood smear initially, are later found to have positive reports, and majority of them with P.vivax. It exhibits that, only by accurate diagnosing procedures, correct identification of the parasites is possible. [69] Therefore for certain cases, rather than single blood film results, multiple samples must be prepared for parasites identification and classification. Based on this, malaria subspecies is detected and out of 404 patients subjected to diagnosis, 253 had P. falciparum, and P.vivax, P.ovale and P.malariae has affected 133, 5 and 1 persons respectively, where 12 patients showed mixed infection. Table 1 defines the result.

[70] Automated hematology analyzer is implemented for malarial detection, in where

the study is undertaken in Korea. In this regard, thin and Giemsa stained thick blood smears are prepared in which the samples are collected from malarial affected and nucleated blood cells of individuals and febrile and healthy controls. The proposed method works well in malarial detection in febrile controls other than nucleated blood cells.

Table 1.Total positive and negative reports [68]

Subgroup	Malaria subspecies	No.	%
Mixed positive blood report	All	404	100
	P.falciparum	253	63
	P.vivax	133	33
	Mixed	12	3
	P.ovale	5	1
	P.malariae	1	0.3
Initial positive report	All	377	100
	P.falciparum	248	66
	P.vivax	112	30
	Mixed	1	3
	P.ovale	5	1
	P.malariae	1	0.2
Initial negative and positive results	All	27	100
	P.falciparum	5	18
	P.vivax	21	78
	Mixed	1	3

3.3. SVM classification

One reason for reduced accessing to malarial diagnosis is limited tools and expertise. So an easy and reliable mechanism in dealing with this treatment is essential. Taking this into account, supervised classification based image analyzing is formulated to identify P.falciparum trophozoites and leukocytes in Giemsa stained thick films. [71] The research is carried out using easily available smart phones, which is also less expensive. SVM classifier is used and the resulted sensitivity and specificity of trophozoites identification is 81% and 94% respectively, whereas for leukocytes, the percentage is 98 and 72 accordingly. Here the proposed work consists of three parts namely,

- Optical magnification,
- Image processing and analysis
- Mobile application

The first component replaces the commonly available microscopes, developed in a way that is adaptable with smartphones. Its additional advantages are 1000x magnification, quality phone camera and self-powered motorized unit that moves the smears and allows for automatic capturing of samples. The second component deals with automated identification of malaria parasites via computer based techniques. For different plasmodium species phase combinations, image processing elements are implemented for detection and counting the specific parasites. Finally the mobile application aids to capture images and analyze such that proper medications can be followed. [72] Whether for optical circle or leukocytes or trophozoites identification, different modules like median filter, Otsu's method, edge-preserving smoothing, adaptive thresholding, blurring and area filtering processes and machine learning classification are applied. Results are measured in terms of specificity, sensitivity and accuracy, and the methodology is based on C++. Table 2 includes the respective average values.

Table 2.Results of machine learning classification [71]

Detection	Sensitivity	Specificity	Accuracy
Leukocyte	98	72	95
Trophozoite	81	94	92

The paper itself suggests that only a single module of mobile based method is implemented and should be combined with image processing in order to detect and count all possible parasites combinations.

3.4. K nearest neighbor and clustering classification

In this regard, [73] has used a detection method based on modified K nearest neighbor classifier and compares with Bayesian technique. Single multi class classification is performed. [74] Computerized image analysis is gaining significance by means of edge based segmentation. Infected red blood cells can be extracted by Fuzzy C-Means (FCM) clustering. The proposed method is also comprised of color space transformation, illumination correction, element analysis, noise minimization and Minimal Perimeter Polygon (MPP). The obtained microscopic blood smear images have distinct color tones. The colored images are then transformed to gray scale since it is simple and convenient for scalar processing. Illumination in these images varies in respect of experimental setup. Since the contrast of the infected RBCs is low, gamma equalization is

used to enhance it. The resulting uncertainty with converted gray images is measured in terms of entropy.

