Design of a Point-of-Care Incubation and Fluorescence Detection Unit for Microfluidic Lab-on-a-Chip Applications

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Mechanical Engineering

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2016

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Abstract

Some parts of the world are underdeveloped and lack access to complete laboratory facilities. Where there is access, laboratory testing is an expensive and time consuming process. The team at Weibel Lab addressed the matter by designing a microfluidic testing system, a device to perform multiple pathogen detection tests using minimal sample size in a short time. This work focused on designing a portable device capable of incubating and extracting data from microfluidic devices, specifically the Quick Chip, a device that simplifies genetic identification processes through RPA and fluorescent indicators but requires the use of a benchtop fluorescence reader.

Device design thus required an optical detection mechanism as well as an excitation system to trigger sample emission. Using an equivalent concentration designated by the team at Weibel Lab, data capture centered on the use of a color CMOS camera using a series of filters. Data was then manually extracted by using ImageJ software.

Resumen

Algunas partes del mundo están poco desarrolladas y no tienen acceso a facilidades de laboratorio completas. En los lugares que si las tienen, las pruebas de laboratorio son costosas y toman mucho tiempo. El equipo en Weiblel Lab se tomó la tarea de atacar el problema diseñando un sistema de pruebas microfluídico capaz de realizar múltiples ensayos de detección de patógenos en corto tiempo y con una cantidad mínima de muestra, el Quick Chip, un sistema que simplifica la identificación de genes utilizando un método de RPA junto con marcadores fluorescentes, pero se limita por el uso de una máquina de lectura de laboratorio.

El diseño de este dispositivo requirió de un sistema óptico de detección además de un sistema de excitación para activar la emisión. Utilizando una equivalencia de muestra designada por el equipo en Weibel Lab, la captura de data se centralizó en el uso de una cámara de color CMOS y un arreglo de filtros mientras que la data se extrajo manualmente utilizando el programa de ImageJ.

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1 CHAPTER – INTRODUCTION

The field of point of care diagnostics has been bombarded by an influx of ideas and approaches. Point of care testing is officially defined as "… tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space, and are performed outside the physical facilities of the clinical laboratories"[1].

Inundated by new attempts at miniaturizing conventional laboratory procedures and taking them to the place where they are most needed. This rapid interest increase surged from the use of devices such as the disposable glucose meter, the most commonly used point of care diagnostics system and an example of a high impact device [2]. Its success is due in part to diabetes patients. While they do not outnumber other device users, they use the meters more often, usually multiple times a day.

Figure 1: A glucose testing kit used by diabetes patients. -Creative Commons

These disposable devices have since become a lucrative business, keeping people healthy and returning their freedom and independence as they can test and regulate their insulin levels without the need of large equipment. While these are limited to people with specific conditions, a different device is the now common pregnancy test. While not as lucrative as the former, it introduces the point of care concept to a wider range of people through convenience [2]. These factors contribute to the growing interest in the field, allowing researchers to delve deeper into potential Point-of-Care applications.

Figure 2: Plain pregnancy test strip. -Creative Commons

Researches strive to find new applications that can be modified and present benefits in becoming free from conventional laboratory limitations. One idea in particular has expansive promise, pathogen detection. Being able to detect foreign agents in samples while eliminating the time consuming procedures resulting from conventional testing yields faster results at fractions of the cost.

One such interested party was the group at Weibel Lab, responsible for the miniaturization of a recombinase polymerase amplification (RPA) based pathogen detection device, the Quick Chip microfluidic assay. Their design eliminates the complexity of DNA amplification and simplifies the identification process, but is limited by the need to use large benchtop incubators and detectors. A proposed device would automate the process, allowing users with little training to reap the benefits of their system.

This work focuses on the development of a functional prototype detection unit that serves to incubate and capture images from the Quick Chip from Weibel Lab. It begins by exploring and testing their initial prototype and defining the issues preventing detection of low concentration fluorescent samples. It leads into defining the constraints for the new device as well as the approach and reasoning for part selection in the final design.

2 CHAPTER – LITERATURE REVIEW

Pathogen detection and diagnostics is a sector largely dominated by localized laboratories where complex tests are performed by trained technicians using sophisticated equipment. While they eclipse the landscape in variety and complexity of procedures performed, and every day new techniques and technologies emerge, there are intrinsic disadvantages to the centralized laboratory concept. An example of such is the delay between ending a sample for testing and receiving the results, which can be in the range of a days to a few weeks [3].

2.1 Point of Care

Microfluidic platforms present the potential to manage large scale testing to detect epidemic risk pathogens, opportunistic infections or chronic problems on a population scale, including advantages due to the low volume involved [4]. Using the same diagnostics platform loaded with different reagents could be used to detect other problems in single patients, such as minor infections or specific conditions [3], [5]. The simplicity and versatility provides an opportunity to change medicine in developing regions. This same technology could eventually reach homes for quick testing and diagnostics in the developed world, liberating laboratories for more complex and pressing matters and streamlining the process from diagnostics, to testing to treatment.

Lateral flow strip tests have been widely used for early detection of an array of sexually transmitted diseases (STDs), allowing for earlier treatment and helping to control the spread of the diseases, as well as vitamin deficiency, pregnancy hormones among other applications [3]. Other versions are designed to detect issues with the environment such as water pollutants and waterborne pathogens [6]. As these technologies are beginning to flood the field with potential new applications and revolutionizing how testing is done, a variety of devices with different approaches are invading the market [2].

2.2 Disposable Devices

Looking to simplify the use of the technology has lead research to move towards complex systems that are easy to operate. While a chip may contain reagents to perform complex tests and reactions, using the device can be as simple as loading the indicated sample and inserting it into a testing device. Some of the major factors in play are the reagents used and byproducts generated.

Disposable systems have a clear advantage in that they are made containing all the necessary reagents in the required distribution ready to use, while the reactions take place inside. Once the results are obtained, what is left is a spent chip that still contains the waste, which is much easier to manage and dispose. An alternative to systems that require users to directly handle the chemicals, which are generally dangerous if managed incorrectly [3]. This approach also prevents cross contamination [7].

As an added benefit, the testing devices can be swapped for new ones to quickly resume testing, allowing operators to handle large quantities of individual tests, such as when testing groups of people during threats of epidemics.

Disposable devices can be made from a variety of materials, the most common of which are paper, plastic, silicone or glass, and poly (dimethylsiloxane) (PDMS) [2]. Each presents advantages for certain kinds of applications and manufacturing methods that can be applied to larger scales.

Table 1: Basic Materials and Advantages

Paper	Printed on, chemically treated, used as membranes
Plastic	Moldable into complex forms, widely available
Silicon/Glass	Machined for complex micro features
PDMS	Gas permeable polymer, simple to mold

1. Paper

   Paper presents a large versatility for designing devices. It can be treated with chemicals, which it absorbs and retains, such as in tests like pH strips. The fibrous nature is perfect for making lateral flow devices while it can be printed on with special inks to produce canals for sample flow or create simple circuits, such as in glucose detectors.
2. Plastic

   Plastic has the main advantage of being widely available and subject to the same manufacturing methods used for other products, such as injection molding, being able to mass produce devices with complex features in an inexpensive manner.
3. Silicon/Glass

Silicon and glass are grouped together due to their similar nature; tough materials which retain their features. This makes them great for micromachining channels and reactors into an unreactive medium while remaining relatively inexpensive.

4. PDMS Microfluidics

   Poly dimethyl siloxane is a gas permeable polymer that can be set onto molds to produce miniature features, entire microfluidic systems. The molds can be made by processes such as soft lithography, through which a single mold can be used for multiple castings. The main advantage is the gas permeable nature of the material. By placing the chip in a vacuum and degassing, the chip is capable of self-loading a liquid sample. As the PDMS is exposed to atmospheric pressure it begins to absorb air. A droplet of sample is placed onto an inlet, sealing the system. As the device absorbs the air inside the chamber, atmospheric pressure propels the sample into the channels and chambers where precise amounts of reagents are present, eliminating the need for a loading mechanism. The laminar flow nature of microfluidics also prevents contamination between wells.

2.3 Examples

Lateral flow tests use membranes or a paper strip to identify the presence of protein markers such as antigens. In a pregnancy test, capillary action drives the sample flow through the system where it is exposed to reagents which, through some mechanism, become an observable band [2].

Glucose detectors use a combination of lateral flow and electrical properties to interact with blood samples and give a quick diagnosis [2], [8].

These are some of the most common, commercially available point of care systems in market. While development is slow due to strict regulations from the FDA, more complex systems are beginning to emerge in the market [2].

2.3.1 Existing Devices

Understanding the quickly developing field of Point of Care Diagnostics is critical to the development of equipment, taking advantage of previous work to gain understanding of the most important factors in the area. It’s vital to understand the parameters of what is considered point of care, going into detail of what makes a device portable, while expanding on the complexity of existing technologies.

In market technologies range in size and utility from hand held devices up to desk size equipment, depending on the assays they're designed for. One example of an existing device is the immune-NASBA chip, which consists of a series of parallel running wells and channels. This device is designed to fit into an existing ELISA microplate reader [6]. Loading the assay chip requires the use of external valves and pumps, resulting in a complex system. The real advantage lies in the amount of complex analysis that are performed simultaneously.

Another detection system is the GeneXpert integrated benchtop analyzer, which uses plastic cartridges to contain and process filter-captured organisms [2]. Solutions are mixed by the user and incubation is done manually before being transferred into the test cartridge.

Figure 3: GeneXpert [2]

A different concept in development aims to develop compact portable devices focused around ease of use and convenience. Devices like the µBar system, which uses a microfluidic chip in conjunction with a phototransistor based design to incubate and read biological assays [9].

Both benchtop devices and ultra-portable systems are two approaches for the development of pathogen detection technology, but specializing devices for the tests to be performed severely reduces the cost and complexity of point of care technology [9].

2.4 Biological testing and Pathogen Detection

Throughout history, the defining factor of medical treatment was been treating the symptoms of illness while the patient recovered. Modern medicine benefits from a large database of information regarding symptoms and their causes. One of the most difficult to identify was the pathogen, a designation for a foreign object which is responsible in some way for the illness, the same term can apply to bacteria, viruses, fungi, whichever is the cause. Identification is a

difficult task, the principal resource consumed is time, which also happens to be critical in most cases. Earlier identification leads to quicker treatment, stunting infection and minimizing the effects. Often the symptoms caused by an illness are dangerous, as is the case of a fever. Fever is a natural immunological response to a foreign agent, the body increases its temperature in an attempt to become inhospitable to the invader, killing it. Unfortunately, the body has a threshold at which cells and protein begin degrading, causing often more harm than the invading pathogen itself. In such cases time is critical, the symptom is combated to prevent damage while the pathogen is identified and neutralized. Such cases are problematic as these symptoms, like the fever, are not unique to a single trigger. Detection methods have improved over the years. These have become faster and more accurate; older methods give way to new techniques [10].

2.4.1 Culture counting

The oldest method for pathogen detection and diagnostics involves growing a culture from a sample, generally fluid collected from an afflicted region, by providing a suitable system for bacterial growth. Given time, the bacteria multiply enough to be seen. Culture counting consists of observing the sample culture, using it as representative of the original source. The amount of bacteria present give a practical indicator of the amount of bacteria in the extraction site, as a mathematical relation can be obtained due to traditional growth studies. This method is time consuming and is susceptible to culture contamination, as any bacteria that mixes into the initial culture will multiply and obscure or destroy the actual pathogen, leading to misidentification.

2.4.2 Immunology Based Systems

This method consists of using the same properties that are used for pathogen detection in nature. Specific antibodies are fixed onto a testing surface or substrate. These adhere to the target bacteria, or antigens, fixing them in place. The targets have at this point been immobilized and can be separated. In order to identify and quantify their presence, an identification technique is still required. A common method is to use more antibodies, this time labeled with a specialized enzyme and washed to remove any residue. The sample should contain antigens fixed in place and marked with enzyme via antibodies. An enzymatic substrate is then added, which reacts to the presence of the specialized enzyme bound to the bacteria antibodies resulting in a color emission. It is this color manifestation that is interpreted to determine the presence and density of the targeted antigen.

2.4.3 DNA Amplification Detection

The goal of nucleic acid amplification techniques revolves around detecting the presence of a genetic sample belonging to a known target. They are generally composed of two individual procedures, a detection method and an amplification technique. Sample amplification means to increase the amount of detectable genetic material by using replication techniques mimicking the way genetic sequences multiply in nature. Most techniques are designed around DNA amplification, but some work with RNA which can be more useful in order to obtain the desired information, especially if a virus is suspected.

2.4.3.1 LAMP

Loop-Mediated Isothermal amplification (LAMP) is a more recent amplification technique. It's characterized by the use of specialized primers to target different regions of the gene in question. Using a strand displacement reaction and targeting eight regions simultaneously, the process proceeds at a constant temperature, normally around 63 degrees Celsius. Like PCR, adding additional primers and markers allows detection to take place, the procedure can then be simplified into a single step [11], [12].

A byproduct of LAMP, pyrophosphate ions, lead to the precipitation of magnesium pyrophosphate, a white substance suspended in the mixture. This can be used as an indicator to track the amplification in real time.

2.4.3.2 RPA

Recombinase polymerase amplification (RPA) is an isothermal DNA amplification technique based on the use of recombinase and polymerase proteins. Recombinase proteins are the proteins that aid in genetic recombination, such as when parent genes mix and interchange genetic material to generate the offspring DNA. They are capable of interacting and separating the DNA strand without the need to denaturalize proteins, operating at constant temperature, hence eliminating the need for thermal cycling. The recombinase is followed by the polymerase, which is the protein responsible for binding the amino acids into the genetic sequence based on the existing strand being copied. Recombinase interacts with primers and allows polymerase to bind nucleic acids into strands corresponding to the strand being replicated without having to fully separate the original strands, requiring less energy than other methods. The number of individual genetic strands increases exponentially as the produced DNA strands are used to repeat the process until all the available amino acids are used. This type of reaction works

efficiently at 37°C and could even work at lower temperatures, albeit at lower rates [13]. This amplification method can be accompanied by fluorophore-quencher pairs bonded with the genetic probes. The quencher prevents the fluorophore, such as 6-fam, from emitting fluorescence. When the probe encounters the matching genetic sequence, the reaction separates the quencher from the fluorophore. The fluorescence no longer inhibited, the solution fluoresces in proportion to the unquenched fluorophores [13].

2.4.3.3 PCR

Polymerase chain reaction (PCR) uses a series of primers to replicate strands of DNA. These primers interact with the target gene sequence, triggering the reproduction sequence in which DNA splits and reacts with amino acids in its environment, resulting in copies of itself. Each copy becomes target for the amplification sequence to repeat, causing an exponential growth in gene population, reaching detectable levels. The main downside to PCR is the need for thermal cycling to trigger the gene reproduction, requiring expensive equipment and a controlled environment. After sufficient sample is prepared, the sample requires a detection method such as gel electrophoresis in order to obtain the results.

Real time PCR introduces another step to simplify the process by adding specific fluorescent compounds that attach themselves to the target sequences, emitting a specific light. The intensity of the emission is directly proportional to the amount of genetic material, allowing the user to keep track of the amplification.

