Development of Automated Microscopy Using Neuro-Fuzzy Logic Controller

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***Abstract*— In this study, the researchers develop an automated microscope wherein each knob has its respective motor that is connected into the motor driver to control the movement of each axis. Fine adjustment will be the one that is responsible for autofocusing. Passive autofocus systems that consist of blur detection method, where brightness and edge are determined by Laplacian Operator, was also used. The mechanism of each motor, assigned in the movement of x, y, and fine adjustment, is specified by Neuro-Fuzzy Logic Controller through microcontroller (Arduino). This device can be controlled using a portable user interface consisting of buttons, manually or automatically, together with the live feed. The whole project is conducted in Processing Programming Language. Knowing that the manual or conventional process of focusing is both labor-intensive and time-consuming. Consequently, this device offers a low cost yet yields an efficient and accurate result.**

***Keywords*—Neuro-Fuzzy Logic Controller; Microcontroller; Laplacian Operator; Processing Programming Language, Automated Microscope**

I. Introduction

There are several microscopes to be used in medical industry. One of them, is the binocular microscope where you can view up to 1000x or even 2000x magnification. This type of microscope has a lens system, illumination structure, and a body containing the body tube, base, and nosepiece. There are also knobs for positioning and focusing a specimen in a microscope. But, knowing that the manual or conventional process of focusing is both labor-intensive and time-consuming, this device offers a low cost yet yields an efficient and less time-consuming way of focusing.

Autofocusing is the moving of the lens in and out automatically until the sharpest possible image of the subject is attained. There are two types of autofocus systems: active and passive autofocus. Active autofocus systems uses emitting signals, usually a red beam, to determine the distance of the object and measuring the delay of the reflected signals. On the other hand, the passive autofocus systems need information from the acquired image data to analyze if the position of the lens is right. As for the microscope, it is not ideal to use active autofocus since sending patterns and getting its reflected one would be difficult. Moreover, since glass cover slips will be used, some patterns will be reflected before it reaches the main object. At this situation, the active method would calculate the wrong distance. Thus, this will use passive autofocus.

Neuro-Fuzzy Logic controller was used for the automation of the microscope which is incorporated in the GUI. The result of a specific specimen is also generated in a GUI together with the live feed. Servo motors will be moving in accordance to the live feed as image processing is running.

II. Literature Review

Binocular microscope has a lens system, illumination structure, and a body containing the body tube, base, and nosepiece. The holder of the eyepiece or also called as the ocular, the coarse and fine adjustment knobs and the objectives are the main components of the lens system. On the other hand, the illumination structure has the condenser, light source, and field and iris diaphragms. Sample specimen to be tested are placed on the motorized stage. This type of microscope has two lens system: the objective which is located near the specimen; and the ocular which is located near the eye piece.

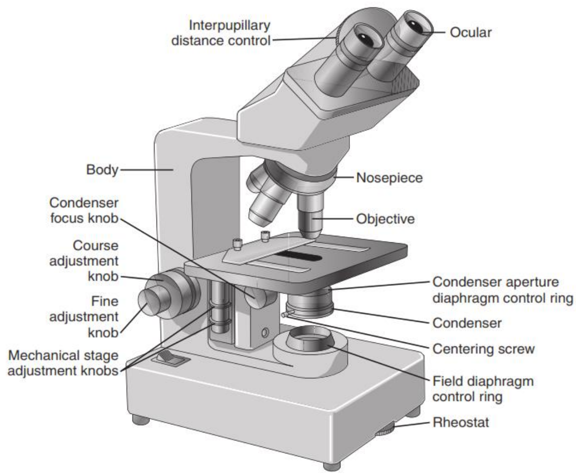


Fig. 1 Parts of a Binocular Microscope
(Source: Strasinger & Lorenzo, 2014)

Passive autofocus systems consist of edge detection method. It is a tool used in image processing, essentially for feature detection and extraction, which main purpose is to identify points in a digital image where brightness of image varies sharply and find discontinuities. It employs different differential operators used in detecting largest intensity changes between gray scaled pixels depicting edges. Edge detection is known for the process of looking for sharp details in an image. It reduces the amount of data in an image contains without changing the significant structural features on that image. Generally, edges are pixels that borders different regions of an image with different changing pixel amplitude attributes. Edge detection operators used to test edges through altering the nature of an image. It can be classified into two main types. One is the first derivative-based edge detection operator. It computes for the image gradient values. These operators are Roberts, Sobel and Prewitt. The other one is Second derivative-based operator which seeks in the second derivative zero-crossing to edge detection. These are Laplacian of Gaussian and Canny Operators.

