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Behavior Analysis for Rapid Screening of Molecular Interactions

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2. Behavioral analysis
3. Receptor-ligand interactions

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Background.....	ZY
Project Strategy.....	DM
Design.....	DM
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Final Design and Validation.....	ZY
Conclusions and Recommendations.....	DM

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Abstract

The purpose of this project was to develop a delivery device to place multiple molecular stimuli in a *C. elegans* culture, then design an experiment to determine receptor-ligand interactions through the behavioral analysis of *C. elegans* using gVision systems and MATLAB algorithms. To do this, the team has designed a Polydimethylsiloxane (PDMS) delivery device. Adult *C. elegans* were transferred to an OmniTray, and were placed under a camera to capture the experiment. Stimuli including IAA and glycerol were transferred into a 96 well plate and the PDMS device was placed into the 96 well plate and stamped onto the OmniTray. The experiment was recorded using gVision software and MATLAB was used to analyze the video experiments. The output of the analysis included heat maps and behavioral plots. These were used in adjunction with one another to analyze the behavioral responses of *C. elegans* to the different stimuli spots. In future applications, this can be used to rapidly identify responses to stimuli exhibited by *C. elegans*, leading to a more complete understanding of receptor-ligand interactions.

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1. Introduction

Many existing neurological disorders have no known cure, and treatment of these disorders relies solely on medicines used to combat the symptoms associated with the disorder. The lack of treatment methods for these is largely due to the fact that many interactions in the brain still remain unknown. About 40% of drugs on today's market are meant to target neurological sites called G-protein-coupled receptors (GPCRs). If more of these site interactions were known, scientists would be able to explore new drug alternatives for treatment of some of these disorders, such as Alzheimer's and Parkinson's disease.

Most GPCRs are identified by their DNA sequences and thus do not have a corresponding known ligand. The identification of the ligands of these orphan GPCRs, deorphanization, is the first necessary step to understand their physiological roles. Deorphanization is a slow process where a single cloned receptor is expressed in a cell line and tested for its response to various synthesized ligands. In order to accelerate this process, genetically-engineered *Caenorhabditis elegans* (*C. elegans*), which have detectable and visible behavioral responses, have become a powerful tool in neural research.

C. elegans, a bacterial feeding nematode, is an ideal system for increasing our understanding of many fundamental biological processes as well as for studying the interactions between genes and neurons. Despite the phylogenetic distance between man and worm, *C. elegans* have been used to understand the state of diseases, mechanisms of drug action and the detection of bioactive compounds. Many researchers have studied *C. elegans*' behavior because it provides information on not only complex motor systems, but also on the indirect physiological relationships between neuronal systems and muscles.

The goal of this project was to design a deliverance system that allows for the identification of multiple molecular receptor-ligand interactions in the same culture. The system was tested through an experiment analyzing the behavioral responses of *C. elegans* using both machine vision technology and MATLAB. To achieve this goal, multiple design alternatives were created and then evaluated to find a final design and a final experimental procedure. The team conducted several experiments to assess the effectiveness of the design and procedure. Our results correctly identified multiple behaviors of *C. elegans* at specific locations on the plate. This allowed for a

baseline experimental procedure which can be a viable option for the future identification of receptor-ligand interactions.

2. Background

2.1. Nervous system

2.1.1. Structure and Components

The nervous system is the control center of the body and includes the brain, spinal cord, and all nerves throughout the body. It controls both voluntary and involuntary movements and coordinates responses to stimuli. The system is split into two separate parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The central nervous system is considered the processing center and works to interpret the signals sent via the peripheral nervous system. The peripheral system includes all nerves and functions to receive the stimuli, send them to the brain, and then initiate the responses (Ophardt 2003).

The central nervous system is composed of the brain and spinal cord and serves as a collection center for the signals sent by the peripheral nervous system. The components of the CNS are the most protected organs in the body, and serve to interpret every signal received, and therefore control all actions of the body both voluntary and involuntary. The functions of this system can be divided up into three sections; sensory, motor, and higher brain functions. The sensory component works to interpret all signals from the senses including auditory, olfactory, visual, and several other systems. The motor component controls the skeletal system, reflexes and other physical responses. The final component is responsible for all higher brain function including personality, language, memory and emotions (Dafny 2013).

The peripheral nervous system consists of all the nerves in the body outside of the brain and spinal cord, and functions to transform stimuli into electrical signals and send them to the brain. After the brain interprets these signals, it then sends a response back to the peripheral nervous system which then carries out the action. The PNS is separated into two separate systems, the autonomic and somatic systems, which control involuntary and voluntary actions respectively. The autonomic system is composed of smooth muscles, cardiac muscles and glands. The somatic system controls the skeletal muscles of the body and the sensory neurons located in skin. The autonomic system is further broken down into the sympathetic system, which increases activity, stress, and emergency situations, and the parasympathetic system which has the opposite effect and controls low energy functions such as digestion (Ophardt 2003).

2.1.2. Structure of Neurons

Neurons are types of cells that are found in the CNS and function by transferring stimuli to other cells. They are classified into three categories: sensory neurons, interneurons, and motor neurons. Sensory neurons conduct sensory information toward the CNS, interneurons work by stimulating other neurons, and motor neurons send motor information from interneurons to muscles or gland cells. Although there are some size and shape differences between different types of neurons, the structure of any neuron consists of four distinct regions. The first region is the cell body (or soma), which contains the nucleus and organelles, and thus is the metabolic control center of the neuron. The second and third regions are processes, which are structures that extend away from the cell body. The second regions are dendrites that function to receive input, and the third regions are axons that allow the flow of outgoing signals to other neurons. The final part is the axon terminal, which is found at the end of the axon and contains neurotransmitters. Neurotransmitters are the stimulants that are manufactured in the cell body and that flow from one neuron to another at chemical synapses (Lodish 2000).

2.1.3. Neuronal Signaling

Synapses are connections between neurons through which information flow occurs. The two main types of synapses include; electric synapse, where two neurons are physically connected through gap junctions, and, the chemical synapse, which is a more common way of signaling, seen in Figure 1 below. Chemical synapse occurs when neurotransmitters are released from the axon terminal of the presynaptic cell to the synaptic cleft, which is the narrow space between neurons. The diffusion of neurotransmitters in the synaptic cleft takes no longer than 0.5 millisecond (ms) and they bind to receptors on postsynaptic neurons (Lodish 2000).

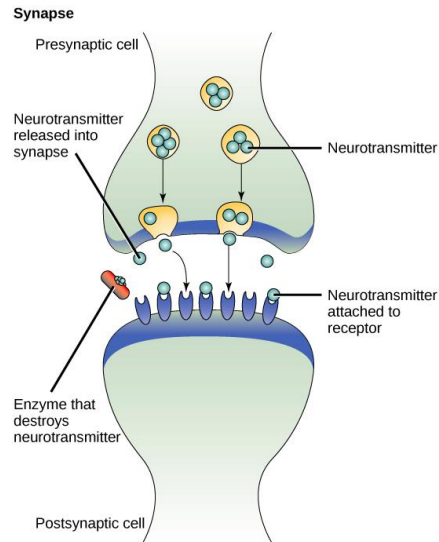


Figure 1: A chemical synapse

In complex multicellular animals, various types of neurons are involved in a single signaling circuit. Multiple sensory and motor neurons are connected to one another by interneurons. Highly specialized sensory receptor cells respond to specific environmental stimuli and send their outputs to receptors.

2.1.4. Neuropeptides

Neuropeptides are small molecules that are used for communication between neurons. They are used to signal neurons throughout the body, and can act as neurotransmitters, neuromodulators or neurohormones (“Neuropeptides.”). Neuropeptides help the brain to develop a connection with the body, and influence the brain’s response to certain signals. Structurally, neuropeptides are short sequences of amino acids and can sometimes function as primary neurotransmitters. There are predicted to be over a hundred neuropeptides in the body, and these are primarily split between two types, insulin-like, and FMRFamide related peptides. Multiple neuropeptides can be neuropeptides expressed in a single cell, and a specific neuropeptide may bind to multiple receptors.

Neuropeptides are formed from a larger molecule which cleaves to form single mature neuropeptide. This cleavage can result in the formation of several distinct neuropeptides, a single

neuropeptide, or different sets of peptides in different cells (Li and Kyuhyung 2008). Many of the functions of these neuropeptides are still unknown, and scientists are looking to further identify them to create advancements in medicine. To identify these, a variety of methods have been explored, including bioassays, radioreceptor assays, and mass spectrometry. (Mains and Eipper 1999).

In brain disorders like Alzheimer's and Parkinson's disease, where abnormal cognition and behavior are observed, the study of neuropeptides is particularly useful because altered neuropeptides can function as biomarkers or they can be targets for novel drugs. It has been observed that in these cases of neurological disorders, there is a reduction in neuropeptide function, however the association of these two variables remains unknown (Beal and Martin 1986). Thus, most downstream neuron targets are unclear and the process of finding the receptor-neuropeptide interactions is time consuming.

2.2. Molecular interactions

2.2.1. G-Protein-Coupled Receptors

G-protein-coupled receptors (GPCRs) are a family of proteins which function to transform external stimuli into signals in the body. They react to a variety of stimuli including light, neurotransmitters, odorants, lipids hormones and several others which have not yet been fully determined. Once stimulated, the GPCRs activate a signal transduction pathway starting with the binding of a ligand to a receptor (Kroeze and Sheffler 2003). GPCRs are also important for drug delivery within the body, and the investigation of many diseases. Several diseases are known to be associated with these receptors and currently, 40% of all drugs on the market target GPCRs. Although there have been recent developments of the identification of receptor interactions, many of them remain unknown, hindering developments in medicine and neuroscience. Scientists and researchers are currently looking into new methods of identification to further these fields (Albrecht 2013).

2.2.2. Receptor Ligand Interactions

All cells in the body rely on a signaling pathway in order to respond properly to stimuli and carry out normal life processes. This intracellular network works with the nervous system to direct the signals throughout the body either through cell to cell contact or through signaling molecules. The

signal sent through these molecules is called the ligand. A ligand binds to a receptor located on a target cell through a “lock and key” method. The ligand is specifically shaped, similar to a key, to fit in individual receptors which work as the lock. The ligand binds to the receptor site and changes the conformation of the receptor, igniting the signaling pathway (Zuk and Geffen 2013). Receptor-ligand interactions play a significant role in many biological processes and aid in the development of new drugs for the treatment of complex neurological diseases such as Alzheimer’s and Parkinson’s. However, many of these receptor-ligand interactions are currently unknown, and most of current methods are limited to *in vitro* testing.

2.3. *Caenorhabditis elegans* (*C. elegans*)

2.3.1. Nervous System

The nervous system of *C. elegans* has been completely mapped out with three hundred and two neurons in their system, and two hundred and eighty two of these neurons belong to the somatic nervous system (Altun and Hall 2011). A mass of these nerve cells, known as ganglia, are located in the head and tail and are then organized into a spinal-cord like ventral nerve cord down the body of the *C. elegans* shown in Figure 2. *C. elegans* have a complex system in wiring from neuron to neuron. Their system also expresses a vast number of signaling molecules, such as neuropeptides and their specific receptors. Of the 1000 G-protein-coupled receptors in *C. elegans*, over 50 of them are likely to be neuropeptide receptors (Bargmann, 1998). Certain studies on the *C. elegans* system have determined a single neuron can express fourteen different receptors and ten different neuropeptides (Hobert 2010). Of the three hundred and two neurons identified in *C. elegans*, eight are dopaminergic neurons. These can easily be highlighted with green fluorescent protein (GFP) and visualized in the transparent body of *C. elegans*. This capacity of determining the presence or absence of neurons in the system is highly useful when studying Parkinson’s disease. Mutations in the α -syn neuron have been found to increase α -syn aggregation and also increase dopaminergic neuronal death. In 2003, a study similar to that of Alzheimer’s disease was done in which the overexpression of α -syn was present under the pan-neuronal promoter (*aex-3*) and the motor neuronal promoter (*acr-2*). Once expressed in the *C. elegans*, a severe reduction in motor movement was observed. This behavioral change establishes *C. elegans* as a good model system to test future drugs in a positive cure for Parkinson’s disease (Harrington and Hamamichi 2010). The system of *C. elegans* processes a

variety of sensory stimuli such as odor attractants or repulsions, water-soluble chemicals, food sources and temperature. These stimuli are processed and then lead to distinct behavioral outputs. Behaviors such as movement, feeding and defecation are all responses to the stimuli, and can change through learning and memory. The neuropeptide and receptor interactions are dedicated to each one of these behaviors, in which can be observed and analyzed in *C. elegans* (Altun and Hall 2011).

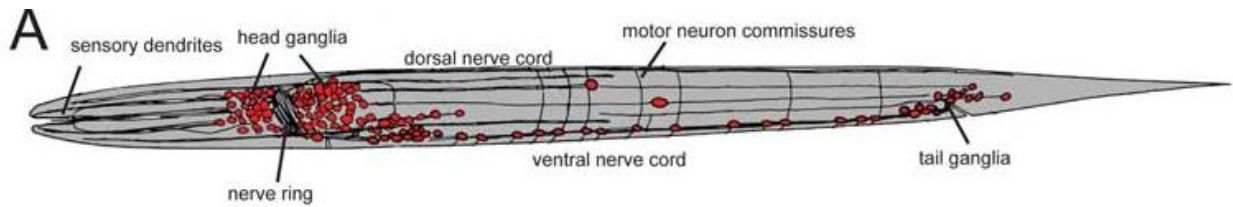


Figure 2: A map of the body of *C. elegans*

2.3.2. Benefit of the *C. elegans* System

The microscopic nematode *C. elegans* is a suitable model for neuropeptide signaling network studies since its isolation in 1963 (Brenner, 1974). In 1998, *C. elegans* became the first multicellular organism to have its entire genome sequenced in a joint project by The Wellcome Trust Sanger Institute and The Genome Institute at Washington University (St. Louis) (*C. elegans* Sequencing Consortium, 1998). Since then, genome-wide comparison of the predicted *C. elegans* genes and their vertebrate equivalents have disclosed significant resemblances between nervous systems. The simple nervous system of *C. elegans* with 302 nerve cells and 8,000 synapses are actually very similar to a vertebrate's nervous system (Hodgkin). It has been found that 60-80% of human genes have corresponding genes in *C. elegans* (Culetto 2000). Other resemblances include the neuronal specification, which is a homeodomain protein code in both, the axonal guidance (UNC-6, UNC-5, UNC-40 etc.) and the neurotransmitters such as acetylcholine and GABA (Hodgkin).

C. elegans are easy to maintain in a laboratory environment using *E. coli* bacteria as a food source at temperatures between 12 °C and 26 °C with a life-span of two to three weeks (Stiernagle, 2006). Frozen strains of *C. elegans* can be stored for more than forty years. The fast life cycle of *C. elegans* is temperature dependent, at 20 °C, embryogenesis occurs in approximately twelve hours and takes about three days for a *C. elegans* to reach adulthood (Frooninckx, 2012). Means of

reproduction for *C. elegans*, a powerful genetics tool that is commonly utilized in high-throughput genetic tests, is either self-fertilization or mating with males. Self-fertilization leads to homozygosity of alleles; hence, new worms are genetically identical to the parent (Sommer, 2005). The development and anatomy of *C. elegans* is well known in detail since the nematode is transparent at all stages of development. The transparency of the worm benefits *in vivo* experiments that use fluorescent proteins to examine the axon guidance, cellular architecture, and neurodegeneration (Chalfie 1994).

Although *C. elegans* have less than a thousand body cells when fully grown, they have well-differentiated muscles, nerve tissues and gut cells. Therefore, it is a simple model compared to more complicated animal models with the same features (Frooninckx 2012). Their small size (about 1 mm) allows researchers to culture the *C. elegans* and observe the nematodes under extensive electron microscopy and high-throughput screening. Animals can also be used in microfluidic devices while drug screening in small volumes (less than 10 μ l) is feasible. Another advantage of *C. elegans* is that the multicellular nematode uses GPCRs expressed in chemosensory neurons in order to detect food and environmental stimuli. This makes *C. elegans* an ideal candidate to study *in vivo* receptor-ligand interactions.

