Portable Polymerase Chain Reaction (PCR) Diagnostics

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***Abstract* — This paper presents a device designed to be a compact and portable machine to perform medical diagnostics by polymerase chain reaction (PCR). The device cyclically heats and cools a sample containing biological reagents in an effort to amplify a DNA sequence. The detection system is based on optical components including an LED to excite the sample and a photodiode to read the fluorescent signal. The compact size of the device is a breakaway from conventional PCR machine technology which is usually large specialized laboratory equipment. Data from the tests is sent to a mobile phone application for data logging.**

***Index Terms* — Portable, polymerase chain reaction, medical diagnostics, optical detection, joule heating, GPS, bluetooth, servo motor, TFT display, microcontroller.**

I. INTRODUCTION

PCR being the key player that it is for molecular biology makes producing a portable PCR machine very appealing. This group desires to produce a device that is small, cheap, and portable. The portability feature by itself, allows for a great impact on the science community and, indirectly, to the world. Being portable, more research can be done around the world. Scientists and forensics don't have the privileges of taking their work or their lab where they need to. For this reason, much of the testing process is delayed, which could have dramatic consequences for individuals with diseases. With a portable device, fast test results would greatly increase and more data from around the world would be acquired, allowing for doctors to better plan strategies for treating disease outbreaks.

Outbreaks are a main focus to molecular biologists and a world concern when they occur. There have been countless times in the United States that an outbreak has caught the attention and stimulated fear of the citizens. Examples such as the Zika virus, Ebola virus, and H1N1 virus are well known. When the United States heard reported cases of the Zika virus in Miami, studies ensued. A portable PCR machine would have been perfect for this situation. Scientists in the area, and many others sent to do studies on the situation, would have the ability to test patients who are suspected of having the virus in a quick manner. Currently the processes is slow. Scientists take samples from the patients then send them to the lab for evaluation. Since a scare is in a specific location, there are a finite number of PCR machines also in that area. Samples must wait to be tested or sent elsewhere, which also hinders results. For any outbreak minimizing delay is crucial. Although our device is capable of running a PCR in a normal operation, our design is specific to viral detection. With quantitative viral detection, our PCR machine will give results to the presence of the virus immediately after running the PCR. Most PCR machines today do not have viral detection and require additional equipment and additional steps to yield those results. Scientists can avoid these hiccups with a portable quantitative PCR device that we are proposing. The device will provide quick results, which allows for more data to be collected over a shorter period of time, reducing risks of the virus spreading.

The hardware included will consist of the materials necessary to complete the basic functions of the PCR machine. The main components of the machine will be two heating elements, two temperature detection devices, two small motors, a photodiode, LEDs, optical filters, a location module, wireless transmitter, a display, 3D printed frame and small parts, and a microcontroller to coordinate it all with a simple user interface.The hardware included will consist of the materials necessary to complete the basic functions of the PCR machine. The main components of the machine will be two heating elements, two temperature detection devices, two small motors, a photodiode, LEDs, optical filters, a location module, wireless transmitter, a display, 3D printed frame and small parts, and a microcontroller to coordinate it all with a simple user interface.

The software will consist of a main program flow to carry out a testing of a sample and a few functions to deal with user input and data processing. There will also be external software development in the form of a mobile app and online database to further process and store data. The software should be able to read, calculate, and represent the data accurately, display the information, and record it ideally indicating whether or not the virus is present in the current sample.

The PCR machine will communicate with a few outside sources wirelessly to achieve the goals for our design. Each component will communicate with the microcontroller to process data and control the machine or provide data remotely (determining location, giving specific settings etc.).

The PCR machine will have at least two options for user interface and control, local and external. Local will include input devices on the module to control the process directly. External controls will be optional to quickly control the module remotely.

