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IRVINE

Spin Stand for Extreme Point of Care Diagnosis

THESIS

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by

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DEDICATION

To: Mom, for helping me with everything from kindergarten homework to college papers and projects! She is my favorite ninja. To: Dad, for nurturing my curiosity about how things work and for encouraging me to take opportunities. To: my siblings, for the laughter that keeps me sane. To: my grandparents and my aunts and uncles, for all the love they give to me.

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Abstract

Title

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Access to clean drinking water is necessary to maintain healthy populations. Remote locations often do not have the equipment or the means to test water quality. A rapid, affordable, user friendly, portable system that can detect and analyze pathogens in water is discussed in this thesis. The system is designed to optically quantify pathogens by digital droplet PCR (Polymerase Chain Reaction) using fluorescence detection and Lab-on-a-Disk technology. A sample microfluidic disk is inserted into the system. The system uses centrifugal forces to break apart cells within the sample, mix reagents with the sample, and generate droplets. Infrared heating is then used to amplify DNA within the sample. Fluorescent images of the droplets within the microfluidic disk are captured. The system functions as designed.

Introduction

The goal of this project is to design the hardware and software for an extreme point of care diagnostic tool. When trying to diagnose disease in remote locations, getting samples to a lab can be non-trivial. By the time a sample gets to the lab, it could be too late to preform proper treatment. In these cases, time is precious; so a way to save time (and, untimely, save lives) will be to cut out the travel time and bring the lab to the point of care. This project builds on the technology developed for lab-on-a-disk applications. It incorporates microfluidic assays, temperature control, PCR and florescence detection, into a single portable system that can transmit data wirelessly. The system is meant to be inexpensive to build and easy to replicate. The project is a collaboration between Michael Hoffman's lab from the California Institute of Technology and Marc Madou's lab from the University of California, Irvine. Development for the system is funded in conjunction with the Reinvent the Toilet Challenge, put on by the Bill and Melinda Gates Foundation. The immediate goal for the system is to detect pathogens in wastewater.

1 Extreme Point of Care, Background and Motivation

The system described in this thesis is a portable pathogen analysis system. It was designed in order to meet specific goals: 1) to be affordable: $2/Test, $5k Capital, 2) to be sensitive: 50 targets/Reaction, 3) to be user-friendly: sample in answer out, 4) and portable. These goals tailor the system to be used in Extreme Point-of-Care (EPOC) applications.

Section 1.1 will look at how the system fits into extreme environments and developing countries. Section 1.2 gives a brief look into Lab-on-a-Disk technology. Section 1.3 reviews digital droplet PCR.

1.1 Extreme Point-of-Care

In a review published in 2016 titled, "CD-Based Microfluidics for Primary Care in Extreme Point-of-Care Settings" by Suzanne Smith et al., the authors report on health care challenges in the developing world [1]. They describe how unreliable electricity, lack of trained staff, harsh environments and the distances patients have to travel affect the ability of patients to receive proper treatment. Smith et al. use these reasons to show that extreme point-of-care solutions are needed by today's world saying, "Providing comprehensive primary care in under-resourced settings is a paramount global challenge, which can be clearly addressed by innovative, effective point-of-care (POC) diagnostic technologies, which are compatible with these extreme environments" [1]. The system discussed in this thesis sets out to be one of those "innovative, effective POC diagnostic technologies" as described by Smith et al.

In a review published in 2016 titled, “Challenges and Opportunities of Centrifugal Microfluidics for Extreme Point-of-Care Testing” by Issac J. Michael et al., the authors look at the current limitations of Extreme Point of Care Testing (EPOCT). They describe how current EPOCT technologies rely on lateral flow strips (LFSs) for disease screening but that centrifugal force-based systems are needed to perform advanced bioassays. Michael et al. look at where research in the field of EPOCT is heading saying, “Recent publications demonstrate significant interest in the microfluidic research community in exploring the advantages of centrifugal microfluidics for diagnostics in developing countries” [2]. The system discussed in this thesis looks to take advantage of “centrifugal microfluidics for diagnostics in developing countries” as mentioned by Michael et al.

1.2 Lab-on-a-CD

In a review article published in June 2015, “Lab-on-a-CD: A Fully Integrated Molecular Diagnostic System” Ling X. Kong et al. describe the current and future states of Lab-on-a-CD technology. Kong et al. describe the forces used in current Lab-on-a-CD technologies saying:

> “A simple motor generates several pseudo-forces on the platform: the centrifugal force, which acts as a liquid pump and generates a force gradient affecting fluids differently at varying radial positions; the Coriolis force, which allows for direction-specific liquid-pumping control; and the Euler force, which can be used to create turbulence during mixing.“

Kong et al. analyze geometries and methods to achieve operations such as storage and dispensation of reagents, efficient sample preparation, nucleic acid (NA) amplification, and rapid detection [3]. The system, described in this thesis, uses some of these geometries and methods to preform assays.

1.3 Digital Droplet PCR

In a paper published in 2015 titled, "Centrifugal step emulsification applied for absolute quantification of nucleic acids by digital droplet RPA," Friedrich Schuler et al. describe the uses and methods of producing aqueous droplets. In their introduction Schuler et al. state:

> "One of the most important applications of aqueous droplets is the use in digital amplification techniques such as digital PCR (dPCR) [4]. Digital PCR offers many advantages over the bulk reaction [5], such as absolute quantification without the need for standards and much higher accuracy and sensitivity."

The system aims to use the advantages of Digital PCR referenced by Schuler et al. [6].

In a paper published in 2016 titled, "Digital droplet PCR on disk" Friedrich Schuler et al. present a disk design that integrates droplet generation, PCR and fluorescence readout. Droplets are generated within a microfluidic disk on a spinning platform. The disk is then transferred to a thermocycler and transferred again into a microarray scanner [7]. This paper shows that digital droplet is possible on a disk. The system described in this thesis

aims to integrate droplet generation, thermal cycling and imaging into a single platform thus removing the need to transfer the disk to multiple platforms.

2 System Overview

The spin stand performs digital droplet Polymerase Chain Reaction (PCR) to detect pathogens in wastewater. The assay includes cell lysis, or the breaking apart of cell walls, mixing, droplet generation, thermal cycling to perform DNA amplification and florescence detection. The spin stand integrates all parts of the assay into a single platform. The individual subsystems are controlled by a central processing unit and displayed on a LCD screen. The subsystems include 1) motor control, 2) temperature control, and 3) florescence detection. The system block diagram is shown in Figure 1.

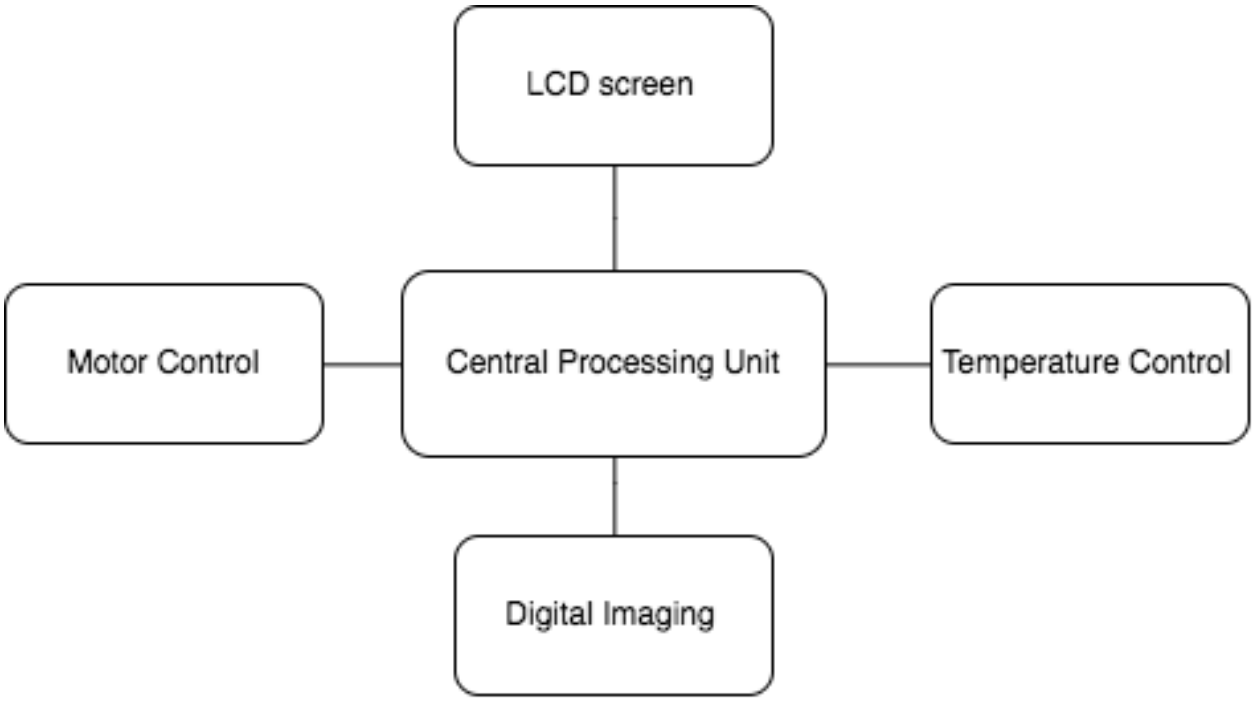

Figure 1: System Block Diagram

2.1 Central Processing Unit

The Raspberry Pi was chosen as the central processing unit (CPU). A Raspberry Pi (Figure 2) is a low cost credit-card sized computer [8], which provides a platform that allows for quick integration of multiple sensors and actuators. The Raspberry Pi, which receives data from the motor, temperature controller and imaging systems, controls motor speed and position as well as CD temperature. The Raspberry Pi also analyses the images to determine the amount of DNA in the sample.

Figure 2: Raspberry Pi 3

2.2 Display

The system uses a 7-inch liquid crystal display (LCD) screen to interface with the system and display data (Figure 3). It is connected to the CPU via DSI interface. The system employs a graphic user interface that allows the user to alter various parts of the assay such as spin speeds, temperature and imaging.

Figure 3: LCD screen

2.3 Motor Control

The motor (Figure 4) is the heart of the spin stand. The disk relies upon centrifugal forces to move through cell lysis, mixing and droplet generation. The CPU communicates with the motor driver board over a USB interface. Position data from an optical sensor, which is attached to the motor shaft, is encoded and sent to the motor driver as feedback for motor position. A block diagram of the motor control subsystem is shown in Figure 5.

Figure 4: Motor

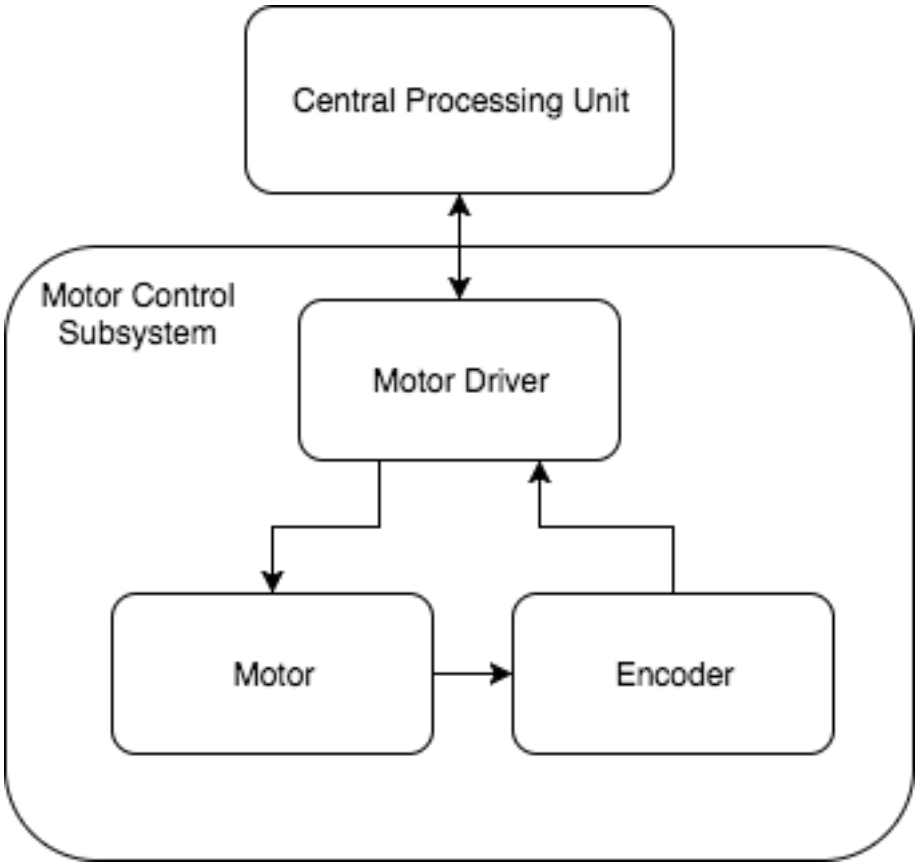

Figure 5: Motor Control Block Diagram

2.3.1 Mechanical Cell Lysis

Within the lysis chamber of a sample CD there are magnetically soft disks. The chambers are passed through a stationary magnetic field and the magnetically soft discs move back and forth within the lysis chamber causing the cell walls to break apart. The spin stand uses an array of neodymium magnets to create a stationary magnetic field. As the CD spins, the discs inside the lysis chamber are pushed radially outward. The magnets are placed radially inward from the chamber. As the chamber approaches the magnets, the

discs are pulled in toward the center of the disk. This results in the magnetically soft disks oscillating within the lysis chamber. As the disks move back and forth with in the sample, the cell walls are broken apart and DNA is released.

2.3.2 Mixing and Droplet Generation

Mixing of the samples with reagents and droplet generation are primarily functions of disk geometry and rotation speed. The spin stand must be able to rotate the CDs at different speeds in order to move the sample from chamber to chamber. Spin speeds are determined experimentally. The reagents allow the specific strains of DNA to fluoresce. Any DNA contained in a droplet will be amplified through PCR and will then be detected through digital imaging.

2.4 Temperature Control

In order to perform DNA amplification through PCR, the system must be able to control the temperature of the samples within the disk. The disk temperature cycles between 60°C and 95°C and then ends with a denature stage at 98°C. The system is made up of Infrared (IR) lamps, and IR sensor and a blower. The Infrared (IR) sensor is connected to the CPU through an Analog to Digital Converter (ADC). The lamps and blower are connected to the CPU through their own individual driver boards. A block diagram of the temperature control system is shown in Figure 6. The CPU uses proportional-integral-derivative (PID) control to move quickly from one temperature to the next.

