CONFOCAL MICROSCOPE Z-STAGE

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Abstract

This project is to redesign the confocal microscope using four piezo electric actuators below the glass slides to control the height of the Z-stage. In order to control the glass slides in nanometer scales and to create a hologram image, the glass slides should constantly oscillate. To accomplish this goal, we aimed to create a sinusoidal wave using the microcontroller unit (MCU). Ultimately, the users can program the MCU and obtain the height they want. Once the user programs the height, it can be verified by using the interferometer. This report begins by introducing the project design, followed by the design processes and testing. Afterwards, the verifications and results are addressed and costs involved in this project. The report ends with conclusion and future works.

Contents

1. Introduction

Confocal Microscopy is an optical imaging technique for obtaining high-resolution image mostly used in biological science [1]. Confocal microscope uses point illumination method and discards any other stray light. Conventionally, Synthetic Optical Holography (SOH) is used for quantitative phase mapping of confocal microscopy by adding a linearly moving reference mirror. Using this method, the reference mirror needs long travel range for creating a linear-phase synthetic reference wave. Two methods are available to improve this drawback. One method is to oscillate the reference mirror sinusoidally and the other method is to tilt and change the height of the glass slide where sample is located. Our design uses the method of changing the height of the glass slide by using four piezo-electric actuators below the glass slides, which reduces the travel range compared to the conventional method. In addition, by programming the MCU, we could generate a sinusoidal wave. By creating the sine wave, we can constantly oscillate the glass slide at the height we want. Consequently, using the interferometer, we could see the phase difference between the incident wave and the reflected wave. This introduces a modulation in the phase of the reflected wave, and ultimately record a hologram image.

1.1 Block Diagram

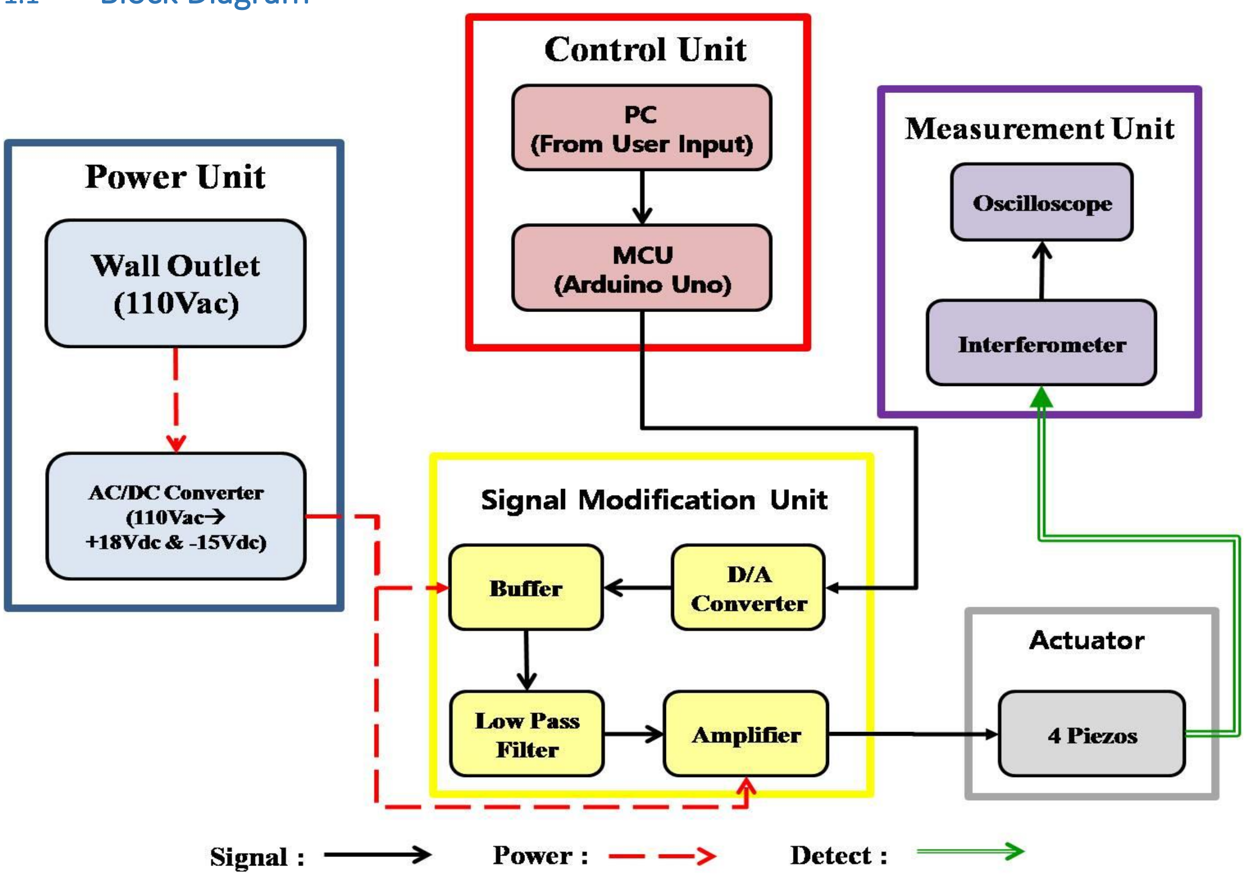

Figure 1. Block Diagram

1.2 Block Descriptions

1.2.1 Power

The power comes from the wall outlet which produces 110V AC. Based on this power, we used a ADC converter which converts 110V AC into 18V DC and -15V DC. The power supply is to supply voltage in the amplifier and the buffer.

1.2.2 Microcontroller

The microcontroller is to produce a sinusoidal wave which is one of the requirements for the project. The sinusoidal wave is to oscillate the glass slides constantly. By producing the sine wave, it produces a modulation in the phase of the reflected wave on the interferometer. Afterwards, we could record the hologram image on the computer using the 'Leica SP8 confocal microscope'.

1.2.3 D/A Converter

Digital to Analog Converter receives 8 bits of digital signal and converts into analog signal. We used R-2R resistor ladder network for the D/A Converter, because using the resistor ladder network is cheaper than the prebuilt D/A Converter. The D/A Converter outputs signal to the buffer.

1.2.4 Buffer

The buffer is inserted between the D/AC converter and the low pass filter. This component acts as a unity gain buffer amplifier. In other words, the operational amplifier does not provide any amplification to the signal. Since the buffer has high input impedance, it draws trivial current and does not disturb the original circuit, giving the same output voltage signal as the input voltage signal.

1.2.5 Low pass filter

The low pass filter receives the input signal from the output signal of the buffer. Since our project aims to obtain precision in nanometer scale, we need precise signals and low noise as much as possible. The filter has frequency range up to 4kHz.

1.2.6 Amplifier

The supply voltage receives power from the power supply and the amplifier should have a gain of 3.44. The choice of gain 3.44 is because the piezo electric drivers need maximum voltage of 13 V in order to obtain 0.5 μm, where the input of the amplifier is 5 V. The input voltage depends on the programming of the MCU and outputs to the piezo drivers.

1.2.7 Piezo electric actuator

There are total of four piezo electric actuators, which control the height of the glass slides. These piezo drivers are placed below the glass slide and depending on the voltage output of the amplifier, the height will be changed.

1.2.8 Interferometer

The interferometer is used to obtain the change in height information. Since the piezo drivers operate in nanometer scale, it is hard to see the change in displacement of the glass slide. Consequently, interferometer is used to verify whether it has met the user input. The interferometer uses HeNe laser source with 532 nm. By measuring the phase shift of the reflected wave from the glass slide, we can calculate the height difference.

2. Design

2.1 Power

The power supply receives the input from the wall outlet, which produces 110V AC. This power supply unit has to convert the 110V AC into 18V DC and -15V DC respectively. Based on datasheet of piezo actuator used, these supply voltages are sufficient to amplify signal to drive piezo electric actuator to 500nm.

