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University Degree in Biomedical Engineering
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Bachelor Thesis

"Development of an electronic driver for piezoelectric actuation in cellular studies"

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Abstract

The behavior of cells has usually been studied regarding biochemical reactions. Nevertheless, new approaches for cellular studies have observed that cells are able to perceive and withstand forces, which can have a huge impact on cellular processes. In the present investigation, it has been developed an electronic driver for piezoelectric actuation in cellular studies, contributing to the emerging discipline of cellular mechanotransduction. Inside the project, the developing of the driver is focused on the generation of the initial signal and its later amplification. There is a great emphasis on the amplification stage, for which a PCB is designed and fabricated. The frequency range of the device will be very important in order to have different mechanical displacements on the piezoelectric material. The last step consists of the stimulation of 28 µm PVDF films.

Results showed the functionality of the driver is possible, as the stimulation of the piezoelectric material does occur. Further studies are needed for the development of the PCB and a design that fixes the whole device into one piece of equipment.

This research has been conducted with the Department of Electronics Technology at the Universidad Carlos III de Madrid, but it was closely run as well with the Department of Biomedical Engineering.

Keywords: piezoelectric material, PCB, bridge-connected circuit, cell mechanical stimulation.

Acknowledgments

With this project, I finish my degree in Biomedical Engineering. The necessary effort to get to this point has been in a great percentage thanks to everyone who has been by my side throughout these four years, and who wanted me to achieve this goal. I can’t finish this work without having some words for all of them.

I would like to start by sincerely thanking my tutor Pablo Acedo, for his support and vote of confidence, and for teaching me the most valuable concepts that an engineer must understand.

Besides, this thesis would not have been possible without the involvement, help and direction of Dahiana. You trusted me to continue with the work you started more than three years ago, and I thank you for all the positive feedback and the great recommendations you have given me throughout the project.

If there is a group of people I would like to highlight in these four years that would be the group of friends I have made, or how we like to call ourselves: “Fiesteo”. I have found in all of you great support to rely on, and these years have been wonderful thanks to all of you. Our time together in class might end, but I am certain that our friendship will last forever. In the same way, I also thank my friends from Colegio Estudio, for keeping our friendship and spending unforgettable times during this last summers.

Lastly, I also thank my family for their support. But more specifically, to mom and dad. Thank you for always being there for me, for helping me when I was frustrated and lost, for loving me so much that I will never be able to reward you.

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1. Introduction

The functionality of cells cannot only be modified chemically but also mechanically. As many recent discoveries state, if cells are brought under some mechanical stress it can be of great influence in processes such as embryo's development [1], angiogenesis and functions in the immune system [2]. The influence of mechanical forces on cells can also be observed in diseases such as cholesterol [3] or cancer [4].

Until very recently, the study of cellular processes was focused on the knowledge of biochemistry. Nevertheless, new fields in the area of biomedical engineering are starting to emerge in order to understand processes that biochemistry did not have answers for, and this is the case of biomechanics [5].

To produce mechanical stress in cells, the necessary forces to do so can come from external devices which can be precisely monitored to perform this function. The effects produced on cells due to the implementation of this mechanical stress is known as mechanotransduction, and it has proven to be very effective in cell regulation and tissue morphology [6].

Piezoelectric materials are characterized for producing an internal mechanical strain due to an applied electric field, being this effect a reversible process (electric charge can be accumulated in piezoelectric materials in response to mechanical stress). Piezoelectric actuators have been widely used in mechanical devices such as printers for a long time now [7]. In the last few years, they have also been used in areas related to medicine [8], which opens a new field of usage for this type of material.

It would be of great interest to try to combine the effect of piezoelectric materials to the mechanotransduction of cells. If cells were able to be attached to a piezoelectric material, it could be studied the behavior of these cells when mechanical stress is applied to them.

1.1 Motivation and objectives

Three years ago, the Department of Electronics Technology and the Department of Biomedical Engineering at the Universidad Carlos III de Madrid started to work on developing a biocompatible piezoelectric device for cellular studies [9]. It was able to come up with the whole schematic of the investigation and thoroughly study each stage. The work was continued by making a deeper study of the effects that piezoelectric materials had on cells [10]. These investigations showed great results when studying the healing process of cells when they were mechanically stimulated. However, they led to the conclusion that an effective electronic driver was needed in order to further investigate this phenomenon. This driver needed to be lightweight so it could be set inside the biological laboratory where cells would proliferate. It also needed an interface capable of letting the user choose between different frequencies and voltage amplitudes, depending on the desired effects that would cause on cells. From the results obtained in the previous investigations, this project focuses on the development of this driver.

In order to produce a mechanical strain in a piezoelectric material, it is first necessary to generate the required electric field that will induce this strain. One main drawback of piezoelectric actuators is the high voltage necessary for them to have a great mechanical change [11]. In most of the piezoelectric materials, the voltage necessary to produce mechanical stress must reach regions close or even higher than 100 volts. Thus, this power cannot be acquired in conventional low-voltage energy sources. Another issue rises up when realizing there must be a stage of amplification of the signal, which will need an operational amplifier capable of dealing with such a high voltage.

Regarding the great potential that piezoelectric materials can have in the study of mechanotransduction of cells, it would be a very good approach to try to stimulate this material with a compact and precise device that can accurately perform its function. The structure of cells that react to different mechanical stimulations can change in large scales with a small change in the magnitude of these stimulations [12].

In this work, it is proposed to use an external power supply that is able to feed the amplification stage of the driver with a great voltage. This amplification stage will have the necessary electronic components that will be able to amplify an initial signal to the required amplitudes. The driver must also be able to generate that initial wave which will be afterward amplified. This signal could easily be acquired in a conventional signal generator, allowing to set up the desired parameters of the sinusoidal. Nevertheless, this work will try to generate a waveform using a simpler device, which will be much smaller in size compared to the waveform generator and it will allow for greater mobility.

The principal objective of this project will be to develop an electronic driver capable of setting a sinusoidal signal and amplify it to a very high amplitude. The final result can be implemented into a piezoelectric material, which in response will be mechanically modified. This mechanical strain will allow to place cells on top of the piezoelectric material and to study their behavior to this change.

There is a great interest in having a deeper knowledge of how mechanotransduction will impact on current diseases. Recent studies have shown that a change in the stiffness of cancer cells can actually reduce its migration considerably [13]. Other studies focus on the great impact that mechanotransduction might have on a quicker regeneration of skeletal cells [14]. This work also suggested that this effect could also be translated to epithelial cells, where mechanotransduction might be of great help for faster healing of wounds or skin-related diseases such as ulcers.

A great motivation of this project would be to be capable of using the electronic driver to study the effect of mechanotransduction on different types of cells such as cancer, skeletal or epithelial cells.

1.2 Planning and development

The classification and distribution of time are observed in Table 1. The project was divided into the two main fields of the electronic driver: software (SW) and hardware (HW). As a more graphical way of showing the organization of the work, it has also been presented a Gantt’s diagram (see Figure 1) indicating the total amount of time invested in this project for each task.

Programmed tasks	Time
Study of the state of the art	**6 weeks**
Board selection and first code	**2 weeks**
Circuit selection and PCB design	**6 weeks**
Final code and parameter modifications	**3 weeks**
PCB welding and checking process	**7 weeks**
Thesis writing	**4 weeks**

Table 1. Programmed tasks and time spent

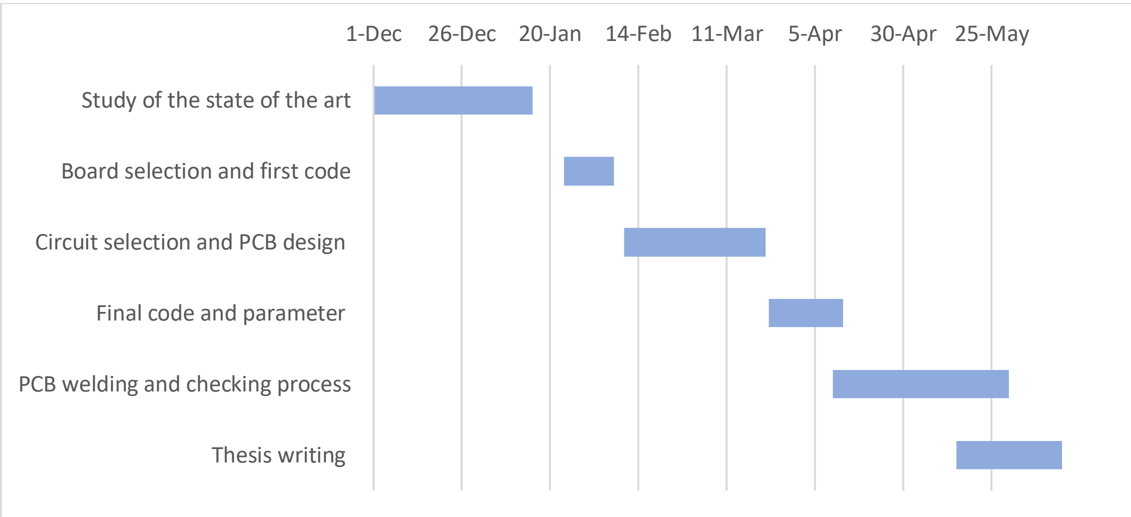


Figure 1. Gantt's diagram of the project

1.3 Document's structure

The electronic driver developed for this project consisted on three clearly divided stages: a first stage where the initial signal is created (corresponding to the software of the device), a second stage where a PCB is designed and built (which constitutes the hardware) and a final stage where the piezoelectric material receives the amplified signal. To easily follow this work, all the subsequent sections will be divided into these three stages. A graphic schematic of these three phases can be observed in Figure 2.

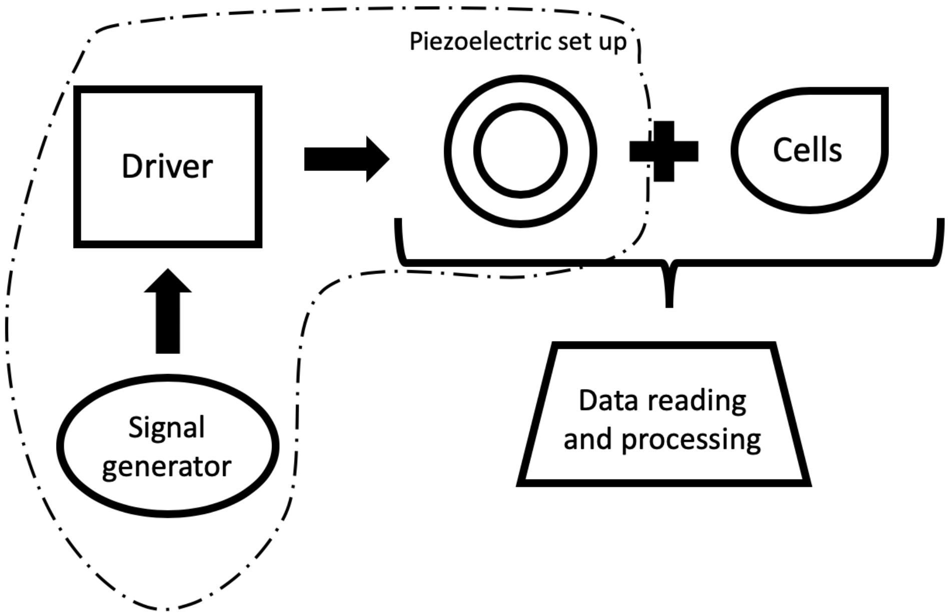


Figure 2. Graphic schematic of the project

1.4 Regulatory framework

This project aims to create a device dealing with high voltages, which can be very hazardous for users if they don't use it correctly. Nevertheless, they must be informed of the risks this device has. Article 51 in the Spanish constitution refers to the protections which must be given to users in the relationship between customers and companies:

"1. The public authorities must guarantee the protection of consumers and users, protecting their safety, health, and legitimate economic interests through effective procedures.

"2. The public authorities must inform and educate consumers and users, foster their organizations, and shall be concerned about those issues brought in by consumers which could affect them under the terms which the law shall establish.

Even if in this project no cells were manipulated, the device has been built to do so. Thus, there are biosafety levels ruled by the European Union which regulate this procedure under *the Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work)*. In the same way, the Biomedical Engineering Laboratories at Universidad Carlos III de Madrid (Molecular Biology and Cell Biology laboratories) also possess some rules that need to be strictly followed, as it is to wear protective equipment or to discard all waste products in appropriate bins.

As technical standards used for the project, Altium was selected as the design automation software for the creation and fabrication of the PCB. Its graphic interface makes it very easy to see how the board would look like, and even though it takes some time to learn its basics, it is a very powerful software recommended by experts in this field. For the programming of the microcontroller board, as Arduino was the selected platform, the programming language used is C++.

This work did not have to deal with ethical responsibilities as there was no need for trials on patients and no cellular components coming from humans or any other animal were used. The current work is on preclinical trials, but in future investigations, this is an issue it must be taken care of. In the same way, if the device ends up being a useful piece of equipment for the investigation, it could be considered to patent it, as there is no similar machine on the market.

1.5 Socioeconomic framework

Pressure ulcers are a great concern in the Spanish health care nowadays. The issue not only relies on the great number of people that suffer from them (more than one hundred thousand people a day) but also the great economic impact that medical attention of pressure ulcers means. This type of ulcers is caused when the skin relies on the same position for a long period of time. Thus, it usually appears on people that lie on a bed or sitting down for long periods of time and usually have mobility difficulties. Depending on the severity of the ulcer, it can be divided into four stages, from grade I to IV, being the last one the worst stage. Ulcers can take a long time to heal, and therefore it increases the cost of treatment.

A thorough study of the economic impact of pressure ulcers in Spain was made by J. Javier Soldevilla Agreda et al. [15], showing very interesting results. According to the study, the cost of treating pressure ulcers in hospitals variates significantly depending on the severity of which the skin is affected. Thus, just a grade I pressure ulcer will mean a 24 € cost to heal, whereas a grade IV can rise up to almost 7000 € to heal. This great difference in cost is greatly related to the healing time, which is superior in the more severe cases. The study also shows that the total cost of pressure ulcers in Spain is 461 million euros. From this expense, 15% comes from the necessary medical equipment to treat the wounds, a 19% goes away to the cost of nursing time and finally, almost a 45% represents the cost of extra stays in the hospital from ulcer-related issues.

Main purchases	**Price (€)**		**Quantity**	**TOTAL (€)**
Arduino DUE board	35		1	35,00
Protoboard	1,6		1	1,60
Printed circuit board	50		1	50,00
Heat sink	22,13		1	22,13
Apex PA79DK	72,7		3	218,10
Altium license (if not a student)	99,99		1	99,99
Power supply IHB200-0.12	40,66		1	40,66
Electronic components	41		1	41,00
Salary of the staff	**Salary (€/hour)** [1]		**Hours worked**	**TOTAL (€)**
Luis del Río	10		560	5600,00
Dahiana Mojena	25		70	1750,00
Pablo Acedo	55		4	220,00
Cleaning personal	5		4	20,00
Equipment used	**Price**	**Lifespan (years)** [16]	**Hours used**	**TOTAL (€)**
Oscilloscope Tektronix TDS 220 [17]	1300	10	75	1,11
ZD-915 desoldering station [18]	140	8	10	0,02
DC Power supply DF1731SB [19]	200	10	50	0,11
Facility costs				**TOTAL + 21% I.V.A (€)**
Access card for the laboratory				5,00
Light	Cost of kWh in Spain (€/kWh)	Consumption (kW)	Hours used	
Room light	0,14	0,324	500	27,44
Oscilloscope Tektronix TDS 220	0,14	0,05	75	0,64
ZD-915 desoldering station	0,14	0,13	10	0,22
DC Power supply DF1731SB	0,14	0,07	50	0,59
TOTAL				**8133,62 €**

Table 2. Budget for the execution of the bachelor thesis

[1] Salary per hour is calculated based on the average wage of an engineer in Spain (depending on its position inside the company) and a person working at a clean service company.

