Low Cost Autofocus System for Optical Microscopes

A Project Report

submitted by

AKSHAY RANGAMANI

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THESIS CERTIFICATE

This is to certify that the thesis titled **Low Cost Autofocus System for Optical Microscopes**, submitted by **Akshay Rangamani**, to the Indian Institute of Technology, Madras, for the award of the degree of **Bachelor of Technology**, is a bona fide record of the project work done by him under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Dr. Mohanasankar Sivaprakasam
Project Guide,
Dept. of Electrical Engineering,
IIT-Madras, 600 036.

Place: Chennai

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Next I would like to thank Dr. Mohanasankar for introducing me to the world of technology in healthcare and helping me discover my passion for this subject.

My family has been very supportive and understanding throughout the duration of this project, as well as the past four years. I would like to place on record my gratitude for their support.

Finally, I would like to thank all the friends I have made over the course of these four years for their companionship. Starting from classmates and hostel-mates to the people I share my lab space with. The past four years would not have been as eventful if not for their company.

ABSTRACT

Rural healthcare centers which typically serve a population of 30,000 people have only one lab technician to collect samples and screen for microbial diseases. In order to remove this bottleneck, an automated system to digitize slides and screen them for pathologies is proposed. Since cost-effective solutions are required, the proposed system is one that modifies existing microscopes into automated ones, making use of the already available resources.

The design and construction of a passive autofocus system is discussed, right from the mechanical design to the electronics and image processing. Five different focus measures are compared and one of them is selected. Different search algorithms are also proposed and two have been implemented. The end result is a low cost autofocus system which is one of the components required for an automated microscope system.

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CHAPTER 1

INTRODUCTION

1.1 Motivation

There are a number of diseases that require laboratory and microscopic methods to diagnose them. Some examples are respiratory diseases like Tuberculosis, a lung infection, and Diphtheria, an upper respiratory tract infection caused by *Corynebacterium diphtheria*; intestinal diseases such as Cholera (*Vibrio cholera*) and Dysentery; STIs such as Gonorrhea and Syphilis. Many of these diseases cause widespread havoc even today. According to the World Health Organization (WHO), there were an estimated 9.27 million incident cases of TB in 2007 [1]. Every year around 100,000 to 120,000 people die of Cholera [2]. Around 200,000 people died of typhoid in 2000 [3]. These statistics reveal that microbial communicable diseases are extremely widespread in the developing world.

Microbial diseases such as these are usually manually screened using microscopes. This process is intensive in terms of both the time required and trained personnel who need to be on-site. In each primary healthcare center, there is only 1 laboratory technician present [4]. A primary health care center is meant to serve a population of 30,000 people [4]. There is a very large gap between the demand for technicians and the number available.

An automated microscope solution will help bridge this gap and will expedite the rate at which patient samples are screened and analyzed. In addition to this, an automated microscope system will be able to collect digital images which can be sent over the Internet to tertiary healthcare centers, usually located in cities. Moreover, the process of diagnosing diseases can also be automated by using image processing algorithms to identify micro-organisms. Automated systems will also be faster than manual operation of microscopes by lab technicians. These systems will help fill in the void in the requirement of trained lab technicians.

However, most automated microscope solutions available in the market cost upwards of Rs. 25 lakh. This is certainly not a cost effective solution, and is not affordable for primary healthcare centers. In this thesis, a solution is described, which converts existing microscopes into automated systems. Since every primary healthcare center will have a normal optical microscope, the cost of adapting an existing microscope will be many times cheaper than purchasing a new automated microscope.

An automated microscope system consists of an autofocus system (which controls the z-direction), as well as a system to move the microscope's stage or slides (control in the x-y direction). This thesis is concerned with the design and construction of the former.

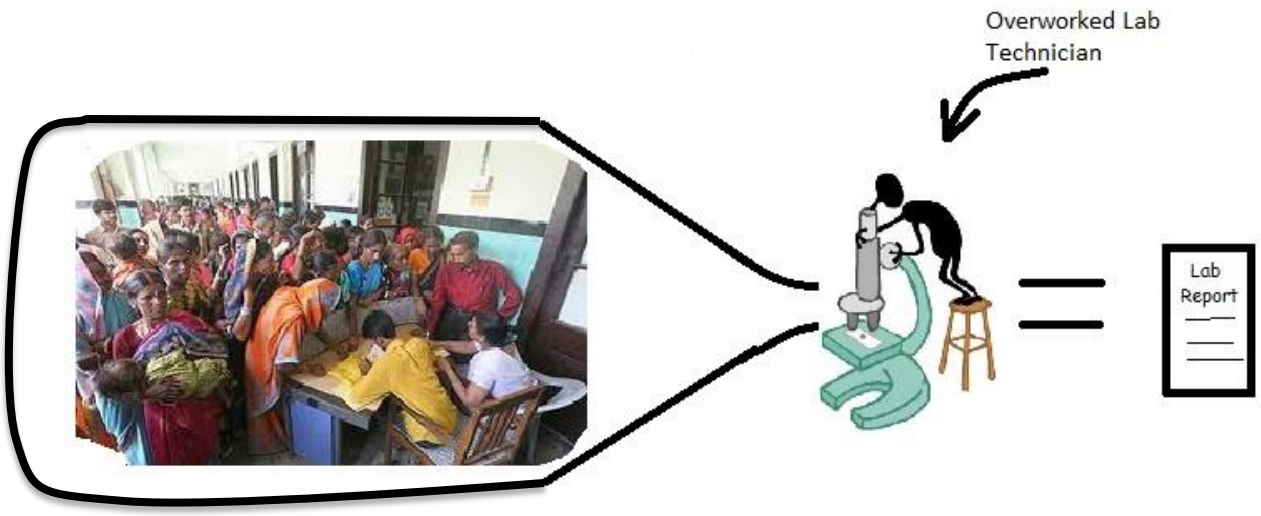


Figure 1: The lack of skilled technicians is the bottleneck in the rural healthcare centers

1.2 Types of Autofocus Systems

There are two different approaches to autofocus systems. One is the active method and the other, the passive method.

The active autofocus method [5] is named so because the camera itself sends a signal (usually infra-red rays or ultrasound waves) and analyzes the received signals, much similar to the technique of SONAR. The time taken for the emitted signal to return, amount of light reflected from the subject and triangulation are some parameters which are used by the active autofocus system to decide how far away the subject is, and which subject is in focus. Depending upon the distance to the subject, the lenses of the camera are adjusted to bring the object into focus. Figure 1 contains a flowchart outlining the process.

Active autofocus is very quick and is not iterative. However, it can be troublesome to use when there is an obstacle (such as glass) between the camera and the subject. In the case of multiple objects in front of the camera, the system has to correctly choose the object to be focused on, which is tricky. An active autofocus system also required extra hardware components, which can be expensive.

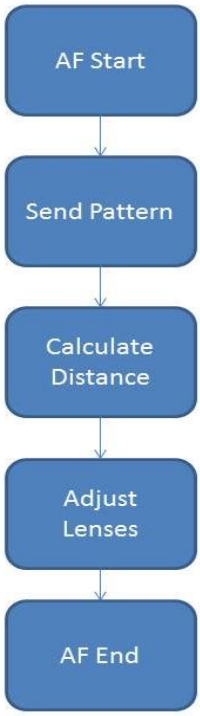


Figure 2: Flowchart of active autofocus method (Adapted from [5])

In contrast, the passive autofocus method [5] does not send any signals from the camera. Instead, the camera analyzes the image capture and computes a value known as focus measure. The lenses are then adjusted so as to make the focus measure maximum. This is usually an iterative process since it is hard to predict the focus value curve (plot of focus measure vs distance from focal plane). Figure 2 contains a flowchart outlining this process.

Passive autofocus is slower than the active method since it is iterative and involves more computation. It is however applicable even if there are multiple objects in the frame. The passive approach does not have any other hardware requirements. However passive autofocus may fail under lowlight conditions where the image is not bright enough to discern focused and unfocused positions.

In the construction of the autofocus system, a passive autofocus approach has been used due to its lower cost as well as ease of implementation. The lowlight conditions will hardly arise while using an optical microscope and thus will not pose a major problem.

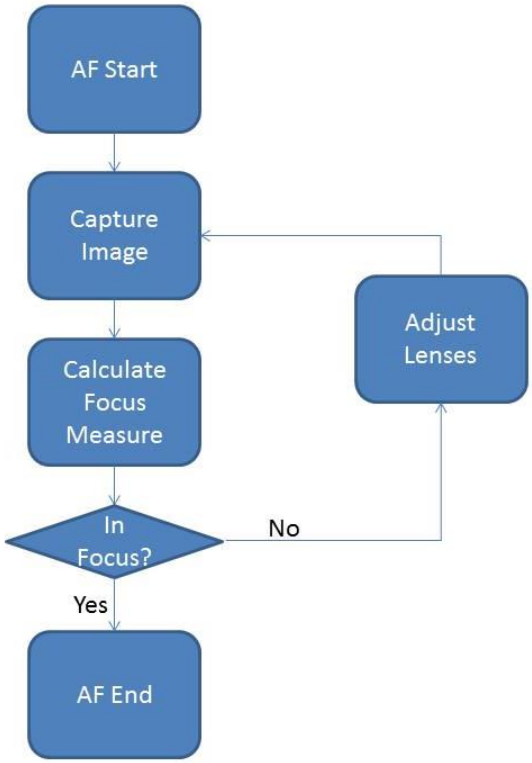


Figure 3: Flowchart of passive autofocus method (Adapted from [5])

CHAPTER 2

DESIGN & CONSTRUCTION

The autofocus system for a microscope consists of the following subsystems:

1. Mechanical (to control the coarse and fine adjustment knobs)
2. Electronics (to control the mechanical system)
3. Imaging (to acquire images required for evaluating the focus measure)
4. Software (to evaluate the focus measure as well as decide how to adjust the lenses)

For now we will focus on the mechanical and electronic design. The Software subsystem will be approached later in this thesis.

2.1 Mechanical Design

The mechanical subsystem is concerned with the problem of adjusting the distance between the objective and the sample under observation. The components which make up the mechanical system are:

1. Stepper Motors (Vexta PH268-23 2 phase unipolar motor, 1.8^{o} per step, 24V, 0.34A)
2. Shaft Couplers

The stepper motors are used to turn the coarse and fine adjustment knobs by the exact degree specified by the software. This particular motor (Figure 4) was chosen for its high torque which enables it to turn the coarse and fine adjustment knobs of the microscope most easily. The resolution of the stepper motor (200 steps/revolution) enables the smallest possible displacement of the objective to be as small as 0.5μm. This value however depends upon the microscope itself.

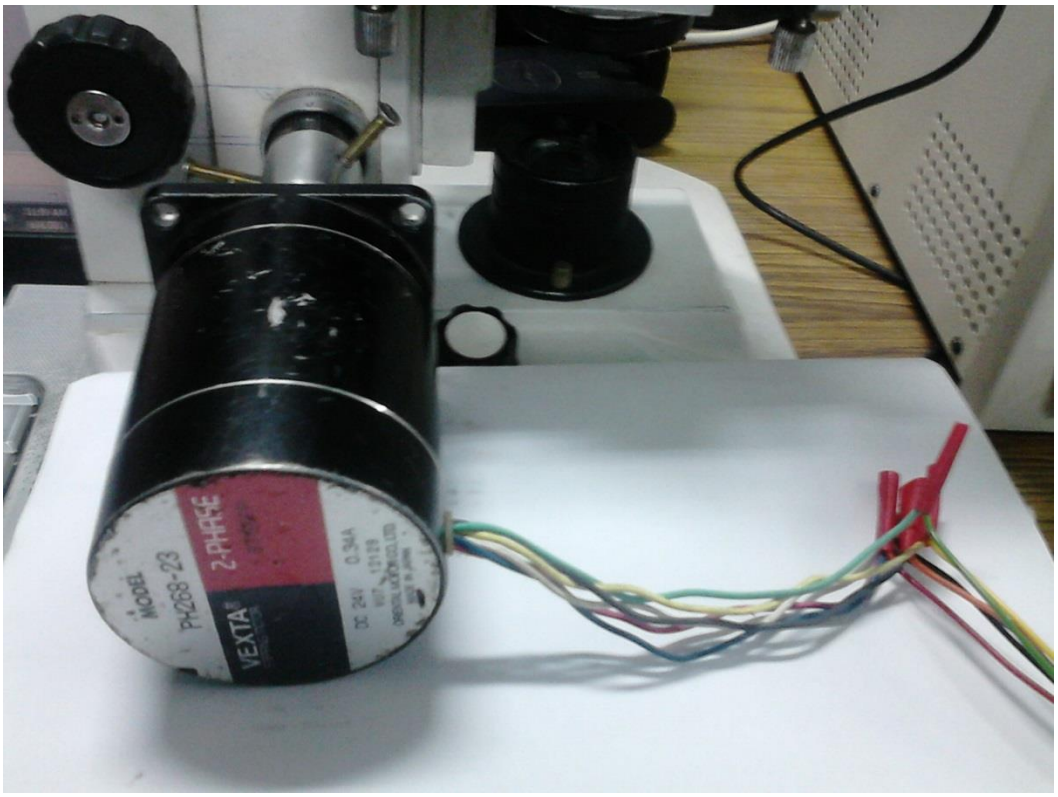


Figure 4: Stepper Motor attached to Fine Adjustment Knob

The shaft couplers are used to connect the shaft of the stepper motor to the coarse and fine adjustment knobs. These were designed and manufactured for the purpose of this project. The shaft couplers were machined out of aluminum in order to be as light as possible. However, mild steel (MS) can also be used to reduce costs. The exact dimensions are given in Figures 5 and 6. The shaft coupler is designed to fit any knob as well motor shaft whose diameters are less than the given dimensions, since it has 3 holes for 4mm screws (with mm threading) placed 120^{o} apart, which can be used to fit the coupler to the desired appendage. Figure 7 is a visual of the coarse adjustment knob shaft coupler.

