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Deep Brain Stimulation Device for *C. elegans*

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Abstract

Deep Brain Stimulation (DBS) is a surgical procedure that utilizes electrical stimulation to affect neural activity and has a mechanism that is currently unknown. To address this problem, a stimulation device for a model system (*C. elegans*) had to be designed. This was composed of an electrical stimulator, a microfluidic device and a mechanism for automation. To meet this goal a number of parameters needed to be determined, including the minimum viable electric field which was 0.28 V/mm. The device created was capable of a range of 3.3 – 30V, 1 Hz -150 kHz, and a minimum pulse width of 6.67 ns. Using automation, these parameters could be easily and accurately manipulated during experiments. The final system, which used a two-channel microfluidic device, simultaneously ran tests against a control group. Through experimentation, the device exhibited the capability of manipulating neural excitability with a 300% increase at 20V and direct activation of the neuron at 30V.

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Glossary

ACC: Anterior cingulate cortex
ASTM: American Society for Testing of Materials
ATN: Anterior thalamic nucleus
*C*BGTC: Cortico-basal ganglia-thalamo-cortical loop
C. elegans: Caenorhabditis elegans
CMTN: Centro median thalamic nucleus
CT: Computed Tomography
DBS: Deep Brain Stimulation
DLPC: Dorsolateral prefrontal cortex
ET: Essential Tremor
FDA: Food and Drug Administration
FRET: Förster resonance energy transfer
GABA: gamma-Aminobutyric acid
GUI: Graphical User Interface
ISO: International Standard Organization
LED: Light-emitting diode
MRI: Magnetic Resonance Imaging
OCD: Obsessive-compulsive disorder
OFC: Orbitofrontal cortex
PCR: Polymerase chain reaction
PD: Parkinson's Disease
PDMS: Polydimethylsiloxane
RNAi: RNA interference
STN: Subthalamic Nucleus
2D: Two dimensional
3D: Three dimensiona

1 Introduction

The human brain is a complex organ that is responsible for all functions of the human body. It both interprets and receives information while also controlling thoughts and emotions. Since the brain completes a significant number of tasks simultaneously, it is vital to ensure that it functions quickly and automatically. However, when damage to specific brain locations occurs it can affect certain impulsive movement behaviors and can lead to neurological disorders. There are a few ways today that neurological disorders are treated, one of which is Deep Brain Stimulation (DBS). DBS is a procedure which involves implanting electrodes into the brain to apply electric impulses to different regions of the brain. This procedure is used to treat a number of neurological disorders where issues of neural activity are present, including both overactive and underactive neurons [Gardner, 2013]. The five disorders currently approved for treatment through the use of DBS include Parkinson's disease (PD), Dystonia, Epilepsy, Obsessive compulsive disorder (OCD) and Essential Tremor [Deep brain stimulation - Mayo Clinic, n.d]. DBS has been successful at alleviating symptoms for all of these different disorders however, why it is able to alleviate these symptoms is unknown.

The physiological responses on the neural level to DBS is still not understood by researchers due to the complex pathways and workings of the human brain. It is clear that this treatment is showing dramatic effects on the symptoms, especially in the case of PD, but what changes are occurring to the neurons is unknown. Due to this lack of knowledge, there are no set parameters for treatment of diseases using DBS. Instead, during a DBS procedure the surgeon/doctor will manipulate the electrical stimulus characteristics such as voltage, pulse width and frequency until the optimum response is observed. To start to address these gaps in knowledge DBS can be conducted on a model organism to better monitor the neural response. An ideal model organism for this task is *C. elegans* which are microscopic worms that have similar homology in their neural circuit to that of a human with similarities in synapses, ion channels, neurotransmitters and genes [Apfeld & Alper, 2018].

To use *C. elegans* for this purpose a stimulation device similar to that used in DBS must be created. This was the goal of the project to create an automated system that is capable of stimulating the neurons of *C. elegans*. By creating this device and using neural imaging software, the effects on neural activity in the *C. elegans* during electrical stimulation could be monitored. As well, using optogenetic stimulation before and after electrical stimulation trends and changes in neural activity could be observed. An example of an experiment of how the effects of DBS would be conceptually studied in vivo is shown in **Fig 1.1**. Allowing with determine an ideal experimental concept a number of design requirements also had to be determined in order to reach this goal.

