

Microbial Pill Sensor

DESIGN DOCUMENT

sdmay25-17

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Executive Summary

Excessive nitrate concentration in bodies of water, often a result of agricultural runoff, poses significant environmental hazards, endangering both aquatic life and the lives of those consuming the water. Current methodologies for identifying nitrate concentration in agricultural runoff are intensive, expensive, and inefficient, often requiring manual sampling and off-site analysis. By providing more efficient, cost-effective, and autonomous nitrate detection, the microbial pill sensor provides continuous monitoring of nitrate concentrations for both environmentalists and farmers. Improved access to environmental conditions allows users to make more informed decisions regarding the health of waterbodies and fertilization processes.

The microbial pill sensor uses a novel biosensing detection mechanism, relying on bioengineered microbes to identify the presence of nitrate in the environment. The microbes used by the microbial pill sensor have been genetically engineered to express **green-fluorescent protein (GFP)** due to the presence of nitrate. The concentration of nitrate in the environment can be determined through the fluorescent response of the expressed GFP, which is initiated, measured and transmitted via the electronic components of the microbial pill sensor.

The microbial pill sensor is a $5 \times 5 \times 10 \text{ mm}^3$ capsule broken into distinct modules. The bioengineered microbes are contained within a housing chamber, and the solution flows in through a selective membrane located at the top of the capsule. The electronic components are situated directly below the housing chamber and arranged in three distinct optical detection, microcontroller, and temperature control PCB layouts. A 488 nm wavelength LED is required to excite the expressed GFP, resulting in the emission of 532 nm wavelength light. The emitted light is captured by a photodetector, resulting in a photocurrent proportional to the presence of nitrate in the environment. A microcontroller is responsible for the activation of the LED at the desired interval and the transmission of the photocurrent value to an external device via low-power Bluetooth. An external GUI application processes the measurement and displays recorded nitrate concentrations for evaluation by the user. Additional temperature control is required to maintain the viability of microbes within the housing chamber.

The modular design of the microbial pill sensor enables the development of individual modules simultaneously, with each member assigned a particular module. Potential LED and photodetector components have been obtained, and the ESP32-C3 microcontroller has been selected for the MCU component. PCB design of the optical detection and microcontroller modules has been completed via Ki-CAD and an initial design of the external capsule has been generated via Fusion 360. Further fabrication and testing are required to assess the functionality of current PCB designs.

A breadboard prototype has been produced, integrating an ESP32-C3 dev kit, RGB LED, and photodetector to provide a proof of concept for the microbial pill sensor design. External data transmission via low-power Bluetooth on the ESP32-C3 has been achieved, and the recorded data is viewable on the first iteration of the external GUI application. The next steps consist of validation of the PCB design and integration into the external capsule. Functional testing will be conducted with fluorescent beads to reproduce the functionality of the bioengineered microbes.

Learning Summary

Development Standards & Practices Used

Circuit, Hardware, and Software Practices

Part decompositions of existing developments to meet requirements

PUN and PDN in circuit design

Use of meaningful commenting in code

Engineering Standards

IEEE 802 Nendica Report: Flexible Factory IoT: Use Cases and Communication Requirements for Wired and Wireless Bridged Network

IEEE Standard for User Interface Elements in Power Control of Electronic Devices Employed in Office/Consumer Environments: IEEE 1621-2004

IEEE Standard for a Real-Time Operating System (RTOS) for Small-Scale Embedded Systems: IEEE 2050-2018

IPC-221 Standards in PCB Design

Summary of Requirements

F.1 House specified bio-detection microbe on necessary amount of agar gel to maintain microbe culture for desired length of operation

F.2 Activate LED component at specified interval to disperse light uniformly in housing chamber

F.3 The dispersed wavelength of light emitted from the LED produces fluorescent emission via GFP protein expressed by bio-detection microbe upon the presence of the desired analyte in the chamber

F.4 Photodetection component produces photocurrent proportional to the concentration of analyte via fluorescent emission from microbe

F.5 Filter membrane allows the solution containing the desired analyte to flow into the detection chamber.

F.6 Temperature control system will maintain the housing chamber within a viable temperature range for specific microbe

F.7 Recorded voltage and temperature measurements are wirelessly transmitted to external device for processing via Bluetooth Low-Energy

F.8 A graphical user interface will display analyte concentration and temperature measurements on external device via processing of recorded voltage values

F.9 The microbial pill sensor will record analyte concentrations within an accuracy of 95%

P.1 The microbial pill sensor should be no larger than **10 x 10 x 5 mm³**

P.2 Properly contained housing chamber that can be removed to replace specific microbe

P.3 Properly contained compartment for holding batteries that can be replaced or recharged when needed.

E.1 The Housing will be composed of environmentally safe materials

E.2 The microbe housing container does not allow other bacteria to enter the chamber due to risk of conjugation

E.3 The microbe housing container does not allow microbe to exit the housing chamber due to risk of uncontrolled mutations

E.4 The battery housing component will prevent environmental contamination resulting from degradation of batteries during the product lifecycle

U.1 The external GUI will display analyte concentration in chamber

U.2 The external GUI will display the temperature of the chamber

U.3 The external GUI will produce a warning message when temperature of chamber exceeds viable ranges for contained microbe

U.4 The external GUI will record temperature and analyte concentration over a specified interval as a function of time to monitor the time evolution of the system.

Applicable Courses from Iowa State University Curriculum

- EE 2300: Electronic Circuits and Systems
- CPRE 2880: Embedded Systems 1: Introduction
- EE 2850: Problem Solving Methods and Tools for Electrical Engineering
- EE 3300: Integrated Electronics
- EE 3320: Semiconductor Materials and Devices
- EE 3330: Electronic Systems Design
- EE 4140: Microwave Engineering
- EE 4500: Biosensors

New Skills/Knowledge acquired that was not taught in courses

- 3D Cad Design
- 3D Printing
- Creating a functional Graphical User Interface
- Programming MCU dev kit in Arduino IDE
- Understanding of genetic memory circuits in bioengineered bacteria

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Definitions

Bluetooth Low Energy (BLE):

A wireless personal area network technology providing considerably reduced power consumption and cost while maintaining a similar communication range as classic Bluetooth. It is independent of classic Bluetooth Basic protocol but uses the same 2.5 GHz radio frequencies.

Green-Fluorescent Protein (GFP):

A protein that exhibits green fluorescence when exposed to blue light. The specific version of GFP employed by microbial pill sensors has an excitation wavelength of 488 nm and an emission wavelength of 532 nm.

Universally Unique Identifier (UUID): 16 (short) or 128 (long) bit number that uniquely identifies an object for BLE services.

1. Introduction

1.1. PROBLEM STATEMENT

Biosensing is a rapidly developing industry where new technologies for the detection of pathogens, contaminants, and other analytes are constantly being developed and improved upon. Biosensing technologies optimally provide quick, accurate, automated, and noninvasive alternatives for detection compared to manual techniques. Biosensing technologies provide continuous and autonomous monitoring of environments, providing a path to improved response and action. Optical detection mechanisms, which make use of changes in the intensity or wavelength of emitted light, provide superior selectivity and sensitivity compared to alternative biosensing mechanisms, improving the ability to monitor and respond to the presence of analytes in the environment. Optical biosensors also provide cost and size benefits when compared to alternative biosensing mechanisms such as electrochemical, impedance measurement, or acoustic technologies.

Excessive nitrate concentrations in bodies of water damage aquatic life and generate unsafe consumption conditions. Additionally, excessive nitrate concentration in agricultural runoff is indicative of inefficient fertilization processes. Available methodologies for identifying the concentration of nitrate in waterways require expensive manual sampling and external laboratory processing, making assessment of environmental and fertilization outcomes expensive and inefficient. Our project aims to provide a novel miniaturized nitrate biosensing technology through a microbial pill sensor for the efficient, autonomous, and economically viable detection of nitrate in waterways. Microbes contained within the microbial pill sensor have been genetically engineered to express **green fluorescent protein (GFP)** under the transcription control of the target analyte nitrate. Through excitation by 488 nm wavelength light, GFP produces a fluorescent response at the 532 nm wavelength. Through measurement of the fluorescent response of the expressed GFP, the microbial pill sensor enables quick, accurate, and autonomous determinations of environmental nitrate concentrations and provides an improved, cost-efficient and environmentally friendly alternative to current nitrate detection technologies.

The microbial pill sensor requires an optical detection module to measure the fluorescent response, a microcontroller module to transmit recorded measurements, and a temperature control module to maintain the viability of the microbes contained within an environmentally friendly housing. Transmission via **Bluetooth low-energy (BLE)** reduces power consumption, enabling extended device lifetime for continuous environmental monitoring. An external GUI application provides processing and visualization of recorded concentration measurements for analysis by the user. While the microbial pill sensor is intended for application in nitrate sensing, the design aims to be configurable to different applications. The microbial pill sensor provides functionality for the detection of different target analytes provided that bioengineered microbes exhibiting GFP in the presence of the target analyte exist. The development of genetic memory circuits for analyte detection is a rapidly developing field, providing a feasible route to the development of additional compatible microbes. The microbial pill sensor's low power consumption, miniaturized size, autonomous operation, and wireless data transmission make it a novel biosensing technology with clear application in environmental monitoring.

1.2. INTENDED USERS

To understand potential users of the microbial pill sensor, the team used empathy maps and personas. Through this exercise, the team generated the empathy map shown in Appendix A. From this empathy map create the following user personas were created, each with distinct needs and potential benefits from the project's implementation:

- Bioengineering Researchers
- Bioengineering Students
- Environmentalists

The first group includes bioengineering researchers. These users typically work on developing bioengineered bacteria for various applications and require a low-energy system to monitor these bacteria. Traditional systems rely on Wi-Fi for data transmission, leading to high energy consumption, which is not suitable for small-scale, continuous monitoring tasks. Our product addresses this need by using a low-power Bluetooth transmission system, offering a more energy-efficient alternative. This approach directly aligns with the microbial pill sensor's problem statement, which focuses on creating a low-power solution for bioengineered bacteria monitoring.

Another important user group is bioengineering students, who often experiment with different types of bioengineered microbes. These students need a versatile sensor package to test their microbes and determine whether the microbes are functioning as intended. Our product aims to allow for flexible testing of various GFP-emitting microbes, eliminating the need for multiple sensor packages. This benefits the students by providing a cost-effective and adaptable solution for their experimentation. The design supports multiple biosensing microbes, connecting directly to our problem statement, which aims to create a flexible, low-power sensor package that can be used across a range of microbial sensing applications in addition to direct application in nitrate detection.

Additionally, environmentalists also stand to benefit from our product. They are responsible for detecting pollutants or contaminants in natural water bodies to ensure ecosystem health. Their need is for a sensor that can perform long-term environmental monitoring without requiring frequent maintenance or battery replacement. Our low-power design allows the sensor to remain operational for extended periods—weeks or even months—before the batteries need to be replaced. This feature enables continuous monitoring and aligns with the need for sustained, uninterrupted data collection in the environment, directly supporting the overarching goal of creating a sustainable, energy-efficient monitoring system. The microbial pill sensor's direct application in nitrate detection provides a viable alternative for application in improving the health of waterways, increasing environmentalists' access to valuable information.

Finally, the microbial pill sensor is advantageous for farmers using nitrogen fertilizer. Assessment of nitrate runoff from the fertilization process enables improved assessment of fertilization processes. Improvement in fertilization processes boosts environmental health both in and surrounding agricultural fields and reduces unnecessary costs to the farmer. Application by farmers requires the microbial pill sensor to be an economically competitive solution to nitrate detection, requiring a cost-aware design of the microbial pill sensor.



Figure 1-1: Empathy Map

2. Requirements, Constraints, And Standards

2.1. REQUIREMENTS & CONSTRAINTS

The following list of functional, physical, environmental, and user experience-related requirements has been created to ensure the development of the desired microbial pill sensor with complete specified functionality.

Functional Requirements:

- F.1 House specified bio-detection microbe on necessary amount of agar gel to maintain microbe culture for desired length of operation.
- F.2 Activate LED component at specified interval to disperse light uniformly in housing chamber.
- F.3 The dispersed wavelength of light emitted from the LED produces fluorescent emission via GFP protein expressed by bio-detection microbe upon the presence of desired analyte in chamber.
- F.4 Photodetector component produces photocurrent proportional to the concentration of analyte via fluorescent emission of bio-detection microbe.
- F.5 Filter membrane allows solution containing the desired analyte to flow into the detection chamber.
- F.6 Temperature control system will maintain the housing chamber within a viable temperature range for specific bio-detection microbe
- F.7 Recorded voltage and temperature measurements are wirelessly transmitted to external device for processing via Bluetooth.
- F.8 A graphical user interface will display analyte concentration and temperature measurements on external device via processing of recorded voltage values.
- F.9 The microbial pill sensor will record analyte concentrations within an accuracy of 95%

Physical Requirements:

- P.1 The microbial pill sensor should be no larger than $10 \times 10 \times 5 \text{ mm}^3$ (constraint)
- P.2 Properly contained housing chamber that can be removed to replace specific microbe.
- P.3 Properly contained compartment for holding batteries that can be replaced or recharged when needed.

Environmental Requirements

- E.1 The housing will be composed of environmentally friendly material.
- E.2 The microbe housing container cannot allow for other bacteria to enter the chamber due to risk of conjugation.
- E.3 The microbe housing container cannot allow for the bio-sensor microbe to exit the housing chamber due to risk of uncontrolled mutations
- E.4 The battery housing component will prevent environmental contamination resulting from degradation of batteries during product lifecycle

User Experience Requirements

U.1 The external GUI will display analyte concentration in chamber

U.2 The external GUI will display the temperature of chamber.

U.3 The external GUI will produce a warning message when temperature of chamber exceeds viable ranges for contained bio-detection microbe

U.4 The external GUI will record temperature and analyte concentration over a specified interval as a function of time to monitor the time evolution of the system.

2.2. ENGINEERING STANDARDS

Standards play a crucial role in engineering. They ensure the consideration of safety, quality, and consistency throughout the development of new technologies. Engineers are given clear guidance on creating reliable products and systems that meet industry and regulatory expectations when following these established guidelines. Standards help improve efficiency, encourage global collaboration, and prevent accidents by ensuring engineering designs are understandable by all and implemented correctly. Most importantly, they protect public health, safety, and the environment, all while promoting innovative designs and processes. The following standards have been selected for our microbial pill sensor project due to their relevancy in creating an effective and secure product:

- IEEE 802 Nendica Report: Flexible Factory IoT: Use Cases and Communication Requirements for Wired and Wireless Bridged Network
 - The application of this standard to our project revolves around the aspects of real-time monitoring, automated data collection, and processing. The standard also indicates the need for security in data transmission to ensure there is no outside interference, which could jeopardize not only the data but also the monitoring system. To conform to the standard, a strong and secure connection between the microbial pill sensor and the external GUI application is necessary.
- IEEE Standard for User Interface Elements in Power Control of Electronic Devices Employed in Office/Consumer Environments: IEEE 1621-2004
 - The main parts of this standard that apply to the project are focused on the user interface and how to make that interface accessible and consistent for users. By creating an accessible system that clearly informs users what features are used and how the data is perceived, this being the GUI system to display concentration and temperature values, the user is guaranteed a consistent experience.
- IEEE Standard for a Real-Time Operating System (RTOS) for Small-Scale Embedded Systems: IEEE 2050-2018
 - This standard applies to the task management mechanisms employed by the project, which break into managing functions based on timers and time-related functions, and memory management. Following a standard that helps simplify and organize the many interconnected systems that are incorporated in the Microbial Pill sensor will be crucial to creating an effective product.

These standards are very applicable to the project as they relate to the front-end user interface, managing data functions to minimize disruptions and possible errors, and ensuring there is no interference with transferring data through Bluetooth. IEEE 1621-2004 reflects on the interaction that the project will have with users. The microbial pill sensor has potential application as a universally assessable biosensor system, meaning that depending on what analyte a user intends to detect and what biosensor they use, the system should still perform the same. Following a standard that focuses on creating a consistent experience will guide the foundation of the GUI. IEEE 2050-2018 reflects on creating a system that manages all the parts of a system while being efficient. Since the microbial pill sensor will have excitation and emission detection circuits, temperature monitoring and controls circuitry, and a data transmission microcontroller, creating an efficient and well-structured system is a must. Lastly, IEEE 802 reports on the importance of creating a secure connection between a device and its recipient. If an opportunity for an attacker to interfere with data transmission were to arise, this could result in false detection and in turn could jeopardize research. The project must protect itself and the user's data.

A standard that was previously selected to be reviewed but was rejected is the IEEE/IEC 62704-2-2017 standard, which is IEEE/IEC International Standard Determining the peak spatial-average specific absorption rate (SAR) in the human body from wireless communications devices, 30 MHz to 6GHz. The reason this was previously considered is due to the long-term goal of the product range of uses. This standard covers the research conducted into looking at how transmitting Wi-Fi signals through human tissue can cause complications with signal processing and concerns for human tissue. This standard wasn't selected due to two main factors: the first being that the project's requirements must use a low-energy data transmission system, which Wi-Fi is not, and the second being that the project's scope is limited to environmental monitoring, not internal health monitoring. There have been multiple similar biosensor systems that have been developed to be used inside the gastrointestinal tract of a human or livestock. However, this project does not aim to meet those requirements.

