Multimed Tools Appl
DOI 10.1007/s11042-017-4495-2

A review on automated diagnosis of malaria parasite in microscopic blood smears images

Zahoor Jan[1] · **Arshad Khan**[1] · **Muhammad Sajjad**[1] · **Khan Muhammad**[1,2] · **Seungmin Rho**[3] · **Irfan Mehmood**[4]

Received: 13 June 2016 / Revised: 15 November 2016 / Accepted: 8 February 2017

Abstract Malaria is a life-threatening disease caused by parasite of genus plasmodium, which is transmitted through the bite of infected Anopheles. A rapid and accurate diagnosis of malaria is demanded for proper treatment on time. Mostly, conventional microscopy is followed for diagnosis of malaria in developing countries, where pathologist visually inspects the stained slide under light microscope. However, conventional microscopy has occasionally proved inefficient since it is time consuming and results are difficult to reproduce. Alternate techniques for malaria diagnosis based on computer vision were proposed by several researchers. The aim of this paper is to review, analyze, categorize and address the recent developments in the area of computer aided diagnosis of malaria parasite. Research efforts in quantification of malaria infection include normalization of images, segmentation followed by features extraction and classification, which were reviewed in detail in this paper. At the end, of review the existent challenges as well as possible research perspectives were discussed.

Keywords Malaria parasite · Red blood cells · Parasite segmentation · Thin blood smear · Classification

✉ Irfan Mehmood
irfan3797@gmail.com

[1] Digital Image Processing Laboratory, Department of Computer Science, Islamia College Peshawar, Peshawar, Pakistan

[2] Intelligent Media Laboratory, Digital Contents Research Institute, College of Electronics and Information Engineering, Sejong University, Seoul, Republic of Korea

[3] Department of Media Software, Sungkyul University, Anyang, Republic of Korea

[4] Department of Computer Science and Engineering, Sejong University, Seoul, Republic of Korea

Published online: 09 March 2017

1 Introduction

The term "malaria" is derived from the Italian word *mal'aria*, meaning "bad air". Malaria is a serious public health problem in many parts of the world, causing millions of deaths every year in more than 90 countries. According to World Health Organization (WHO) report about 3.2 billion humans (approximately half of the world's population) are at risk of malaria, causing about one million of people death every year [45]. According to the latest WHO estimates, released in September 2015, there were 214 million cases of malaria in 2014 and 438,000 deaths [45]. Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and, to a lesser extent the Middle East and parts of Europe, are also at risk [45, 84]. In Pakistan, 95 million people i.e., roughly 60% of the total population, live in malaria endemic regions. Malaria in Pakistan is typically unstable and major transmission period is post monsoon i.e. from August to November [34].

Malaria is caused by parasite (a small living organism) of genus plasmodium and transmitted by infected female Anopheles mosquitoes which carry plasmodium sporozoites in their salivary glands. When an infected mosquito bites a person, the plasmodium parasites enter the blood and travel to the liver where it grows. After development in lever, parasite leaves liver and travels back to blood stream and attack Red Blood cells (RBC) also called Erythrocytes [52, 62]. Symptoms of malaria typically develop within 10 days to four weeks following the infection. Common symptoms of malaria are high fever, shivering, headache, vomiting, muscle pain and pain in joints [3, 44, 82]. There are more than 50 species of *plasmodium*, only four of which cause human malaria are: *plasmodium falciparum*, *plasmodium* vivax, *plasmodium malariae* and *plasmodium ovale* [3]. *Plasmodium vivax* is the most common type of malaria and usually causes a slight and very rarely mortal form of malaria. Similarly, *plasmodium ovale* causes a mild infection. *Plasmodium malariae* causes a severe fever, but it is not usually life threatening. While, *plasmodium falciparum* is considered as a most deadly species that kills millions of people every year worldwide. The malaria parasite appears in four stages in human blood i.e. ring, trophzoite, shizont and gametocyte [8]. Four species of malaria parasite along with their corresponding life-stages are shown in Fig. 1 and morphological variation of different species and life stages of malaria parasite are presented in Table 1.

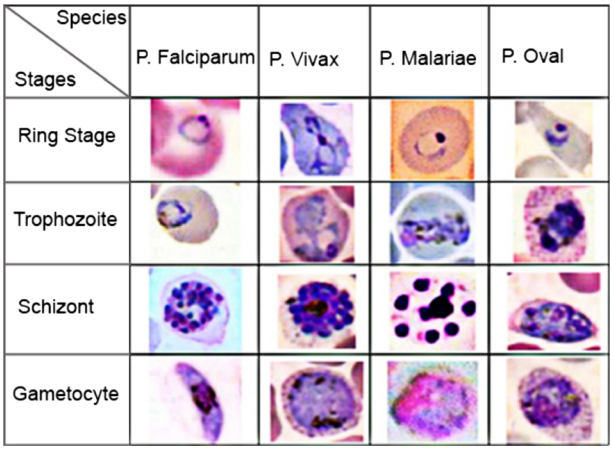

Fig. 1 Sample views of four different malaria parasite species and their life-stages

Table 1 Morphological variations of different stages of malaria parasite

Plasmodium Species	Stages Found in blood	Appearance of RBC	Appearance of Parasite
P. Falciparum	Ring	normal; multiple infection of RBC more common than in other species	delicate cytoplasm; 1–2 small chromatin dots; occasional applique forms
	Trophozoite	normal; rarely, Maurer's clefts	seldom seen in peripheral blood; compact cytoplasm; dark pigment
	Schizont	normal; rarely, Maurer's clefts	seldom seen in peripheral blood; mature =8–24 small merozoites; dark pigment, clumped in one mass
	Gametocyte	distorted by parasite	crescent or sausage shape; chromatin in a single mass or diffuse ; dark pigment mass
P. vivax	Ring	normal to 1–1/4 X, round; occasionally fine Schuffner's dots; multiple infection of RBC not uncommon	large cytoplasm with occasional pseudopods; large chromatin dot
	Trophozoite	enlarged 1–1/2–2 X; may be distorted; fine Schuffner's dots	large ameboid cytoplasm; large chromatin; fine, yellowish-brown pigment
	Schizont	enlarged 1–1/2–2 X; may be distorted; fine Schuffner's dots	large,may almost fill RBC; mature =12–24 merozoites; yellowish-brown, coalesced pigment
	Gametocyte	enlarged 1–1/2–2 X; may be distorted; fine Schuffner's dots	round to oval; compact; may almost fill RBC; chromatin compact, eccentric or diffuse ; scattered brown pigment
P. ovale	Ring	normal to 1–1/4 X, round to oval; occasionally Schuffner's dots; occasionally fimbriated; multiple infection of RBC not uncommon	sturdy cytoplasm; large chromatin
	Trophozoite	normal to 1–1/4 X; round to oval; some fimbriated; Schuffner's dots	compact with large chromatin; dark-brown pigment
	Schizont	normal to 1–1/4 X; round to oval; some fimbriated; Schuffner's dots	mature =6–14 merozoites with large nuclei, clustered around mass of dark-brown pigment
	Gametocyte	normal to 1–1/4 X; round to oval; some fimbriated; Schuffner's dots	round to oval; compact; may almost fill RBC; chromatin compact, eccentric or more diffuse scattered brown pigment
P. Malariae	Ring	normal to 3/4 X	sturdy cytoplasm; large chromatin
	Trophozoite	normal to 3/4 X; rarely, Ziemann's stippling (under certain staining conditions)	compact cytoplasm; large chromatin; occasional band forms; coarse, dark-brown pigment
	Schizont	normal to 3/4 X; rarely, Ziemann's stippling	mature =6–12 merozoites with largenuclei, clustered around mass of coarse, dark-brown pigment; occasional rosettes
	Gametocyte	normal to 3/4 X; rarely, Ziemann's stippling	round to oval; compact; may almost fill RBC; chromatin compact, eccentric or more diffuse; scattered brown pigment

