Automatic Malaria Diagnosis system

3 authors, including:

Arash Mehrjou
University of Tehran
19 PUBLICATIONS   **53** CITATIONS

SEE PROFILE

Tooraj Abbasian
University of Tehran
6 PUBLICATIONS   **53** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   Advanced Control of Electric Motor Drives View project

Proceeding of the 2013
RSI/ISM International Conference on Robotics and Mechatronics
February 13-15, 2013, Tehran, Iran

Automatic Malaria Diagnosis System

Arash Mehrjou, MSc Student
ECE Department – Faculty of Engineering
University of Tehran
Tehran, Iran
a.mehrjou@ut.ac.ir

Tooraj Abbasian, Assistant Professor
ECE Department – Faculty of Engineering
University of Tehran
Tehran, Iran
najafabadi@ut.ac.ir

Morteza Izadi, Associate Professor
Health Research Center
Baqiyatallah University of Medical Sciences
Tehran, Iran
morteza_izadi@yahoo.com

I. *Abstract—* **Malaria Diagnosis is normally accomplished by visual microscopy which is time consuming and offers low accuracy because of the operator's tiredness and lack of profession in job. To overcome this liability, we designed an automatic system which is able to take photos of blood smears automatically in high rate using motorized sophisticated microscope. After gathering enough samples for microscopy, Image processing task is launched which is the core of our job in this paper. Finally, results of the whole process are reported to physician for prescribing the best cure. Because of the importance of correct diagnosis stage of Malaria and parasite type in curing process, this system can attract a great deal of attention in Malaria Diagnosis task.**

II. *Index Terms—***Malaria, Parasites, Motorized microscope, Image Processing, Segmentation.**

II. INTRODUCTION

There are several diseases which cause some effects on peripheral blood smears and the best way to identify them is microscopic inspection. Diseases like Malaria, Acute lymphocytic leukemia, and chronic lymphocytic leukemia can be classified under this group. Detection of these diseases is time-consuming and requires intensive work for a human operator. Each of them can be detected via taking blood smears and watch them under microscope in order to find some specific features or colors which can be identified as signs of that disease. Sample images which are taken from each of these infections suggest an image processing method to detect specific features which is normally done by a human operator. (Fig. 1) The method which is suggested in this paper is based on image processing concepts, especially color differences or morphological properties which can be used as a criterion to differentiate infection from healthiness. The method is General and can be used in any of the diseases with microscopic detection, but in this paper we used Malaria to test our method on a real example.

Malaria is a serious global disease and a leading cause of morbidity and mortality in tropical and sub-tropical countries.

It affects between 350 and 500 million people and causes more than 1 million deaths every year [1]. Malaria is caused by protozoan parasites of the genus Plasmodium. There are four species of Plasmodium that infect man by an Anopheles mosquito and result in four kinds of malarial fever: P. falciparum, P.vivax, P. ovale, and P. malariae [2]. The most serious and virulent forms of the disease are caused by Plasmodium falciparum which contributes to the majority of deaths associated with the disease. Others only cause milder diseases in humans. Each malaria parasite life cycle can be divided into four stages
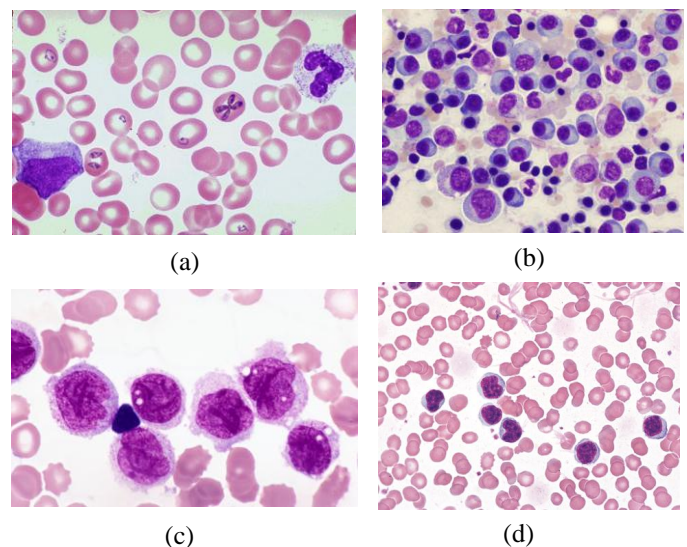

Fig.1. Blood smears from disease with microscopic diagnosis method (a) Malaria (b) Chronic lymphocytic leukemia (c) Acute lymphocytic leukemia

which are merozoites, ring stage trophozoites, mature stage trophozoites, and then schizonts or gametocytes sexual erythrocytic stages[3]. As the therapeutic success is linked to an adequate diagnosis, the World Health Organization recommends that the search for early and precise diagnosis should be a priority action. Unfortunately, the early diagnosis

is very difficult to achieve in cases in which the individual has asymptomatic forms of malaria [1].