The system applies adaptive median filter to deal with noise factors. [75] Generally the malaria affected cells tend to be the darkest in where the edge improvement enhances the contrast between affected RBCs edges and the background pixels so as to improve segmentation process. Using FCM, malaria infected RBCs are extracted from microscopic images. Then connected component analysis is done by including morphological operation (elimination of extra RBC parts) and hole filling (in order to retrieve the correct infected cells, any holes in RBCs are to be filled), followed by MPP that segments the infected cells. After then the infected cell edges are obtained and subjected to malaria diagnosis. [76] has employed K means clustering to separate infected cells from the image and tested using k nearest neighbor classifier. The accuracy and sensitivity are not more than 90% each. [77] However by applying right algorithm like Adaboost, the success rate can be improved.

3.5. Neural network

[78] Using the image analysis process combining the absence of gradients and Nernstian equilibrium stripping along with morphological gradient, malarial parasites detection is carried out. The principal stages of this system include wavelet based feature retrieval, neural network categorization and principal component analysis. PCR can substitute microscopy and is found to be superior but costly. In practical studies, RDTs [79] would be fruitful. In this approach, initially the digitized malarial blood smear is subjected to image selection, where plasmodium falciparum/vivax are the parasites detected. Then it undergoes segmentation phase that involves image processing techniques, followed by morphological gradient and K-media methods. By the combination of neural network, fuzzy interface unit and Support Vector Machine (SVM), [80] has proposed a strategy to classify malaria parasites. Factors like skewness, kurtosis, and standard deviation are considered to extract features using histograms. Of the three algorithms, SVM is proved to be better. This method would further be effective by minimizing redundancy in the dataset and enhancing pre-processing methods. [81] has carried out malaria severity detection using Jordan-Elman neural network. When this is compared with SVM, the performance of the proposed method is improved, and can be implemented in tropical and sub-tropical areas for faster identification of parasites in respect of the feature extraction from thick films.

[82] Parasites analyses based on computer technology have paved ways for early detection of infection. To deal with the challenges of cell segmentation and morphological analysis that arise out of cell complexities, artificial neural network is introduced, which also includes Rao's segmentation (cell shapes, light variations, cell overlapping and noises are really challenging; therefore proper segmentation method has to be followed) and bounding box methods. Initially, the smears are preprocessed to enhance image quality, where median filter is employed to filter noise. Furthermore, forward discrete curvelet transform is used that senses the contours and extract features. Then adaptive equalization is applied to improve contrast. In order to distinguish healthy cells and infected ones, textural and color features are vital, where Gray Level Cooccurrence Matrix (GLCM) modules are applied.

Table3.Rules for parasites detection [82]

Feat-ure	P.falci-parum	P.vivax	P.ovale	P.malar-iae
Size	Not enlarged	Enlarged	Enlarged	Enlarged
Shape	Round crescent gameto-cyte	Round or oval	Round or oval amoeboid	Round
Dots	Large red	Small red	Small red	Few tiny dots

Certain procedures to identify plasmodium species are tabulated in table 3. Segmentation of parasites is improved, where only three features are taken into account. It would be better if the same improvement could be possible with more features. Basically color features should have been also included.

3.6. Computer vision based segmentation

[83] has reported computer vision to classify malarial pathogens in RBCs. Linear programming based numerical analysis is

introduced to resolve medical imaging difficulties. The mathematical model is initially formulated based on the collected data and then solved using graphical method (species classification). Here all parasite species is considered as a single variable. Alternatively further study can be made with specifying variables for each parasite separately using other methods. [84] has evaluated computer vision system to detect parasites in digitized blood films, which is potential enough to improve the throughput in malarial diagnosis. Using computer algorithms, detection in thin and thick blood smears would produce 92% sensitivity and 90% specificity. As it not fully automated, difficulties arise during decision making and thus likely for misclassification.

