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Image Analysis and Machine Learning for Detecting Malaria

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Abstract

Malaria remains a major burden on global health, with roughly 200 million cases worldwide and more than 400,000 deaths per year. Besides biomedical research and political efforts, modern information technology is playing a key role in many attempts at fighting the disease. One of the barriers towards a successful mortality reduction has been inadequate malaria diagnosis in particular. To improve diagnosis, image analysis software and machine learning methods have been used to quantify parasitemia in microscopic blood slides. This paper gives an overview of these techniques and discusses the current developments in image analysis and machine learning for microscopic malaria diagnosis. We organize the different approaches published in the literature according to the techniques used for imaging, image pre-processing, parasite and cell segmentation, feature computation, and automatic cell classification. Readers will find the different techniques listed in tables with the relevant papers cited next to them, for both thin and thick blood smear images. We also discussed the latest developments in sections devoted to deep learning and smartphone technology for future malaria diagnosis.

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1. Introduction

Malaria is caused by protozoan parasites of the genus Plasmodium that are transmitted through the bites of infected female Anopheles mosquitoes and that infect the red blood cells. Most deaths occur among children in Africa, where a child dies almost every minute from malaria, and where malaria is a leading cause of childhood neuro-disability. According to the WHO World Malaria Report 2015 [1], an estimated 3.2 billion people in 95 countries and territories are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). There were about 214 million cases of malaria globally in 2015 and about 438 000 malaria deaths. The burden was heaviest in the African region, where an estimated 92% [2] of all malaria deaths occurred, and in children aged under 5 years, who accounted for more than two thirds of all deaths (see also the malaria death rates from an earlier WHO report in Figure 1). Typical symptoms of malaria include fever, fatigue, headaches, and in severe cases seizures and coma leading to death.

Hundreds of millions of blood films are examined every year for malaria, which involves manual counting of parasites and infected red blood cells by a trained microscopist. Accurate parasite counts are essential not only for malaria diagnosis. They are also important for testing for drug-resistance, measuring drug-effectiveness, and classifying disease severity. However, microscopic diagnostics is not standardized and depends heavily on the experience and skill of the microscopist [1]. It is common for microscopists in low-resource settings to work in isolation, with no rigorous system in place that can ensure the maintenance of their skills and thus diagnostic quality [1]. This leads to incorrect diagnostic decisions in the field [1]. For false negative cases, this leads to unnecessary use of antibiotics, a second consultation, lost days of work, and in some cases progression into severe malaria. For false positive cases, a misdiagnosis entails unnecessary use of anti-malaria drugs and suffering from their potential side-effects, such as nausea, abdominal pain, diarrhea, and sometimes severe complications.

This sober analysis of malaria diagnosis has prompted efforts to perform malaria diagnosis automatically. Automatic parasite counting has several advantages compared to manual counting: i) it provides a more reliable and standardized interpretation of blood films, ii) it allows more patients to be served by reducing the workload of the malaria field workers, and iii) it can reduce diagnostic costs. Several key processing steps are typically required to quantify parasitemia automatically. First, digital blood slide images need to be acquired, which often requires preprocessing to normalize for lighting or staining variations. In a second step, blood cells and/or parasites need to be detected. For blood cells, this typically implies cell segmentation to identify individual cells in cell clumps to obtain accurate cell counts. In a third step, after cell detection and segmentation, features are computed to describe the typical visual appearance of infected and uninfected blood cells. In a final classification step, a classifier, who has been trained on an independent and typically manually annotated training set then discriminates between infected and uninfected cells. Once the number of infected and uninfected cells are known, computation of parasitemia is a straightforward mathematical equation, which includes clinical parameters such as hematocrit value for example.

The prospects of automating malaria diagnosis with its obvious advantages has attracted many researchers, especially in the last decade. The publications reflect all the major developments we have seen in the areas of automatic pattern recognition and machine learning in the last years. Our paper will give an overview of the papers that have been published, using the processing steps mentioned above as a framework and guide. This is not the first survey paper on the subject. In fact, several survey papers have already been published before, which bears testimony to both the importance of automated malaria diagnosis and the research dynamics and rapid system development. We refer readers in particular to the following surveys for additional information about the background of automatic malaria diagnosis and the image processing and machine learning methods used for automated microscopy diagnosis of malaria: [3, 4, 5]. In addition, more specific surveys have been published on cell features for malaria parasite detection [6], on malaria diagnosis [7], on malaria diagnostic tools [8], and on alternatives to conventional microscopy [9]. The purpose of our paper is not to replace these surveys, but rather to complement them and to provide the latest update of the state-of-the-art in image analysis and machine learning for malaria diagnosis as it presents itself at the end of the Year 2017. With about 160 literature citations, we have collected more references compared to the other surveys. We had the goal to include also maybe lesser known publications to provide a historical documentation of the work done. In addition, we included a section on deep learning, which is the latest development in malaria diagnosis and which arguably has the potential to render many of the old approaches obsolete, similar to the development in other imaging application areas. There have also been many developments in hardware for automatic malaria diagnosis, which are however out of the scope of this paper and deserve a separate paper. Nevertheless, we devote a section to rapid diagnostic tests (RDTs) for malaria diagnosis because they are also widely used in the field. The bulk of our papers have been collected from the Journal of Microscopy, Malaria Journal, and PLOS ONE, including a few papers from Nature and others. We have also collected publications from IEEE conferences and other proceedings published by Springer and Elsevier. Furthermore, we have organized the papers into sections for preprocessing, cell detection and segmentation, feature computation, and classification. We have also added a separate section about deep learning and an extensive section about mobile smartphone applications for malaria diagnosis. A discussion of the latest developments and our conclusion mark the end of this paper.

2. Malaria

There are five Plasmodium species that cause malaria in human: Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi. The two most common species are P. falciparum and P. vivax. P. falciparum is the most severe form and is responsible for most malaria-related deaths globally [1].

Plasmodium falciparum is the most prevalent malaria parasite in sub-Saharan Africa, accounting for 99% of estimated malaria cases in 2016. Outside of Africa, P. vivax is the predominant parasite in the WHO Region of the Americas, representing 64% of malaria cases, and is above 30% in the WHO South-East Asia and 40% in the Eastern Mediterranean regions [162].

Each of these parasite species goes through stages during their development cycle (48 hours), which gives the parasites a different visual appearance that can be observed under the microscope. In chronologic order, these stages are the ring stage, trophozoite stage, schizont stage, and gametocyte stage. Figure 2 shows typical examples of all stages for each species.

In non-severe malaria mostly the young stages (< 24 hours old) of P. falciparum are present in the peripheral blood, while for severe malaria all stages can be present in the peripheral blood. For P. falciparum, the trophozoite-infected red blood cells disappear from the peripheral blood circulation by attachment to the walls of capillaries inside vital organs, which is a process called sequestration. If the capillaries are blocked for newly infected cells by already attached cells, more mature parasite stages (trophozoites and schizonts) will be visible in the peripheral blood, which indicates a severe infection and a bad prognosis.

For P. falciparum, ring stages have a visible cytoplasm and one or two small chromatin dots. The infected blood cells are not enlarged but can feature multiple infections. P. falciparum trophozoites are rarely seen in peripheral blood smears. The cytoplasm of mature trophozoites tends to be more dense than in younger rings, while growing trophozoites can appear slightly amoeboid in shape (CDC). P. falciparum schizonts are also seldomly seen in peripheral blood. They are displaying several dark pigments (merozoites), which are clumped in one mass. Gametocytes of P. falciparum have a crescent, or sausage shape and can be seen in the blood smear one weak after a parasite infection. The chromatin is visible as a single mass or is diffuse. For more information about P. falciparum morphology, see for example the following references [163, 164]. Similar observations can be made for the stages of the other parasite species. For example, for P. vivax, hosts cells are often enlarged and have irregular shape. Trophozoites are amoeboid in shape and for severe infections multiple infections of single blood cells are not uncommon. For P. malariae, host cells are not enlarged. Trophozoites have a strong tendency to form as a band across the diameter of infected red blood cells. Multiple infections are extremely rare for P. malariae. On the other hand, for P. ovale, host cells are slightly enlarged and have an oval shape with tufted ends, often fimbriated. Parasites are slightly enlarged and trophozoites are amoeboid in shape. Multiple infections of a single cell are more common than for P. vivax. For P. knowlesi, infected red blood cells do not appear enlarged. The parasite erythocytic cycle is only 24 hours, which is shorter than P. falciparum's cycle (48 hours) and much shorter than P. malariae's cycle (72 hours), which will lead to the same stage seen in peripheral blood every day at a given time. The morphology of P. knowlesi parasites is similar to P. malariae. Trophozoites can feature pigments spread as a band like P. malariae, but their cytoplasm is more irregular and multiple parasites infecting one single red blood cell can be seen like in P. falciparum.

Figure 3 shows two examples of different parasite stages in the same thin blood slide image. In the first slide image, P. falciparum trophozoites and gametocytes can be seen together with white blood cells. The latter are larger and have a pronounced nucleus compared to the many red blood cells in the image. In the second image, P. falciparum ring stages are together with schizonts. In addition, other objects such as parasite outside cells and staining noise are visible in both images. Staining noise in particular can be confused with parasites by an unexperienced microscopist.

3. Malaria Diagnosis

Malaria is a curable disease with drugs available for treatment, including drugs that can help prevent malaria infections in travelers to malaria-prone regions. However, there exists no effective vaccine against malaria yet, although this is an area of active research and field studies. Once infected, malaria is a rapidly progressing disease, with a serious risk of developing into severe and cerebral malaria with neurological symptoms for P. falciparum infections. Therefore, a timely diagnosis of malaria is very important. Although malaria can be diagnosed in many different ways, there is room for improvement for current malaria diagnostic tests including reducing cost, increasing specificity and improving ease of use. Because automated malaria diagnosis for resource-poor settings is the main topic of this survey, we have devoted two subsections to light microscopy and Rapid Diagnostic Tests (RDTs), which are by far the two most heavily used diagnostic means in these areas. We also briefly discuss the other options for malaria diagnosis, although they are arguably less suited for the conditions in remote malaria regions. For more information about malaria diagnosis, we refer readers to the surveys in [7] and [9], and the following references: [8, 13, 16].

Detecting the presence of parasites is the key to malaria diagnosis. In addition, identifying the parasite species and presence of potentially mixed infections is important, as well as the observation of the stage development of P. falciparum parasites in relation to the severity of the disease. Counting parasites for determining the level of parasitemia is not only important for identifying an infection and measuring its severity, it also allows monitoring patients by measuring drug efficacy and potential drug-resistance.

