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Blood Smear Counter for Malaria Parasite Diagnosis

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Abstract

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a certain type of mosquito which feeds on human blood. People who get malaria are typically very sick with high fevers, shaking chills, and flu-like illness. Although malaria can be a deadly disease, illness and death from malaria can usually be prevented through early diagnosis and treatment. Although manual counting is relatively inexpensive to implement, it is tedious and time-consuming. Its adequate sensitivity requires proper training and supervision of technicians. Hence, a blood smear parasite detection system using image processing is used to classify the presence of the malaria parasite in a micrograph of a blood smear image. In this study, we improved parasite mask processing and circle detection by using two different candidate masks, one more selective than the other, to detect the sharp points in an outline formed when two circles overlap. MATLAB has been used in every procedure. The three main steps – pre-processing, individual candidate parasite detection, and cell detection - were taken using image processing techniques such as edge and corner detection, thresholding and segmentation. We aim to obtain more accurate and robust results by implementing this project in the screening of malaria infections in microscope images of thin blood film smears.

Keywords: Segmentation; Thresholding; Blood smear; Malaria parasite

Introduction

The gold standard test for malaria is the hundred-year-old method of preparing a blood smear on a glass slide, staining it, and examining it under a microscope to look for the parasite genus *plasmodium*. While several rapid diagnostic tests are also currently available, they still have shortcomings compared to microscopic analysis [1]. In the regions worst affected by malaria, reliable diagnoses are often difficult to obtain, and treatment is routinely prescribed based only on symptoms. Accurate diagnosis is clearly important, since false negatives can be fatal, and false positives lead to increased drug resistance, unnecessary economic burden, and possibly the failure to treat diseases with similar early symptoms such as meningitis or typhoid [2]. The scale of the problem is huge: annually there are 300-500 million cases of acute malaria illness of which 1.1-2.7 million are fatal, most fatalities being among children under the age of five [3-5]. The lack of access to diagnosis in developing countries is largely due to a shortage of expertise, with a shortage of equipment being a secondary factor. For example, a recent survey carried out in Uganda [6] found 50% of rural health centres to have microscopes, but only 17% had laboratory technicians with the training necessary to use them for malaria diagnosis. Even where a microscopist is available, they are often oversubscribed and cannot spend long enough examining each sample to give a confident diagnosis. This situation has prompted an increasing interest in their research findings.

State of the art

Conventional practices involves manual counting by a laboratory technician or another individual, who can distinguish staining artefacts from actual nuclei, white blood cells, and (depending on specific requirements) the life cycle and species of malarial parasites [7]. Although manual counting is relatively inexpensive to implement, adequate sensitivity requires proper training and supervision of technicians. This poses problems for both medical care providers in impoverished regions of the world as well as laboratory settings which may benefit from automation of a tedious and time-consuming task [8].

The absence of human resource for performing the diagnosis of malaria in these setting is one of the reasons for the development of alternatives methods of diagnosis [6]. This includes the development of several rapid diagnostic tests (RDTs) for malaria, methods for automating the microscopic examination with image processing, and other forms of diagnosis. Rapid diagnostic tests (RDTs) for malaria have been a great success in reducing the disease burden following the change in policy by WHO [9,10]. RDTs, based on testing for antigens produced by the immune system in response to plasmodium, have high sensitivity for parasite concentrations of over 500/μL. For smaller concentrations the sensitivity of RDTs becomes too low to be used reliably, however [11]. Test results are usually available in 5-20 minutes; do not require capital investment, electricity, or extensive training for laboratory staff, although individual tests are more expensive with RDTs than microscopic analysis [12]. Apart from the issues of inadequate sensitivity for low parasite concentrations, there are other concerns about the discriminative effectiveness of RDTs in specific situations. These include frequent false positive results in areas of low transmission [13] and false negatives for individuals with asymptomatic infections or multiple organism infestations [14]. Overall, RDTs are successful in a number of situations, but the gold standard for diagnosis for malaria remains the microscope—especially in those instances such as treatment failures or low parasitemia where RDTs will not work [15,16].

