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Red Blood Cells and White Blood Cells Detection, Differentiation and Counting using Image Processing

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Abstract: Urinalysis is a diagnostic test that evaluates a sample of the urine for detection and assessment of a wide range of disorders such as urinary tract infection (UTI), kidney diseases and diabetes. Epithelial (or flat cells), red and white blood cells may be seen in the urine. The high amount of red blood cells (RBCs) in the urine may indicate infection, trauma, or kidney stones. Since the urine is a sterile body fluid, the presence of white blood cells (WBCs) or bacteria in it is considered abnormal and may indicate a urinary tract infection. The appearance of these cells can be observed through a high power field microscopic examination and is quantitatively recorded based on the manual counting performed by a registered medical technologist. In this study, an automated detection and counting of red and white blood cells in the urine using image processing was proposed. Sample images were captured looking at the microscope used in a standard diagnostic laboratory. The images were processed and a blob detection algorithm was used to detect and differentiate RBCs from WBCs. A cell counting method was also used to provide an actual count of the RBCs and WBCs detected. The automation comes with a graphical user interface backed-up with a working database system to keep the records of the users (e.g. patients, respondents). The performance of the system was statistically described as compared to the manual method of counting. Results show an accuracy of 93.64% for RBCs and 91.6% for WBCs. Hence, the proposed system can benchmark with the manual methods of detection and counting of RBCs and WBCs in urine samples.

Key Words: image processing; microscopic examination; cell radius; cell counting; database

1. INTRODUCTION

Public health is one of the main concerns of the society with which most detection and diagnosis are carried out through laboratory tests and examinations. Several biochemical tests are available and are typically done for continuous monitoring or for assessment of certain diseases. Although there are different body fluids which can be examined, blood and urine are two of the most commonly analyzed specimens due to its simplicity in sample acquisition. Urine is one of the most easily accessible biological samples and it provides a treasure trove of molecules essential for clinical diagnostics (Rai, 2010). This examination is thoroughly done through one of the most prominent clinical laboratory examination, urinalysis.

Urinalysis is a diagnostic test which can provide useful relevant information in a wide

spectrum of clinical solution (Fogazzi & Garigali, 2003). Urinalysis can be performed by direct observation, dipstick analysis and microscopic analysis (Lockwood, 2011). Among these, the microscopic analysis which deals with the identification of casts, cells, crystals and bacteria aids is an indispensable part of urinalysis (Simerville et al., 2005). The presence and concentration of urine sediments can result to various diseases such as urinary tract infection (UTI), renal diseases, bladder defects and other illnesses that can be detected through a thorough examination that requires scanning of a low power and high field microscopes (Bartges, 2012). Infections are caused by fungi, viruses and bacteria. It typically occurs when bacteria enter the urinary tract through the urethra. The bacteria then multiplies in the bladder and may result to an infection (JJustad, 2010).

In practice, UTI detection is done in a clinical laboratory but with the advancements in technology and the availability of image processing techniques, more revolutionary means of detection were made possible. An improved Sobel operator, Hough transform, principal component analysis (PCA) and linear discriminant analysis (LDA) was used by Cao et al. (2009) for the detection of red blood cells in urine image captured under microscope. An automatic detection and recognition of casts in urine sediment images was proposed by Li et al. (2009) which involves a 4-direction variance mapping image acquired from a gray scale image. An improved adaptive bi-threshold segmentation algorithm was used to obtain binary image and the five textures and shape characteristics of casts are extracted from both gray scale image and binary image. A decision-tree classifier was used to distinguish casts from other particles in the image. Results show that the method produces satisfactory segmentation and an improved recognition performance. The blob detection algorithm of Gupta (2012), which consists of resizing, flipping, changing the color space, comparing and separation, smoothing and rendering can be used for cell detection. Halim et al. (2012) created an algorithm for counting pus cells and epithelial cells in sputum which uses K-means clustering and color thresholding. An artificial neural network (ANN) – based algorithm was proposed by Tangsuksant et al. (2013) for the segmentation and detection of RBCs and WBCs in urine sediment images which resulted to an average percentage error of 5.28 and 8.35, respectively. Automated recognition techniques for urinary epithelial cells (Almadhoun, 2013) were even found to have very good performance.

This study aims to create an executable software equipped with RBC and WBC detection and counting algorithm. The software comes with a database system to keep the records of the patients for future reference. The ability of the algorithm to detect and count blood cells was compared to the manual counting of a registered medical technologist (RMT) aiming to have less than 10% margin of error and more than 90% level of accuracy.

2. METHODOLOGY

2.1 Software design

The software includes three significant parts: the graphical user interface (GUI), the image processing block and the database.

