# ME 450-006 FALL 2021

# Nematode Detection & Imaging System (NemaDIm)

# In conjunction with the Gourgou Research Group

# Team 22

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#### **PROBLEM DESCRIPTION**

The Gourgou group is conducting research about the *C. elegans* migration and behavior in a controlled arena. Our group was tasked with the development of a system that can monitor the migration process and detect the presence of the nematodes when they cross the channel for migration and capture an image. The arena consists of two chambers connected by a hallway as shown below on Figure 1. The walls of the arena are 3D printed and the interior filled with Nematode Growth Medium (NGM, an agar gel). Our goal is to design a device that can detect and record nematode migration. The design is proposed to be created by mounting a sensor on the arena (without interfering with nematode motion) and a camera mounted on the microscope. Having an open-to-air design allows our sponsor, Dr. Eleni Gorgou, and future bioscience researchers to directly observe the path of migration.





#### Caenorhabditis elegans

Nematodes are a type of roundworms that move and live freely in soil or water as shown in Figure 2. The type of nematode we are experimenting with is *Caenorhabditis elegans*. They are non-parasitic, non-infectious, and non-toxic making them safe to handle while experimenting. [1] Also, we are only interested in the migration of adult *C. elegans* which are approximately 1 mm in length and 50  $\mu$ m in diameter. [2] The lifespan is only about two to three weeks, so they can be easily harvested in research labs without requiring vast resources. Ease of harvesting, and their short life cycle, make *C. elegans* beneficial to researchers looking to study cell interactions and genetic behavior across multiple generations. [6]



Figure 2. Image of C. elegans. (https://news.wisc.edu/newsphotos/kimble10.html)

## Research

This species of nematode began its academic notoriety when Dr. Sydney Brenner, a prominent microbiologist, selected *C. elegans* as his subject organism for his new study of genetics and cellular lineage in 1963. By 1986, Dr. Brenner published the first complete description of an organism's nervous system. [4] This founded much of the modern research in connectomics and how we understand mutations. Since this publication, significant contributions have been made to both the biomedical sciences and public health, including the awarding of multiple nobel prizes to those who have studied this species. [3]

Modern use of *C. elegans* in research has focused on studying the aging of cells, and what parallels can be made to organisms other than *C. elegans*. Additionally, research has been conducted on how cells behave in a different environment. Such research can help society by studying human cell aging [4] and even nicotine dependency of humans that are based on cell behaviors. Therefore, it is important to study the factors that influence decision making in *C. elegans* to allow conclusions to be drawn in similar organisms. In addition, since *C. elegans* have relatively simple cell systems (1000 cells) which allows researchers to easily identify and visualize the research of cell interactions. Also, *C. elegans* is a primitive organism that shares essential biological characteristics that can be applied to problems of human biology. [6] In addition, *C. elegans* have high homology with human genes, the first completely mapped nervous system, and sequenced genome such that researching them can be highly applied to human physiology. [10] This makes our project capable of applying to address human health problems such as aging and causes of cancer. [6]

## **Intellectual Property**

Intellectual properties of this project consist of the custom housing design and the algorithm for detecting the nematodes. All the copyrights are reserved to the team members of Team 22.

#### Social Context Assessment

The Gourgou Group and other bio researchers will be positively affected by the outcome of our project as it'll allow them to conduct research in an advanced manner. Manufacturers and supplies can also benefit by recreating and further improving our system and making it available in the market. Our stakeholder map can be seen below in Figure 2.



Figure 2: Stakeholder Map

#### Library

We have used a variety of methods to gather references to learn more about the existing *C*. *elegans* research and known facts about them. Although we did not directly contact a librarian, we gathered important information and references from our sponsor Dr. Eleni Gourgou. We searched extensively for online resources that are relevant to our project. Some of the more viable resources included the CalTech WormLab and the Hobert lab at Columbia University. These resources provided extensive background on the biology of *C. elegans* and more importantly, the role they have played in research. This allowed the team to consider the broader context of how this research, as well as our design, will be used.

The most significant challenge the team encountered was researching biosensors on a comparable scale, both in terms of subject size and time. Many of the sensors that already exist were either an order of magnitude too large or too small. In the latter, they typically operated by measuring transient properties of large groups of organisms over extended periods of time (e.g. monitoring the gases emitted by a bacterial colony over several months). Without an analogous design, the team identified the characteristics of these designs that might scale to the project and incorporated them into the design generation process.

Lastly, the final design sensor chosen relied heavily on the application of computer vision and image analysis techniques. Although the team had some experience with this, an expert at the department of Computer Science Engineering in the University of Michigan (Professor Anhong Guo) was interviewed to discuss the direction of the project and provide insight into some tasks that were less clear. This background provided clarity that helped maintain project timelines.

## Public Health

Our design can help biomedical researchers learn about *Caenorhabditis elegans (C. elegans)* behavior. The findings from this type of research have been used to provide insight into human cell aging, nicotine addiction, and discover more about human health. In addition, our design enables research of cell behavior with the relatively low cost of our design (~\$500) for researchers without access to expensive research equipment (which can approach tens of thousands of dollars). With a modular design between the 3D printed components and tower height, researchers will be able to tailor the system to specifically address their needs.

## **Global Context**

Our design can help the researchers who are studying nematodes and other tiny organisms as our design can be easily integrated and operated. While such a design isn't currently available in the market, our design can be a powerful tool that is capable of operating independently and efficiently.

## Social Impact

Our design can have significant social impact as *C. elegans* have a similar nervous system to humans and through the study of their migration and behavior with aging as the lifespan of a nematode is about three weeks, a relatable conclusion can be made regarding human aging and might further be applied to control aging. [6]

## Economic Impact

With our relatively low cost compared to full scale research equipment, biomedical researchers and students can learn about *C. elegans* and cell behavior. Therefore, our design would help advance the medical technology of economically disadvantaged communities in our country and abroad.

#### Inclusion and Equity

In this project, we received stakeholder feedback and inputs about our design. As our project did not directly involve the interactions between the end users, our design mainly focused on how biological researchers are able to use our design. Also, we expected the end users to be non engineers, so the ease of troubleshooting by someone who is not an expert in troubleshooting. This enables the inclusion of users throughout the design process so that people without engineering knowledge can use our design. Lastly, our design enables the affordability of nematode migration research, which gives access to more end users who are looking to learn about it. Since everyone is from a diverse economic background and the worsened economy, we gave the priority of a maximum budget of \$1,000 so researchers won't have to budget a large sum of money to study nematodes. Our stakeholder was also looking for this requirement, so we were able to balance this requirement with our design.

#### **Ethics**

Throughout our project, we strived to respect the resources and the work that are presented to us. We properly acknowledge the information that was provided to us at the References section. Also, during the experiment of nematode migration, our team ensured that we aren't causing harm to the nematodes as they are living organisms with life. To ensure we don't unexpectedly harm them, we came up with specifications of our design to avoid harming nematodes. Lastly, we kept our expectations of our professional practices by being honest and truthful about the reports of our design.

## **Design Process**

During the design process, we followed steps of designing where we came up with preliminary design, then revised our design for the second half phase of the project. The reason we followed such a process was because we initially thought the design would work as we expected, but actually did not work as we expected. Therefore, revisions were necessary throughout the design process. For example, we scrapped the concept of having electromagnetic wave detection of nematodes because this concept wasn't feasible for detecting microorganisms. In addition, trial and error process was used when improving the design of the 3D printed camera mount and tower.

## **REQUIREMENTS & ENGINEERING SPECIFICATIONS**

To detect and capture the nematode migration, we have come up with a set of requirements and specifications to be integrated into our system as shown in Table 1. The first requirement is to capture a top view image where the camera will be mounted on a microscope that's observing the migration of the behavioral arena. The second requirement is to have a system that's easy to repair and perform maintenance by non-specialist, an undergraduate for example in a quick and timely manner. This requirement is mainly given due to the short lifespan of a nematode which's two to three weeks thus if something fails, it's important that maintenance is performed in a timely manner. If something breaks during an experiment, the part should be easily accessible and quickly integrated. The third requirement is to maintain a maximum budget of \$1,000 for all components in the system. Fourth, to have a small error rate of 1%, since the system will be operating independently, so a bigger error cannot be afforded.

The last requirement is to not interfere with the migration of the nematodes. The purpose of this study is to observe the independent decision making of the nematodes, so it's vital that the sensor does not contaminate the data. Therefore, the temperature and vibrations due to sound must fall within a nominal range. Additional specifications might need to be defined if the mode of detection requires it (e.g. a capacitance based sensor would necessitate limits on capacitance). This final requirement also aims to reflect the fact that this study concerns living organisms whose health should be respected. These requirements and specifications were defined from interviews with the project sponsor as well as background research into the species, studies, and comparable solutions. The following table summarizes the requirements and specifications as they have been stated to ensure the successful completion of the project and the preservation of the *C. elegans*.