Moreover, a blur detection using discrete wavelet transform was presented in an image quality assessment based on perceptual blur metric. The proposed method was to merge the quality score obtained by the index of structural similarity with the blur amount measured in order to obtain a quality measure that takes account the perception of blur. The idea was to extract local structural attributes of the image from which each block is described by its brightness, contrast and structure [1].

Neuro-fuzzy Controller utilizes the neural network learning techniques to solve the problem of tuning the membership functions of fuzzy logic controller while keeping the semantics intact [2]. The Fuzzy Logic is a problem-solving control system that has a tactic to mimics how a person would make decisions, only much faster and it can be implemented in hardware, software or combination of both. Moreover, Fuzzy Logic requires Fuzzy Rules and Inference to convert fuzzy input sets to crisp output [3].

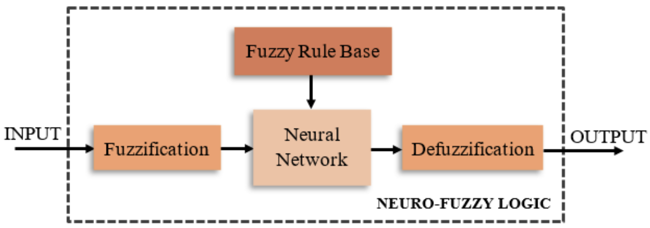


Fig. 2 Block Diagram of Neuro-Fuzzy Logic Controller

For the implementation of a Neuro-fuzzy Logic Controller in connection with this paper, a similar development of fuzzy logic algorithm using Arduino is used in the proposed study of F. Gomide et al. This study analyses the fundamental concepts and applications of fuzzy and neural control frameworks. It also emphasized the development of neuro-fuzzy control and decision systems. The key ideas behind these schemes are delineated and is a currently available equipment and support materials portrayed. Lastly, it is also recommended on how neuro-fuzzy systems might be utilized to develop control systems with innovated capabilities. In addition to that, a study from Usoro et al, demonstrate the effect of the Proportional Integral Derivative (PID) and fuzzy logic controller (FLC) in the control capability of DC motor by means of MATLAB. Based on the results of the performance of fuzzy logic controller constructed on Mamdani type fuzzy inference system (FIS), it is concluded that the efficiency of fuzzy speed-controlled DC motors is better. For the implementation of a Fuzzy Logic Controller in connection with this paper, a similar development of fuzzy logic algorithm using Arduino is used in the proposed study of Mira Zara Fernandez et al [4, 5].

In addition, to identify the immobile condition, using sensor readings to determine the object's dynamic state an expert system was used. Simultaneously, methods based on fuzzy logic, which allow processing of information from several sensors, are able to consider and detect the indistinct boundaries between dynamic states and at transitional points, for example, rotation, or uniformly accelerated linear movement under uncertain conditions [6].

Adaptive Neuro Fuzzy Inference System (ANFIS) was used for the modeling of radial friction welding by considering two input parameters and one output parameter. It was integrated for the prediction of the tensile strength. This technique used was made for a method of fuzzy modeling procedure to learn data set information to determine the membership function parameters that is ideally suited to fuzzy inference system to monitor the given data input/output. And that method was the same to that neural networks [7]. Parameters of membership function were balanced using a hybrid system with the combination of back propagation and least squares type method. The parameters for membership function would be change in learning process. Calculation of these parameters was made easier by gradient vector, which measures how well the fuzzy inference system is input/output data modeling for a given set of parameters. Upon obtaining the gradient vector, any one of the various optimization routines could be used to change the parameters to lower some measure of error. The system is Sugeno-type based system, and the mapping relationship between input and output can be stimulated and analyzed data from hybrid learning to assess how membership function is best distributed [8].

Lastly, included in this framework is the study of Adaptive Neuro-Fuzzy Interference Systems. This study gives a smart decision support system to various home automation services using fuzzy logic. Implementation of fuzzy control is for handling qualitative information and solving difficulties in tuning complexity of home appliances to make it smarter, safer and automated. The proposed system is built with ANFIS algorithm for control operations and decision systems [9].

III. Methodology

A. Hardware Development

Installation of motors, gears and belts is performed for the mechanism of microscope – x, y and fine adjustments. Each knob has its respective motor that is connected into the motor driver to control the movement of each axis. Motor A for the x-axis, Motor B for the y-axis and Motor C for the fine adjustment.