If more than 200 million euros are spent to treat pressure ulcer issues due to the long time they take to heal, it would be of great interest to try to reduce this healing time. By decreasing this time, the annual cost would be reduced significantly but it will also allow for patients to leave hospital rooms earlier, allowing more severe patients to occupy them.

Bearing in mind the idea of the last paragraph, it has also been calculated the budget necessary for this project to be achieved. As seen in Table 2, the total cost of this investigation would mean an investment of over 8000 euros. The difference in value between the cost of this project and the money saved if the healing time of pressure ulcers is reduced, makes this work a great opportunity that can be worth it.

1.6 State of the art

1.6.1 Software

1.6.1.1 Microcontroller board platforms

Microcontroller boards are found in many electronic devices nowadays. It consists of a printed circuit board (PCB) with circuitry and hardware that is designed to facilitate experimentation with several features. These boards are also designed with a processor, memory and a chipset, and might also include LCD screens, USB port, serial port, Ethernets and others. The idea of microcontroller boards has allowed the world of electronics to expand into new fields, giving place to new gadgets in the last few years such as drones [20].

These boards are also very useful for studying purposes in engineering. As it is not necessary to have very deep knowledge in programming to create a simple code, these boards allow for engineering students to use them in laboratories. The boards allow the acquisition of signals to be analyzed (behaving as an oscilloscope), to generate output signals and many other functions. Related with biomedical engineering, a microcontroller board also can allow creating a device capable of monitoring heart rate and body temperature [21].

During the last years, many different platforms have decided to create their own microcontroller board, and each of them presents their own features as well as some drawbacks. In the same way, some development platforms are better than others depending on the certain project they want to be used for.

As a first example, there are DIY (do it yourself) microcontroller boards. As the actual name indicates, these types of boards are made by the own user and can adapt to the specific function they are being built for. It is necessary to buy the different electronic components, as well as the actual microcontroller and some external peripherals [22].

Then it is Arduino. It is one of the most popular open-source electronic prototyping platforms. It was also one of the first microcontroller-based development platforms. What makes Arduino the first choice when choosing a microcontroller board is mainly the characteristic of being an open source IDE (integrated development environment) to develop sketches, which has a simple syntax based on C++ language, and it is easy to learn its code. Arduino already possesses many different types of boards, but the most popular one is the Arduino UNO, which makes it very familiar for both a beginner and an expert. The main characteristics of this board are [23]:

- Microcontroller: ATmega328P
- 32 KB of Flash memory
- Operating Voltage: 5V
- Input Voltage (recommended): 7-12V
- Input Voltage (limits): 6-20V
- Digital I/O Pins: 14 (6 pins provide PWM output)
- Analog Input Pins: 6
- DC Current per I/O Pin: 40 mA
- DC Current for 3.3V Pin: 50 mA.

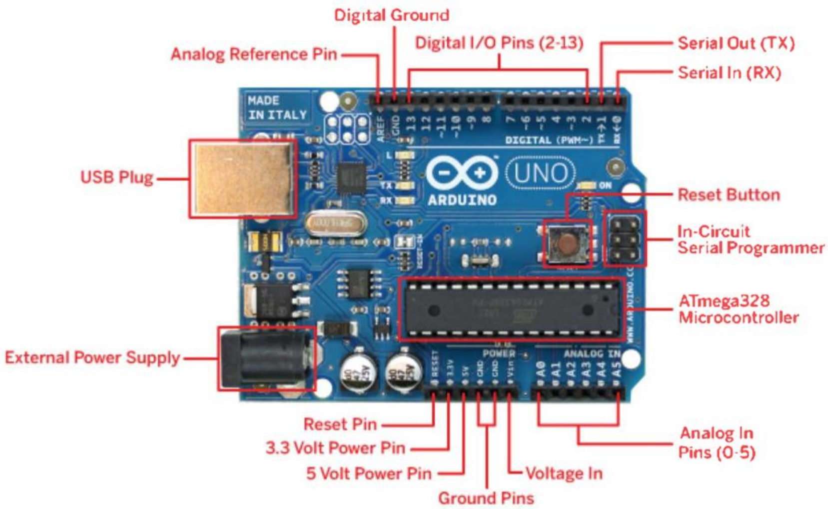


Figure 3. Arduino UNO [24]

Another microcontroller board platform that is very popular among the electronic community is the Raspberry Pi. It is much smaller in size than Arduino and it allows for many of the Arduino application, and its size allows it to be plugged into a computer or configure a home security control [25]. Raspberry Pi 3 is the most powerful and affordable of the microcontroller boards for this platform, whose last version included all the following characteristics [23]:

- Processor: 1.2GHz, 64-bit quad-core ARMv8 CPU
- 802.11n Wireless LAN
- Bluetooth 4.1
- Bluetooth Low Energy (BLE)
- 1GB RAM
- 4 USB ports
- 40 GPIO pins
- Full HDMI port

Figure 4. Raspberry Pi [25]

1.6.1.2 Generation of signals using microcontroller boards

It can be very useful to be able to generate signals from the own microcontroller board. Usually, these types of boards possess digital pins that can be configured to be output or input pins. If the first case is chosen, these pins will only be able to generate a HIGH and a LOW voltage (usually 5 volts for HIGH and 0 volts for LOW) [26].

This project, however, is looking for a microcontroller capable of generating an electrical analog signal. In an analog signal, its voltage value is constantly changing with time and it can take any value between a certain range. A control system (such as a microcontroller board) is not able to work with analog signals, so it needs to convert this signal into a digital one in order to work with it. These boards are not capable of generating a pure analog signal from its pins either, so to fix this problem they use the PWM technique. Pulse Width Modulator (PWM) is a technique that allows getting analog results from digital means. This method allows simulating voltages in between the full power (usually 5 volts) and zero volts by changing the time the signal spends on versus the time that the signal spends off. To get different voltages, the pulse width (time of the signal is spent on) can be varied. In Figure 5 it can be observed different variations of the pulse width, which will in return give as an output different analog signals in amplitude. The frequency for all of them is set to 500 Hz, but this parameter may also be modified if needed.

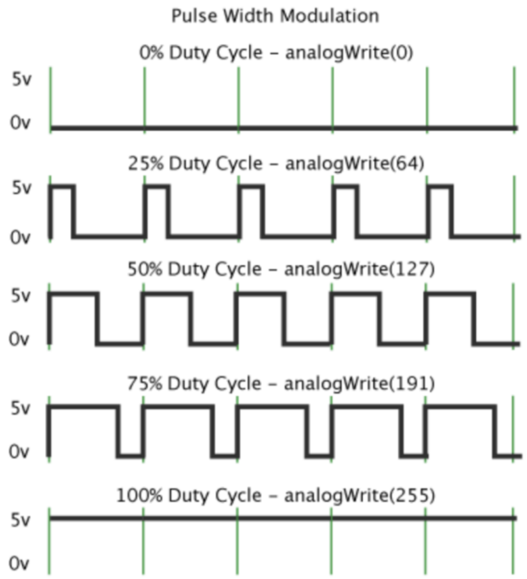


Figure 5. Pulse Width Modulation (PWM) technique [27]

The PWM has been the most common technique used when trying to generate an analog signal from a microcontroller board, but this technique possesses some drawbacks. If a microcontroller board was to be programmed to generate an analog signal, it will only be able to generate a signal with a specific frequency, not allowing to vary it unless the code was to be reprogrammed again. Also, the resolution of a signal generated with the PWM method is not very good, and if it was to be amplified, the output would show that is not a pure analog signal.

Luckily, new types of microcontroller boards are coming out to the market every year. In 2012 a new type of Arduino board was released. What made this microcontroller board so special was the addition of two new Digital-to-Analog Converter (DAC) pins. These two pins were able to generate an analog signal without the need of using the PWM technique. This board seemed to be very appropriate for the project as it provides the main requirement that a microcontroller board must have.

However, Arduino DUE is also a great microcontroller board for other characteristics that it has [28]:

- Arduino DUE's pins go from 0 to 53, which can be used as digital input or output. Some of these pins have specific functions:
  - Pins 2 to 13 are output PWM (Pulse Width Modulation) pins, with a resolution of 8 bits. This type of output pins uses a technique for getting analog results with digital means. However, as this board possesses pins specifically thought to generate analog signals, this technique is not used.
  - Input analog pins from A0 to A11.
  - **DAC0 and DAC1**. These two pins supply an analog output signal with a resolution up to 12 bits (4096 levels), as the resolution can be modified with the function *analogWriteResolution(bits).*
- It is a microcontroller of 32 bits, which already improves the capacities of previous models like Arduino UNO (8 bits).
- The resolution of both input and output analog signals is 12 bits, as well as a sampling rate of 1000 ksps (kilo samples per second), much higher than the Arduino UNO (15 ksps).
- The input/output pins work at a voltage of 3.3 V.
- It possesses two micro-USB ports: *"Programming"* and *"Native"*. The first one is thought to be used for programming and communication, while the *"Native"* port is used to connect external USB peripherical, such as a mouse or a keyboard.

Some projects have already tried to use Arduino DUE for the generation of analog signals. As an example, a project shows how as initial parameters all it is necessary to set is the number of samples of the desired signal and the frequency of the sinusoidal. Given these two parameters, the final vector follows the shape of a sinusoidal signal very accurately as it can be seen in Figure 6. It can be observed that the sinusoidal has only positive values, as Arduino has voltage values from 0 to 3.3 volts. These types of examples can be useful when creating the Arduino code for the generation of sinusoidal signals.

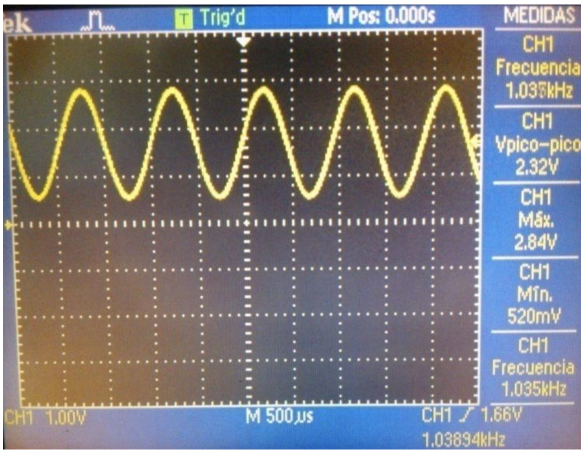


Figure 6. Obtained signal for a 1 kHz frequency [28]

1.6.2 Hardware

1.6.2.1 Signal amplification

An amplification stage is necessary before a piezoelectric actuator is electrically stimulated. As the main drawback of this type of material is the high power it needs in order to have a mechanical strain. To obtain such an amplification from an initial signal, two main components are needed: a power source and an amplifier.

A power source is a device capable of delivering a specific power to an electric load. Usually, this device obtains an initial electric power from the power mains and converts it into the correct voltage, current and frequency to power the load. Power supplies may be separate pieces of equipment or they can be integrated into the load they power. There are many types of power supplies depending on what they are needed for. In conventional electronic laboratories, they usually possess programmable power supplies. This type of power source allows remote control of its output through an analog input or a digital interface. It is very useful for short laboratory practices, but its big size doesn’t allow it to be the first choice when building a device where just a fixed power source is needed. If all is needed in an amplification stage is to have a fixed DC voltage, the best approach nowadays would be to choose an AC to DC power source.

DC power sources use AC mains electricity as their source of energy, and it takes a few steps to convert from one current to the other. First of all, the AC voltage is converted to a higher or lower voltage using a transformer. Afterwards, a rectifier is able to transform AC into a varying DC voltage, which then passes through an electronic filter to finally set it to an unregulated DC voltage (see Figure 7).

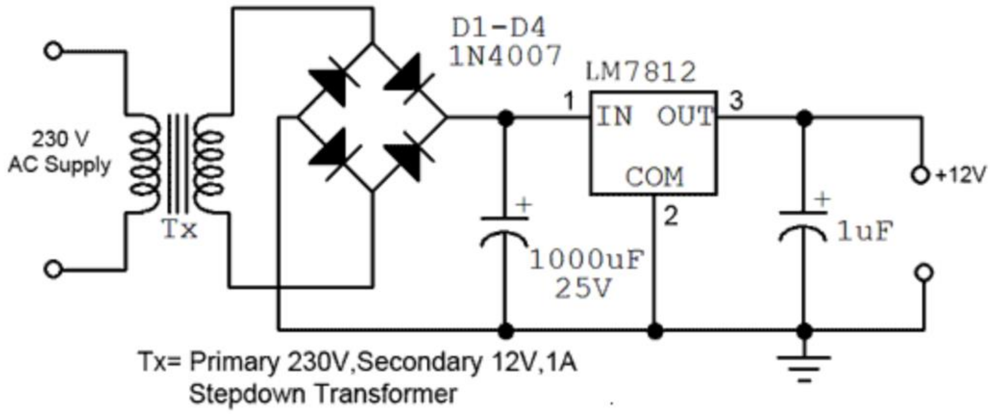


Figure 7. Conversion of 230V AC to 12V DC [29]

The other main component in an amplification stage is the amplifier chosen for the electronic circuit. This device is able to increase the amplitude of a signal using the energy coming from a power supply. The magnitude of this amplification is given by the gain, which is the ratio in amplitude between the input and the output signal.

However, the power amplifiers needed to electrically stimulate a piezoelectric material have to be very efficient to deliver the necessary signal at the same time as dealing with a very high voltage source. The issue coming with this high power is the heat concentrating on the amplifier, which was an issue it had to be dealt with since piezoelectric material started to be used [30].

More recent investigations regarding the electric stimulation of piezoelectric actuators have been able to reduce heat dissipation considerably of both the amplifier and the piezoelectric actuator [31]. These advances allow finding better amplifiers for the objective of this project.

1.6.3 Piezoelectric actuators

Even though the project is not focused on the study and selection of piezoelectric materials, the electronic driver is being made to stimulate such a material, so it is very important to understand its characteristics and how it works.

The phenomenon of piezoelectricity describes the interrelationship between mechanical strain and its electrical performance. As a reversible process, it is possible to distinguish between the direct and the reverse piezoelectric effect. The direct electric effect occurs when the material transforms a mechanical change into an electrical signal. On the other hand, when dealing with a reverse piezoelectric effect, the material will transform electrical into mechanical energy. In this second effect, if the applied voltage variates with time, the mechanical changes produced will oscillate with the same frequency as the electrical signal applied.

These two effects that piezoelectric actuators posses can be put into a mathematical equation when considered as directions: an electric field in one direction will be leading to a mechanical change in any direction. Thus, these equations are expressed using tensors, but to avoid a complex expression, the reaction produced in the actuator will only be able to follow a parallel or a perpendicular direction. By taking these facts into consideration, the direct and reverse piezoelectric effect can be expressed in equation (1) and (2) respectively [32].

$$\vec{D} = d_1\vec{T} + \epsilon^T\vec{E} \quad (1)$$

$$\vec{S} = s^E\,\vec{T} + d_2\vec{E} \quad (2)$$

$\vec{D}$: electric displacement

d_1 and d_2: piezoelectric charge coefficients, respectively for the direct piezoelectric effect and the converse piezoelectric effect

$\vec{T}$: mechanical stress

ϵ^T: permittivity at constant stress

$\vec{E}$: electric field

$\vec{S}$: mechanical strain

s^E: mechanical compliance

Equation (2) shows the effect of interest in this project. As depicted in Figure 8, when a piezoelectric actuator is subdued to a voltage applied longitudinally, there is a displacement (ΔL) of the material under the same axis. Most of the piezoelectric materials are able to withstand a change in length of 0.1%. This change in length can actually be approximated from equation (2), and it will only depend on the different characteristics of the material and the forces applied, as shown in equation (3).