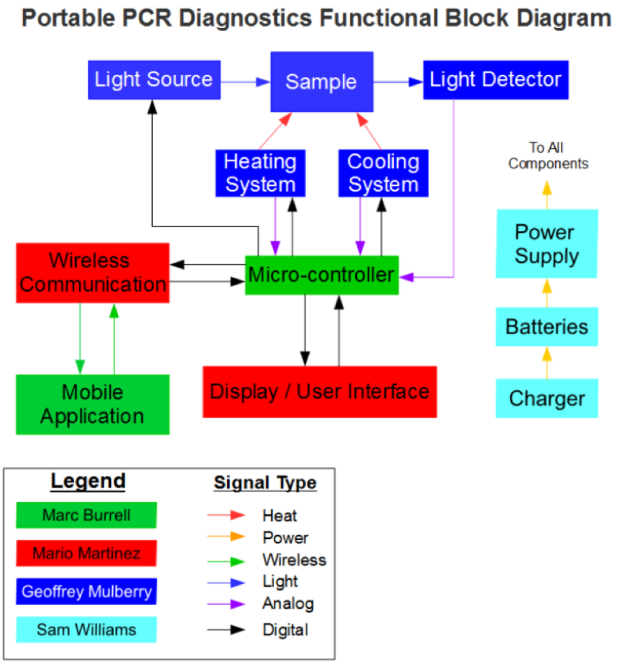


Fig.1 Block diagram of the device

The proposed system consists of the above elements. The main component of the device is the sample, which contains (or does not contain) the virus being detected. Two systems, the heating and cooling systems are controlled by the microcontroller and are responsible for the transfer of heat into and out of the sample to facilitate thermocycling, which is required PCR. The light source and light detector, work together to determine the concentration of target DNA in the sample. This data is recorded by the microcontroller and sent to the mobile application for additional processing via the wireless communication system. The system also consists of a display and user interface so that the device is still functional without the use of a mobile phone.

II. COMPONENTS

As mentioned previously, the device is made of several subsystems each consisting of carefully selected components.

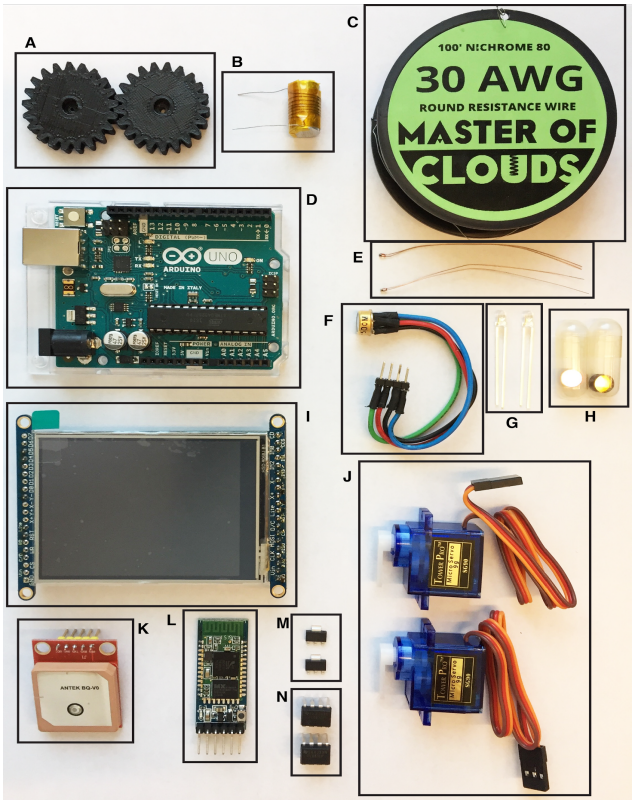


Fig.2 Components chosen to implement the device

Component **A** is an important example relevant to this project, as it shows a set of 3D printed gears. 3D printing will be used to produce and construct all of the components in the design that are not purchased, as a result, most of the parts will be 3D printed in the final product. The gears are shown to represent these parts which will be designed.

B is an example of the heating elements. This part is made from an aluminum rod and is wrapped with polyimide tape for insulation and NiCr resistance wire to form the heat source.

C is the NiCr resistance wire which will be used to construct the heating elements.

D is an Arduino Uno, which is a development board which contains an ATmega328 microcontroller and will be used in the breadboard phase of this project to control all of the electronic components until a custom PCB is designed.

E are the thermistors that will be used to determine the temperature of the heating elements.

