

Autofocus Microscope System using Contrast Measurement Approach

Thang Ehang, Mohd Afzan Othman, Nasrul Humaimi Mahmood, Mohd Azhar Abdul Razak*

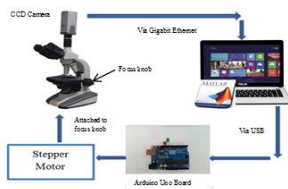
Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

*Corresponding author: mohdazhar@utm.my

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Graphical abstract



Abstract

Cell counting is a method used to quantify cells for disease diagnosis and medical research. Generally, hemocytometer is used to count cells as it is a cheaper and simple method. Specimen contains cells is loaded into the hemocytometer and placed on a microscope. The coarse adjusting knobs of microscope are turned to focus the cells. For a beginner user, to get focused cells is time-consuming. If the user too harsh with the adjusting knob, it may loosen the knob gear. Moreover, if the cells are too over focused, it may cause damage to the objective lens and the hemocytometer. In order to overcome these problems, an autofocus system is developed to control the movement of adjusting knob in achieving the focused image automatically. A CCD camera is attached to the microscope to capture images of cells via gigabit Ethernet. The images are then analyzed using contrast measurement method. To control the movement of knob, an attachable stepper motor is used. The movement is stopped when the focused image is achieved. As a result, the autofocus system could assist a user to focus cells faster and make the microscope become more user-friendly.

Keywords: Hemocytometer; focused image; microscope; contrast measurement

Abstrak

Pengiraan sel adalah satu kaedah yang digunakan untuk mengira sel-sel, diagnosis jenis penyakit dan ianya juga diaplikasikan dalam penyelidikan perubatan. Secara umumnya, "hemocytometer" digunakan untuk mengira sel-sel kerana harganya yang murah dan kaedah penggunaannya mudah. Spesimen yang mengandungi sel-sel dimasukkan ke dalam "hemocytometer" dan diletakkan di bawah mikroskop. Pemutar kasar di mikroskop dilaraskan untuk memfokus sel-sel. Bagi pengguna baru, untuk mendapatkan imej sel-sel yang jelas memerlukan masa yang panjang. Jika pengguna memusing pemutar dengan terlalu kasar, ia mungkin akan melonggarkan gear pemutar. Selain itu, apabila sel-sel terlebih fokus, ini boleh menyebabkan kerosakan pada kanta mikroskop dan "hemocytometer". Untuk mengatasi masalah tersebut, sistem "autofocus" dibina untuk mengawal pergerakan pemutar supaya imej yang fokus boleh didapatkan secara automatik. Sebuah kamera CCD diletakkan kepada mikroskop untuk menangkap imej sel-sel melalui "gigabit Ethernet". Imej-imej ini kemudiannya dianalisa menggunakan kaedah ukuran kontras. Sebuah motor digunakan untuk mengawal pemutar. Pergerakan motor akan berhenti setelah imej yang focus diperolehi. Akhirnya, sistem "autofocus" boleh membantu pengguna memfokus sel dengan lebih cepat dan menjadikan mikroskop lebih mesra pengguna.

Kata kunci: Hemocytometer; imej fokus; mikroskop, ukuran kontras

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1.0 INTRODUCTION

Autofocus optical systems use control systems that consist of a sensor and a motor to get a focused image automatically. Basically, autofocus system is developed based on three methods namely active, passive or hybrid. Active autofocus system utilizes active components, such as infrared or ultrasonic; to determine the distance to the subject [1]. Passive autofocus system determines correct focus point by using light in order to do the analysis on images. There are two parameters could be determined in passive autofocus namely phase detection and contrast measurement [2]. For the hybrid

autofocus system, focused image can be achieved by combining either active and passive methods or phase detection and contrast measurement.

A research to produce an auto-focus system for biomedical digital microscope was demonstrated by [1]. The system consists of a lens, CMOS sensor and electronics part to focus the image on the sensor. The lens formula and the Discrete Cosine Transform are applied in the study to produce the auto-focus algorithm.

