DESIGN AND FABRICATION OF HANDHELD CONFOCAL MICROSCOPY HEAD

by

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DESIGN AND FABRICATION OF HANDHELD

CONFOCAL MICROSCOPY HEAD

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ABSTRACT

DESIGN AND FABRICATION OF HANDHELD CONFOCAL MICROSCOPY HEAD

Confocal Microscopes have advantageous results compared to Wide Field Microscopes while imaging the cellular structures of thick biological specimens. The aim of this thesis work is to design and fabricate a handheld confocal microscopy head in order to enable of imaging from different depths. The handheld confocal microscopy head is composed of three main parts which are; fiber optical bundle, the designed and fabricated mechanical housing and the embedded system.

The fiber optical bundle is used to increase the flexibility of the tabletop confocal microscope. In other words, the fiber optical bundle used to ease the visualization of the biological specimens that are not easily accessible. By connecting the fiber optical bundle to the end of the objective lens in confocal microscope, the handheld microscopy head can be used in a broad range of areas.

The mechanical design is made by taking the efficiency of the handheld confocal microscopy head into consideration during the imaging of biological specimens. The dimensions of the device are suitable for hand held usage. On the other hand, the embedded system design is implemented carefully to produce a back and forth movement of the fiber optical bundle at different step sizes due to the selection of the user. The dynamic range of the confocal microscopy head is 1 μm to the 2 cm which is appropriate for the use of imaging of the biological specimens.

ÖZET

ELDE TAŞINABİLİR EŞ ODAKLI MİKROSKOP BAŞI TASARIMI VE ÜRETİMİ

Eş odaklı mikroskoplar geniş alanları mikroskoplara kıyasla hücresel seviyede kalın biyolojik yapıların görüntülenmesinde daha avantajlı sonuçlara sahiptir. Bu tezin amacı elde taşınabilir bir eş odaklı mikroskobu başı tasarlamak ve üretmektir. Elde taşınabilir eş odaklı mikroskop başı temel olarak 3 ana kısımdan oluşur, bu ana kısımlar fiber optik kablo, tasarlanan ve üretilmiş mekanik sistem ve de gömülü sistemdir.

Fiber optik kablo masaüstü eş odaklı mikroskopların elastikiyetini arttırmak amaçlı kullanılmıştır. Diğer bir deyişle fiber optik kablo ulaşımı zor olan bölgeleri görüntülenmesini kolaylaştırır. Eş odaklı mikroskopta fiber optik kabloyu objektif lensin önüne koyarak görüntüleme alanları genişletilebilir ve bir çok spesifik alanlarda kullanılır.

Mekanik dizayn, biyolojik numunelerin görüntülenmesinin verimliliğine dikkat edilerek yapılmıştır. Dizayn edilen parça boyutlarına göre elde taşınabilmek için uygundur. Öteki yandan, bütünleşik sistem tasarımı fiber optik kablonun ileri ve geri yönde kullanıcının seçtiği adım sayısına göre hareketini sağlamak amacıyla dikkatle yürütülmüştür. Elde taşınabilir eş odaklı mikroskobun dinamik çalışma aralığı biyolojik yapıların görüntülenmesine uygun olarak 1 µm ve 2 cm arasındadır.

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LIST OF SYMBOLS/ABBREVIATION

LSCM	Laser Scanning Confocal Microscope
PMT	Photon Multiplier Tube
FOV	Field of View
DAQ	Data Acquisition
NI	National Instruments
NA	Numerical Aperture
Ω	Ohm
λ	Wavelength

1. INTRODUCTION

1.1. CONFOCAL MICROSCOPE

According to the World Cancer Report on 2014, cancer, which is being one of the primary diseases that affects human health, is associated with 14 million newborn cancer incidences for the next 20 years. Cancer cases will be expected to jump to approximately 22 million for the same interval of time [1]. As we can realize by looking up to the statistic of the cancer on worldwide, one can state that in order to improve the life quality of cancer patients and to reduce the cost of the treatment, early diagnosis is crucial [1] [2]. Moreover, the rate of the cancer cells that are originated from epithelial cells, consists the 85% of the total and to detect the formation of the tumor cells the cellular level of diagnosis is required [1].

There are a lot of methods that are used in the imaging of biological specimens on cellular level, such as magnetic resonance and ultrasound imaging, but these technologies are resolution limited [1]. On the other hand, confocal microscopy has the opportunity of imaging biological specimens in real time at cellular level with a sub-micron resolution [1] [3] [4] [5] [6] [7] [8].

Confocal Microscopy has two major advantages compared to wide field microscopy, which can be seen in Figure 1.1. Firstly, in the confocal microscopy, a spatial pinhole is used to eliminate the out of focus of light in order to prevent the glaring of the biological specimens, whose thicknesses exceeds the relative immediate plane of focus [9] [10] [11]. On the other hand, in conventional microscopy the whole specimen is simultaneously flooded with excitation source and this results in the defocusing of the microscope.

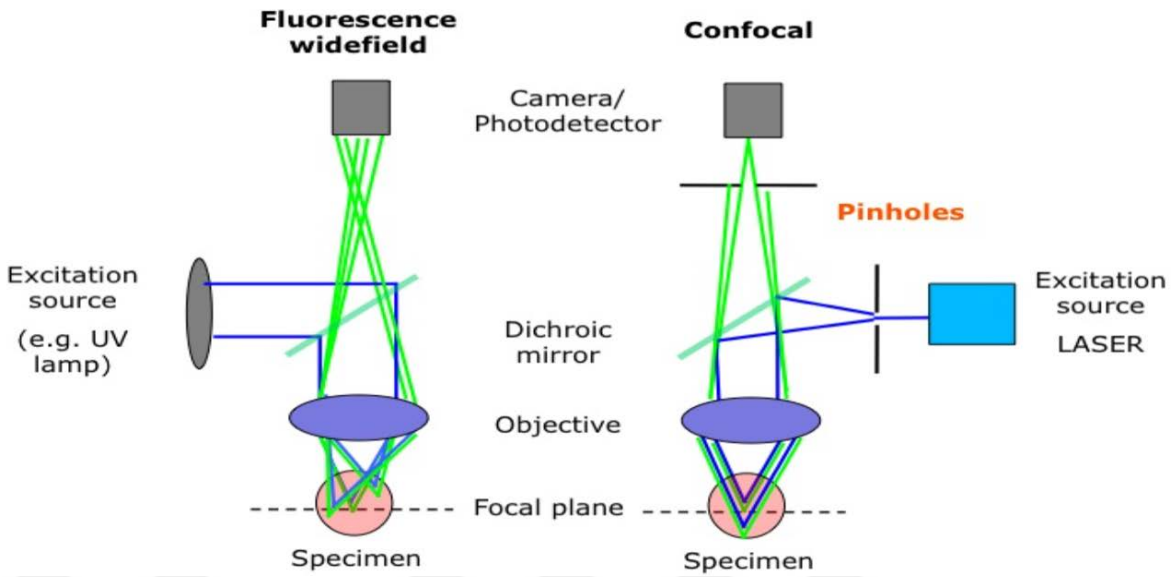


Figure 1.1. Comparison of the Fluorescence Wide field and Confocal Microscopy [12]

As a consequence, achieved resolution is smaller compared to confocal microscopy. In other words, because of the part that the emission of the secondary fluorescence occurs at high levels for the thicknesses that are greater than the 2 µm throughout the volume of excitation on the objective focal plane, the resolution of the images are low [13]. To exemplify this situation Figure 1.2. can be seen.

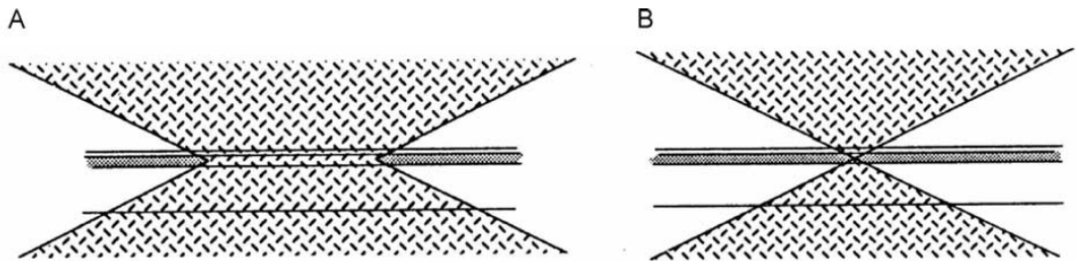


Figure 1.2. Comparison of the immediate plane of focus: (A) Conventional Fluorescence Microscopy; (B) Confocal Microscopy [4]

As in the Figure 1.2., the comparison of the achieved resolution between conventional and confocal microscopy is illustrated. Since in confocal microscopy a pinhole is used to focus,

the resolution is higher. The usage of a pinhole is a fundamental improvement of the confocal microscopy thanks to its ability to capture images with higher quality [10] [14]. Since the imaging capability in confocal microscopes has improved to the cellular level imaging, confocal microscopy is a valuable tool for imaging of biological specimens. Moreover, with the use of a, the signal to noise ratio, image contrast and axial resolution are enhanced because the pinhole prevents the out of focus of the light on the biological specimen [1] [2] [15].

Secondly, Confocal Microscopy gives the ability of composing 3-dimensional imaging of the biological specimens with very high accuracy from the combination of 2-D images at different depths which is called as optical sectioning [4] [8]. The optical sectioning ability, z-slicing, in which image slices that are parallel to the surface of the targeted specimen are taken from different tissue depths [4] [14] [13]. As a result, the optical sectioning makes the in vivo imaging of thick biological specimens possible without physically cutting and slicing the tissue as opposed to conventional microscopy [1] [4] [11] [8] [14]. In addition, the optical sectioning ability of the confocal microscopy can be used in determination of the suspected lesions and elimination of biopsies that may be painful for the patient [5] [8] [9] [16]. Moreover, the LSCM is also useful in the imaging of frozen pathology [16]. To summarize, the optical sectioning and 3-Dimensional configuration can be seen in Figure 1.4.

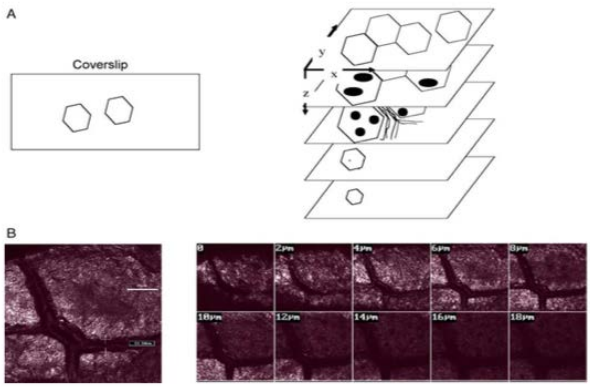


Figure 1.3. (A) The xy images from different depths(z); (B) Acquired porcine skin images via Confocal Microscopy from different depths [4]

As you can see, Confocal Microscopy is advantageous in the characterization of the cell morphologies of the biological specimens via non-invasive methods both in vivo and in

vitro, in which the cell information is used in the determination of the precancerous cells with respect to the images that are analyzed by the doctors [1] [4] [9]. Thus, the use of confocal microscopes could be time saving in the treatment of cancerous cells [17]. On behalf of the advantage of time saving, due to the early detection of the cancerous cells, the possibility of any disabilities in neurological system that stems from cancers, could be prevented or abated [18].