2 Scope of this review

Computer aided diagnosis of malaria parasite and recognition has opened a new area for early malaria detection that showed potential to overcome the drawbacks of manual strategies. The scope of this paper is to review and analyze the recent work of different researchers in the area of malaria parasite recognition using computer vision. This paper provides a good basis for researchers who are starting to investigate the computer aided malaria diagnosis methods. In this paper, a review and analysis of computer vision and image analysis studies which addresses the automated diagnosis of malaria on blood smear images and its necessary supporting functions is provided. Brief features of this paper are as under:

- Medical background of malaria disease is explained comprehensively.
- Morphological variations of different species and life stages of malaria parasite is discussed.
- Microscopic diagnosis method for malaria is presented in detail.
- General architecture of automated diagnosis of malaria is presented.
- The contribution of different researchers is demonstrated and summarized in context of general architecture of automated diagnosis of malaria.
- Different techniques used by various researchers in each step of automated diagnosis of malaria are presented in tabular form.
- Performance comparison of different research works is presented.
- At the end of review, some major challengings and future directions are suggested.

3 Malaria diagnosis techniques

One would think that the symptoms of malaria, including chills, fever, and pain etc. would be a good indication of the disease. However, there are a number of other diseases, such as severe nephritis, that could cause the same symptoms. Thus dominant diagnosis techniques are required that detect malarial plasmodium in patient accurately. WHO recommends that all cases of suspected malaria must be confirmed using parasite-based diagnostic testing (either microscopy or rapid diagnostic test) before administering treatment. Many techniques have been developed for malaria diagnoses such as flow cytometry, fluorescent microscopy, polymerase chain reaction (PCR) etc. However, microscopy is still considered as a golden standard for laboratory confirmation of malaria [8].

3.1 Microscopic diagnosis of malaria

Detection of malaria parasites by light microscopy is still considered the primary method for malaria diagnosis in health clinics and hospitals throughout the world. An accurate laboratory diagnosis is essential as false negatives can result in untreated malaria patients, causing severe consequences [53]. The WHO practical microscopy guide for malaria provides detailed procedures for malaria diagnosis [6]. Using a microscope, visual discovery and identification of the parasite is possible and efficient via a chemical process called staining. Giemsa is a popular and cost effective stain that is generally used during staining process [81]. Giemsa stain slightly colors RBCs but highlights the parasites, white blood cells (WBC), platelets, and

various artifacts. In order to detect the infection the stained objects could be divided into two groups i.e. parasite and non-parasite (Fig. 2).

Slides for microscopic diagnosis of malaria can be prepared in two different methods namely thick and thin blood slides. Samples of thick and thin blood smear are shown in Fig. 3. A thick blood smear is a drop of blood on a glass slide. It is most useful for detecting the presence of parasites, because they examine a larger sample of blood. It is dried for 30 min and mainly used to detect infection and to estimate parasitemia. Infected species of malaria cannot be detected in this method. On contrary, thin blood smear is a drop of blood that is spread across a large area of the slide which is dried for 10 min and fixed in methanol. This can be done by either dipping the thin smear into methanol for 5 s or by dabbing thin smear with a methanol-soaked cotton ball. While fixing the thin smear, all care should be taken to avoid exposure of thick smear to methanol. Thin blood smears help doctors discover what species of malaria are causing the infection [81]. The variation between thick and thin blood smear for malaria infection evaluation is discussed in Table 2.

Advantages of microscopy are possibility of distinguishing species of Plasmodium, quantifying parasitemia, observing asexual stages of parasites as well as having low material cost. Besides numerous advantages of microscopic diagnosis of malaria, there are some weaknesses. It heavily depends on the skills and expertise of pathologist/Technician. It was observed in several studies that manual microscopy is not a reliable screening method when performed by a non-expert [7]. Additionally, confirming negative status of malaria slide take considerable time and efforts. Furthermore, it is difficult to observe each blood smear with full concentration where a pathologist has to conduct many tests. Therefore, an automated image analysis system would improve the performance of microscopy by avoiding its main limitations in term of dependency on the ability of laboratory technician to diagnose blood images accurately.

3.2 Computer aided diagnosis of malaria

Computers play a vital role in the medical field and without it, proficiency and productivity would decline markedly. Computers are already playing a major role in variety of medical diagnosis applications such as digital X-ray, magnetic resonance imaging (MRI), computed tomography (CT Scan), Ultrasound and many others. Computerized diagnosis of malaria is a microscopy diagnosis technique by the use of computer vision and machine learning methods. It can be used as an aid or a complete automated diagnosis technique, which replaces the manual microscopy examination. Tek et al. [77] presented review papers which address the

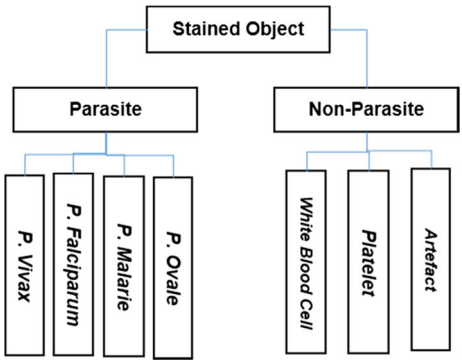

Fig. 2 Stained object classes: Parasite and Non-Parasite

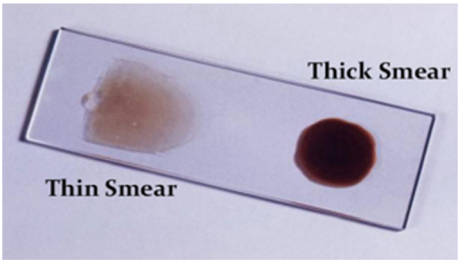

Fig. 3 Thick and thin blood smear for microscope

automated diagnosis of malaria based on computer vision using microscopic slide images. An automated malarial diagnosis system can be designed by understanding the diagnostic expertise (hematologist knowledge) and representing it by specifically tailored image processing and pattern recognition algorithms. Image processing based malaria diagnosis methods have been widely studied to provide early and accurate detection of malaria parasite. Computer aided malaria diagnosis system must be capable of differentiating between malaria infected cells and healthy blood components. Generally, there are five major steps for analyzing microscopic images namely: image acquisition, pre-processing, segmentation, features extraction and classification [77]. The general architecture of automated diagnosis of malaria through image processing adopted in several studies is explained in Fig. 4.