2.4.3.4 RT-PCR

One of the big issues with nucleic acid amplification is that any target material is amplified and detected without information on the viability or status of the cells from which it originated. This includes information such as whether the cell is dead or alive. Reverse Transcript PCR addresses this issue by basing the process on RNA amplification and detection instead. RNA is the genetic sequence produced by the cell nucleus to transcribe the information from the DNA, hence it is only produced by living cells [14]. The detection mechanism similar as for PCR.

3 CHAPTER – PORTABLE OPTICAL DETECTION DESIGN PROCESS

3.1 Focus

We want to design a portable fluorescence detection unit for use with a self-loading PDMS microfluidic device that has been functionalized with all the reagents necessary for DNA detection at the point-of-care. Quick Chip, a self-loading PDMS microfluidic device developed at the Weibel lab [15] is simple to work with and convenient for use in point-of-care applications. The concept is to develop a comprehensive portable system capable of sample acquisition and detection while using a microcontroller and open source tools for control and analysis.

3.1.1 Quick Chip

The research focused around the Quick Chip device designed by a team at Weibel Lab at University of Wisconsin, Madison [16], [17], [15]. The Quick Chip is design to perform RPA, requiring an incubation time of 30 minutes at 37 degrees Celsius. The chip uses reagents and genetic primers and probes to detect the presence of specific genetic sequences. These genetic sequences are representative of specific strains of bacteria, and can be individually detected. The Quick Chip device can be designed to house multiple wells, currently 16. Each well inside the chip contains the necessary reagents for the RPA assay, including the primers and probes at sequences specific for a target bacteria. Each well can have different primer and probe sequences, allowing to test a sample fluid for multiple individual target pathogens. In order to expedite testing between UPRM and UW-Madison, the teams at Weibel Lab and at UPRM have established a fluorescent equivalence of 6-FAM to represent a positive RPA result.

A positive RPA reaction inside Quick Chip emits a signal similar to that of fluorescein at a concentration of 100 nano Molar in water, which set the benchmark for development. To increase the emission they also included a concentration of 14 milli Molar of magnesium acetate tetra hydrate in solution. Using the PDMS degas self-loading technique greatly simplifies The sample loading process into these portable systems. In market, each device would be vacuumed and sealed before being distributed for use. Where conventional methods involve handling samples and inserting them into large systems or using complex mechanisms to load

microfluidic devices, this is all greatly simplified by these self-loading devices, becoming a simpler practice for microfluidic chip design and prototyping [18].

3.1.1.1 Microfluidic Chip

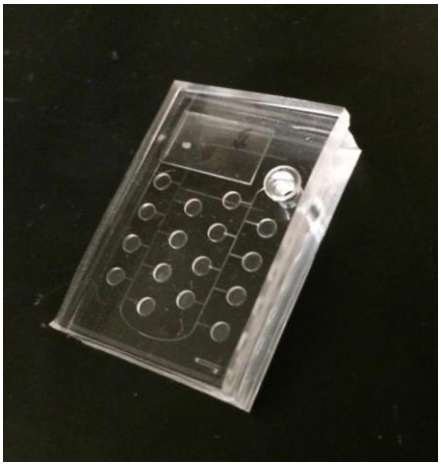


Figure 4: Quick Chip design cast in PDMS

The Quick Chip is also knows as the B-Chip in the Weibel Lab, for bacterial detection chip, figure 4. The chip consists of 16 1µL wells, a reservoir chamber and an inlet chamber. The reservoir chamber assists in loading of the chip, which will be discussed later in detail. A serpentine channel, 45 mm long, connects the inlet to the reservoir. Short 1.5 mm channels connect the wells to the serpentine. All channels are 150 µm wide and 50 µm tall. This is an arbitrary arrangement of wells, both the size and location of the wells could be modified, a fact to be taken into account when designing the reading device.

3.1.1.3 Loading Mechanism

PDMS is a gas permeable polymer, when it is placed in a vacuum for a specific period of time it releases the gas from within, generating a gas absorbing potential. An analogue to this would be a sponge in water, where the vacuum is squeezing the sponge before submerging it in water. When removed from the vacuum and exposed to atmospheric pressure it slowly absorbs air through every exposed surface.

While the device is absorbing air, placing a fluid sample in the inlet seals the interior from the atmosphere. As the PDMS absorbs air through every surface, the interior surfaces of the

channels and wells absorb the air trapped by the sample, creating a pressure differential that drives the fluid into the chip. The well walls absorbing the air drives the sample to completely fill each well [19][20].

3.1.2 Purpose

These devices are aimed to be a marketable product, aimed at a sector where the use of fast DNA based detectors would leave a definite imprint, such as developing and underdeveloped countries. Specific sectors into which the team is looking onto are food testing and medical diagnostics, where it presents several interesting possibilities.

3.1.2.1 Food

Food approval refers to companies that require constant sampling, such as import authorities and the FDA. These are responsible for ascertaining the quality of the products at different stages of their shelf life. The problem is this, the targeted product is usually a perishable product with a relatively short shelf life. The more time it spends getting to the consumer, the less time left for the product to be sold and consumed. One example would be the meat industry where tons of meat is transported. Meat has a life of a few weeks if properly refrigerated. It has to be tested and approved for consumer use such as when it leaves the production plant or when it's imported into a country. The meat, a perishable whose quality diminishes due to freshness has to wait up to days for testing and results with conventional laboratory testing, resulting in reduced shelf life and possible loss to the industry. Point of care testing means that a batch can be tested and approved in a matter of hours, resulting in longer life and a better product for consumers.

3.1.2.2 Medicinal

A portable system based around a Smart Phone presents an opportunity to communicate the data through the cellular network. While an untrained user can operate the detection device, the results can be transmitted to a specialist that can perform more advanced diagnostics, such as a doctor. A patient could process their own sample and transfer the information to his doctor, who can then suggest treatment, or monitor a patient's progress when dealing with an existing illness. The data can also be stored in a central database, to be used as record or to be processed further.

3.1.2.3 GeoMapping

Point of care pathogen detection combined with real time data transmission presents an interesting opportunity. By sending the diagnostic data from each device with the GPS coordinates from the smart phone used, a central service can combine it with data from other devices to form an area of effect map, and keep track of disease propagation. This would be also very valuable for environmental analysis, depending on the performed tests.

3.1.3 Constraints

Portable devices are a broad description for devices included in point of care technologies. It includes those devices that can be transported and do not require complex infrastructure and can be operated with little or no modification to the target location. This hypothetical description would include, as an example, any device that can be moved and operated with access to any electrical outlet. A visual aid, a microwave can be moved with relative ease and works with any standard power outlet. Certain additional constraints make the device not only portable but also more convenient. The desired size device should be carried with ease by one person using both hands. It should be possible to make it function independent of the power grid in order to work where power is unavailable or inconsistent. Convenience includes simplicity, hence the device is to be as easy to operate as possible, requiring minimal training. The device must then be compact, able to run on battery powered and easy to use. The goal of the design is to create a product to compliment the Quick Chip design.

3.1.4 Sample Equivalence

Biological testing requires rigorous procedures and various permits depending, especially when dealing with infectious diseases and bacteria. It also requires complex equipment, a heavily controlled environment and handling techniques, increasing costs and difficulty. Sample preparation and handling would be an issue as mishandling or errors in preparation could lead to false data, compromising the design process.

Through statistical analysis, the team obtained a calibration curve of fluorescence emission relating to the assay outputs performed in the chip and determined that a device capable of detecting fluorescence from a 100 nM concentration of 6-fam in distilled water would be able to detect the output from the assays. They recommend the use of a 14 mM concentration of magnesium acetate tetra hydrate to increase fluorescence emission. This equivalence made samples easy to prepare without the need of extremely advanced chemistry laboratory

equipment. The resulting solution is to be kept dark as to not bleach the fluorescent compound, a reaction caused by exciting the samples for a long period of time which permanently reduces fluorescent emission.

3.1.5 Weibel Lab's Design

A preliminary device was designed as a proof of concept to demonstrate the use of the chips and how the final device would operate. While the device was designed to work in theory, it was constructed and tested but did not achieve the desired results.

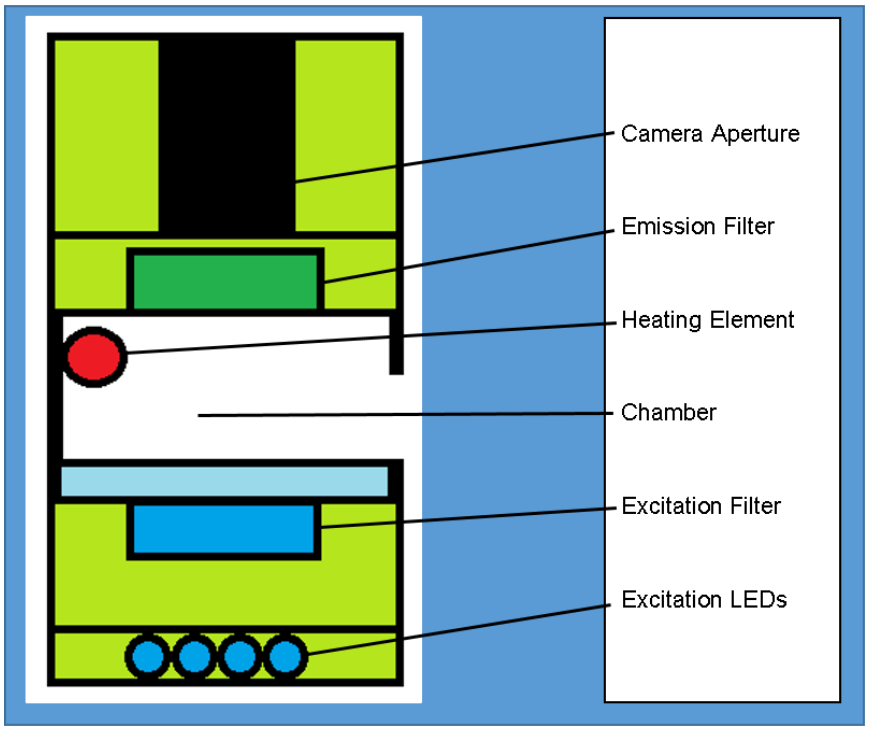


Figure 5: Sketch of Weibel Lab's initial device design.

Starting from an existing prototype design adds the challenge of gauging the capabilities of the initial system and comparing them to the desired outcome. This involved developing an understanding of the design, its performance and the theoretical principals upon which it should work.

3.2 Problem definition

A portable pathogen detection system characterized by its small size and ease of use has the potential to revolutionize industries. While the testing chips are small and easy to operate and are designed to replace laboratory testing, they require laboratory equipment to be incubated and analyzed. These machines, incubators and the like, are far from portable or raise the cost to prohibitive levels. The problem is the incubation and diagnostics equipment, the goal is to create a portable, relatively inexpensive device that is easy to use, requiring little training to make these technologies accessible. The device will be developed for the Quick Chip, but it could be adapted for other systems.

3.2.1 Process Requirements

In accordance to the specifications of the Quick Chip team, the device should be able to excite and detect emission, they established an equivalence to fluorescein to simplify this process. They have stated that the chip can operate at room temperature, but the reaction is accelerated by incubating the chip in 37 degrees Celsius. Since convenience is a requisite, incubation was deemed necessary. Data had to be extracted from the chips after incubation, to be processed and interpreted, in an uncomplicated manner such that it was simple to perform and possibly automated. Based on these conditions, the device was divided into core systems, incubation, excitation, data capture and control systems.

3.2.1.1 Incubation

Maintaining an environment favorable to the various chemical reactions is vital to the functioning device, in this case this means the ability to control and maintain chamber temperature is necessary. Some tests require constant temperatures, while others may require complex processes such as temperature cycling (PCR). Ideally, the incubation system would allow for heating, cooling and accurate temperature control for extended periods of time in order to be programmed for various microfluidic chip systems. In this case however, the Quick Chip uses RPA, which occurs at room temperature, but is accelerated by maintaining an environment close to 37 degrees Celsius, making heating and maintaining the temperature a priority while active cooling is a desirable function.

3.2.1.2 Excitation – Fluorescein Properties

The challenge is to have a system that can register an identifiable fluorescence emission from 1µL 100 nM fluorescein samples inside a small chip. The first step is to acknowledge the factors that influence fluorescein emission. Fluorescein responds to a narrow excitation range, referred to as the excitation wavelength. This occurs as photons at or close to that particular wavelength hit the fluorescein molecules and are absorbed. The absorption increases the vibratory state of the molecules, which is associated with the electronic state. In the simplest models, the atom then decays to the ground state, releasing a photon.

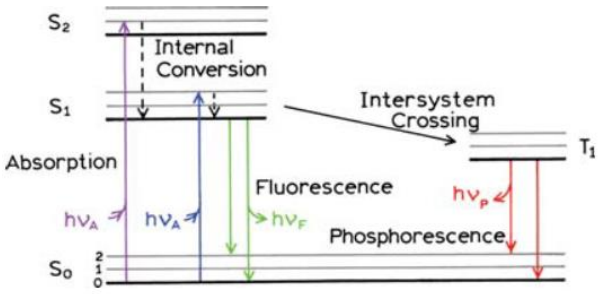


Figure 6: One version of a Jablonksi Diagram [21] Energy excites the fluorescent molecule from the ground state S_0 into an excited state S_1, S_2, etc. Through internal processes, such as thermal emission and atomic geometry changes, the energy state generally drops to S_1, then drops to the ground state as the atom decays and emits fluorescence.

Relaxation is when the internal structure of the atom consumes some of the absorbed energy, slightly deforming the nuclear geometry before decaying, causing the emitted photon to have less energy, and thus a higher wavelength than the excitation source. This shift in wavelength is the Stokes Shift, or the difference between excitation wavelength and emission wavelength measured at its peaks.

Fluorescent emission can be reduced or even prevented when a fluorescent molecule comes into contact in the solution with another molecule known as a quencher. Quenchers allow the compound to drop in excitation state without the emission of photons in a process known as quenching [21].

Fluorescence excitation and emission are related by the absorption of the excitation energy, which is described by the Beer-Lambert equation

$$log\frac{I_0}{I} = \varepsilon cd = optical\ density$$

Where I_0 is the intensity entering the medium and I is the intensity exiting from the other side, ε is the decadic molar extinction coefficient, c the concentration in moles/liter and d the thickness of the sample [21]. This means that the excitation is related to the sample concentration and distance the energy penetrates. This also means excitation reduces as distance within the sample increases, so too large a volume would not be excited all the way through, too little volume and a large part of the excitation energy goes through the sample without excitation. This relation also applies to the emission when the wavelength is close enough to the excitation wavelength, causing large volumes to absorb the emission from a part of its fluorescent compound, dimming the emission.

The light emitted from this fluorescence also has a specific range with an intensity proportional to the excitation intensity, around 488 nm, hence higher intensity yields a larger response [22]. Volume is a key factor as a larger sample means more fluorescein excited, thus more response. While concentration and volume are determined by the chip design, the device controls the parameters referring to the excitation energy, the excitation light wavelength and intensity.

3.2.1.3 Data Capture

Various alternatives exist for capturing the data from the testing chips. Each has a set of advantages and disadvantages. The most appealing are using photodetector units comprised of phototransistors or using camera systems complimented by software [8], [23]. The original device called for an inexpensive webcam type color camera because of size and cost constraints.