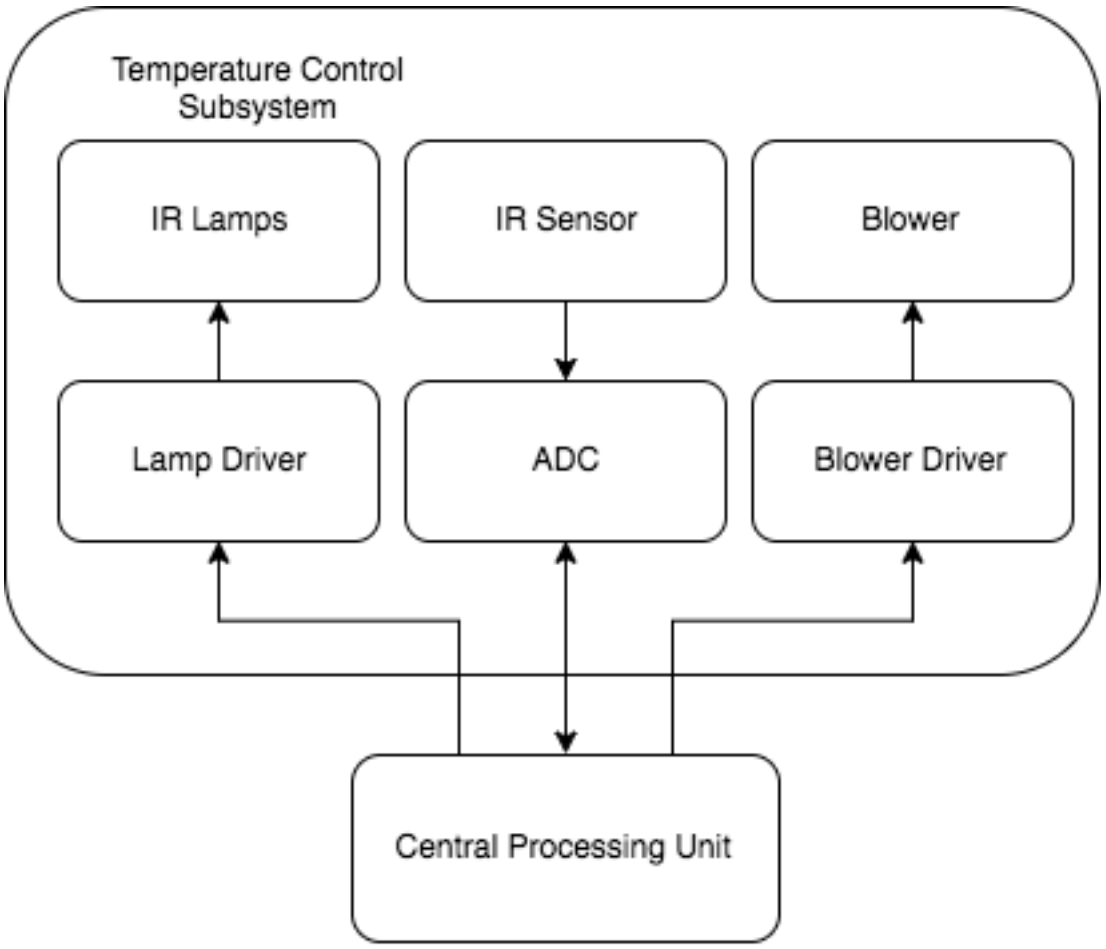

Figure 6: Temperature Control Subsystem

2.4.1 Infrared Lamps

An array of 5W Infrared (IR) lamps is used to heat the radius of the disk that houses the droplet chambers. The infrared lamps are connected to the CPU through a driver board. The CPU controls the IR lamps using a pulse width modulated (PWM) signal.

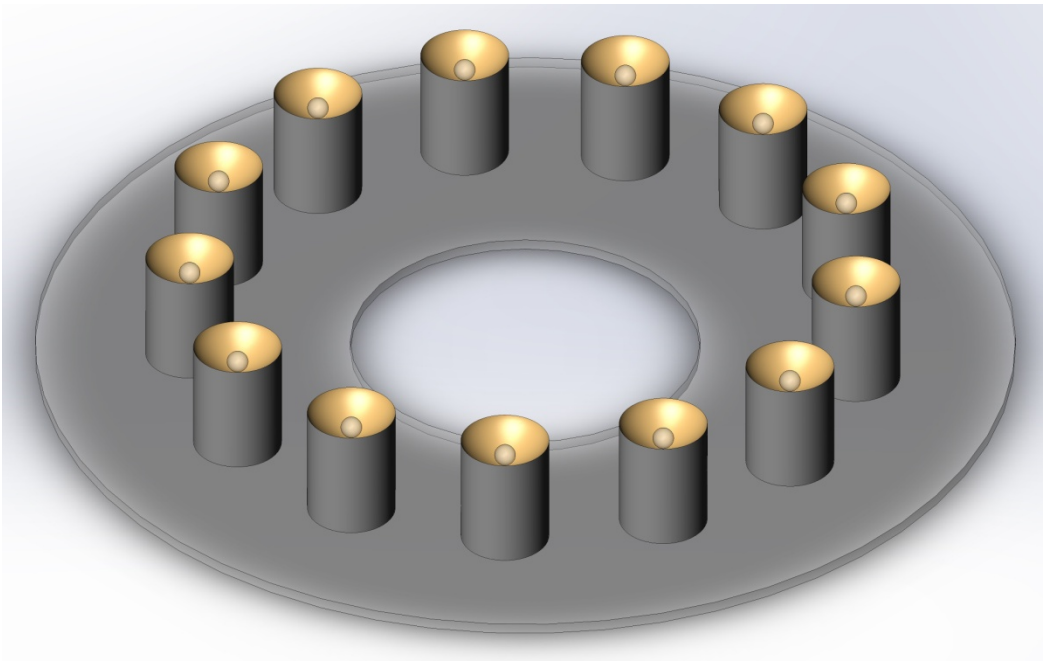

Figure 7: IR Lamp Array

2.4.2 Blower

A 12V blower as shown in Figure 8 is used to remove heat. This blower is connected to the CPU through a driver board and is controlled by a PWM signal.

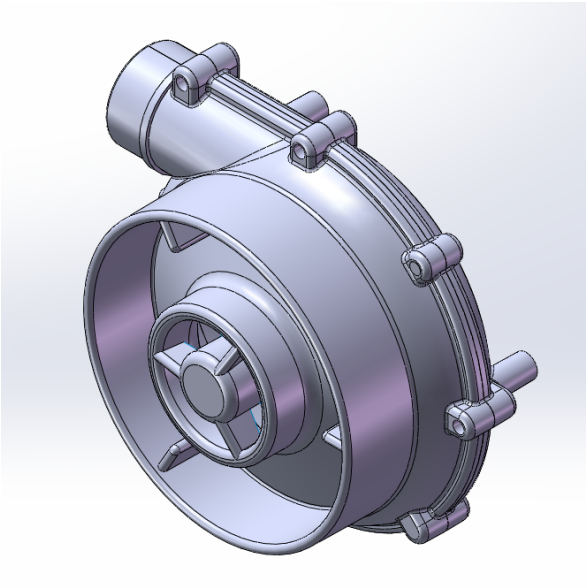

Figure 8: 12V Blower

2.4.3 IR Sensor

An IR sensor, as shown in Figure 9, gives feedback to the temperature controller. The analog signal from the IR sensor is converted to a digital signal and read by the CPU.

Figure 9: Infrared Sensor

2.5 Florescence Detection

Once the DNA has been amplified the chambers are ready to be imaged. The imaging detection subsystem includes excitation LEDs, thin-film dichroic filters and a camera. The LEDs are turned on and off by relays controlled by the CPU. The filters are on a slider that is controlled by relays. The camera is connected to the CPU via USB interface. The block diagram for the florescence detection subsystem is shown in Figure 10.

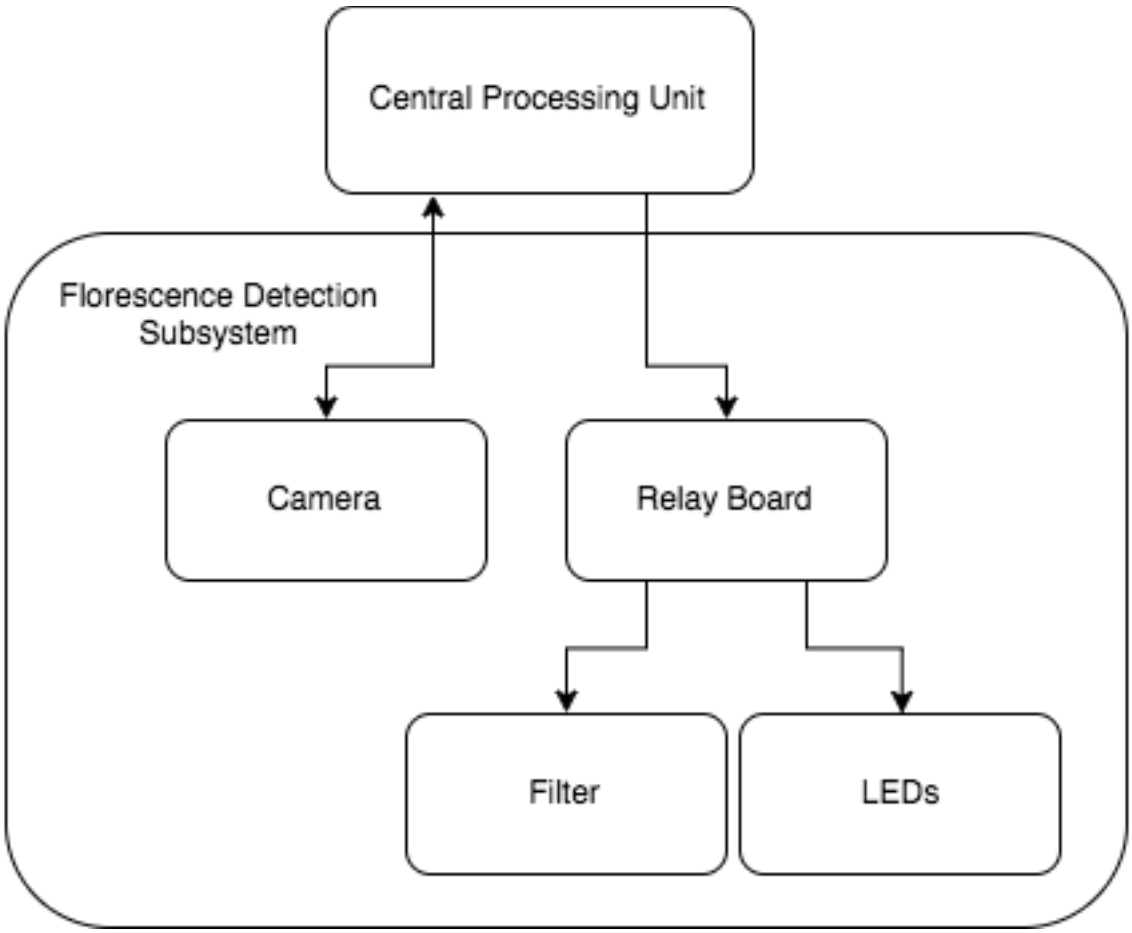

Figure 10: Florescence Detection Subsystem

2.5.1 Excitation LEDs

The droplets are excited by LEDs. There are two wavelengths of excitation: 450nm and 525nm. The LEDs operate between 900mW and 1020mW. They are mounted on a Metal-Core Printed Circuit Board (MCPCB) as shown in Figure 11. The MCPCB is designed to provide high-power output in a compact package [9].

Figure 11: LEDs

2.5.2 Filters

Because the droplets fluoresce at a different wavelength compared to their excitation, thin-film dichroic filters allow for cleaner imaging of the droplets. There are two filters that correspond to the two wavelengths of LEDs. For green fluorescence, the absorption maximum is at about 450nm and the emission maximum is an about 500nm. For the orange fluorescence, the absorption is at about 530nm and the emission at about 590nm.

The dichroic filters block the lower wavelengths of the LEDs and let the higher wavelengths of the florescent droplets pass to the camera. The filters are on a linear slider as shown in Figure 12. The CPU controls a motor, which actuates the slider.

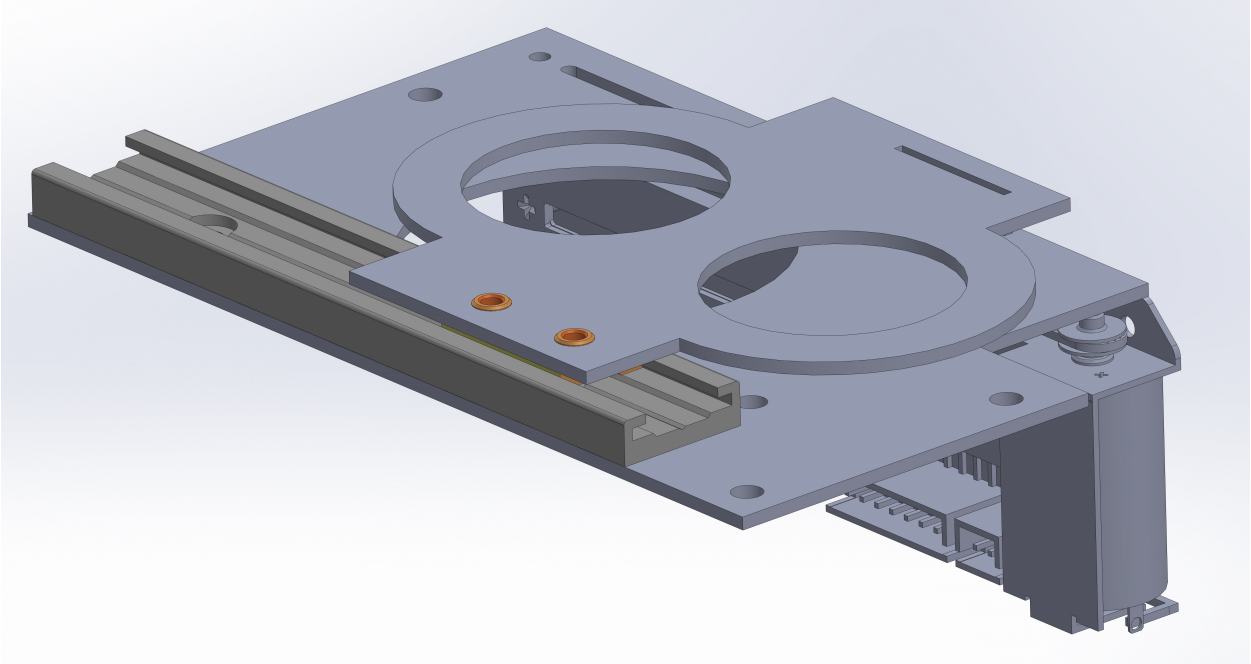

Figure 12: Filter Holder and Slider

2.5.3 Camera

The camera for this system is a Raspberry Pi Camera as shown in Figure 13. This camera was chosen for ease of integration with the Raspberry Pi. Open source libraries and tutorials make this camera ideal for rapid prototyping. The camera is connected to the CPU via CSI interface; it has a 5-megapixel resolution, and has a fixed focus lens onboard. In terms of still images, the camera is capable of 2592 x 1944 pixel static images, and also supports 1080p30, 720p60 and 640x480p60/90 video[10].

Figure 13: Raspberry Pi Camera

3 Hardware

3.1 Power Management

There are 4 voltage levels used by the system: 12V, 36V, 5V and 3.3V. The system takes in 12V and powers the IR lamps and the blower. The motor is powered by 36V. The control for the lamps, blower and filter slider are given by 3.3V PWM signals. All other subsystems are powered by 5V.

3.1.1 12 Volts

The system is designed so that it can be plugged into a car cigarette lighter. A 12V 15Amp Power Converter, as shown Figure 14, is used to simulate the power supplied by a cigarette lighter. 12V from the converter powers the IR lamp driver and the blower.

Figure 14: 12V 15Amp Power Converter

3.1.2 36 Volts

A DC-to-DC converter, as shown in Figure 15, is used to convert 12V from the supply to 36V for the motor.

Figure 15: 12V to 36V converter

3.1.3 5 Volts

A 12V DC to 5V DC Step Down Converter, as shown in Figure 16, is used to supply the Raspberry Pi, LCD screen, filter slider, camera, IR sensor and motor driver.

Figure 16: 12V to 5V Converter

3.1.4 3.3 Volts

The Raspberry Pi outputs 3.3V PWM signals. Three separate 3.3V signals are used to switch the filters, control the brightness of the IR lamps, and regulate blower speed.