Requirements	<b>Engineering Specifications</b>
• Capture a top view image of the migrating nematodes across a 2D connection channel within a behavior arena	<ul> <li>Camera compatible with <i>Diagnostic</i> <i>Instruments TLB D4.1 Illuminator</i> microscope [10]         <ul> <li>Desirable: Modular mount for mounting on other microscopes</li> </ul> </li> </ul>
• Doesn't interfere with nematode travel	<ul> <li>Steady state temperature no higher than 10°F from ambient [2]</li> <li>&lt; 50 dB</li> <li>Pending the mode of detection - external stimuli doesn't contaminate data</li> <li>Sensor maintains internal dimensions of the channel</li> </ul>
• Accurately detect nematodes moving	• Trigger an image signal within 1%

**Table 1.** Sensor and image capture system design requirements and specifications. These requirements are ordered by importance.

through the channel	error (i.e 10 errors for every 1000)[10]
• Easy to repair and replace parts in a timely manner in case of failure by students and researchers in the biofield	<ul> <li>Standard replacement part acquisition is no more than 2 weeks</li> <li>≤ 2 specialized tools to repair</li> <li>Don't need to remove sensor for maintenance</li> </ul>
• Sense and capture nematode migration	<ul> <li>Integrable to provided environment (potentially modular)</li> <li>Operate independently for at least 36 hrs</li> <li>Can detect mutations of <i>C. elegans</i> (no morphological change)</li> </ul>
Maintains budget	• \$1,000

## **CONCEPT GENERATION**

#### **Generation** Method

After developing our requirements and specifications, we spent time generating and exploring a wide variety of potential ideas. Our main concept generation method we used was the morphological chart that allowed us to generate ideas in an analytical and systematic manner. Our slimmed down morphological chart can be seen in Figure 3. As we mentioned this is our slimmed down version that was pre-screened and does not include all of our ideas.

r	1	1	1	1	1
Detection Method	Sub-NGM Extensometer	Change in Electromagnetic field	Video Processing	Capacitive Touchpad	Light Gate/Photoelectric
Capture Method	1 Camera	2 Camera	GoPro	Arduino/Raspberry Pi Compatible	Webcams
Electrical Housing and Mounting	PolyCarb Enclosure -existing product/easily accessible -cheap part (in scope) -designed for electrical housing	3D-Printed Housing -Custom Fit -Optimize heat flow -Cheapish -Might be excessive	3D-Printed Camera Mount -Might be necessary for arduino/Pi	<u>QR/Swivel Mounts</u> -widely purchasable -Dynamic set-up -Easy to change battery/sd/settings	Remote Trigger -IR -Camera specific -Bluetooth
Integration (power supply, housing, processor)	Arduino -sd card -camera -digital -power supply	Raspberry Pi -HD Camera(+ lenses) -sd card -digital	Analogue switch on breadboard -simple -MOSFET -lot of options	USB 5V power bank -can get high quality -Desired amount of operating hours between charge	AC-DC <u>Wall plug</u> -lot of power -more dangerous circuit -restricted to an outlet

Figure 3. Morphological chart for ideation

Our morphological chart consists of four sub functions: detection method, capture method, electrical housing and mounting, and integration. For each sub function we generated components that would realize the sub function. This is where we pre-evaluated all of the components that we came up with and disposed of the ideas that we didn't believe would fulfill our specifications. These disposed ideas just didn't go to waste however, they helped us cross-pollinate and iterate upon ideas leading to more and better quality ideas. Solutions can then be generated by selecting one component from each of the sub functions. Our evaluation method was to use pugh charts to rank each component in a variety of categories. We go into detail on our evaluation and selection process later in our report in the *Concept Selection Process* section.

## **Detection** Method

For detecting the *C. elegans*, we can use an inductance coil and probe on either side of the channel as shown in Figure 4. We can measure the nominal electromagnetic field (EM field) without the *C. elegans* presence. Also, the EM field per *C. elegans* must be measured and analyzed in the sensing process. The good news is that the hardware would be relatively easy to manage and digital sensors can account for state change for error handling. The biggest drawback is that the lab environment has a lot of background EM fields from electronic devices and equipment, which could contaminate the data unexpectedly. Lastly, the EM field is sensitive to noise and surrounding unwanted EM interference.

Figure 4. Rough schematic image of the EM field detection method.



We can also install a small metal plate (sensor) under the NGM gel and measure the change in surface capacitance like in Figure 5. This method is similar to how a laptop touchpad functions, where the surface capacitance of the laptop touchpad changes if there is a human hand touching it. When using this method, the difference in capacitance with and without the presence of *C*. *elegans*. By observing such a change in capacitance, we can tell that there are *C*. *elegans* on the surface of the NGM Gel. The downside is that we are unsure about the feasibility of the sensor to be embedded under the NGM gel. Also, we have not tested that the capacitance change would be large enough for a capacitance sensor to detect. In addition, there may be unknown effects to the nematode travel, so extensive testing is needed when using this system. Finally, the computational cost would be large.

Figure 5. Rough schematic image of the capacitance detection method.



## **Capture** Method

If we use either Arduino Uno or Raspberry Pi, there are many software compatible cameras available on the market. For Arduino Uno, we would purchase a camera that can be controlled from Arduino Uno directly and program a computer vision software on the camera. Also, if the camera is compatible with an Arduino Uno, then there is a chance that we have more efficiency in data communication with the computer from the Arduino Uno compatible camera that captures the nematode migration. Also, there are many open source softwares in the Arduino community, so we can possibly use image processing software encoded into the Arduino compatible camera by utilizing C++.

The idea would be similar for the cameras compatible with Raspberry Pi 4 where we can use Raspberry Pi 4 Cameras as seen on Figure 6. The good news is that Raspberry Pi compatible cameras have very high quality video feed and have an established community with aftermarket lenses just like the Arduino. Lastly, the Raspberry Pi 4 supports computer vision libraries in Python.

**Figure 6.** Image of the Raspberry Pi compatible camera. (https://www.element14.com/community/docs/DOC-94932/l/raspberry-pi-high-quality-camera)



The number of cameras was a significant parameter when we developed our initial Alpha Design. We have the option to mount one camera or two cameras on a microscope. Having one camera would mean that such a camera has to cover all areas of interest of *C. elegans* migration as shown on Figure 7. The red area on Figure 7 is the possible area of focus when having one camera. Whether or not we only use one camera is dependent on the resolution of the chosen camera. Specifically, if the camera has a high enough resolution to take a detailed image of the entire area that allows for the identification of the *C. elegans*.





In addition to just having one camera, adding another camera would enable us to focus on two areas of interest as shown on Figure 8. Further, when having two cameras, one can be devoted for capturing at the entrance of the migration hallway (red area of Figure 8) and another can be devoted for capturing at the exit of the migration hallway (blue area of Figure 8). Having two cameras would be more beneficial if one camera does not have sufficient resolution to take a detailed image of the entire arena.





#### **Electrical Housing and Mounting**

For containing and protecting the electrical components of the system, one option is purchasing a Polycarbonate Corrosion-Resistant Washdown Enclosure from McMaster Carr as shown in Figure 9. McMaster Carr has various size options for the enclosures, varying sizes and protection standards are readily available. The enclosure is relatively cheap (\$20), but would require further machining in order to make it compatible. In addition to McMaster Carr, there are many companies offering electrical housing enclosures online and in hardware stores, so the enclosure is widely available on the market for relatively low cost. The downside of this type of enclosure is they don't account for protection from overheating and it is hard to visually inspect the components unless the enclosure is opened.

Figure 9. Image of the Polycarbonate Corrosion-Resistant Washdown Enclosures from McMaster Carr. (https://www.mcmaster.com/69945K91/)



The second option for sourcing the enclosures for the electrical components of the design is to 3D print the enclosure. There are several open-source, Arduino and Raspberry Pi compatible, enclosures that wouldn't require additional machining. An example is shown on Figure 10. Since our project is on a tight schedule, that is very beneficial. The good news is that we can fully customize the enclosure to allow optimal heat flow and allow easy modification of design if needed. Also, PLA has great insulation properties under 70°C, however, PLA is flammable and lacks in strength possibly making it a hazard and susceptible to cracking.

Figure 10. Image of the 3D printed enclosure. (https://www.thingiverse.com/thing:4341737)



## Integration Method

Arduino Uno, seen in Figure 11, can be used for integrating and controlling the camera and sensors as well as storing the images. This makes the controlling process of the design much more simple than having separate controlling systems for camera and sensor. Arduino Uno is relatively inexpensive (\$23) when compared to the Raspberry Pi 4 (\$35-\$75). Also, Arduino Uno can be powered by USB cable and produces an output voltage of 5 volts. This output voltage can be used to supply power for cameras and sensors. However, Arduino Uno can be only

programmed with C or C++, so someone with a knowledge of either is required to set up the system. Also, the design must be able to be troubleshooted by non-engineers who do not know C/C++ programming, which may cause some issues if something is wrong with the code.



**Figure 11.** Image of Arduino Uno. (https://store-usa.arduino.cc/products/arduino-uno-rev3/?selectedStore=us)

The Raspberry Pi 4 ,seen in Figure 12, is also an option for integrating the cameras and sensors. Like Arduino Uno, there are many available Raspberry Pi 4 compatible cameras and lenses. Also, Raspberry Pi 4 has USB or USB-C compatible power supply with voltage up to 5 volts. Raspberry Pi 4 supports a variety of programming languages, so it is capable of handling more software than Arduino Uno. The Raspberry Pi 4 comes in 2, 4, and 8 GB RAM options, giving us more computing power if we need it. Again same as Arduino, the process of troubleshooting the design with Raspberry Pi 4 must be able to be addressed without modifying the code as we expect non-engineers to handle this design.

Figure 12. Image of Raspberry Pi. (https://en.wikipedia.org/wiki/Raspberry\_Pi)



#### **CONCEPT SELECTION PROCESS**

Detection selection process was evaluated by using a matrix, shown in Table 2, that consisted of a variety of the detection option such as EM-Field, Photoelectric Retroreflective, Computer Vision (Video Processing), and Touchpad and ranked them in terms of Potential Stimuli to Nematode, Ease of Operation, Susceptibility to Noise, and cost. Computer Vision scored the highest followed by a tie between the Capacitive Touchpad and the Photoelectric Retro-reflective Sensor. Those scores were the total number that was given based on the four evenly weighted factors that they were ranked based on.