3.2.1.4 Extraction

An image captured from the test chip contains all the information but needs still needs to be converted into useful data, this involves processing the image to obtain quantified information to be interpreted as test results. This data can then be interpreted, stored and shared globally.

3.3 Initial Testing

The conclusion from Weibel Lab, in accordance with the information provided, stated that the device did not function with their chips, they were unable to detect fluorescence using the

device. In order to understand what was happening with that design, the parts were printed using a 3D printer and preliminary runs were conducted.

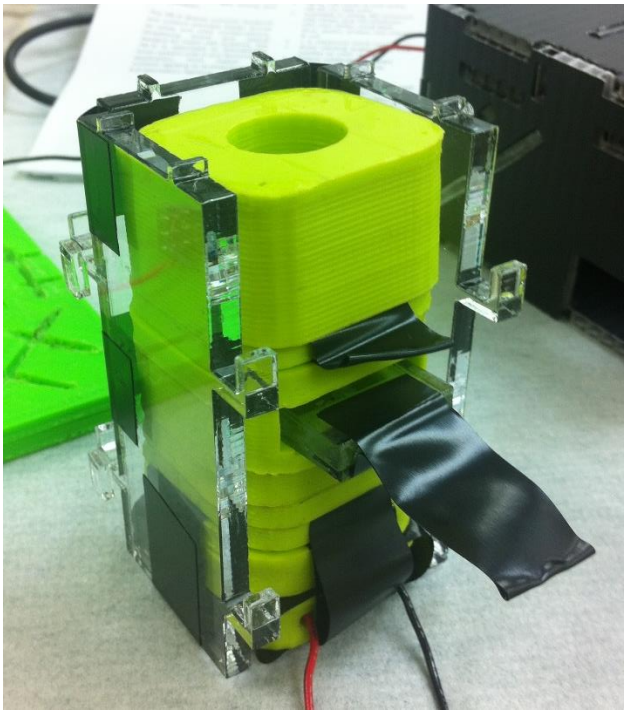


Figure 7: Prototype of the original device loaded with an acrylic test slide.

An acrylic slide with a drop of 6-Fam solution at 500 nM served as a preliminary sample to observe the effects of the proposed LEDs on a sample.

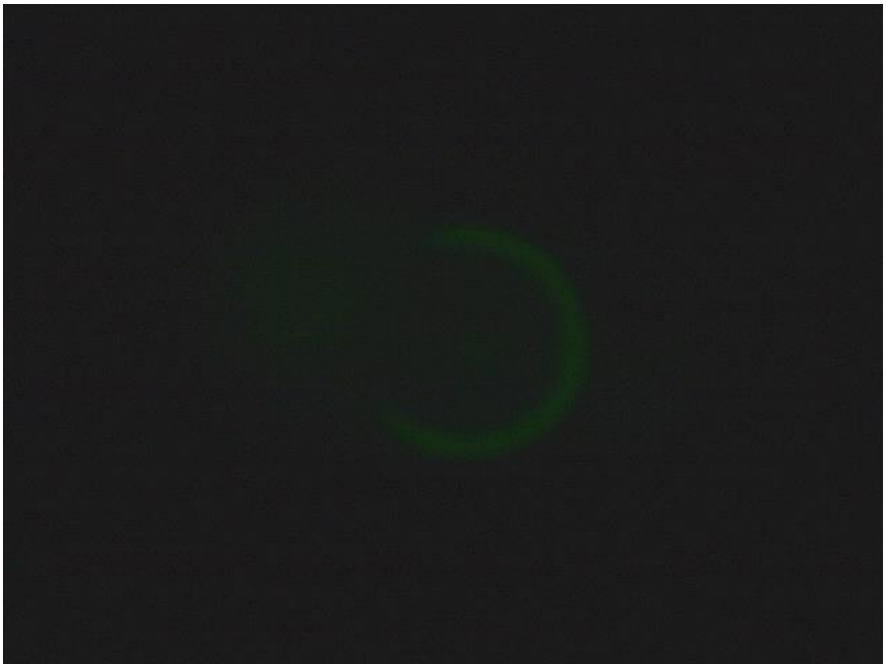


Figure 8: 500 nM 6-fam sample on acrylic slide. Two fluorescent signatures are present from a single droplet of sample. The dimmer one on the right was the sample as excited from the bottom. The second signal was the reflection on the excitation filter.

Observation through the camera revealed two signatures registered in the image, a dim circular area and a brighter, sharper shape overlapping. After repositioning the sample and multiple runs it became apparent that the dimmer signal was the emission from the sample that was expected. The brighter region was the other side of the sample as reflected on the excitation filter. The sample was emitting stronger fluorescence on the side it was being excited but that signal did not show through the fluid as though it was blocked and dimmed by the sample.

3.4 Limiting factors of initial design

The original design supplied by the team was a small vertical device, about the size of a 10 ounce cup implementing a vertical system in which the sample was inserted into the middle of the device through a slot in the side.

To maximize the use of space, the sample was excited from the bottom using super bright LEDs along the vertical plane. These were specific to the 480 nm wavelength, seen as blue light. Hypothetically, the excitation method allowed the chip to be bombarded with illumination, penetrating the liquid sample completely and exciting the fluorescein. The sample emits the fluorescent response in form of a light around 530 nm in wavelength. The design called for a

535 nm bandpass filter set between the camera and the chip filters the light, to eliminate any light from the LEDs, leaving the light from the fluorescence to be registered by the camera.

Incubation would be achieved by using a resistive wire heater in the sample chamber, controlled using a temperature sensor and regulated by switching the heater on and off at the desired temperature. Passive cooling was achieved via natural hear conduction through the device walls with no active cooling component.

The prototype was designed to be powered using Li-Po batteries and controlled through an Arduino microcontroller. While it had not yet developed to this point, it was meant to send images from the camera to a smartphone, where they would be analyzed and diagnostics interpreted.

3.4.1 Conclusions as to why it failed

Insufficient excitation could be one of the primary factors as the emission is directly proportional to the intensity of the excitation energy used on the sample. The filters block out any light that's not of the desired wavelength. While the LEDs peak at the desired spectrum, enough of their emission is away from it such that the filters blocking reduces the intensity too much. This is in addition to what the filters already reduce. Eliminating the excitation filter results in too much of the LED light coinciding with the emission wavelength and passing the emission filter, interfering with the captured image.

Another possibility was that the camera adjusts automatically to the brightest object in the image. Aiming the LEDs into the camera caused it to adjust to them instead of to the sample, possibly reducing the apparent intensity in the final image.

Absorbance played a major role in preventing the device from detecting any emission; the phenomenon of how a solution absorbs light. As light passes through the solution, it's absorbed by the fluorescein, exciting the sample. It also means that fluorescein closest to the excitation source absorbs a higher intensity light than the rest of the sample. Being excited from the bottom, part of the light never reaches the camera side of the sample, causing uneven excitation. Emission from the excited bottom half is absorbed and dimmed by the top side, preventing part of emission from reaching the camera. The result is a sample that emits light to the LEDs instead of the camera.

Absorbance also adds an additional consideration to the design. A higher sample volume should result in higher emission, the absorbance of the sample causes the fluorescein to progressively receive less intensity as it penetrates the sample. This means that there is a limit to the increase in emission that can be achieved by changing the volume alone. Increasing the volume in the direction of the excitation source would present diminishing returns. Increasing the depth of the wells to increase volume is counterproductive.

An alternative is to increase volume perpendicular to the excitation source, thus increasing the exposed surface. Increasing the radius of the wells would increase the exposed surface area without increasing absorbance, but it reduces the space on the chip, presenting a chip design constraint. Relocating the excitation source to be at an angle, exciting the sample from the side, allows the depth of the wells to be increased while minimizing the negative effects.

The thermal system wasn't tested as I was unable to get it to work and concluded that there had to be a simpler way, prioritizing the sample excitation and image capture over the incubation.

3.4.2 Chip Changes – Increased Well Depth

During the initial testing, one of the main variables that affected emission was the excitation intensity. To augment the amount of energy absorbed by the sample, it was necessary to increase the cross-sectional area perpendicular to the excitation light. The well area presented a large area for top or bottom excitation, but the alternative being considered required the LEDs to excite from the side, which meant a need to increase the depth of the wells. The team at Weibel Lab complied by increasing the depth of the wells to 500 µm which greatly helped in absorption.

3.5 Design Changes – Additional Constraints

Conclusions from initial testing presented a narrow list of factors that prevented the device from functioning as intended. A list of additional constraints composed from the observations further guided the design process.

Vertical illumination through the chip didn't excite the sample enough to be captured sufficiently by the camera. Removing either of the filters increased the excitation intensity but caused interference with the automatic adjustment of the camera. The LEDs had to be reoriented as to excite the sample with minimal interference. The filters consume a portion of the excitation energy from the LEDs in proportion to the amount generated outside of the desired wavelength.

LEDs with narrower emission spectrum were needed, or eliminating one or more of the filters. These changes required an extensive redesign and slight size increase.

3.6 Design Process

In order to design a device capable of performing the required tasks, it was necessary to divide the process to understand how each section would work.

3.6.1 Excitation

Fluorescence is dependent on excitation intensity, the higher the intensity of the excitation light the higher the emission. The key here is to observe how fluorescein behaves. Higher intensity LEDs yield better excitation and emission. Normal LEDs have an optimal emission wavelength at which they emit most of their light.

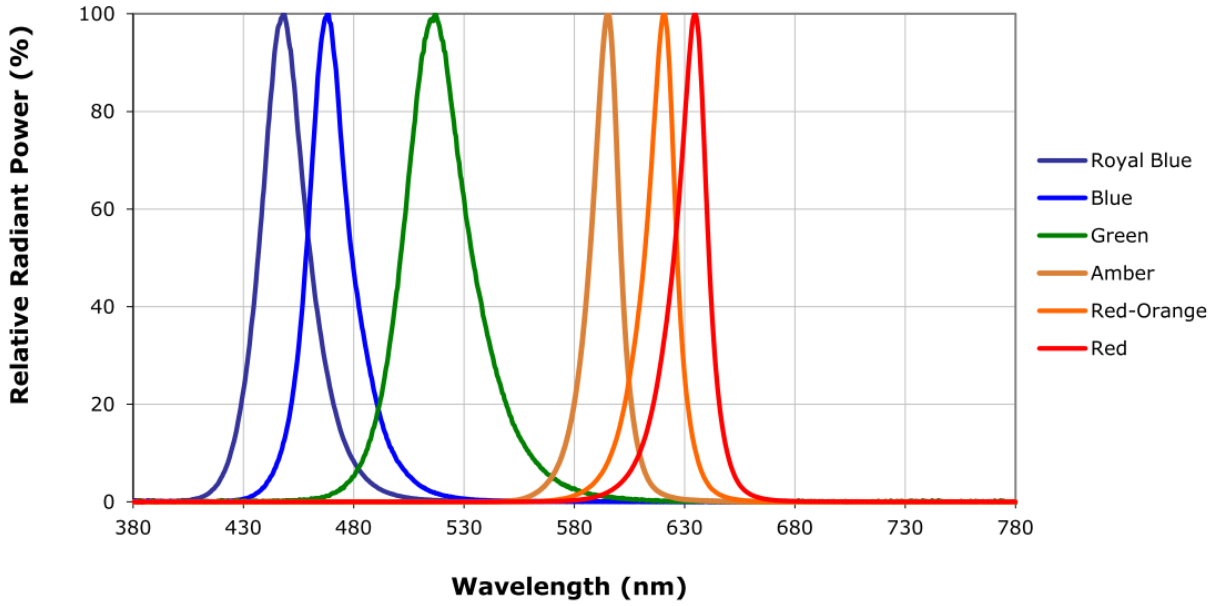


Figure 9: Emission spectrum for the Cree Xlamp XB-D LEDs. The closest peak to the desired wavelength corresponds to the Blue LED [24].

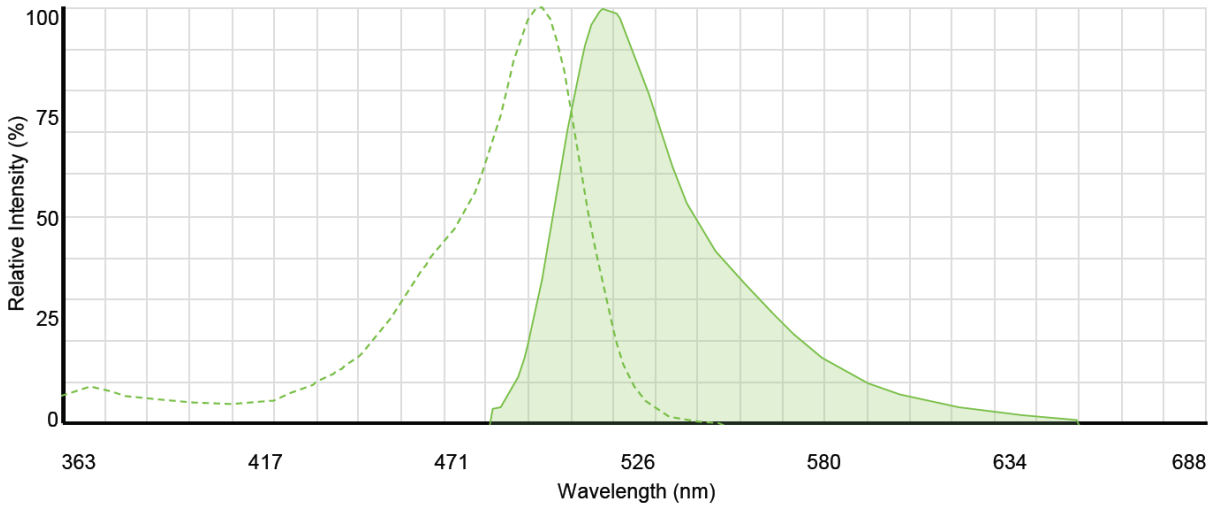


Figure 10: 6-Fam Excitation and Emission curves.

Fluorescein responds more to a specific wavelength, and the emission wavelength coincides at some regions. One problem is that a region of the LED spectrum coincides with the spectrum of the fluorescein that is trying to be detected. This overlap gives false positives and floods the image with a signal indistinguishable from the fluorescein. While it represents a small intensity of the total emission from the LEDs, that intensity is comparable to the low intensity signal from low concentration solutions, generating noise that makes the signal undetectable. This noise easily goes through the emission filter and using a narrower excitation filter causes a large drop in intensity that also drops the detectable signal. And even the error margin on the filter presents enough light to interfere with detection.

One solution is to use narrower emission LEDs, these have a sharper cutoff point in the spectrum, meaning that more intensity comes off as the desired wavelength. This means less light is blocked by the excitation filter, higher intensity reaches the sample. It also reduces the intensity of the light that wrongfully passes the filter, due to the margin of error, keeping it low enough as to no interfere with the signal.

The next issue to address was ensuring that the entire samples are excited uniformly, that every well on the chip receives similar excitation light from the LEDs. The selected approach was to use 4 LEDs located one in each corner of the chamber. These are set at a height to coincide with the microfluidic chips as to illuminate into the sides of the chip. The transparency of the PDMS serves to transmit the excitation light to all the wells. This arrangement takes advantage of the conical shape of the emission pattern from the LEDs. Having 4 cones aimed at the center

covers the entire chip while the center of the chip receives light from all 4 LEDs. This overlap helps compensate for the drop in intensity caused by the increased distance, as well as the loss of intensity due to interference from the other wells between the LEDs and the center region.

