

Autofocus Web Application for Portable Darkfield Microscope

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Abstract

A darkfield microscope is required equipment for studying the structural and embryo development, which requires an expert in focus adjusting. The microscope weight cause inconvenience in carrying on the field and includes the high cost of the microscope. In this project, we develop an autofocus web application for a microscope using the contrast detection autofocus (CDAF) method in capturing images and calculating contrast value by finding the maximum sum of differences between each before and next pixel of the whole image which indicate the focus point. In building the web application using HTML, CSS, and JavaScript. Then connect to the Realtime-database feature of Firebase for communicating and controlling the NodeMCU board (ESP2866 V3 Wi-Fi) via Wi-Fi. NodeMCU board can adjust the stage by controlling the motor to autofocus the image. The web application design for smartphones uses a polymer lens (Epoxy resin) in each magnifying 10x, 40x, and 100x as an objective lens by replacing on webcam surface. Redesigning the microscope model to be portable using a light scattering technique to originate dark field property requires the sample to be in the middle of the slide, the skating model using the FreeCad program, and then printing out the 3D model. The image under defocus parameter ($D = -10$), the contrast value ($C = 253027.24$) after increasing defocus parameter the graph has an upward trend until ($D = 0$), the contrast value ($C = 635163.00$) then the graph shown downward trend comparing between ($D = 0$) and ($D = 10$). The maximum contrast value at ($D = 0$) proves the focus point.

In applying IoT to solve the difficulty in focus adjusting, this project is able to process in light microscopes in other formations. The quality of the image depends on the mechanical resolution, program setting, lens, and smartphone position.

KEYWORDS: Autofocus, web application, Darkfield microscope, Contrast detection

1 INTRODUCTION

An embryo is the initial stage of development of a multicellular organism, which has transparent properties. So a darkfield microscope is commonly required equipment for embryonic structural and development studies in embryology because the darkfield property causes a detailed and conspicuous image, which is convenient for correctly understanding

and describing the organelles and embryo development in each stage. The condition of using the microscope under darkfield property requires the expert to set up the pieces of light block filter and lens using the scattering of a filtered light beam to originate darkfield property. The cause of its fragile of lens, weight, and size cause inconvenience and difficulty in carrying on the field. Recently, the usage and development of computer processing ability have been applying various fields, including military, medicine, biology, and social media, including implementing image processing in autofocus.

Autofocusing is the advantage of improving image processing to generate fine and sharp images, which this technology has developed in digital cameras, smartphones, and webcams and not only for the development of computer processing ability but also the internet and device development in these years. The internet is a gateway between devices for transferring data via Wi-Fi signal to construct communication between devices into the IoT system or internet of things, the collective network of connected devices and the technology that facilitates communication between devices and the cloud. For example, Smart refrigerators, medical sensors, and fitness trackers, so deciding to use the advantage of computer processing ability, the image processing combined with IoT systems, the communication between interactive web applications and devices requires client, server, and device coding in contributing the autofocus web applications for the darkfield microscope.

Digital autofocus camera development usage in image processing techniques. The phase detection autofocus method (PDAF) uses sets of dedicated light sensors detecting refraction rays from the lens, then converting them into the signal to measure defocus distance. This technique takes less time to focus and works great for tracking objects but for the contrast detection autofocus method (CDAF), using the contrast between the edges in the image to find a focus point. Contrast detection analyses the contrast value on pixels on the camera's sensor and moves the lens back and forth until finding the right focusing point. This process takes more time to find the focus point, but great results with the non-movement object. But under the condition of developing a web application to apply with devices. The most simple autofocus technique is the contrast detection autofocus method (CDAF) because the phase detection autofocus method (PDAF) requires specially dedicated light sensors for measuring a defocused point, which is highly costly. Based on the background acknowledgment and issue. This article uses the contrast detection autofocus method (CDAF) with an IoT system to build an autofocus web application for the portable darkfield microscope to solve the difficulty in manual focus adjusting without expertise, including minimizing the weight and size for convenience in carrying on the field.