3.2.1 Image acquisition

Most of the studies on malaria detection have considered thin blood smear images; whereas only a few studies have used thick blood smear images. Ross et al. [61] acquired images of thin stained slides by using a charge-coupled device (CCD) camera with full 4 × optical zoom connected to the light microscope with 1000 magnifications. Images were captured in the JPEG format at the maximum resolution of the camera, 2048 × 1536 pixels. Images in [38, 68] were captured by 3-CDD color video camera (JVC, Japan) connected to Olympus BX60 microscope under an oil immersion objective (100×). Same procedure was observed in several studies where images were captured by charge-coupled device (CCD) camera connected with

Table 2 Staining variation of blood smear

S.N.	Thick Blood Smear	Thin Blood Smear
1.	Thick blood smears are most useful for detecting the presence of parasites.	Thin blood smears helps to discover which species of parasite is causing the infection.
2.	A thick blood smear is a drop of blood on a glass slide.	A thin blood smear is a drop of blood that is spread across a large area of the slide.
3.	The blood films must be laked before or during staining to rupture all the RBC so that only WBC, platelets and parasites are visualized.	The purpose is to allow malarial parasites to be seen within the RBC and to assess the size of the infected RBCs compared to uninfected RBCs
4.	Thick smears allow a more efficient detection of parasites (increased sensitivity 11 times than thin smear).	Less sensitive than a thick film especially where there is a low parasitemia.
5.	It is not fixed in methanol.	It is fixed in methanol.
6.	Thick smears are mainly used to detect infection and to estimate parasitemia.	Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes.

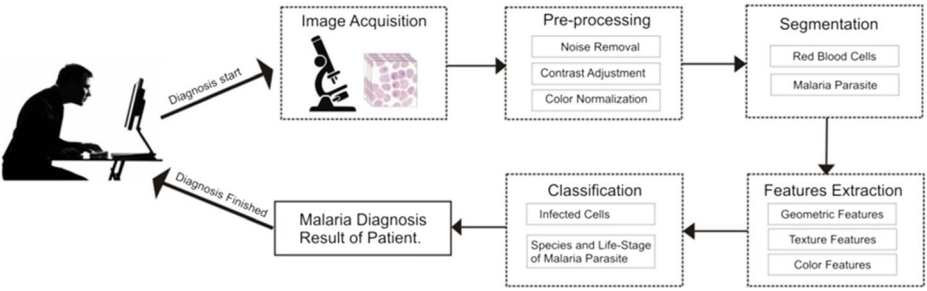

Fig. 4 General architecture of automated diagnosis of malaria

microscope. However, in several studies slide images were downloaded from websites where it is available free of cost for research purpose. Gitonga et al. [21] obtained thin blood smear images from two sources, namely Center for Disease Control (CDC) and Kenya Medical Research Institute (KEMRI).

3.2.2 Pre-processing

The main purpose of pre-processing step is to generate low noise high contrast images for the further processing. Due to staining variability of blood smear and camera adjustment, changes occur in illumination of the microscope images. This particular problem erects difficulties for classification of blood cells since it is hard to deal with proper segmentations of objects with quite similar colors. Various studies have presented different methods to deal with pre-processing issues such as illumination, noise reduction etc. Combination of different filters can be used to reduce the illumination effect from both microscope and camera side. Even though, it is possible to overcome the illumination issue somehow but still human factor is involved in the preparation of blood slides that is due to the non-homogeneous and non-standard staining concentration and appearances. Different techniques for image enhancement were presented in [67]. Different solutions were proposed by researchers to address the enhancement and noise problems in automated diagnosis of malaria.

Abdul-Nasir et al. [1] suggested solution to low contrast images of malaria blood slides. Four contrast enhancement techniques namely global, linear, modified global and modified linear contrast stretching were presented. It was observed from the results that modified global and modified linear yield better results than conventional global and linear stretching. Hanif et al. [24] presented dark stretching technique to enhance and segment the plasmodium falciparum based on thick blood smear images. Dark stretching is a process that uses auto scaling method which is a linear mapping function mostly used to enhance the brightness and contrast level of the image. The approach was capable to enhance the image quality and segment out region of interest in malaria slide images based on thick blood smear.

Sio et al. [68] used adaptive histogram equalization for image enhancement whereas Diaz et al. [17] applied low pass filter to correct luminance differences on luminance channels. Filter was designed for a window size which contained the largest image feature, i.e., a typical erythrocyte size. Khan et al. [31] used non-linear filter SUSAN for noise removal, edge finding and corner finding. Several researchers [18, 21, 43, 46, 61, 63] used median filter to remove noise form smear images. For contrast adjustment of images, several researchers [42, 57, 65, 72, 80] used histogram equalization method. Median filter is a non-linear digital filtering technique used for noise reduction.

Morphological operation is well suited for biological and medical image analysis. It offers a powerful tool for extracting image components that are useful for representing shape, size and color of target regions. Morphological operators have been extensively used as preprocessing for image enhancement in major studies [15, 16, 29–31, 61, 78, 80]. Erosion and dilation operations on raw smear images allow discarding undesired patterns [25] and help in the selection of required cells or regions of interest. Pre-processing techniques used by different researchers during automated diagnosis of malaria are summarized in Table 3.

3.2.3 Segmentation of RBC and parasites

Segmentation is one of the most vital tasks in image processing and computer vision. It is defined as the process of partitioning an image into a set of non-overlapping regions whose union is the entire image. In the analysis of automatic classification of malarial parasite procedures, most important and difficult stage is the accurate segmentation of blood smear image into various elements such as RBC, WBC, malaria parasites etc. Image segmentation identifies and segments possible parasites and erythrocytes (RBC) from thin blood smear

Table 3 Image pre-processing techniques applied in various studies

References	Pre-processing techniques	Remarks
Ross N.E. et al. [61], Gatc, J., et al. [18], Savkare, S. and S. Narote [63, 64], Anggraini et al. [4], Vishnu V. Makkapati et al. [43], Malihi, L., K et al. [46], Di Ruberto, C., et al. [15, 16], Gitonga, L., et al. [21], Ghosh M. et al. [19], Yi-Wen Hung et al. [26]	Median filter or Mean Filter	Ability to remove noise and preserve sharp edges
Diaz et al. [17]	Low pass filter	Used for removal of high frequency components
Ross N.E. et al. [61], F.Boray Tek et al. [78], Di Ruberto, C., et al. [15, 16], Tsai M-H. et al. [80], Khan, M.I., et al. [31], Kareem et al. [29, 30], Khatri K et al [33].	Morphological Filtering	Useful for removal of unwanted objects, holes filling, splitting, thinning and thinking.
Aimi Salihah A-N et al. [2],	Contrast enhancement based on partial contrast stretching technique	Useful for increasing contrast of the images
Sio S.W. et al. . [68], Purwar et al. [57], Meng- Tsai M-H. et al. [80], Sheeba et al. [65], Suradkar, P.T. [72], Maiseli, B., et al. [42], Somasekar, J. and B.E. Reddy [69], Arco, J., et al. [5]	Adaptive Histogram Equalization or Local Histogram Equalization	Effective for low resolution images
Savkare, S. and S. Narote [63, 64], Kaewkamnerd et al. [27]	Laplacian filter	Used for sharping the edges in image
Khan, M.I., et al. [31], Soni, j. [71]	Non Linear Filtering: SUSAN	Useful for noise filtering, edge finding and corner finding
Das et al. [13]	geometric mean filter	Removal of Gaussian noise to preserve edges.
J. Somasekar et al. [70]	Gaussian low pass filters	Effective for removing Gaussian noise
Rakshit, P. and K. Bhowmik. [58]	Wiener filter	Used for removal of blur in images due to linear motion or unfocussed optics.

image. To extract the infected erythrocytes, it is necessary to identify them from the combination of parasites and erythrocytes in the image, and then segment them from the background. Cell segmentation can either be inductive or deductive. In inductive, the stained objects are located first by using color intensity values and then regions that contain stained are segmented, while in deductive method, the image is first segmented into background and foreground before segmenting the stained object [77]. An adequate segmentation may result in efficient detection and classification of malaria parasite. Segmentation process for detection of RBC and parasite is illustrated in Fig. 5.