In order to assemble the mechanical subsystem, one must screw the shaft coupler onto the motor shaft first and then attach it to the coarse/fine adjustment knob of the microscope. One issue which arose while assembling the mechanical subsystem was ensuring that the alignment of the motor shaft was exactly at the center of the shaft coupler. This could be done only by trial and error. One way to verify if the motor shaft is at the center is to check the rotation of the coupler along with the motor. Irregular motion would mean that the shaft is not centered.

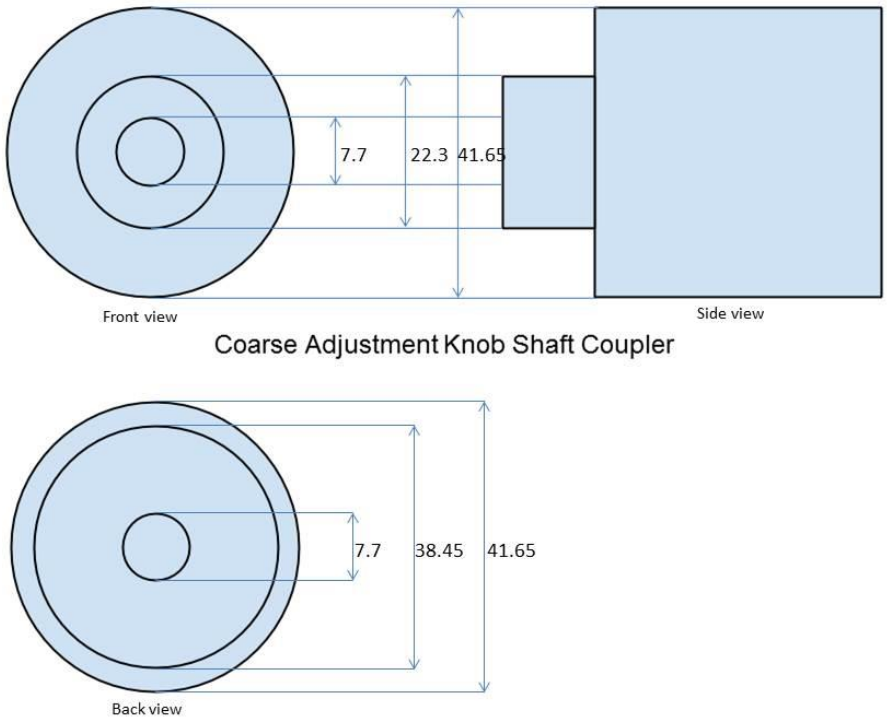


Figure 5: Coarse Adjustment Knob Shaft Coupler Dimensions

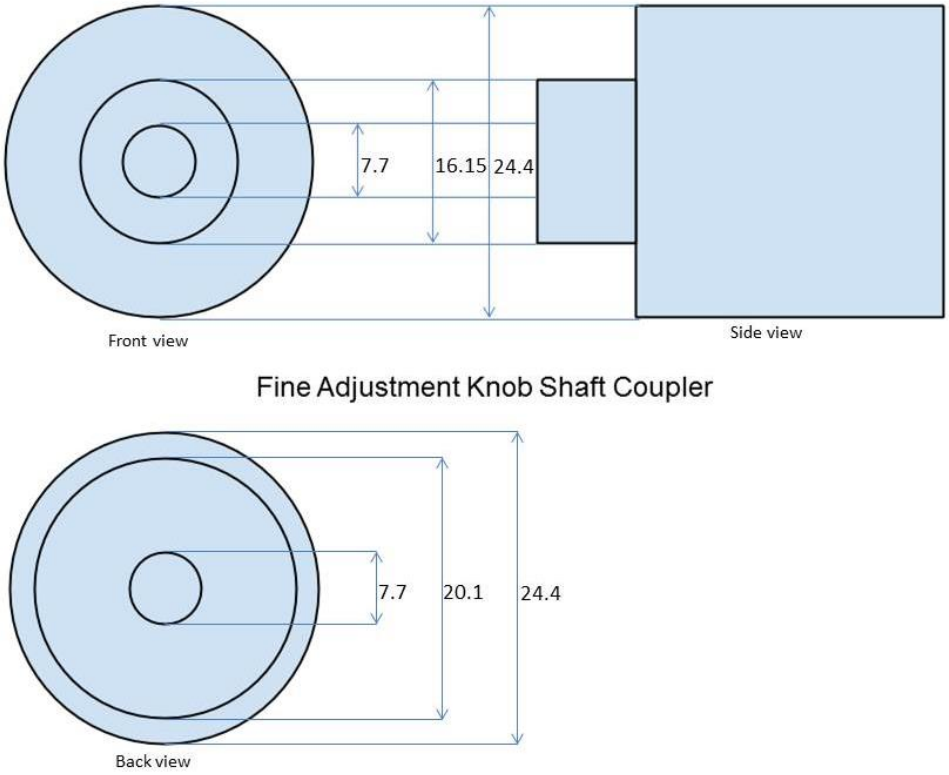


Figure 6: Fine Adjustment Knob Shaft Coupler Dimensions

Figure 7: Coarse adjustment Knob Shaft Coupler

2.2 Electronic Design

The electronic subsystem is the one which is responsible for controlling the mechanical subsystem according to the inputs from the autofocus algorithms. The components which make up the electronic subsystem are:

1. Arduino UNO
2. ULN2803

The Arduino UNO is a microcontroller board based on the ATmega328. This is used to provide the signals to control the 2 phase unipolar stepper motor described in the previous section. The code used to control the stepper motor makes use of the existing Stepper Arduino library. The Arduino communicates with the computer over a serial port (9600 baud) and receives the number of steps to be taken from the autofocus algorithm. According to the data received, the Arduino excites the coils of the motor for the appropriate duration in the required direction.

The ULN2803 is an 8 channel Darlington array IC which is used to drive the stepper motor. Since the Arduino UNO can only source a maximum of 40mA from its digital I/O pins, a Darlington array is used to drive the stepper motor from a separate 24V power source. The driver circuit is given in Figure 8. The actual circuit along with the Arduino can be seen in Figure 9.

Though the motor has only 4 inputs, the high current requirement causes the IC to get heated up quickly. In order to solve this, the 4 channel input was split across 8 channels in order to reduce the current drawn per channel. Another issue which arose during development was the requirement of pull-up resistances. Since the Darlington array has

only open collector outputs, a pull-up resistance was required to maintain the output at 24V.

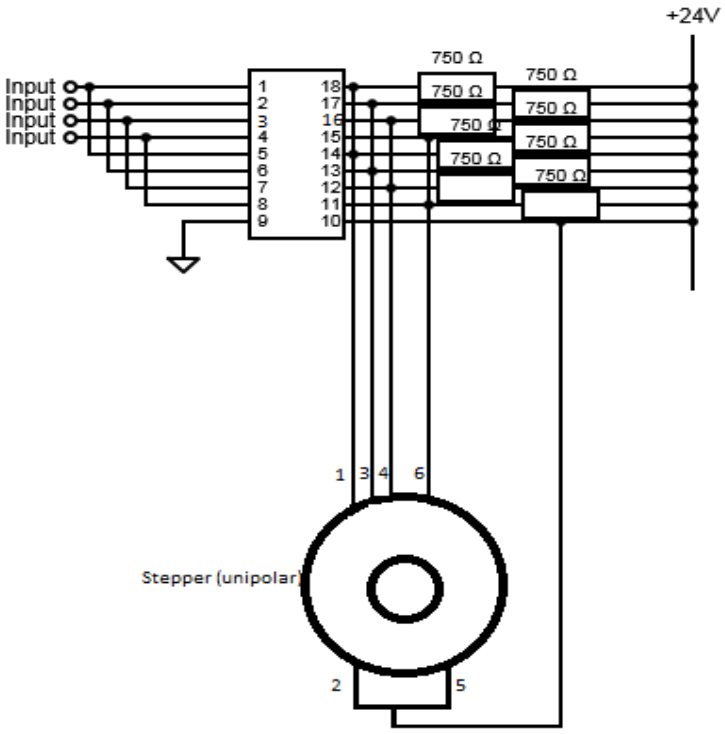


Figure 8: Driver circuit for stepper motor (Input denotes input from Arduino)

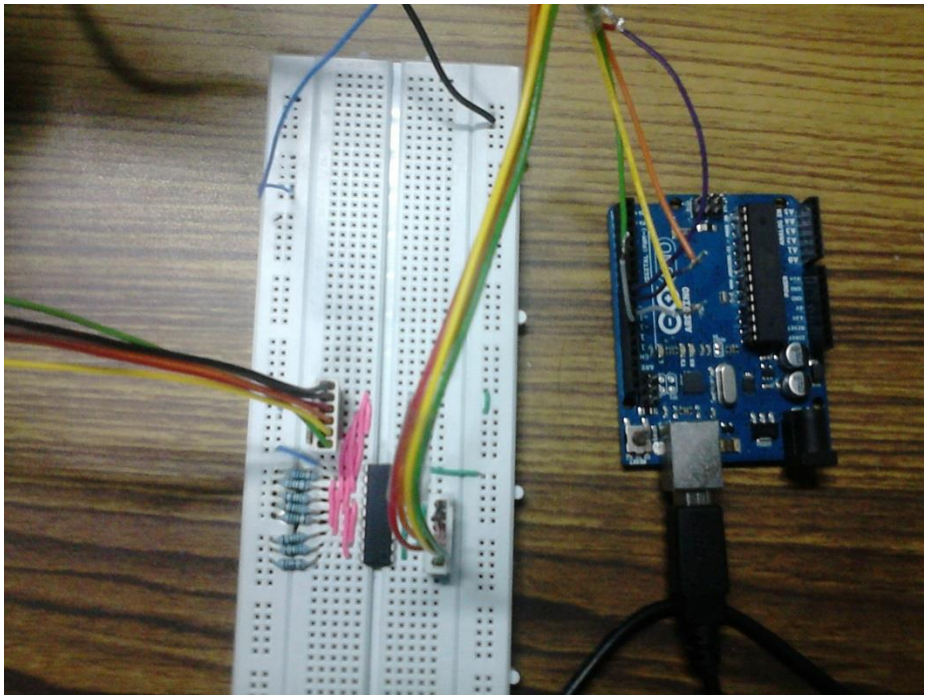


Figure 9: Driver circuit for stepper motor and Arduino UNO

2.3 Imaging Subsystem

The imaging subsystem is used to capture the pictures which are used to determine if the sample under observation is in focus. It is also used to capture the final focused images of the sample for further analysis.

The imaging subsystem contains only one component, the digital imager. The imager chosen for this project was a Celestron Digital Microscope Imager. This is designed to replace the eyepiece of microscopes and fits in microscope with eyepiece diameters between 23mm and 30mm. The imager has a magnification of 15x and has a resolution of 2MP. The size of the image sensor is however small. Both this and the higher magnification result in a highly reduced field of view. In this particular arrangement, the field of view of the microscope using a 10x eyepiece was 2.5mm wide. However the field of view when seen through the imager was between 0.5mm and 1mm wide. The imager communicates with the computer through a USB2.0 cable. Figure 10 shows the imager purchased.

Figure 10: Celestron Digital Microscope Imager

Details of procedure followed for image capture:

- Images were captured using three different objectives (10x, 45x, 100x)
- For all three objectives, the coarse adjustment has to be kept at an extreme position, and is never used in the focus adjustment. It is only the fine adjustment knob that is used during the autofocus procedure
- Images were captured at different regions
- The images were captured within a 400μm range of the focal plane, 200μm in both directions from the focal plane
- While capturing images, three different values of the interval between subsequent images were used (5μm, 2.5μm, 1μm)

The fully constructed system can be seen in Figure 11.