2.4 Current Techniques to Identify Receptor-Ligand Interactions

Current methods to study receptor-ligand interactions in mammals are mostly *in vitro* systems, which do not always represent an accurate reflection of *in vivo* interactions. Thus, the use of *C. elegans* to investigate these interactions is valuable.

In order to detect peptide ligands for a particular receptor, researchers have developed a technique called ‘deorphanization’. Deorphanization describes an orphan receptor by identifying its endogenous ligand. Orphan receptors are defined by their sequence only, thus information on their ligand and physiological role is unknown. Research on ligand identification may have various difficulties such as identifying the receptor itself, developing a specific assay and gathering information on the nature of the ligand.

One approach to find the activating ligands of a GPCR is to use reverse pharmacology by expressing the orphan GPCR in a heterologous expression system. The recombinant system of choice is often the Chinese hamster ovary (CHO) or human embryonic kidney (HEK) since these

are easy-to-use and show functional GPCR expression (Szekerers 2002). The receptor expressing cells are tested with a library of compounds based on bioinformatic predictions and activation of GPCR is measured.

One of the most widely used methods for receptor deorphanization is the calcium mobilization assay based on detection of intracellular calcium that is released when a receptor is activated. This method can be combined with the co-expression of a prominent G protein, which can direct intracellular signaling of the activated receptor through a calcium flux (Offermanns and Simon 1995) or with chimeric G proteins that can guide the signal cascade to the desired pathway. After the calcium mobilization assay, the resulting calcium flux is detected by bioluminescent proteins or by fluorescent calcium indicators. Omitting the prominent G-alpha16 protein identifies the endogenous G-alpha signaling protein.

The first neuropeptide GPCR to be deorphanized in *C. elegans* is the neuropeptide receptor 1 (NPR-1) (Kubiak et al., 2003; Rogers et al., 2003). NPR-1 is the homolog to the vertebrate NPY receptor family that is associated to physiological processes such as food intake and stress (Heilig, 2004; Arora et al., 2006). It has been found by various studies that the NPR-1 in *C. elegans* is implicated in food-dependent behaviors, thermal avoidance, ethanol tolerance and innate immunity (Bargmann et al., 1998).

A study by Chalasani et al has found that, when the food source is taken away from the worms, *C. elegans* will search for the food by a turning behavior with increased rates. Another group of *C. elegans* were glutamate-depleted mutants and no increase in turning was recorded. These results demonstrated that the *C. elegans* behavior is dependent on the activity of AWC olfactory neurons, which release glutamate and also the neuropeptide nlp-1. So, the authors concluded that glutamate was necessary for increased turning rates when looking for food and the nlp-1 decreases glutamates effect.

Teng et al. used *C. elegans* in order to analyze the expressed mammalian GPCRs in the ASH and ADL gustatory neurons in *C. elegans*. They chose ASH and ADL neurons for various reasons. These neurons are directly exposed to the environment, which makes them accessible protein and peptide ligands to the heterologous receptors. They also express a large variety of G-alpha subunits that enhance the possibility of GPCR-G-alpha protein interaction. ASH and ADL neurons drive repulsive responses to an experimental setting made by robust behavioral assays, this way the

receptor activation can be observed when ligand exposure response is avoided. *C. elegans* avoid toxic chemicals by reversal behaviors which are arbitrated by sensory neurons such as the ASH neurons (Bargmann, 1990).

Studies on *C. elegans*, such as the ones described above, provide information on unknown peptide-receptor interactions and their signal transduction pathways. By testing the behavioral responses of *C. elegans* to human ligands, the interaction of receptors to drug candidates can be tested and novel drugs can be discovered for neurological diseases such as Alzheimer's and Parkinson's.

2.4.1. Behavioral Assays

Different goals in research led to *C. elegans* experiments in various environments, such as a soil-system or a liquid environment. The latter was performed in order to gather information on the organism's behavioral responses to external stimuli, which is also what our project is searching for. Behavioral responses reflect activity in the nervous system and are powerful tools to understand neuronal function. The earliest examinations of *C. elegans* behavioral responses to chemicals used point sources of concentrated stimuli to generate broad spatial gradients by chemical diffusion through agar or air. However, these gradients were variable over time and the behaviors of nematodes were affected by small changes, so the data wasn't consistent. In order for behavioral assays to work successfully and to achieve statistical significance, multiple assays need to be done. The feeding status and cultivation conditions should be consistent in all experiments; possible environmental factors such as room humidity, temperature, and assay plate dryness should be controlled and recorded. *C. elegans* behavioral assays can be combined with current molecular biological techniques to understand the behavioral responses to specific neurons and consequently, to identify unknown ligand-receptor interactions.

2.4.2 Tracking Systems to Analyze *C. elegans* Behavior

In order to find out more about the genes and ligand-receptor interactions, behavioral assays are combined with tracking systems and recording techniques. Tracking systems work by measuring certain parameters that define behavior; such as the average speed and the fraction of *C. elegans* in a specific spot. All trackers rely on high contrast images, which can be generated by a simple microscope. Existing trackers can be classified according to the information used from the video

frames about worms such as the centroid position (Dusenbery 1985, Bargmann 1998), or a curve that corresponds to the central skeleton of the worm's image (Cronin 2005, Baek 2002, Geng 2003). Centroid-based trackers can define multiple worms at low magnification. They can follow single animals over minutes or hours. The limitation of centroid-based trackers is that they only provide limited information about the details of worm posture and they cannot easily distinguish between forward and reverse movements of the *C. elegans*. On the other hand, skeleton-based trackers operate at high magnification and can derive a skeleton of each worm from segmented binary images (Cronin 2005, Feng 2005, Tsibidis 2007). The skeletons of the *C. elegans* provide information about the posture and also they are widely used to classify mutants that interrupt locomotion (Geng 2004). In order to ameliorate the tracker function, research has moved on to implement tracker systems that would be able to identify multiple animals at once. MATLAB®, a numerical computing environment and a fourth- generation programming language, has been widely used by researchers to implement and record multiple worms in sequential video frames. Tracks were used to compute worm speed, angular velocity and behavioral responses, as well as the turning events or pirouettes. However, current trackers still lack the ability to track multiple stimuli at once.

2.5 Behavioral Analysis for Rapid Screening of Molecular Interactions

Due to the thousands of unknown receptor-ligand interactions, there is a need for a method to rapidly identify these, as the current method can only examine one at a time. As shown by Teng et al. it is possible to identify molecular interactions through the change in behaviors exhibited by the animals. The aim of this project is to identify multiple molecular interactions simultaneously as well as to develop a quantitative behavioral analysis to increase the efficiency of identifying the thousands of potential receptor-ligand interactions. In order to achieve this, a delivery device needs to be designed to deliver multiple stimuli to a *C. elegans* culture simultaneously. Then behavioral experiments need to be conducted to analyze the behavioral responses of *C. elegans* to different stimuli, which will be recorded by machine vision systems and analyzed through MATLAB. If this project is successful, this method can be used by researchers to analyze behavioral responses to different stimuli and to identify unknown receptor-ligand interactions *in vivo*.

3. Project Strategy

3.1. Initial Client Statement

Professor Albrecht developed an initial client statement to narrow the scope of the project. He was interested in a novel way to test multiple neuropeptides or other ligands in a single system as the system currently in practice by colleagues utilizes a binary comparison. Since it is only able to identify one receptor-ligand interaction at a time, it has proven to be time consuming and inefficient. The goal of this project is to develop a system to test multiple receptor-ligand interactions in parallel within *C. elegans* and analyze the data to determine positive or negative interactions. The initial client statement created by Professor Albrecht is as follows:

“Design, implement, and characterize a system to identify multiple molecular receptor-ligand interactions in parallel by behavioral analysis of genetically-engineered C. elegans nematodes.”

3.2. Objectives

A series of objectives for the project was derived from the client statement. The team created an objectives tree to organize primary objectives and sub-objectives. The objectives tree of the design and experiment can be seen in Appendix A. In order to simplify the project the objectives were ranked by design and experimental subsets. The design subset incorporates our engineering device while the experimental subset incorporates our biological testing with the system and MATLAB. The primary objectives for the design subset are that the system needs to be spaced evenly, reusable and simple. The primary objectives for the experimental subset are that it needs to be reliable and consistent.

The team utilized a pairwise comparison chart (PCC) to determine the order of importance for our main objectives. A pairwise comparison chart is created by the objective in the row receiving a 1 for being more significant, or a 0 for being less significant than the objective in the column. The objective receives a 0.5 if the objectives are of equal importance.

	Spaced Evenly	Reusable	Simple	Total
Spaced Evenly		0	0	0
Reusable	1		1	2
Simple	1	0		1

Table 1: Pairwise comparison chart

The team determined, through use of the pairwise comparison chart shown in Table 1, that it is most important for our design to be reusable. The system created must be easy to clean and reused to repeat experiments. Simplicity was determined to be the second ranked objective and it was defined to make the experiments easier and more time efficient. The third ranked objective was that the delivery of the stimuli be spaced evenly so the samples do not interact with one another.

The objectives for the experimental subset were determined by the team as one being more important than the other. The main objective for the experiment is that it must be reliable. The experiment must be highly selective, but also have the ability to accomplish the goals set. Secondly ranked was consistency because the experiment needs to deliver the same results through multiple trials.

3.3. Functions

To ensure that the project meets the objectives set, there are a number of functions that the design and experiment must perform. For the design, the system overall must be able to deliver multiple stimuli simultaneously. This is to determine if specific concentrations and if multiple stimuli can affect the behaviors of *C. elegans*. The design must also not penetrate the culture surface of the *C. elegans*. If the design penetrates the culture, the *C. elegans* can burrow into the agar, and their behavior cannot be determined, thus data will not be collected. Lastly, the *C. elegans* must have independent stimuli interactions. This specifies that the response from the *C. elegans* to the stimuli must be independent and not affected by the surrounding stimuli. The primary function for the experimental design is that it must detect correct behavioral responses. The MATLAB coding has

to recognize the *C. elegans* behavior to the stimuli so the team can correctly analyze a positive or negative behavioral response.

3.4. Constraints

Certain constraints were determined in order to develop the best design for the experiment. One restriction is the lens size of the camera that will be used to capture the experimental procedure. Currently, the lens size available is not able to view a larger plate that is envisioned for the experiment. The code is also a constraint as it does not give complete analysis about the behavior of the *C. elegans* at specific locations. This portion of the code will need to be added if possible to convey the information necessary for the behavioral analysis. The final constraint determined is the diffusion of the liquid stimuli. The liquid has the potential to diffuse faster than desired causing the experimentation time to decrease.

3.5. Revised Client Statement

The initial client statement was reviewed to better incorporate all aspects of the project. The revised client statement now includes the device created to deliver the stimuli and the designed experimental procedure.

“Design, implement, and characterize a deliverance system to place multiple molecular stimuli in a culture. Then design an experiment to determine receptor-ligand interactions through the behavioral analysis of genetically-engineered C. elegans using machine vision systems and MATLAB to identify these responses.”

3.6. Project Approach

3.6.1. Technical

In the technical approach to the project, we decided to separate it into two sections: device design and experimental design. This was done to break up the project and better understand our approach to each of the challenges given in the project.

The team wanted to first design a deliverance system to place multiple stimuli in an OmniTray plate containing a *C. elegans* culture. An OmniTray is a petri dish that can hold a *C. elegans* culture and is large enough for 96 stimuli to be delivered to. It is the ideal plate for our experiments to be

conducted on due to its properties. In order to satisfy the objectives of our design, the team's approach is to use a 96 well plate as a mold. This mold will ensure the spots of the stimuli are spaced evenly on the plate and that they will not interfere with each other. The interference of these spots would skew our data on the behavioral interactions of the *C. elegans*. Using an accessible mold would also make our design simple and easy to rebuild again if it fails with multiple experiments. As mentioned previously, the design cannot puncture the agar of the *C. elegans* culture, so the team plans on using a soft and flexible material to deliver the stimuli. To meet all the objectives, the design will also have to be reusable so the material can be washed off to repeat experiments easily and in short time periods. The team will use the design evaluation matrix, shown in Appendix B, to narrow down possible options for a design. The top row explains the basic design choices, while the left column contains our objectives and functions. The two are then compared to find the design that best satisfies the objectives and functions.

With the use of our device, the team will perform experiments with the *C. elegans* to analyze their responses to the stimuli present. The design created would extract stimuli from a 96 well plate and place those stimuli on the *C. elegans* culture plate at the start of the experiment. The experiments would then be captured by machine vision systems and later analyzed. MATLAB will be used because that is the preference of our client, and it also is widely used in biomedical applications to analyze data. The team has received a MATLAB code from Professor Albrecht's colleague, Navin Pokala (Pokala 2014) which can be modified in order to fit our experiment. The team will need to modify the code so it calibrates to the scale of an OmniTray plate that is desired for the project. The original code outputs several PDF plots of the behavior of the *C. elegans* along with a series of variables for each of the animals such as the speed, X and Y coordinates, the behavior, the size of the nematodes, and several other variables. The team is most focused on the coordinates and the behavior of the nematode when in contact with the stimuli, therefore new scripts would be added to the code to visually represent these variables.

3.6.2. Management

In order to manage project tasks, the team has developed a Gantt chart, which is a common technique that represents the phases and activities of a project along with a work breakdown structure (WBS). A Gantt chart and the WBS can be found in Appendix C and D respectively.

The team met weekly with the advisor (client), throughout the duration of the project. During the first meetings, the initial client statement was proposed to the team, the team had the chance to generate questions and conduct a client interview, which led to a revised client statement. As the project moved on, design alternatives, as well as the experimental approach were reviewed with the client. At the end of the project, the results gathered from the experiment, which was conducted by using the designed device, were presented to the advisor.

The team conducted literature reviews and background research throughout A term in order to learn more about the background of the project and be able to develop the design alternatives. The team started working in the lab in B term in order to generate the design alternatives and have hands-on experience with the *C. elegans* and the experimental setup. The team became confident with transferring worms to maintain a culture that would be essential for conducting the experiments. Design alternatives were created and measured to find the best option. Working on MATLAB started at the beginning of C term. As the project moved on and more data was gathered, the MATLAB portion of the project became essential. The team re-explored the code that was given, made some changes and went to MATLAB Help hours in order to find the best way to analyze and represent the results. During D term, the team gathered all the results, analyzed and interpreted them. The report was finalized and a PowerPoint presentation was given at the WPI Project Presentation Day, on April 24, 2014.

3.6.3. Financial

The project cost was broken down to determine where the money in the budget would be designated. The team planned on casting our mold with a polymer solution in order to make the design flexible. Casting a polymer solution is roughly \$10 each time, and the team casted the mold 5 times to obtain the final design. This total would come out to be about \$50 for casting the polymer solution. Another cost to be considered is the plates used to conduct the tests and the agar needed to fill them. “Worm plates” will be used for preliminary testing, which will amount to \$36 for 500 plates. We estimate that only one set of worm plates will be needed to maintain our *C. elegans* culture. Further testing on a larger scale will require OmniTrays, which have the same dimensions as a typical 96 well plate. Sixty of these could be purchased for about \$200, and this would be a sufficient amount to conduct our testing. Finally agar will be needed for each of the plates, costing approximately 13 cents for approximately 35ml. About 90 ml of agar is poured into the

OmniTrays, and the team poured about 10-15 OmniTrays of agar for the experiments. Just for the agar poured into the OmniTray, the cost is about \$4. The MATLAB certificates and code will not be a cost to the team and neither will the supplies readily available in the lab.