Recent studies have suggested several segmentation methods for blood cells. Di Ruberto et al. [15] used green components to isolate RBC followed by opening a non-flat disk shape structure element. Then watershed algorithm was used for segmentation followed by separation of overlapped cells. Before applying watershed algorithm, they used disk shaped structuring element to enhance the roundness and compactness of cells to avoid the incorrect segmentation. Khan et al. [31] used several algorithms for image segmentation because of image complexity. They have used Otsu method [54] along with local and global threshold for RBC and parasite segmentation, respectively. Then marker controlled watershed algorithm was used to segment the touching cells whereas they have used clump splitting algorithm for overlapped cells.

Sio et al. [68] suggested combination of edge detection, edge linking and clump splitting for segmentation of RBCs. The edges were linked together at their terminal points to form closed boundaries around the cells and then linked together if their terminal points were in close proximity. Afterwards, the parasite detection is done with the help of binary mask. Savkare et al. [63, 64] used Laplacian filter on green channel of blood smear image and then applied Otsu threshold to get binary image of original slide image. Objects having area less than average area of RBCs were removed using morphological opening with disk shaped structuring element. Kaewkamnerd et al. [27] segmented the background by using histogram on HSV color format. After background segmentation, image was partitioned into small windows of 300 × 300. Finally, malaria parasites were identified based on their size. Aimi Salihah A-N et al. [2] applied K-Mean on to the three color space RGB, HSV and CY followed by seeded region growing area extraction in order to segment the infected cells. Form the results, it was observed that segmentation using saturation component of C-Y color model provided best results.

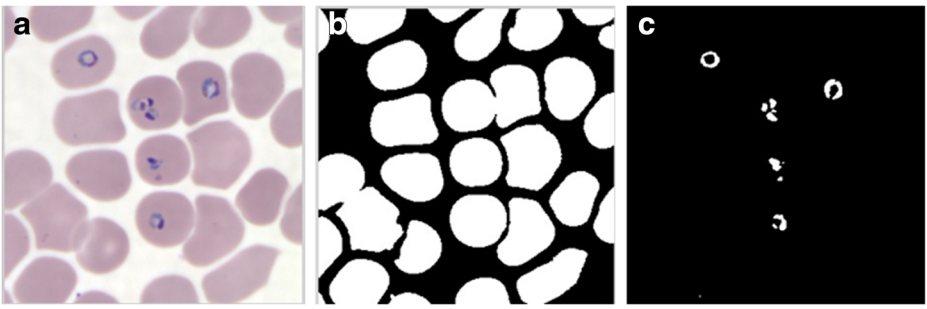

Fig. 5 Illustration of blood smear image segmentation. **a**. Original image, **b**. Segmentation of Red Blood Cell, **c**. Segmentation of malaria parasite

Lee and Chen [40] presented a method for segmentation of overlapped blood cells by the combination of canny edge detectors and Otsu algorithm. Das et al. [13] applied marker controlled watershed algorithm for segmentation of blood cells and possible parasites. Kumarasamy et al. [38] presented a four stage-based segmentation method namely edge detection, edge linking, clump splitting, and parasite detection. Somasekar and Reddy [69] presented an edge-base segmentation method for segmentation of infected erythrocytes, providing a consistent and robust segmentation of parasite infected erythrocytes. Fuzzy C-means clustering was then applied to extract infected erythrocytes. Arco et al. [5] proposed an adaptive threshold technique for segmentation of blood cells and malaria parasite by selection of local features which significantly improved the accuracy of the algorithm as compare to other approaches.

Segmentation techniques used by different researchers are summarized in Table 4 and it can be observed that most of the researchers applied Ostu algorithm [54] for segmentation of RBCs and malaria parasites. Likewise watershed and marker controlled watershed algorithm were used by various researchers at segmentation stage. Watershed algorithm provides best results for overlapping cells. Mandal et al. [47] used the normalized cut (NCut) algorithm and tests it over various color spaces. The results exhibited that the performance of the NCut algorithm is best in HSV color space.

3.2.4 Features extraction

Feature extraction is the process of image representation in non-visual form. It is a critical step in most computer vision and image processing solutions because it marks the transition from pictorial to non-pictorial data representation [50]. Parasites and other stained components are flexible objects with large variations in the shape, size, and morphology. The color information is valuable but is not adequate to distinguish between the other stained objects and plasmodium, and also within the different species. The features which give dominant difference between normal cells and infected cells are identified as feature set. Most of the studies have reported both texture as well as geometric features for describing malaria infection stages.

Geometric features Geometrical features remain very important for complex shape recognition and many researchers have used them for malarial parasite recognition. Area and perimeter are the features used to represent the size of the cells while shape features can be grouped into region and boundary-based features. To extract the features, cell image is converted into binary image where cell pixels are represented by non-zero value. In context of automated diagnosis of malaria parasite, geometric features are used for classification of malaria species and life-stages of each species regarding shape of parasite. However, geometric features are not suitable for classification of infected RBC and un-infected RBC. Zhang, D. and G. Lu [86] presented review on shape representation and description techniques. The shape and size features which were used by majority of researchers in automated diagnosis of malaria are briefly discussed below.

- ***Area****:* Area of the cell or malaria parasite is represented by the total number of non-zero pixels within the cell boundary.
- ***Perimeter:*** Perimeter is the total length of the object boundary. It is calculated by measuring the sum of the distances between successive boundary pixels of blood cells or malaria parasite.