3.1. Light Microscopy

The current gold standard method for malaria diagnosis in the field is light microscopy of blood films, which is the main focus of this paper. Although other forms of diagnosis exist and have become popular in recent years, in particular Rapid Diagnostic Tests, microscopy remains the most popular diagnostic tool, especially in resource poor settings. With microscopy, all parasite species can be detected. It allows computing the level of parasitemia, clearing a patient after a successful treatment, and monitoring drug-resistance. Furthermore, it is less expensive than other methods and widely available. However, its biggest disadvantages are the extensive training required for a microscopist to become a proficient malaria slide reader, the high cost of training and employing, maintaining skills, and the large component of manual work involved.

To diagnose malaria under a microscope, a drop of the patient's blood is applied to a glass slide, which is then immersed in a staining solution to make parasites more easily visible under a conventional light microscope, usually with a 100x oil objective. Two different types of blood smears are typically prepared for malaria diagnosis: thick and thin smears. A thick smear is used to detect the presence of parasites in a drop of blood. Thick smears allow a more efficient detection of parasites than thin smears, with a 11 times higher sensitivity [5]. On the other hand, thin smears, which are the result of spreading the drop of blood across the glass slide, have other advantages. They allow the examiner to identify malaria species and recognize parasite stages more easily.

The actual microscopic examination of a single blood slide, including quantitative parasite detection and species identification takes a trained microscopist 15-30 minutes. Considering that hundreds of thousands of blood slides are manually inspected for malaria every year, this amounts to a huge economic effort required for malaria diagnosis.

3.2. Rapid Diagnostic Tests

The main advantage of microscopic malaria diagnosis lies in its low direct cost, which gives it a distinct advantage in resource poor settings [1]. Other existing diagnostic methods, and any new method, have to prove that they can provide the same ease of use and price point as microscopy given the limited financial resources typically available in malaria-prone regions. Arguably the only and main competitor in this sense are Rapid Diagnostic Tests (RDTs). They detect evidence of malaria parasites (antigens) and take about 10-15 minutes to process. Their detection sensitivity is lower but comparable to manual microscopy, and they do not require any special equipment and require only minimal training.

Although RDTs are currently more expensive than microscopy in high burden areas [165], a valid question is whether these tests can replace microscopy in the near future. At the time of this writing, according to WHO [1], more countries use microscopy more than they use RDTs [2]. RDTs are used more in rural areas where microscopy is not available. About 47% of malaria tests in malaria endemic countries worldwide were made by RDT [2].

The use of RDTs, however, does not eliminate the need for malaria microscopy. A major disadvantage is that RDTs do not provide quantification of the results. Therefore, at this point in time, microscopy and RDTs are more complementing each other than one replacing the other.

3.3. Other Tests

Several methods for diagnosing malaria are available. Important criteria are cost per test, sensitivity and specificity of the method, time per test and the required skill level of the user. Furthermore, quantification of the number of infected red blood cells is important as a prognostic indicator [64].

• Polymerase Chain Reaction. A molecular method called Polymerase Chain Reaction (PCR) has shown higher sensitivity and specificity than conventional microscopic examination of stained peripheral blood smears [7]. In fact, it is considered the most accurate among all tests. It can detect very low parasite concentrations in the blood and can differentiate species. However, PCR is a complex high-cost technology that takes many hours to process by trained staff. According to [7], PCR is not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely. Quality control and equipment maintenance are also essential for the PCR technique, so that it may not be suitable for malaria diagnosis in remote rural areas or even in routine clinical diagnostic settings.

• Fluorescent Microscopy. Quantitative buffy coat (QBC) is a laboratory test to detect infection with malaria or other blood parasites, using fluorescent microscopy. A fluorescent dye makes parasites visible under ultraviolet light. According to [166], this test is more sensitive than the conventional thick smear. Nowadays, portable fluorescent microscopes with fluorescent reagent to label parasites, are available commercially. Although the QBC technique is simple, reliable, and user-friendly, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites [7].

• Flow Cytometry. This is a laser-based cell counting and detection methods that allows to profile thousands of cells per second. Although flow cytometry offers automated parasitemia counts, this is offset by a rather low sensitivity. Flow cytometry is less suitable as a diagnostic technique in the field, when a direct answer is required for treatment decisions. However, in developed countries it can be applied in the clinical setting for accurate counting of parasite numbers, for instance in the follow-up of drug treatment (van Vianen et al., 1993).

4. Staining methods

Over 100 years ago, Giemsas stain (1902) was applied for the first time for the diagnosis of malaria. Since then, it received increased attention. Because of its low cost, its high sensitivity and specificity, it is currently widely used in microscopical malaria examinations (Keiser et al., 2002). However, Giemsa staining requires multiple reagents, experienced personal, and is labor intensive and time consuming (it typically requires at least 45 min to stain a slide; Keiser et al., 2002).

Other stains have been used, too, like Field stain that significantly reduces the staining time, although it requires drying of samples before and during staining (Houwen, 2002). However there are also disadvantages with Field's stain, especially in under-resourced health centers in which the stain might be used. Poor blood preparations often result in the generation of artifacts commonly mistaken for malaria parasites, such as bacteria, fungi, stain precipitation, dirt, and cell debris. These can frequently cause false positive readings.

Another stain is Leishman's stain (1901), which has a high sensitivity, is cheap, and relatively easy to perform. Among the other stains being used is, for example, the Wright-Giemsa stain, which is a combination of Wright and Giemsa stain, and where the former facilitates the differentiation of blood cell types.

In 1970s, Sodeman et al. [167] investigated the effect of Fluorochrome staining in identifying the malaria parasites at low-level infection. It has been shown that Fluorochrome staining is more sensitive and less time-consuming than Romanowsky and Giemsa staining methods [60, 168, 169] but requires considerable practice and training, and suffers from artifacts including photobleaching and phototoxicity [170, 171]. Moreover fluorescence microscopes are more expensive than standard light microscopes, which is a factor in tropical resource-poor regions where malaria is endemic [167, 172, 168].

Table 1 shows the blood smear types and staining techniques used for the approaches published in the literature. Clearly, the vast majority of publications has been for thin smears. Certainly, one reason for this lies in the fact that thin smears allow to determine the parasite species and stages more easily, in addition to the parasitemia. So, in some sense, thin smears are more versatile and contain more information. Another important reason is probably that the presence of red blood cells gives the problem of parasite detection more structure, and makes the problem easier to a certain degree, as parasites need to be detected only inside cells. For thick films, parasite detection may be harder due to noise and staining artifacts that can lead to false positives. Nevertheless, because of the importance of thick smears for practical malaria diagnosis, it is very likely that more approaches for thick films will be implemented in the future. However, if convincing optical hardware solutions are found to scan multiple fields in thin smears and achieve a sensitivity comparable to thick smears, then this may be a moot point [37, 122].

Table 1 also shows that the majority of approaches, for both thin and thick smears, have adopted the most popular stain in practice, Giemsa. While stains like Leishman provide very good results for malaria parasites, Giemsa stain has proved to be the best all-round stain for the routine diagnosis of malaria. It has the disadvantage of being relatively expensive, but this is outweighed by its stability over time and its consistent staining quality over a wide range of temperatures.

5. Automated Diagnosis of Malaria

This section provides the core information of our survey, namely a compilation of references that should cover the vast majority of papers ever published on automated microscopy for malaria diagnosis, with the bulk of the papers published in the last ten years. The work that has been done in this area is quite diverse. Nevertheless, a system for automated cell microscopy usually implements a sequence of key processing steps that can serve as a guideline. Therefore, each of the following subsections will focus on one specific aspect of the processing pipeline.

The first step is usually the acquisition of digital images of blood smears, which largely depends on the equipment and materials being use. Section 5.1 breaks down the different approaches for the different types of microscopy, blood slides (thin or thick), and staining.

Following image acquisition, most systems perform one or several pre-processing methods to remove noise and to normalize lighting and color variations inherent in the image acquisition and staining process. Section 5.2 sorts the publications according to the pre-processing methods implemented.

The next step usually involves the detection and segmentation (outlining) of individual blood cells and maybe other objects than can be visible in a blood slide image, such as parasites or platelets. Section 5.3 gives an overview of all the segmentation methods that have been used for microscopic malaria diagnosis.

For most papers, cell segmentation is followed by the computation of a set of features, which describe the visual appearance of the segmented objects in a mathematical succinct way. Section 5.5 presents the different features and potential feature selection strategies that can be found in the literature.

In the last step, a mathematical discrimination method that classifies the segmented objects into different classes based on the computed features is implemented. For example, labeling each red blood cell as either infected or uninfected is a key classification task performed in this step, which then allows to compute the parasitemia. Section 5.6 lists all the classification methods used in the literature for malaria diagnosis.

Later in the paper, in Section 6, we will present references for the latest classification trend, deep learning, which skips the feature computation step and sometimes even the segmentation step. Furthermore, in Section 7, we will discuss how smartphones can be used for microscopic malaria diagnosis and list the systems that have already been implemented and published.

5.1. Image Acquisition

Table 2 lists all published systems according to the type of microscopy used. Because light microscopy is the most common form of malaria diagnosis in resource poor settings, where automation will also have the largest impact on healthcare and economy, it is not surprising that most authors implemented systems for standard microscopy. We have also added all other imaging techniques that we found in the literature and for which automated systems have been developed. For more detailed information about these approaches we refer to the references listed in the table and the reference list at the end of this paper [7, 9, 13, 16, 8].

5.2. Pre-processing

Table 5.2 lists all preprocessing approaches that have been applied to automatic analysis of digital blood slide images.

Pre-processing is mainly applied to improve the quality of the image and to reduce variations in the images that would unnecessarily complicate the subsequent processing steps. Three key objectives can be identified: noise removal, contrast improvement, illumination and staining correction.

For noise removal, the most popular approaches have been well-established filters, such as mean and median filters, or Gaussian low pass filtering. In addition, applying morphological operations is very popular. For contrast improvement, contrast stretching techniques, and histogram equalization in particular, have been the most popular approaches. For illumination and staining variations, color normalization techniques have been applied, including the popular use of gray-scale colors.

5.3. Red Blood Cell Detection and Segmentation

Table 4 shows the different segmentation techniques applied to thin smears. The vast majority of these techniques are thresholding techniques, such as Otsu thresholding in combination with morphological operations. However, these techniques may not be dominating because of their superior performance compared to other methods but rather because of their relative simplicity. Other methods include Hough transform, which makes assumptions about the blood cell shape, and unsupervised k-means pixel clustering. Cell segmentation needs to be accurate to compute the correct parasitemia. However, touching cells in particular complicate the identification and segmentation of individual cells. For this problem, methods like Watershed and active contours have been applied.