Besides, a passive auto-focus camera control system was introduced by [2] utilizing Discrete Wavelet Transform (DWT) and also morphological edge enhancement algorithm. To control

the adjustable lens in achieving the focused position, Self-organizing Map (SOM) neural network controller was developed. Furthermore, a multi-focus image fusion was introduced by [3]. In this study, a new method of combining the Contourlet Transform with image blocking fusion was introduced. [6] explored on-Chip Voice Coil Motor (VCM) used for mobile auto-focus. A systematic operation of the VCM driver and Image Signal Processing (ISP) were required in order to keep a good focus. A new auto-focus method based on focal window searching and tracking approach for digital camera was introduced by [7]. This algorithm can achieve the auto-focus function in high motion and low motion sequences.

A fuzzy search method introduced by [8] was used in the motion control system of the microscope. Other new auto-focus algorithm that combines initial search for direction, a search fine and a rough search was introduced by [9]. The sum of gradient magnitude was proposed due to the advantages of fast response and less computation. [10] developed a compact camera module having auto-focus actuator and mechanical shutter system. The function of auto-focus actuator is to move a lens module. Meanwhile, the mechanical shutter was used to adjust an intensity of light and focus length.

Autofocus takes an important role in cell counting application. This is because, a focused cell image is a necessity before the activity of cell counting is takes place [11-13]. Nowadays, numerous procedures in medicine and biology require the cell counting as a part of diagnostic tools [11]. A device used to count cells is called hemocytometer. The hemocytometer is loaded with cells and placed onto the stage of microscope. In practice, user will manually turn the microscope knob to focus the cells image. However, a common problem has been discovered especially for beginner users; they tend to take longer time in adjusting the knob of microscope to get a focused image. Hence, it causes a waste of time just to get the focused image. Furthermore, for beginner users the microscope is not convenient to be used. This is because they do not have enough experience to handle compared to those experience users. Sometimes when the microscope is exaggeratedly focused; it would break the hemocytometer and at the same time could damage the microscope lens. Moreover, if the user is too harsh with the adjusting knob, it may loosen the knob gear. Time is important when doing the cell counting and it is dependent on how fast the focused image can be captured. For example, finding the concentration of bacteria, virus or pathogens in the blood by using cell counting can give us some reveal information about the progress of an infection disease [13]. If the time usage is short, probability of a patient to recover from a disease will be increased.

Therefore, the development of autofocus microscope system using contrast measurement method is needed to solve the problem of getting focused image and to avoid human error. Furthermore, microscope may become convenient and user-friendly to researchers because the process of getting the focused cell image can be done automatically.

2.0 METHODOLOGY

2.1 Hardware

Figure 1 shows the work flow of the autofocus microscope system. The system consists of an upright microscope (Radical,

India), a CCD camera (Basler Ace, Germany), a computer with MATLAB platform, unipolar stepper motor and an Arduino-Uno board. The Arduino-Uno board was chosen due to its ability to control the movement of stepper motor easily. To operate the system, the position of microscope stage was set at the lowest position. Two experimental setups were conducted to test the operation of the system.

First experiment was carried out by putting the hemocytometer without yeast cells on the microscope stage. The CCD camera was attached to the eyepiece of microscope to capture series of images via gigabit Ethernet. Each image was analyzed using contrast measurement algorithm written in MATLAB software. The stepper motor was attached to the microscope's focus knob to rotate the knob clockwise. For each step of rotation moves the hemocytometer approximately 0.0167 mm towards the objective lens. The process of image acquisition, analysis and moving the microscope stage up were repeated until a focused image was obtained. A green LED will light up as a notification to the user that the process was completed.

Then the system was verified with the hemocytometer which was loaded with yeast cells. The process of image acquisition, image analysis and stage moves up was continued until focused image of cells was obtained.

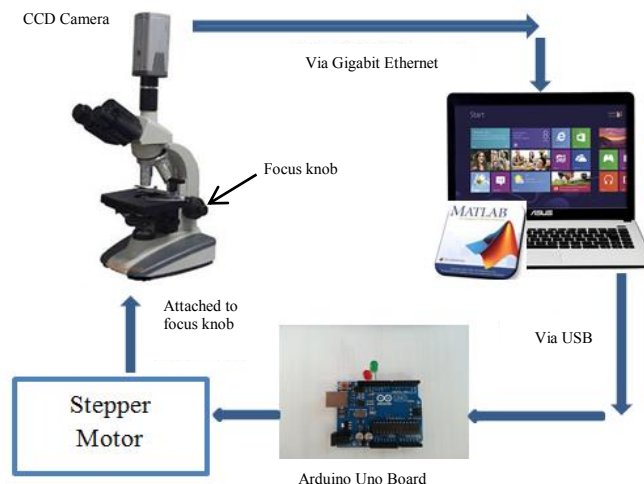


Figure 1 System overview

2.2 Software

Contrast measurement is a passive autofocus method implemented in the algorithm to find the focused image. It is simple and requires no active component. The value of contrast (C) for each image is calculated using Equation (1).