Table 4 List of techniques used in various studies for segmentation of RBC and parasites in microscopic blood smear images

References	Techniques used for Segmentation of RBC & Parasite	Remarks
Lee, H. and Y.-P.*P. Chen* [40], Gatc, J., et al. [18], Savkare, S. and S. Narote [63, 64], Nugroho, A.S., et al. [51], Sheeba et al. [65], Anggraini et al. [4], Vishnu V. Makkapati et al. [43], Malihi, L., K et al. [46], Kumar, A., et al. [37], Ghosh S et al. [20], Mas D. et al. [48], Rosado, L., et al. [60]	Otsu thresholding	Classify the pixels through optimal threshold value.
Aimi Salihah A-N et al. [2], Khan, N.A et al. [32]	K-Mean clustering	More homogeneous regions are obtained
Prasad et al. [55], Toha, S.F. and U.K. Ngah [79], Halim, S., et al. [23], Somasekar, J., et al. [70], Suwalka, I., et al. [74], J.E. Arco [5], Linder, N., et al. [41]	Histogram threshold	Selection of threshold is crucial, wrong choice may result into over or under segmentation.
Sio S.W. et al. [68]	Rule-based approach (edge detection, edge linking and clump splitting)	Very effective for overlapping cell segmentation but shape information may distorted
Tek et al. [78]	Morphological top-hat operation	Useful in non-uniform illumination condition
Purwar et al. [57],	Chan-Vese segmentation	Not useful for overlapping cell segmentation
Tsai M-H. et al. [80], Hung, Y.-W., et al. [26]	Region growing method	Fast and reliable methods for uniform images but inconsistent for the images having considerable variability
Suradkar, P.T. [72], Kumarasamy S.K et al. [38], Komagal . E et al. [35], Rakshit, P. and K. Bhowmik. [58], Maiseli, B., et al. [42], Somasekar, J. and B.E. Reddy [69]	Edge detection algorithms	good for images having better contrast between objects
Damahe, L.B et al. [12], Chakrabortya, K., et al. [9], Le M.-T., et al. [39],	Zack threshold	Evaluate optimal threshold values
Sri Widodo et al. [43], Gual-Arnau et al. [22]	Active Contour base segmentation	Major limitation of multiple overlapping objects.
Di Ruberto, C., et al. [15], F. Tek, F.B et al. [76]	Granulometry	Effective for object with regular size
C. Di Ruberto et al. [16], Devi RR et al [14]	Watershed Transform	Work well for overlapping objects
Khan, M.I., et al. [31], Das et al. [13], J. Soni [71]	Marker controlled watershed with morphological approach	Effective for overlapping cell segmentation and may fail to segment highly overlapped cell segmentation
Zou, L.-h., et al. [87]	Circular Hough Transform	Required knowledge about the radius of the blood cell
Kareem et al. [28, 29, 30], Dallet, C., et al. [11]	Angular ring ratio	Useful only for circular shapes
Chayadevi, M. and G. Raju [10]	Fuzzy rule base segmentation	Designing of rule base is very complex
Suryawanshi M.S. and V. Dixit [73]	Poisson distribution thresholding	Not effective for overlapping cell segmentation
Yunda et al. [85]	Morphological gradient and K-Median	Initialization of K is trivial task
Ghosh M. et al. [19]	Fuzzy divergence	Useful where uncertainty is present
Mandal, S., et al. [47]	Optimized Normalized Cut	Computationally expensive

- ***Eccentricity:*** Eccentricity of an object is defined as the ratio of the major and minor axes of the object and defined as

$$\text{Eccentricity} = \frac{\text{Length of Major Axis}}{\text{Length of Minor Axis}} \tag{1}$$

- ***Convex Hull:*** Smallest convex polygon that can contain the region.
- ***Convex area:*** In some cases, convex hull is calculated and its area is termed as number of pixels inside its boundary.
- ***Solidity:*** Solidity is the ratio of actual cell area to convex hull area as

$$\text{Solidity} = \frac{\text{Area}}{\text{Convex Area}} \tag{2}$$

- ***Compactness:*** Compactness is the ratio of area and square of the perimeter

$$\text{Compactness} = \frac{\text{Area}}{\text{Perimeter}^2} \tag{3}$$

- ***Circularity:*** Circularity measurement of the cell is defined as the ratio between the blood cell or parasite area and the square of its perimeter as given in eq. 4. With the help of this feature circularity of blood cells or malaria parasite are evaluated which is further used in classification stage.

$$\text{Circularity} = \frac{4\pi A}{P^2} \tag{4}$$

- ***Orientation:*** Angle between the x-axis and the major axis of the cell is known as orientation.
- ***Rectangularity:*** Rectangularity of an object is defined by the ratio A/A_m of the object area (A) and minimal bounding box area (A_m). This ratio is 1 if the object is a rectangle, and smaller for all other shapes. With the help of this feature, rectangularity of cell and parasite are evaluated.

Texture features Texture is a powerful descriptor of an image that describes the spatial distribution of intensity or color in a particular region. The texture of a healthy red blood cell shows uniform intensity across the cell surface under microscopic image. Texture features discussed below are used for identification of infected RBCs and their classification into two classes i.e., infected and non-infected. However, it is a difficult task to classify parasite species and life-stages by using only texture features. For classification of parasite species and life-stages geometric features are used along with texture and color feature. Image texture is well described by properties like uniformity, coarseness, roughness and regularity. Some basic texture features used by researchers for classification of malaria are defined below:

- ***Mean:*** Mean value of gray level of pixels (μ) inside red blood cell or malaria parasite can be calculated by sum of all the gray level values of the cell pixels divided by the number of cell pixel

$$\mu = \frac{\sum_{i=1}^{n} xi}{n} \tag{5}$$

Where X_i is the gray level of i^{th} pixel inside cell and n is total number of pixels.

- ***Variance:*** Variance is the average of the squared differences from the mean:

$$\sigma^2 = \frac{\sum_{i=1}^{n} (Xi - \mu)}{n-1} \tag{6}$$

- ***Standard deviation:*** It is defined as square root of variance. The standard deviation provides a concise representation of the overall contrast of blood cells. Mathematically standard deviation is:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (Xi - \mu)}{n-1}} \tag{7}$$

- ***Skewness:*** Skewness is a measure of the asymmetry of the data around the sample mean. The skewness of the normal distribution is zero. The skewness of a distribution

$$\text{Skewness} = \frac{E(x-\mu)^3}{\sigma^3} \tag{8}$$

where σ is the standard deviation of x and E (t) is expectation operator.

- ***Entropy:*** The entropy measures the randomness of the intensity showing in the blood cell region and measured as

$$\text{entropy} = -\sum_{j=0}^{L-1} p(r_j)\ log_2\ [p(r_j)] \tag{9}$$

- ***Energy:*** Uniformity within the blood cell can be measured by energy which is calculated as

$$\text{energy} = \sum_{0}^{L-1} [p(r_j)]^2 \tag{10}$$

- ***Correlation:*** Correlation between pixel values and its neighborhood is represented

$$\text{Correlation} = \frac{\sum_{i=0}^{N-1} \sum_{j=0}^{N-1} (i,j) P(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y} \tag{11}$$

where σ_x , σ_y, μ_x and μ_y indicate the standard deviations and means of P_x, P_y; whereby P_x, P_y correspond to the partial probability density functions. $P_x(i)$ = i^{th} entry in the marginal-probability matrix obtained by summing the rows of P(i, j). Das et al. [13] computed a set of 96 features textural and morphological features. They extracted 80 textural (entropy, Haralick textural features, local binary pattern, fractal dimension, histogram based features, gray level run length matrix based texture) along with 16 morphological features (shape features and Hu's moment) to discriminate six types of infected and non-infected erythrocytes.