1. Materials and Equipment

a. Binocular Microscope

Quantum Binocular Microscope displays a binocular microscope which has wide field eye pieces (16X and 10X), four achromatic objectives (4X, 10X, 40X and 100X), mechanical stage with scales, coarse & fine focusing, Abbe NA 1.25 condenser with Iris Diaphragm & Filter condenser and variable intensity halogen transmitted illumination system. This microscope's fine adjustment knob has a focusing range of 30 mm with focusing interval of 0.002 mm.

b. Microscope Camera

A DigiEye USB Digital Microscope Camera is compatible with Windows XP, Vista, 7, 8 and 10 Mac OS and Linux operating system. This microscope digital camera is a USB 2.0 interface and has a resolution of 1280x1024 pixels (1.3 MP). It captures still images and streams live videos on computers. This camera can be attached to any microscope with 23.2mm eyepiece tube.

c. Microcontroller

The Arduino UNO is an open-source microcontroller board that is based on the Microchip ATmega328P microcontroller. It is consisting of sets of digital and analog input/output pins which can be interfaced to various expansion boards and other circuits.

d. Motor Driver

2CH Tiny Motor Driver is a simple motor driver board and small package based on A3906 Allegro's low voltage dual DC motor driver IC. It is designed for PWM control of low voltage motors (5 to 7 VDC) and it is compatible in all gizDuino boards.

e. Digital Servo Motor

SG-90 160deg Servo Motor has a torque output despite of its weight and can rotate approximately 180 degrees, making 90 degrees in each direction. It is compatible with any servo code, hardware, and library by any Arduino board.

2. Hardware Design

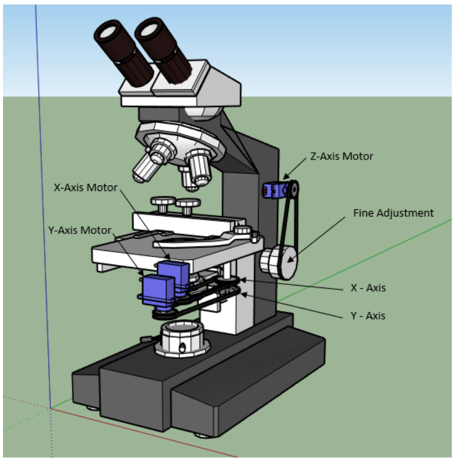


Fig. 3 Mechanical Design of Automated Microscope

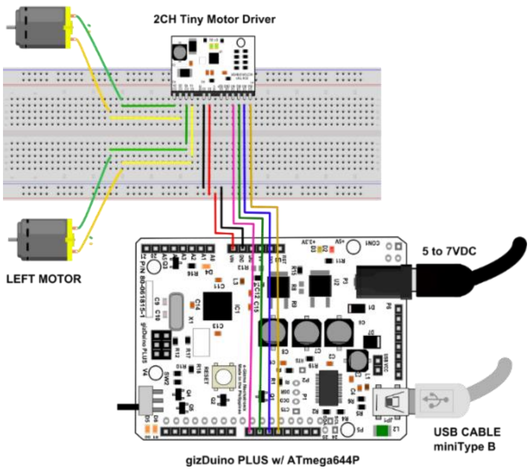


Fig. 4 Wiring Diagram for motor, motor driver and microcontroller (Retrieved from https://github.com/e-Gizmo/2-Channel-Tiny-DC-Motor-Driver-)

Fig. 5 Actual Automated Microscope

B. Software Development

To manually or automatically control the knobs and focus the lens of the microscope, the proponents develop a graphical user interface. A combination of Fuzzy Logic System and Artificial Neural Network called Adaptive Neuro-Fuzzy Inference System. This concept has been used for decision rule based in Arduino.

1. *Graphical User Interface*

Fig. 6 GUI Controls and Buttons

The proponents developed a graphical user interface, consisting buttons together with the live feed, to control the mechanism of the microscope, manually or automatically. It can also capture an image and record a video for compilation. These are all conducted in Processing Programming Language.

2. *Manual control of x, y and z-axis motors*

The manual control of x, y and z-axis motors was defined by the buttons in GUI. The button that is activated will be the serial input in the Arduino to do a specific instruction.