$$C = \sum_{j=1}^n \sum_{k=1}^m \left[\frac{(X(j)Y(k) - X(j)Y(k+1))}{+(X(j)Y(k) - X(j+1)Y(k))} \right] \quad (1)$$

where m and n is the number of pixel of an image in vertical and horizontal axis, respectively. The contrast value of an image is obtained by adding the summation values of pixel difference in horizontal and vertical. Theoretically, an image that has a

highest contrast (C) value is determined as a focused image. Figure 2 shows the flow of obtaining the focused image by implementing the contrast measurement method.

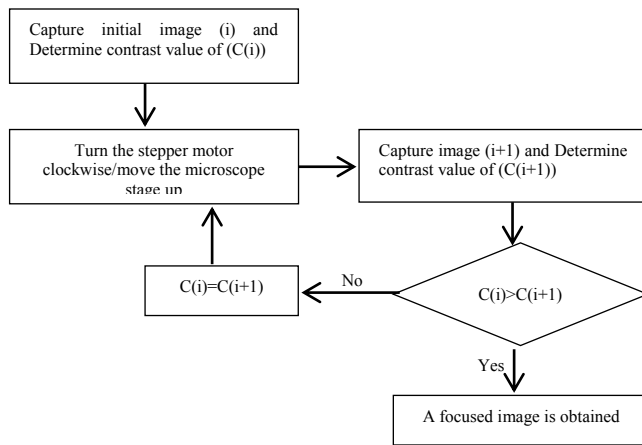


Figure 2 Flow chart to determine the focused image

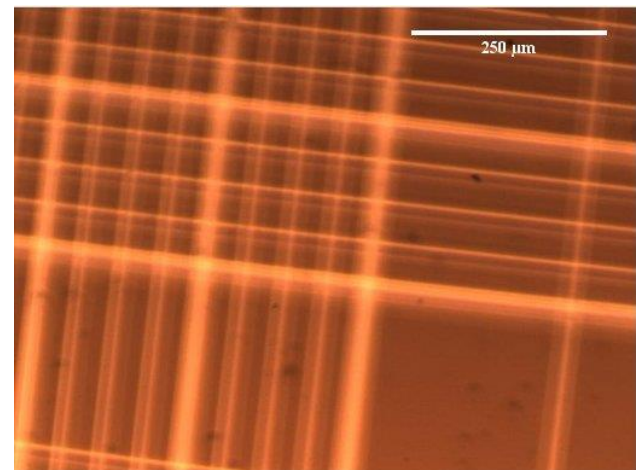
Referring to Figure 2, once the hemocytometer with or without yeast cells was placed under the microscope, an initial image was captured and the value of contrast measurement $C(i)$ was calculated using Equation 1. Next, the stepper motor rotates the focus knob to move up the microscope stage and specimen. At this time, second image was captured and analyzed to determine the new contrast value $C(i+1)$. The new contrast value was compared to the previous contrast value $C(i)$. The process of moving up, capturing and analyzing the specimen image was repeated until the $C(i)$ was bigger than $C(i+1)$ value. The focused image was achieved and displayed once the $C(i)$ value was highest among the captured images. The obtained focused image can be used in further analysis such as cell counting and morphological analysis for disease diagnosis.

