DESIGN AND PROTOTYPE LOW-COST EDUCATIONAL VERSION OF EXISTING LABORATORY INSTRUMENT

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis Related Assignments.

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Abstract

Multi-well plate readers have become an integral part of any laboratory conducting biological research because of their ability to detect and quantify biological, chemical, or physical events within the wells of a microplate (Jones et al., 2004). However, current plate readers are large and very expensive, therefore they are not easily accessible for educational purposes, especially in a high school classroom. Because the high school biology and life sciences curriculum in Virginia Schools focus primarily on qualitative analysis and lack a thorough method for teaching quantitative analysis, this Capstone project aims to develop a miniaturized plate reader and accompanying module to explore an improved method of teaching quantitative analysis. After interviewing local high school teachers and receiving feedback on the project, the authors developed a computational 3-D prototype which would have been incorporated into the classroom. Due to the current pandemic situation, the project aims were shifted to focusing on the accompanying module as the part of the final deliverable.

Introduction

Educational technology has been shown to provide many benefits to a classroom and can enhance the learning of the students. Sarah Butzin, an author and scholar who has focused on how learning environments are created for improved educational experiences, was able to show technology's benefits on education through her 2001 study that focused on the implementation of computers into elementary school classrooms. By placing computers into the classroom, and modifying the curriculums to effectively incorporate them, students were shown to have better test scores for all subjects when compared to their peers who did not have access to the technology (Butzin, 2001). Multi-well plate readers are important laboratory instruments used for collecting quantitative data for a variety of biological and chemical events. However, these devices are typically large and very expensive which makes them inaccessible for most high school classrooms. Making a plate reader that was designed with the needs of a high school in mind would allow students to collect quantitative data using a common piece of scientific technology.

One Charlottesville biotechnology company, Cerillo, has created a miniaturized multi-well plate reader that addresses the issue of size, but their device was still not ideal for use in a high school classroom. While being the smallest plate reader on the market, it is still too expensive to be a viable option for most high schools. The focus of this project was to design a multi-well plate reader that was based on Cerillo's device and make it an effective choice for educational settings. Along with the actual device, an experimental module would be developed to ease the integration of the device and make it as effective as possible. After initial meetings with teachers from local high schools to determine their specific needs, a prototype was designed and in the beginning stages of development. However, the

project shifted focus and an experimental module was instead developed with the specific needs of a high school classroom still in mind.

Results

Interviewing Teachers and Designing the Prototype

Pain Points

From the interviews conducted with the high school teachers from Albemarle High School, Monticello High School, Murray High School and St. Anne's Belfield School, several pain points were discovered within the classroom. Albemarle High School addressed a need for quantitative, "ready-to-go" experiments or kits that lasted one to two class sessions. The Monticello High School teacher, Jeremy Dove, mentioned the students' lack of understanding for math concepts within Biology and for multi-step biological processes. Murray High School addressed the need for more in-depth, quantitative experiments for the students compared to the more qualitative experiments that are currently incorporated into the curriculum. St. Anne's Belfield School mentioned no current methods for addressing bacterial growth or antibiotic resistance, which are concepts taught in the SOL curriculum. The biggest pain point for these schools was a lack of quantitative experiments that could be taught within one to two class periods and could cover concepts of the SOL curriculum.This led to the development of the prototype design of the miniaturized reader along with an accompanying experimental module to teach quantitative analysis to the high school students.

Design Features

For the design of the prototype, several features were included to address minor pain points and to accommodate for classroom use. The device would be used to detect absorbance of 24-well plates rather than the standard 96-well plates. This feature accounted for the classroom sizes and provided each