Related work in computer vision diagnostics has emerged recently. A number of studies have looked at image processing and computer vision methodology for automated diagnosis of malaria from blood smears. In vision terms this is an object detection problem, and some previous work is reviewed in [17]. There has also been work in comparing these methods with other forms of diagnosis [18,19] uses neural networks with morphological features to identify red blood cells and possible parasites present on a microscopic slide. The results obtained with this technique were 85% recall and 81% precision using a set of 350 images containing 950 objects.

This paper describes some basic image processing algorithms implemented in MATLAB which detects Plasmodium falciparum parasites within a micrograph of a blood smear slide and attempts to determine which red blood cells in an image are infected. Efficacy of this algorithm is demonstrated.

Proposed Methodology

Overall, the main processing steps used through this report are grouped into three: pre-processing, parasite candidate detection, and cell segmentation. MATLAB is used in every process carried out throughout the project. The program flow concept diagram can be viewed in Figure 1.

Pre-processing

Blood smear micrographs are first transformed into the hue-saturation-value (HSV) colour space using the rgb2hsv function. This transforms a standard red-green-blue image (such as those obtained

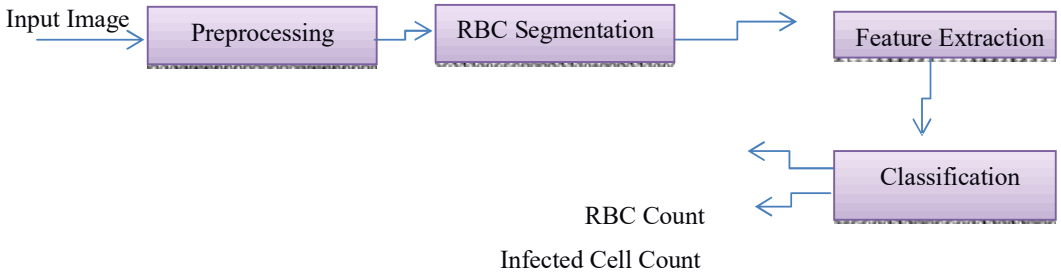

Figure 1: Program flow concept diagram.

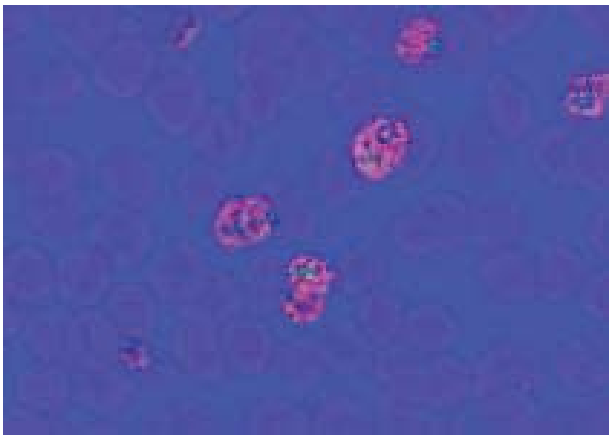

Figure 2: HSV colour space image.

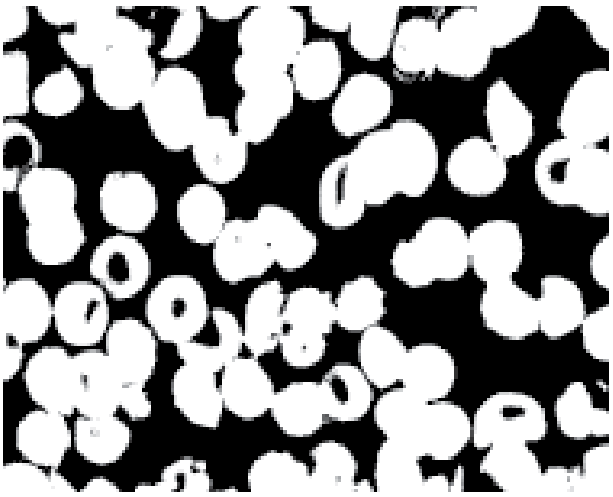

Figure 3: RBC area mask.