2 PRINCIPLE AND METHOD

Overview of processing and method

This project is cooperating of three sections, the autofocus web application using HTML and CSS to design the web page of the application, using JavaScript to control the processing and calculating. The Real-time database feature of Firebase for communicating between the web application and devices. A NodeMCU board is the microcontroller board for controlling the motor mechanic. The autofocus web application is designed for the smartphone, and to find the focus point, this web application uses the contrast detection autofocus (CDAF)

method.

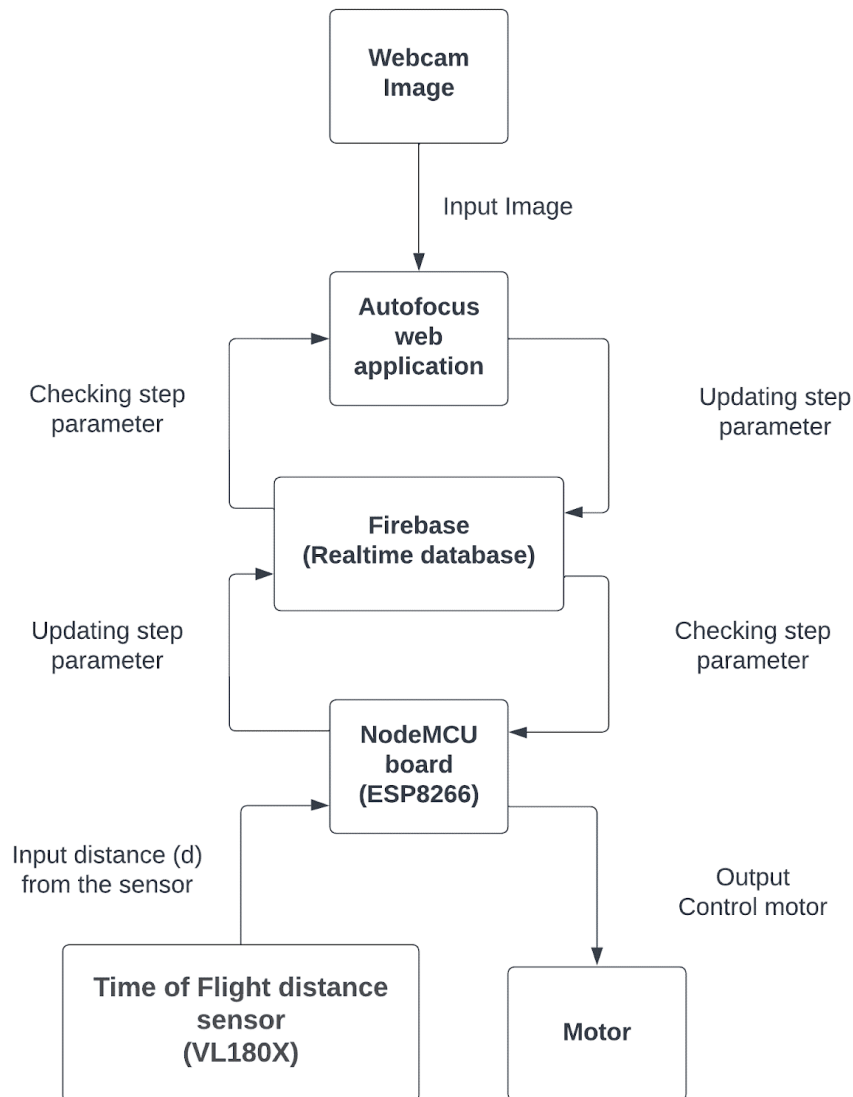


Figure 1: Overview of connecting between web application, Realtime database, and NodeMCU board

From the figure 1, The program will capture the recent image from the webcam and access the pixel data in arrays, then convert it to grayscale by changing the RGB value in pixel into the average. After changing the image into grayscale, access the pixel value and calculate the contrast value by finding the sum of differences between each before and following pixel of the whole picture. Then collect the contrast value in the dictionary type variable with the distance value from the real-time database as the key and the contrast value as the value in the dictionary and update the step parameter on the real-time database. The NodeMCU board will call the step parameter with the distance value from the time-of-flight distance sensor (VL180X) to check the condition to control the motor in the model shown at figure 2.