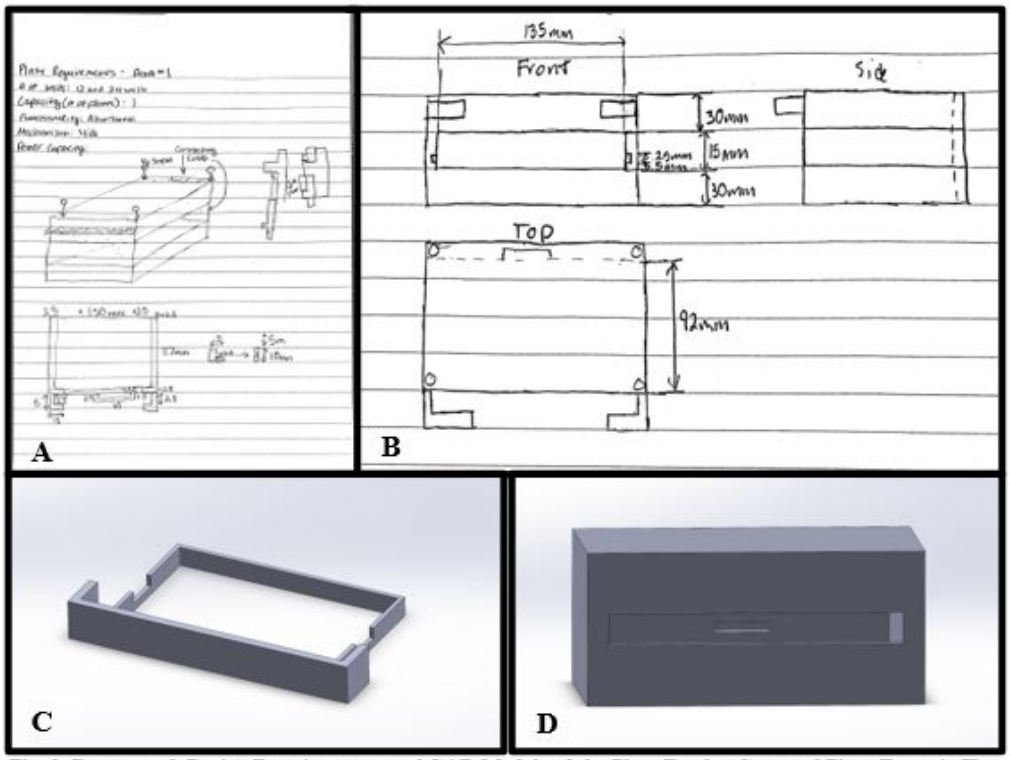

Fig. 1. Prototype 1 Design Requirements and CAD Models of the Plate Reader Case and Plate Tray. A. The first prototype requirements were discussed and developed alongside the help of the local, high school teachers and Cerillo. **B.** Isometric views of first prototype design. **C.** The tray on which the multiwell plate would rest. It would include a transparent film on the bottom to allow the LEDs to show through into the plate. **D.** The case where the components, including the tray and PCB, would be housed.

student individual wells to perform independent experiments. Another feature included enclosing the circuit components to limit access to students, which would prevent tampering of or damage to the electronics. A sliding tray mechanism for the microplate was provided for ease of use and durability throughout classroom use. Finally, the overall cost to develop the device was decreased due to in-house 3-D printing of the components at Cerillo. The basic design and 3-D model of the prototype are shown in **Figure 1**.

Project Shift

Due to the unforeseen circumstances caused by the COVID-19 pandemic, the prototype was unable to be completed. This meant that validation testing and gaining student and teacher feedback could not be accomplished. Therefore, the project shifted focus and the experimental module became the

Fig. 2. Timeline for Capstone Project for the 2019-2020 School Year. The timeline incorporates scheduling interviews and receiving feedback from local high school teachers. Due to the pandemic situation, the timeline was adjusted to account for not being at the University. **A.** The expected timeline from the Fall Semester. **B.** The shifted timeline after Spring Break.

main goal for the final aim. The original and shifted timelines can be seen in **Figure 2.** Originally meant as a secondary component of aim 3, the experimental module was intended to accompany the finished prototype device as a means of easily integrating the device into the classroom. It focused on two subjects that were determined to be ideal fits for both the high school classrooms and curricula that were examined during aim 1, as well as, for highlighting the potential use of the device. Those two subjects were bacterial growth and antibiotic resistance, and the experimental module that was developed was designed as a two part experiment that focused on bacterial growth for the first part and antibiotic resistance for the second.