The GUI is composed of computer generated codes that identifies the parameters of the objects present in the working interface. It prompts for inputs and whenever wrong inputs are placed, an error-handling function is used until a correct input is entered. The GUI prompts for multiple input information fields which includes the ID No. name of the patient/respondent, gender and age. In addition, images and cell (RBC and WBC) count are also displayed together with the function buttons Upload, Count, Reset and Save. If the fields are insufficient and the Count or Save button is pressed, the GUI will display an error message. The sample GUI is shown in Fig 1.

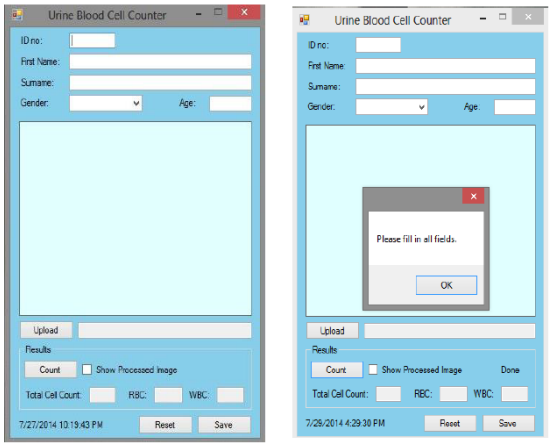


Fig. 1. Urine Blood Cell Counter GUI

Image processing is the core of the software with the help of the GUI. The image processing algorithm detects and counts the cells present in an uploaded image captured from a 400x microscope. Provided that the input fields are correctly filled-up and an image is uploaded, by pressing the Count button, the software will display the number of RBCs and WBCs and their total count. The user can reset

and repeat the process again or save the results which can be stored in a database that is accessible on-line using MySQL connections. The general flow of the processes in the Urine Blood Cell Counter (UBCC) is shown in Fig 2.

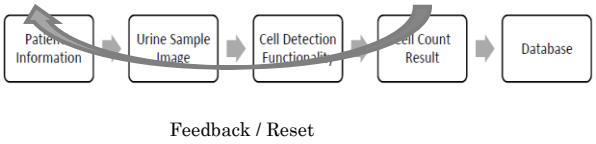


Fig. 2. UBCC Process Flow Diagram

2.2 Sample Preparation and Image Acquisition

The process for preparing the samples and acquiring the images of the samples are as follows: 1) The respondents were asked to urinate to fill the urine container provided, 2) A medical technologist performs a reagent strip test on the sample urine, 3) The urine sediment is prepared for microscopic urinalysis by centrifuging the sample, 4) After an average of five minutes, the unnecessary parts of the sample were disregarded, 5) The parts were transferred to a glass slide for viewing under a microscope, 6) Capture the image using a mobile phone camera that is placed 2cm in front of the eyepiece of the microscope. The camera setting is at 50% magnification while the eyepiece has 40x objective lens and 10x magnification.

2.3 Image Processing

When the input fields are complete and the respective image is acquired, the image processing procedures will then follow. The image processing flow diagram is shown in Fig 3.

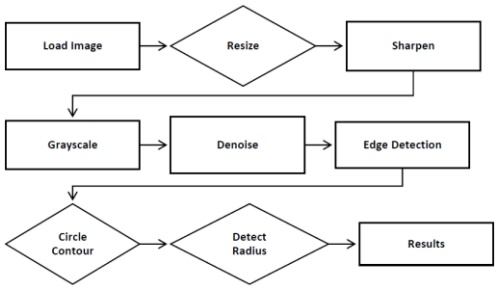


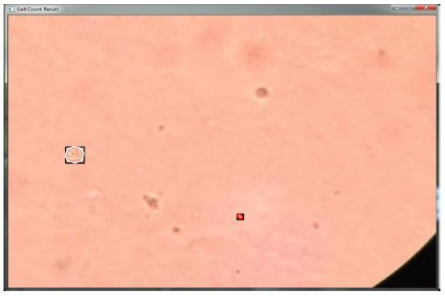
Fig. 3. Image Processing Flow Diagram

The image is sharpened before turning it into grayscale in order to increase the texture and detail of the image for edge attribution (Amin, 2012). A slightly blurred version of the original image is subtracted from the original image to detect the edges. With this enhancement, the edges of the cells will be clearer and shadowed cells will be hazier. A Gaussian blur was used as a filter to sharpen the image, remove some noise and reduce detectable unwanted edges from the image.

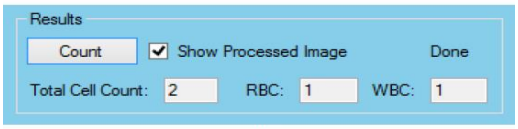
The parameters used for fast non-local means de-noising include the source, destination, template window size and h (filter strength for luminance). The template window size is the size in pixels of the patch used to compute weights and was set to a default value of 7. The search window size is used to compute the weighted average given per pixel and is set to 15 to reduce computing time. The parameter regulating the filter strength for luminance is h. The higher the value of h, the better it removes noise. However, it may also remove some image details. It was set to 5 for balance purposes. This de-noising method averages three pixels using colors of nearby pixels (Buades, et al., 2011).

