©2025 Published in 11th International Symposium on Intelligent Technologies in Engineering and Science 16-18 November 2025 (ISITES2025 Diyarbakır - Turkey)

Embedded System-Based Autofocus Interface Design for Microscopes

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Abstract:

Optical microscopes are essential tools widely used in clinical and laboratory research. The quality of images depends on various software and hardware criteria, with the focus point being a key factor. Accurate focus, determined by movement along the vertical axis, is crucial for precisely observing microscopic details, particularly cellular structures. A properly focused image enhances research reliability and repeatability. This study developed an automation system integrated into a graphical user interface (GUI) to automatically determine the focus distance of optical microscopes using gradient and intensity functions. A motorized mechanical system controlled by an embedded computer moves the microscope stage. The GUI evaluates real-time images, which adjusts the system to the correct focus point. Calibration tests with a 0.01 mm spaced cover slip confirmed the system's high accuracy and precision, enabling a reliable universal autofocus solution for optical microscopes.

Keywords: Autofocus Algorithms, Microscopy Automation, Real-time Image Analysis, Digital Microscopy, Embedded Computer

1. Introduction

Microscopes play a significant role in observing and explaining phenomena occurring in various fields of science. It is widely used in industry, genetics, archaeology, geology, and biomedical [1]. In biomedical sciences, microscopy is crucial in characterizing various samples, from subcellular structures to organisms [2]. There are many types of microscopes according to their areas of use. An optical microscope is used depending on the environment's brightness level (light type). These are light, dark field, fluorescence, phase contrast, and electron microscopes. It is the most widely used and most straightforward type of microscope. They are the most commonly used tools in evaluating and examining tissues and cancer cells, especially in clinical and laboratory environments. Optical microscopes consist of a tube that mechanically carries the eyepiece and objectives, the arm that holds the microscope, the table, and the base that allows the microscope to sit on the ground. The arm for holding and lifting microscopes can be half-moon or straight [3]. At the same time, the mechanisms on the arm allow the object's distance to the tube to be adjusted. The microscope table is round or four-cornered, and the sample to be examined is placed on it. In some microscopes, the table is fixed; in others, it can move up and down. The optical part consists of lenses and eyepieces. The lens is the part of the optical part closest to the object. The objectives

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are fixed on a table under the microscope tube and can rotate around the center axis. It has different magnifications and is composed of many lenses [4]. On them are rovelver, which reports the magnification rates. The eyepiece forms the part of the optical part that is looked at by the eye and is located in the upper part of the tube. Its task is to enlarge the image of the object and correct some errors in the lens. Microscopes have single or double eyepieces. It allows the object to be illuminated in the lighting section. It consists of a mirror that reflects the light source onto the object and a capacitor that collects the light on the object. There are special diaphragms in the light source illumination that provide the necessary light and adjust this light. Mirrors mounted on the microscope reflect the rays from the source onto the object. In some cases, the mirror can be found inside the microscope. Blue, green, or matte filters that filter the rays coming from the light source are placed, and the unique and ring-shaped place under the capacitor provides the best image [5]. A diaphragm under the capacitor ensures that the light from the lamp reaches the capacitor at a specific rate, depending on its level. The condenser's primary function in a microscope is to collect and illuminate the light on the object. Capacitors, usually consisting of two lenses, enable up and down movement with the button and good focus of the light. The working principle of the light microscope is based on the light being focused by the capacitor, reflected from the sample, and refracted at the focal point of the lenses, creating an image on the retina. Light is first refracted in the objective lens. With the second refraction of the ocular lens, an enlarged image of the sample is formed on the retina. As the objective lens magnification increases, the visible surface area decreases, allowing the sample to be examined in detail [6]. There are two important concepts related to imaging in microscopes: Magnification and resolution. Magnification is a measure of how many times the sample is enlarged. Multiplying the magnification ratio of the objective lens and the magnification ratio of the ocular lens calculates the magnification value of the microscope [7]. Resolution is defined as the separation of power. It measures how images of different points are seen separately from each other in the microscope. The resolution of the microscope varies depending on the wavelength of light. Another critical concept in microscope imaging is contrast. In microscopic images, a color contrast between the sample and the background is necessary to distinguish the sample from its surroundings.