Shown in **Figure 3** is an overview of the experimental procedure for the first part of the module. Beginning with an empty 24 well plate, 3 mL of Luria broth, or similar nutrient media, would be loaded into each of the wells. Next, colonies of E. coli would be selected from an agar plate and placed into well columns 1-5 for each row. The 6th column will contain no E. coli or chosen antibiotic in order for it to act as a negative control. The next step is to add the appropriate amount of the chosen antibiotic to the

different wells. We have indicated that Ampicillin would be an appropriate choice given that E. coli is known to have some resistance towards Ampicillin (Vranic & Uzunovic, 2016). In order to study the effects of antibiotics on bacterial growth and antibiotic resistance, different concentrations of the antibiotic will need to be added to each well. According to a protocol from Addgene, 100 microgram/mL of Ampicillin is the ideal amount to cause resistance in E. coli (*Addgene: Protocol - How to Inoculate a Bacterial Culture*, n.d.). Beginning in well 1 and moving across the row there should be 6 microliters of the 1000X stock Ampicillin, 3 microliters of the 1000X stock Ampicillin, 1.5 microliters of the 1000X stock Ampicillin, 0.3 microliters of the 1000X stock Ampicillin, and no Ampicillin should be added to wells 5 or 6 so that they can act as positive and negative controls, respectively. Once the appropriate components are added to each well, the plate can be placed into the finished version of the device and data can be collected that measures the rate of bacterial growth in each well. Once the recommended incubation period is over there should be four different strains of E. coli that each have varying levels of antibiotic resistance to Ampicillin, and that can be used for part two of the module.

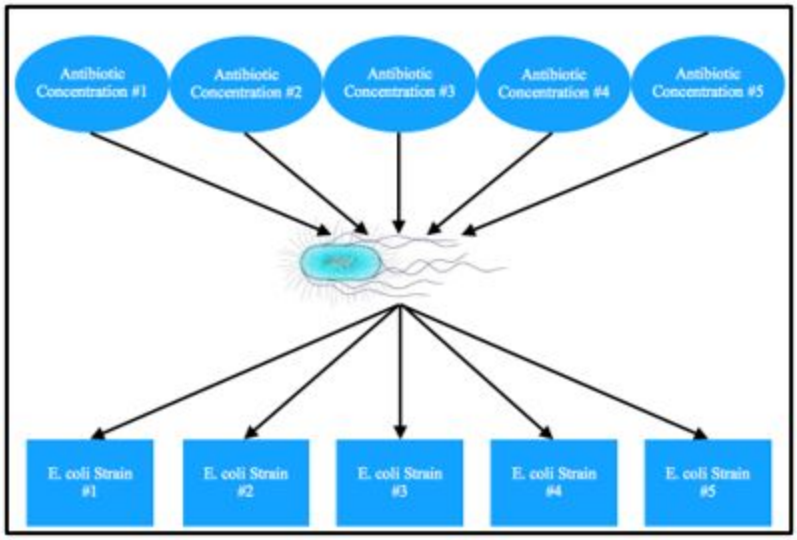

Fig. 3. Guide for Part One of the Experimental Procedure. Pipeline for the first part of the experimental procedure. Five different antibiotic concentrations will be used for inoculation of E. coli, resulting in five different E. coli strains that will be used for the next portion of the experiment.

The second part of the module focuses on the topic of antibiotic resistance and the outline for the experiment can be seen in **Figure 4**. Using the E. coli strains from the previous part of the module, the

experiment shows how resistance in a bacteria can affect the efficacy of the treatment drugs. Starting with a new 24 well plate, Luria broth, or the chosen nutritional media, should be added just as in the last part of the module. From there a sample of each of the strains of E. coli should be added to wells 1-4 with well 5 containing a sample of E. coli from the original stock to act as a positive control, and well 6 containing no E. coli to act as a negative control. This time the same amount of Ampicillin should be added to wells 1-5 so that the efficacy of it can be tested on each different strain. Six microliters of the 1000X stock Ampicillin should be added to attain a 200 microgram/mL concentration. No Ampicillin should be added to well 6 in order for it to act as a negative control. Again the plate can be placed in the finished version of the device and data can be collected that reflects the efficacy of the antibiotic as it acts on the resistant strains of bacteria. Both parts of these modules should provide ample quantitative data for the classes to use in their analysis, and the potential of the plate reader device is highlighted.