The tabletop confocal microscopes made great contributions to the biological imaging field but there is also a necessity of handheld devices with smaller dimensions to use in the clinical experiments [16]. In other words, the miniaturization of the medical devices became a required specification [19]. This requirement stems from the rigid pattern of the benchtop confocal microscopes; the practical usage is limited in terms of the sample position [16]. Therefore, the miniaturization of the confocal microscopes is necessary.

Arrassmith et. al. presented a portable handheld confocal microscope that can be clinically used. As shown in Figure 1.5. , in their device they used a MEMS-based micro mirror as the scanning unit and for axial resolution they chased to actuate the objective lens [16].

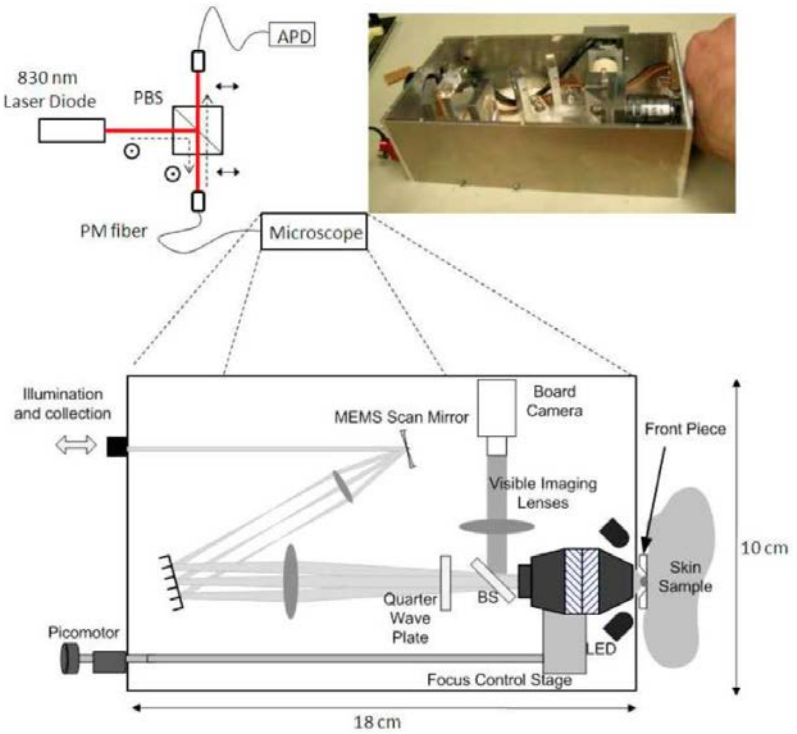


Figure 1.4. The Handheld Confocal Microscpe [16]

The dimension of the proposed handheld LSCM is 10 cm x 18 cm. A laser diode with a wavelength of 830 nm is connected to the microscope via single mode polarization maintaining fiber bundle (PM). Arrassmith et. al. used Pico motor to actuate objective lens.

On the other hand, Sung and Liang designed and fabricated miniature objective mechanism, which was connected to a syringe pump. The syringe pump is controlled via a step motor and with the applied pressure tissue could be placed to the immediate plane of focus and also with its movement axial resolution is achieved [20].

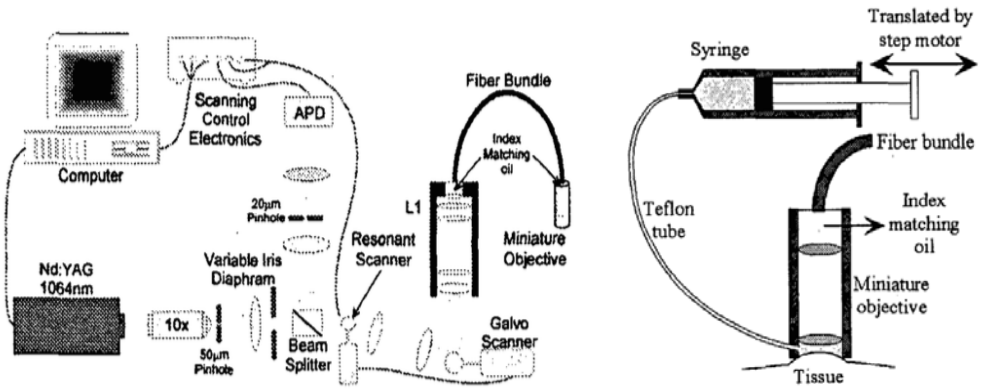


Figure 1.5. ; 1.6. The Schematic Confocal Microscope and Axial scanning mechanism [20]

The proposed two examples are used fiber optical bundles in their systems for a lot of different aims such as giving flexibility to the system or increasing the field of view and data acquisition. Therefore, the fiber optical bundles are important for optical systems. To clarify the usage, fiber optical imaging bundles are light transmission channels and used in the design of the specialized high resolution imaging probes in a various of applications such as confocal microscopy and endoscope [1] [21] [22]. In other words, The fiber optical bundles serve as an extension unit for the microscopes [22]. Thanks to the fiber optical bundles, the size restriction of the tabletop microscopes could be eliminated and miniaturization of the microscopes could be successfully achieved [22] [19]. As a result, increased flexibility and access lead to improvements in imaging biological samples [22].

The placement of the fiber optical bundle is very critical for the conductance of light. The fiber optical bundle must be placed on the focal point of the objective lens in order to take the image of every fiber core in the bundle. Wang et. al. designed a confocal endoscope and imaged the end face of the fiber optical bundle [1].

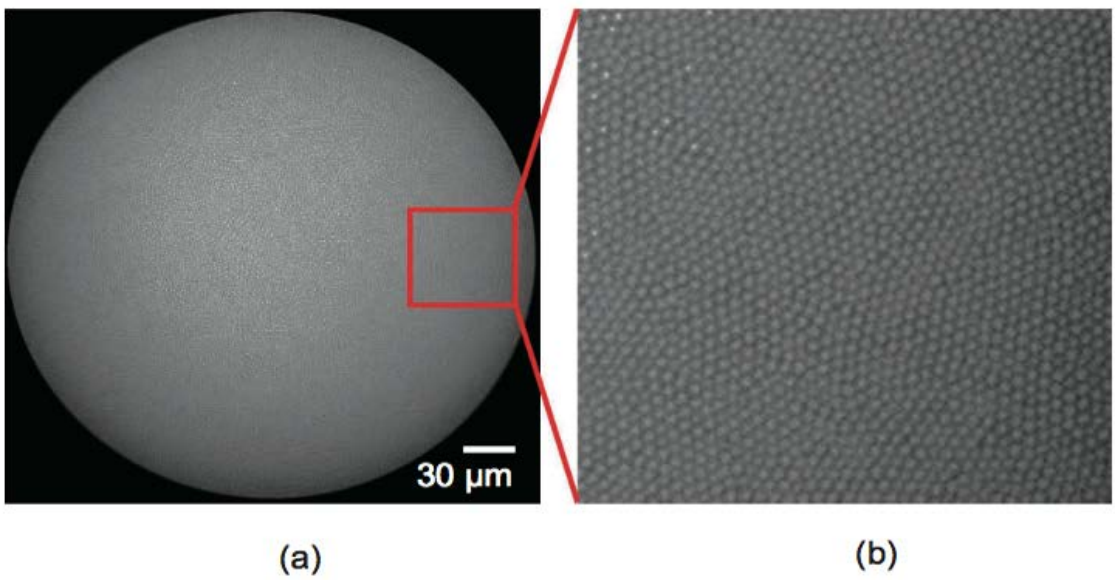


Figure 1.6. (a) The end face of the fiber optical bundle [1]; (b) enlarged view of the red area in (a).

Wang et al used contact imaging technique because high-resolution images can be taken from the desired biological samples. In contact imaging technique, the fiber optical bundle is opposed directly to the focal plane of the sample and due to the small diameter of the fiber optical bundle high-resolution images could be taken [22]. To make it clear, the subsurface images of the biological specimens are taken via combining the focus of the objective lens and the distal end of the fiber optical bundle [15]. Moreover, by using skin contacting device, the location of the area that is wanted to be imaged is found with high accuracy [9].

There are a lot of ways for achieving axial resolution in Confocal Microscopy setups. In this thesis, we aimed to design and fabricate a handheld confocal microscopy head, in which the fiber optical bundle is able to move in selected step sizes. The aim of making a handheld device is explained as miniaturizing of the tabletop confocal microscopes for clinical settings and make a flexible device to use in a lot of fields such as skin, uterine, lung, colon as well as stomach cancers. On the other hand, the reason behind making an embedded control system with 1 µm sensitivity is to increasing the axial resolution and increasing the depth of focus. Thus, improving the optical sectioning ability and to ease the analysis of cellular structures. Also, because the field of view is limited with 100 µm, the range 1-100 µm must be imaged.

1.2. OPERATION PRINCIPLE

In this thesis project, a handheld confocal microscopy head will be designed and fabricated. The design is made with the aim of actuating the fiber optical bundle resulting a back and forth movement with 1 µm step size. The proposed system is shown in Figure 1.7.

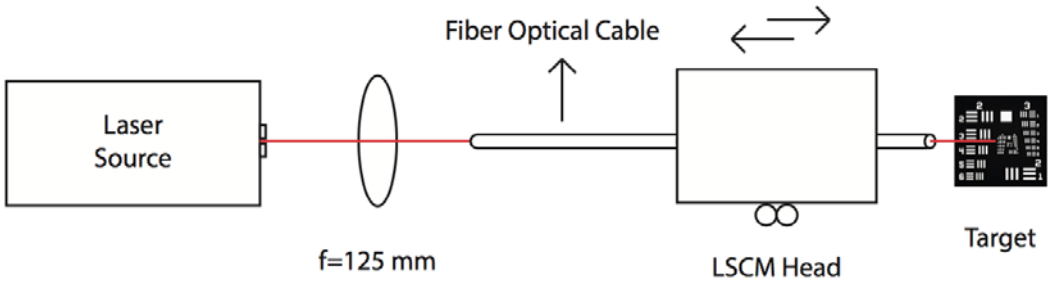


Figure 1.7. The Schematic of the Setup

The proposed system is aimed to calibrate the LSCM head and measure the differences in the size of the laser beam. In the test setup, the laser source with wavelength of 663 nm, will be focused with the aid of a relay lens into the fiber optical bundle. The distal end of the fiber optic bundle will be connected to the fabricated confocal microscopy head and will be positioned to the target. Both of the displacements of the head and beam size will be recorded with a video camera under constant step sizes. After that, the video will be divided into frames and the displacement will be calculated via using the software ImageJ. After the tests completed, comparison graphs related to the displacement of the beam size and the confocal microscopy head will be made. In conclusion, the dynamic range of the device will be found.

1.2. TABLE TOP CONFOCAL MICROSCOPY SETUP

During the thesis, two table top confocal microscopy setup will be constituted. Firstly, images from Thorlabs USAF 1951 resolution target will be taken. Afterwards, the alignment of the fiber optic bundle will be achieved.

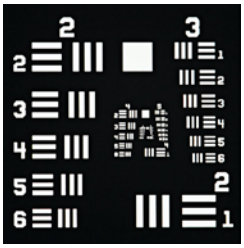


Figure 1.8. The Thorlabs USAF 1951 Resolution Target

The Figure 1.8. shows the Thorlabs USAF 1951 Resolution Target. Each number and the columns have different resolutions.