3.7. Adaptive thresholding

[85] also has stated that although microscopic diagnosis was the gold standard approach, it is complex and tiresome. Therefore an alternative computational method is employed involving segmentation and detection processes. Six-sigma and Kapur's threshold methods are followed to detect and count red blood cells, and infected cells respectively. In addition, chessboard distance and Hough transform are also used that finds RBCs from the derived image data. Purchased blood is used for malaria drug discovery. At first Giemsa stained blood smears [86] are prepared followed by image acquisition and standardization and then subjected to software processing. It is deduced that categorization in terms of entropy measures is effective. The process is not simple as there are various complex approaches and computations. [87] provides a critical review on image processing functions like adaptive thresholding, histogram equalizations, morphological computations and connected component analysis to estimate parasitic infection by rapidly quantifying the number of parasites in digital images. Figure 9 illustrates the image processing steps. Similar to [88], this also recommends not to use Otsu's method in image segmentation because of the image characteristics. Additionally, global threshold is also of no value due to broad range of gray scale intensity and complexity. Therefore adaptive threshold would be a promising method that improves the accuracy and shows effective results than other prevailing strategies.

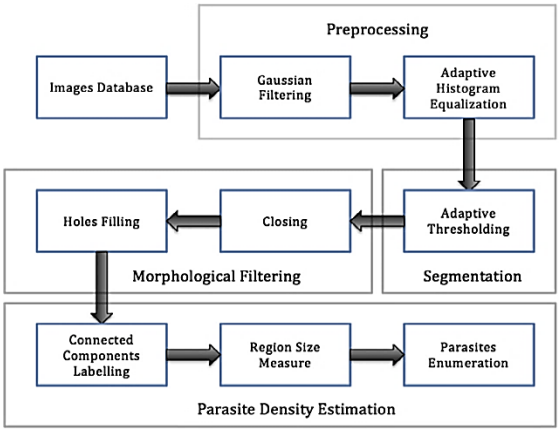

Figure 9.Processing stages [87]

3.8. Quantitative buffy coat thick films in parasite detection

[89] has found out a novel method named single step amplification to identify intra and intergenic multiloci of nucleotides of P.falciparum and P.vivax, thus to detect the two malarial parasites. Genome sequence data of both the parasites are obtained from public database, and chromosomes are analyzed using bioinformatics tools. This method has focused on new target DNA sequence identification in the parasite genetic material. QBC is the strategy adopted to detect malarial parasites and for DNA isolation, standard chelex system is used. Further DNA is taken from healthy persons also for the estimation of analytical specificity and cross reactivity. The analysis proves the suitability of the recognized targets to be utilized as a useful diagnostic device in upgrading the performance of molecular treatment approaches in malaria identification especially in case with low parasitemia and for epidemiological investigations that signifies high throughput evaluation. [90] has carried out buffy coat blood films to detect malarial parasite with negative thick smears. More importantly, it is concluded that this blood films in terms of capillary tubes are beneficial and cost effective, and has detected around 30% of the malarial victims which could not be diagnosed by classical thick films. [91] has compared QBC results with that of Giemsa stained thick smears. The buffy coat test fails in accurately differentiating the parasite species. Using Giemsa stained thick films,

around 87% of malaria victims have had P.falciparum, 7% is with P.malariae and 6% have had combined species. Even though the buffy method is rapid and simple, it cannot replace the other. Still it could be beneficial in certain occasions. [92] conducted QBC and it is highly specific and sensitive. Nevertheless, its limitations are,

- Costlier
- Unable to quantify parasitaemia
- Unstable if the storage is longer

[93] Modified QBC can be an alternative for conventional microscopy only when appropriate laboratory setups are available. In a place without sufficient facilities, advantage mal card can be applied. [94] When QBC is compared with RDTs, the latter one is better with improved specificity and sensitivity. [95] has conducted a survey between QBC and conventional thick smear. The resultant values show that QBC is rapid but provided only 56% sensitivity; but it is more specific (95%). The results of QBC are unsatisfactory for malarial species detection. Also, its quantification of parasitemia is difficult; at the same time, the specimens cannot be stored for further use. Therefore it supports conventional thick blood smears.

The next section holds a brief study on the more suitable method among the reviewed works of parasite detection.