3.2 Central Processing Unit – Raspberry pi

As previously mentioned, the Raspberry Pi controls the system. The functionality of the Raspberry Pi exceeds the needs of the project but the ease of integration and the low cost make it the ideal for early prototypes. Open source software as well as onboard components (such as: the camera interface, USB ports, Wi-Fi, Bluetooth and a DSI port) allow for incorporation of all parts of the system with relative ease as compared to building a processor from scratch or using a system without such components. The Raspberry Pi was also chosen based on the familiarity that the designers have in using said platform for other embedded systems projects. As the system matures and needs are solidified, it will be possible to move to a cheaper micro-controller. However, for the early iterations, the versatility of the Raspberry Pi justifies the extra cost.

3.3 LCD screen

The LCD screen was basically plug and play. There was no programming necessary and within minutes the Raspberry Pi combined with the screen essentially functions as a tablet. The screen operates with a 5V supply that can be provided by the Raspberry Pi GPIO pins. The only difficulty in working with the LCD screen to date is that the screen is not sensitive to double-click. This means that executing programs will either take tapping the screen repeatedly or a wireless mouse will be required for double clicking on

applications. Eventually this will not be a problem because the application will be programmed to open at start up.

3.4 Temperature control

3.4.1 Peltier Cells

Peltier cells, as seen in Figure 17, were used in the initial design for temperature control. When two dissimilar metals are joined at a junction and a current is passed through the junction a temperature gradient is generated across the junction [11]. This effect, which was discovered in 1834 by Jean-Charles-Athanase Peltier, can also be seen when joining two dissimilar semiconductors. Peltier cells utilize this property by putting an array of junctions together to get an appreciable temperature difference between two plates.

The direction of current determines which plate gets hot and which gets cold. The idea was to use the Peltier cells as heaters and then reverse the current direction and use them as coolers. Peltier cells were abandoned in the design for two main reasons: 1) Low efficacy 2) complexity of contact heating and cooling a spinning disc. In terms of efficiency, each Peltier cell drew around 0.5Amps so the number of PCR chambers that could be put on a disk was limited to the number of Peltier cells that could be powered; the design would have been limited to 2-4 cells. In terms of contact heating, a design was proposed to employ a custom micro-controller that was inductively powered and could spin with the microfluidic disk. Designing and testing the custom microcontroller proved

to not be worth the extra cost when compared to the simplicity of non-contact heating that could be done by infrared lamps.

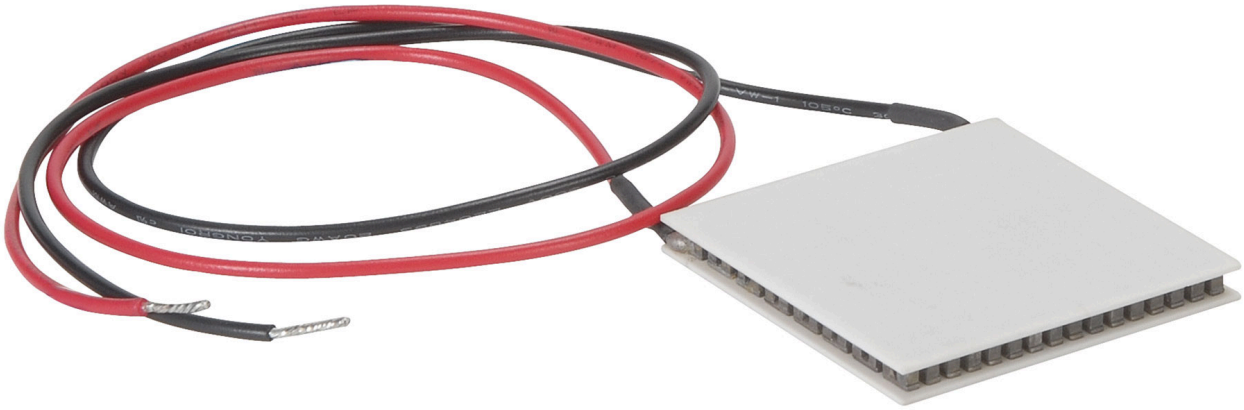

Figure 17: Peltier Cells

3.4.2 IR Lamps

Infrared lamps were chosen as an alternative to Peltier cells. IR lamps do not need to be in direct contact with the PCR chambers and thus do not require a separate micro-controller. A large single IR lamp had been used on earlier spin stands in the BioMEMS Lab. However, in this design an array of small lamps is used, making heating more uniform. The IR lamps are set in aluminum parabolic reflectors, as shown in Figure 18, that direct the light and heat in parallel rays. Initially the desire was to have the lamps placed evenly around the circumference of the disk, as shown in Figure 7, but in order to fit other components (camera, IR sensor, blower) the lamps were arranged in a half circle.

Figure 18: IR Lamps

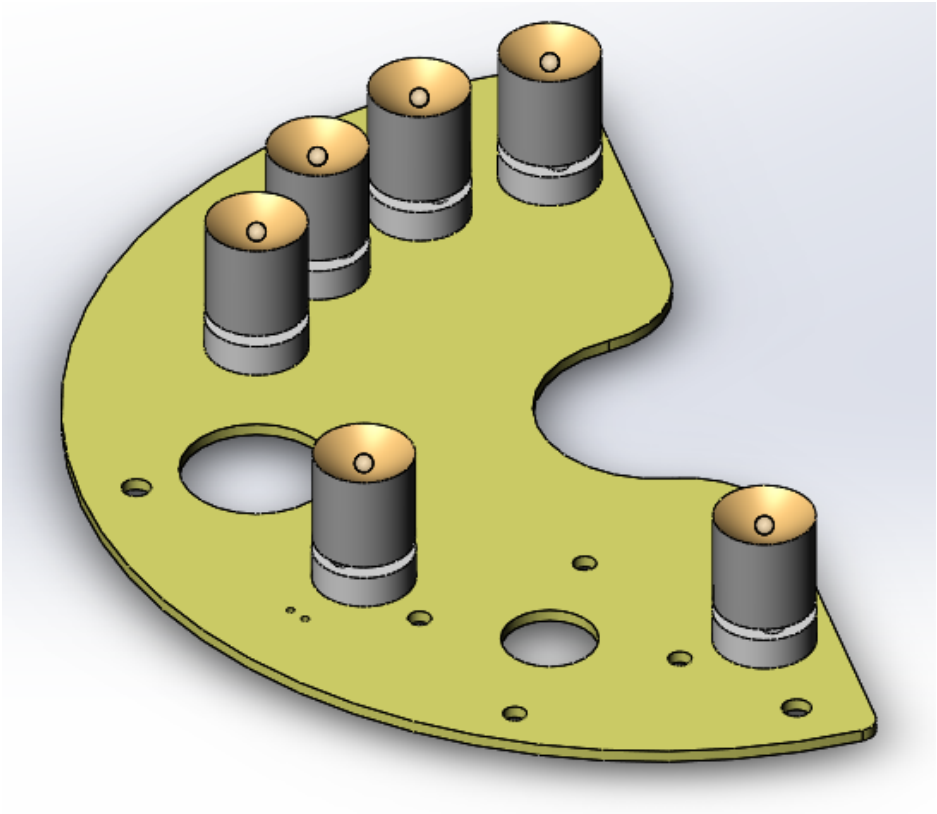

Figure 19: IR lamps and lamp holder

3.4.3 Thermistor

When contact heating and cooling with Peltier cells was part of the design, contact temperature sensing was achieved by thermistors. A thermistor is a circuit element that changes resistance according to temperature. A thermistor was to be placed in contact with each PCR chamber. A 10KΩ thermistor was placed in series with a 10KΩ resistor and the node between the two elements was connected to an analog input of an Arduino Uno. A simple tutorial of how to use a thermistor with an Arduino was found in a tutorial by Adafruit [12]. The thermistor circuit was to be built into the custom spinning micro controller. Once the decision was made to move away from contact heating, the Peltier cells and the thermistors became obsolete but the code, which was developed with these elements, persisted through consecutive designs.

3.4.4 IR sensor

The IR sensor was purchased from Process Sensors Corporation. It is capable of operating in analog and digital modes. The IR sensor's analog data is connected to an 8-bit analog to digital converter. The analog data from the IR sensor ranges from 0V to 5V. The Raspberry Pi only accepts a high of 3.3V but the system should not get hot enough to produce a 5V reading from the IR sensor. As a precaution, a 3.3V zener diode is placed across the output of the sensor to protect the Raspberry Pi.

3.4.5 IR controller board

The IR controller board was based off a controller board from a previous BioMEMS Lab spin stand. The updated board used all surface mount components, not including the

header. The previous generation controller board is 4″ by 8″ while the new board is 1.5″ by 1.7″. The schematic and board layout (Figure 20) was done using Eagle Cad software. The board was fabricated by OSH Park and assembled at UC Irvine.

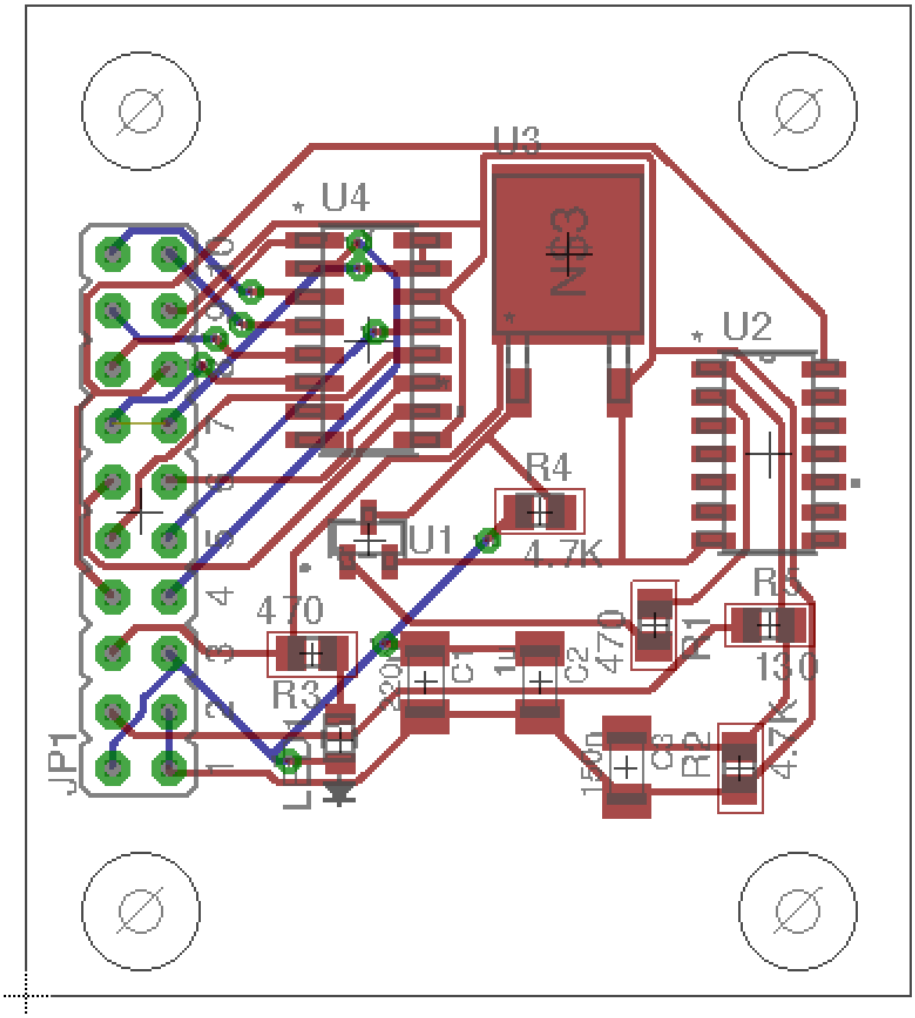

Figure 20: IR Driver Board Layout

3.5 Motor and Encoder

3.5.1 Motor

The motor, as shown in Figure 4,was purchased from Anaheim Automation. It is a 36V motor that has an upper-end speed of 4000rpm and an upper torque of 31oz-in. The speed and torque are more than what will be needed by the final assay but having extra torque and speed is helpful for experimenting with different micro-fluidic designs. Once the assay is tuned, a less powerful motor can be used.

3.5.2 Encoder

An optical encoder, as shown in Figure 21, detects motor position. The encoder senses 4000 pulses per revolution and is powered by the 5V coming from the motor driver board. The encoder allows the system to be programmed to stop the disk at precise locations in order to image the PCR chambers.

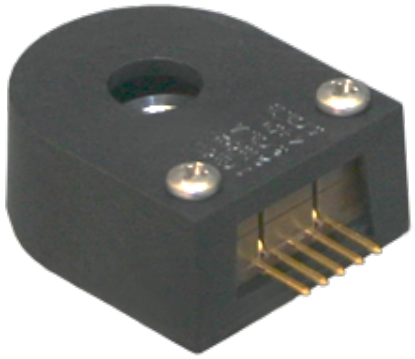

Figure 21: Motor Position Encoder

3.6 Florescence Detection

3.6.1 Cell Phone Florescence

Initial system designs included a cellphone as the CPU and the cellphone's camera as the imaging device. Initial florescence detection using a cellphone was demonstrated as shown in Figure 22. The design of the system moved away from using a cellphone because of two main unwanted characteristics: 1) cellphones become outdated annually and 2) cellphone dimensions change yearly and differ by brand. It proved more challenging and more expensive to design around cellphones compared to purchasing a much cheaper microprocessor (Figure 2) and camera (Figure 13). Working with the initial cellphone proof-of-concept was helpful in follow up iterations as it allowed the designers to become familiar with florescence and optical filtering.

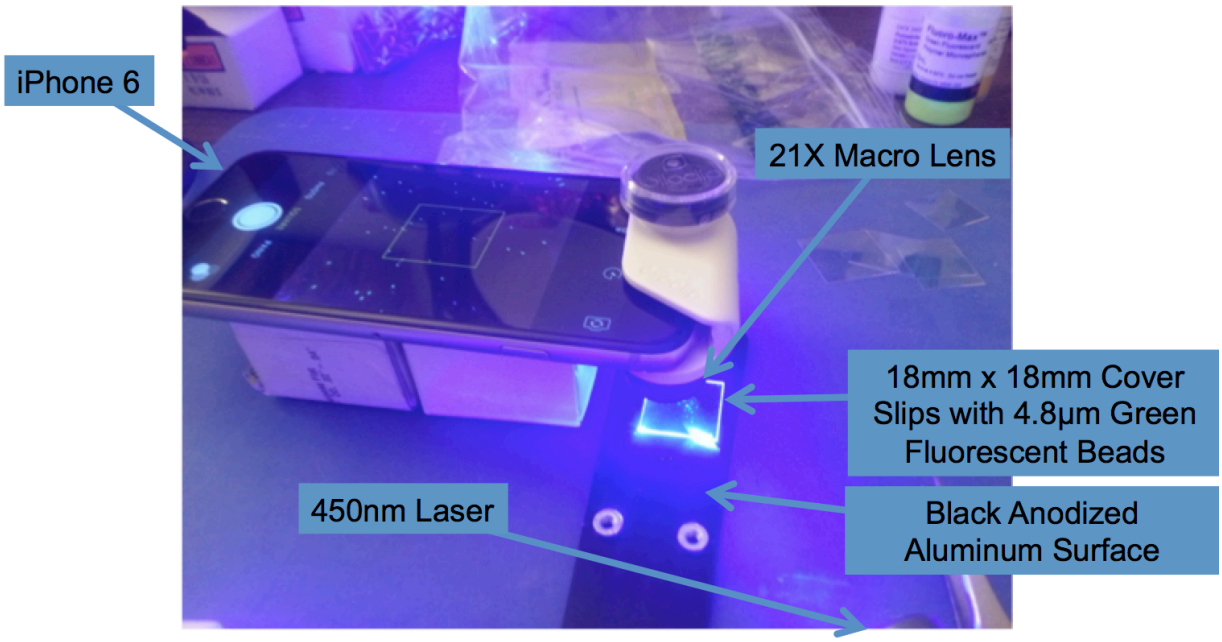

Figure 22: Cellphone Florescence Detection

3.6.2 Excitation LEDs and Filters

The system is made to detect orange and green fluorescence. For orange fluorescence, the absorption maximum is at about 530nm and the emission at about 590nm. For green fluorescence, the absorption maximum is at about 450nm and the emission maximum is an about 500nm. Light from a green LED of 530nm and a blue LED of 450nm shine onto the edge of the disk during imaging. The disk acts as a wave-guide allowing the light to illuminate the PCR chamber. Reagents are specifically chosen to allow for particular strands of DNA to be excited by the chosen wavelengths. Droplets with those particular strands of DNA will fluoresce. In order to see the florescent droplets, filters are placed in front of the camera to block the light from the LEDs. A deep amber filter was chosen to block out the light form the green LED and a golden amber filter was chosen to block out the light from the blue LED. The absorption spectra of the golden and deep amber filters are shown in Figure 23 and Figure 24, respectively.

