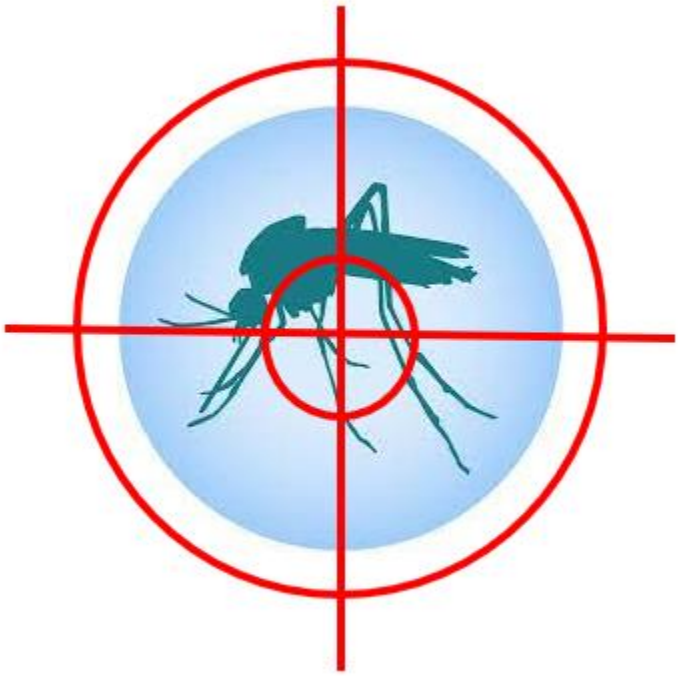
Bug Bane
Target Malaria



ME 4723-A & BMED 4873-A Interdisciplinary Capstone
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3. Executive Summary

Malaria is a rampant disease that specifically plagues many underdeveloped nations in sub-Saharan Africa. The primary reason for this localization is the mosquito; though the insect exists worldwide, epidemics of malaria, dengue fever, West Nile virus, Zika, and yellow fever are largely attributed to the specific species of mosquito native to these geographical locations. Historically, efforts to control such diseases through manipulating local mosquito populations are often labor intensive, difficult to implement, and unreliable. Recently, a modern solution has been developed which utilizes genetically modified mosquitoes, which are unable to breed viable offspring, to control the prevalence of malaria-carrying insects in the wild. However, the production of transgenic mosquitos is not yet reliable, and a robust approach to select the transgenic mosquitos is needed.

Currently, the Centers for Disease Control and Prevention (CDC) implements a system whereby transgenic mosquito larvae are fluorescently marked and separated by hand from their wildtype counterparts and retained for study and/or release into the target ecosystems. However, this system is slow, non-scalable, and taxing on the researcher. Commercially available equipment for biocompatible macro-organism sorting exists (e.g., Union Biometrica's COPAS), but these devices are overly sophisticated and cost prohibitive. Therefore, it has been proposed to design, fabricate, and validate a simple robust device that can detect and separate fluorescent mosquito larvae at a rate greater than 1 larva per second with an error margin less than 1%. This proposal implies coordination of two subsystems. After individualization is achieved in a constant flow stream of mosquito larvae, one subsystem must excite and detect fluorescence in the larvae while a subsequent subsystem must sort the larvae into transgenic and wildtype output reservoirs. These can be identified as a "sensing" subsystem and a "sorting" subsystem, respectively. The sensing subsystem will function using a laser and luminous intensity sensor chip, and the sorting subsystem will function using actuated flow displacement.

By creating a low-cost, reliable, easy-to-use device that efficiently sorts transgenic mosquito larvae based on fluorescence, the CDC will receive a tool that grants two major benefits: increased researcher productivity and elimination of tedious manual sorting. Together, these benefits will enable transgenic mosquito sorting in developing countries for on-site malaria mitigation and further international efforts to address epidemics affected by infectious mosquito populations in tropical and subtropical regions.

The requested device will use a combination of optical sensing and mechanical actuation to sort the larvae. To accomplish the former, factors such as fluorescence intensity/purity, media clarity, sensor capability, and post-signal processing will be considered, which will define design parameters such as material selection, fabrication strategies, and experimental setup. To define the same respective parameters for mechanical sorting, factors such as cycle frequency, specimen sensitivity, fluid dynamics, and robustness will be considered. For both sensing and sorting, flow rate will have a significant impact on design. Because of tradeoffs between reliable source pressure, cost and complexity, and ease of flow rate manipulation, some optical sensors that may operate optimally when the specimen is still as well as mechanical actuators that move slowly might become infeasible.

The main goal of the Target Malaria project is to balance the requirements of high-throughput sensing/sorting with the design challenge to minimize device complexity. By creating a function tree, we have determined a set of tools that will best serve us in the design

of this device. First, we will utilize either gravity fed flow, a pump, or hand fed to control flow. Secondly, an excitation laser will be needed to force fluorescence of the DsRed protein within transgenic larvae. Then, an optical sensor that can detect red fluorescence will communicate through an Arduino microcontroller to send a signal downstream to the sorting mechanism, which will include either a mechanical actuator, air blast sorter, or will physically move the reservoirs to separate out the fluorescent from the non-fluorescent larvae.

Inspiration for the design was influenced heavily by Dr. Kyle Gabriel's Automated Particle Counter (APC), which utilizes a similar sensor chip to count larvae as an LED is blocked during flow. However, the APC is unable to detect fluorescence or differentiate between transgenic and wildtype larvae. It is expected that the final design strategies reported here will be adapted to upgrade the APC, enabling it to both count and separate fluorescent and non-fluorescent larvae samples.

4. Nomenclature & Glossary

Glossary:

1. ATLAS: Automated Larvae Sorter
2. APC: Automated Particle Counter; can be programmed to aliquot a specified number of larvae from a batch into a new reservoir
3. COPAS: Complex Object Parametric Analyzer and Sorter
4. CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; gene editing technology
5. FACS: Fluorescence-Activated Cell Sorting
6. CDC: Centers for Disease Control and Prevention
7. RUO: Research Use Only

5. Main Body

5.1 Introduction and Background

Malaria is a rampant disease that commonly plagues many underdeveloped nations in sub-Saharan Africa.[1] The primary reason for this localization is the mosquito; though the insect exists worldwide, epidemics of malaria, dengue fever, West Nile virus, Zika, and yellow fever are largely attributed to specific species of mosquito native to these geographical locations. In 2019, Malaria infections alone numbered 229 million, causing over 400,000 deaths worldwide; over 95% of these deaths occurred in Africa.[1,2] Controlling such diseases is often accomplished through controlling local mosquito populations via fumigation or habitat destruction, but these efforts are labor intensive, difficult to implement, and unreliable.[3] Recently, a modern solution has been developed which utilizes genetically modified mosquitoes to artificially control the prevalence of malaria-carrying insects in the wild.[2,4] This modification causes most of the mosquitos' offspring to be male; male mosquitos of the relevant species are incapable of transmitting Malaria through their bite[2]. The laboratory process for transfecting and proliferating these modified mosquitos is not perfect, and some of the mosquitos do not receive the gene needed to control the population. Because of this, the genetically modified mosquito larvae must be sorted from the wildtype mosquito larvae that did not successfully inherit the modified gene. Ensuring that only modified mosquitos are released is essential to successful population control. The modified mosquito larvae have an added protein, DsRed, which allows them to fluoresce or glow under a certain wavelength of light so they can be identified and distinguished from the non-transgenic mosquitos.[4] Because this method of mosquito population control is novel, commercial tools have not yet been developed to quickly separate transgenic mosquitos from the wildtype population in the lab. Currently, the sorting process is performed by hand. The physical act of manual sorting individual mosquito larvae is both mentally and physically draining over the span of hours. For the large-scale release of genetically modified mosquitos, a device is needed which can quickly and efficiently sort the genetically modified mosquito larvae from the wildtype larvae.

This Automated Larvae Sorter (ATLAS) is intended to be developed initially for use at the CDC, but its design must consider future deployment in labs in rural Africa for localized sorting and distribution of modified mosquito populations. Localized sorting is an important consideration because of the short lifespan of mosquitoes, usually 1-2 weeks depending on species. The ATLAS can be designed to be used in a laboratory setting, meaning that extensive containment and casing for the device is a low priority. However, intended users of the ATLAS include scientists in rural Africa, meaning the device must be user-friendly, low-cost, robust, and easy to repair.

The main functions of the proposed Automated Larvae Sorter will be a sensing and sorting function. The system must be able to (a) excite the modified mosquito larvae for detection, (b) detect the fluorescently marked larvae, and (c) appropriately sort the marked and unmarked larvae. The ATLAS must also be designed to interact with an individualization device developed by the CDC which turns a reservoir of larvae into an individualized stream of larvae with an inconsistent individualization rate. However, larvae density in the stream can be controlled indirectly by diluting the inlet reservoir.

The main goal of the Target Malaria project is to balance the requirements of high-throughput sensing/sorting with the design challenge to minimize device complexity. By creating a low-cost, reliable, easy-to-use device that efficiently sorts transgenic mosquito larvae based on fluorescence, the CDC will receive a tool that grants two major benefits. Researcher productivity will be increased, as the need for tedious manual sorting will be eliminated in the lab. Additionally, transgenic mosquito sorting will be available in underdeveloped countries for on-site malaria mitigation. Together, these benefits will further international efforts to address epidemics affected by infectious mosquito populations in tropical and subtropical regions.

5.2 Existing Products, Prior Art, Applicable Patents

There is currently a device that could carry out the desired functions for the Target Malaria project called the Complex Object Parametric Analyzer and Sorter or COPAS for short. While the COPAS meets the technical needs of the project, it costs ~$400,000 to buy and install and the yearly maintenance costs are ~$80,000. This price excludes the use-case for malaria sorting in the needed regions of the world. However, some of its individual components may be valuable to serve as references and design inspirations to develop a simpler, cheaper, and more robust solution system. Namely, its fluidic strategy wherein pneumatic pressure drives liquid droplets containing the specimen through the channel could be adapted and implemented in the Target Malaria device. Additionally, its sorting mechanism moves a collection reservoir below a stationary outlet, which is unique from the current setup and similar existent products, which attempt to divert flow into separate stationary collection reservoirs. Diving deeper into the functionality of this sorting technique could provide us with strategies to simplify sorting to differentiating one from another. The COPAS's software parameters and multiple configuration options for sorting criteria should be considered, especially if the Target Malaria project is to be extended to sorting of other macro-organisms or to separate mosquitoes based on sex as well as transgenic status.

Prior art includes microfluidic research by Hang Lu, a professor in the Georgia Tech School of Chemical and Biomolecular Engineering. Dr. Lu and her team reported automated sample handling, high resolution microscopy, phenotyping, and sorting of numerous small organisms[7]. Her team was able to develop a method to screen *C. elegans* by cellular and subcellular phenotypes with 95% success at the rate of several hundred worms per hour[8]. While this method of sorting was not utilized for species of mosquito larvae, the steps that the researchers took to arrive at their device could provide insight into potential steps for the Automated Larvae Sorter.

