Short Communication

Automated Camera Lucida Method with Colored Images through Integration of Hardware and Software in Microscopic Zooming

Abstract

Automating the camera Lucida method which is a standard way for focusing microscopic images is a very challenging study for many scientists. Hence, actually combining hardware and software to automate microscopic imaging systems is one of the most important issues in the field of medicine as well. This idea reduces scanning time and increases the accuracy of user's results in this field. Closed-loop control system has been designed and implemented in the hardware part to move the stage in predefined limits of 15°. This system produces 50 consecutive images from parasites at the mentioned spatial distances in two directions of the z-axis. Then, by introducing our proposed relational software with combining images, a high-contrast image can be presented. This colored image is focused on many subparts of the sample even with different ruggedness. After implementing the closed-loop controller, stages movement was repeated eight times with an average step displacement of 20 μm which were measured in two directions of the z-axis by a digital micrometer. On average, the movement's error was 1 μm. In software, the edge intensity energy index has been calculated for image quality evaluation. The standard camera Lucida method has been simulated with acceptable results based on experts' opinions and also mean squared error parameters. Mechanical movement in stage has an accuracy of about 95% which will meet the expectations of laboratory user. Although output-focused colored images from our combining software can be replaced by the traditional fully accepted Camera Lucida method.

Keywords: *Automatic focusing, camera Lucida, closed-loop control, edge intensity energy, optical microscope, the stack of images*

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Introduction

Microscopies images are one of the most usable images for pathological studies, diseases diagnosis, and medical experiments. In these images, different technology gathered to have a focused image. However, it is obvious that, based on samples volume, every image could be focused only on some parts of it and not for complete. In previous studies, it is mentioned that "detailed description" of these images is important because of the nature of their analysis.[1-4] Different microscope types present various images of samples for better consideration of them.[5-7] In many studies, specialists want to study different focused parts of the sample in one image to analyze details.[5] However, it could not be achieved because of the different depth of the samples parts. They are composed of different parts which have various depths. Hence, specialists could not achieve all focused parts in one image unless doing it through handmade images with camera Lucida method.[8] It could be done manually with time-consuming, human errors, and boring that is painting of monochromic handmade images. Despite these disadvantages, these images are focused on different parts. A specialist must change the microscope setting and complete the painting picture through stages movement. This method is Camera Lucida, which is an acceptable method to have these images.[8,9]

The implemented system is capable of presenting microscopically colored focused images. You can focus on images in its desired internal parts too. This process is completely automatic. This is a challenge in microscopic images. Camera Lucida is a gold standard method for this aim.

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However, it is a manual painting monochrome picture of a sample with no quality measurement. It must be mentioned that this manual method could not present images with focused special internal parts. So having automatic "digital" "focused" "colored" images with capability of measuring parameter of resolution are the good outcomes of this project.

Automatic-focused microscopic images would be much useable for specialists. Although many recent studies are working on increasing the quality of images, it could not be achieved as an automatic colored image with focusing on many subparts.[10-12]

Recent studies show that accurate scanning and creating high-quality images of microscopic samples are important for diagnostic applications. One of the challenges in microscopic images is a lack of accurate access to details at the full slope.[4,7] Recent research and inventions include systems with specific automation applications such as automatic slide scanning to access all regions and slide subsets in focus to overcome this challenge.[3]

Methods

Camera Lucida is a standard tool used as an optical device for drawing images in the microscope. This method helps to see both a sample on stage and drawing paper. By this method, they could be superimposed in one field of view (FOV). For this method at the side of the microscope place, a paper with a pencil on, lying beneath the mirror. Temporarily, the user place a small piece of paper on the stage to covering the specimen sample and block out the image and bring the Camera Lucida top lens into position and look through them. Then, the user must move the stage to have a view of focused parts of the sample. At each focused position, the focused part of the sample must be painted on the paper by the user. This manual method could be time-consuming, boring, and mid-human errors. Although resulted images are monochromic which have no intensity nor color information. However after these disadvantages, the camera Lucida idea is a simple way to paint a three-dimensional sample in two-dimensional paper with focusing subparts of the sample which has a different depth. Hence, in this paper, we have tried to automate this system and have automation-colored focused microscopic images. Based on our result, it could be automatic-colored version of standard method camera Lucida with much quality. Hence, the manual camera Lucida idea could be used to automate system movement and capturing the digital-focused colored image. Numerical results and specialist opinions show that the suggested system works even better than the manual method. Although, one of the other advantages of the presented system is that, after focusing the image, the user can select internal part of previous view as a new FOV to focus. Automatically, it focused based on the standard camera Lucida method but with more resolution and in color level. This subsurface of the image is focused like the suggested method and could be down sampling and fractal interpolation. This focused part could be placed in its position of the initial image. However, it must be mentioned that if the subpart has a very low surface with high downsampling, the resolution could not increase well.