To ensure the incorporation of these IEEE standards considered in the microbial electronic sensor pill project design, we can modify the design to improve data transmission connectivity and device functionality. For example, implementing encryption protocols and authentication mechanisms for Bluetooth data transmission will ensure the design exhibits secure communication between the pill sensor and the user interface. Adding specific user-friendly elements to the user interface design, such as easily visible alerts and notifications as well as power status indicators, would ensure that the project design abides by these selected focal engineering standards.

3 Project Plan

3.1 PROJECT MANAGEMENT/TRACKING PROCEDURES

The project management style that the team is adopting is a blend between waterfall and agile. The beginning of the project follows an agile style of management, with each individual team member making progress on different subsystems. The modular design of our microbial pill sensor enables team members to work independently on different portions of the system, such as the external housing design, PCB design, or GUI implementation.

The project then shifts to a waterfall style of project management following the completion of individual module design. A shift in project management style is required due to the dependency on integrating the different subsystems together. Once a functional prototype has been created, linear testing of the individual modules and the entire integrated system will take place. If a fault or potential improvement in the microbial pill sensor design is noted, the team will consolidate and reach an appropriate fix.

The team's progress has been documented through the use of the Weekly Reports and individual notes recorded by each member as they progress through their individual portions of the project. These weekly reports and notes have been documented on the Microsoft Teams channel. From the beginning of the project, in alignment with the team contract, each member has communicated responsibilities, accomplishments, and setbacks. Weekly meeting with the client advisor provides determination of progress.

3.2 TASK DECOMPOSITION

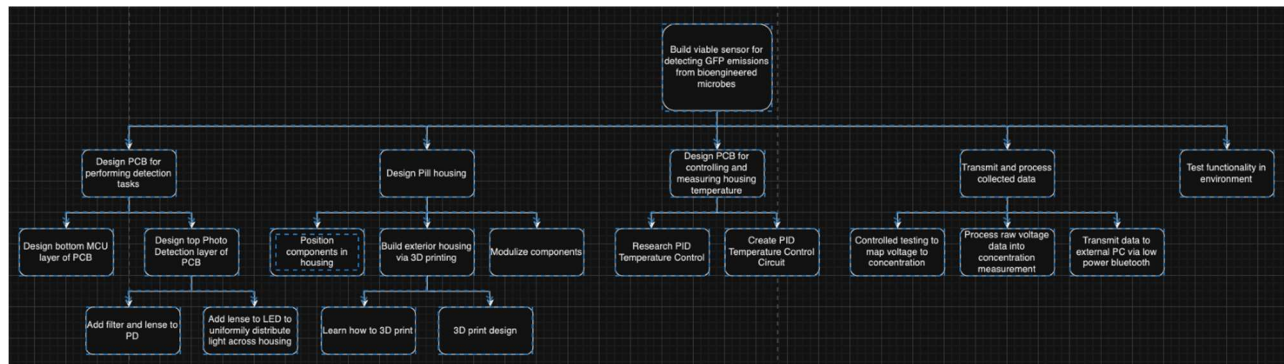


Figure 3-1: Task Decomposition Chart

The task decomposition chart depicted in Figure 3-1 was created to help boil down specific tasks into more manageable ones. This will allow for an easier understanding of each specific task required to complete the overarching goal of the project. Without this decomposition, coming up with attainable short-term goals would prove difficult due to only looking at the project from a big picture perspective.

3.3 PROJECT PROPOSED MILESTONES, METRICS, AND EVALUATION CRITERIA

Milestone 1: Develop a proof-of-concept prototype using BLE transmission of the light intensity of an LED via a photodetector component.

Milestone 2: Identification of all commercially available components used for the development of the final microbial pill sensor.

Milestone 3:

Milestone 3a: Development of 3d printed housing chamber prototype for final microbial pill sensor.

Milestone 3b: Development of optical PCB component for final microbial pill sensor.

Milestone 3c: Development of microcontroller PCB component for final microbial pill sensor.

Milestone 3d: Development of GUI for display of recorded measurements made by final microbial pill sensor.

Milestone 4: Assembled prototype produces measurement corresponding to 95% accuracy when applied to fluorescent beads resembling genetically engineered microbes used for final device.

Milestone 5: Development of temperature control module to finalize complete prototype.

Milestone 6: Testing and revision of final microbial pill sensor in functional environment produces accurate concentration measurements with device error within 1%.

The milestones depicted represent the flow of project management implemented throughout the project as discussed in section 3.1: *Project Management*. Milestone 3 depicts the portion of the project development process where an agile project management style is being used, where each individual member is working on the completion of a sub-milestone. The other identified milestones display the use of a waterfall project management style, where the entire team is actively working toward the completion of the same shared goal. As the project develops, future milestones now identified as needing a waterfall style of project management may transition to an agile style as subtasks are identified for the completion of the milestone.

Milestones 1, 2, 3, and 5 all correspond to the physical development of a component of the project or the physical development of a complete prototype. The evaluation of the completion of these milestones will be qualitative, where we will be able to visually characterize the completion of these tasks, such as visually examining the GUI, the fabricated housing chamber or PCB components. Testing will be conducted on these components to ensure they are functional, such as ensuring that a voltage is produced by the photodetector component contained within the photodetector PCB, but the establishment of accuracy or targeted value metrics are not applicable to these milestones.

Quantitative milestones cannot be identified until the fabrication of the first complete prototype, which requires the completion of the major project and design component in milestone 2. Upon the completion of a prototype, testing will allow for further evaluation criteria to be identified. Further discussion of meaningful evaluation criteria is being conducted, and this section will be updated with the identified evaluation criteria. The evaluation criteria for this project will concern the ability of the device to properly characterize the concentration of analyte in the functional environment.

3.4 PROJECT TIMELINE/SCHEDULE

An important part of project management and documentation is to develop a specific timeline for completing tasks and milestones. By decomposing each task into different variables, the team is allowed to create an accurate timeline and allocate resources to the areas of focus that require it. Properly accessing the situation and responding accordingly to a Gantt chart document will improve the team's efficiency. This specific Gantt chart, figure 3-2, follows the sprint schedule model, with each specific task having a dedicated timeline. In this chart, there is documentation of the performed task on the left axis. The designated time allocated for the tasks is shown. This model follows the spring style, as overlapping progression occurs.

A future Gantt chart creation that divides each task into appropriately decomposed parts would be helpful as it would document specific progress more accurately than the current Gantt chart. This documentation would show the incorporation of both the agile management style of the entire project's progression and the waterfall management style that's used for progression through tasks and integration.

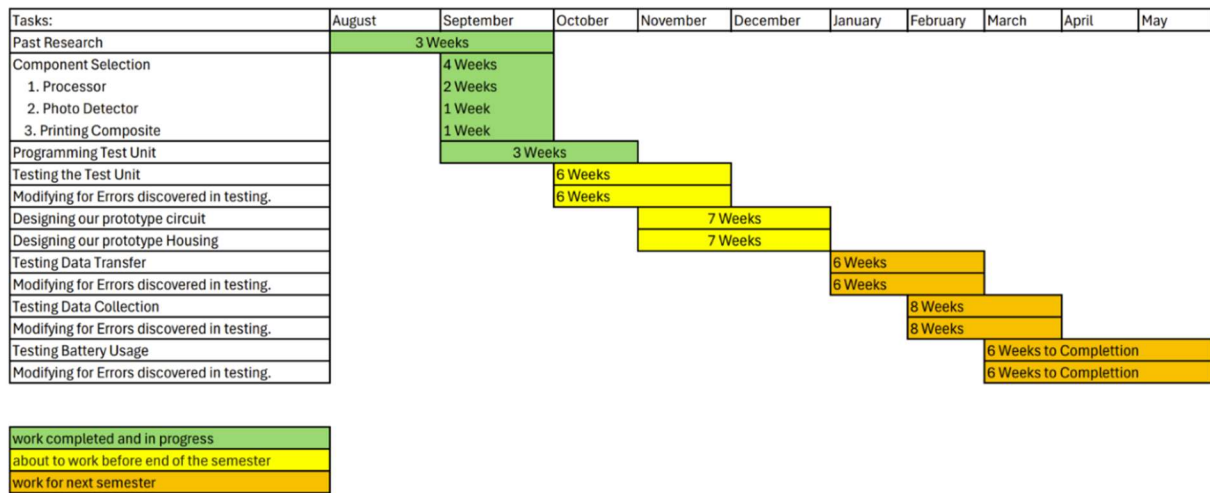


Figure 3-2: Gantt Chart

3.5 RISKS AND RISK MANAGEMENT/MITIGATION

The task decomposition chart identifies six main subtasks that must be completed for this project to function:

- ST1: Design PCB for performing detection tasks
- ST2: Design pill housing
- ST3: Design PCB for controlling and measuring internal housing temperature
- ST4: Transmission of data to external device via BLE
- ST6: Implementation of GUI to display transmitted data
- ST5: Test functionality in environment

For ST₁, the design of the optical detection PCBs could suffer from random dispersion of the emitted fluorescent response, reducing the amplitude of the incoming signal. A weak signal limits the detection limit and sensitivity of the microbial pill sensor and reduces the functionality of the device. This risk can be mitigated by introducing a lensing system into the design that could be bought commercially and would focus the produced fluorescent response onto the photodetector for increased signal strength.

A potential risk for ST₂ is contamination or release of the microbes from the containment. The microbial pill sensor will house bioengineered microbes that could rapidly reproduce if released into the environment the user wants to test. Therefore, insurance that the housing design properly contains the microbes is necessary. Ensuring the durability and permeability of the housing properly contains the microbes will be required through the exploration of potential material options. Additionally, with environmental improvement as a motivation for the microbial pill sensor, the device must be environmentally friendly. Therefore, ensuring the material used to build the housing chamber prevents damage to the interior electronics by the external environment while not harming the environment is a requirement of the microbial pill sensor.

For ST₃, a potential risk associated with this task is the control system not operating correctly, leading to the premature death of the bioengineered microbes inside and the sensor not working. To minimize this risk, the development team will need to complete rigorous amounts of testing on the PID control scheme used in the temperature control PCB. If this testing is done and errors are still introduced after ensuring the control scheme is working properly, choosing a different control scheme or method for controlling the internal housing temperature might become necessary.

ST₄ poses a potential risk of data not being sent to the GUI correctly, giving the user misleading information about the concentration of analyte in the environment. One way this risk can be mitigated is by adding a data checker to the data processing done in the GUI, ensuring that the data that gets transmitted to the GUI is within a specific range of realistic values before more processing is done and the results are shown to the user.

Final system testing in the functional environment brings complexities that may not be adequately represented by testing. These risks are represented in ST₅, accounting for the complexities of submerging the sensor within the solution. Functional testing in a mock environment is required to assess risks to electronic components, transmission reliability, and intake membrane permeability. Additionally, the functional environment poses the same risk of allowing the microbes to escape the sensor capsule, potentially contaminating the environment. Thorough testing in a mock environment, truly representative of the functional environment, will be performed to mitigate these risks.

3.6 PERSONNEL EFFORT REQUIREMENTS

Table 3-1 below shows an example of a weekly report table in which each member describes their individual contributions to the specific portions of the project on which they are designated to work in accordance with the Gantt chart and task decomposition. The team has concluded that this is the optimal way to document weekly progress as well as time invested into different tasks. If a specific task has been completed or reached a milestone listed above in section 3.3 *Project Proposed Milestones, Metrics and Evaluation Criteria*, it shall be noted on the Gantt chart so that accurate documentation can be created.

<u>NAME</u>	<u>Individual Contributions</u> (Quick list of contributions. This should be short.)	<u>Hours this week</u>	<u>HOURS cumulative</u>
Cade Kuennen	Contributed to the extensive amounts of class-related assignments. Learned how to import KiCad files into Fusion 360. Looked for schematic of Arduino dev kit used in our breadboard prototype.	6.5	37
Alex Upah	Assistance and troubleshooting with Wes on low-power Bluetooth operation. Extensive contribution to class-related assignments such as the design document.	6	33.5
Wes Ryley	Completed the Arduino code to connect and send data that's collected on the Photodetector through BLE. Resourced materials that will be used in KiCAD to construct a first edition PCB.	8	36
Rakesh Penmetsa	Worked on converting kicad files to fusion 360	5	30

Table 3-1: Weekly Progress Documentation

Progression is tracked to the very specific tasks, and progress is documented by the hourly time invested. The team has concluded that documentation of personnel effort in the weekly reports and the personal notes, which are generated through the completion of the individual task, works optimally for the project's needs and the currently employed management style.

3.7 OTHER RESOURCE REQUIREMENTS

The financial resources made available by our faculty advisor and client, Dr. Lu, will provide the team with the ability to obtain all the necessary resources to design, purchase, and fabricate the necessary electronic components. For the electronic components, the project required the purchase of ESP32-C3 microcontroller units, as well as the identified photodetector, LED, lensing, and filtering components. Fabrication of our PCB design containing these identified and purchased components will be handled via a professional service.

On-campus 3D printing resources will allow the team to produce the necessary prototype housing chamber, providing an easy route to testing. Final production of the housing chamber may occur off campus through a professional service that will be available via the financial backing for the project. Laboratory facilities sponsored by Dr. Lu and the department will provide the necessary soldering, assembly, and testing resources to ensure the design meets the intended functionality.

Upon the completed design of the prototype, fluorescent beads will be made available that will allow for the testing of the microbial pill sensor in a functional environment, where the fluorescent beads will simulate the response of the would-be bioengineered microbe. Once the necessary prototype and testing have been completed, the genetically engineered E. Coli will have to be provided to complete the necessary biosensing functionality testing of our product.

4 Design

4.1 DESIGN CONTEXT

4.1.1 Broader Context

Farmers in the United States use around 150 pounds of nitrogen per acre. Average nitrate runoff is around 20 pounds per acre, contaminating waterways and public bodies of water. Excess nitrate and nitrogen levels in water bodies lead to excessive algal blooms, a process known as eutrophication. Excessive algal blooms reduce oxygen levels, damaging fish and other aquatic life. Additionally, high levels of nitrate also pose health risks to humans and other lifeforms consuming contaminated water. Current commercially available measurement methods for nitrate levels in runoff require sampling and off-site testing, limiting the frequency and availability of testing. The recent development of advanced technologies in nitrate detection has increased the autonomy of nitrate detection but at a significant increase in cost with a reduced lifetime. Our device aims to provide an easier route to determine nitrate concentration in agricultural runoff and other water bodies by providing continuous, cost-effective, and easy detection. Through continuous monitoring, farmers can more accurately assess fertilization strategies and amounts. Our device also provides environmentalists with more frequent information about hazardous conditions in waterways, providing a route to increase the quality of our waterways and limiting public health crises.

Area	Description	Examples
Public health, safety, and welfare	Provides environmentalists and farmers with real-time information regarding nitrate levels that allows for the improvement of waterway health.	Farmer John realizes that the nitrate levels in runoff to the south of his field are very high and addresses the problem through changes in fertilizer application.
Global, cultural, and social	Our project aims to improve environmental monitoring and make sure to use environmental protective practices.	Our capsule will be sealed so that no degradation of battery capsule results in environmental damage.
Environmental	Our project aims to improve environmental monitoring and make sure to use environmental protective practices. By providing improved environmental monitoring, we provide professionals with the proper tools to identify environmental issues as quickly as possible.	By identifying high levels of nitrate in a public waterway, environmentalists can work to improve the water quality of that water body.
Economic	Our product will be low cost and require no installation cost. This provides a cheaper alternative to current nitrate monitoring method.	Our capsule is cheaper than sending an environmental water sample to a professional lab for analysis, providing farmers and environmentalists cheaper, faster, and more effective sensing solutions.

Table 4-1: Design Implications

4.1.2 Prior Work/Solutions

Our biosensor capsule is a novel product, as no research or product has been documented using bioengineered bacteria for the detection of nitrate via an electronic capsule pill. Previous research has been conducted to demonstrate the ability of bioengineered bacteria to act as biosensors for nitrate, but none of these groups have integrated these bacteria into an electronic capsule for continuous environmental monitoring. Similar electronic biosensing capsules containing bioengineered bacteria have been documented but have different analytes and different application environments.

A similar biosensor [1] using bioengineered microbes integrated into a miniature electronic capsule to detect gastrointestinal inflammation using bioluminescence in the presence of the target analyte provides the motivation for the design. Like our product, the capsule uses a membrane to intake the solution into the microbe housing chamber and uses a proprietary threshold-based bioluminescence IC integrated with four photodiodes to identify the concentration of three different analytes in the solution simultaneously. Our product provides sensing capabilities for a different target analyte and uses BLE results rather than Wi-Fi. By using BLE, the microbial pill sensor will exhibit lower power consumption, which is advantageous for our application in the continued monitoring of agricultural runoff.