Although the qualities of optical microscopes have improved from the past to the present, their working logic has generally remained the same. While images are obtained in microscopes, information resulting from the interaction of medical samples placed on the sample plane with light is collected. Obtaining the highest level of information from the samples to be displayed depends on optimizing many parameters. Generally, the main problems are limited field of view and depth of field, manual control of focus, and high cost. The image is obtained by passing light through the lens system and reflecting it on the screen. The resulting image is formed at a fixed distance from the lens, depending on the distance of the lens to the object [8]. There are two ways to focus. First, the lens system is kept constant and adjusted according to the distance of the object from the lens system. The second way is to focus by changing the distance of the lens system to the object.

It is crucial to determine the focal point correctly in image quality. This study used focus algorithms to control focus as a focus criterion. In this study, a GUI and automation system was developed to determine the automatic focusing distance of optical microscopes. Focus control is provided by focus algorithms. A motorized mechanical system and a corresponding electronic system have been designed for the vertical axis movement of the microscope table. The system includes circuits consisting of the stepper motor, motor driver cards, and a power supply. In the real-time system, the embedded computer and GUI work synchronously. A 0.01 mm spaced calibration target was

used as a reference image. This study developed a universal autofocus method for conventional optical microscopes, providing focusing with high accuracy and precision.

2. Materials and Method

2.1. Optic System

A microscope with a CMOS-3.0 USB camera was used in the optical system. The image resolution is 5 Megapixels. Calibration coverslips with 0.01 mm intervals were used as reference images. Objective lenses with four different magnifications of 10x, 20x, 40x, and 100x were used during the experiments. In this way, observations could be made to varying levels of detail. It is optimized with the high-quality glass lenses and special coatings used and offers clear and sharp images. The eyepieces provided a 10x magnification ratio, supporting images to appear larger and clearer. The lighting system is LED-based, and the light is focused effectively on the object with the capacitor.

2.2. Mechanical System

A mechanical system specially designed for the stepper motor used in the electronic system has been developed. The system's main components are designed in the form of closed cubes at the top and bottom with a superstructure suitable for the stepper motor socket connection. In addition, a ramp is included in the system to be placed under the microscope. The ramp is designed in such a way that the mechanical system remains stable.

2.3. Electronic System

The electronic system consists of a Nema 17 stepper motor, Arduino Uno board, A4988 motor driver board, independent stepper motor control card, and power supply. Control of the stepper motor was carried out in a special software environment; How much movement the engine will move at each step is determined by calculating the amount of advance of the engine. During the image acquisition process, the movement of the motor was optimized, the second's value was reduced, and calibration was performed again. The connections between the Arduino Uno board and the A4988 driver board were first designed using a special software application, and the connection diagram was arranged using this software. An independent stepper motor control card was also used in the system, thus providing a cleaner and more organized installation without the need to use breadboard. Arduino Uno and the microscope are connected to the computer via USB. Storing images and focusing control is done through the designed GUI; The user performs this process via a button. With this system, precise control of motor movement and focusing is ensured and the performance of the system is increased.

2.4. Focus Algorithms

Focus algorithms play an important role in evaluating the performance of a system or design to ensure control of focus as a focus criterion. These functions are used to measure the accuracy of

focusing in the field of image processing and microscopy. Image sharpness-based algorithms are used to measure the difference in sharpness between defocused and focused images. Edge detection and characteristics are poor in unfocused images, with unclear transitions between dark and bright objects. Focused images are images obtained by capturing the same scene at different focal lengths. The focus determination criteria used in this study are mentioned in the following sections.

2.4.1. Thresholded Absolute Gradient Function

The Thresholded Absolute Gradient function basically works on the principle of measuring the gray level differences between two pixels in an image. This function is used to detect large differences between pixel values and is effective in determining focused regions [9]. The mathematical equation of the Thresholded Absolute Gradient function is given in Equation 1.

$$AG = \sum_{M}\sum_{N}|f(i,j+1) - f(i,j)|$$

$$|f(i,j+1) - f(i,j)| \geq T \tag{1}$$

2.4.2. Square Gradient Function

The Square Gradient function also calculates the differences between two pixels, but unlike the MG function, it aims to calculate larger gradient values. This method helps to identify focused regions more clearly [10]. The mathematical equation of the Square Gradient function is given in Equation 2.