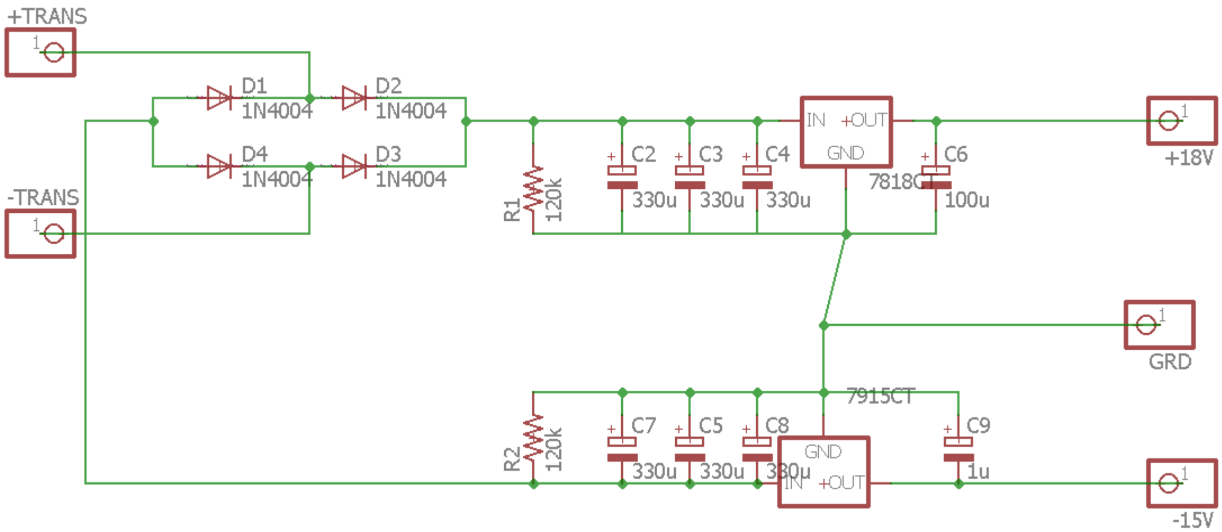

Figure 2. Design of Power Supply

According to figure 2, there are three parts in power supply unit. In order to produce the expected values, first, 117V to 25.2V center-tapped transformer (P-8180) is used. Second, the positive terminal and negative terminal of transformer goes into full wave rectifier using four diodes (1N4004) to produce DC power. Afterwards, two voltage regulators (LM7818 [2] and LM7915 [3]) are used to regulate the outputs of full rectifier to 18V DC and -15V DC respectively.

2.2 Microcontroller

Microcontroller provides user input for generating different output voltages and different frequencies of the microcontroller. The output voltage ranges from 0V to 5V and the frequencies ranges from 10Hz to 3000Hz. For the microcontroller and software, we used Arduino Uno and the Arduino software to generate code for the digital bit output signal. We designed the code to generate 8-bit digital signal, and when all of the 8-bits are high, we get 255/256th of the reference voltage. When the most significant bit is low and all the other bits are high, we get 127/256 reference voltage. From the code, which is on appendix Figure B.1, user can change the value for gain from 0 to 1 in steps of 0.05 to change the voltage output of the microcontroller. In addition, user can change the value of the delay to change the frequency from 10 Hz to maximum of 3.1 kHz.

2.3 D/A converter

From the digital 8-bit output from the microcontroller, D/A converter receives the digital bits and converts the signal into analog signal. The most inexpensive way to achieve this conversion was using resistor ladder network, so called R-2R resistor ladder network. R-2R resistor ladder requires 2n resistors, where n is the number of bits, so we used 16 resistors for our 8-bit digital signal.

2.4 Buffer

The usage of the buffer is to create a unity gain buffer amplifier. This component isolates the signal from the D/AC converter and the low pass filter. Since it has a high input impedance, it is necessary to place a buffer between the D/AC converter and the low pass filter. The buffer outputs the same as the input voltage and reduces noise. Since this project deals with nanometer scale precision, the usage of buffer is essential. In addition, since we are using one power supply, the supply voltage should meet the requirement of the power supply. Consequently, the choice of the buffer is to use 'TL082 operational amplifier' [4], which has a supply voltage of +18V & -15V.

2.5 Low pass filter

One of the requirements for the low pass filter is to have a cutoff frequency of 4 kHz. Consequently, we used a first-order filter, which has RC components. Equation (2.1) is used to obtain the resistance and capacitance values.

$$f_c = \frac{1}{2\pi RC} \tag{2.1}$$

Where the cutoff frequency is 4kHz. Using the resistance value as 10kΩ, we obtained the capacitance as 4nF.

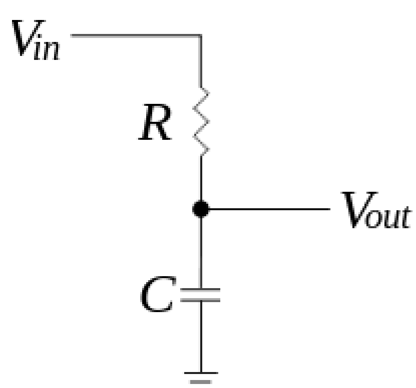

Figure 3. First-order Low Pass Filter

2.6 Amplifier

The amplifier receives input from the output of the low pass filter. The voltage range of the input is from 0V to 5V. By using a gain of 3.44, we can output a voltage range of 0V to 17V. In addition, similar to the buffer component, the supply voltage range should be within the power supply. Consequently, we chose LN741 operational amplifier [5] for the design. According to the datasheet of LN741, this op-amp has a supply voltage of +18V & -15V and input voltage of +/- 15V, which is more than enough for the requirement. The resistors of the amplifier are 8.2kΩ and 20kΩ to obtain a gain of 3.44. Equation (2.2) shows the calculation of the gain obtained.

$$\frac{V_{out}}{V_{in}} = \frac{R_1+R_2}{R_2} = \frac{20k+8.2k}{8.2k} = 3.44 \tag{2.2}$$

2.7 Circuit and PCB design

The overall circuit schematic and the PCB design are shown in the appendix figure C.1 and C.2. The circuit schematic is created using the Eagle program and then converted into a PCB layout. The PCB results in much neatly wiring because most of the wires are replaced by the traces on the board. Using PCB instead of the breadboard also prevents the wires from getting disconnected. The components in the PCB are buffer, D/A converter, amplifier, and low pass filter. The output of the amplifier goes to the input of the four piezo drivers.

2.8 Piezo electric actuator

There are total of four piezo electric actuators used under the glass slide on the confocal microscope. The requirement of the maximum displacement of the piezo electric actuator is 0.5 μm and the displacement should change linearly with the voltage applied. Consequently, we chose the piezo actuator, which has a maximum displacement of 2.8 μm and a voltage range of 0 to 75 V [6]. According to the datasheet of the piezo actuator, when exceeding 75 V, the device's lifespan will decrease and may possibly cause mechanical failure. The maximum voltage from the amplifier is 18V, which is the input voltage for the piezo actuator and the piezo actuators are expected to lift the glass slides by 0.5 μm with the maximum amplifier voltage.

2.9 Metal plate

The metal plate is placed on the confocal microscope and four piezo electric actuators are placed on top of the plate. On top of the four piezo drivers, the glass slide will be placed where the sample is located. In order to obtain the dimensions of the metal plate, we had to accurately measure the dimensions of the confocal microscope. By using a caliper, we obtained 6.26 inch horizontally and 3.20 inch vertically. We chose the thinnest possible metal, available in the ECE machine shop, which would not bend. We decided to choose the thickness to be 0.125 inch. The error range is 0.05 inch because if the plate is smaller or bigger than the error range, the metal plate will not fit on the microscope. The completed metal plate is shown in appendix D. Moreover, the material is made of aluminum because it is the lightest but also the cheapest to purchase.

In addition to the dimensions of the horizontal and vertical, we had to consider a square hole in the middle of the metal plate. This location is where the piezo drivers and glass slides will be placed. Since we are using 3 x 1 inch glass slide, the x-axis of the square hole has to be smaller than 3 inch and y-axis has to be larger than 1 inch. Figure 4 shows the overall dimensions of the metal plate.