The main advantages that the Computer Vision has over the other detection options is its ability to detect very small objects with limited susceptibility to noise. It also has essentially no potential to provide a stimulus to the Nematode and affect its behavior. It may be expensive relative to the other methods but since our detection method is the most important component of our system, we are willing to spend the most money on it while staying within the budget.

By using the concept selection matrix on Table 2, we can conclude that the computer vision for nematode detection would be most suitable for our project. The reason being is that the computer vision method does not stimulate or contact directly with the *C. elegans* in which is best desired for our design. Our goal is to not have any external stimuli to *C. elegans* in order to better assess the migration as they are very sensitive to environmental changes. Also, using computer vision methods greatly reduces the needs of accounting for noises that are present in the environment. By taking a direct image of nematode migration, we are able to directly see the noise unlike EM-field detection or touchpad detection methods. Both EM-field detection and touchpad detection methods involve a very small difference in capacitance when the nematode is present and not present. These methods are likely to induce small errors from the environment such as static electricity. In summary, we are using computer vision methods for the alpha design as well as the final design. The biggest reason is that we can avoid stimulating the nematodes and also ease of detecting noises.

*Rated from 1-5, 5 being best for the project	Potential Stimuli to Nematode	Ease of Oper.	Susceptibility to Noise	Cost	Total
EM - Field	1	2	2	5	10
Computer Vision	5	4	5	2	16
Touchpad	3	5	1	4	13
Photoelectric Retroreflective	4	3	3	3	13
Shape Sensing Fiber Optic	2	1	4	1	8

Table 2: Concept Selection: Detection Method

Capture selection process was evaluated in a similar manner to the detection selection by also using a matrix as shown in Table 3 that ranked the capture options that included GoPro, Arduino, Raspberry Pi, and WebCam. The highest ranking was given to the Raspberry Pi followed by the GoPro then Arduino.

The main advantages that the Raspberry Pi has over others are the High-quality video feed and the established community with aftermarket lenses, inexpensive, and supports a variety of programming languages. While the GoPro is durable, battery and tethered power, and a variety of trigger selection such as signal or IR light. On the other hand, the main disadvantages of the Raspberry Pi is that it requires a unique housing and tethered to the processor. While the con of the GoPro is that it's relatively super expensive to obtain.

In addition, webcam and DSLR cameras would be less suitable on our design because they are not compatible with Raspberry Pi, so this gives a disadvantage compared to the Raspberry Pi compatible camera assuming our design operates with Raspberry Pi. Also, the resolution of the webcam is very low compared to any other capturing methods although they are inexpensive than GoPro and Raspberry Pi cameras. GoPro offers a great ease of operation and high quality images, but costs as much as \$450 so we decided not to incorporate it with our design. Therefore, we decided to outsource Raspberry Pi compatible cameras for ease of operation and integration with Raspberry Pi. Also, the cost was much less expensive (\$90) than the standard DSLR or GoPro cameras.

*Rated from 1-5, 5 being best for the project	Compatible Lenses	Ease of Oper/Imp.	Resolution	Cost	Total
GoPro	3	5	5	2	15
Arduino	4	2	2	4	12
Raspberry Pi	5	3	3	5	16
Webcam	2	4	1	3	10
DSLR	1	1	4	1	7

Table 3: Concept Selection: Capture Method

## **CONCEPT DESCRIPTION**

As mentioned above, our chosen final design concept is as follows: Detection Method  $\rightarrow$  Computer Vision Capture Method  $\rightarrow$  Raspberry Pi Compatible Camera Housing Method  $\rightarrow$  Custom 3D Printed Integration Method  $\rightarrow$  Raspberry Pi 4 Microprocessor (8GB RAM)

## **Detection** Method

The detection method for our final concept is computer vision. More specifically, it is an image processing algorithm. The image processing algorithm takes a live video feed from our capture method, breaks it down into frames, and then uses an algorithm to analyze the frame determining whether or not a nematode is present. If there is a nematode present, the frame is saved for future visual inspection from a lab attendant or researcher. The full algorithm is written in Python using the OpenCV library and is run on the Raspberry Pi 4, our integration method.

#### **Capture** Method

The capture method is a Raspberry Pi compatible camera that connects directly to the Raspberry Pi using a ribbon cable. The camera is also equipped with a compatible Telephoto lens that uses a reverse lens macro kit for zoom functionality. It is in charge of sending a live video feed to the Raspberry Pi. The Raspberry Pi 4 is able to control the camera using the PiCamera Python interface.

## Housing Method

Our housing method consists of 4 custom 3D printed components which are the tower, arena base, tower cap, and camera mount which we talk about in more detail in our *Final Design* section and Appendix 3: Manufacturing Plan. The main function of our housing is to block the ambient noise and light. The housing components are assembled together to form a system block external effect on the experiment while providing access to the camera adjustment knobs for better focusing and zooming. Figure 15 in the final design section shows the housing components.

## Integration Method

Our final concept integration method is the Raspberry Pi 4 Microprocessor. It is the center of our system and serves several different functions. First, it controls and provides power to our camera. It uses the PiCamera Python interface to convert the live video feed into frames. Second, it runs our image processing algorithm which decides whether or not a nematode is present in each frame using the OpenCV library. Finally, it saves and stores the images on a flash drive to later be used for visual inspection by a lab attendant/researcher.

#### **ENGINEERING ANALYSIS**

One of the most impactful design parameters lies in the implementation of the computer vision algorithm. A specification for this project, as communicated directly from the sponsor (Dr. Eleni Gourgou), requires the system to be operable continuously for up to 36 hours. An experiment that runs this long might require considerable computational power to process the images as well as sufficient storage to save them. These were driving factors in two significant design decisions. First, the computational complexity of the algorithm isn't known for certain at the time of part acquisition. Because of this, it is not known the required processing power to meet the prescribed error rate. Therefore, to ensure that this design can function with algorithms of varying complexity, the team has elected to purchase the 8 GB Raspberry Pi 4 (the most gigabytes available). In future iterations after the methodology has been refined, an analysis of the floating point operations per second (FLOPS) can be performed to get a better understanding of the minimum computation power necessary. This would allow smaller sized processors to be purchased, potentially saving money on future designs.

Second, the number of images taken over a 36 hour period will require considerable storage. The Raspberry Pi chosen comes with a 128 GB storage drive. A factor considered by the design team is the convenience of being able to quickly swap out SD cards for these extended trials without removing the arena from the enclosure. To further minimize the necessary storage space the team has elected to perform the image classification in real time as opposed to after the fact. By performing the classification as images are being taken, negatives can be discarded and not saved to the storage. The researcher still might desire the negatives for visual inspection (particularly in early iterations) but this allows them to choose whether or not they should be saved. Additionally, after discussions with the project sponsor it was determined that a real-time analysis presented no significant concerns and that classification after the fact (via post-processing), although it allowed for more computational power, only added a step to the process for the researcher. Following engineering best practices and believing that the simpler solution is generally the better one also contributed to the decision to perform the classification concurrent with the experiment.

The size of the arena was also a control variable within the system. The original arena has a channel width (perpendicular to nematode travel) of 22 mm which results in a "skinny" control area. Consider the depiction in Figure 13.

Figure 13. Drawing of camera field of view showing the nematodes entering the channel.



As one can see, the relative length of the *C. elegans* as they enter the control area is considerably smaller than the width of the channel. Since noise in the signal comes in the form of irregularities in the images (bubbles/eggs/streaks/etc.), the team considered different methods of shrinking the control area into more manageable sizes where the noise was considerably smaller. Two primary ideas were analyzed: segment the control area and have a rolling window that moves down an image looking for a nematode. However, as this began to increase the complexity of the system it was determined that a more effective solution would be to scale down the size of the arena considerably. After discussing the viability with our project sponsor and considering different scaling factors, the final arena was chosen such that the width of the channel is 3 mm. This results in a small enough control area that it only needs to be checked once, without the need for segmentation.

Figure 14. Sample image taken using ambient lighting, Olympus DP23 camera, and final arena scale.



As discussed in Appendix 1A, the team is considering a variety of image processing methods to convert the RGB image to the desired signal type. Some of these methods include: edge detection, morphological operations, and simple binarization. While researching these methods as well as considering sample images provided by the sponsor (like the one above in Figure 14) it quickly became apparent that a procedure would need to be standardized to account for consistent lighting and orientation with respect to the camera. An algorithm could be made more robust to handle things such as shadows and instances where the arena is askew to the camera, but as previously discussed, robustness increases computational complexity. The team decided that an arena housing would be built to accomplish three key objectives: minimize ambient light, increase contrast between nematodes and NGM, and standardize methods of orientation. The arena housing will be completely closed off from external light sources so shadows aren't varying between trials. The housing will have a cutout in it's base that is nearly the same size of the arena that will utilize the illuminator in the microscope to project light from beneath the arena up through the NGM. This light will provide increased contrast in the images, improving the ability of the algorithm to distinguish between nematodes and NGM. Lastly, this housing will fix the arena and camera to a common datum so we will be able to guarantee consistent positioning of the arena within the field of view. This allows the control area to be set directly in the algorithm ("hard-coded") as opposed to requiring some input from the researcher.

After discussing different variations of the arena housing, one notable problem could be identified. By enclosing the arena and the nematodes, the temperature change of the environment becomes more of a concern. The first specification of our second requirement limits the change from ambient temperature to 10 degrees fahrenheit. The halogen lamp used in the illuminator certainly can experience changes outside this range so the team began considering various adaptations, namely: optimizing geometry (volume to surface area/wall thickness) for heat transfer or adding a vent with a fan to move air out of the housing. We presented these ideas to our project sponsor for feedback and were informed that the heat from the illuminator is also a concern of theirs for their own research, independent of the previously mentioned arena housing, and an LED based illuminator had been purchased. Since the main heat source will no longer be a factor, the team believes confidently that the temperature will maintain within the tolerable range, even for extended periods of testing.