The process will redo over again until process all images at each distance, then find the focus point.

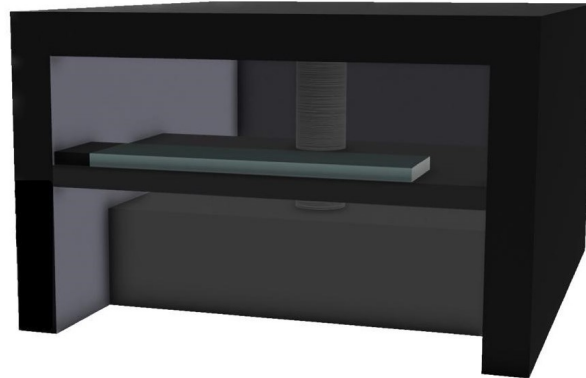


Figure 2: *Prototype model sketch*

Web page building

Build an autofocus web page using HTML and CSS to design the web page. The web page requires a monitor screen showing the real-time image from the autofocus image and the start button for the user to click to start autofocus shown on figure 3. However, designing the web page could be in any pattern or format, depending on the developer’s creativity.

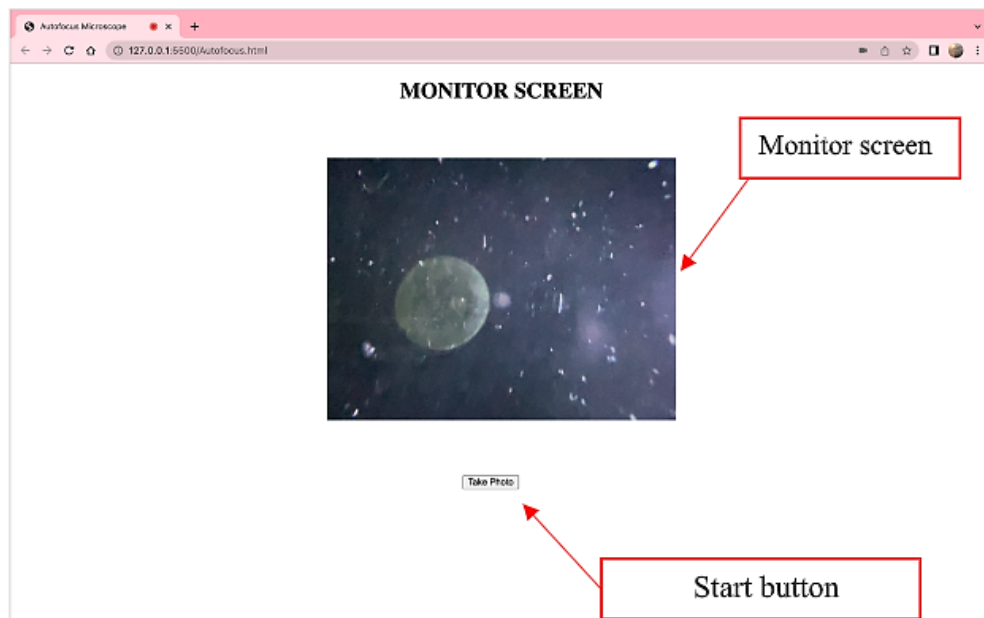


Figure 3: *The autofocus web application web page*

Contrast calculation

When clicking the start button on the web application at the first calculation, the web application will capture the image it will be present in the two-dimensional array format containing the rows and columns shown in figure 4. However, the array pixels have only one dimension in storing colour values in a linear sequence shown in figure 5, which starts to read the RGBA value at the first left top array at the corner and continues following the row (X-axis). This project uses the RGBA colour model for image processing, and the RGBA stands for red, green, blue, and alpha. Each pixel contains three colour channels, combining the three colours, red, green, and blue. The alpha channel presents the opacity in each pixel. After reading all RGBA values in each pixel and storing them in the array format to convert the image into grayscale, change the colour value in each colour channel into the average of three channels except the alpha channel, following the equation (1). Then update the colour value of three channels in each original pixel to generate the grayscale image.