Bioengineered microbes provide significant advantages in specificity and robustness compared to current biosensing alternatives, such as electrochemical, microfluidic, or acoustic devices. *Escherichia coli* [2] has been bioengineered to detect nitrate through regulated expression of GFP. While the techniques, methodologies, and principles of bioengineered bacteria are beyond the scope and responsibilities of this project, the active development of microbial-based biosensing solutions compatible with the microbial pill sensor increases the device's functionality.

A commercially available nitrate sensor is produced by *Clear Water Sensors* [3]. This sensor uses advanced microfluidic lab-on-chip technology to perform nitrate and nitrite monitoring in rivers, wastewater, oceans, and autonomous vehicles with a maximum depth of 6000 m. The device has a total height of 56 cm, which is much larger than our proposed design. With a power consumption of 1.8 W and a limit of detection of .05 μM , this device provides a performance standard our microbial pill sensor should reach in terms of sensitivity and power consumption. The device also has a lifetime of 1000 readings per canister, which results in 250 hours' worth of data collection per canister. Our microbial pill sensor should have an extended lifetime to provide a cheaper continuous monitoring biosensing solution. This device, while relying on a different detection mechanism, provides market performance standards our microbial pill sensor should meet to provide a viable alternative in the market.

4.1.3 Technical Complexity

The microbial pill sensor is a complex and novel engineering solution for a direct application in improving environmental monitoring strategies. The team aims to generate a first-of-its-kind solution that is not currently available in the market. The design consists of multiple components, identified as a housing chamber, an optical detection module, a microcontroller module, a temperature control module, and an external GUI application. Each module is complex and presents its own subsets of challenges. Size restrictions require a well-designed housing chamber, along with an extensive evaluation of available components for the optical detection module. The optical detection module also poses challenges related to optical lensing and filtering, with our excitation and emission wavelengths being relatively comparable. Reduction of a commercially available MCU board to fit our available size requirements requires an understanding of circuit and PCB design. Additionally, the programming required for the successful function of the MCU and the GUI requires significant hardware, software, and programming skills.

4.2 DESIGN EXPLORATION

4.2.1 Design Decisions

The microbial pill sensor's physical system layout was the most influential and first choice made in the design process. The team opted for a vertical design with the modular systems stacked, with each module component responsible for a specific function in the design. Modular design allows for a further breakdown of individual members' responsibilities to a singular component. Following the design choice of physical system layout, future design decisions related to selecting the necessary components for the microcontroller and optical detection modules.

Considering system requirements in BLE transmission, reduced power consumption and sizing, along with user needs and economic cost factors, the team decided that the ESP32 C3 microcontroller was optimal for the microbial pill sensor. Within the optical detection module, component choices regarding the LED, photodetection component, and necessary optical components have been identified but require further testing to finalize the selection. These component choices are crucial to the ability to meet the system requirements and functionality while maintaining the economic feasibility of our microbial pill sensor. Additional decisions regarding lensing and filtering components are necessary for the complete functionality of the device. Further discussion of electronic components included in the design will take place in section 4.3: *Proposed Design*. To continue in the development of the microbial pill sensor, PCB design layout decisions were made once the necessary electronic components were identified. The layout of the PCBs will be further discussed in section 4.3: *Proposed Design*. Through optimal PCB layout, the team aims to ensure the device's functionality while reducing the physical footprint of the device. Along with the necessary physical design choices involved in designing and ultimately demonstrating functional biosensing capabilities, our microbial pill sensor requires the ability to process and display the recorded data. By making the active design choice to handle the processing and display of recorded data measurements through an off-platform GUI, the team optimized the microbial pill sensor's ability to meet system requirements in sizing and power consumption while meeting user needs in clear and functional display of concentration measurements.

4.2.2 Ideation

Creating a functional project inside a capsule is incredibly difficult. Factors the team has considered include the physical space, orientation of components, and modular connections inside the project. The original proposed idea was to surround the housing chamber with all of the sensor monitoring systems. This would mean the bacteria housing chamber extended deep into the center of the project. After reviewing the design with Dr. Lu, simplifying the system into modules would improve the design. This decision resulted in discussions about requirement needs and how to meet these needs while keeping a simplified design. Creating a vertical system that breaks down into separate compartments and PCBs simplified the physical and spatial design. Initially, the options revolved around organizing the modular PCBs into a functional order. Understanding that the GFP sensor PCB should be placed directly below the housing container and by implementing an optical lens that will uniformly disperse the excitation light and a focal lens to collect GFP emissions directly into the phototransistor, the design reached closer to the current design. Another design feature that was considered and is waiting to be implemented into the design is the use of double-sided PCBs to maximize space. Since the GFP sensor system will be controlled by the microcontroller PCB, combing these two systems onto one board simplifies the project. By brainstorming user needs and requirements of the projects monitoring system, the group has been able to generate ideas of how to improve and implement functional sections.

4.2.3 Decision-Making and Trade-Off

Weighted Decision Matrix					
	Economic Consideration	Technical Needs	Simplification	Meeting Requirements	Totals
Weights	3	4	2	5	14
Central Housing Chamber	1	2	1	1	18
Vertical Pill Chamber	1	2	2	2	25
Modular System Breakdown	2	2	3	3	35
Focal Optical Lensing	3	4	4	4	53
Double-Sided PCB	3	5	4	5	62

Table 4-2: Weighted Decision Matrix for Ideated Design Considerations

Table 4.2 shows the weighted decision matrix for the project's design considerations. It follows the order of design features as they were created and implemented. A key feature of this project, which varies from others, is that our project doesn't have a very wide range of options to consider, so rather than generating and scrapping past ideas, the team has prioritized implementing useful considerations and revising them for improvements. The design considerations in the table all fall under the umbrella of system layout. Referencing the ideation of each consideration, it can be concluded that as each portion brought about positives, the next ideation works to fix the negatives of the previous creation.

This can be seen in the figure as the most recent feature implemented into the design is the double-sided PCB and it has the highest weighted total. This total is a representation of how the team feels the ideation impacts the project's demands and design steps. Having a higher value results in a larger positive impact on the outcome of the project. The reason that the team has valued this final design idea for the spatial considerations is due to how it meets and improves all the physical and technical considerations, as well as improving the simplification of the design will reduce the amount needed for multiple PCBs. Integration of ideas through considerations and analyzing the impact an idea will have on a final product is crucial to creating a smooth, constantly developing design process.

4.3 PROPOSED DESIGN

4.3.1 Overview

The desired physical microbial pill sensor must detect the desired analyte concentrations in the functional environment while meeting sizing, power consumption, and BLE transmission requirements. The following microbial pill sensor design considers system requirements, as well as user needs, environmental considerations, and economic feasibility. Figure 4.1 provides a physical 3D view of the microbial pill sensor. Figure 4.2 depicts a system sketch overview of the design, detailing the connections between the individual modules. The proposed design breaks the entire system into multiple subsystems or modules, each with a desired and specific functionality. These modules are a housing chamber designed to contain the microbes while flowing in the solution, an optical detection module to excite and measure the GFP fluorescent response, an MCU module to transmit the recorded voltage produced by the optical detection module, a temperature control module to maintain environmental conditions suitable for the microbes, and a battery housing component to provide power. The physical 3D design vertically integrates these modules, meeting sizing requirements while providing easy access to the battery and microbe housing chambers. Providing access to battery and microbe housing chambers, while not incorporated in our system requirements, satisfies user needs relating to the continued operation of the microbial pill sensor, allowing users to easily replace the batteries or microbe nutrient gel.

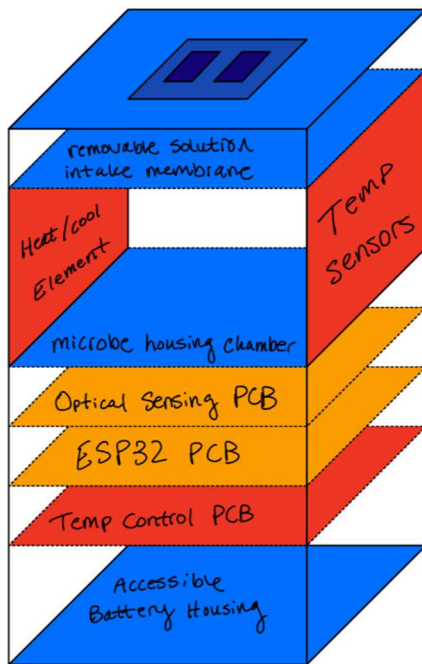


Figure 4-1: 3D Design Sketch

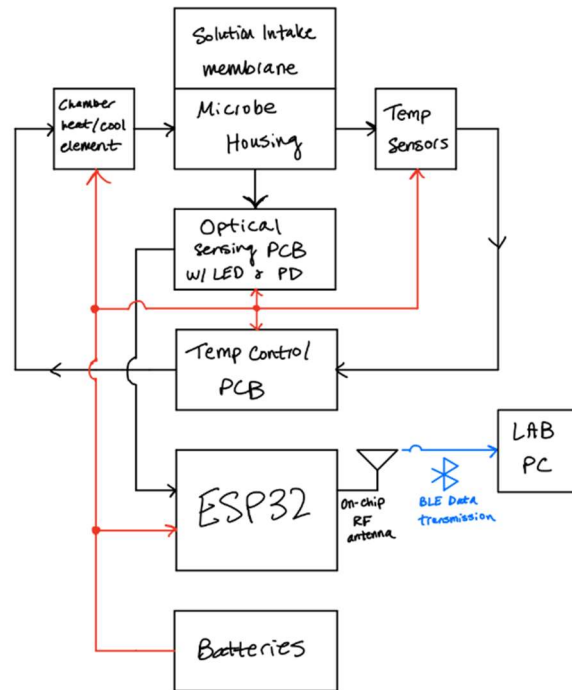


Figure 4-2: Schematical System Breakdown

4.3.2 Detailed Design and Visual(s)

As previously described and visualized in 4.3.1: *Overview*, the design of the microbial pill sensor is modular, resulting in the integration of multiple distinct components into the final device. The following sections will be broken down into describing the design of these individual modules. At this point in the design process, not all these modules have been thoroughly designed or had their development prioritized. This section of the document will reflect current progress in each of the modules and will be updated as the design of these modules progresses throughout the project. Prioritization within the early portion of the design has been focused on the optical and MCU modules and components due to their crucial nature to the system's functionality. Once sufficient design finalization and component selection for these crucial modules are completed, individual focus will be applied to the remaining component design.

Exterior Housing Design

A CAD model of the exterior housing for the microbial pill sensor has been designed, viewable in **Figure 4-3**. The design follows the physical layout depicted previously in Figure 4.1. The initial design task involved defining precise dimensions for the device, with an initial proposed size of $5 \times 5 \times 10 \text{ mm}^3$. The housing model is divided into distinct compartments for the battery, microbe housing, and MCU. The battery cell dimensions were designed as $3(+0.2) \times 5.3 \times 5.3 \text{ mm}^3$, with 0.2 mm height allocated to connect the battery cell to the MCU housing, and the dimensions of the MCU cell housing are $3.2 \times 5.3 \times 5.3 \text{ mm}^3$. The microbe housing measured $3.8(+0.2) \times 5.3 \times 5.3 \text{ mm}^3$, capped by a $2.6 \text{ mm} \times 5.3 \times 5.3 \text{ mm}^3$ detachable lid. Additional consideration regarding material and coating to fabricate the housing module is required. This section will be updated as material and coating discussions take place.

After prototyping using a filament 3D printer, it was evident that the initial dimensions were too small to be 3D printed effectively. Furthermore, feedback from our advisor revealed that the initial size needed to be increased to accommodate the MCU and sensor PCBs. This led to an updated prototype design with significantly larger dimensions of $22 \times 22 \times 24 \text{ mm}^3$ for the entire module. The final housing design now includes a $22 \times 22 \times 7 \text{ mm}^3$ battery compartment, with an additional 2 mm height to join it to the microbe housing to the MCU cell of dimensions $22 \times 22 \times 8 \text{ mm}^3$. The microbe housing has been redesigned to measure $22 \times 22 \times 9 \text{ mm}^3$, with a detachable cap measuring $22 \times 22 \times 3.5 \text{ mm}^3$ for easy access and maintenance. The increased size ensures sufficient component space while maintaining a compact and modular form. The revised housing has been prototyped, but challenges with the filament 3D printer persist, particularly for fine detail and accuracy. A switch to a resin 3D printer is under consideration to achieve the required precision and reduce the housing design to the desired size. These advancements ensure that the design now effectively meets the spatial requirements of the project prototype.

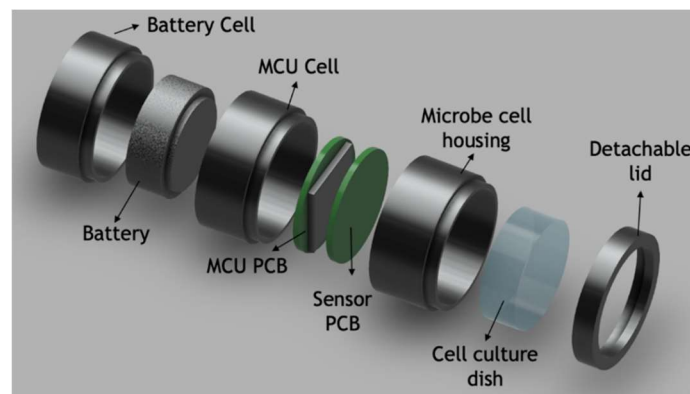


Figure 4-3: Exploded view of the Microbial Pill Sensor Housing design

Microbe Housing Chamber

The microbe housing chamber has been significantly refined to address functional and fabrication challenges. The chamber contains bioengineered microbes on agar gel while enabling efficient biosensing through a transparent bottom interface. Initially, the chamber was designed with dimensions of $3.8(+0.2) \times 5.3 \times 5.3 \text{ mm}^3$, including a detachable cap of $2.6 \text{ mm} \times 5.3 \times 5.3 \text{ mm}^3$. However, this design proved impractical for 3D printing and failed to meet the spatial requirements. Responding to these issues, the chamber's dimensions were increased to $22 \times 22 \times 9 \text{ mm}^3$, with a detachable cap of $22 \times 22 \times 3.5 \text{ mm}^3$. The updated design allows sufficient space for microbial growth, nutrient gel replacement, and the integration of optical sensors for GFP fluorescence measurement. The detachable cap provides easy access for maintenance, ensuring extended operational life for the device.

To optimize prototyping, the chamber design was 3D printed using filament technology, but the precision required for such small-scale components led to poor results for smaller dimensions. Future iterations will utilize a resin 3D printer or alternative options to improve accuracy and achieve the desired fit and finish. By addressing fabrication challenges and spatial constraints, the updated microbe housing chamber design ensures functionality and manufacturability while supporting the project's overall goals.

Optical Detection PCB

The optical detection module is the most crucial component to the functionality of the microbial pill sensor. Without the proper design of the optical detection module, the sensor will lack the ability to correctly detect the desired analyte in solution, rendering the device ineffective or even useless. The following circuit diagram depicts the system-level design of the optical detection module.

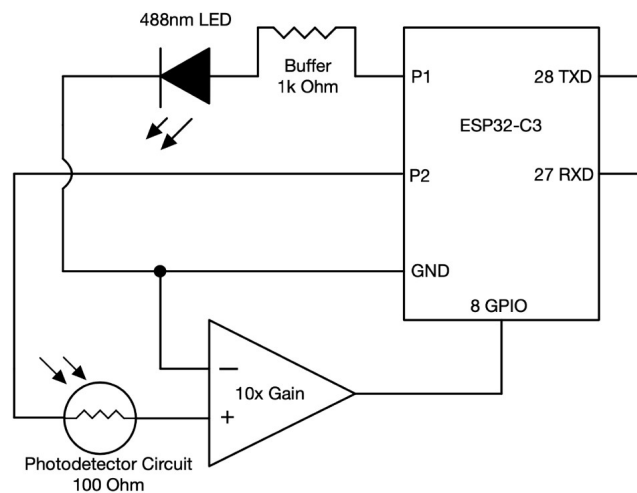


Figure 4-4: ESP₃₂-C₃ PCB Prototype Schematic

Figure 4-4 depicts the necessary connections between the ESP₃₂ C₃ microcontroller and the LED and photodetection component. The ESP₃₂ C₃ activates the 488 nm LED component at the desired timing interval, exciting the microbes with the necessary blue wavelength. A buffer resistor is included to protect the integrity of the LED. The recorded fluorescent response is measured by the photodetection component, which produces a voltage proportional to the light intensity produced by the excited GFP expressed by the microbes. This voltage, in turn, is proportional to the concentration of nitrate. Amplification of the recorded voltage to a meaningful value is performed by a LM324 operation amplifier. This voltage value is then passed to the ESP₃₂ C₃ microcontroller through GPIO port 8.