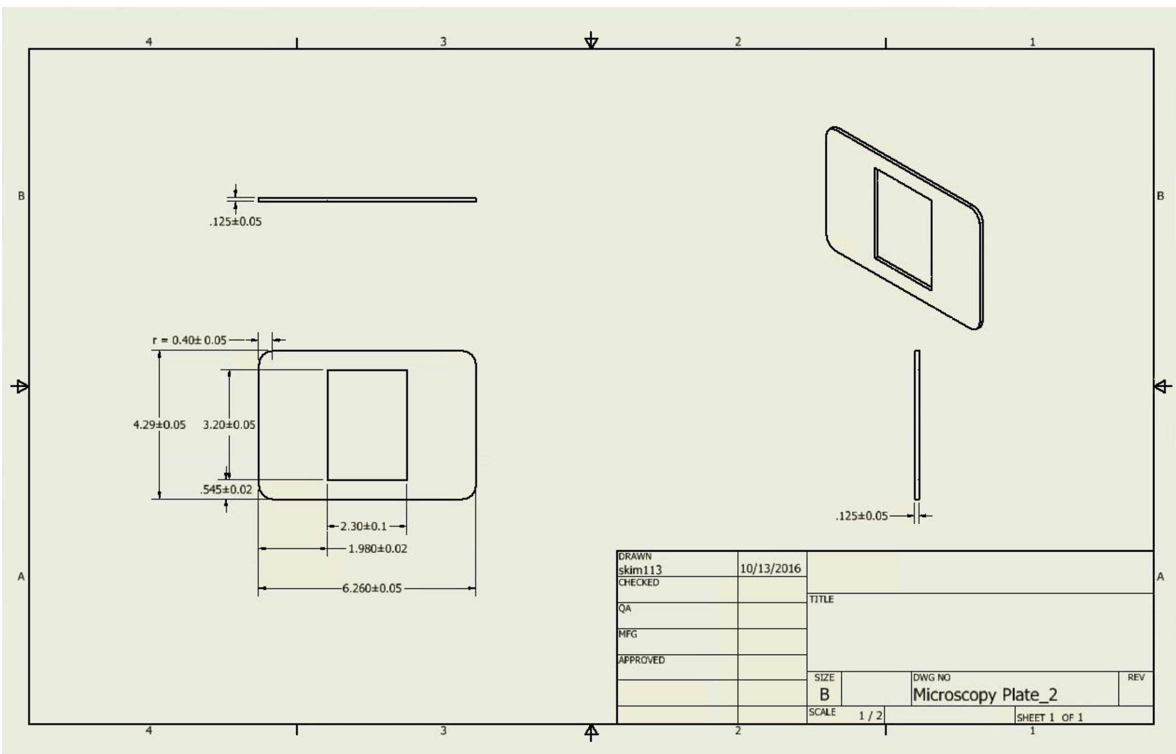

Figure 4. Metal Plate Dimensions

2.10 Interferometer

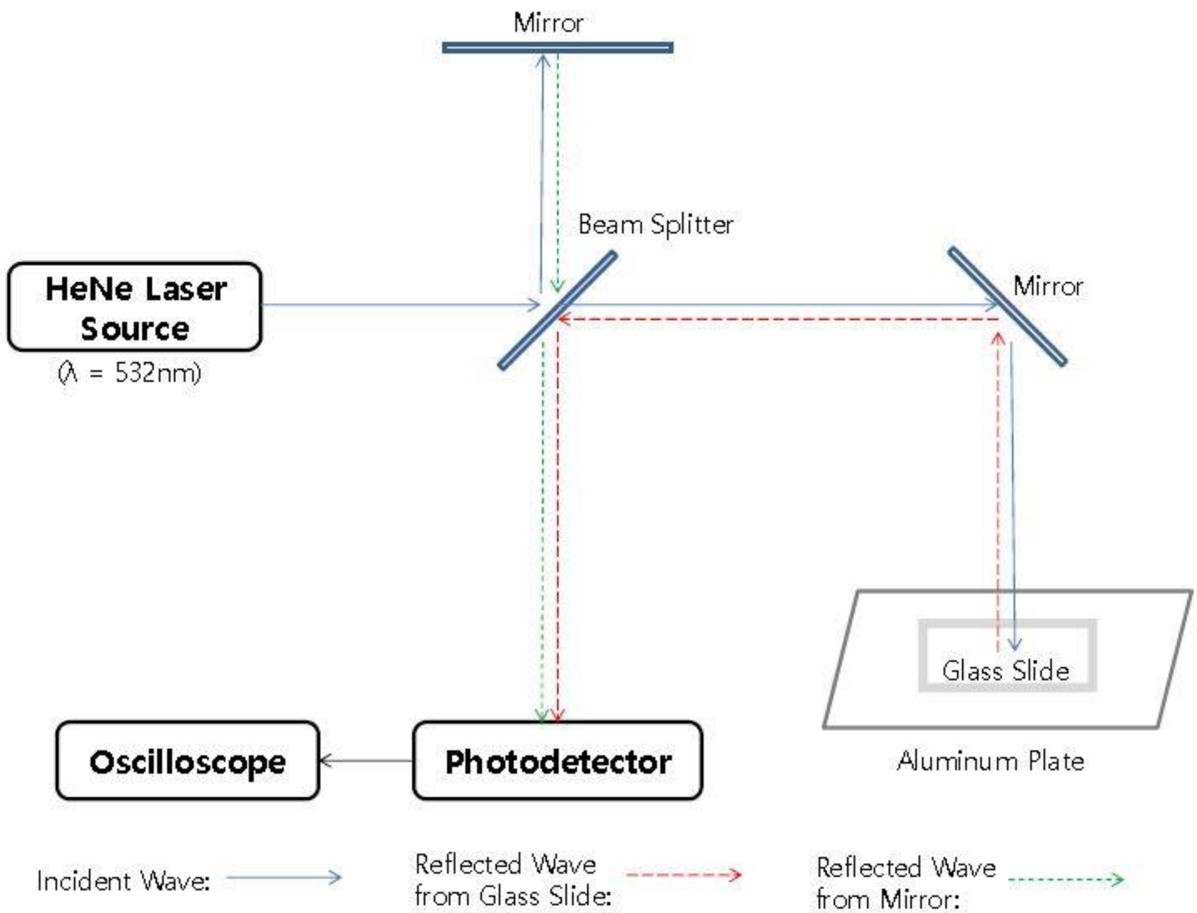

Figure 5. Interferometer Setup

Figure 5 shows the setup of the interferometer. The laser source uses HeNe 532 nm wavelength. The incident wave, which is the output of the amplifier, is split by the beam splitter and hits the glass slide on the confocal microscope. The reflected wave, which is shown in red dotted line in Figure 5, is the detect signal we measured. The photo-detector will detect both incident and reflected waves and show the results on the oscilloscope. By using the excel file, we obtain the phase difference between the two waves. From these results, we can obtain the height difference calculated in equation (2.3)

$$\text{Change in displacement (nm)} = \frac{\Delta\varphi}{2\pi} * \frac{\lambda}{2} \qquad (2.3)$$

Where $\Delta\varphi$ is the phase difference between the incident wave and the reflected wave, and λ is the wavelength of the laser source which is 532 nm. We need to divide lambda by two because the wave travels twice when it hits the glass slide. Based on the settings of Figure 5, we are able to verify the change in height of the glass slide and satisfy what the user has input.

3. Design Verification

3.1 Power

In order to verify whether the power supply performs as expected, we connected the output of the power supply and measured the output voltages. As stated previously, the required output voltages should be at least 15V DC and -15V DC.

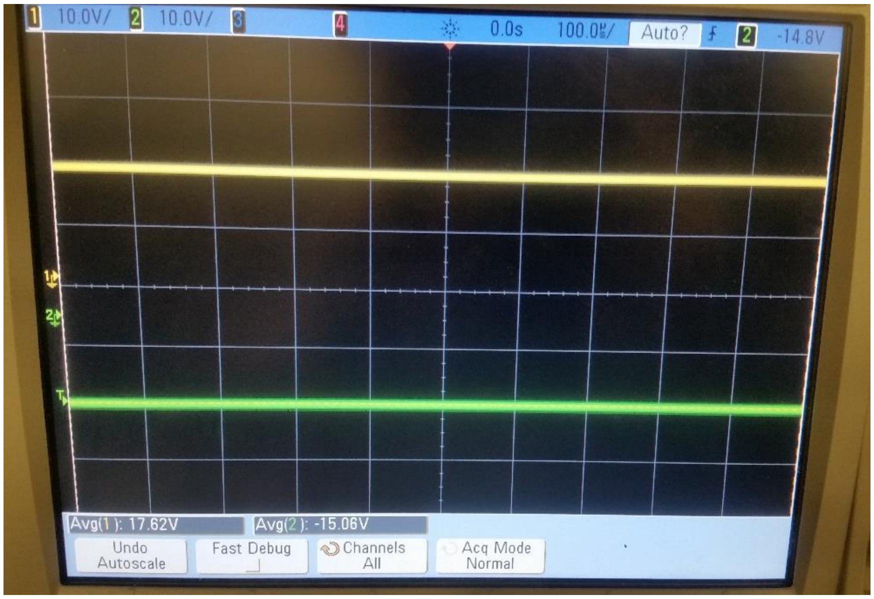

Figure 6. Power Supply Output

Yellow signal in Figure 6 represents the output voltage from the LM7818 voltage regulator. As shown in Figure 6, the average value of yellow signal is 17.62V. Therefore, positive output voltage is high enough to supply at least 15V DC to amplifier and buffer. In addition, the green signal represents the output from LM7915 and shows -15.06V. Consequently, our power supply can produce sufficient output voltages to amplifier and buffer and it shows that the requirements of power supply are satisfied.

3.2 Microcontroller Unit

Eight output ports are in microcontroller, and the output signals are hard to interpret when the output signals when we measure the output signal directly from the output ports of the microcontroller. Therefore, we verify the requirements on the output of the Digital to Analog converter.

3.3 D/A Converter

We can verify the requirements for the microcontroller from the output of the Digital to Analog converter. The requirements for the output of the D/A converter are outputting sine wave, with maximum voltage of at least 2.5 volts peak-to-peak and maximum frequency of at least 3000 Hz. The maximum values for the voltage and frequencies can exceed the values specified, because the voltage limit and frequency limit for the piezo actuators are way above the voltage we can generate. From Figure 7 and 8, we can verify that the output signals are sine wave, and have peak-to-peak voltage of 5V and maximum frequency of 3.13 kHz, which satisfies the requirements.