$$\text{Grayscale value} = \frac{R + G + B}{3} \quad (1)$$

2 dimension array

X →

		Column 1	Column	Column	Column
Y ↓	Row 1	0	1	2	3
	Row	4	5	6	7
	Row	8	9	10	11
	Row	12	13	14	15

1 dimension array

	Column 1	Column 2	Column 3	Column 4	Column 5	...	Column 14
Row 1	0	1	2	3	4		15

Figure 4: Two-dimension array and one dimension

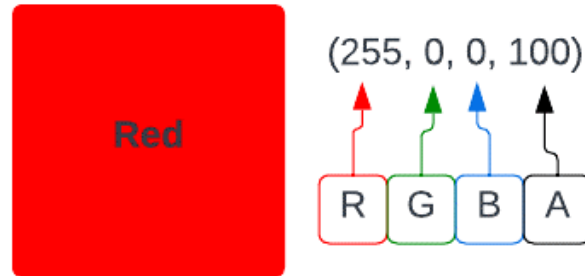


Figure 5: *RGBA palette of red colour in the pixel*

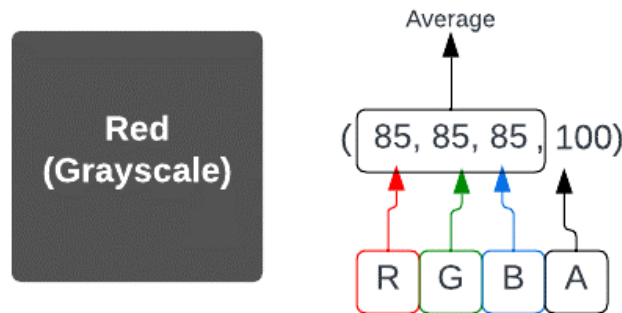


Figure 6: *Grayscale palette of red colour in the pixel*

After converting the RGBA image into grayscale, calculating the contrast value, find the sum of differences between each before and next pixel of the whole image following the below equation.

$$\text{Contrast value} = \Sigma \left| a_{\max(i)j} - a_{\min(i)j} \right| + \Sigma \left| a_{(i-1)j} + a_{ij} \right| \quad (2)$$

When the i variable is the columns, and the j variable is the rows in matrix $i \times j$. When the web application keeps processing each image to find the maximum contrast value from each distance, which proves the focus point. The NodeMCU will control the motor to move to capture the continued image, causing the blurred images in each defocus distance.

Proving the method

Proving method for the equation using python programming and OpenCV library when generating the grayscale image, then blurring the images with Gaussian blur function in each parameter with the absolute of odd number in the range $[-n, n]$, when is the positive number. The newly generated image will be even more blur from 3 to n and declare the list to contain