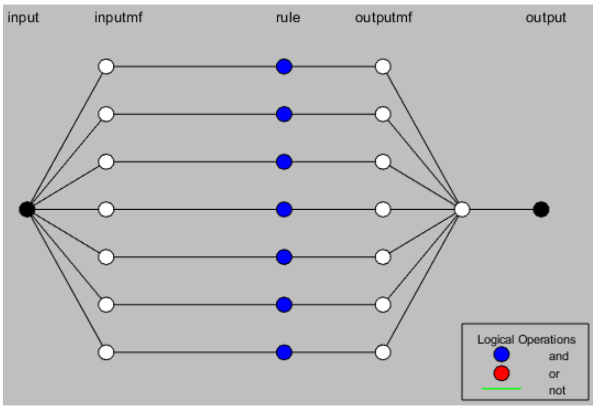


Fig. 7 ANFIS Model Structure for Manual Control

ANFIS information for this ANFIS model is as given below:

Number of nodes: 32
Number of line parameters: 14
Number of nonlinear parameters: 21
Total number of parameters: 35
Number of training data pairs: 7
Number of fuzzy rules: 7

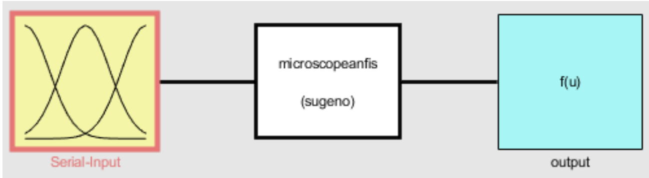


Fig. 8 Fuzzy Inference System (SISO)

The Fig. 8 shows real input and real output. It is a SISO (single input single output) system with an input parameter of pointer's position in the pixel of GUI and an output parameter of desired rotation of each motor. Input and output parameters are given below:

Pointer's position (Serial-Input) = 0 to 0.84

Motor N's rotation = x, y or z, clockwise, counter-clockwise or stop

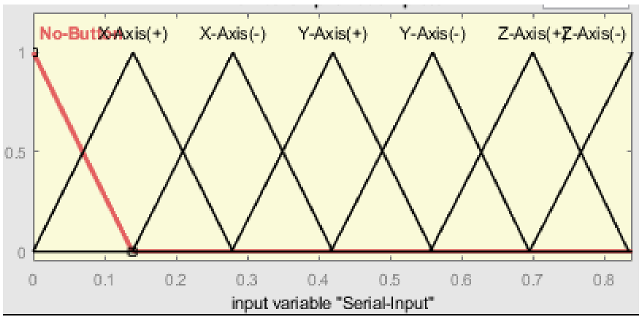


Fig. 9 Triangular membership function for input variable "Serial-Input"

TABLE I. FUZZY INPUT VARIABLE "SERIAL-INPUT" IN ITS UNIVERSE DISCOURSE (0 TO 0.84)

Name of Linguistic Level	Membership Function	Range
No button	Constant	0
X-Axis (+) button	Constant	0,14
X-Axis (-) button	Constant	0.28
Y-Axis (+) button	Constant	0.42
Y-Axis (-) button	Constant	0.56
Z-Axis (+) button	Constant	0.70
Z-Axis (-) button	Constant	0.84

TABLE II. FUZZY OUTPUT VARIABLE "OUTPUT" IN ITS UNIVERSE DISCOURSE (0 TO 6)

Name of Linguistic Level	Membership Function	Range
Stop	Constant	0
X-axis motor, Clockwise	Constant	1
X-axis motor, Counterclockwise	Constant	2
Y-axis motor, Clockwise	Constant	3
Y-axis motor, Counterclockwise	Constant	4
Z-axis motor, Clockwise	Constant	5
Z-axis motor, Counterclockwise	Constant	6

The output indicates what motor to be used and its rotation, either x, y, or z-axis and clockwise or counterclockwise. From table 2, 0 is for stop, 1 and 2 are for x-axis, 3 and 4 are for y-axis, and 5 and 6 are for z-axis, clockwise and counterclockwise, respectively.

3. *Autofocus*

The fine adjustment or the z-axis motor will be the one that is responsible for autofocusing. Passive autofocus systems that consist of blur detection method, where brightness and edge are determined by Laplacian Operator, was used.