5.3 Codes and Standards

This device is not intended for any clinical diagnoses, it is for research use only (RUO) and should be labeled as such. Labeling requirements include writing somewhere on the device stating it is for RUO, such as a sticker or engraving.[12] Any electrical device falling under the RUO category is subject to regulatory testing requirements set under IEC-61010-1 for basic device safety standards. This standard is set by the IEC System for Conformity Testing and Certification of Electrotechnical Equipment and Components. This standard examines safety properties of research devices such as voltage requirements, fire risk, spill risk, structural

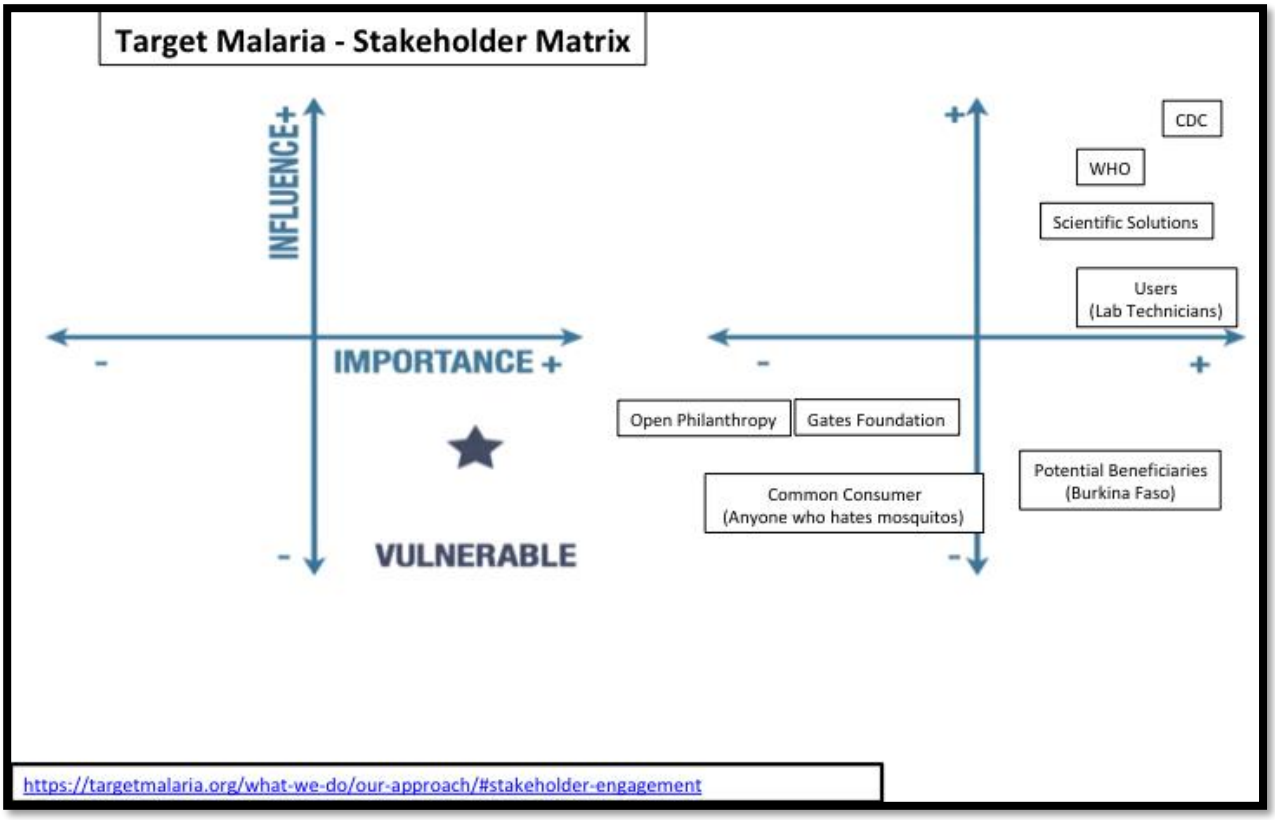
integrity, etc. Please see Testing and Measuring Equipment document provided by IEC for full list of testing requirements. These requirements were used to guide our overall design to make sure the device was safe for everyday use in a lab environment.

With the inherent hazard present whenever utilizing laser technology, it is important to recognize and educate the end-users of the ANSI Z136 series of laser safety standards. These consist of general safety practices to avoid laser damage to the eyes or skin of a user.

5.4 Design Inputs

Target Malaria is an international research consortium that spans many different institutions and sponsoring organizations, including the CDC, the Bill & Melinda Gates Foundation, and Open Philanthropy. Additionally, Target Malaria's phased development pathway follows guiding protocols recommended by organizations including the WHO[9]. At the initial stages of this project, the sponsors provided the design team with specifications and customer needs that depicted what a feasible, valuable solution would look like to the client. To complement this information, the team drafted a stakeholder matrix **(Figure 1)** that identified what groups that would interact with the final product were high priority and which had high influence on the direction the project would go. Much of the stakeholder information is based upon sponsors and interested parties in the Target Malaria project as a whole; many of these groups are not involved in the specific project described here. For the development of the ATLAS, the CDC is a high priority and influence stakeholder that should be interacted with heavily through the semester as well as the fact that the true end-user, the country of Burkina Faso, has very little input to the project aside from the final approval for mosquito release by the local governing parties.

Figure 1: Stakeholder Matrix



This initial information from the client was then compiled into a specification sheet along with requirements found by the design team during background research. The full spec sheet can be seen in **Table 1** and was used to identify what design constraints exist. The full spec sheet is present in the Appendix: Table 1.

Table 1

				Issued: 9/21/2021		
			For: Bug Bane, solution device for Target Malaria Project		**Page: 1**	
			Specification			
No.	**Date**	**D/W**	**Requirements**	**Responsible**	**Source**	**How Validated**
General						
1	9-Sep	D	sense fluorescent protein markers in transgenic mosquito larvae travelling in a flow	product manager / testing lead	CDC	validated during testing phases
2	9-Sep	D	concurrently sort transgenic larvae from other larvae	product manager / testing lead	CDC	validated during testing phases
Physical Characteristics / Production Geometry						
3	9-Sep	D	actuator properly pauses / diverts flow through the tubing	Fluidics Lead / product manager	CDC	derive requisite actuator power and rigidity to oppose fluid pressure
4	9-Sep	W	apparatus does not create back pressure in the tubing	Fluidics Lead / product manager	CDC	derive non-larvae-enriched fluid pressure created when actuator is activated
5	13-Sep	W	less than half of parts are externally sourced	Prototyping Lead	M-Design	utilize part designs / configurations that are easily produced in-house
6	13-Sep	W	housing for externally-sourced parts can be modelled and prototyped in a campus makersp	Prototyping Lead	M-Design	ensure constant velocity during actuation
7	13-Sep	W	no more than 6 significant parts	Prototyping Lead	M-Design	minimize redundancies in part production and assembly
8	13-Sep	W	minimum tolerances between actuator(s), housing, and tubing	Prototyping Lead	M-Design	coordinate with CDC contacts and teammates on dimensioning
9	13-Sep	W	minimum tolerance between optical sensor and larva flow	Prototyping Lead	M-Design	coordinate with CDC contacts and teammates on dimensioning
Electrical						
10	15-Sep	D	minimal power draw from outlet	Lead	CDC	probe power cord with Ohm-meter
11	15-Sep	D	compatible with African wall outlet	Lead	CDC	source proper adapter(s)
12	15-Sep	D	operate within safe voltage and current ranges	Lead	CDC	probe components with a Voltmeter and Ohm-meter during test trials
Mechanical						
13	9-Sep	D	strong enough force magnitudes to enact proper flow regulation	Lead / fluidics lead	CDC	derive requisite forces via approximation of fluid pressure
14	9-Sep	D	throughput >1 larva / second	Lead	CDC	ensure high velocity of actuation and detection when sourcing parts
15	15-Sep	W	minimum weight required for sysyem stability	Mechatronics Lead	M-Design	ensure ability of actuactor(s) to reliably return to initial orientation, and of whole apparatus to not drift after each sorting iteration
16	15-Sep	W	no symptoms of resonance in the apparatus	Lead	M-Design	ensure minimal to no damage to non-contact parts during endurance testing
Energy						
17	15-Sep	W	proper ventilation of heat generated from actuation	Lead	M-Design	calibrate tolerances to minimize friction between actuator parts and housing / tubing
18	15-Sep	W	proper ventilation of heat generated from microcontroller	Lead	M-Design	ensure a heat shield is added to controller if deemed necessary
19	15-Sep	W	efficient power storage and distribution to peripheral subsystems	Lead / perception lead	M-Design	probe each peripheral component with a Voltmeter during test trials

5.5 Market Research

The fatal effects of Malaria in sub-Saharan regions of Africa have motivated significant efforts to eradicate the disease. According to the World Malaria report 2020, total funding for Malaria control was $3 Billion USD, with 73% of this funding benefitting African regions.[1] It is important to specify that this figure is only for Malaria control and prevention, not treatment. Investments in vector control products, which include genetically modified mosquitos, totaled $453 million from 2007 to 2018 and averaged over $41 million USD per year.[1] The ATLAS will be a valuable addition to these disease control efforts, and past financial investments in these efforts suggest that an automated sorting system will have high monetary value to researchers.

Current devices which can sense and sort larvae like COPAS cost upwards of $400,000 dollars with $80,000 yearly maintenance requirements, which is expensive even for many labs within the United States. Similarly, fluorescence-activated cell sorting (FACS) systems are characterized by high precision, adjustability, and throughput necessary for biological sorting, but these devices are infeasibly expensive for many labs[10,11].

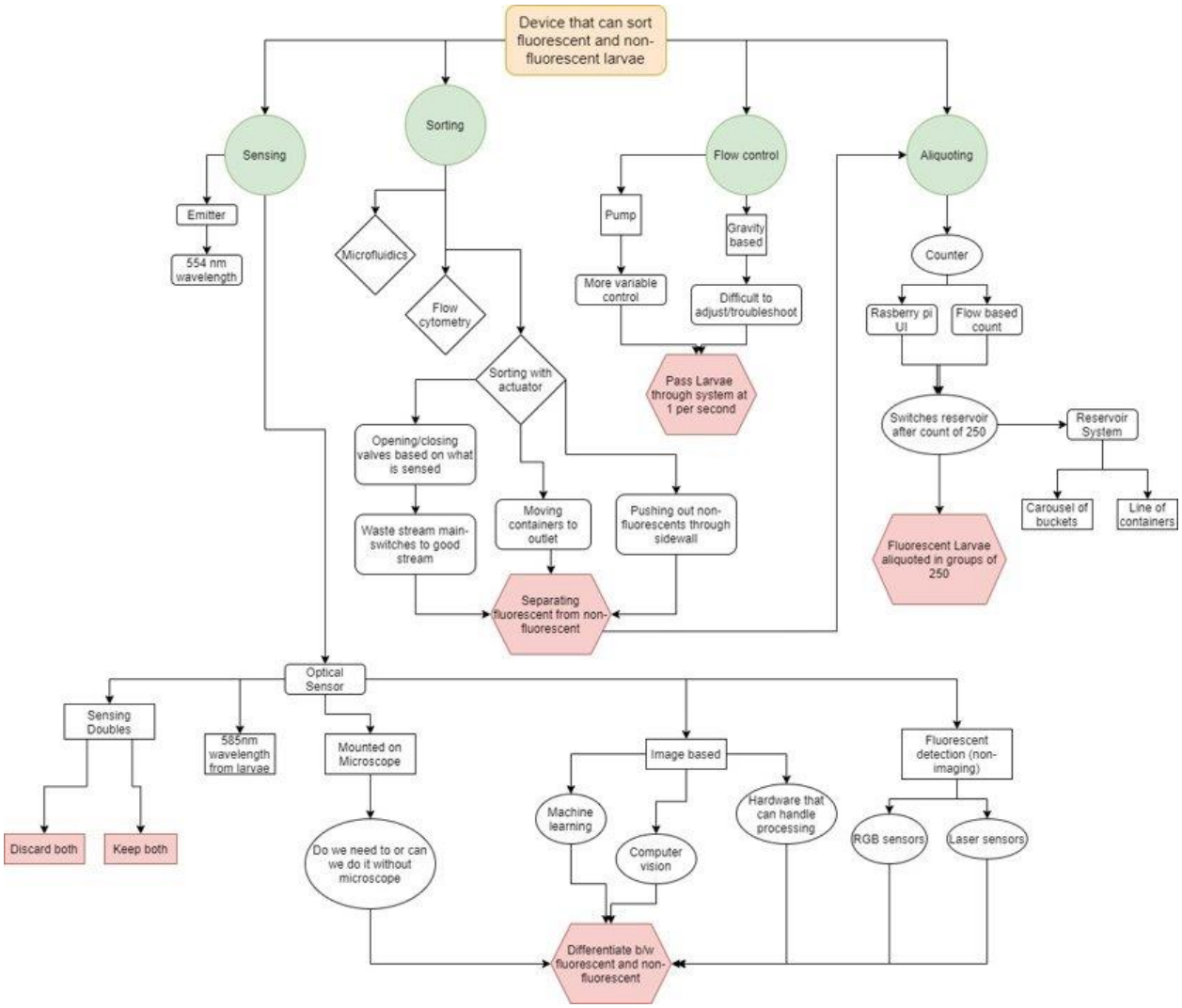
Presently there is no commercially available product capable of efficient fluorescent sorting that is also financially attainable for large-scale use, especially in African laboratories. By integrating the high-quality design techniques and capabilities of FACS systems and COPAS with the affordability and simplicity of the Automated Particle Counter (APC), the larvae sorting device presented here would capitalize on an unfilled niche in the market. This indicates a promising potential for the ATLAS as a profitable product targeted for customers that have already exhibited a willingness to make significant financial contributions to Malaria relief.

Price for our device would be determined by manufacturing costs and is preferred to be less than $1000 per unit.