#### **FINAL DESIGN**

Our final design is slightly different from our alpha design that was previously presented in DR2. Our design consists of 4 components: a Raspberry Pi 4 microprocessor, a Raspberry Pi compatible camera, a custom 3D printing housing that allows mounting of the camera and arena, and finally an SD card to save the images. From a holistic birds eye view, the camera will provide a constant video stream to the Raspberry Pi 4 which will be running an image processing algorithm. The algorithm will break down the video stream into frames that will then be analyzed to determine if a nematode is present in the frame or not. If there is a nematode present, the image will be saved to the SD card and if there isn't it will be discarded.

## **Detection** Method

For our detection method, we will be using the Image Processing to perform the task of detecting the presence of the nematodes with an algorithm written in Python using the OpenCV library that will be uploaded and run as hardware on the Raspberry Pi 4. At an overview, the algorithm will take a video feed, convert it to still image frames, complete the rest of the image processing, and finally save the images where a nematode is present. For more detail on the image processing algorithm please refer to the *Engineering Analysis* section.

## Integration & User Interface

We will use the Raspberry Pi 4 microprocessor to integrate our system components which are the camera, user interface, and a flash drive. All 3 of these components have direct connection to the Raspberry Pi 4. The Raspberry Pi 4 will also be our processing center and will run our image processing algorithm written in Python using the OpenCV library as well as the PiCamera interface to control the camera. The main reason we chose the Raspberry Pi 4 over the Arduino Uno is due to its ability to run Python which is what the OpenCV library is written in as well as it's large Random Access Memory (RAM) which is 8GB.

The goal of our user interface is to provide a simple process for the user, who will likely not have any engineering knowledge. The user interface is made up of a small circuit that includes a push button and an RGB bulb. Our user will boot up the Raspberry Pi and use the terminal to run a command that starts our first script. This will only involve a single word (i.e. "startExperiment.py"), in order to keep the process simple. This first script initializes the system and will wait for the user to push the button. The button serves as the start of the experiment. Once the button is pushed the first script will automatically call the second script which is where the system will begin taking a live video feed, convert it to frames, and process those frames using our image processing algorithm. Once the experiment is over, the user will push the button again which will signal the system to stop running.

Throughout the process, the RGB light will be used to provide visual confirmation to the user of which step the system is currently in. Green will be used to signal that the system is on and the first script is running. Blue will be used to signal that the button has been pressed and that the second script has been started. Red will be used to signal that the second script is running and an experiment is currently being recorded. Finally, no light signal will signal that the system is either not on or has completed the experiment.

## **Capture** Method

The video feed of the *C. elegans* will be taken by *Raspberry Pi High Quality HQ Camera - 12MP* that is directly compatible with the Raspberry Pi 4. We chose this camera due to its compatibility with the Raspberry Pi 4 and its high resolution. We are confident in this camera choice as it has twice the resolution (12MP) of the camera that was provided to us by Dr. Eleni Gourgou, the *Olympus DP23* microscope camera. The Raspberry Pi camera is paired with the compatible *16mm 10MP Telephoto Lens* equipped with a reverse lens macro kit that allows zoom capability.

#### Housing

We will 3D print a custom housing, seen in Figure 15, that will provide mounting places for the camera as well as the arena. We chose to create a custom housing for 2 reasons. First, the main role of the housing is to block ambient light from affecting the video feed taken by the camera. This allows us to give a more consistent image to our algorithm and this will help us achieve a lower error rate. Second, since our camera and arena are specific to our system, there are no available housing options that would allow for mounting without a lot of extra manufacturing. 3D printing the housing lets us quickly create the housing without extra manufacturing and also quickly iterate upon our design if need be. Second, the main role of the housing is to block ambient light from affecting the images



#### Figure 15. Custom housing

## Assembly

Although there is no manufacturing required nor the use of machine shops, we have steps that we have to take to assemble the imaging assembly. (1) The Raspberry Pi 4 is connected with the Raspberry Pi camera by a ribbon cable. (2) Wide angle lens is attached to the Raspberry Pi camera. (3) Samsung 128GB SD card is inserted into the MicroSD slot of the USB MicroSD card reader, then connected to the Raspberry Pi 4. (4) The camera is connected to the camera mount and tower cap and secured in place via screws, nuts/bolts. (5) The secured camera and two holders inserted to the top of the tower. (6) The arena is placed in the arena base and then inserted into the bottom of the tower. The schematic image of the first three steps are shown in Figure 17. And steps four through six are shown in figure 18. Further, the whole system is placed on the stage of the microscope for the LED light that's needed to provide illumination to take good images.

Figure 17. Schematic image of the first three assembly steps of the image processing assembly. (Parenthesis refers to step number).







## VERIFICATION AND VALIDATION

#### **Overview**

In order to assess our systems ability to meet the requirements and specifications, we will utilize both theoretical and empirical analysis supported by our knowledge of engineering fundamentals. We will begin our analysis and testing on the detection method which is our system's most critical component and main design driver. The selection of our detection method will drive the selection of the capture, integration, and mounting & housing methods. Our chosen detection method is image processing which allows us to complete verification & validation in a variety of ways. Verification involves verifying that the image processing can indeed detect a nematode while validating involves making sure that the image processing can fulfill our 1% error rate specification. From an overview, our verification plan starts out simple and increases in complexity until we eventually test the full system on a pseudo experiment similar to that of the experiments that Dr. Eleni Gourgou intends to use our system for. For more detail on the analysis and verification of our image processing algorithm itself, see the *Engineering Analysis* section. Our original plan for verification and validation can be found in *Appendix 1B*. An image of our system can be found in Figure 19.





## **Detection** Method

To begin, we wrote our image processing algorithm in Matlab and tested the algorithm against pre-taken images. Once this was successful, we moved onto testing our algorithm in Matlab against a video feed which involved breaking the video feed into frames. It is important to note that the video feed was not taken with our chosen camera, but rather with the *Olympus DP23* microscope camera that Dr. Eleni Gourgou provided us. The results of this testing were a 100%

success rate with 0% error on 190 images. The Matlab image processing algorithm and testing is detailed below.

After testing the computer vision algorithm on the frames extracted from a video provided by our sponsor, it was determined that the average ratio of black pixels to white pixels in positive frames is  $0.0087 \pm 0.0027$  and in negative frames is  $0.0012 \pm 0.0009$ . These images were processed using an adaptive threshold (to binarize the image) but no additional measures were necessary (e.g. morphological operations). Additionally, a reference negative frame was used to subtract off some of the black pixels present due to shadows and noise. Using a pixel-ratio-threshold of 0.0025 (greater indicates the presence of a nematode) the algorithm was able to accurately classify all 190 images as positives or negatives. This is a small dataset and a larger one must be considered, but with an improvement in resolution and image quality (due to arena housing) the team anticipates this method of detection to be viable.

## Capture Method

Our next step is to complete the same testing with the Matlab algorithm however instead of using the *Olympus DP23* we want to use our *Raspberry Pi High Quality HQ Camera*. Originally we didn't think this would be a problem at all since our camera is twice the resolution (12 MP) of the camera provided to us by Dr. Eleni Gourgou, however, ran into some issues with the image quality using the compatible lenses with the *Raspberry Pi High Quality HQ Camera*. More specifically, the *6mm 3MP Wide Angle Lens* that we originally chose did not have sufficient zoom capability to provide enough detail to identify the *C. elegans*. In order to solve this problem we have purchased the *16mm 10MP Telephoto Lens* along with a reverse macro lens kit. This lens should provide sufficient zoom to successfully identify the *C. elegans* as visually inspected by Dr. Eleni Gourgou and the Gourgou Research Group researchers.

## Integration & User Interface

We have completed significant verification testing of the Raspberry Pi 4 Microprocessor and the user interface that goes along with it. To begin, we've installed OpenCV and PiCamera on the Raspberry Pi. We've confirmed that we are able to control the camera through the empirical testing including the ability to convert a live video feed into frames. We've also coded our entire system, including the user interface, other than the actual image processing algorithm that needs to be converted from Matlab to OpenCV. With the research we've conducted, we don't foresee this being an issue since Matlab and OpenCV are very similar and allow for simple conversion. Moreover, this system has been used to complete the testing of our capture method although the capture method testing is not fully complete.

## Housing

Along with verification testing of the Raspberry Pi 4 and user interface, we have also integrated all components of our design including the Raspberry Pi 4, housing, and camera. All components are compatible and the connections work as intended. The housing blocks out ambient light and provides a robust and sturdy mount for both the camera and arena. As we tested and verified our system as a whole, we made improvements to our housing along the way. Thanks to Dr. Eleni Gourgou, we had 24/7 access to a 3D printer that allowed us to iterate and update our design very

quickly. To satisfy the minimum distance required for the wide angle lens, we initially made a tower that was 9 inches long but we ran into a few issues mainly, light reflecting off of the walls of the tower and the inability to zoom. To overcome this, we made a new tower that's 5 inches long, but we were unsuccessful in focusing the image as we still weren't able to zoom in enough. We even went a step further and tried a 2 inches tower that gave a better image with the wide angle lens but this still was not sufficient. After purchasing a new lens, we had to develop another camera mount and tower cap in order to fit it. Finally, we made three iterations of our arena mount to fit the arena snuggly. The first two iterations were to get used to the 3D printed material shrinking so we had to make some small adjustments. The third iteration was to increase the size of the arena mount in order to limit the amount of light that could leak through along the edges.