A number of new methods have been developed in recent years for the diagnosis of malaria. These include the use of fluorescent microscopy, rapid antigen detection methods and polymerase chain reaction (PCR) - based techniques that detect specific nucleic acid sequences [4]. Despite these advances, malaria diagnosis by means of light microscopy remains the most widely and commonly used method [5].

Generally speaking, the diagnosis of malaria involves two or more of the following tasks [6]: (1) to determine the percentage of parasitaemia. (2) To identify the species and the life-cycle stages of the detected parasites.

Usually, these jobs are conducted by experienced microscopists manually.Microscopic diagnosis entails examining thick and thin blood smears for the presence of Plasmodia. It is the most efficient and reliable diagnostic technique and is very sensitive and highly specific [7]. There are numerous advantages to microscopy: it is possible to differentiate between species, quantify parasitaemia and observe asexual stages of the parasite [5, 7]. Low material costs [8] means that the marginal costs of tests are very low. Unfortunately, there are also disadvantages to the method: substantial costs are incurred purchasing and maintaining microscopes and training technicians [8], the technique is labor intensive and time-consuming [5] and the accuracy of the final diagnosis ultimately depends on the skill and experience of the technician and the time spent studying each slide [7]. Compared with expert microscopy, standard laboratory microscopy has a sensitivity of approximately 90%, a figure which drops dramatically in the field [5]. Variable smear quality and slide degeneration with time [9] are also problematic. Besides, a recent study on the field shows the agreement rates among the clinical experts for the diagnosis are surprisingly low [10]

In this research we proposed a completely automatic mechatronics system to detect malaria plasmodium which is able to eliminate the most important limits of microscopic method, that is: (1) Time-consuming and tiring job (2) Low accuracy even in experts. Here in our research we mainly focus on the task of determining the percentage of parasitaemia, because it is the most essential and time-consuming item in diagnosis of malaria. Also, we propose a motorized microscope which is fully matched with image processing procedure to make the whole diagnosis process automatic. Furthermore, it means that the physician just puts the blood smear under the lens of microscope and runs the system; after a few minutes the report, which includes the number of RBCs and parasites, is issued.

Stained blood smear images have been addressed in several works using different approaches. In [11] a novel image processing algorithm is developed to realiably detect the presence of malaria parasites from Plasmodium falciparum species in this smears of Giemsa stained peripheral blood samples.
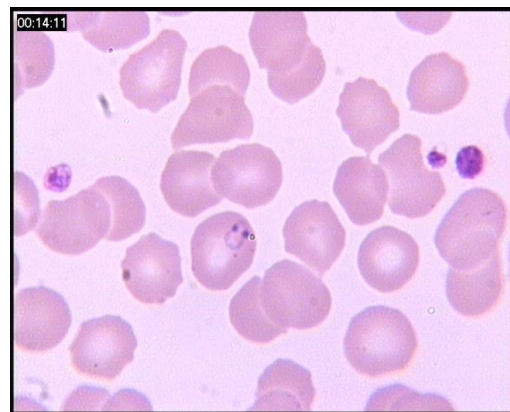

Fig. 2- a sample slide taken from infected blood smear

The preprocessing and the image analysis parts follow, to a certain extent, the works of Di Ruberto [12, 13]. The image analysis step includes calculation of the size and eccentricity of the erythrocytes and differentiating free-standing cells from the overlapping ones. In the image segmentation step, potential parasites and erythrocytes are identified and segmented from the background. In the works of Di Ruberto et al. [12, 13], objects have been detected by means of an automatic thresholding on single components of the RGB and HSV histograms based on a morphological approach. Automated image analysis-based software "MalariaCount" for parasitemia determination, i.e. for quantitative evaluation of the level of parasites in the blood, has been described in [14]. The presented system is based on the detection of edges representing cell and parasite boundaries. The paper by Díaz et al. [15] evaluates a color segmentation technique for separation of pixels into three different classes: parasite, red blood cell and background, based on standard supervised classification algorithms. The paper by Tek et al. [16] presents a method to detect malaria parasites using a Bayesian pixel classifier to first separate stained and non-stained pixels and a distance weighted K-nearest neighbor classifier to further classify the stained pixels as parasites or non-parasites. The second classification is performed using four selected features: color histogram, Hu moments, shape measurements, and color auto correlogram, which are all rotation and scale invariant. According to the results of the study, the most successful feature to classify the stained objects as parasite/non-parasite was the combination of correlogram, Hu moments and relative shape measurements.