5.6 Design Concept Ideation

Ideating solution mechanisms involved distinguishing 3 separate subsystems constituting the ATLAS system: sensing, sorting, and flow control. (**Figure 2**). From ideation, 6 solutions were considered for sensing, 4 solutions were considered for sorting, and 3 solutions were considered for flow control. Any three solutions comprising each of the subsystems may be combined to define the complete ATLAS system, implying 72 versions. Aliquoting was initially considered as a subsystem, however after discussion with the CDC advisor we determined that it was only necessary to collect the larvae at the end of the process.

Figure 2: Function Tree

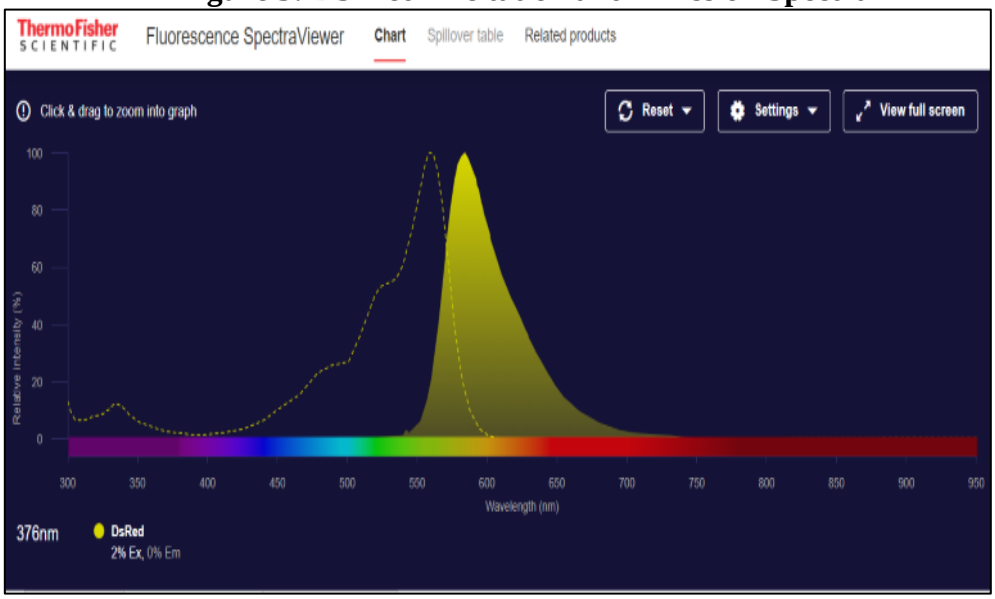


For sensing, the potential solution mechanisms included a variety of commercially available optical sensor products of varying sensitivities, resolutions, and processing speeds. These included a CI-6604 photodiode, a PS-3213 wireless light sensor, a TCS34725 RGB sensor, a MAX30101 photodetector, a TSL2591 diode sensor, and a VEML7700 ambient light sensor. Each sensor listed showed promise in being able to meet the required sensing specifications. However, concept ideation for the sensing subsystem included more than simply selecting an appropriate sensor for purchase.

Additional factors such as excitation, optical focusing, and signal processing were also considered in the design process. Excitation of DsRed is essential to cause the fluorescence to be detectable by any kind of sensor. DsRed's maximum excitation wavelength is at 554 nm (green) and its emission wavelength is 584 nm (red). Lasers that emit a beam at 554 nm wavelength are extremely rare and expensive. A common, commercially available laser is the 532 nm laser. At 532 nm, DsRed intensity is around 60% of its maximum as can be seen in the DS-red emission spectra in **Figure 3**. Using a 532 nm laser should excite the DsRed protein enough to cause it to be differentiable from non-fluorescence. Designing the sensing subsystem involves exact placement of the sensor relative to the tubing and detector sensitivity. For a

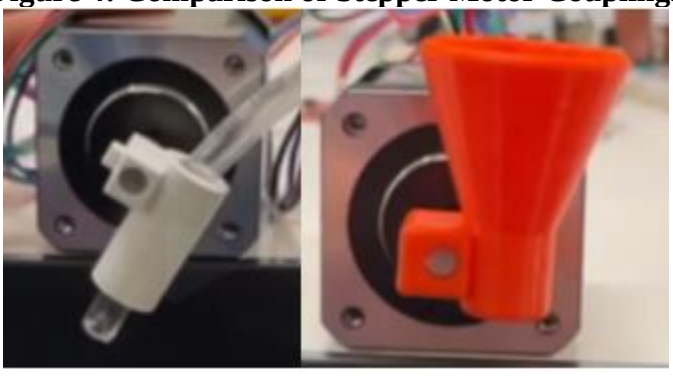
detector with low sensitivity (>0.1 Lux minimum), it may be necessary to focus the larvae's emission signal via lenses. Initial ideation suggests adapting the sensor to be used in junction with the microscope's optical path and with an excitatory laser. However, if the sensor is sufficiently sensitive, the system may be operable without optical focusing by a microscope's lenses. This is preferable, as the device's portability, accessibility, and cost of manufacturing would be significantly reduced.

Figure 3: DS-Red Excitation and Emission Spectra



For sorting ideation, we initially considered using an internal valve to divert the larvae one way or the other. We also considered the same pneumatic blast method that the COPAS currently uses. However, based on design considerations, we later decided to avoid interfering with the fluid itself and instead to manipulate the tubing outlet to deposit larvae in one of two well-placed reservoirs. This added the stepper motor and solenoid as solution mechanisms. Similarly, a solution mechanism was considered which translates the collection reservoirs themselves via a conveyor belt. Therefore, 4 solution mechanisms have been considered for sorting: a pinch valve for diverting flow, a solenoid and stepper motor for translating and rotating the outlet respectively, and a motorized conveyor for translating the collection reservoirs. Both the stepper motor and solenoid introduce a new design challenge, as the coupling component (as seen in **Figure 4**) that allows interface between the actuator and tubing is a crucial component for maintaining sufficient power and speed during operation.

Figure 4: Comparison of Stepper Motor Couplings



Communication between the sensing and sorting subsystems will be facilitated by an Arduino Uno microcontroller board. As more components have been added to the system, consolidation of circuit components has become preferable, and design has begun on a customized printed circuit board (PCB) layout to reduce the size of the ATLAS system. In later prototyping stages, it is expected that a user interface system will be powered by a 2A Raspberry Pi 3 B+ with touchscreen display, as employed by the APC currently. Due to the similarities here, an integration between our system and the APC has been discussed with Dr. Gabriel, leaving Bug Bane's system to only need the functionality of outputting large batches of larvae that would then be sent though the APC to produce the required batch sizes. Current design focuses are on integrating essential circuit components with shared binary data structure communication from the sensor to the sorter, transferred by the Arduino Uno. While experimentation is focused on testing several different solution mechanisms, it is important to maintain adaptability as new circuit components are added/removed frequently. Until a solution mechanism has been finalized for each subsystem, a permanent PCB or circuit layout will not be employed. Additional considerations include power supply for extra system components that do not communicate electronically with the system, such as a stirrer motor to avoid larvae clumping or standalone laser for constant fluorescence excitation.

After initial prototyping and calculations of sensor controls such as integration rate, we realized that we needed to add a subsystem for flow control. We had to consider how to drive and control flow as a larva passes over the sensor to ensure the sensor has time to see it. Engineers at the CDC have assumed responsibility of designing a system for larvae flow and individualization before reaching the sensing subsystem; therefore, these design goals are outside the scope of the solution mechanisms presented here. However, optimizing flow where the larvae pass the sensor is critical. Flow speed can impact both the ability of the sensor to detect fluorescence and the ability of the sorter to react quickly enough for accurate separation; data from flow speed analysis is presented in **Appendix B**. For our testing purposes, we acquired a simple aquarium pump to drive flow and we can adjust height of tubing to change flow rate. The requirement for tubing is that enough fluorescent signal can reach the sensor through the tubing. Our current options for tubing include translucent PVC tubing, transparent PVC tubing, and glass capillaries. In the APC, a glass capillary is employed during the sensing component to maximize the signal's transmission. Sensor testing was done using all three tubing types as it was easy to switch between them. Flat glass slides were also considered but were not tested as cylindrical tubing was sufficient.

5.7 Concept Selection and Justification

To reduce the complexity of design choices, we chose to pursue the highest rank choice from each subsystem. Because the evaluative criteria for each solution mechanism was informed by the functions of the other subsystems, this resulted in a holistic concept selection process that will yield the best possible combination of components for the ATLAS system from those presented here.

Initially, sensing conceptualization involved a high-speed camera mounted to a microscope. However, initial experimentation revealed that the rate of information transfer between the camera and a computer was far too slow for effective sorting speeds. Therefore, design moved to prefer a sensing system that operated with dedicated optical intensity/

wavelength sensors instead of a camera. Most of the initial sensing experimentation was conducted using the TCS34725 RGB sensor. At close ranges and with powerful optical signals, the sensor exhibits sufficient sensitivity and speed for the ATLAS's design goals. However, further experimentation with fluorescent larvae at the CDC revealed its sensitivity is not high enough to detect actual larvae emission with the current flow system setup. Optical intensity from the larvae is approximately 0.1 Lux, which is below the specifications of the TCS34725 RGB sensor. Therefore, new research was conducted into alternative sensors. From this research, the CI-6604 photodiode, the PS-3213 wireless light sensor, and the MAX30101 photodetector have been considered. The technical specifications of the PS-3213 such as integration time, sensitivity, and lens properties were superior to the other two sensors, and we reached out to the company to ask for a loaner sensor. However, the interface part for the sensor proved too difficult to obtain and the other sensors proved too expensive. Therefore, we decided to research a fast, cheap, highly sensitive light sensor. We discovered the TSL2591 diode sensor. While the TSL2591 could detect as low as 0.00018 Lux, its integration time of 100ms was too slow for our needs. Finally, we discovered the VEML7700 ambient light sensor which can detect as low as 0.0036 Lux at an integration time of as low as 25ms which is fast enough to detect larvae travelling by.

In addition to sensor selection, experimentation has confirmed that the standalone laser design strategy is sufficient for fluorescent excitation. This is a valuable discovery for the ATLAS system, as it indicates the sorter may be able to operate independently from a microscope or other high-powered and expensive optical setup. Current design employs a common, commercially available 532nm line laser to excite DsRed in the larvae (which has an optimal excitation wavelength of 554nm). Upon exposure to the 532nm laser, DsRed is expected to fluoresce at approximately 60% its maximum brightness (**Figure 3)**. In experimentation with the 532nm standalone laser, fluorescence is bright enough to be easily identifiable by the naked eye (**Figures 5-6)** and distinguishable from non-fluorescent samples.

Figure 5: Visible emission from fluorescent beads after excitation by 532nm standalone laser. A red acetate filter blocks the green laser light from reaching the camera.