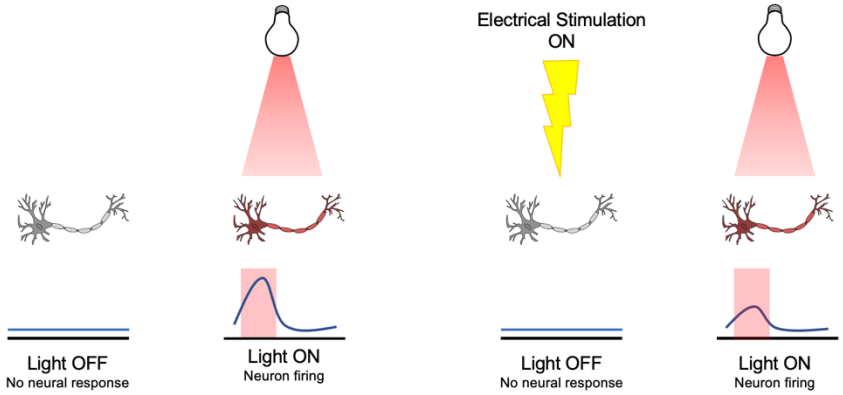


Figure 1.1: Conceptual experimental outline for studying effects in vivo

When determining the design requirements, the device being designed can be broken up into three different parts. These include the stimulation device, the microfluidic device and the serial control of the system. Unlike the human DBS system, this electrical stimulation system must be external due to the size of the *C. elegans*. By using a microfluidic housing device, the electrical stimulus is able to be applied across the area where the animals are housed, stimulating their neurons in the process. In terms of the stimulation device the main parameters were the ability to manipulate and reach certain values for the frequency, pulse width and voltage. Finally for the serial control, all of the parameters of the stimulus were required to be automated so that the system could rapidly and effectively manipulate parameters with little oversight needed. The team went through a number of steps to build this device and verify its ability to function properly.

The following sections will discuss the steps the team went through to accomplish this goal of creating a functioning device. As outlined in the following sections the first step in the process required doing extensive research into the current practices and research being conducted on this topic. From here a project strategy was determined to identify the best way to create this device. Then the team began the design process which required multiple iterations and changes being made to the device. After this came putting the device together with all of the different components needed to conduct experiments on live animals. From here experiments were conducted and results were collected. These results were analyzed and used as verification that the device was working as anticipated. The results obtained where then discussed pointing out important conclusions which were made, and information learned from the experimentation. Finally, conclusions were drawn about the research and recommendations for future directions of research with the device were laid out.

2 Background

2.1 Deep Brain Stimulation

DBS is a neurosurgical procedure which involves the placement of a medical device called a neurostimulator into the brain. Throughout history this device had evolved and turned into what is now called a DBS device. This device once implanted will send electrical impulses to specific target regions of the brain. The components and more detailed procedure for this device will be discussed further in the following sections. Many different diseases and disorders can be treated through DBS and multiple studies have been conducted into how DBS is being used. Although DBS can be used to treat multiple neurological disorders, it most commonly is used for movement disorders such as PD.

2.1.1 History of Deep Brain Stimulation

Early DBS devices were either modified cardiac pacemakers or based on cardiac pacemakers. The basic components of a cardiac pacemaker are the power source, timer, output driver, and electrodes. While more advanced pacemakers contain more components, these four are always present [Nguyen et. al., 2016]. Although pacemakers were the baseline for modern day DBS devices there are some differences in functionality as DBS pulse stimulators need more precision when it comes to voltage, pulse width, and frequency [Pacemakers, n.d]. DBS sees its roots in stereotactic or functional neurosurgery which was popularized in the 1940s.