Diaz et al. [17] used mean, standard deviation, skewness, kurtosis and entropy as basic descriptors of the histogram properties. Lee and Chen [40] used shape features such as cell

circularity, medial axis ratio, cell deform ratio, eccentricity and Hausdorff distance along with texture features such as mean intensity, variance, smoothness of the cell and entropy for classification of infected and uninfected red blood cells. Ruberto et al. [15] used combination of color and morphological features for analysis and classification of infected cells. Raviraja S et al. [59] used the invariants moments for detection of infected erythrocytes. Rosado et al. [60] used combination of geometric, color and texture features with two classessupport vector machine (SVM) classifier. Generally features used in analyzing red blood cells can be grouped into geometric features and texture features as shown in Table 5.

3.2.5 Classification

A good segmentation and feature extraction process greatly simplifies the design of the classifier. A comprehensive review on classification techniques was presented by Kotsiantis et al. [36]. Classification process in automated diagnosis of malaria is generally adopted for two purposes: for deciding whether or not an erythrocyte was infected and for classification of species and life-stage of malaria parasite. List of various classifiers used by different researchers for classification of infected cells or life-stage and species is described in Table 6.

Tek et al. [76] used distance weighted K-nearest neighbor classifier to differentiate between parasites and other stained components or artifacts. However, the classification of species and life stage was not implemented. Lee and Chen [40] used a hybrid neural network architecture for classification of healthy RBC and infected RBC. However classification of parasite species and life-stage were also passed over in this method. Similarly, Mushabe al. [49] used K-nearest neighbor and linear Bayesian classifier for classification infected RBCs. However, from the diagnosis point of view the essential task is to identify parasites in the presence of other stained structures, artifacts, and then finally identify the species and life-stage of parasite as applied in [13, 15, 17, 31, 61, 78]. Diaz et al. [17] carried out parasite classification in two steps: deciding about the status of RBC (infected/non-infected) and identification of infected stage. Two classifiers were evaluated for these phases: a multilayer perceptron neural network (MLP) and SVM.

Table 5 Categorization of features used for classification of erythrocytes and malaria parasite

References	Category	Name of features
Devi RR et al [14], Tek, F.B et al. [76, 78], Lee, H. and Y.-P.P. Chen [40], Ross N.E. et al. [61], Di Ruberto, C., et al. [15], Savkare, S. and S. Narote [63, 64], Malihi, L., K et al. [46], Di Ruberto, C., et al. [15], Das et al. [13], Gitonga, L., et al. [21], Kumarasamy S.K et al. [38], Raviraja S et al. [59].	Geometric Features	Area, perimeter, area ratio, convex area, solidity, form factor, moments, compactness, eccentricity, number of lobes, circularity, orientation, rectangularity, symmetry, concavity, and elongation
Tek, F.B et al. [76, 78], Lee, H. and Y.-P.P. Chen [40], Díaz et al. [17], Prasad et al. [55], Ross N.E. et al. [61], Savkare, S. and S. Narote [63, 64], Widodo, S [83], Miss. S Annaldas et al. . [66], Khan, M.I., et al. [31], Das et al. [13], Gual-Arnau et al. [22], Chayadevi, M. and G. Raju [10], Gitonga, L., et al. [21], Yunda et al. [85]	Texture or color features	Mean, variance, standard deviation, skewness, smoothness, entropy, energy, homogeneity, correlation, regularity, coarseness, and color

Table 6 Different Classification techniques used by researchers for classification of infected erythrocytes and classification of species, life-stage of malaria parasite

References	Classification Technique	Remarks
Díaz et al. [17], Tek, F.B et al. [76, 78], Malihi, L., K et al. [46], Gual-Arnau et al. [22]	K-nearest neighbors classifier (KNN)	The classification of unknown sample is done simply based on comparison with stored training data.
Díaz et al. [17], Savkare, S. and S. Narote [63, 64], Malihi, L., K et al. [46], Widodo, S [83], Miss. S Annaldas et al. [66], Das et al. [13], Chayadevi, M. and G. Raju [10], Kumarasamy S.K et al. [38], Linder, N., et al. [41]	Support Vector Machine (SVM)	SVMs are well suited to deal with learning tasks where the number of features is large with respect to the number of training instances.
Lee, H. and Y.-P.P. Chen [40], Miss. S Annaldas et al. [66], Gitonga, L., et al. [21], Yunda et al. [85]	Artificial Neural Network (ANN)	It uses Nonparametric approach. Performance and accuracy of classification depends upon the network structure and number of inputs.
Díaz et al. [16], Das et al. [13]	Naive Bayes	The main advantage of the naive Bayes classifier is its short computational time for training.
Tek, F.B., et al. [78], Malihi, L., K et al. [46]	Fisher linear discriminant (FLD)	Perform classification of the objects on the basis of learning and minimization of some error criterion.
Tsai M-H. et al. [80],	Genetic algorithm	Result depends on the chosen chromosome encoding scheme, crossover and mutation strategies as well as fitness function.
Purwar et al. [57],	K-Mean Clustering	K-means clustering is unsurprised classification method which cluster unknown pixels in number of classes.
Tek, F.B., et al. [78], Khan, M.I., et al. [31], Ross N.E. et al. [61], Chayadevi, M. and G. Raju [10]	Back propagation neural network (BPNN)	It overcomes the limitations that single-layer networks have.
Leila Malihi et al. [46]	Nearest Mean Classifier (NM)	Useful in situations with few samples and large number of features
Premaratne, S.P., et al. [56]	Feed Forward neural network	The training subset is used in optimization and the validation subset to estimate the generalization error.
Miss. S Annaldas et al. et al. [66]	Adaptive Neuro Fuzzy interface System	It applies a hybrid-learning algorithm, the gradient descent method and the least-squares method, to update parameters.
Suryawanshi M.S. and V. Dixit [73]	Decision tree using Euclidean Distance	Provides hierarchical associations between input variables to forecast class membership and provides a set of rules.

Boray et al. [78] compared three different classification models for species and life-cycle-stage identification. The first model (20-class) considered performing detection, species, and life-cycle-stage recognition in a single classification, which also allows differentiating within the non-parasite classes. The second and third model considered performing a binary detection previously followed by a single 16-class classification or two 4-class classifications for

identification, respectively. They implemented the mentioned classes by using KNN, fisher linear discriminant (FLD) and the back propagation neural network (BPNN) classifiers in which KNN gave better result. Ross et al. [61] used back propagation feed forward neural network for classification of infected erythrocyte and species of parasite if infected. The species for every infected erythrocyte is determined, and the sample species are those having highest number of parasites in the sample. The accuracy of this classifier was claimed to be 73%.

4 Discussion

In this paper, a systematic review of automated diagnosis of malaria based on microscopic blood smear images has been presented. A complete malaria diagnosis system must have the ability to perform image acquisition, pre-processing, segmentation and classification task. In order to perform diagnosis on malaria blood smear images, diagnosis system of malaria requires the ability to detect the presence of parasite in a blood sample by differentiating between non-parasitic stained objects (artifacts, white blood cell, and red blood cell) and malarial parasites. To specify the infection, an additional process of species and malaria parasite development stages identification by differentiating species and development stages is also required if the blood sample is diagnosed as positive. However, majority of existing malaria-related image analysis studies fail to fulfill above mentioned requirements.