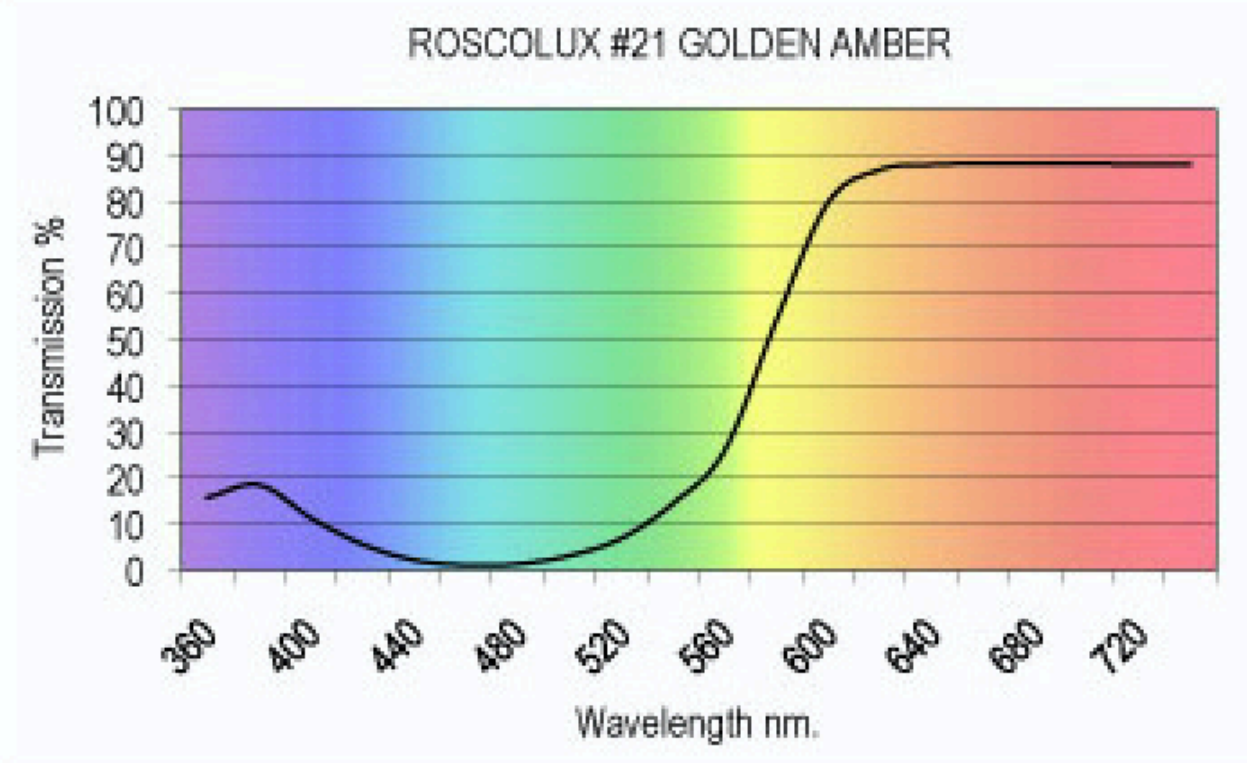

Figure 23: Golden Amber filter spectrum

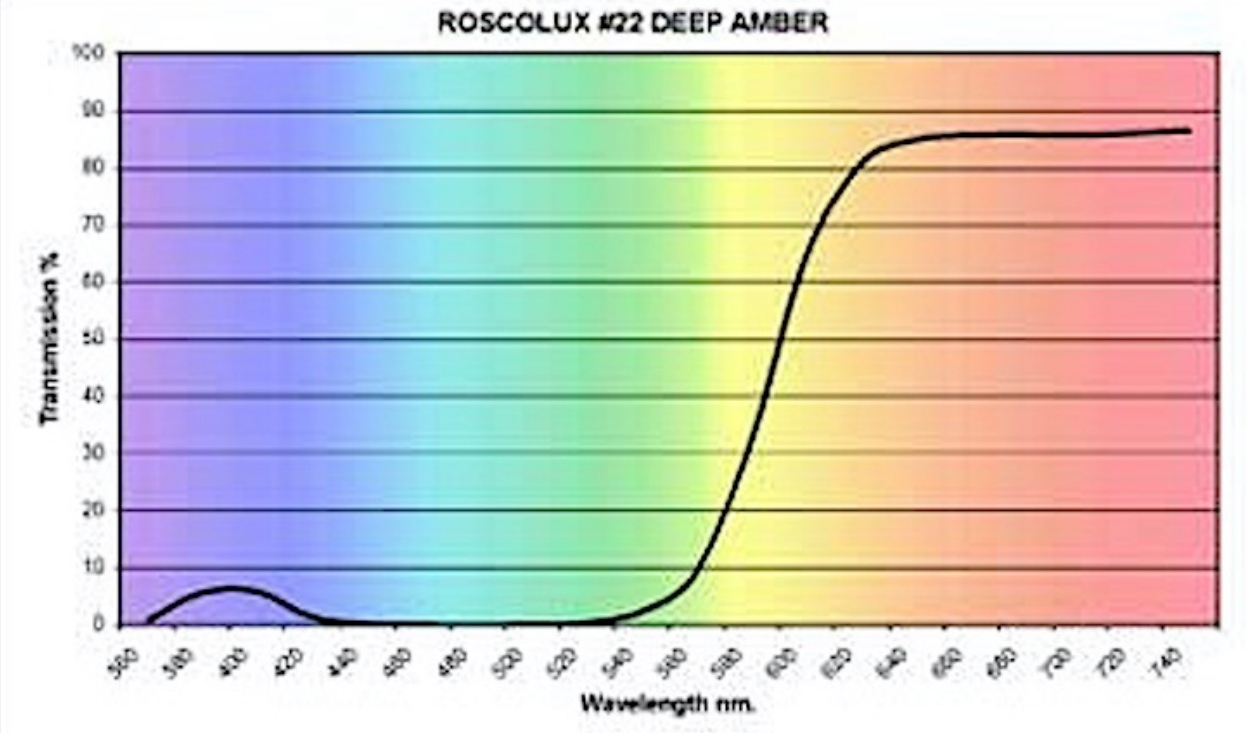

Figure 24: Deep amber filter spectrum

3.6.3 Camera and Lens

The camera, as shown in Figure 13, was purchased from Arducam. It seamlessly integrated with the Raspberry Pi. The camera has a fixed focus lens on board so an additional lens was needed to be able to focus on the droplets. The lens used is a Tamron 12VM412ASIR 1/2" 4-12mm F/1.2 Infrared Manual C-Mount Lens as shown in Figure 25.

Figure 25: Lens

4 Software

4.1 Central Processing Unit– Raspberry pi and Display

The Raspberry Pi is running the latest version of NOOBS (New Out Of the Box Software). The desktop environment on the Raspberry Pi is PIXEL (Pi Improved Xwindows Environment, Lightweight). The assay application is an executable file that is programmed in Python. The graphical user interface for the assay application is developed using the Tkinter module which is the standard Python interface to the Tk GUI toolkit [13].

4.2 Temperature control

The temperature controller uses Proportional, Integral and Derivative (PID) control logic. The code used for the PID control of the IR lamps was developed by Ivmech Mechatronics Innovation Ltd as open-source software [14].

4.2.1 IR Lamps

The IR Lamps are controlled by a PWM signal from the CPU. The commands to control the output are taken from an open source library named RPi.GPIO [15]. The functions used from this library are “setup” and “PWM”. The “setup” function tells the CPU how to allocate the General Purpose Input/Output (GPIO) pins as either an input or an output. The “PWM” function allows duty-cycle changes of desired signals. The changes in the duty cycle are determined by calculations from the PID control logic.

4.2.2 IR sensor

The IR sensor outputs voltage between 0V and 3.3V and is nearly linear with respect to temperature. It is connected to the ADC, which maps the output voltage to a digital signal, ranging from 0 to 1023. Software for reading the ADC was taken from the Adafruit website [16].

4.2.3 Blower

The blower is controlled by a PWM signal from the CPU. The same functions that are used for pulse width modulation of the lamps are used for the blower.

4.3 Motor

The motor driver accepts ASCII commands. The application sends the motor driver the serial commands that correspond to the speed and/or positions each step of the assay. The baud rate of communication between the CPU and the driver board is 9600 bits per second.

4.4 Florescence Detection

4.4.1 Camera

The commands to control the camera are taken from an open source library named Picamera [17]. The two functions used from this library are “preview” and “capture”. The “preview” function displays video from the camera onto the LCD screen and “capture” function saves camera images to the Raspberry Pi Desktop.

4.4.2 Excitation

Each excitation LED is controlled by an output signal from the Raspberry Pi. The commands to control the output are taken from the RPi.GPIO library [15]. The functions that are used from this library are “setup” and “output.” The “setup” function tells the CPU how to allocate the General Purpose Input/Output (GPIO) pins as either an input or an output. The “output” function sends an on or off signal that changes the voltage on the corresponding GPIO pin to either 0V or 3.3V.

4.4.3 Filter Slider

The filter slider is controlled by three output signals from the CPU. Similar to the LEDs, the signals for the filter slider are simply on or off. One of the signals turns the slider DC motor on and off. The other two signals determine which direction the current flows through the motor, which corresponds to forward and reverse.

5 CAD Assembly

Horacio Kido PhD designed the system’s assembly in SolidWorks. Figure 26 through Figure 29 show different CAD views of the system. The system is divided into three compartments: the disk compartment, motor compartment and CPU compartment.

5.1 Case and Frame

The case was laser cut from black acrylic sheets. Aluminum extrusions form the frame of the structure. The LCD screen holder, as shown in Figure 30, was 3D printed from polycarbonate.