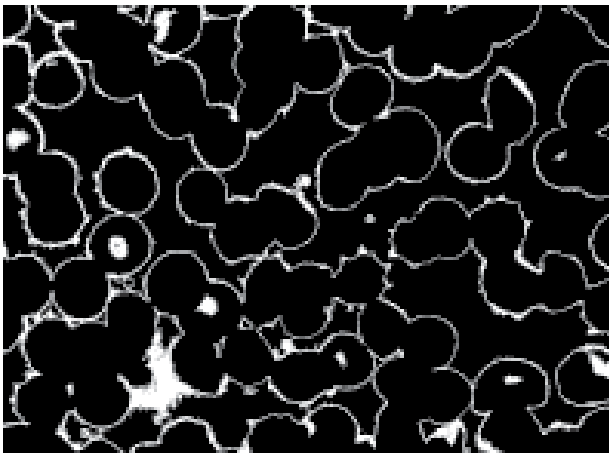

Figure 4: RBC outline mask.

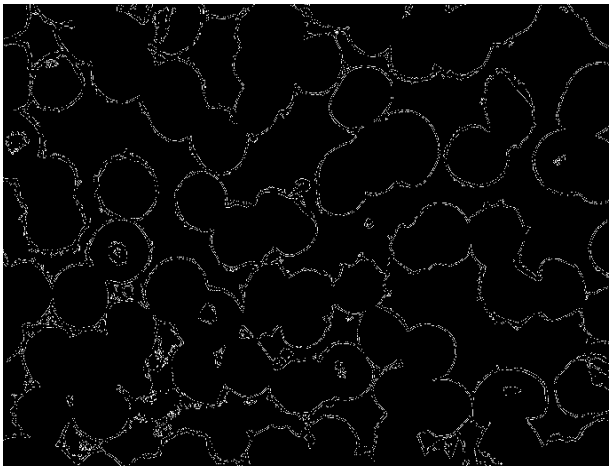

Figure 5: Edge detection (portion).

from digital cameras) into a three-dimensional vector whose dimensions correspond to the hue (H), saturation (S), and value (V) of each pixel in the image (Figure2).

Two masks are created from the V component of the image, which is relatively insensitive to the presence or absence of purple stain areas. The V component is subjected to histogram equalization (histeq). Histogram equalization tends to 'spread out' the range of grayscale values comprising an image and provides a form of normalization that allows both edges and nuclei to be more easily thresholded across differently lit images. Because the interiors of red blood cells are significantly darker than the surrounding background, Otsu thresholding is carried out to create an area mask which determines whether or not a particular region of the image lies within a red blood cell [19] (Figure 3). It is possible to exploit optical artefacts in the V component by conducting thresholding on a band of values (for histogram-equalized images, between around 0.6 and 0.7) to produce a reasonable approximation of the edges of the cells (Figure 4).

This outline mask is then subjected to Canny edge detection [4] to create an edge map comprised of single-pixel edges (Figure 5). It is also possible, depending on the characteristics of the map, to perform morphological dilation and erosion on the area mask to create this outline. A particularly complex example is provided in Figures 6 and 7. In this example, it is conceivable that morphological erosion could be carried out on the area mask and then applied to the edge mask in order to create a significantly cleaner edge mask devoid of the 'noise' beyond the edges of the cells.

Parasite Candidate Detection

The H and S components are used to detect and differentiate regions which have been stained (including candidate parasite nuclei). The H and S components are subjected to histogram equalization and then thresholded to produce a mask consisting of all purple regions in the input image, each of which is believed to correspond to either a parasite or part of a parasite (Figure 8). Small region removal is carried out to suppress extremely small signals caused by poor image quality or noise

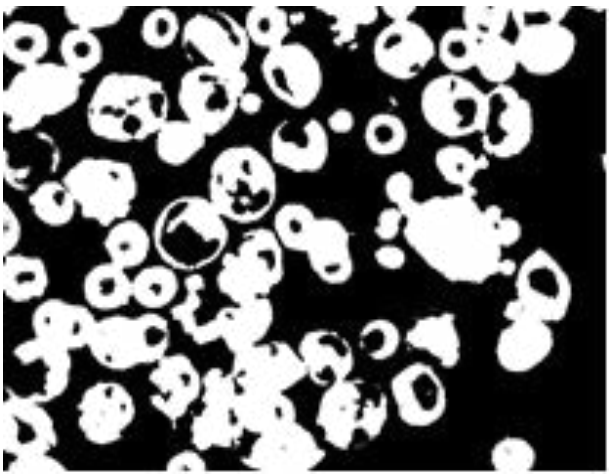

Figure 6: Poor-quality area mask.