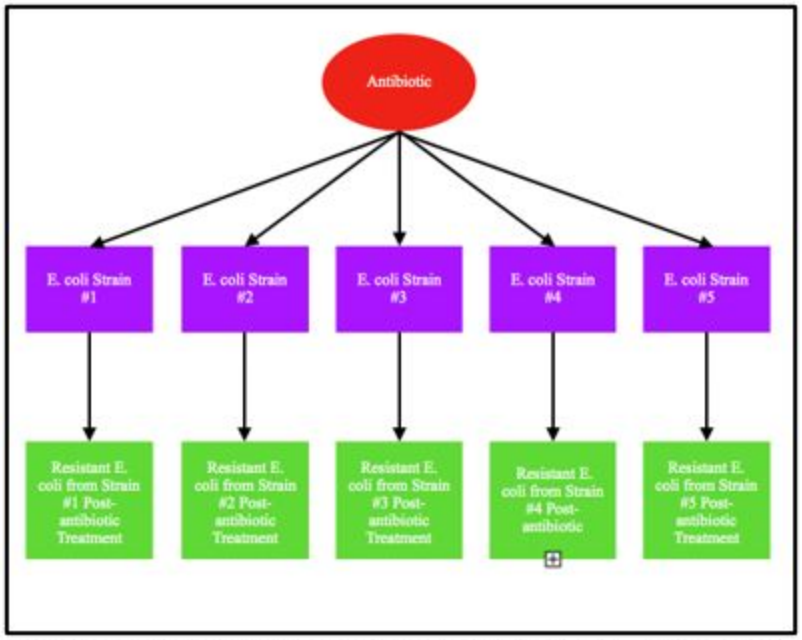

Fig. 4. Guide for Part Two of the Experimental Procedure. Pipeline for the second part of the experimental procedure. The five different E. coli strains gathered from part one will be cultured and then treated with the same level of an antibiotic, resulting in different amounts of remaining resistant E. coli.

If the plate reader device is completed in the future, the original goal for aim 3 would have been validation testing. This testing was originally going to be done using Cerillo's Stratus model plate reader as the comparison device and results of at least 85% accuracy would have been the goal. After

confirming the ability of the device through validation testing it would have been presented to some of the teachers and students that we had interviewed for aim 1, and feedback would have been gathered in the form of a survey. Those results would have helped us either make more improvements, or shown that the device was a viable product and Cerillo could look into it as a potential market.

Discussion

After interviewing the teachers and designing the prototype, the project was temporarily paused due to the current pandemic situation. The initial aims for the Capstone were re-evaluated and shifted to focus primarily on the accompanying module because physical progress for the prototype was no longer possible.

Lessons Learned

This project thoroughly covers the customer development process starting with customer development and ending with the product delivery. Understanding how to professionally collaborate with a Biotechnology Startup proved to benefit the progress of the project. Having the resources and knowledge from the original developer of the mintarurized plate reader greatly improved the design and progress of the prototype. Ultimately, being able to shift and pivot were essential for the project during this pandemic situation.

Future Work

With the current progress of this Capstone, this should be considered as a continuation project next academic school year. With the approval of Cerillo, the work could potentially begin over the summer with receiving teacher input about the current prototype. Some of the potential responsibilities of the next team would include improving the 3-D design and electronic circuit design, developing more

thorough supplemental modules and looking into avenues of affordability for these schools to implement after the completion of the project.

Materials & Methods

Researching Target Customers and Interviewing Stakeholders

To better understand the current Biology and Life Sciences curriculum, which biological concepts the curriculum aims to teach and the classroom environment in the Virginia high schools, interviews were scheduled with teachers at Albemarle High School (Tony Wayne), Monticello High School (Jeremy Dove), Murray High School (Jenna Barazi) and St. Anne's Belfield School (Dr. Bob Troy). After learning about the curriculum goals, two biological concepts were identified which included bacterial growth and antibiotic resistance. These two concepts would be the focus of our plate readers capabilities and the focus of the accompanying module.