A bandpass filter, passing ranges 532 ± 20 nm, must be used to ensure that the produced voltage is not generated by any additional wavelengths of light other than those of GFP response due to nitrate present in the solution. Additional lensing will provide a more consistent response by focusing the emitted light on the photodetector. The addition of these optical components will increase sensitivity to nitrate concentration, making the microbial pill sensor a more effective biosensing solution. Selection of filtering components is an ongoing process. Concerns exist regarding cost and availability of filtering options within desired wavelengths and size constraints. Prototype testing with PCB will be used to determine the necessity for filtering and lensing components.

The current choice for the LED component is the SK6812MINI. This device provides a controllable RGB Led in a $3.5 \times 3.7 \times .95$ mm top SMD, meeting both our wavelength and sizing requirements. This device was integrated into the dev kit currently used for prototyping, meaning that our MCU code already correctly handles the digital control of the LED. This device also provides complete RGB wavelength control, allowing our design to be applicable to different excitation wavelengths beyond our initial application in nitrate sensing. Further details regarding the component, including depictions of pin layouts, optoelectronic characteristics, and spectral emission, can be found in Appendix A.

The current choice for the photodetector is the EPIGAP OSA Photonics EOPD-525-1-0.9 525 nm photodiode. With a peak sensitivity at 525 nm, sizing of $3.2 \times 1.6 \times 1.2$ mm and a responsivity of $.3$ A/W, this photodiode provides the desired functionality of the module. Further testing must be conducted within the module to demonstrate device functionality. Further details and specifications regarding the device can be found in Appendix B.

Once components were decided upon, custom PCB design for the optical sensor portion of the project could commence. To start, a schematic was created denoting the electrical connections between components to be placed on the board. Figure 4-5 depicts the designed KiCad schematic, utilizing the LM324 10x gain amplifier, the SK6812MINI RGB LED, the EOPD-525-1-0.9 525 nm photodiode, and other supporting passive components such as resistors and ferrite beads. With all connections made, PCB layout footprints were assigned to all components based on their corresponding datasheet sizing requirements.

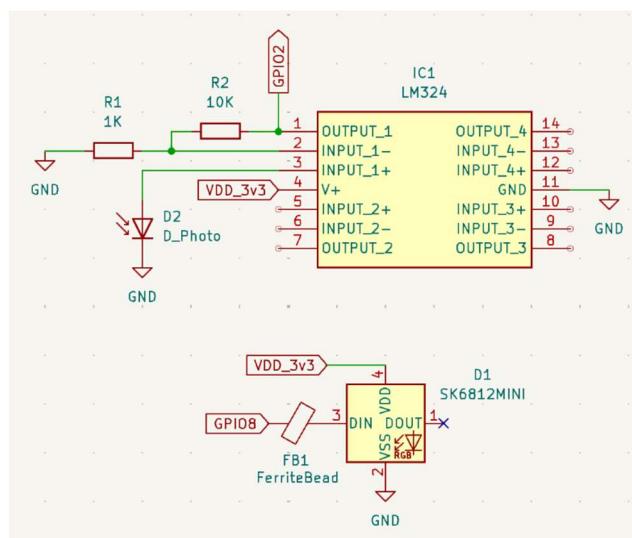


Figure 4-5: Custom Optical Sensor PCB Schematic

With connections made and footprints specified, the PCB layout shown in Figure 4.6 was laid out and routed. The outer dimension of this custom PCB is 20 mm. Although this is larger than the minimum sizing requirement, the team believes this to be a good first draft for the custom design as it allows us to be able to test functionality without the team's ability to solder extremely tight points hindering the device operation. Note that D2 (the photodiode) and D1 (the RGB LED) are spread out to allow room for the inclusion of lensing and filtering, which will be implemented later. Another important clarification to make is the reasoning behind the three open pads on the back copper of the PCB. These have been placed as solderable jumper points between the optical sensor PCB and the microcontroller PCB, which is to be discussed in the following section.

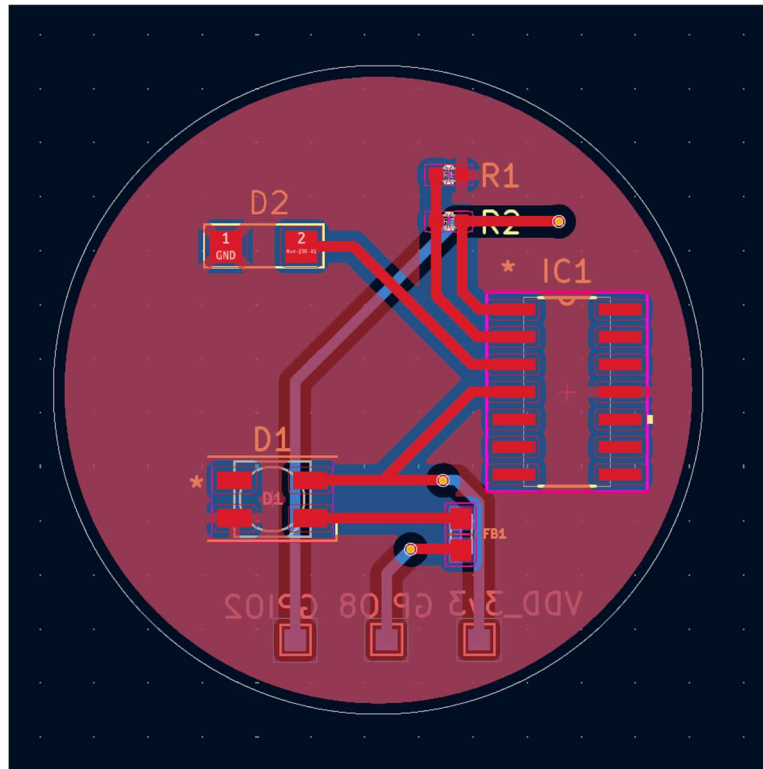


Figure 4-6: Custom Optical Sensor PCB Layout

Microcontroller PCB

Another crucial design aspect of this project is the selection of a microcontroller unit (MCU) and the design of a custom PCB utilizing said MCU. For this project, the Espressif ESP32-C3-DevKitC-02 was chosen as the starting point for our implementation due to the dev kit being very similar to the implementation the team would realize. A parts decomposition on the Espressif ESP32-C3-DevKitC-02 schematic (see Figure 4-7) was completed to identify the components from the dev kit that were necessary to carry over into the custom PCB implementation.

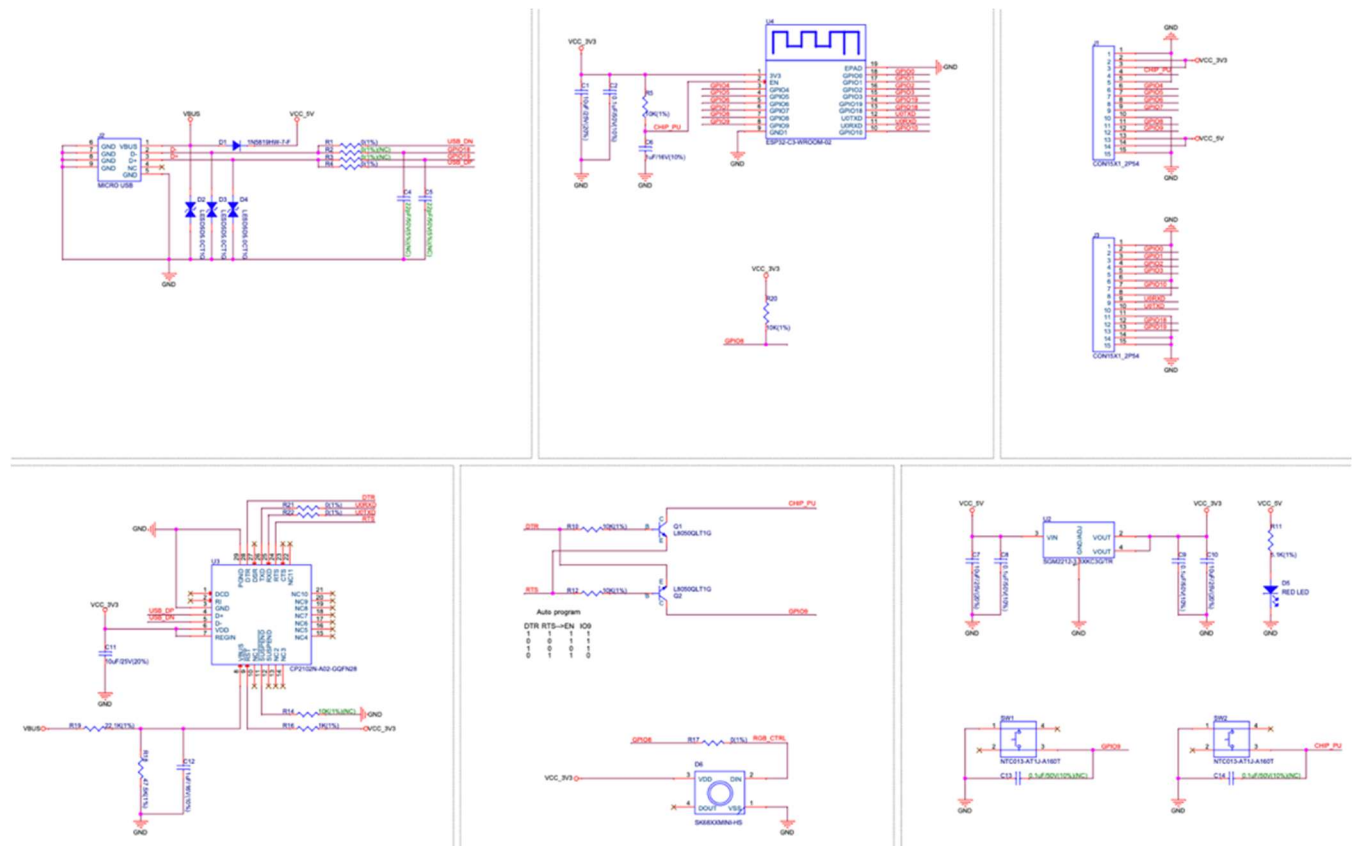


Figure 4-7: Espressif ESP32-C3-DevKitC-02 Schematic

After a discussion with our advisor Dr. Lu, the team decided to utilize the USB power option that comes with the dev kit for the first round of prototyping of the PCB implementation, allowing us to focus more time on the optical sensors portion of the design. From the parts decomposition, the team concluded that the only unnecessary components on the dev kit are the push buttons and their corresponding transistor amplifiers, which will allow for the required reduction in size for the custom PCB implementation. This custom PCB implementation will be responsible for routing signals to and from the optical sensor portion of the project; it will turn the excitation OFF and ON at our desired frequency and collect the data from the photodetector to be sent via BLE.

After completion of the parts decomposition done on the ESPRESSIF ESP32-C3-DevKitC-02 and component selection was solidified, a custom PCB design for the microcontroller module was conducted. Figure 4.8 depicts the designed electrical schematic showing pin connections between devices and components. The main differences between this schematic and the ESPRESSIF dev kit are the removal of the push buttons and corresponding switching current amplifiers. The linear regulator used in the design was also changed from the SGM2212-3.3 to the LD1117S33 due to stock limitations of the original regulator. This new regulator was chosen due to having very similar operational, sizing, and packaging characteristics to the SGM2212-3.3. Another important note is that the capacitor values for both oscillator crystals used in this design still need to be specified. This will be done using the equation shown under the crystals in Figure 4.8 and tuned utilizing a spectrum analyzer to make the oscillations more precise. With all connections made, PCB layout footprints were assigned to all components based on their corresponding datasheet sizing requirements.

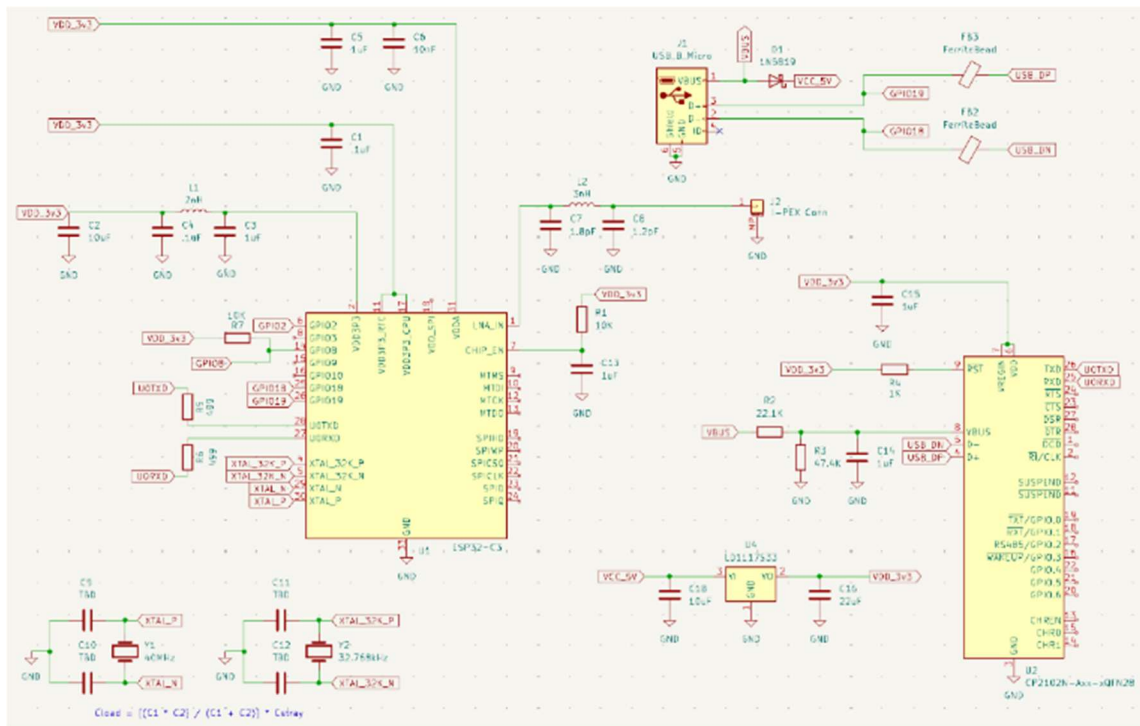


Figure 4-8: Custom MCU PCB Schematic

With connections made and footprints specified, the PCB layout shown in Figure 4.9 was laid out and routed. The outer dimension of this custom PCB, like the optical sensor PCB, is also 20 mm. Again, although this is larger than the minimum sizing requirement, the team believes this to be a good first draft for the custom design as it allows us to be able to test functionality without the team's ability to solder extremely tight points hindering the device operation. One major consideration that must be noted is again the use of solder pads for jumping necessary lines between the separate MCU and Optical Sensor custom PCB designs inside the housing. More work will be done to both this custom PCB and the Optical Sensor PCB next semester to reach the minimum sizing requirement after testing has been completed on the larger version and the correct operation is verified.

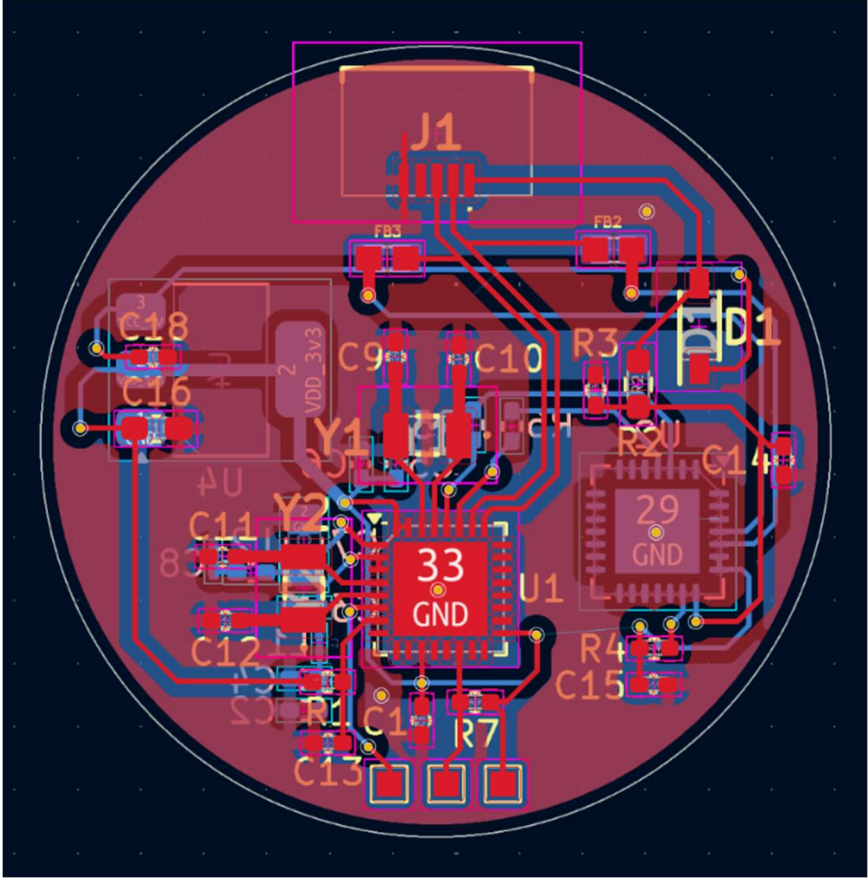


Figure 4-9: Custom MCU PCB Layout

Software and GUI Development

For the program development of the microbial pill sensor both Arduino IDE and VSCode hosting Python are used. The Arduino IDE is used to program an MCU using a vast quantity of MCU chips, Devkit Boards, and libraries for specific MCUs and their functions. The vast array of resources for Arduino IDE has led to the programming being simplified down to a few key features. For the ESP32-C3 in the microbial pill sensor design, the main functions are to control the optical circuits, power on the excitation LED, collect an amplified response from the PD, and transfer the collected data via BLE to an external GUI application. The Adafruit NeoPixel library is used to operate the RGB LED. The BLEServer, BLEDevice, and BLEUtils libraries are used to create a BLE Server, register a device, and set custom UUIDs for the BLE connection. All other operations used in the program are methods developed in the Arduino Software. The current prototype functions on a ten-second loop that runs continuously to the advertising of the BLE connection. Once a connection has been made, the data collected at the end of the loop will be transmitted to the connected device and repeated. Future implementations of this program will require the BLE connection to be established before the optical components become operational in a way to minimize wasted power.