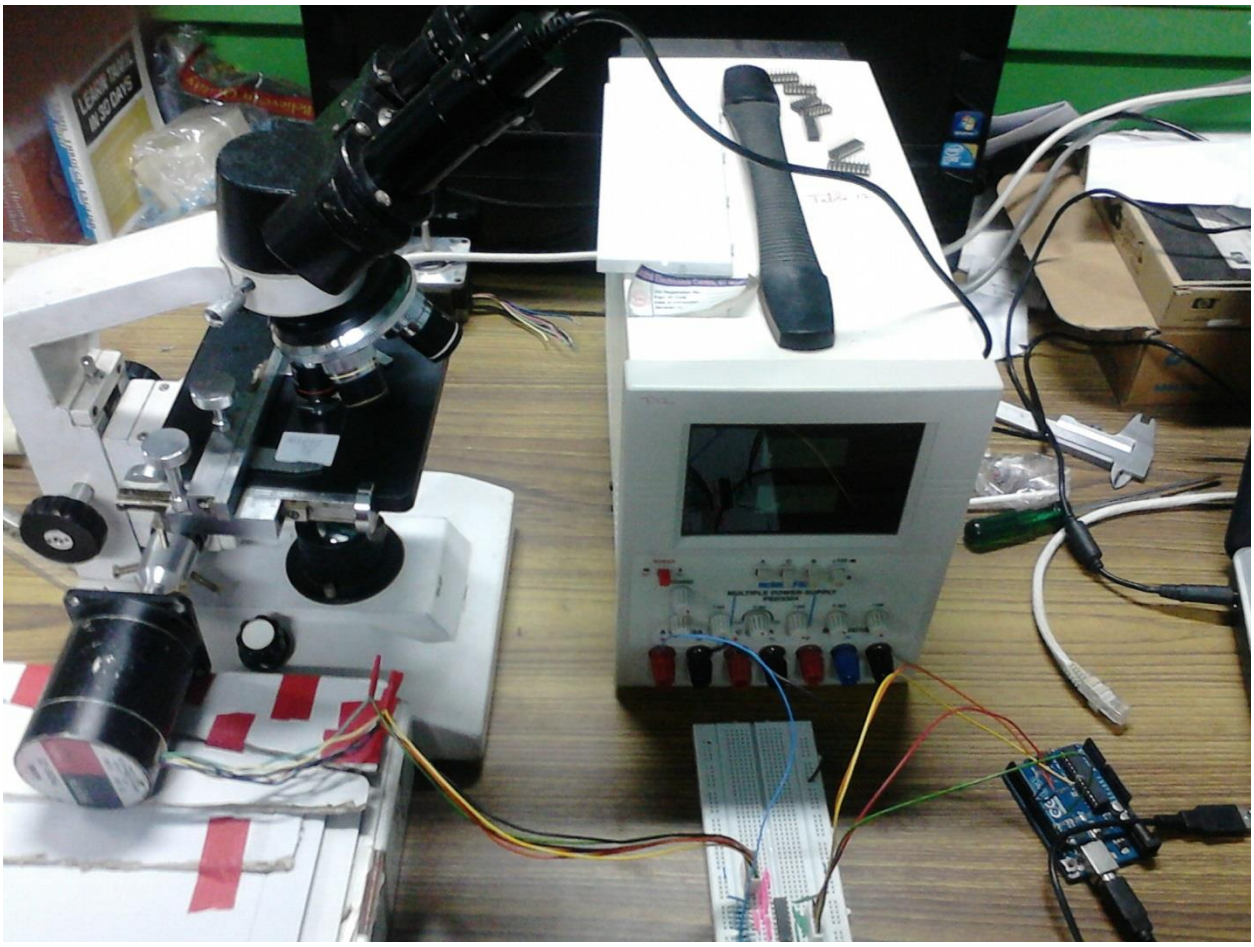


Figure 11: Final setup of autofocus system

CHAPTER 3

IMAGE COMPUTING

The design and construction of the mechanical and electronic subsystems have been described thoroughly in the previous chapter. Now we move on to the software subsystem which has the following functionalities in the passive autofocus system.

1. Evaluate whether image captured by the imager is focused or not
2. Send commands to the mechanical system in order to capture a focused image

These lead to the two components of the autofocus algorithm:

1. Focus measure and Focus Value Curves
2. Search algorithms to find the maxima of the focus value curve

3.1 Focus Measures and Focus Value Curves

Focus measures are functions of images which take the image (usually grayscale) as an input and return a number which tells us how focused or unfocused an image is. A focus measure should be content independent, i.e. should not be based on any particularly bright structures in the image; it should be monotonic with respect to blur; and it should be robust to noise. There are different types of focus measures present [6]. We will focus only on two types in this thesis.

1. **Derivative based measures:** These take advantage of the fact that since focused images are sharp and clear, they have a lot of high frequency content. The derivative based algorithms involve taking different derivatives of the image (passing the image through a high-pass filter) and calculating the energy of the derivative image (output of the convolution). These algorithms are usually sensitive to high frequency noise.
2. **Statistical measures:** These measures, instead of using high pass filters, use the variance and auto-correlation of the image in order to arrive at a focus measure. These are less susceptible to noise than the derivative based algorithms.

We will now look at 5 different focus measures [6].

1. **Energy of the Laplacian:** In this measure, the image is passed through the mask given by $K = \begin{bmatrix} 0 & -1 & 0 \\ -1 & 4 & -1 \\ 0 & -1 & 0 \end{bmatrix}$ The energy of the output is then taken as the focus measure.

$$F = \sum_{i,j} S(i,j)^2$$

2. **Prewitt's Operators:** The Prewitt focus measure is given by:

$$F = \sum \sqrt{{p_x}^2 + {p_y}^2}$$

Where p_x and p_y are the results of passing the image through the below two masks:

$$P_x = \begin{bmatrix} -1 & 0 & 1 \\ -1 & 0 & 1 \\ -1 & 0 & 1 \end{bmatrix}$$

$$\text{and } P_y = \begin{bmatrix} -1 & -1 & -1 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \end{bmatrix}$$

3. **Tenenbaum Gradient:** The Tenegrad function is given by

$$F = \sum \sqrt{{S_x}^2 + {S_y}^2}$$

Where S_x and S_y are the resultant images by convolving the image with the Sobel operators:

$$S_x = \begin{bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{bmatrix}$$

$$\text{and } S_y = \begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{bmatrix}$$

4. **Variance:** This measure computes the variations in gray levels among the pixels of the image. W denotes the width of the image and H denotes the height.

$$F = \frac{1}{WH} \sum \sum (i(x,y) - \mu)^2$$

5. **Normalized Variance:** This measure compensates for the difference in average intensities of the images.

$$F = \frac{1}{WH\mu} \sum \sum (i(x,y) - \mu)^2$$

These are the five different focus measures which were experimented with on the database of images collected.

Focus Value Curves are curves of focus measures vs distance from the focal plane. For very good focus measures, the focus value curves have a global maximum at the origin. Focus value curves tend to have local maxima other than the global maximum at the origin. These may correspond to different portions of the image coming into focus. A representative focus value curve is given in Figure 12. The focus value curves for the above five mentioned focus measures were plotted. These are discussed in the next section.

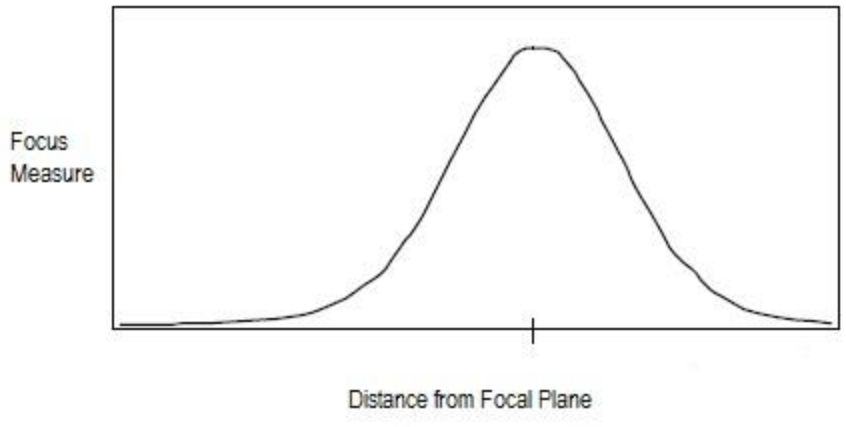


Figure 12: Representative focus value curve when only one object is in the field of view

3.2 Search Algorithm

The second component in the software subsystem is the search algorithm which is used to adjust the distance between the objective and sample in order to advance along the focus value curve and eventually settle at the global maximum (focal plane). There are a number of different search algorithms which are available. As part of this thesis, two search algorithms have been implemented, one is a simple local maximum search and the other is a more useful global maximum search.

In both algorithms, the first six images are used to decide which direction to move the lenses. The first focus measure is compared to the mean of the next five measures. If the first value is greater, the direction is reversed. Otherwise, the algorithm continues as it is.

In the local maximum approach, the algorithm keeps stepping with a constant step size (of 2.5μm) until it finds a maximum. As soon as a maximum is found, the algorithm captures the final image and terminates.

The second algorithm is the global search, which is more useful since it will bring the objective to the exact distance from any initial position. As in the previous case, the first six steps are used to set the direction of stepping. The algorithm keeps computing the focus measures and stepping by a fixed amount (2.5μm). Every time a local maximum occurs, the algorithm waits for ten more steps (25μm) before declaring that maximum a global maximum.

Another approach would be to keep stepping until the focus measure falls within a threshold of the initial focus measure and finally move to the global maximum. The first approach will avoid unnecessary steps, while the second approach will definitely lead to the global maximum every time.

CHAPTER 4

RESULTS AND DISCUSSION

We have seen the design and construction of the autofocus system for the microscope, both the hardware and the software components. Now we will discuss the performance of the system. The system was tested using a cervical Pap smear sample (prepared beforehand) as the test sample.

4.1 Focus Value Curves

As described in the Design and Construction chapter, images were captured using three different objectives (10x, 45x, 100x). The five focus measures described in the previous chapter were computed for each of those images and the plots were obtained. These plots are displayed below in figures 13-17.

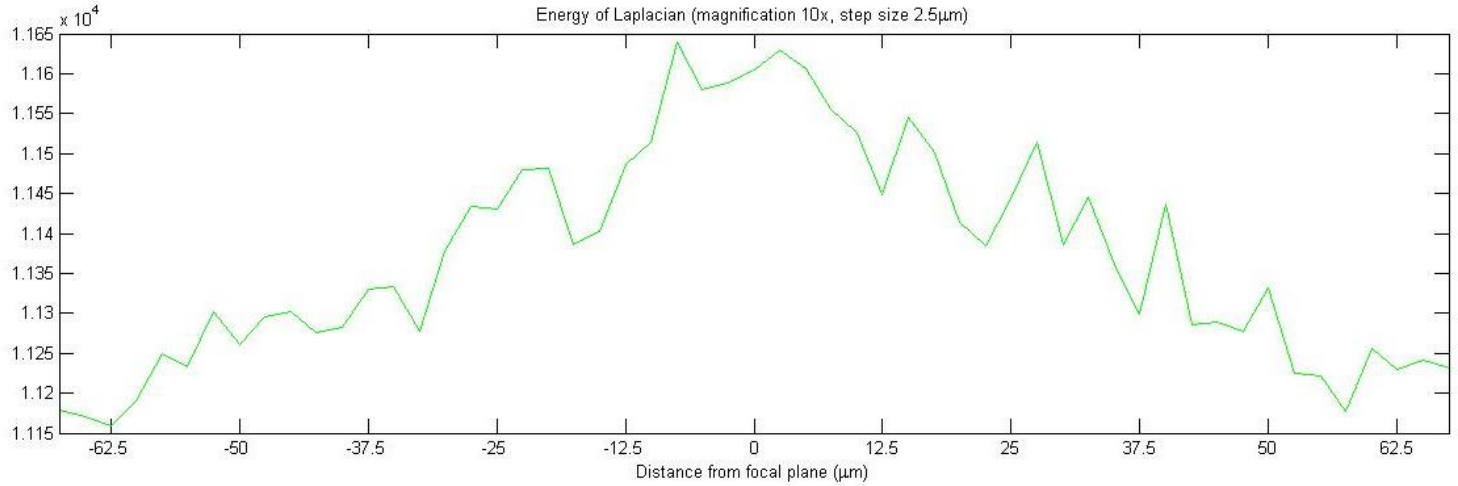


Figure 13: Energy of Laplacian (objective 10x, step size 2.5µm)

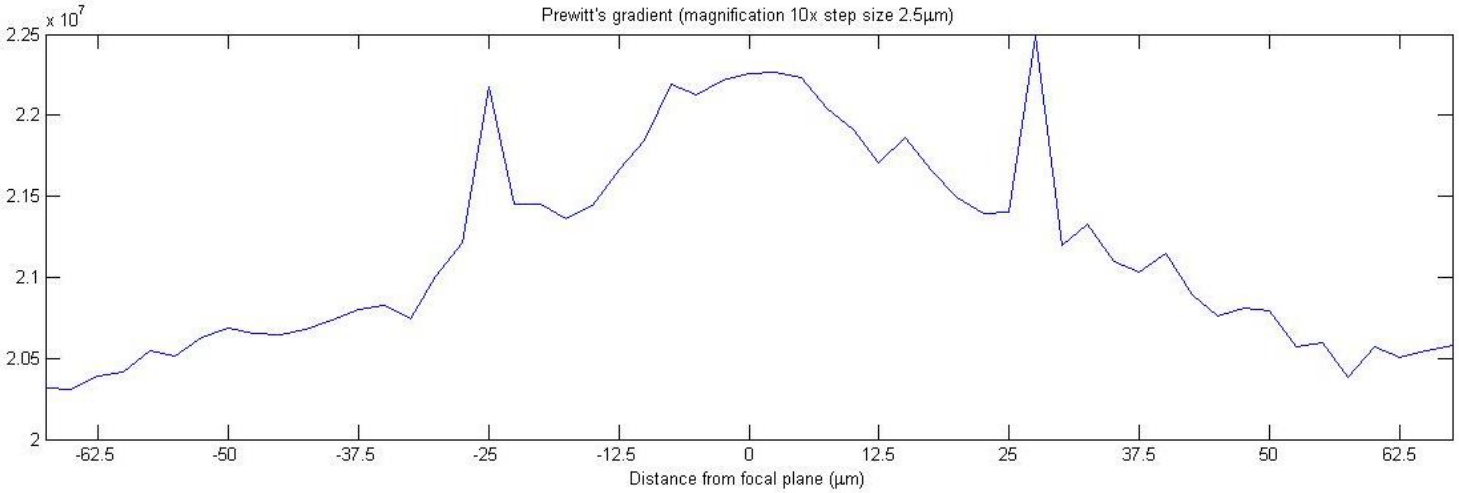


Figure 14: Prewitt's Gradient (objective 10x, step size 2.5µm)