Other costs to be taken into account include prototyping and manufacturing costs, and consumer prices. The estimated manufacturing cost of each deliverance system is about thirty dollars, which includes the cost of one casting of PDMS and any additional materials needed. Finally, the estimated consumer price of the system is about \$70, which accounts for manufacturing cost while still allowing for a profit margin gained.

4. Design

4.1. Device Design

One of the most important steps in the design process is generating design alternatives. Having multiple options prevents design failure and as a result, allows the optimal design to be as flawless as possible. This chapter illustrates multiple design options for the device, which were generated by the group through brainstorming, basic illustrations, and a design evaluation matrix. This section will also outline the experimental design generated through seeking the best methods in making the experiment the most accurate and efficient.

On the market now, there is a device called a 96 Well Transfer Device shown in Figure 3. This device can transfer liquid from a 96 well plate. The device uses small needles on the end to pull up the liquid and transfer it. This device would be perfect for our experiment, but the problem is that it costs about \$1,000 which is expensive since we want our device and procedures to be reproducible. Also, the needles on the end would puncture our culture surface preventing the *C. elegans* from being tracked if they burrow into the agar. Our team decided to base a cheaper, feasible and reproducible device based on the 96 Well Transfer Device that is on the market. The group decided to divide the delivery device by the categories shown in Table 2 to focus on each part separately and make the selection process easier.



Figure 3: One Lambda Device www.onelambda.com

Use of a mold
Material used for the mold
Shape and dimensions of the mold
Number of tips
Distance between tips

Table 2: Categories for device design

Each of the categories was broken down into candidate materials that could be used for the final design. Alternatives were evaluated based on their satisfaction to the team’s objectives, functions and the revised client statement. Specifically, the design needed to accomplish the system’s main function of delivering multiple independent stimuli at once.

The team decided to use a mold for the delivery device to make the design simple and easy to rebuild if multiple devices are needed or if device fails with multiple experiments. The device could then be made out of a material which flexible enough to be dipped in the stimuli, yet stiff enough to be handled without bending. The team was looking for a mold shape that could be handled easily and also had multiple tips to deliver multiple stimuli. The team came to conclusion that with some minor adjustments a 96-well optical reaction plate would work. A 96-well optical

reaction plate has 96 tips that could potentially deliver up to 96 stimuli spots. This plate was an ideal mold because the stimuli spots spaced evenly when delivered.

The material selected for the device was Polydimethylsiloxane (PDMS) mainly because of its flexibility and availability in the lab. As mentioned earlier, the device cannot puncture the agar of the *C. elegans* culture, so the device needed to be soft and flexible for the delivery of stimuli. PDMS is a widely used non-biodegradable silicon-based organic polymer. It is a clear viscoelastic compound that is inert, non-toxic and non-flammable. The external surface of a solid PDMS sample is hydrophobic; therefore aqueous solvents do not infiltrate or swell the material. PDMS can be washed off to repeat multiple experiments, or autoclaved providing a reusable device for multiple experiments.

4.1.1. Design Alternative 1

The first design alternative was to mold the 96-well plate as it is, as seen in Figure 4. The team followed the steps shown in Appendix E.

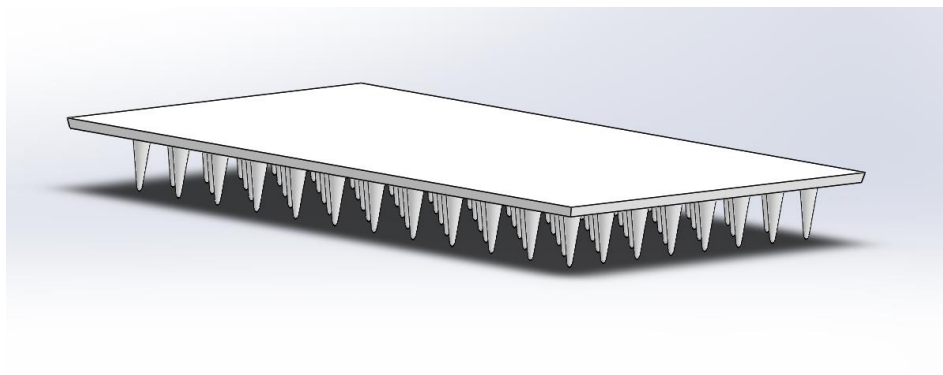


Figure 4: CAD representation of design alternative 1

When taking the PDMS out of the 96-well optical reaction plate, the edges were stuck to the plate and thus some of the tips ripped off. This can be seen in Figure 5, where the device ripped and therefore did not contain all 96 stimuli spots.

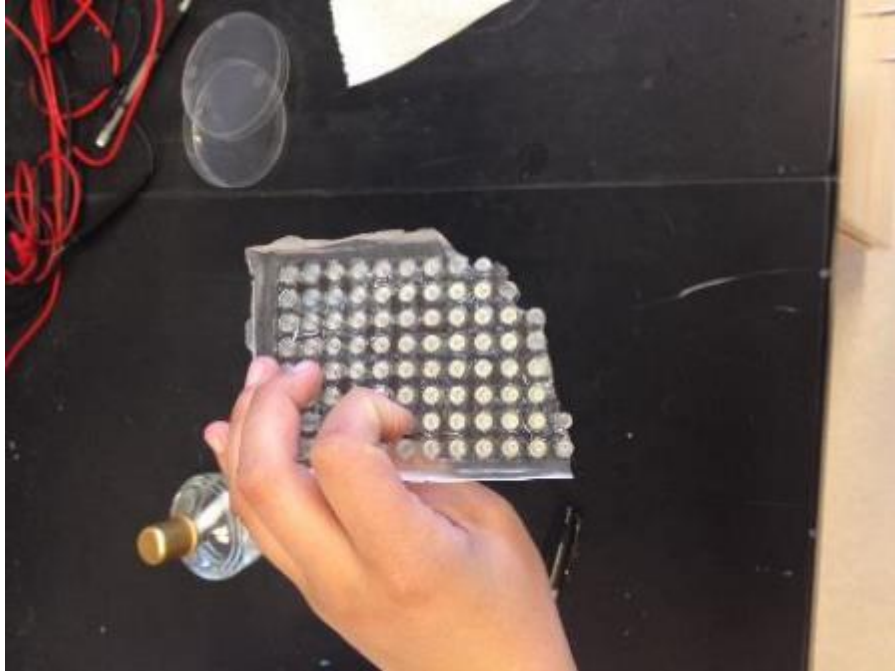


Figure 5: Ripped design alternative 1

The PDMS was casted one more time to ensure the device was carefully removed from the 96 well plate mold. Below in Figure 6 is a picture of the device removed from the mold. Although the device did not rip, the device was extremely flexible and unable to be held to keep the tips equidistant.

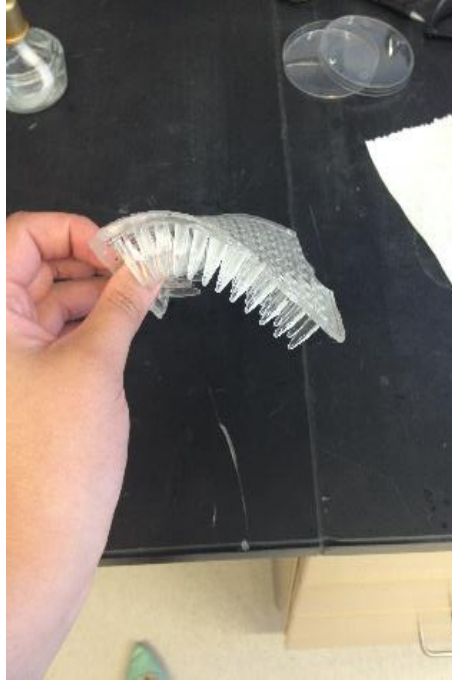


Figure 6: Flexibility of design alternative 1

4.1.2. Design Alternative 2

The next alternative was to improve the stiffness of the PDMS device by a glass sheet on top of the surface of the current device. There would be another layer of PDMS on top of the glass sheet. This design alternative is illustrated in Figures 6 and 7. This design alternative was eliminated because it was determined that the team would not be able to remove the PDMS from the 96 well plate mold due the flexibility being reduced.

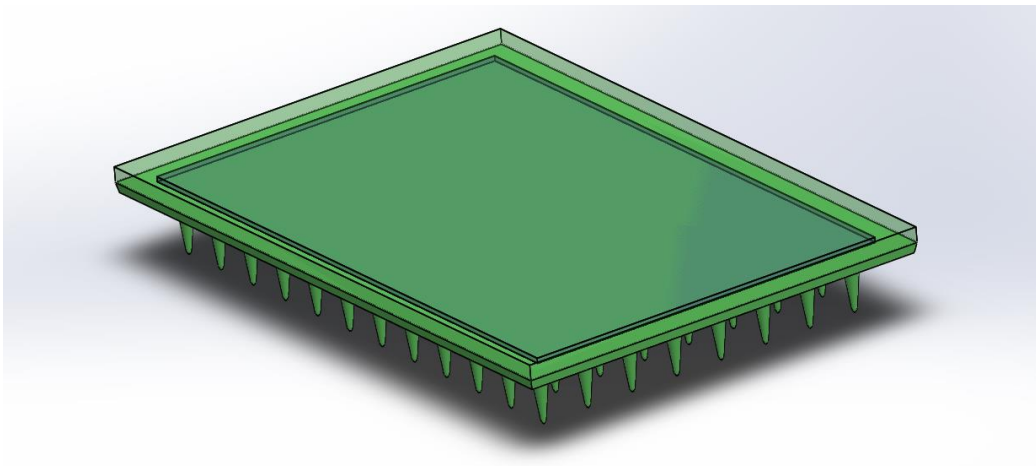


Figure 7: Top view of design alternative 2

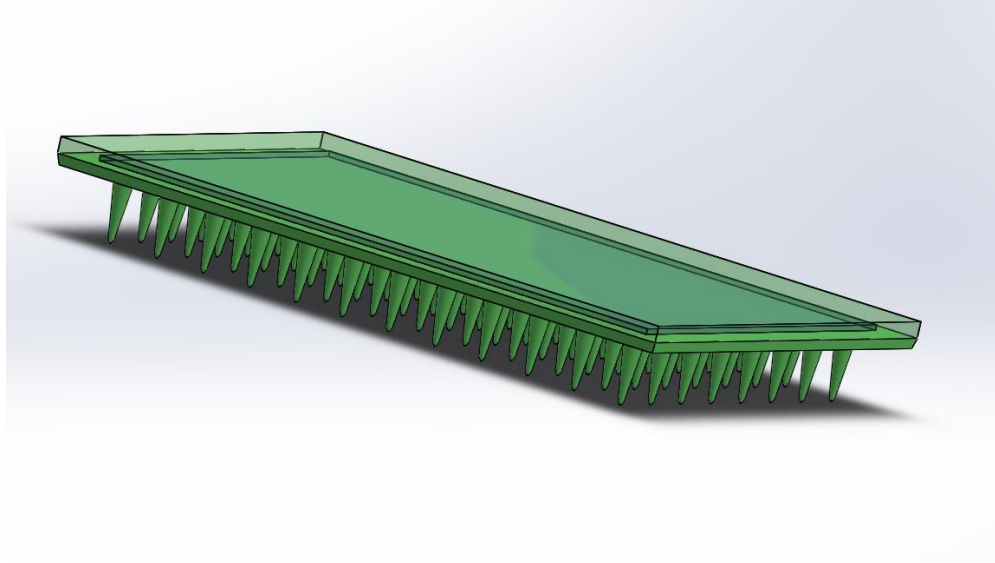


Figure 8: Side view of design alternative 2

4.1.3. Design Alternative 3

The third design alternative was to make the PDMS mold stiffer by changing the molding steps that are described in Appendix E. Instead of molding the PDMS with 90% of the silicon elastomer base and with 10% of the silicon elastomer curing agent, the team decided to do the molding with 88% of the base and 12% of the curing agent. This alternative was not carried out in the lab as changing the proportions of the components could potentially affect other properties of PDMS such as making the PDMS brittle and not successfully removing the full device from the mold.

4.1.4. Design Alternative 4

The final design alternative was to still improve the stiffness of the design by giving more thickness to the 96-well plate, as seen in Figure 9. This way, the mold could be taken out easily without ripping off any of the tips and also there would be less bending that will make the device easy to handle. In order to solve this problem, the team decided to tape the edges of the plate and cast it following the same steps.

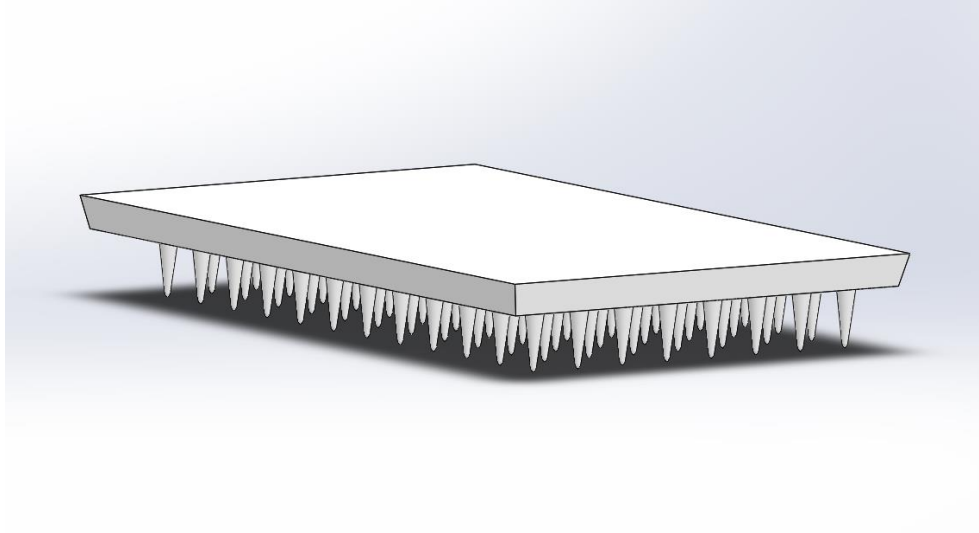


Figure 9: CAD representation of design alternative 4

Using this design, the device was easily pulled out of the mold without damaging any of the tips. This design provided a much stiffer mold, and the tips were able to be properly inserted into a 96 well plate, therefore, this alternative is a viable option for the delivering stimuli. The finished product is shown in Figure 10.

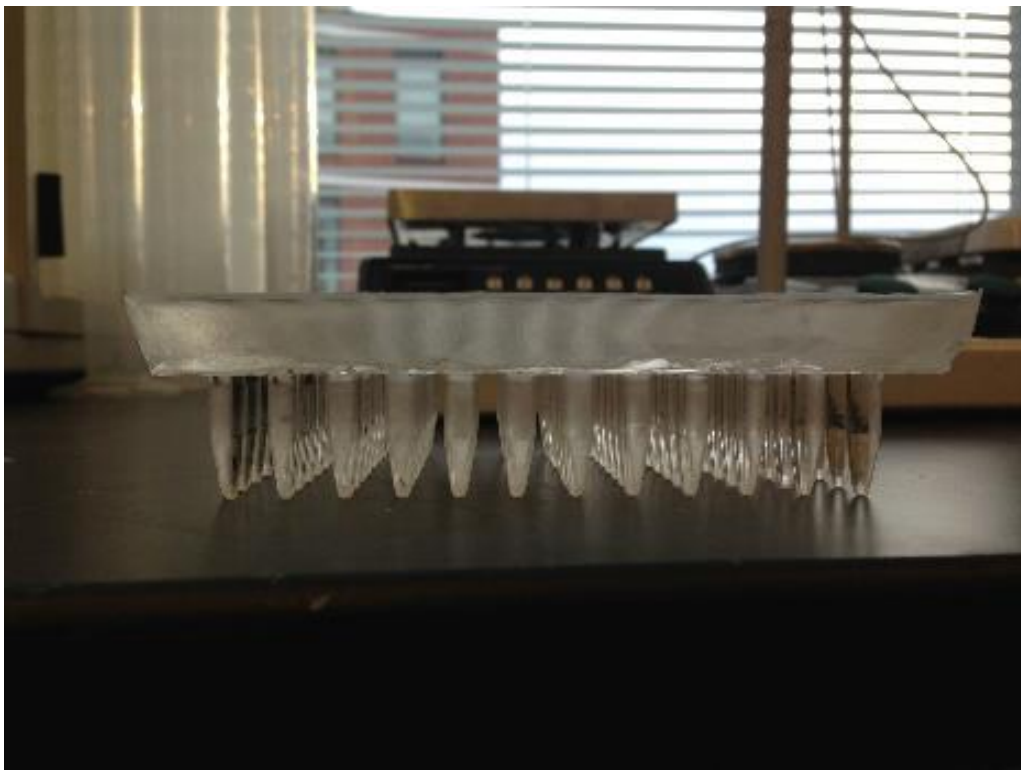


Figure 10: Design alternative 4

4.2. Experimental Design

The next part of our project was designing an experiment that could be used to successfully track the behavior of the *C. elegans* at each stimuli spot. The parts considered were the most successful way to transfer the *C. elegans*, the transfer of the stimuli, capturing the experiment and analyzing the experiment.