1.2.1. Imaging from USAF 1951 Resolution Target

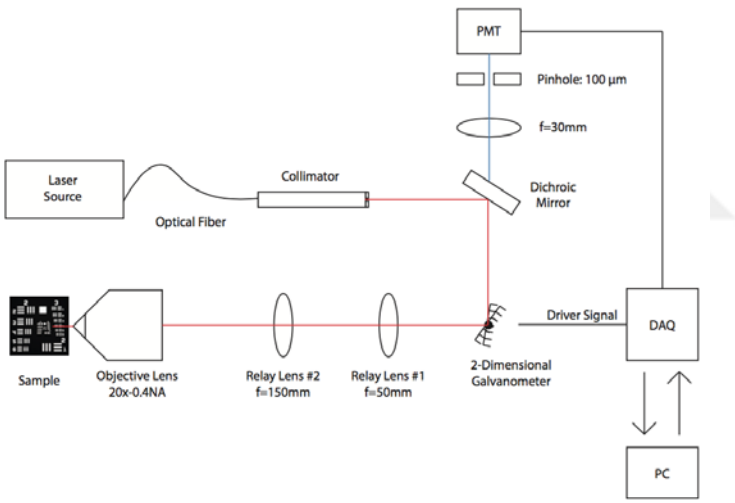


Figure 1.9. The Schematic of the Confocal Microscopy Setup

To begin with, the first aim is capturing the images of the USAF 1951 target via using tabletop LSCM. In Figure 1.7., the schematic of the laser scanning confocal microscope is shown. As it is illustrated, the tabletop confocal microscope mainly composed of a 658 nm wavelength laser source (LP 660-SF60 Thorlabs*)*, dichroic mirror (Thorlabs DMLP638*)*, galvanometer (GVS002/M Thorlabs), relay lenses, Data Acquisition Card (DAQ), Photon Multiplier Tube (PMT) and PC. Each element is explained in detailed.

The pigtailed laser diode (*LP 660-SF60 Thorlabs*), which has 658 nm wavelength is connected to the pigtailed laser mount. The pigtailed design of the mount, which is closely

resemble to a clamshell, brings a lot of advantages to the pigtailed laser diode, such as working as a protection layer from both physical and thermal damaging. To drive the laser diode, *Thorlabs ITC4001* Benchtop Laser Diode Control unit, which constitutes the key part of using lasers, is used to control the precision and stability of the current that the laser diodes has been driven. The ITC4001 model have capability to drive the laser diodes at currents up to 1 A and drive different laser diodes with different wavelengths. This ability of the laser diode can be used in different other optical setups.

The laser diode is collimated through the *Thorlabs DMLP638- Ø1"* Long pass filter dichroic mirror. The purpose of selecting long pass filter comes from its ability to behaving highly reflective below the cutoff wavelength, conversely being highly trans missive above the cutoff wavelengths.

A galvanometer, *GVS002/M Thorlabs*, is consisted of two separated silver coated mirrors one on the top of other to provide the movement on x and y axis. Each mirror has a separate motor to be driven. The actuation of galvanometric mirrors is done via National Instruments DAQ card (USB-X Multifunction) together with a developed MATLAB program. Moreover, the galvanometer has the advantage fast scanning frequencies of 1 Hz and 100 Hz with respect to y and x axis and give the capability of the scanning field of view as 1000 µm x1000 µm.

The raster scanning will be made with galvanometer. The raster scanning pattern can be seen in Figure 1.7.

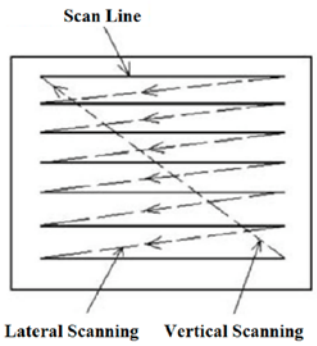


Figure 1.10. The Raster Scanning Pattern

In the setup two separate relay lenses, whose focal lengths are 50 mm and 150 mm, are used in order to increase the beam size of the laser from 2 mm to 6 mm. After the relay lenses, the *RMS20X Olympus Plan Achromat Objective Lens* is placed. The objective lens, which has a working distance of 1,2 mm and numerical aperture of 0,40, focusses the light onto the biological sample and gathers the reflected light back from the sample.

The reflected light from the biological sample sent back to the photon multiplier tube (PMT), as indicated as the blue signal in Figure 1.7. The PMT is a light sensitive detector that perceives the light and has the capability of the measuring the input as light intensity and giving the output as an analog value via National Instrument Daq card. Since the output of the PMT is taken as current value, it is converted to voltage with the aid of 50 k Ω (ohm) resistor. The conversion operation current to voltage via using resistor can be lead to noisy images. As a solution for the noisy images, the analog input data is converted to digital output data, which constitutes the input data for our MATLAB code. NI-DAQ cards has two main duties which are; providing the data acquisition (DAQ) and the actuation of the galvanometer with the MATLAB program are controlled by National Instrument DAQ card NI-USB 6356. The frequency that is needed to be applied for the actuation of the galvanometer, the amplitudes of the applied signals, frame rate, the storage of the taken images as well as field of view are able to controlled via using National Instruments Software.

On the MATLAB code, the assigned values for the imaging operation will be entered to the Daq card and the imaging operation will be started.

1.2.2. Fiber Optical Bundle Alignment

As the second step, the fiber optical bundle will be placed to the working distance of the objective lens. Simultaneously, the distal end of the fiber optic cable will be positioned parallel to the mirror. To test the working of the fiber optical bundle and its located position, the imaging end face of the fiber bundle is processed. The Figure 1.11., shows the schematic of the tabletop LSCM with connection of fiber optical bundle.

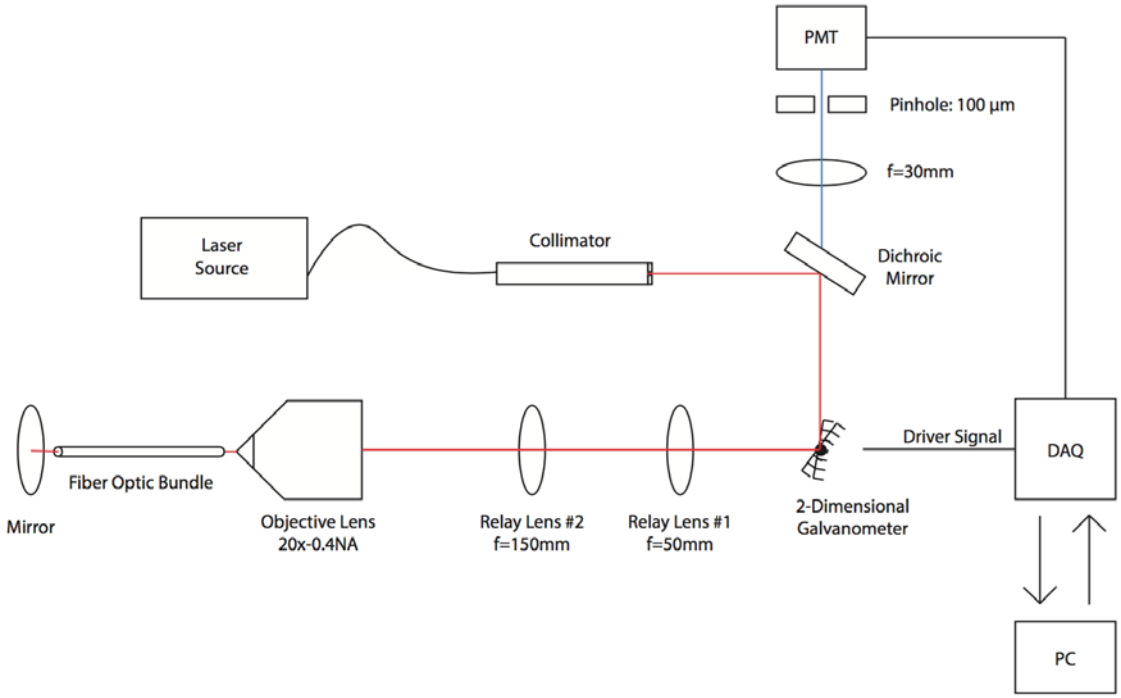


Figure 1.11. The Schematic of the LSCM with Fiber optical bundle Connection

The novelty of this project is to design and fabricate handheld confocal microscopy head with providing 1um movement of the fiber optical bundle on z direction. Therefore, The Fujikura FIGH-50-1100N image fiber optical bundle, which is consisted with 50,000 picture elements and 1,101 mm fiber diameter, will be located at the working distance of the objective lens as in Figure 1.10.

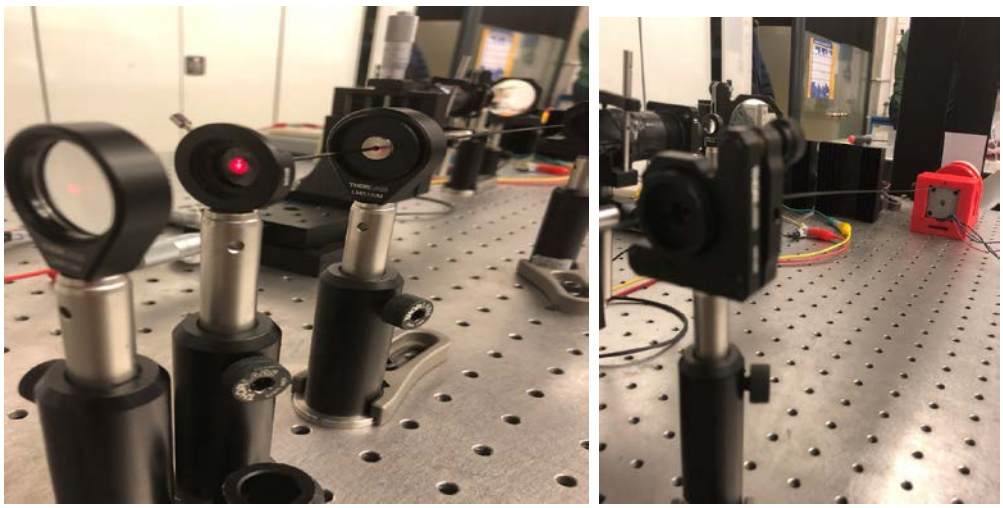


Figure 1.12. ; Figure 1.13. Fiber Optic Bundle

One end of the fiber is opposed to the objective lens and the other distal end of the fiber optical bundle is placed into the handheld microscopy head. Moreover, the properties of fiber optical bundle are given on Table 1.1.

Table 1. Properties of the Fiber Optical Bundle

ITEM	FIGH-50-1100N
Pixel Number	50,000 ± 5,000
Image circle Diameter (μm)	1,025 ± 80
Fiber Diameter	1,100 ± 80
Coating	1,200 ± 100
Coating Material	Silicone resin (Black)
Lattice Defect (%)	<1
Uncircularity (%)	<5

2. MACHINE DESIGN

In this section, the design of the confocal microscopy head is explained in detailed. The machine is used to move the fiber optical bundle with 1 µm increments back and forth in order to get images on the biological specimens from different level of depths. The confocal microscopy head, which is consisted of various parts, is designed on Solidworks software tool. The head is composed of cover parts for the step motor, stationary part, movable parts, cover parts for the machine and finger holding part.