$$SG = \sum_{M}\sum_{N}|f(i,j+1) - f(i,j)|^2$$

$$|f(i,j+1) - f(i,j)| \geq T \tag{2}$$

2.4.3. Brenner Function

The Brenner function is used to detect focused regions by squaring the difference between two pixels. A large gradient difference between pixels indicates that the focus is good [11]. The equality of the Brenner function is given in Equation 3.

$$B = \sum_{M}\sum_{N}|f(i,j+2) - f(i,j)|^2$$

$$|f(i,j+1) - f(i,j)| \geq T \tag{3}$$

2.4.4. Tenengrad Function

The Tenengrad function calculates the gradient magnitude of the image through its convolutions with the Sobel function. This function determines focused regions and evaluates the focus quality [12]. The equality of the Tenengrad function is given in Equation 4.

$$T = \sum_{M} \sum_{N} g(i,j)^2$$

$$g(i,j) = f_x^2(i,j) + f_y^2(i,j) \tag{4}$$

These functions have been used to objectively assess the focusing quality of images taken under the microscope. The advantages of each function are used to make up for the shortcomings of the other function and to determine the most accurate focal point.

2.5. GUI Desing

The GUI transfers images from the microscope to the user in real-time. This interface makes it easy for the user to interact with the microscope, allowing them to manage operations such as focusing and improving image quality. The interface presents snapshots to the user in a large window and allows the user to perform various functions through the buttons on the right. The "Start" button activates the camera and starts the real-time image stream. The "Set Focusing Distance" button is used for the focusing process. This button moves the motor in certain steps, allowing photos to be taken at each step. If the user wants to reset the focus, the "Leave Microscope Distance" button stops the motor and initializes the focus setting.

To increase image clarity, four criteria are presented to the user in the interface: Thresholded Absolute Gradient function, Square Gradient function, Brenner function, and Tenengrad function. The user can get the focused image by choosing one of these criteria. Images evaluated and optimized according to the selected criteria can be saved with the "Save" button.

The GUI also works very flexibly in the process of connecting microscope cameras to the computer. USB microscope cameras are usually automatically recognized when connected to the computer, and the necessary drivers are installed. The movement of the microscope motor is controlled with the COM connection established between the Arduino Uno board and the computer via USB 2.0. The buttons on the interface are equipped with the necessary software to control motor movements.

Finally, when the user has completed all his operations, the "Shut Down" button closes the interface window, ending the program. This system provides the user with efficient and effective control to obtain the best-focused images of the specimen under the microscope. The designed GUI shown in Figure 1 allows the user to easily perform these functions. The developed GUI is shown in Figure 1.