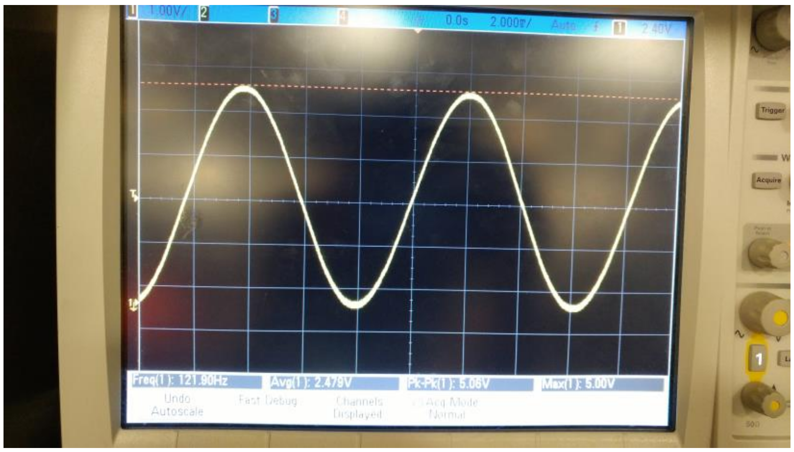

Figure 7. Voltage Output DAC Low Frequency

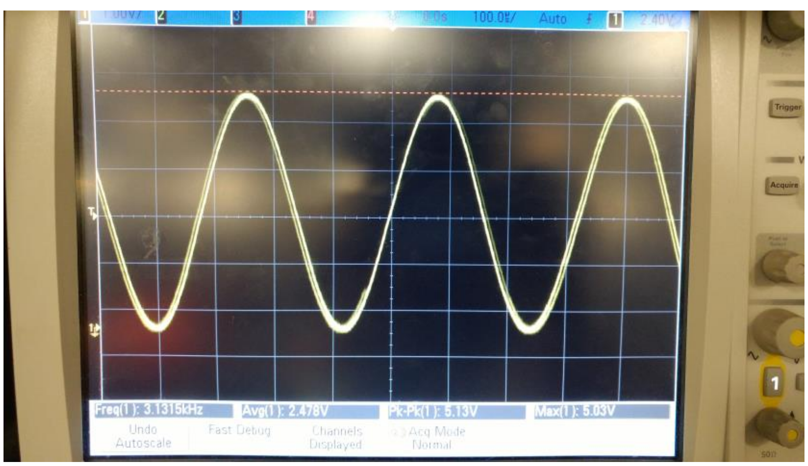

Figure 8. Voltage Output DAC Max Frequency

3.4 Amplifier

We verified the amplifier by using the breadboard. As it was mentioned in section 2.5, the cutoff frequency of the low pass filter is 4 kHz. Consequently, we tested whether the amplifier functions properly with different frequencies. As shown in the left image of figure 9, when the frequency is given at 121.7 Hz from the MCU, the output voltage, shown in yellow signal, is 19.4V. The input to the amplifier is 5.19V. As a result, the gain could be calculated as $\frac{19.4}{5.19} = 3.72$. Afterwards, the right image of figure 9 shows when the frequency is at high frequency, 3.12 kHz. The input voltage was given the same as with the medium frequency, 5.19V, and the output voltage showed identical result. Overall, the performance of the amplifier did not depend on the frequency below the cutoff frequency as expected.

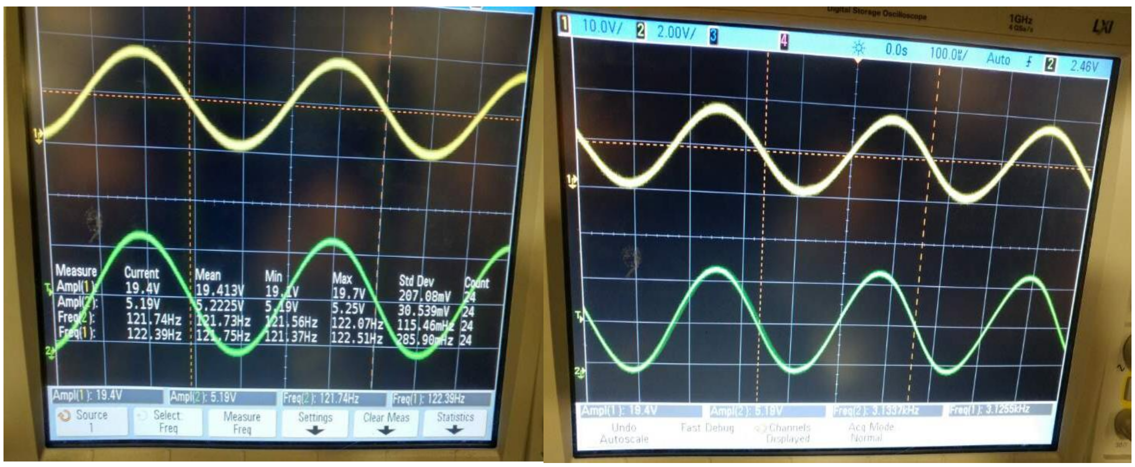

Figure 9. Amplifier Output (Left: medium frequency, Right: High frequency)

In addition, one of the requirements, shown in appendix A, for the project is to have a stable frequency throughout the whole circuit. As shown in figure 9, the yellow signal is the output from the D/A converter and the green signal represents the output from the amplifier. The image on the left side of figure 9 shows that the output frequency of the D/A converter is 121.75 Hz and the output frequency of the amplifier shows 121.73 Hz. These results show that the frequency did not change more than 1% and satisfied the requirement. Similarly, the image on the right side of figure 9 also shows that the frequency did not change more than 1%. Last requirement for the amplifier is to output current up to 5mA. If the current is higher than the required current, then the amplifier will not function properly. Consequently, we showed that the amplifier works and proves that it satisfies the requirement.

3.5 Piezo electric actuator

One of the requirements for the piezo electric actuator is to have a maximum displacement of 0.5μm as shown in the requirement and verification table in appendix A. Consequently, using the datasheet of the piezo electric actuator, we can determine the required input voltage to obtain the 0.5μm displacement. According to Figure 10, the black colored circle represents the point how much the voltage is required. To obtain a displacement of 0.5μm displacement, 13V input voltage is needed. Consequently, the output of the amplifier must satisfy at least 13V. Since the output voltage of MCU is 5V peak to peak, and the gain of the amplifier is 3.44, we can obtain a maximum output voltage from amplifier as calculated in equation (3.1).

$$\text{Maximum Output Voltage from Amplifier: } 5 \times 3.44 = 17\text{V} \tag{3.1}$$

Since the output of the amplifier is 17V, we achieved more than enough voltage requirement. Using this setup, we can now verify whether our design has met the required displacement using the interferometer.

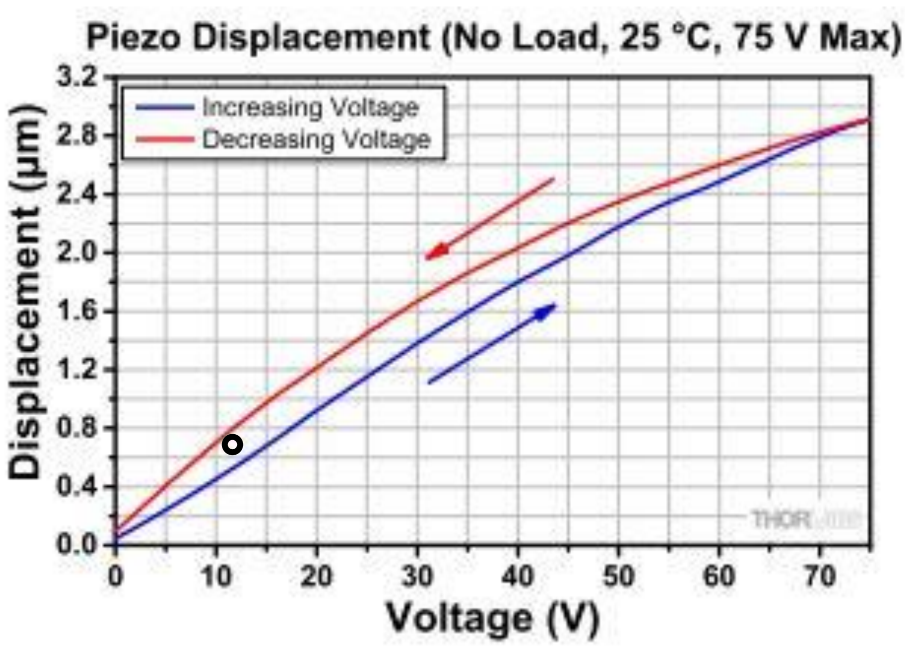

Figure 10. Piezo Displacement [6]

3.6 Interferometer

Interferometer is used to monitor the behavior of Piezo electric actuators. By using data obtained from interferometer, the displacement of glass slide, which has 4 Piezo electric actuators underneath, can be calculated. Because it is impossible to visualize displacement of glass slide directly from interferometer, we select data in random position while piezo electric actuators are oscillating in z-direction.

