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Literature Review

An Approach for Initial Screening of Malaria Infected Slides

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Abstract

Image processing and image analysis is also core area of application in computer science for medical diagnosis. The images are collected by modern digital media and these require processing so that meaningful information can be retrieved and collated. Computerized algorithms play a significant role to achieve this goal. Malaria disease identification is an age-old problem that significantly affects a huge population. The paper presents an approach for pre-processing of Malaria infected slides.

Keywords: *Pathology; Histological Samples; Female Anopheles Mosquito; Schizogony*

Introduction

Pathology is a branch of medicine that combines the science of disease, their cause, effect and diagnosis. A Pathologist determines the cause of a particular disease conditions based on certain prescribed tests (chemical/clinical/microscopy), for accurate diagnosis and provide relief to the suffering patient. Most of this test is conducted by automated equipment using body fluids/tissue sample extracted from the patient. Digital Microscopy plays a vital role in disease determination for cases of parasitic invasion within tissues and to locate abnormality in histological/cytological body samples.

Microscopic examination of cellular and histological samples is widely used as a basis for disease detection. However, with the introduction of advanced digital microscopy and high resolution scanners the approach towards pathology had a paradigm shift towards pre-processed malaria images.

Malaria is the oldest and cumulatively the deadliest of the human infectious diseases and is a primary cause of child mortality. The disease is predominantly widespread in tropical climatic regions. Female Anopheles mosquito is the reason for the protozoan infectious disease. In this paper, CAD based pre-processing system for Malaria disease detection have been proposed.

Different methods used

The images obtained require processing so that meaningful information can be retrieved and collated. Computerized algorithms play a significant role to achieve this goal. Medical images obtained from different equipment/vendors and of different modalities have been

standardized by the Digital Imaging and Communications in Medicine (DICOM) standard. The use of digital imaging has also opened a new technological dimension for pathology. The images can be processed with Artificial intelligence and machine learning algorithms in Computer Aided Diagnosis Systems or CAD system.

The Etymology of Malaria originated from Italian 'mal aere', that has the meaning of 'bad air' found in the book entitled 'Scitture della laguna' of Marco Cornaro [1], published in 1440 in the city of Venice. However, the term Malaria got its introduction in English literature from the letters of Horace Walpole to his cousin [1]. The word got associated to the specific disease in the publication of a book by Guido Baccelli called 'La malaria di Roma' in 1878 [1]. There are 5 species that cause Malaria infection to human beings. The female Anopheles mosquito is key to the widespread dispersion of the Malaria disease. Infected mosquitoes that had sucked the blood of an infected patient initiates becomes the carrier of the disease without itself being affected by the disease.

Fertilization occurs to form a zygote which matures to form an Ookinete. This process occurs within the mid-gut of mosquito. The diagnosis of malaria identifies the presence of malaria parasite cells, antigens and antibodies within the human blood. Different diagnostic systems are now described.

The clinical diagnosis of malaria is done by a medical practitioner, very often at low cost or sometimes even free of cost in government hospitals and medical centres. The laboratory diagnosis of malaria is the most widely used procedure for malaria detection in the region where malaria commonly occurs. The laboratory techniques vary in the nature of tests conducted but generally, they involve the collection of blood sample from the affected patient for identification the presence of malaria parasite. The combination of Giemsa-stained thick smear slide for initial screening and thin smear slide for species identification still remains the 'gold standard' for laboratory diagnosis of malaria. Both type of smear, thin and thick can be used to calculate Parasitaemia. The quantitative buffy coat test was devised to simplify and enhance detection of malaria parasite. This method involves the collection of blood in haematocrit capillary tube coated with anticoagulants and Acridine orange fluorescent dye. The method is simple, dependable and user-friendly. However, the equipment is costly. The method cannot calculate the Parasitaemia or determine the species. Moreover, the detection rate decreases with non-falciparum species. The Rapid Diagnostic Test (RDT) kit detects malaria quickly. The RDT's chemicals mark the presence of such antigens in the blood sample provided. Single species or multiple species can be detected. The results are shown immediately. For RDT the Mean Operational Sensitivity with reference to microscopy was 64.8%. Higher Sensitivity of RDTs was proportional to increasing in Parasitaemia. Even with poor slide quality the Specificity of 87.8% was achieved. Serological tests refer to detection of specific antigens and antibody from chemical assay of blood plasma. Immunofluorescence Antibody Testing (IFA) for malaria detection has been proved relevant in contemporary findings.

The recent advancement in molecular biology and biotechnology has contributed to the development of modern techniques and machines that are used for different types of pathological testing and determination of diseased conditions. These modern techniques involve costly machines that can be adapted to detect malaria infection. Some of these techniques are described here.