## Final Validation

Although we were not able to fully complete our final validation before submitting this report, we still wanted to touch on how we planned on going about it. To begin, we would fully assemble our entire system with all components. We would then go through the entire process of booting up the system and running the software just like how our end user would. Finally, we wanted to conduct a pseudo experiment similar to the experiments that Dr. Eleni Gourogu plans to use our system for. The pseudo experiment is as follows:

We will drop  $\sim 50$  *C. elegans* on one side of the arena and bait them with food (or another positive stimulus) on the other side of the arena. We will let our system run and once the experiment is complete, we will visually inspect the images and video feed with the researchers. This will allow us to compare the saved images, with nematodes present, to the live video feed. Although this is a lengthy process, it is necessary to ensure that our system will achieve an error rate of 1%.

#### DISCUSSION

#### **Problem Definition**

Due to the inherent time constraint of this project being a semester long, there are many aspects of the project that we were unable to explore and would want to if we had more time and resources. First, we would further investigate what types of external stimulus are acceptable to the *C. elegans*. This goes along with our engineering requirement of "Doesn't interfere with nematode travel." This requirement was one of our most important as it directly affected which type of detecting method we could use and for our specific system, whether or not we needed to use a housing. To further investigate this we would have taken two paths. The first one would be to read and collect more data from research on nematodes that has to do with that type of topic. Since nematodes are a very popular organism to research and conduct experiments on, we are very confident that we would be able to find useful information. The second path would be to conduct more empirical testing both on our own and with Dr. Eleni Gourgou to answer specific questions that we had that we were unable to find the answers to through other sources.

#### **Design** Critique

Now that we have had the chance to complete the entire design & manufacturing process, from problem definition to verification & validation, there are a few improvements we would have liked to make and things that we would have done differently. The first aspect we would have done differently has to do with the reason why we do not have a fully functioning version of our system at the time of turning in this report which is our capture method. We underestimated the importance of researching and acquiring knowledge in this area due to a single assumption that we made. This assumption was that the camera we purchased (12MP) would provide us with images similar to or significantly better than the test images that were taken with the (6MP) microscope camera that was provided to us. The lack of research in that domain left us unprepared when it came to the verification & validation of our system and more specifically choosing a compatible lens. We ended up having to order 2 different lenses as well as a reverse lens macro kit to convert one of the lenses into a macro lens. With our time constraints and slow shipping times we did not receive all of the parts in time to verify & validate our system before submitting this report.

The next improvement we would have liked to make if we had more time is to investigate whether or not it's necessary to block out ambient light and isolate our system from potential disturbances. We decided to develop a custom housing that allowed us to block out ambient light as well as isolate our system from potential disturbances such as air particles. Our idea was that this would help our camera provide a more consistent and noise free image to our image processing algorithm. However, throughout our verification & validation process, blocking out the ambient light has only caused more issues such as reflections off of the side of the tower and inconsistent lighting of the arena. With more time we would like to perform a variety of different tests differing the amount of light provided, tower heights, and amount of zoom from the lens. We would also like to investigate whether or not the tower is needed in the first place. The microscope camera that was provided to us, and that is currently used by our Dr. Eleni Gourgou, does not block out ambient light or other disturbances and is still able to provide a detailed image. On top of that we used a live video feed from that camera to run tests with our algorithm

which we were able to achieve a 100% success rate with as mentioned in the *Verification & Validation* section.

The final improvement we would have liked to make if we had more time is to develop a lighting component for our system instead of relying on the microscope base to provide the light. First, this would allow our system to be completely stand alone and allow it to be used by a stakeholder even if they don't have access to a microscope stage. Next, with our own lighting system we would be able to provide more consistent lighting and it wouldn't be necessary to tune the LED every time you use the system like we currently need to do with the microscope stage.

## **Post-Processing Solution**

In order to best meet the needs of our sponsor and provide as close to a final solution as possible, an alternative design was produced that utilized many of the same key features of our final design. Without a viable lens that could focus on the surface of the NGM (where the *C. elegans* traversed), real-time classification was not possible. However, since the detection algorithm had been tested successfully on the videos provided by the project sponsor (using the Olympus DP23 camera) a series of Matlab files were written to allow post-processing of the experiment. The scripts consist of one main file, a function to export the frames from the videos, and another to apply the detection algorithm. A description of this process can be found below.

The file structure for processing an experiment begins with the scripts 'NemaDIm.m', 'exportFrames.m', and 'nemaDetect.m' in the same folder (in the Matlab path). In this folder, there is a subfolder titled 'Data' which will contain the trial videos. The file type necessary for the scripts are '.avi' files, the only exportable type from the Olympus DP23. Once processing is complete, a folder titled 'Extract-NameOfVideoFile' will be created and can be found in the 'Data' folder. This new subfolder will contain the original video file as well as the folders 'Positive' and 'Positive\_Crop'. These contain the positively detected *C. elegans* as: the original frames with a red box around the control area and the frame cropped down to the control area, respectively. The figure below demonstrates these differences.

Figure 20: Samples of positively defined original frames (left) and cropped (right).



Once the file structure has been appropriately configured, the processing of the videos can begin by opening and running the 'NemaDIm.m' script. This will prompt the user to select the desired

'.avi' video file in the 'Data' folder (via a modal dialogue box). After a moment, the first frame of the video will appear on screen. The user will then be prompted to use their cursor to click and drag on the frame to draw a rectangle that defines the control area. This rectangle will remain on screen and can be moved or scaled to allow adjustments to be made. Once the user is satisfied with the location, they will double click on the interior (shaded) portion of the rectangle and the processing of the video will begin. The time it takes to completely analyze a video will be approximately 75% of the length of the video. The limiting factors on the processing time is the Matlab function 'read' which acts to extract the frames from the video as well as how many worms cross the boundary over the course of the video. The primary variables the user can adjust (if desired) are the pixel ratio threshold (used in the detection method) and the number of frames to skip when extracting. The number of frames to skip is currently set to twenty (the script analyzes every twentieth frame) which was determined based on the maximum speed of the *C. elegans* and the frame rate of the camera in use. Sample images of the control area selection can be found below.

**Figure 21:** The user defined control area will be set on the first frame. This location/frame will serve as a reference in the background subtraction used in the detection algorithm.



Through testing, a few important notes arose. First, arenas that had been sitting in place for a while (12+ hours) and had accumulated more artifacts (bubbles, streaks, eggs, etc.) had a higher rate of false positives. We believe this is due to the natural flickering that is noticed in frames creating momentary blurs in portions of the image. These blurs can shift the pixel ratio enough that the detection algorithm signals a positive. This sensitivity also works to the system's advantage as it is also capable of identifying younger, and thus smaller, *C. elegans*. Further tuning of the threshold value can increase or decrease this effect. It is worth noting that in the testing that occurred there were zero false negatives for the given threshold (0.0025).

#### RECOMMENDATIONS

The most significant recommendation for further development of this project is a deeper analysis of the lighting conditions and lens combination that allows the algorithm to best identify the *C. elegan.* By further considering the factors of light intensity, diffusivity filters, and object distance the data set would be further optimized to the most successful test conditions. In a similar manner, adding some sort of adjustable rail system on the tower (similar to a microscope focus or macro lens focusing rail) would allow the user to finely tune the focus and magnification of the lens. This improved data-set would allow for a more precise threshold with lower error rates, allowing the detection algorithm to minimize the false positives without compromising the low number of false negatives.

Another option to decrease the error rate is to implement further image classification by training a neural network to identify nematodes within the binary images. This method may be too slow to be applied to every frame but could be applied to the positively identified frames, also decreasing the rate of false positives.

The arena housing could be re-designed to accommodate more control over the environmental factors contributing to the motion of the worms. Temperature control and diffuse top-down lighting could improve the analysis and interpretation of the frames when being inspected after the trial is complete. This could also provide opportunity for further variations of the experiment. Another factor that could be redesigned is the reliance on the LED in the microscope stage. A simple design of a small box containing LEDs and the necessary filters could provide comparable trials without requiring the use of a dedicated microscope that could then be used for other tests.

Finally, once the design and scripts are optimized, a thorough analysis of the computational complexity can be performed so the optimal Raspberry Pi can be purchased. Currently, the team implemented an 8 GB Raspberry Pi 4 (the most available) to ensure this would not be a limiting factor. However, determining the minimum necessary size of processor would decrease the overall cost per unit as well as likely improve availability.

## CONCLUSION

The goal of this project is to detect and photograph the *C. elegans* migration from a chamber on the left side to a chamber on the right side via a narrow channel in the middle of the arena as seen on Figure 1, on page 1. *C. elegans* travels on the surface of Nematode Growth Medium (NGM) inside the arena as they graze for bacteria on its surface. Unlike many nematodes and roundworms, *C. elegans* impose no significant safety hazard and are non-parasitic to humans, making them safe to handle in a lab setting. [1] Also, their lifespan is as short as a few weeks, so studying aging, cell behavior, and decision-making across multiple generations makes *C. elegans* a model organism for learning about other organisms including humans. From these facts, studying about *C. elegans* migration would be a significant benefit to addressing many of the obstacles we experience in society, including causes of cancer. [6] However, major research about *C. elegans* did not start until 1963 when Dr. Sydney Brenner selected this organism for study about genetics and cellular lineage. [3] Currently, *C. elegans* can be visualized and identified, which is a great advantage to study about *C. elegans* for helping address social health issues such as aging. [4]

This system will be able to capture 2D nematode migration images via a camera mounted on top of a *Diagnostic Instruments TLB D4.1 Illuminator* microscope. Also, the system must be easy to handle by non-engineers, easy to repair (repairing timespan being less than 2 weeks), and requires less than two specialized tools without the need of removing the sensor. The total budget for this project should not exceed \$1,000. Most importantly *C. elegans* are a very small worm with 1 mm in length, so they are sensitive to vibrations, sounds over 50 dB, electric currents, and temperature changes. To ensure useful data is acquired, the system must not make such environmental changes within the migration arena. In addition, we are not planning on relying on dyes or bioluminescence to detect *C. elegans* (as this isn't a universal characteristic), so the system must be able to capture transparent *C. elegans* without the need for such methods.