3.6.2 Incubation

For this particular case it's necessary to be able to heat the sample to 37 degrees Celsius. The first alternative to consider was a conventional resistance wire coil. Heating of the chamber is achieved by running a current through a length of wire, which heats up due to the resistance. This is the same mechanism used by conventional ovens. Temperature regulation is achieved by cutting the current when the chamber reaches the target temperature, when it drops under the threshold connecting it again. This cycle keeps the chamber in the desired range. In this method, cooling is dependent on heat conduction through the chamber walls, a form of passive cooling.

The second alternative is a transparent heating element comprised of a glass slide coated with a transparent resistive film that heats up when current is applied. This is the system used in cameras to prevent fogging in cold climates. Regulation and cooling worked the same as with the resistive wire.

To address the heating element, we chose a Peltier heat pump, also known as a thermoelectric cooler. It's a solid state electronic component that forces heat from one of its surfaces to the other, resulting in a hot surface on one side and a cold surface on the other. This kind of heat transfer device works via the Peltier Effect.

The Peltier Effect is observed as heating or cooling at a conductive junction between two semiconductors, an N-type and a P-type when a voltage is applied across them. The direction of the heat flow depends on the current path, where the current travels from N to P, the junction cools and the device is pulling the heat in. Where the junction makes the current flow from P to N, the heat is expelled, creating a heated surface. One complete device consists of a large array composed of many sections, orienting all the N to P on one face and all the P to N on the other.

Using this type of component addresses another issue that's not necessarily relevant for this application, active cooling. The target chip only requires incubation at a constant temperature, thus cooling can be left to natural conduction and convection. But it the device were to be applied to another kind of test, it may require the ability to both heat and cool, which would

normally require separate components. Since the temperature orientation is dependent on current direction, active cooling can be achieved by reversing the current direction on the heat pump. The heater becomes an active cooler.

3.6.3 Temperature Control

A temperature sensor is needed to control the chamber conditions. A One Wire temperature sensor was used due to its advantage of transmitting temperature data directly with Arduino libraries readily available, unlike simple thermocouples which require additional components and calibration. The temperature sensor communicates directly with the main control unit, which through basic logic activates or deactivates the heat pump.

During incubation it's engaged when the temperature inside the chamber drops below a set threshold, heating the surface, and deactivates it when it goes above the set temperature.The sensor is clamped onto on corner of the heating surface, wires running to the control Arduino.

For simplicity, this version of the device does not use the heat pump as a cooling unit, but it should be noted that by reversing the current in the heat pump it can work as an active cooler. More precise temperature control can be achieved by using more advanced thermodynamic analysis to compensate for the heat capacitance of the system and more accurately regulate the temperature. This involves using more complex equations in the programming to alter the voltage and polarity for increased accuracy, but it goes beyond the scope of this investigation.

3.7 Data Capture

With this device, data has to be extracted from a sample chip. Fluorescence emission means a positive response, the presence of the target gene for a particular well. There are various methods used for this, two common approaches are, using phototransistors or using a camera system.

Phototransistors are electronic components that react to photons in the same way a transistor responds to a current in its gate. LEDs are commonly known to generate light, but a less known fact is that they can also capture photons to generate a small current. This charge is large enough to trigger a small transistor, hence combining the two creates a transistor that responds to light. Photons cause the component to allow the flow of a current, which can be directly interpreted as data. Using the diodes to capture the photons presents a challenge. The photon emission of the samples needs to be large enough to generate a current. To maximize the

effectiveness of the phototransistors they have to be directly aligned to the sample wells and in close proximity. One component is required per section to be analyzed, limiting chip arrays to arrays matching the sensors. The chips would have to be designed to fit the sensor array and limited to a maximum number of chip sampling sections or wells, severely limiting versatility for future chip makers.

Another alternative is to use a camera to capture an image of the chip [25]. Using a camera allows the analysis of the entire field of vision, but has lower sensitivity than other methods. Capturing an image of the entire chip leaves the analysis and extraction entirely up to the image analysis software, minimizing the constraints in chip design and geometry. Fluorescein emission would be shown as a glow in the sections of the image corresponding to the wells. A color tool allows further analysis of an image as the color filter separates part of the background light.

3.7.1 Image capture

Using an image based system allows for freedom of chip design, presenting a wider variety of design parameters that become available when designing chips to be used with the detection device. Making such an open device focuses priority on image quality.

3.7.1.1 CMOS vs CCD (Complementary metal-oxide semiconductor vs charge-coupled devices)

The first decision within image capturing technology is what kind of image sensor is to be used. The main alternatives to be considered are charge-coupled device (CCD) and complementary metal-oxide semiconductor (CMOS). Charge-coupled device chips use a series of pixels that capture light intensity as a voltage. [CCD Ref] The charge from each pixel travels along the surface of the chip to a registry that analyzes each voltage in order and converts it into a signal. This specific measurement of the voltages allows for better pixel performance and higher capture quality. The surface transfer system prevents noise, creating sharper images. These type of sensors require special manufacturing methods that increase the difficulty and as a consequence the cost of production, making CCD sensors relatively expensive.

Complementary metal-oxide semiconductor chips work by having a photodetector with a small circuit in each pixel. Each pixel responds to light, each circuit translates the response into an electric signal which travels to the signal output. The signal from the individual pixels combines

in the bus, through which the combined result travels to the output. This method of combining makes the system prone to errors and noise [CMOS Noise Ref], unlike the more orderly sequenced signal interpretation method offered by the CCD. The major advantage is that CMOS chips can be manufactured using ordinary chip manufacturing facilities, drastically reducing costs. The largest downside is in the design, using space on each pixel to fit the detector and relevant circuitry means less space available for the light sensor, and smaller sensing area means less signal generated. This downside is in large part offset by the use of lenses over the detectors that focus light onto the detection area. As it stands, CMOS cameras are less effective at light capture than CCD but much more inexpensive, making CMOS the system used for consumer cameras while CCD is used mostly in laboratory equipment, where the sensitivity requirement offsets the increase in price [23].

3.7.1.2 Bayer Filter

Both systems capture images in grayscale as the sensors detect the presence of light but have no way of determining the colors. To capture color images it is necessary to understand how the images work. Color images are composed of combinations of red, green and blue pixels, the primary component colors of light. They are primary because they cannot be created through the combination of any colors but different combinations of them can be used to create any color. A Bayer filter is a device composed of a fixed array of red, green and blue color filters. Each filter section is aligned with a photodetector such that each sensor captures the intensity of light in its corresponding filter wavelength. The result is an array of colors that the eye blends together to form the complete color image.

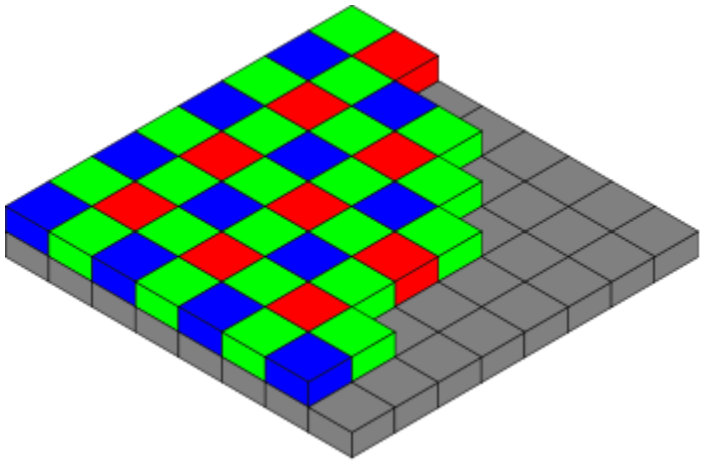


Figure 11: Bayer filter array - en:User:Cburnett

ttps://commons.wikimedia.org/wiki/File:Bayer_pattern_on_sensor.svg), „Bayer pattern on sensor“,

https://creativecommons.org/licenses/by-sa/3.0/legalcode. The filter has twice as many green segments to imitate human eyesight. Humans detect green more than the other colors, more green pixels makes for more accurate images to the human eye. This is what allows the images to be separated into RGB components later.

Being designed to generate color images to be observed by the human eye. Human eyes are more sensitive to the color more so than to other colors. In order to generate better images, the filters are made to capture more green light. This means dedicating more space to the color, hence having more pixels dedicated to green. As a consequence, Bayer filters make color cameras more sensitive to green light, a coincidence that makes them more plausible for detecting the green emission from fluorescein compounds.

3.7.2 Data extraction

The camera provides an image of the chip in the device. This image contains the relevant information, it has to be interpreted in order to extract useful data in a quantifiable form. Image analysis is accomplished through the use of specialized software, such as ImageJ. ImageJ can be used to process and measure pixel color and intensity in any section defined by either a user or a previously recorded macro sequence, a simple program that automatically applies the recorded steps. Quantification is achieved by measuring pixel intensity on a scale of 0 to 255. The values are related to the standards of programming, in which one byte in binary reaches up to the number 255. By having the software define the sections that are to be examined it limits the geometry and arrangements of compatible chips only by software, meaning that as new devices are designed the software can be updated to accommodate the new additions. Future projects could further expand the applications of the device to detect color changes and even motion or particle propagation.

3.7.2.1 Image Analysis and ImageJ

Extracting the data involves analyzing the emission from the chip, manifested on the image as the color green. The average pixel intensity in the areas pertaining to the well should be higher than the regions not pertaining to wells. In practice, the well intensity was to be compared to the background pixel intensity to determine if it was higher or equal, a positive result being significantly higher than the rest of the chip.

The procedure depends on the kind of image being analyzed. For images using the emission filter, everything detected by the camera is part of the signal since the filter blocks anything else. These images are analyzed directly. For images not using the emission filter, the device

depends on the Bayer filter used in color cameras. Color images are composed by three kinds of pixels, red, green and blue. The proportions of these make up all visible colors in the images. Using ImageJ, we separate these pixels into three separate component images, the red image, green image and blue image. Each consists of the pixels of that color. The excitation light corresponds to the blue spectrum and is thus caught mostly in the blue image, there is little or no signal in the red image and the green fluorescence is shown in the green image. Since all colors and variations are created by these three specific colors, they don't exactly match with the tones used by the LEDs nor is the tone of the emission, so a bit of everything is seen in each. To minimize these effects, the image needs to be cleaned up, which will be discussed later in detail. The green component image will be analyzed similarly to the filtered image to extract the data. While this is more extensive than simply using the emission filter, all of this is software related and easily automated. The filter being one of the more costly component presents interest, as cost reduction, if it can be eliminated.

A designer creates a chip to be used with the device, in this case the Quick Chip by Weibel Lab. They then create the software that defines the sections of their chip to be analyzed and what image analysis method to use, along with any incubation instructions and when to incubate or capture an image. The software tells the device what to look for and how to look for it. This is done by specifying the relevant regions when the chip is designed, the software then calculates the average pixel intensity in each specified area yielding a numeric value. This value is what the programs interprets as a positive result, negative result, or whatever the test was designed to measure based on the designer's specifications. This research is limited to the device development and basic functionality testing, it will not include automated analysis and diagnostics of the sample images, which are beyond the scope but are necessary for real world implementation.

In order to further simplify the device design, two approaches were tested as to discern the necessity and importance of the emission filter. The first was to use the filter, capture the green spectrum from the sample and analyze the image. Even though the filter blocks everything that's outside the desired range, the image is still a color image, showing green sections for fluorescence.

Two alternatives that were available to change the image into grayscale, the first was to change it directly into grayscale, in which all the pixels would be converted to their gray, black or white counterparts depending on the intensity. The second was to separate the image into its

component images, red, green and blue (RGB) images, or an RGB image stack. Each component appears as a grayscale but it only considers the corresponding color for the change. This method was selected as any errant wavelengths that may have come through the filter due to its error margin would be reflected in their corresponding color, where for the direct grayscale method, they would be considered in the grayscale and could alter the results.

The second approach was to forgo the emission filter completely and directly capture an unfiltered color image of the chip. In these images, the chip appears clearly along with the light from the excitation system. The idea was to apply the RGB image stack technique, any light from the emission filter was captured as part of the blue image, there was little or no color in the red image, and the fluorescence emission would be captured in the green image. The data was then to be extracted from the green image, which is already a grayscale representing the desired wavelength. This approach is only possible because the excitation and emission wavelengths are different enough as to be captured on separate color layers of the image.

The keystone of the entire process depends on analyzing the image obtained from the camera into useful data the device can interact with. This is achieved through image analyzing software that can convert the image into values as assigned by the user. One such program is Image J, which is open source software, meaning it's free and open to third parties to develop additional software. This is important since open programs tend to have a larger user base and community support, meaning that there is a large amount of additions or alterations available for the source program. What Image J allows us to do is select any section of a JPEG file and apply a series of processes and alterations, one of which is calculating the average pixel intensity inside the designated region. It takes the selected area into account and measures the pixel intensity on a scale of 0 to 255, o corresponding to what would be a completely dark pixel and 255 complete white. It gives you the highest and lowest single pixel intensity and calculates an average between all the pixels in the region. This average, from 0 through 255, is the value that the program would interpret as positive, negative or whatever it is programmed to say, and associates it with data in memory to determine the chip results. It also allows preprocessing of the images for easier analysis, such as changing from a color image to the component images in grayscale.

The strongest argument for this program during development is the simplicity in programming macros. Macros are a series of commands that are programmed to run automatically, mimicking the actions of a user. They are used to automate repetitive tasks. Ideally, a macro would be

made for varying chip designs such that the program can interpret the desired information. A portable version of this software can also be installed on smaller microcontrollers, such as a Raspberry Pi, a microcomputer the size of an Arduino.

3.8 Control System

Arduino Uno – The microcontroller used to manage the entire system. This open source piece of equipment allows enough freedom to design this kind of device.

The brains behind the device's basic systems is an Arduino Uno, an open source and widely supported microcontroller specifically designed to be used as a prototyping platform. It can perform linear operations, but can only perform one task at a time. Through its many I/O pins, it can interact with a large variety of electronic devices, sensors, motors, etc. The pins are limited to a voltage of 0 to 5 volts, analog pins, which are special pins, allow any value between the limits and can sense voltage levels if used as inputs. The digitals pins serve as Boolean communicators, setting a voltage of 0 to function as a low signal or even to function as ground, while 5 volts is used as a high signal. When set as an input they can detect a voltage but not the magnitude of said voltage, unlike analog pins, which assign a value of 0 to 255 in accordance with how it compares to 0 or 5 volts. This is enough to trigger the power systems, but the available amperage is low. Both the heating and excitation systems require a larger amount of power than what can be managed by the Arduino alone, hence a power management system is necessary. A Mosfet is an electronic switch capable of handling higher currents by connecting it directly to a power source, it can then be controlled from the voltage from a single Arduino pin. Using one of these for the excitation and another for the heater unit would allow decent control, but to be able to use the heat pump as a cooling unit, a desired feature, the current needs to be invertible. A simple method to invert the current direction is by using an H-bridge.