We have designed and implemented a developed microscope with closed-loop control of a motor to move the stage in predefined fine intervals. A servo motor MG945 has been attached to fine adjusting screw on one side and at another side, an encoder KY-040 has been attached. On another side, an encoder has been measured motor rotation degrees then these data are used to control motor for stage movements. The motor starts to move up or down based on user selection in software, servo motor rotation degrees have been controlled based on predefined input pulse width. Hence, in predefined positions, images have been taken and archived. These images have been captured in predefined positions or even by user-selected locations. In every cycle of the stage movement from down to up, 25 images were captured. Raspberry Pi camera is used to capture images and videos of the samples. These images are sent to a Raspberry board for processing.

In this study, slides of different specimens like Dicrocoelium dendriticum (a kind of Parasite) with different degrees of unevenness have been used. The picture of this parasite is shown in Figure 3 In this regard, the specialist parasitologist was asked to determine the areas that are different in terms of unevenness to evaluate the performance of the system. Images that have been approved manually by the user are also prepared and compared with software results. The depth limit range of the samples was set by Motic software. Motic is the application software in this field. This range which is required to examine their components was 30–360 μ.

However, based on the amount of motor movement error, which is equal to a maximum of one micrometer, it is clear that this error does not change the overall performance of the system, and components with different roughness will be imaged in their respective places.

In our design, the stage moves in three dimensions to capture accurate parts of the sample to see. Their movements are about in micrometer ranges through DC and servomotors. Using an encoder in closed-loop control taking images could be done incorrect positions. We have successfully designed and test the system with pleasure results. This system is evaluated by caliper Digital in mechanical movements, standard protocols, and accepted reference images with specialists. To minimize the human error in using the caliper, we test our system several times with different samples and our reported measurements are the average of recorded data. Furthermore, we try to do measurement based on the instrument manual user. The schematic scheme of this system is shown in Figure 1. It
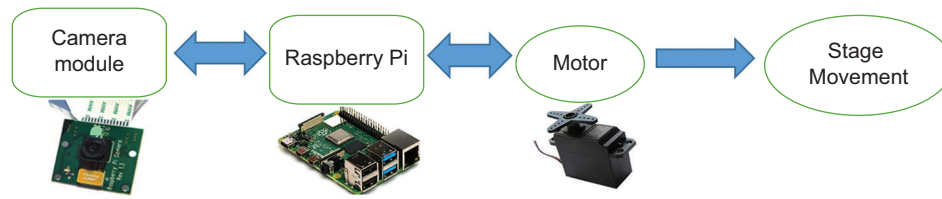
Figure 1: Our implemented system, its components and connections
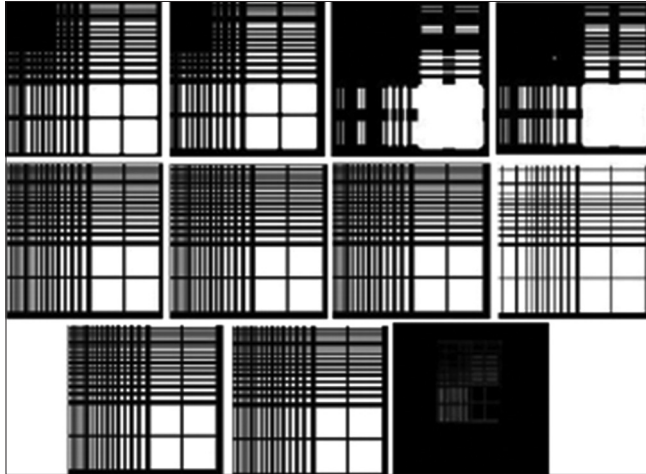

Figure 2: The first and second rows are references images, in the third row left image is the original image, the center image is the output image, and the right image is a different image between the two

represents the different parts and their connections to have good performance in our design.