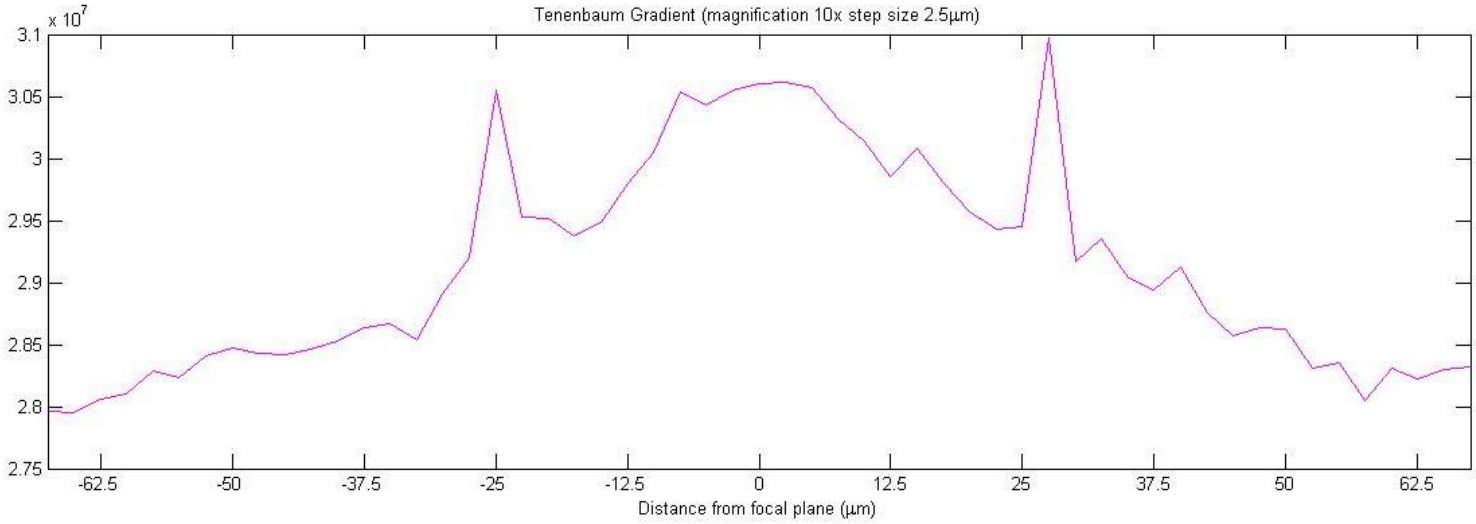


Figure 15: Tenenbaum Gradient (magnification 10x, step size 2.5µm)

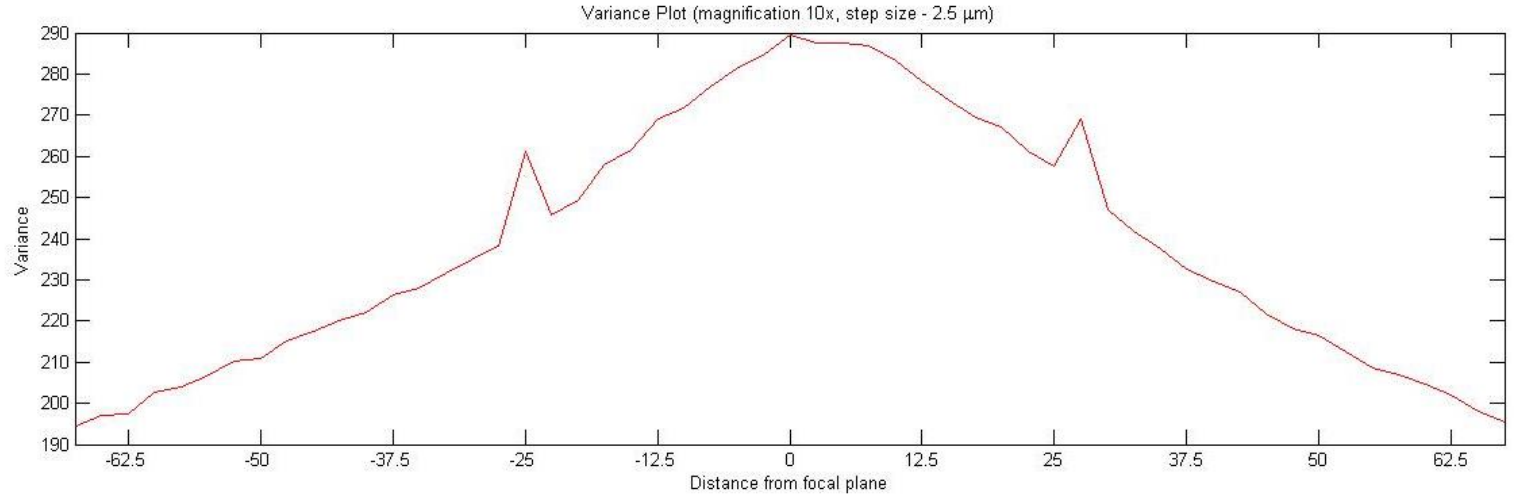


Figure 16: Variance (magnification 10x, step size 2.5µm)

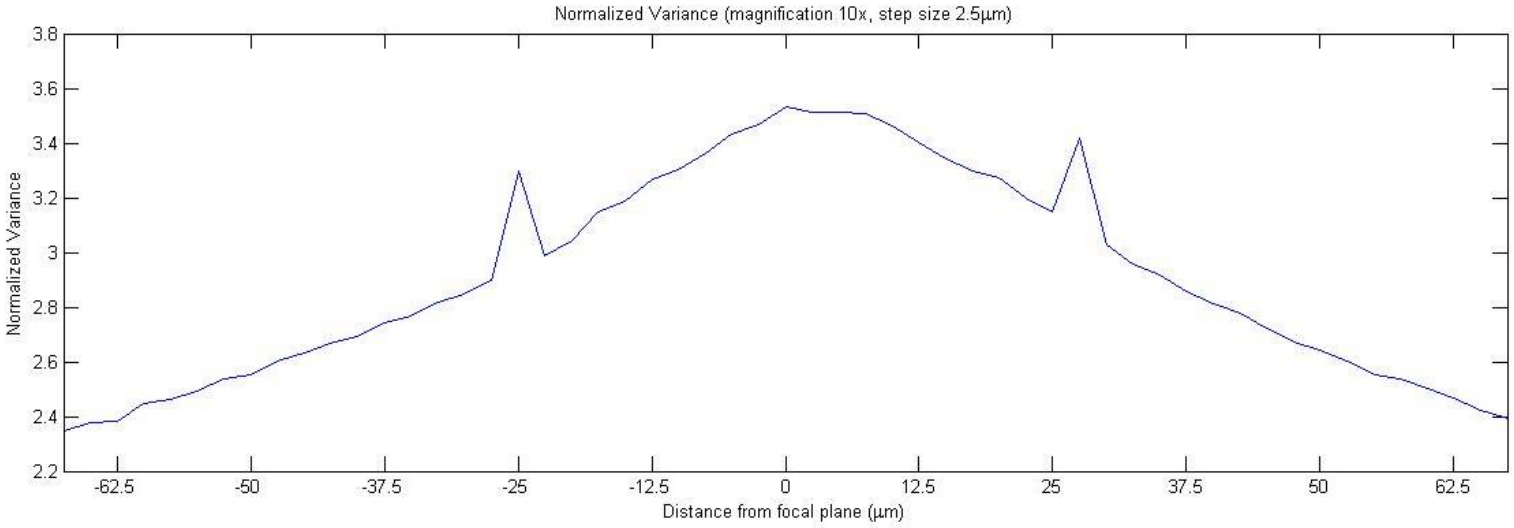


Figure 17: Normalized Variance (magnification 10x, step size 2.5µm)

Some desirable characteristics of focus value curves are that they should be monotonic away from the focal plane, and that they should have a global maximum at the origin (focal plane). None of the graphs are monotonic away from the origin and have local extrema, this is because there are multiple objects in the field of view which give rise to different extrema. By the above two criteria, it can be seen that the derivative based measures are less desirable than the statistical measures. The global maxima in the case of the derivative based measures are away from the origin. In addition to this, the focus value curves have multiple local maxima even at distances far away from the focal plane.

We have seen the focus value curves from images captured using a 10x objective. Now we will look at the focus value curves from 45x and 100x objectives. These reiterate the fact that the statistical measures are better than the derivative based ones.

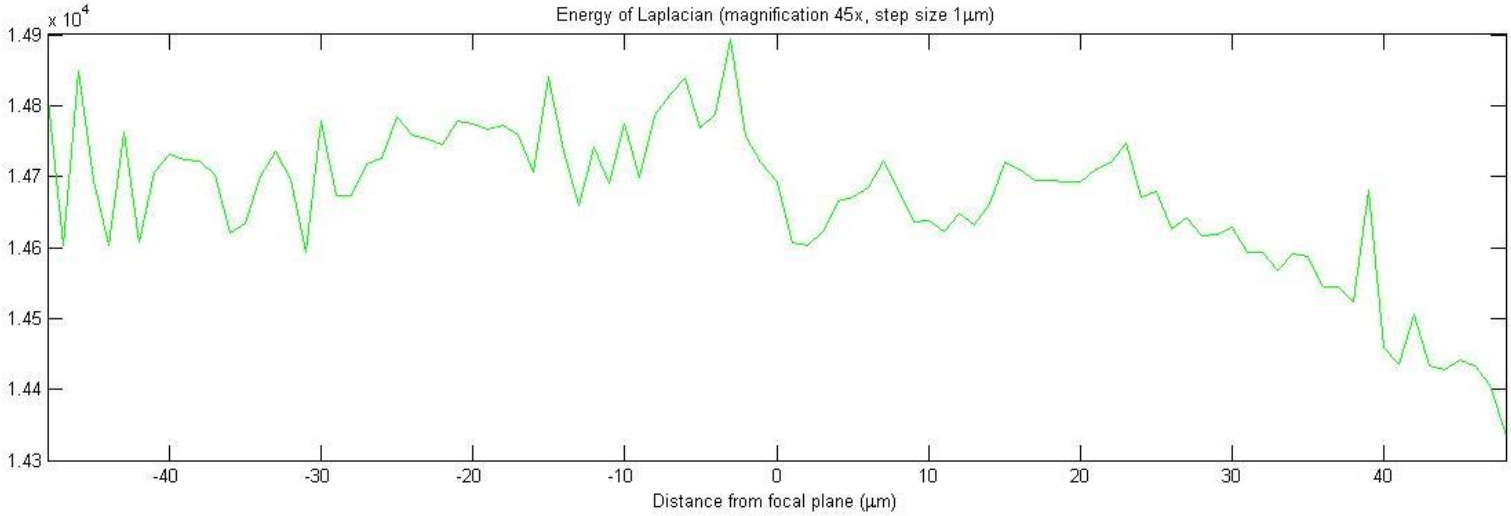


Figure 18: Energy of Laplacian (objective 45x, step size 1µm)