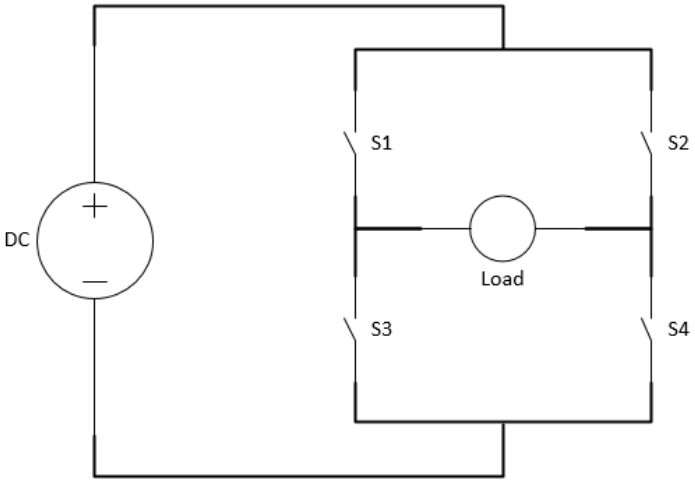


Figure 12: Basic H-Bridge Diagram The H-bridge consists of 4 switches. Enabling the correct sequence of switches reverses the current flow through the load. Enabling S1 and S3 or S2 and S4 will create a short circuit.

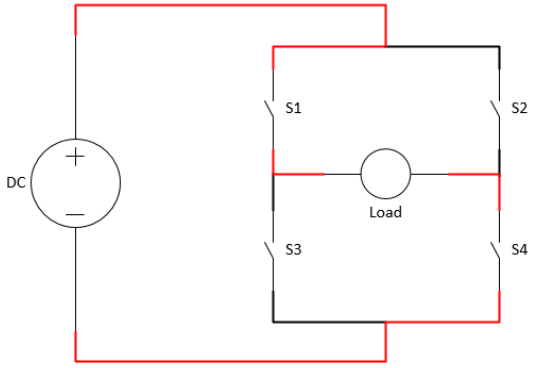


Figure 13: H-Bridge Enabling S1 and S4. Enabling S1 and S4 causes the current to flow from the left side of the load to the right.

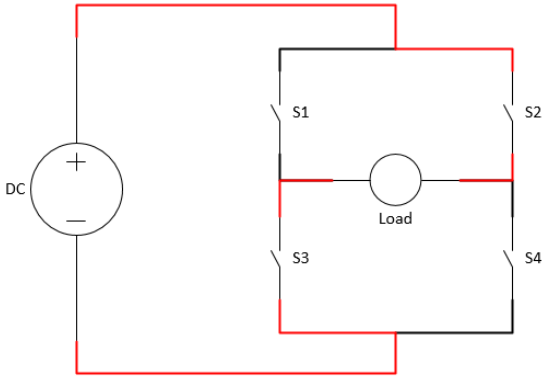


Figure 14: H-Bridge Enabling S2 and S3 Enabling the S2 and S3 switches allows the current to flow from right to left on the motor.

Seeeduino Motor Shield V2.0 –

One of the advantages of using Arduino is the wide array of components available designed to be easily integrated into an Arduino product. Expansion shields are complex chips that are connected directly onto the Arduino pins to add additional features. The Seeeduino Motor Shield V2.0 is one such add on chip. It was originally designed to control motors, controlling higher current levels and allowing current inversion. Here it is used to control the heat pump and the LEDs. The shield is designed to convert a 3.3 to 5 volt input from the Arduino into a higher voltage and up to 2 amps through four special output pins. By connecting a component's poles between two of these pins, current inversion can be done and controlled. It gives high enough power to use the heat pump and simplifies the LED wiring. The special power pins also allow the controller to invert current polarity, which in conjunction with the Peltier pumps, allows the heater to function as a cooling system, allowing for complex programs to more accurately regulate the chamber temperature.

The final control system consisted of an Arduino Uno with the Motor Shield. The high power outputs of the shield were connected to the heat pump and the LED assembly. The Arducam shield with the OV5642 camera module would be powered from the main Arduino Uno. While originally intended to be battery powered, the testing system was powered using a 12V power supply through the Motor Shield in order to eliminate battery power as a factor during testing.

4 CHAPTER – MANUFACTURE

This design was not meant to be a final production model, the purpose was to create a prototype for concept validation that should work with the Quick Chip. Developing fast prototypes would allow for earlier testing and adjustment, defining the manufacturing process.

4.1 Parts

The brains behind the device's basic systems is an Arduino Uno, an open source and widely supported microcontroller specifically designed to be used as a prototyping platform. Through its many Input/output pins, it can interact with a large variety of electronic devices, sensors, motors, etc.

Each pin can be set to function as an input through which it reads electrical signals or as output to send signals to other parts. The pins are limited to a voltage range from 0 to 5 volts and are mostly binary pins, capable of delivering 0 or 5 volts as a digital 0 or 1. Certain pins can function as analogue pins, special pins that can emit and read voltages between 0 and 5 volts. Using the digitals pins as outputs serve as Boolean communicators, setting a voltage of 0 to function as a low signal or even to function as ground, while 5 volts is used as a high signal. When set as inputs, 0 volts is read as low while any voltage is interpreted as high.

Analog pins can read the magnitude of the voltage value by assigning a value of 0 to 255 in proportion with the voltage read from 0 or 5 volts. While this is enough to trigger the power systems, the available amperage is low. Both the heating and excitation systems require a larger amount of power than what can be managed by the Arduino alone, hence a power management system is necessary.

All the custom parts were printed using a Lulzbot Taz generation 1 printer using 3mm PLA plastic.

4.2 Electronics

The electric circuit consists of the Arduino Uno with the Motor shield connected to the excitation and incubation systems. Shown are the pins used from the Motor Shield in order to simplify the diagram.

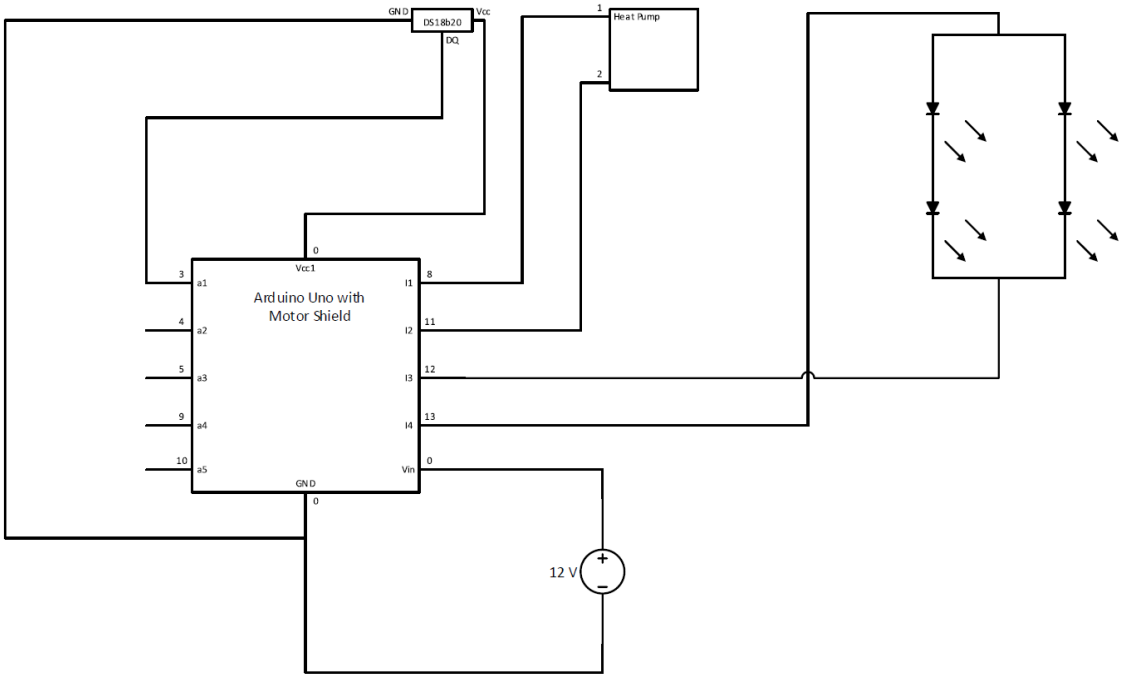


Figure 15: Basic control circuit diagram

Each LED has a forward voltage of 3.5 to 3.7 volts and draws 350 mA of power. This configuration requires around 7.5 volts to power the LEDs, a 1 ohm resistor is recommended after each set of series LEDs. Using a power supply, the current drawn by the Peltier heat pump was measured to be 2 Amps at 12 volts. Both voltages would be supplied by the controller.

Table 2: Arduino shield pin assignments

Pin #	Description
3	Data line for the DS18b20 temperature sensor
4	Assigned to trigger the camera controller but not used during the experiments
5	Assigned for a push button to activate the heater run
8	Motor Shield's high power output leading to the heat pump
9	Referenced in the code to allow heater to be activated. Required by the Shield code
10	Referenced in the code to allow heater to be activated. Required by the Shield code
11	Motor Shield's high power output leading to the heat pump
12	Motor Shield's high power output leading to LEDs
13	Motor Shield's high power output leading to LEDs
Vcc	Constant 5 volt output pin
Vin	12 volt input to power the system

Connected on top of the Arduino, the motor shield extends the Arduino pins and adds four high power pins assigned to pins 8, 11, 12 and 13 by the hardware. It also has a power connector and regulator that uses a 12 to 15 volt input to power the system, including the Arduino Uno.

The Shield requires the assignment of pin 9 to allow pins 8 and 11 to be enabled as a form of redundancy, but these were assigned and hard coded so no electrical component needs to be connected. By connecting one terminal of the heat pump to pin 8 and the other to pin 11, the system can switch 8 or 11 to serve as voltage source or ground, controlling the direction of the current through the pump. Pins 12 and 13 are connected to the LED circuit and activate the LEDs when pin 12 is the voltage source and 13 is set to ground. Since LEDs are unidirectional, reversing the current has no effect, but they were connected to both pins to prevent issues with the high current in other pins.

Pin 4 is assigned in the code to actuate a relay that triggers the camera system to maintain the option, but it wasn't used in any of the experiments. For the incubation system trials, a push button was connected between pin 5 and 5V. Pressing the button pulled the pin to 5 Volts, or HIGH, which the system used as signal to start the temperature run.

4.3 Final Design

Several iterations after defining the main issues to be addressed resulted in a design that proved to be simple to operate and covered the basic requirements. The latest version was designed to work with a computer.

Figure 16: Final device concept

4.3.1 Design Model

The final design consists of a main chamber composed of a stationary base with chamber walls and roof mounted on a hinge. Inserted in the square chamber hull there's a mount that holds and aligns four excitation filters and the corresponding LEDs at a distance such that they align with the sample when the chamber is closed.

The OV5642 camera module, connected to the Arducam shield, fits into the hole on the top of the chamber cap. Inserting the camera makes the focus wheel of the module fit tightly into the focusing wheel and aligns the system with the emission filter mount. These components are all fixed onto the chamber and move with it when the device is opened.

On the base there's a hollow pedestal onto which the heat pump fits into. The hollow chamber connects to the outside of the device through channels under the base. This is to allow the heat to be absorber or dissipated into the ambient when the device is heating or cooling the chamber. The temperature sensor is fixed to the top of the heat pump using a small clip.

Wiring runs from the controller outside into the chamber through an orifice under the hinge in the back. Temperature control wires run parallel to the base and up the heat pump pedestal while the LED wiring goes up the wall and around the chamber. An outside box serves as a battery case, for battery operation, doubling as a base for the Arduino main controller.

Incorporated onto the hinged chamber are the LEDs and filters which move with the chamber when the device is open. A hollow pedestal on the base serves as a mounting surface for the heat pump and as a duct for air to pass as the heat pump moves heat into the chamber. An exterior mount serves as a battery pack, with wiring holes and doubles as a base for the Arduino controller. The Arducam system and CMOS module fit into the orifice on the roof of the chamber where the OV5642 camera aligns and fits snug into the focusing wheel. This is aligned with the emission filter mount inside.