The aim of our system is to quantify the number of Red Blood Cells and determine if they are infected by malaria parasites or not. In most recent researches done in this subject the slides gathering step is ignored and the majority of researchers only focus on the image processing step. But we implemented a system which can take photos using a CCD automatically and send them to a computer to perform the image processing task. Furthermore, a GUI based program is developed which receives data from microscope and analyzes them visually. In any step, the system operator can pause the procedure and conduct parts of the measurement task manually, if necessary.

In fact, it is a user friendly software which enables operators to dedicate their time to more challenging jobs for curing the patience instead of counting the RBCs and parasites. This paper is organized as follows:

Section IV describes the mechanical parts of our system which is equipped with motorized microscope to match with the developed method of malaria diagnosis. Section V presents the pre-processing method preparing the images for analysis. The method for segmenting the RBCs is illustrated in section VI and section VII is devoted to the method we used for detecting overlapping RBCs. Detecting infected RBCs using feature extraction is described in section IX. Finally, section X concludes this paper.

IV. MECHANICAL PARTS

The first requirement in this research is blood smears which are Giemsa stained microscopic slides prepared from blood samples that allow microscopically examinations of blood cells. [17] Thin blood smears allow better species identification, because the appearance of the parasites is better preserved in this preparation. Thick blood smears allow screening of a larger volume of blood , therefore, they can give more than a ten-fold increase in sensitivity over thin films. In this research, morphological properties are important for us, hence we used thin films.

For malaria diagnosis, blood films should be prepared as soon as possible after blood samples are taken. Such films adhere better to the slides; leave a clearer background after drying, thus, parasite and red cell changes are minimal [18]

After preparing blood films they should be examined by microscope. Overall scheme of our diagnosis system is a complete cycle (Fig .3).
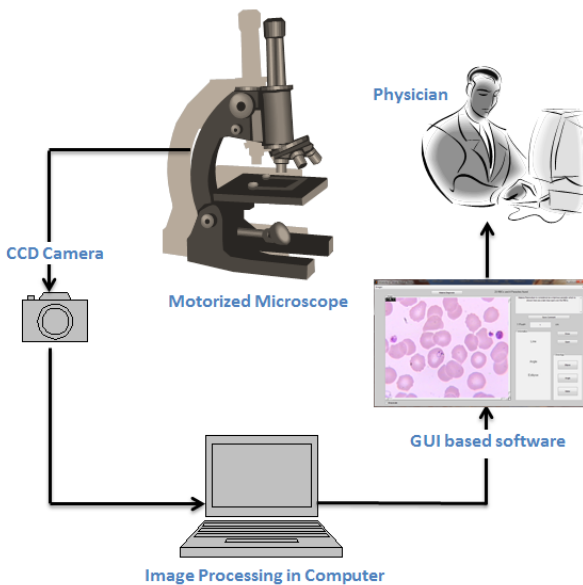

Fig. 3 overall scheme of our proposed Malaria diagnosis automated system

A microscope is equipped with two stepper motors which move the blood samples under lens quite smoothly. The amount of movement in each direction is calculated by a microcontroller installed on the microscope board to avoid taking overlapped photos. This also helps avoid calculating each RBC more than once. The photos taken by CCD are transmitted to the image processing software running on a computer. RBCs and infection are detected simultaneously and the final report is issued. The GUI based program developed for this purpose could be used for manual measurement of the properties of RBCs, for example: the radius of RBCs which is an important factor in some cases of infection, or the elongation of parasites which is another important factor for analysis of the type of infection.

V. PRE-PROCESSING

To count the number of cells in a slide and extract their features, we need to separate them from background and also from each other. The segmentation of an input image is a crucial step in almost all image analysis tasks.

The segmentation of red blood cells can be performed via thresholding with an adaptive estimated threshold. The presented segmentation technique consists of several steps. The original image is an RGB raw image in TIFF format. Our method is very susceptible to true selection of threshold which is used for segmentation. Non-uniform illumination of the image caused by using Omni light under the blood smear instead of flat one leads to wrong selection of threshold and afflicts our method. To correct this effect, at first we need to extract background pixels from cells. If we observe histogram of a given image of a blood smear slide, its typical pattern is like Fig. 4. Two peaks in the histogram represent background pixels and RBCs. RBCs are darker than background to the extent that they can be seen intuitively.