4. AUTOMATIC COMPUTERIZED VISION BASED IMAGE PROCESSING-BETTER CHOICE IN MALARIA DIAGNOSIS

Computer vision and image process aim at automatic diagnosing. Generally this process includes multi parts like image acquisition/ segmentation and pattern categorization, [96-99] where pre-processing has a great role. Various works validate automated malaria diagnosis. Acquiring microscopic images may include variation, and that could be addressed by preprocessing. Other than these, stain identification is also important. Statistical learning based methods helps in differentiating patterns. In image acquisition, the captured images are magnified to become 25 times larger. Blurring of images should be avoided. This process may use Xenon strobe light, but is expensive. In thick blood smears, if parasites are noticed in an area, parasitaemia can be assessed based on several other areas. Recent innovative advances would meet the speed requirements. The system robustness can be improved by addressing the image variations. Local illumination variations results in drastic issues. In this, filtering approaches would be beneficial. As color play a great role in distinguishing the parasites species, color based parameters, calibration and constancy should be addressed. Numerical modeling of the staining concentration transmittance function aids digital modification. Further, granulometric assessment estimates the cell size; but the arising abnormality causes irregular cell shapes and hole formation, thus degrading its efficiency. Studies have been formulated to address this also [100].

Automatic diagnosis aids in improving accuracy, time/cost saving and reduces human errors. To overcome the limitations of previous methods like technical drawbacks and human inconsistency and to improve the accuracy and performance in classifying pathogens using digital image processing, automatic device is presented. [101] As similar method has already been developed using thin films, where there is difficulty in detecting the presence of parasites owing to its scarcity, the proposed method has considered automated identification and categorization of plasmodium species on thick blood smears. It includes the design of motorized system to control the specific motions of objective lens and microscope. The modules comprise image acquisition and image analysis. Strategies to evaluate image acquisition unit and image enhancement are implemented. [102] In image analyzing, the classification of malarial parasites is based on the chromatin size. This research has employed 20 and 40 parasites-negative and positive thick blood films respectively; also the corresponding identification accuracy is 69% and 95%. As the morphology of Plasmodium falciparum and Plasmodium vivax closely resembles each other, improved image acquisition process and precise feature identification are necessary to overcome the complexity in distinguishing these two species. In spite of its machine technics, sometimes unusual changes based on unknown species demands manual examination which may make the process more complicated. [103] An unsupervised and sensitive malaria screening method is developed taking into account of

manual microscopy that is prone to human errors [104]. The proposed system automates enumeration and identification concentrating on thin blood slides. This image based screening diagnosing process would be simple and beneficial in laboratory prospective. [105] Segmenting the regions enable image partitioning like foreground and background, static and dynamic areas or objects with specific characteristics. Hierarchical partitioning, deductive strategies and other segmentation methods are available. The methods should concentrate on high concentrated fields of thick smears as well. [106] Segmentation is a common procedure for any diseases. [107] supports computer aided automatic tools for segmentation. An automated live imaging system has been presented in [108] that standardizes experiments, increases throughput and overcome the demerits of live optical microscopy too.

K means clustering has been adopted by [109] to segment image, which additionally uses subtractive clustering model. [110] has applied genetic algorithm based k means clustering for classification. [111] has used deep learning algorithms for image classification and segmentation. [112] has attained better results with cascaded moving k-means and FCM clustering algorithms. Both the algorithms together yields more specificity and accuracy than the algorithms used separately. The proposed method is effective for better segmentation process. [113] has presented enhanced k-means clustering, and has validated that the results are more efficient than Otsu's thresholding and k-means clustering. It has resulted in 99% segmentation accuracy, 88% sensitivity and 99% specificity. To address the problems (inaccuracy and high computational cost) of Otsu algorithm, [114] has enhanced conventional Otsu algorithm and proposed fast image segmentation. Its advantages are precise segmentation and less time consumption. [115] has focused on combined algorithm using morphological functions and color based pixel differentiation for parasites detection of thick films. It has high prediction rate with low False Positive Rate (FPR). Furthermore it does not depend on any training set that makes the model unsupervised and simple. But possible interface with supervised technique can be used to further enhance prediction rate and lower FPR. Moreover better results could be achieved by interfacing this proposed model with smart machines. [116] has discussed on color image segmentation to identify parasites utilizing color models and unsupervised technique based k-means clustering. Images are enhanced using partial contrast stretching before segmenting the infected red cells. Moreover median filtering and seeded region growing area retrieval methods are also applied.