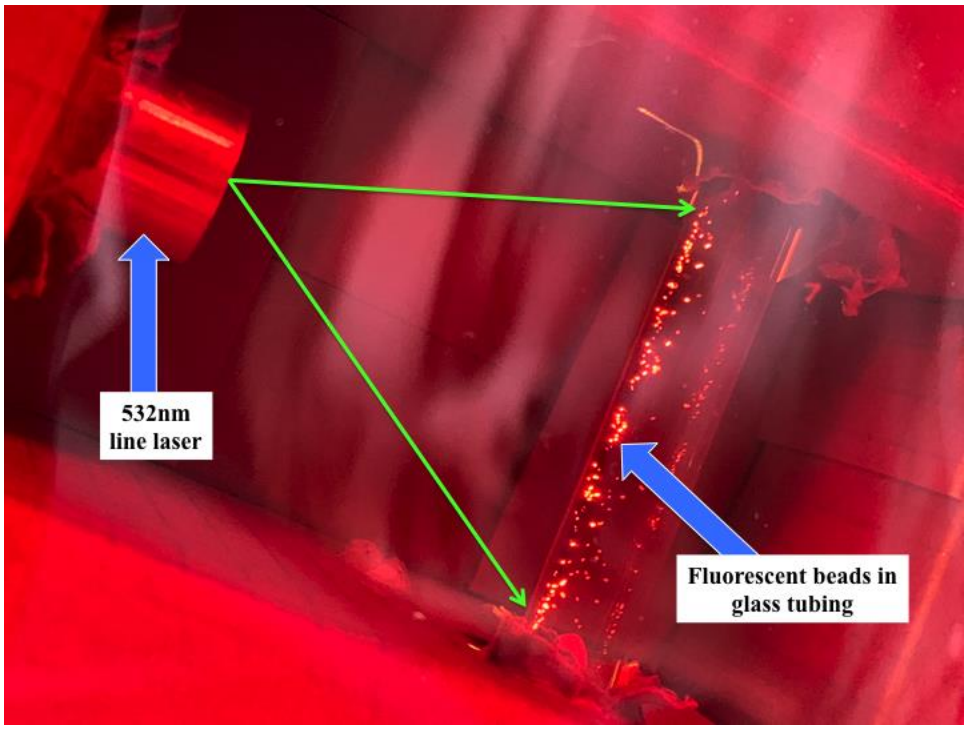
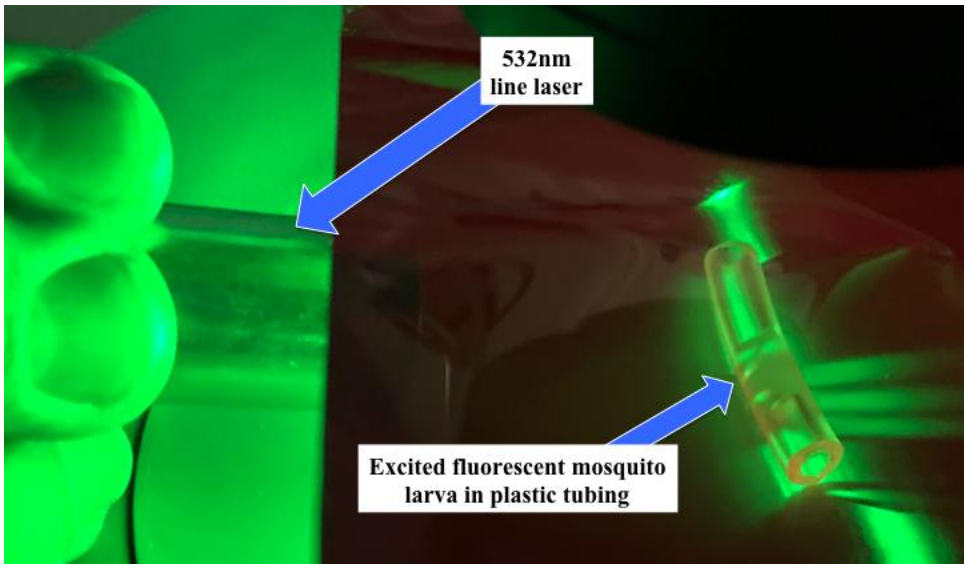


Figure 6: Stationary Excitation of DsRed in fluorescent mosquito larva using standalone 532nm laser. Excitation is seen from the orange-red glow emanating from the tube under the acetate sheet



While initially we were focused on an internal diversion system for sorting, we discovered that a stepper motor that rotates the tubing outlet above two separate collection reservoirs is a more viable option. **Table 3**, is a design concept evaluation matrix that highlights justification for this selection. The stepper motor's advantageous speed is complemented by its ability to interact indirectly with the flow – thereby avoiding any damage to the larvae themselves that

might occur with a pinch valve or diverter. The solenoid for outlet translation, while similar in design, is limited by its weaker power output compared to the stepper motor and its tendency to overheat when activated for extended periods of time. A conveyor system for translating the collection reservoirs would similarly work well, as it would not affect the fluid flow stream at all, but it would require a high amount of power and could potentially cause spillage. Therefore, a sorter subsystem that employs a stepper motor for outlet rotation is the current design selection. Further optimization will involve precise design of a coupler to facilitate an efficient interface between the stepper motor and the tubing. Each sorting solution mechanism represents the largest system component in terms of power consumption; most options – including the stepper motor – operate on a minimum of 9V.

Table 2: Sorting Subsystem Evaluation Matrix

R = Rating / WT = Weighted Total | Solenoid | Pinch | Conveyor| Servo Motor

Criteria	Importance	R	WT	R	WT	R	WT	R	WT
Fast Trigger (>1 per second)	10	5	50	4	40	3	30	5	50
Successfully Blocks/Allows Flow	10	4	40	4	40	5	50	5	50
Inexpensive	8	4	32	4	32	4	32	5	40
Comes in Needed Size	9	5	45	5	45	5	45	5	45
Connects Securely with Tubing	8	4	32	5	40	5	40	5	40
Microcontroller Compatible	10	5	50	5	50	5	50	5	50
Safety	10	5	50	5	50	4	40	4	40
Aesthetics	4	4	16	4	16	5	20	5	20
Setup Time	7	5	35	5	35	3	21	5	35
Resistant to Cyclical Wear	9	5	45	5	45	3	27	4	36
Total		403		393		355		406	
Relative Total		395/425 =.948		393/425 =0.925		355/425 =0.835		406/425 =0.955	
Rank		2		3		4		1	

After the stepper motor was chosen, we designed two designs for couplings; a cylindrical coupling that fits over the tubing, and a conical coupling that rotates below the outlets stream. We chose to pursue both designs to determine which would be the better choice. The two designs can be seen connected to the stepper motor in **Figure 4.** Testing for couplings will be covered in **Section 5.9**

From the beginning of ideation, it has been preferred to use a gravity-based system to power the flow in the ATLAS. This is conducive to both user needs and efficient design. The currently employed APC also uses a hand-fed, gravity-powered flow system, making it simple to use for all users and easy to calibrate. The design concept evaluation matrix in **Table 5** supports the selection of a gravity-powered flow system. With the acquisition of the VEML7700 sensor, which is highly sensitive and incredibly fast, we also chose to attempt to use the aquarium pump to create a looped testing system which can be seen in section 5.9.

Table 3: Flow Subsystem Evaluation Matrix

R = Rating / WT = Weighted Total | Pump | Gravity | Suction

Criteria	Importance	R	WT	R	WT	R	WT
Flow Rate Fast Enough for Goals	10	4	40	5	50	2	20
Limited Pressure on Tubing	10	4	40	4	40	5	50
Inexpensive	7	5	35	4	28	5	35
Power Efficient	8	5	40	4	32	5	40
Reduces Doubles in Flow	6	3	18	5	30	5	30
Safety	10	5	50	5	50	5	50
Aesthetics	4	4	16	4	16	3	12
Setup Time	7	4	28	4	28	5	35
Resistant to Cyclical Wear	9	5	45	5	45	3	27
Total		312		319		299	
Relative Total		312/355 =.879		319/355 =0.899		299/355 =0.842	
Rank		2		1		3	

Current options for tubing material include translucent plastic tubing, transparent plastic tubing, and glass tubing. The APC also uses a clear glass capillary over the sensing component, which connects to translucent plastic tubing during other parts of the flow. A similar setup is proposed for the ATLAS system. By utilizing plastic tubing over most of the flow, the flow rate can be precisely controlled by increasing the effective flow distance without increasing the size of the ATLAS itself. Additionally, plastic tubing is more robust than glass tubing and will provide a superior interface with the sorting subsystem. However, glass tubing might reduce loss of optical intensity from the excited fluorescent larvae. Both plastic and glass tubing will be tested with the hypothesis that glass tubing will provide better sensing results. The results will be discussed in Section 5.9.

5.8 Industrial Design

Additionally, human factor considerations have been made about the end user of the product. This ATLAS is intended to be used in a lab by technicians with limited electrical experience, and as such will be mostly contained with a simple, independent interface which can be used to calibrate and start the ATLAS.

To create the housing unit, it was easiest to use a 3D printer and thus PLA was used as the material. We chose to use black material so that light is not reflected around the chamber when sensing is active. We also made the housing modular and split it into various parts so that height adjustments could be made for the tubing and the laser. We also decided on using glass tubing instead of plastic for sensing so that a clearer image would be available to the sensor. A further consideration for containment of the larvae for sensing is a glass box in the case that glass tubing causes excessive refraction of light.

5.9 Engineering Analyses and Experiments

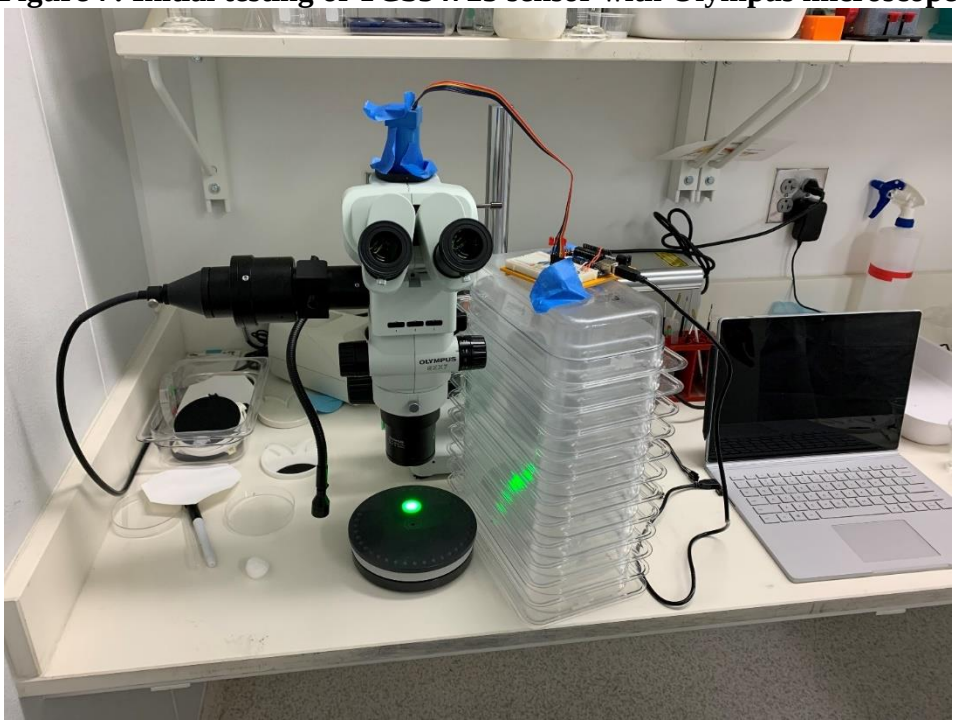
Engineering analysis and experiments can be divided into three initial subsections, sensing experimentation, sorting experimentation, and flow control.

The main objective of testing the sensing subsystem of the ATLAS is determining the sensitivity of the sensor under various testing conditions and understanding the emittance of the DsRed fluorescent marker. The laser and red acetate system were taken to the CDC to compare the effectiveness of the laser/red acetate system to an Olympus fluorescent microscope, which is the microscope most used to sort larvae by hand. It was discovered that the DsRed proteins appeared visually identical under the Olympus microscope as they did when excited with the 532nm laser and filtered with the red acetate sheet under a standard optical microscope with the same magnification. Fluorescence in the larvae could also be seen without magnification. This confirmed that a system does not need to be built around an Olympus microscope system, as excitation of the larvae can be achieved without it.

The TCS34725 sensor was tested by making a small housing to fit the sensor into the eyepiece of the microscope, the sensor was also rigged onto a secondary camera mount located on top of the microscope (**Figure 7)**. In both scenarios, the TCS34725 RGB sensor was not able to pick up any illumination from the excited larvae. A lux sensor was used by our CDC advisor to measure the light being emitted from the larvae and found it to be <0.1 lux, which proved to be too little light for the RGB sensor. The experiment was repeated with laser/acetate apparatus without the microscope, but instead of illuminating mosquito larvae, 150µm

fluorescent microbeads were used to test the sensor. The microbeads have approximately the same emission profile as DsRed proteins in the larvae and are being used as substitutes for preliminary testing outside of the CDC when mosquito larvae are not available. In this preliminary testing, the sensor was able to detect slight variations in color between the fluorescent and non-fluorescent beads when stationary. When in flow, the sensor could not identify the fluorescent beads.

Figure 7: Initial testing of TCS34725 sensor with Olympus microscope



Experience with initial testing of TCS34725 promoted a need for a system to align and hold the laser, acetate, tubing, and sensor. A simple casing was modeled and printed (**Figure 8&9)** to everything together for testing.