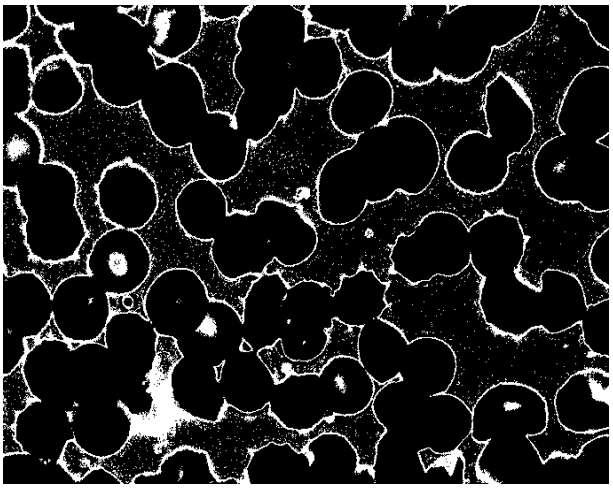

Figure 7: Edge mask.

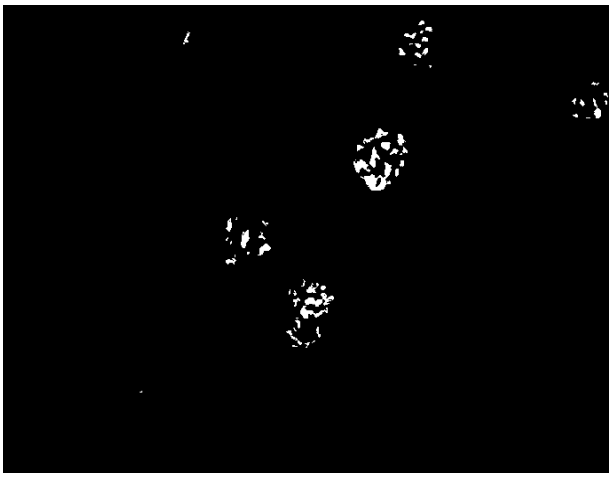

Figure 8: Candidate region mask (portion).

from imprecise threshold choice. Once this is complete, the remaining regions in the image can be identified and labelled using bwlabels.

Cell Segmentation

Once parasite nuclei candidates have been isolated and identified, it is necessary to examine each one in order to determine whether or not they lie within the confines of a red blood cell, and if so, to estimate the centre and approximate extent of the red blood cell. This task is complicated by certain common image properties – overlapping and deformed blood cells, low contrast, and blur from poor focusing.

To overcome this problem, a Gaussian-weighted method was applied. This method consists on giving weights to pixels according to their distance to the window center p(xi, yi). Each window is associated with a Gaussian kernel of the same size computed using Equation 2.1. This kernel type was preferred due to its isotropic nature.

$$G_i(x, y) = (1/2\pi\sigma^2)e^{-}(x - x_i)^2 + (y - y_i)^2/2\sigma^2 \quad .....2.1$$

Therefore, pixels closer to the window centre are given higher weights than distant ones. Hence, pixels from overlapping regions are assigned different threshold values, each one with its respective weight. To elucidate this concept, a given pixel $p(x, y)$, which is in the overlapping region between window 1, w_1, and window 2, w_2, is considered. Both threshold values are computed using Equation 2.2, as well as both Gaussian values, $G_i(x, y)$.

$$T_i(x, y) = \sum_{\omega i} \omega(x, y)\, p(x, y) / \sum_{\omega i} \omega(x, y) \quad ..2.2$$

Finally, the threshold value for $p(x, y)$ is computed using Equation 2.3. If $p(x, y)$ is closer to w_1's centre than w_2's, the threshold value from w_1 is given higher weight in the calculation of the threshold value assigned to $p(x, y)$. Additionally, this method enables to overcome discontinuity problems that might occur otherwise.

$$T(x, y) = \sum_i G_i(x,y)\, T_i(x,y) / \sum_i G_i(x, y) \quad ..2.3$$

Results

The results achieved by this method were 74% recall and 88% precision using a set of 250 images containing 850 objects.