Then comes classification. It could be based on KNN classifier. Advancement in joint classification model based on generalized features also improves the expandability and scalability of malarial diagnosis. If the processing speed of thick blood smear matches with that of thin films, then it would be really a great achievement. [117, 118] Hybrid method of SVM and firefly algorithm enables better classification than Artificial Neural Network (ANN), auto regressive moving average model and SVM. Similarly [119] has presented computational fuzzy expert system based on Visual Basic. NET (VB.NET) aiming to reduce mortality rate by enabling right drug prescription. Such a way computer based expert systems are suggested by many others as well [120-122].

In addition to these advancements, mathematical modeling [123] could also contribute to evidence based decision making to keep the disease in check. [124] has achieved 97% sensitivity by using computer based two-stage algorithm (detection and false positive reduction) to automatically detect plasmodium parasites in thick smears. Yet improvements require in terms of attainment of training and test data sets, thus to enhance system robustness. [125, 126] have presented a list of contribution on computer based malaria diagnosis (computer vision and machine learning). It prefers multi-class classification rather than using binary features. According to [127], automated computerized image mining methods are effective in providing multi-parametric and accurate data on plasmodium species responses. [128] has adopted constrained clustering aiming to enhance the accuracy in classification of parasites. [129] has developed an image analysis based system using MalariaCount software that automatically and accurately determines

parasitemia. Most of the computer aided techniques works well and more suitable for malaria diagnosis.

5. SUMMATION

The identification and classification of malarial parasites demand more improvements and testing. The summary of the review is available in table A1. In most of the cases, thick blood smears are considered, though more researches concentrate on thin films. So it is recommended to highly focus on thick smears despite processing of thick samples are significantly challenging. [71] has attempted to formulate a reliable system (free software and semi automation) that digitally counts pathogens aiming to overcome the expensive computer controlled motorized microscopic phases. It shows that it could improve the accuracy and reproducibility, but unable to replace the training of microscopist. [73] has adopted microscopy imaging to segment infected red cells. The so used Gamma equalization and confusion matrix enhances segmentation, and it employs fuzzy C-means clustering that extracts affected red cells. The overall results are very effective.

[103] also has researched based on automated and unsupervised mode using digital image processing that enumerates and identifies parasites. This method reduces human intervention, which is also consistent. 50-88% sensitivity is achieved through this method considering all plasmodium species and its specificity is 100%. This is the one of the methods in the literature with maximum sensitivity. On accounting its specificity, still more improvement is expected.

According to [80], morphological and novel threshold selection methods would be helpful in identifying red cells and the pathogens. Comparatively SVM shows better accuracy than neural network and adaptive fuzzy. Its performance could be still improved by minimizing redundancy and advance pre-processing methods. [83 and 84] have classified the pathogens based on computer vision. [83] has applied linear programming and graphical models to detect parasites apart from the applications of image segmentation and morphological analyses. Its future scope lies in developing the concept further with separate variables for different species, as in this paper only one variable is considered. [84] has implemented a decision support system to identify parasites in respect of computer vision algorithm. Mainly it focuses to deal with ring phase parasites. This method would be useful for the works that face problems in detecting younger parasites.

[85] has described an automated high performance technique with minimal manual methods to detect and quantify infected erythrocytes. Here Kapur's entropy measure finds application in distinguishing parasitized cells from healthy ones. The article holds good for providing better malaria diagnosis besides its complicated process. No information regarding cost function is depicted. [101] has developed an automated detection and classification of malaria parasites using motorized arrangement. [102] has come forward with improved image acquisition method; but the demand of manual examination, sometimes leads to difficulties. [103, 104] have developed simple mechanism for malaria diagnosis intending to overcome the demerits of manual microscopy. K means clustering algorithm finds application in detection and classification of plasmodium species and has been suggested by many authors [109, 110, 112, 113, 116]. All uses this algorithm along with other methods like subtractive clustering model, Otsu's thresholding or with genetic or FCM clustering algorithms for better performance. Likewise computer vision and deep learning algorithms are constructed along with numerical models to ensure their quality and accuracy. Hybrid models have also been followed by [112, 115, 117, 118]. Some papers have dealt with enhanced k-means clustering and Otsu algorithm [113, 114] to address the shortcomings of their respective traditional models. [119, 124-127]