4.2.1. Transfer of Worms

The first part in our experiment was determining the best way to deliver the *C. elegans* from one plate to another. This is accomplished first by a technique called “worm picking.” Our team used this technique multiple times to grow a *C. elegans* culture. It allows transferring worms by using a picking tool from a seeded plate or *E. coli* containing plate to another seeded plate. This would feed the *C. elegans* and allow them to grow and reproduce. When the plate was starved, meaning no food was left; the *C. elegans* would die or not grow into the adults that were useful for our experiments. In order to keep a culture of worms that were useful for our experiments, the team “worm picked” about every 2 days.

For our experiments, the team wanted to deliver multiple spots to a large agar plate. The worms were grown on a 60 mm petri dish to maintain the culture but the team wanted a larger plate to deliver the 96 spots at once. It was decided to use OmniTray plates that are 128x86 mm and would fit all 96 stimuli spots. Nematode Growth Medium (NGM) agar was prepared and poured into the OmniTray plates; the protocol can be seen in the Appendix F for the NGM agar. The OmniTray plates containing the agar could then be stored and used when needed for experiments.

The team would keep about 3-5 cultures of the *C. elegans* in the 60mm petri dishes and allow them to grow into adults to use for experiments. The next task was to transfer the worms from the seeded culture plates to the OmniTray. We first tested adding stimuli to a *C. elegans* seeded culture. It was determined that the *C. elegans* were attracted to their food, so when running stimuli experiments on the worms they did not deliver other responses. The team then determined that our experiments needed to be run on unseeded plates to eliminate the attraction and congregation of worms to the *E. coli*. Adult worms were picked from the seeded 60mm petri dishes to an unseeded 60mm petri dish. The worms were transferred to another 60mm plate first to reduce the risk of puncturing the OmniTray agar and therefore the worms burrowing into the agar eliminating

detecting their behavior. Adult worms were also used because they are the standard in that they respond the most to external stimuli and they are also the easiest to track. Once the numbers of adult worms that we wanted to use were transferred to the unseeded plate, the buffered solution S. basal, a standard worm buffer, was added to the 60mm petri dish. The plate was then swirled to lift the worms off the agar. With the use of a syringe, the *C. elegans* along with the S. basal could be pulled up and transferred easily. This method allows all the worms to be transferred over to the OmniTray successfully without puncturing the agar. The syringe was then pushed to release the S. basal along with the worms onto the OmniTray. The excess S. basal was then pulled up through the syringe and discarded. The *C. elegans* were now free roaming in the agar OmniTray and the next step in the experiment could be performed.

4.2.2. Transfer of Stimuli

The next procedure in completing the experiment was to transfer the stimuli. Since our device was created from a 96 well plate, the most logical plate to hold the stimuli and allow for efficient transfer was a 96 well plate. The team assumed the stimuli could be transferred from the 96 wells to the OmniTray by dipping our device into the well, and stamping it onto the OmniTray. First the wells had to be loaded up with stimuli. The team performed many tests with three main stimuli: Isoamyl Alcohol (IAA), Glycerol, and S. basal. These three stimuli were chosen because the behaviors of the *C. elegans* with the stimuli were already known; IAA has a positive response, glycerol has a negative response and S. basal was used as a control.

Both IAA and glycerol are extremely potent stimuli so the *C. elegans* stay away from both stimuli and do not roam around the plate as much. Therefore, the team performed 1:10 dilutions on the IAA and the glycerol with each experiment. These dilutions were conducted in a 2mL microcentrifuge tube and about 10 μ L of dye was added to each stimulus and mixed to increase visibility. When the stimuli was mixed it could then be transferred into the 96 well plate in any configuration and in the number of stimuli spots wanted for the experiment.

4.2.3. Capturing the Experiment

Our team wanted to use a method that quantitatively determined the behavior of the *C. elegans* and did not use the standard method of counting the number of *C. elegans* approaching a stimuli

as described previously. To quantitatively analyze our experiments, they needed to be captured on video first. The set-up of the experiment can be seen below in Figure 11.

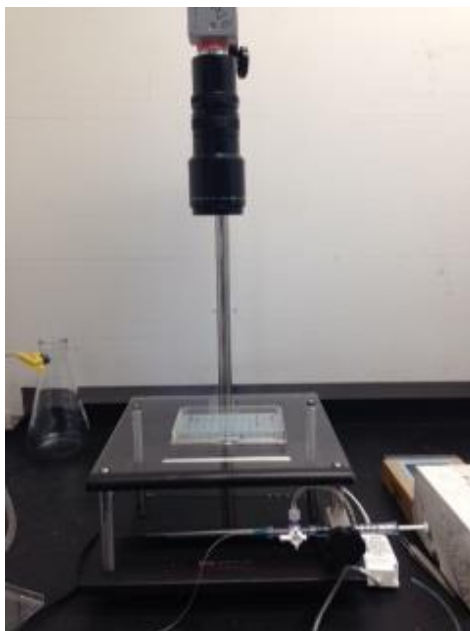


Figure 11: Video set up in the lab

At the top of the picture is a PixeLINK PL-A741 camera fixed on a stand. The stand allows the camera to be adjusted closer or further away from the stage. The camera could capture the experiment from an aerial view. The experimental OmniTray can be seen placed on the stage with a light source underneath to allow for contrast between the worms and the agar. The camera was then connected to the computer through a firewire cable. An application called gVision, developed by Gus K Lott III, PhD, was used through MATLAB. gVision allows videos to be captured, compressed and saved. The frames per trigger, trigger repeats and cap intervals could be entered into the application to fit the desired criteria of the user. The device was then placed into the 96 well plate of stimuli and stamped onto the OmniTray containing the *C. elegans*. The camera was ensured to be in focus and the video was ready to be run. The gVision application saves the videos as an AVI file.

4.2.4. Analyzing the experiment

The team needed to use a code that would track the *C. elegans* in a free roaming agar plate. Since we knew a code that would track the *C. elegans* would be difficult for our group to create in the

time frame we had, Professor Albrecht reached out to his colleague Navin Pokala from the University of Rockefeller to allow access to his code. Navin's code tracks each of the animals in each frame of the video. First, the Navin code is run on MATLAB which then allows all the Navin code files to be accessed and used in analyzing, the activation and set up of the code can be seen in Appendix G. The command "TrackerAutomatedScript" is then used to upload the saved video file captured from gVision. Our team then had to modify and calibrate the scaling of the video so the code could detect the *C. elegans* along with their positions on the plate. For the code to detect the number of worms on the plate, 'numworms' is used in conjugation with 'TrackerAutomatedScript' for the user to communicate an estimate of the number of worms on the plate. Once all has been entered, the code is then run to start analyzing the video captured of the experiment. The code then takes individual frames of the video, tracks the worms in that frame, and then detects the *C. elegans* state of behavior, X and Y coordinates, speed, length etc. Since the video is analyzed in frames, tracking *C. elegans* can be difficult because the track can get lost. To account for this, the code then links the tracks from each frame together by what the code believes to be the same *C. elegans* track. This compresses the size of the data from fragments of tracks of the *C. elegans* to linking the tracks.

The data for each of the linked tracks was then useful for our group to compile and map out the tracks of the *C. elegans* using plot commands in MATLAB. The team was most concerned with the X and Y coordinates of the *C. elegans* along with their state of behavior at each coordinate. The ideal output the team wanted to create was a visual map that identified each state of behavior that correlated with an X and Y coordinate of each of the animals on the plate.

5. Verification

5.1. Device Design Verification

In order to ensure our design was feasible, various tests and observations were performed on the devices created to ensure the objectives were met.

First, the device was tested to evaluate if the transfer of stimuli from the 96 well plate to the agar OmniTray plate was successful. Below is a figure of the final device being placed into 96 wells all containing 75 μL of diluted dye. The alternative design chosen to be the most feasible in delivering the 96 stimuli spots was design alternative 4. The design is an 8x12 matrix that can transfer the stimuli to a 128x86mm OmniTray plate.

Once the device was placed into the plate, it was then removed and stamped onto the OmniTray. Below in Figure 12 is a picture of the agar OmniTray with the spots transferred successfully to the plate.



Figure 12: Stimuli being transferred to the OmniTray

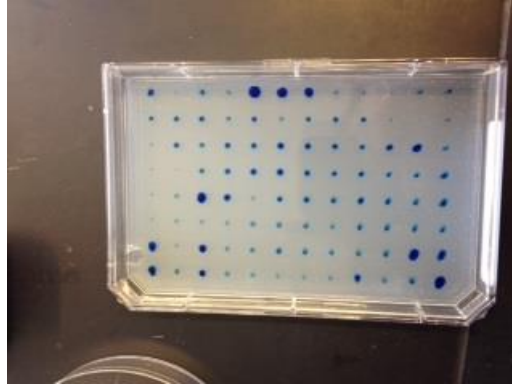


Figure 13: Stimuli spots do not interact with one another

Our team was most concerned with the spots not touching or interacting with one another, which we can conclude they do not by Figure 13. As seen in the figure, the spots are not uniform in size, however this was not a primary objective as the behavior of the animals is mainly dependent on the type and concentration of the stimuli rather than the amount of the stimuli present.

The team then placed multiple spots of stimuli on a *C. elegans* culture using our device and observed the movements of the *C. elegans* using the gVision system application through MATLAB. Isoamyl Alcohol (IAA) has a positive response so the *C. elegans* are attracted to it while Glycerol has a negative response and the *C. elegans* move away from it. By placing these two stimuli next to each other, we could then observe if the spots were too close together that the *C. elegans* did not respond with an aversive or non-aversive behavior to the respective stimuli. A video was captured and observed by the team and ensured the *C. elegans* were able to have independent interactions with each of the stimuli spots they came in contact with. This was verification that the tips of the device delivered the stimuli were far enough away from each other that the *C. elegans* were interacting with each stimuli spot separately.

The team then tested the volumes of each stimulus that could be added into the 96 well plate. This was a concern because when the PDMS device was placed into the 96 well plate, the stimuli would sometimes spill over into other wells. This would create a mixture of stimuli and would not allow the individual stimuli interactions necessary for the experiments. The volumes could also not be too little because the device will not reach to the bottom of the well touching the stimuli. The team determined that 90 μL of IAA and S. basal and about 120 μL of glycerol could be added to each well that would allow the stimuli to be transferred properly. It is important to keep in mind that

the glycerol is a viscous stimulus. Our team performed a 1:10 dilution on the glycerol reducing the viscosity.

Each of the tests performed above was verification for the team that the alternative design number 4 was best for delivering the multiple stimuli properly to the OmniTray plate. The team then had to verify the experimental procedure created was suitable in detecting the behaviors of the *C. elegans* at each spot.

5.2. Experimental Design Verification

The first results obtained by the team were observational results. The team placed stimuli onto a plate of *C. elegans* in various experiments. This was done to verify that the *C. elegans* were responding with either an aversive or non-aversive behavior to the appropriate stimuli. The team took several videos using Isoamyl Alcohol (IAA) studied as a positive response and glycerol studied as a negative response. These observations allowed the team to view if the stimuli in the lab would be successful in displaying the behaviors the team was expecting. The team observed reversals and turns at the glycerol spots, while in the IAA, the team observed forward motions by the *C. elegans* and pauses in the specific IAA spot. This was to be expected, as animals near the IAA typically exhibit positive behaviors and the glycerol generally elicits negative behaviors. This was observed in all experiments conducted by the team. The *C. elegans* aggregated in the area of the IAA, and in all cases avoided the glycerol through various turns and reversals.

The team decided we needed to first run and modify Navin Pokala's code to ensure it was running properly and that it was able to be modified for our experiments. A 60 mm plate of *C. elegans* with a spot of stimuli was captured using gVision systems as a test video to run through the code. The Navin code was then activated in MATLAB to analyze the video. The team then calibrated the scale using a picture of a holepunch into a piece of paper. The code already knows the dimensions of a standard holepunch, therefore it scaled the video using the dimensions of the holepunch. The code was run and then completed proving that the code was able to be run using videos from our lab from the gVision system application.

The team encountered issues with the Navin code after the initial test was run. The team used a different computer in the lab to analyze the experiments, but it was determined that the Navin code

would not run on the latest version of MATLAB (2013), it would only run on the MATLAB (2012) version. The code needed to be run on 2012 or all of the commands in the Navin code needed to be changed to satisfy the 2013 version. When loading the video into the code using “TrackerAutomatedScript,” the command “numworms” was not allowing the code to run properly. When the team would run the code, we would eliminate the use of “numworms” to run the code properly. When the “numworms” command was deleted a picture would show up on the monitor. This picture was a depiction of the initial frame of the video that the code viewed when it was about to start analyzing the video. It would then ask the user to right click on the worms to add worms and left click to omit worms. The worms would be lighter than the background because of the contrast between the agar and the worms created by the camera and light source. Once all the worms were clicked on by the user, the code continued to run and analyze.

The team had three different camera lenses to choose from that allowed for wider or smaller ranges of area to be covered and they were also of different resolutions. Each of the cameras were placed above an OmniTray of *C. elegans* and previewed using gVision to determine which lens was the best to capture and analyze the videos. One of the results we determined from this was that not all 96 stimuli spots could be seen with any of the camera lenses. This determined our experiment needed to be cut down to less than 96 stimuli spots and to the highest number of spot that could be captured and analyzed. The best camera determined was the PixeLINK PL-A741 shown above in Figure 11. This camera allows the view of a 6x6 matrix. The worms were transferred onto the OmniTray, the stimuli were placed on the OmniTray and the experiment was captured using gVision of the 6x6 matrix. The video was then run through the Navin code in MATLAB. When the first frame of what the code viewed from the video in MATLAB showed up to click on the *C. elegans* in the plate, it resulted in a dark picture and the contrast between the worms and the agar made the animals difficult to see. The code could only track a few of the worms on the plate. This determined that the 6x6 matrix was still too far away for the code to track the *C. elegans* properly. This resulted in cutting the matrix of stimuli spots seen by the camera to a 3x4 matrix. When the 6x6 matrix was cut down to the 3x4 matrix, it resulted in a better contrast and the worms were tracked more accurately by the code.

Another result the team found was that the length of the gVision AVI video file determined the length of the running code. The team wanted to take videos of the experiment for up to an hour to

analyze if interactions still occurred after longer periods of time. An issue determined was that a twenty minute long experimental video ran for about thirteen hours. This resulted in the team only capturing videos for no more than ten minutes so that the code did not take a long time to run and more results could be obtained.