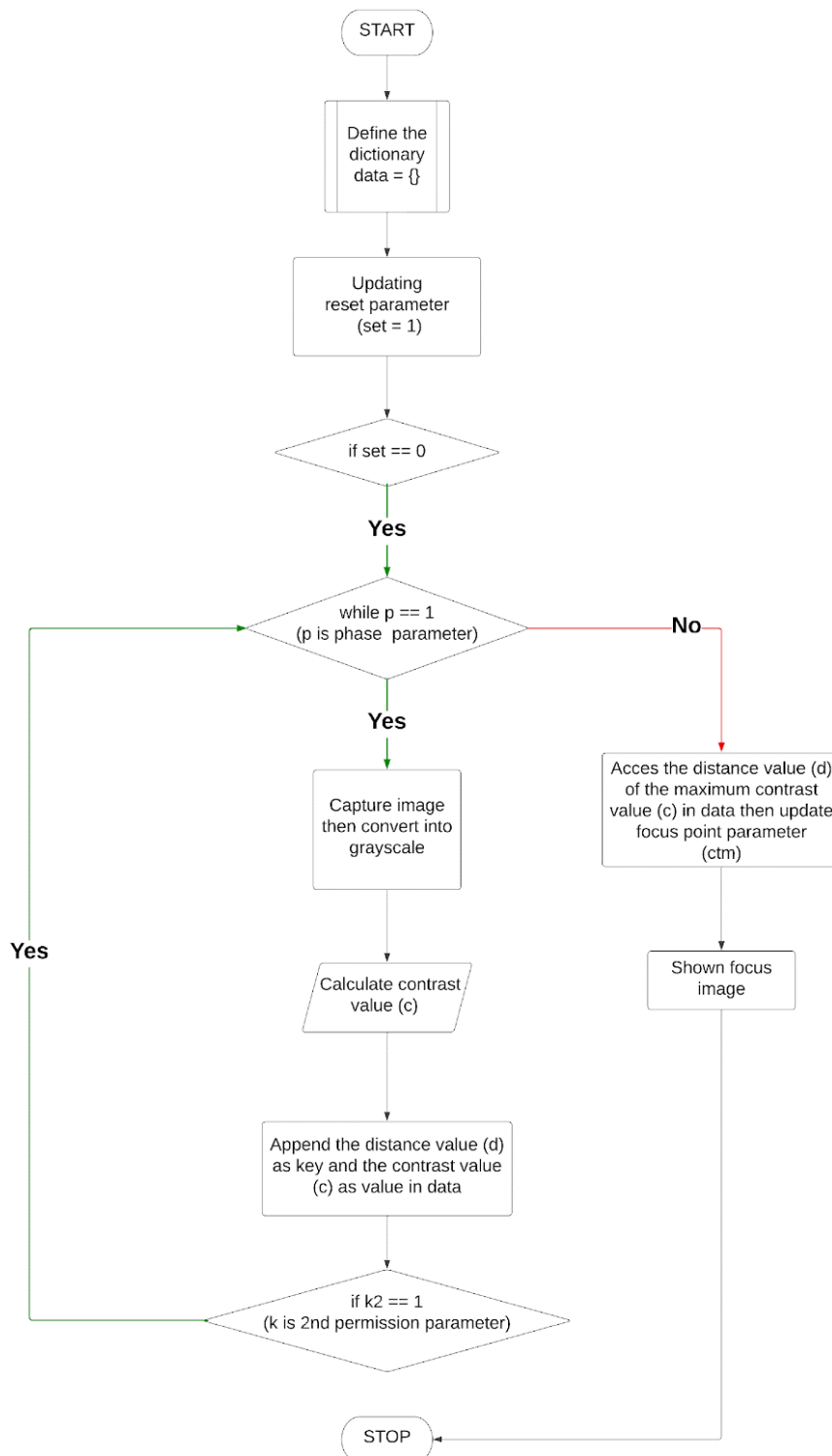


Figure 7: Web application processing flowchart

the contrast value by calculating the contrast value with the equation (2) and plotting the graph, the defocus (axis = X), and the contrast value (axis = Y).

3 RESULT AND DISCUSSION

From the image processing technique in converting the RGBA image into grayscale to calculating the contrast value shown in figure 8 on the right compared to the original RGBA image on the left.

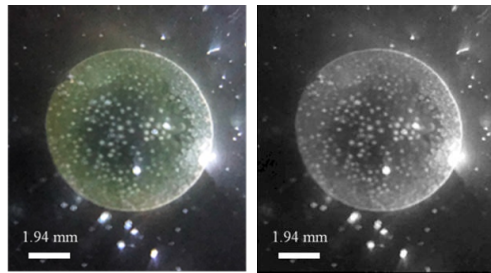


Figure 8: Original RGBA frog embryo image (Left), and Grayscale fog embryo image (Right).