In a nutshell, it is validated that, most of the reviewed article focused on Giemsa staining. In addition, automatic computerized vision based image processing method would be fruitful along with supplementary functions like advanced clustering, threshold models and hybrid systems.

6. CONCLUSION

Almost all the reviewed articles are taken into consideration for the study to emphasize the seriousness of this threatening disease and report its victims and deaths

caused by it. This urgent demand of proper diagnostic measures to treat the fatal disease prompted to review the prevailing techniques and this work would be a tool to identify the specification of each method and in near future a more promising device would benefit millions of people around the world. Besides detecting and classifying malarial parasites to provide right treatment, steps ought to be taken to block their capability to actively pass and interact with host elements. Preventive factors begin from skin as it is the first mammalian part to obstruct sporozoites. Antibodies mediated responses could prevent infections. Thick blood smear test is the easiest test in malaria parasite detection and is also very significant. As its role is important, more training is needed for prompt and effective analysis. Several techniques to detect parasites in thick smears are concentrated, even though a few deals with thin films.

To conclude, extensive studies of image processing techniques to detect and classify plasmodium species have been made successful due to its accuracy, reliability and time-saving nature. While certain methods provided less specificity and accuracy, certain others are expensive or tedious. In some researches, several strategies are elaborated that makes the entire process complex whereas in several others it is limited with classical manual methods making the system error prone. On the contrary, computer aided algorithms that are able to differentiate parasites life stages and also they can effectively identify the infected cells, are described along with upgraded techniques like improved clustering, thresholding and hybrid models that could be very efficient in parasite detection. Besides it acts as a useful tool in diagnosing malaria parasites, it could also be utilized in other digital image processing sectors. The work reviewed here indicates that several imaging techniques have a crucial role in improving the pathophysiological study of malarial parasites, aiming to critically influence its future assessment and treatment.

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APPENDIX

Table A1.Summary

Segmentation/ Algorithm/Method	Features Staining/ Classifier	Advantages	Limitations	References
Computerized image analysis	KNN FCM clustering	Enhanced edge – based segmentation Consistent, robust 98% sensitivity 93.3% specificity	Specificity can be further increased	[73]
Computer based automated detection	Color space transformation Gamma equilization SVM supervised classifier Giemsa stained	94% specificity 81% sensitivity	Needs improvement in sensitivity	[71]
Comparison	Modified K nearest neighbor classifier	Better than Bayesian classification, KNN classifier adapts easily to thick blood smears	Tedious	[73]
Unsupervised and image based screening	Giemsa stained	Better than manual microscopy 100% sensitivity Less time consuming Effective at laboratory studies	50-80% specificity	[103]
Morphological and novel thresholding Neural network Adaptive neuro fuzzy network	SVM classifier	Effective training by the use of hybrid algorithms SVM better among the three	Still needs improvement in performance	[80]
Computer vision algorithm	Color, object size, image features Giemsa stained SVM classifier	92% sensitivity 90% specificity Reduction of visual examination High throughput	Enabling full automation prevents misclassification	[84]
Six-sigma, modified Hough and Kapur's threshold	Giemsa stained Kapur's entropy classification	Effective classification based on entropy Less computation time High accuracy	Too many computations	[85]
Improved image acquisition process feature identification	Chromatin size	Effective to distinguish P.falciparum and P. vivax	Demands manual examination	[102]
Automated live imaging system	-	Increases throughput Overcomes the demerits of live optical microscopy	-	[108]
K-means clustering plus supportive algorithms	Otsu's thresholding	High accuracy, sensitivity and specificity	-	[109,110, 112, 113]