Our final design utilizes a Raspberry Pi 4 as the central processor and 'sensor'. A Raspberry Pi HQ camera with a 16 mm telephoto lens is used to capture images and send them in real time to the raspberry pi. External factors are constrained using: a 2" square plastic extrusion to block ambient lighting, a 3D printed tray holds the arena to block microscope stage light from entering the walls of the arena as well as to constrain the orientation of the arena, and 3D printed mounts are used on top of the plastic extrusion to mount the camera and provide access for the lens to the enclosed arena. Once the images are sent to the Raspberry Pi, an algorithm uses adaptive thresholding and image binarization to convert the grayscale image to binary. Further analysis calculates the ratio of white pixels to black pixels within the user selected control area and subtracts off the pixel ratio from a negative frame in the same control area. Once this adjusted pixel ratio is calculated it is compared to a threshold value and if greater, the image is saved to a folder. If it falls below the threshold, the image is discarded to maintain storage space.

The individual components of this design have been validated to confirm that: the Raspberry Pi properly interfaces with the scripts and camera to read in image data and send it to the algorithm, the algorithm is able to accurately detect the presence of *C. elegans* with <10% false positives and zero identified false negatives (where a worm is present but the algorithm doesn't detect it), the arena enclosure (consisting of the plastic extrusion and 3D printed parts) doesn't impact

worm motion while blocking ambient light, and the camera module can take pictures at the necessary rate. The biggest critique of the design (and where it fails) is in the lenses tested. Both the 16mm telephoto lens and the 6mm wide angle lens had too large of a minimum object distance, where the close distance necessary to view the worms led to an inability to focus on the *C. elegans*. Without a lens functioning within these requirements the completely integrated system was not able to successfully identify the *C. elegans*. To correct this, adapters were purchased (37mm-49mm reverse macro ring, 49mm-Pentax K mount adapter, K mount to C mount adapter) to implement a macro lens through reverse telephoto lens techniques. This will allow the lens system to focus on the worms at a close enough distance they can be identified. The parts for this have not arrived at the time of this report, but the team is confident in the viability of this solution and plans on spending the coming days implementing it for our sponsor.

With significant portions of our system functioning properly, a back-up solution was developed to allow our project sponsor to still accomplish the goal of detecting *C. elegans* over the course of a trial. Matlab scripts were developed to utilize the same detection algorithm on the existing camera (Olympus DP23) by post-processing the trial videos. These videos (.avi file-type) are the same ones used to initially develop the detection algorithm so a high degree of confidence exists for this solution to work. The initial desire to avoid post-processing was due to the added steps and increased complexity, so these factors were considered in designing the Matlab scripts. This solution is able to import the video.avi files, extract the desired frames, determine the presence of a worm, and then save the frame to the appropriate folder. This process requires only three button clicks (run main script, select video.avi, confirm control area) and then is able to process and save the frames without additional input from the user. In the testing performed with this design, zero false negatives were identified and <10% false positives were identified on average. These false positives are likely due to camera flickering or undesired capture of small artifacts. A larger dataset will provide an increased confidence interval in the threshold value chosen and help to minimize these false positives without compromising the accuracy of detection.

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#### **APPENDICES**

#### Appendix 1: PRIOR REPORT PROGRESS

#### A. ALPHA DESIGN

As discussed in the Concept Selection section, it was soon identified that both the scale and biological aspect of this design would be notable constraints on the solution space. However, given the uniqueness of the subsystems (relative to the overall design) the possible solutions within the space were highly divergent. Since many of the subsystems could potentially be combined to form a functional (although not necessarily optimal) solution, the driving functions for the design were used to select the less significant systems. Thus, for the alpha design computer vision was chosen as the detection method and a Raspberry Pi 4 Camera Module was chosen for image capture. This camera will require a custom mount to fit the hardware to the microscope. These selections led to the choice of a Raspberry Pi 4 as the processor for the design as well as polycarbonate housing for electrical integration. An overview of the system can be found in Figure 22.

Figure 22: Overview drawing of alpha design (outlined in red). Notice the camera mounted to the neck of the microscope but still external to the arena.



#### **Detection Method: Computer Vision**

Computer vision as a method for aiding the research of C. elegans is well established [11][12]. Many algorithms rely on a variety of machine learning techniques to determine a variety of desirable parameters considering a nematode's locomotion and genetics [13]. However, many existing solutions rely on algorithms that have a much higher computational cost than would be desirable for simply detecting the presence of a nematode. Therefore, in an effort to minimize this cost while building on the work previously done, the fundamentals that allow these algorithms to function will be researched further. For starters, the language this algorithm will be implemented in is likely to be Python. Although other languages are certainly viable, the Raspberry Pi is specifically designed to support this language. Just as importantly, Python contains an expansive computer vision library (particularly OpenCV [14]) which provides many of the functions that most likely will be utilized.

There are two advantages to this detection method that stood out among the rest. First, the system is scalable to sizes of the order of magnitude of negative three meters. As opposed to many of the other methods considered, the most significant obstacle anticipated in scaling this method is determining the appropriate camera resolution and/or lenses necessary to adjust the field of view. This will directly impact the ability to achieve the required error rate of 1%. Second, computer vision would combine the detection and capture functions into a single subsystem. Aside from the gains of a minimalist design, this would remove the stimuli that a sensor with direct contact might introduce. There would be no anticipated increase in temperature outside of the specified bounds as well as no direct impedance of *C. elegans'* locomotion.

However, there are some disadvantages that require further examination. The computational cost of computer vision is considerable when compared to many of the other methods previously discussed, where a signal meeting a threshold might be the only necessary requirement. In a similar manner, these thresholds could simplify many fringe cases, where tangled nematodes might be difficult to decipher. These considerations, as well as others, have made it clear that moving forward, the specific algorithms chosen must be implemented with efficiency in mind. Team 22 acknowledges that there are aspects of this design still being considered and disadvantages potentially not yet known.

One method being considered for optimizing the detection is minimizing the field of view. Since the scope of this project concerns the detection of a nematode entering some control area, it is not of importance what occurs immediately following the entering of the area. As shown below in Figure 23, by reducing the width of the image being processed to 3-5x the length of an adult *C. elegan* the computational power and time of the detection will be significantly reduced from processing an image square with the height of the channel.

Figure 23: Method to be implemented to optimize computational cost by minimizing the area



#### **Image Processing**

This method seeks to analyze a given image for the presence of a nematode. The first task in implementing this is image conditioning. This process involves applying the appropriate filters

so that the image is ready to be processed. There are a variety of processes that can be used to accomplish this, but considering the relative opacity of the NGM to the nematode, the initial concepts being analyzed revolve heavily around modern edge-detection algorithms. These edge-detection filters generally work by comparing the gradient of light intensity for a given pixel to the gradients of neighboring pixels [14], like the Sobel method shown in Figure 24.a.

**Figure 24:** Grayscale image of a nematode on NGM, taken under creative copyright use for initial analysis methods. (https://en.wikipedia.org/wiki/Caenorhabditis\_elegans)



Figure 25. Edge detection methods from left to right: (a) Sobel, (b) Canny, (c) Prewitt, (d) log, (e) zerocross.



Additionally, these pre-processing conditions algorithms can be compounded, to provide larger (yet cruder) edges. It's important to remember how the errors are compounded by iterating these algorithms, however for simple detection these might be manageable. Figure 25 below shows how implementing a morphological opening, using a linear structural element, can increase the density of the edges detected.

**Figure 26.** A sobel edge detection applied to the base gray-scale image (top) and then in addition, a morphological opening using a linear structural element to fill holes (bottom).



From this point, image classification using a multi-layer perceptron will be used to determine the presence of a nematode. To accomplish this. The perceptron will be trained using images collected from the lab of *C. elegans* traversing the arena. An important aspect of acquiring this training data will be consulting the project sponsor to ensure accurate nominal orientations are considered [12].

Ultimately, this method would consist of three key steps: image conditioning apply the appropriate filters to the image, a convolution neural network to separate the object from the background, and a multi-layer perceptron to classify this image as a nematode or not as a nematode. Upon classifying an image as containing a nematode, the image would be saved to the local storage. If the output is negative, the image can either be discarded or saved for further verification.

## B. VERIFICATION & VALIDATION \*As of 11/23/2021

In order to assess our systems ability to meet the requirements and specifications, we will utilize both theoretical and empirical analysis supported by our knowledge of engineering fundamentals. We will begin our analysis and testing on the detection method which is our main design driver. The selection of our detection method will drive the selection of the capture, integration, and mounting & housing methods. Since our chosen detection method is image processing, we have a variety of ways to verify and validate it. Verification involves verifying that the image processing can indeed detect a nematode while validating involves making sure that the image processing can fulfill our 1% error rate specification. From an overview, our verification plan starts out simple and increases in complexity until we eventually test the full system on a pseudo experiment similar to that of the experiments that Dr. Eleni Gorgou intends to use our system for. For more detail on the analysis and verification of our image processing algorithm, see the *Engineering Analysis* section.