Table 5 shows the different segmentation techniques in the literature for thick smears. The segmentation situation for thick smears is different in that white blood cells and parasites need to be segmented. However, white blood cells are bigger than red blood cells and have more texture, which makes their segmentation much easier. Furthermore, white blood cells just need to be identified and not to be processed or classified further. In addition, parasites are very small and their reliable identification is most important. Therefore, the detection of these objects is practically more important than their segmentation, which may explain again the dominance of thresholding techniques and morphological operations.

5.4. Feature Extraction and Selection

Table 6 lists the different features used in the literature to describe the appearance of red blood cells, infected and uninfected, in thin smears. Obviously, because parasites have been stained, color features are most natural and indeed used by many papers. In addition, several texture and morphological features have been used to describe the inside of red blood cells. The idea is that in case of infected cells, these features can pick up the typical appearance of ring structures with visible cytoplasm and other unique parasite characteristics. Generally speaking, most of the features used are tried and trusted features that have already been applied in other, often non-medical, application domains. For example, Haralick’s texture features, Local Binary Patterns, co-occurence matrices, Histogram of Gradients (HOG), and many others have been successfully used across a wide range of applications. This also includes morphological shape features and moments.

Most notably here is the use of different color spaces, which leads to sets of more malaria-specific features, depending on the color space used. While most papers remain in the standard RGB color space, we think that there is a perfectly good reason to use a different color space better suited to extract the typical staining colors, which often range from a blue or purple to brownish shade. The HSV color space is favored by many papers, and several other papers use the green channel of RGB to extract staining-related color information in gray-scale.

Table 7 shows the features used for thick smears. Because of the smaller number of publications for thick smears, a smaller number of features has been experimented with in the literature. Nevertheless, authors have used similar, if not identical, features compared to the ones used for thin smears, experimenting with established features as well as different color spaces.

5.5. Feature Extraction and Selection

Some papers compute a large set of many different features and then for practicality reasons cut down on these features by selecting the most discriminative feature subset using feature selection strategies. Specifically, the feature selection techniques used to reduce feature dimensionality include Principal Component Analysis (PCA), F-statistic, One-way-Anova, information gain, and SVM-based recursive feature elimination [51, 65, 72, 109, 138, 143, 147].

However, such classical approaches to feature computation and selection run the serious danger of being superseded soon by techniques not relying on hand-crafted features, such as Deep Learning in particular, which we will discuss in Section 6.

5.6. Parasite Identification and Labeling

Table 8 lists all classification methods that have been used for either discriminating between infected and uninfected red blood cells in thin smears or identifying parasites in thick smears.

Virtually all classification methods popular in the last decade have been applied to malaria diagnosis, ranging from decision trees and basic artificial neural networks over support vector machines to random tree classifiers. Very few papers have developed classification technologies specifically for cell discrimination or parasite detection. Most of the malaria-specific domain knowledge lies in the interplay of segmentation, features, and classification.

Comparing the performance of the published systems is very hard. The systems have been evaluated on blood slides from entirely different origins with largely varying parameters for image acquisition and slide preparation. Very often the evaluation set is too small or too limited to allow making a statement about the general system performance. Currently, there exists no publicly available image benchmark set, small or large, which could be used for fair comparisons of systems. Therefore, although many papers are reporting quite high performance numbers in terms of accuracy, sensitivity, specificity, and area under the ROC curve (AUC), we prefer not to compare these numbers in this survey paper.

We can observe a trade-off between the processing pipeline's run-time performance and its accuracy. Typically, as the accuracy of a technique increases, its computational complexity increases all the same. For example, sophisticated level-set methods for cell segmentation perform better than Otsu thresholding but also require a longer runtime. Furthermore, feature computation can affect system efficiency. Some papers therefore apply feature selection methods to reduce feature dimensionality and remove non-discriminative features, which can improve both accuracy and efficiency. Finally, the runtime of cell classification depends on the classification architecture used. For example, a support vector machine's classification is much faster than the classification by a deep neural network. While many papers do not report runtimes for their systems, we think that most of the cited systems will perform their task many times faster than a microscopist, or at least will perform faster than a human after a little optimization of their implementation. We have also found two papers in which the authors developed dedicated hardware devices with motorized stage units to increase throughput [173, 122].

In combination with software, this will fully automate the slide screening process so that a microscopist does not need to move the microscope dish to take an image of the next field. This will also result in a higher throughput that can increase the sensitivity of the system by allowing to inspect more fields in the same time.

To improve system accuracy, there seems to be a trend to follow the mainstream classification method at the time of publication to take advantage of the latest classification architecture and performance improvements it brings. Consequently, we are now seeing the first Deep Learning papers entering the scene, as listed in the next section.

6. Deep Learning

Deep learning is the latest trend in machine learning, which has already boosted the performance in many non-medical areas. Deep learning can be seen as an extension of the well-known multi-layer neural network classifiers trained with back-propagation, except that many more layers are used. There are also different kind of layers that are used in typical successions. Because deep learning is supervised learning, it requires large training sets. This is the reason why medical applications have been among the last applications to adopt deep learning, as annotated training images are significantly harder to obtain due to expert knowledge requirements and privacy concerns. The first paper to apply deep learning to malaria diagnosis is by Liang et al. [47], who use a convolutional neural network to discriminate between infected and uninfected cells in thin blood smears, after applying a conventional level-set cell segmentation approach. This is an ideal application for deep learning because images of segmented red blood cells are a natural input for a convolutional neural network. Deep learning does not require the design of handcrafted features, which is one of its biggest advantages. Other authors who have applied deep learning to cell segmentation are Dong et al. [43, 46] and Gopakumar et al. [173], who used Convolutional Neural Networks, and Bibin et al. [48], who used Deep Belief Networks.

Since deep learning is the overarching machine learning technique nowadays, we can expect many more publications to appear soon for cell classification, cell staging, cell segmentation, and other sub-problems in automated malaria diagnosis.

7. Mobile Smartphones for Malaria Diagnosis

The ideal hardware solution for microscopic malaria diagnosis in resource-poor settings would be a small portable slide reader into which a blood slide could be inserted and which would then output the parasitemia. While modern technology is heading this way, we are still far from having a field-usable device. In particular, the relatively high optical magnification needed (up to 1000x) for malaria diagnosis in combination with oil immersion is a major miniaturization obstacle, unless alternatives are found. The next best solution are small camera-equipped computing devices, such as smartphones, which can be attached to a magnifying device and can then compute the parasitemia automatically, using image analysis and machine learning. Modern smartphones have become powerful computing devices and their cameras provide sufficient resolution for malaria diagnosis. Moreover, Android phones have become relatively cheap and are often already in the possession of healthcare workers, even in resource-poor settings. While cellular network connectivity can help with the information exchange between field workers and hospital, it is not immediately needed for malaria diagnosis and the actual cell counting. Small magnifying devices that can be attached to a smartphone's camera, allowing true optical magnification compared to mere digital zooming, are commercially available. However, from the authors' experience, these devices are still lacking in the image quality provided. Therefore, a more practical approach is to simply attach the smartphone to the eyepiece of a regular microscope with an adapter so that blood slide pictures can be taken with the smartphone's camera.

A few experimental set-ups along these lines have been reported in the literature. In [15], Breslauer et al. built a mobile phone mounted light microscope and demonstrated its potential for clinical use by imaging P. falciparum-infected and sickle red blood cells in brightfield and M. tuberculosis-infected sputum samples in fluorescence with LED excitation. In all cases resolution exceeded that necessary to detect blood cell and microorganism morphology. For tuberculosis samples, they took advantage of the digitized images to demonstrate automated bacillus counting via image analysis software.

In [94], Pirnstill and Cote present a cost effective, optical cell-phone based transmission polarized light microscope system for imaging the malaria pigment known as hemozoin, which is a disposal product of the parasite's blood digestion. It can be difficult to determine the presence of the pigment from background and other artifacts, even for skilled microscopy technicians. The pigment is much easier to observe using polarized light microscopy. However, implementation of polarized light microscopy lacks widespread adoption because the existing commercial devices have complicated designs, require sophisticated maintenance, tend to be bulky, can be expensive, and would require re-training for existing microscopy technicians. The cell-phone based polarimetric microscopy design presented by Pirnstill and Cote shows the potential to have both the resolution and specificity to detect malaria in a low-cost, easy-to-use, modular platform.

Rosado et al. presented an image processing and analysis methodology using supervised classification to assess the presence of P.falciparum trophozoites and white blood cells in Giemsa stained thick blood smears [77]. Using a support vector machine (SVM) and a mix of geometric, color, and texture features, their automatic detection of trophozoites achieved a sensitivity of 80.5% and a specificity of 93.8%, while their white blood cell detection achieved 98.2% sensitivity and 72.1% specificity.

In [85], Quinn et al. presented their 3D-printable design of an adapter to attach a smartphone to a microscope, although all images for their experiments were taken with a dedicated microscope camera, which offered a higher pixel resolution than their smartphone camera. They presented a workflow for automated analysis of thick blood smears, which involved the computation of morphological and moment features and an ensemble tree classifier trained on these features to discriminate between abnormal patches containing parasites and normal patches. The performance they reported was 97% AUC (area under the ROC curve).

Skandarajah et al. built a custom mobile phone microscope that is compatible with phones from multiple manufacturers [14]. They demonstrated that quantitative microscopy with micron-scale spatial resolution can be carried out with multiple phones and that image linearity, distortion, and color can be corrected as needed. Specifically, they showed that phones with greater than 5 MP cameras are capable of nearly diffraction-limited resolution over a broad range of magnifications, including those relevant for single cell imaging. Furthermore, they found that automatic focus, exposure, and color gain standard on mobile phones can degrade image resolution and reduce accuracy of color capture if uncorrected, and they devise procedures to avoid these barriers to quantitative imaging.

Dallet et al. describe a mobile application platform for Android phones that can diagnose malaria from Giemsa-stained thin blood film images [52]. The main imaging component consists of elaborate morphological operations that can detect red and white blood cells, and identify parasites in the infected cells. The application also recognizes the different life stages of parasites and calculates the level of parasitemia. The application takes less than 60 seconds to give a diagnosis, and has been tested and verified on several version and types of Android mobile phones and tablets.

The authors of this survey paper have developed a smartphone application to compute parasitemia in Giemsa-stained thin blood film images [174, 47, 175]. In order to segment individual red blood cells, we applied Marker-Controlled Watershed *MC-W* to thin blood smears to efficiently detect and segment individual cells, separate touching cells, and meet the demand of real-time processing. In the cell detection step, we apply a multi-scale Laplacian of Gaussian (LoG) filter on the green channel of an RGB color slide image. The local extrema of the LoG response indicate the approximate centroids of the individual cells that will serve as the approximate centroids for the *MC-W* segmentation step. The cell foreground mask is estimated using Otsu thresholding and cell edges are extracted by computing the gradient magnitude over the minimum values of the green and blue channels. Then, in the segmentation step, we apply watershed transform on cell markers, foreground masks, and edge information to segment and separate touching cells. For cell classification, we follow a deep learning approach and use a convolutional neural network for discriminating infected from uninfected cells [47, 175].