In pre-processing, median filter has been found to be very effective for reducing impulse noises from the microscopic images. Local histogram technique was widely used for enhancing the microscopic blood smear images. Abdul-Nasir et al. [1] used a modified image enhancement techniques i.e., modified global and modified linear contrast stretching with conventional global and linear contrast stretching. This method enhanced the image from the luminance information of an entire image. Image with a high global contrast will cause a global feeling of a detailed and variation-rich image. The results showed that modified global and modified linear contrast stretching techniques have successfully improved the contrast of the parasites and the infected red blood cells. Hanif et al. [24] presented dark stretching technique to enhance and segment the malaria parasite blood smear images. In dark stretching method auto scaling which is a linear mapping function mostly used to enhance the brightness and contrast level of the image. Results showed that the approach is capable to enhance the image quality and segment the regions of interest in malaria blood smear images.

Segmentation is considered as a critical step in automated diagnosis of malaria parasite. A good segmentation simplifies the process of parasite identification and feature extraction. From Table 4 it has been expressed that most of researchers used Otsu threshold [54] for segmentation of erythrocytes and malaria parasite. The benefit of Otsu threshold is that it selects optimal threshold based on minimization of a criterion function. However, Otsu threshold fails in segmentation of overlapping cells. For segmentation of overlapping cells Di, Rubeto et al. [16] applied watershed segmentation. Applying a watershed transform on the image directly is generally useless unless the objects are flat or at least smooth grey level regions. Hence, a marker controlled transform is usually preferred which basically replaces the regional minima with the externally supplied markers [75]. Researchers [13, 31, 71] applied marker controlled watershed for separation of overlapping cells. Similarly, circular hough transform, K-mean clustering, edge detection algorithm and zack algorithm were used in various studies in segmentation.

Texture, geometric and color features have been evaluated for classification of infected erythrocytes and infection stages of malaria. In majority of the existing methods, researchers have used combination of texture and geometric features at feature extraction stage [13, 40, 78]. However, some have used either texture features or geometric features for classification purpose. It has been observed from various studies of automated diagnosis of malaria that texture or color features are used for classification of infected and healthy erythrocytes while geometric features are used for identification of parasite species and life-stage. Comparative evaluation of the performance of reported malaria detection methods is shown in Table 7. Boray et al. [78] used a concatenated feature of color histogram, local area granulometry and shape measurements vector. They used 20 classes for classification of four stages of each species, white blood cells, artifacts and platelet. The results were evaluated with three different classifiers including fisher linear discriminant (FLD), back propagation neural network (BPNN) and KNN with accuracy 90.1, 92.0 ± .4 and 93.3, respectively. Diaz et al. [17] applied two classifiers including multilayer perceptron neural network (MLP) and SVM during classification and acquire the best performance by SVM with a polynomial kernel, which exhibited an effectiveness of 0.95, a sensitivity of 94%, and a specificity of 99.7%. Das et al. [13] compared the performance of SVM and Naive Bayes classifier for Vivax and Falciparum infection stage classification by using geometry, intensity and texture information. The accuracy of SVM and Naive Bayes were 76% and 84%, respectively in classification with top 19 features. Lee. and Chen [40] used hybrid neural network based classifier for red blood cells, based on the shape and texture features. Their system showed comparative advantages over the conventional neural network classifier with single input layer, which usually requires an implementation of feature selection strategy to improve classification results. As exhibited in Table 7, SVM has been used by most of the researchers. The main advantage of the SVM is its extraordinary generalization ability and extremely powerful learning rate, leading to the global minimum of the defined error function. From classification point of view, several classifiers have been presented for the automatic classification of malarial parasites in the presence of other stained objects in blood smear images. However, these studies rarely focused on the life-stage classification. It is better to find life-stage of parasite and this can be solved using the multi-class classification instead of binary class problem i.e., parasite or non-parasite. Various methods of automated parasitemia counting have been reported by different researcher as summarized in Table 8. They evaluated the performance of their proposed algorithms of parasitemia count by comparing with the manual counting procedure.

4.1 Possible directions for future research

Automated detection and classification of malaria parasite can help the pathologists in the disease identification and drug development. Although, a significant amount of work has been done in this field, but still there are some challenges which lead to lower accuracy in identification of malaria parasites. Therefore, improvements are required to fulfill the expectations of pathologists, which can reduce the problems faced in manual analysis. From the literature review, it has been observed that most of the studies are limited to detection of malaria parasite in blood smear image. Identification of species and life-stage of malaria parasite have been unheeded in most of studies. However, researchers in [21, 46, 78, 80] diagnosed four species of malaria parasite but the results are still not according to expectation

Table 7 Comparative study of performance of various malaria detection methods

Authors	Classes/ group for Malaria Parasite	Performance statistics (%)
Aimi Salihah A-N et al. [2]	Two classes (malaria infected and non-infected)	Accuracy: 99.46; F-score:93.70
Diaz et al. [17]	Two classifiers: one for infected RBC and other for life stage of malaria parasite.	For infected RBC the specificity of 99.7% and a sensitivity of 94%. The infection stage was determined with an average sensitivity of 78.8% and average specificity of 91.2%.
Tek, F.B., et al. [78]	20 classes (04 stages of each species)	Sensitivity: 72.4; Specificity: 97.6
Das et al. [13]	Six classes for two species of malaria P. vivax and P. Falciparum	Sensitivity: 99.72; Specificity: 84.39; PPV: 98.64 and Accuracy: 96.3
Tek, F.B et al. [76]	Two classes parasite /non-parasite	sensitivity:74%, specificity:98%
Ghosh M. et al. [19]	Two classes (malaria infected and non-infected)	Accuracy: 98
Halim, S., et al. [23]	Three classes (Gametocyte, haemozoin and schizonts)	Precision: 89.42; Recall: 91.65
Kareem et al. [29]	Two (malaria infected and non-infected)	Sensitivity: 90; Accuracy: 87
Prasad et al. [55]	Two (malaria infected and non-infected)	Accuracy:96
Ross N.E. et al. [61]	Three classifier (infected, non-infected and species of malaria)	85% and a PPV of 81%
Gatc, J., et al. [18]	Two (malaria parasite and non-parasite)	Sensitivity: 85.52; PPV: 92.85
Purwar et al. [57]	Two (malaria infected and non-infected)	Sensitivity: 100; Specificity:50–80
Gitonga, L., et al. [21]	12 (04 stages of each species)	Accuracy: 99 for recognizing stages and 96.2 for malaria species
Khan, M.I., et al.44]	Two-stage tree classifier (P. Falciparum, P.Vivax, P. Ovale or P. Malariae)	Sensitivity: 85.5% and PPV: 81%.
Leila Malihi et al. [46]	Four (P. falciparum, P. vivax, P. ovale, P. malariae)	Accuracy: 91
Kumarasamy S.K et al. [38]	Three (ring, trophozoite, gametocyte)	Accuracy: 86
Somasekar, J. and B.E. Reddy [69]	Parasite/ Non-parasite	sensitivity: 98%, specificity: 93.3%

of pathologists. The accuracy of malaria parasite identification may be affected by human factor in preparation of blood slides, microscope, noise and several other factors such as scale correction and color normalization. To overcome under- or over-staining condition of blood slides, color features, hyperspectral imaging could be used. The different spectral ranges of blood sample images are very helpful for the extraction of meaningful regions. One of the major challenges that exist in segmentation of malaria parasite is to distinguish parasite from WBC and other staining objects as they have the same color and intensity. Existing methods based on segmentation are not applicable to all fields of a blood slide. Global segmentation can be replaced by localized malarial parasite analysis. Thus, it may be possible to perform the malarial parasite classification without segmenting them into infected cells. The segmentation method can be enhanced to such level that it process efficiently blood smear image without noise removal and contrast adjustment. Similarly, in case of parasitemia measurement, exact numbers of healthy erythrocytes and infected erythrocytes need to be counted, thus, overlapping cells may yield to inconsistent results. Therefore, a suitable algorithm can be used for counting overlapping cells