The gray scale version, g, of a given blood smear image, f , is thresholded using threshold θ_b based on the observation that the imaged objects are darker in nature than the background. The actual value for θ_b is obtained by automatically detecting the minimum between the two characteristic peaks in the image histogram. Considering those peaks as probability density functions and modeling them by equally likely Gaussian functions with $\sigma^2 = \sigma_1^2 = \sigma_1^2$, then the optimal threshold is given by:

$$\theta_b = \frac{\mu_b + \mu_f}{2} + \frac{\sigma^2}{\mu_b - \mu_f} \qquad (eq.1)$$

Where μ_b and μ_f are the means of the probability density functions corresponding to background and foreground respectively. g is threshold by θ_b and the result will be:

$$b(x,y) = \begin{cases} 1 & g(x,y) > \theta_b \\ 0 & otherwise \end{cases} \qquad (eq.2)$$

The primary binary mask of the image is extracted but illumination is affected by the intensity property of the image, as a result RGB color space doesn't work well here. The image is converted to HSV color space and its Intensity is extracted as a 2D matrix represented by i . Due to radial effect of omni lights, the image is converted to polar
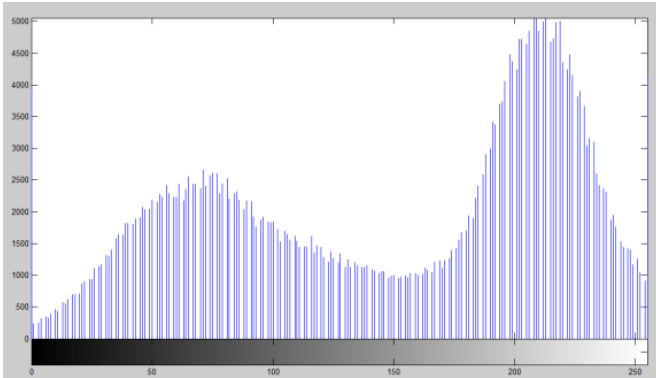
representation i^o . Proposed illumination correction function is:

$$i^o(r, \alpha) = average(i^o(r, \alpha): b^o(r, \alpha) = 1, \forall \alpha) \qquad (eq.3)$$

Where the average function is taken over the whole pixels of the image and the function considers the whole range of alpha which is the second argument, columns, of each pixel notation means that average is taken over the whole columns which
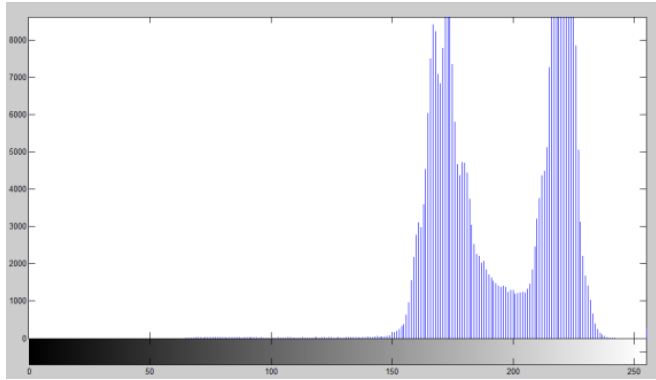
4(a)

4(b)

Fig .4. (a) histogram before correction (b) histogram after correction

is the average of all the background pixels intensity. After illumination correction the image is converted to RGB color space again and its green channel is extracted as a 2D matrix to be used in next steps because of its higher contrast comparing other two channels. Then the noise in the 2D image is smoothed by a median filter using 3x3 window. In this method the value assigned to each pixel is the median of its neighborhood that causes removing pixels with extreme values such as salt and pepper noise.

The last and most important work that is done before beginning image analysis is improving the contrast. To get the best result we used adaptive histogram shaping function instead of a flat one. The whole image is divided to many several tiles and histogram shaping is applied to any of the tiles separately. Then bilinear interpolation is applied to any adjacent tiles to eliminate artificially induced boundaries. The histogram pattern which is desired in any of the tiles is included in the histogram shaping function. In this research we used uniform

histogram shaping for each tile that leads to best results compared to bell shape, Rayleigh, etc. the histogram of a typical blood smear image is shown in Fig. 4.