One important aspect of the transfer of stimuli analyzed was the possible diffusion of the stimulus into the agar, and also if the outward diffusion leads to the mixing of separate stimuli. To examine this, the various stimuli used were dyed and placed on the agar through the use of the transfer device. Pictures were taken from above at minute intervals for a period of ten minutes to determine the outward spreading, as this is a typical time for experiments to be conducted. The agar was then cut into after an hour to determine the diffusion into the agar, as this was the point in time where there was no remaining liquid stimuli on the surface of the agar. Figure 14 shows pictures after the first and last minute of outward diffusion.

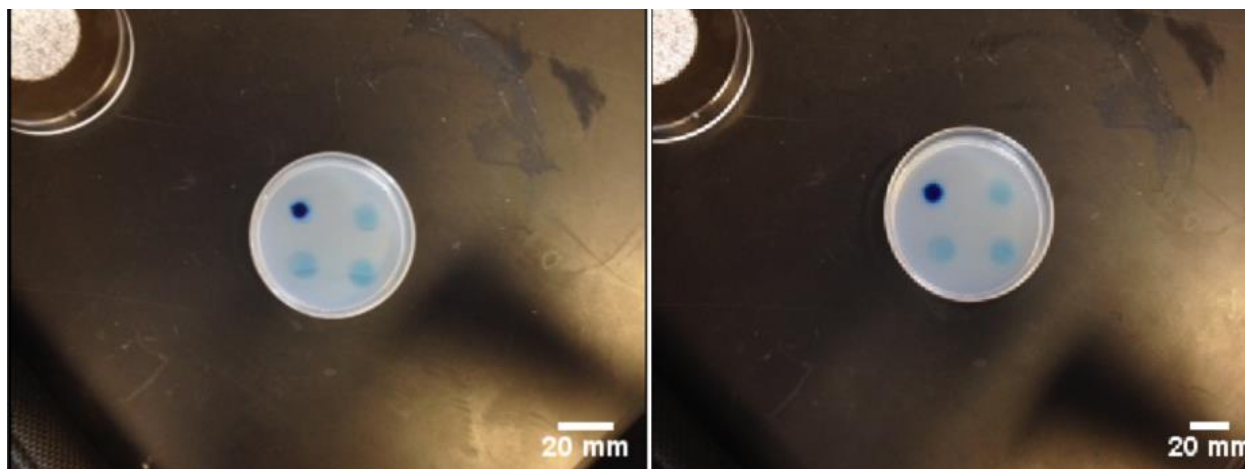


Figure 14: Stimuli spots at 1 (a) and 10 (b) minutes of observation

As seen in Figure 14, the spots did not visibly interact with one another, fulfilling the requirement that the spots must be kept separate in order to obtain accurate results.

After an hour, each stimuli had completely diffused into the agar, as seen in Figure 15. This proves that an experiment cannot be run for that length in time, as the *C. elegans* would no longer be in contact with the stimuli. This limitation adds to the team's earlier conclusion that long videos are not feasible to run for these types of experiments.

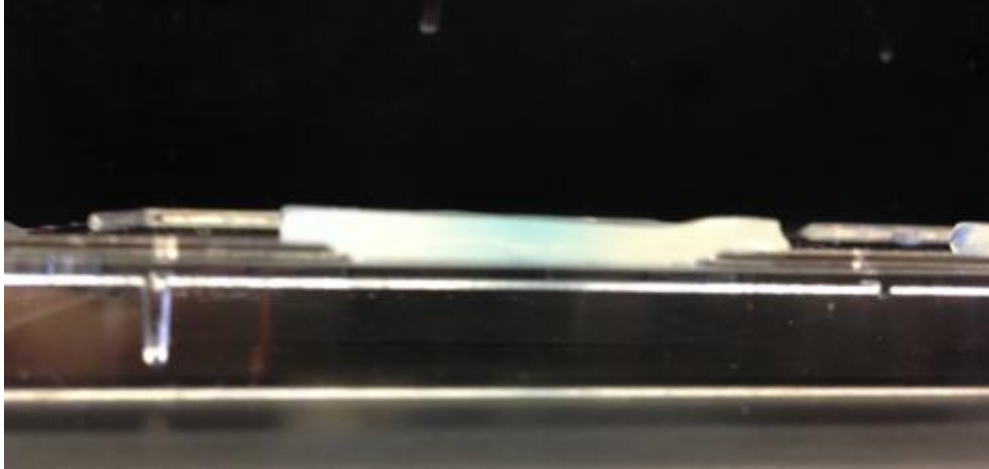


Figure 15: Section of agar showing complete diffusion into agar

The MATLAB code output different variables for each of the *C. elegans*, so for a typical video analyzed, there could be hundreds of vectors of the same variable for each of the *C. elegans* found. In order to analyze the behavior of all the *C. elegans* at once, the variables were combined into a single vector using the `struct2matrix` command. This function was developed by Dirk Albrecht and can be seen in Appendix G. Using the vectors from all of the X and Y coordinates from a single video, the team developed a code for a two-dimensional histogram, the code for which can be seen in the Appendix G. This heat map can display the frequency of the presence of an animal in a specific area.

The team performed multiple experiments and ran these videos through the Navin code. Once the videos were complete being analyzed, the heat map code was then run on the x and y outputs of the code. Below in Figure 16 is a heat map that was created with one of our videos.

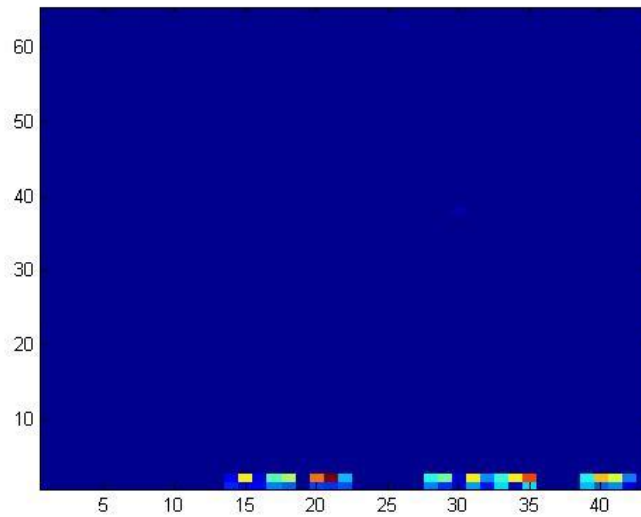


Figure 16: Heat map with timestamp included in video

As can be seen from the heat map, it shows that the most frequented spot was at the bottom of the plate. From video to video, each heat map had the three groups on the bottom that depicted *C. elegans* frequented the most in that area. This did not accurately convey the data shown in the videos, so after discussion with our advisor, it was realized that the timestamp on the videos was causing this because the code was detecting the timestamp along with tracking the *C. elegans*. Since the time stamp was constantly changing, it took away from actually tracking of the *C. elegans* in the plate. To eliminate this problem, the videos were cropped using an application called VideoMach. VideoMach allowed our videos to be loaded into the application and cropped out the timestamp. The edited videos could then be saved and run through the Navin code eliminating the movement and tracking of the timestamp. Figure 17 below shows a screenshot of the video used to create the heat map. The placements of the stimuli are designated by the colored circles.

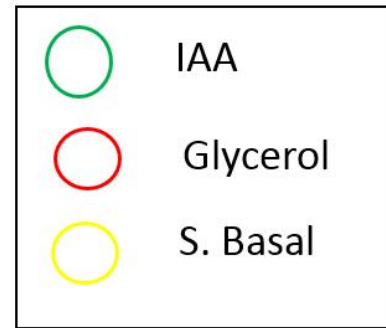
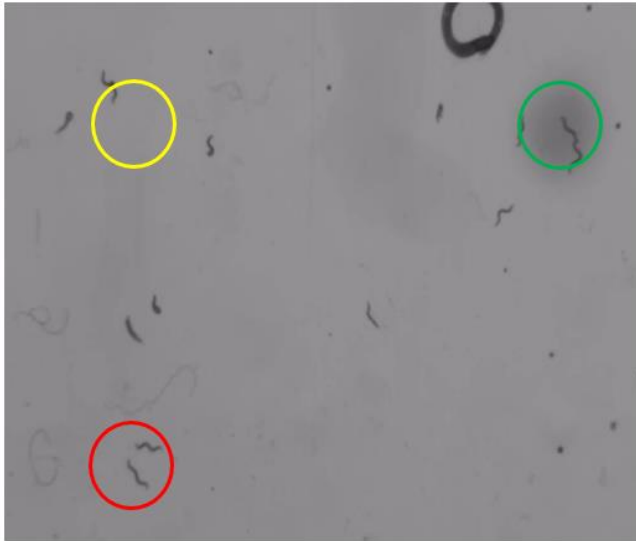


Figure 17: Screenshot of video analyzed

Below in Figure 18 is another heat map created with the edited video shown above in Figure 17. Table 3 describes the qualitative observation of the frequencies found in each of the stimuli spots.

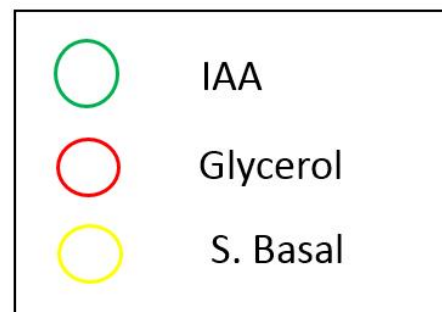
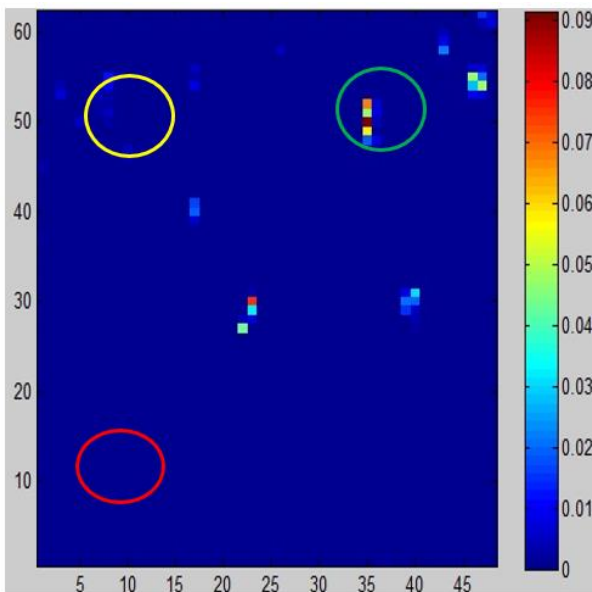


Figure 18: Heat map

Stimulus	Qualitative observation of frequency
Isoamyl alcohol (IAAA), 10% concentration	High
Glycerol, 10% concentration	Low
S. basal	Low

Table 3: Qualitative observation of each stimulus

This heat map shows the stimuli that were used in the experiment circled and designated by color. The edited videos eliminated the frequency caused by the timestamp and tracked the *C. elegans* properly. The red designated the most frequented areas by the *C. elegans* and the blue designates the least frequented areas. As shown, the most frequented area was at the IAA which has a positive response by *C. elegans* while the glycerol which has a negative response is the least frequented.

The team also wanted to create a visualization of the state of behavior correlating with the X and Y coordinates of each of the *C. elegans*. The Navin code outputs a state of behavior at each X and Y coordinate frequented by an animal. The team created a code that allowed a color to designate the state of behavior at an X and Y coordinate on a plot. However, because there is such a wide variety of behaviors that can be displayed, many of the behaviors were grouped together so only four general behaviors are represented; forward, reversal, pause, and turn. The code can be seen in the Appendix G. When all of the behaviors are plotted on the same graph, it is difficult to determine the behavior due to the overlapping of the colors. To make increase the visibility of the behaviors, only one was expressed in each figure using an altered code found in Appendix G. Below in Figures 19, 20 and 21 are the state plots using the video from Figure 17. Table 4 represents the most prominent behavior in the area of each stimulus.

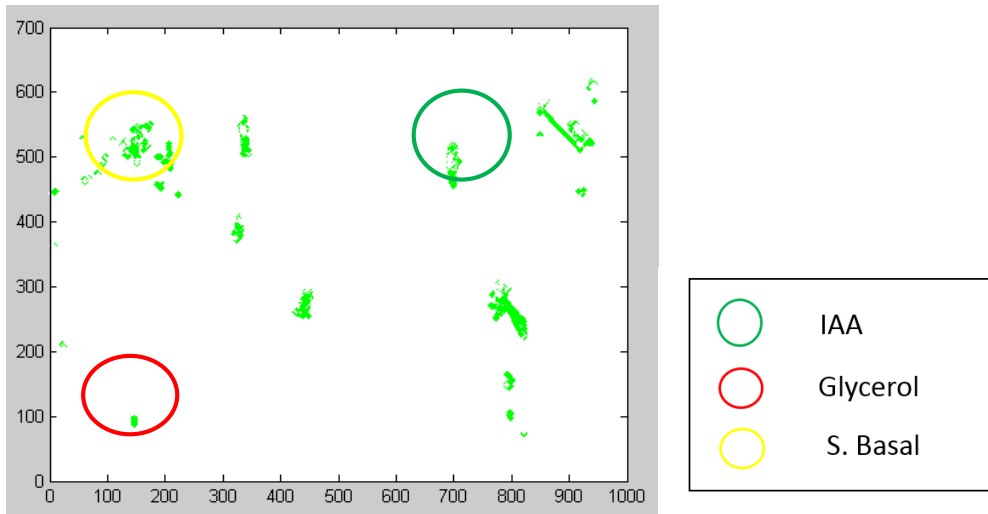


Figure 19: Forward behaviors

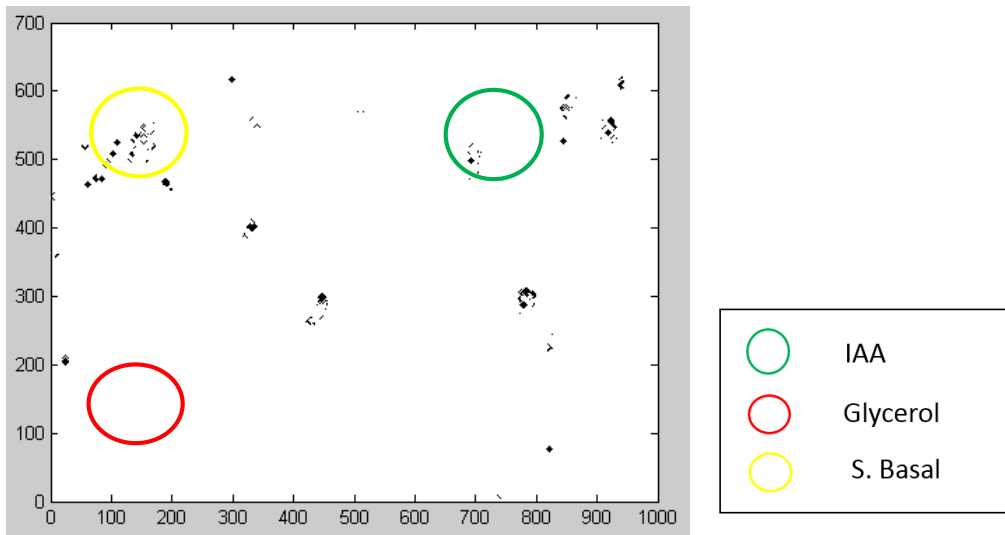


Figure 20: Pause behaviors

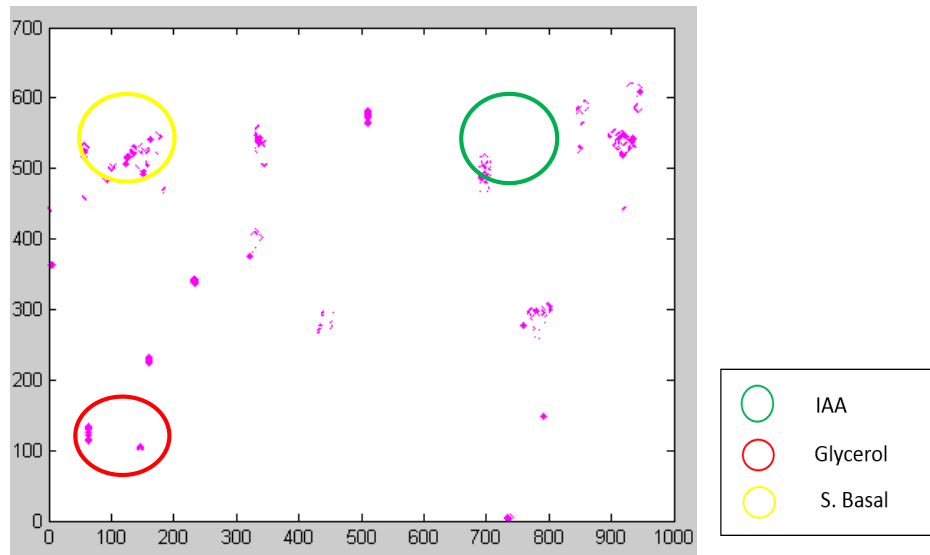


Figure 21: Turn behaviors

Stimulus	Most prominent behavior
Isoamyl alcohol (IAA), 10% concentration	Forward
Glycerol, 10 % concentration	Turns
S. basal	All behaviors prominent

Table 4: Most prominent behavior for each stimulus

The state plots above designate the state of behavior of all the *C. elegans* correlating with the X and Y coordinate the behavior occurred at. From these plots, the behaviors the *C. elegans* exhibited can correlate where the stimuli are placed. For example, a lot of green is shown the map indicating there are a lot of forward behaviors. This can determine if there is a positive stimulus on the plate, as forward motion toward a stimuli indicates that it is most likely positive. Reversals would be indicated by red, however there were no reversals recorded from this experiment, possibly indicating the *C. elegans* did not come in close contact with the glycerol to exhibit a reversal behavior to the negative stimulus.