To begin, we wrote our image processing algorithm in Matlab and tested the algorithm against pre-taken images. Once this was successful, we moved onto testing our algorithm in Matlab against a pre-taken video feed which involved breaking the video feed into frames. It is important to note that the pre-taken images and video feed were not taken with our chosen camera, they were taken with the camera that Dr. Eleni Gourgou provided us. This is where we currently stand in our plan. In the near future, we plan on taking images and video with our chosen camera and essentially re-completing the testing that is mentioned above. We don't think this will be a problem at all since our camera is twice the resolution of the camera provided to us by Dr. Eleni Gourgou. On top of the testing above, we will take these images to Dr. Eleni Gourgou and the researchers in the Gourgou lab so they can visually inspect the images ensuring that they are of sufficient resolution to identify the *C. elegans*. This is the end of our verification stage.

After testing the computer vision algorithm on the frames extracted from a video provided by our sponsor, it was determined that the average ratio of black pixels to white pixels in positive frames is  $0.0087 \pm 0.0027$  and in negative frames is  $0.0012 \pm 0.0009$ . These images were processed using an adaptive threshold (to binarize the image) but no additional measures were necessary (e.g. morphological operations). Additionally, a reference negative frame was used to subtract off some of the black pixels present due to shadows and noise. Using a pixel-ratio-threshold of 0.0025 (greater indicates the presence of a nematode) the algorithm was able to accurately classify all 190 images as positives or negatives. This is a small dataset and a larger one must be considered, but with an improvement in resolution and image quality (due to arena housing) the team anticipates this method of detection to be viable.

Once we know that our algorithm is working with pre-taken images and video from our chosen camera, we will move onto validating our 1% error rate by testing our system as a whole rather than just the camera and image processing algorithm. First, we will re-write our Matlab algorithm in Python using the OpenCV library. We will then take this algorithm and upload it to the Raspberry Pi 4. At this point we will also need to incorporate our camera control which involves breaking the video feed into frames and saving the images to the SD card. Second, we will need to put our entire system together. This involves connecting the camera, SD card, and power supply to the Raspberry Pi 4. Once everything is connected, we will mount our camera to the housing and the housing to the microscope base. This is when we will conduct our pseudo experiment detailed below.

We will drop  $\sim 50$  *C. elegans* on one side of the arena and bait them with food (or another positive stimulus) on the other side of the arena. We will let our system run and once the experiment is complete, we will visually inspect the images and video feed with the researchers. This will allow us to compare the saved images, with nematodes present, to the live video feed. Although this is a lengthy process, it is necessary to ensure that our system will achieve an error rate of 1%.

We plan to use theoretical methods to analyze the other 2 methods which are the integration and electrical mounting & housing methods. For the integration method will we utilize the specifications of the micro controller and the capture method to ensure that they can be connected and are compatible. Success will be determined by whether or not they are compatible

with each other. For the electrical mounting method we will use CAD software to model the component ensuring that both the arena and camera will fit. Theoretical analysis was chosen for these methods since they are the quickest, easiest, and cheapest way to sufficiently determine if the methods will work in the intended way. The electrical housing method does not require analysis since we purchased it as part of a kit with our Raspberry Pi 4 so we know that it will be compatible.

## C. PROBLEM ANALYSIS \*As of 10/26/2021

In order to achieve our project goals, we will need to utilize our knowledge of engineering fundamentals to analyze our design and overcome challenges along the way. The most crucial part of our design and our system as a whole is the method of detecting the motion of the *C. elegans*. This is mainly due to the size of both the *C. elegans* and the environment in which the experiments will take place, as well as the strict error of 1% that we need to achieve. Although detection and imaging systems do exist, they have not been created on the size scale of our system and are not easy to use without an engineering or computer science background. In order to achieve our project goals, we plan on analyzing these larger systems as well as conduct in depth research on detection methods and more specifically, millimeter scale detection methods.

There are a few potential obstacles that we expect to encounter. The first obstacle that we are expecting is with interfacing our detection method with our imaging system. Given that we are all Mechanical Engineers and don't have immense knowledge in electronic systems, we will need to learn about these systems as our project progresses in order to create a robust and simple interface. Additionally, if we choose to use computer vision as our detection method, we will need to gain sufficient knowledge in how it works and how to implement it. In order to learn more about electronics, computer vision, and ensure our success, we plan on utilizing resources such as professors and faculty members and publishing journals and articles. Another obstacle that we expect to encounter is ensuring that the detection method can handle the behavior of C. *elegans* since that is something we cannot control. There are a few specific instances we've brainstormed that we feel are likely to occur. First, if a C. elegans stops in the way of the detection method and triggers it multiple times, we need to make sure that duplicate images aren't taken or that we can filter those images out with our image processing. Finally, if a C. elegans triggers the detection method but then turns around and goes back to the area it came from, we want to be able to give the researcher the data/images that show that the C. elegans did indeed turn around.

Given that our detection method is the most crucial part of our system, we have already begun some initial concept exploration and benchmarking. When our project was first assigned our initial concept was the use of an external sensor, such as an ultrasonic sensor, interfaced with a webcam that takes an image when triggered. Although this concept still remains a viable option, we have been able to build upon this idea as well as come up with completely different ideas. We've researched several different types of external sensors such as pressure transducers that go under the Nematode Growth Medium, Passive Infrared Radiation Sensors that mount to the microscope, and Photoelectric Sensors that mount to the 3D printed environment. As we continue to do more research and as our project develops we plan on exploring many more potential solutions as well as build upon our current ones. Benchmarking has allowed us to explore some different options, specifically, computer vision software. Computer vision software would only require a camera and would not involve an external sensor. Originally, we were skeptical about using computer vision and image processing to detect nematodes due to our limited experience in that area. However, with some research, learning, and advice from EECS professors and professor Perkin's, we feel completely different about it. We are now confident that we will be able to develop an algorithm using computer vision and image processing that will successfully detect nematodes.

## D. PROBLEM ANALYSIS \*As of 11/23/2021

Although we hope for the rest of our project to go smoothly and our system to work perfectly, that is not likely. We would be doing ourselves a disservice if we did not expect things to go wrong and have a plan for when they do.

As mentioned previously in our report, our image processing method is our most critical component and design driver, thus, if something goes wrong with our current plan we need to have an answer. Our current plan is to use the simplest algorithm that successfully accomplishes our goal error rate of 1%. We will continue to develop our algorithm which will become more complex until that goal is reached. Thankfully, with image processing there are many different algorithms and we are confident that we will be able to find one that accomplishes our goal.

Another potential problem that we are considering has to do with the developing and manufacturing of our mounting component. As mentioned previously our mounting component is going to be custom designed and 3D printed. We expect to have to make iterations upon our original design and with our quickly approaching deadlines this could lead to a potential time constraint issue. The main goal of our housing is to block out ambient light from reaching our arena so we have come up with a quick alternative solution to accomplish this if we are not able to 3D print our custom housing. We will obtain cardboard from a local store and essentially form it into the same shape as our custom housing. Although it may not be as aesthetically pleasing, it will still accomplish our main goal for the housing and allow our system to fulfill the requirements and specifications.

## E. PROJECT PLAN \*As of 11/23/2021

We plan on performing a few tests to determine the sound threshold that the nematodes can take and measure their velocity as this information will help us build and choose the components for our system. We'll also be developing a sensor design for the sensor that will be implemented in the arena to ensure that it's not interfering with the travel and behavior of the nematodes.

Our long term goal is to implement a detection method and connect it to the mounted camera on the microscope and operate the system to function independently for detecting and capturing an image for the nematodes. A challenge that we're anticipating is a failure of our system while operating to detect and capture an image for the nematodes since no supervision will be applied. This is a challenge because the life-span of the nematodes is about three weeks and a system failure where no supervision is applied will have a big impact on the study. Thus, to overcome this challenge, we're aiming to keep the system simple in terms of assembling and disassembling; as well as, allow for replacement parts to be affordable and easily obtained. Furthermore, we want to potentially include a warning system to send a warning message quickly in case of failure so that the problem can quickly be resolved. Our budget should be maintained within \$1,000 where this would cover the detection method (depending on how many we will use), the camera, the wiring, and the software.

Our short-term plan will be reaching out to EECS professors and other suppliers to identify sensor types that meet our needs. Then, we'll perform sensor testing to confirm the capability of detection. Afterwards, we will validate and verify that the other components are functional such as the electrical housing and mounting. Furthermore, we're planning on working in conjunction with Team 21 towards using a computer vision (CV) as a possible detection method.

We're still on schedule with our project plan as shown in Figure 27 and will start performing tests to validate and verify the capability of our new alpha design of detecting and capturing a nematode.