Cesario et al. discuss mobile support for vector-borne diseases in areas where specialist healthcare is scarce [134]. They focus on the image analysis and classification component of a system that aims to reduce the chance of misdiagnosing less common diseases as malaria and to assist health professionals. Their paper largely describes work in progress towards the image analysis and classification component, but feedback from health care professionals has been generally positive.

Herrera et al. tested the diagnostic performance of a device for automated interpretation of RDTs, which uses smartphone technology and image analysis software [87]. The diagnostic performance of the device was comparable to visual interpretation of RDTs; without significant differences for P. falciparum and P. vivax. Providing standardized automated interpretation of RDTs in remote areas, in addition to almost real-time reporting of cases and enabling quality control, would greatly benefit large-scale implementation of RDT-based malaria diagnostic programs.

In similar work, Mudanyali et al. demonstrated a cellphone-based rapid-diagnostic-test (RDT) reader platform that can work with various lateral flow immuno-chromatographic assays and similar tests [113]. Their compact and cost-effective digital RDT reader attaches to the existing camera unit of a cellphone, where RDTs can be inserted to be imaged. Captured raw images of these RDTs are then digitally processed through a software application running on the cellphone for validation of the RDT and for automated reading of its diagnostic result. In addition, this smart RDT reader platform running on cellphones provides real-time spatio-temporal statistics for the prevalence of various infectious diseases, which allows tracking epidemics.

8. Discussion

From the very different methods published during the last ten years, we can see that there has been a lot of experimenting done to reach the current state-of-the-art. However, despite the large number of publications, the performance numbers that have been published are very unsatisfying from a clinician's point of view. It is actually very hard to quantify the current state-of-the-art. Many of the papers just present performance numbers in terms of sensitivity and specificity for classification, representing only one operating point among many on a receiver operating characteristic, which would present a more complete evaluation of any method for different sensitivity requirements. Furthermore, the data used for evaluation has very often been simply too small to allow a convincing statement about a system's performance. Many different training and test sets have been used to evaluate the proposed methods, but the lack of uniformity and standardization across all papers makes a fair comparison almost impossible. Extensive field studies on patient level or for tracking disease severity over time are needed to establish a baseline for standardized comparisons in the future.

A well-performing system will require the interplay of several factors, such as the characteristics of the microscope, the type of staining, the slide preparation, and the image analysis and machine learning software. However, no clear winners for each of these factors have emerged yet.

Nevertheless, progress has been made as can be seen by the natural development of methods used for image analysis and machine learning. In fact, this development has largely followed the development in other fields and has adopted major techniques and successfully applied them to malaria diagnosis. Many of these methods are general-purpose methods that are independent from the application domain. This being said, there has been a lot of fine-tuning of these methods to make them perform better for blood smear images, and more so for the image analysis methods than for machine learning. There is certainly the potential that some of these methods gain importance outside malaria diagnosis, in particular for pre-processing and for detecting and segmenting red blood cells in other applications.

For example, the filters used for preprocessing, as listed in Table 3, are a good example of known methods applied to malaria diagnosis. The same holds for the detection and segmentation methods in Tables 4 and 5, with established methods like k-means clustering, Hough Transform and active contour models, among others. Watershed in particular was a preferred technique to split touching cells. For feature computation, we can find the whole gamut of features used in other computer vision areas, ranging from the first Haralick features and chain codes to established and widely-used local binary patterns and other texture measures. The same holds for the classification methods in Table 8, which nicely reflect the historical development of classification methods over the last ten years. We can see the older decision tree methods, followed by the then-popular Ada-boost classification strategy and support vector machines, culminating in the modern deep learning networks.

9. Conclusion

We wrote this survey paper on image analysis and machine learning methods to give an update on the latest development in automated malaria diagnosis with image analysis and machine learning. This is a very dynamic area of research that has seen an extensive number of publications in the last decade. However, with the advent of new deep learning approaches, which have already left a deep impression, the research is seeing a new exciting development that is nothing short of a revolution. So far, only a few papers have been published, but it is already evident that this will be the dominating technique in the foreseeable future. This will render many of the former classification approaches dispensable. Moreover, because deep learning takes the difficult task of designing features for classification from the user, many of the handcrafted features used so far may become useless. In addition, because deep learning can be used not only for cell classification but also for cell segmentation, many of the cell segmentation approaches presented so far could become outdated very soon. Even the pre-processing techniques, which play an important role, are not safe from this development. One way of thinking is that neural networks can learn how to process different staining and lighting variations if only enough training data is being presented to the network. Given the recent developments and future possibilities, there is in fact a good chance that most of the papers referenced in our and other surveys will become a mere historical side note very soon, describing the state-of-the-art before the advent of deep learning. All of the deep learning papers published so far have concentrated on thin blood smears, but it is very likely that we will see papers for thick films very soon. Given the wide acceptance of deep learning, the importance of large annotated data image repositories for training is now widely understood, leading to a great support of data acquisition efforts. This will likely lead to larger test suites on patient level, allowing for more standardized evaluations and extensive field testing. Given these developments, automated microscopy is very much in the race towards a cheap, simple, and reliable method for diagnosing malaria.

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References

[1] WHO. Malaria microscopy quality assurance manual-version 2. World Health Organization; 2016.

[2] WHO. World malaria report 2016. World Health Organization; 2016.

[3] Tek FB, Dempster AG, Kale I. Computer vision for microscopy diagnosis of malaria. Malaria Journal. 2009;8(1):153.

[4] Das D, Mukherjee R, Chakraborty C. Computational microscopic imaging for malaria parasite detection: A systematic review. Journal of microscopy. 2015;260(1):1–19.

[5] Jan Z, Khan A, Sajjad M, Muhammad K, Rho S, Mehmood I. A review on automated diagnosis of malaria parasite in microscopic blood smears images. Multimedia Tools and Applications. 2017;p. 1–26.

[6] Devi SS, Sheikh SA, Laskar RH. Erythrocyte Features for Malaria Parasite Detection in Microscopic Images of Thin Blood Smear: A Review. International Journal of Interactive Multimedia and Artificial Intelligence. 2016;4(2):34–39.

[7] Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: A brief review. The Korean journal of parasitology. 2009;47(2):93.

[8] Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). The American journal of tropical medicine and hygiene. 2007;77(6_Suppl):119–127.

[9] Hänscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. International Journal of Laboratory Hematology. 1999;21(4):235–245.

[10] Park HS, Rinehart MT, Walzer KA, Chi JTA, Wax A. Automated Detection of P. falciparum using machine learning algorithms with quantitative phase images of unstained cells. PloS one. 2016;11(9):e0163045.

[11] Yang D, Subramanian G, Duan J, et al. A portable image-based cytometer for rapid malaria detection and quantification. PloS one. 2017;12(6):e0179161.
[12] Eshel Y, Houri-Yafin A, Benkuzari H, et al. Evaluation of the Parasight Platform for Malaria Diagnosis. Journal of clinical microbiology. 2017;55(3):768–775.
[13] Parsel SM, Gustafson SA, Friedlander E, et al. Malaria over-diagnosis in Cameroon: diagnostic accuracy of Fluorescence and Staining Technologies (FAST) Malaria Stain and LED microscopy versus Giemsa and bright field microscopy validated by polymerase chain reaction. Infectious Diseases of Poverty. 2017;6(1):32.
[14] Skandarajah A, Reber CD, Switz NA, Fletcher DA. Quantitative imaging with a mobile phone microscope. PloS one. 2014;9(5):e96906.
[15] Breslauer DN, Maamari RN, Switz NA, Lam WA, Fletcher DA. Mobile phone based clinical microscopy for global health applications. PloS one. 2009;4(7):e6320.
[16] Lee SA, Leitao R, Zheng G, Yang S, Rodriguez A, Yang C. Color capable sub-pixel resolving optofluidic microscope and its application to blood cell imaging for malaria diagnosis. PloS one. 2011;6(10):e26127.
[17] Mavandadi S, Dimitrov S, Feng S, et al. Distributed medical image analysis and diagnosis through crowd-sourced games: a malaria case study. PloS one. 2012;7(5):e37245.
[18] Moon S, Lee S, Kim H, et al. An image analysis algorithm for malaria parasite stage classification and viability quantification. PloS one. 2013;8(4):e61812.
[19] Linder N, Turkki R, Walliander M, et al. A malaria diagnostic tool based on computer vision screening and visualization of Plasmodium falciparum candidate areas in digitized blood smears. PLoS One. 2014;9(8):e104855.
[20] Mohammed HA, Abdelrahman IAM. Detection and classification of Malaria in thin blood slide images. In: International Conference on Communication, Control, Computing and Electronics Engineering (ICCCCEE). IEEE; 2017. p. 1–5.
[21] Zhang Z, Ong LS, Fang K, et al. Image classification of unlabeled malaria parasites in red blood cells. In: 38th Annual International Conference of the Engineering in Medicine and Biology Society (EMBC). IEEE; 2016. p. 3981–3984.
[22] Tek FB, Dempster AG, Kale I. A colour normalization method for giemsa-stained blood cell images. In: 14th Signal Processing and Communications Applications. IEEE; 2006. p. 1–4.

[23] Makkapati VV, Rao RM. Segmentation of malaria parasites in peripheral blood smear images. In: International Conference on Acoustics, Speech and Signal Processing. IEEE; 2009. p. 1361–1364.

[24] Di Rubeto C, Dempster A, Khan S, Jarra B. Segmentation of blood images using morphological operators. In: 15th International Conference on Pattern Recognition. vol. 3. IEEE; 2000. p. 397–400.

[25] Halim S, Bretschneider TR, Li Y, Preiser PR, Kuss C. Estimating malaria parasitaemia from blood smear images. In: 9th International Conference on Control, Automation, Robotics and Vision. IEEE; 2006. p. 1–6.

[26] Anggraini D, Nugroho AS, Pratama C, Rozi IE, Pragesjvara V, Gunawan M. Automated status identification of microscopic images obtained from malaria thin blood smears using Bayes decision: a study case in Plasmodium falciparum. In: International Conference on Advanced Computer Science and Information System. IEEE; 2011. p. 347–352.

[27] Mandal S, Kumar A, Chatterjee J, Manjunatha M, Ray AK. Segmentation of blood smear images using normalized cuts for detection of malarial parasites. In: Annual India Conference. IEEE; 2010. p. 1–4.