The Python GUI program was created after the implementation of the Arduino IDE program. This was done as a way to verify the BLE connection would transmit data before beginning to attempt to connect the MCU to the custom program. The included libraries are tkinter, matplotlib, and pytz to help operate the create and update the GUI with accurate recorded data, and the bleak library which is used to connect the program to the MCU's advertising signal.

As a user begins to run the program, an interactive window opens, which allows a user to scan for BLE devices. Figure 4-10 (left) shows a view of this window once it has been created. It is important to note that the next window will appear after the scan has been completed, so it is crucial to wait for this to execute before clicking the scan for devices button again.

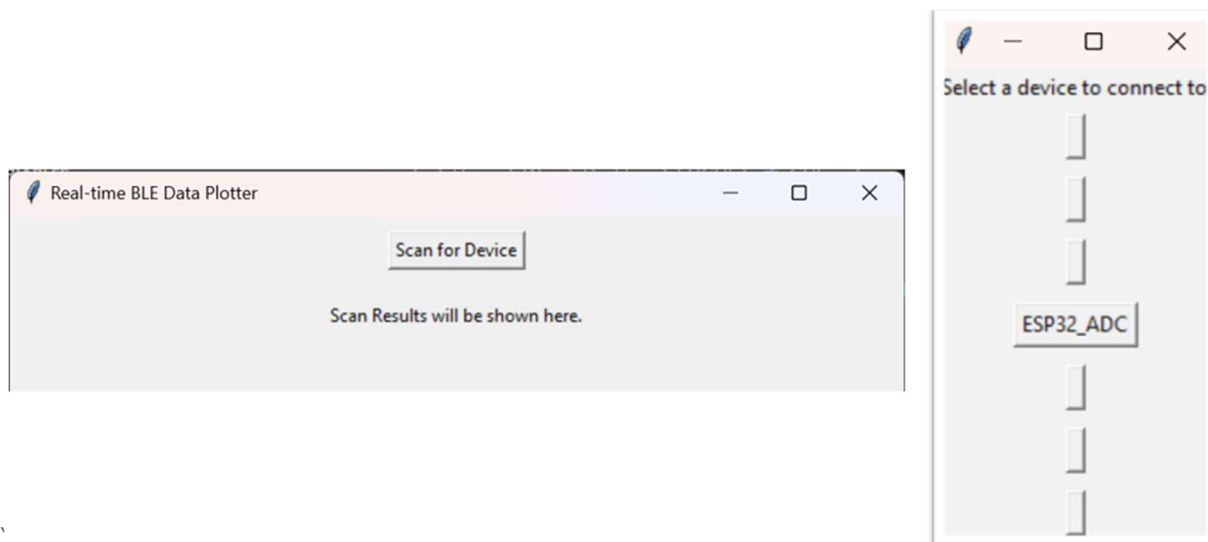


Figure 4-10: GUI Start-Up and Select Device Window

Once the scan is completed, a list of scanned devices opens in an additional window in which a user can select the specific device. Figure 4-10 (right) contains a screenshot of the detected devices window. The spaces with no titles are devices which are broadcasting a signal, but do not contain a specified name to connect to. The project's name currently is ESP32_ADC.

Upon establishing a connection, the original window changes into a time series of recorded amplified voltages. Additional testing of the functional biosensor will provide the necessary response curve to process recorded voltage values into measurements of nitrate concentration. Figure 4-11 depicts a voltage response graph with four data points being recorded from the photodetector. This example was produced using the prototype discussed in *Section 6 Implementation*. The dips in recorded voltage correspond to physical covering of the LED and a reduction in light intensity.



Figure 4-11: Real Time Amplified Voltage Graph

Temperature Control PCB

Due to discussions with our client Dr. Lu, design and implementation of the temperature control PCB has been assigned low priority in the design of the microbial pill sensor. Discussions with the client have determined that a functional prototype of the other integrated PCB components should be provided prior to the development of the temperature control PCB, making it the last task in the development of the microbial pill sensor. This design prioritization decision was made due to both the complexity and less crucial nature the temperature control PCB serves to the functionality of the device.

Battery Housing

Similarly to the temperature control PCB, the design and implementation of the battery housing is considered to be low priority in the development of the microbial pill sensor. No work has been done regarding battery component selection or housing design. We have established that we want the battery component to be easily accessible and replaceable, continuing the life of the fabrication microbial pill sensor beyond the length of the battery. Ease of battery replacement will provide increased ease of use for our users, but little work has been done to implement this in the design. The physical design of this component will be incorporated into the overall exterior housing design, but consideration of the battery component and component protection will not occur until a functional prototype integrating the MCU and optical detection modules is demonstrated per discussion with the client.

4.3.3 FUNCTIONALITY

The initial application for the microbial pill sensor is detecting levels of nitrate in agricultural field run-off to assess pesticide application and environmental impacts. The microbial pill sensor is intended to be submerged in agricultural run-off in which the desired test solution will flow into the microbial pill sensor. The concentration of nitrate in the run-off will be detected via measuring the GFP fluorescent response of bioengineered microbes. A voltage value will be transmitted BLE to an external platform where it will be converted to a concentration value and displayed via a GUI, allowing researchers, farmers and other users to assess nitrate concentrations in agricultural run-off to better inform environmental and farming practices. Additional testing of the final device will establish reasonable lifetimes and transmission ranges of the final device, ultimately impacting the final functional interaction with the user.

Figure 4-12 shown below depicts a storyboard depiction of the microbial pill sensor functionality with the user. Once placed, the microbial pill sensor functionality will be automated through the activation of the LED component at a specified timing interval and recording the produced voltage. The transmission and processing of the data will occur automatically and will populate the GUI with up-to-date, real-time concentration measurements. The GUI will also be designed to provide system-level feedback regarding device connectivity as well as temperature warnings if the built-in temperature control system fails.

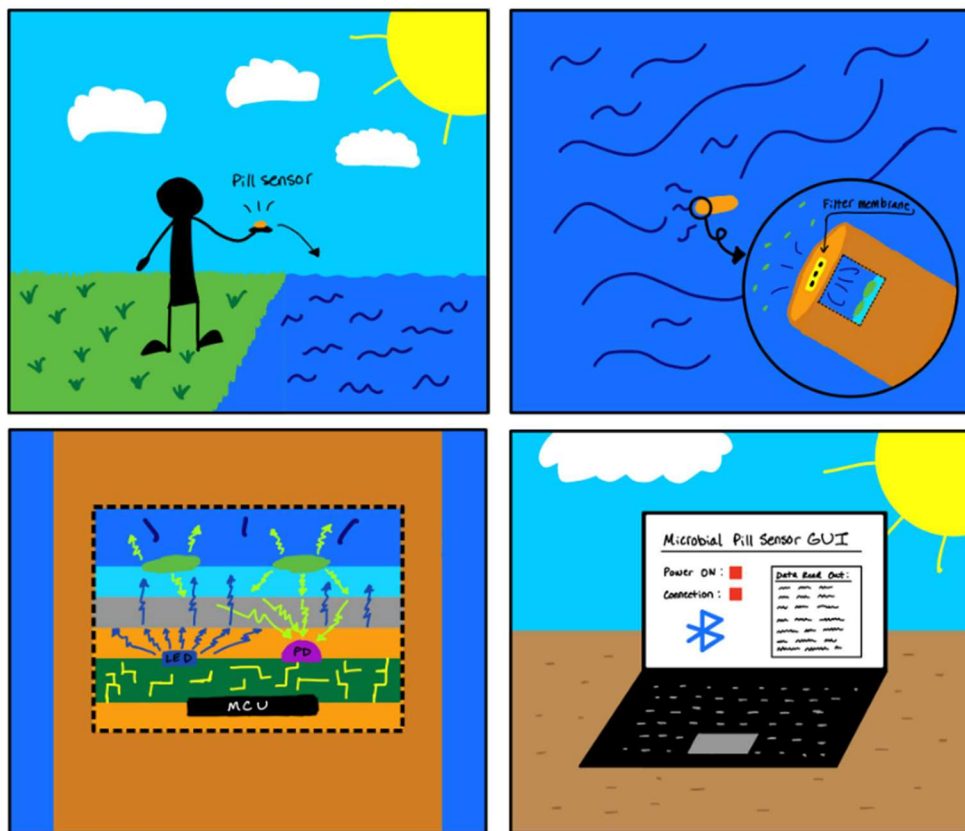


Figure 4-12: User Functionality Storyboard

4.3.4 Areas of Concern and Development

Verification of our design's ability to satisfy our functional requirements and meet user needs is currently not available in complete form. A functional breadboard prototype has been built and tested using an ESP 32C dev kit, LED and an alternative photodetector, providing proof of concept for our design. Components for the LED and photodetector have been selected as discussed in *Section 4.3: Detailed Design*. Initial PCB design has been finalized but will require fabrication by a professional service. Upon receiving fabricated PCBs, further testing will verify the functionality of the current module designs.

Users will interact with the microbial pill sensor through the off-platform GUI and exterior housing. Initial 3D design and prototyping of the housing is being performed and the GUI is actively being developed. Feedback throughout the prototyping process will be used to ensure these products meet user needs and provide easy to use platforms to achieve system functionality. Completion of the physical housing design will require PCB design and component selection to be completed to determine overall sizing of the housing.

The addition of optical components such as lenses and filters is an active area of development in overall microbial pill sensor design. Additional discussions with Dr. Lu will be necessary to develop an understanding of these components and their incorporation into the current design. The use of simulation tools is being considered to provide alternative testing environments for the optical detection mechanism, specifically in the verification of lensing and filtering functionality. However, simulation tools provide a layer of complexity at this point in the design that may be harmful to the continued development of the project, so further discussion regarding their use is required. Concerns with the current design consider functional range, operating lifetime and the ability to perform continuous automated testing with the device. Prototype and final testing will establish the functional transmission range and operating lifetime of the device. Concerns regarding the ability of the device to exchange the analyzed solution without a fluid pumping component exist, but will be tested later in the development of the device.

4.4 TECHNOLOGY CONSIDERATIONS

In designing the microbial pill sensor, several key technologies were selected to effectively meet the project's functional and design needs. Fusion 360 was chosen for its robust 3D modeling capabilities and cloud-based accessibility, allowing seamless collaboration across the team. This software enables easy import of PCB designs from KiCad, simplifying the integration of electronic and physical design stages. Additionally, Fusion 360 supports individual part design and assembly, making it ideal for creating and refining the modular components in the pill sensor. This cloud-based tool also facilitates the transition to 3D printing, supporting the development of the pill's physical structure. While AutoCAD is an alternative option, Fusion 360's integration with KiCad and user-friendly interface makes it a more practical choice for this project.

KiCad was selected for designing the PCB layouts and schematics, mainly due to the team's prior experience and the open-source nature of the software. KiCad offers simplified PCB design workflows, extensive online resources, and handy tutorials for prototyping with the ESP32 C3 microcontroller. Additionally, KiCad's compatibility with Fusion 360 supports an efficient transfer of designs between PCB and physical model development. While KiCad lacks some advanced features in commercial PCB software, its open-source accessibility and robust community support make it a strong choice for the project's current needs. Fusion 360 could be a backup option for basic circuit sketches, but it does not offer the specialized electronics design tools available in KiCad.

The team selected Arduino IDE to program the ESP32 C3 microcontroller and manage Bluetooth data transmission. This open-source platform is known for its ease of use and strong community support, making it ideal for handling the sensor's BLE transmission and real-time data monitoring. Although Arduino IDE requires hard-wiring of components, which can introduce connection issues that are sometimes challenging to debug, its simplicity aligns with the project's focus on creating a reliable yet straightforward device. PlatformIO was considered an alternative due to its sophisticated debugging capabilities, but its added complexity was deemed unnecessary for this project phase.

For the graphical user interface (GUI), PySimpleGUI was selected for its ease of use and quick learning curve. This library enables the rapid development of a functional interface for displaying real-time data from the sensor. With minimal coding requirements, PySimpleGUI provides an accessible solution for creating an intuitive and responsive GUI. However, it does have limitations in customization and scalability, which might affect its viability in a more complex or polished application. PyQt was considered as an alternative due to its extensive widget options and customization capabilities. However, its steeper learning curve and potential licensing requirements for commercial applications made PySimpleGUI the preferred choice for this project phase.

4.5 DESIGN ANALYSIS

A breadboard prototype to demonstrate proof of concept of the microbial pill sensor design has been implemented using an Espressif ESP32-C3-DevKitC-02 with the SK6812MINI LED integrated into the board, as well as a photodetector component obtained from Dr. Lu's lab. The breadboard prototype, programmed using Arduino IDE, was able to activate the LED every ten seconds, record a photovoltage value, and transmit that photovoltage to the external GUI application via BLE. Further details regarding the breadboard prototype can be found in *Section 6: Implementation*. This prototype serves as a demonstration of BLE transmission of a photodetector-recorded input voltage using the ESP32-C3 MCU, verifying the high-level system design of the microbial pill sensor. Incorporated with the breadboard prototype is the functional demonstration of a working GUI application, as discussed in *Section 4.3: Detailed Design*. Further work is required to ensure the GUI application provides an optimal user experience.

As discussed in *Section 4.3: Detailed Design*, a 3D CAD model of the microbial pill sensor capsule has been developed and 3D printed using resources on campus. Upon fabrication of the PCBs, evaluation of the 3D design will be possible regarding sizing, structural support, and fastening of PCB boards within the capsule. Through visual evaluation, on-campus 3D printing resources have been determined not to be capable of fabricating the external capsule at the necessary quality. This has been assessed as a fault of printer quality rather than the design of the capsule. Further research and evaluation is required to identify the material and coating for the external capsule.

Section 4.3: Detailed Design details the PCB designs developed for both the photodetector and MCU modules. Evaluation and testing of the physical PCBs is required to verify the design developed so far. Testing of the PCB modules will be used to determine the necessity of lensing components. An optical filtering component is still required to ensure the proper functionality of the device. The demonstration of the breadboard prototype provides a strong foundation for the validity and feasibility of the microbial pill sensor design. As discussed, future testing will provide the necessary further analysis of the module design.

5 Testing

5.1 UNIT TESTING

As previously noted in section 4: *Design*, the microbial pill sensor has been broken down into simplified modules. These modules stand as the simplest systems needed to operate functionally and within requirements before the full microbial pill sensor can be integrated and operated. Testing each individual module's ability to operate successfully based on the given requirements and metrics will ensure a successful integration.

A prototype of the final housing module design discussed in section 4.3: *Detailed Design* has been fabricated using on-campus 3D printing resources. The final housing module will be fabricated professionally to ensure the quality of the housing module. Upon fabrication of PCB modules, testing of dimensions and placement of the PCB modules within the housing will be manually conducted. Further evaluation of the physical housing strength and environmental safety verification plans will occur after material and coating selection. The housing exterior's permeability to water will be tested to ensure the safety of the electronic modules housed within the capsule. Verification of the permeability of the solution intake membrane will also need to be verified. The testing plan for the exterior housing will be updated upon its development.

Upon fabrication of the PCB modules, verification of their electronic functionality will be conducted. While considered separate modules, the photodetection PCB will be tested in conjunction with the MCU PCB. The functionality of the LED within the photodetection PCB requires programming from the MCU module, meaning the LED functionality must be tested with both modules. Physical verification of photodetector response will be conducted through exposure to defined light intensities. The MCU functionality will be verified through recorded outputs in Arduino IDE. As discussed in section 6: *Implementation*, the code to program the ESP32-C3 to transmit data through BLE to the external GUI application has been verified on the breadboard prototype. Verification of this code functionality will be tested on the MCU PCB with the currently functional GUI application.

As discussed in section 4.3: *Detailed Design*, little consideration of the design of the temperature control module has taken place due to established design and demonstration priorities. Upon completion of the design and demonstration of the functionality of the prioritized modules, this section will be updated with the necessary testing plan for the temperature control module.