VI. CELL SEGMENTATION

In this section the cells should be segmented from the background. RBCs are our regions of interest in this application, thus, we need to eliminate the artifacts which are included in blood smears.[20] By shaping the image histogram as mentioned in the previous section, modified image histogram can be like Fig .4a. It is clear that this histogram has two sharp peaks and the threshold is θ_b in eq. 2 which is calculated using Otsu's method [21]. The Result of applying eq.2 to a sample blood smear slide is depicted in Fig. 5.
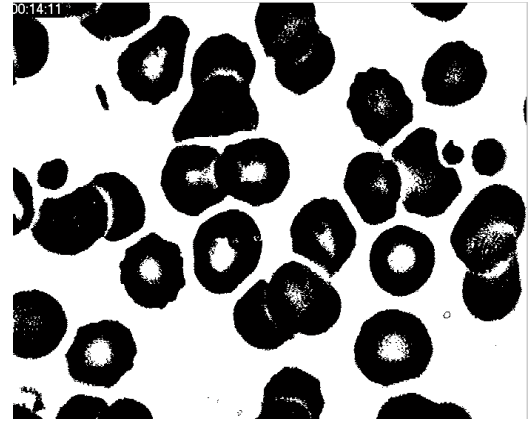

Fig .5. Binary image resulted by adaptive thresholding

For cell segmentation, two more steps should be done: (1) Removing artifacts (2) Filling the holes.

Artifacts are the objects that remain as foreground pixels in the resulted photo of thresholding. We define a set of connected pixels "island". To remove the artifacts, we remove all the islands with an area less than a specific proportion (0.1 works good in our application) of the average area of the RBCs. The area of the RBCs is calculated using a database with which the program should be trained before starting to analyze slides.

As depicted in Fig.6 red blood cells in some blood smear images have ring-like shapes or the corresponding binary mask obtained by thresholding of such an image may contain holes in the centers of the cells. Such holes have to be filled in order to obtain correct masks of the red blood cells and to allow the subsequent steps to work properly.

A hole in a binary image is a set of pixels of background surrounded and enclosed by pixels of foreground. Since in all images the background is connected to the edges of the image, we can use a flood-fill operation starting with the border pixels of the background to fill the background area connected to the edges and identify the holes in the image as background pixels that cannot be reached by flood-fill operation [22].Thus, filled malformed RBCs represents a concave area (Fig6).

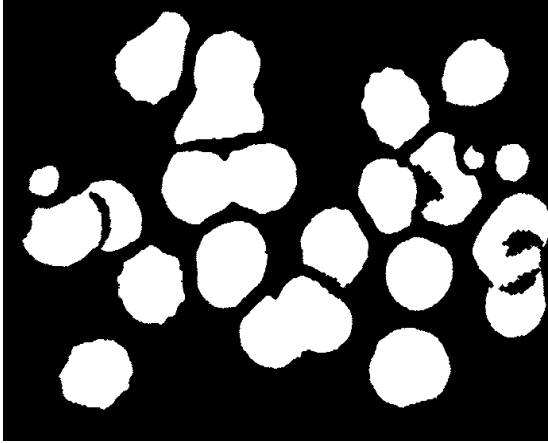*Fig .6 a) Ring-like RBC b) Corrected ring-like RBC using convex hull*

Fig. 7 Images after filling holes and removing artifacts

Such holes can be filled by simply inverting the values of the pixels that were not reached by the flood-fill operation marking them as foreground. Furthermore, to eliminate counting a RBC twice in adjacent slides, the RBCs which touch the border are removed. The final resulted image is shown in Fig. 7.

VII. ADDRESSING OVERLAPPING CELLS

The aim of the segmentation is to isolate each individual red blood cell in the image. Some connected regions in the binary image of the red blood cells with filled holes already represent individual cells. The rest of the regions are overlapping cells that form clusters which need to be separated. To distinguish between single cells and cell compounds, two simple criteria with empirically estimated parameters were used: the relative area and the elongation of the object. In this step, also objects that are too small to represent a cell are removed. Objects are connected pixels which form "islands" determined by labeling each of them. In the area method the sum of pixels of any of the objects is calculated and normalized with the average area which is a parameter calculated from database. In our project the values less than 0.5 are ignored for being a RBC. The values over 1.5 are potentially corresponding to overlapped RBCs. Another property which helps to determine if an object is an overlapped RBC is the elongation which is calculated by:

$$E = \frac{length}{breadth} \qquad (eq.3)$$

The value of E can be referred to as a criterion that can determine if an "island" is a single RBC or overlapped one.
E > 1.5 can be assumed as overlapping where "R" is the average radius of RBCs according to our prepared database.
The method we used in this research uses a mathematical transform that can be useful to determine the number of cells. Because for our purpose, the number of RBCs is more important than separating them, therefore, this method can be useful for counting RBCs while the processing load is lower than other intensive methods. Each island is separated firstly and cropped (Fig .8a, b, c, and d).
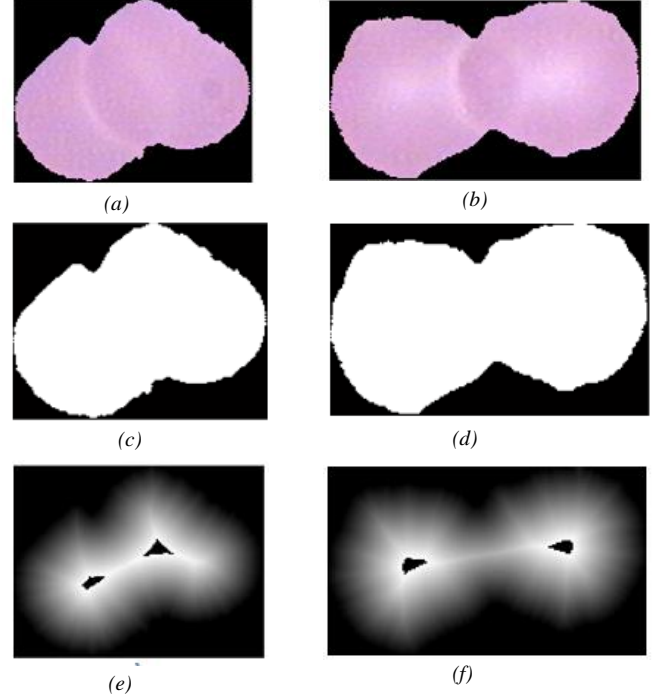

Fig. 8 our idea for counting the overlapped RBCs.
a, b) original image   c, d) masked image   e, f) result

Then we complement the binary image in order to swap foreground and background colors. Now a distance transform is applied to the image. For each pixel in the binary mask, the distance transform assigns a number that is the distance between that pixel and the nearest nonzero pixel of the mask. Due to the typical forms of overlapped RBCs, which are like two or many circles partially overlapping each other, local maxima of the transformed image can be a representation of any of the overlapped RBCs which is shown with black spots in the center of the cells. (Fig. 8.e, f)
The number of cells in any overlapped island is calculated as follows:

number of RBCs= separated black regions – 1

1 is subtracted from separable black regions because of the background which is resulted in black according to the distance transform.

IX. FEATURE SELECTION

In order to distinguish between infected and non-infected red blood cells, we need to extract feature from the image array and compute new variables that concentrate information to separate classes. Such feature set has to consist of features leading to large between-class distance as much as possible to make the differentiating process feasible. Raw images cannot be used directly as features due to high variations in morphology and colors which are coupled with arbitrary rotations, scales and color distribution respectively. So, the raw images contain large amount of data, but relatively little information. This is the aim of feature extraction to transform the input data into a reduced set of features that extract the relevant information from the input data. Feature extraction can be divided into two main tasks: (1) Determine the region of interest (2) Transform data into proper form. This is the process generally followed in this work. Since we were working with the original images, the pre-processing step was added to correct some deficiencies in the input images. Namely, illumination correction and noise filtering were performed depending on the particular set of features. The zone of measurement was in our case the whole area of the cell, which was defined by the mask obtained as a result of the preceding segmentation. Color images usually have to be transformed to gray-scale according to a certain color system, e.g. RGB or HSV. The actual choice depends on the physical properties of the stain used. We used the green channel of the RGB image in the segmentation method, because the Giemsa staining solution has a dark purple color. However, also other types of color transformations are used in this work and the features extracted are evaluated and compared. After segmentation of RBCs the transformation to gray-scale with HSV color space is used to obtain more contrast between RBC plain area and the parasites on it. In our case, the task is to distinguish whether or not a red blood cell is infected by malaria. Therefore, the selected features must provide information with which it is possible to carry out such classification. While extracting features for the subsequent classification, it is advantageous to apply expert, a priori knowledge to a classification problem.