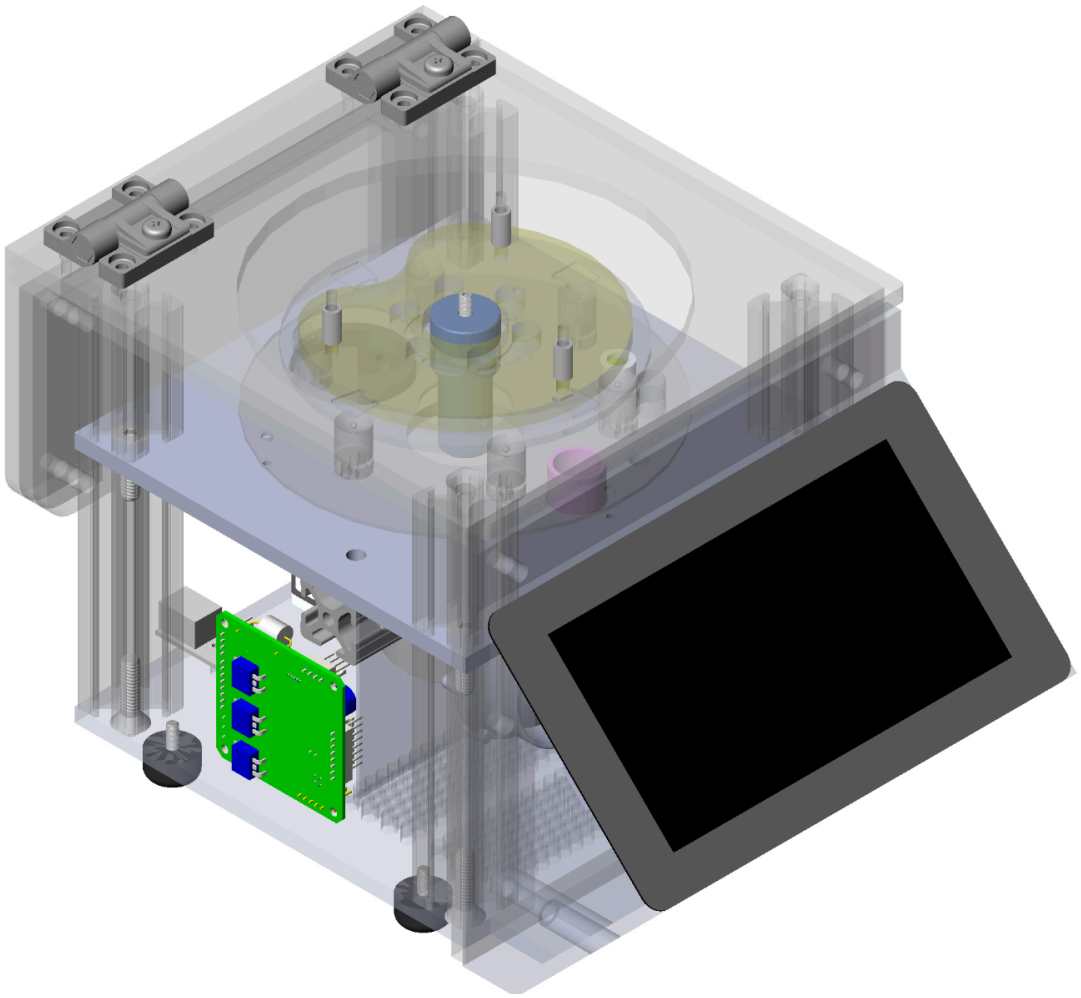

Figure 26: CAD isometric view of the system

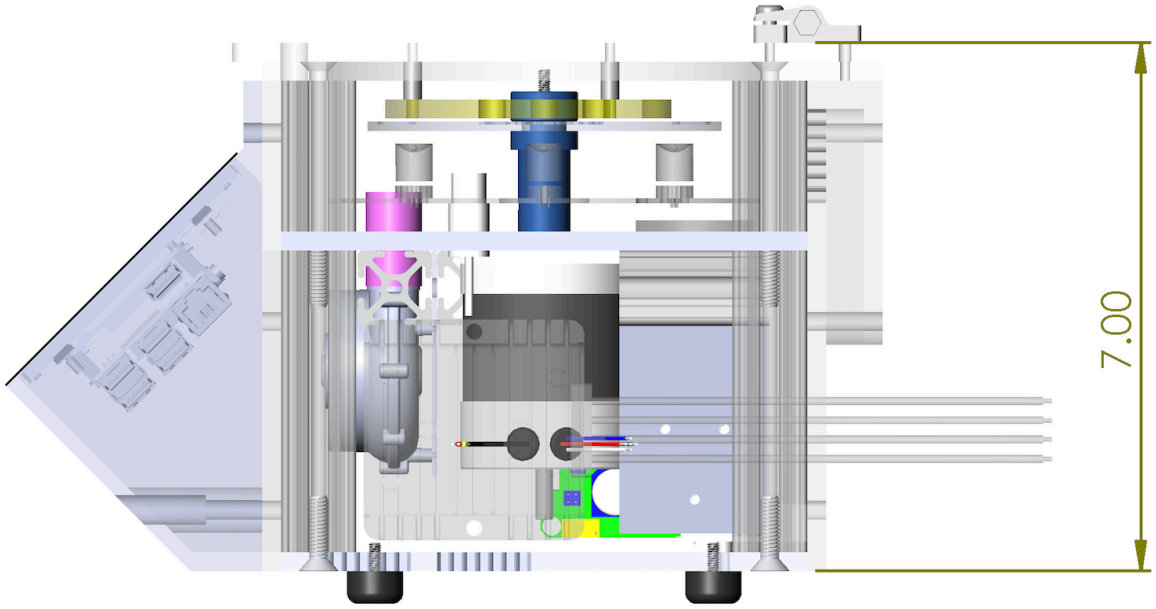

Figure 27: CAD right side view of the system

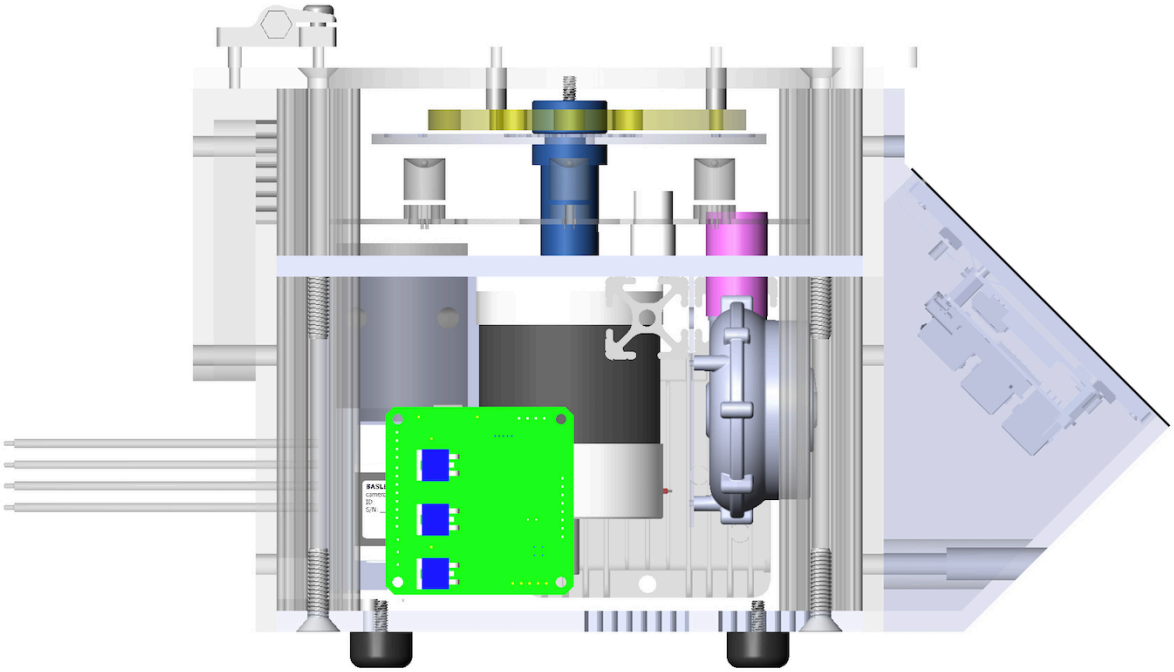

Figure 28: CAD left side view of the system

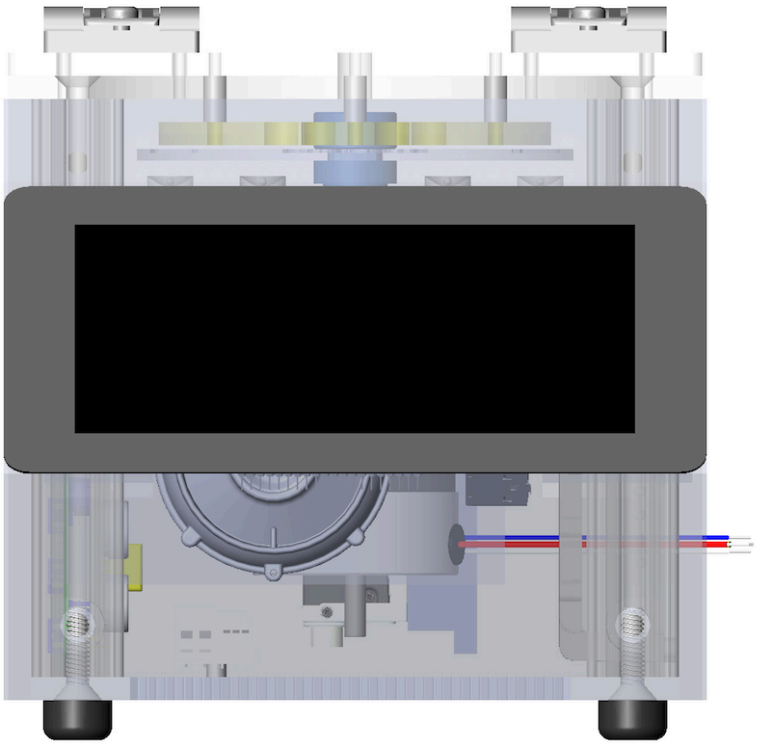

Figure 29: CAD front view of the system

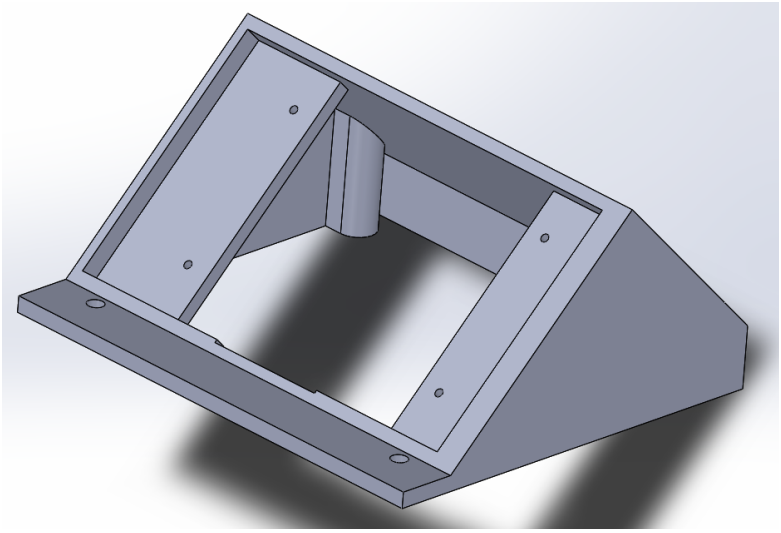

Figure 30: LCD screen holder

5.2 Disk Compartment

The disk compartment contains: the disk, spin chuck, IR Lamps, IR sensor, filters and filter slider, and magnets. A cut-a-way view of the disk compartment is shown in Figure 31. The magnets are not shown in this figure as they are suspended from the lid of the system. Figure 32 shows the disk compartment during assembly.

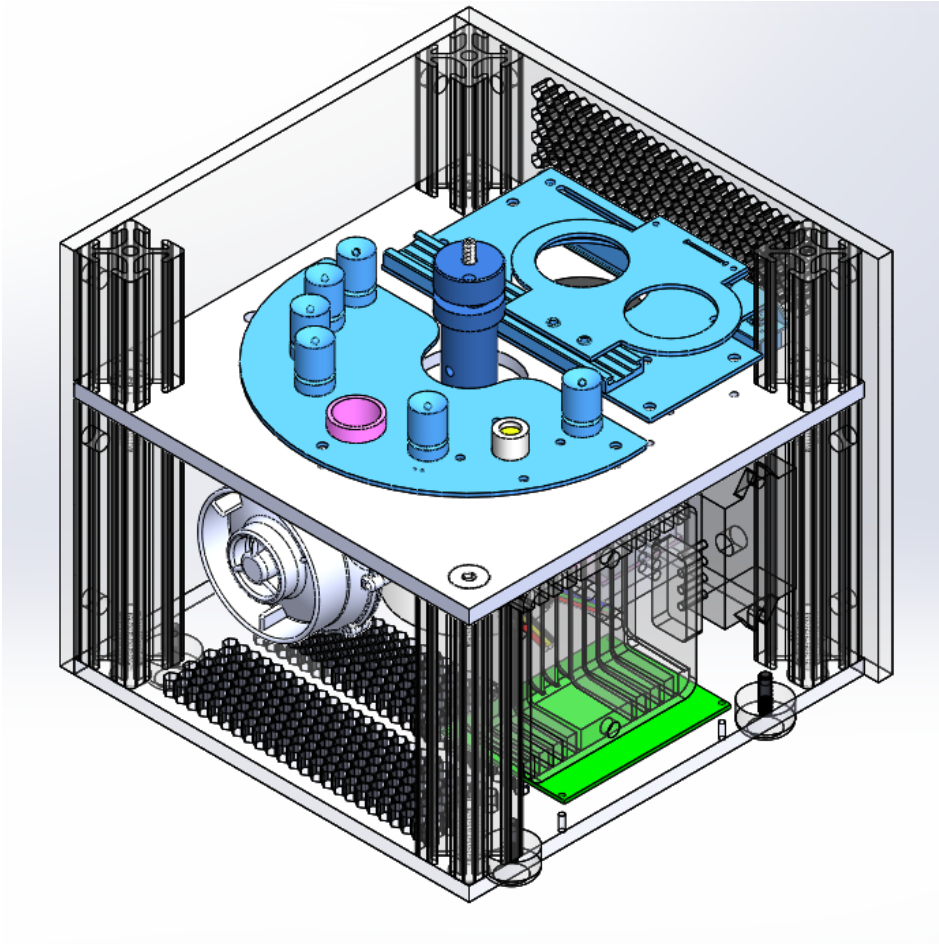

Figure 31: CAD view of disk compartment

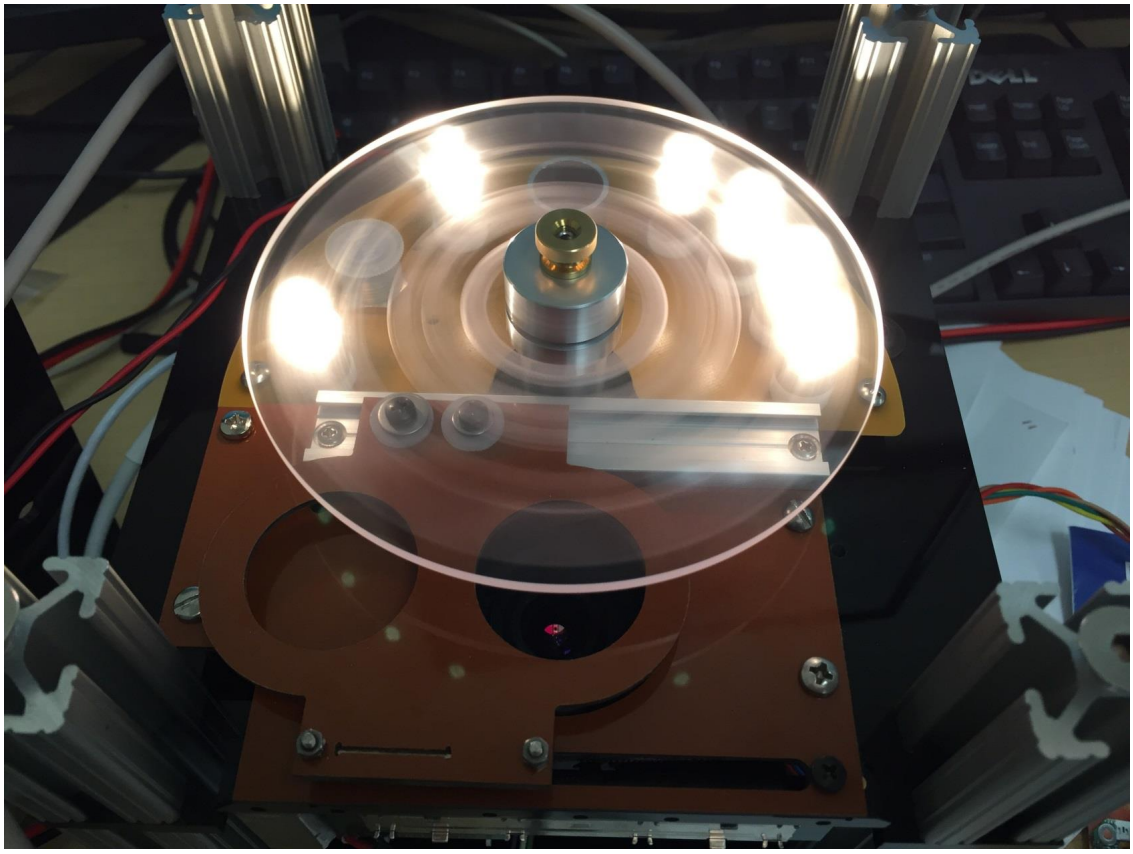

Figure 32: View of disk compartment during assembly

5.3 Motor Compartment

The motor compartment sits directly below the disk compartment. It includes the motor and motor driver, camera and lens, DC-DC converters, the blower and the blower driver. A cut-a-way view of the motor compartment is shown in Figure 33.

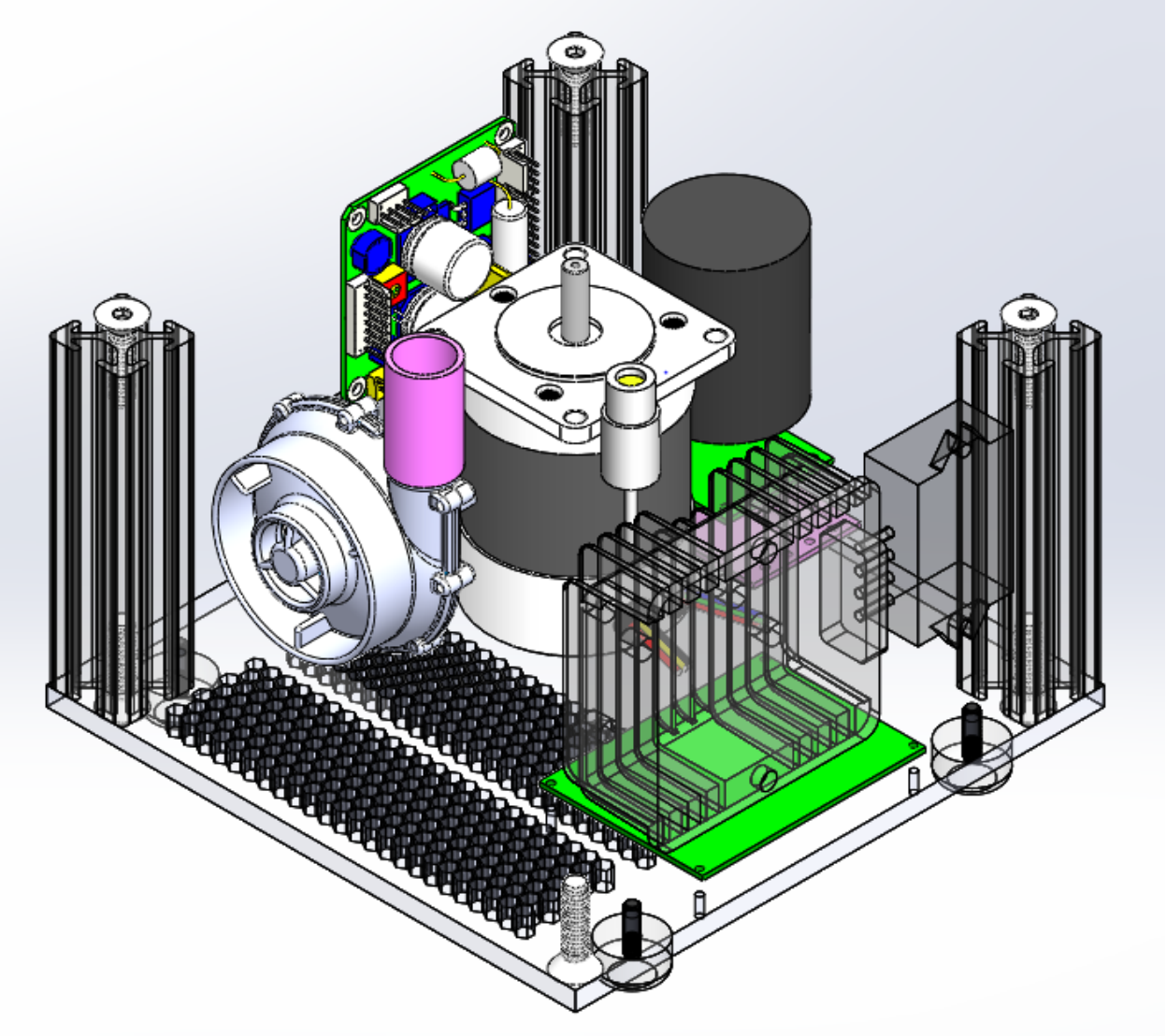

Figure 33: View of motor compartment

5.4 CPU Compartment

The CPU Compartment is inside the LCD screen holder. It houses the Raspberry Pi and the IR lamp driver board. A cut-a-way view of the CPU compartment is shown in Figure 34.