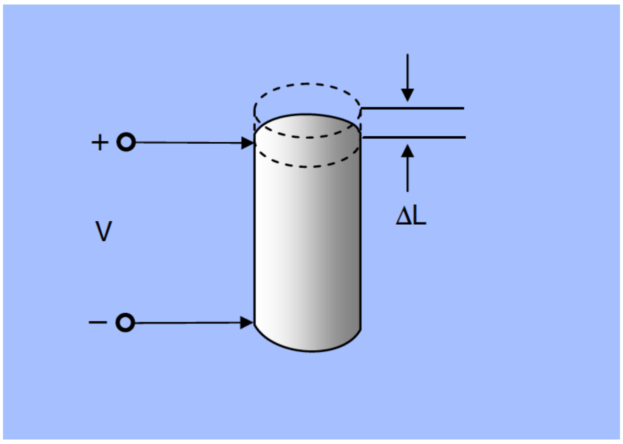


Figure 8. Linear piezoelectric actuator [33]

$$\Delta L = SL_o \approx E d_{xx} L_0 \quad (3)$$

Equation (3) shows how the displacement of an actuator depends on the electric field strength (E), the initial length of the actuator (L_0) and a piezoelectric coefficient (d_{xx}) whose first sub-index indicates the axis of the electric field and the second one indicates the axis of the displacement [33].

The piezoelectric material used for this project is PVDF. From previous investigations to this work, it was studied the mechanical displacement produced on the PVDF film at different frequencies. From Figure 9 it was observed that the maximum displacement of the piezoelectric material occurred at low frequencies. Special attention was given to a displacement peak produced at a frequency of around 80 Hz, after which the displacement lowered down considerably until a new peak appeared at 3 kHz approximately. The reason behind the 80 kHz peak is that this is the resonance frequency of PVDF, therefore producing a greater strain in the material than at any other frequency.

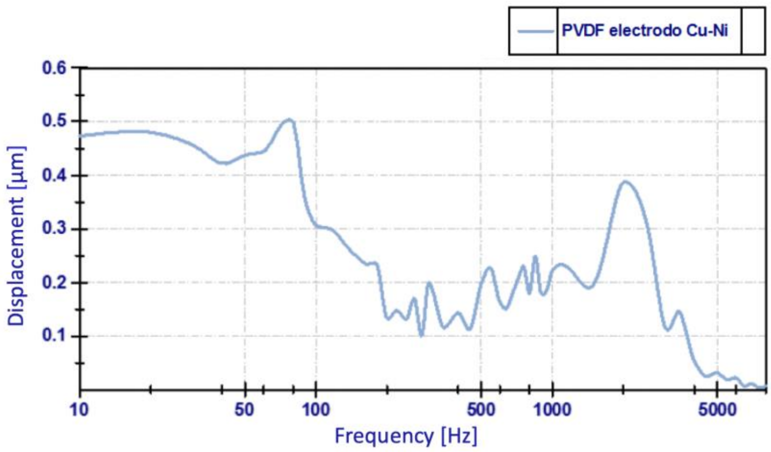


Figure 9. PVDF frequency response analysis [9]

In the same way, it was studied how the piezoelectric material changed to variations in the amplitude of the sinusoidal. Figure 10 shows how an increment in the electric tension will produce a higher displacement in the PVDF material. The displacement is directly proportional to the value of the voltage.

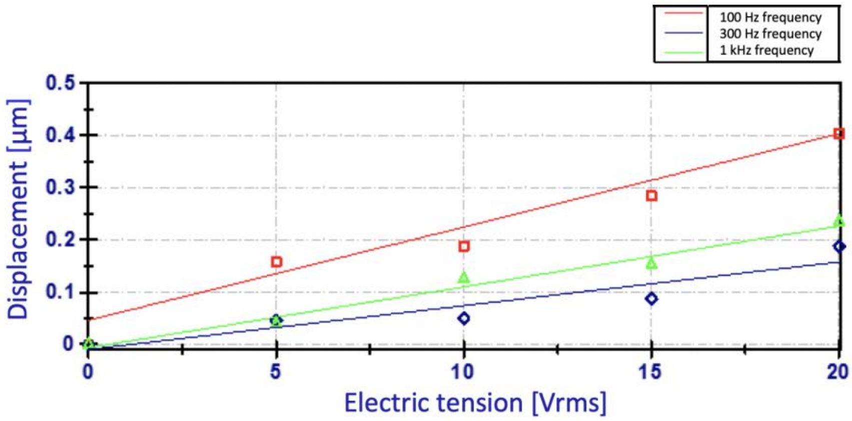


Figure 10. PVDF electric tension analysis [9]

As already mentioned, cells are not only stimulated due to biochemical reactions but can also have significant changes if mechanical forces are applied to them [3, 4, 5, 6]. In this area was the study done by previous investigations related to this project [10]. The response of cells was studied for two specific frequencies: 1 Hz and 80 Hz. The 1 Hz frequency seemed to be a good choice as it produces a considerable displacement on the PVDF film. Moreover, this frequency is also in the order of frequency of many natural physiological processes such as heart rate or breath. The 80 Hz frequency is the resonance frequency of PVDF, was also studied to see if a greater displacement of the piezoelectric material was an advantage or disadvantage for the healing process of cells. The results (see Figure 11) showed that the 1 Hz frequency had a higher healing rate than the 80 Hz stimulation rate.

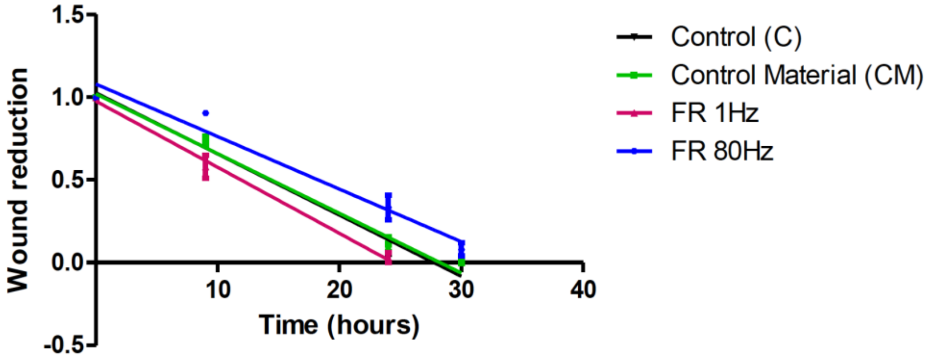


Figure 11. Area decrease in scratch assay due to the migration process [10]

From these previous studies urged the necessity of developing an electronic driver that lets analyze cell response in a broader amount of frequencies and amplitudes, and also allowed to have this device right next to the culture while it is growing.

2. Materials and methods

2.1 Software

2.1.1 Arduino DUE

In order to generate the initial sinusoidal wave that will later be amplified, it was necessary to choose a microcontroller capable of completing this task. There are many microcontroller board brands in the market, one of the most popular being Arduino.

Arduino is made of both hardware and software, based on a microcontroller with both input and output signals. This platform has also the advantage of being an open system, meaning it can be used without having to purchase any license. Another benefit of Arduino is that it can be used in either Windows, Mac OS or Linux. All these benefits added to the fact of already knowing the software that Arduino uses, as well as that it has several great boards currently in the market. Thus, it was very easy to think Arduino was the best choice for the microcontroller board.

It then came to the decision of choosing between the wide selection of boards that Arduino has. The main characteristic needed was to be able to generate as an output an analog signal, as well as to be able to set the main characteristics of this sinusoidal wave. It was discovered that Arduino had recently put out into the market a new board: Arduino DUE. The main characteristic of these boards was that it possesses two analog outputs. This new addition was not in the other Arduino boards and made it the best option for this project.

Figure 12. Arduino DUE

2.1.2 Signal creation

After the microcontroller was finally chosen, it was time to start creating a piece of code capable of producing a signal, just to check that it was possible to obtain it. To do so, a sinusoidal of 1 kHz in frequency and 2.24 $V_{pk\text{-}pk}$ in amplitude was created [28]. This signal is sent as an output through the DAC0 pin, whose output can be checked in Figure 13.

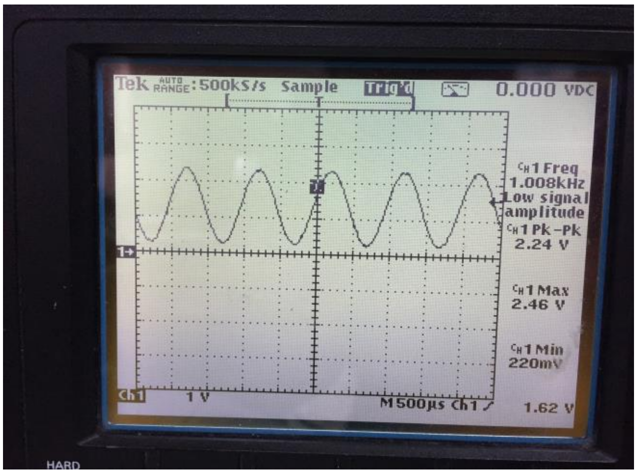


Figure 13. 1 kHz sinusoidal signal obtained from DAC0 pin of Arduino DUE

The next step consisted of checking that this signal was capable of being amplified. To do so, a small non-inverting circuit with a gain of 2.5 V/V was created in a protoboard. The signal exiting the DAC0 output pin was set to the input signal of this simple circuit, and its output was checked using an oscilloscope. The results can be seen in Figure 14. It can be observed that the signal still has the same frequency and the voltage has been amplified correctly, obtaining a final value of 5.6 $V_{pk\text{-}pk}$.

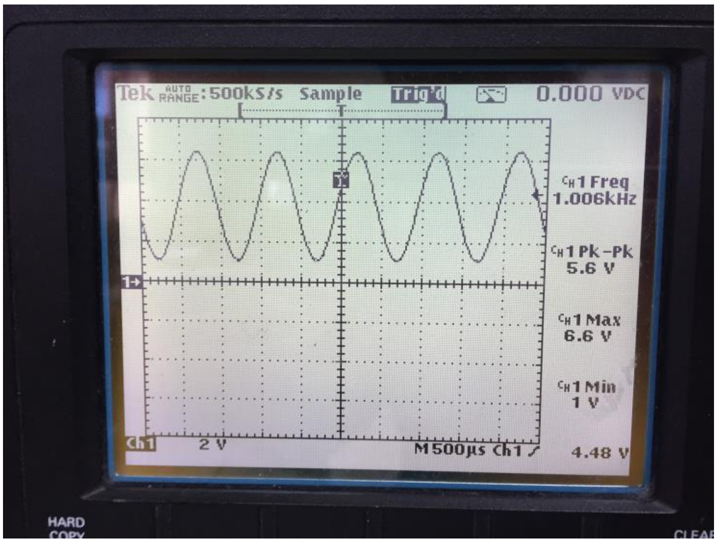


Figure 14. Amplified signal using a non-inverting amplifier

$$Non-inverting\ amplifier\ gain\colon\ G = 1 + \frac{R_1}{R_2} = 1 + \frac{1.5\ k\Omega}{1\ \text{k}\Omega} = 2.5\ \frac{V}{V}$$

$$V_{out} = V_{in} * G = 2.24\ V\ (2.5\ \frac{V}{V}) \quad \rightarrow \quad V_{out} = 5.6\ V\ (as\ expected)$$

Once it was already checked that is was possible to amplify a signal created by Arduino DUE, the code needed to be improved in order to allow the user to choose between a variety of signals. To do so, the idea was to create a small circuit with some buttons and potentiometers that allowed to change between types of waves and also the frequency of interest. As an initial objective, there was also interest in being able to change the voltage amplitude of the initial wave. However, as Arduino DUE has a very narrow voltage range (0 to 3.3 volts) and the signal was already going to be amplified to a great value during the next stage, this initial objective was disregarded.

As an open system, Arduino has the great advantage of having multiple codes that can be used by the user for their own projects. This is the case of a tutorial found on Arduino's website, consisting of a waveform generator with Arduino DUE which allowed to change the waveform thanks to a pushbutton and also variate its frequency by using a potentiometer [34]. As seen in Figure 15, the initial idea of this project was to have two output signals, both for DAC0 and DAC1. Nevertheless, for the sake of our project just one output signal was enough. The code was also modified for the sake of the project's interest.

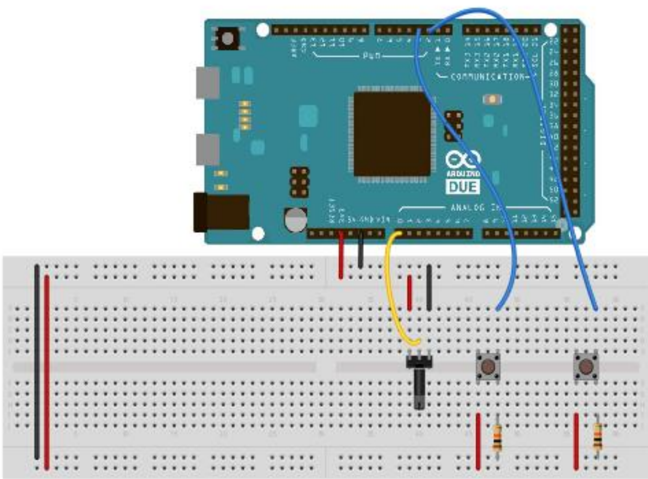


Figure 15. Illustrative schematic circuit for the waveform generator

The components needed for the waveform generator to work were:

- Arduino DUE
- 10 kΩ potentiometer
- 1 push button
- One 10 kΩ resistor
- Jumper wires

The push button allows changing between different types of waveforms: sinusoidal, triangular, sawtooth and square wave. Every time the push button is pressed, the voltage read by a digital pin on the board observes a rising edge momentarily, which as a consequence will rise a counter, changing the waveform type.

The potentiometer middle pin is connected to an analog input on the Arduino DUE, while the other two sides are connected one to power and the other to ground. This technique allows that every time the potentiometer value is changed, the sample rate variates and so does the frequency. The time needed for instructions to be read as well as the time needed for the analog input allows to have a variation in frequency from 1 Hz to somewhere over 500 Hz.

2.2 Hardware

2.2.1 Bridge-connected driver circuit

A piezoelectric material needs of a high sinusoidal wave in amplitude to produce a displacement of the material. In order to obtain such a high wave, a bridge-connected driver circuit is used.

This circuit is composed of two operational amplifiers, and its basic concept and advantage are that it is capable of providing an output voltage swing double in value of just one operational amplifier, as seen in equation (4). The most common diagram showing a bridge connection is shown in Figure 16. A1 is set to be the master amplifier, whereas A2 is the slave one. The total gain across the load will be twice the gain set for the master amplifier. To connect both amplifiers the output of the master op-amp will be driven into the slave one by a unity gain inverting configuration. Thanks to this type of circuit the wave's amplitude can be increased to the necessary values for the piezoelectric circuit to be stressed.