F is the photodiode that will be used for the detection of the fluorescent emission from the samples.

G are the blue LEDs that will be the source of light for the excitation of the fluorescent probes in the sample.

H are the optical filters used to eliminate crosstalk between the excitation and emission wavelengths of light

in the optical system.

I is the LCD display and touchscreen assembly which will be used for user input and output in order to operate the device.

J are the servomotors which will form the basis of the mechanical system that will move the PCR tube between heating and cooling elements.

K is the GPS module which contains the GPS antenna and GPS radio used to communicate with GPS satellites to determine the device's location on the surface of Earth.

L is the Bluetooth module that will be used to communicate with a smartphone for the data logging and analysis smartphone application.

M are MOSFETS that will be used to enable and disable the heating elements, providing temperature control.

N are the operational amplifiers that will be used to amplify the signal from the photodiode as well as remove the signal offset before being sent to the microcontroller. These components were used during the breadboard testing phase of the project in order to confirm that the design was functional before designing a PCB.

III. SYSTEMS DESIGN

Design is an important process required to produce a final product. As such much effort has been placed into the design of systems for the device. In this section, we will describe in detail the elements that have been designed for the project.

A. Heating Element Design

In order to realize a practical heating element, some mechanical properties must be taken into consideration. Firstly, the materials used must be available and easily manufactured. For example, arbitrary sizes of raw materials should be avoided because they are usually available in standard sizes. Because of this, aluminum rod with a diameter of ⅜ inch was chosen to form the main body of the heating element because it is small enough to allow for the device to stay small, but large enough for the PCR tube to fit inside. The basic structure of the device consists of a short section of the aluminum rod wrapped with NiCr wire that will provide heat to the rod by Joule heating. This structure is shown in Fig. 3. The length of the rod, 0.75 inches, was chosen so that there would be enough space to wrap several turns of the NiCr wire around the rod without shorting out between turns, while still being compact. Temperature feedback will be provided to the microcontroller using a thermistor attached to the aluminum rod. The temperature of the rod will be controlled by the microcontroller and a MOSFET using a PID feedback system for an accurate and stable temperature.