The figure 9 below, the image under defocus parameter ($D = -10$), the contrast value ($C = 253027.24$) after increasing defocus parameter the graph has an upward trend until ($D = 0$), the contrast value ($C = 635163.00$) then the graph shown downward trend comparing between ($D = 0$) and ($D = 10$). The maximum contrast value at ($D = 0$) proves the focus point.

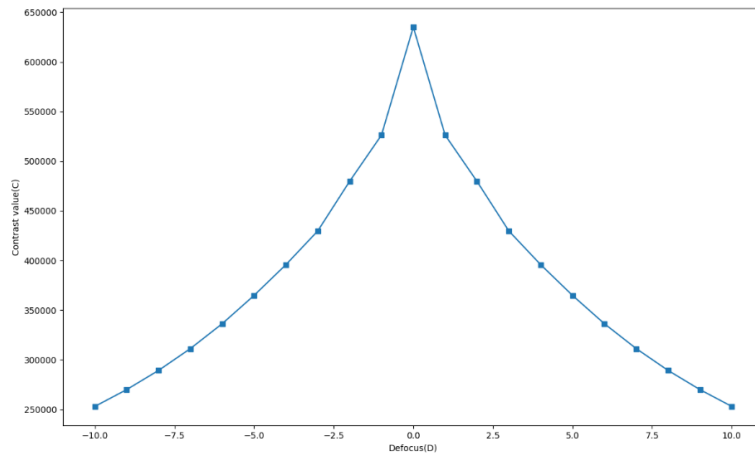


Figure 9: Contrast value with defocus

The proving method show that the sum of differences between pixel neighbourhoods indicating a significant to the contrast value, when the sum of differences between pixel neighbourhoods increasing shown that contrast value also increases and at the maximum of sum of differences between pixel neighbourhoods at ($D = 0$) is the focus point. The figure 10 shown the set of defocus image in each point on the graph.