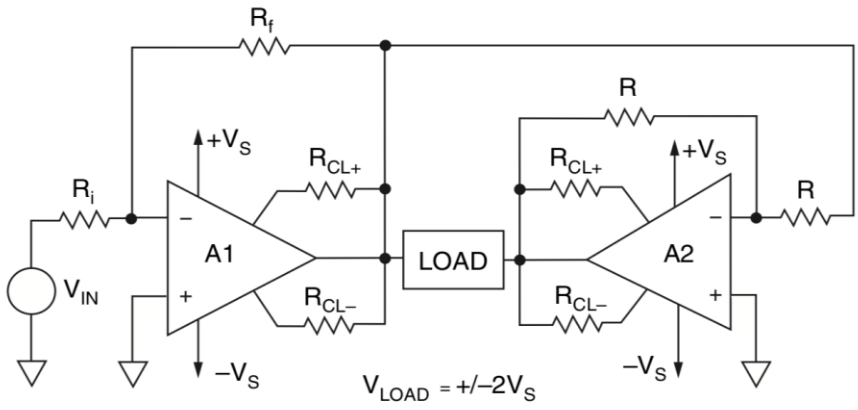


Figure 16. Schematic of a common bridge-connected circuit [34]

$$G_{A1} = -\frac{R_f}{R_i}\left[\frac{V}{V}\right] \qquad ; \qquad G_{A2} = -\frac{R}{R} = -1\left[\frac{V}{V}\right]$$

$$V_{out_{A2}} = V_{out_{A1}} * G_{A2} = -V_{out_{A1}}$$

$$V_{LOAD} = V_{out_{A1}} - V_{out_{A2}} = V_{out_{A1}} - (-V_{out_{A1}}) = 2\,(V_{IN} * G_{A1}) \qquad (4)$$

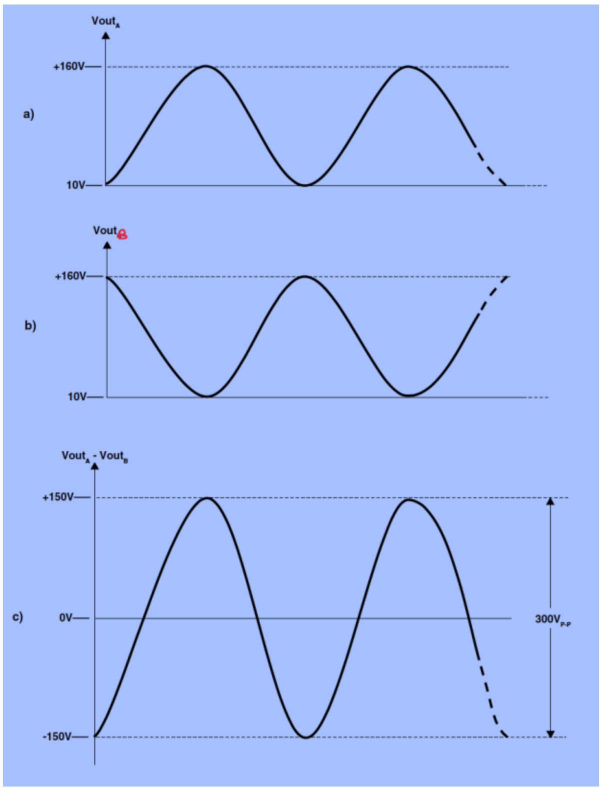


Figure 17. Output of the bridge-connected driver circuit [32]

2.2.2 Operational amplifier: PA79

The selection of the operational amplifier for the circuit was the next step. An op-amp capable of dealing with a very high voltage was the main requirement. However, the market nowadays does not deal with many operational amplifiers which show this characteristic, so the selection was narrowed down to just two of them: LTC6090 or PA79. One main drawback of LTC6090 was its maximum input voltage, which was ± 70 V. In comparison with PA79 (±175 V) this op-amp possessed a lower maximum input voltage. Even though this voltage could be high enough for the piezoelectric actuator, it would be necessary a new transformer with that amplitude, or an integrator that would lower down the voltage supplied by the linear power supply IHB200-0.12 which was already acquired. The second issue regarding LTC6090 was that two op-amps of this type were necessary, in comparison to just one PA79 (two PA78 op amps are included inside). For these reasons, PA79 was the operational amplifier chosen for the driver.

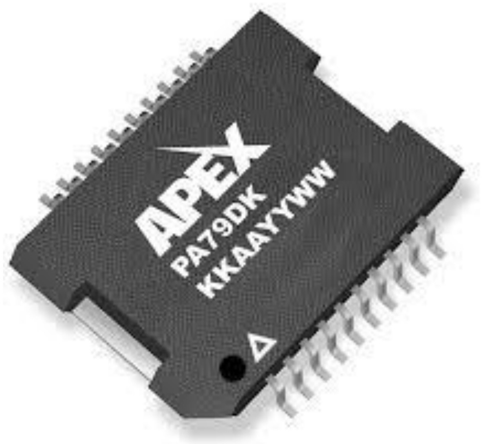


Figure 18. PA79 [35]

The own manufacturer of PA79 (Apex), explains how this op-amp is specially focused on piezoelectric positioning and actuation, among other applications [36]. For this reason, it is already proposed a bridge-connected driver circuit using this op amp (see Figure 19).

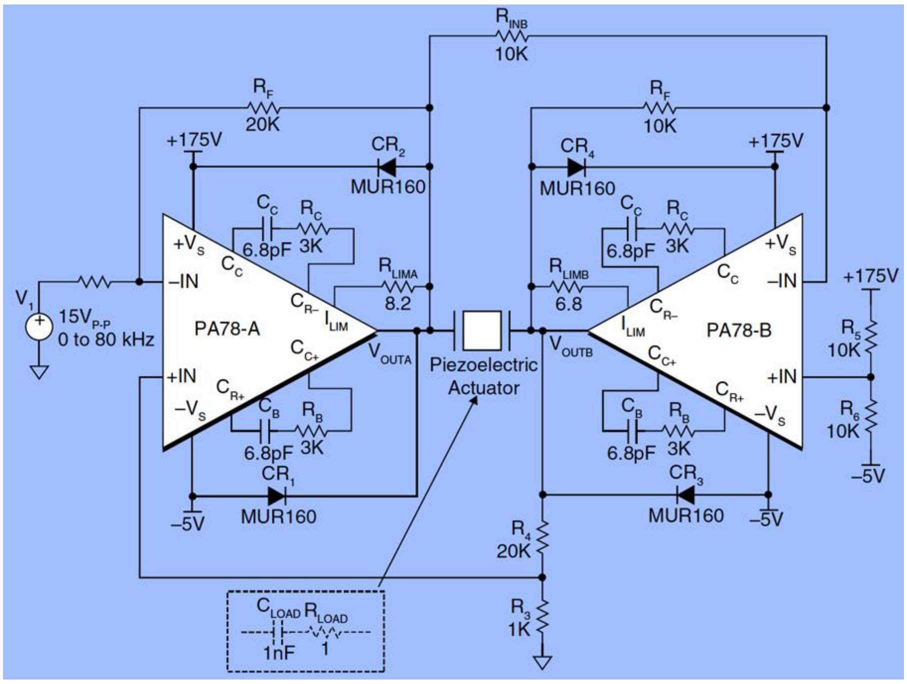


Figure 19. Bridge-connected driver circuit for PA79 [33]

2.2.3 Altium Designer®

In order to make the PCB, it was necessary to choose the software where the buildup was going to take place.

There is some decent open software such as KiCad. This software was downloaded to check it and observe if it had all the requirements to build the necessary PCB. Some issues encountered were that, as KiCad is open software, its library is very limited, and it did not have many of the components needed to build the board. Another issue observed was that it was very hard to find tutorials to understand how the software worked, which would have made the task to slow down considerably.

Once it was clear open software had many disadvantages when trying to build a good PCB, the focus was to choose between the best ones on the market. The main problem with working with this type of software is that the best ones are usually not free. That is the case of Altium Designer, consider by many experts the best PCB and electronic design automation software. However, Altium's drawback relies on its price, which can be very expensive depending on the package. Regardless of this fact, Altium is usually the software used in most companies where electronic boards need to be designed. It is also considerably easy to use after a few days studying video tutorials. The decision was finally taken when it was discovered there was a free-month trial for engineering students, allowing to build the PCB during that time.

Altium Designer is composed of four main functional areas: schematic capture, 3D PCB design, Field-programmable gate array (FPGA) development and release/data management. The interest of the PCB build-up only relied on the first two areas: schematic and PCB design.

2.2.3.1 Schematic capture

In order to build the PCB, it is first necessary to draw a schematic of the circuit that wants to be implemented on the board. As already explained, the desired circuit consists of a bridge-connected driver, which is shown in Figure 19. However, there are previous steps that need to be taken until the final result is acquired.

As previously mentioned, this type of software needs of multiple libraries in order to obtain the necessary characteristics for the components that the circuit requires. This step is essential if a simulation wants to be performed in Altium, showing the hypothetic output obtained. Thus, a few libraries had to be downloaded, as some of the components were not found in the libraries already provided by Altium Designer.

Analyzing the circuit proposed for the driver, there are several parameters that were necessarily taken into account. One of those characteristics is the placement of four diodes that don't allow any voltage to travel backwards into the output of the amplifier. The reason for these diodes is related to the function of the piezoelectric materials: the same way as this material can produce mechanical from electrical energy, the inverse situation can also happen. Thus, if by any means the piezoelectric material is mechanically stressed, this transducer can produce a lot of energy that could travel into the operational amplifiers. This situation can be easily avoided by the placement of these diodes.

Regarding now the input voltage used for PA79, the chosen values were +175V for the positive input and -5V for the negative one. These values allow producing a sinusoidal wave of 360 Vpk-pk, amplitude more than enough to produce a mechanical displacement in the piezoelectric actuator.

It was also observed how the manufacturer of PA79 suggests using the possible maximum current limit resistor R_{lim}. However, the initial circuit was proposed using a current limit resistors of 50 Ω. By observing Figure 20, provided also by the manufacturer, the decision of using a higher resistor value of 100 Ω was made. By doing so the current limit is decreased, and it is also followed the recommendation of using the possible maximum value for the resistor.

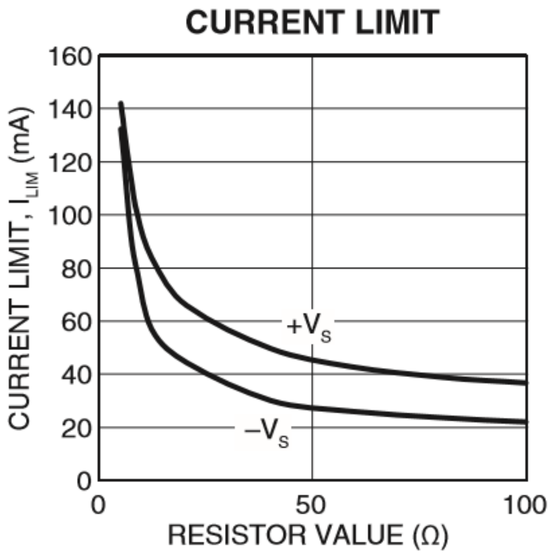


Figure 20. Current limit of PA79 [36]

Regarding the compensation capacitors (C_C), they directly affect the open loop gain and phase performance as it is observed in Figure 21. Following these performances and the parameters suggested for the compensation capacitors, its value is changed from 3.3 pF to 4.7 pF. The reason to do so is to obtain a closer behavior to the curves observed in the graphs for a value of 5 pF. There were also no specific reasons to have chosen the value initially proposed by the manufacturer, so this value could be changed without worrying about any drawbacks.

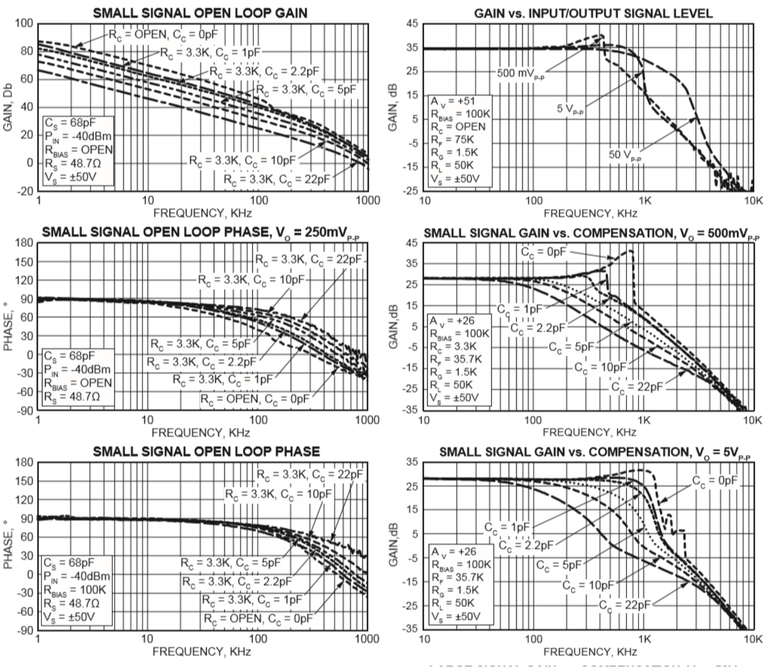


Figure 21. Small signal characteristics of PA79 [36]

After all these parameters and components were thoroughly studied and modified in some cases, the schematic of the circuit was created using Altium (see Figure 22).

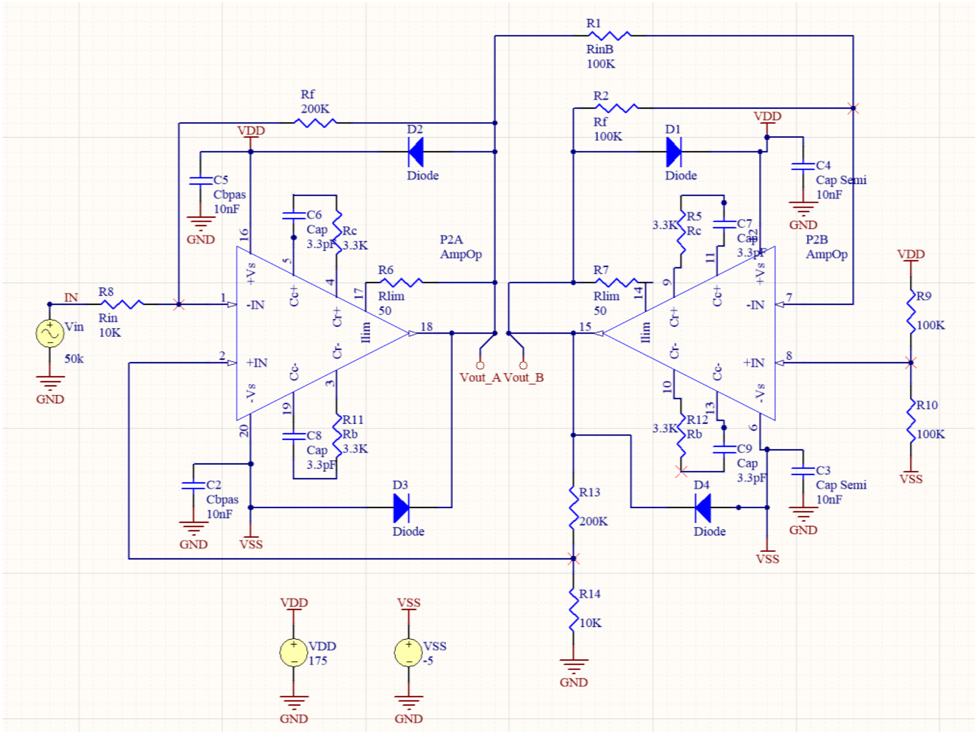


Figure 22. Schematic of the circuit

The gain of the amplification stage in this circuit can also be calculated:

$$G_{A1} = -\frac{R_f}{R_{in}} = \frac{200\ k\Omega}{10\ k\Omega} = 20 \left[\frac{V}{V}\right] \quad ; \quad G_{A2} = -\frac{R_2}{R_1} = -\frac{100\ k\Omega}{100\ k\Omega} = -1 \left[\frac{V}{V}\right]$$

$$V_{out_{A2}} = V_{out_{A1}} * G_{A2} = -V_{out_{A1}}$$

$$V_{LOAD} = V_{out_{A1}} - V_{out_{A2}} = V_{out_{A1}} - (-V_{out_{A1}}) = 2\ (V_{IN} * G_{A1}) = 40 * V_{IN} \qquad (5)$$

This circuit allows having a voltage increase of 40 times the initial signal. As observed in equation (5), the gain of this amplification completely relies on the value of R_f (if R_{in} remains constant), as it sets the value for the gain of the first amplifier A_1.