Throughout this time imaging allowed for different parts of the brain to be identified and associated with specific disorders. These advances also decreased the mortality rate of the procedure from 15% to 1% and within 10 years over 40 locations across the globe had begun adapting these procedures. The growth of this technology was charged by neurological disorders that did not have any other treatments. Before the 1950's when antipsychotic drugs were introduced, parts of the brain that were associated with neurological disorders were destroyed. Parkinson's disease was one of the primary conditions stereotactic surgery was utilized for. During this time doctors were also able to investigate the effects of stimulation on areas deep within the brain. Throughout this time, they were also able to draw connections between areas of the brain and symptoms of disorders such as Parkinson's [Gardner, 2013]. A timeline of the important events associated with DBS can be seen in **Fig. 2.1** below.

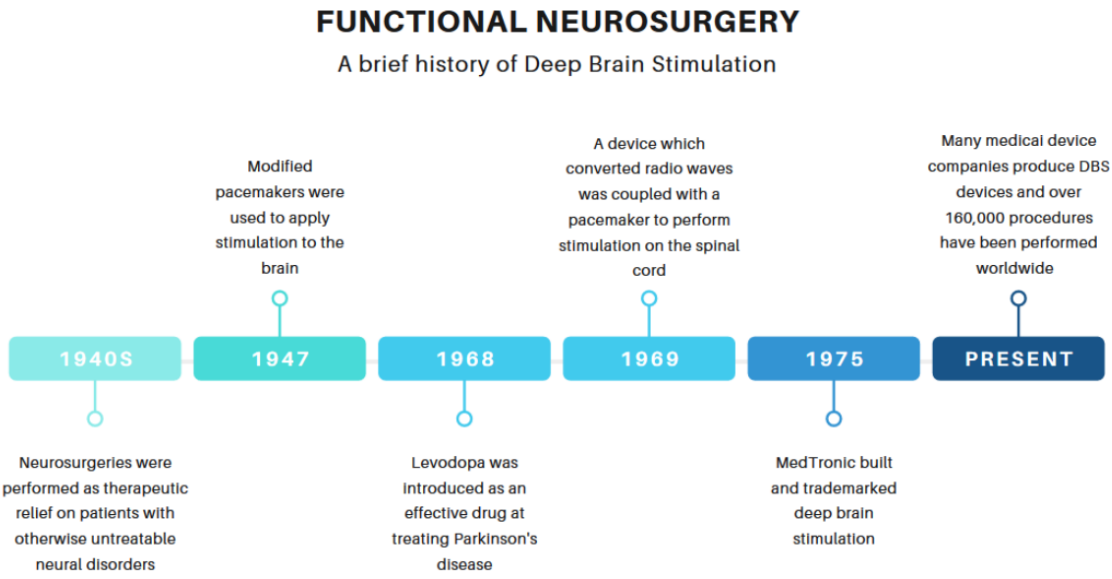


Figure 2.1: Timeline of functional neurosurgery and how DBS got to where it is today

2.1.2 DBS Components and Procedure

DBS consists of three main components: 1) electrodes, 2) wires, 3) internal pulse generator. First is the electrode that is placed in targeted sections of the brain to achieve stimulus. This electrode can also be connected to a lead that sits on the surface of the brain. The second primary component is the wire that connects the electrode in the brain to an additional device placed in the chest. The third component is the neurostimulator or internal pulse generator which controls the stimulus to the brain [Pilitsis, Khazen & Patel, n.d]. The neurostimulator in the chest is controlled by a handheld device that allows for the patient to control when the DBS is on or off [Mandybur, n.d].

The general DBS procedure can be broken down into two surgical procedures. The first is the brain surgery throughout which the brain activity is monitored. Prior to the actual surgery, the patient has an MRI to map out the brain and identify locations for electrode placement. There are two different strategies for electrode placement; 1) multiple electrodes that target a specific area of the brain, 2) a lead can be placed on each side of the brain. These leads are connected to a wire that runs underneath the skin to the pulse generator.

The second procedure is to place the pulse generator in the chest of the patient. The generator is placed underneath the skin on the chest near the collarbone. Weeks after this procedure is complete a doctor will activate the pulse generator with specific settings for stimulation. It can take many months to determine the optimal stimulation settings which requires regular visits to the doctor. The stimulation can be on for 24 hours a day however, it is dependent on the condition and specific patient needs [Deep brain stimulation, n.d].