Measures of parasites and infected red blood cell morphology that are commonly used by technicians for manual microscopic diagnosis can be utilized. It is desirable to focus on these features, because it is already known that they are able to differentiate between infected and not infected red blood cells and between species of malaria. Such features, which were suggested in [11], may include, for example, the relative size of the infected red blood cells; the relative eccentricity of the infected cells; smoothness of the cell margin; the relative color of infected red blood cells; and texture information of infected cells, i.e. presence of stippling. Another set of features can be based on image characteristics that have been used previously in biological cell classifiers [11, 19, and 16].
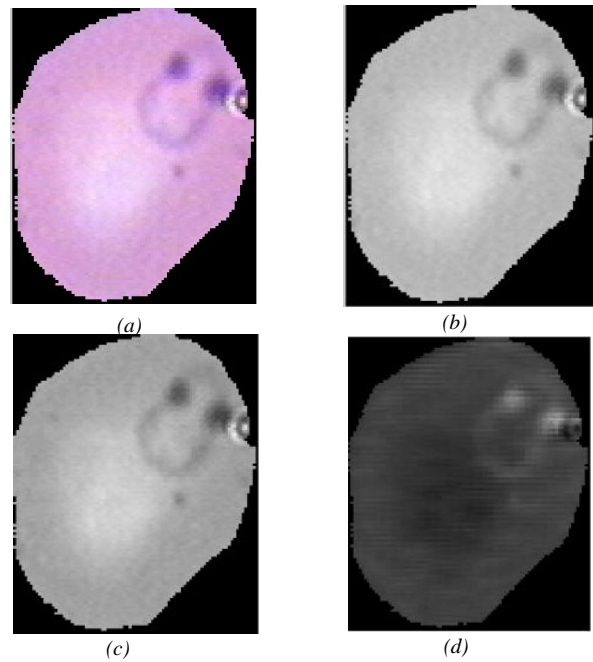

Fig. 9 Segmented cells after transformation to gray-scale
a) Original b) Gray-scale c) Green channel
d) Saturation (from HSV)

This set includes various features that are for the purpose of this work grouped into shape features, intensity features, and texture features. The features of the last group have been found the most useful ones in many applications, but at the same time the most difficult to define and the most difficult to understand intuitively [22].

In contrast to the texture features used by technicians for manual microscopic diagnosis, texture features used in image processing and classification are often difficult or even impossible to comprehend and visualize.

Another problem with textural features is the missing relation of specific feature values to appearance and function of cells.

In our case the region of interest is the area of each RBC which is segmented in preceding section. In this stage we have a picture like (Fig. 9) in hand to extract its features. This segmented cell is extracted from original pre-processed images and cropped via binary masks made in previous sections. We used Green channel of true-color images to segment the RBCs in preceding stages and the result was so satisfying that assures us to use this kind of gray-scale transformation. But in current stage the purpose is to detect whether a segmented cell is infected or not. The proposed method is to transform the segmented cell image to HSV color space and separate its components: Hue, Saturation, and Value. In this case our interested component is saturation which intensifies the contrast between parasite's color and background color which is RBC plain surface in this section. The results of applying each gray-scale transformation to original segmented cell are shown in (Fig.9)

The HSV color space (Hue, Saturation, Value) is often used by people who are selecting colors (e.g., of paints or inks) from a color wheel or palette, because it corresponds better to how people experience color than the RGB color space does. As hue varies from 0 to 1, the corresponding colors vary from red through yellow, green, cyan, blue, magenta, and back to red, so that there are actually red values both at 0 and 1.0. As saturation varies from 0 to 1, the corresponding colors (hues) vary from unsaturated (shades of gray) to fully saturated (no white component). As value, or brightness, varies from 0 to 1, the corresponding colors become increasingly brighter. Figure.10 illustrates the HSV color space. By using this method a simple thresholding can be applied to determine infection of healthiness of an interested RBC. The proper threshold can be derived from the database or even set intuitively. Once you select a proper threshold, it will be identical if you don't change the condition of microscope or illumination and also staining. However, it can be regulated manually even if staining material is changed.
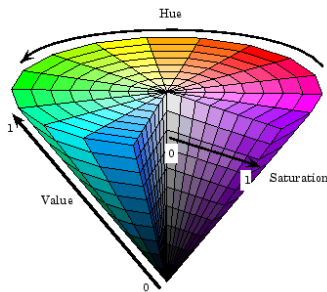

Fig.10 Hue-Saturation-Value Color space visual diagram

X. CONCLUSION

In this paper an automatic sophisticated mechatronics system is designed and implemented for Malaria parasite diagnosis. A motorized fully automated microscope is designed and manufactured along Image Processing software which interacts with microscope concurrently. Some innovative Image processing ideas is mentioned in this paper which facilitate the task of counting RBCs and detecting infections within cells. Our system is tested and the result was properly near to the labels issued by a sophisticated operator. This system is applicable in hospitals and especially distant areas in where human operators are not present.