In order to better understand the behavior of the circuit and check that the principal connections are properly placed, Altium allows running simulations of the schematic circuit. To do so, several points of interest need to be set: an initial signal has to be created at the input of the circuit (V_{in}), both power supplies need to be set (V_{DD} and V_{SS}) and the output points (V_{out_A} and V_{out_B}) where the signal will be evaluated. However, all components in the circuit also need to have the necessary information for the simulation to work. An error appeared when trying to run the simulation of the circuit, stating that there was no simulation information for PA79. This problem comes from the manufacturer of PA79, which only uploaded the schematic of the amplifier but not its simulation function.

Nevertheless, a simpler simulation wanted to be performed to check out that the theory of a bridge-connected circuit did work on practice. As it can be seen in Figure 23, a bridge-connected circuit is designed following the same connections as in the final circuit shown in Figure 22. In this case, it is established a gain of 25 V/V for the master operational amplifier. The input signal has an amplitude of 2 V, which will be amplified, obtaining two final signals: V_{out_A} and V_{out_B}. The final idea of this circuit configuration is to subtract both output signals, obtaining the desired output signal as shown in Figure 24. This signal will be twice in amplitude of the ones obtained using just one operational amplifier.

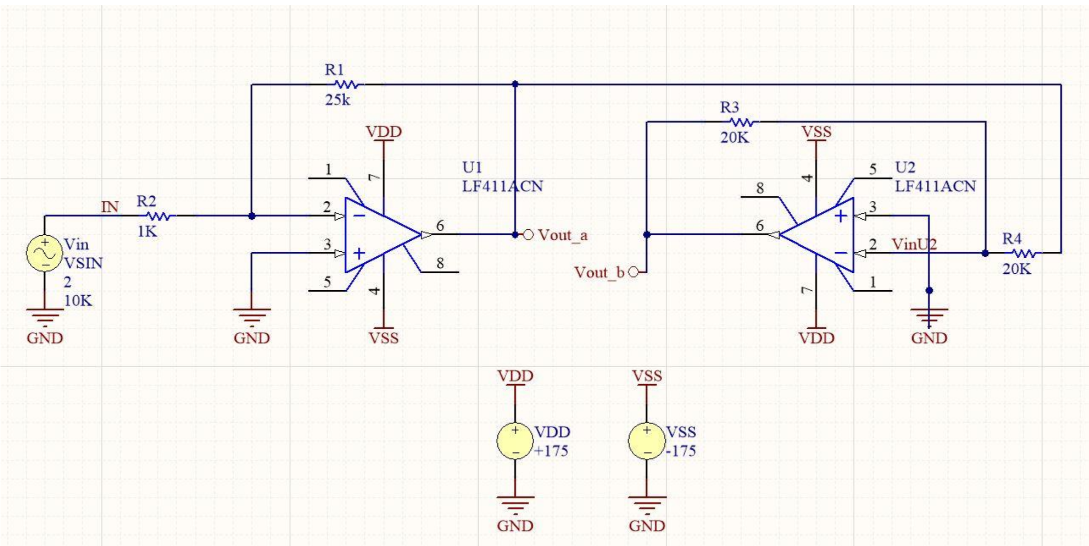


Figure 23. Bridge-connected circuit using Altium

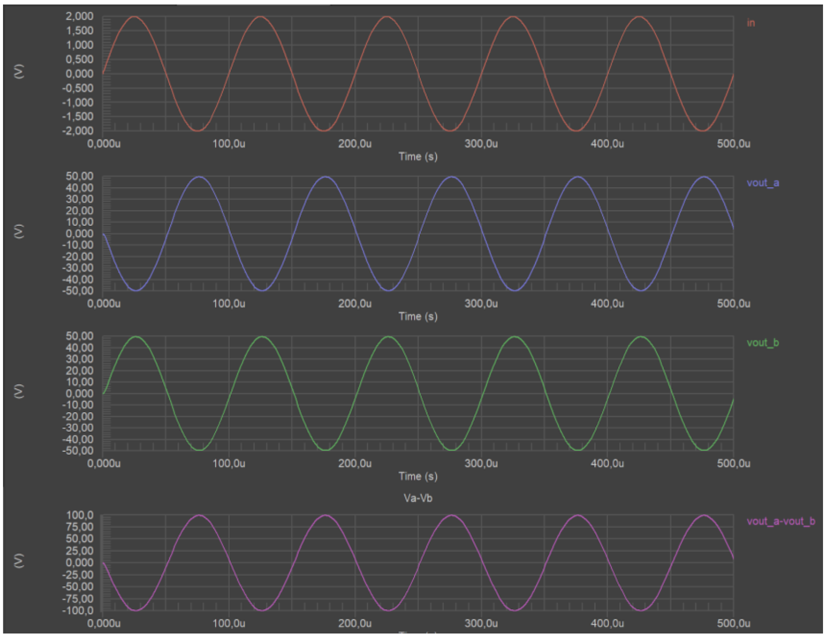


Figure 24. Simulation of the bridge-connected circuit done with Altium

In Figure 24 four different sinusoidal signals can be observed. The red sinusoidal refers to V_{in}, which only has an amplitude of 2 volts. Both blue and green signals correspond to V_{out_A} and V_{out_B} respectively, and as it can be observed, they have been amplified to 50 volts (gain of 25 V/V) and they are inverse functions. Finally, the purple signal corresponds to the operation: V_{out_A} - V_{out_B}. This operation allows having a sinusoidal of up to 100 volts in amplitude.

Once it was checked the configuration of the bridge-connected driver circuit worked perfectly, it was time to reproduce the schematic of the circuit shown in Figure 22 in an actual PCB.

2.2.4 PCB design

For this step, some video tutorials were also necessary to understand how Altium Designer worked when building a PCB for fabrication. The software allows translating the physical surface that each component occupies from the schematic window to the PCB design one. However, all the necessary tracks to connect the whole circuit is performed by the user.

The idea of how a PCB works relies on the definition of many tracks that connect the whole circuit together. These tracks are independent of one another and they are made of fiberglass and copper. The idea of a PCB is also supported by the concept of multiple layers. For the purpose of this board, two layers are enough: a top and a bottom layer. Thus, if two tracks (e.g. Track 1 and 2) of the circuit were to cross each other on the top layer, Track 2 will be set to go to the bottom using a small hole covered also with fiberglass and copper, to then go underneath Track 1 on the bottom layer. Also, the components that were welded afterwards are surface-mount technology, so all of them needed to be mounted on the top layer. Thus, all tracks need to finish at the top.

Figure 25 shows the different tracks and surface-mount components that were created in Altium Design in order for the circuit to work.

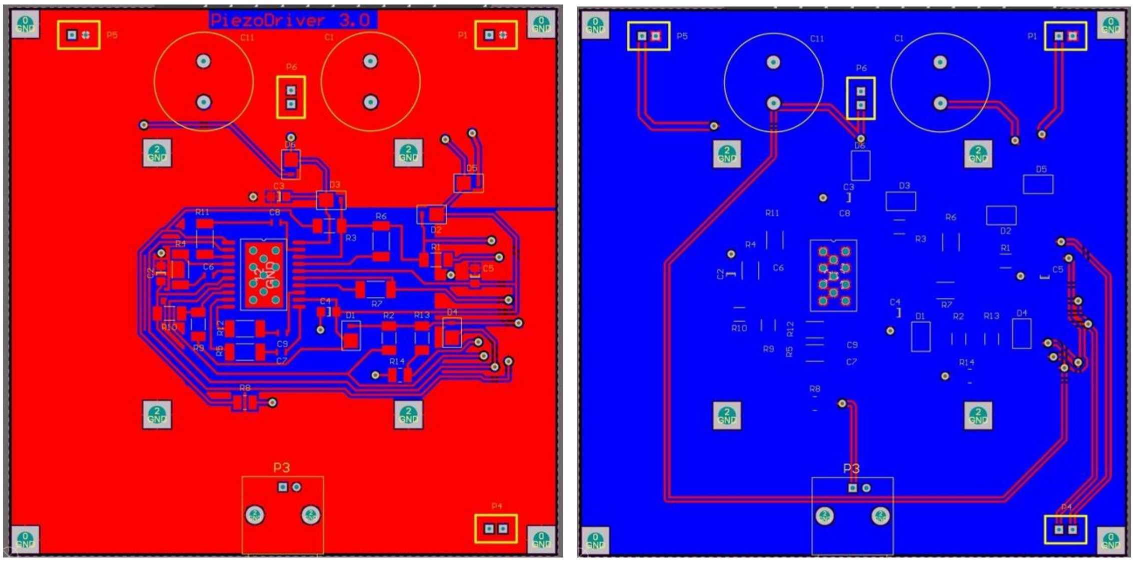


Figure 25. PCB design using Altium of the top view (left) and bottom view (right)

The final step consisted of welding all electronic components to the PCB. The total list of these components was the following:

- 2 electrolytic capacitors of 220 µF
- 4 ceramic capacitors of 10 nF
- 4 ceramic capacitors of 4.7 pF
- 6 avalanche diodes
- PA79
- 3 2-pin header wire-to-board connectors
- 1 BNC elbow connector
- 2 SMD resistors of 200 kΩ
- 4 SMD resistors of 100 kΩ
- 4 SMD resistors of 10 kΩ
- 4 SMD resistors of 3.3 kΩ
- 2 SMD resistors of 100 Ω

2.2.5 Heat sink

Another focus when designing the driver was on the necessary attention that heat dissipation needs, especially when regarding power amplifiers focused on electronically stimulating piezoelectric actuators [30]. Like any other electronic device, PA79 also releases heat, but even more when it has to deal with a very high voltage. According to the manufacturer, the operational amplifier improves its thermal performance to 25 °C/W when the solder connection of the heat slug is connected to a 1 square inch foil area on the printed circuit board [36]. To allow this improvement several holes were done in the PCB where the PA79 was welded, in order to better dissipate the heat to the bottom of the board, as the heat sink will be placed there.

By following the equation of power dissipation, it could be calculated the maximum possible temperature the operational amplifier might reach, so it could be found a heat sink that was able to dissipate most of the high temperature produced by the amplifier.

$$P_{max-dissipated} = V * I = 175\,V * 200 \cdot 10^{-3}\,A = 35\,W \qquad (6)$$

Knowing already the power dissipated, all it was left to do was to choose a heat sink that could fit in the PCB and dissipate that power. The first approach was to screw the heat sink to the PCB so it was well attached and making sure it would not fall off. For this reason, the PCB was made with 4 holes surrounding the power amplifier PA79 but also taking care no track was passing nearby (see Figure 26).

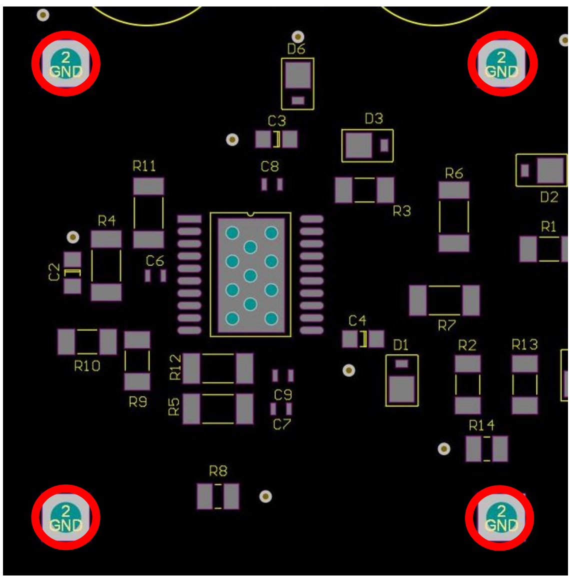


Figure 26. Holes made for the heat sink to be screwed to the PCB

However, having the heat sink screwed to the PCB was an issue, as the holes had to be at some distance to the location of PA79. Thus, the heat sinks found were of considerable size in order to have holes that could be screwed to the PCB. The heat sink chosen was HS202 (see Figure 27). It has the perfect dimensions to be screwed, as it possesses holes on top of it. It also has a thermal resistance of 2 ºC/W, therefore the maximum possible temperature it could reach would be around 70 ºC as seen on equation (7). This temperature is still under a decent temperature range, so the heat sink found was a good fit.

$$T_{max} = P_{max-dissipated} R_{thermal} = (35\ W)\ 2\ {}^{º C}/_{W}\ A = 70\ ºC \tag{7}$$

Nevertheless, as it will be seen in the results section, it was found that the heat sink was too big for the PCB, so a new heat sink needed to be found.

Figure 27. Heat sink HS202 [37]

A different approach was taken: instead of screwing a big heat sink to the PCB, a smaller one would be glued to PA79, which it will not be as efficient for heat dissipation, but it will make the entire device much lighter and easier to be manipulated. Luckily, in an electronics store next to Universidad Carlos III in Leganés, the owner had a useless heat sink that was given away for free to be used in the project. As seen in Figure 28 this heat sink was much smaller in size than HS202. However, being a smaller heat sink implies a lower heat dissipation, but its thermal resistance could not be found as it does not state its serial number on it.

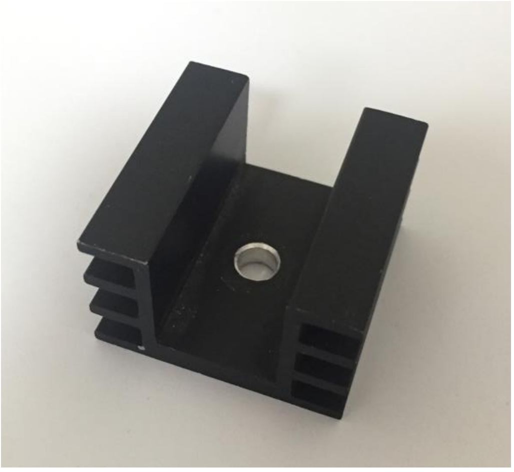


Figure 28. Smaller heat sink

2.2.6 Power supply IHB200-0.12

Regarding the PCB, everything had already been taken into account. However, in order for the amplification stage to work, it is also necessary to use a power supply that feeds the entire system. The circuit designed was configured to support a double supply of +175 V and -5 V.

Regarding the 175 V, it clearly was necessary an external power source that was able to supply such a high voltage. For this reason, it was looked for an AC to DC power supply which was also able to regulate the output voltage source in case a different voltage wants to be supplied. Power supply IHB200-0.12 gathered all these characteristics and seemed to perfectly fit for the project (see Figure 29). It also has a potentiometer that enables to regulate the output voltage from a range of 175 V to 215 V. The only drawback is its weight, being around 1.2 Kg and making the device heavier.

Figure 29. Power supply IHB200-0.12

However, power supply IHB200-0.12, being sold internationally, is not specific for the electric current of a certain country or region. Therefore, it is necessary to jumper several wire inputs depending on the voltage of the plug connection. Knowing that the voltage in Spain 220 V and 50 Hz and looking at Figure 30, it is necessary to fuse wires 2 & 3 as well as 1 & 5, leaving unfused wire number 4.

	AC Input		47-63-Hz	
For use at	100 VAC	120 VAC	220 VAC	230/240 VAC
JUMPER	1 & 3 2 & 4	1 & 3 2 & 4	2 & 3	2 & 3
Apply AC	1 & 5	4 & 1	1 & 5	4 & 1

Figure 30. AC connection and fusing table for power supply IHB200-0.12 [38]

Regarding now the -5 V power, this one can actually be obtained from the own Arduino DUE, as it has a power supply of both 3.3 and 5 volts. The only previous step is to invert this signal so it gives a negative voltage to the PCB. However, the circuit does not necessarily need a negative feed, and that is the reason for the P6 input which can be seen in Figure 25. This 2-pin header is placed to act as a way to connect the -5 V to ground (jumper), as a simple wire which connects both pins can perform this action. This feature allows having other approaches to the amplification of the sinusoidal wave.