Figure 8: CAD model of Alignment Casing

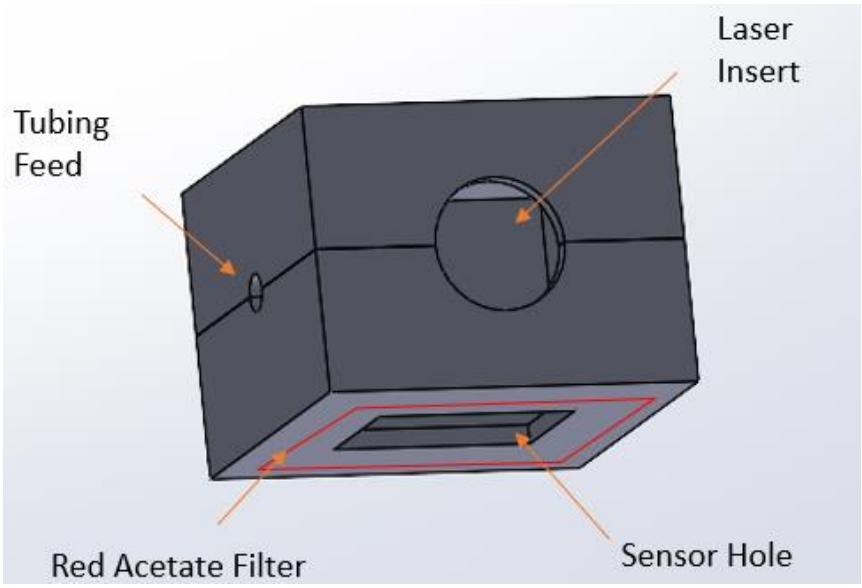
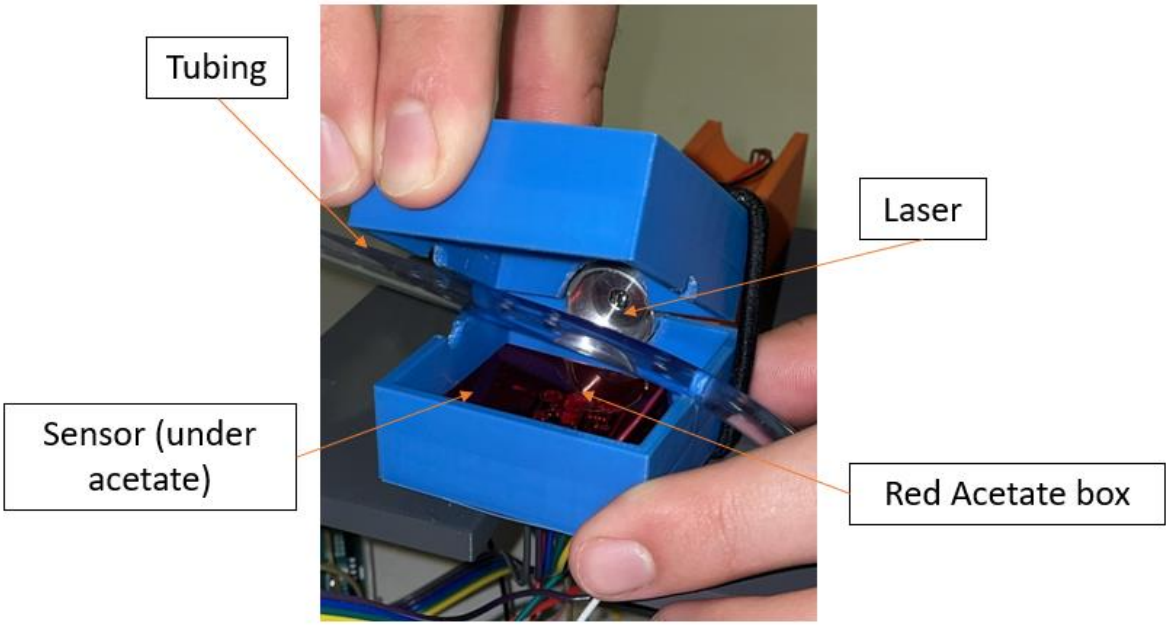


Figure 9: Alignment Casing



The VEML7700 lux sensor was used next identify fluorescent beads. The sensor was first placed under a red acetate bounding box and exposed to stationary fluorescent beads. The sensor was tested using a piece of PDMS with fluorescent beads suspended in it. The sensor

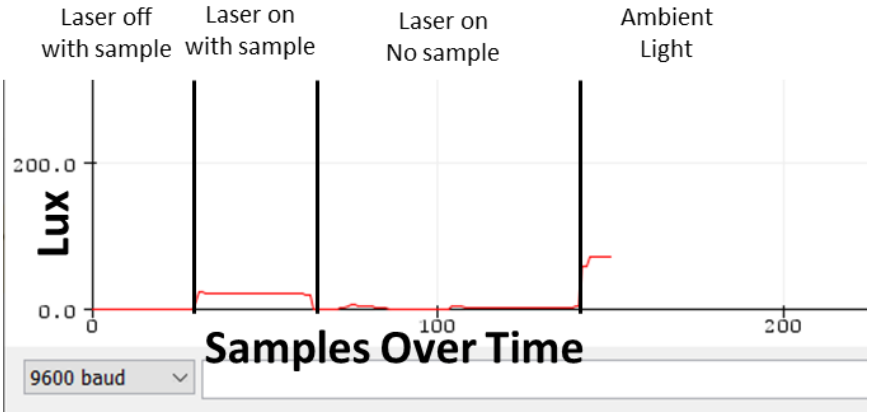
was tested under four test cases: with and without beads and with and without laser. The results of this testing are shown in **Table 4.**

Table 4: Initial Stationary Testing of VEML7700

Condition	Lux Reading
No Laser, PDMS	**0**
With Laser, PDMS	**26.4**
No Laser, No PDMS	**0**
With Laser, No PDMS	**0 – 0.06**

A second test using only a single showed the lux reading from a single bead to be 0.12 Lux. 26.4 Lux is divisible by 0.12, and from this we can estimate the number of beads in the PDMS chunk to be 220. A time plot of these conditions can be seen in **Figure 10.**

Figure 10: Plot of Lux readings in different test conditions



Next, we needed to test our VEML7700 sensor with flowing beads. To simulate flow, we used an aquarium pump in a closed loop, a t-joint at which we could inject fluorescent beads into the transparent tubing, and the alignment box (**Figure 11**) holding the VEML7700 sensor, red acetate, and laser. We were monitoring the sensors output using the serial monitor for the Arduino Uno. Results can be shown below in **Table 5**. The next test was to circulate beads through the system and test the error of the sensor for both our demo design and the final design (**Table 5)**. To do this we used the point laser and red acetate to visually detect when a bead passed over the sensor and then verified on the serial monitor whether the sensor detected it or not.

Table 5: Confusion Matrices of Classification Performance

Demo Design

Confusion Matrix (n = 440)	Measured: No	Measured: Yes
Actual: No	True Negative: 298	False Positive: 2
Actual: Yes	False Negative: 23	True Positive: 117

Final Design

Confusion Matrix (n = 450)	Measured: No	Measured: Yes
Actual: No	True Negative: 300	False Positive: 0
Actual: Yes	False Negative: 3	True Positive: 147

We tested the sorting subsystem experimentally to determine true actuation speed, lag between direction changes, lag in communication, and torque capabilities. Testing of both the stepper motor and solenoid actuator determined the maximum controllable actuation speed of the NEMA stepper motor was 300RPM, which translates to 0.17ms to turn 30 degrees. Lag in communication was found to be essentially negligible as the stepper motor was running on a dedicated microprocessor with a dedicated driver. Lag between direction changes was found to be less than 0.1 seconds and could likely be further optimized with the control code.

The two couplings were tested to determine how they affect the speed of the motor and observed qualitatively to determine how couplings affect real flow from the system. Both couplings met the actuation time requirement of <1 second. As can be seen in **Table 6**, the funnel coupling was faster on average than the other coupling, however this speed advantage was not statistically significant. Because there was not a significant difference in the speed of the motor with each coupling, the flow from the end of each coupling was compared. The flow comparison can be seen in **Figure 8.** The cylindrical coupling does not actually touch the flow stream, only the tubing, and therefore had smoother flow than the funnel coupling which interacts directly with the water.

Mean Actuation Time Comparison of Motor Couplings

	Cylindrical Coupling	Funnel Coupling	Uncertainty due to Frame Rate	Two-Sample Equal Variance T-Test P Value
Unhindered	0.1172 s	0.1138 s	0.017	0.369467464
Hindered	0.1206 s	0.1182 s	0.042	0.450103548

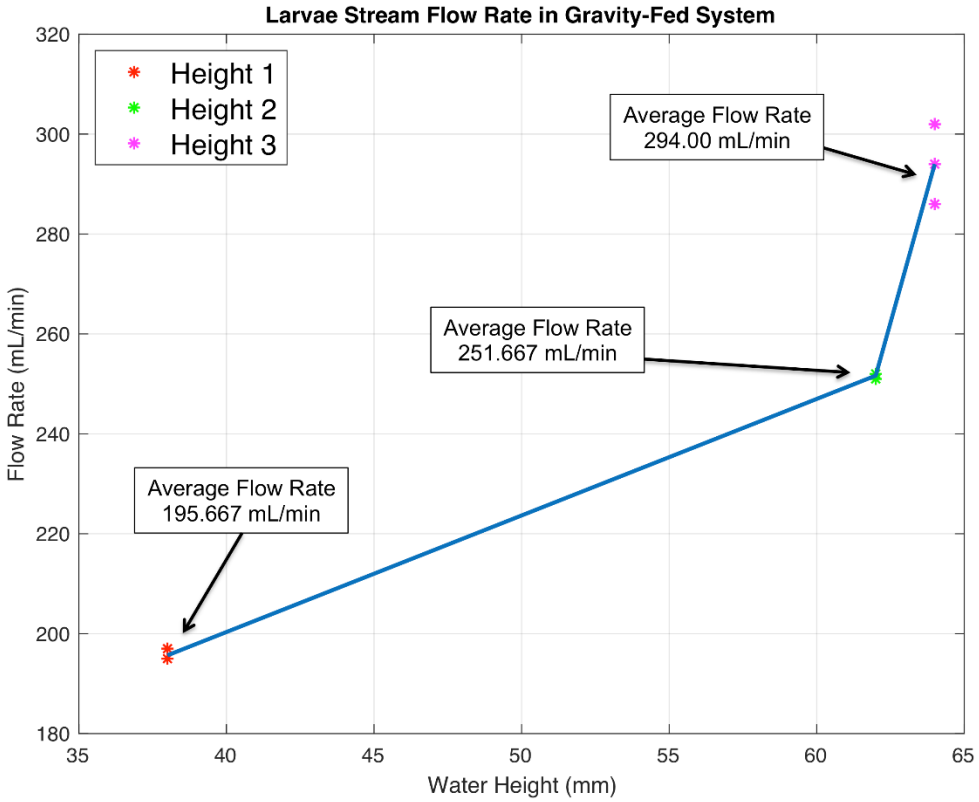
Table 6: Mean Actuation Time Comparison of Motor Couplings. Hindered actuation indicates that tubing was connected to the coupling during testing; unhindered actuation indicates the motor and coupling were activated while isolated from the system.

Figure 11: Water Flow Coupling Comparison

A 5N solenoid actuator was also tested and found to have a 4mm actuation distance and actuated in roughly 50 ms under load. It was also found that the actuator performs best when not initially under load. Additionally, the solenoid actuator was found to have heating issues when activated for extended periods of time. These findings show the stepper motor is a better choice.

The last subsystem requiring extensive testing is flow control. Based on the evaluation matrix in **Table 5**, gravity was selected as the simplest and likely best solution for controlling flow. With a gravity fed system, flow rate of the water moving through the system can easily be estimated based on the height of the initial water reservoir using the equation $V = \sqrt{(2gh)}$ where V is velocity, g is acceleration due to gravity, and h is height. This equation is derived from a simplification of Bernoulli's equation and assumes conservation of energy, negligible friction, laminar flow, steady state and shows that flow speed and tubing height are non-linearly related. The velocity can then be multiplied by the cross-sectional area of the tubing to determine flow rate. Because this is an approximation that does not account for friction, flow rates were experimentally confirmed at three distinct heights, this can be seen in **Table 7**.

Table 7: Average Flow Speed Vs Height



Lastly, tubing material and geometry must be considered. Pliable transparent plastic tubing will be used for a majority of the length of tubing. Testing was conducted comparing translucent plastic tubing, transparent plastic tubing, and glass tubing. Translucent plastic tubing yielded the worst results while glass tubing and transparent plastic tubing yielded similar results, therefore, due to its pliability, and to eliminate complication with changing tubing material in the flow stream, the transparent plastic tubing was the best choice to continue testing with.