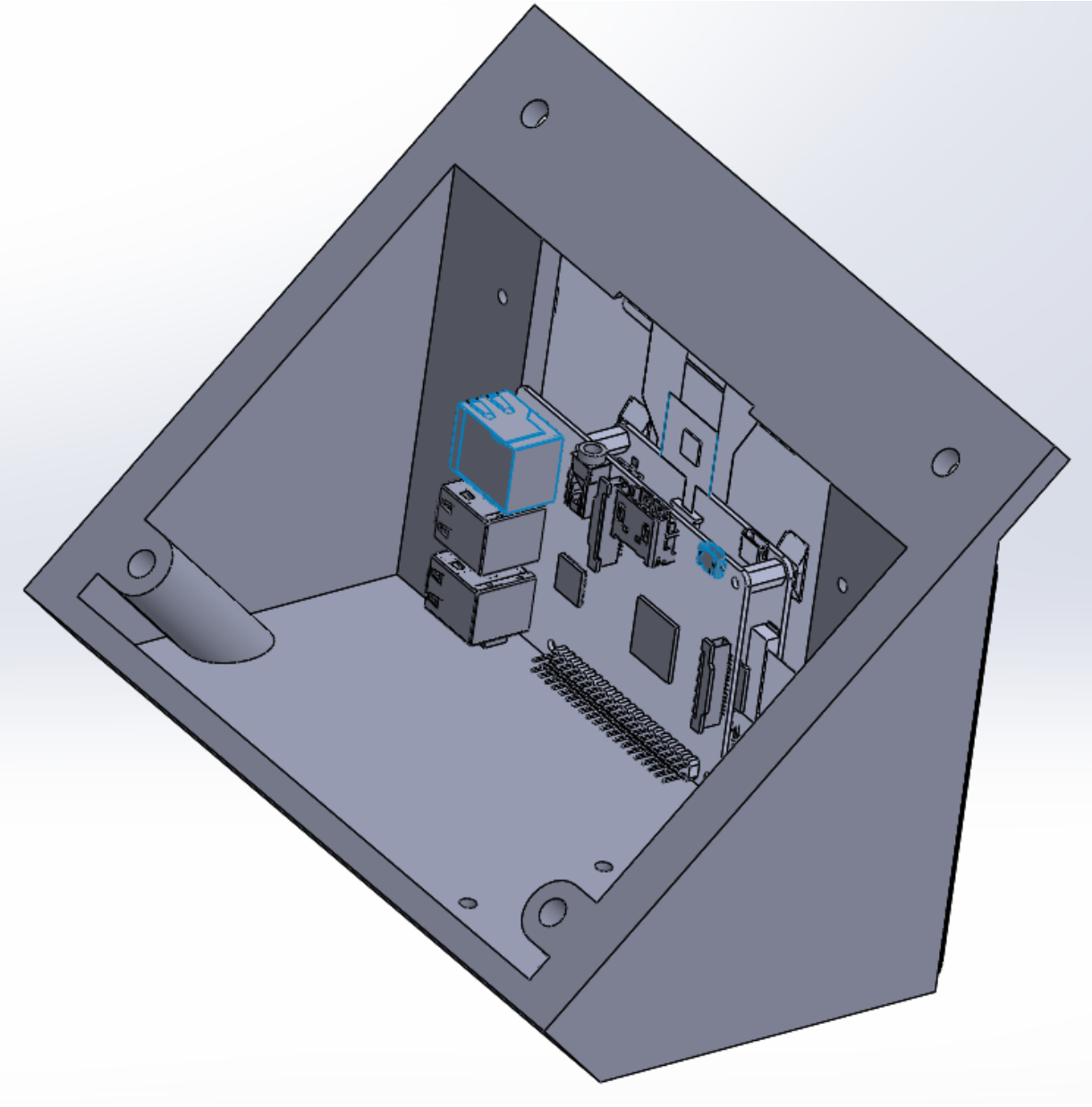

Figure 34: CPU compartment

5.5 Assembled system

Figure 35 through Figure 39 show various views of the assembled system.

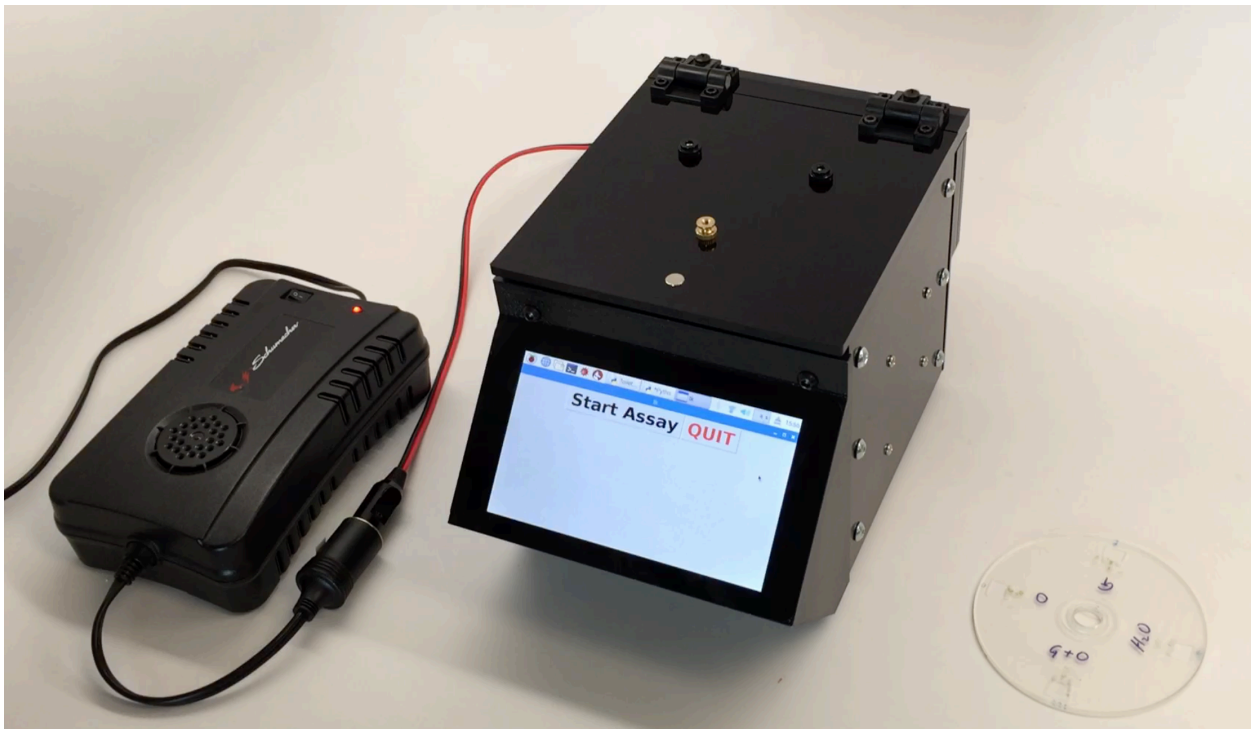

Figure 35: Completed system, test disk, and power supply

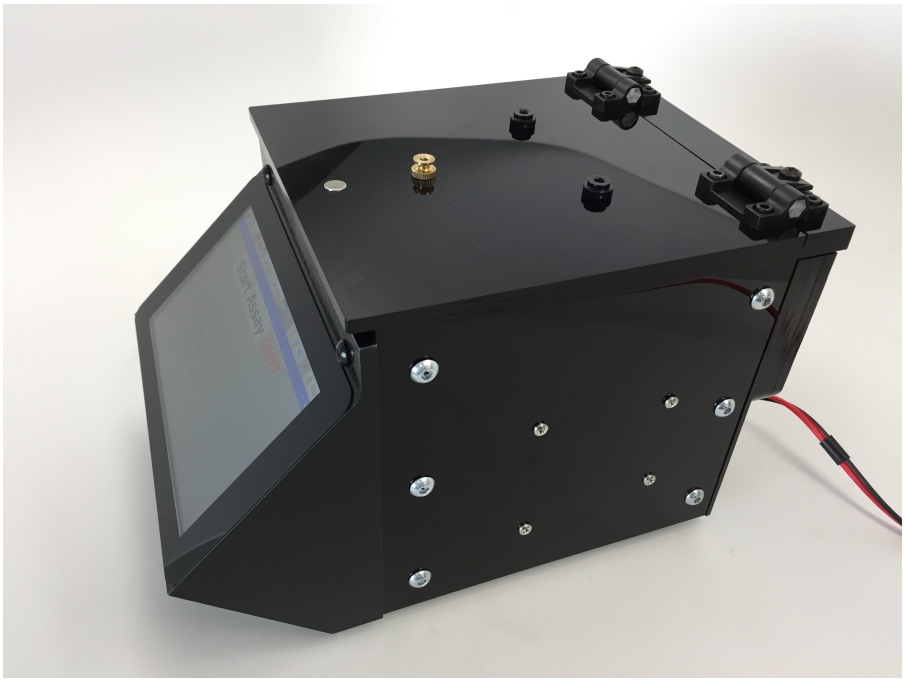

Figure 36: Right side view of the system

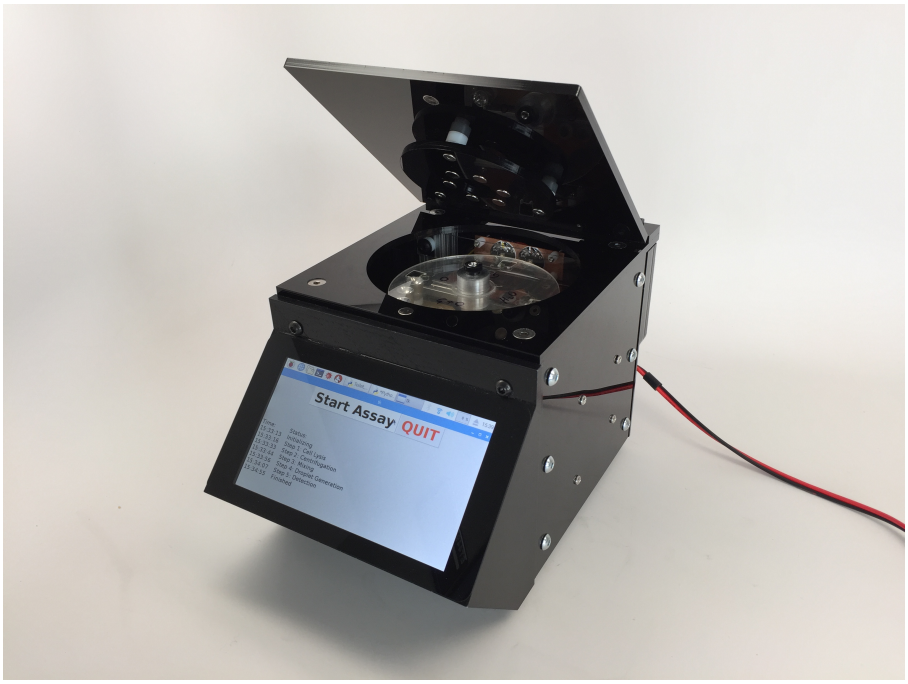

Figure 37: View of system with lid open

Figure 38: View of the disk compartment

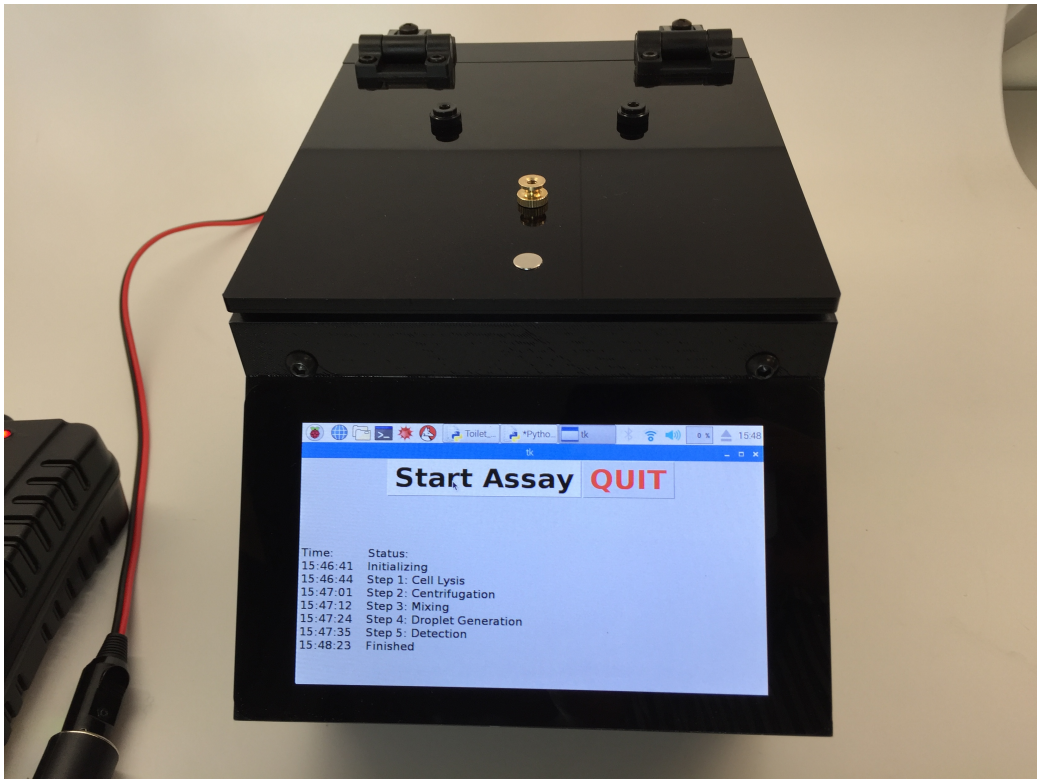

Figure 39: Front view with GUI

6 Data and Analysis

6.1 PID Temperature Control

6.1.1 Temperature Control Using a TMP36 for Detection

Early testing of PID control used a temperature sensor that was in direct contact with one side of a Peltier cell. The other side of the cell was attached to a heat sink. The temperature sensor was a TMP36 from Analog Devices. Figure 40 shows an initial warm up and four cycles between 60°C and 95°C.