Polymerase Chain Reaction (PCR) method can amplify trace amounts of DNA present in any body fluids for diagnostic purpose. Traces of elements containing segments of DNA or RNA can be multiplied by copying the strands thus increasing the probability of detection of the small amount of pathogenic DNA. Such techniques have helped in molecular diagnosis of malaria particularly in cases where they are admixed with other pathogens and cases of very low Parasitaemia levels. The advantages of PCR technique are in achieving best possible sensitivity and specificity values, its ability to detect at low Parasitaemia levels and detect drug resistance. The disadvantage of PCR is however that it requires complex equipment that is costly and requires trained experts.

The Loop Mediated Isothermal Amplification (LAMP) is very similar to PCR and performs gene amplification. It has been widely used for early detection of microbial diseases. This is a relatively low cost method for malaria diagnosis. The LAMP technique detects the presence of falciparum parasite by locating the occurrence of 18S ribosome RNA gene. LAMP exhibits high values of sensitivity and specificity for other species of malaria. Being a more reliable and cost-effective method can easily replace PCR for screening in malaria affecting regions. The only disadvantage is that the chemicals require cold storage facilities and further clinical trials are required.

A Flow Cytometer is a device based on Coulter principle is a laser or impedance based cell counting and sorting machine where the cells are suspended on a solution stream. The use of such cytometer to detect haemozoin formed as a consequence of malaria parasites digesting RBC. When Haemozoin flows through cytometer depolarization of laser is detected. The performance analysis for the use of cytometry yields sensitivity value range of 49 - 98% and the range for specificity value of 82 - 97%, in case of malaria detection. Such methods are susceptible to error prone reporting of cases when there are different types of infections. Moreover, the systems are very costly and are unaffordable in under-developed countries.

The Cell-Dyn is a popular cell counter that uses laser light multiple-angle polarized scatter for WBC separation and analysis. They have been found to detect haemozoin containing monocytes and granulocytes. High sensitivity and specificity of 81.3 and 80.1% are obtained for malaria infection. A DNA microarray is also known as biochip. A particular genomic expression or DNA is embedded on a region containing picomoles as probes or short fragments of DNA. The probe and target hybridization reaction can be detected as fluorophore. These microarrays are future diagnostic mechanisms. Multiple probes can be embedded within a chip for detection of multiple conditions or targets. Such miniaturized chips can be automated for the development of microarray and falciparum malaria has been detected in clinical specimens.

Mass Spectrometry (MS) is a technique that ionizes chemical and sorts these ions and a plot is obtained depending on their mass which is proportional to the charge. Such ion charges are obtained when any kind of matter is bombarded with electrons which gets distributed in an electrical/magnetic field based on the mass-to-charge. The mass of atoms and molecules are constant and known and are used to label by comparing the mass pattern. This method can be employed for detection of malaria parasite *in-vitro* even at Parasitaemia levels of 10 parasite/µl.

Conventional microscopy utilizes normal light microscopy. The accuracy of detection is heavily dependent on the heuristic knowledge of the pathologists. Evaluation of slides by pathologists requires time and often prone to error of judgement. Other techniques like the use of RDTs though very popular and provide a rapid diagnosis, but the sensitivity is lower than microscopic evaluation. Moreover, they are specific for a particular species. Other techniques described require costly setup and equipment. For efficient malaria detection using the gold standard required a technological upgradation. Advancement in microscopy, coupling them with a digital camera and connecting it with the computer system using firmware has ushered a digital era in the microscopic evaluation. Contemporary microscope does not use reflected light rather they have built in Light Emitting diode (LED) lighting systems with an array of light filters and fluorescent microscopy. Most of the problems associated with illumination in conventional microscopy are done away with the use of inbuilt illumination system. Modern digital camera with very high resolution can capture minute objects. Modern microscope systems also contain motorized stage for fully automated image capturing. Moreover, in addition to this there are slide scanners or WSI that can capture the entire slide surface in a large image file. They mostly operate in batch processing mode which makes it suitable for mass analysis and hence lucrative to pathological industry. High resolution images generated by these scanners allow the pathologist to have the entire view of the slide on their computers instantly. With the development of microscope technology, computer software and algorithms are required for efficient identification of parasites. The development of CAD software for malaria detection and diagnosis is one of such many applications of digital microscopy.

Literature Review

The intervention of technology to assist pathologists and medical practitioners is vital for the fight against malaria and its early diagnosis to prevent mortality. The biologists and the chemists were busy discovering means to control the disease. Advancement in microscopy and computer technology has bolstered this effort. Several works can be found in the research domain that have significant contribution. Several authors have tried to compare different methodology both in terms of detection technology and computational methods to establish better ways to identify and diagnose malaria.

Pre-processing of digital images are important feature of any image processing algorithm. Images obtained from digital capturing equipment often suffer from illumination and noise related issues. Before application of any automated algorithm it is pertinent to make

adjustment to the images in terms of illumination correction and noise removal. The illumination of images and colour density may vary intra/inter dataset. This arises due to different staining methods, stains and drying time. Moreover, the image capturing medium also utilises different lighting condition giving rise to illumination differences among images. Moreover, color images vary based on staining methods used. For execution of an algorithm, it often requires some correction to maintain parity of conditions in the images. Tek., *et al.* [2,3] used image subtraction from a known image and low pass filtering whereas in the latter work they assumed the gray world normalization where they used a reference image. The images will have a constant gray value corresponding to a particular known image as the reference. Das., *et al.* adapted the 'Gray World assumption' as proposed by Lam., *et al.* for illumination correction [4,5]. According to this approach the average of each channel is calculated. While keeping the green channel constant the gain in other two channels are computed.