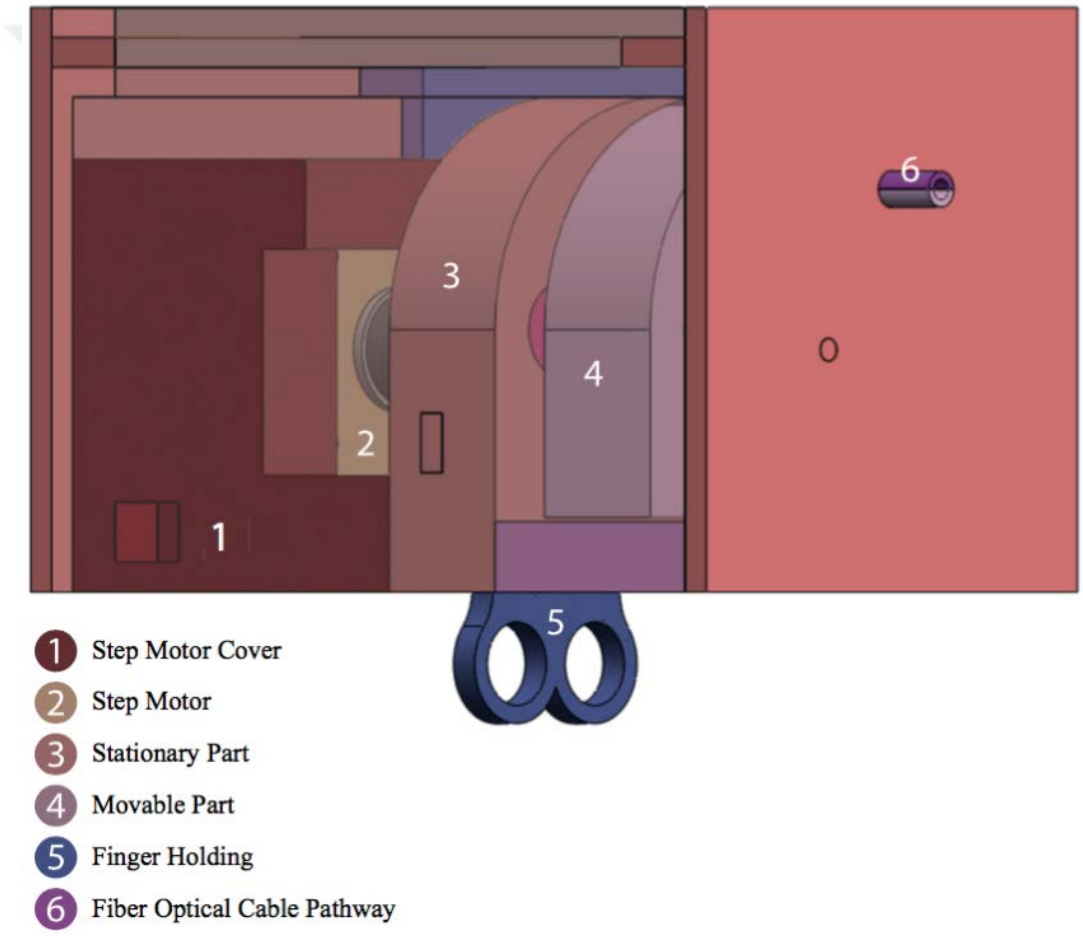


Figure 2.1. *The Oblique View of the Design of the Confocal Microscopy Head*

In Figure 2.1., the major parts are shown in bullet points. As it is seen, the design has covers around all the way of its circumference. This helps to protect the inner parts from the risk of damaging and ease the mobilization. There is a finger holding part on the outer

bottom base of the head. Moreover, there is a continuous fiber optical cable holding line along the head in order to protect the fiber optical cable from any inflection that can be harmful.

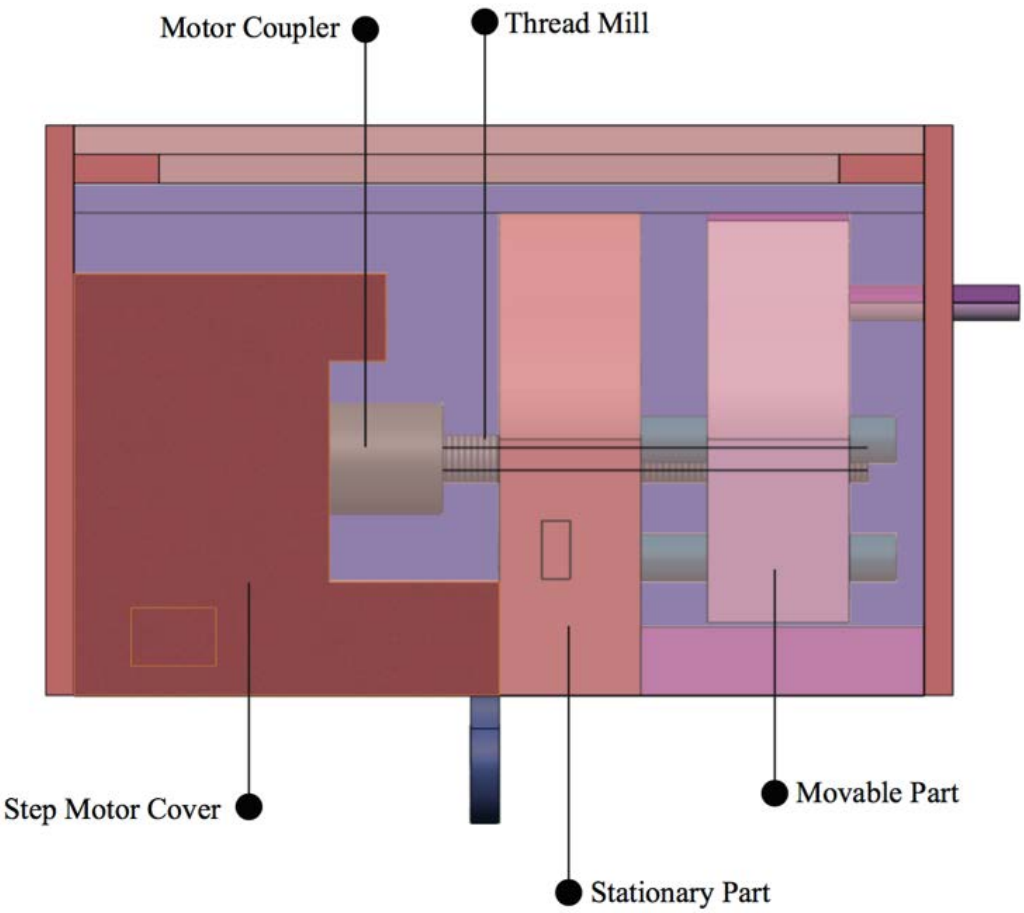


Figure 2.2. The Right-hand Side View of the designed Confocal Microscopy Head

All of the parts can be seen with the Figure 2.2. The operation principle of this machine is simple. The stepper motor progress step by step. With the aid of motor coupler, the rotation and progress of the stepper motor transferred to the thread mill. With the turning of the thread mill, the movable part start to move because of the hex nut. The direction of the movement could be controlled with the turning direction of the stepper motor.

The necessary equipment list for the assembly is shared in the Table 1.2.

Table 2. The Necessary Equipment List for the Assembly

	Parts	Details
1	Step Motor	Bipolar NEMA 17 200 Step 2.8 Step Motor
2	Step Motor Driver	AMIS 30543 PL-2970
3	Power Supply	12V 3A Metal Switch Adaptor
4	Thread Mill	5 mm Dia
5	Motor Coupler	5*5 Dia Coupler
6	Linear Mill	(7.5 mm Dia)*3
7	Linear Ball Bearing	LM 8 UU
8	Hex Nut	5 mm Dia

All of the parts are designed as the complementary in itself to ease the implementation. The 3-Dimensional model files are converted to the STL file and fabricated using a 3-D Printing Machine with filling ratio of 30% and 200 μm sensitivity. The dimension of this head is 98 mm x 88 mm without the finger holding part. With the finger holding part included, dimension of the design became 110 mm x 88 mm. The details of each designed are explained in following sections.

1.1. STEP MOTOR COVERAGE

The step motor coverage is composed of three major parts in order to reduce the required time by the 3-Dimensional Printing Machine and protect every detail on each sides during the fabrication.

1.1.1. Base Part

The base part constitutes the cover for the bottom part of the step motor. It has two intertwinement fingers on the upper part, to merge with the step motor coverage top part.

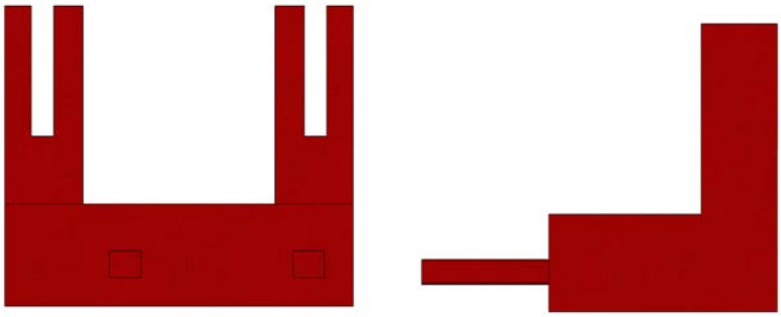


Figure 2.3. The Front and Right-hand Side Views of the Base Part

In addition, there are two extensions that are shaped as rectangular prisms parellel to the base. All of the edges of the rectangles are filletted to ease the interwinement with the stationary part.

1.1.2. Top Part

The top part has a hole with 3,5 mm diameter on the front side of the part to the end of the part to constitute a linear pathway for the fiber optical bundle. There are also two extensions as rectangles to intertwinement with the base part.

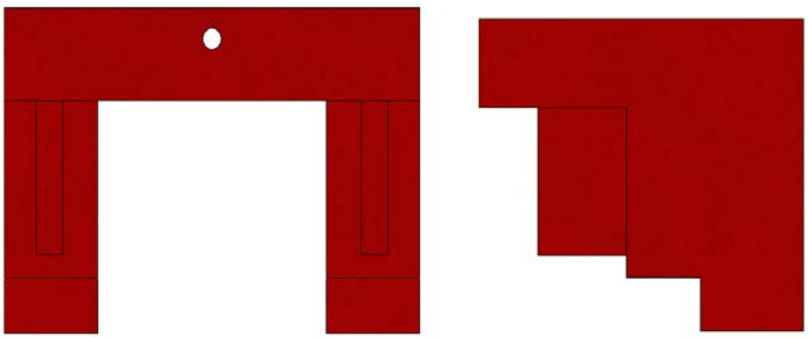


Figure 2.4. The Front and Right-hand Side Views of the Top Part

The right-hand side view of the top part resembles to steps of a ladder. The goal behind this design is to make the assembly as strict as it can be and abstain from the possibility of any vibration.

1.1.3. Rear Part

The rare part has three holes on its front, right hand and left hand sides. Each extruded block on the part is made for relative covers of the handheld confocal microscopy head.

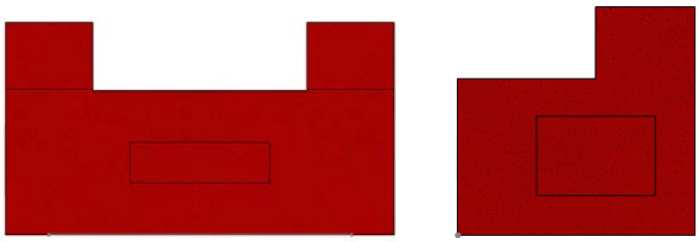


Figure 2.5. The Front and Right-hand Side Views of the Rear Part

As the goal behind the ladder appearance is told in the section 2.1.2 Top Part, the rare part has a ladder structure that works as a clips between the coverage parts.

1.2. STATIONARY PART

The stationary part is the part that combines the step motor coverage and the movable part. One of the major unwanted consequences that was encountered in the mechanical assembly is the sensitivity losses, which can be stems from vibration during the movement in which the error rates are increased. Because of our systems is designed for 1 µm sensitivity, the machine must have zero vibration. In other words, the mechanical design must be strict as it is integrated. In order to eliminate the vibrational effects, both of the stationary and the movable parts are bounded with linear bearings. During the design, the selection of the linear bearing is made through choosing the one, which has minimum circular diameter and length. Thus, as a linear bearing, which has 8 mm inner and 16 mm outer diameter with the length of 25 mm, is used. In the design of the part the width is arranged as the same as the length of the linear bearing.