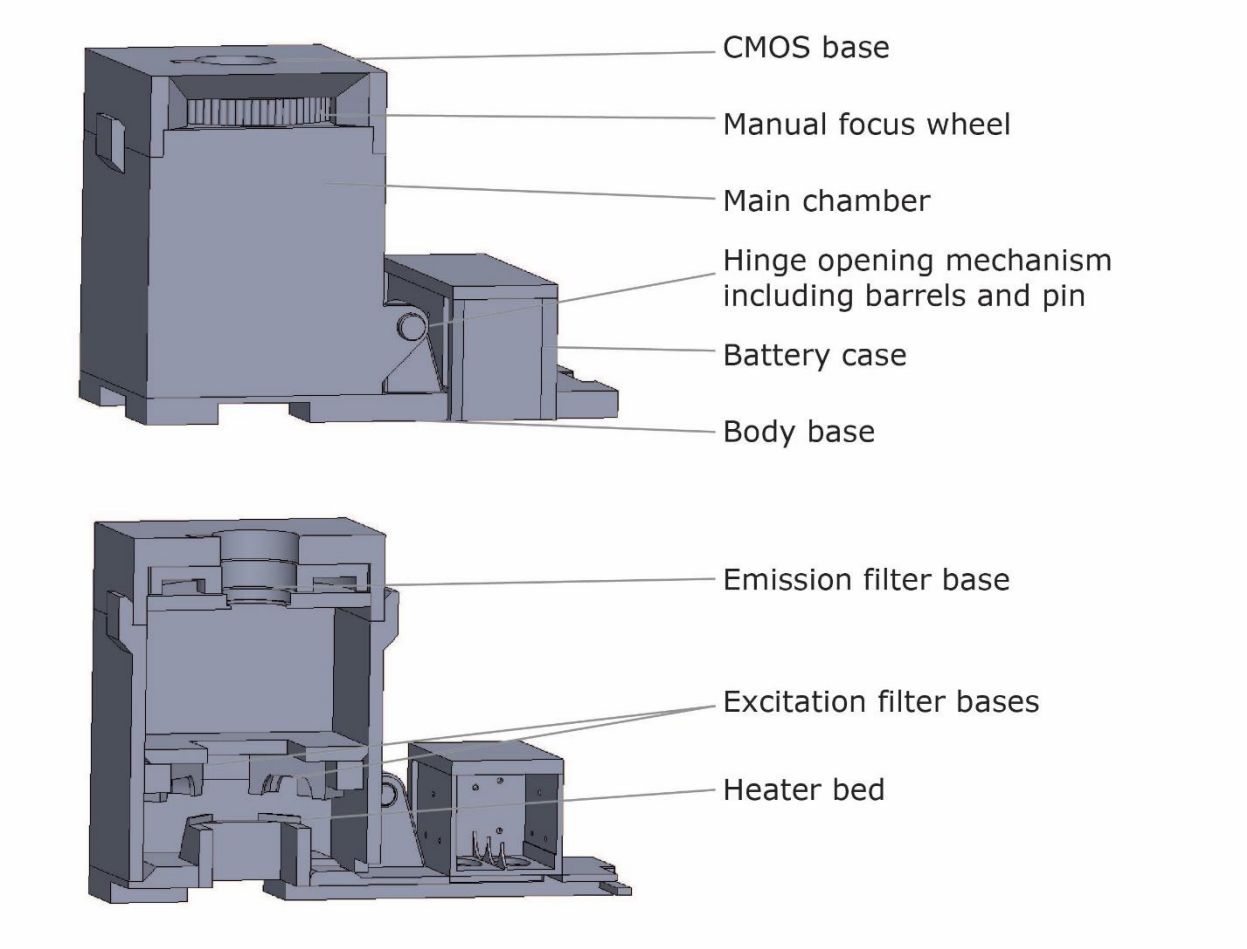


Figure 17: Final device design CAD and Cross-section

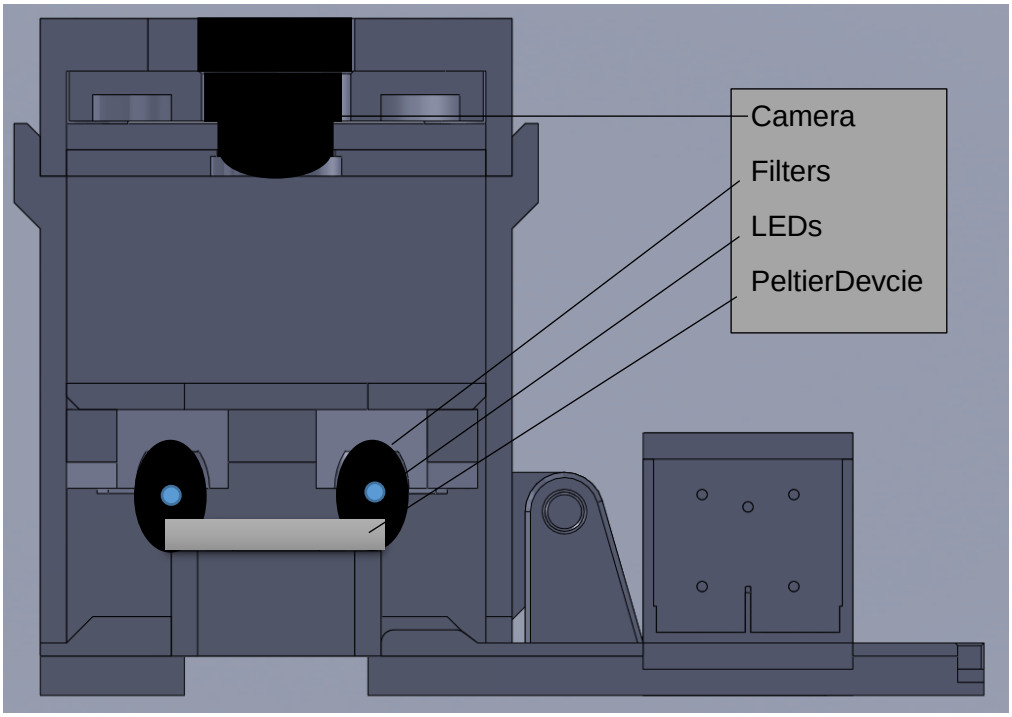


Figure 18: Device Cross-section Arrangement

4.3.2 Parts List

Table 3: Electronic Components

2	Arduino Uno
1	Seeeduino Motor Shield
4	Mounted Cree XLamp XB-D Blue LEDs
4	Excitation Filter, 480 nm bandpass filter
1	Emission Filter, 535 nm bandpass filter
1	Arducam Shield Module
1	OV5642 Camera Module
1	Peltier heat pump, 40x40 mm thermoelectric cooler, painted black
1	DS18S20 OneWire temperature sensor
1	12 v 2000 mA Power supply, wall outlet transformer

Table 4: Printed Parts

1	Device Base
1	Heat pump mount
1	Arduino Mounting Base
1	Mounting base cover
1	Chamber body
2	Hinge pins
1	LED and Excitation filter mount
1	Emission filter mount
1	Camera Mount
1	Focus wheel
1	Temperature sensor clip

4.3.3 Functional Summary

The chamber design is focused around ease of use. Keeping the LEDs and camera on the swinging chamber keeps everything aligned through repeated use while granting easy access to the sample. Opening the chamber moves the LEDs and filters away from the sample, leaving just the heater surface in place.

A user would open the chamber by swinging the chamber back and place the chip onto the heat pump surface and close the chamber. A programmed profile for the chip would automatically do the rest, incubate the sample and capture the images of the chip, hence loading the chip onto the device is the most complicated operation directly performed by the user.

5 CHAPTER – EXPERIMENTATION

Prototype testing was performed to demonstrate that the device meets the requirements to function with the Quick Chip system.

- Detectable emission using the LEDs and CMOS camera. Detectable emission is defined as enough of a difference between the well pixel intensity from the pixel intensity on the rest of the images, corresponding to background noise.
- Uniformity among the wells, which means a consistent excitation field in the testing chamber. This is measured by comparing the pixel intensity among the wells belonging to the same chip.
- Analysis by color separation. Using the Bayer filter from the CMOS camera, separating the component color imager to extract the data from the green image.
- Incubation temperature control. Uniform temperature in the testing chamber over a period of time, tested by measuring the deviation of the chamber temperature with respect to a set target.

5.1 Experimental Setup

The experiments are divided into either optical or thermal category, using slightly different setups. For the optical systems, the designed device was assembled, powered with a 12 volt power supply as to eliminate the need to recharge batteries after repetitions. The OV5642 camera module was set in the Arducam system along with a 2 GB micro SD memory card. The Excitation filters mounted oved the LEDs were 490 nm wavelength bandpass filters. The heating bed was a Peltier heat pump with a temperature sensor on the surface. The emission filter, a bandpass of 535nm wavelength, was set over the testing chamber in front of the camera module for some specified tests, removed for others as specified. The LEDs and heating system were connected to the Seeeduino Motor shield on the Arduino Uno while the camera was triggered manually from the Arducam. The captured images were stored onto the SD card from where they were transferred to the computer for analysis.

5.2 Fluorescein Sample Preparation

Optical experiments are based around a series of microfluidic chips, each is loaded with a different 6-fam solution. Each solution consists of a different concentration of 6-fam in solution with water and magnesium acetate tetra hydrate. For a successful detection, the device must be

able to capture the required 100 nM of 6-FAM with 14 mM magnesium acetate in water, an emission equivalent to that of the Quick Chip system.

5.2.1 Concentrations and Dilutions

Stock solution was prepared using 6 - Carboxyfluorescein powder at 2 mM in distilled water. This was the base for dilution. The magnesium acetate tetra hydrate was prepared in a similar fashion as a 140mM stock. Both were combined in the appropriate proportions in 50 mL test tubes and filled with distilled water, yielding concentrations of 200, 100 and 50 nM each with 14 mM magnesium acetate tetra hydrate.

Each sample was loaded into a different chip through the self-loading mechanism. The chips were placed in vacuum at 0.01 atm for one hour, degassing the PDMS. Immediately after removing the chips from vacuum, each was loaded with a drop of sample on the inlet and labeled accordingly. They were stored in a petri dish and covered in aluminum foil to prevent exposure to light until they were used shortly after.

5.3 Temperature Experiment

Incubation requires controlled temperatures for periods of time. For Quick Chip, the reaction is accelerated at 37 degrees Celsius and should take around 30 minutes for incubation. These are the goal parameters, using the Peltier heat pump in conjunction with the temperature sensor and control to maintain a steady temperature for a prolonged period. A run time of over an hour was used to observe the temperature variation while using the simplest control method, engaging the heater while under a set threshold and disengaging it when it meets the desired temperature. The graph should oscillate between high and low values. To understand the chamber behavior it’s necessary to measure the set temperature in the chamber and measure it to the actual temperature felt by the chip. If the behavior of these is similar then it’s only a matter of calibration to set the appropriate value in the program to obtain the desired chamber temperature.

To measure the temperature inside a PDMS chip, a special chip was made by encasing a temperature sensor inside a slab of PDMS in the same approximate dimensions as a microfluidic chip. It was loaded onto the chamber and the system was set to run at 40 degrees Celsius. The temperature was measured by connecting the sensor to the Arduino which was set to export the information and the readout from the thermal control sensor through the Serial

Monitor. The readouts were taken at one measurement for both sensors each second and presented a column of readings for each.

5.4 Optical Experiments

Fluorescence based tests consisted of capturing images of the chips containing the different concentrations. For the various tests, the experimental setup was identical, unless otherwise specified, since the difference was in the data extraction and analytical methods.

5.4.1 Concentration Detection

Optical testing was conducted using the device configuration using both excitation and emission filters. To test the effect of concentration, a sample chip was placed in the chamber and an image captured during excitation. First, the 50 nM solution chip is placed in the chamber, the LEDs activated and an image was captured using the Arducam, using the OV5642 module onto a micro SD card. This process was repeated for the 100 nM and 200 nM chips. Resulting images were transferred via the micro SD card to the computer for analysis.

5.4.2 Emission Filter

In order to test if it is possible to extract data from an image using only RGB decomposition, comparable images were taken consisting of sets of two images. For the first, a sample chip was captured using both the excitation and emission filter. For the second image the emission filter was removed, capturing a full color image of the chip.

Capturing both kinds of images, with and without emission filter, was repeated for a 200 nM sample chip, a chip containing distilled water and one containing just a 14 mM MAT concentration. The water and MAT chips were used as control chips to determine their respective effects on pixel intensity and observe the amount of noise generated from negative chips. The comparison between the control chips and the 200 nM would determine plausibility for filter-less data extraction.

5.4.3 Uniformity

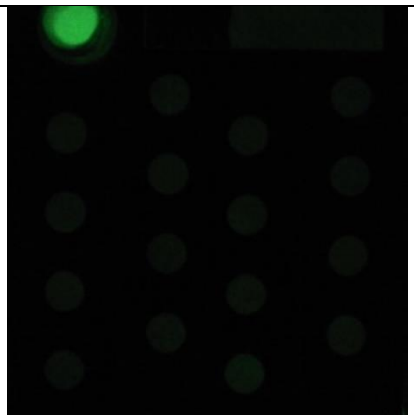
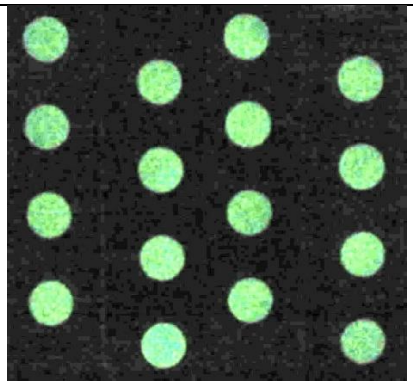
In order to test the uniformity of the excitation field, a comparison between the intensity emitted from each well was compared to the intensity of the rest of the wells on the same chip. Since each individual chip contains only one concentration sample throughout all the wells, the variation in emission would be attributed to the difference in excitation. The largest difference

would be expected between the innermost and outermost wells, so it was on this comparison that the analysis would focus. The setup for capturing these images consisted of the complete system using both excitation and emission filters.

5.5 Image Analysis

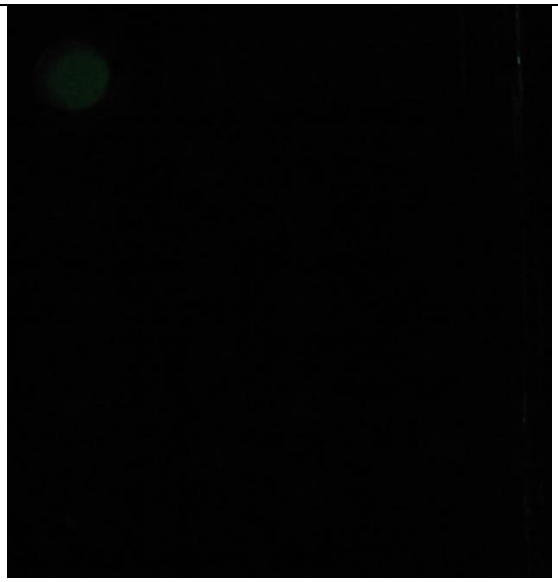
Having the captured images, the data was extracted by using an automatic enhancement tool. The filtered images contain only the desired light spectrum, they can be analyzed directly.

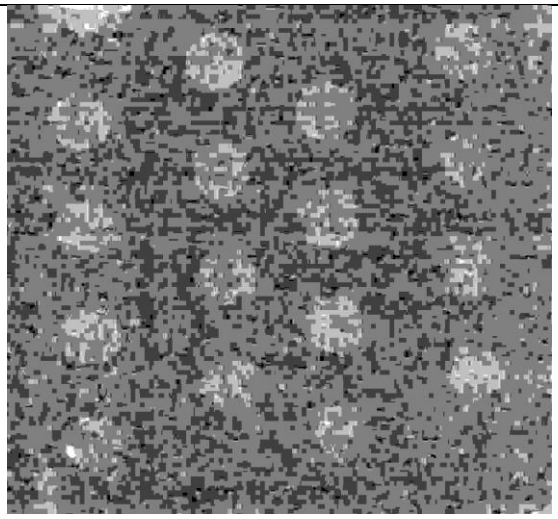
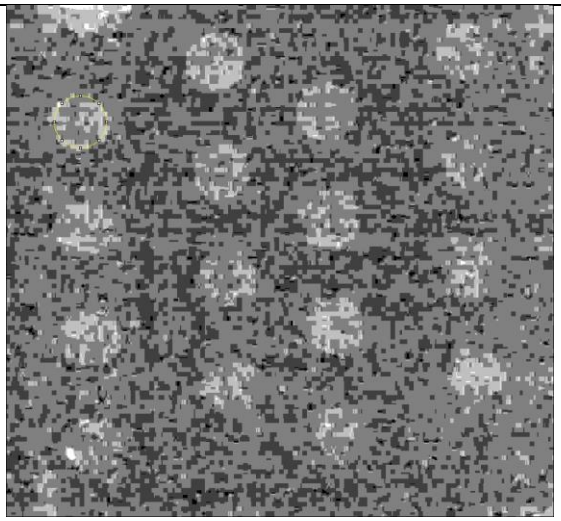
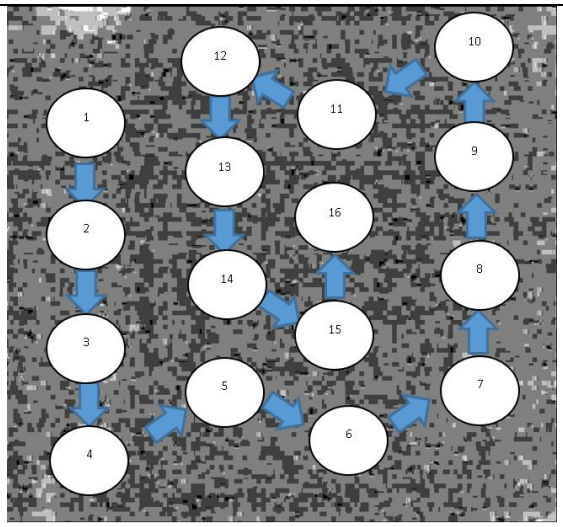
Table 5: Image progress for 200 nM.

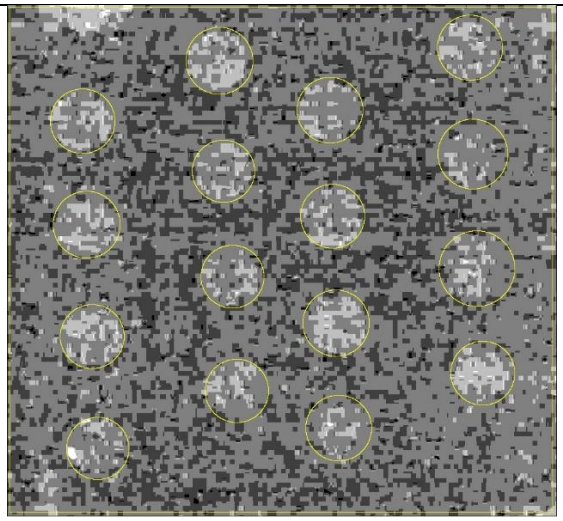
	Initial image of a 200 nM sample.
	Cropped image, eliminating unnecessary sections to keep them from interfering with the next step.
	Enhanced image of the 200 nM sample using the Automatic enhancement tool set to 0.4% in ImageJ.