Table 8 Performance based comparison of parasite count methods

Authors	Method	Performance Remarks
Purwar et al. [57]	Probabilistic k-means clustering	Sensitivity: 100 and Specificity: 50–88
Diaz et al. [17]	support vector machine (SVM)	Sensitivity of 94% and a specificity of 99.7%.
Sio S.W. et al. [68]	Morphological approach	Discrepancy: 2.04 ± 2.86 for poorly separated cell and 0.25 ± 0.18 for well separated cell
Kumarasamy S.K et al. [38]	support vector machine (SVM)	Accuracy: 80
Halim, S., et al. [23]	color co-occurrence matrix (CCM)	precision:92% and recall rates: 95%
Arco, J., et al. [5]	Morphological approach	Discrepancy:3.54
Gitonga, L., et al. [21]	Artificial neural network	Accuracy:79
Savkare, S. and S. Narote [63]	SVM with RBF kernel	Sensitivity: 93.12 and Specificity: 93.17
Linder, N., et al. [41]	SVM	Sensitivity 95% specificity 100% and correlation coefficient between manual and automated count 0.97

correctly. Likewise, classification stage needs to be improved so that it could better classify infected cells as well as identify species and life-stage of parasite by using optimal features.

5 Conclusion

This paper provides a basis to researchers who want to start research in the area of automated diagnosis of malaria based on microscopic blood smear images. The focus of this article is to review, analyze and categorize malaria recognition algorithms, techniques and methodologies and uncover existing limitations. The problems faced by pathologist are also discusse. The review is presented for four significant stages of automated malaria parasite diagnosis namely image pre-processing, parasite segmentation, feature extraction and classification.

In preprocessing, problems concerning to color variations, illumination variations, and presence of noise in the stained microscopic images are discussed. To overcome under or over-staining condition of blood slides, color features, hyperspectral imaging can be used. Different spectral ranges of blood sample images are very helpful for the extraction of meaningful regions. Segmentation is the second major step of malaria parasite classification and highly affects the performances of the classifiers. Global segmentation can be replaced by localized malarial parasite analysis. Thus, it may be possible to perform the malarial parasite classification without segmenting them into infected cells. Similarly, the color and texture along with morphological features is valuable feature information. In classification point of view, it may be better to add contextual knowledge into the classification for malarial parasites. This can be solved using the multi-class classification instead of binary class problem, i.e., parasite or non-parasite.

This review may aid researchers to go through the state-of-the-art methods presented in last two decades along with their limitations. Since, automated analysis of malaria cannot be achieved only through computer vision scientists but also required the involvement of pathologists. Collaboration between the two communities will lead development of more robust and effective computer aided pathological image analysis techniques.

Acknowledgements This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1A09919551).

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Zahoor Jan is currently holding the rank of an associate professor in computer science at Islamia College Peshawar, Pakistan. He received his MS and PhD degree from FAST University Islamabad in 2007 and 2011, respectively. He is also the chairman of Department of Computer Science at Islamia College Peshawar, Pakistan. His areas of interests include image processing, machine learning, computer vision, artificial intelligence and medical image processing, biologically inspired ideas like genetic algorithms and artificial neural networks, and their soft-computing applications, biometrics, solving image/video restoration problems using combination of classifiers using genetic programming, optimization of shaping functions in digital watermarking and image fusion.

Arshad Khan received his BCS degree in Computer Science from Islamia College, Peshawar, Pakistan. Currently, he is pursuing MS degree from the same university. He is working as a researcher at DIP Lab since 2015. His research interests include image and video processing, medical image analysis, and diagnosis of malaria parasite from microscopic blood smear images.

Muhammad Sajjad received his Master degree from Department of Computer Science, College of Signals, National University of Sciences and Technology, Rawalpindi, Pakistan. He received his PhD degree in Digital Contents from Sejong University, Seoul, Republic of Korea. He is now working as an assistant professor at Islamia College Peshawar, Pakistan. He is also the head of "Digital Image Processing Laboratory (DIP Lab)" at Islamia College Peshawar, Pakistan. His research interests include digital image super-resolution and reconstruction, sparse coding, medical image analysis, video summarization and prioritization, image/video quality assessment, and image/video retrieval.

Khan Muhammad received his BCS degree in Computer Science from Islamia College, Peshawar, Pakistan in 2014 with research in information security. Currently, he is pursuing MS leading to PhD degree in digital contents from Sejong University, Seoul, Republic of Korea. He is working as a research associate at Intelligent Media Laboratory (IM Lab) since 2015. His research interests include image and video processing, image and video steganography, video summarization, diagnostic hysteroscopy, wireless capsule endoscopy, and CCTV video analysis. He has published 20+ papers in peer-reviewed international journals and conferences such as Future Generation Computer Systems, Biomedical Signal Processing and Control, IEEE Access, Journal of Medical Systems, Pervasive and Mobile Computing, Multimedia Tools and Applications, SpringerPlus, KSII Transactions on Internet and Information Systems, International Journal of Applied Pattern Recognition, Journal of Korean Institute of Next Generation Computing, NED University Journal of Research, Technical Journal, Sindh University Research Journal, Middle East Journal of Scientific Research, MITA 2015, PlatCon 2016, and FIT 2016. He is a student member of IEEE.

Seungmin Rho is a faculty of Department of Media Software at Sungkyul University in Korea. In 2012, he was an assistant professor at Division of Information and Communication in Baekseok University. In 2009-2011, he had been working as a Research Professor at School of Electrical Engineering in Korea University. In 2008-2009, he was a Postdoctoral Research Fellow at the Computer Music Lab of the School of Computer Science in Carnegie Mellon University. He gained his B.S degree in Computer Science from Ajou University. He received his MS and PhD degrees in Information and Communication Technology from the Graduate School of Information and Communication at Ajou University, South Korea. He visited Multimedia Systems and Networking Lab in University of Texas at Dallas from Dec. 2003 to March 2004. Before he joined the Computer Sciences Department of Ajou University, he spent two years in industry. His current research interests include database, big data analysis, music retrieval, multimedia systems, machine learning, knowledge management as well as computational intelligence.

Irfan Mehmood received his BS degree in Computer Science from National University of Computer and Emerging Sciences, Pakistan. He completed Ph.D. degree from Sejong University, Seoul, Korea. Dr. Irfan is Assistant Professor in College of Electronics and Information Engineering at Sejong University, Seoul, South Korea. His research interests include video and medical image processing, big data analysis, and visual information summarization. He is a member of the IEEE and corresponding author of this paper.