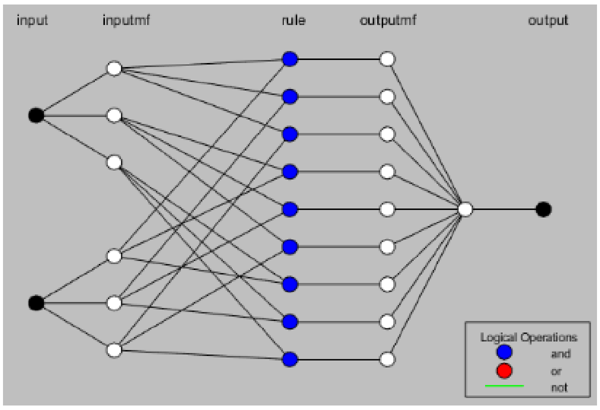


Fig. 10 ANFIS Model Structure for Autofocus

ANFIS information for this ANFIS model is as given below:

Number of nodes: 35
Number of line parameters: 9
Number of nonlinear parameters: 12
Total number of parameters: 21
Number of training data pairs: 16
Number of fuzzy rules: 9

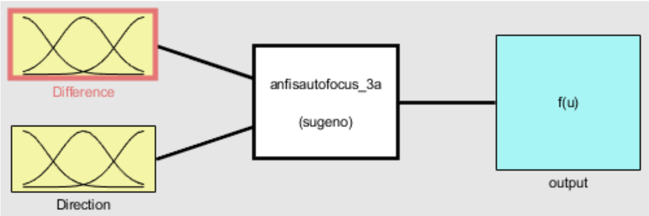


Fig. 11 Fuzzy Inference System (MISO)

The Fig. 11 shows real inputs and real output. It is a MISO (multi input single output) system with two input parameters of input 1 and input 2 and an output parameter of desired rotation of z-axis motor. Input 1 is the difference between the highest-valued gray pixel and the current value of the gray pixel displayed in the live feed, while Input 2 is the previous motor's rotation. Input and output parameters are given below:

Difference = 0 to 50

Direction (Previous) = clockwise, counter-clockwise or stop

Z-Axis Motor's rotation = clockwise, counter-clockwise or stop

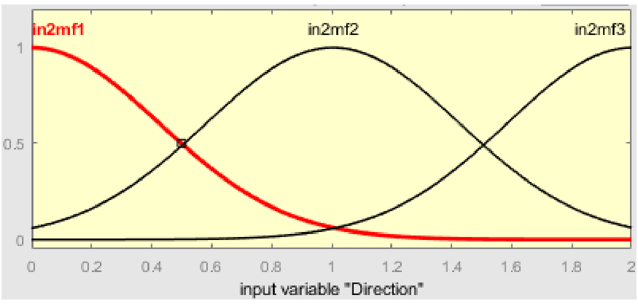


Fig. 12 Gaussian membership function for input variable "Difference"

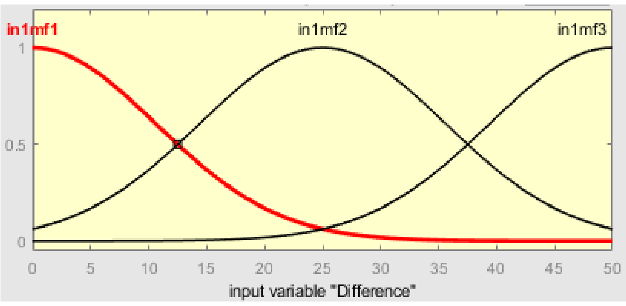


Fig. 13 Gaussian membership function for input variable "Direction"

TABLE III. FUZZY INPUT VARIABLE "DIFFERENCE" IN ITS UNIVERSE DISCOURSE (0 TO 0.84)

Name of Linguistic Level	Membership Function	Range
in1mf1	gaussmf (Gaussian MF)	0 to 25
in1mf2	gaussmf (Gaussian MF)	0 to 50
in1mf3	gaussmf (Gaussian MF)	25 to 50

TABLE IV. FUZZY INPUT VARIABLE "DIRECTION" IN ITS UNIVERSE DISCOURSE (0 TO 0.84)

Name of Linguistic Level	Membership Function	Range
in2mf1	gaussmf (Gaussian MF)	0 - 1
in2mf2	gaussmf (Gaussian MF)	0 - 2
in2mf3	gaussmf (Gaussian MF)	1 - 2

TABLE V. FUZZY OUTPUT VARIABLE "OUTPUT" IN ITS UNIVERSE DISCOURSE (0.33 TO 0.99)

Name of Linguistic Level	Membership Function	Range
out1mf1	Constant	0.99
out1mf2	Constant	0.99
out1mf3	Constant	0.99
out1mf4	Constant	0.66
out1mf5	Constant	0.66
out1mf6	Constant	0.66
out1mf7	Constant	0.33
out1mf8	Constant	0.33
out1mf9	Constant	0.33

From Table V, 0.33 means clockwise, 0.66 means counterclockwise and 0.99 means stop.