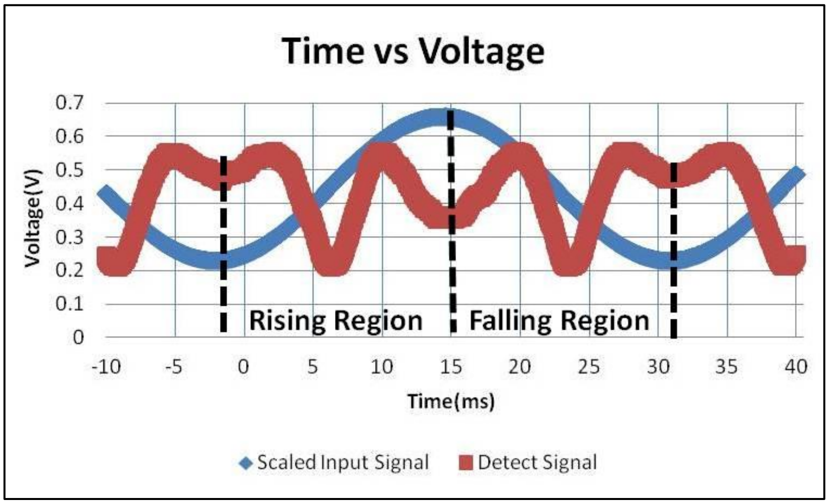

Figure 11. Scaled Input Signal and Detect Signal vs Time Graph

Figure 11 shows data obtained from interferometer when maximum amplitude of input signal is applied to Piezo electric actuators. Blue line is scaled input signal of piezo electric actuator to compare with detect signal. Original amplitude of input signal is 17V and the amplitude of input signal is compressed to 0.5V. Also, the red line indicates detected waveform of laser(HeNe) in interferometer. While input signal is increased, HeNe signal is traveled roughly in 1.5 of period. Also, the detected signal while input signal is decreased is symmetric with detected signal in rising region because the HeNe laser traveled same distance in rising region and falling region in oscillating motion.

$$\text{Displacement} = \frac{\lambda \times \mathrm{T}}{2} \tag{3.2}$$

To calculate displacement glass slide moved, equation (3.2) is used when λ and T indicate the wavelength and the number of period of HeNe laser respectively. Usually the distance can be calculated by multiplying the number of period and wavelength of wave. However, photo-detector in Figure 5 detects HeNe laser, which is reflected to glass slide. Therefore, the laser travels twice as much as the displacement of glass slide moves. So, the half of travel range is used for displacement of glass slide in equation (3.2). Because the wavelength of HeNe is 532nm, the number of period is only one factor we need to know for calculation of displacement in equation (3.2). However, it is difficult to figure out the number of period of detect signal in Figure 9 because piezo movement is accelerated in the middle of rising and falling region and decelerated as input signal approaches to maximum or minimum points. And it causes the detect signal to be not a perfect sinusoidal wave but a tilted sinusoidal wave in each region.

For better precision of indicating the number of period of detect signal, reference sine wave, which has same peak to peak voltage and offset with detect signal and has period of 2π ms is used. By comparing the detect signal with reference sine wave, it is possible to make tilted sine wave to perfect sine wave by finding two points, which have same voltage with tilted sine wave and pass the same number of critical points.

$$\mathrm{T} = \frac{\Delta \mathrm{t}}{\mathrm{T_o}} \tag{3.3}$$

According to equation (3.3), T, Δt , T_o indicate the number of period, time difference of two points and the period of waveform respectively. Because we already know that the value of T_o is 532nm, Δt is the only factor we need to know for calculation of the number of period.

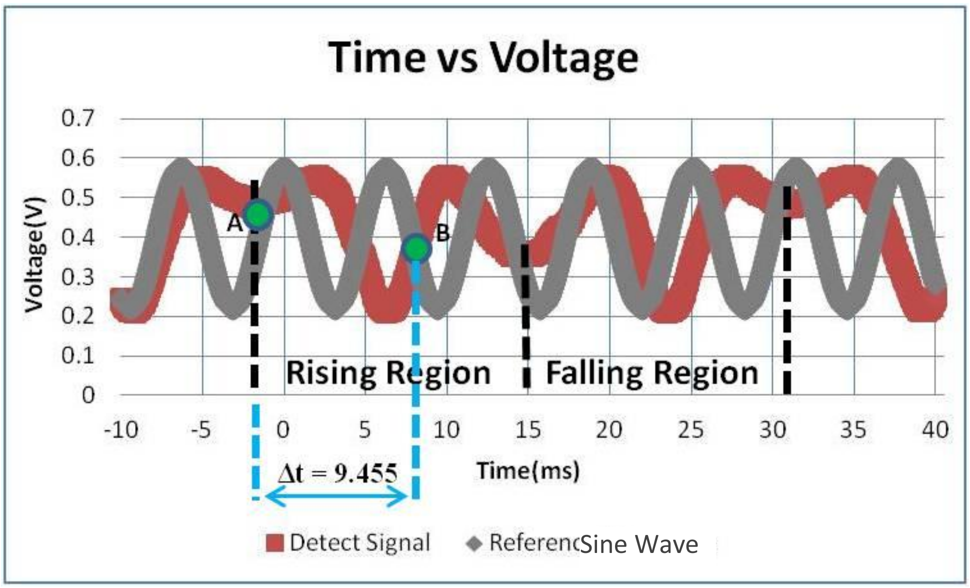

Figure 12. Detect Signal and Reference Sine Wave vs Time Graph

Red line and grey line in Figure 12 indicate the detect signal and reference sine wave respectively. To calculate Δt, initial point and terminal point of reference sine wave are needed. The initial point of reference sine wave become the point at the beginning of rising region because it has same voltage with detect signal at the beginning of rising region. And the initial point is the left handed side green circle labeled as A. The terminal point of detect signal is at the end of rising region and it passed two maximum points and one minimum point from initial point. Therefore, the terminal point of reference sine wave is when it passed two maximum points and one minimum point from initial point and has same voltage with terminal point of detect signal. And the terminal point of reference sine wave is right handed side green circle labeled as B. Therefore, the time difference between point A and B can be obtained as 9.455 ms. According to equations (3.2) and (3.3), the displacement of glass slide moved can be calculated by obtained data upward.

$$\mathrm{Displacement} = \frac{\lambda \times \mathrm{T}}{2} = \frac{\lambda \times \frac{\Delta \mathrm{t}}{\mathrm{T_o}}}{2} = \frac{532\mathrm{nm} \times \frac{9.455\mathrm{nm}}{2\pi\mathrm{nm}}}{2} = 400.28\ \mathrm{nm} \tag{3.4}$$

According to equation (3.4), the displacement of piezo electric actuator moved while maximum input signal is applied to piezo actuator is 400.28 nm. With same procedures above, it is possible to calculate the displacement of piezo actuator when different input signals are applied as following.

Gain	Voltage Applied (V)	Displacement Glass Slide Moved (nm)
1	17.1	400.27
0.95	16.65	386.25
0.9	15.6	362.15
0.85	14.7	352.2
0.80	13.65	331.65
0.75	12.9	313.86
0.70	12.1	274.89
0.65	11.26	247.77
0.60	10.27	206.38
0.55	9.61	195.55
0.50	8.36	180.44
0.45	7.46	170.69
0.40	6.74	149.0955
0.35	5.9	121.14
0.30	4.66	129.01
0.25	4.14	118.59
0.20	3.33	89.37
0.15	2.52	74.12
0.10	1.62	66.48
0.05	0.83	58.29

Table 1. Calibration Table of Displacement of Glass Slide vs Voltage Applied to Piezo

According to Table 1, the gain refers the variable user can adjust in MCU by PC. From gain of 0.05 to 0.1, the displacement of glass slide is increased to 8.19nm. Therefore, the resolution of piezo movement is smaller than 8.19nm which is much better than our requirement that resolution should be smaller than 20nm. Moreover, glass slide is lifted up to 400.27nm when maximum input signal is applied to Piezo. Our original requirement is to reach 500nm. Even though it does not satisfy our requirement fully, our design mostly satisfy the requirement.

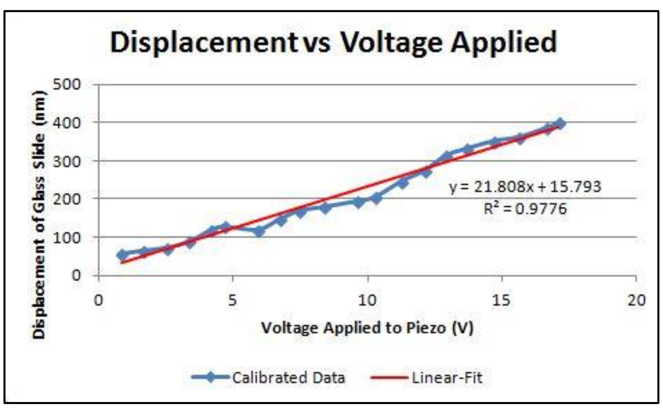

Figure 13. Linearity of Displacement vs Voltage Applied

In Figure 13, blue line shows the displacements vs voltage applied to piezo according to Table 1. The red line shows linear fit of blue line. Because R^2 is 0.9776, the data in Table 1 has good linearity and displacement of glass slide is increased as voltage applied to piezo is increased.