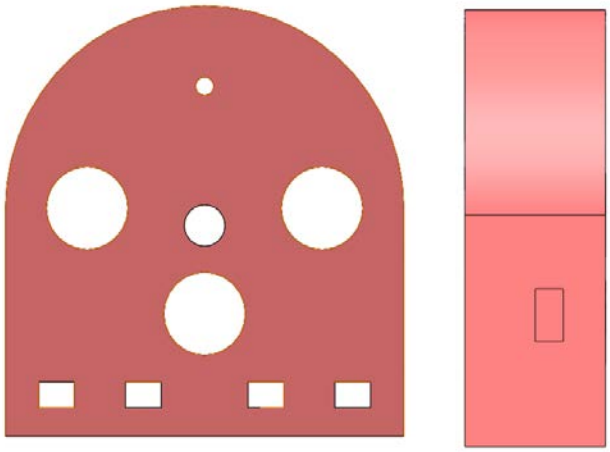


Figure 2.6. The Front and Right-hand Side Views of the Stationary Part

As it can be seen, the three bigger holes are used for the linear bearings to limit the movement on x-axis. The upper hole is made for the pathway of fiber optical bundle, as it made on the step motor coverage top part. In addition, there are four rectangular gaps for intermeshing of the step motor coverage front part and movable parts' base. Finally, the stationary part has an extruded part as diameters 5 mm x 10 mm on its right hand side in order to pave the way of the right-hand side cover parts connecting.

1.3. MOVABLE PART

1.3.1. Base Part

The movable part has designed with nearly the same logic as the stationary part in terms of prevention from vibrational effects. The bigger three circular holes are designed for linear bearings.

There is an extruded part on the upper side, which can be seen in section 2.3.3 Top Part, is the linear pathway of fiber optical bundle.

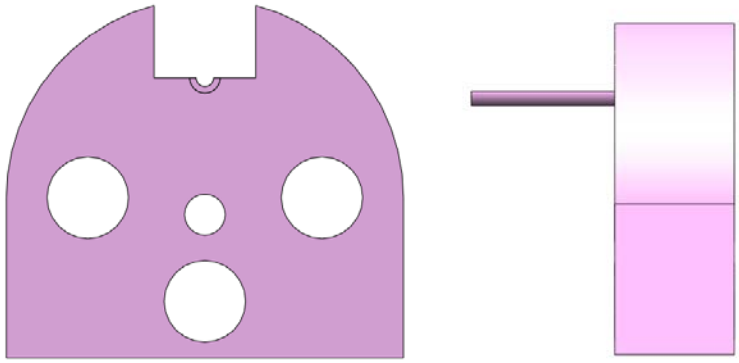


Figure 2.7. The Front and Right-hand Side Views of the Base Part

Moreover, there is an extruded part on the upper side which is used for the calibrating the exact location of the fiber optical bundle. The details of the part can be seen in 2.3.3 Top Part. There is also an extension on the front view of the base part that resembles to a half torus shape with inner 1,75 mm and outer 3 mm radius. The aim of this extension is to ease the accessing the biological specimen.

1.3.2. Bottom Part

This part is used to the protect the point of the center of mass in order to abstain from the swinging of the design during the movement of the movable part to forward direction. The length of the bottom part is designed with the same length of the stationary part. However, the height of the part is 11.75 mm which is less than the step motor base part in order to save space throughout the motion of the movable part without any friction. The rectangular extensions are filleted with 0,3 mm radius with the same goal as the 2.1.1. Base Part The extensions have dimensions 7 mm x 5 mm. Their width is designed as 25 mm to fulfill the intermeshing of stationary part and fix the fitting.

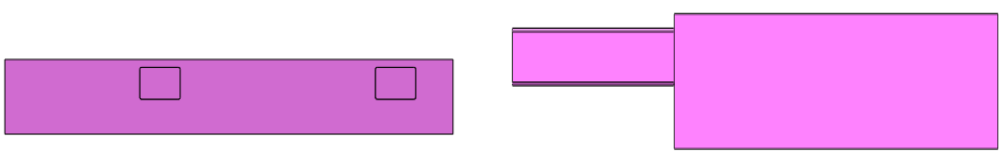


Figure 2.8. The Front and Right-hand Side Views of the Bottom Part

As it is seen, the fitting of the parts is tried to be strict as it can be in to prevent any unwanted consequences such as effecting the sensitivity of the motor steps.

1.3.3. Top Part

The top part is used to control the location of the fiber optic cable in a precise and safer manner as it is told in the section 2.3.1 Base Part. The top part has also a half-torus shape as it seen with inner and outer radiuses as 1,75 mm and 3 mm.

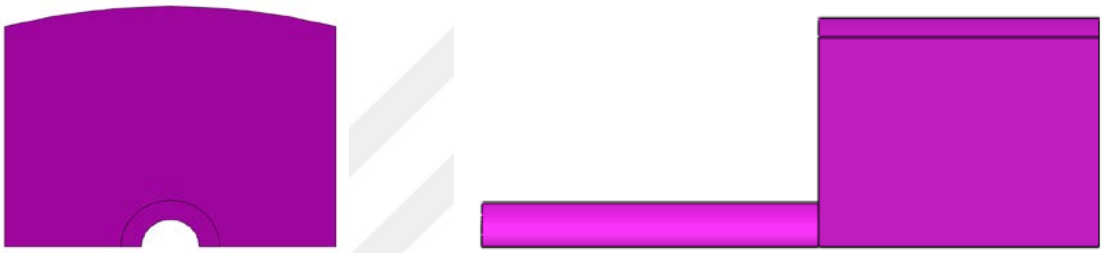


Figure 2.9. The Front and Right-hand Side Views of the Top Part

1.4. OUTER COVERS OF THE CONFOCAL MICROSCOPY HEAD

The covers are designed as puzzle parts with its intermeshing principle. The front and rear covers have rectangular block extensions on their upper side. On the other hand, the right and left hand side covers have extruded blocks on their upper side which serves as the merge point for the front and rear covers.

1.4.1. Front Cover

The front cover has dimensions 98 mm x 88 mm. It has 6,20 mm diameter hole on its upper side to constitute the exit of the movable parts circular end. the fiber optical bundle.

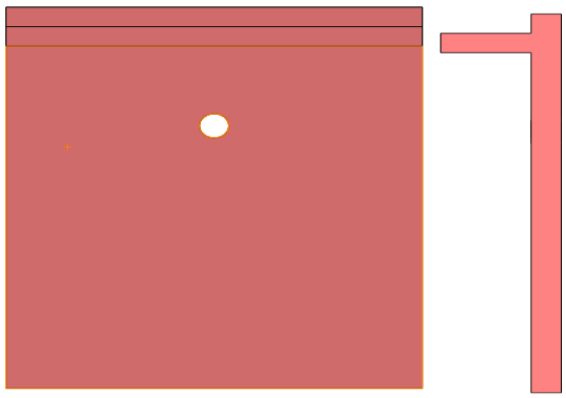


Figure 2.10. The Front and Right-hand Side Views of Front Cover

With the aid of this circular hole we can easily visualize the movement of the step motor which results in the movement movable part and the location of the fiber optical bundle. Calculations related to the step sizes and process time could be done as taken the reference point as front cover.

1.4.2. Rear Cover

The rear cover has same dimensions with 2.4.1. Front Cover which are 98 mm x 88mm. There exists a 3,50 mm diameter conversely smaller than the one that exists in front cover because in this part there is only need of a path for the entry fiber optical bundle.

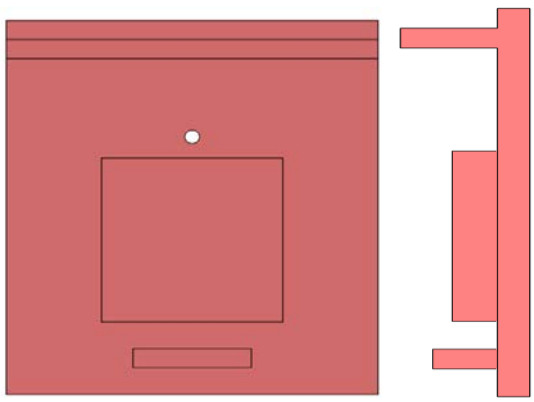


Figure 2.11. The Front and Right-hand Side Views of the Rear Cover

In addition, there are two extruded rectangular blocks numbered as 1 and 2 on the inner side of the rear cover with dimensions 43,1 mm x 43 mm x 7 mm and 28 mm x 5 mm x 7

mm relatively. The number 1, is made for stick the step motor in its coverage and to hold it still. On the other hand, the number 2 block is made for the meshing with the part 2.1.3 Rear Part.

1.4.3. Right Hand Side Cover

The right hand side cover has dimensions 150 mm x 98 mm x 5 mm. The right and left hand side covers are wider compared to front and rear covers due to the arrangement of the design. The right hand side cover has also two extruded rectangular prisms on the inner side.

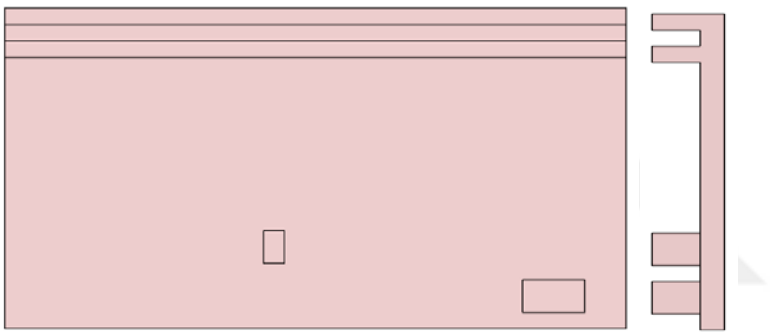


Figure 2.12. The Front and Right-hand Side Views of the Right Hand Side Cover

The lower rectangular prism has dimensions 15mm x 10mm x 10mm and made for the connection with the part 2.1.3 Rear Part. On the other hand, the upper rectangular prism has dimensions 5 mm x 10 mm x 10 mm and used in the connection with the part 2.2. Stationary Part.

1.4.4. Left-Hand Side Cover

The left hand side cover has the same dimensions with the 2.4.3 Right Hand Side Cover as 150 mm x 98 mm x 5 mm. Similarly, there is a rectangular extension with 15 mm x 10 mm x 10 mm dimensions for the part 2.1.3. Rear Part.

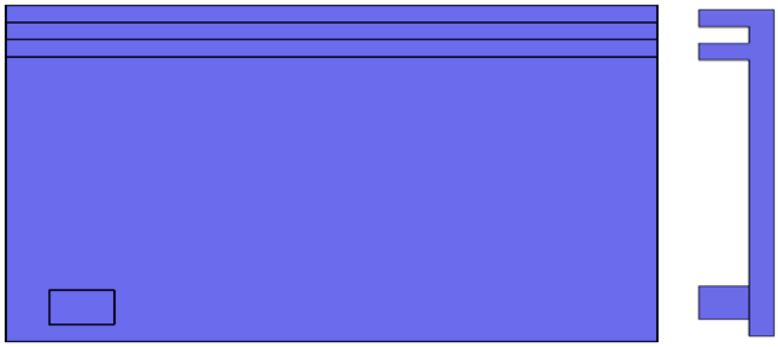


Figure 2.13. The Front and Right-hand Side Views of the Left Hand Side Cover

1.4.5. Top Cover

The top cover has the duty of combining the all covers. The top cover resembles like the combination of two separate rectangular prisms. The upper prism has dimensions 150 mm x 58 mm x 5 mm and used to combine the left and right hand side covers.