The output indicates the direction of z-axis motor's rotation, either clockwise or counter-clockwise. And when the motor stops, it indicates that the knobs are in position of being in the focus state.

C. *System Synchronization*

The system synchronization is establishing a connection between the hardware and the software part of the system. Specifically, the connection of the mechanism of the microscope, Arduino microcontroller, Neuro-Fuzzy Logic Controller, and the GUI. This device can be controlled using a portable user interface consisting of buttons, manually or automatically, together with the live feed. The whole system is conducted in Processing Programming Language.

1. *Block Diagram*

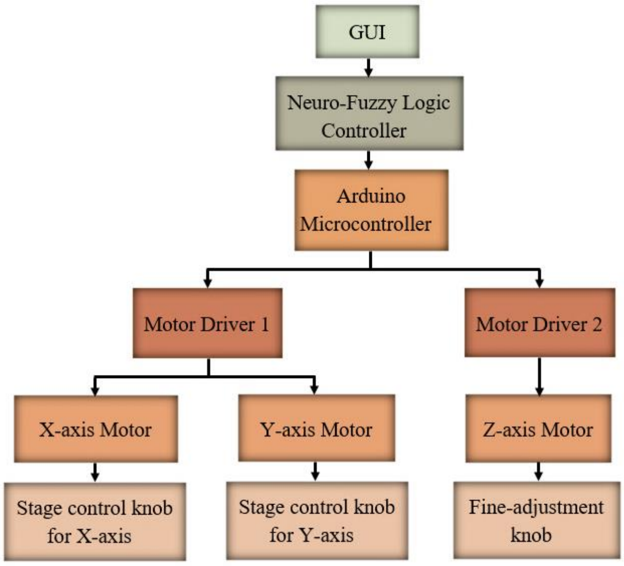


Fig. 14 Block Diagram of Automated Microscope

The mechanism of each motor, assigned in the movement of x, y, and fine adjustment, is specified by Neuro-Fuzzy Logic Controller through microcontroller (Arduino). This device can be controlled using a portable user interface consisting of buttons, manually or automatically, together with the live feed.

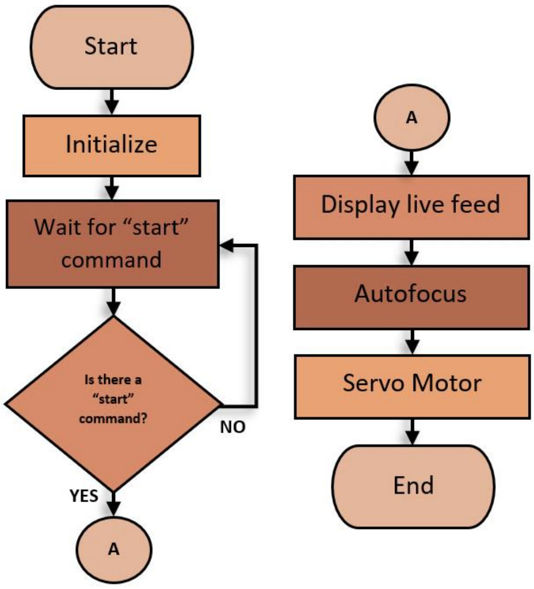


Fig. 15 Overall System Flow Chart

Fig. 15 shows the flowchart of the overall system. First, the system will initialize and wait for the commands. When the "start" command has been received, the system will display a live feed. It will then undergo to the processes of autofocus, for checking if the image is clear, and servo motor, for adjustments.

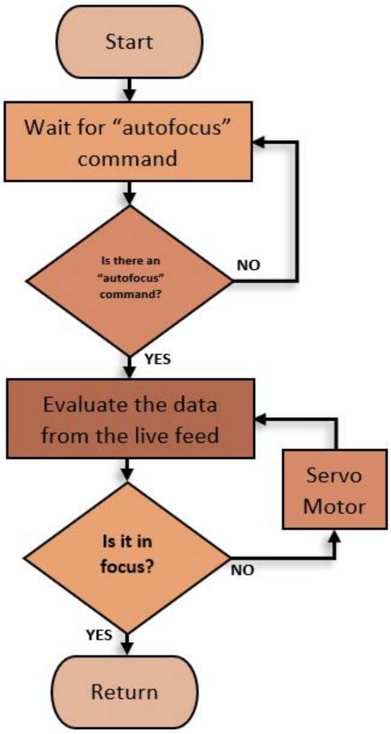


Fig. 16 Autofocus Subroutine Flow Chart

Fig. 16 shows the flow chart of the autofocus subroutine. Once it starts, when the commands are already received, it will control the stepper motors connected to the knobs of the microscope to move in accordance with the instruction. When all the conditions are met, the subroutine will end and return to the main loop.