Having the power supply such an important role in the amplification stage and taken into account the high voltages that have to be dealt with, it was thought to design a circuit that protects this stage. This circuit can be observed in Figure 31, which is composed of two electrolytic capacitors of 220 µF (support up to 220 V) as well as two diodes that prevent the current from going backward.

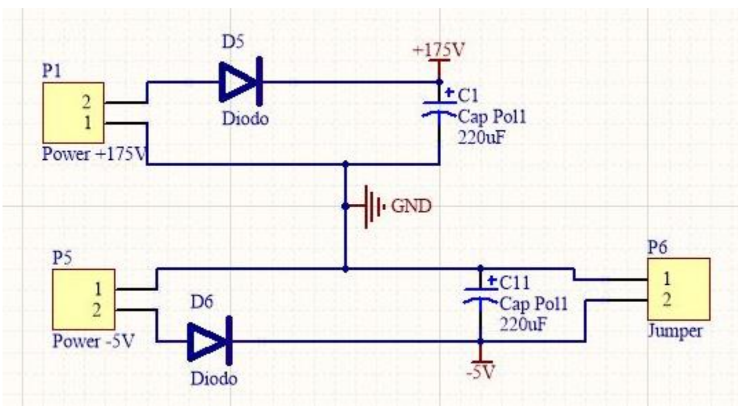


Figure 31. Protection circuit from the power supply

2.3 Piezoelectric set up

In this set up it will be placed the piezoelectric material that will be stimulated, as well as the cell culture that wants to be studied. This set up was used from a previous investigation [9], but it will be explained how it was made and its characteristics.

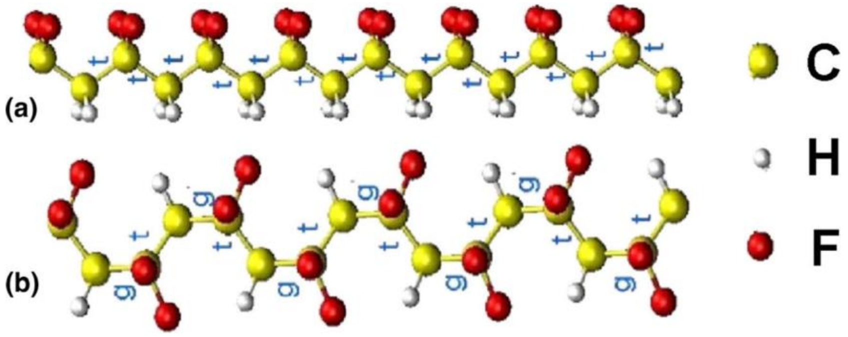


Figure 32. Beta phase (a) and alpha phase (b) of PVDF [39]

The piezoelectric material used for this investigation is called polyvinylidene fluoride, or polyvinylidene difluoride (PVDF). This material is a fluoropolymer, characterized by having a great piezoelectric coefficient (6.7 pC/N), being that number 10 times greater than any other polymer. PVDF can be found in three different crystal phases: alpha, beta and gamma. The second one (beta) is the only one with a piezoelectric behavior, which is actually due to the disposition of its atoms (see Figure 32). The great electronegativity that fluorine atoms have in comparison to the hydrogen ones makes each side of the polymer chain to have a dipolar moment perpendicular to the main backbone.

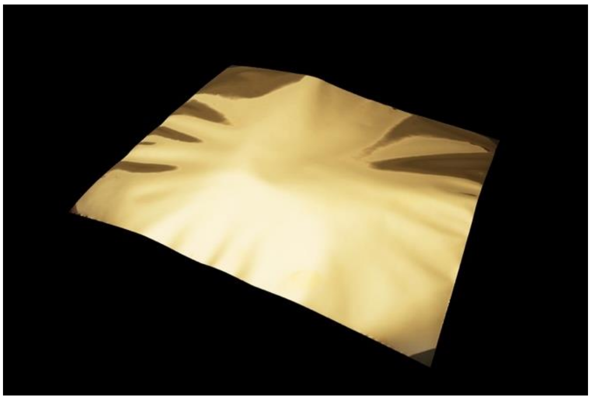


Figure 33. PVDF film of 28 µm in thickness [40]

For the purpose of this project, it was used a 28 µm thick film of PVDF (see Figure 33). This film is also coated with Ni-Cu metalized layers. However, in order for the set up to work, the PVDF film needed to be attached to proper copper wires to receive the electrical stimulation. Another issue was related to the attachment of cells to the material, as it first needed to be insulated

Regarding the connection problem, the PVDF had no external electrodes. Therefore, a copper conductive strip is used. This strip is attached to the film, and on top of it, external cables are welded. This way the input voltage can be connected to the wires, in order to stimulate the material.

Once the electrical problem was overcome, there was a more related biological issue. It cannot be forgotten that on top of the PVDF strip it will be placed cells in order to study their reaction to mechanical strain. However, these cells need a culture medium in order to survive and grow, and it is mainly composed of water. As water is a great conductive material for electricity, if cells lied on top of the piezoelectric material, they will probably die. Therefore, the PVDF film needed to be insulated, and to do so it is used one-sided adhesive polyester film. This polyester material is a great insulator and completely biocompatible, allowing cells to grow on top of it.

The last step consisted of placing the PVDF film already insulated and wired into a petri dish. This last step allows having some height on the setup so that the culture medium doesn't spread out. In order for the wires to go through the petri dish, two holes were also made. Finally, PDMS was chosen to hold in place all components of the device, act as an insulator and favor the growth of the cell culture. PDMS is poured around a vial which served as a mold, in order to surround the PVDF film but making sure the polymer did not exceed the petri's height.

3. Results

3.1 Software

3.1.1 Signal generator

For the generation of the signal, it was used a waveform generator algorithm in Arduino. The final circuit built can be observed in Figure 34, while the code used is provided in the Annex.

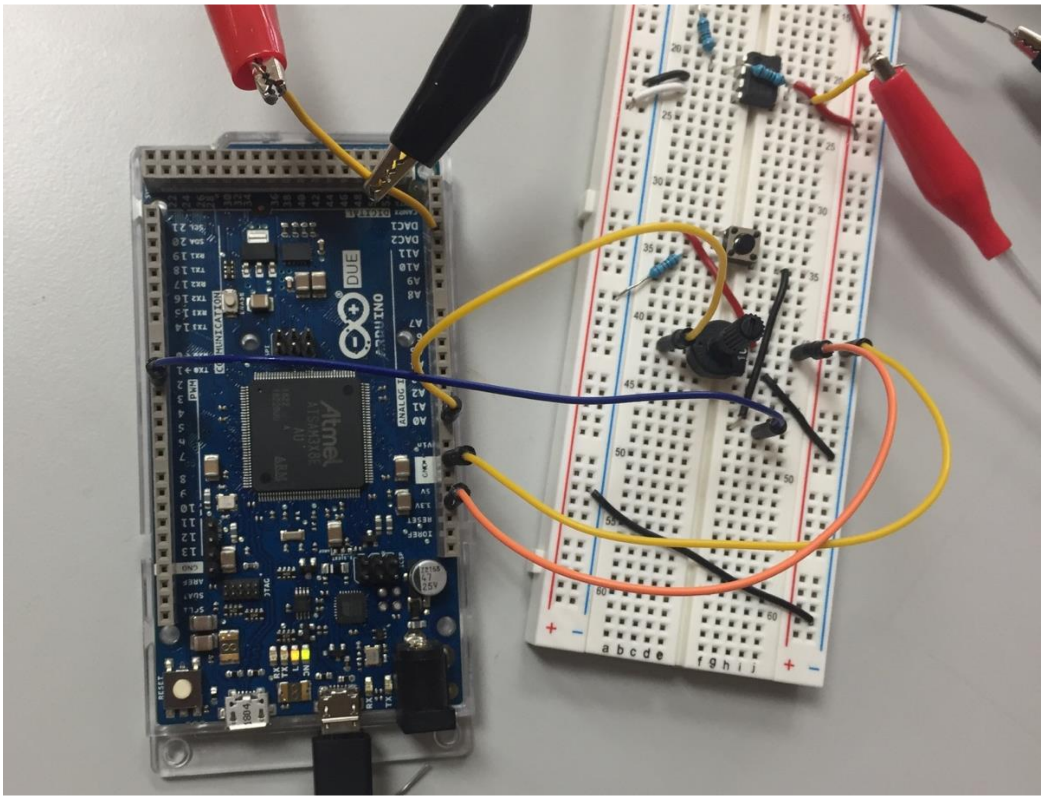
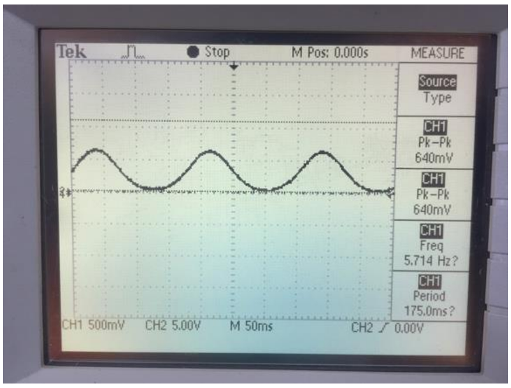
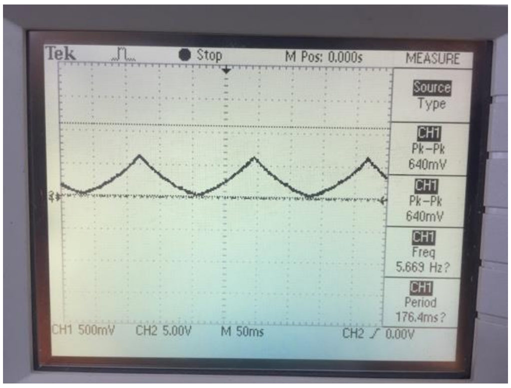


Figure 34. Waveform generation using Arduino DUE

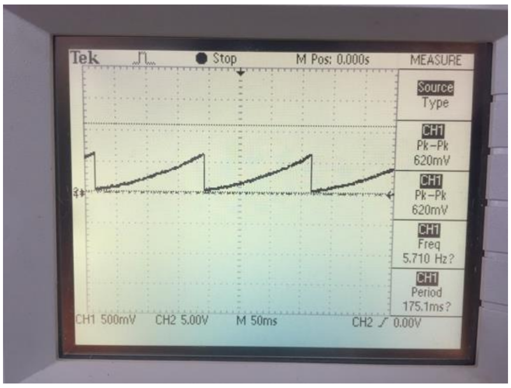
To check that the set up worked perfectly, the output signal was evaluated using the oscilloscope Tektronix TDS 220. It was checked that all four different types of waveforms worked, as well as the frequency range (1 Hz – 500 Hz).



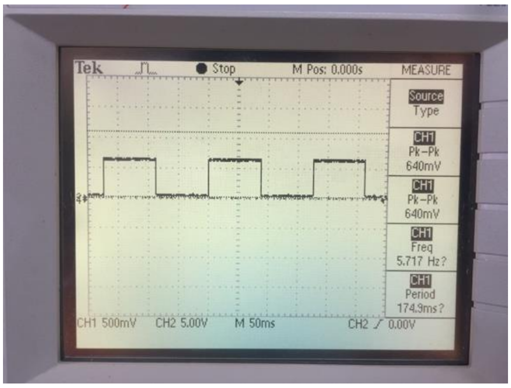
Sinusoidal wave of 5 Hz



Triangular wave of 5 Hz



Sawtooth wave of 5 Hz



Square wave of 5 Hz

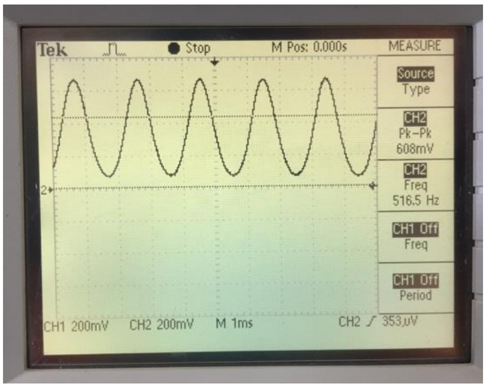
Figure 35. Output signals from DAC0 with different waveforms at 5 Hz

Lower than 5 Hz the oscilloscope is not able to acquire a clear signal, even if the potentiometer could still be fixed to have a lower resistance. The Arduino code, in theory, allows having signals with a minimum frequency of 1 Hz. Nevertheless, the lowest possible frequency acquired was around 5 Hz for all waveforms.

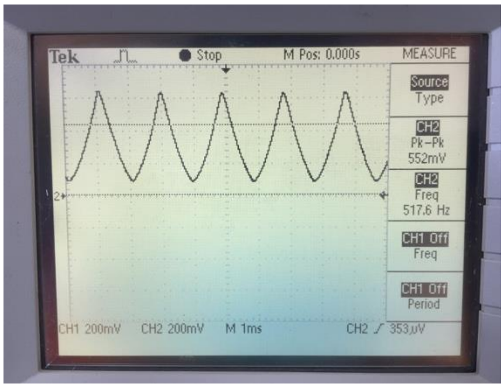
In Figure 36 the maximum possible frequency is also evaluated. In this case, the greatest value reached was around 500 Hz, as the 10 kΩ potentiometer had already moved up to its top. This result does coincide with what it was expected when the code was executed.

For all output signals, the amplitude obtained was around 0.6 $V_{pk\text{-}pk}$. As previously mentioned, there is no interest in being able to modify the amplitude of this signal, as in the next stage it will be highly amplified. In this case, the final value of the signal can be seen in equation (8).

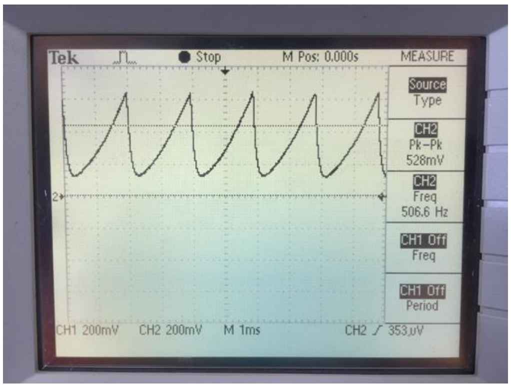
$$V_{LOAD} = 40(V_{in}) = 40(0.6) = 24\ V \qquad (8)$$



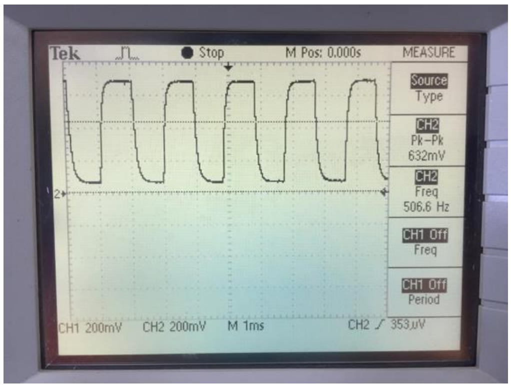
Sinusoidal wave of 500 Hz



Triangular wave of 500 Hz



Sawtooth wave of 500 Hz



Square wave of 500 Hz

Figure 36.Output signals from DAC0 with different waveforms at 500 Hz

3.2 Hardware

3.2.1 PCB

Once it was checked in Altium that all tracks were correctly placed, as well as every component, the PCB design was sent to be built by the electronic department of Universidad Carlos III de Madrid. It took some time for the PCB to be built, as the process is not easy, and every step has to be accurately performed. The PCB built can be observed in Figure 37.

Afterwards, all components had to be welded to the PCB. The final result can be observed in Figure 38.