Figure 2.14. The Front and Right-hand Side Views of the Top Cover

On the other hand, the lower prism has dimensions 120 mm x 88 mm x 5 mm and used in the augmentation of the front and rear covers.

1.4.6. Finger Holding

The finger holding is designed to ease the holding of the confocal microscopy head during the imaging. It has dimensions of 30 mm x 22 mm x 5 mm.

Figure 2.15. The Front and Right-hand Side Views of the Finger Holding

3. IMPLEMENTATION

3-Dimensional Printing Machines have a lot of advantageous such as rapid prototyping and lower cost compared to other fabrication techniques. The usage area of the 3-Dimensional Printers is extensive from medicine to defense technologies. Moreover, the operation principle is very simple. The 3-Dimensional Printing Machine uses molten silicon in order to build up the 3-dimensional model layer by layer. After each layer is printed, the head of the printer goes up a layer and printing continues so on. Also, the printed plastic is cool down by automatic fan.

In this thesis, the 3-Dimensional Printing Machine is used in the fabrication of the machine design parts. To fabricate each part, Zaxe X1 3-Dimensional Printing Machine is used in the Mechatronics Lab. Zaxe X1 is able to print the layers with thicknesses between 50 µm to 300 µm which is enough our parts. The designed parts are printed with filling ratio of %30 and 200 µm resolution.

After the fabrication of each part, the assembly is started to construction. Firstly, each part is cleared from supports. Secondly, step motor cover, stationary and movable parts are assembled relatively. To interconnect the machine, linear bearing and linear shafts are assembled as one group. After that, the stepper motor, motor coupler and thread mill connected as a second group. When the assembly is completed, each part also glued together.

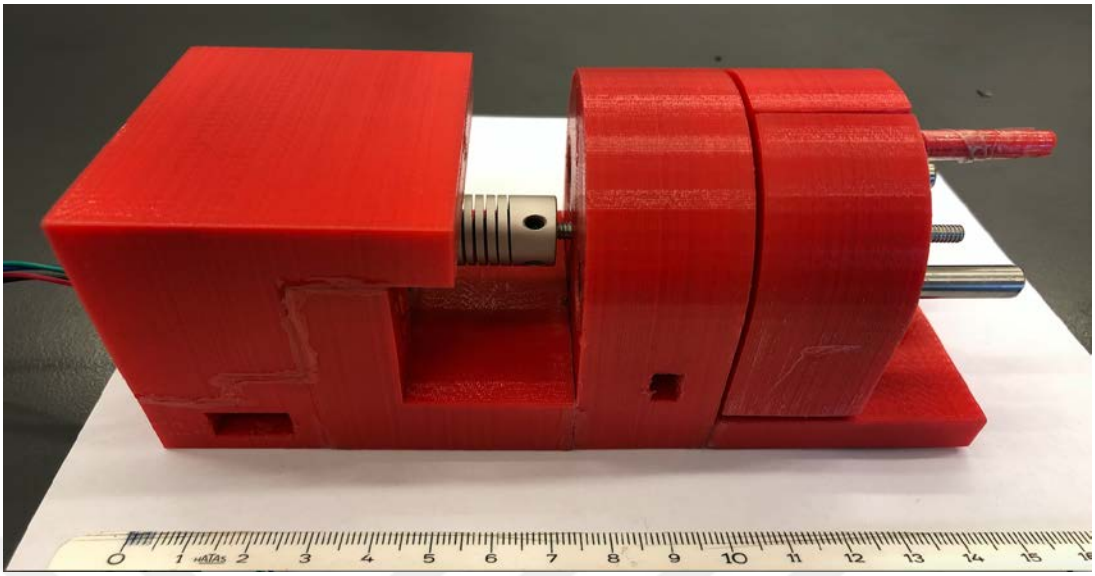


Figure 3.1. *Right-hand Side View of the Confocal Microscopy Head.*

All of the fabricated parts could be seen with Figure 3.1. which shows the right hand side view of the Confocal Microscopy Head. On the other hand, in the Figure 3.2., the front view of the confocal microscopy head is shown. As you can see, the movable part is connected with three linear shafts to limit movement of the machine only to x-axis. Moreover, in the middle of the part, the thread mill is connected to the hex nut in order to move the part back and forth with the progress of the stepper motor.

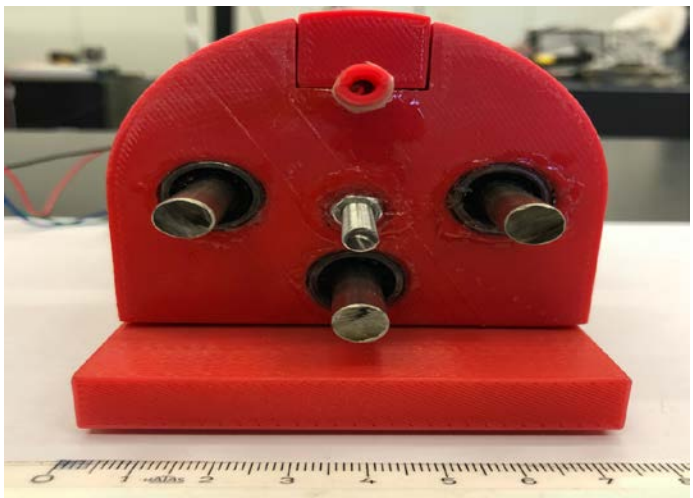


Figure 3.2. The Front View of the Confocal Microscopy Head

4. EMBEDDED SYSTEM

The embedded system is responsible from the movement of the fiber optical bundle with 1 µm step sizes. Due the fact that, the step sizes are important while taking images from different depth levels, stepper motor is the most appropriate motor type in this project. There are a lot of advantages of using step motors. To make it clear, stepper motor consists of a group of multiple coils, which called as phases. With the energization of each phase, the motor rotates one step at a time. Via using a computer and a microcontroller, the velocity and the positioning of the stepper motor is controlled. Therefore, the step motors are very useful in the applications in which high sensitivity is expected.

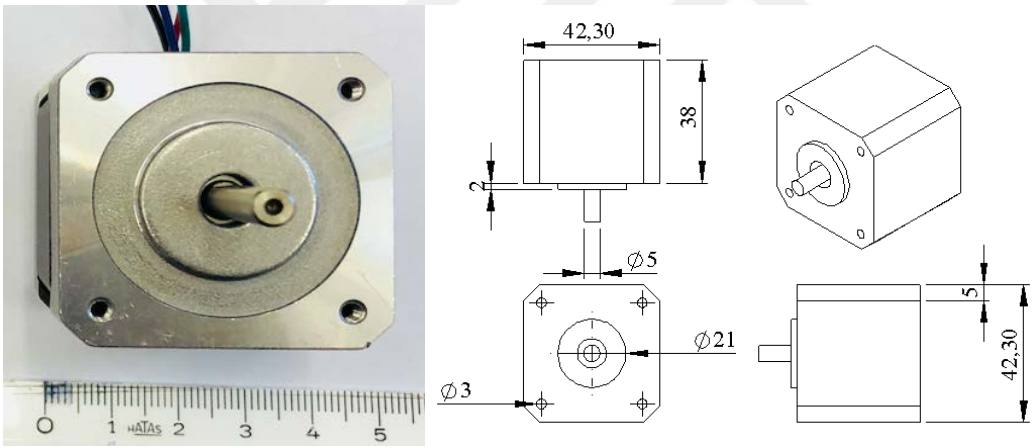


Figure 4.1.; Figure 4.2. Step Motor and Layout

Stepper motors requires motor drivers within itself for actuation. The motor drivers, send pulses to the step motors and when the step motor took the pulse, moves a step. Step motors differs relatively depending on their pitch angle. The pitch angles can be range between; 90°, 45°, 1.8°, 7.5° and 1.8°. In this setup, Bipolar NEMA 17 2.8 V type step motor is chosen relative to its 1.8 angle of pitch because the aim is dividing the step size as much as we can to 1 µm step size. The equation of the pitch angle is expressed in;

$$1.8° = \frac{360°}{200}$$

As we can see, the step motor has 200 step size, in other words, the step motor could progress 200 steps in one fully turn.

The step motor is driven with AMIS-3053 Step Motor driver card. Due to the ability of the motor driver, the steps could be divided in different eleven modes starting from 1 to 1/128. In the setup, the step size is divided with 1/128 to get higher sensitivity. Moreover, to give power to the systems, with the properties of 12 Volt and 3 Ampere metal switch mood adaptor is used.

As a microcontroller Arduino Nano is chosen based on its micro step technique and easier to programming. Also, an LCD is augmented with the setup to show the step value during the imaging operation.

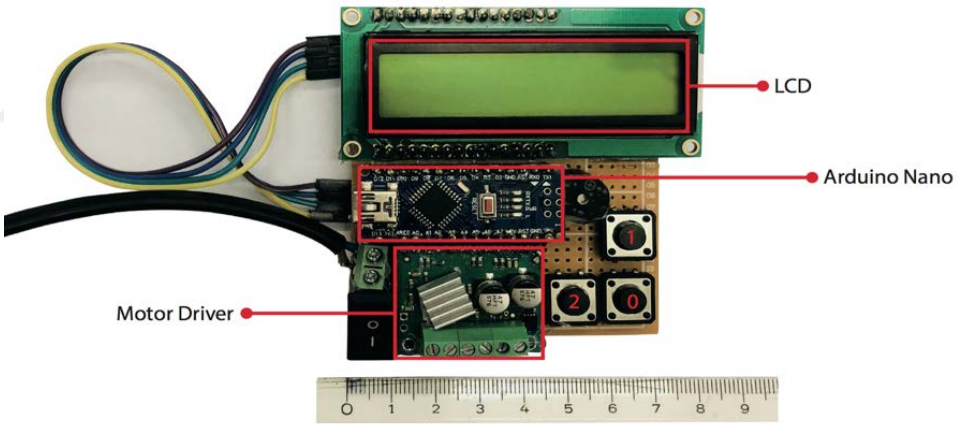


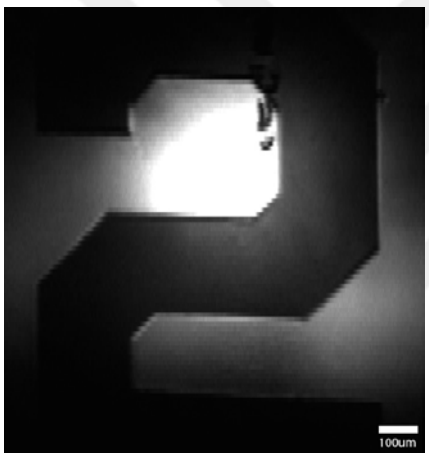
Figure 4.3. The Embedded Control System

Figure 3.2., shows the structure of the embedded system. The working principle of the code is very simple. If the power button, which is placed next to the motor driver, is pushed the LCD asks for the selection of the step values. There are three buttons to control the movement. When the power is on, the right two buttons is used for determination of the step value. With upper button, the step value increases as one. Controversially, if the lower button is pushed than the step value decreases by one. After the selection of the desired step value, the left button must be pressed for the approval. To clarify, the code is given in Appendix: A

5. EXPERIMENTAL RESULTS

5.1 Confocal Microscopy Setup

In this thesis, the designed and fabrication of a Confocal Microscopy Head is aimed. To begin with, imaging of USAF 1951 Target and fiber optic bundle alignment via using confocal microscope is planned. Two images taken from USAF 1951 Resolution target. The resolutions of each image is illustrated on its bottom side. Two major images that taken with this alignment is shown in Figure 5.1. and 5.2.