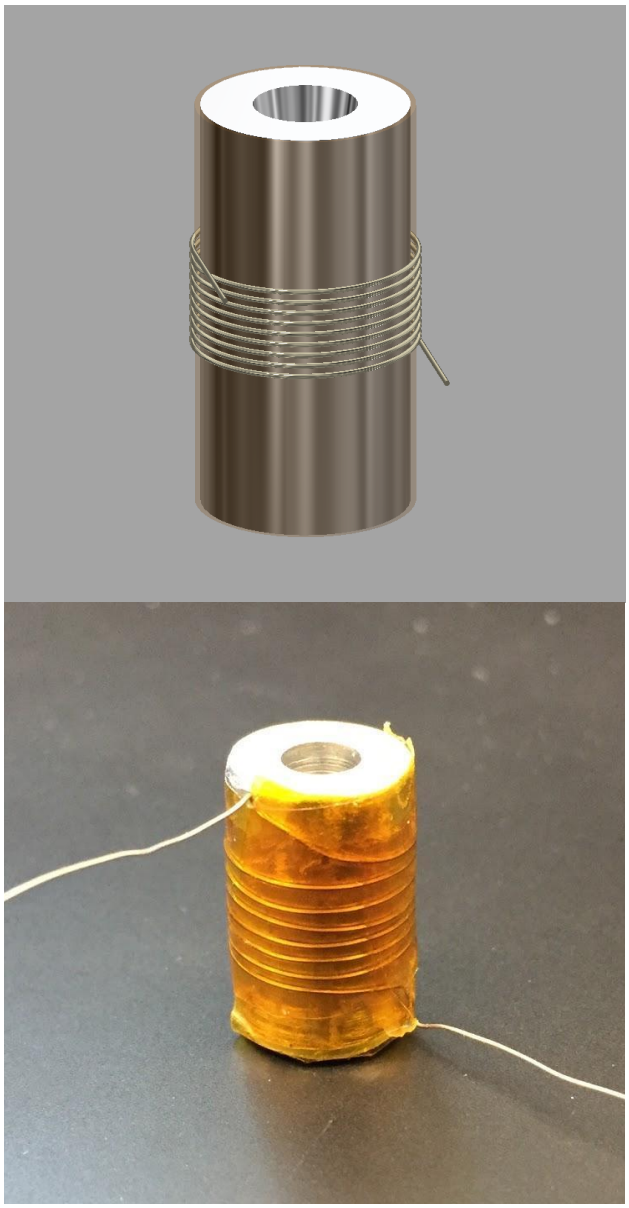


Fig.3 Top: The heating element as designed in Inventor. Bottom: The heating element as constructed using NiCr wire and aluminum rod.

B. Optical Design

Besides the thermocycling, the most important system of a qPCR device is the ability to quantify the amount of target DNA present in the sample. This feature is what differentiates a qPCR machine from a standard PCR thermocycler. There are a number of ways to determine the target concentration, including electrochemical

systems, however the most widespread and effective method is one which relies on optics. The method works by placing a specific primer into the PCR sample. When bonded to DNA, this primer fluoresces if excited by a particular wavelength of light and emits light on another wavelength. In physical implementations of a fluorescent detection system, the sample is excited by an LED or laser usually passed through an optical bandpass filter. This is so the excitation occurs on the exact wavelength required by the specific primer. The system then observes the sample using a photodiode or similar detector behind another optical bandpass filter centered on the specific wavelength emitted by the primer. Since the primer will only bond to a specific sequence of DNA, a PCR system equipped with a fluorescent detection system can be extremely sensitive at measuring very specific target DNA. Since the primers can be designed to bond to any sequence of DNA, a primer can be made that will bond to a sequence only found in a virus, such as HIV. Using this feature, a PCR device can be made to search for and detect any virus present in a sample, thus allowing for accurate and reliable diagnosis of a patient. The optical detection system will form the heart of the qPCR machine.

To design an appropriate optical system, several challenges were faced. The main issue with the design is placing all of the necessary optical components without interfering with the heating element. To face this challenge, the layout shown in Fig. 4 was designed. In this design, only a small additional feature must be added to the heating element, that is a small hole must be drilled into the side to allow for the entrance of light. The source of light is the LED located towards the bottom of the system and perpendicular to the PCR tube. When a measurement is to be taken, the microcontroller enables the LED and light is emitted. This light travels through the excitation filter which filters out undesired wavelengths. This light is now of a very narrow band of wavelengths and is blue in color. Since the wavelength is quite small, 470 nanometers, the energy carried by it is quite high. As such, when the blue light strikes the fluorescent probes in the sample, the probes are excited and emit light at a lower energy, or longer wavelength, in this case 518 nanometers, a wavelength in the green portion of the spectrum. This green light travels up through the lid of the PCR tube and through the emission filter. The emission filter is another narrow bandpass optical filter centered at 518 nanometers. This filter serves the purpose of making certain that all of the light which reaches the photodiode is green in color. In this way, crosstalk introduced by the blue emission light reaching the photodiode is at the lowest possible value and the reading from the photodiode is a more accurate representation of the amount of target DNA in the sample. The transmission curves of these filters are shown in Fig. 5. Notice that there is no overlap in the passbands, thus, crosstalk is eliminated.