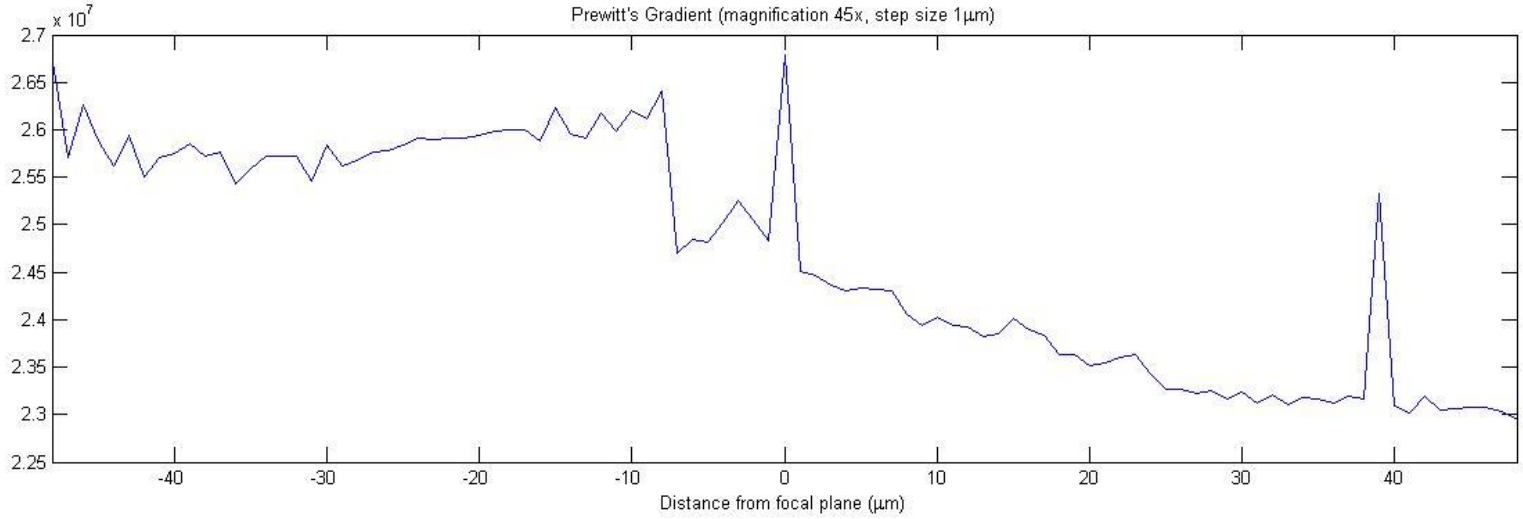


Figure 19: Prewitt's Gradient (objective 45x, step size 1µm)

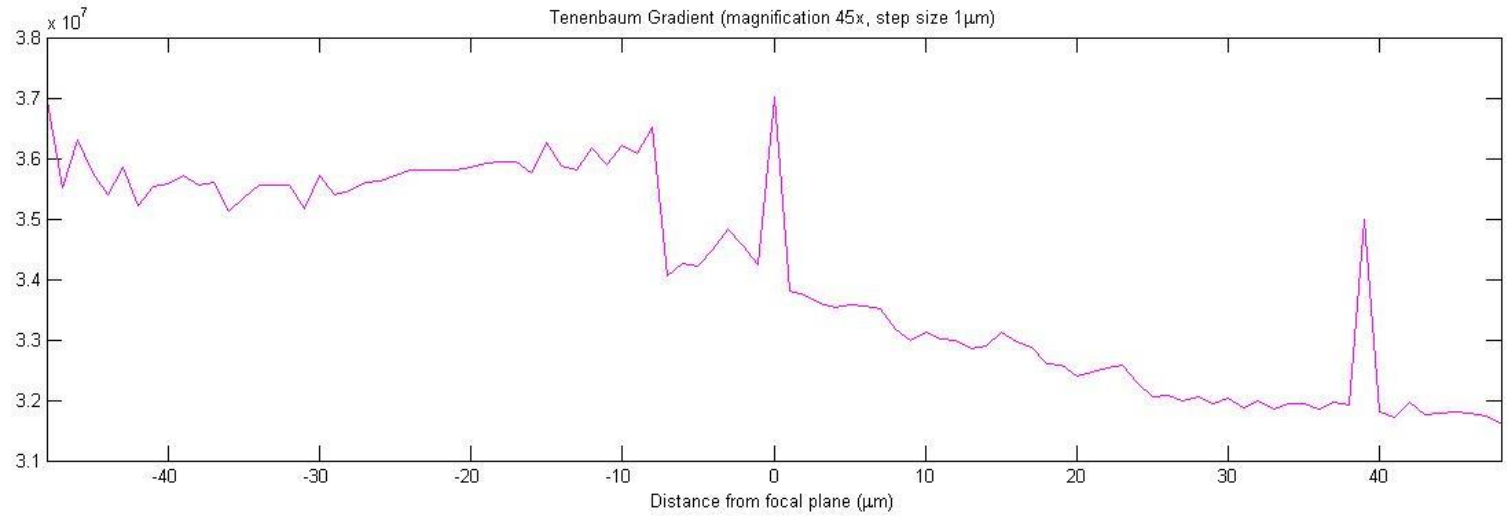


Figure 20: Tenengrad function (objective 45x, step size 1µm)

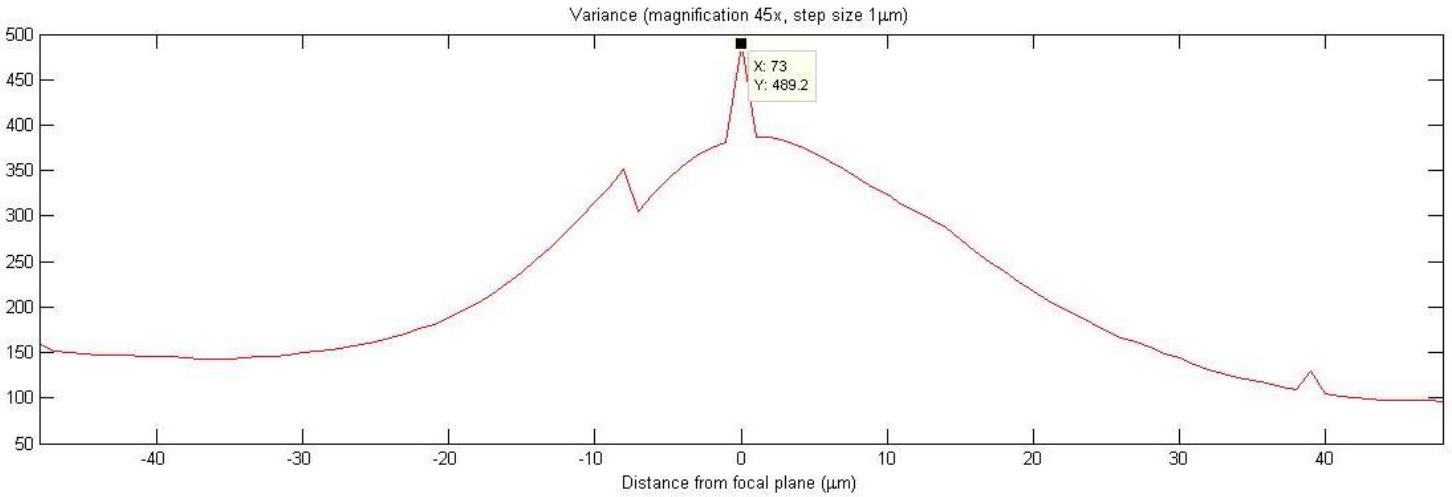


Figure 21: Variance (objective 45x, step size 1µm)

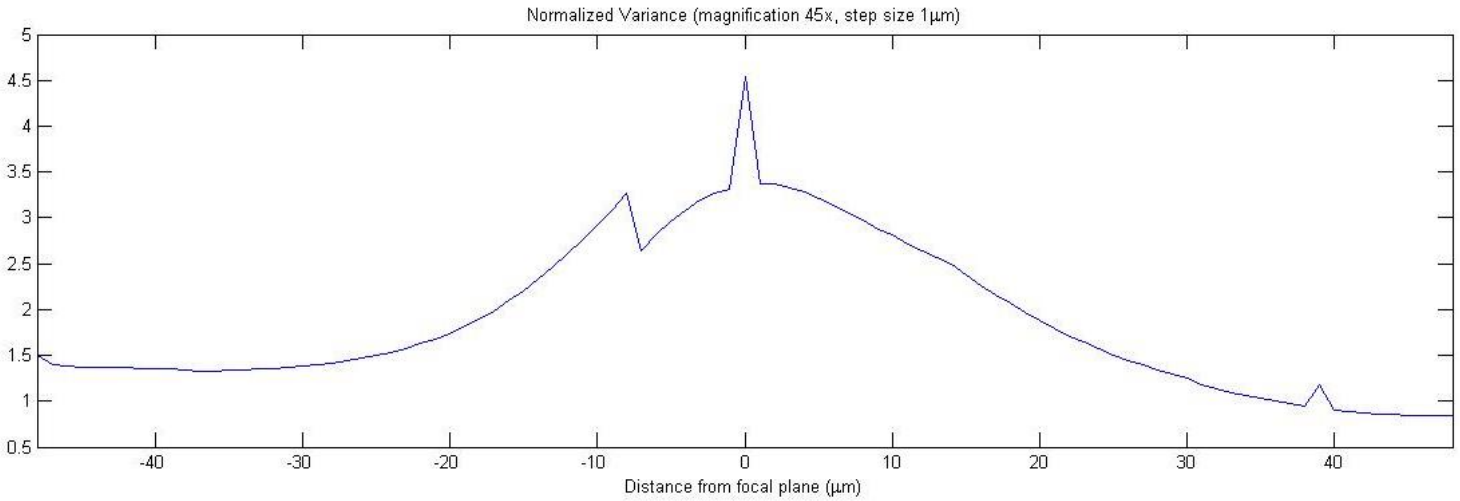


Figure 22: Normalized Variance (objective 45x, step size 1µm)

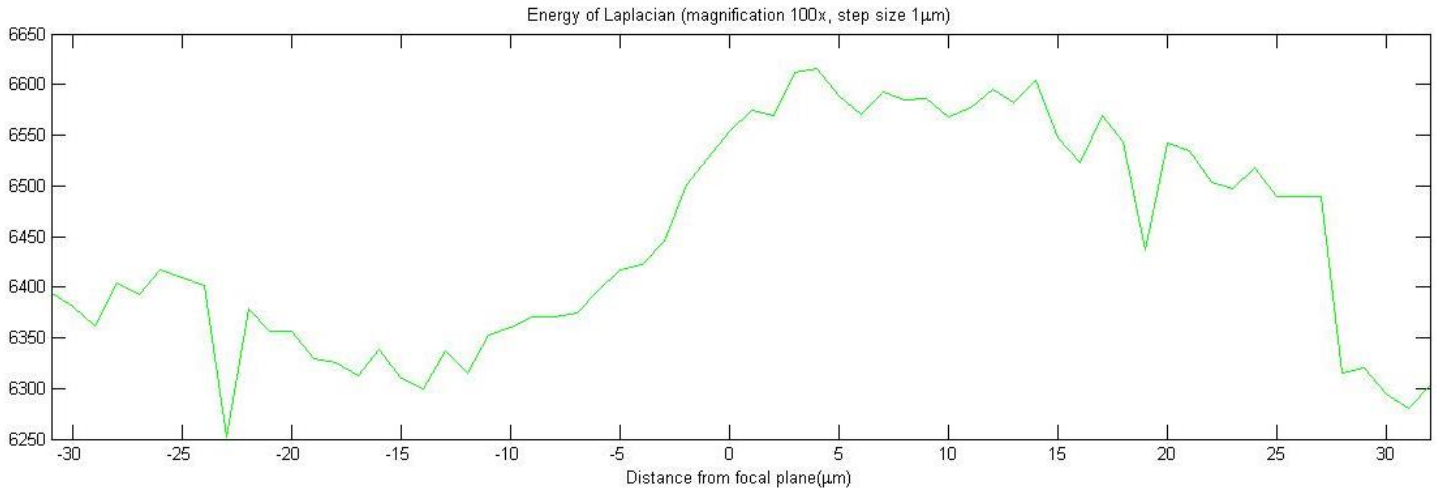


Figure 23: Energy of Laplacian (objective 100x, step size 1µm)

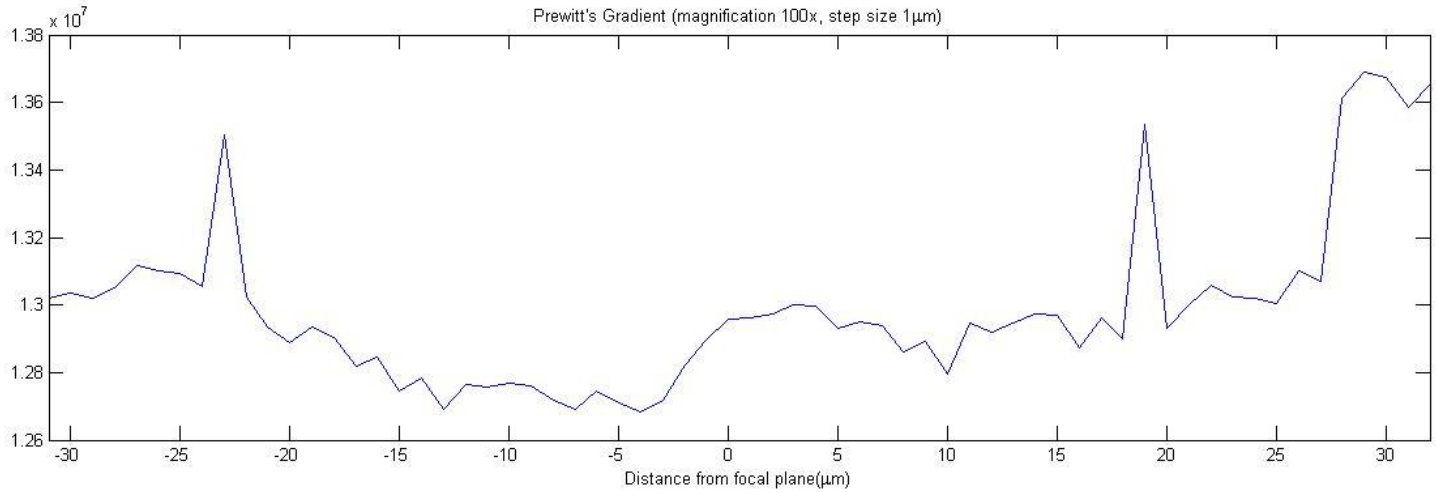


Figure 24: Prewitt's Gradient (objective 100x, step size 1µm)