Figure 27. Floject schedule							
Week of	11/15	11/22	11/29	12/6	12/13		
DR 3 Presentation							
Dr 3 Report							
Verification&Validation							
Test Image Taking&Familiarize							
with Raspberry Pi							
Safety/Risk Assessment							
Prep for Design Expo							
Design Expo							
Final Report							

Figure 27. Project schedule

Appendix 2: BILL OF MATERIALS

Figure 28. Itemized bill of materials

Team 22 Bill of Materials	Last Updated: 12/	8/2021	]	Budget (\$)	1,000.00	Total Cost (\$) 483.8	7	
			Cost Per		Remaining	Item No (Part		
Part No Item Name	Item Description	Quantity	Unit (\$)	Cost (\$)	Budget (\$)	No) Supplier	Status	Link
CanaKit Raspberry Pi 4 8GB Extreme Kit - 1 128GB Edition (8GB RAM)	Includes Parts #2 to #10	1	169.59	169.59	830.41	B08B6F1FV5 Amazon	Received	https://w
Includes Raspberry Pi 4 8GB Model B with 2 1.5GHz 64-bit quad-core CPU (8GB RAM)	Raspberry Pi 4 board	1	n/a	n/a	n/a	n/a Amazon		
Includes 128GB Samsung EVO+ Micro SD 3 Card (Class 10) Pre-Loaded with NOOBS	Raspberry Pi OS preloaded	1	n/a	n/a	n/a	n/a Amazon		
4 USB MicroSD Card Reader		1	n/a	n/a	n/a	n/a Amazon		
CanaKit Premium High-Gloss Raspberry Pi 5 4 Case with Integrated Fan Mount	Will not be used	1	n/a	n/a	n/a	n/a Amazon		
6 Micro HDMI to HDMI Cables - 6 ft		2	n/a	n/a	n/a	n/a Amazon		
7 CanaKit Low Noise Bearing System Fan	Possibly used for cooling	1	n/a	n/a	n/a	n/a Amazon		
CanaKit 3.5A USB-C Raspberry Pi 4 Power 8 Supply with Noise Filter		1	n/a	n/a	n/a	n/a Amazon		
Set of heat sinks (one each for large, 9 medium, small)	Possibly used for cooling	1	n/a	n/a	n/a	n/a Amazon		
CanaKit USB-C PiSwitch (On/Off Power 10 Switch for Raspberry Pi 4)		1	n/a	n/a	n/a	n/a Amazon		
Raspberry Pi High Quality HQ Camera - 11 12MP	Raspberry Pi Camera	1	61.28	61.28	769.13	4561 Adafruit	Received	https://w
6mm 3MP Wide Angle Lens for Raspberry 12 Pi HQ Camera - 3MP	Lens	1	30.64	30.64	738.49	4563 Adafruit	Received	https://w
13 3D printed nematode migration arena		1	n/a	n/a	738.49	n/a Dr. Gourgo	u Received	
Flex Cable for Raspberry Pi Camera or 14 Display - 2 m	Ribbon cable extension	1	18.85	18.85	719.64	Adafruit	Received	https://w
Chemical-Resistant Rectangular PVC Tube 15 2" x 2" Outside Size, 5 Feet Long	Camera tower	1	66.89	66.89	652.75	85095K82 McMaster	Received	https://w
White Delrin® Acetal Resin Tube,								
16 Long		1	16.83	16.83	635.92	8627K149 McMaster	Received	<u>https://w</u>
18-8 Stainless Steel Hex Nut, 2-56 Thread 17 Size	Nut	1	5.26	5.26	630.66	91841A003 McMaster	Received	https://w
18-8 Stainless Steel Button Head Hex Drive 18 Screw, 2-56 Thread Size, 1/2" Long	Screw	1	11.91	11.91	618.75	92949A081 McMaster	Received	https://w
16mm 10MP Telephoto Lens for Raspberry 19 Pi HQ Camera - 10MP	Lens	1	65.53	65.53	553.22	4562 Adafruit	Received	https://w
20 Camera Mount	Camera Holder	2	n/a	n/a	n/a	n/a Team 22	Manufactured	
21 Screws and nuts		1	9.59	9.59	543.63	Home Depo	ot	
22 3D printed camera ring		1	17.99	17.99	525.64	n/a Team 22	Manufactured	
22 3D printed compare have support	Camera base		-		-	n/a Taam 22	Manufactured	
24 Foto Macro Reverse Adapter	40mm K-Mount	1	n/a 8.42	1/a g //2	535.20	Amazon	Ordered	
25 Pentax Pk to C Mount	Adapter	1	19.07	19.07	516.13	n/a Amazon	Ordered	

## Appendix 3: MANUFACTURING PLAN

Our project didn't involve any parts that needed to be manufactured in the shop, however, for our custom housing, we used SolidWorks to design the Arena Base, Tower Cap, and Camera Mount. Those parts were 3D printed using a 3D printer in the lab of our sponsor, Dr. Eleni Gourgou. Once the design was completed on SolidWorks, we converted the design into an STL file type then uploaded into a software for the 3D printer to slice the part and choose other parameters. The arena itself was provided by our sponsor, but was also a 3D printed component. Other components were purchased from McMaster-Carr, Adafruit, and Amazon. A detailed list of the specific parts and where they were purchased can be seen above in Figure 27.

The tower was manufactured from Chemical-Resistant Rectangular PVC Tube 2" x 2" with a length of 5'. The PVC Tube was cut to length using the band saw in the machine shop with various lengths of 2 inches, 5 inches, and 9 inches which were filed down to remove all excess debri. The various lengths were made to perform multiple tests for the purposes of our

experiment. The spaces were manufactured from White Delrin Acetal Resin Tube with dimensions <sup>3</sup>/<sub>8</sub>" OD, <sup>1</sup>/<sub>4</sub>" ID, and a length of 3'. From the stock, four pieces were cut to 1.5" using a special scissor from the machine shop then we used a lathe run at 1000 rpm to face each piece and get a uniform contact surface on both sides. An exploded view of the components mentioned above can be seen below in Figure 29a and an image of our system can be seen in Figure 29b.





## Assembly Instructions

In order to assemble our system, complete the steps below in order.

- 1. Start by connecting the Camera to the Raspberry Pi via the ribbon cable. To connect the ribbon cable:
  - a. Lift the black plastic piece
  - b. Insert the ribbon cable
  - c. Push the black plastic piece down
- 2. Connect the Raspberry Pi to the display screen via the HDMI cord (using the port closest to the USB-C power port) and plug in the Raspberry Pi Power Supply to the USB-C port.
- 3. Secure the camera to the camera mount using the small bolts/nuts.
- 4. Connect the camera secured to the camera mount to the tower cap

- a. Insert the 4 <sup>1</sup>/<sub>2</sub>" long Hex Drive Screw through the camera mount and tower cap
- b. Place a spacer and nut on each of the Drive Screws securing them to the tower
- 5. Upon successful completion of assembling the camera, camera mount, and tower cap insert that whole assembly into the top of the tower.
- 6. Place the arena into the arena tray then place that inside the bottom of the tower.

Following those steps ensures the successful assembling of our system. To run and operate our system, an operating manual guide was generated and can be used to provide assistance in running our system.

## BIOS

## Alex Jordan

I'm a senior studying mechanical engineering. I'm originally from Jordan and moved to the U.S. after high school. I have attended Washtenaw Community College and took most of my pre engineering courses there. I wasn't sure what I wanted to study initially, but I realized that I have a passion for math and science as I was at WCC so I thought that mechanical engineering would be the most suitable for me. Before transfering over to Michigan, I participated in UROP and worked in the Randall Laboratory on Developing a Phantom for Ultrasound Heart Imaging. Also my first year at Michigan, I stayed involved with UROP and selected another project for the whole academic year about Powertrain Strategies for the 21st century which is a yearly project that gets presented at the NCRC. After graduation, I hope to work in the auto industry as I developed a deep interest in the automotive industry while doing my research and over the summer I like to work on cars at my friend's shop. For fun, I like biking a lot and it's my main way for commuting and also exploring new places.

## **Nick Kirkpatrick**

I am a senior studying Mechanical Engineering with a minor in Mathematics at the University of Michigan, Ann Arbor. I've bounced around the Midwest throughout my life but have lived in SE Michigan for the past few years. My whole life I've really enjoyed trying to understand the finer points of what makes things work. Like many others, I'd often find myself taking things apart and asking question after question to whoever would listen. Towards the end of high school I really fell in love with math and enjoyed partaking in 'the language of the universe'. All of this, and many other things, have pushed me to study Mechanical Engineering here at U of M. I've had many positive experiences over my academic career but have mostly enjoyed the passion my peers bring to every subject. When I graduate in December, I hope to pursue a career and continue education in computational neuroscience so that I can continue to tackle complex problems and apply the background of an ME. Some of my hobbies include fishing, biking, and working on 3D Printers.

## **Brendan Miesch**

I am a senior studying Mechanical Engineering and minoring in Computer Science. I am from Macomb Township, Michigan. Ever since I was little, I've enjoyed taking things apart and learning how they work. Upon researching the different disciplines of engineering during my application process, I thought Mechanical Engineering would be the best fit for me and I haven't turned back since. As I've learned more about Mechanical Engineering and begun the job search process, my interests have taken me toward a technology and/or product development path. Through these paths, I hope to integrate both my knowledge of Mechanical Engineering and Computer Science. As of now I hope to work in either the aerospace & defense industry or the automotive industry. In the future, I plan on obtaining a Master's in Business Administration. Outside of class, I enjoy exercising/working out and actually used to be a Personal Trainer for the University of Michigan Recreational Sports Department. I also enjoy golfing, fishing, and poker.

#### Shungo Okubo

I am a senior Mechanical Engineering student from Orange, California. When I was in elementary school, I lived in Tokyo, Japan for five years where almost all the methods of transportation are electric trains, instead of cars here in the United States. I saw the difference in transportation methods in the U.S. and Japan to be very interesting, then started to learn more about trains when I was in Tokyo. When I was little, I was always curious about the high speed train system in Japan, especially the high-speed bullet train that travels as fast as 300 km/h. In my first two years at UM, I participated in the Michigan Hyperloop student project team where I started to learn CAD, basic machining, and engineering designing from upperclassmen then started to be interested in Mechanical Engineering. In 2018, our Hyperloop team got selected as the top 20 teams for the designs of the Hyperloop pod for the international SpaceX Hyperloop competition. Furthermore, we showed our actual Hyperloop pod to a general audience and even to Elon Musk at SpaceX headquarters in California. My future plan after graduation is to be an Engineering/IT Consultant. Outside of my engineering career, I enjoy playing board games, video games, and programming simple games.