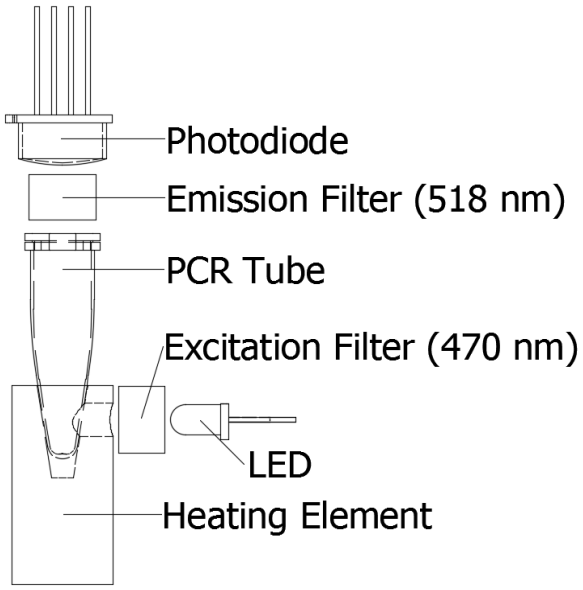


Fig. 4 Optical components of the qPCR device

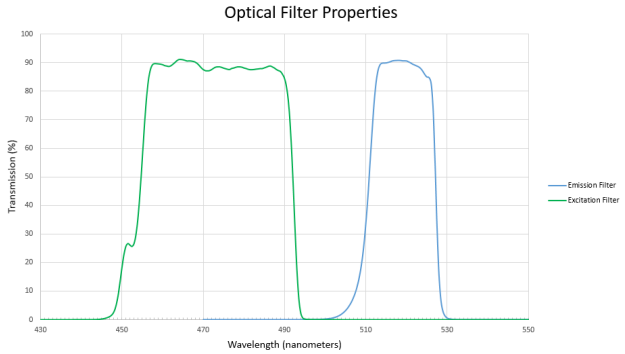


Fig. 5 Plots showing the passband characteristics of the chosen optical filters. [1] Note that there is no overlap of the two bandpass filters

IV. SOFTWARE DESIGN

The software will be responsible for taking in inputs from a few sources, interpreting them correctly, and using that data to quickly and accurately send output signals to control the main processes to be checked again. The initial and most important inputs are those from the user interface. Weather they are buttons, a touch screen, or from the android application should not affect the progress but the code will account for inputs from different areas coming from simple button signals, serial interface information from a UART connected to a touch display, or signals from Bluetooth connections. After processing these signals the code should tell the system to start the main process, sending signals to the heating blocks to being to up the current dedicated to them to begin heating.

The program will begin reading signals from the Thermistor to process with PID control to determine the current temperature and adjust output signals to the heating blocks accordingly. Once at the desired point, the program should recognize it is time to flash the led, sending a quick signal to it. Simultaneously, the program should take a quick reading of the photodiode and use the data to calculate the DNA concentration in the sample. Also at about the same point it will begin to signal the servo to move the sample to the next temperature block to repeat the process. As the process is repeated, the MCU will keep track of the cycle count and graph the results slowly, sending the information to be displayed on the user interface either through the UART for the screen or Bluetooth to display on the app. Fig. 6 shows the software flow of the program.

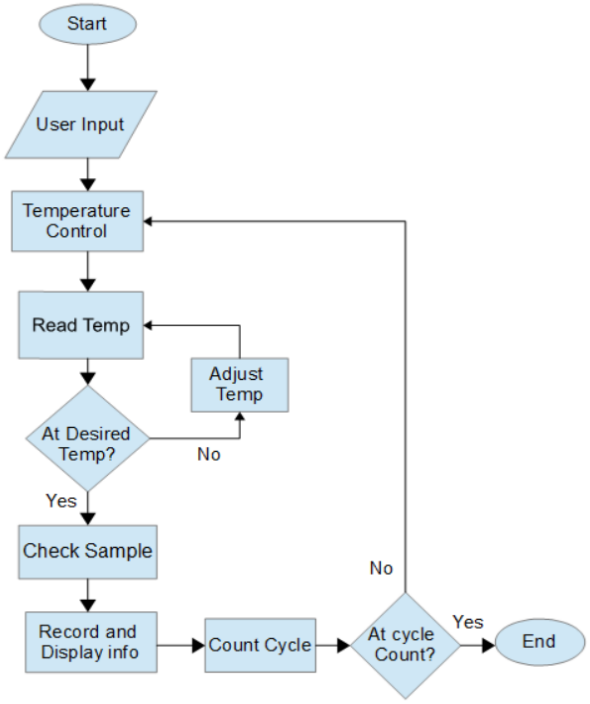


Fig. 6 This flowchart shows the program flow used to control the PCR machine.