4. Cost

4.1 Parts

Part	Source	Part Number	Quantity	Cost
Transformer	ECE Supply	p-8180	1	$20.00
Diode	ECE Supply	1N4004	4	$0.64
Resistor	ECE Supply	120 kΩ 5% 1/4W	2	$0.75
Resistor	ECE Supply	20 kΩ 5% 1/4W	9	$0.75
Resistor	ECE Supply	10 kΩ 5% 1/4W	9	$0.75
Resistor	ECE Supply	8.2 kΩ	1	$0.75
Buffer	ECE Supply	TL082	1	$0.86
Amplifier	ECE Supply	LN741CN	1	$0.65
Voltage Regulator	ECE Supply	7818CT	1	$1.00
Voltage Regulator	ECE Supply	7915CT	1	$1.00
Capacitor	ECE Supply	330 μF	6	$1.95
Capacitor	ECE Supply	100 μF	2	$1.95
Capacitor	ECE Supply	1 μF	1	$1.00
Capacitor	ECE Supply	1 nF	1	$1.95
Piezo Electric Actuator	Thorlabs	TA0505D024W	5	$160
PCB	PCBWIN	PW291A1208	1	$214
Microcontroller	Arduino Store	Starter Kit	1	$100
Metal plate	ECE Machine Shop		1	$80
Total				$588

Table 2. Parts Cost

4.2 Labor

Student	Hourly Rate	Total Hours Invested	Total*2.5
Hyunjae Cho	$30	250	$18750
Sung Hun Kim	$30	250	$18750
Ye Hyun Kim	$30	250	$18750
Total		750	$56250

Table 3. Labor Costs

4.3 Grand Total

Section	Total
Labor	$56250
Parts	$588
Grand Total	$56838

Table 4. Total Cost

5. Conclusion

5.1 Accomplishment

The goal of this project was to generate sinusoidal wave from the MCU and to control the height of the piezo electric actuators. In order to perform the expected output wave, we have used several signal modification unit other than the MCU. It involved using buffer, amplifier, low pass filter, and D/A converter. When testing the signal modification unit on the breadboard, every components functioned as expected. Consequently, we have used the completed circuit on the breadboard and tried to verify the change in height information of the piezo electric actuators using the interferometer. As mentioned in section 3.6, we have successfully verified that the piezo electric actuators performed well. The requirements for verifying were to show that the resolution is within 20nm range, and have a maximum displacement of 0.5μm. After seeing that our results were consistent with the expected values, we have used a PCB. However, whenever we were using PCB, the results were not as expected. The output of the amplifier did not show the expected values when it was in high frequency. In addition, when we used this PCB on the confocal microscope, the image shown on the microscope did not show the correct hologram image.

5.2 Safety Consideration

There are two safety considerations in our project. One involved using the power supply and the other is when we used an interferometer using HeNe laser source. For safety consideration, we used a wall outlet to power the transformer of our power supply design, and unplugged any unnecessary electric devices from the wall outlet. In addition, although the wavelength of the laser source is 532nm and is not considerably dangerous to human eyes, we have used a black colored curtain behind the interferometer just in case for anybody else to get injured by looking at the laser source.

5.3 Future Work

Our goal was to change the displacement of the piezo electric actuators up to 500nm. However, our theoretical calculations and the data given by the datasheet for the piezo electric actuators were not accurate experimentally. We will need a better amplifier and power supply so that we can generate sine wave with more peak-to-peak voltage than what we have designed.

Full product was working in the breadboard correctly, so we moved every parts to the PCB we ordered and soldered the parts to the PCB. We tried to run the whole product at once without checking each component, and few components burnt. We realized it is important to test each part before running the full product.

When the metal plate and the piezo electric actuators were in the confocal microscope, we were almost out of range for the working distance of the confocal microscope, meaning that we needed the location of the piezo electric actuators to be higher than what we designed.

Our product includes Arduino Uno in the design, but including Arduino Uno's ATMEGA only for our product would make the product cheaper and smaller in design. Additionally, sealing the whole product in the future will be required if we wanted to fit the product inside the microscope.

6. References

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[6] TA0505D024 Piezoelectric Chip, datasheet, Thorlabs, 2014. Available at: https://www.thorlabs.com/drawings/f5db5188c41ee18a-9660343F-5056-0103-7981F2E89848C1A1/TA0505D024-SpecSheet.pdf

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Appendix A Requirement and Verification Table

Requirement	Verification	Points
1. Piezo Drivers Piezo drivers must have a minimum displacement of 0.1 micrometers and maximum displacement of 0.5 micrometers (travel range of the piezo drivers)	1. Piezo Drivers Calculate the maximum displacement of the piezo drivers using the signal obtained from the interferometer	0.1um~0.2um: 5 points 0.1um~0.3um: 10 points 0.1um~0.4um: 15 points 0.1um~0.5um: 20 points
2. Piezo Drivers Piezo drivers need to have resolution of 20 nanometers	2. Piezo Drivers Calculate the minimum displacement of the piezo drivers using the signal obtained from the interferometer (displacement of the piezo drivers of the minimum increment of input voltage)	Below 40 nm: 5 points Below 30 nm: 10 points Below 20 nm: 15 points
3. Metal Plate Weigh less than 800g and dimensions of 160mm*110mm +/- 5% with square shape	3. Metal Plate Measure the dimensions of the plate using caliper and measure the weight and check whether the plate fits in the microscope	10
4. Metal Plate Must have a hole smaller than the glass top of 3inch*1inch for imaging and have thickness less than 7mm to suite microscope's working distance	4. Metal Plate Verify microscope is functioning properly with the plate inside the microscope and be able to image through the hole	5
5. MCU Must be able to produce sine wave output with amplitude of maximum 2.5 Vpp and maximum frequency 3kHz	5. MCU Directly connect the output of the MCU to the oscilloscope and measure the amplitude and frequency	0V~1.5Vpp: 3points 0V~2Vpp: 6 points 0V~2.5Vpp: 9 points Up to 1kHz: 3 points Up to 2kHz: 6 points Up to 3kHz: 9 points
6. Amplifier Must be able to output up to 15V +/-5% with 5mA +/-5%	6. Amplifier Check the output of the amplifier with multimeter	10

7. Frequency Frequency should not change more than 1% of the original frequency for all components (output of the MCU, input and output of the amplifier, low pass filter, buffer	7. Measure the frequency for each components and check the change in frequency using oscilloscope or multimeter	10
8. Power Supply From the wall outlet (110V AC), converter must be able to output 15V(+/- 10%) AC and +/- 15V(+/- 10%) DC for the amplifier supply voltage	8. Power Supply Measure the output of the transformer for the 15V AC and measure the output of the voltage regulators for the +/-15V DC using oscilloscope	12

Table 5. R&V table

Appendix B Microcontroller unit (software)

```
int sine[255];
int *p; //access the data in the array by using pointers
       //set this to the address of 0th element of the sine array
void setup() //store results in a global array
{
  pinMode(0, OUTPUT); //specify pin to behave as output
  pinMode(1, OUTPUT);
  pinMode(2, OUTPUT);
  pinMode(3, OUTPUT);
  pinMode(4, OUTPUT);
  pinMode(5, OUTPUT);
  pinMode(6, OUTPUT);
  pinMode(7, OUTPUT);
  pinMode(9, OUTPUT);

  float x;
  float y;
  float gain;
  gain=1;

  for(int i=0;i<255;i++)
  {
    x=(float)i; //float value for values between -128 and 128
    y=gain/2*sin((x/255)*2*PI); //sine wave with 256 time-steps per cycle
    sine[i]=int(y*128)+128*gain/2;
  }
  p=&sine[0];

 }

void loop()
{
  for (int i=0;i<255;i++)
  {
    PORTD=*(p+i); //port register D, faster than DigitalWrite
    delayMicroseconds(64); //change the frequency. 0delay=3kHz, 64delay=60Hz
                           //1228delay=30Hz
   }
}
```

Figure B.1 Sine digital output code [7]