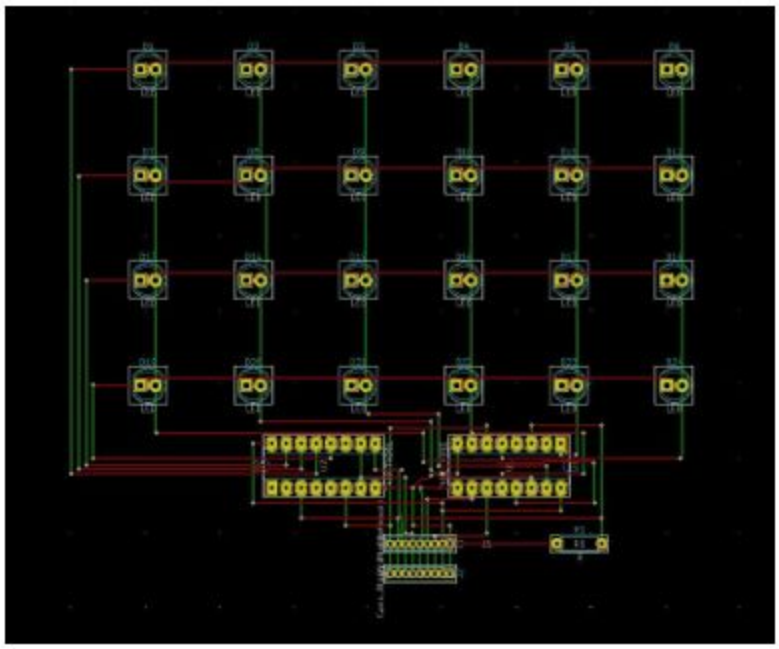

Fig. 5. Final Multiplexer Circuit used in Plate Reader Design. The final circuit design includes 24 LEDs and 24 phototransistors, which are read simultaneously at specific points controlled through the Arduino code. The final circuit was converted into a printed circuit board using the KiCAD program.

Designing the Prototype

After receiving input from the teachers, careful considerations were made for the design requirements of the miniaturized plate reader. A basic plate reader contains several components: light-emitting diodes (LEDs) for producing a light across the microplate, phototransistors to read the absorbance of the microplate, multiplexers to read individual wells, and a motherboard to control the microplate. To

develop a functioning plate reader, three programs were used including SolidWorks Autocad software to develop the 3-D model, Arduino software to develop the code for functionality and KiCAD software to design the circuit boards to control the LEDs and phototransistors to take readings of the absorbance of the microplate. To maintain simplicity while configuring the electronic circuit for the device, the first iteration of Cerillo's circuit was used in the prototype. The circuit was first completed using testing equipment provided by Cerillo and converted to a printed circuit board (PCB) design in KiCAD (**Figure 5**).

Technical Report References

Addgene: Protocol—How to Inoculate a Bacterial Culture. (n.d.). Retrieved April 16, 2020, from https://www.addgene.org/protocols/inoculate-bacterial-culture/

Butzin, S. M. (2001). Using Instructional Technology in Transformed Learning Environments. *Journal of Research on Computing in Education*, *33*(4), 367–373. https://doi.org/10.1080/08886504.2001.10782321

Jones, E., Michael, S., & Sittampalam, G. S. (2004). Basics of Assay Equipment and Instrumentation for High Throughput Screening. In G. S. Sittampalam, A. Grossman, K. Brimacombe, M. Arkin, D. Auld, C. Austin, J. Baell, B. Bejcek, J. M. M. Caaveiro, T. D. Y. Chung, N. P. Coussens, J. L. Dahlin, V. Devanaryan, T. L. Foley, M. Glicksman, M. D. Hall, J. V. Haas, S. R. J. Hoare, J. Inglese, … X. Xu (Eds.), *Assay Guidance Manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences. http://www.ncbi.nlm.nih.gov/books/NBK92014/

Vranic, S. M., & Uzunovic, A. (2016). ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI STRAINS ISOLATED FROM URINE AT OUTPATIENT POPULATION: A SINGLE LABORATORY EXPERIENCE. *Materia Socio-Medica*, *28*(2), 121–124. https://doi.org/10.5455/msm.2016.28.121-124