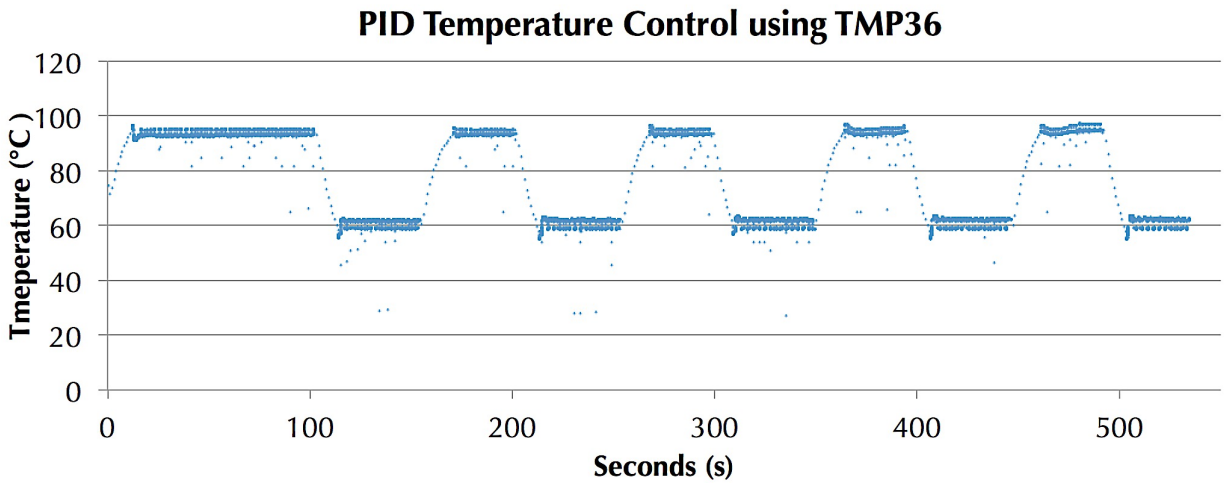

Figure 40: Temperature control using TMP36

6.1.2 Temperature Control Using a Thermistor for Detection

PID control testing was also done using a thermistor as the temperature sensor. One side of the Peltier cell was in contact with the thermistor; the other side was in contact with ambient air. Figure 41 shows an initial warm up and three cycles between 60°C and 95°C.

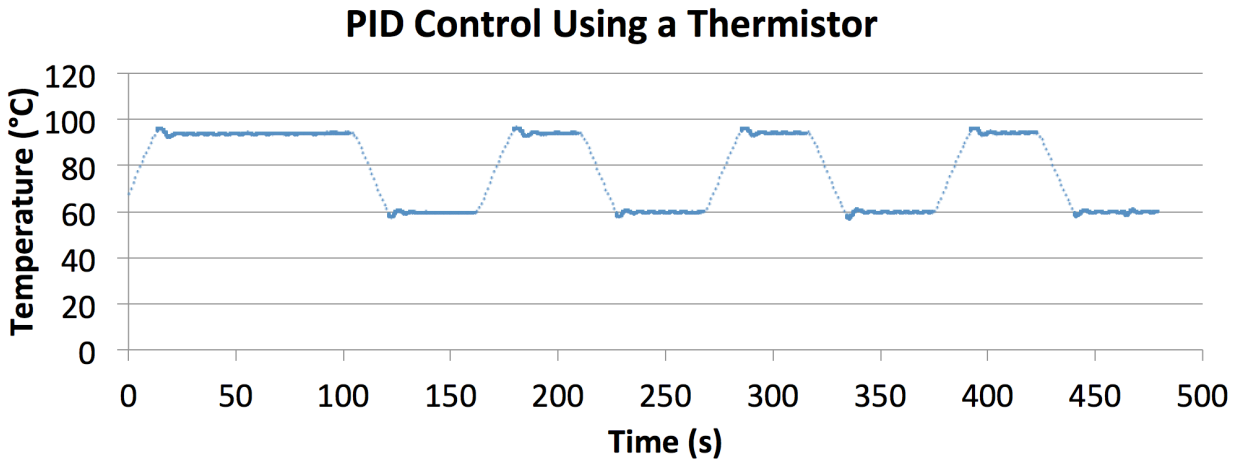

Figure 41: Temperature control using a thermistor

6.1.3 Temperature Control with Peltier and Thermistor Attached to the Disk

Once the Peltier cell was attached to a disk, the time to ramp up to 95°C remained approximately the same but the time to cool down to 60°C was extended, as shown in Figure 42.

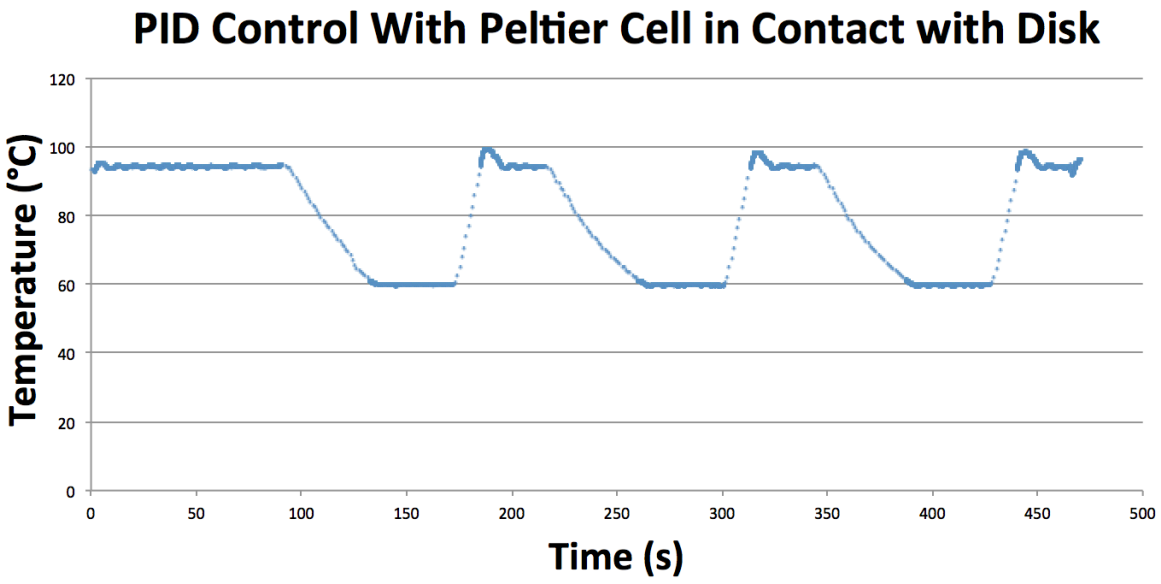

Figure 42: Temperature control with Peltier cell attached to disk

6.2 Imaging

Orange and green fluorescent micro beads were used to test the system's ability to view droplets. The test disk, as shown in Figure 43, is loaded into the upper compartment of the system. Figure 44 shows one of the test chambers being excited by the blue LED; the filters can be seen through the clear test disk. During assay execution the lid is closed and the Raspberry Pi camera captures the images. Each chamber is imaged twice, once with green excitation and a second time with blue excitation.

The test disk includes four chambers. The first chamber contains no micro beads and contains only water. The second chamber contains orange fluorescent beads. The third chamber contains green fluorescent beads. The forth chamber contains orange and green fluorescent beads. Figure 45 through Figure 52 are images of each of the four chambers when excited by wavelengths of 530nm and 450nm, respectively. Each image is saved to the Raspberry Pi as a JPEG file.

Orange fluorescence was much harder to see so the brightness of the green LED was increased to be significantly greater then the blue LED. This is why the images are almost completely black when the blue LED is used for excitation and there are no green fluorescent beads present as shown in Figure 46 and Figure 48.