A comparison between the pixel intensity of the corresponding well regions in contrast to the background pixel intensity serves as a method to determine intensity increase due to fluorescence. This method allowed for fluorescence detection at low concentrations down to 50 nM.

Table 6: Step by step image enhancement and data extraction.

	Original image of sample chip cropped to the size of the chip. The wells are not visible in this sample as it best demonstrates the impact of this process. The green circle is the inlet well through which the sample was inserted. The higher fluorescence is due to its large volume and interferes with with the automatic enhancement tool, so it must be cropped out.
	The image is cropped to include only the area pertaining to the Wells, eliminating any unnecessary sections as these interfere with the processing.

	Use the Enhance Contrast tool set to 0.4%, increasing contrast until this percentage of pixels reach saturation or 255 intensity. This is where the unnecessary sections would have interfered as the software would have taken them into consideration in the contrast.
	The desired testing location is selected, in this case the inside of the well, using a 90 pixel circular selection. The measurement is taken avoiding the edge to maintain a clear measurement. The average intensity is measured from this area.
	The selection and measurement is repeated for each well in a counterclockwise spiral order. This groups the more outer wells from 1 to 12 and the inner wells from 13 to 16.

	The entire chip is selected using a square section and each well is excluded from the selection. The areas excluded are slightly larger than the wells in order to eliminate the well edges to ensure a clear measurement. The average intensity of this complex selection is measured, this represents the average intensity for noise and background effects.

Comparing each region to the background demonstrates that the positive is visible. For individual well analysis a better measure would be to compare the well intensity to a more localized background measurement like the area immediately surrounding the specific well in question.

6 CHAPTER – RESULTS

6.1 Concentration

Analyzing the filtered images yields the following results. The difference between background intensity and well intensity gives the effect of the fluorescence on the pixel intensity. A correlation was apparent between pixel intensity and fluorescein concentration, the water and magnesium acetate samples were taken as controls to observe the effects of these compounds on the detection. The difference measured in the non-fluorescent samples gave a baseline to compare and define the pixel difference that could be interpreted as a positive result. The high intensity measured in the non-fluorescent regions, such as H2O, MAT and background measurements, are due to captured noise from the camera later interacting with the automatic enhancement tool.

Table 7: Pixel intensity per well for each sample chip.

Well #	50 nM	100 nM	200 nM	H2O	MAT
1	152.023	162.078	183.468	96.851	113.169
2	156.952	162.208	179.078	91.572	112.942
3	156.785	171.724	200.066	78.068	107.743
4	143.421	171.443	181.349	81.641	102.851
5	149.215	157.495	204.222	73.295	112.756
6	144.457	186.067	217.581	77.441	92.452
7	167.212	174.439	200.683	82.067	136.712
8	152.031	167.465	195.728	91.039	110.383
9	140.963	167.046	174.311	97.451	107.21
10	152.169	189.79	180.544	86.706	116.648
11	144.559	157.292	181.144	98.118	102.824
12	159.716	157.827	175.146	91.732	110.036
13	146.643	170.249	203.822	79.694	92.945
14	142.965	163.835	190.185	79.574	98.625
15	164.017	173.611	210.823	75.921	105.064
16	146.697	167.644	183.545	75.628	95.372
Average	151.2391	168.7633	191.3559	84.79988	107.3583

Background	108.676	65.377	45.538	87.227	106.9

Table 7 shows the values for average pixel intensity for each well in each chip, where a different chip was used for each solution. The H2O and MAT values are due to the noise generated by the camera. These values seem large due to the automatic enhancement tool used on the images, which brings the highest 0.4% of pixels to saturation, 255, and adjusts the rest of the pixels in proportion to this new scale. This results in augmented noise. Since there is no fluorescence in the image, the intensity in the wells is close to the background. Having a higher background intensity is due to the nature of noise after being enhanced.

Figure 19: Increase in pixel intensity per sample concentration

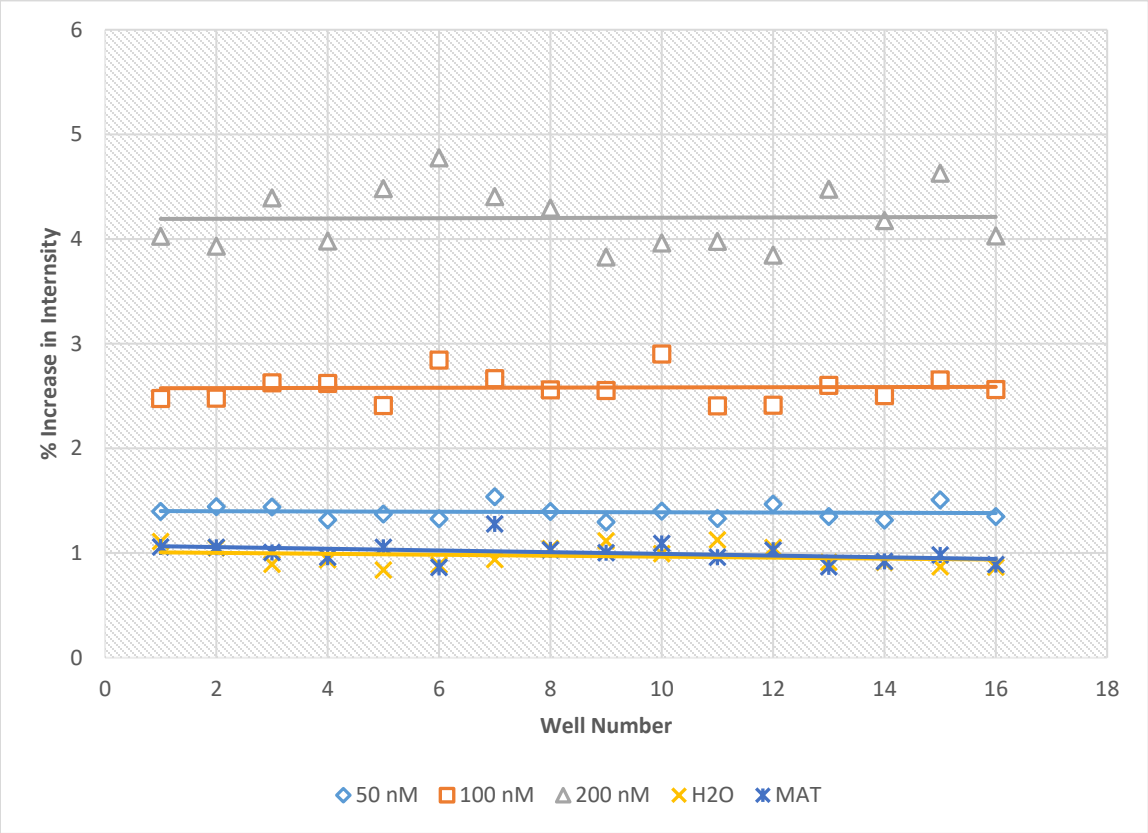


Table 8: Intensity Difference between Well Regions and Background

Well #	50 nM	100 nM	200 nM	H2O	MAT
AVG - BG)/BG	0.391651	1.581387	3.202116	-0.02783	0.004287
% Change	39.1651	158.1387	320.2116	-2.78254	0.428672

Table 9: Percentage Change from Background Intensity

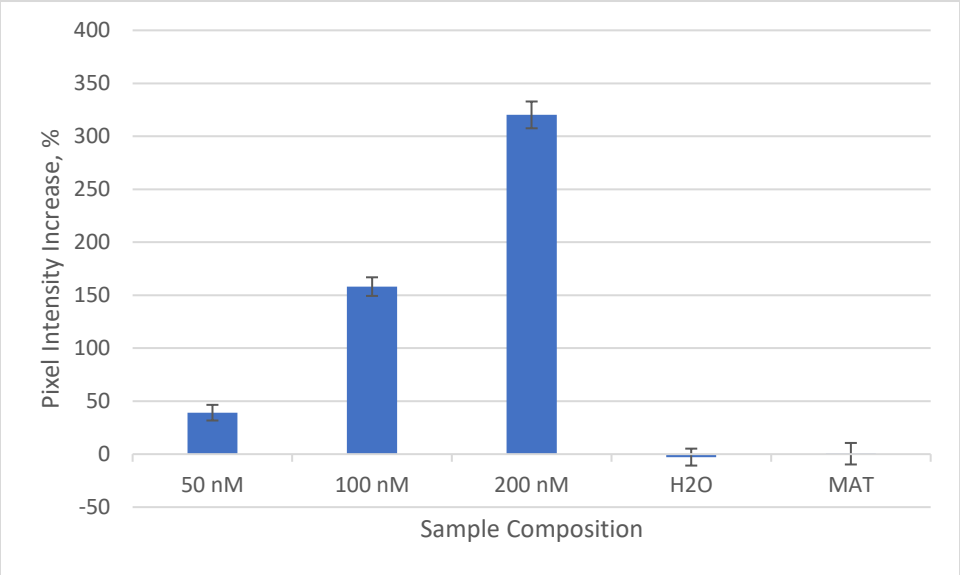


Table 9 shows the increase in fluorescence emission in comparison to the background intensity in each chip by comparing the well intensity of each well to the background of the pertinent chip. Standard deviation was calculated using n = 16, 16 wells per chip where different chips were used for each molarity.

6.2 Illumination Field

The relative uniformity of the excitation field certifies that wells get even excitation, this is validated by measuring the intensity of the wells and comparing the difference between the center wells and those closest to the edge of the chip. Due to the numbering convention set to identify the wells, well numbered 1 through 12 correspond to the edge wells while 13 through 16 are the inner wells. Comparing the average pixel intensity of the outer wells to the average of the inner wells and obtaining the difference between the two sets of values serves as a measure

of the difference in emission. Since the sample concentration is the same, the variation is due to the difference in the excitation field.

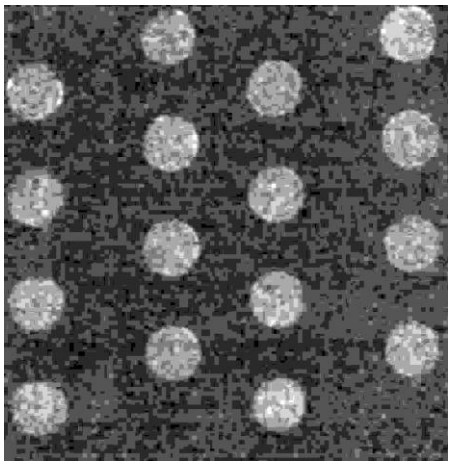


Figure 20: 100 nM Enhanced Contrast

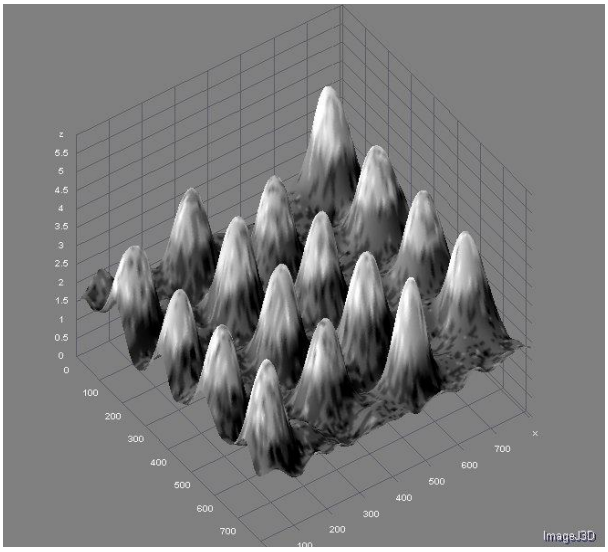


Figure 21: Surface Plot 100 nM Enhanced Contrast

Table 10: Well comparison

	50 nM	100 nM	200 nM
Outer Wells	151.6253	168.7395	189.4433
Inner Wells	150.0805	168.8348	197.0938
Difference	1.54475	0.09525	7.650417
%Difference	1.018795	0.056448	4.038367

The difference between the averages from the outer wells to the inner wells are at most 4% for 200 nM and around 1% for 50 nM.

The field appears to be uniform enough to excite the wells evenly enough as to not produce major difference in their emission.

6.3 Filters

In order to test the validity of using software processing to detect fluorescence in the images, a simple test was designed, capturing an image of 200 nM sample and comparing it to a control image containing water. After RGB decomposition of the image, the water chip should only show the noise generated by the system, generated by the error light from the LEDs and refracted wavelengths on the transparent chip. The purpose is to show that there is a measurable difference between the two chips and thus demonstrate that the software analysis is a viable method to detect fluorescence emission eliminating the need of an emission filter.

Both imaged were captured using the device prototype and the OV 5642 camera module using the excitation filters but no emission filter capturing a clear color image of the system.

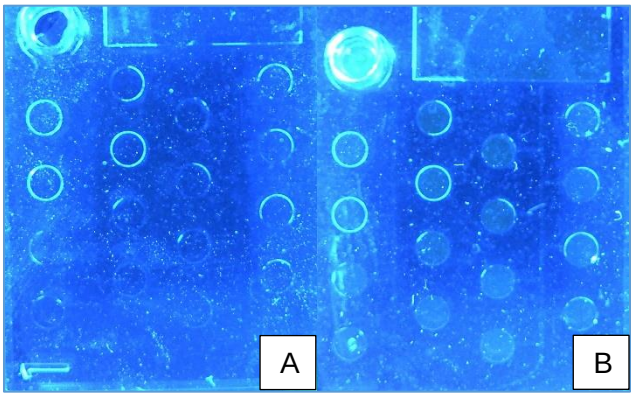


Figure 22: A) H2O B) 200 nM Both with No Emission Filter

After being loaded onto ImageJ, both images were separately processed. The first step was to eliminate part of the noise which would affect the readings by removing outlying pixels that exceed the a localized average value by comparing each pixel to an average of a surrounding radius of pixels, in this case 10 pixel radius. If a pixel surpasses the localized average by a threshold amount, in this case an intensity threshold of 5 units, it is replaced by the average value of that circular region. This smoothens the image. The Image is separated into the RBG

component images, isolating the green image and extracting the background to increase the contrast of the well regions.

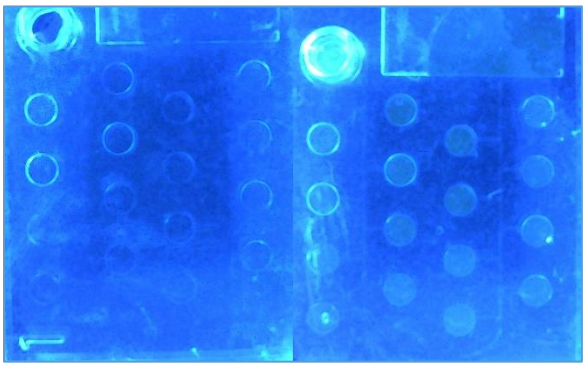


Figure 23: Clean Image. Outliers have been replaced by the average intensity of their surrounding region.