6. Discussion

Initially, the team set out to create a delivery vehicle that places multiple stimuli on a *C. elegans* culture. The success of the project was based on the initial objectives, functions, and constraints. The final design delivers a predetermined amount of stimuli simultaneously to the culture without causing penetration. The delivery device is reusable, sterile and simple. In addition, it is spaced evenly so that different stimuli do not interact with one another. Another part of the project is the experimental design where MATLAB and machine vision systems are used to identify behavioral responses of the *C. elegans*. The experiment needs to be reliable and highly selective and deliver same results throughout multiple trials. Based on the design process and the experimental results fulfilled by the team, the PDMS stimuli delivery device has successfully met the objectives and functions. Further description of the final design and the experimental setup is discussed in this chapter.

6.1. Device Design

The team's decision to use PDMS, as opposed to a rigid material allowed the final design to be a soft, reusable and flexible delivery device. The softness of the material is especially important since the delivery vehicle shouldn't penetrate the culture. The advantage of using a rigid material would be that the different tips that correspond to different stimuli of the device wouldn't intervene with each other. However, if a rigid material were to be used, the *C. elegans* culture would be penetrated and the design wouldn't be functioning properly. Thus, PDMS, which is a soft, flexible material was chosen. On another note, although PDMS is a flexible material, the flexibility can be altered with different molding dimensions. Thus, after various design alternatives, a reasonable degree of flexibility was chosen so that the tips of the device could not bend and allow the tips to touch one another.

6.2. Experimental Design

The heat map and behavioral plot created in the additional MATLAB scripts can be used to better understand the behaviors of *C. elegans* in response to various stimuli. Upon a brief examination of the figure, it can be determined which areas on the map the animals most frequented. Using this map alone, it can give an indication of areas containing positive or negative stimuli. For example, using the same heat map as mentioned earlier, in Figure 22, the *C. elegans* appear to be

highly frequenting in the IAA area, correctly identifying this as a positive stimuli, where there is not much attraction to the areas containing glycerol and S. basal.

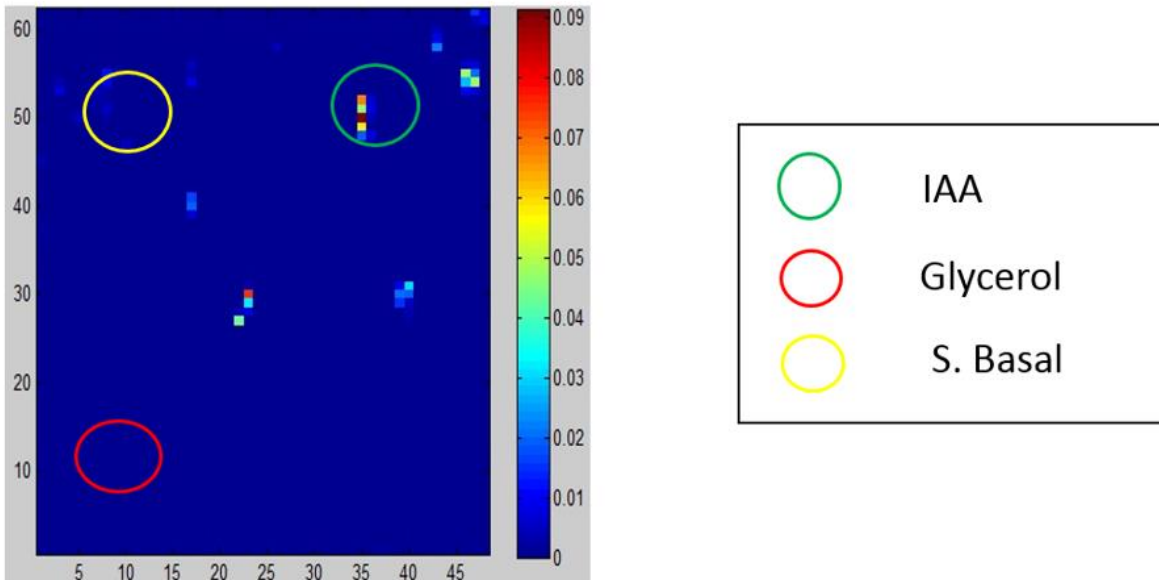


Figure 22: Heat map

The behavioral map alone can also give an indication of what kind of stimulus is present in an area. Upon examination of Figure 23 below, there is evidence of a large amount of forward motion toward the positive stimuli. This forward motion can indicate the animals are attracted to this specific stimuli and are moving to that area and away from the aversive stimulus. It can also be noted in Figure 24 that there are mostly turns in the glycerol spot, with few variation in behavior, which could indicate the animal is trying to turn to get away from the stimulus because of its negative effects.

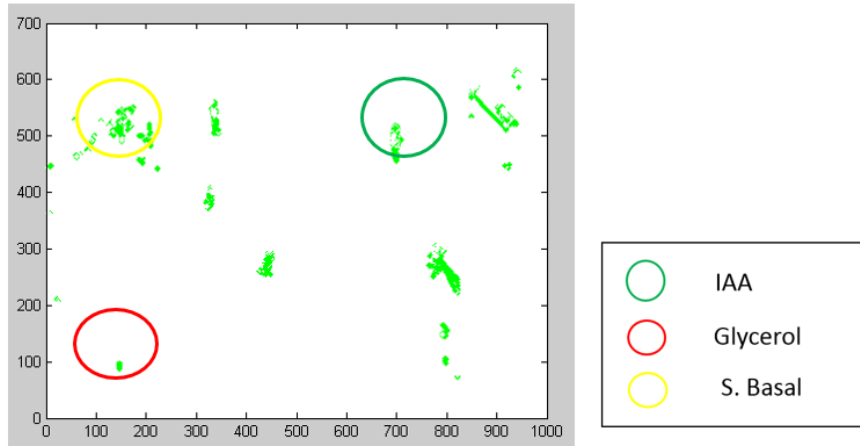


Figure 23: Forward motion towards a positive stimulus

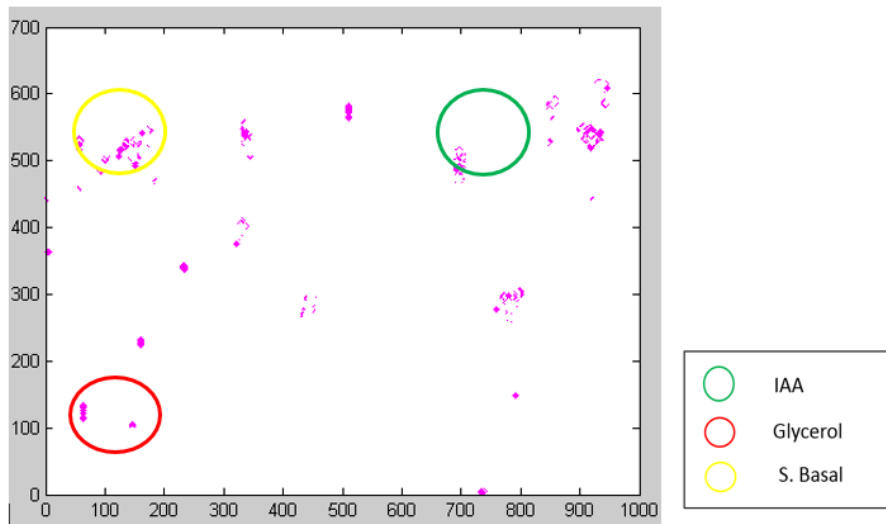


Figure 24: Many turns located in the negative stimulus

These two maps should be used in adjunction with one another to gain the best understanding of the *C. elegans* for a specific experiment. Using each one separately could give an adequate analysis of the behaviors, but may be deceiving for some behaviors. For example, by looking at the state plot, it appears that there is a lot of activity, including forward motion, in the area of the S. basal. Using this map alone it could be possible to infer that is a positive stimuli. However, upon further investigation of the heat map, that area was not one highly frequented by the animals. Therefore although there was a lot of noted behavior in that area, it was only for a brief amount of time, indicating that there is not a strong correlation for the identification of a positive stimulus in this area. The IAA area however, appears to have a positive response by the standards of each of the

maps, justifying that behavior. Table 5 shows how the maps can be used in adjunction with one another to properly identify the stimuli.

Stimuli	Qualitative observation of frequency	Most prominent behavior
Isoamyl alcohol (IAA), 10% concentration	High	Forward
Glycerol, 10% concentration	Low	Turns
S. basal	Low	All behaviors prominent

Table 5: Identification of stimuli

This can be useful in future applications when trying to identify an unknown stimulus as a positive or negative one. If an unknown stimulus is placed on the plate, it could be identified through the use of the two maps. To use the heat map for this, the area around the stimulus would be examined for the frequency of the animals. The state plot would then be examined for the behavior of the animals, looking for forward motion toward the stimuli, possible reversal and turns in the vicinity, and any pauses in the area. These examinations of the maps outputted by the code can lead to the identification of the effects of the stimulus to the *C. elegans*.

When the team received the outputs of the code from the videos and created our state plot, it was noticed that no reversals were detected for the videos analyzed. The team then reevaluated the videos through observation and determined that there were reversals from the *C. elegans* specifically in the glycerol spots. This meant that there was a discrepancy between what was observed and what was analyzed. The team decided this discrepancy could have been related to three things: the resolution of the camera, the code detecting the dye instead of worms, and the grouping of behavioral activity.

The code that Navin Pokala allowed the team to use is a very complex and specific code. This code was created for the specific resolution camera that is used in Pokala's lab. Since our team used a different resolution, our experimental set up was not specific to the parameters the code is designed for. This makes a difference when detecting behaviors in the *C. elegans*. For example, the code analyzes the videos and detects behavior by the centroid of the animal. With different resolution

compromising the scaling, a slight movement could be determined as forward behavior in Pokala's experiments, but as a pause in our experiments. The mismatch in resolution or scaling could make for incorrect analysis of behaviors with such small animals.

One of the experiments run used the 6x6 matrix of glycerol in columns 1 and 3, and IAA in column 2. The video was analyzed through the code and the outputs were converted to the heat map and state plot. Below in Figure 25 the heat map is shown. On the left side of the figure there are 3 outlines of circles which show an aggregation of *C. elegans*, however these spots are the locations of the glycerol. The code identified the outer edge of the dyed stimuli spots as animals, which misconstrued the data. This was noted as the heat map displays almost perfect circles where the dyed stimuli was placed. This could be attributed to the contrast between the agar surface and the edge of the stimuli spots or the resolution at which the experiment is captured at. It was determined that the worms were too small to detect with a lower resolution camera in our lab.

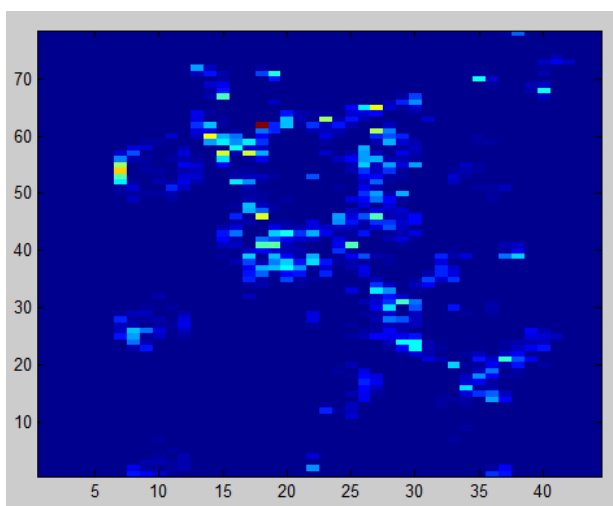


Figure 25: Heat map of low resolution video

The last factor that could have determined the discrepancy between observations and the outputs of the code was the way the team decided to group the behaviors. As mentioned previously, the team grouped all behaviors from the Navin code into four main behaviors: forward, turns, pauses, and reversals. Upon discussion with Professor Albrecht, it was noted that *C. elegans* can create marginal behaviors such as a slight reversal followed by an immediate turn. Since the team grouped all turns together, this would make the reversal behavior exhibited by the *C. elegans* nonexistent

in the state plot. In future applications of the code, this can be further examined and corrected in order to better display reversal behaviors.

6.3. Device and Experimental Design Limitations

A limitation of the device that we have created is that it does not deliver a specific amount of stimuli to the OmniTray plate. As previously shown in our design verification, the stimuli spots are delivered, but they are not the same size. This means the delivered volume of stimuli varies from spot to spot. Our experiments omitted this as a factor, but further modification of the device could allow the possibility of equal volumes delivered. The primary limitation of the experiment is that all 96 spots cannot be seen due to the limitation in camera lens size. Without the proper camera to zoom out to all 96 spots, the design is only suitable for smaller areas and few different spots of stimuli. The resolution on the camera also is a limitation because it did not allow even the 6x6 matrix to be tracked by the code. With better resolution and a wider lens range, the contrast can be improved and all 96 spots can potentially be analyzed. Although not all 96 spots were used for our experiments, there was a big improvement as the device allowed 12 spots to be delivered and tracked compared to the current standard of just one. In addition, while stamping the PDMS device on the *C. elegans* culture, it is important to place the stimuli in the right spot where *C. elegans* are present. Since only a 3x4 matrix was used, the stimuli spots needed to be placed in the correct dimensions of the OmniTray that the gVision application is viewing. Sometimes our stimuli spots were delivered outside the view of the camera causing the team to reset or modify the experiment. The final limitation is the team's MATLAB knowledge. While we were familiar with MATLAB, it took the team time to understand and run the code. If the team was more experienced in MATLAB, we could have accomplished coding and analyzing faster leading to more results.

6.4. Manufacturability

The team's design can be manufactured as a kit for academic or commercial use. The steps to make a mold using PDMS can be accessed in Appendix E. Once dried, PDMS can be removed from its mold and the device would be ready to use. Furthermore, if the shape of the device needs to be

altered, a different mold could be used with the same molding steps. The primary market for this product is small academic laboratories and research institutions interested in testing the behavioral responses of *C. elegans*. This product can also be used in other stimuli delivery applications.