While developing microscope in hardware part of our implemented system, we have improved system in software part for gathering datasets. In our study, we have designed a Graphical User Interface (GUI) to control this developed microscope in different intervals. Furthermore, the operator can see the sample in various fields of view through stage movements and even take a picture at any time that prefers. Then, different images through movements have been used to reconstruct an image with the most focused parts in many windows of it. This has been achieved in our study by choosing focused windows of captured images and pushes them into one image in their accurate coordinates. This idea was based on acceptable manual method "Camera Lucida." That was wonderful because it could help specialists to have automated colored images with approximately all focused parts, which was a good idea to automate camera Lucida method.

For having better evaluation of results, appropriate reference images with horizontal and vertical lines have been used. In this image, 16 areas have been selected and 8 different images have been created from these 16 areas by creating blurring in 14 different areas by opaque filters.

For every sample, eight images are given as input to the system, and its output image is compared with original one without blurring.

The subtraction of these two images shows good performance of combined program in creating an image with a resolution of images close to the original image without blurring.

Figure 2 shows reference images, output images, and different images between original one and suggested system output. The suggested system output is reconstructed from blur images with idea of camera Lucida method.

To test our implemented system, 400 microscopic parasite samples have been prepared, and their captured image resolution has been evaluated by edge strange intensity parameter. This parameter is mentioned as relation 1 to evaluate the image-focusing quality:

$$Q^{AB/F} = \frac{\sum_{m=1}^{X}\sum_{n=1}^{Y}(Q^{AF}(m,n)w^{A}(m,n) + Q^{BF}(m,n)w^{B}(m,n))}{\sum_{m=1}^{X}\sum_{n=1}^{Y}(w^{A}(m,n) + w^{B}(m,n))} \tag{1}$$

Where $Q^{AF}(m,n) = Q_{g}^{AF}(m,n)Q_{\alpha}^{AF}(m,n)$ is containing edge information of the image, $Q^{AF}(m,n)$ and $Q^{BF}(m,n)$ are criteria of image boundaries with weight $w^{A}(m,n)$ and. $w^{B}(m,n)$ X and Y stand for dimensions of the image.

An expert pathologist helps us to check focused images. Based on his adjustment, at the center of stage movement limit, captured image has higher resolution and by move far from this point, image resolution is decreased.

Figure 3 shows focused parasite image by user and output automated program image based on camera Lucida method. Due to the simulation camera Lucida method in this study, obtained result has acceptable accuracy, which is approved by experts too. Although calculated numerical results show that, this system can focus different areas of samples with various roughness.

Table 1 presents the results of the system review to evaluate it according to user's opinion at four levels. The value of mean square error was obtained from final image of software. Mean squared error is also calculated for user-selected image according to the following equation.

Figure 4 shows the edge-strength intensity parameter as software output for different images. The results show changes in this parameter for different positions. These graphs are for 1–25 images of the user-adjusted focusing
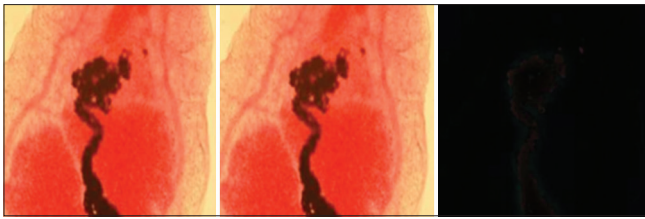
Figure 3: The left image is the focused image by the user, the center image is the output image of suggested software, and the right image is their difference