5.10 Final Design, Mockup, and Prototype

Figure 12: Final Design Rendering

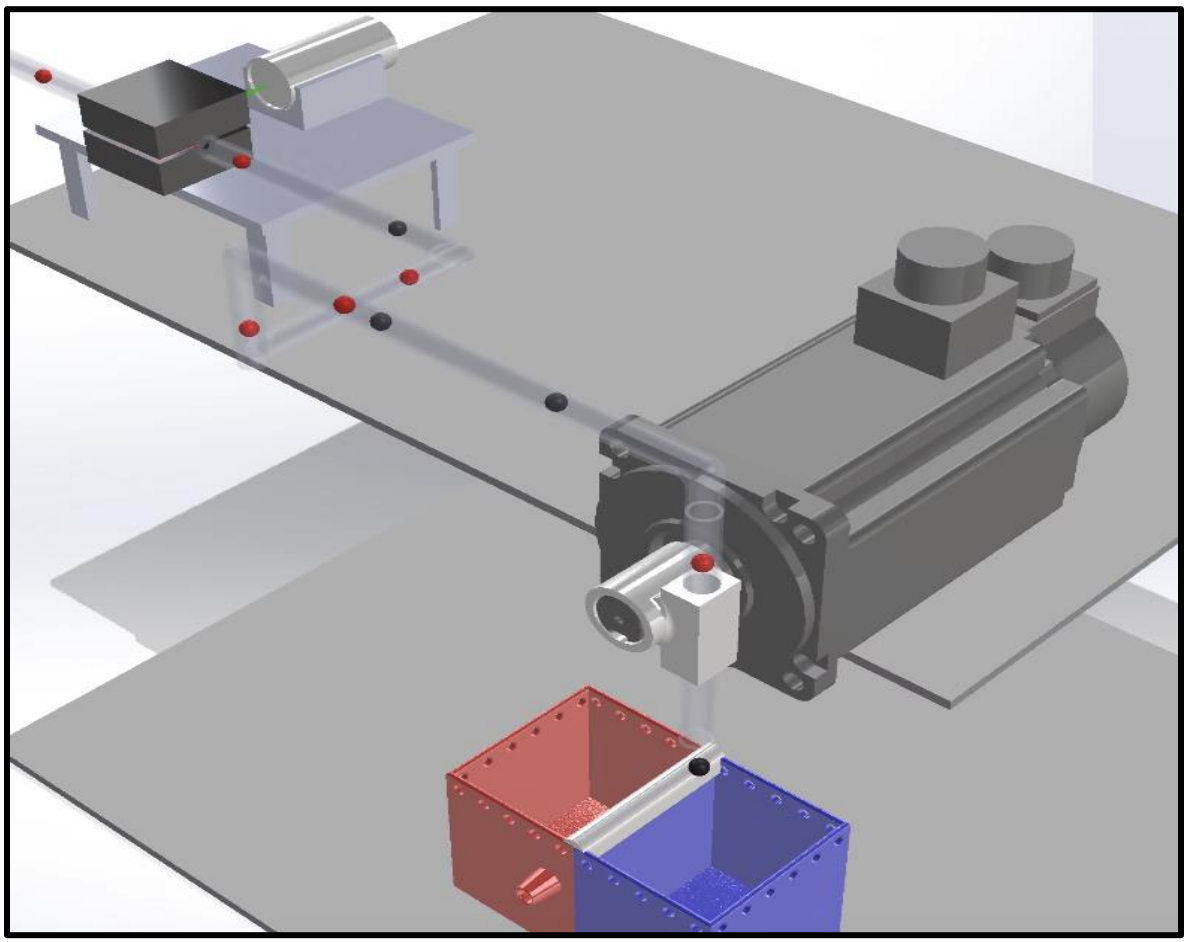
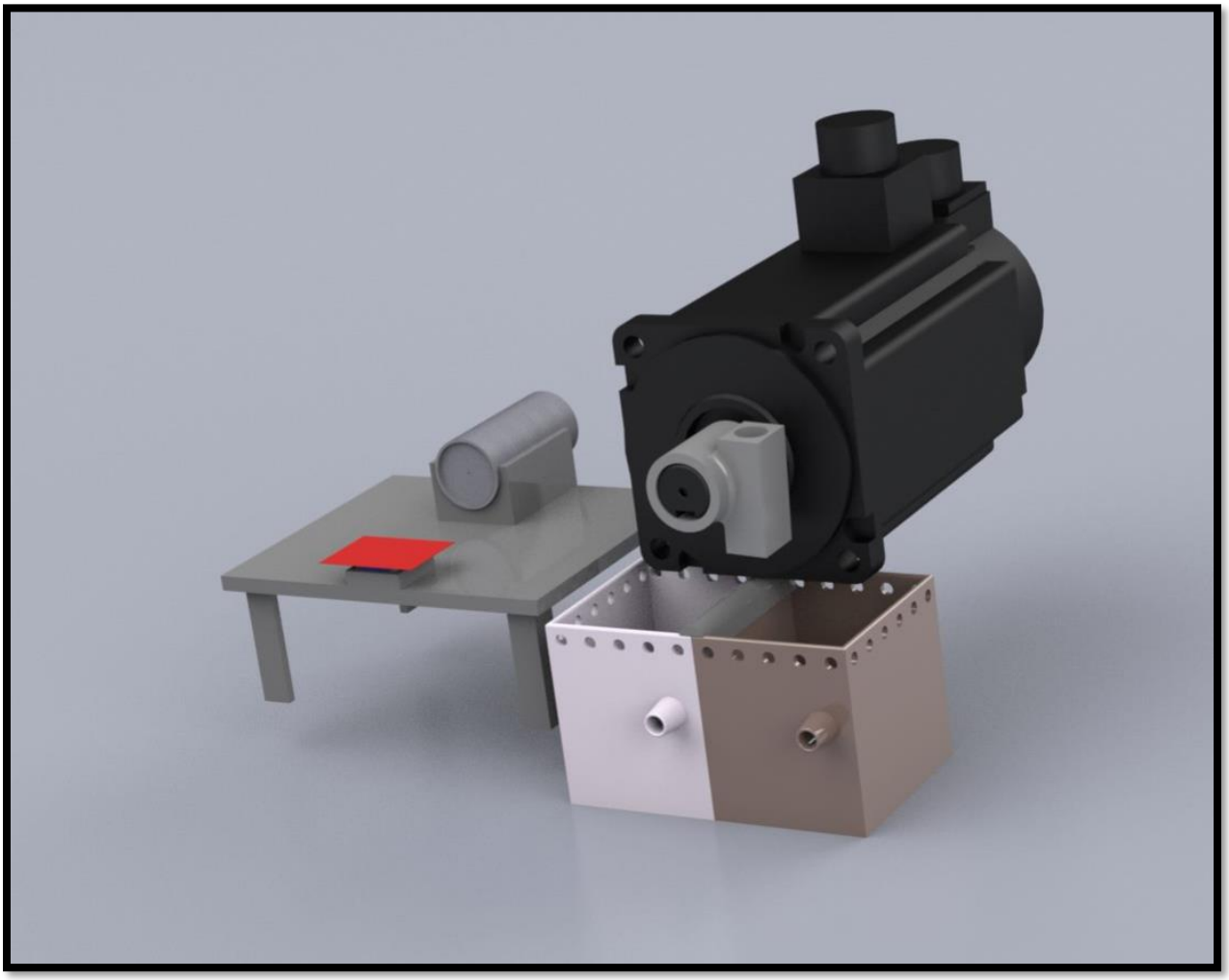
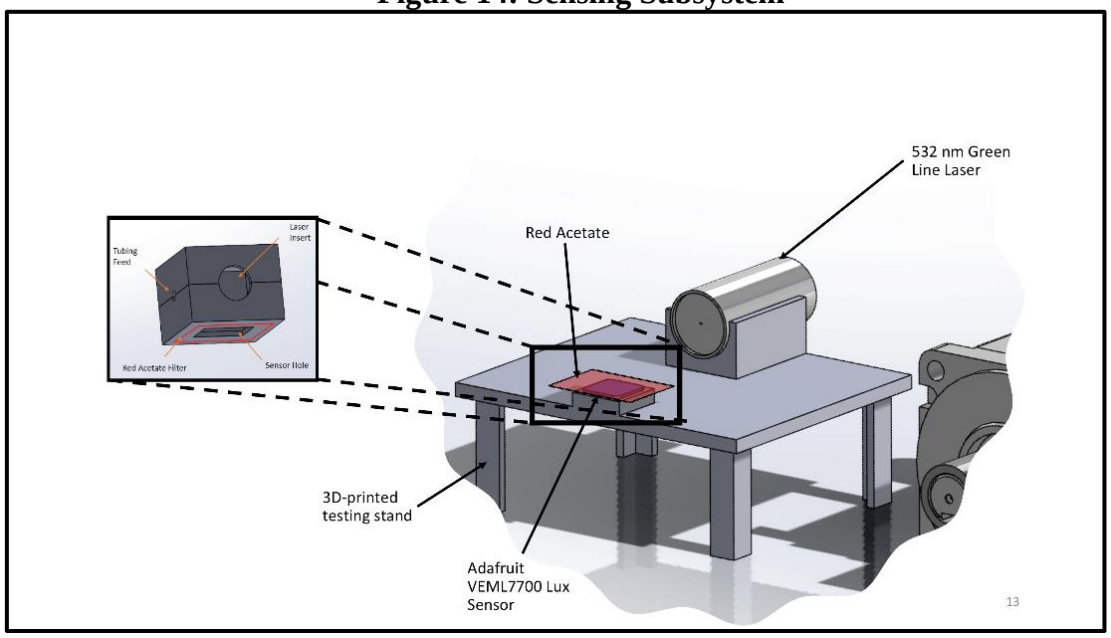


Figure 13: Final Design Rendering 2



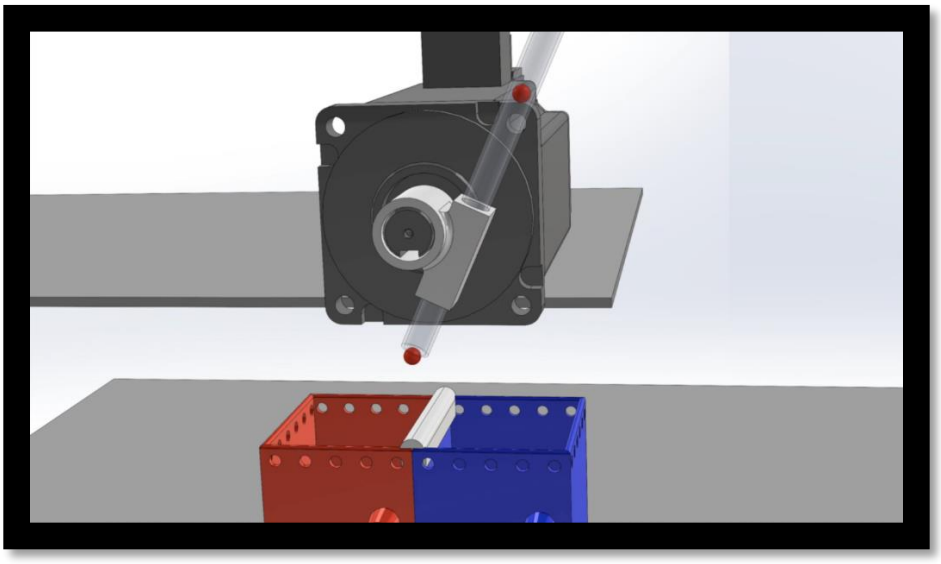
The sensing subsystem (**Figure 11)** in the latest iteration of the ATLAS is comprised of an adjustable, 3D-printed sensing platform, a 532nm line laser, and flexible silicone tubing. Our final design includes the newest sensor that we tested, the VEML7700 because it is both sensitive enough and fast enough to identify the flowing fluorescent beads. We also switched out the sheet of red acetate for a box of red acetate that envelopes the entire sensor to filter out undesired exposure to ambient light and reflections of the laser beam. Additionally, we added a new sensor housing alignment case (**Figure 12**) that reduces the ambient light that can reach the sensor as well as perfectly aligning the tubing, laser, and sensor. While we were using an aquarium pump and a peristaltic pump to simulate flow for testing purposes, the final design will utilize the CDC's gravity fed individualizing system. Therefore, the final design will be fed in a similar manner to the APC. Larvae will be individualized, aligned with the laser and sensor covered by a red acetate filter, and its fluorescent signal will be quantified and reported to the Arduino. The Arduino will then communicate with the sorter to sort the larvae into the correct reservoir.

Figure 14: Sensing Subsystem



The final sorting subsystem design employs a voltage divider circuit, the NEMA-17 stepper motor, a cylindrical motor coupling to manipulate the angle at which the flow exits, and a dual-reservoir design to collect both positive and negative samples as depicted in **(Figure 15)**. The reservoirs are designed to be used with a form of fine netting to collect the larvae batches for removal from the reservoir itself and easy transferal to the APC for batch size control. In terms of integration with the sensing system, communication is through a binary signal. When a transgenic mosquito is detected, the motor is sent a high signal, turning the motor coupling, and angling the tubing so that the larva is deposited into the appropriate bin. After enough time has passed for this to occur smoothly, a low signal is sent. This mechanism relies on the success of individualization from the gravity-fed system created by the CDC and proper spacing between the sensing and sorting subsystems.