6.5. Economic Impact

The main goal of this project was to aid the research for neurological diseases using a cost-effective, simple, effective and reliable design. *C. elegans* are used in neurological research because they are easy to access and very economical. Due to the selected material for the delivery device being PDMS, the design is cost efficient and easy-to-access. PDMS is available at various suppliers nationwide and worldwide. MATLAB is the most common coding program used for *C. elegans* research due to its advanced image processing algorithms. Most research laboratories and institutes use MATLAB in various applications and have the certificate. Currently, there is no patented, simple and cost-effective device that tests the behavioral responses of *C. elegans*. Thus, the delivery device designed in this paper would economically help opening the way of research that identifies neurological responses.

6.6. Environmental Impact and Sustainability

This system uses PDMS and agar culture to design a method that detects the behavioral responses of the nematodes. The agar culture of *C. elegans* is made out of NaCl, peptone, agar, H₂O, 1M CaCl₂, 1M MgSO₄, 1M H₂KO₄P and cholesterol, as can be seen in Appendix Eii. The very small amounts of chemicals that make the agar culture do not have a harmful effect on the environment. PDMS is a common material that is used in a variety of applications such as household and automotive care products, softeners for skin care products, component of surface treatments, manufacturing textiles and paper. PDMS is non-volatile so it doesn't evaporate into the atmosphere. Some PDMS fluids enter into soil or wastewater. When in contact with soil, PDMS will degrade into lower molecular weight compounds, primarily Me₂Si(OH)₂, this is a widespread phenomenon in nature (Buch et al, 1979). It has been found that PDMS fluids pose no known hazard to the environment, but even if they enter an aquatic environment, the natural cleansing process of sedimentation removes them.

6.7. Societal Influence

The research from this project could potentially aid in research of neurological diseases and also provide new information on different responses to unknown stimuli. If the design is eventually released commercially, a culture of genetically engineered *C. elegans* can provide information on the behavioral responses to stimuli and help in formation or modification of certain drugs. This research paper also contains an experimental design that can aid researchers in their experimental protocols.

6.8. Political Ramifications

Currently, the device has no foreseeable political ramifications, as the *C. elegans* are widely accepted nematodes that are used in research and public communities. However, in the future, formation or a modification of a neurologic drug using the designed device would endorse a big impact on the distribution of research funds.

6.9. Ethical Concerns

The device is designed to test the behavioral responses of *C. elegans*, which are multicellular organisms living in the soil and that are easy and safe to use in research. They are used in a large variety of research, from high-school experiments to Nobel Prize winning research projects. Although most vertebrates are either very expensive or pose ethical dilemmas, experiments with *C. elegans* are free of ethical concerns. However, when using the designed device, the team has made sure to use the nematodes under scientific and valid objectives, as should any other researcher.

6.10. Health and Safety Issue

This system is not designed to be used on humans; therefore there is no potential for health and safety concerns. In addition, harmful or toxic substances were avoided while designing and using this system. Furthermore, while designing and using the delivery device and experimenting with *C. elegans*, all safety protocols were implemented according to the protocols at Worcester Polytechnic Institute's Gateway Park Laboratories.

7. Final Design and Validation

Based on the testing and validation of the four conceptual designs, the team was able to develop a design that satisfied all the objectives and performed all of the desired functions. The initial idea was to build a device with multiple tips that would be able to deliver stimulus without interacting with one another. The experiment should then verify that the delivery device works and also be able to analyze correct behavioral responses of *C. elegans* correlating with their position on the OmniTray plate. In this chapter, we will first describe the reasons why the final prototype was chosen, and then discuss the methods used to construct the device and set up the experiment. Finally, we will discuss the MATLAB analysis we have used to verify that our final design functioned correctly.

7.1. Device Material and Shape

In order to build the final design within our objectives and functions, a specific material for the delivery device needed to be chosen. The group researched to find a soft, flexible and biocompatible material that could be used to deliver the stimuli to the culture. It was found that PDMS would be functional and advantageous because it was already present in our working lab.

The team focused on the primary function of the design, which is to deliver multiple stimuli at the same time, when determining the shape of the delivery device. Thus, the team chose to use a 96 well plate as a mold. Creating a thicker surface provided a stiffer device that does not bend and delivers stimuli equidistant from one another. In order to recreate our device, see Appendix E. A modification made to create the device was to first tape a layer around the top of the 96 well plate to allow a thicker surface than what is currently there. When the thicker layer is not present, the device becomes floppy and useless for our experiments. Also, ensure the PDMS is fully in the wells of the 96 well plate by vacuuming the PDMS in a weigh boat and then again when it is poured into the mold. This removes the air bubbles that can form at the bottoms of the wells of the mold leading to the tips not being fully formed.

7.2. Experimental Set-Up

As described in Chapter 5, the materials needed for the experimental part of our project were: a camera, an OmniTray, adult *C. elegans*, chemical stimuli and the PDMS delivery device. The applications that made it possible to analyze the responses of *C. elegans* included gVision and

MATLAB. The primary function of the experiment was to record and analyze the behaviors of *C. elegans* as they encounter the attractant, repellent or neutral chemical stimuli in an OmniTray. The *C. elegans* were transferred to the OmniTray using a standard worm buffer, S. basal. Adults worms were first transferred from their seeded *E. coli* plates to unseeded, no *E. coli*, plates. The S. basal was then added to the unseeded plates and swirled to lift the worms off the agar. A syringe was used to pull up the worms along with the S. basal and then transfer both to the OmniTray plate. Excess S. basal was pulled up into the syringe and discarded leaving only the *C. elegans* on the agar. The stimuli our team used were Isoamyl Alcohol (IAA) which has a positive response, glycerol which has a negative response, and S. Basal which was used as our control. The stimuli was diluted and dyed if necessary, then placed into the 96 well plate in any configuration needed for the experiment. The delivery device was placed into the 96 well plate and stamped on the spot where *C. elegans* were placed in the OmniTray. Once in focus, the camera recorded the movements of *C. elegans* using the gVision application. In order to meet our experimental objectives, we conducted multiple experiments to make sure that the results gathered from the experiments were reliable and consistent. At times, the team used different stimuli and compared the behavioral responses of *C. elegans* to the literature.

7.3. MATLAB Analysis

Once the videos were recorded with *C. elegans* responding to different stimuli, they were uploaded into MATLAB and the behavioral analysis of nematodes was done by the use of MATLAB algorithms retrieved from Navin Pokala. Inputs of the MATLAB code can be seen in Appendix G. With the outputs of the Navin code, the team decided to create heat maps and plots to visually analyze the behavior of the *C. elegans*, these functions can be seen in Appendix G. Heat maps and state plots were used together in order to correlate the X and Y coordinates and state of behavior of the nematodes. Heat maps were used as a visual of the frequency of *C. elegans* in a specific spot on the plate. If there was a higher frequency in a certain spot, we conducted that an attractant stimulus was stamped in that area. On the other hand, if there were only a few *C. elegans* in a spot, the delivered stimulus was considered a repellent one. The state of behavior plots allowed us to correlate x and y positions with the behaviors of the *C. elegans* in the plate. When these two maps were put together, we could determine attractive or repellent stimuli by analyzing how *C. elegans* behaved when in contact with the stimuli.

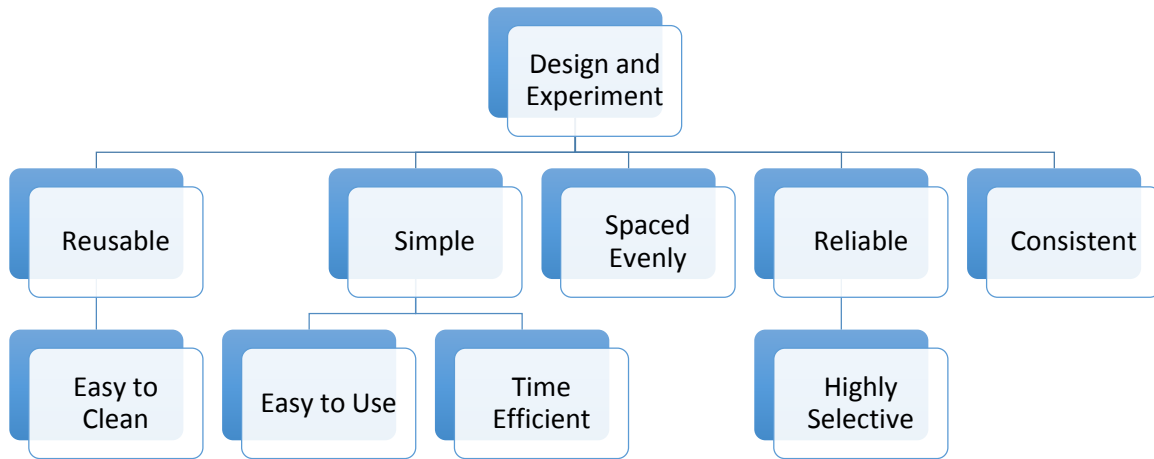
8. Conclusions and Recommendations

The device and experiment that our team designed has created a baseline in expediting the research of molecular interactions through by the behavioral analysis of *C. elegans*. Some future recommendations are first to retrieve a camera with a higher resolution. As mentioned previously, a higher resolution camera will allow for better contrast and therefore lead to analysis of more *C. elegans* and more stimuli spots. Another recommendation would be to perform surface chemistry on our PDMS device to make it more hydrophilic. Since our device is hydrophobic, the stimuli are being transferred from the 96 well plate to the OmniTray by surface tension. By making the device more hydrophilic, the spots delivered could potentially be of a more equal volume than with our hydrophobic device. The next recommendation would be the placement of the *C. elegans*. In our experiments, we placed the *C. elegans* in the middle of the frame of the video. Due to this, they avoided most of the spots they were not attracted to, such as the glycerol, and traveled directly to the IAA, which they are attracted to. This resulted in few than expected aversive behaviors detected. Our recommendation is to find a system in which the *C. elegans* will be fully spread across the OmniTray plate so that they can come in contact with all the stimuli instead of moving straight to the attractive stimuli. Another future recommendation would be using this system on genetically engineered *C. elegans*. First, sensory null *C. elegans* would be used because their survival senses such as food and attractive odors are wiped and only a specific receptor in the *C. elegans* can be expressed. With this system, the *C. elegans* can be tracked using the device and experimental procedure to determine molecular interactions between receptors and ligands based on the behavioral analysis.

In conclusion, our team has created a device and experimental procedure baseline to expedite the identifications of receptor-ligand interactions. The behaviors of *C. elegans* can be used to identify the interactions that cause neurological diseases such as Alzheimer's and Parkinson's disease. If more of these molecular interactions are known, advancement in drugs targeting these molecular interactions can be developed to treat or relieve symptoms in neurological diseases.

Appendices

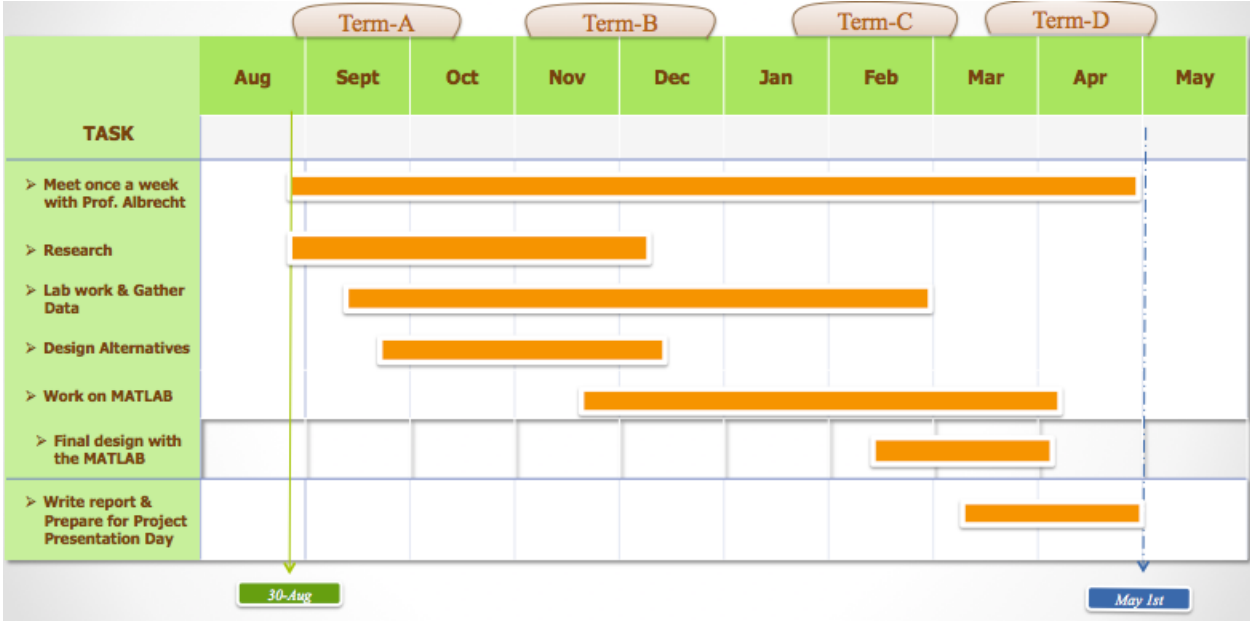
A. Objectives Tree



B. Design Evaluation Matrix

	Molded Silicone	Metal	Molded Silicone Syringe System	Metal syringe system	Molded Silicone dipped into stimuli
<u>Objectives</u>					
Reusable	Y	Y	Y	Y	Y
Simple	Y	Y	Y	N	Y
Spaced Evenly	Y	N	Y	N	Y
<u>Functions</u>					
Test Multiple Stimuli	Y	Y	Y	Y	Y
Independent Stimuli Interactions	Y	Y	Y	Y	Y
Not penetrate the culture surface	Y	N	Y	N	Y

C. Gantt Chart



D. Work Breakdown Structure

1. Design a system to identify behavioral analysis of <i>C. elegans</i>
1.1. Design Process
1.1.1. Initial Client Statement
1.1.2. Generate questions for the client interview
1.1.3. Conduct client interview
1.1.4. Develop pairwise comparison chart
1.1.5. Develop objectives tree
1.1.6. List functions for experimental setup and design separately
1.1.7. List constraints for experimental setup and design separately
1.1.8. Revise client statement for clarity and completeness
1.2. Research
1.2.1. Background
1.2.1.1. Nervous systems
1.2.1.2. Molecular interactions
1.2.2. <i>C. elegans</i>
1.2.2.1. Neurodegeneration in <i>C. elegans</i>
1.2.3. Current techniques
1.3. Developing Design Alternatives
1.3.1. Brainstorm individually
1.3.2. Brainstorm as a team
1.3.3. Review design alternatives with client
1.3.4. Review design alternatives based on literature reviews
1.3.5. Review design alternatives based on initial testing
1.3.6. Review design alternatives based on subsequent testing
1.3.7. Select final design
1.4. Documentation
1.4.1. Weekly progress reports from each team member
1.4.2. Notes taken during weekly meetings with advisor
1.4.3. Literature reviews and research notes

1.4.4. BME430X Presentations
1.4.4.1. BME430X Presentation 1: Background, client statement, significance
1.4.4.2. BME 430X Presentation 2: Detailed background, objectives, functions
1.4.4.3. BME 430X Presentation 3: Project Approach
1.4.5. MQP report chapters
1.4.7. IEEE abstract for Project Presentation Day
1.4.8. Final presentation for Project Presentation Day
1.4.9. Self and team evaluations
1.4.10. Final MQP Report
1.5. Project Management
1.5.1. Have weekly meetings
1.5.2. Organize electronic files in “MQP” Dropbox folder
1.5.3. Time Management
1.5.3.1 Create and periodically modify Gantt chart, WBS, to-do lists
1.5.4. Financial
1.5.4.1. Cost analysis
1.5.4.1.1. Manufacturing costs
1.5.4.1.2. Prototyping expenses
1.5.4.1.3. Consumer prices
1.6. Laboratory Work
1.6.1. Design the stimuli delivery device
1.6.1.1. Mold PDMS in the design of choice
1.6.1.2. Dip the delivery device tips to different stimuli
1.6.2. <i>C. elegans</i>
1.6.2.1. Practice the transfer of <i>C. elegans</i>
1.6.2.2. Prepare NGM agar plates
1.6.3. Machine vision systems
1.6.3.1. Analyze frames of video
1.6.4. Modify MATLAB code so that it calibrates to a larger scale

1.6.4.1. Determine the state and behavior of <i>C. elegans</i>
1.6.4.2. Identify the frequency of <i>C. elegans</i> in stimuli spots
1.6.4.3. Identify the response of the <i>C. elegans</i> to specific stimuli

E. PDMS protocol

1. Wear gloves and clean up working area.
2. Pour 90 grams of PDMS onto a clear container on the electronic weighing machine.
3. Carefully add 10 grams of PDMS plastomer curing agent onto the PDMS container. (Ratio of PDMS to curing agent should be 9:1.)
4. Place the mixture on the lab counter. Whisk this mixture vigorously with a spatula or with 2 plastic pipettes for 10 minutes. Thorough mixing is needed to make sure that the curing agent is uniformly distributed, so that the PDMS mold is uniformly cross-linked.
5. The final mixture will have lots of air bubbles from the whisking process, which need to be removed before curing.
6. Place the mixture in a common bell-jar dessicator connected to a vacuum pump for 1 hour to make the final mold bubble free.
7. Pour the mixture in a mold that you would like it to be shaped as.
8. Place the mixture in the mold in the vacuum again for 5 minutes.
9. Place the mixture in an incubator at 56 °C, let stand for 3 hours.
10. Once the PDMS is cooled down, take out the PDMS from its mold by tweaking around the edges to get a grip of the PDMS at the edge, and then pull the whole PDMS out.