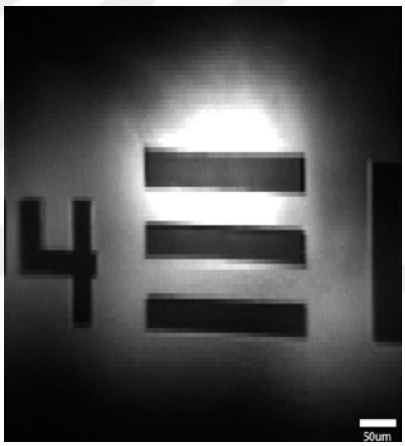


Figure 5.1.; Figure 5.2. Images of USAF 1951 Resolution Target

After the USAF 1951 target images, the alignment of the fiber optic bundle is placed at the focal plane of the objective lens. To scan the end face of the fiber optic bundle, a mirror is placed to the distal end of the fiber optic bundle. The used schematic of the confocal microscopy can be seen in Figure 5.3. and relatively the taken image from the enface of the fiber optic bundle is shown in Figure 5.4.

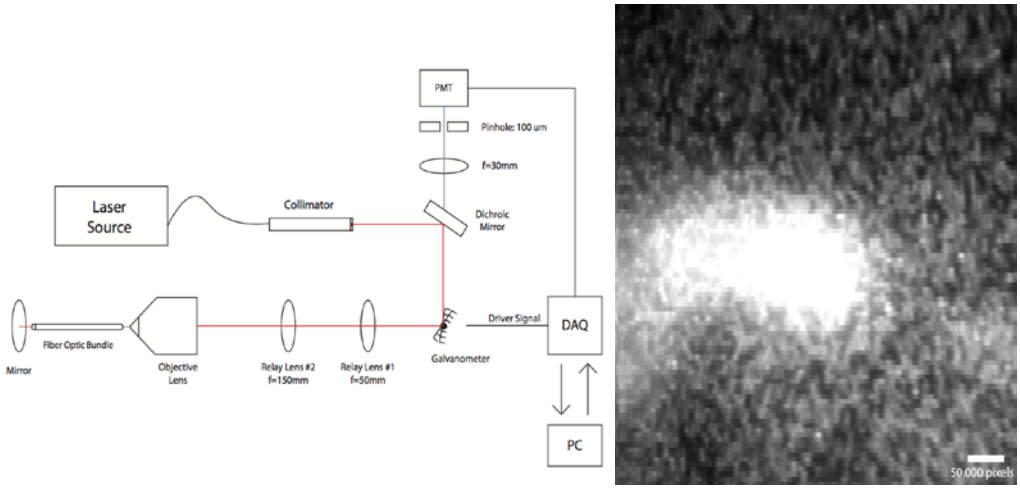


Figure 5.3. ; Figure 5.4. The schematic for the Scanning of the End Face of the Fiber Optic Bundle and, End Face Image of Fiber Optic Bundle with 50.000 cores.

5.2 Handheld Confocal Microscopy Head

The goal of this tests is to control the working capability of the handheld confocal microscopy head under different step sizes. Test setup is shown in the Figure 5.5.

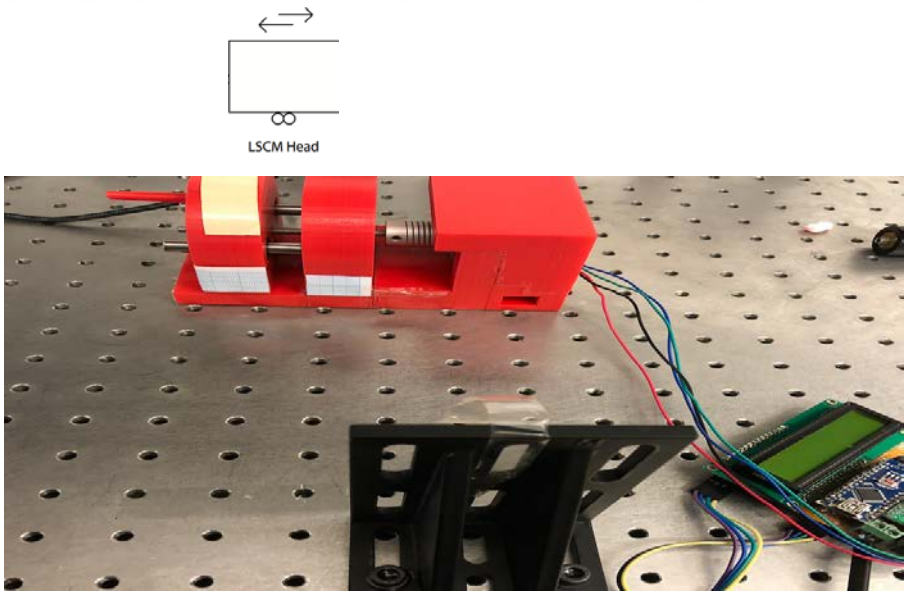


Figure 5.5. The Test Setup

As it is told, a camera, I phone X, which has resolution of 2436 x 1125, able to is placed on the optical test setup to measure the location of the movable part throughout the process. 1

mm distanced calibration pages were implemented on the stationary and movable part. A video is recorded during the movement. Afterwards, the video is divided into frames for every one second. The location of the movable part reference to the stationary part is measured with ImageJ software for every one second. In each test, 1000 mA current is applied to the system, the only changeable variable is the step sizes that selected by the user.

Firstly, to calibrate the handheld confocal microscopy head, 1 mm distance tests were made. To process 1 mm, 26 is selected as a step size in the Arduino code. In the Figure 5.6. the results of the 1 mm distance tests are compared. Each result is similar to each other as expected.

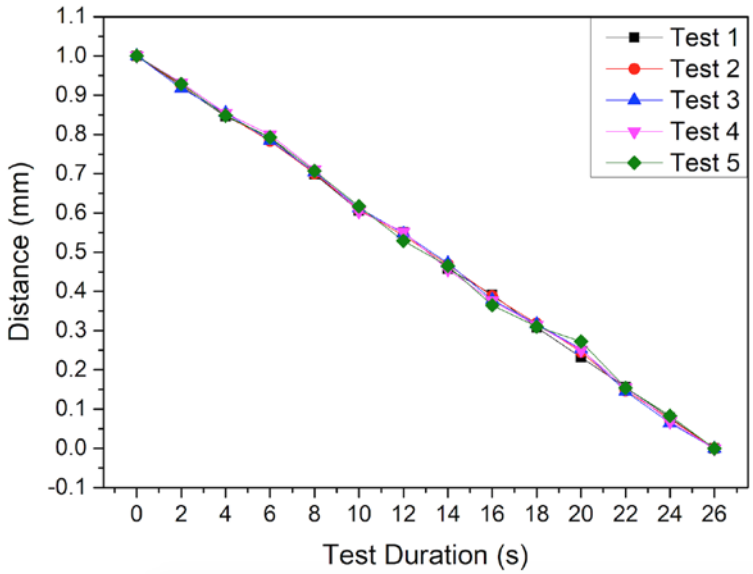


Figure 5.6. 1 mm Distance Tests

On the other hand, in the Figure 5.7. the average of the 1 mm distance tests depicted with error bars. The error rate is increased in the times that are closed to the shutdown of the stepper motor.

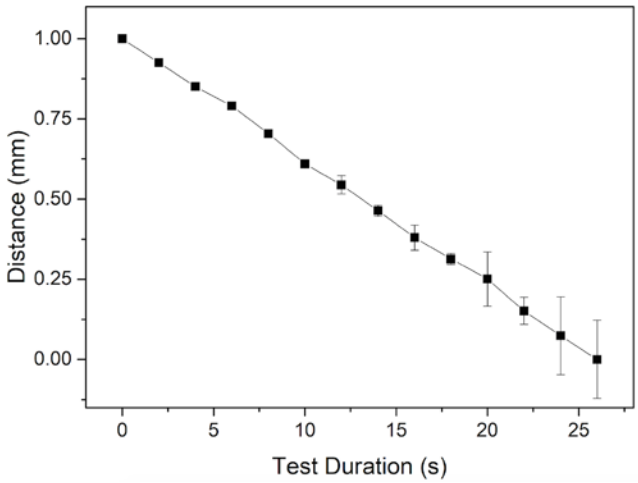


Figure 5.7. 1 mm Distance Tests with Error Bars

Secondly, to calibrate the handheld confocal microscopy head, a 500 µm distanced forward and backward movement processed. This reverse movement repeated for 10 times in each direction. The aim of this first test is to observe the existence of any type of inaccuracies of the handheld microscopy head.

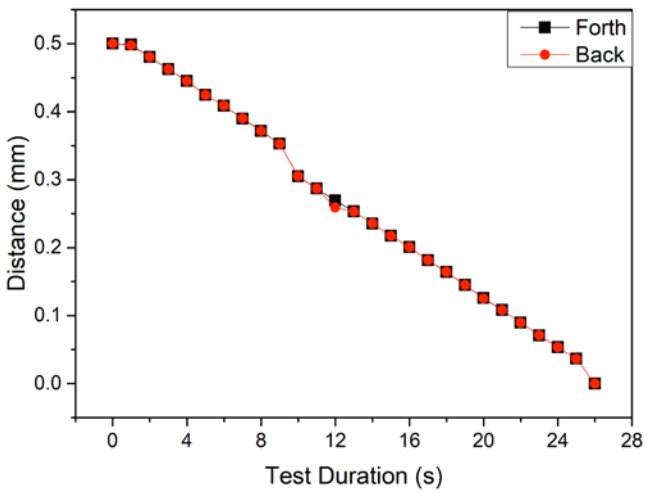


Figure 5.8. The Hysteresis Test

The Figure 5.7. shows the results of the reversed test. The figure consists of the average of 10 tests of both in forward and backward direction. In the tests, 13 steps, in other words the equivalently 500 µm path processed. The position of the movable part is calculated. The

forth and back movements are colored differently in the figure to make comparison between each other. According to the graph, the results are as expected. The movement in each direction similarly the same. In the both directions, there is a drop between 8^{th} and 10^{th}

seconds. This drops stems from the inaccuracy on the threaded mill that used in the handheld confocal microscopy head.

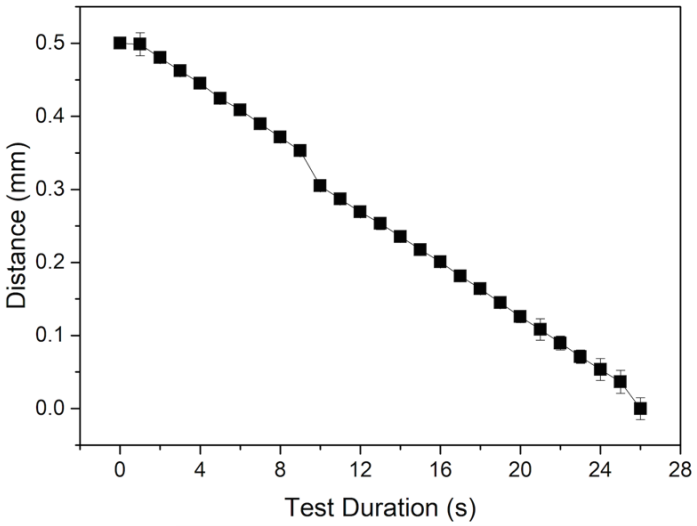


Figure 5.9. The Average of the 10 tests of 500 µm Distance versus The Error

In Figure 5.8., the results of the 10 tests in the forward direction is compared with the errors. As it can be seen, there is an increased error in the end of the tests as similar as the results of the 1 mm distance tests.