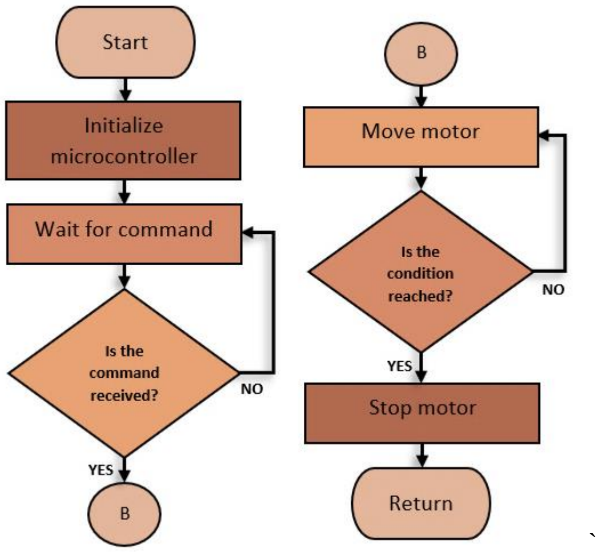


Fig. 17 Servo Motor Subroutine Flow Chart

Fig. 17 shows the flow chart of the Servo motor subroutine. Once it starts, the microcontroller which manipulates the function of the stepper motors will initialize and wait for the commands. When the commands are already received, it will control the stepper motors connected to the knobs of the microscope to move in accordance with the instruction. When all the conditions are met, the subroutine will end and return to the main loop.

D. Troubleshooting, Training and Testing

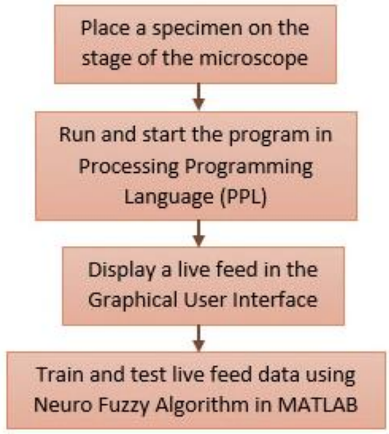


Fig. 18 Block diagram for training and testing

Specimen slide to be used for training was placed on the stage of the microscope. Then, the program runs and starts at displaying a live feed. Lastly, the data collected in the live feed will be trained and tested through ANFIS in MATLAB.

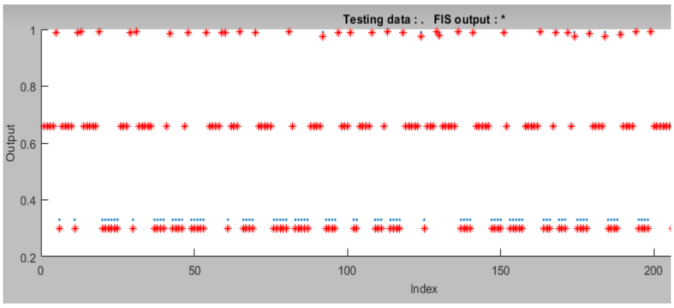
IV. RESULTS

TABLE VI. TESTING DATA GATHERED FROM THE LIVE FEED (90 OUT OF 221)