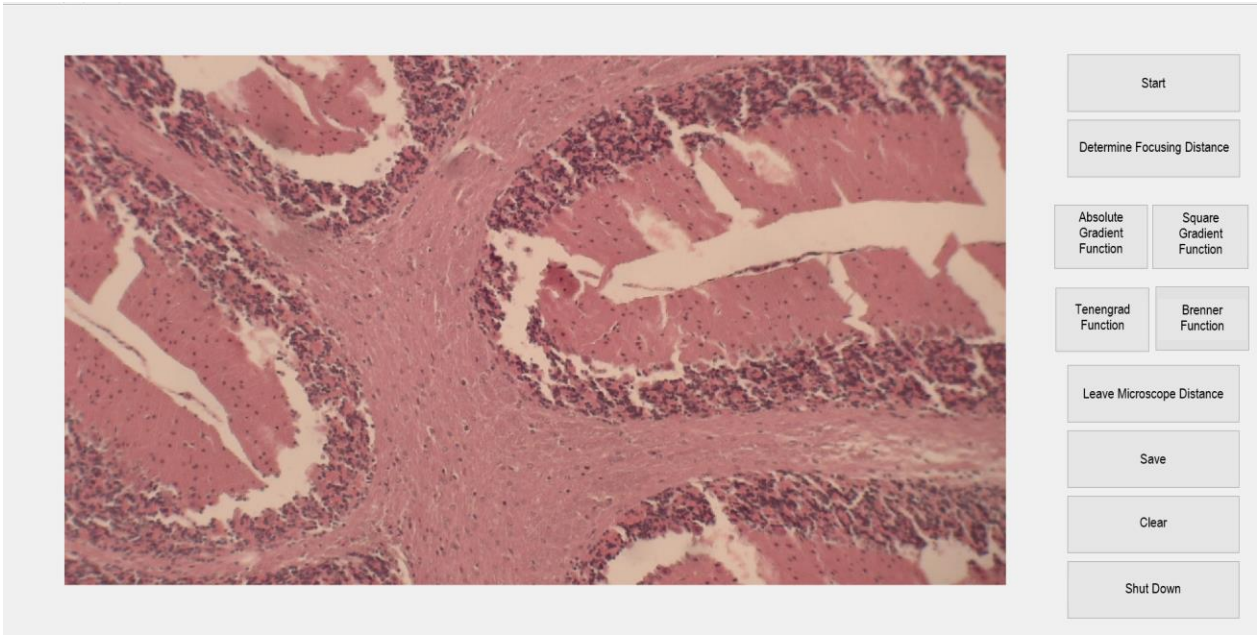

Figure 1. Developed GUI screen

2.6. Autofocus Process

Focus quality is directly related to the clarity and sharpness of an image. Sharp edge transitions and distinct color differences are observed in a correctly focused image. As the focusing quality decreases, edge transitions become smoother and color differences decrease. An edge detection filter is applied to the image to evaluate the focusing quality, and the color transition levels between pixels are measured. High pass levels indicate well-focused areas, and low pass levels indicate poorly focused areas. However, comparing edges on the entire image may reduce processing speed and accuracy. Therefore, it is necessary to select specific areas to focus on. Especially when examining under a microscope, as the magnification coefficient increases, ensuring clarity in the entire image becomes more challenging. For example, on non-uniform crystal surfaces, some areas may be close to the lens, and some may be far away. In this case, only a specific area needs to be focused. If a large surface needs to be imaged clearly, the multifocal image fusion technique should be used. During the automatic focusing process, the sharpest image is obtained by making sharpness measurements in the determined focusing areas on the image. This way, the areas to focus on are accurately determined and optimized. Focus criteria, which are essential in determining the depth of focus of images taken from the microscope, are integrated into the interface. With the help of the designed algorithm, color images were first converted into gray level images. On these gray-level images, a user-selected function is applied to the gradient values of each pixel. These values are summed and divided by the total matrix size, and a graph is created with the sharpness value obtained as a result of this process. The algorithm determines the correct focus point by detecting this graph's local maximum and local minimum points. This determined value represents the most clearly focused image. Four criteria are presented to the user: Thresholded Absolute Gradient function, Square Gradient function, Brenner function, and Tenengrad function. When one of these criteria is selected, the application determines the focal

distance using the specified algorithm, increasing the image's focus quality. The GUI interacts with electronic and mechanical systems. The camera becomes ready when the “Start” button is pressed on the GUI screen. When the "Set Focusing Distance" button was pressed, the motor was made to take 30 steps forward. Photos are taken at each step. The engine stops automatically after 30 photos are taken. After the engine stops, one of the focusing criteria is selected. Following the chosen criteria, the best-focused image among 30 photographs is displayed on the screen. Information on which photo is the best in focus and the gradient value calculated according to the selected criterion is also included. The Square Gradient value obtained by pressing a function button chosen was calculated using a brain cell sample. This calculated value and the best-focused image obtained when the Square Gradient function is pressed are shown in Figure 2. At the same time, the local maximum and minimum points on the graph for the Square Gradient function are shown in Figure 3.