Figure 15: Sorting Subsystem



The final design meets the design requirement of the CDC to sort greater than or equal to 1 larva per second. They also requested <1% error; between false positives and false negatives our error is ~2% with false negatives accounting for most of the error. This is preferrable, as transgenic batch purity is maintained. Our final design is also drastically cheaper than alternative methods of sorting as described below.

Table 8: No False Positives

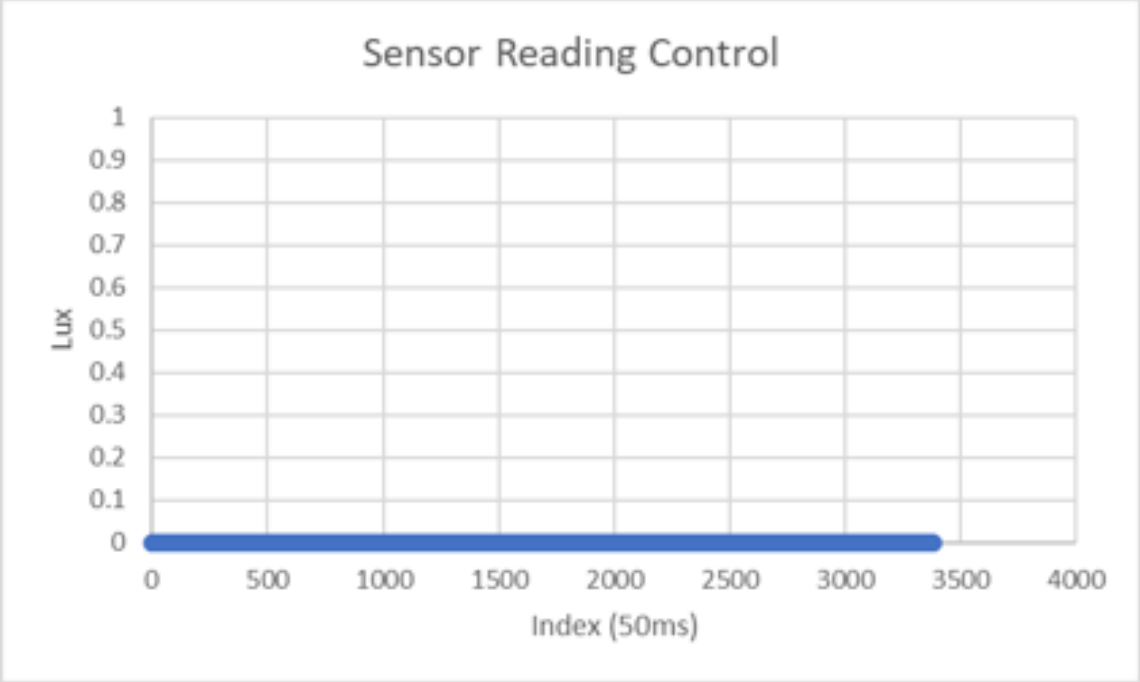


Table 9: Final Design Classification Error Analysis

Confusion Matrix (n = 450)	Measured: No	Measured: Yes
Actual: No	True Negative: 300	False Positive: 0
Actual: Yes	False Negative: 3	True Positive: 147

To evaluate the functionality of the finished prototype, a cost analysis was conducted to compare the current CDC practice of hand sorting larvae to the new proposed practice utilizing Bug Bane's design. We first assumed that a technician would sort by hand for 8 hours and could sort 960 larvae in that time, and that the same technician could run 10 ATLAS machines for that same 8 hours and each machine can sort 1 larva per second. Under these assumptions, our ten machines can sort 288,000 larvae in 8 hours. Assuming the technician gets paid $20/hour for 8 hours a day totaling $160 a day in both scenarios, to sort 1,000 larvae, by hand it costs $167 per 1000 larvae, with our 10 devices it costs $0.55 per 1000 larvae. This is 300 times cheaper and 300 times faster than the current hand sorting method.

5.11 Societal, Environmental, and Sustainability Considerations

The Target Malaria project is subject to ethical considerations regarding genetically modified organisms (GMOs), the term under which the transgenic mosquitoes are categorized, and many barriers to deployment have lain in lack of education about the relevant science. Anti-GMO groups often cite environmental concerns revolving around potential harm to the ecosystems in which the GMO's may outcompete wildtype organisms for resources.[13] However, these concerns do not apply to Target Malaria's transgenic mosquitoes as the self-limiting gene depletes the population of only the targeted mosquito species without affecting populations of adjacent mosquito species or the larger environment,[4] and the fluorescent marker is only visible to the naked eye when subjected to laser excitation.

Relevant societal considerations for Target Malaria include ethics of interfering with nature and freedom of choice,[13] as the members of the communities in the targeted areas must give their consent prior to deployment. However, the release of genetically engineered mosquitoes has been permitted by Burkina Faso's national biosafety authority according to Delphine Thizy, Target Malaria's director of stakeholder engagement.[14] The lead researcher in Burkina Faso, Abdoulaye Diabate, has also been addressing the concerns of those living in the targeted communities by assuring that "If any [transgenic females] bite a human, they will not pass on any genetically modified material" and dispelling concerns that "sterile males might somehow pass on that sterile status to humans." [14]

Critics of gene-drive organisms fear that they have the potential to "run amok and wreak havoc if they were ever released into the wild," via "eliminating important pollinators" and consequently "inadvertently have a negative effect on crops." [15] However, while gene drive mosquitoes pass on variant traits to offspring indefinitely, Target Malaria's transgenic mosquitos stops carrying the genetic variant after a few generations "are not intended to have a lasting impact on the insect population," [4] in order to ensure that any negative side effects of deployment are able to be mitigated, just as the intended population decrease is.

5.12 Team Member Contributions

Matthew was responsible for Sponsor, Advisor, and other forms of external communications, setting up meeting times for the group and searching for available equipment needed for the project. They were, along with William and Nathan, responsible for establishing the problem statement, conducting market research, and researching prior art. Due to their strong background in fluidics, Matthew led the research efforts on spectroscopy and fluidics as well.

Nathan was responsible for, along with William and Matthew, the bulk of the CAD models for the system, including motor couplings, component housings, and more. Along the same lines, 3D printing efforts were also led by this subgroup. Along with Tyler and Yanni, Nathan drafted the preliminary ideation report that outlined what brainstorming occurred in the early days of the project during the developmental stage. Along with Nathan and Matthew, Tyler was also the lead on the formation of the Morphological Chart. William was responsible for creating the function tree with assistance from Nathan and for search efforts across campus for higher quality light sensors that may be better suited for the team's purposes. Along with Tyler, Yanni drafted the Specifications Sheet and House of Quality in the preliminary ideation stage.

Yanni was crucial in the development of circuitry for both mechanisms, utilizing their strong mechatronics background and Arduino experience. For the majority of the last half of the

project, Yanni spearheaded the sorting prototyping and testing along with Will and Tyler. Will designed the couplings for the servo motor and Yanni built the circuit required for it. Because sensing was such a large part of the project, the entire team participated in developing testing and prototyping. Nathan and Tyler conducted a large portion of initial sensor testing. Will, Tyler, and Nathan developed the closed loop pump system for testing. Matthew developed the method for coupling the tubing with the T-joint and created PDMS with beads to simulate transgenic mosquito larvae for testing purposes. Nathan designed the sensing stand, and Will designed the alignment box. Matthew created a CAD design for another housing unit for the sensor and tubing and animated all the CAD for the team's final video presentation. Tyler came up with the idea to create a box of red acetate, and he found the VEML7700 sensor included in our final design. Yanni led the integration of the sorter with the sensor and developed code to scale and filter the sensor outputs.

Work that was evenly distributed amongst the entire team includes reports and presentations, sustainability and societal impact research, analysis of the actuation mechanism for sorting, and familiarization with needed tools and software relevant to the project.

5.13 Summary & Future Work

Since being tasked to assist the CDC with overcoming the throughput and cost issues, Bug Bane has successfully produced a prototype that is 300 times more efficient than the current CDC procedure of manual sorting at a microscope. The most prominent benefit generated by our design is the elimination of a microscope as a necessary component in the sensing subsystem. By proving that DsRed excitation and emission detection can be accomplished without a laboratory microscope, the ATLAS distinguishes itself as a promising solution for low-resource areas like sub-Saharan Africa. This has allowed our solution to be vastly superior to machines like COPAS, which are inaccessible to the Target Malaria project's beneficiaries. Currently, the CDC is beginning initial testing using our design strategy for DsRed detection in adult mosquitoes using an acetate filter with an excitatory LED. The setup presented by our team is ideal for implementation in the field. Future steps regarding project ownership include the transition of this project to Dr. Gabriel and Dr. Benedict for further development with all CAD files, code, and final prototype materials released to them. The next steps in this product's design life include testing at the CDC with larvae specimen for further proof of concept validation, the integration of both the sensing and sorting systems into a singular microcontroller for simplicity, the transition to finalized PCB designs to consolidate the circuitry, and further aesthetic work. The major project deliverables regarding throughput, cost, and error of outputted batch were all accomplished in Bug Bane's final prototype, bringing the future success of this project and the joint mission between Target Malaria and the CDC closer to fruition.

6. References

1. "World Malaria Report 2020." https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020 (accessed Sep. 21, 2021).
2. "Target Malaria | Together we can end malaria," *Target Malaria*, https://targetmalaria.org/ (accessed Sep. 21, 2021).
3. *Malaria*. (n.d.). Retrieved September 23, 2021, from https://www.who.int/news-room/fact-sheets/detail/malaria
4. *Genetically Modified Mosquitoes | Mosquitoes | CDC*. (n.d.). Retrieved September 23, 2021, from https://www.cdc.gov/mosquitoes/mosquito-control/community/sit/genetically-modified-mosquitoes.html
5. FAQ - American Mosquito Control Association. (n.d.). Retrieved September 20, 2021, fromhttps://www.mosquito.org/page/faq
6. *SUB-SAHARAN AFRICA*. (n.d.). ABC-CLIO's World Geography Website. Retrieved September 23, 2021, from https://www.fortbendisd.com/cms/lib/TX01917858/Centricity/Domain/1006/SSA%20Overview.pdf
7. Hwang, H., & Lu, H. (2013). Microfluidic tools for developmental studies of small model organisms – nematodes, fruit flies, and zebrafish. Biotechnology Journal, 8(2), 192-205. doi:10.1002/biot.201200129
8. Chung, K., Crane, M. & Lu, H. Automated on-chip rapid microscopy, phenotyping and sorting of C. elegans. Nat Methods 5, 637–643 (2008). https://doi.org/10.1038/nmeth.1227
9. "Our Approach," *Target Malaria*, https://targetmalaria.org/what-we-do/our-approach/ (accessed Oct. 20, 2021).
10. *Fluorescence-activated Cell Sorting (FACS) | Sino Biological*. (n.d.). Retrieved September 23, 2021, from https://www.sinobiological.com/category/fcm-facs-facs
11. *Bigfoot Spectral Cell Sorter | Thermo Fisher Scientific - US*. (n.d.). Retrieved September 23, 2021.
12. *Distribution of In Vitro Diagnostic Products Labeled for Research Use Only or Investigational Use Only Guidance for Industry and Food and Drug Administration Staff Preface Public Comment*. (n.d.). Retrieved October 28, 2021, from http://www.regulations.gov.
13. Lewis, R. (2019). Transgenic Organisms: Ethical Issues. Encyclopedia.com. Retrieved October 29, 2021, from https://www.encyclopedia.com/medicine/medical-magazines/transgenic-organisms-ethical-issues.
14. Swetlitz, I. (2018, September 4). Researchers to release first-ever genetically engineered mosquitoes in Africa. STAT. Retrieved October 29, 2021, from https://www.statnews.com/2018/09/05/release-genetically-engineered-mosquitoes-africa/.
15. Swetlitz, I. (2018, September 4). Researchers to release first-ever genetically engineered mosquitoes in Africa. STAT. Retrieved October 29, 2021, from https://www.statnews.com/2018/09/05/release-genetically-engineered-mosquitoes-africa/.