[28] Kareem S, Morling RC, Kale I. A novel method to count the red blood cells in thin blood films. In: International Symposium on Circuits and Systems (ISCAS). IEEE; 2011. p. 1021–1024.

[29] Toha S, Ngah U. Computer aided medical diagnosis for the identification of malaria parasites. In: International Conference on Signal Processing, Communications and Networking. IEEE; 2007. p. 521–522.

[30] Kareem S, Kale I, Morling RC. Automated malaria parasite detection in thin blood films:-A hybrid illumination and color constancy insensitive, morphological approach. In: Asia Pacific Conference on Circuits and Systems (APCCAS). IEEE; 2012. p. 240–243.

[31] Nasir AA, Mashor M, Mohamed Z. Segmentation based approach for detection of malaria parasites using moving k-means clustering. In: EMBS Conference on Biomedical Engineering and Sciences (IECBES). IEEE; 2012. p. 653–658.

[32] Malihi L, Ansari-Asl K, Behbahani A. Malaria parasite detection in giemsa-stained blood cell images. In: 8th Iranian Conference on Machine Vision and Image Processing (MVIP). IEEE; 2013. p. 360–365.

[33] Das D, Ghosh M, Chakraborty C, Maiti AK, Pal M. Probabilistic prediction of malaria using morphological and textural information. In: International Conference on Image Information Processing (ICIIP). IEEE; 2011. p. 1–6.
[34] Berge H, Taylor D, Krishnan S, Douglas TS. Improved red blood cell counting in thin blood smears. In: International Symposium on Biomedical Imaging: From Nano to Macro. IEEE; 2011. p. 204–207.
[35] Di Ruberto C, Dempster A, Khan S, Jarra B. Automatic thresholding of infected blood images using granulometry and regional extrema. In: International Conference on Pattern Recognition. vol. 3. IEEE; 2000. p. 441–444.
[36] Kareem S, Kale I, Morling RC. Automated P. falciparum detection system for post-treatment malaria diagnosis using modified annular ring ratio method. In: International Conference on Computer Modeling and Simulation. IEEE; 2012. p. 432–436.
[37] Zou Lh, Chen J, Zhang J, Garcia N. Malaria cell counting diagnosis within large field of view. In: International Conference on Digital Image Computing: Techniques and Applications. IEEE; 2010. p. 172–177.
[38] Mushabe MC, Dendere R, Douglas TS. Automated detection of malaria in Giemsa-stained thin blood smears. In: International Conference Engineering in Medicine and Biology Society (EMBC). IEEE; 2013. p. 3698–3701.
[39] Savkare S, Narote S. Automated system for malaria parasite identification. In: International Conference on Communication, Information & Computing Technology. IEEE; 2015. p. 1–4.
[40] Mehrjou A, Abbasian T, Izadi M. Automatic malaria diagnosis system. In: International Conference on Robotics and Mechatronics (ICRoM). IEEE; 2013. p. 205–211.
[41] Gatc J, Maspiyanti F, Sarwinda D, Arymurthy AM. Plasmodium parasite detection on red blood cell image for the diagnosis of malaria using double thresholding. In: International Conference on Advanced Computer Science and Information Systems. IEEE; 2013. p. 381–385.
[42] Nanoti A, Jain S, Gupta C, Vyas G. Detection of malaria parasite species and life cycle stages using microscopic images of thin blood smear. In: International Conference on Inventive Computation Technologies. vol. 1. IEEE; 2016. p. 1–6.
[43] Dong Y, Jiang Z, Shen H, et al. Evaluations of deep convolutional neural networks for automatic identification of malaria infected cells. In: EMBS International Conference on Biomedical & Health Informatics (BHI). IEEE; 2017. p. 101–104.

[44] Maiseli B, Mei J, Gao H, Yin S. An automatic and cost-effective parasitemia identification framework for low-end microscopy imaging devices. In: International Conference on Mechatronics and Control (ICMC). IEEE; 2014. p. 2048–2053.
[45] Salamah U, Sarno R, Arifin A, et al. Enhancement of low quality thick blood smear microscopic images of malaria patients using contrast and edge corrections. In: International Conference on Knowledge Creation and Intelligent Computing. IEEE; 2016. p. 219–225.
[46] Dong Y, Jiang Z, Shen H, Pan WD. Classification accuracies of malaria infected cells using deep convolutional neural networks based on decompressed images. In: SoutheastCon, 2017. IEEE; 2017. p. 1–6.
[47] Liang Z, Powell A, Ersoy I, et al. CNN-based image analysis for malaria diagnosis. In: International Conference on Bioinformatics and Biomedicine (BIBM). IEEE; 2016. p. 493–496.
[48] Bibin D, Nair MS, Punitha P. Malaria Parasite Detection from Peripheral Blood Smear Images using Deep Belief Networks. International Journal of Applied Engineering Research. 2017;5:9099–9108.
[49] Adi K, Pujiyanto S, Gernowo R, Pamungkas A, Putranto AB. Identifying the Developmental Phase of Plasmodium Falciparum in Malaria-Infected Red Blood Cells Using Adaptive Color Segmentation And Back Propagation Neural Network. International Journal of Applied Engineering Research. 2016;11(15):8754–8759.
[50] Devi SS, Roy A, Sharma M, Laskar R. kNN classification based erythrocyte separation in microscopic images of thin blood smear. In: 2nd International Conference on Computational Intelligence and Networks (CINE). IEEE; 2016. p. 69–72.
[51] Muralidharan V, Dong Y, Pan WD. A comparison of feature selection methods for machine learning based automatic malarial cell recognition in wholeslide images. In: International Conference on Biomedical and Health Informatics (BHI). IEEE; 2016. p. 216–219.
[52] Dallet C, Kareem S, Kale I. Real time blood image processing application for malaria diagnosis using mobile phones. In: International Conference on Circuits and Systems. IEEE; 2014. p. 2405–2408.
[53] Elter M, Haßlmeyer E, Zerfaß T. Detection of malaria parasites in thick blood films. In: International Conference on Engineering in Medicine and Biology Society. IEEE; 2011. p. 5140–5144.

[54] Fang Y, Xiong W, Lin W, Chen Z. Unsupervised malaria parasite detection based on phase spectrum. In: International Conference on Engineering in Medicine and Biology Society. IEEE; 2011. p. 7997–8000.

[55] Ghosh M, Das D, Chakraborty C, Ray AK. Plasmodium vivax segmentation using modified fuzzy divergence. In: International Conference on Image Information Processing (ICIIP). IEEE; 2011. p. 1–5.

[56] Hanif N, Mashor M, Mohamed Z. Image enhancement and segmentation using dark stretching technique for Plasmodium Falciparum for thick blood smearImage enhancement and segmentation using dark stretching technique for Plasmodium Falciparum for thick blood smear. In: International Colloquium on Signal Processing and its Applications (CSPA). IEEE; 2011. p. 257–260.

[57] Makkapati VV, Rao RM. Ontology-based malaria parasite stage and species identification from peripheral blood smear images. In: International Conference on Engineering in Medicine and Biology Society. IEEE; 2011. p. 6138–6141.

[58] Nguyen NT, Duong AD, Vu HQ. A new method for splitting clumped cells in red blood images. In: International Conference on Knowledge and Systems Engineering. IEEE; 2010. p. 3–8.

[59] Sharif JM, Miswan M, Ngadi M, Salam MSH, bin Abdul Jamil MM. Red blood cell segmentation using masking and watershed algorithm: A preliminary study. In: International Conference on Biomedical Engineering. IEEE; 2012. p. 258–262.

[60] Suwalka I, Sanadhya A, Mathur A, Chouhan MS. Identify malaria parasite using pattern recognition technique. In: International Conference on Computing, Communication and Applications. IEEE; 2012. p. 1–4.

[61] Thung F, Suwardi IS. Blood parasite identification using feature based recognition. In: International Conference on Electrical Engineering and Informatics. IEEE; 2011. p. 1–4.

[62] May Z, Aziz SSAM, et al. Automated quantification and classification of malaria parasites in thin blood smears. In: International Conference on Signal and Image Processing Applications. IEEE; 2013. p. 369–373.

[63] Mulay HD, Murthy TD, Nerune SM, Amrutha M. New Methylene Blue Stain for Malaria Detection on Thin Smears. Journal of Krishna Institute of Medical Sciences (JKIMSU). 2017;6(1):76–81.

[64] Vink J, Laubscher M, Vlutters R, et al. An automatic vision-based malaria diagnosis system. Journal of microscopy. 2013;250(3):166–178.

[65] Das D, Maiti A, Chakraborty C. Automated system for characterization and classification of malaria-infected stages using light microscopic images of thin blood smears. Journal of microscopy. 2015;257(3):238–252.

[66] Das D, Chakraborty C, Mitra B, Maiti A, Ray A. Quantitative microscopy approach for shape-based erythrocytes characterization in anaemia. Journal of microscopy. 2013;249(2):136–149.

[67] Sheikhhosseini M, Rabbani H, Zekri M, Talebi A. Automatic diagnosis of malaria based on complete circle–ellipse fitting search algorithm. Journal of microscopy. 2013;252(3):189–203.

[68] DABO-NIANG S, Zoueu J. Combining kriging, multispectral and multimodal microscopy to resolve malaria-infected erythrocyte contents. Journal of microscopy. 2012;247(3):240–251.

[69] Janse CJ, Van Vianen PH. Flow cytometry in malaria detection. Methods in cell biology. 1994;42:295–318.

[70] Díaz G, González FA, Romero E. A semi-automatic method for quantification and classification of erythrocytes infected with malaria parasites in microscopic images. Journal of Biomedical Informatics. 2009;42(2):296–307.

[71] Tek FB, Dempster AG, Kale I. Parasite detection and identification for automated thin blood film malaria diagnosis. Computer vision and image understanding. 2010;114(1):21–32.

[72] Das DK, Ghosh M, Pal M, Maiti AK, Chakraborty C. Machine learning approach for automated screening of malaria parasite using light microscopic images. Micron. 2013;45:97–106.

[73] Bhowmick S, Das DK, Maiti AK, Chakraborty C. Structural and textural classification of erythrocytes in anaemic cases: a scanning electron microscopic study. Micron. 2013;44:384–394.

[74] Arco JE, Górriz JM, Ramírez J, Álvarez I, Puntonet CG. Digital image analysis for automatic enumeration of malaria parasites using morphological operations. Expert Systems with Applications. 2015;42(6):3041–3047.

[75] Brückner M, Becker K, Popp J, Frosch T. Fiber array based hyperspectral Raman imaging for chemical selective analysis of malaria-infected red blood cells. Analytica chimica acta. 2015;894:76–84.