A. Application Structure

The application designed to work with the PCR machine will be a linear string of prompts and commands with an option to cancel the present test at any time. This process is shown in Fig. 7. The sequence will consist of five main steps:

- Connecting via Bluetooth
- Inputting the settings and user information
- Running the test
- Showing results to analyze
- Submitting results and an option to repeat the process.

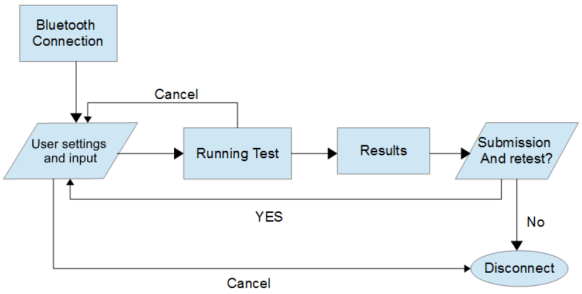


Fig. 7 This flowchart shows the program flow of the Android application connected via Bluetooth.

B. Online Databases

A major focus for the portable PCR machine is to be able to gather information anywhere with ease and send this data to a single large database for future studies. The database will be used to study trends in location, age, gender, and even over the course of certain time periods (Seasons, weeks, months, years, etc.). Analysts and doctors can use the information to track the progress of the diseases and take action accordingly. This could reveal hidden seasonal activity, trends in age groups, show where outbreaks may be concentrated, or even reveal how different cultural groups and areas may suffer differently from exposure to the diseases.

V. SCHEMATICS

To use the selected photodiode with the chosen microcontroller an amplifier was required. This amplifier is used in a configuration which is known as a difference amplifier. It allows for the amplifier to produce the required gain which can be set by the resistors, but not amplify the offset voltage caused by the photodiode biasing configuration. This circuit is shown in Fig. 8.

To supply power to the board and various components a Switch Mode Power Supply (SMPS) will be used in the Buck-Boost topology. A switched-mode power supply regulates either output voltage or current by switching ideal storage elements, like inductors and capacitors, into and out of different electrical configurations. Ideal switching elements (e.g., transistors operated outside of their active mode) have no resistance when "closed" and carry no current when "open", and so the converters can theoretically operate with 100% efficiency (i.e., all input power is delivered to the load; no power is wasted as

dissipated heat) The designed power supply is shown in Fig. 9.

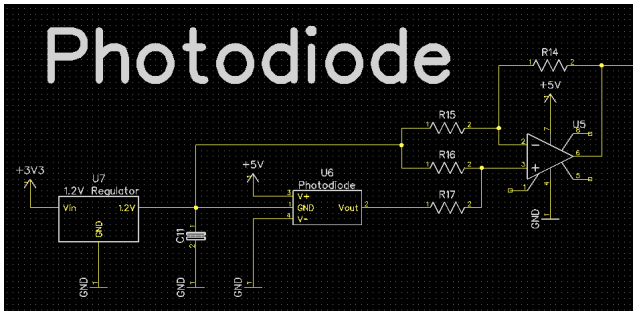


Fig. 8 Schematic of the photodiode difference amplifier

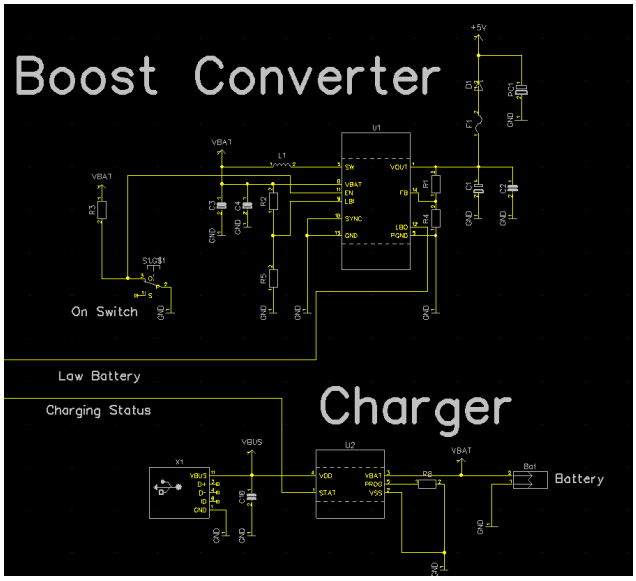


Fig. 9 Schematic of the boost converter and charger

VI. 3D MODEL

For the housing of the device, 3D printing will be used to create the parts out of plastic. During the design process, Autodesk Inventor CAD software was used to design the parts and ensure that the structure would work in 3 dimensional space. The model is shown in Fig. 10. This model was used to print the 3D printed parts.