To achieve noise elimination, Median filtering has been adapted by most authors like, Ruberto., *et al,* Ross., *et al,* Anggraini., *et al,* Rosado., *et al,* Predanan., *et al,* Bahendwar., *et al,* Makkapati., *et al,* Gitonga., *et al.* and Nugroho., *et al* [6-14]. Authors Dave., *et al.* and Savkare., *et al.* have used a combination of Median filtering with Laplacian filter for noise removal along with enhancement of the edge region [15,16]. Adaptive and local histogram equalisation method is used for illumination correction by Sio., *et al,* Purwar., *et al.* and Somasekar., *et al* [17-19]. Gaussian filter is employed by Arco., *et al,* Somasekar., *et al.* and SUSAN filter by Ahirwar., *et al,* Khan., *et al.* for noise reduction [20-23]. The authors Reni., *et al.* performed contrast enhancement on grayscale image by finding optimum weights for R, G and B channels [24]. Author Diaz., *et al*. performed low pass filtering for noise removal [25].

Morphological operations have also been used by authors for image pre-processing. The morphological operations of dilation and erosion helps in removal of unwanted artefacts and noise from the image. Such morphological operations are performed by several authors [26-28].

Pre-processing of malaria images

Illumination correction and noise removal are used pre-processing pertaining to initial screening phase. Illumination correction has been implemented by Diaz., *et al,* Tek., *et al* [29-32]. A Kruskal Wallis H test performed on a randomly selected set of three images revealed that there is significant difference in luminance distribution in the images, $\chi^2(2) = 21.706$, $p = 0.000 < 0.01$. Thereby, suggesting significant difference in luminance among images in the MaMic dataset. For the datasets under consideration, automated luminance correction of image is performed to extend the applicability of the algorithm across multiple datasets. Figure 1 depicts an image from the MaMic dataset whose luminance has been particularly marred to test the performance of the algorithm in question. A single or the same image was used as the reference luminance for all images in the dataset. The ratio of the difference in the standard deviation of the test ($\sigma test$) and reference image ((σref) against the standard deviation for the reference image was used to increase or decrease the luminosity of the RGB image under consideration.

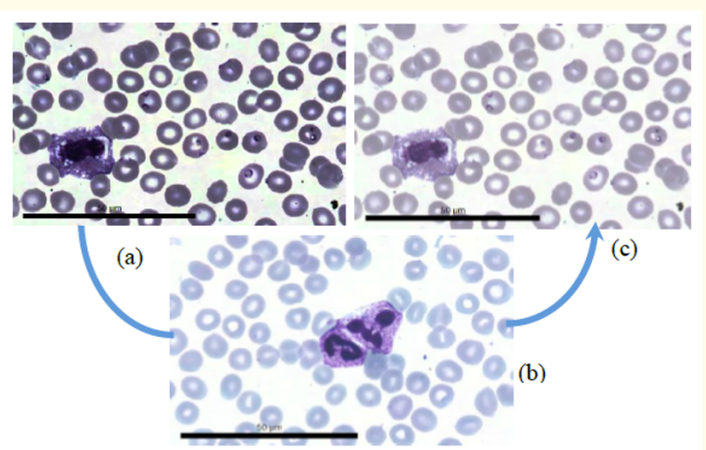

Figure 1: *(a) An image whose luminance has been degraded on purpose to test whether the algorithm works on other image datasets with bad luminance. (b) A reference image from the MaMic database to improve the illumination of image (c) Luminance improved image.*

It must be noted that, even such dynamic illumination correction of image based on a reference image does not particularly work in case of a differently stained digitized thin smear image. So for each dataset differing from the other in terms of colour composition, the illumination correction phase had to be customized using a reference image from the concerned database.

To correct salt pepper noise 2D Median filtering with 3 by 3 window was performed. Once noise corrected, the RGB images were converted to Lab Colour Space image. Based on the a and b components, unsupervised K-means clustering was performed to segment out Red Blood Cells from the Geimsa stained thin blood smear images.

Background separation is integral towards labelling of the cellular components while negating out the spurious pixels in the blood serum along with the unwanted image artefacts, namely, the reference scale.

Conclusion

The staining colour for the images as also illumination for the images was found to vary in both inter and intra database images. Again intra cellular color variation for RBC cell in a particular image was also recorded. Owing to the oval, discoid shape of the red blood corpuscle, statistically significant colour variation was recorded for a particular cell represented in RGB colour space. This paper is mainly aimed to specify different methods that are used for the detection of disease. Also, initial screening of malaria images has been proposed.

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Volume 7 Issue 5 May 2020