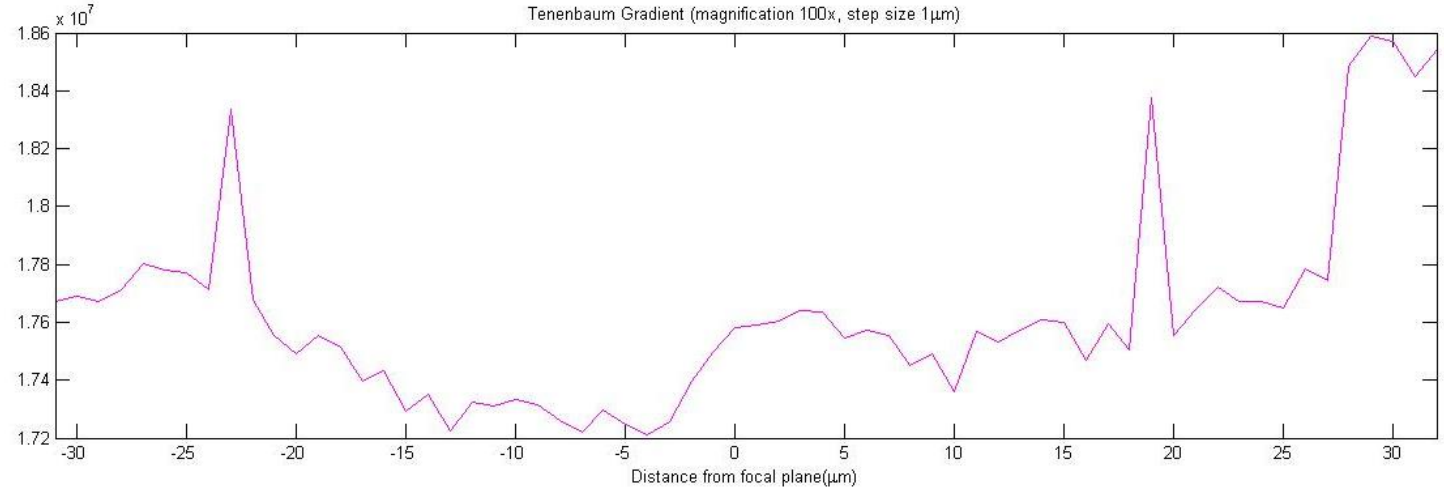


Figure 25: Tenengrad function (objective 45x, step size 1µm)

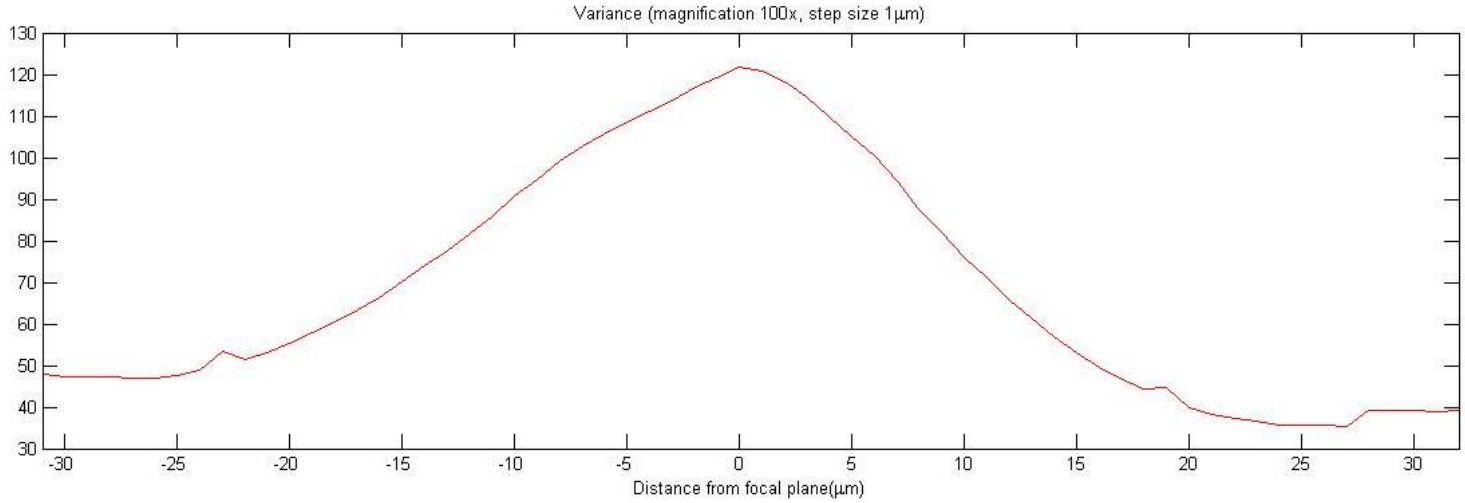


Figure 26: Variance (objective 100x, step size 1µm)

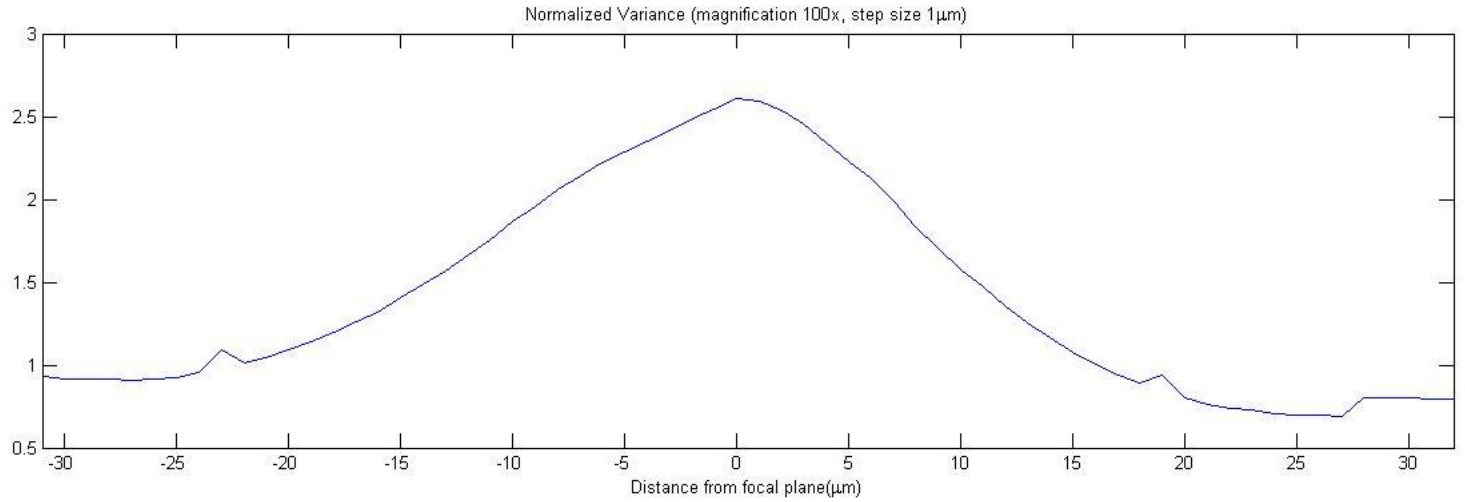
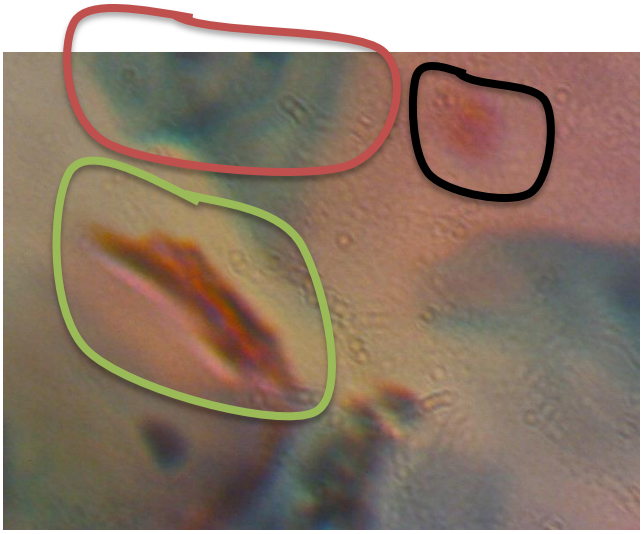


Figure 27: Normalized Variance (objective 100x, step size 1µm)

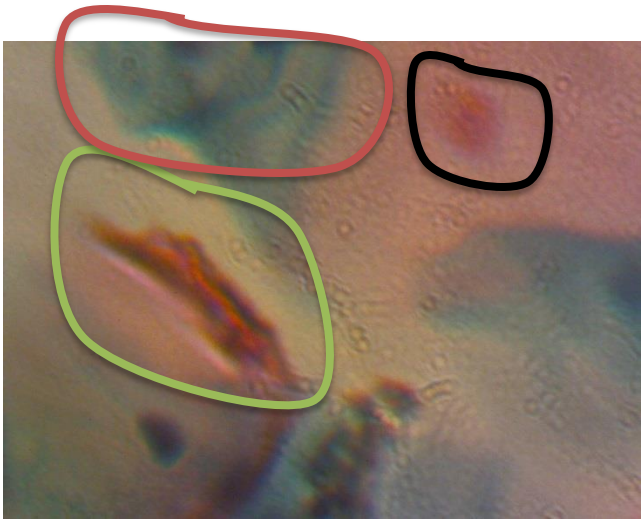
4.2 Discussion of Results

From the previous section we have established that statistical measures are better suited for autofocus algorithms. Normalized Variance was finally chosen as the focus measure for this autofocus system. This was because the difference between the global maximum of the focus value curve and the best focused image as determined by inspection was at most 2µm. This difference was the least among the five focus measures proposed.

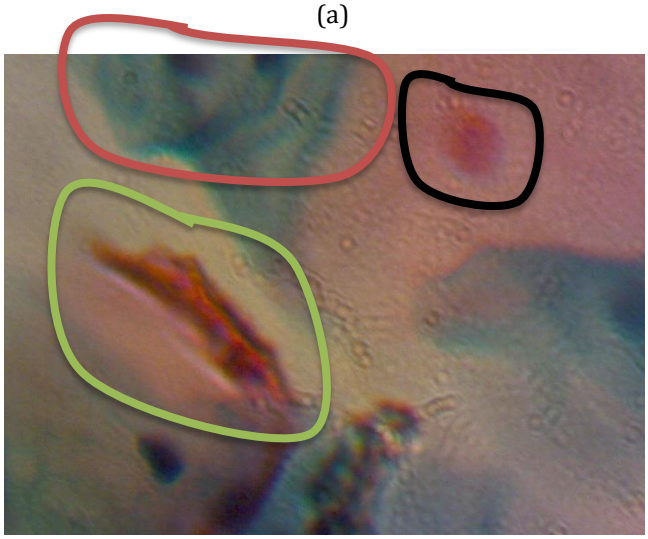
Both the local maximum search and the global search were implemented and tested. Below, you can see a sequence of images taken as the local maximum algorithm progresses from an unfocused image to a focused one. The objective lens used was the 45x objective and the step size taken was 1µm.