F. Preparation of Nematode Growth Medium (NGM) Agar

Equipment and Reagents:

- NaCl
- Agar
- Peptone
- 5 mg/mL cholesterol in ethanol
- 1M KPO₄ buffer pH 6.0 (108.3 g KH₂PO₄, 35.6 g K₂HPO₄, H₂O to 1 liter)
- 1M MgSO₄
- OmniTrays

Methods:

1. Mix 3 g NaCl, 17 g agar, and 2.5 g peptone in a 2 liter Erlenmeyer flask. Add 975 mL H₂O. Cover the mouth of the flask with aluminum foil.
2. Autoclave for 1 hour.
3. Cool flask in 60 °C water bath for 15 mins.
4. Add 1 mL cholesterol (5mg/mL in ethanol), 1 mL 1M CaCl₂, 1 mL 1M MgSO₄, 25 mL 1M KPO₄ buffer, in the same order. Swirl to mix well.
5. Using sterile procedures dispense the NGM solution into the OmniTray. Each OmniTray should have approximately 90 mL of agar.
6. Leave OmniTrays at room temperatures for 2 days to allow excess moisture to evaporate. (For long-term storage; store in air-tight containers at room temperature)

G: MATLAB Coding and Functions

Navin Code Activation:

```
>> navin
>> TrackerAutomatedScript ('moviefile.avi', 'scale', 'scalevideo.avi', 'numworms', 'number of
worms in the plate')
    %moviefile.avi- this is the name of video of the experiment captured
    %scale- used to tell the code to scale for proper coordinates and outputs
    %scalevideo.avi- a video file of a holepunch in a piece of paper used as dimensions for
the code to scale to
    %numworms- used to tell the code the number of worms in the video for analyzing
    %number of worms in the plate- can type in the number of worms in the plate for the
code to track
```

Struct2mat function: (this function was developed by Professor Dirk Albrecht)

```
function output = struct2mat(dim,structure,index,fields)
```

```
if ~iscell(fields) fields = {fields}; end
if isempty(index) index = 1:numel(structure); end
```

```
output = [];
for i = 1:length(index)
    substructure = structure(index(i));
    for j = 1:length(fields)
        substructure = getfield(substructure,char(fields(j)));
    end

    output = safecat(dim,output,substructure);
end
```

% the struct2mat function combines all similar fields into a single vector

Heat map Code:

```
load('glyceroltestomnitray.linkedTracks.mat')
AllX = struct2mat(2,linkedTracks,[],{'SmoothX'});
AllY = struct2mat(2,linkedTracks,[],{'SmoothY'});
imagesc(hist2D(AllY',AllX',5,5))
% linkedTracks.mat is an output matrix of the code, this heat map code used the SmoothX and
SmoothY coordinates
%struct2mat combines all SmoothX and SmoothY into a single vector
```

State Plot Code:

```
function [] = draw_worm(x,y,s)
load('glyceroltestomnitray.linkedTracks.mat')

AllX = struct2mat(2,linkedTracks,[],{'SmoothX'});
```

```
AllY = struct2mat(2,linkedTracks,[],{'SmoothY'});  
AllS = struct2mat(2,linkedTracks,[],{'State'});
```

```
x=AllX;  
y=AllY;  
s=AllS;
```

```
x=double(x);  
y=double(y);
```

```
N = length(x);
```

```
s = round(s*10);
```

```
for n=1:N
```

```
    switch(s(n))  
        case 10  
            cs = '.g';  
        case 13  
            cs = '.g';  
        case 40  
            cs = '.r';  
        case 50  
            cs = '.r';  
        case 11  
            cs = '.k';  
        case 30  
            cs = '.m';  
        case 20  
            cs = '.m';  
        case 70  
            cs = '.m';  
        case 80  
            cs = '.m';  
        case 47  
            cs = '.m';  
        case 74  
            cs = '.m';  
        case 43  
            cs = '.m';  
        case 34  
            cs = '.m';  
        case 53  
            cs = '.m';  
        case 35
```

```

        cs= '.m';
    case 41
        cs= '.m';
    case 31
        cs= '.m';
    case 51
        cs= '.m';
    case 71
        cs= '.m';
    case 12
        cs= '.m';
    case 57
        cs= '.m';
    otherwise
        cs = '.w';
end

plot(x(n),y(n),cs);
hold on
end

```

% linkedTracks.mat is an output matrix of the code; this state plot code used the SmoothX and SmoothY coordinates and the State

% struct2mat combines all SmoothX, SmoothY, and State into a single vector

% 10, 13, 40 etc. represent states of *C. elegans*, numbers are converted to states of behavior using 'num_state_convert'

References

- [1] Seung-Ho Kang, Sang-Hee Lee, Tae-Soo Chon. Exploring the behavior of *Caenorhabditis Elegans* by using a self-organizing map and hidden Markov model. *Journal of the Korean Physical Society* 2012, Vol. 60, Issue 4, pp 604-612.
- [2] Kotarsky K, Niclas E.N. *Reverse Pharmacology and the deorphanization of 7TM Receptors*, Elsevier 2004
- [3] Teng et al., Expression of mammalian GPCRs in *C. elegans* generates novel behavioural responses to human ligands, *BMC Biology* 2006, vol. 4 no 22.
- [4] Ophardt, Charles E. "Nervous System-Overview." *Virtual Chembook Elmhurst College*. 2003. Web. 10/13/13. <www.elmhurst.edu/>
- [5] Dafny, Nachum. "Chapter 1: Overview of the Nervous System." *Neuroscience Online*.2013. Web. 10/13/13. <neuroscience.uth.tmc.edu/>
- [6] Li, Chris and Kyuhyung Kim. "Neuropeptides." *Wormbook*. 2008. Web. 10/13/13. <wormbook.org/>
- [7] Chen, Pan et al. "Metal-induced neurodegeneration in *C. elegans*." May 20, 2013. Web. 9/26/13.
- [8] Hobert, Oliver. "Neurogenesis in the nematode *Caenorhabditis elegans*." 2010. *Wormbook*. Web. 9/26/13.
- [9] Albrecht et al., High-content behavioral analysis of *Caenorhabditis elegans* in precise spatiotemporal chemical environments, *Nature Methods* 2011, vol. 8 no.7 p. 599-606
- [10] Link, Christopher "C. elegans models of age-associated neurodegenerative diseases: Lessons from transgenic worm models of Alzheimer's disease" *Experimental Gerontology* 41 (2006): 1007-1013
- [11] "What is Parkinson's Disease?" *Parkinson's Disease Foundation* (2012) Web 10/9/2013<<http://www.pdf.org/>>

- [12] Harrington, Adam and Hamamichi, Shusei “*C. elegans* as a Model Organism to Investigate Molecular Pathways Involved with Parkinson’s Disease” *Developmental Dynamics* 239 (2010): 1282-1295
- [13] Altun, Z.F. and Hall, D.H. “Nervous system, general description” *WormAtlas* (2011) Web 10/14/2013<<http://www.wormatlas.org/>>
- [14] Hobert O. “Neurogenesis in the nematode *Caenorhabditis elegans*” *WormBook* (2010) Web 10/14/13<<http://www.wormbook.org/>>
- [15] Brenner, S. “The genetics of *Caenorhabditis elegans*.” *Genetics*, 1974, vol. 77, 71–94.
- [16] WormAtlas, Altun, Z. F., Herndon, L. A., Crocker, C., Lints, R., and Hall, D. H. (eds). <<http://www.wormatlas.org/>>
- [17] Szekeres, P. G. Functional assays for identifying ligands at orphan G protein-coupled receptors. *Recept. Channels* 2002, vol. 8, 297–308.
- [18] Kubiak, T. M., Larsen, M. J., Nulf, S. C., Zantello, M. R., Burton, K. J., Bowman, J. W., et al. Differential activation of social and solitary variants of the *Caenorhabditis elegans* G protein-coupled receptor NPR-1 by its cognate ligand AF9. *J. Biol.Chem.* 2003, vol. 278, 33724–33729.
- [19] Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P., et al. Inhibition of *Caenorhabditis elegans* social feeding by FMRFamide related peptide activation of NPR-1. *Nat.Neurosci.* 2003, vol. 6, 1178–1185.
- [20] Offermanns, S., and Simon, M. I. G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C. *J. Biol.Chem.* 1995, vol. 270, 15175–15180.
- [21] Heilig, M. The NPY system in stress, anxiety and depression. *Neuropeptides*, 2004, vol. 38, 213–224.
- [22] Arora, S., and Anubhuti. Role of neuropeptides in appetite regulation and obesity—a review. *Neuropeptides* 2006, vol. 40, 375–401
- [23] Davies, A. G., Bettinger, J. C., Thiele, T. R., Judy, M. E., and McIntire, S. L. Natural variation in the npr- 1 gene modifies ethanol responses of wild strains of *C. elegans*. *Neuron* 2004, vol. 42, 731–743.

- [24] Gray, J. M., Karow, D. S., Lu, H., Chang, A. J., Chang, J. S., Ellis, R. E., et al. Oxygen sensation and social feeding mediated by a *C. elegans* guanylatecyclase homologue. *Nature* 2004, vol. 430, 317–322.
- [25] Cheung, B. H. H., Cohen, M., Rogers, C., Albayram, O., and de Bono, M. Experience-dependent modulation of *C. elegans* behavior by ambient oxygen. *Curr.Biol.* 2005, vol. 15, 905–917.
- [26] Rogers, C., Persson, A., Cheung, B., and de Bono, M. Behavioral motifs and neural pathways coordinating O₂ responses and aggregation in *C. elegans*. *Curr.Biol.* 2006, vol. 16, 649–659.
- [27] Gloria-Soria, A., and Azevedo, R. B. npr-1 regulates for aging and dispersal strategies in *Caenorhabditis elegans*. *Curr.Biol.* 2008, vol.18, 1694–1699.
- [28] Styer, K. L., Singh, V., Macosko, E., Steele, S. E., Bargmann, C. I., and Aballay, A. Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science* 2008, vol. 322, 460–464.
- [29] Glauser, D. A., Chen, W. C., Agin, R., Macinnis, B. L., Hellman, A. B., Garrity, P. A., et al. Heat avoidance is regulated by transient receptor potential (TRP) channels and a neuropeptide signaling pathway in *Caenorhabditis elegans*. *Genetics*, 2011, vol. 188, 91–103.
- [30] Milward, K., Busch, K. E., Murphy, R. J., de Bono, M., and Olofsson, B. Neuronal and molecular substrates for optimal foraging in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 2011, vol. 108, 20672–20677.
- [31] Jang 2012 Jang, H., Kim, K., Neal, S. J., Macosko, E., Kim, D., Butcher, R. A., et al. Neuro modulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in *C. elegans*. *Neuron* 2012, vol. 75, 585–592.
- [32] The *C. elegans* Sequencing Consortium. Genomesequencingofthe nematode *C. elegans*: aplatformfor investigatingbiology. *Science*, 1998, vol. 282, 2012–2018.
- [33] “Neuropeptides.” *Neuropeptide Database*. Web. 10/13/13. <www.neuropeptides.nl/>
- [34] Kroeze, Wesley K., Sheffler, Douglas J., and Roth, Bryan L. “G-protein-coupled receptors at a glance.” *Journal of Cell Science* 116 (2003): 4867-4869. Web. 10/13/2013. <jcs.biologists.org/>

- [35] Stiernagle T. Maintenance of *C. elegans*. Wormbook, ed. The *C. elegans* Research Community, WormBook. February 11, 2006. <<http://www.wormbook.org>.>
- [36] Culetto E, Sattelle DB, Review A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. *Hum Mol Genet.* 2000 Apr 12; 9(6):869-77.
- [37] *C. elegans* Sequencing Consortium, Review Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science.* 1998 Dec 11; 282(5396):2012-8.
- [38] Chalasani,S. H.,Kato,S.,Albrecht,D. R., Nakagawa,T.,Abbott,L.F.,and Bargmann,C.I. Neuropeptide feedback modifies odor-evoked dynamics in *Caenorhabditis elegans* olfactory neurons. *Nat.Neurosci,* 2010, vol. 13, 615–621.
- [39] Sommer, R.J. *Evolution of development in nematodes related to C. elegans (December 14, 2005), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.46.1, <<http://www.wormbook.org>/>*
- [40] Hodgkin J. *C. elegans as a model organism, Genetics Unit, Department of Biochemistry, University of Oxford*
- [41] Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. *Science.* 1994; 263:802–5.
- [42] Lotte Frooninckx, Liesbeth Van Rompay, Liesbet Temmerman, Elien Van Sinay, Isabel Beets, Tom Janssen, Steven J. Husson and Liliane Schoofs. Neuropeptide GPCRs in *C. elegans*. *Neuroendocrine Science* 2012, vol 3, article 167.
- [43] Zuk, Patricia and Geffen, David. “Receptor-ligand interactions-cell signaling, adhesion, motility, cell migration.” Web. 10/13/13. <www.ipam.ucla.edu>
- [44] Albrecht, Dirk. “Engineered Animals for rapid screening of molecular interactions. September 1, 2013.
- [45] Mains, Richard E. and Eipper, Betty A. “The Neuropeptides.” *John Hopkins University of Medicine.* 1999. Web. 9/26/13.
- [46] RR Buch and D.N. Ingebrigtsen, “Rearrangement of Polydimethylsiloxane Fluids on Soil,” *Environmental Science and Technology* 13, 676, 1979.

[47] Pokala, Navin. Silencing of *Caenorhabditis elegans* neurons in vivo with histamine-gated chloride channels. *Proceedings of the National Academy of Sciences*, vol.111, no.7. 2770-2775.

[48] Lodish H, Berk A, Zipursky SL, et al. *Molecular Cell Biology*. 4th edition. New York: W. H. Freeman; 2000. Section 21.1, Overview of Neuron Structure and Function. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK21535/>

[49] Beal, MF and Martin, JB (1986). "Neuropeptides in neurological disease." *Ann Neurol* **20** 547-65