The image processing pipeline was applied to five test images. Candidate nuclei within 250 pixels of the edge of the image were intentionally ignored due to time constraints and limitations with the test harness. These images were chosen due to their lack of visual noise (red blood cells are evenly coloured, no background artefacts) in order to carry out a baseline performance evaluation and identify strengths and weaknesses in the pipeline. Test images 2 and 3 exhibit the highest performance. Test image 2 (Figure 9) demonstrates the identification of four red blood cells containing parasites and estimates a boundary for each of the cells. These boundaries fall into four distinct clusters, which could be correlated through further analysis (consolidating cells with very close centres together). In test image 3 (Figure 10), seven cells containing parasite nuclei are identified, and the borders of the predicted red blood cells are clearly delineated. Test images 1 (Figure 11) and 4 (Figure 12) demonstrates several limitations of the system as it currently exists. Test image 1 is dominated by parasites in the schizont stage, which appear as round purple clusters containing many dark purple spots. Each of the spots within the schizont is currently detected as a separate candidate and analysed as such. The three schizonts in the middle of the image are clearly detected, but a large number of spurious detections characterize both these three schizonts and the fourth one to the upper right. These spurious detections are

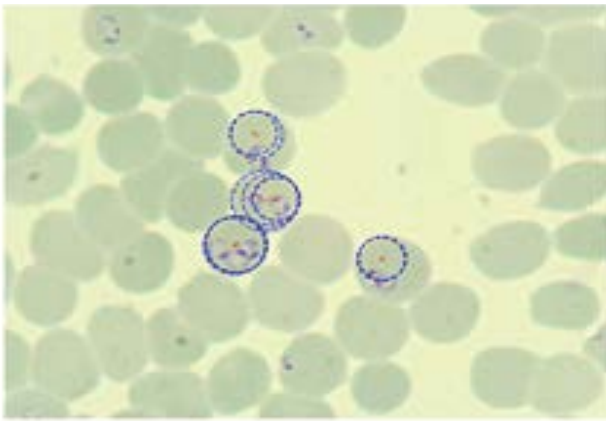

Figure 9: Image 2, categorised.

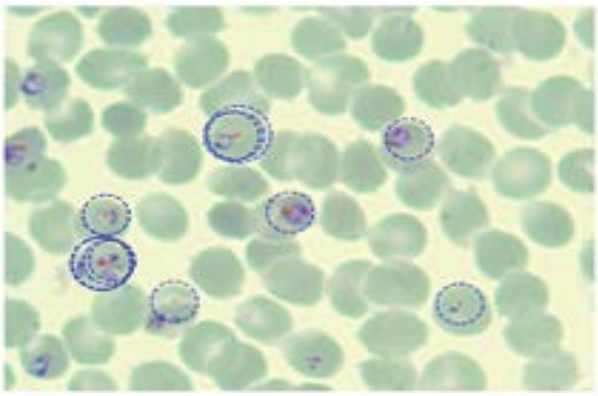

Figure 10: Image 3, categorised.

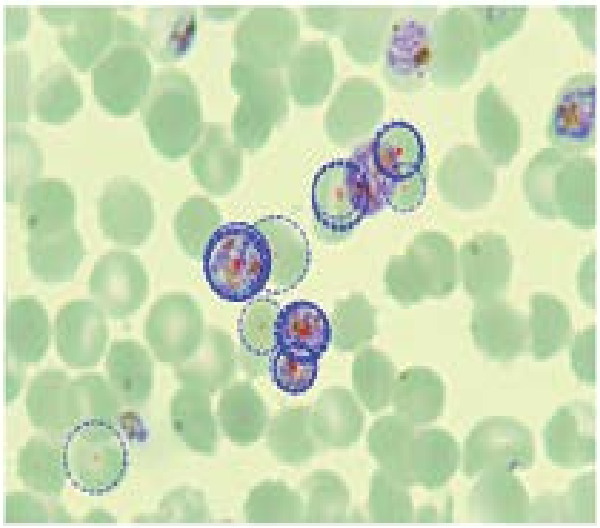

Figure 11: Image 1, categorised.