As seen from the figures the LCD is going to be on the top with the PCB right below the LCD. This section will be the area at which our device can be opened to be able to insert and retrieve the PCR tube. The Bluetooth Module as well as the GPS will will be on the PCB. The servos, battery, PCR tube heating elements, LED, Photodiode, and filter will be on the the second half of the housing, beneath the LCD and PCB.

For the process of thermocycling, it is necessary to move either the PCR tub or the heating and cooling elements with respect to each other to control the sample's temperature. We have decided to use the former method where the PCR tube will remain stationary and the heating and cooling elements will move with respect to the PCR tube. This decision was made so that the optical system can remain stationary and therefore, less prone to errors. The system we have designed consists of two servomotors that move the mechanical components. One of the servos controls the angular position of an arm that will hold the heating and cooling elements, allowing for the selection of either heating, or cooling. The other servo controls the vertical position of the arm, this allows for the heating and cooling elements to be raised to the position of the PCR tube. Combining these two actions allows for the temperature of the PCR tube to be controlled by selectively placing the tube in either the heating or cooling mode of operation. These two operations are shown in Fig. 10. For the user to insert the PCR tube in order to perform an experiment, the top part of the device opens up to allow access to the internals. The figure also shows the location of the custom PCB which holds all of the electronic control components.

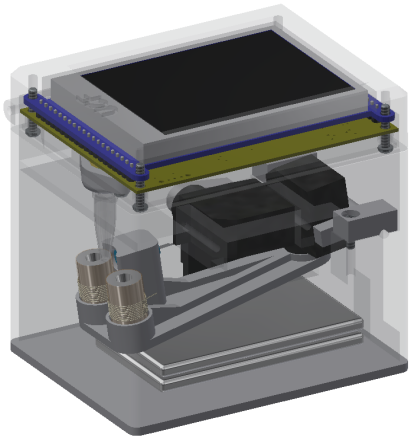


Fig. 10a The 3D model representation of the device in its closed configuration

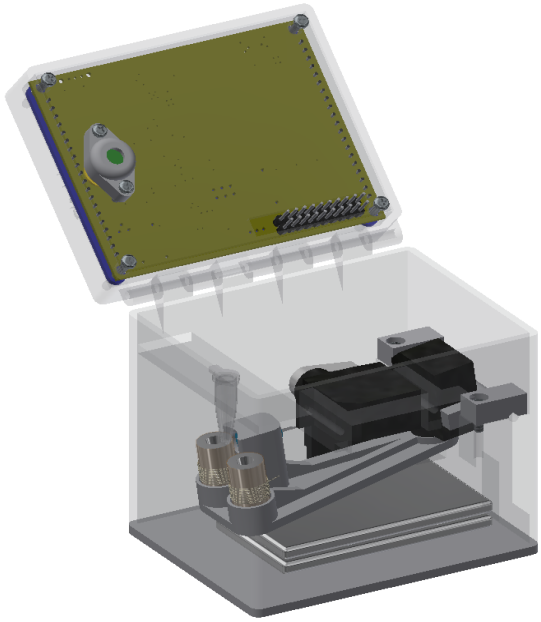


Fig. 10b The 3D model representation of the device in its open configuration

VII. PCR TESTING

The overall goal of the project is to design and build a device which is able to perform quantitative PCR. In order to be certain that the qPCR device is able to detect the presence of a virus, tests will need to be performed on samples which contain a known virus quantity, as well as be able to differentiate positive samples and controls. Traditionally, tests of real time PCR machines use test runs of multiple virus concentrations in order to create a plot of multiple concentrations on the same graph. Usually, the difference in virus concentration between these tests is a factor of 10. Because of the characteristic of PCR where the number of DNA sample doubles with each cycle, the number of DNA molecules in the sample with respect to a given number of cycles n is:

$$\#DNA_{final} = \#DNA_{initial} * 2^{n}$$

So if the same final point is referenced, the equation can be rewritten to solve for the cycle number where the final DNA concentration is crossed:

$$n = log_2(\#DNA_{final} / \#DNA_{initial})$$

Thus, if the final concentration is the same value for two experiments and the initial concentration is an order of magnitude, or ten times more dilute, then the cycle where the threshold is crossed will shift by ~3 cycles.