Figure 43: Test disk

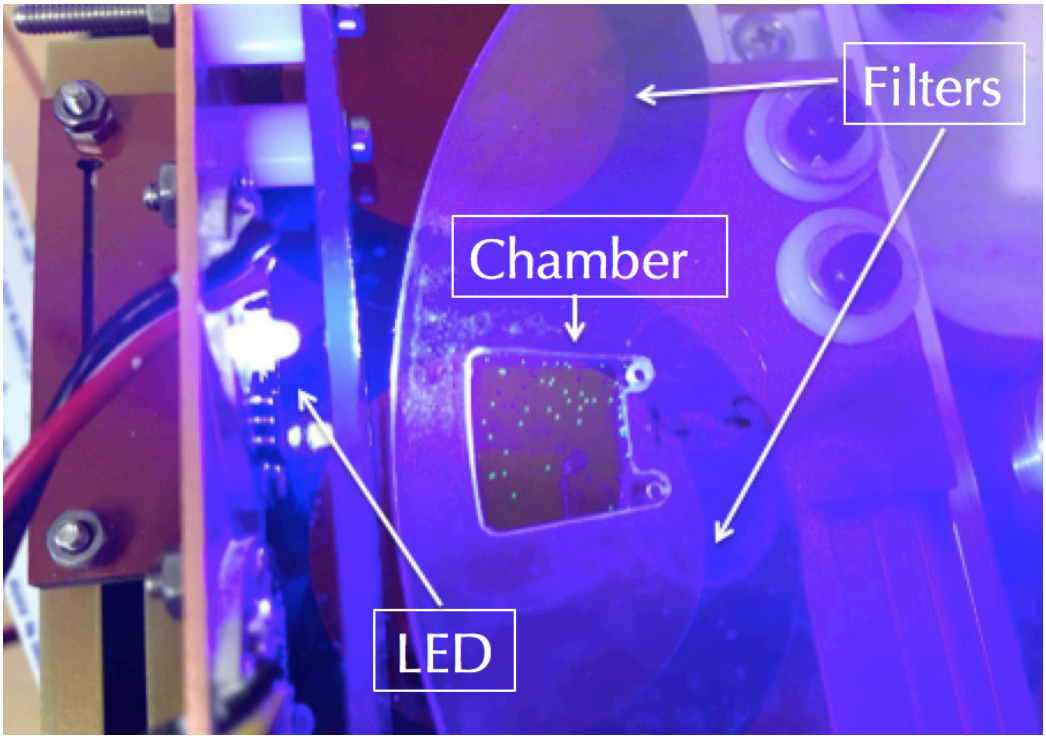

Figure 44: Test disk, blue excitation LED, Filters

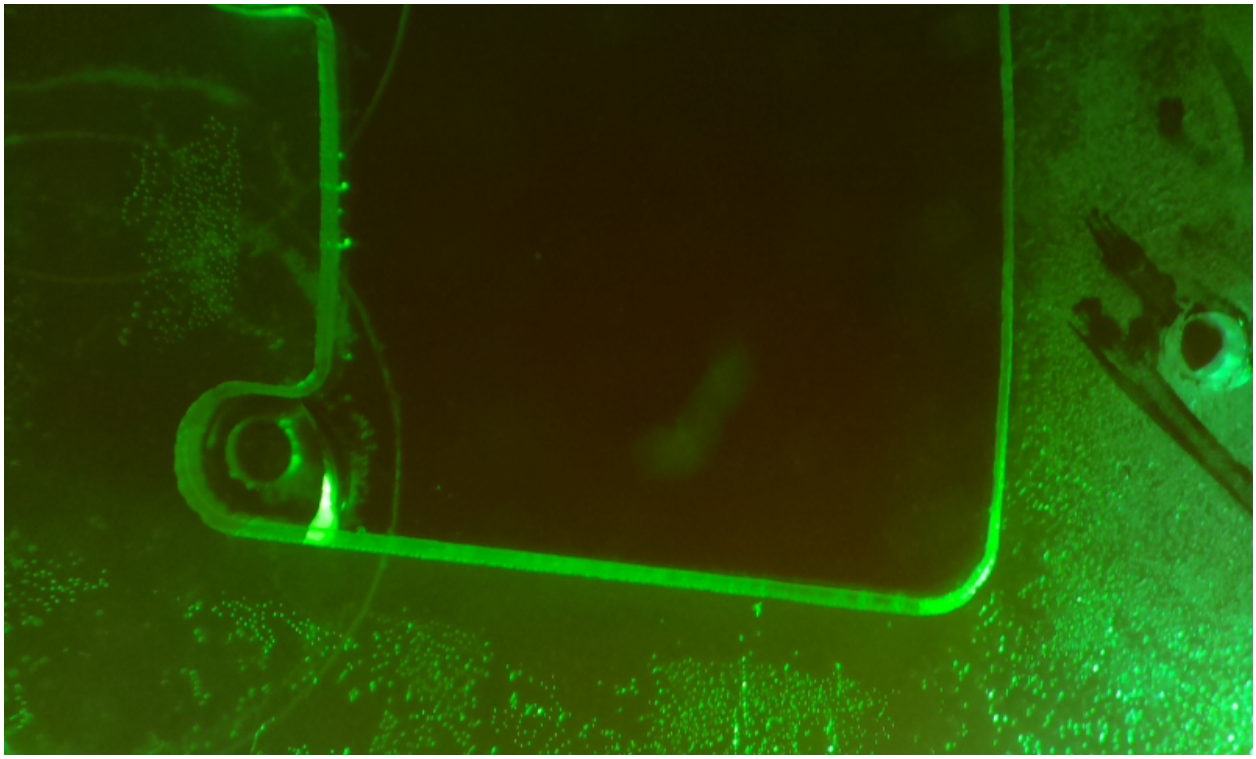

Figure 45: Water chamber with green excitation

Figure 46: Water chamber with blue excitation

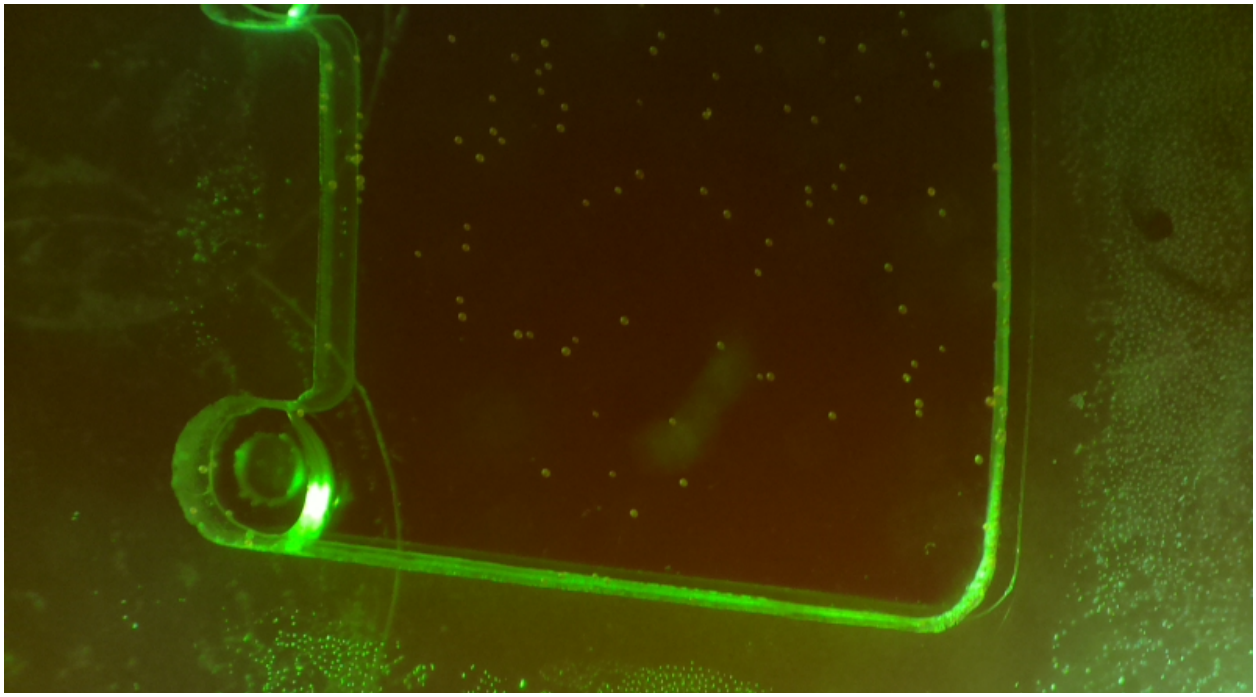

Figure 47: Chamber with orange Fluorescent beads and green excitation

Figure 48: Chamber with orange fluorescent beads and blue excitation

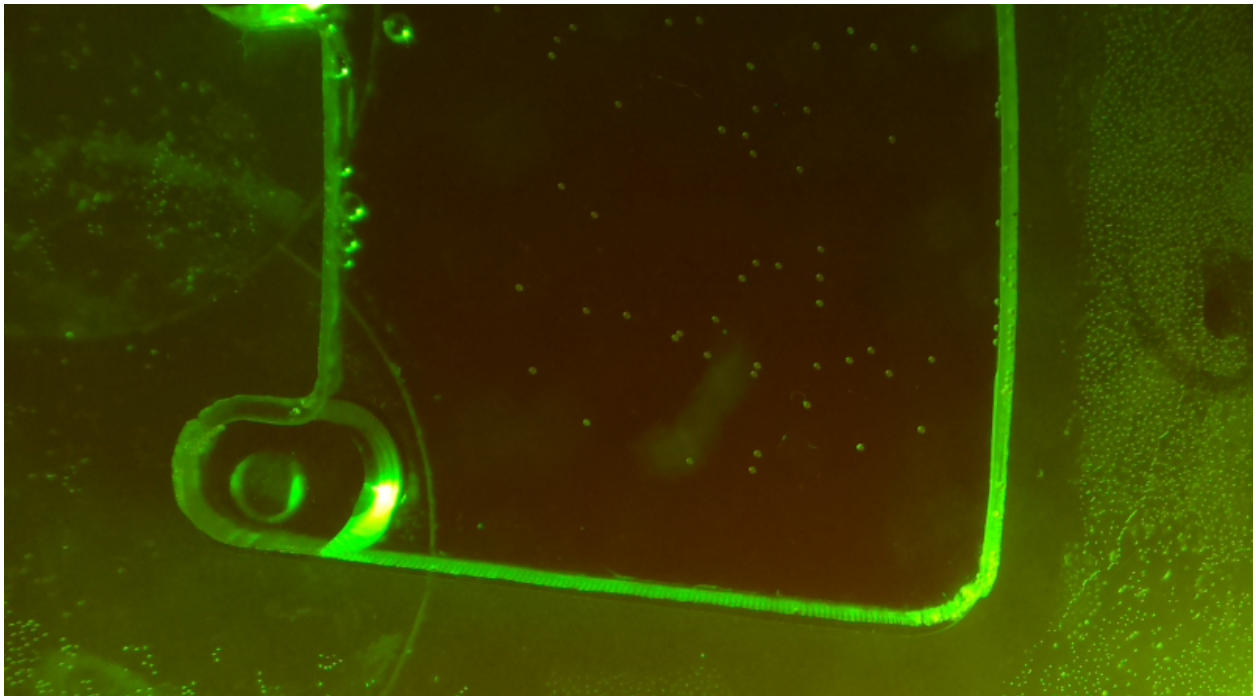

Figure 49: Chamber with green fluorescent beads and green excitation

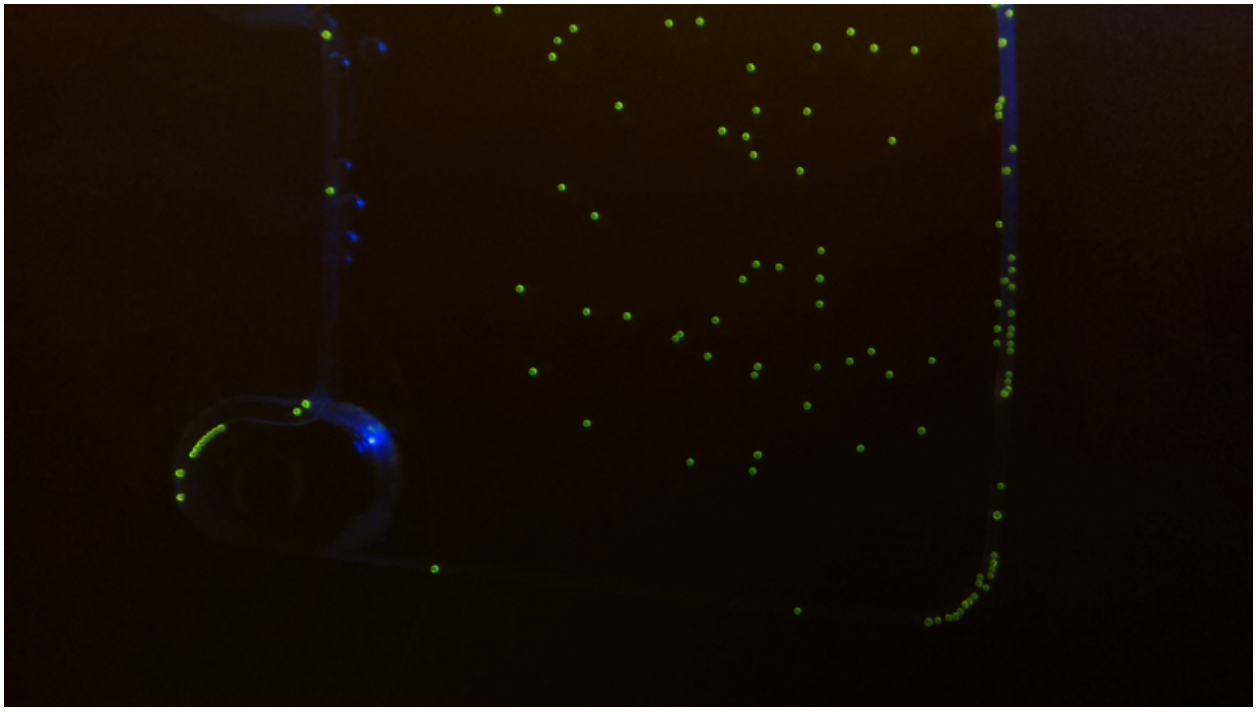

Figure 50: Chamber with green fluorescent beads and blue excitation

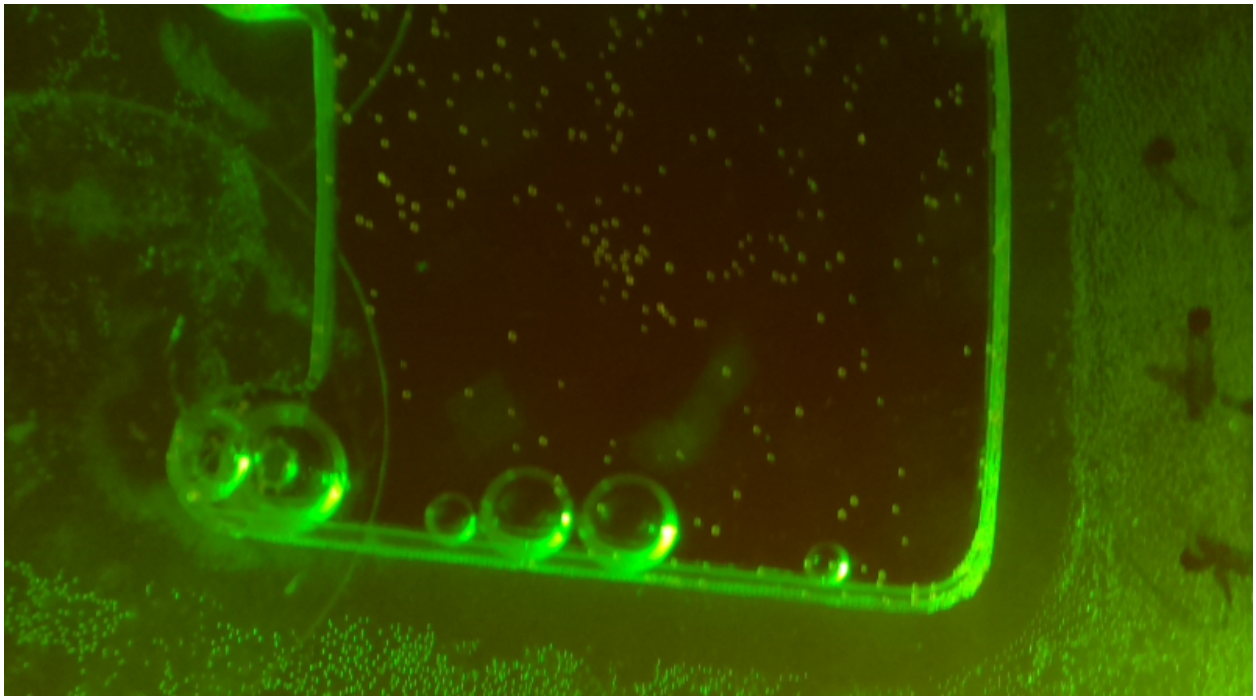

Figure 51: Chamber with orange and green fluorescent beads and green excitation

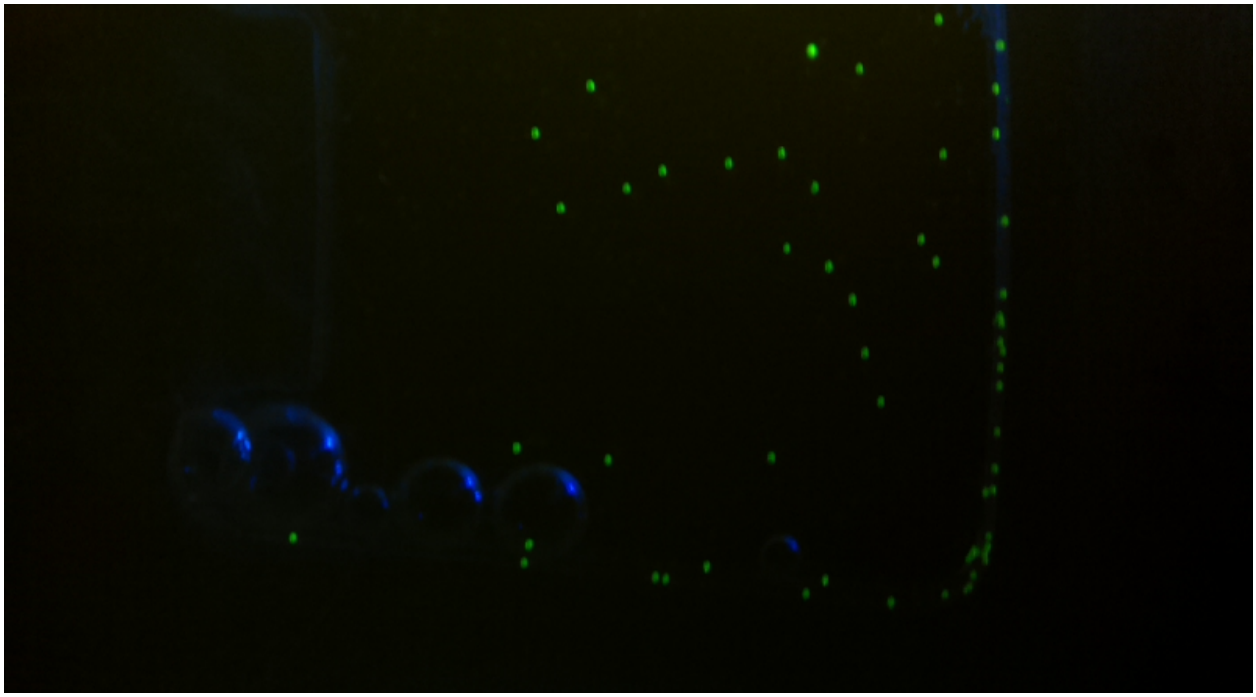

Figure 52: Chamber with orange and green fluorescence and blue excitation

7 Conclusion and Future Work

The system was designed according to the needs of the project. It is a portable device that is capable of cycling through temperatures for PCR. It is also capable of detecting fluorescence.

When building a system and fitting the components together for the first time, there are aspects of the hardware that the designers plan to improve in later iterations of the prototype. Many of these improvements are discussed in section 7.1.

All the subsystems are functional and many parts of the assay are integrated into software but there are two software components that still need to be implemented into the main executable file. The integration of these components is discussed in section 7.2.

Image processing is needed to analyze the fluorescent images. This is discussed in section 7.3.

In order to know if the system will work with pathogens in wastewater, there is still work to be done with the microfluidic sample disk. The system will allow the disk designers to rapidly test their designs, however; a working sample disk is not operational at this point in time. Progress on the disk design is discussed in section 7.4.

7.1 Hardware Updates

7.1.1 Driver Boards

There are multiple driver boards and each are connected to the GPIO pins by jumper wires. Future plans include combining all the driver boards onto one PCB and connecting the driver board to the Rasbperry Pi using a ribbon cable. This will clean up the space inside the CPU compartment and improve assembly.

7.1.2 Filter Slider

The filter slider is currently controlled by relays. The relay board was an off-the-shelf quick fix that was implemented in order to meet a demonstration deadline. It uses full power to move the slider between positions. Future plans include replacing the relays with an H-bridge motor driver. It will use less power and less force to move the slider back and forth.

7.1.3 Case

All the components fit tightly in the motor compartment. During assembly there were non-essential components that we removed in order to fit everything in. Non-essential components included extension chords for the HDMI and USB ports to the case, and an additional 5V DC-to-DC converter so that the LCD screen and CPU could have separate power supplies. The system still works without these components however being able to reach the ports, without having to disassemble the system, will be advantageous. The extra power supply was recommended by the LCD screen manufacturer but not required. A larger case will allow these items to be implemented to the next design.

7.1.4 Fluorescence

When the blue LED is turned on, only the green fluorescent beads appear on the image; however, when the green LED is turned on, both the green and orange beads are visible. The quantity of orange beads can still be found using the current system by subtracting the green beads from the total number of beads shown. Tuning the brightness of the LEDs and finding sharper filters may be beneficial to truly multiplex multiple types of fluorescence. Experimentation with different types of fluorescence is also needed.

7.2 Software Updates

7.2.1 Temperature Control

Initial testing of temperature control on the system has shown promise. Reaching and holding temperatures between 95°C and 60°C has been achieved but cycling between temperatures still needs to be implemented into the main code. Additionally, the PID parameters still need to be tuned.

7.2.2 Graphic User Interface

Currently the GUI just displays the steps and the images. Future plans are to allow the user to input changes to steps and be able to tune parameters without having to open up the source code.

7.3 Counting Droplets

The image-processing component of the project still needs to be completed. Software will be developed that can look at the images and count bright spots. Information from this code will go into quantifying pathogens in a given sample.

7.4 Microfluidic Sample Disk

The sample disk is still in the design phase. There are two main problems with the microfluidic assay that still need to be solved: 1) droplets tend to coalesce during thermal cycling and 2) there is a large bubble in the PCR chamber.

7.5 Final Note

The system operates as designed. Whether or not the system will be able to detect pathogens is still yet to be determined.

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