5.2 INTERFACE TESTING

Since we are operating an ESP32-C3 MCU, the team will be utilizing a few different interfacing components. The MCU powers circuits and collects ADC values through GPIO Ports (General Purpose Input/Output). These GPIO parts are designated for specific functions such as Power Supply, UART Transfer and Receive, and ADC Channels. By cross-referencing between the ESP32-C3 and the Espressif ESP32-C3-DevKitC-02 datasheets, successful pin implementation can be created in the software programming for the MCU and can be tested on the breadboard system by collecting voltage values in a multimeter throughout the system and converting ADC values back to voltages by having a constant source voltage from a Lab Power Source. In addition to the GPIO Ports of the MCU, another interface which must operate successfully for the project to work is the MCU BLE connection to the Python Processing and GUI. This system relies on both the programming of the MCU through the Arduino IDE Programming Software to be correct in having the ESP32-C3 MCU advertise for a connection and having the Python Script run a detection for BLE devices through the bleak and asyncio libraries in Python. Testing for this interface relies heavily on reviewing errors within each program, ensuring the libraries needed are properly installed and called, and running test results sending different messages through the BLE connection, in our specific case the data sent were ADC values and verification of establishing a connection between devices.

5.3 INTEGRATION TESTING

For the physical hardware of the project, integrating the different custom PCBs inside of the compartmentalized housing unit is the largest integration. This involves combining multiple simplified PCBs with different functions together while maintaining physical size limitations. As the PCBs come in, the designated components for each board will be soldered onto the boards and will be tested to collect resistor and capacitance values, as well as verifying MCU functions. These PCBs, after passing the required functionality testing, will be inserted into the housing container and wired vertically along with batteries to complete the full system. Since the PCBs will be tightly secured inside, tests which collect any faults or errors that may occur after shaking the structure would help verify the stability of the PCBs. Similarly, when the final housing compartments have been created, ensuring that the sealing mechanisms for both the compartments and bacteria housing chambers are waterproof will be conducted by dunking the container into a water solution to ensure that it meets the requirements.

Another aspect of integration is integrating the hardware's data into the software. Once the physical system has been constructed, tested, and verified that it meets requirements, ensuring that the programming of the MCU is correctly created and the functionality of the processing and GUI are simplified and working. Although most of the testing of the software will be done digitally when debugging the code, it is important to verify results with constant values. This can be done by using lab equipment to simulate different situations such as having a constant higher light intensity and slowly raising the light intensity to verify the integration.

5.4 SYSTEM TESTING

Overall, each step in testing adds to the overall system testing. As results come back, the project can begin moving into the next phase of testing or can be improved upon before taking the next steps forward. By ensuring that each module works before being integrated together, and after this integration has happened, ensure that the system works as intended. Meeting physical size and sealing requirements would provide positive unit tests, proper MCU and GUI programming functionality passes the requirement of interface tests, and integrating these modules together to verify results meets the integration tests.

Once the full system has been made, system level testing can commence. These tests would look to analyze how the entire system operates. For example, understanding the power consumption of each subsystem which operates in this system would possibly give results as to where battery life can be saved. Through running demonstrations on the operation of the project, areas that need improvement that were not previously affected or thought of may become exposed and improved upon.

Testing of the finalized system will first be performed with fluorescent beads to model the fluorescent response of the microbes. Final functional testing with the bioengineered microbes is required to generate a characterizable response curve. With the final response curve of the microbial pill sensor, performance metrics such as sensitivity, specificity and limit of detection can be evaluated against competitive market alternatives.

5.5 REGRESSION TESTING

As the project has progressed, the team has opted to work in both an agile and waterfall management style. By breaking down the project into modules, each member has been tasked with creating a different component of the project. Throughout this process collaboration has allowed for mostly seamless integration. Luckily for this project, the different modules operate together in very simple manners, such as placing components inside of the housing or connecting the MCU to GUI via a BLE connection. Even though these connections are simple, ensuring the unit, interface, integration, and system tests result in positive results the project can continue to move towards a final design.

The main features which must remain functional in order to achieve a successful project with regards to the requirements are to create a small housing system which will host a chamber for the custom PCBs and a chamber for hosting the biosensor, an operating optical monitoring system on the PCB, and an operating electrical system to interface with the optical monitoring system.

The largest amount of regression occurs during software generation. As a user creates more complicated software, the likelihood of errors arising between methods and calling systems rises. During the creation of the Arduino IDE software, which operates the Espressif ESP32-C3-DevKitC-02, many issues arose. For example, understanding how to integrate the Espressif Bluetooth Library with this specific devkit and implementing that into MCU which needs to also power an amplifying circuit for the PD and program an RGB LED to light with a specific brightness and wavelength can be overwhelming at first. But through error notifications and additional libraries which simplify functions, a successful program could be made. Similarly, when programming the Python code which would create a functional GUI which would allow a user to select a BLE device to connect with and perform processing equations to convert ADC values to their corresponding voltages when they were received can be a large task. By breaking down this task into subsystems, like BLE connection, processing, and GUI creation, slowly improving each subsystem and attempting to integrate them together can be achieved. Overall, debugging inside Arduino IDE and Python was mandatory to improve the system and conducting other system tests to help verify the results.

5.6 ACCEPTANCE TESTING

Since our project's client and advisor and both Dr. Lu, acceptance testing is quite simple. During each step of the process, the group has been very vocal with Dr. Lu about our progress, concerns, and areas which members may need additional knowledge or assistance. This relationship with Dr. Lu has been incredibly helpful in understanding our requirements as well as the path that both parties feel should be taken to accomplish these goals.

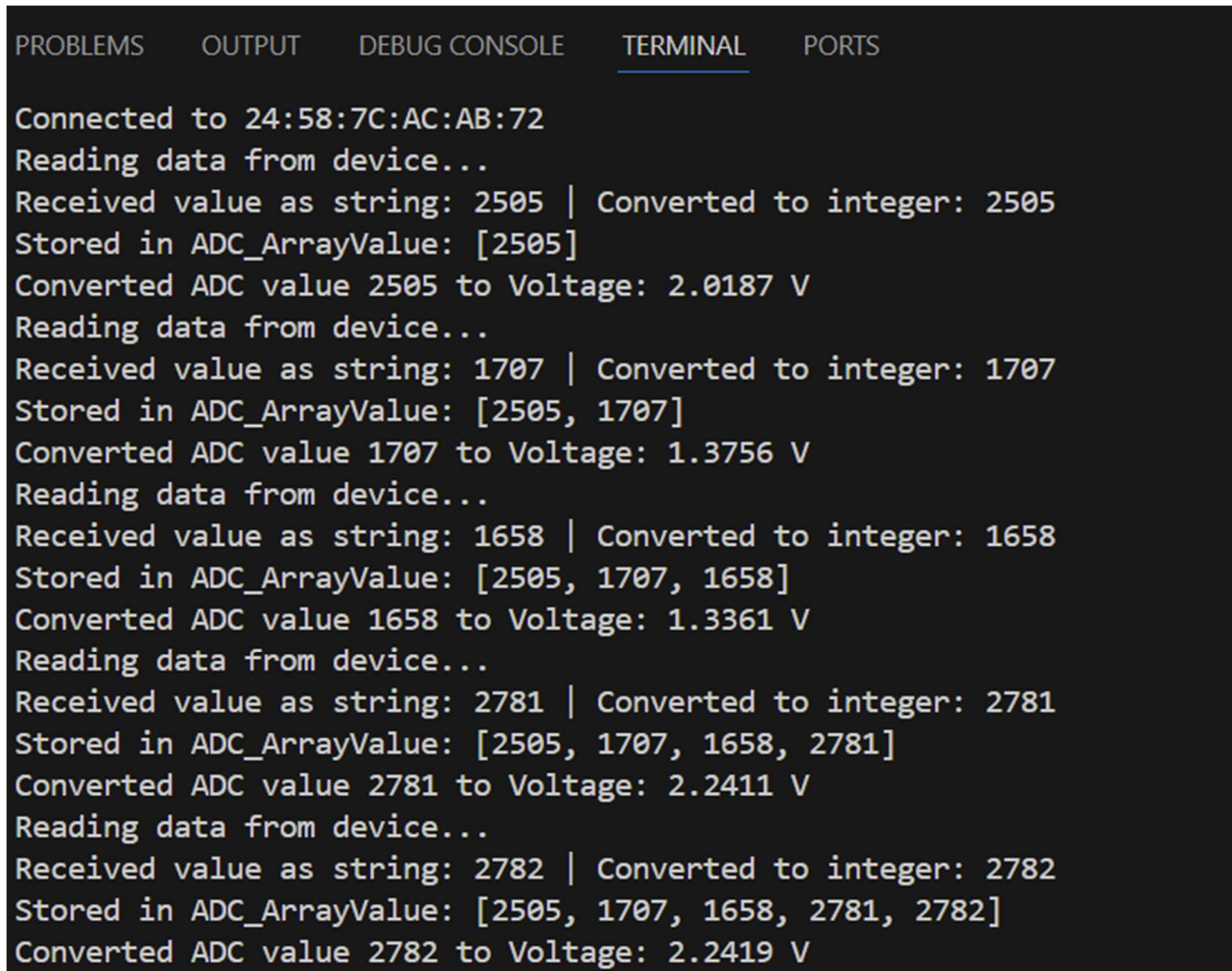
5.7 RESULTS

During the first iterations of the Lily-Go Dev Kit, testing the operational functions of the MCU and the TSL2591 photodetector circuit to get an understanding of limitations and areas of improvement for future designs was crucial. Once the MCU was properly programmed and the system was implemented onto a breadboard, testing included a collection of lux values sent via BLE to a connected device, which monitored UART commands and transferred messages. This initial design worked well to collect lux values which the TSL2591 generated but lacked the ability to transfer the photocurrent or photovoltage data that was generated from the photodetector. Programming the ESP32's to transfer data via BLE was successful, but in this current design, the power being drawn through the MCU to the TSL2591 was noticeably larger than anticipated. This power consumption led to the idea of doing off-chip processing which would operate as data was transferred to the interactive GUI.

Once the Espressif ESP32-C3-DevKitC-02 and a single photodetector arrived, the next iteration of this module could be created and tested. After the MCU had been properly programmed for the new system and the PD and amplifier circuit was implemented onto a breadboard, the team began collecting data. The team looked into the power consumption of the new model, this being both the circuit and RF power consumption, the ability to transfer data at different ranges according to the Espressif ESP32-C3-DevKitC-02 datasheet, and the ADC sensitivity of collecting amplified PD voltages. While the system was operational, meaning the PD and LED components were engaged and the BLE data transfer was advertising, the team collected an average power usage of 431.2mW. This can be broken down to using 130mA for Active BLE advertising, 0.183mA for the circuit components, and 0.182mA for the Op-Amp supply. Through these power tests, the team has been able to understand the need of implementing a Light-sleep state for the MCU to operate under until the BLE advertising is needed.

With the current Espressif ESP32-C3-DevKitC-02 system, the MCU has a data transferring range of ~220-meters once a connection has been established. In order to establish a successful connection, it is recommended to be within a ~100-meter range to give the highest probability of a successful connection on the first attempt. This data, although useful in the current design, will have to be recollected and will serve as a great goal to reach with the custom PCB system.

While researching through the ESP32-C3 datasheets, the team discovered that the ADC Calibration results can induce a $\pm 10\text{mV}$ error in a recorded value. This problem can be easily solved by introducing an amplifier into the PD circuitry, which was already implemented beforehand. This test confirmed our previous idea that improving the readability of the PD's output would be beneficial for both processing and operating the MCU's functionality. Figure 5-1 shows the terminal view of the Python program computing the voltages from the approximate ADC value. This view paired with a multimeter can confirm the correct conversions and approximate error percentages.



```
PROBLEMS  OUTPUT  DEBUG CONSOLE  TERMINAL  PORTS

Connected to 24:58:7C:AC:AB:72
Reading data from device...
Received value as string: 2505 | Converted to integer: 2505
Stored in ADC_ArrayValue: [2505]
Converted ADC value 2505 to Voltage: 2.0187 V
Reading data from device...
Received value as string: 1707 | Converted to integer: 1707
Stored in ADC_ArrayValue: [2505, 1707]
Converted ADC value 1707 to Voltage: 1.3756 V
Reading data from device...
Received value as string: 1658 | Converted to integer: 1658
Stored in ADC_ArrayValue: [2505, 1707, 1658]
Converted ADC value 1658 to Voltage: 1.3361 V
Reading data from device...
Received value as string: 2781 | Converted to integer: 2781
Stored in ADC_ArrayValue: [2505, 1707, 1658, 2781]
Converted ADC value 2781 to Voltage: 2.2411 V
Reading data from device...
Received value as string: 2782 | Converted to integer: 2782
Stored in ADC_ArrayValue: [2505, 1707, 1658, 2781, 2782]
Converted ADC value 2782 to Voltage: 2.2419 V
```

Figure 5-1: Terminal ADC Data Conversion

6 Implementation

As discussed in 4.5 *Design Analysis*, a prototype breadboard version of our final prototype has been fabricated. Further work is required to miniaturize the design through custom PCB fabrication. A larger-scale version of our exterior capsule has been 3D printed. A professional service will be required to fabricate the miniaturized version at the necessary quality. An implementation of our GUI has been completed, providing visualization of measurement data transmitted through low-power Bluetooth.

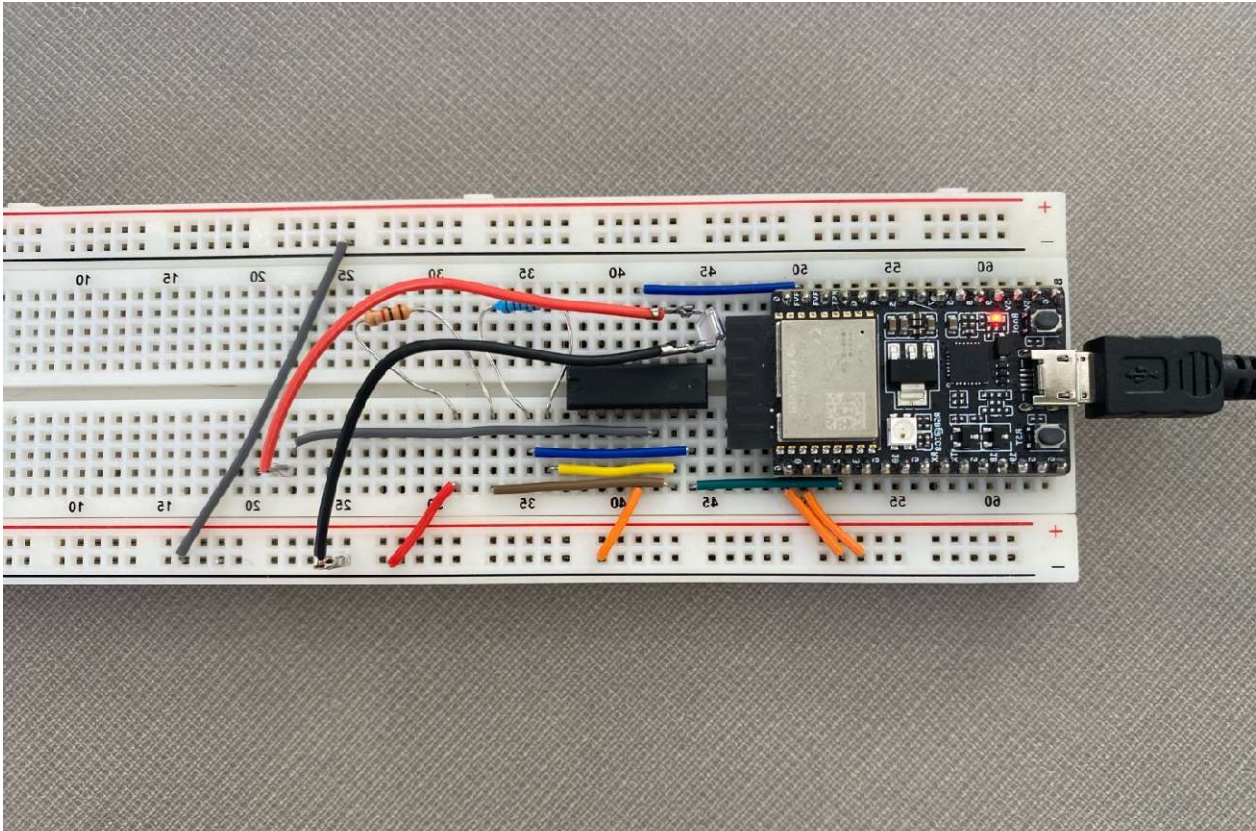


Figure 6.1: Current Prototype

Figure 6-1 shows the current breadboard prototype that the team has created. The PD which is on the end of the red and black wire prongs. This component discharges a voltage through the non-inverting amplifier circuit which is just left of the RF antenna. This amplified voltage travels through the green wire just below the devkit to the ADC pin which generates the ADC which is sent via BLE to the Python program. The excitation LED is the white square found in the bottom center of the devkit. This RGB LED is programmed to produce a cyan Lightwave of 488nm.