position at the center and the images taken at other steps. Edge-strength intensity is a good parameter to introduce image resolution. As it can be seen in Figure 4, for image 13 which is in center position of stage movement limit, this parameter has maximum value. This position is where the user selected as focusing position for that sample. So by moving far away from this point in any direction, this parameter goes to decrease.
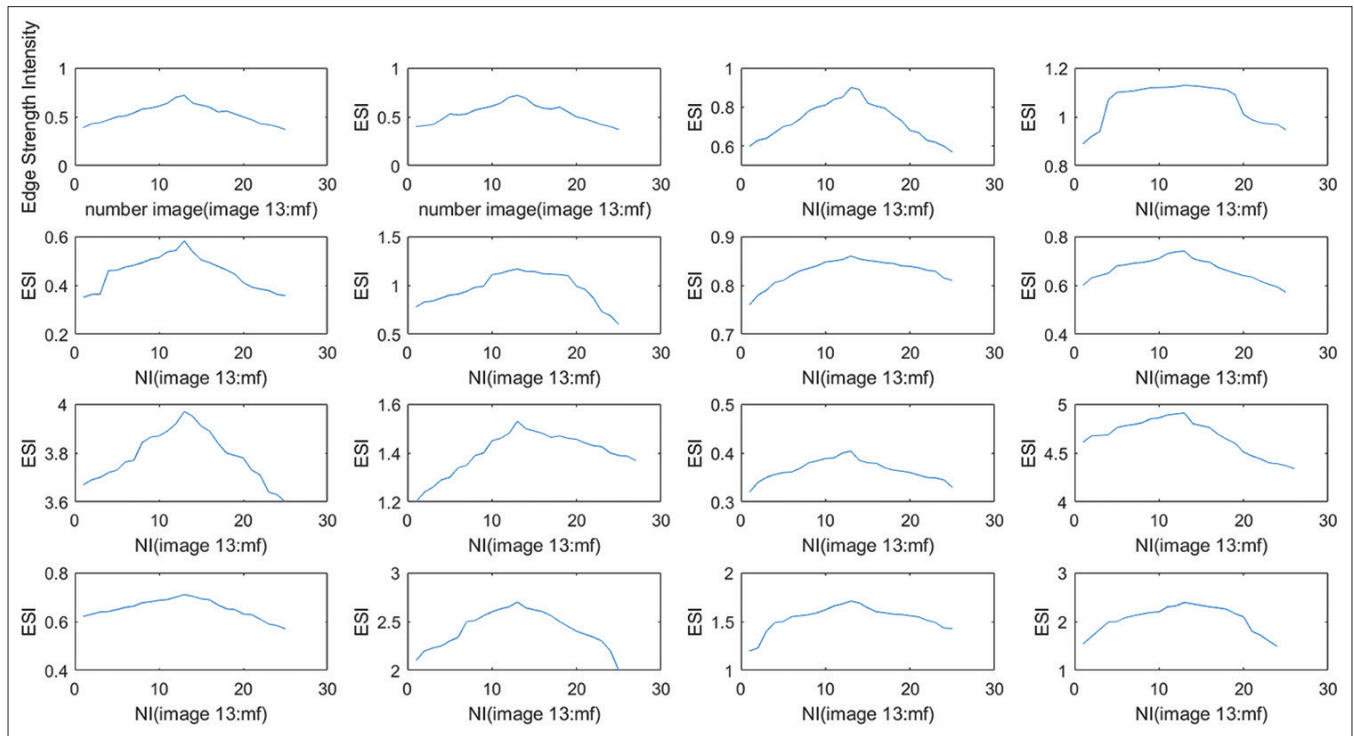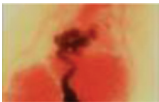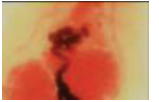

Figure 4: Edge strength intensity of 16 samples from all 400 laboratory samples, vertical shows the value of edge strength intensity, and the horizontal axis shows the number of images in every step while staging movement

Table 1: Automated focused image versus manually focused one				
Software output image and manually focused one	**Level of stage movement**	**MSE**	**Degree of motor movement**	**Parasite images**
	1	37.8	45	
	2	40.5	90	
	3	39.5	−135	
	4	40.2	−180	

MSE: Mean squared error

Results and Discussion

The proposed system is combined of hardware and software device with very simple compatible using. In our designed GUI, 16 submenus were introduced which include all control operations such as motion of screening stage, zooming images, transferring and storing images, show samples images through stage movement in the monitor, and capturing online images. The results of combining the performance of the purposed system which is presented in this paper are provided an opportunity to simulate Camera Lucida method with acceptable comparable colored results. These images have higher quality in detail. This system prevents time-consuming and user boring with low cost versus manual standard camera Lucida method.

Conclusion

The proposed automated focusing images system is a user-friendly device in some developing and even developed countries. Usually microscopic images are focused manually via camera lucida method. This method reduces speed and accuracy when focusing has been repeated.

In this study, we have designed and implement a system for zooming images with different depths such as camera Lucida standard method idea. The final designed system includes a GUI with 16 menus and each menu includes different distances of the microscope stage, image capturing part, and zooming images part. Due to the assessment's obtained results in z-axis movement, the system achieves 95% accuracy, which expects laboratory users. The output obtained from our combined system can be replaced by traditional Camera Lucida method in parasite-colored images with better performance even with focusing more on additional inner areas.

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Conflicts of interest

There are no conflicts of interest.

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