Data #	INPUTS		OUTPUT	Data #	INPUTS		OUTPUT	Data #	INPUTS		OUTPUT
	Diff.	Dir.			Diff.	Dir.			Diff.	Dir.	
1	347	1	0.66	31	2	0	0.99	61	343	2	0.33
2	347	1	0.66	32	347	1	0.66	62	347	1	0.66
3	346	1	0.66	33	347	1	0.66	63	347	1	0.66
4	244	1	0.66	34	344	1	0.66	64	109	1	0.66
5	13	0	0.99	35	81	1	0.66	65	5	0	0.99
6	24	2	0.33	36	29	1	0.66	66	347	2	0.33
7	347	1	0.66	37	347	2	0.33	67	346	2	0.33
8	347	1	0.66	38	347	2	0.33	68	239	2	0.33
9	345	1	0.66	39	347	2	0.33	69	190	2	0.33
10	51	1	0.66	40	195	2	0.33	70	7	0	0.99
11	99	2	0.33	41	198	1	0.66	71	347	1	0.66
12	10	0	0.99	42	17	0	0.99	72	347	1	0.66
13	1	0	0.99	43	347	2	0.33	73	254	1	0.66
14	347	1	0.66	44	347	2	0.33	74	172	1	0.66
15	347	1	0.66	45	347	2	0.33	75	30	1	0.66
16	347	1	0.66	46	178	2	0.33	76	347	2	0.33
17	184	1	0.66	47	190	1	0.66	77	347	2	0.33
18	88	1	0.66	48	13	0	0.99	78	241	2	0.33
19	4	0	0.99	49	347	2	0.33	79	162	2	0.33
20	347	2	0.33	50	347	2	0.33	80	32	2	0.33
21	346	2	0.33	51	346	2	0.33	81	2	0	0.99
22	344	2	0.33	52	218	2	0.33	82	341	1	0.66
23	76	2	0.33	53	115	2	0.33	83	347	2	0.33
24	45	2	0.33	54	8	0	0.99	84	347	2	0.33
25	24	2	0.33	55	347	1	0.66	85	135	2	0.33
26	347	1	0.66	56	347	1	0.66	86	88	2	0.33
27	347	1	0.66	57	347	1	0.66	87	21	2	0.33
28	284	1	0.66	58	232	1	0.66	88	347	1	0.66
29	7	0	0.99	59	14	0	0.99	89	347	1	0.66
30	30	2	0.33	60	7	0	0.99	90	255	1	0.66

TABLE VII. TRAINING DATA FOR FIS

Difference	Direction	Output
0	0	**0.99**
1	0	**0.99**
2	0	**0.99**
3	0	**0.99**
4	0	**0.99**
5	0	**0.99**
10	1	**0.66**
20	1	**0.66**
30	1	**0.66**
40	1	**0.66**
50	1	**0.66**
10	2	**0.33**
20	2	**0.33**
30	2	**0.33**
40	2	**0.33**
50	2	**0.33**



* ANFIS output in MATLAB * ANFIS output in program

Fig. 19 Plot of ANFIS autofocus output using MATLAB

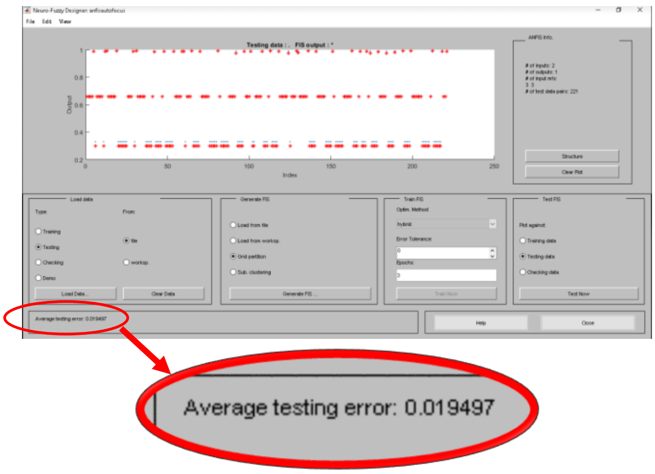


Fig. 20 Average Testing Error Result

After FIS training in MATLAB, the model was validated using a testing data gathered from the live feed shown in Table VI, that differs from the training data used to train the ANFIS in MATLAB shown in Table VII. The average testing error of the testing data is 0.019497 that has been shown in Fig. 20.

V. CONCLUSION

The microcontroller (Arduino) has been successfully integrated to the microscope for the automation of its mechanism. The algorithm, Neuro-Fuzzy was programmed for the decision rule-based in controlling the Arduino. The GUI was successfully modified by adding buttons for manual control of the microscope's knobs, autofocus of the lens, and for the automatic movement of the stage of the microscope. The program for both manual and automatic mechanism of the microscope was also integrated in the GUI. Overall, the device was tested for accuracy.

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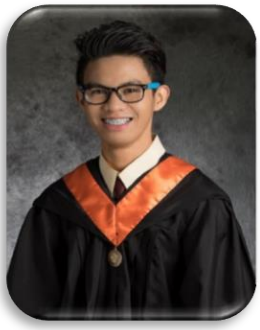

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