[76] Somasekar J, Reddy BE. Segmentation of erythrocytes infected with malaria parasites for the diagnosis using microscopy imaging. Computers & Electrical Engineering. 2015;45:336–351.
[77] Rosado L, da Costa JMC, Elias D, Cardoso JS. Automated detection of malaria parasites on thick blood smears via mobile devices. Procedia Computer Science. 2016;90:138–144.
[78] Chen F, Flaherty BR, Cohen CE, Peterson DS, Zhao Y. Direct detection of malaria infected red blood cells by surface enhanced Raman spectroscopy. Nanomedicine: Nanotechnology, Biology and Medicine. 2016;12(6):1445–1451.
[79] Sakaguchi M, Miyazaki N, Fujioka H, Kaneko O, Murata K. Three-dimensional analysis of morphological changes in the malaria parasite infected red blood cell by serial block-face scanning electron microscopy. Journal of structural biology. 2016;193(3):162–171.
[80] Di Ruberto C, Dempster A, Khan S, Jarra B. Analysis of infected blood cell images using morphological operators. Image and vision computing. 2002;20(2):133–146.
[81] Sio SW, Sun W, Kumar S, et al. MalariaCount: an image analysis-based program for the accurate determination of parasitemia. Journal of microbiological methods. 2007;68(1):11–18.
[82] Savkare S, Narote S. Automatic system for classification of erythrocytes infected with malaria and identification of parasite's life stage. Procedia Technology. 2012;6:405–410.
[83] Snounou G, Pinheiro L, Gonçalves A, et al. The importance of sensitive detection of malaria parasites in the human and insect hosts in epidemiological studies, as shown by the analysis of field samples from Guinea Bissau. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1993;87(6):649–653.
[84] Frean J. Improving quantification of malaria parasite burden with digital image analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;102(11):1062–1063.
[85] Quinn JA, Andama A, Munabi I, Kiwanuka FN. Automated blood smear analysis for mobile malaria diagnosis. Mobile Point-of-Care Monitors and Diagnostic Device Design. 2014;31:115.
[86] Srivastava B, Anvikar AR, Ghosh SK, et al. Computer-vision-based technology for fast, accurate and cost effective diagnosis of malaria. Malaria Journal. 2015;14(1):526.
[87] Herrera S, Vallejo AF, Quintero JP, Arévalo-Herrera M, Cancino M, Ferro S. Field evaluation of an automated RDT reader and data management device for Plasmodium falciparum/Plasmodium vivax malaria in endemic areas of Colombia. Malaria Journal. 2014;13(1):87.

[88] Omucheni DL, Kaduki KA, Bulimo WD, Angeyo HK. Application of principal component analysis to multispectral-multimodal optical image analysis for malaria diagnostics. Malaria Journal. 2014;13(1):485.

[89] Prescott WR, Jordan RG, Grobusch MP, et al. Performance of a malaria microscopy image analysis slide reading device. Malaria Journal. 2012;11(1):155.

[90] Purwar Y, Shah SL, Clarke G, Almugairi A, Muehlenbachs A. Automated and unsupervised detection of malarial parasites in microscopic images. Malaria Journal. 2011;10(1):364.

[91] Frean JA. Reliable enumeration of malaria parasites in thick blood films using digital image analysis. Malaria Journal. 2009;8(1):218.

[92] Ma C, Harrison P, Wang L, Coppel RL. Automated estimation of parasitaemia of Plasmodium yoelii-infected mice by digital image analysis of Giemsa-stained thin blood smears. Malaria Journal. 2010;9(1):348.

[93] Proudfoot O, Drew N, Scholzen A, Xiang S, Plebanski M. Investigation of a novel approach to scoring Giemsa-stained malaria-infected thin blood films. Malaria Journal. 2008;7(1):62.

[94] Pirnstill CW, Coté GL. Malaria diagnosis using a mobile phone polarized microscope. Scientific reports. 2015;5:13368.

[95] World Health Organization. Microscopy for the detection, identification and quantification of malaria parasites on stained thick and thin blood films in research settings. WHO. 2015.

[96] Daniel T, Pierre E, Emmanuel T, Philippe B. Automated Diagnosis of Malaria in Tropical Areas Using 40X Microscopic Images of Blood Smears. International Journal of Biometrics and Bioinformatics (IJBB). 2016;10(2):12.

[97] Kim JD, Nam KM, Park CY, Kim YS, Song HJ. Automatic detection of malaria parasite in blood images using two parameters. Technology and Health Care. 2016;24(s1):S33–S39.

[98] Abbas N, Saba T, Mohamad D, Rehman A, Almazyad AS, Al-Ghamdi JS. Machine aided malaria parasitemia detection in Giemsa-stained thin blood smears. Neural Computing and Applications. 2016;p. 1–16.

[99] Di Ruberto C, Dempster A, Khan S, Jarra B. Morphological image processing for evaluating malaria disease. In: Visual Form 2001. Springer; 2001. p. 739–748.

[100] Prasad K, Winter J, Bhat UM, Acharya RV, Prabhu GK. Image analysis approach for development of a decision support system for detection of malaria parasites in thin blood smear images. Journal of digital imaging. 2012;25(4):542–549.

[101] Bibin D, Punitha P. Stained blood cell detection and clumped cell segmentation useful for malaria parasite diagnosis. In: Multimedia Processing, Communication and Computing Applications. Springer; 2013. p. 195–207.
[102] Ghosh P, Bhattacharjee D, Nasipuri M, Basu DK. Medical aid for automatic detection of malaria. In: Computer Information Systems–Analysis and Technologies. Springer; 2011. p. 170–178.
[103] Daz G, Gonzalez F, Romero E. Infected cell identification in thin blood images based on color pixel classification: comparison and analysis. Progress in Pattern Recognition, Image Analysis and Applications. 2007;p. 812–821.
[104] Khot S, Prasad R. Optimal computer based analysis for detecting malarial parasites. In: Proceedings of the 3rd International Conference on Frontiers of Intelligent Computing: Theory and Applications. Springer; 2015. p. 69–80.
[105] Kumarasamy SK, Ong S, Tan KS. Robust contour reconstruction of red blood cells and parasites in the automated identification of the stages of malarial infection. Machine Vision and Applications. 2011;22(3):461–469.
[106] Raviraja S, Bajpai G, Sharma SK. Analysis of detecting the malarial parasite infected blood images using statistical based approach. In: 3rd Kuala Lumpur International Conference on Biomedical Engineering. Springer; 2007. p. 502–505.
[107] Ross NE, Pritchard CJ, Rubin DM, Duse AG. Automated image processing method for the diagnosis and classification of malaria on thin blood smears. Medical and Biological Engineering and Computing. 2006;44(5):427–436.
[108] Somasekar J, Reddy ARM, Reddy LS. An efficient algorithm for automatic malaria detection in microscopic blood images. In: Global Trends in Information Systems and Software Applications. Springer; 2012. p. 431–440.
[109] Devi SS, Sheikh SA, Talukdar A, Laskar RH. Malaria Infected Erythrocyte Classification Based on the Histogram Features using Microscopic Images of Thin Blood Smear. Indian Journal of Science and Technology. 2016;9(45).
[110] Tek FB, Dempster AG, Kale I. Malaria Parasite Detection in Peripheral Blood Images. In: BMVC; 2006. p. 347–356.
[111] Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bulletin of the World Health Organization. 1988;66(5):621.

[112] Le MT, Bretschneider TR, Kuss C, Preiser PR. A novel semi-automatic image processing approach to determine Plasmodium falciparum parasitemia in Giemsa-stained thin blood smears. BMC Cell Biology. 2008;9(1):15–27.

[113] Mudanyali O, Dimitrov S, Sikora U, Padmanabhan S, Navruz I, Ozcan A. Integrated rapid-diagnostic-test reader platform on a cellphone. Lab on a Chip. 2012;12(15):2678–2686.

[114] Savkare S, Narote S. Automatic detection of malaria parasites for estimating parasitemia. International Journal of Computer Science and Security (IJCSS). 2011;5(3):310–315.

[115] Suradkar PT. Detection of malarial parasite in blood using image processing. International Journal of Engineering and Innovative Technology (IJEIT). 2013;2(10):124–126.

[116] Aimi Salihah AN, Yusoff M, Zeehaida M. Colour image segmentation approach for detection of malaria parasites using various colour models and k-means clustering. WSEAS Transaction on Biology and Biomedicine. 2013;10(1):41–55.

[117] Luengo-Oroz MA, Arranz A, Frean J. Crowd sourcing malaria parasite quantification: an online game for analyzing images of infected thick blood smears. Journal of medical Internet research. 2012;14(6):1–19.

[118] Khan MI, Acharya B, Singh BK, Soni J. Content based image retrieval approaches for detection of malarial parasite in blood images. International Journal of Biometrics and Bioinformatics (IJBB). 2011;5(2):97.

[119] Khatri K, Ratnaparkhe V, Agrawal S, Bhalchandra A. Image processing approach for malaria parasite identification. In: National Conference on Growth of Technologies in Electronics, Telecom and Computers Indias Perception; 2013. p. 5–7.

[120] Yunda L, Ramirez AA, Millán J. Automated image analysis method for p-vivax malaria parasite detection in thick film blood images. Sistemas & Telemática. 2012;10(20):9–25.

[121] Hirimutugoda Y, Wijayarathna G. Image analysis system for detection of red cell disorders using artificial neural networks. Sri Lanka Journal of Bio-Medical Informatics. 2010;1(1):35–42.

[122] Kaewkamnerd S, Uthaipibull C, Intarapanich A, Pannarut M, Chaotheing S, Tongsima S. An automatic device for detection and classification of malaria parasite species in thick blood film. Bmc Bioinformatics. 2012;13(17):S18.

[123] Frean J. Microscopic determination of malaria parasite load: role of image analysis. Micrsocopy: Science, technology, Applications, and Education. 2010;p. 862–866.

[124] Somasekar J, Reddy BE, Reddy EK, Lai CH. An image processing approach for accurate determination of parasitemia in peripheral blood smear images. International Journal of Computer Applications. 2011;p. 23–28.

[125] Razzak MI. Malarial parasite classification using recurrent neural network. International Journal of Image Processing (IJIP). 2015;9(2):69.

[126] Ajala F, Fenwa O, Aku M. Comparative Analysis of Different Types of Malaria Diseases using First Order Features. International Journal of Applied Information Systems (IJAIS). 2015;8(3):20–26.

[127] Pamungkas A, Adi K, Gernowo R. Identification of plasmodium falciparum development phase in malaria infected red blood cells using adaptive color segmentation and decision tree based classification. International Journal of Applied Engineering Research. 2015;10(2):4043–4055.