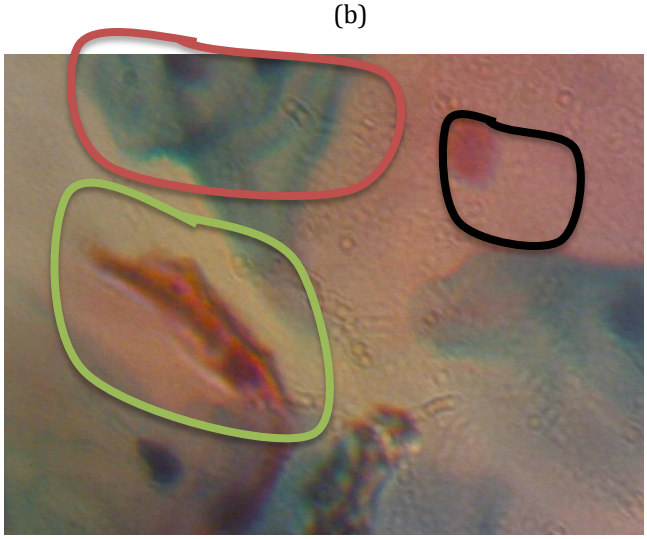
(a)



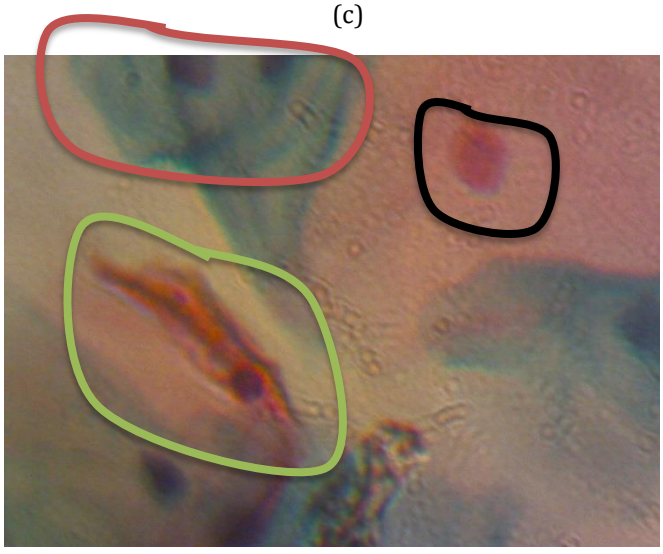
(b)



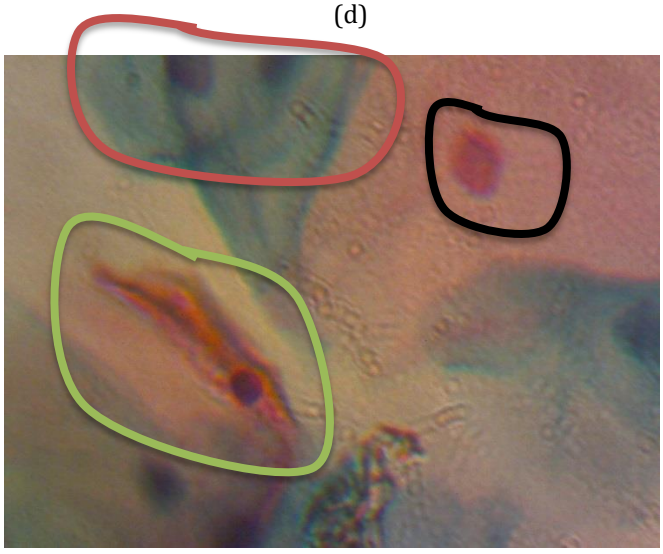
(c)



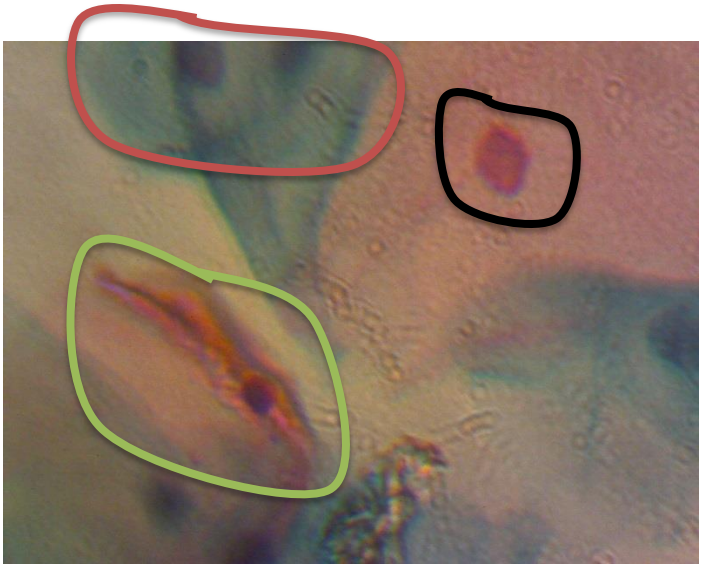
(d)



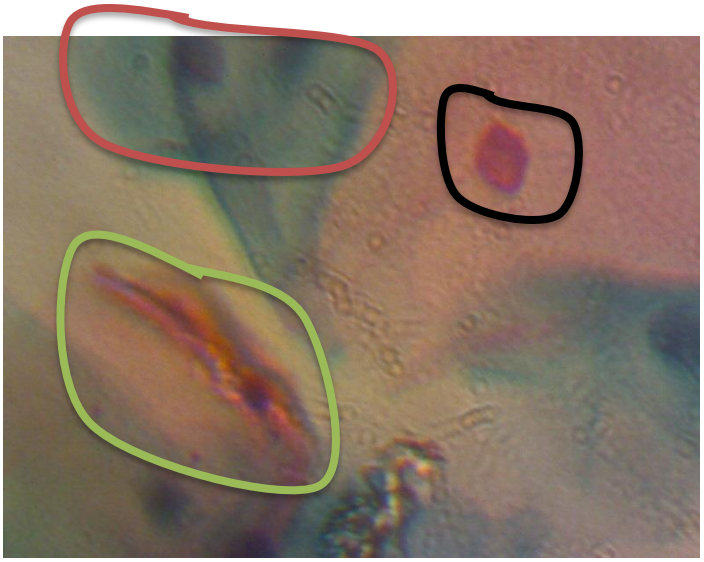
(e)



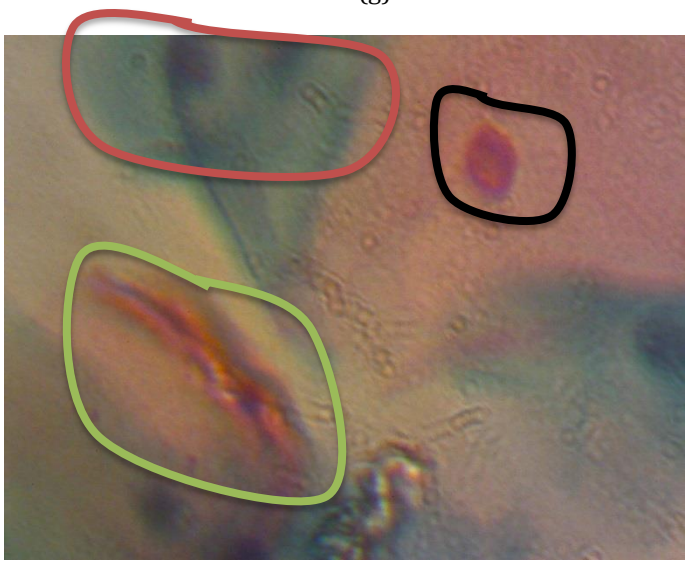
(f)



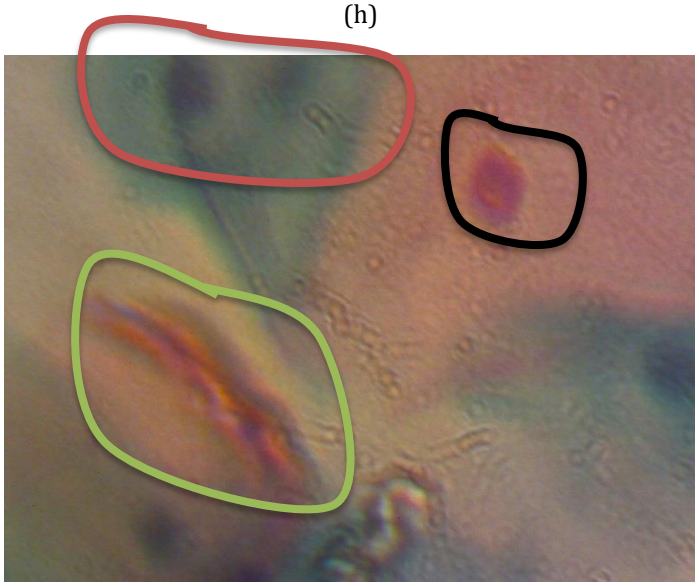
(g)



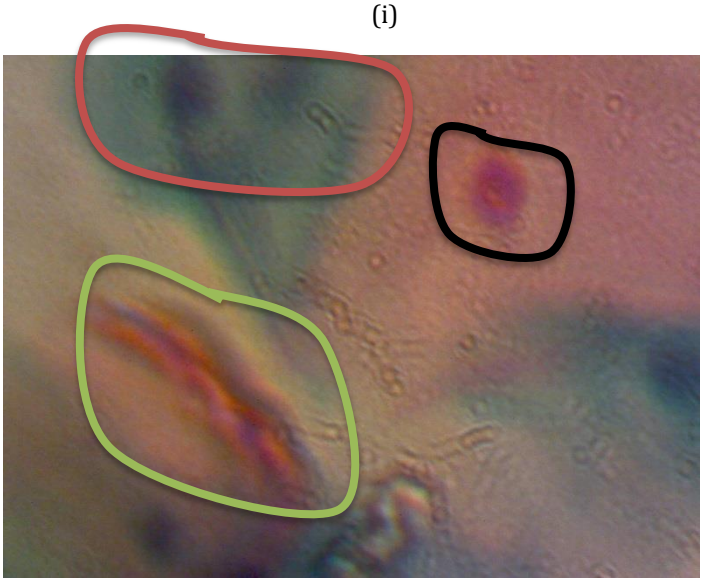
(h)



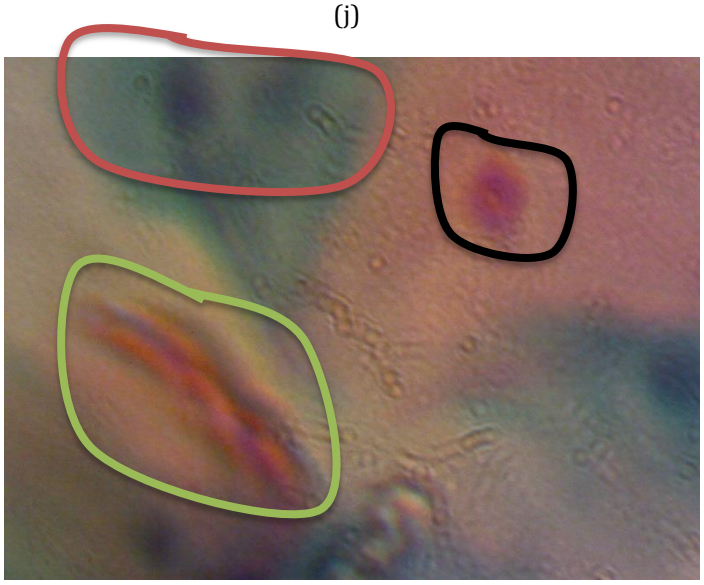
(i)



(j)



(k)



(l)

Figure 28: Sequence of images taken from local maximum performed on the Pap smear sample with a 45x objective using a step size of 1µm

In Figure 28 (a) to (k), one can see the progression from an unfocused image to a focused one. Though there are multiple components to the image, which have been circled in red, green and black, the cell circled in green is the central focus of the image. Figure 28 (f) is the image returned by the autofocus algorithm as the best focused image. One can observe how the different components of the image come into and go out of focus with each step of the stepper motor.

The local maximum search as described in the previous chapter works perfectly when the objective lens is within 24μm from the focal plane. This distance was established by repeatedly displacing the microscope from the focused position by various distances, small and large. This value was found to be consistent as it was tested at different regions within the sample. Some more examples of results are shown in Figures 29 and 30. Within this range, the system took between 60 and 90 seconds to arrive at the best focused position.

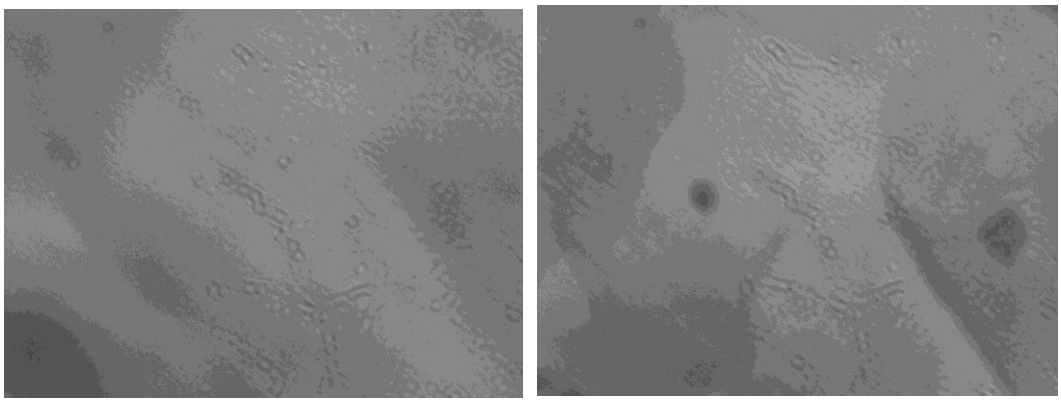


Figure 29: Initial (left) and Final (right) images when system was tested using 45x objective

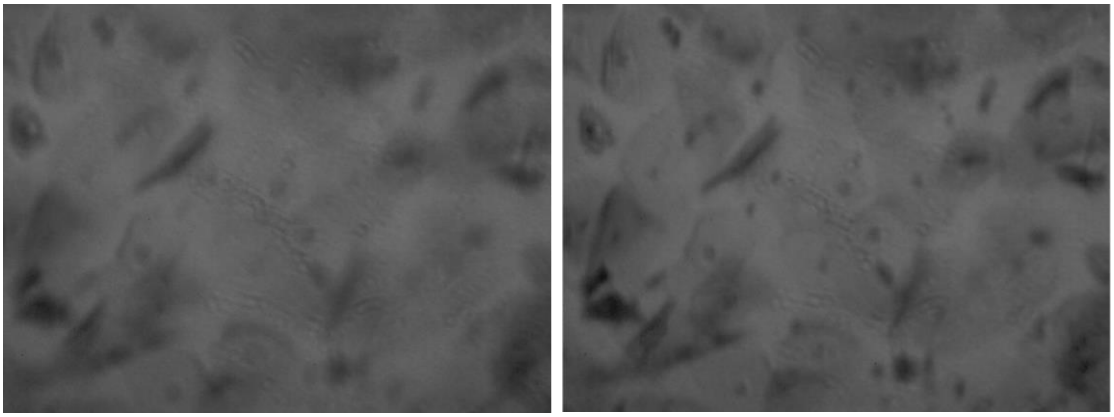


Figure 30: Initial (left) and Final (right) images when system was tested using 10x objective

The global search technique also finds the best focus position, but is extremely slow due to the fact that it has a fixed step size and a very large range (~400μm) to search in. However, the global search manages to find the focal plane, whatever the initial position. The global search algorithm takes about 2 minutes to attain the focus position when placed at an extreme.