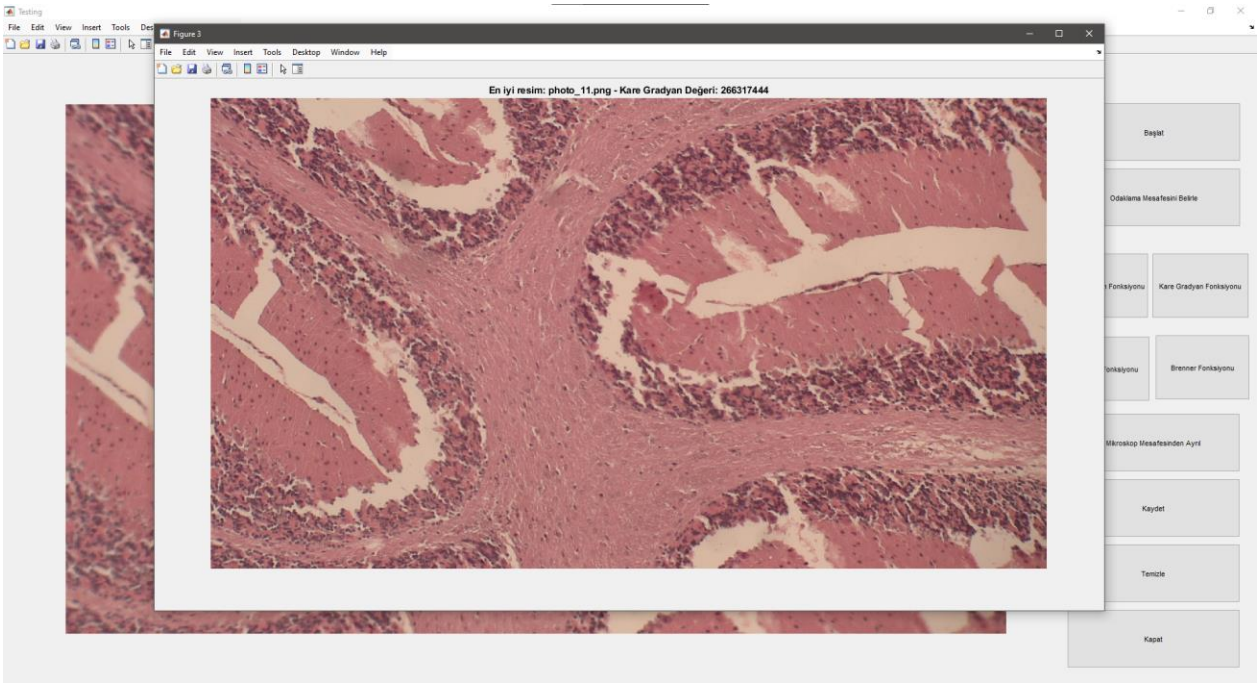

Figure 2. Demonstration of the image obtained by using the Square Gradient Criterion function of the designed automated system on the GUI screen