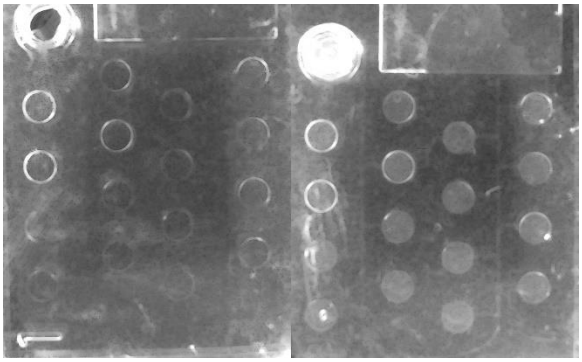


Figure 24: Green component images after RGB decomposition

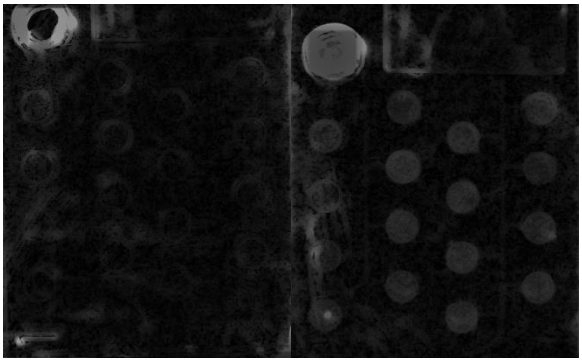
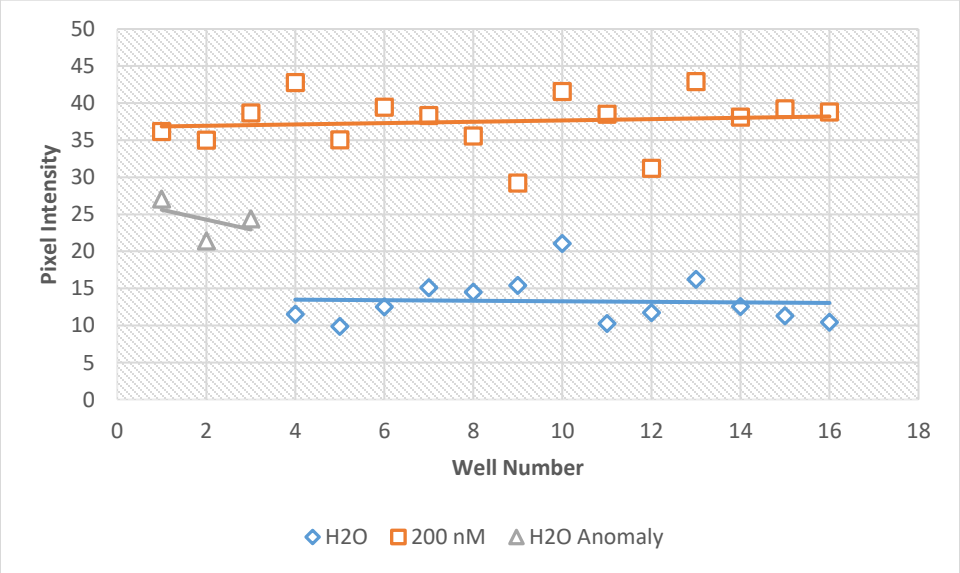


Figure 25: Images after using the automatic background extraction tool

Well	H2O	200 nM
1	27.05	36.185
2	21.398	35.003
3	24.386	38.662
4	11.5	42.779
5	9.87	35.024
6	12.509	39.44
7	15.112	38.313
8	14.469	35.556
9	15.405	29.21
10	21.053	41.551
11	10.242	38.486
12	11.729	31.196
13	16.232	42.886
14	12.528	38.11
15	11.275	39.231
16	10.417	38.819
Average:	15.32344	37.52819

Figure 26: Average well pixel intensity. Wells are numbered 1 through 16 in a counterclockwise spiral from the inlet down.

Table 11: No-Emission Filter Comparison



A clear difference is observed between the 200 nM chip and the H2O control. Fluorescence is observable using the software analysis method hence the device can function without the emission filter as the 200 nM sample gives over twice the pixel intensity as the water.

6.4 Temperature

Temperatures measured within the PDMS chip reached the target temperature within 2 minutes of incubation starting from around 27 degrees Celsius to 40 degrees Celsius. While the temperature measured by the device stabilized around the 40 degree Celsius mark, the temperature within the chip continued to rise, stabilizing around 43 degrees Celsius, remaining within one degree of variation.

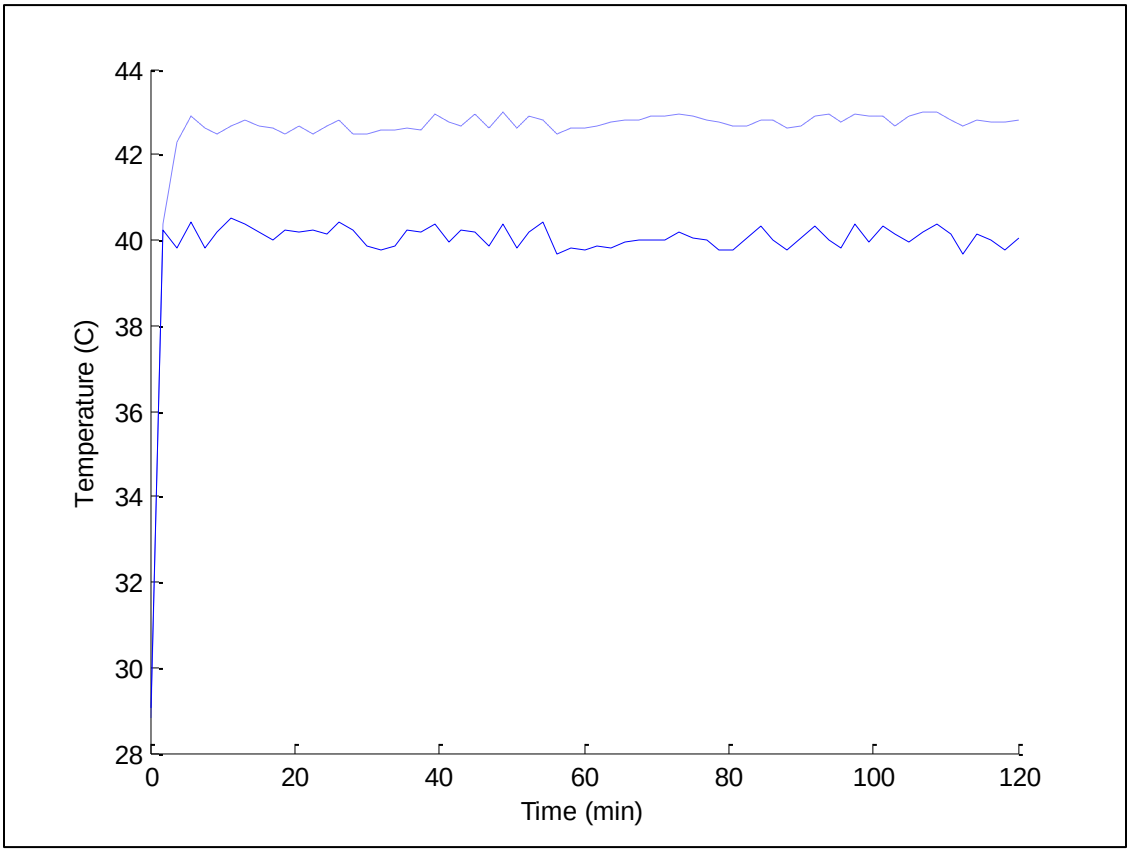


Figure 27: Temperature measurement Deviation = 0.44576

The continuous line in Figure 27 is the temperature as set in the device control and measured by the temperature sensor controlling the thermal cycles. Whenever the heater reaches the set temperature it is disabled and enabled when the temperature drops below that temperature. The dashed line represents the actual temperature inside a PDMS chip as measured by the PDMS

encased sensor in the temperature testing chip. The difference was due to the thermal properties of the PDMS as it does not cool as quickly as the chamber, hence the heat was engaged before it dropped to the chamber temperature. This behavior stabilized at a higher temperature.

6.5 Quick Chip Validation Testing – ESKAP Bacteria Measurements

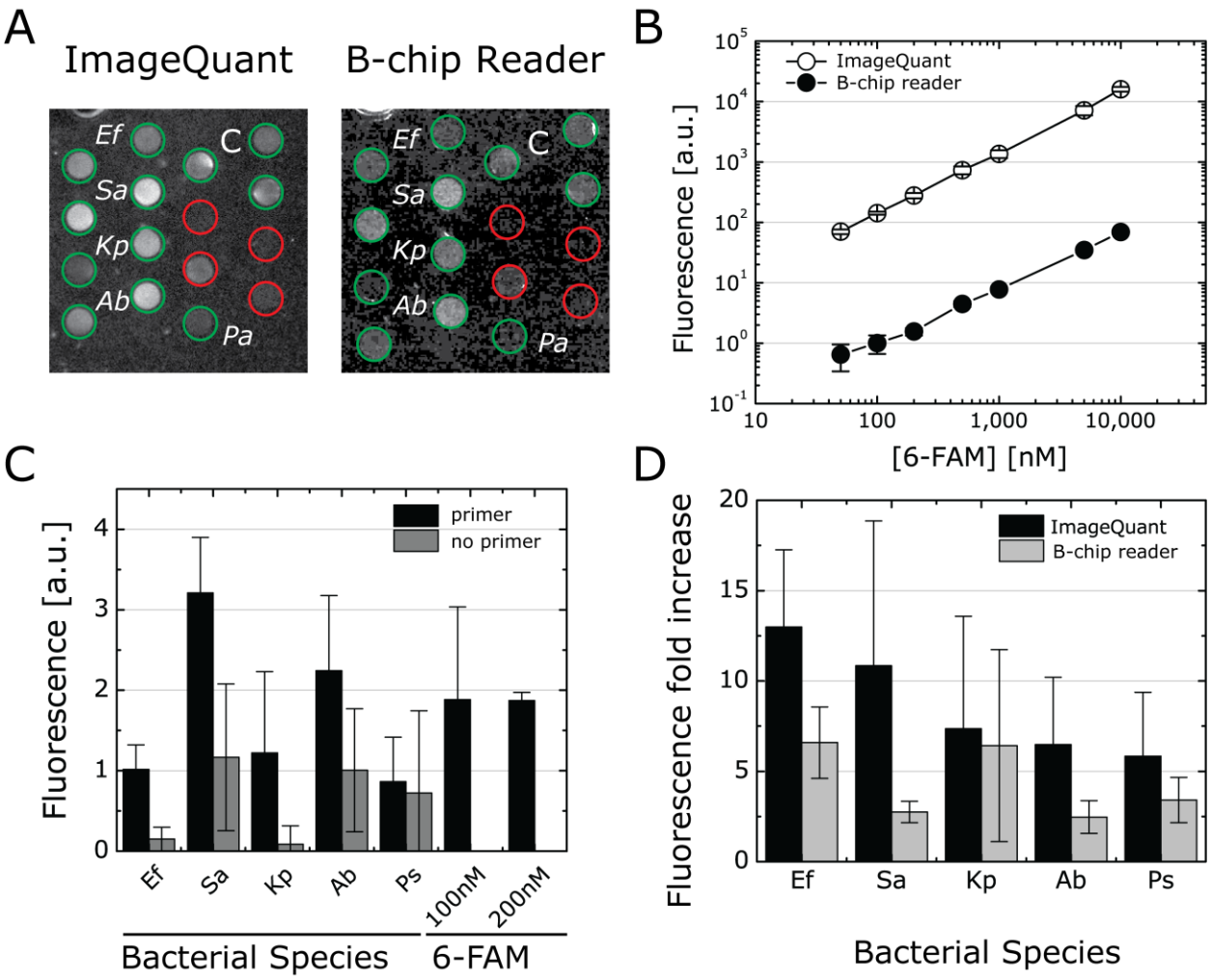


Figure 28: RPA Functionalized Chip Comparison. - [15] A) Image captured using a commercial desktop detector, ImageQuant, for a series of pathogens compared to the same chip captured using the designed device, labeled the B-chip Reader. B) Fluorescence detection comparison versus concentration for both machines, demonstrating fluorescence behavior for the chip in both machines. C) Fluorescence detection levels for the bacterial species using the reader with 100 and 200 nM as control for 6-fam intensity D) Increase in fluorescence between samples with primers and without primers, essential for DNA replication to observe the difference in detection signal caused by the DNA amplification. A. baumannii (Ab), E. faecium (Ef), K. pneumoniae (Kp), P. aeruginosa (Pa) and S. aureus (Sa).

Figure 28 shows a comparison between data extracted from a functional B-chip using the newly designed reader and data obtained using a commercial Image Quant fluorescence reader. Unlike the samples used for device testing, this B-chip is a prototype chip functionalized with genetic probes for a series of genetic sequences and loaded with a sample containing all the relevant sequences to obtain positive results in the respective wells. It demonstrated that the reader was able to detect the various degrees of fluorescent emission from the actual chip to a degree comparable to the machine that was used in the lab.

7 CHAPTER – CONCLUSION

We successfully designed, developed and built a portable DNA amplification fluorescence detector capable of measuring positive isothermal DNA amplification results from an RPA assay which produced great results when used with RPA assays in collaboration with the team at Weibel Lab. Detection was achieved for samples as low as 50 nM 6-FAM using a single image. The only change done to the chip was a small change in well height to 500 µm, increasing the sample surface exposed to the excitation light, a change suggested to the team, which became part of the final chip design.

These fluorescence results could be enhanced by changing the image capturing method, using multiple image stacking or increasing aperture time for the camera. The original target was detection of 100 nM, clearly detected using the emission filter.

The difference in intensity between the inner wells and outer wells in a chip demonstrated the uniformity of the excitation field, which minimizes variation within the chips.

While the emission filter was needed to detect 100 nM and 50 nM signals, using 200 nM samples demonstrated that it is possible to detect fluorescent signals using image decomposition in this particular case, but is impractical using this setup as the noise in the image is increased and alters the results of the image processing. Variations in the background and excitation also alter affect the quality of the data while the overall pixel values are much lower than those obtained in the concentration experiment, likely due to drops in relative intensity from RGB decomposition. This method could become practical after further tuning of the excitation system and processing methods of the images.

Incubation proved possible in the chamber by demonstrating the capacity to sustain temperatures for extended periods of time with temperature deviations of around one degree.

While the achieved temperature was off, this can be addressed with calibration by altering the expected values in the control program and was likely due to not taking into consideration the thermal properties of the microfluidic chip. Finer control should be possible by using more complex control equations that regulate the power to the heat pump considering the thermal properties of the entire system. Reversing the current in the heat pump allows for active cooling and more accurate temperature control, something not tested here, but achievable using the hardware as is.

The device was demonstrated capable of sustaining incubation temperature as well as providing sufficient excitation energy to warrant an emission. The camera system proved adept at capturing the signals, which were processed using a computer. The next step for the device would be to develop a control system that automatically performs incubation, excitation and image analysis with the goal of using a smartphone to carry out the image processing, but this is outside the scope of this investigation.

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