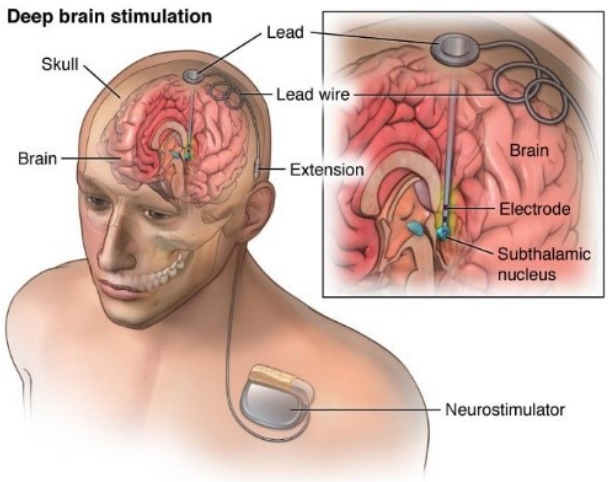


Figure 2.2: Example of DBS and how the device appears when it is inserted in the brain along with a breakdown of the major components of the device [Deep Brain Stimulation – Advantages, Risks and Conditions Treated, n.d]

DBS devices currently on the market are composed of the same main components and work in the same manner. The differences come in design and specific functionality of the pulse generator. The two main competitors of this device are Medtronic and Boston Scientific. A comparison between the different devices available from these two companies can be seen in the **Table 1** below.

*Table 2.1: Comparison of major DBS devices on the market today [*Deep Brain Stimulation Systems, n.d], [Vercise DBS Clinical Study Data, n.d] and [Neurological Surgery: DBS and SCS Products, n.d]

Device	**Company**	**Number of Channels**	**Rechargeable**	**Other Features**
Activa™ SC	Medtronic	single channel	non-rechargeable	Ideal for moderate energy level use and limited to single side stimulation
Activa™ RC	Medtronic	dual channel	rechargeable	Used in PD and ET patients with high energy needs, has a battery lifespan of 15 years
Percept™ PC	Medtronic	dual channel	non-rechargeable	Only device with sensing technology, can capture and record brain signals while providing stimulus, battery lifespan of >5 years
Vercise™ DBS	Boston Scientific	dual channel	rechargeable	Has the ability to stimulate finite areas of the brain, longest battery life on the market

2.1.3 Studies of Deep Brain Stimulation and Treatment of Specific Disorders

Since the 1940s when deep brain stimulation was first used there have been numerous studies and clinical trials to advance the procedure. Worldwide there are 700 different centers which are able to perform deep brain stimulation. Most of the time deep brain stimulation is being used in patients who suffer from movement disorders such as Parkinson's and essential tremor. Therefore, many of the studies and research that has been conducted involved those two disorders, especially Parkinson's disease which is the most commonly treated disorder using DBS procedures. A study conducted by Harmsen and associates [Harmsen et. al., 2020] looked at all of the ongoing and previous clinical trials pertaining to deep brain stimulation and recorded numerous different parameters. There have been 384 different DBS trials since 1997 targeting 28 disorders. The majority of the clinical trial focused on movement disorders at 62% of all the clinical trials. Parkinson's disease alone accounts for 151 trials and 39.3% of all the studies. In these studies, there have been 26 different regions of the brain that were targeted. The most commonly targeted area is the subthalamic nucleus (STN) with 33% of all trials. Majority of the trials however, 57%, target regions involved in the cortico-basal ganglia-thalamo-cortical motor circuit (CBGTC loop).

In a study conducted by Lozano and Lipsman [Lozano et. al., 2019], they studied how different target areas helped different neurological disorders including the five discussed in this report. However, a more in-depth knowledge of what precise areas to target the lead for not only the illness but the particular patient would be key to truly making the treatment more effective seen in **Table 2.2** below.