The Canny edge detection algorithm was proved to be one of the most efficient detection algorithms because of its low error rate, well organized points and gaining unity response to a single edge (Maini & Aggarwal, 2011). The generated image is now ready to be defined. The function Contours finds all points in the image and determine which of them will have a vertex of 6 or more. The number of vertices entails if the point forms an arc or resembles a circle (Amin, 2012).

Small and non-convex objects were skipped. Once the contour of the cells is detected, the center and radius is determined. If the radius is less than 15 pixels, the contour is a red blood cell and is marked with a red point. On the other hand, if the radius is greater than 14.99 pixel, the contour is a white blood cell and is marked with a white point (Cao, et al., 2009).A sample result is shown in Fig 4a. All the counts are collected and displayed on the Results Panel (Fig 4b).



(a)



(b)

Fig. 4. (a) Processed Sample Image, (b) Results Panel

2.4 On-line Database

The MySQL database consists of columns for ID number, Name, Surname, Age, Gender, RBC, WBC, Date and File Path. These inputs can be filled-up through the GUI as discussed in Section 2.1. There are security features in the MySQL workbench which provide enough security to keep the confidentiality of the data. Once the database has been opened, the left side panel shows four panels: Management, instance, Performance and Schemas. Under Schemas: select databasefinal, select Table, then select personalinformation. This is where final results are stored.

The data type and other parameters (Fig. 5) for each column in the database, it is set by right clicking on personalinformation and clicking "Alter Table". The ID Number is set as the primary key. The primary key is the unique valued variable in the database.

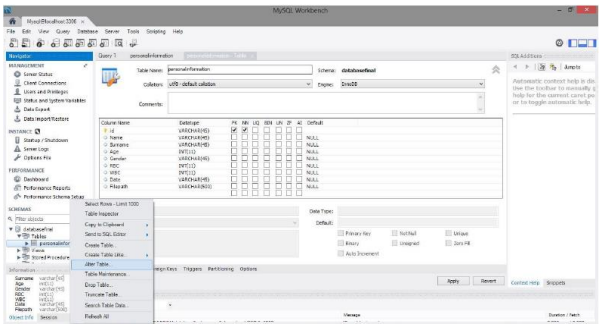


Fig. 5. Database Header Parameters

In order to transfer the data output from the GUI to the database, the command "insert info" was used. This can be found in the text boxes variables with respect to their column names in the MySQL database (Oracle 2008).

MySQL has a feature where the database may be accessible online as long as the server is online. The server should contain the images captured from the microscope which can be accessed if the client has the following: MySQL connection, the server name, database name, username, password, port number (3306 for default) and MySQL Command-on-connect, for the database.

3. RESULTS AND DISCUSSION

The main functionality of the software is the image processing part which utilizes OpenCV libraries. The GUI was created using Visual Studio 2012 and the database was based on MySQL. The OpenCV using C++ interfaces and MySQL open source database were successfully integrated to Visual Studio 2012 Windows forms application by setting up parameters and changing some windows default settings.

The images were captured at a microscopic level and a rectangular-shaped region of interest was considered based on where the most concentration of cell is found as identified by a registered medical technologist. The processes involved in image processing were carried out as shown in Fig. 6.

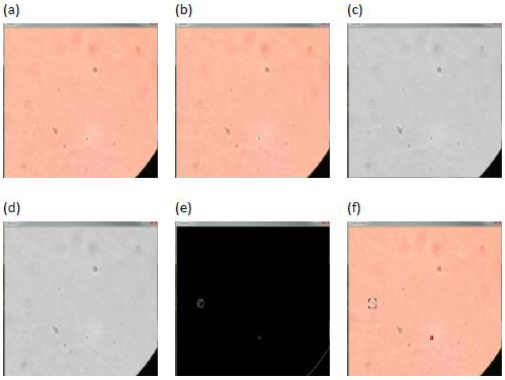


Fig. 6. (a) Original image, (b) Sharpen mask, (c) Gray scaled image, (d) Fast means non-local de-noising, (e) Canny edge detection, (f) Final processed image

Cell size was chosen over cell color since the RMT did not use a dye to put color to the cells. The function minEnclosingCircle takes the matrix vector which contains the array of values that has six vertices and then outputs its center and radius. These values were used to locate the center of the cell and determine the type of cell detected. The first output is the abscissa and ordinate of the center

which also determines the location of the cell. The second output is the radius.