$$n = log_2(1 / 0.1) = 3.01$$

To further illustrate what is expected to see, the following plot is provided by Dr. Kim's lab. These tests were run using three different virus concentrations of $2x10^7$, $2x10^6$, and $2x10^5$; as well as a control, containing no virus. It is clear what it meant by this shift of three cycles by looking at the graphical representation in Fig. 11. Notice that each curve with the exception of the control, is approximately three cycles to the right of the one before it. This indicates that the concentration for each run is 10 times more dilute than the one before it.

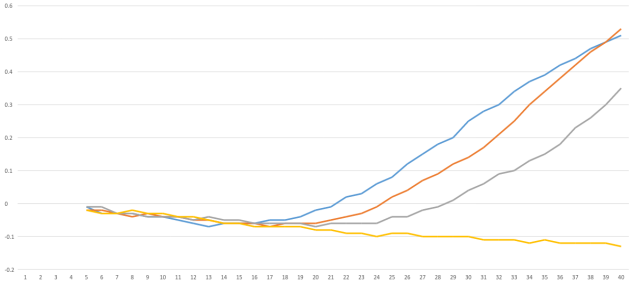
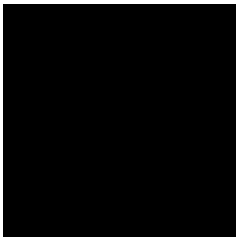


Fig. 11 Experimental data that should be replicated by new PCR machine. The four curves, blue, orange, gray, and yellow, are the samples containing 10^7, 10^6, 10^5, and the control respectively.

VIII. SPONSORSHIP

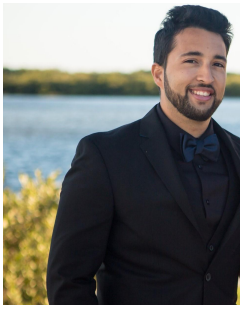
Through the course of our project we have worked with a UCF faculty member. Dr. Brian N. Kim has sponsored our efforts. Dr. Kim's lab specializes in the field of bioelectronics which includes diagnostic devices similar to the one being proposed by the group. Additionally, Dr. Kim's lab is located at the Burnett School of Biomedical Sciences Lake Nona Campus, which allows the group to perform medical experiments in the lab in order to confirm the functionality of the device. Without access to a biosafety lab, these experiments would not be able to be performed. Dr. Kim is appointed by both the College of Engineering and Computer Science and the College of Medicine.

IX. BIOGRAPHY

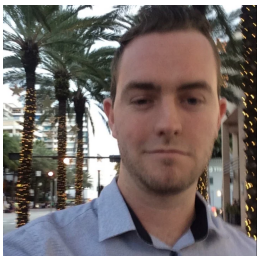


Geoffrey Mulberry will graduate from the University of Central Florida in the summer of 2017 with a B.S. of Electrical Engineering. He is currently an undergraduate research assistant in Dr. Kim's lab and will go on to graduate school at UCF beginning in fall of 2017. He is especially interested in the

integration of multiple disciplines such as mechanical, electrical, and optical engineering into one functional device.



Mario Martinez will graduate from the University of Central Florida in the fall of 2017 with a B.S. in Electrical Engineering. He will begin interning at a company of his choice. His focused discipline is in renewable energy and hopes to help to contribute aiding the world of the energy issues.



Marc Burrell will graduate this summer from the University of Central Florida with a B.S. in Electrical Engineering and Computer Engineering. He is currently working for Lockheed Martin and will possibly go to graduate school for Biomedical Engineering.

X. REFERENCES

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