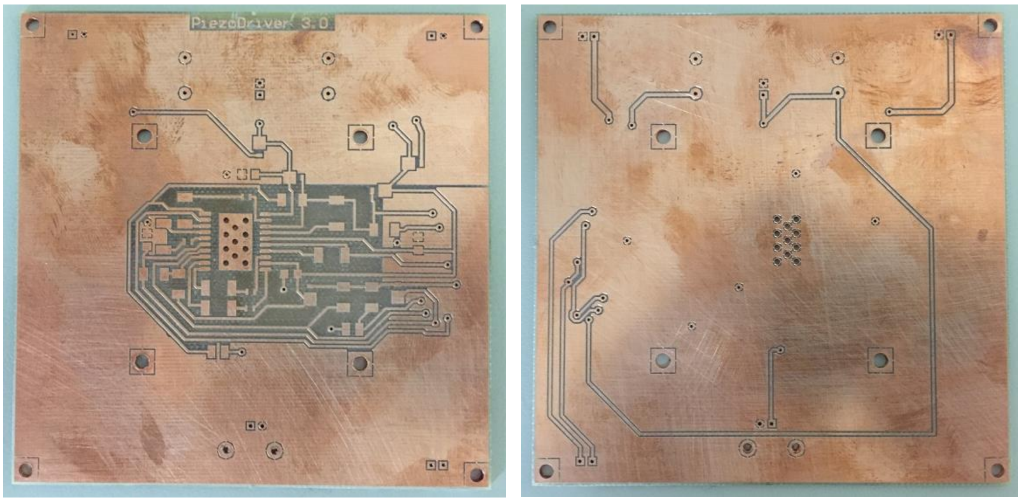


Figure 37. PCB from the top view (left) and bottom view (right)

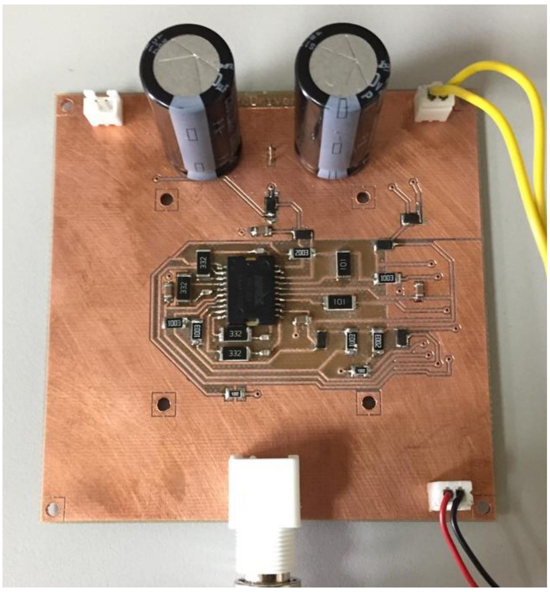


Figure 38. PCB with all components welded

The following step consisted of checking that the PCB worked. Firstly, the power supply IHB200-0.12 was plugged into the 2-pin header connector. Once the power supply was connected to the plug it was checked if there was a shortcut on the circuit, and unfortunately, there was. The multimeter showed a drop of the power supply to a few volts, meaning that there was a shortcut somewhere in the circuit. In order to try to find this issue on the board, the entire circuit was evaluated. This evaluation started by checking the connectivity of all tracks, to see if there was some tin that was badly welded and was acting as a bridge between two tracks. This process allowed to fix a few welding mistakes that were defective and were acting as that mentioned bridge. Once again, the power supply was connected and plugged in, and once again there was a shortcut in the circuit.

Discarded that the problem was coming from the circuit, there were only two other possible sources of the shortcut: the power supply and the amplifier. It was easy to check if the problem was coming from the power supply, as all it was done was to connect this voltage source to the oscilloscope. The screen showed a steady voltage at around 175 V, so the power source worked perfectly. This fact led to conclude that the problem was coming from the power amplifier PA79. The decision was to buy a new amplifier to substitute the broken one.

Once the new PA79 arrived, the amplifier was welded on its spot in the PCB. As expected, this time there was no shortcut when the power supply of 175 volts was connected, as there was no voltage drop. The same test was run for the other voltage supply input of -5 volts, obtaining the same successful result. Nevertheless, when measuring the 175 volts on the tracks of the PCB, a human mistake was produced, and both the track and the ground were touched with the multimeter probe. This mistake produced a shortcut, which could be told by the loud noise produced and the shiny sparkle, which ended up burning a part of the track. As the track was burned, an external wire had to be welded to go across the broken area. Sadly, after connecting again the power source, there was a new shortcut, meaning the amplifier was again broken, so a new one had to be purchased.

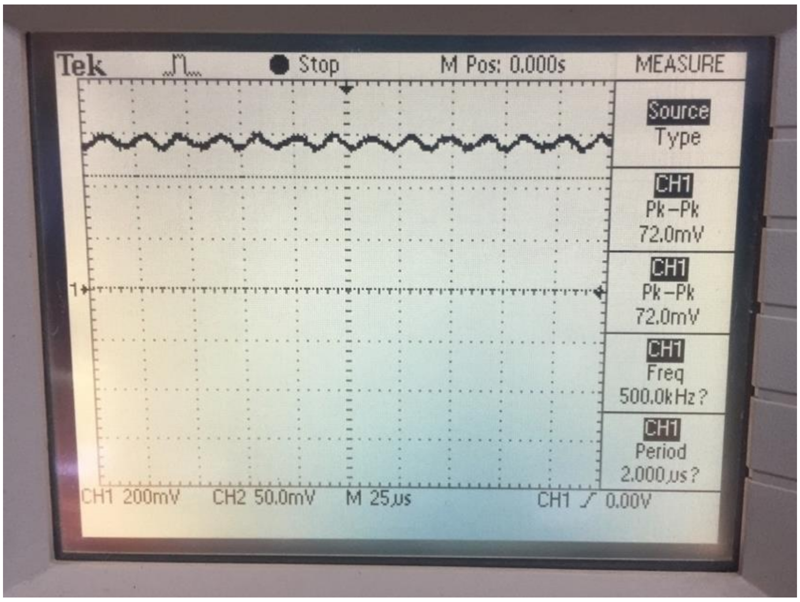


Figure 39. Output of the PCB when a sinusoidal of 1 kHz want to be amplified

Finally, the new PA79 was welded and there was no shortcut, so the PCB was ready for the next step: checking that the board amplified a sinusoidal wave. A new disappointment appeared when it was seen on the oscilloscope that there was no signal amplification, as seen in Figure 39.

Due to the discontent after not being able to make the PCB work, it was decided to build a bridge-connected circuit on a protoboard (see Figure 40) to at least check and see how this amplification process worked.

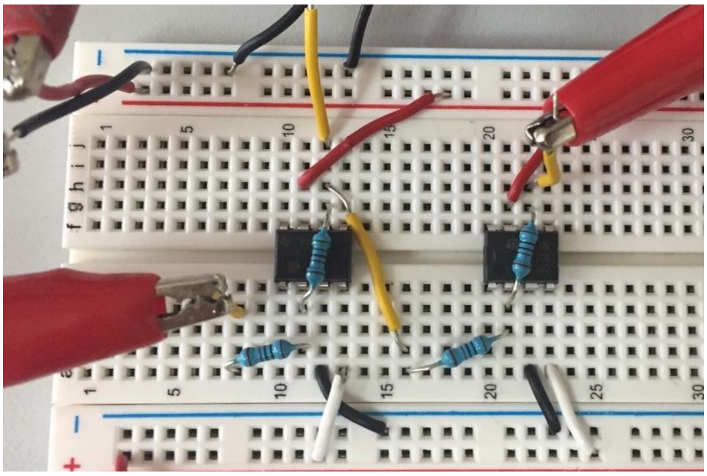


Figure 40. Simple bridge-connected circuit

An initial signal of 1 $V_{pk\text{-}pk}$ is implemented in the circuit shown in Figure 40. From Figure 41 it can be observed both outputs that this type of amplification process gives. By the simple subtraction of one wave from the other, it is obtained a wave doubled in amplitude. That was the outcome it should have been expected from Figure 39, but by this simple experiment at least it can be checked that it is possible to achieve that result.

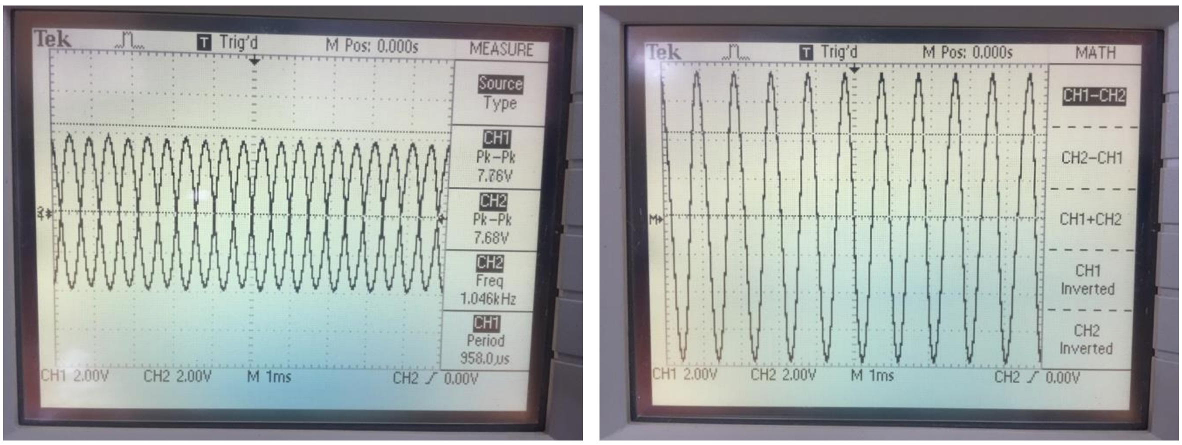


Figure 41. Outputs coming out from both amplifiers (right) and subtraction of signals (left)

3.2.2 Heat sink

The holes made around the circuit allowed for heat sink HS202 to be screwed to the PCB. This fact allowed the heat sink to be in contact with the bottom of the PCB, and particularly to the bottom of PA79. The holes also made at the bottom of the power amplifier allowed a better heat dissipation of the entire system (see Figure 42).

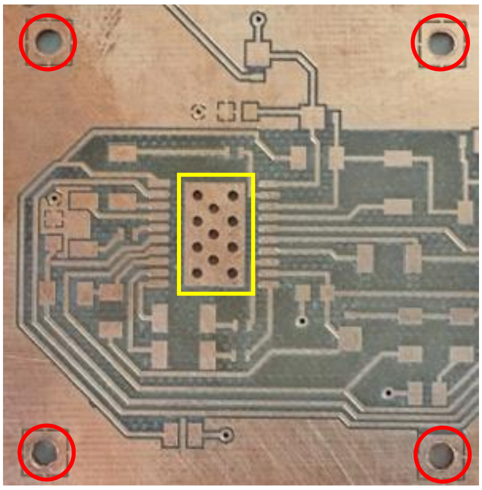


Figure 42. Holes on each corner for heat sink attachment (red) and holes under PA79 for a better heat dissipation (yellow).

The final result of having HS202 can be seen in Figure 43. As observed, the heat sink was too big in size and was also very heavy, contributing in excess to the final weight of the system.

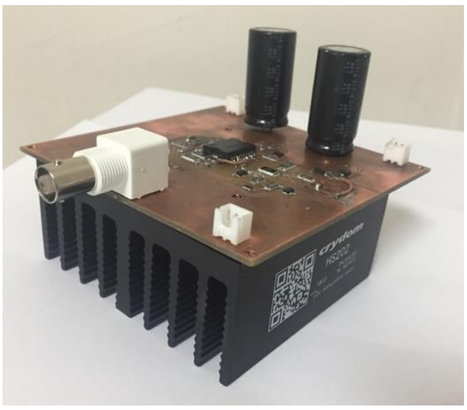


Figure 43. Heat sink HS202 screwed to the PCB

As already explained, the next approach was to find a smaller heat sink that would be glued to the PCB instead of screwed. The results can be observed in Figure 44, which allows to either glue it on top of PA79 or to the bottom of the PCB, right under the power amplifier.

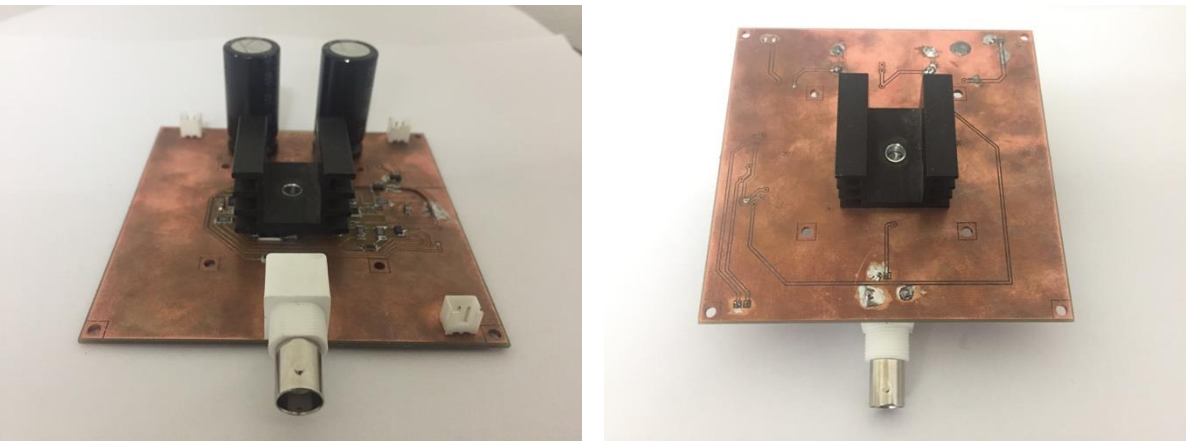


Figure 44. Smaller heat sink glued to the top (left) or bottom (right) of PA79

3.3 Piezoelectric set up

The piezoelectric set up used for this project can be observed in Figure 45. As previously mentioned, it has an isolated and wired PVDF film on the bottom of a petri dish. The copper wires exit the petri dish thanks to two holes made. It is also observed that the PVDF film is circularly surrounded by PDMS, which allows cell growth, fixes the whole system and avoids the cell culture to spread to areas where the film is not underneath.

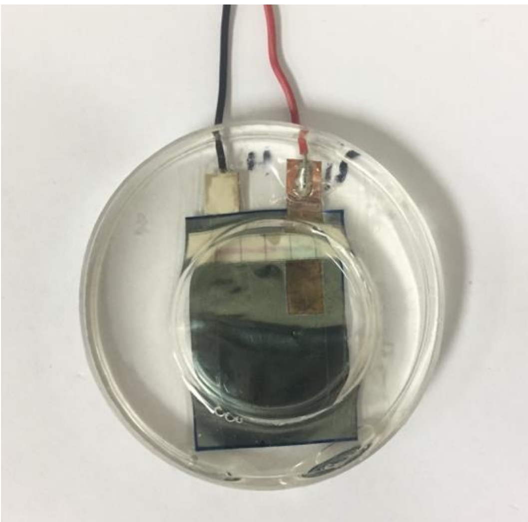


Figure 45. Piezoelectric set up

In order to check if a piezoelectric material works, an easy and effective method can be very helpful to do so. The material is connected to a signal generator and it is fed with a high voltage amplitude and a frequency in the hearing range (20 to 20 kHz approximately). If it is actually possible to hear a noise coming from the piezoelectric material, then the set up works satisfactorily.

The mentioned method was performed in the piezoelectric set up, and at 10 kHz in frequency it could be perfectly heard a buzzing coming from the PVDF film, confirming that the set up was working correctly.

3.4 Electronic driver

The final result of this project can be observed in Figure 46. It shows how all sections previously mentioned and described are fixed together to form one final device: an electronic driver which stimulates a piezoelectric material.