Figure 6-2 shows the current housing compartments. The leftmost black compartment is the battery housing compartment. This is where the batteries will sit and this section can be detached to replace or recharge whatever batteries are used to power the system. The central black module is the housing for the PCBs. The two white discs represent the custom made MCU and optical detection PCBs. These will be placed into the central module and secured. The right black module will be the bacteria housing chamber. This compartment will hold a clear disc which the bacteria will sit on top of in an agar gel. The optical detection system will monitor activity from directly below the clear dish.



Figure 6-2: Current Housing

All of the subsystem modules have been partially created, tested, and implemented into the current prototype, with improvements being made as manufactured parts arrive and research data has been collected. A future improvement of the design will involve collecting concentrations of nitrate through Voltage conversions. These will be done in the Python program and will connect the final objective of verifying nitrate concentrations with the use of optical sensors, through converting a voltage to a light intensity to a concentration. This will not require any extra hardware but will require test data and software development to be collected and implemented.

7 Ethics and Professional Responsibility

7.1 AREAS OF PROFESSIONAL RESPONSIBILITY/CODES OF ETHICS

Area of Responsibility	Definition	Relevant IEEE Code Clause	Team Interaction
Work Competence	Performing tasks with expertise and diligence to ensure high-quality results.	To undertake only those tasks for which they are qualified	Team members focus on their areas of expertise, such as PCB design or GUI development, while learning new tools (e.g., Fusion 360, Arduino IDE) to maintain technical proficiency and high project standards.
Health, Safety, and Welfare	Safeguarding the well-being of all stakeholders and the environment.	To hold paramount the safety, health, and welfare of the public.	The team has prioritized rigorous testing in controlled environments to ensure the microbial containment chamber functions effectively reducing ecological or health risks during use or testing.
Communication Honesty	Transparent and truthful reporting of progress and findings.	To be honest and realistic in stating claims or estimates.	Challenges in size constraints and material fabrication have been openly communicated with the advisor, enabling transparent progress tracking and iterative design improvements.
Sustainability	Ensuring the environmental friendliness and longevity of designs	To hold paramount the safety, health, and welfare of the public	Environmentally safe materials have been selected for housing, with continuous efforts to minimize ecological impact through design refinements, such as reusable components and sustainable packaging.
Social Responsibility	Contributing to societal and environmental improvements.	To improve the understanding of technology and its applications.	By addressing nitrate pollution in water, the project supports sustainable agriculture and ecosystem health, benefitting farmers, environmentalists, and broader communities reliant on clean water systems.

Table 7-1: Areas of Professional Responsibility

The team has demonstrated strengths and areas for improvement in adhering to various professional responsibility dimensions throughout the microbial pill sensor project. One notable strength is the team's Communication Honesty, exemplified by its commitment to transparency in addressing challenges encountered during the project. The team faced difficulties establishing a Bluetooth connection and transferring data from the TTGO LillyGO T-Display module early in the development process. Through open communication with the advisor, additional resources were identified, ultimately resolving the issue. Similarly, the team encountered challenges designing the cell housing to meet size requirements. Initial attempts at 3D printing the housing failed due to the small dimensions, resulting in incomplete and unusable prototypes. By discussing these issues with the advisor and reevaluating the design requirements based on the PCB and microcontroller dimensions, the team collaboratively redesigned the housing to ensure it met all functional and manufacturability requirements. This open and iterative approach highlights the team's strong performance in maintaining clear and effective communication throughout the project.

While the team has succeeded in communication honesty, the team has not performed well in the area of sustainability. The microbial pill sensor has a direct impact in environmental health, and while the teams aim to make a sustainable and environmentally friendly device, little work has actively been done to ensure the sustainability of the device. While the team has not actively made design or testing decisions to harm our responsibilities in sustainability, no work has been actively conducted to promote the sustainability of the microbial pill sensor. The team plans to address this deficiency following the completion of the modules at the beginning of the second semester of the senior design course.

7.2 FOUR PRINCIPLES

Table 7-2 assesses the four principles in regard to the microbial pill sensor and its public health, global, environmental and economic impacts. The four principles assessed are beneficence, nonmaleficence, respect for autonomy, and justice. The broader context-principal pair of Environmental and Beneficence is incredibly important to our project, as it provides the entire motivation for the microbial pill sensor. Our biosensor is intended to improve environmental outcomes related to excessive nitrate runoff in waterways, providing a continuous monitoring system for early detection of hazardous conditions or inefficient fertilization processes. If our final product does not provide beneficence in environmental outcomes, our product has failed in its intended functionality.

The microbial pill sensor does lack consideration of particular factors in application within the broader context of Environmental and Nonmaleficence. While the microbial pill biosensor is not intended to obstruct natural environmental processes, interfere with natural ecosystems, or harm the environment or its inhabitants, little thought has been dedicated to the application of the biosensor in the environment. A potential issue facing the microbial pill sensor is animal consumption of the biosensor, as little thought has been placed into how the biosensor will be fixed in a dedicated position. Displacement of the microbial pill sensor could generate misleading readings, which could prompt environmentally disrupting action. As the project enters a more functional and final phase, engineering consideration will be applied to the application of the biosensor in the intended environment, alleviating any potential unintended environmental maleficence from the displacement of the biosensor.

	Beneficence	Nonmaleficence	Respect for Autonomy	Justice
Public health, safety, and welfare	Enhances public safety by creating a biosensing system that monitors health indicators and contaminants effectively.	Avoids harm by ensuring safe application of bioengineered bacteria and preventing misuse.	Provides accurate, accessible health data for informed decisions by users.	Ensures equal access to the technology for all communities regardless of socio-economic status.
Global, cultural, and social	Promotes better global health outcomes by detecting pollutants and diseases in diverse contexts.	Avoids harm by considering cultural sensitivities in system design and deployment.	Respects cultural autonomy by tailoring features to community-specific needs.	Distributes benefits equitably, ensuring marginalized groups also gain from the innovation.
Environmental	Reduces environmental risks by detecting harmful pollutants quickly and efficiently.	Prevents ecological harm through sustainable material sourcing and manufacturing.	Respects environmental autonomy by avoiding interference with natural ecosystems.	Balances environmental benefits across regions and protects resources for future generations.
Economic	Reduces health monitoring costs through efficient and affordable technology.	Avoids financial harm by ensuring affordability and transparency in costs for users.	Empowers users to make cost-effective decisions with reliable, accessible information.	Balances economic benefits for all stakeholders, including underprivileged communities.

Table 7-2: Four Principles

7.3 VIRTUES

With the application of the microbial pill sensor in improving environmental outcomes, our team has prioritized the virtue of commitment to the public good. Our device is intended to provide paths to improving the quality of public waterways, so design decisions or practices that result in damage to the public good fundamentally contradict the motivation for the project. The team has prioritized our commitment to the public good by ensuring the product is environmentally friendly. Concerns regarding contamination of the environment through poor membrane and material selection will be prioritized by the team as the design of the individual modules is finalized. In tandem with a prioritization of commitment to the public good is a commitment to quality. The team strongly believes that our microbial pill sensor has a strong application in improving continuous monitoring and biosensing technologies. Producing a low product quality damages the applicability of our device. Through our product research, we have established performance metrics that the final microbial pill sensor should reach to serve as a competitive biosensing solution in the market. Ensuring our product exceeds these benchmarks through extensive testing and strategic design decisions ensures the quality of the microbial pill sensor. Our team also prioritizes clear and thorough documentation through the weekly reports, project scheduling and tracking of accomplishments through the team channel. Producing a high-quality version of this design document provides thorough documentation of the design, ensuring our team is reaching our dedication to documentation through the development of the microbial pill sensor.

Cade Kuennen:

Throughout my senior design work, I have consistently demonstrated a strong commitment to quality. This virtue is important to me because I believe that quality reflects the integrity of both the individual and the work itself. Delivering high-quality results ensures the solution we provide is reliable, effective, and impactful for stakeholders. I have exemplified this commitment by carefully reviewing project deliverables, ensuring designs meet both functional and aesthetic standards, and incorporating feedback from team members and mentors. For example, when completing the parts decomposition of the MCU dev kit, one of the potential dev kits had poor documentation. Instead of guessing on what components were missing, I talked with my team and advisor and was able to find a dev kit with better documentation that could also work for the project. By prioritizing quality, I aim to not only meet, but exceed project expectations. This dedication strengthens our team's outcomes and prepares us for the professional challenges that lie ahead.

Cooperation is a virtue I deeply value, yet I recognize that I haven't fully demonstrated it during my senior design work thus far. Effective collaboration is essential in any team-based effort because it fosters open communication, ensures diverse perspectives are considered, and strengthens the overall quality of the final product. While I have contributed individually and engaged in discussions, I see room for improvement in proactively seeking input from teammates and understanding the inner workings of their individual sprint portions of the project. To demonstrate greater cooperation, I plan to become more familiar with the development process of my team members' portions of the project and ask for team member feedback more often than during our weekly meeting times. By enhancing my cooperative approach, I can help create a more unified team dynamic and contribute to a stronger, more cohesive project.

Rakesh Varma Penmetsa:

From the beginning of my senior design class project, the development of the microbial pill sensor, I have consistently demonstrated a dedication to detail and adaptability. These virtues are essential to me because they ensure the creation of high-quality, reliable designs that meet the project requirements. In my work on the 3D design of the cell housing, these traits were crucial for securely housing the bioengineered microbes while ensuring proper integration with other system modules. Early in the process, I faced significant challenges with the small size of the housing, which made accurate 3D printing difficult. Prototypes produced with filament-based printers failed to meet precision requirements, resulting in unusable designs. Recognizing the need for improvement, I collaborated with the advisor and my teammates to refine the design. By increasing the housing dimensions to account for PCB and microcontroller sizing, I developed a more functional model. I also incorporated a detachable cap feature to allow easy maintenance and replacement of the nutrient gel, improving the usability and longevity of the housing.

While I have demonstrated dedication to detail and adaptability, I have yet to exhibit the virtue of cooperation in my work thoroughly. Cooperation is essential to me because it fosters a more potent team dynamic, ensures diverse perspectives are considered, and leads to better overall project outcomes. Although I collaborated during design challenges, I have yet to actively seek feedback or engage with teammates' work on their modules, such as PCB design or microcontroller programming. To demonstrate greater cooperation, I plan to spend more time understanding the other project modules during the assembly process and ask for feedback more frequently outside our regular meetings. By strengthening my cooperative approach, I aim to create a more unified team dynamic and contribute to a more cohesive final product.

Wes Ryley:

A virtue that I believe to be crucial to a successful group project is the need for cooperation and clear documentation. Since our project has been broken down into separate modules, with each team member taking a specific area of focus, being able to communicate effectively and allow collaboration to help design the functions of the project has helped simplify the process and improve our team's efficiency. As the Data Transmission Design Lead, I've been tasked with programming both the MCU and creating the Python code which does the system's processing and GUI. Although my work doesn't interact with the PCB design or housing modules, through collaboration our team has been able to verify that the software that has been developed will work with the new PCB boards and that the housing modules will be able to have an antenna for BLE connectivity.

One virtue that I feel I haven't been as accepting of during this process is the virtue of liberality. Since the project is linear in nature, having the PCB's collect data, have that data be sent via BLE, and having a program process and display the data, the need to be open to interesting and new ideas has been limited. Although I don't directly think this is a negative virtue, I believe that being focused on specific aspects of the project rather than trying to constantly innovate sporadically has been beneficial for myself and the rest of the team. If I were to include this virtue into my design, I would actively search for innovations, no matter how distant they might be from the current design, if they are useful and improving the project.

Alex Upah:

Honesty is an incredibly important virtue in ensuring the quality completion of a major project. Failing to be honest with teammates and myself regarding accomplishments, setbacks or the quality of the work and documentation provided is damaging to the successful completion of the project. I have demonstrated honesty through the revision of my own work in the system's design, the design document and my documentation procedure. I also believe I have provided honest and fair criticism, when necessary, regarding the work of my teammates.

Attentiveness to both your own work and the work of your teammates is a valuable virtue in the completion of a major project. The agile workflow implemented by the team this semester has resulted in individuals spending most of their time working on their own individual components. With separation from the design of the other modules, I have not focused on understanding the inner workings of the GUI or MCU code. I plan to take the time to develop a better understanding of the entire system, specifically the code behind the GUI and MCU operation, as the team enters the next stage of development.

8 Closing Material

8.1 CONCLUSION

Our microbial pill sensor aims to develop a novel biosensing technology for the detection of nitrate in agricultural field runoff using bioengineered microbes. The microbial pill sensor uses the fluorescent response of GFP expressed by the bioengineered microbes in the presence of nitrate to produce a photocurrent proportional to the concentration of nitrate. The photocurrent value is transmitted to an external device via Bluetooth low-energy and processed and displayed by our external GUI application. Our design is modular, broken into an external housing module, an optical detection PCB module, a microcontroller PCB module, a temperature controller module, and an external GUI application.

For the choice of microcontroller, the ESP32-C3 has been selected for its reduced size and capability to transmit data via Bluetooth low-energy. Photodetector and LED components for the optical detection module have been identified and purchased. A breadboard prototype of the system has been developed, integrating an ESP32-C3 dev kit, an RGB LED, and a photodiode to replicate the microbial pill sensor functionality. An initial version of our GUI application has been developed, and data transmission via BLE to our external GUI application has been achieved. Future improvement of the GUI application is required to fulfill user needs.

PCB design of the optical detection and microcontroller modules is currently in progress. Upon fabrication and testing of the necessary PCBs, the optical detection and microcontroller modules will be integrated into the external capsule for functional testing of the device. Development of the temperature control module has been delayed until the demonstration of device functionality following a discussion of design priority with our faculty advisor. Much additional work and development is required to complete the functional microbial pill sensor, but the achievements and functionality of the breadboard prototype, along with the identification of the necessary components, provide a foundation from which to complete the goals of the project in the remaining time.

8.2 REFERENCES

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- [4] Espressif Systems, “ESP32-C3 Series Datasheet v2.0,” ESP32-C3 datasheet, 2024.
- [5] EPIGAP OSA, “EOPD-525-1-0.9 Specifications”, EOPD-525-1-0.9 datasheet, Aug. 2023.
- [6] Dongguang OPSCO Optoelectronics CO., LTD, “SK6812MINI: Integrated Light Source Intelligent Control of Chip-On-Top SMD Type LED,” SK6812MINI datasheet, Sept. 2015.
- [7] Espressif Systems, “ESP Hardware Design Guidelines: ESP32-C3 Schematic Checklist” *Espressif Systems*, 2024. [Accessed Dec. 2, 2024]

8.3 APPENDICES

Appendix A: SK6812MINI

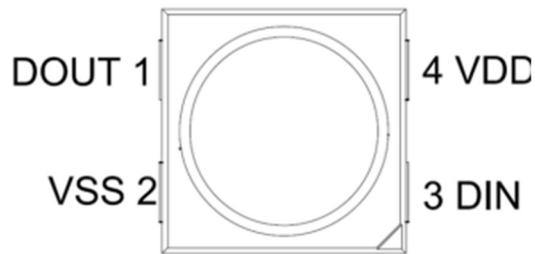


Figure A-1: SK6812MINI Pin Outs

Parameter	Symbol	Range	Unit
Power supply voltage	VDD	+3.5~+5.5	V
Logic input voltage	V_{IN}	-0.5~VDD+0.5	V
Working temperature	T _{opt}	-40~+85	°C
Storage temperature	T _{stg}	-50~+150	°C
ESD pressure	V _{ESD}	4K	V

Figure A-2: SK812MINI Electrical Characteristics

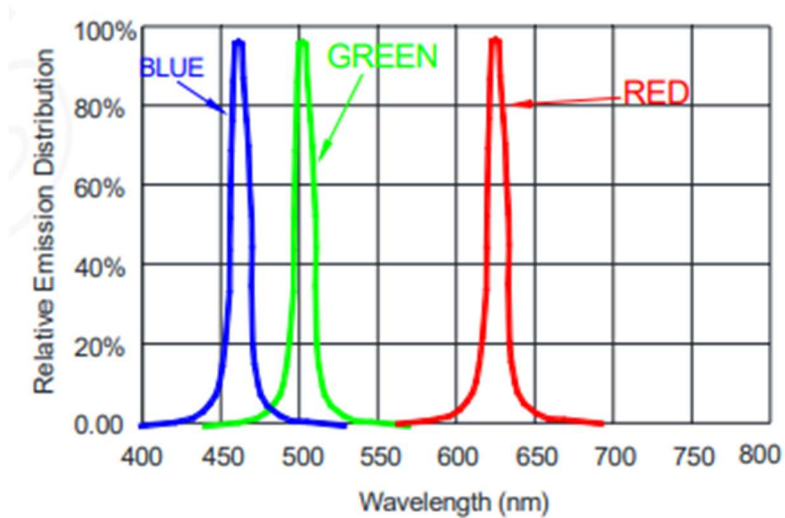


Figure A-3: SK812MINI Wavelength Emission Characteristics

The complete specifications can be found in the product datasheet: https://cdn-shop.adafruit.com/product-files/2686/SK6812MINI_REV.01-1-2.pdf

Appendix B: EOPD-525-1-0.9

Table B.1 depicts the optoelectronic characteristics of the device. A peak sensitivity wavelength of 525 nm provides excellent performance with our fluorescent excitation wavelength at 523 nm. A responsivity of 0.3 A/W is acceptable for this project, with the maximum demonstrated responsibility demonstrated in any photodiode being 0.5 A/W.