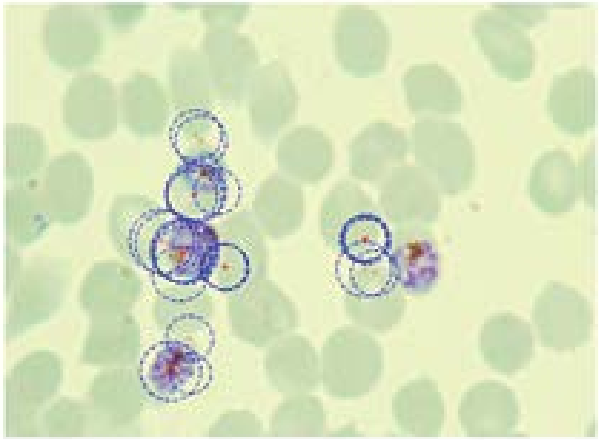

Figure 12: Image 4, categorised.

caused when cell segmentation is carried out on some of the schizont spots near the edge of the cluster. Test image 4 exhibits similar issues with schizont detection, exhibiting two high accuracy detections of schizonts and a large number of close-by spurious signals. Test image 5 (Figure 13) poses significant complications for detection, as a large number of red blood cells are clumped together and edges between adjacent cells are indistinct to non-existent. There are three strong infected cell correlations, one strong correlation with a spurious stain (due to insufficient candidate processing), and a number of highly imprecise detections which could not properly characterize the cell within which a parasite resided. Finally, of particular note are the two candidates near the lower right section of the image. Both these

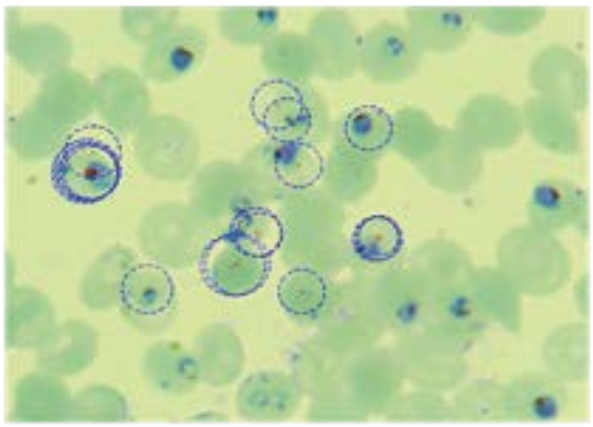

Figure 13: Image 5, categorised.

candidates lie within a group of cells which touch in multiple places and have no visible internal edges to aid with cell segmentation. These candidates demonstrate the weakness of an approach (such as this one) which exclusively tries to fit parasite candidates into blood cells without additional heuristics.

Conclusion

Blood tests are one of the most commonly requested diagnostic tests since most dysfunctions induce fluctuations from normal blood biochemical, molecular and cellular levels. In particular, the cellular information can be retrieved from complete blood count tests which nowadays are preferably performed through automated analysers instead of the labour-intensive manual count through microscopic analysis. Considering that this work is part of the broader project, Malaria Scope, aiming to assess the malaria parasitaemia through automated devices, not only are the automated analysers inadequate for the assessment of the parasites but also the malaria-affected regions lack such health resources. Thus, this work focuses on image processing approach for the automatic analysis of blood cells in microscopic images. Interest in computer vision approach, as faster alternatives for labour-intensive time-consuming visual blood analysis has been growing. However, their limited efficiency for processing low quality images and computational cost for implementation in an automated-device framework makes them unsuitable for the project. In this work, we presented a method for automatic detection of Plasmodium falciparum using MATLAB.

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