RBCs and WBCs may be seen as concentric circles depending on the view of the captured image. The function Contours determines which of the two concentric circles will be detected and it will only consider them as a single cell. The outer or the larger circle is considered and detected even if the center of the inner circle is not aligned with the center of the outer circle.

A total of forty-five (45) samples were used in this study. Fifteen (15) samples were used for training and calibration and thirty (30) samples were used for testing. The samples were taken in collaboration with MediCard Philippines, Inc. – Festival Mall branch. Each urine samples was prepared and cultured by a registered medical technologist.

Table 1 shows a sample data set obtained from the laboratory and the counter count output of UBCC. Each sample was given an ID number to keep the confidentiality of the person to whom the samples were taken. The manual counting conducted by the RMT was recorded the same as with the UBCC counting performed by the developed software. The data acquisition was done for two (2) days. The possibility of getting a zero RBC or WBC count is highly probable due to the randomness of the medical situation of the respondents.

Table 1. Manual count and UBCC count output

SAMPLE (ID No)	Manual Count		UBCC Count	
	WBC	RBC	WBC	RBC
TRS01	3	0	3	0
TRS02	1	1	1	1
TRS03	1	0	1	0
TRS04	1	6	1	6
TRS05	0	0	0	0
TRS06	1	0	1	0
TRS07	2	2	2	2
TRS08	1	0	1	0
TRS09	1	0	1	0
TRS10	2	0	2	0
TRS11	0	13	0	12
TRS12	0	16	0	14
TRS13	1	9	1	9
TRS14	1	6	1	6
TRS15	0	19	0	18

The overall percentage accuracy of the UBCC as compared to the manual counting is shown in Fig. 7.

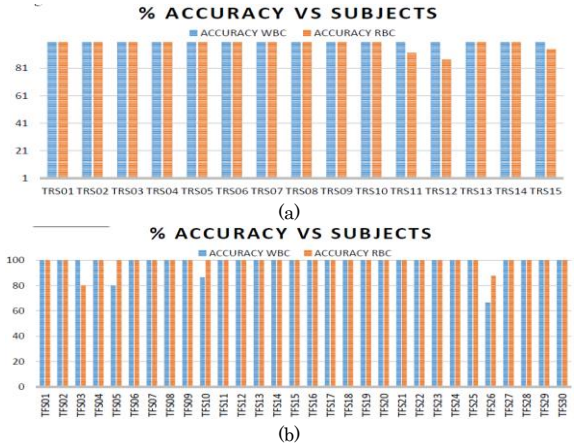


Fig. 7. Percent Accuracy (a) training set, (b) testing set

The percent accuracy obtained for each subject ranges from 66.67% up to 100%. Results show an accuracy of 93.64% for RBCs and 91.6% for WBCs. The percent margin of error was also considered and was calculated using (Eq.1) with 90% level of confidence.

$$ME_p = 1.645\sqrt{\frac{(E_p)(A)}{n}} \qquad \text{(Eq. 1)}$$

where:
ME_p – margin of error
E_p – percent error
A – accuracy
n – number of samples

The computed percent margin of error for WBC was 0% and 9.79% for RBC in the training set. In the testing set, the computed percent margin of error for WBC was 8.33% and 7.33% for RBC.

The Kruskal-Wallis (KW) test was used to determine if there is no significant difference in the results of the manual count and the automated count using all the samples used in this study. The KW test was used because the data obtained was not normal. Using Minitab, the results are obtained as shown in Table 2.

Table 2. Kruskal-Wallis Test on WBC & RBC Manual and Automated Counting Schemes

WBC		RBC	
H	p-value	H	p-value
0.00	*0.955	0.00	*0.981
0.00	**0.953	0.00	**0.978

** unadjusted for ties*
**adjusted for ties*

For WBC & RBC count, the test statistics (H) had a p-value of 0.955 and 0.981 (unadjusted for ties) and 0.953 and 0.978 (adjusted for ties), respectively. These results indicate that there is no significant difference between the manual counting and automated counting for an α-level of 0.05 since it is less than the computed p-values.

4. CONCLUSIONS

The implementation of the image processing software for red and white blood cell detection and counting in urine samples was made possible with a high level of accuracy. The UBCC software application was able to meet a margin of error which is less than 10%. Results show an accuracy of 93.64% for RBCs and 91.6% for WBCs. Overall, the average accuracy of the UBCC software application was 94.99% and a percentage difference margin within 0% to 10% with 90% level of confidence. Statistical results confirm that there is no significant difference between the manual and automated counting of red and white blood cells in the samples at $\alpha = 0.05$.

Furthermore, improved accessibility and portability of the results were obtained through the on-line database support of the software.

5. ACKNOWLEDGMENTS

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