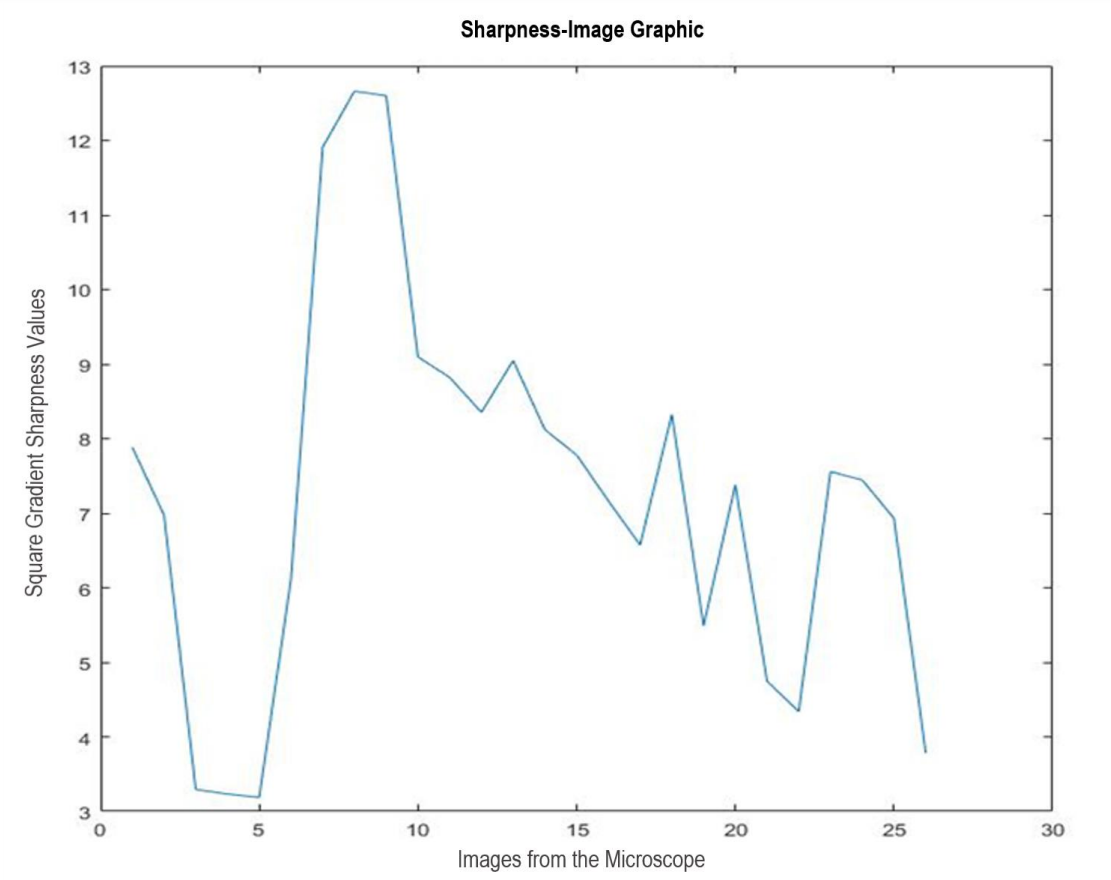

Figure 3. Maximum and minimum values of focus value calculated using the square gradient function

3. Results

Optical microscopes are critical tools widely used in clinical and laboratory research. Image quality depends on software and hardware compatibility. In this study, a graphical user interface (GUI) was developed that automatically determines the microscope focusing distance. Using gradient and tendency functions, images taken from microscope cameras were transferred to the interface and evaluated. The system works in real-time with a motorized mechanical structure and electronic components, providing high accuracy and precision autofocus. The interface allows users to select different focusing criteria and improve image quality. The application's modular structure adapts to various microscopes and imaging systems, making it easily adaptable to user requirements. While widespread use in industrial microscopy applications is targeted, future studies are planned to improve the user experience and optimize image processing algorithms. The general view of the system is shown in Figure 4.

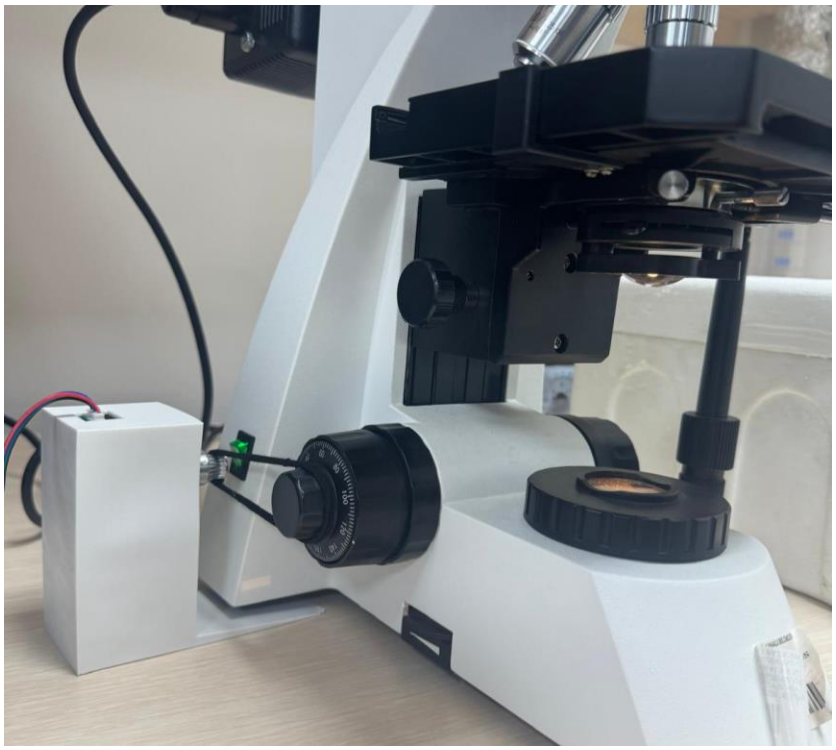

Figure 4. Integration of the designed system into an optical microscope.

Acknowledgments

This study was supported by TUBITAK 2209-A University Students Research Projects Support Program.

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