The autofocus algorithm does not work when the object content in the frame is very low. This is evidenced by the fact that working with the 100x objective is difficult. The 100x objective results in highly zoomed images which hardly have one cell in them. This proves hard for the algorithm, since the focus value curve is fairly flat.

4.3 Limitations and future work:

4.3.1 Limitations of the system in its current form:

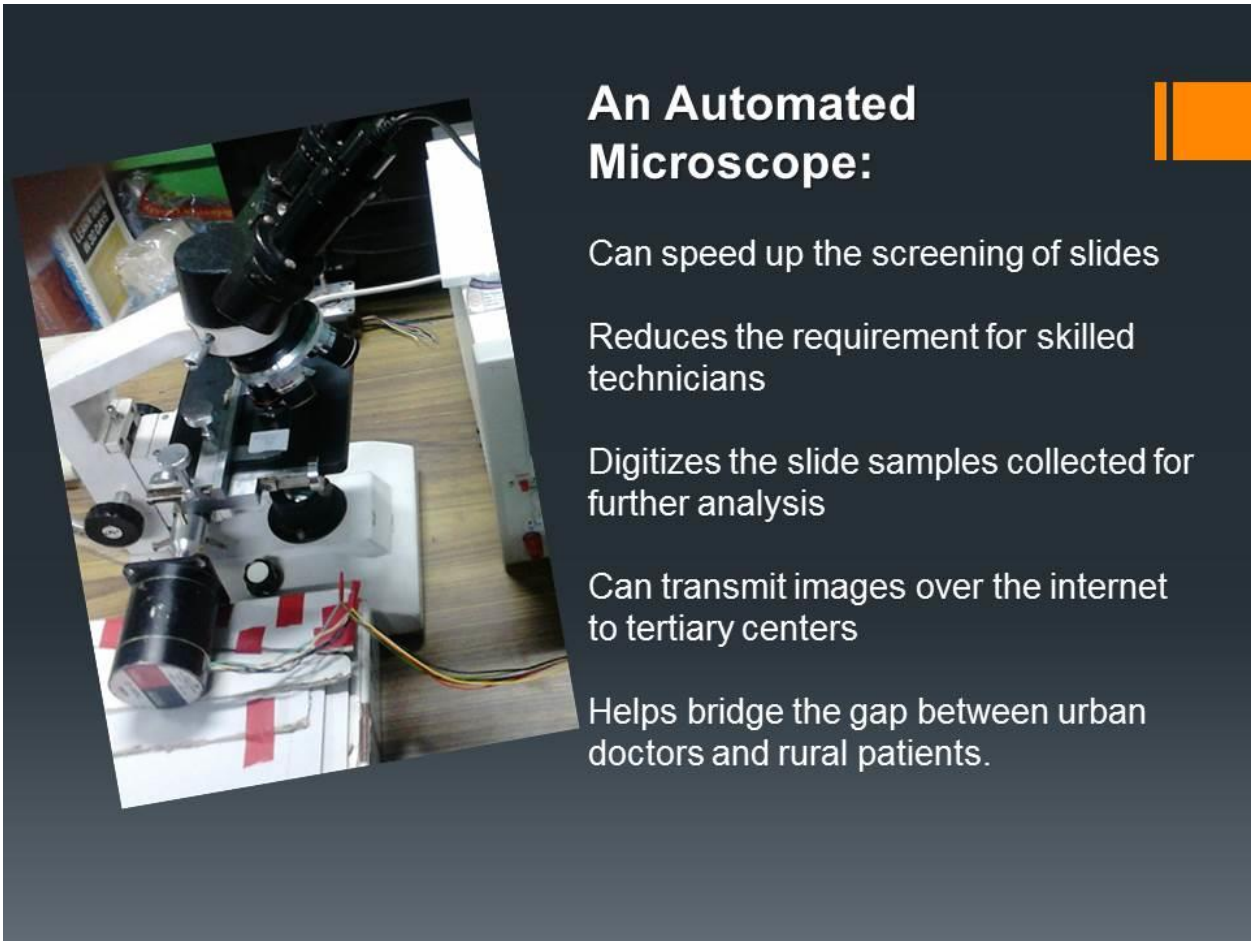
1. Autofocus works only when the objective is within a 24μm of the focal plane.
2. The whole system is extremely slow. This can be attributed to both the motor rpm as well as the small step size (2.5μm). The motor rpm has been fixed at 10 rpm to achieve higher torque, but can be increased to expedite the autofocus.
3. The digital imager has a poor resolution (2MP) and the images captured are of inferior quality.
4. The optical system of the microscope needs to be cleaned, there are certain details which are common to all the images captured which are not part of the sample under observation
5. Ensuring that the motor shaft and the adjustment knob of the microscope are in the same line is a cumbersome task, but most important to the functioning of the system.
6. For now the system is using a variable DC power supply to provide 24V to the motor. A battery pack needs to be designed or purchased to ensure that the system is portable.

4.3.2 Future work:

1. A better search algorithm needs to written in order to make the system faster. Possible alternatives are:
    a. Adaptive Step Size Search which changes the step size depending upon how far the objective lenses are from the focal plane [7]
    b. Adaptive step size to narrow the range followed by quadratic curve fitting with three points within a 20μm range [8]
2. Purchasing a better imager to get high quality images. Possible alternatives include digital cameras for microscopes from Olympus and Nikon.
3. Testing the autofocus system on different microscopes and studying the adaptability of the system to different brands of microscopes.
4. Possible redesign of the shaft coupler to make it easier to align the motor shaft with the microscope knobs.
5. Designing or purchasing a battery pack that can supply 24V at 0.34A for the stepper motor.
6. Extending the automated microscope system to include control over the x-y movement of the stage.
7. Transferring the driver circuit from bread board to PCB.

CONCLUSION

The end result of this thesis is a cost-effective, automated solution to remove the bottleneck in rural healthcare as depicted at the beginning. This is a simple yet effective tool which will release the pressure on the single lab technician present at primary healthcare centers. One of the most salient features of the approach presented is that it can be fitted onto existing microscopes which are present at the healthcare centers. The autofocus system is also very cheap, with the total cost of the system to be attached to the microscope coming to less than Five Thousand Rupees.



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7. Chia-Hao Chang, Chiou-Shann Fuh, *Auto Focus Using Adaptive Step Size Search and Zoom Tracking Algorithm*, Department of Computer Science and Information Engineering, National Taiwan University, Taipei, Taiwan

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9. Y. Zhai, Y. Liu, D. Zhou, S. Liu, *Automatic Identification of Mycobacterium Tuberculosis from ZN-stained Sputum Smear: Algorithm and System Design*, Proceedings of the 2010 IEEE International Conference on Robotics and Biomimetics

APPENDIX

A. Codes used in the project

1. Arduino code:

```
#include <Stepper.h>
const int stepsPerRevolution = 200;
const int bufferLength = 10;
char inBytes[bufferLength] = {0};
int steps_to_take = 0;
Stepper myStepper(stepsPerRevolution,11,10,9,8);
void setup(){
        myStepper.setSpeed(10);
        Serial.begin(9600);
        Serial.println("Ready for transmission");
}
void loop(){
        if(Serial.available()>0)
        {
        Serial.readBytes(inBytes,bufferLength);
        steps_to_take = atoi(inBytes);
        myStepper.step(steps_to_take);
        Serial.write("Microscope stepped ");
        Serial.write(inBytes);
        Serial.println(" steps");
        for(int i=0;i<bufferLength;i++)
        inBytes[i] = 0;
        }
}
```

2. Code to capture images sequentially

```
%Open the arduino serial port
arduino = serial('COM4');
fopen(arduino);

%Open the camera
vid = videoinput('winvideo',2,'YUY2_1600x1200');
set(vid,'ReturnedColorSpace','rgb'); %use 'grayscale' alternatively
```

```
for i = 1:140
    %get snapshot
    data = getsnapshot(vid);
    %save snapshot to file
    if(i<10)
        filename = ['0506_45x_5step\image00' int2str(i) '.jpg'];
        imwrite(data,filename,'jpg');
        fprintf('Saved file %d\n',i);
    else if(i>=10 && i<100)
            filename = ['0506_45x_5step\image0' int2str(i) '.jpg'];
            imwrite(data,filename,'jpg');
            fprintf('Saved file %d\n',i);
        else
            filename = ['0506_45x_5step\image' int2str(i) '.jpg'];
            imwrite(data,filename,'jpg');
            fprintf('Saved file %d\n',i);
        end
    end
    %step the motor
    fprintf(arduino,'-5');
end
fclose(arduino);
fopen(arduino);
fclose(arduino);
```

3. Autofocus Search algorithm – local maximum

```
%Open the arduino serial port
arduino = serial('COM4');
fopen(arduino);

%Open the camera
vid = videoinput('winvideo',2,'YUY2_1600x1200');
set(vid,'ReturnedColorSpace','grayscale');
fprintf('Devices connected\n');

% ------ Autofocus Algorithm goes here------- %
in_focus = 0;
i = 1; %iteration
step = 5;
init_image = getsnapshot(vid);
```

```
while(~in_focus)
    img = getsnapshot(vid);
    imshow(img);
    F = var(double(img(:)))/mean(double(img(:)));
    if(i<=6)
        if(i==1)
            f = F;
        else
            f = [f F];
        end
        fprintf(arduino,int2str(step));
    else if(i==7)
            if(f(1)>mean(f(2:end)))
                i = 0;
                step = -step;
            else
                f = [f F];
                fprintf(arduino,int2str(step));
            end
        else
            f = [f F];
            if(F == max(f))
                fprintf(arduino,int2str(step));
            else
                [value max_index] = max(f);
                diff = abs(i-max_index);
                fprintf(arduino,int2str(-diff*step));
                final_image = getsnapshot(vid);
                in_focus = 1;
            end
        end
    end
    fprintf('completed iteration number %d\n',i);
    i = i+1;
end
fclose(arduino);
fopen(arduino);
fclose(arduino);
figure; imshow(init_image);
```

```
figure; imshow(final_image);
```

4. Autofocus Search algorithm – global maximum

```
%Open the arduino serial port
arduino = serial('COM4');
fopen(arduino);
%Open the camera
vid = videoinput('winvideo',2,'YUY2_1600x1200');
set(vid,'ReturnedColorSpace','grayscale');
fprintf('Devices connected\n');
% ------ Autofocus Algorithm goes here------- %
in_focus = 0;
i = 1; %iteration
step = 5;
init_image = getsnapshot(vid);
j = 0; %keeps count of local maxima
while(~in_focus)
    img = getsnapshot(vid);
    F = var(double(img(:)))/mean(double(img(:)));
    if(i<=6)
        if(i==1)
            f = F;
            f_diff = 0;
        else
            f = [f F];
            f_diff = [f_diff F-f(i-1)];
        end
        fprintf(arduino,int2str(step));
    else if(i==7)
            if(f(1)>mean(f(2:end)))
                i = 0;
                step = -step;
            else
                f = [f F];
                fprintf(arduino,int2str(step));
                f_diff = [f_diff F-f(i-1)];
```

```
            end
        else
            f = [f F];
            f_diff = [f_diff F-f(i-1)];
            if(F == max(f))
                fprintf(arduino,int2str(step));
            else if(F~=max(f) && max(f)<3.2)
                    fprintf('local maximum found, continuing...\n');
                    fprintf(arduino,int2str(step));
                    j = j+1;
                    i = i+1;
                    if(j>10)
                        [value max_index] = max(f);
                        diff = abs(i-max_index);
                        pause(2);
                        fprintf('shifting to max position\n');
                        fprintf(arduino,int2str(-2));
                        fprintf(arduino,int2str(-diff*step));
                        fprintf('shifted\n');
                        final_image = getsnapshot(vid);
                        in_focus = 1;
                    end
                    continue;
                else
                    [value max_index] = max(f);
                    diff = abs(i-max_index);
                    fprintf(arduino,int2str(-diff*step));
                    final_image = getsnapshot(vid);
                    in_focus = 1;
                end
            end
        end
    end
    fprintf('completed iteration number %d\n',i);
    i = i+1;
    j=0;
end
fclose(arduino);
fopen(arduino);
fclose(arduino);
```

```
figure; imshow(init_image);
figure; imshow(final_image);
```

B. Budget

Item	Cost
Driver Circuit + Bolts	Rs 100
Motor	Rs 500
Shaft Coupler	Rs 200
Arduino UNO	Rs 1360
Celestron Imager	Rs 2400
Total	Rs 4560

The entire autofocus system costs less than Rs. 5000.