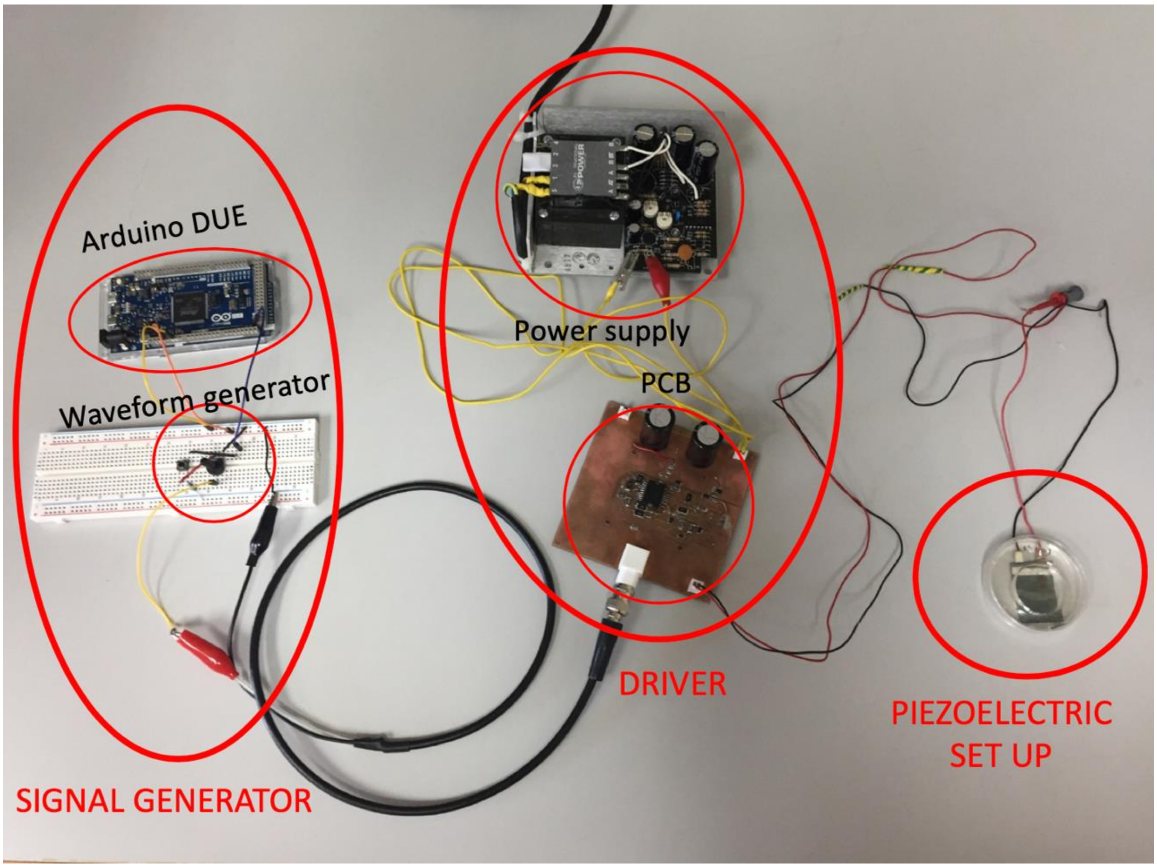


Figure 46. Final set up of the electronic driver

4. Discussion of results and conclusion

In order to further study how cells reacted to mechanical stimulations due to displacements of piezoelectric materials, it was necessary to develop an electronic driver that could attend the necessary electrical, mechanical and biological requirements.

The first stage consisted of the generation of the signals. To do so, Arduino DUE was used as the microprocessor board. The addition of the initial circuit for waveform generation allowed to change both the shape and frequency of the signal. The range of frequencies allowed to go from 5 Hz all the way up to 500 Hz. By checking Figure 9, this range of frequency allows for a broad difference of displacements produced on the PVDF film. The higher displacements would be achieved in the 1 Hz to 80 Hz range, being the 80 Hz the resonant frequency analyzed in previous studies [10]. However, the frequency range also allows to study the behavior of cells in the 100-500 Hz, where the piezoelectric displacement is lower, but it could potentially have a great impact on cells too.

Regarding the voltage of this signal, it remains constant at around 0.6 $V_{pk\text{-}pk}$. After its amplification, this value would increase to 24 V. This value can be enough to stimulate the piezoelectric material, but it would be better to obtain an even higher one. This issue will be referred to as future work in the next pages.

On the other hand, there was the proper elaboration of the driver. This stage was the most complex of the study and the one where most time was spent. The design and build up of the PCB had to be addressed with special attention, as the minimum error could mean that the entire system would not work. Nevertheless, as it was the first time dealing with such a complex work, many failures had to be dealt with: irregular welding of some electrical components led to shortcuts on the board. It was especially difficult to work with the delicate power amplifier PA79. As a power amplifier with twenty "legs", it had to be carefully welded not only to avoid connections between them, but also as not to overheat the amplifier. This amplifier is very fragile to heat changes and any anomaly in the circuit, and twice in the project, it had to be substituted as it broke down, causing a shortcut in the system. By the end of this project, it was finally achieved to have a driver where no shortcuts were produced. Unfortunately, the system was not able to amplify the initial signal, as it can be observed in Figure 39.

The best approach after reaching this point would be to build a new PCB. Like any other new board that it is trying to be made and make it work, there is no ideal first version. Thus, future work relies on developing this board with higher quality and accuracy. It is most probable that while trying to make it work, some irregularities were made on the circuit which cannot be fixed anymore. However, by looking at Figure 41 it can be checked that the idea of amplifying the signal using a bridge-connected circuit does work.

One last issue of this worked had to deal with was the heat dissipation of the system. Dealing with high voltages makes the power amplifier to raise its temperature, so a heat sink is crucial to make sure the temperature does not reach values too high. With the two heat sinks found on this project, this task was achieved, and the selection of one or the other depends on what function is more important for the device: a better heat dissipation or a lower weight.

This work has been able to come up with a device capable of generating a signal, and it potentially will also be able to amplify it. Moreover, this device will allow choosing a frequency and an amplitude for the sinusoidal wave that will stimulate the piezoelectric material. Its lightweight compared to other mechanisms with similar functions will allow the device to be moved from one workstation to another, allowing to lie next to the cell culture that it is being stimulated. The initial objectives of this project were highly achieved. The creation of an electronic driver for piezoelectric actuation makes possible to continue studying how cells behave to mechanical stimulations, and it makes this bachelor thesis an improvement in the studies of this field.

5. Further studies

As a future approach to improve the electronic driver presented in this work, the most demanding request would be to get all electronic devices seen in Figure 46 inside a unique box. The only necessary connection would be to plug in the power supply to the electric outlet. As outputs from this box, it could be found the two connections necessary for transmitting the sinusoidal wave to the piezoelectric material. In each output would travel the two waves created, that would be subtracted in order to generate that final wave doubled in amplitude.

In order to variate the voltage of the signal, the best idea is to have this set up in the PCB. As already shown in equation (5), the main component for the gain of the amplifier to be modified is the value of R_f. Therefore, this resistance could be changed by a potentiometer of high value. In the same way, the value of R_{in} could be decreased, in order to increase the gain of the system. The voltage of the sinusoidal can make a great difference in the displacement of the piezoelectric material. As seen in Figure 10, this new parameter in the driver will allow studying how cells react to different mechanical strains by keeping the same frequency constant.

Regarding the set up for the wave generation of the signal, the implementation of a digital interface is a great advance. This screen would show the actual frequency and voltage amplitude values of the output signal, and each of these parameters could be fixed with a wheel which will variate the potentiometer's values. As future developments of the wave generation, it could be tried to achieve higher frequencies to have a wider range of studies.

The next step of improvement for the amplification of the signal would rely on reviewing the PCB created for this project and send a new board to be made. As already mentioned, the welding step would be crucial in order to avoid any human errors as it happened on this project. An even better approach would be on sending the board and all its components to be welded by a professional machine, which already exists in the market and they make no mistakes.

Once the electronic driver has accomplished these improvements, its great potential will be ready to be used in the biological laboratory for cell investigation. Until today, the investigation has been focused on specific piezoelectric materials and cells, but there are many other fields in the biological investigation where piezoelectric actuation will sure have a great impact. To do so, the electronic driver created in this project will allow it to happen.

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Annex

Arduino code for the waveform generation

WaveformGenerator_ArduinoDue.ino

```
/*
 * connect two push buttons to the digital pins 2 and 3
   with a 10 kilohm pulldown resistor to choose the waveform
   to send to the DAC0 and DAC1 channels
  * connect a 10 kilohm potentiometer to A0 to control the
   signal frequency

#include "Waveforms.h"
#define oneHzSample 1000000/maxSamplesNum  // sample for the 1Hz
signal expressed in microseconds

const int button0 = 2;
volatile int wave0 = 0;
int i = 0;
int sample;

void setup() {
  analogWriteResolution(12);  // set the analog output resolution to
12 bit (4096 levels)
  analogReadResolution(12);   // set the analog input resolution to 12
bit

  attachInterrupt(button0, wave0Select, RISING);  // Interrupt
attached to the button connected to pin 2
}

void loop() {
  // Read the the potentiometer and map the value  between the maximum
and the minimum sample available
  // 1 Hz is the minimum freq for the complete wave
  // 170 Hz is the maximum freq for the complete wave. Measured
considering the loop and the analogRead() time
  sample = map(analogRead(A0), 0, 4095, 0, oneHzSample);
  sample = constrain(sample, 0, oneHzSample);

  analogWrite(DAC0, waveformsTable[wave0][i]);  // write the selected
waveform on DAC0

  i++;
  if(i == maxSamplesNum)  // Reset the counter to repeat the wave
    i = 0;

  delayMicroseconds(sample);  // Hold the sample value for sample time
}
// function hooked to the interrupt on digital pin 2
void wave0Select() {
  wave0++;
  if(wave0 == 4)
    wave0 = 0;
}
```

Waveforms.h

```
#ifndef _Waveforms_h_
#define _Waveforms_h_

#define maxWaveform 4
#define maxSamplesNum 120

static int waveformsTable[maxWaveform][maxSamplesNum] = {
  // Sin wave
  {
    0x7ff, 0x86a, 0x8d5, 0x93f, 0x9a9, 0xa11, 0xa78, 0xadd, 0xb40,
0xba1,
    0xbff, 0xc5a, 0xcb2, 0xd08, 0xd59, 0xda7, 0xdf1, 0xe36, 0xe77,
0xeb4,
    0xeec, 0xf1f, 0xf4d, 0xf77, 0xf9a, 0xfb9, 0xfd2, 0xfe5, 0xff3,
0xffc,
    0xfff, 0xffc, 0xff3, 0xfe5, 0xfd2, 0xfb9, 0xf9a, 0xf77, 0xf4d,
0xf1f,
    0xeec, 0xeb4, 0xe77, 0xe36, 0xdf1, 0xda7, 0xd59, 0xd08, 0xcb2,
0xc5a,
    0xbff, 0xba1, 0xb40, 0xadd, 0xa78, 0xa11, 0x9a9, 0x93f, 0x8d5,
0x86a,
    0x7ff, 0x794, 0x729, 0x6bf, 0x655, 0x5ed, 0x586, 0x521, 0x4be,
0x45d,
    0x3ff, 0x3a4, 0x34c, 0x2f6, 0x2a5, 0x257, 0x20d, 0x1c8, 0x187,
0x14a,
    0x112, 0xdf, 0xb1, 0x87, 0x64, 0x45, 0x2c, 0x19, 0xb, 0x2,
    0x0, 0x2, 0xb, 0x19, 0x2c, 0x45, 0x64, 0x87, 0xb1, 0xdf,
    0x112, 0x14a, 0x187, 0x1c8, 0x20d, 0x257, 0x2a5, 0x2f6, 0x34c,
0x3a4,
    0x3ff, 0x45d, 0x4be, 0x521, 0x586, 0x5ed, 0x655, 0x6bf, 0x729,
0x794
  }
  // Triangular wave
  {
    0x44, 0x88, 0xcc, 0x110, 0x154, 0x198, 0x1dc, 0x220, 0x264, 0x2a8,
    0x2ec, 0x330, 0x374, 0x3b8, 0x3fc, 0x440, 0x484, 0x4c8, 0x50c,
0x550,
    0x594, 0x5d8, 0x61c, 0x660, 0x6a4, 0x6e8, 0x72c, 0x770, 0x7b4,
0x7f8,
    0x83c, 0x880, 0x8c4, 0x908, 0x94c, 0x990, 0x9d4, 0xa18, 0xa5c,
0xaa0,
    0xae4, 0xb28, 0xb6c, 0xbb0, 0xbf4, 0xc38, 0xc7c, 0xcc0, 0xd04,
0xd48,
    0xd8c, 0xdd0, 0xe14, 0xe58, 0xe9c, 0xee0, 0xf24, 0xf68, 0xfac,
0xff0,
    0xfac, 0xf68, 0xf24, 0xee0, 0xe9c, 0xe58, 0xe14, 0xdd0, 0xd8c,
0xd48,
    0xd04, 0xcc0, 0xc7c, 0xc38, 0xbf4, 0xbb0, 0xb6c, 0xb28, 0xae4,
0xaa0,
    0xa5c, 0xa18, 0x9d4, 0x990, 0x94c, 0x908, 0x8c4, 0x880, 0x83c,
0x7f8,
    0x7b4, 0x770, 0x72c, 0x6e8, 0x6a4, 0x660, 0x61c, 0x5d8, 0x594,
0x550,
    0x50c, 0x4c8, 0x484, 0x440, 0x3fc, 0x3b8, 0x374, 0x330, 0x2ec,
0x2a8,
    0x264, 0x220, 0x1dc, 0x198, 0x154, 0x110, 0xcc, 0x88, 0x44, 0x0
  }
  // Sawtooth wave
  {
```

```
    0x22, 0x44, 0x66, 0x88, 0xaa, 0xcc, 0xee, 0x110, 0x132, 0x154,
    0x176, 0x198, 0x1ba, 0x1dc, 0x1fe, 0x220, 0x242, 0x264, 0x286,
0x2a8,
    0x2ca, 0x2ec, 0x30e, 0x330, 0x352, 0x374, 0x396, 0x3b8, 0x3da,
0x3fc,
    0x41e, 0x440, 0x462, 0x484, 0x4a6, 0x4c8, 0x4ea, 0x50c, 0x52e,
0x550,
    0x572, 0x594, 0x5b6, 0x5d8, 0x5fa, 0x61c, 0x63e, 0x660, 0x682,
0x6a4,
    0x6c6, 0x6e8, 0x70a, 0x72c, 0x74e, 0x770, 0x792, 0x7b4, 0x7d6,
0x7f8,
    0x81a, 0x83c, 0x85e, 0x880, 0x8a2, 0x8c4, 0x8e6, 0x908, 0x92a,
0x94c,
    0x96e, 0x990, 0x9b2, 0x9d4, 0x9f6, 0xa18, 0xa3a, 0xa5c, 0xa7e,
0xaa0,
    0xac2, 0xae4, 0xb06, 0xb28, 0xb4a, 0xb6c, 0xb8e, 0xbb0, 0xbd2,
0xbf4,
    0xc16, 0xc38, 0xc5a, 0xc7c, 0xc9e, 0xcc0, 0xce2, 0xd04, 0xd26,
0xd48,
    0xd6a, 0xd8c, 0xdae, 0xdd0, 0xdf2, 0xe14, 0xe36, 0xe58, 0xe7a,
0xe9c,
    0xebe, 0xee0, 0xf02, 0xf24, 0xf46, 0xf68, 0xf8a, 0xfac, 0xfce,
0xff0
  }
  // Square wave
  {
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff, 0xfff,
0xfff,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0,
    0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0, 0x0
  }

};

#endif
```