Future studies can be devoted to detecting the types of infection. Four types of malaria parasites can infect human cells that their identification and characterization is of much interest to both medical researches and mechatronics systems.

REFERENCES

[1] WMR UNICEF, World Malaria Report. Technical Report, WMR and UNICEF, 2012.

[2] Smyth, J. D.,Introduction to Animal Parasitology. Cambridge University Press, Cambridge, 1994.

[3] World Health Organization, "Basic Malaria microscopy,Learner's guide", Part I

[4] M. Haditsch, "Quality and reliability of current malaria diagnostic methods," *Travel Medicine and Infectious Disease*, Volume 2, Issues 3–4, August–November 2004, Pages 149-160

[5] Thung, F.; Suwardi, I.S.; , "Blood parasite identification using feature based recognition," *Electrical Engineering and Informatics (ICEEI), 2011 International Conference on* , vol., no., pp.1-4, 17-19 July 2011

[6] World Health Organization. "What is malaria?" sheet no94. *http://www.who.int/mediacentre/factsheets/fs094/en/.*

[7] " Techniques for the diagnosis of malaria." S Afr Med J 74(2):55–57

[8] Oaks SCJ, Mitchell VS,Pearson GW, Carpenter CCJ (eds) (1991) "Malaria: "obstacles and opportunities," National Academy Press, Washington, DC.

[9] Ohrt C, Purnomo, Sutamihardja MA, Tang D, Kain KC (2002) "Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials." *J Infect Dis* 186(4):540–546

[10] K. Mitiku, G. Mengistu, and B. Gelaw. "The reliability of blood film examination for malaria at the peripheral health unit." *Ethiop.J.Health Dev*, 17(3):197–204, 2003.

[11] Anggraini, D.; Nugroho, A.S.; Pratama, C.; Rozi, I.E.; Iskandar, A.A.; Hartono, R.N.; , "Automated status identification of microscopic images obtained from malaria thin blood smears," *Electrical Engineering and Informatics (ICEEI), 2011 International Conference on* , vol., no., pp.1-6, 17-19 July

[12] Di Ruberto, C., et al: "Morphological Image Processing for Evaluating Malaria Disease." *IWVF4*.2001, 739-748.

[13] Di Ruberto, C., et al: "Analysis of infected blood cell images using morphological operators." *Image and Vision Computing* 20(2). 2002, 133-146

[14] Sio, W.S.S, et al: "MalariaCount: An image analysis-based program for the accurate determination of parasitemia." *Journal of Microbiological Methods*. 2006

[15] Díaz, G., Gonzalez, F., Romero, E., "Infected Cell Identification in thin Blood Images Based on Color Pixel Classification: Comparison and Analysis." *Springer Berlin*, 2007, pp. 812-821

[16] Tek, F., Dempster, A., Kale, I.: "Malaria parasite detection in peripheral blood images*." Proceeding of British Machine Vision Conference*. 2006

[17] Mui, J.K., Fu, K.S.: "Automated classification of nucleated blood cells using a binary tree classifier.", *IEEE Trans. Pattern Analysis and Machine Intelligence* 2(5), 1980, 429-443

[18] Warhurst, D.C., Williams, J.E.: "Laboratory diagnosis of malaria." *J Clin Pathol* 49, 1996, 533–38

[19] Rodenacker, K., Bengtsson, E.: "A feature set for cytometry on digitized microscopic images.", *Analytical Cellular Pathology* 25, 2003, 1-36

[20] Sharif, J.M.; Miswan, M.F.; Ngadi, M.A.; Salam, M.S.H.; Mahadi bin Abdul Jamil, M.; , "Red blood cell segmentation using masking and watershed algorithm: A preliminary study," *Biomedical Engineering (ICoBE), 2012 International Conference on* , vol., no., pp.258-262, 27-28 Feb. 2012

[21] Otsu, N., "A Threshold Selection Method from Gray-Level Histograms.", *IEEE Transactions on Systems, Man, and Cybernetics*, Vol. 9, No. 1, 1979, pp. 62-66.

[22] Soille, P.: "Morphological Image Analysis: Principles and Applications." *Springer-Verlag, Heidelberg*, 2003.