[128] Razzak MI. Automatic detection and classification of malarial parasite. International Journal of Biometrics and Bioinformatics (IJBB). 2015;9(1):1–12.

[129] Hiremath P, Bannigidad P, Geeta S. Automated identification and classification of white blood cells (leukocytes) in digital microscopic images. IJCA special issue on recent trends in image processing and pattern recognition. 2010;p. 59–63.

[130] Abbas N, Mohamad D. Microscopic RGB color images enhancement for blood cells segmentation in YCbCr color space for k-means clustering. Journal of Theoretical and Applied Information Technology. 2013;55(1):117–125.

[131] Ahirwar N, Pattnaik S, Acharya B. Advanced image analysis based system for automatic detection and classification of malarial parasite in blood images. International Journal of Information Technology and Knowledge Management. 2012;5(1):59–64.

[132] Anand PR, Bajpai G, Bhaskar V, Job SM. Detection of the malarial parasite infected blood images by 3D-Analysis of the cell curved surface. In: 4th Kuala Lumpur International Conference on Biomedical Engineering. Springer; 2008. p. 166–169.

[133] Annaldas S, Shirgan S, Marathe V. Automatic identification of malaria parasites using image processing. International Journal of Emerging Engineering Research and Technology. 2014;2(4):107–112.

[134] Cesario M, Lundon M, Luz S, Masoodian M, Rogers B. Mobile support for diagnosis of communicable diseases in remote locations. In: Proceedings of the 13th International Conference

of the NZ Chapter of the ACM's Special Interest Group on Human-Computer Interaction. ACM; 2012. p. 25–28.

[135] Chavan SN, Sutkar AM. Malaria disease identification and analysis using image processing. International Journal Latest Trends Engineering Technology. 2014;3:218–223.

[136] Chayadevi M, Raju G. Usage of art for automatic malaria parasite identification based on fractal features. International Journal of Video and Image Processing and Network Security. 2014;4(4):7–15.

[137] Damahe LB, Krishna R, Janwe N. Segmentation based approach to detect parasites and RBCs in blood cell images. International Journal Computing Science and Applications. 2011;4:71–81.

[138] Das DK, Maiti AK, Chakraborty C. Textural pattern classification of microscopic images for malaria screening. Advances in Therapeutic Engineering. 2012;p. 419–446.

[139] Dowling M, Shute G. A comparative study of thick and thin blood films in the diagnosis of scanty malaria parasitaemia. Bulletin of the World health Organization. 1966;34(2):249.

[140] Ghate DA, Jadhav C. Automatic detection of malaria parasite from blood images. International Journal of Computer Science & Applications (TIJCSA). 2012;1(3).

[141] Ghosh M, Das D, Chakraborty C, Ray AK. Quantitative characterisation of Plasmodium vivax in infected erythrocytes: a textural approach. International Journal of Artificial Intelligence and Soft Computing. 2013;3(3):203–221.

[142] Gitonga L, Memeu DM, Kaduki KA, Kale MAC, Muriuki NS. Determination of plasmodium parasite life stages and species in images of thin blood smears using artificial neural network. Open Journal of Clinical Diagnostics. 2014;4(02):78.

[143] Khan NA, Pervaz H, Latif AK, et al. Unsupervised identification of malaria parasites using computer vision. In: International Joint Conference on Computer Science and Software Engineering. IEEE; 2014. p. 263–267.

[144] Komagal E, Kumar K, Vigneswaran A. Recognition and classification of malaria plasmodium diagnosis. International Journal of Engineering Research & Technology (IJERT). 2013;2(1):1–4.

[145] Kumar A, Choudhary A, Tembhare P, Pote C. Enhanced identification of malarial infected objects using otsu algorithm from thin smear digital images. International Journal of Latest Research in Science and Technology ISSN. 2012;p. 2278–5299.

[146] Maitra M, Gupta RK, Mukherjee M. Detection and counting of red blood cells in blood cell images using hough transform. International journal of computer applications. 2012;53(16):18–22.

[147] Maity M, Maity AK, Dutta PK, Chakraborty C. A web-accessible framework for automated storage with compression and textural classification of malaria parasite images. International Journal of Computer Applications. 2012;52(15):31–39.

[148] Memeu DM, Kaduki KA, Mjomba A, Muriuki NS, Gitonga L. Detection of plasmodium parasites from images of thin blood smears. Open Journal of Clinical Diagnostics. 2013;3(04):183.

[149] Panchbhai VV, Damahe LB, Nagpure AV, Chopkar PN. RBCs and parasites segmentation from thin smear blood cell images. International Journal of Image, Graphics and Signal Processing. 2012;4(10):54.

[150] Parkhi V, Pawar P, Surve A. Computer automation for malaria parasite detection using linear programming. International Journal of Advanced Research in Electrical, Electronics and Instrumentation Engineering. 2013;2(5):1984–1988.

[151] Premaratne SP, Karunaweera ND, Fernando S, Perera WSR, Rajapaksha R. A neural network architecture for automated recognition of intracellular malaria parasites in stained blood films. In: Proceedings of APAMI & CJKMI-KOSMI Conference; 2003. p. 1–4.

[152] Soni J. Advanced image analysis based system for automatic detection of malarial parasite in blood images using SUSAN approach. International Journal of Engineering Science and Technology. 2011;3(6):5260–5274.

[153] Soni J, Mishra N, Kamargaonkar N. Automatic difference between RBC and malaria parasites based on morphology with first order features using image processing. International Journal of Advanced Engineering Technology. 2011;1(5):290–297.

[154] Suryawanshi MS, Dixit V. Improved technique for detection of malaria parasites within the blood cell images. International Journal of Scientific Engineering Research. 2013;4(7):373–376.

[155] Tulsani H, Saxena S, Yadav N. Segmentation using morphological watershed transformation for counting blood cells. IJCAIT. 2013;2(3):28–36.

[156] Wahyuningrum RT, Indrawan AK. A hybrid automatic method for parasite detection and identification of plasmodium falciparum in thin blood images. International Journal of Academic Research. 2012;4(6):44–50.

[157] Walliander M, Turkki R, Linder N, et al. Automated segmentation of blood cells in Giemsa stained digitized thin blood films. Diagnostic Pathology. 2013;8(1):S37.

[158] vonMühlen A. Computer image analysis of malarial Plasmodium vivax in human red blood cells. California Polytechnic State University, San Luis Obispo; 2004.

[159] Špringl V. Automatic malaria diagnosis through microscopy imaging; 2009.

[160] Memeu DM. A rapid malaria diagnostic method based on automatic detection and classification of plasmodium parasites in stained thin blood smear images; 2014.

[161] Ahmed LJ. Virtual microscopy for the assessment of competency and training for malaria diagnosis. Manchester Metropolitan University; 2012.

[162] WHO. Malaria microscopy quality assurance manual-version 2. World Health Organization; 2017.

[163] Silamut K, White N. Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1993;87(4):436–443.

[164] Silamut K, Phu NH, Whitty C, et al. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. The American journal of pathology. 1999;155(2):395–410.

[165] WHO. Determining Cost Effectiveness of Malaria Rapid Diagnostic Tests in Rural Areas with High Prevalence. Available from: http://www2.wpro.who.int/sites/rdt.

[166] Adeoye G, Nga I. Comparison of Quantitative Buffy Coat technique (QBC) with Giemsa-stained Thick Film (GTF) for diagnosis of malaria. Parasitology international. 2007;56(4):308–312.

[167] Shute G, Sodeman T. Identification of malaria parasites by fluorescence microscopy and acridine orange staining. Bulletin of the World Health Organization. 1973;48–50(5):591–596.

[168] Kawamoto F. Rapid diagnosis of malaria by fluorescence microscopy with light microscope and interference filter. The Lancet. 1991;337(8735):200–202.

[169] Wongsrichanalaia C, Kawamotob F. Fluorescent Microscopy and Fluorescent Labelling for Malaria Diagnosis. 2014.

[170] Diaspro A, Chirico G, Usai C, Ramoino P, Dobrucki J. Photobleaching. In: Handbook of biological confocal microscopy. Springer; 2006. p. 690–702.

[171] Waters JC. Accuracy and precision in quantitative fluorescence microscopy. Rockefeller University Press; 2009.

[172] Guy R, Liu P, Pennefather P, Crandall I. The use of fluorescence enhancement to improve the microscopic diagnosis of falciparum malaria. Malaria journal. 2007;6(1):89–96.

[173] Gopakumar GP, Swetha M, Sai Siva G, Subrahmanyam S. Convolutional neural network-based malaria diagnosis from focus stack of blood smear images acquired using custom-built slide scanner. Journal of Biophotonics. 2017.

[174] Poostchi M, Ersoy I, Bansal A, et al. Image analysis of blood slides for automatic malaria diagnosis; 2015. p. MoPoster04.22.

[175] Sivaramakrishnan R, Antani S, Jaeger S. Visualizing Deep Learning Activations for Improved Malaria Cell Classification. In: Medical Informatics and Healthcare; 2017. p. 40–47.

Figure 1: World-wide malaria death rates (Source: World Malaria Report - WHO).

Figure 2: Five different human malaria Plasmodium species and their life-stages in thin blood film (Source: K. Silamut and CDC).

Figure 3: Parasite stages in a single thin blood smear.