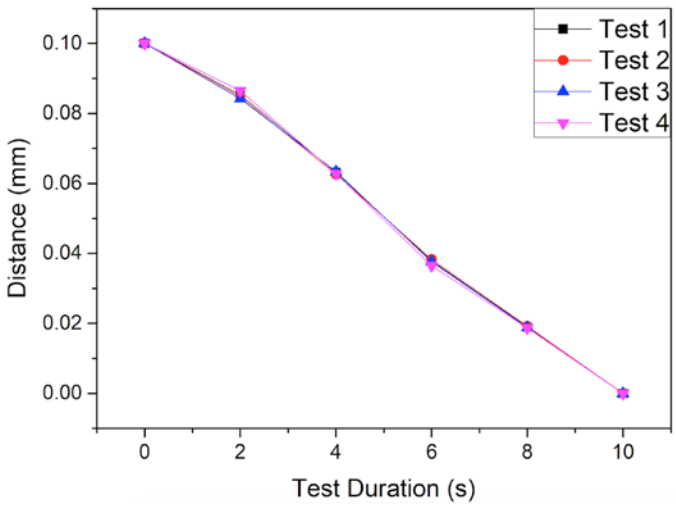


Figure 5.10. 100 µm Distance Tests

Finally, 100 µm distance tests is made as in Figure 5.9. To compare the results with the error rates, a new graph with error bars is made, which also can be seen in Figure 5.10. Conversely to the other tests, the error bars are much clear. Because of the test duration is really low compared to other tests due to the selected step size, the disturbance effects at the close to shut down time are observable.

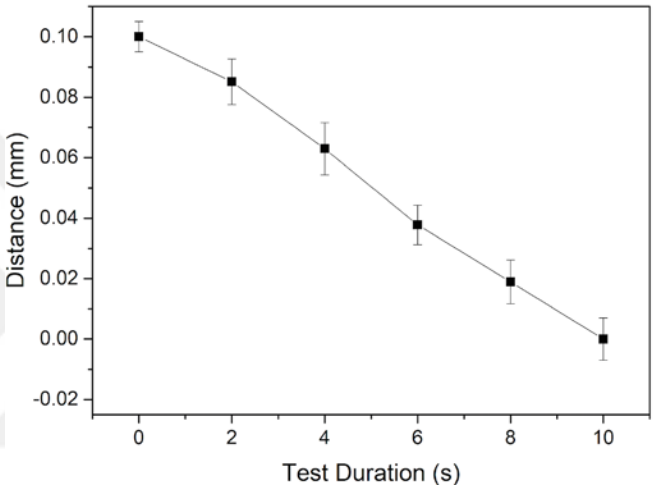


Figure 5.11. The Average of the 4 tests of 100 µm Distance with Error Bars

6. SUMMARY AND CONCLUSION

In this thesis, the design and fabrication of a handheld confocal microscopy head is aimed. The design is made via using the Solidworks and each part is fabricated via 3-Dimensional Printing Machine with 100 μm sensitivity and filling ratio of 30%. Simultaneously, an embedded controlling system is designed with Arduino. The embedded system is responsible from the back and forth movement of the stepper motor at selected step sizes by the user.

To test the fabricated system, a 1 mm calibration sheet is implemented on the handheld confocal microscopy head. An I phone X camera is used during each test to record the movement of the movable part with respect to the stationary part. Via ImageJ software location of the movable part is calculated for every one second throughout the recording. The results are graphed with the figures.

The disturbances at the end of the tests, can be related to the filling ratio of the fabricated part and the industrial parts that are used in the confocal microscopy head. There is an observed flexibility at the end of the handheld confocal microscopy head, which stems from the filling ratio of the material. The results are suitable for our tests but if further enhancements on the results wanted to be made, especially for the distances under 100 μm, the filling ratio of the handheld confocal microscope could be increased and the flexibility of the material could be decreased further.

The designed and fabricated confocal microscopy head, has dimensions of 88 cm x 16 cm. The dimensions of the designed part are nearly as 90% smaller compared to the Arrassmith et. Al works, which is 10 cm x 18 cm. Moreover, with the acquired test results, the handheld device has capable of the movement of 1 μm step size in both back and forth directions and has a dynamic range of 2 cm relative to the dimensions of the head.
Finally, with combining confocal microscopy head with appropriate GRIN lenses, imaging of the biological tissues on z-axes could be achieved. Thus, the early signs of the cancerous cell could be imaged via using confocal microscopy head.

7. REFERENCES

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APPENDIX A: MOTOR DRIVEN CODE

```
#include <SPI.h>
#include <AMIS30543.h>
#include <LiquidCrystal_I2C.h>

const uint8_t amisDirPin = 8;
const uint8_t amisStepPin = 7;
const uint8_t amisSlaveSelect = 10;
byte buttonPins[3] = {4, 5, 3};
unsigned int x = 0;
int button = 6;
int rotationalValue = 32;

int speedValue = 0;
float stepValue = 26;
int locationValue = 0;
//int stepNumber = 5;

AMIS30543 stepper;
LiquidCrystal_I2C lcd( 0x27 , 16 , 2);

void setup() {

  SPI.begin();
  lcd.begin();
  for ( byte i = 0; i < 3; i++ ) {
    pinMode(buttonPins[i], INPUT );
    digitalWrite(buttonPins[i], HIGH);
  }
   pinMode(button , OUTPUT);
```

```
stepper.init(amisSlaveSelect);
digitalWrite(amisStepPin, LOW);
pinMode(amisStepPin, OUTPUT);
digitalWrite(amisDirPin, LOW);
pinMode(amisDirPin, OUTPUT);

delay(5);

stepper.resetSettings();

stepper.setCurrentMilliamps(1000);
stepper.setStepMode(128);

stepper.disableDriver();


stepSelection();

stepper.enableDriver();
/*
  lcd.clear();
  lcd.home();
  lcd.print("fast");
  lcd.setCursor(0, 1);
  lcd.print("calibration...");
*/
fastCalibration();
delay(700);
digitalWrite(button , HIGH);
delay(100);
```

```
 digitalWrite(button , LOW);
 /*
   lcd.clear();
   lcd.home();
   lcd.print("slow");
   lcd.setCursor(0, 1);
   lcd.print("calibration...");
   slowCalibration();
   lcd.clear();
   lcd.home();
   stepper.setStepMode(1);
   // applyTime();*/
lcd.clear();
 lcd.print("applying...");
 delay(400);
 runStepper();
 /* rotationalForward();

   digitalWrite(button , HIGH);
   delay(1000);
   digitalWrite(button , LOW);

   rotationalBackward();

    digitalWrite(button , HIGH);
   delay(100);
   digitalWrite(button , LOW);
 */
 lcd.clear();
 lcd.home();
 lcd.print("done!xx");
```

```
  stepper.disableDriver();

}

void loop() {}

void step() {

  digitalWrite(amisStepPin, HIGH);
  delayMicroseconds(1000);
  digitalWrite(amisStepPin, LOW);
  delayMicroseconds(1000);

  delayMicroseconds(1);
}

void setDirection(bool dir) {
  delayMicroseconds(1);
  digitalWrite(amisDirPin, dir);
  delayMicroseconds(1);
}

void fastCalibration() {
  while ( !digitalRead(buttonPins[2]) == 0)
  {
    stepper.setStepMode(1);
    // lcd.clear();
    //  lcd.print("press key");
    //  lcd.setCursor(0,1);
    //  lcd.print("to move!");
```

```
    if ( !digitalRead(buttonPins[0]) == 1 ) {
      //   stepper.enableDriver();
      setDirection(1);
      step();
      x = x + 1;
    }

    if ( !digitalRead(buttonPins[1]) == 1 ) {
      // stepper.enableDriver();
      setDirection(0);
      step();
      x = x + 1;
    }

    else
      // stepper.disableDriver();
      x = 0;


  }
}

void slowCalibration() {
  while ( !digitalRead(buttonPins[2]) == 0)
  {
    stepper.setStepMode(32);
    // lcd.clear();
    //  lcd.print("press key");
    //  lcd.setCursor(0,1);
    //  lcd.print("to move!");
```

```
    if ( !digitalRead(buttonPins[0]) == 1 ) {
      //   stepper.enableDriver();
      setDirection(1);
      step();
      x = x + 1;
    }

    if ( !digitalRead(buttonPins[1]) == 1 ) {
      //   stepper.enableDriver();
      setDirection(0);
      step();
      x = x + 1;
    }

    else
      // stepper.disableDriver();
      x = 0;
    //  delay(250);
  }
  // stepper.enableDriver();
}

void speedSelection() {
  lcd.clear();
  lcd.home();
  lcd.print("select speed:");

  while (!digitalRead(buttonPins[2]) == 0) {

    if (!digitalRead(buttonPins[0]) == 1) {
      lcd.clear();
```

```
      lcd.home();
      lcd.print("selected speed: ");
      speedValue = speedValue - 100;
      lcd.setCursor(0, 1);
      lcd.print(speedValue);
      delay(200);

    }
    if (!digitalRead(buttonPins[1]) == 1) {
      lcd.clear();
      lcd.home();
      lcd.print("selected speed: ");
      speedValue = speedValue + 100;
      lcd.setCursor(0, 1);
      lcd.print(speedValue);
      delay(200);
    }
    if (speedValue == 0)
      speedValue = 1;
  }
  lcd.clear();
  lcd.print("passed");
  delay(400);

}

void stepSelection() {
  lcd.clear();
  lcd.home();
  lcd.print("select step:");
```

```
while (!digitalRead(buttonPins[2]) == 0) {

  if (!digitalRead(buttonPins[0]) == 1) {
    digitalWrite(button , HIGH);
    delay(100);
    digitalWrite(button , LOW);
    lcd.clear();
    lcd.home();
    lcd.print("selected: ");
    stepValue = stepValue - 0.02;
    lcd.setCursor(0, 1);
    lcd.print(stepValue);
    lcd.print(" step");
    delay(200);

  }
  if (!digitalRead(buttonPins[1]) == 1) {
    digitalWrite(button , HIGH);
    delay(100);
    digitalWrite(button , LOW);
    lcd.clear();
    lcd.home();
    lcd.print("selected: ");
    stepValue = stepValue + 0.02;
    lcd.setCursor(0, 1);
    lcd.print(stepValue);
    lcd.print(" step");
    delay(200);
  }
  if (stepValue == 0)
```

```
    stepValue = 0.02;
  }
    digitalWrite(button , HIGH);
    delay(100);
    digitalWrite(button , LOW);
  lcd.clear();
  lcd.home();
  lcd.print("calibrating...");
  delay(400);

}


 void rotationalBackward()
{
 rotationalValue = 32;
  stepper.setStepMode(rotationalValue);

  digitalWrite(button , HIGH);
  delay(100);
  digitalWrite(button , LOW);

  setDirection(0);
  for ( int i = 0 ; i < 5 ; i++ ) {
    for ( x = 0; x < stepValue * 1000; x++) {

      step();

    }
  }
}
```

```
void runStepper()
  stepper.setStepMode(128);
  setDirection(0);

    for ( x = 0; x < stepValue *1000  ; x++) {
      step();
    }

  digitalWrite(button , HIGH);
  delay(100);
  digitalWrite(button , LOW);
}
```