Appendix C Circuit schematic and PCB layout

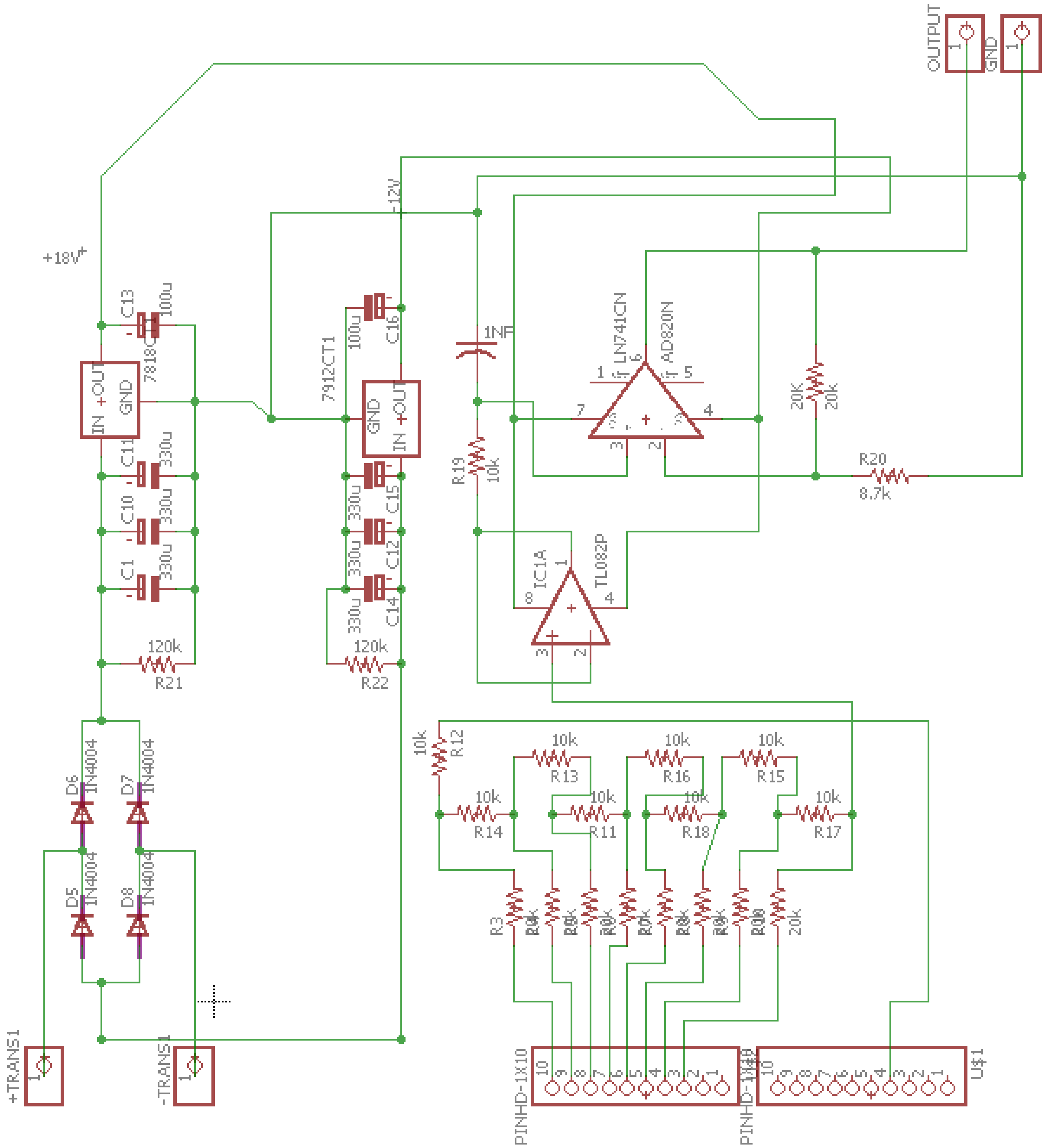

Figure C.1 Circuit schematic

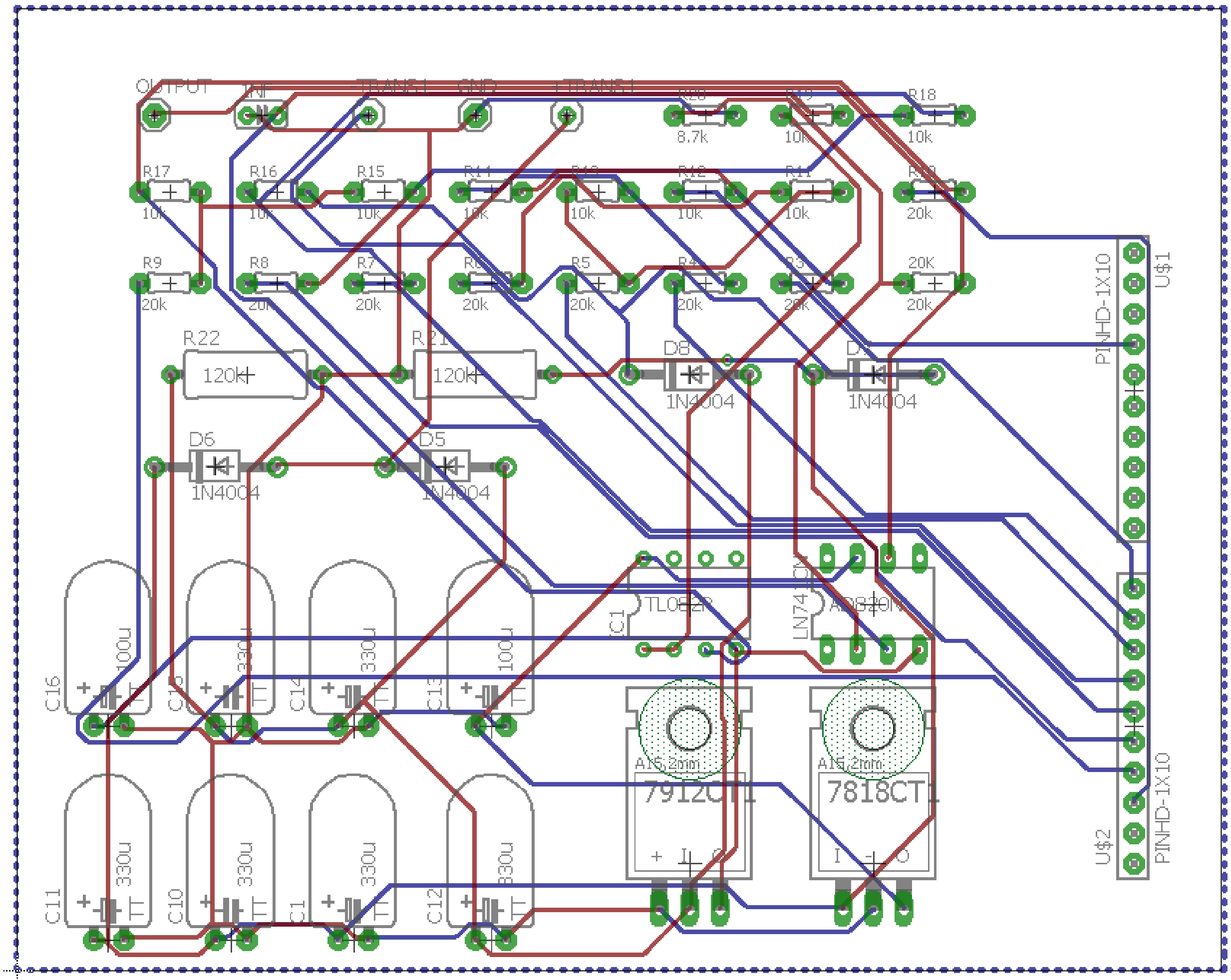

Figure C.2 PCB design

Appendix D Metal Plate Dimensions

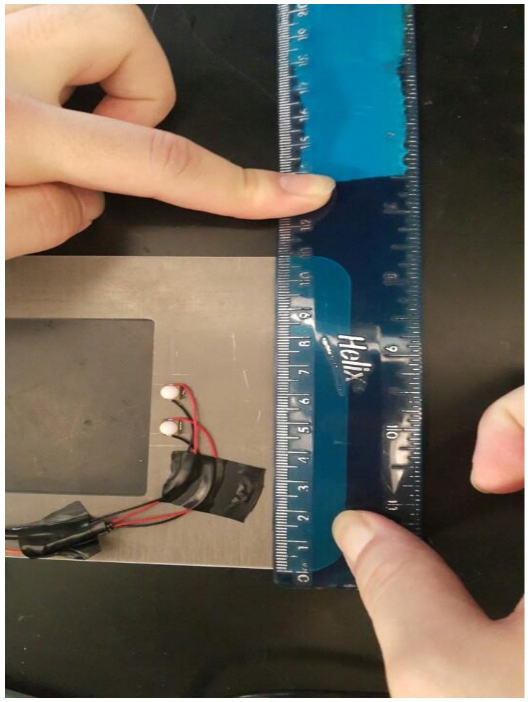

Figure D.1. Metal Plate Vertical Dimension (110mm)