3.0 RESULTS AND DISCUSSION

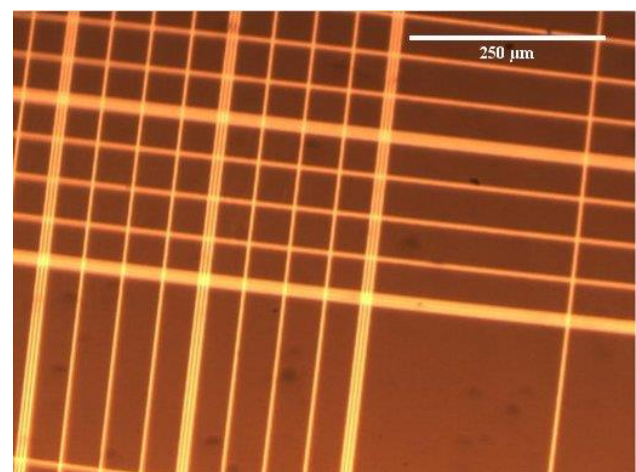
A simple Graphical User Interface (GUI) with start button was created to operate the autofocus system. Once the button was pressed, the system starts with image acquisition, determination of contrast value, $C(i)$ and stage moves up until the focused image was found.

Figure 3 and Figure 4 show images of hemocytometer without and with yeast cells respectively. The images without yeast were viewed at $10\times$ magnification objective lens meanwhile the images with yeast were viewed at $20\times$ magnification. Higher magnification is required to view the image of yeast cells as the diameter of the cells is small ($\approx 8\mu\text{m}$) However, the yeast cells in the image were unobservable due to the improper preparation process. The cells were not gone through washing process to remove unviable cells and debris.

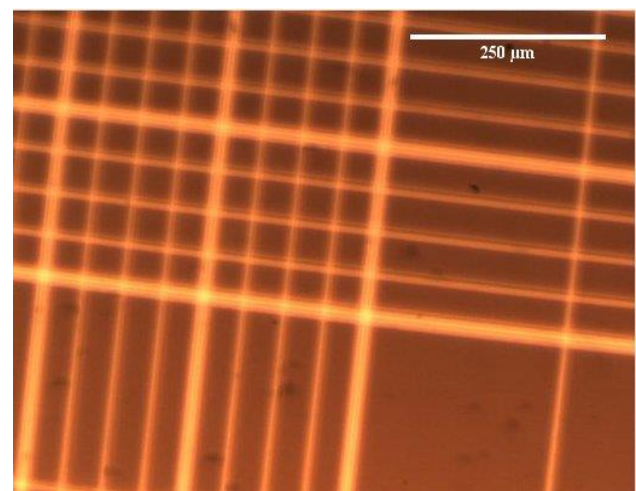
The C values for those images are calculated and tabulated in Table 1. As mentioned earlier, highest value indicates clearer image.



(a)



(b)



(c)

Figure 3 The images of hemocytometer without yeast cells under three conditions; (a) Under focused, (b) Focused, (c) Over focused