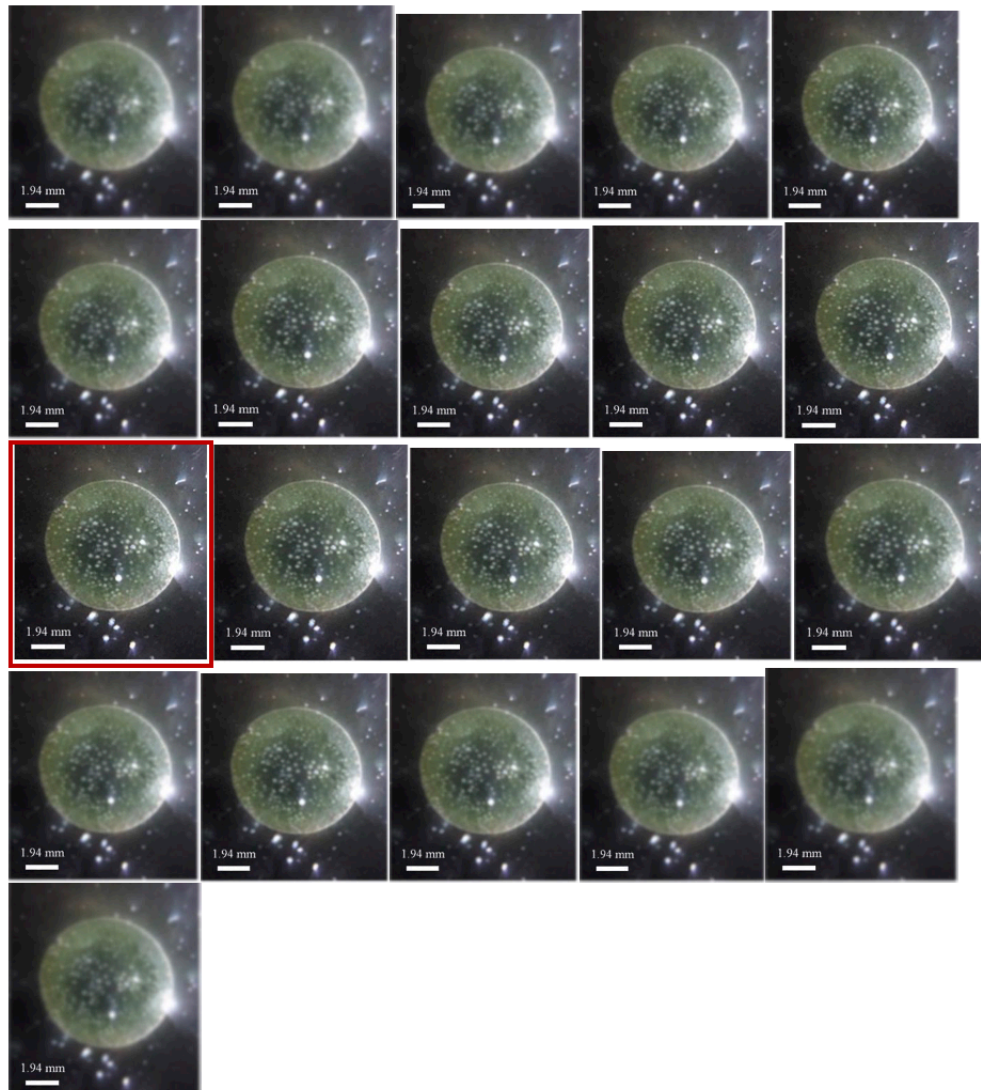


Figure 10: Set of blur frog embryo image in each defocus from -10 to 10 , following the alphabet (a - u), the border image (k), (defocus = 0) is the most in focus and sharpest image

Figure 11 compares the ostracod image from the original method and the autofocus web application. The image from the original method is more blurred than the image from the autofocus web application, including the small difference between each defocus image from figure 10. The Autofocus web application method still found the closest focus point indicating the efficiency and precision of this method.

4 CONCLUSION

Nowadays, IoT systems and image processing are developed and applied in various fields. Medical and biological research, which is used manually in focusing on traditional techniques, causes difficulty in achieving the best focusing position quickly and accurately and requires

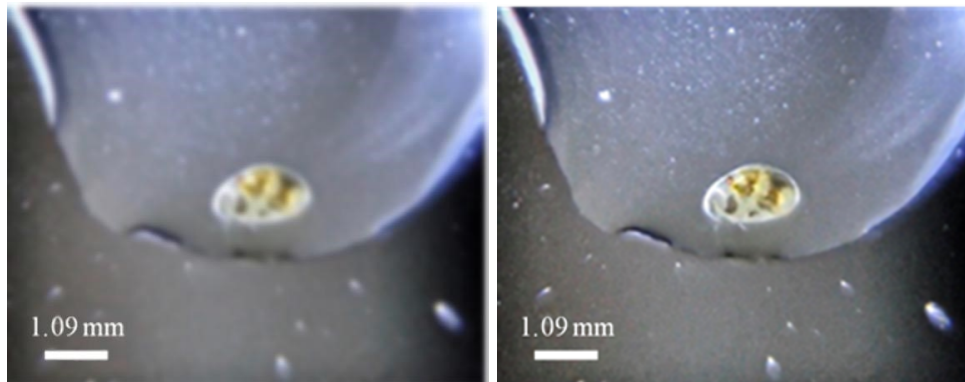


Figure 11: The ostracod image from original method (Left) and autofocus web application (Right)

experts with experience. But an autofocus web application applying IoT to solve the problem of focus adjusting, using the contrast detection autofocus (CDAF) method and image processing to find the focus point, which can find the focus point accurately and efficiently shown on the results. This project aims to develop a portable dark field microscope functionality by the web application with an IoT system for convenient user usage and carrying on the field.

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