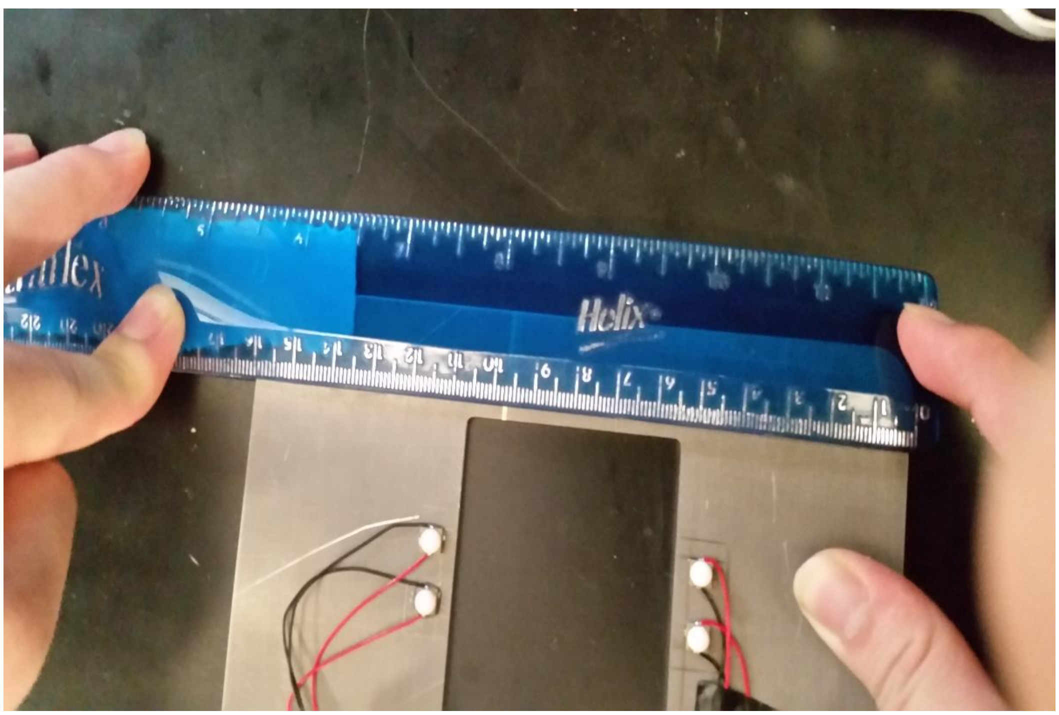

Figure D.2. Metal Plate Horizontal Dimension (160mm)

Figure D.3. Metal Plate Thickness (0.32mm)

Figure D.4. Metal Plate Weight (125.5g)

Appendix E Final Product

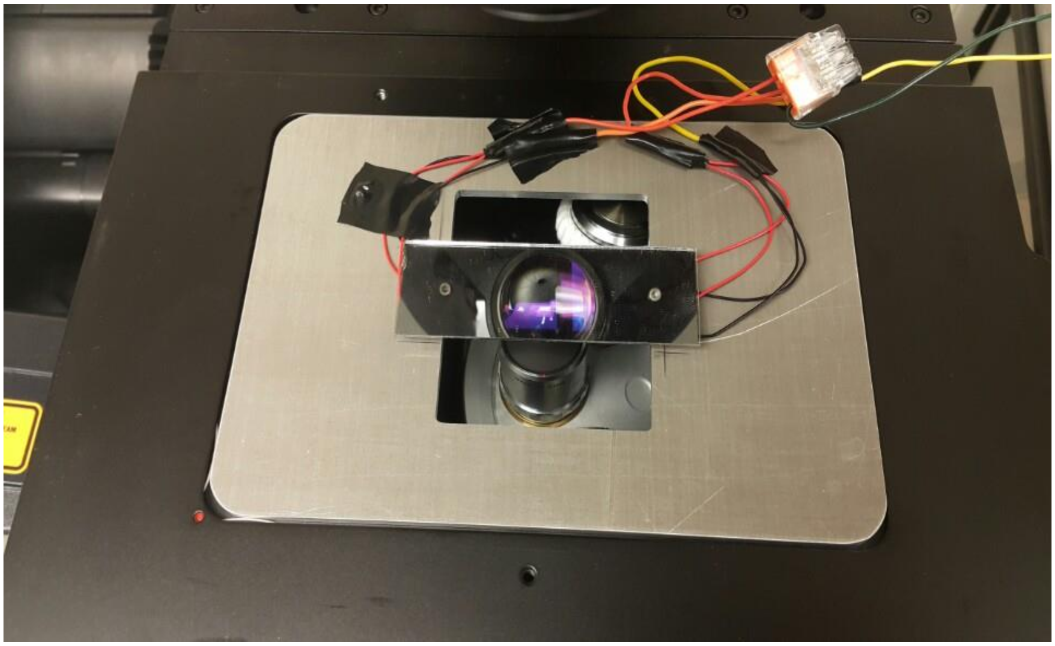

Figure E.1 Metal Plate

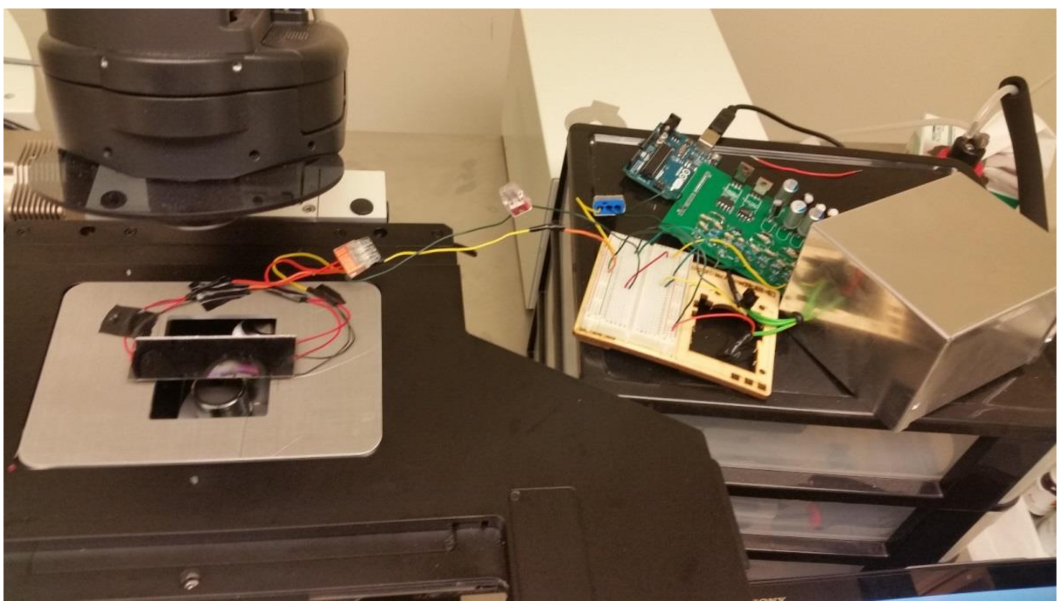

Figure E.2 Final Product on Confocal Microscope

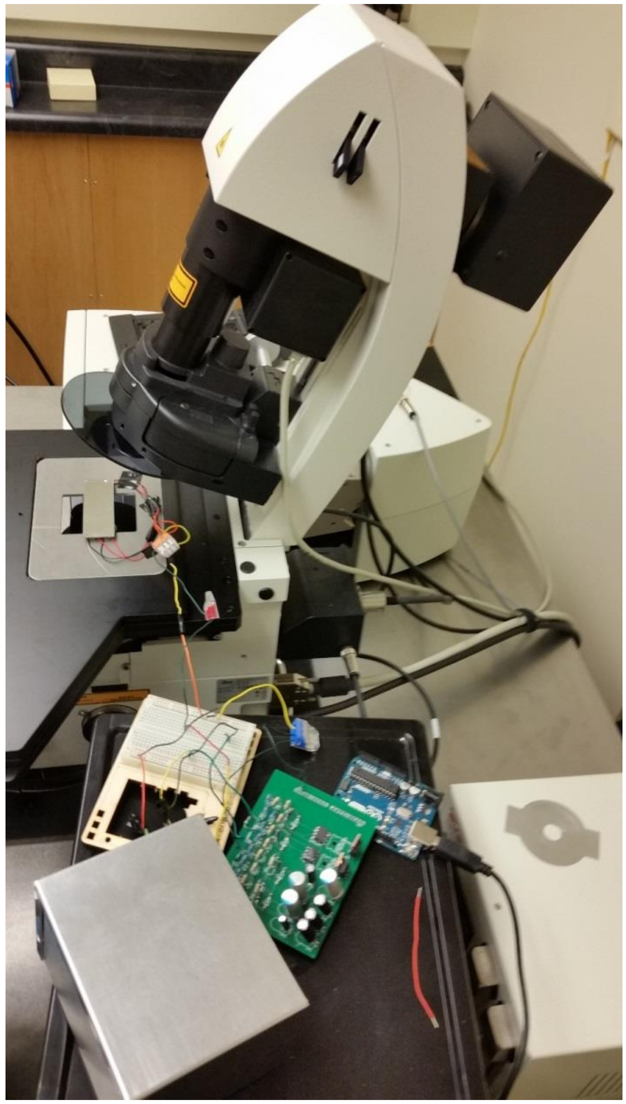

Figure E.3 Final Product