Measurement conditions Messbedingungen		T _{ambient} = 23 °C; t _{test} ≤ 60 ms				
Parameter	Symbol	Condition	Min	Typ	Max	Unit
Emitting Color Farbe				Photo Diode Photodiode		
Peak Sensitivity Wavelength Peak Empfindlichkeitswellenlänge	λ_p	$U_R = 0\text{ V}$		525		nm
Sensitivity Range at 1% Empfindlichkeitsbereich bei 1%	λ	$U_R = 0\text{ V}$	410		580	nm
Spektral Bandwidth at 50% Spektrale Bandbreite bei 50%	$\Delta\lambda$	$U_R = 0\text{ V}$		70		nm
Responsivity at λ_p Empfindlichkeit bei λ_p	S_λ	$U_R = 0\text{ V}$		0.3		A/W
Dark Current Dunkelstrom	I_D	$U_R = 5\text{ V}$		5	30	pA

Table B-1: EOPD-525-1-0.9 Optoelectronic Characteristics

Figure B.1 depicts the graph of sensitivity as a function of wavelength. A moderately high sensitivity at 488 nm motivates our inclusion of a filtering component in the design to remove unwanted response to our LED.

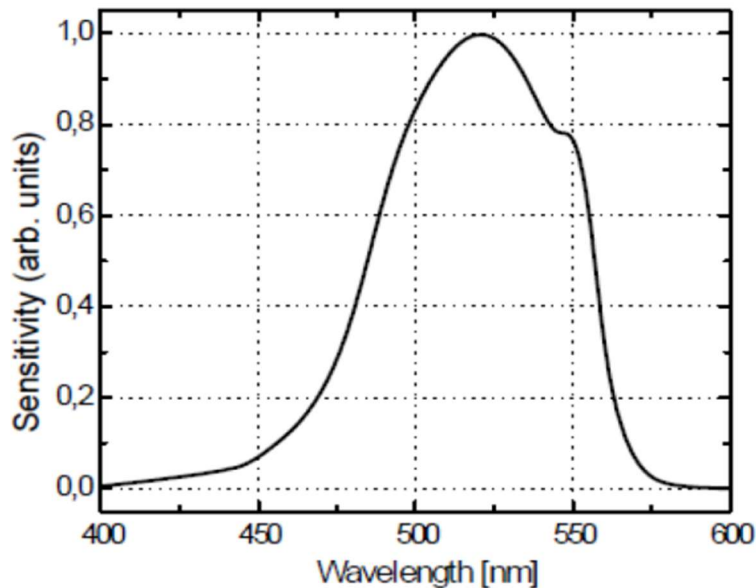


Figure B-1: EOPD-525-1-0.9 Wavelength Sensitivity

The complete specifications regarding sizing, soldering, and temperature dependence can be found in the product datasheet: <https://www.epigap-osa.de/wp-content/uploads/2023/09/EOPD-525-1-0.9.pdf>

Appendix C: ESP32-C3

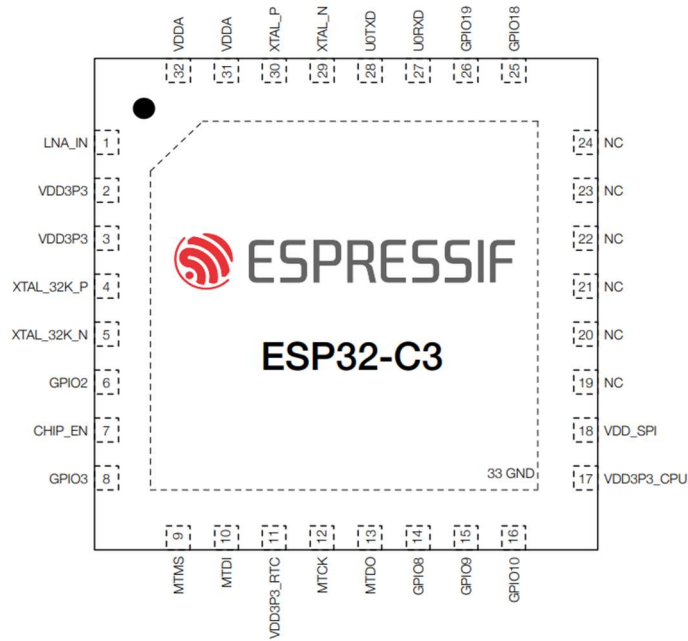


Figure C-1: ESP32-C3 Pin Layout

Pin No.	Pin Name	USB Serial/JTAG	JTAG	ADC1	ADC2	UART0 ¹	SPIO/1 ¹	SPI2 ¹	UART1	I2C	I2S	TWAI	LED PWM	RMT
1	LNA_IN													
2	VDD3P3													
3	VDD3P3													
4	XTAL_32K_P			ADC1_CH0		GPIO0	GPIO0	GPIO0	GPIO0	GPIO0	GPIO0	GPIO0	GPIO0	GPIO0
5	XTAL_32K_N			ADC1_CH1		GPIO1	GPIO1	GPIO1	GPIO1	GPIO1	GPIO1	GPIO1	GPIO1	GPIO1
6	GPIO2			ADC1_CH2		GPIO2	GPIO2	FSPIQ	GPIO2	GPIO2	GPIO2	GPIO2	GPIO2	GPIO2
7	CHIP_EN													
8	GPIO3			ADC1_CH3		GPIO3	GPIO3	GPIO3	GPIO3	GPIO3	GPIO3	GPIO3	GPIO3	GPIO3
9	MTMS		MTMS	ADC1_CH4		GPIO4	GPIO4	FSPiHD	GPIO4	GPIO4	GPIO4	GPIO4	GPIO4	GPIO4
10	MTDI		MTDI		ADC2_CH0	GPIO5	GPIO5	FSPiWP	GPIO5	GPIO5	GPIO5	GPIO5	GPIO5	GPIO5
11	VDD3P3_RTC													
12	MTCK		MTCK			GPIO6	GPIO6	FSPiCLK	GPIO6	GPIO6	GPIO6	GPIO6	GPIO6	GPIO6
13	MTDO		MTDO			GPIO7	GPIO7	FSPiD	GPIO7	GPIO7	GPIO7	GPIO7	GPIO7	GPIO7
14	GPIO8					GPIO8	GPIO8	GPIO8	GPIO8	GPIO8	GPIO8	GPIO8	GPIO8	GPIO8
15	GPIO9					GPIO9	GPIO9	GPIO9	GPIO9	GPIO9	GPIO9	GPIO9	GPIO9	GPIO9
16	GPIO10					GPIO10	GPIO10	FSPiCS0	GPIO10	GPIO10	GPIO10	GPIO10	GPIO10	GPIO10
17	VDD3P3_CPU													
18	VDD_SPI					GPIO11	GPIO11	GPIO11	GPIO11	GPIO11	GPIO11	GPIO11	GPIO11	GPIO11
19	SPIHD					GPIO12	SPIHD	GPIO12	GPIO12	GPIO12	GPIO12	GPIO12	GPIO12	GPIO12
20	SPIWP					GPIO13	SPIWP	GPIO13	GPIO13	GPIO13	GPIO13	GPIO13	GPIO13	GPIO13
21	SPiCS0					GPIO14	SPiCS0	GPIO14	GPIO14	GPIO14	GPIO14	GPIO14	GPIO14	GPIO14
22	SPiCLK					GPIO15	SPiCLK	GPIO15	GPIO15	GPIO15	GPIO15	GPIO15	GPIO15	GPIO15
23	SPiD					GPIO16	SPiD	GPIO16	GPIO16	GPIO16	GPIO16	GPIO16	GPIO16	GPIO16
24	SPIQ					GPIO17	SPIQ	GPIO17	GPIO17	GPIO17	GPIO17	GPIO17	GPIO17	GPIO17
25	GPIO18	USB_D-				GPIO18	GPIO18	GPIO18	GPIO18	GPIO18	GPIO18	GPIO18	GPIO18	GPIO18
26	GPIO19	USB_D+				GPIO19	GPIO19	GPIO19	GPIO19	GPIO19	GPIO19	GPIO19	GPIO19	GPIO19
27	U0RXD					U0RXD	GPIO20	GPIO20	GPIO20	GPIO20	GPIO20	GPIO20	GPIO20	GPIO20
28	U0TXD					U0TXD	GPIO21	GPIO21	GPIO21	GPIO21	GPIO21	GPIO21	GPIO21	GPIO21
29	XTAL_N													
30	XTAL_P													
31	VDDA													
32	VDDA													
33	GND													

¹ For UART0, SPIO/1, and SPI2 interface, the signals routed to fixed pins via IO MUX can also be routed to any GPIO pins via GPIO Matrix.

Figure C-2: ESP32-C3 Peripheral Pin Assignments

The complete specifications: https://www.espressif.com/sites/default/files/documentation/esp32-c3_datasheet_en.pdf

9 Team

9.1 TEAM MEMBERS

Cade Kuennen (Electrical Engineering)

Rakesh Penmetsa (Electrical Engineering)

Wes Ryley (Electrical Engineering)

Alex Upah (Electrical Engineering)

9.2 REQUIRED SKILL SETS FOR YOUR PROJECT

- Identification of necessary components for the system
- 3D CAD Design
- 3D Printing
- PCB Design
- Programming of MCU
- Programming functional GUI
- BLE transmission
- Biosensing data analysis
- Familiarity with photodetection

9.3 SKILL SETS COVERED BY THE TEAM

- Identification of necessary components for the system: **Team**
- 3D CAD Design: **Rakesh Penmetsa**
- 3D Printing: **Rakesh Penmetsa**
- PCB Design: **Cade Kuennen**
- Programming of MCU: **Wes Ryley**
- Programming functional GUI: **Wes Ryley**
- BLE Transmission: **Wes Ryley**
- Biosensing data analysis: **Alexander Upah**
- Familiarity with photodetection: **Alexander Upah**

9.4 PROJECT MANAGEMENT STYLE ADOPTED BY THE TEAM

As discussed in section 3.1: *Project Management*, our team is employing a mixture of waterfall and agile project management. Through the development and design of the individual modules, the team is employing agile project management. In the integration of the complete modules into the functional microbial pill sensor, the team is employing a waterfall management style. For further details, reference section 3.1: *Project Management*.

9.5 INITIAL PROJECT MANAGEMENT ROLES

Cade Kuennen: PCB Design Lead

Rakesh Penmetsa: External Housing Design Lead

Wes Ryley: Microcontroller and GUI Design Lead

Alex Upah: Biosensing System Lead, Documentation Lead

9.6 Team Contract

Team Members:

- 1) Cade Kuennen 2) Rakesh Penmetsa
- 3) Wes Ryley 4) Alexander Upah

Team Procedures

1. Day, time, and location

- The team will meet weekly on Wednesdays from 12 – 1 pm in TLA or Lab 1125.
- The team will meet weekly with Dr. Lu on Mondays from 12 – 1 pm in a reserved conference room.
- Additional meetings may be scheduled through additional weekly communication.

2. Preferred method of communication updates, reminders, issues, and scheduling

- The team will use Microsoft Teams and Discord to communicate.
- Meetings will take place face-to-face, but virtual meetings are acceptable provided a valid reason for failure to make meeting in person.
- A team schedule has been created containing each individual's schedule, which serves as a tool for scheduling additional meetings outside of arranged weekly meeting times.

3. Decision-making policy (e.g., consensus, majority vote):

- The team will use a majority rules policy when making decisions.
- Split decisions will be decided by the individual responsible for the module/component.

4. Procedures for record-keeping

- The team will use shared documents saved to a team One Drive to record and archive meeting minutes.
- Each individual has edit and read privileges for every document within the One Drive space.

Participation Expectations

1. Expected individual attendance, punctuality, and participation at all team meetings:

- Team members must attend all agreed-upon meeting times on time and actively participate in them unless a valid reason for absence is presented.
- Should a team member not be able to attend a team meeting, the team member should notify the team of their absence as soon as possible.

2. Expected level of responsibility for fulfilling team assignments, timelines, and deadlines:

- All team members are expected to fulfill communicated and agreed-upon commitments and responsibilities on various assignments and deadlines.
- If external events present reasonable challenges to fulfilling assignments, timelines, or deadlines, the team member is expected to notify the team as soon as possible or at least three days before the deadline.

3. Expected level of communication with other team members:

- Team members are expected to check Teams or Discord once a day for updates.
- Team members will send updates on project work if any major milestones are met or if completed work assists another team member in the completion of their responsibilities.
- Team members will send updates on project work if any major setbacks occur.

4. Expected level of commitment to team decisions and tasks:

- All decisions agreed upon by the team are final unless a change in the course of action is decided by the team.
- All team members are expected to fulfill assigned responsibilities to the best of their abilities.

Leadership

1. Leadership roles for each team member

- Cade Kuennen: PCB Design Lead
- Rakesh Penmetsa: Capsule Housing Lead
- Wes Ryley: MCU and Data Transmission Lead
- Alex Upah: Optical Detection Lead

2. Strategies for supporting and guiding the work of all team members:

- If a team member needs assistance or guidance, additional time outside of set meetings may be scheduled to reach set milestones.
- Team members will provide collaborative support when necessary.
- Dr. Lu will provide available assistance and support when the team faces challenges.

3. Strategies for recognizing the contributions of all team members:

- Team members will show approval and acknowledgment and ask questions regarding the independent work of individuals.
- Communication of completion of major milestones provides a route to recognition of contributions of members.

Collaboration and Inclusion

1. Describe the skills, expertise, and unique perspectives each team member brings to the team.

- Cade Kuennen:
 - Experience in PCB design for low and high-frequency systems
 - Experience thinking about design from an end-user-oriented perspective
- Rakesh Penmetsa:
 - Experience in circuit design
 - Experience using cadence software
- Wes Ryley
 - Understanding of power usage to help preserve battery life and potential heating issues
 - Understanding of data transfer methods, specifically through Wi-Fi
- Alex Upah
 - Academic coursework in biosensing, bio-detection mechanisms, and related biosensing data analysis
 - Academic coursework in optical components such as photodiodes
 - Knowledge of signals and communication of modulated signals
 - Extensive experience in a research environment working directly with faculty members.

2. Strategies for encouraging and supporting contributions and ideas from all team members:

- Each team meeting will involve a discussion of current progress and a discussion of future tasks and steps forward.
- Discussion of future steps forward provides opportunities for contributions and ideas of all team members to be heard and supported.

3. Procedures for identifying and resolving collaboration or inclusion issues

- The team has agreed upon an open communication policy. If a team member sees an issue with how the team is operating, whether it is obstructing or limiting their ability, team members are encouraged to openly share constructive feedback.
- The team open communication policy expects that each team member is responsive to reasonable and constructive criticism.
- If an issue has been raised and a resolution has been discussed, yet no progress for improvement has been made, the team will contact the course instructors for assistance.

Goal-Setting, Planning, and Execution

1. Team goals for this semester:

- Create an initial design of the system by November
- Order and receive the necessary components by the end of Thanksgiving break.
- Large-scale breadboard prototype of the system and initial version of functional GUI by the end of the first semester.
- Initial PCB design completed by the end of the first semester

2. Strategies for planning and assigning individual and teamwork:

- The team will assign individual and team responsibilities during weekly meetings

3. Strategies for keeping on task:

- During the weekly meeting, the accomplishments of the previous week will be discussed.
- If team members decide work appears to be off task, discussion will take place in adherence to the team's open communication policy.

Consequences for Not Adhering to Team Contract

1. How will you handle infractions of any of the obligations of this team contract?

- Minor infractions not deemed detrimental to the team's ability to complete the project will be discussed within the group and with Dr. Lu.
- If infractions of the team contract are deemed detrimental to the ability of the team to complete project assignments within the expected quality and deadline, the team will notify Dr. Lu and course instructors of the infractions and their impact.

2. What will your team do if the infractions continue?

- The team will approach course instructors to discuss potential next steps regarding future action, specifically regarding course penalties and potential separation from the team.

a) I participated in formulating the standards, roles, and procedures as stated in this contract.

b) I understand that I am obligated to abide by these terms and conditions.

c) I understand that if I do not abide by these terms and conditions, I will suffer the consequences as stated in this contract.

1) Cade Kuennen

DATE: 9/11/2024

2) Rakesh Varma Penmetsa

DATE: 9/11/2024

3) Wes Ryley

DATE: 9/11/2024

4) Alexander Upah

DATE: 9/11/2024