Appendix

Figure 4: Servo motor coupling to optimize interface between sorting subsystem and tubing

Tables and Charts

Table 1: Specification Sheet

				Issued: 9/21/2021		
			For: Bug Bane, solution device for Target Malaria Project		Page: 1	
			Specification			
No.	Date	D/W	Requirements	Responsible	Source	How Validated
General						
1	9-Sep	D	sense fluorescent protein markers in transgenic mosquito larvae travelling in a flow	product manager / testing lead	CDC	validated during testing phases
2	9-Sep	D	concurrently sort transgenic larvae from other larvae	product manager / testing lead	CDC	validated during testing phases
Physical Characteristics / Production Geometry						
3	9-Sep	D	actuator properly pauses / diverts flow through the tubing	Fluidics Lead / product manager	CDC	derive requisite actuator power and rigidity to oppose fluid pressure
4	9-Sep	W	apparatus does not create back pressure in the tubing	Fluidics Lead / product manager	CDC	derive non-larvae-enriched fluid pressure created when actuator is activated
5	13-Sep	W	less than half of parts are externally sourced	Prototyping Lead	M-Design	utilize part designs / configurations that are easily produced in-house
6	13-Sep	W	housing for externally-sourced parts can be modelled and prototyped in a campus makerspa	Prototyping Lead	M-Design	ensure constant velocity during actuation
7	13-Sep	W	no more than 6 significant parts	Prototyping Lead	M-Design	minimize redundancies in part production and assembly
8	13-Sep	W	minimum tolerances between actuator(s), housing, and tubing	Prototyping Lead	M-Design	coordinate with CDC contacts and teammates on dimensioning
9	13-Sep	W	minimum tolerance between optical sensor and larva flow	Prototyping Lead	M-Design	coordinate with CDC contacts and teammates on dimensioning
Electrical						
10	15-Sep	D	minimal power draw from outlet	Lead	CDC	probe power cord with Ohm-meter
11	15-Sep	D	compatible with African wall outlet	Lead	CDC	source proper adapter(s)
12	15-Sep	D	operate within safe voltage and current ranges	Lead	CDC	probe components with a Voltmeter and Ohm-meter during test trials
Mechanical						
13	9-Sep	D	strong enough force magnitudes to enact proper flow regulation	Lead / fluidics lead	CDC	derive requisite forces via approximation of fluid pressure
14	9-Sep	D	throughput >1 larva / second	Lead	CDC	ensure high velocity of actuation and detection when sourcing parts
15	15-Sep	W	minimum weight required for sysyem stability	Mechatronics Lead	M-Design	ensure ability of actuactor(s) to reliably return to initial orientation, and of whole apparatus to not drift after each sorting iteration
16	15-Sep	W	no symptoms of resonance in the apparatus	Lead	M-Design	ensure minimal to no damage to non-contact parts during endurance testing
Energy						
17	15-Sep	W	proper ventilation of heat generated from actuation	Lead	M-Design	calibrate tolerances to minimize friction between actuator parts and housing / tubing
18	15-Sep	W	proper ventilation of heat generated from microcontroller	Lead	M-Design	ensure a heat shield is added to controller if deemed necessary
19	15-Sep	W	efficient power storage and distribution to peripheral subsystems	Lead / perception lead	M-Design	probe each peripheral component with a Voltmeter during test trials
Materials						
20	19-Sep	D	sanitary, not cytotoxic (to the malaria) / must not harm larvae	Prototyping Lead	CDC	investigate requisite materials using literary resources and relevant vendors
21	19-Sep	D	not toxic to humans or mosquitoes under operating conditions	Prototyping Lead	CDC	
22	19-Sep	D	Be aBle to stand up to cleaning with ethanol	Prototyping Lead	CDC	
Signals / Ergonomics						
23	13-Sep	D	user interface is simplistic enough for 4 points of human interaction	Perception Lead	CDC	implement best practices within hardware configuration
24	13-Sep	W	signal transfer between devices take up minimal storage and power	Perception Lead	M-Design	implement best practices within code and hardware configuration
25	13-Sep	W	minimal storage is taken up on the microcontroller	Perception Lead	M-Design	implement best practices within code
26	15-Sep	D	perception of the larvae is monitored via a digital display	Perception Lead	M-Design	observe behavior of outside users when trying to transport device
27	15-Sep	D	digital display has negligible lag which doesn't accumulate over time	Perception Lead	M-Design	source proper detection equipment with minimal lag
28	15-Sep	W	95% of first time users can calibrate device and carry out experiments without instruction	Testing Lead	M-Design	observe behavior of outside users when trying to interface with device
Safety / Quality Control						
29	9-Sep	D	Accordance with regulatroy safety codes for laboratory equipment	Product Manager	IEEE, ASTM, NIST, CDC, OSHA, CPS	coordinate with CDC contacts on relevant safety codes and compliance methods
30	9-Sep	D	Accordance with EPA guidelines regarding ethical insect treatment	Product Manager	EPA	BSL2 laB will assure these reqs are met
31	9-Sep	D	<1% output error	Testing Lead	CDC	Count sorting errors over many trials
32	13-Sep	D	shows resistance to degradation over infinite use cycles	Testing Lead	CDC	observe wear over 10^6 iterations
Transport						
33	13-Sep	D	dimensions fit a standard oversees shipping container	Prototyping Lead	CDC	observe behavior of outside users when trying to transport device
34	13-Sep	W	ergonomic tear down for transport	Prototyping Lead	M-Design	
35	13-Sep	W	intuitive setup after transport	Prototyping Lead	M-Design	
Operation / Maintenance						
36	15-Sep	D	suitable for use in tropical environments	Fluidics Lead	CDC	ensure high robustness against humidity and heat
37	15-Sep	D	minimal (non-replacement) maintenance required	Fluidics Lead	CDC	observe wear over many trials, noting user opinions of maintenance needs
38	15-Sep	W	90% of detectable wear affecting the actuator(s) and close components	Testing Lead	M-Design	observe wear over many trials, noting incremental wear across all components
39	15-Sep	W	easy dismantling for cleaning of pertinent parts	Testing Lead	M-Design	observe behavior of outside users when trying to clean device
40	15-Sep	W	need for replacement of high-wear components occurs every >10^6 iterations	Testing Lead	M-Design	observe wear over many trials, noting incremental wear across high-wear components
Costs						
41	9-Sep	D	total manufacture/ assembly costs must not exceed $1000 per unit (excluding labor costs)	Prototyping Lead	Dr. Jariwala	search for vendors concurrently during design and prototyping phase
42	10-Sep	W	maximum raw materials for final product housing $200	Prototyping Lead	M-Design	
43	10-Sep	W	sensors and actuators for final product must not exceed $400	Prototyping Lead	M-Design	
44	10-Sep	W	tooling / machining costs and assembly for final product must not exceed $400	Prototyping Lead	M-Design	
Scheduling						
45	9-Sep	D	must be completed prior to Dec 7, 2021	Product Manager	Dr. Jariwala	proper project planning
46	9-Sep	D	project planning and control	Product Manager	Dr. Jariwala	ensuring time efficiency via colla
47	9-Sep	W	all parts for final product can be sourced within a month	Product Manager	M-Design	search for parts vendors concurrently during design and prototyping phases

Table 2: Morphological Chart

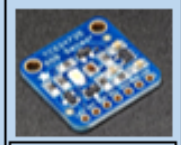
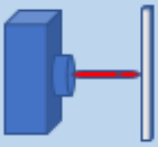
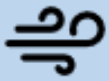
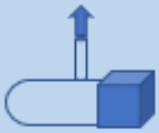
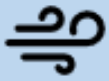
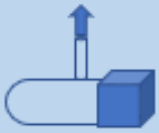
Sub-Function	Idea #1	Idea #2	Idea #3	Idea #4
Pass Larvae Through System	 Gravity	 Pump	 Hand-Fed	
Differentiate Between Fluorescent and Non-fluorescent	 Classification Algorithm	 RGB Sensor w/ Microcontroller	 Sort by Hand (Current Method)	
Separating Fluorescent from Non-Fluorescent	 Solenoid Valve	 Pinch Valves w/ Forked Flow	 Laser Kills Unwanted Larvae	 Conveyor Belt to Move Target Reservoir
Group Fluorescent Larvae into 250 Batch Sizes	 Photo-eye Detector	 Classification w/ Incrementor	Kyle Gabriel's Solution: The APC	
Discard Both Larvae	 Air Burst	 Laser Burst	 Side Cavity Driven by Back Pressure	
Keep Both Larvae and Separate Them	 Air Burst	 Oil Droplet	 Side Cavity Driven by Back Pressure	

Table 3: Sorting Subsystem Evaluation Matrix

R = Rating / WT = Weighted Total | Solenoid | Pinch | Conveyor| Servo Motor

Criteria	Importance	R	WT	R	WT	R	WT	R	WT
Fast Trigger (>1 per second)	10	5	50	4	40	3	30	5	50
Successfully Blocks/Allows Flow	10	4	40	4	40	5	50	5	50
Inexpensive	8	4	32	4	32	4	32	5	40
Comes in Needed Size	9	5	45	5	45	5	45	5	45
Connects Securely with Tubing	8	4	32	5	40	5	40	5	40
Microcontroller Compatible	10	5	50	5	50	5	50	5	50
Safety	10	5	50	5	50	4	40	4	40
Aesthetics	4	4	16	4	16	5	20	5	20
Setup Time	7	5	35	5	35	3	21	5	35
Resistant to Cyclical Wear	9	5	45	5	45	3	27	4	36
Total		403		393		355		406	
Relative Total		395/425 =.948		393/425 =0.925		355/425 =0.835		406/425 =0.955	
Rank		2		3		4		1	

Table 4: Sensing Subsystem Evaluation Matrix

R=Rating / WT = Weighted Total | TCS34725 | CI-6604 | PS-3213 |MAX301010

Criteria	Importance	R	WT	R	WT	R	WT	R	WT
Recognize Fluorescence	10	5	50	4	40	3	30	0	0
Signal to Microcontroller	10	5	50	5	50	0	0	4	40
Inexpensive	6	4	24	5	30	5	30	3	30
Fast Enough for Goals	9	3	27	4	36	3	27	4	36
Recognizes Doubles in Flow	4	5	20	3	12	5	20	4	20
Aesthetics	4	4	16	4	16	3	12	3	12
Setup Time	8	3	24	4	32	5	40	2	16
Total		211		216		159		154	
Relative Total		211/255 =.827		216/255 =0.847		159/255 =0.624		154/255 =0.604	
Rank		2		1		3		4	

Table 5: Flow Control Evaluation Matrix

R = Rating / WT = Weighted Total | Pump | Gravity | Suction

Criteria	Importance	R	WT	R	WT	R	WT
Flow Rate Fast Enough for Goals	10	4	40	5	50	2	20
Limited Pressure on Tubing	10	4	40	4	40	5	50
Inexpensive	7	5	35	4	28	5	35
Power Efficient	8	5	40	4	32	5	40
Reduces Doubles in Flow	6	3	18	5	30	5	30
Safety	10	5	50	5	50	5	50
Aesthetics	4	4	16	4	16	3	12
Setup Time	7	4	28	4	28	5	35
Resistant to Cyclical Wear	9	5	45	5	45	3	27
Total		312		319		299	
Relative Total		312/355 =.879		319/355 =0.899		299/355 =0.842	
Rank		2		1		3	

Mean Actuation Time Comparison of Motor Couplings

	Cylindrical Coupling	Funnel Coupling	Uncertainty due to Frame Rate	Two-Sample Equal Variance T-Test P Value
Unhindered	0.1172 s	0.1138 s	0.017	0.369467464
Hindered	0.1206 s	0.1182 s	0.042	0.450103548

Table 6: Gantt Chart of Phase 4 (Design Iteration)

Task	Responsible	Start Date	End Date
Further investigate spectroscopy and fluidics	Nathan, Matthew	9/24/21	10/1/21
Assemble Sensing Subsystem	Yanni, Tyler	9/24/21	10/1/21
Assemble Sorting Subsystem	Will, Nathan, Matthew	9/24/21	10/3/21
Program Perception	Yanni, Tyler	9/29/21	10/3/21
Program Actuation	Yanni, Tyler	10/2/21	10/6/21
CAD Each Subsystem	Will, Nathan, Matthew	10/2/21	10/12/21
Analyze Sensor Optics	Yanni, Tyler	10/4/21	10/12/21
Analyze Actuation	Everyone	10/4/21	10/14/21
3D-Print Subsystem Housing	Will, Nathan, Matthew	10/4/21	10/14/21
Prepare Presentation #2	Everyone	10/15/21	10/24/21
Prepare Report #2	Everyone	10/15/21	10/24/21

Table 7: Flow Rate Testing Results

Position	Water Height (mm)	Flow Rate (mL/min)
1	64	286
1	64	294
1	64	294
1	64	302
2	62	252
2	62	252
2	62	251
3	38	195
3	38	195
3	38	197