Table 1: Blood Smear types and staining methods for malaria diagnosis

Blood Smear	Staining
Thin	Giemsa [11, 17, 19, 20, 22, 24, 25, 26, 28, 31, 30, 32, 34, 38, 35, 39, 41, 47, 48, 53, 67, 69, 71, 75, 79, 81, 8, 86, 90, 93, 97, 99, 102, 106, 110, 112, 114, 116, 118, 128, 135, 140, 142, 152, 154, 158, 160, 36, 37, 40, 42, 44, 49, 52, 54, 62, 70, 80, 82, 89, 92, 96, 98, 100, 103, 106, 115, 119, 121, 125, 126, 127, 132, 134, 136, 143, 151, 131, 153, 157, 159]
	Leishman [23, 33, 55, 57, 65, 137, 72, 101, 109, 138, 141, 145, 147, 149]
	Leishman-Methylene Blue [63]
	Combination of DNA and RNA fluorescent [12]
	Wright [14, 43, 51]
	Fluorochrome [169, 167, 168, 172, 13, 15, 18, 86]
	Romanowsky [60]
	Acridine Orange (AO) [64]
	DAPI/Mitotracker [18]
	Toluidine blue [16]
	Unstained [10, 21, 73, 88]
Thick	Giemsa [29, 77, 8, 91, 120, 122, 45, 53, 56, 74, 85, 87, 117, 123]
	Leishman [143]

Table 2: Malaria Image Acquisition

Imaging Techniques
Light Microscopy [17, 19, 21, 26, 33, 41, 61, 65, 72, 81, 110, 111, 112, 118, 20, 25, 28, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 57, 59, 62, 63, 67, 70, 71, 74, 76, 77, 80, 82, 85, 88, 90, 91, 92, 93, 97, 98, 99, 100, 102, 103, 104, 105, 107, 108, 109, 116, 114, 115, 117, 118, 119, 120, 121, 122, 123, 124, 125, 127, 128, 132, 135, 136, 138, 141, 142, 143, 145, 147, 148, 149, 150, 151, 157, 158, 159, 160]
Binocolor Microscopy [96, 153, 131, 152]
Fluorescent Microscopy [169, 167, 168, 172, 13, 15, 18]
Polarized Microscopy [94]
Image-based Cytometer [11]
Sub-pixel Resolving Optofluidic Microscopy (SROFM) [16]
Quantitative phase imaging (QPI) [10]
Quantitative Cartridge-scanner System [64]
Scanning electron microscopy (SEM) [73]
Fiber array-based Raman imaging [75, 78]
Serial block-face scanning electron microscopy (SBFSEM) [79]
SightDx Digital Imaging Scanning [86]

Table 3: Image pre-processing techniques applied to enhance malaria blood smear images

Blood Smear	Challenges	Pre-Processing methods	Remarks
Thin	Noise reduction	Mean Filtering [126, 155]	
		Median Filtering [11, 19, 24, 26, 31, 33, 35, 39, 41, 32, 34, 38, 40, 76, 80, 82, 96, 98, 109, 125, 138, 147, 157]	Remove impulse noise and preserve edges
		Geometric Mean Filtering [66, 72, 141]	
		Wiener Filtering [62]	
		Gamma Equalization [76]	
		SUSAN Non-linear Filtering [152, 152, 131]	
		Gaussian Low-pass Filtering [74, 92, 136]	
		Non-linear Diffusion Filtering [67]	
		Gamma	

		Transformation 14]	
		Orthogonal Wavelet-based Threshold [27]	
		Interscale Orthogonal Wavelet-based Thresholding [27]	
		Perona-Malik Denoising Model [44]	
		Morphological Operations [26, 31, 43, 71, 114, 118, 124, 128, 137, 158, 32, 34, 38, 52, 71, 147]	Remove unwanted small objects, hole filling, closing and opening
	Low Image Contrast	Laplacian Filtering [39, 82, 102]	Edge detection
		Adaptive/Local Histogram Equalization [45, 81, 90, 146, 39, 40, 44, 74, 90, 115, 125]	Enhance image resolution
		Forward Discrete Curvelet Transform [125]	
		Contrast	Contrast

		Stretching Techniques [31, 45, 42, 56, 147]	enhancement
	Uneven Illumination	Low-pass Filtering [70, 103]	Remove high frequency components
		Morphological Top-hat Operation [71, 128, 158, 158]	Remove nonuniform illumination effects
	Cell Staining Variation	Linear Model [25]	
		Color Normalization [119]	Illumination correction
		Gray World Color Normalization [22, 110, 66, 72, 109, 121, 134, 138]	Normalization of image color profile
		Histogram Matching	
Thick	Noise reduction	Median Filtering [77, 91]	
		Laplacian Spatial Filter [122]	

Table 4: Segmentation techniques for thin blood smears

Blood Smear	Segmentation Techniques	Remarks
Thin	Otsu Thresholding [18, 26, 33, 39, 41, 114, 145, 158, 18, 26, 33, 41, 114, 145, 158, 26, 32, 40, 50, 62, 82, 145, 147, 149, 157, 173]	Calculates optimum threshold assuming that image contains bi-modal histogram
	(Adaptive) Histogram Thresholding [11, 15, 23, 10, 21, 25, 35, 37, 43, 49, 51, 73, 91, 140, 11, 21, 25, 10, 44, 96, 100, 107, 127]	Difficult to determine the thresholding value
	Zack Thresholding [137]	Triangle-based method particularly effective with a weak peak in the image histogram
	Poisson Distribution Thresholding [154]	Finding a threshold that separates foreground and background using minimum error
	Morphological	Mathematical

	Operation [20, 24, 71, 74, 80, 99, 118, 120, 128, 28, 30, 34, 36, 38, 119, 125, 153, 155]	morphology operations including Granulometry, opening, closing, etc.
	Edge Detection Algorithm [81, 105, 115]	Works well for high contrast images with sharp edges, false edge detections should be filtered out
	Hough Transform [21, 37, 43, 51, 146, 92]	Requires red blood cells circular measures including radius, shape
	K-means Clustering [31, 90, 116, 130, 42]	Unsupervised learning technique that iteratively assigns pixels to K clusters using their feature descriptors
	Watershed Algorithm [33, 59, 97, 114, 159]	Extract continuous boundary regions but over-segmentation is the typical issue
	Marker Controlled	Mostly applied to

	Watershed [65, 73, 66, 72, 73, 109, 138, 173]	separate touching cells
	Active Contour Models [65, 90, 48, 101, 174]	Level-set based approaches that ensures topological flexibility, computationally expensive
	Rule-Based Segmentation [65, 81]	Requires knowledge about cells shape, size, color, etc.
	Fuzzy Rule-based Segmentation, Fuzzy Divergence [55, 136, 141]	Building rules is not easy when uncertainty is high
	Neural Network [160]	Requires discriminative and strong features to distinguish foreground and background pixels
	Template matching [25]	
	Adaptive Gaussian Mixture Model Distance transform [98]	
	Distance Transform	

	[58]	
	Ada-boost [64]	
	Look-Up Table [103]	

Table 5: Segmentation techniques for thick blood smears

Blood Smear	Segmentation Techniques	Remarks
Thick	Otsu Thresholding [158, 77]	Calculates optimum threshold assuming that image contains two classes following bi-modal histogram
	Histogram Theshold [29, 122, 74, 85]	Difficult to determine the thresholding value, Usually fused with other methods to improve performance
	Morphological Operations [158]	Mathematical morphology operations including Granulometry, opening, closing, etc. are useful to characterize and represent blood cells circular

		shape, size, boundaries, skeletons, texture, gradient, etc.
	Normalized-cut Algorithms [27]	Computationally expensive
	Fuzzy C-means [76]	

Table 6: Feature Computation for Malaria Parasite Classification in Thin Blood Smears [6]

Blood Smear	Features Type	Feature	Remarks
Thin	Color	RGB [11, 15, 17, 14, 16, 27, 37, 27, 71, 88, 97, 99, 106, 110, 114, 116, 118, 120, 142, 142, 160, 32, 38, 60, 80, 98, 100, 103, 107, 109, 115, 134, 149, 157, 174]	provide color information
		HSV [31, 35, 27, 137, 23, 30, 40, 48, 70, 136]	
		YCbCr [27, 130, 155]	
		LAB [42, 62, 136]	
		Intensity [39, 26, 41, 65, 88, 90, 124, 34, 92, 119, 151]	
		color correlogram, color co-occurrence matrix [25, 110, 134, 147]	

	Texture	Haralick [33, 51, 48]	characterize the overall shape and size of the erythrocyte without taking the density into account
		Gray-level run length matrices (GLRLM) [51, 72, 138]	
		GLCM [64, 72, 128, 138]	
		Local Binary Pattern (LBP) [19, 48, 72, 138, 147]	
		Fractal [136]	
		Wavelet transform [120]	
		Gradient Texture [102, 120, 17, 32, 159, 173]	
		gray level co-occurrence matrix [128, 48, 141, 153]	
		Entropy [43, 133, 135, 72, 126, 138]	
		HoG [19]	
		Multiscale Laplacian of Gaussian and Gabor [154]	

	Morphological	Shape (area, perimeter, compactness ratio, eccentricity, bending energy, etc.) [12, 18, 10, 39, 51, 67, 71, 73, 91, 97, 99, 106, 110, 110, 114, 124, 133, 140, 142, 30, 36, 50, 58, 64, 66, 72, 80, 96, 121, 123, 125, 128, 135, 145, 153]	encodes the spatial distribution of the intensity in a particular region
		Moments (zero, central, Hu) [43, 51, 110, 66, 72, 126, 132, 138]	
		Area granulometry [71, 152, 28, 32, 82, 107, 123]	
		Roundness ratio [49]	

Table 7: Feature Computation for Malaria Parasite Classification in Thick Blood Smears [6]

Blood Smear	Features Type	Feature	Remarks
Thick	Color	RGB [77]	provide color information
		HSV [122]	
		LAB [143]	
		Intensity [29, 74]	
	Texture	Haralick [53]	characterize the overall shape and size of the erythrocyte without taking the density into account
	Morphological	Shape (area, perimeter, compactness ratio, eccentricity, bending energy, etc.) [53, 77, 85]	encodes the spatial distribution of the intensity in a particular region
		Moment (zero, central, Hu) [53, 85]	
		Roundness ratio [49]	

Table 8: Classification Methods

Blood Smear	Classification methodology	
Thin	Unsupervised	K-mean Clustering [90]
		Quaternion Fourier Transform (QFT) [54]
	Supervised	Thresholding [25, 35, 40, 62, 92, 96, 100, 112, 115, 119, 140, 145, 159]
		Bayesian Classifier [73, 110, 26, 38, 72, 134, 141, 173]
		Annular Ring Ratio Method [36, 52]
		Naiive Bayes Tree [65, 147]
		Logistic regression Tree [10, 33, 66]
		Linear Programming [150]
		Euclidean Distance Classifier [154]
		K-nearest Neighbors classifier [10, 71, 110, 32, 42, 50, 103]
		Decision tree [18, 26, 67, 81, 91, 102, 122,

		127, 153]
		Template Matching [60, 99]
		Ada-boost [21, 64]
		Genetic Algorithm [53]
		Nearest Mean Classifier (NM) [10]
		Fuzzy Interface System [55]
		Normalized cross-correlation [20]
		Support Vector Machine (SVM) [12, 11, 19, 39, 51, 77, 114, 42, 70, 82, 105, 141, 174]
		Linear Discriminant (FLD) [10]
		Neural Network [43, 49, 65, 118, 120, 128, 133, 142, 152, 160, 107, 109, 121, 125, 136, 138, 151]
		Deep Learning [43, 47, 46, 48, 173]
Thick	Unsupervised	K-mean Clustering [143]
	Supervised	Naiive Bayes Tree [65]

		Crowd Source Games [17]
		Randomized Tree Classifier [85]
		Nearest Mean Classifier (NM) [143]
		Thresholding [123, 29, 91]
		Support Vector Machine (SVM) [53, 77]
		Neural Network [120]