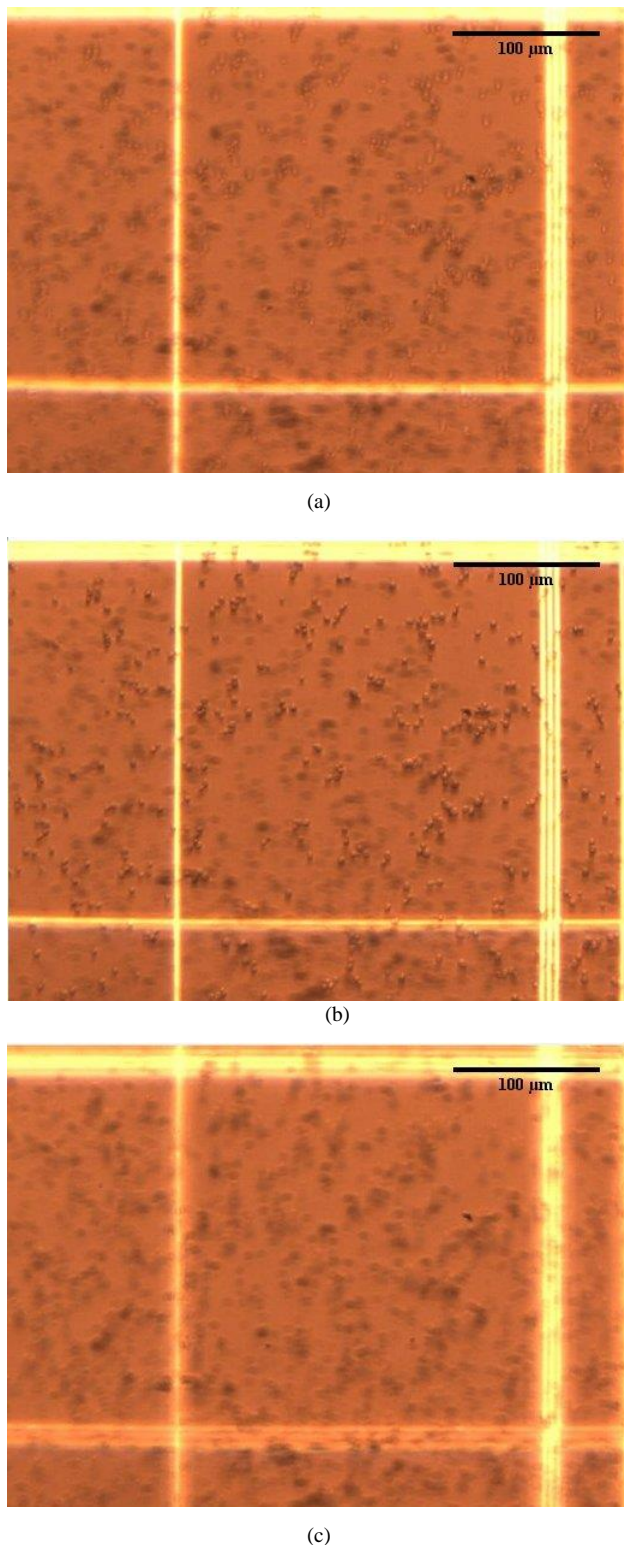


Figure 4 The images of hemocytometer with yeast cells under three conditions; (a) Under focused, (b) Focused, (c) Over focused

Table 1 Contrast value of each image a, b and c

Cell Images	Contrast Value (C)	
	Without yeast cells	With yeast cells
Under Focused (a)	116	146
Focused (b)	244	158
Over Focused (c)	171	154

Based on Table 1, as no yeast cells were loaded into the hemocytometer, the contrast value for focused image recorded a reading of 244 that is the highest compared to under focused and over focused with contrast values of 116 and 171, respectively. Meanwhile, as for hemocytometer with yeast cells, the focused image recorded the highest contrast value (158) compared to under focused and over focused images that recorded contrast values of 146 and 154, respectively. Thus as a result, the developed system displays the image with the highest contrast value at the GUI picture box, i.e. in this case the focused image is selected and displayed at GUI as shown in Figure 5.

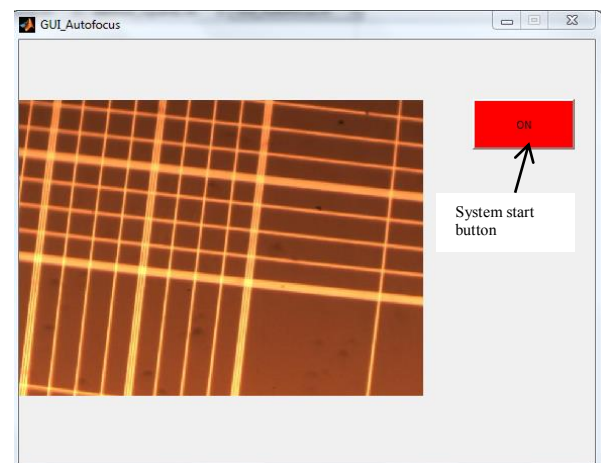


Figure 5 Focused image is displayed on GUI

The focused image takes less than 10 seconds to display on the GUI after start button is pressed. With this autofocus system, user can save time in getting clearest image. Once the image is obtained, it could be used for further analysis e.g. cell counting, morphological analysis in early disease diagnosis.

■4.0 CONCLUSION

In conclusion, an autofocus microscope which fulfills the objective stated in the study has been successfully developed. It is done by writing the contrast measurement algorithm as shown in Figure 2 in MATLAB, in which this algorithm is used to determine the rotation of adjusting knob as it will then captured the focused image and displayed the image on GUI picture box (as in Figure 5) automatically. By developing this system, the microscope becomes more user-friendly as any users (with or without knowledge of using microscope) could use it with hassle free. Besides, the system could avoid the microscope's lens and the hemocytometer/glass slide of specimen from broken. At the same time, it can protect the knob gear of microscope from damage due to harsh user adjusting the knob.

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