Table 2.2: Parameters for DBS treatments based on the neurological order of interest [Groiss, Wojtecki, Südmeyer & Schnitzler, 2009], [Bogdan, van Laar & Oterdoom, 2020] and [Magown, Andrade, Soroceanu & Kiss, 2018]

Disorder	**Frequency**	**Pulse Width**	**Voltage**
Parkinson's Disease	130 - 180 Hz	60–90 μs	2.5 - 3.5 V
ET	130 Hz	40–90 μs	1.5–4 V
Dystonia	131 Hz ± 5 Hz	112 ± 31 μs 203 ± 22 μs 446 ± 8 μs	3.3 V ± 0.6 V

DBS has been FDA approved for treatment of five different neurological disorders. As previously mentioned, these include Parkinson's disease, OCD, Epilepsy, Dystonia, and ET [Deep brain stimulation, n.d]. DBS initially received FDA approval in 1997 for the tremors associated with Parkinson's Disease. In 2002, it then received additional approval for use in advanced Parkinson's. Most recently, in 2016, DBS was approved for usage in the early stages of Parkinson's. Each patient must meet a list of qualifications in order to be a candidate for DBS. Some of these qualifications include having Parkinson's symptoms for over five years, having

dyskinesias, an inability to use medications due to side effects, or that medications are insufficient. If a patient meets these requirements as well as others, then the procedure process is initiated [Deep Brain Stimulation (DBS), n.d].

2.1.4 Neurological Disorders Overview and Treatment

As previously mentioned, five different neurological disorders have been FDA approved for treatment through the use of DBS. These five disorders can be seen in the **Table 2.3** below where the region of the brain which has been affected can be seen along with the effects this damage has on neural activity. An overview of the disorders in terms of activity and region. For the purpose of this study however the main neurological disorder that will be focused on in more specificity is PD. The information on the symptoms, physiological cause and treatment of this disorder can be found in the following section.

Table 2.3: Relevant neurological disorders with the regions of the brain impacted and how those regions are impacted

Neurological Disorder	**Region of the Brain Impacted**	**Reduction/Elevation of Activity**
Parkinson's Disease	Brain stem, cerebral cortex	Reduction in activity, loss of dopaminergic neurons
Essential Tremor	Cerebellar function	Elevation in activity, bilateral cerebral hemispheric activation
Epilepsy	Glutamate and gamma-aminobutyric acid (GABA) neurotransmitter pathways	Elevation and reduction in activity, depending on glutamate and GABA levels
Dystonia	Damage to multiple brain regions including basal ganglia, thalamus, brainstem, parietal lobe, and the cerebellum	Reduction in activity, inhibition and sensory motor integration
OCD	Dorsolateral prefrontal cortex (DLPC), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC)	Elevation and reduction in activity, DLPC and OFC inhibited and ACC increased activity

2.1.4.1 Parkinson's Disease

Parkinson's disease (PD) is a common neurological disorder, especially in the elderly population. PD is characterized by neurodegeneration that initially impairs gait and movement

function and progresses to eventually affect the cognitive function of individuals as well. There currently is no cure for PD and the main route of action is improving symptoms through physical therapy, speech therapy and mental health counseling. Since PD is a neurodegenerative disease, it is classified by neuron loss which begins in the brainstem and advances to eventually reach the cerebral cortex. More specifically there is a loss of dopaminergic neurons from the substantia nigra located in the midbrain [Hawley, Armsrong & Weiner, 2014]. The damage which occurs to the substantia nigra occurs in a systematic manner where the lateral ventral tier of the pars compacta suffers from the most damage. The main options for treatment are based on pharmacological strategies, such as Levodopa (this drug has seen the greatest effect at treating PD patients). These treatments target the loss of dopaminergic cells in the substantia nigra by replacing or enhancing dopamine levels in the basal ganglia. By targeting this pathway, the symptoms which are usually improved include tremor, bradykinesia, rigidity and gait impairment.

For the treatment of Parkinson's disease, noninvasive diagnostic imaging is performed using magnetic resonance imaging (MRI) or computed tomography (CT) scanning. The target area of the brain is thus identified for the surgery. Specifically, for treatment of Parkinson's disease, DBS targets the part of the brain that plays a role in the control of movement including the thalamus, which involves sensory and motor information, the subthalamic nucleus (STN), which helps direct movement, or the globus pallidus, which helps regulate intended movement. [Deep Brain Stimulation for Movement Disorders, n.d.] The required parameters for stimulation in these area can be seen in **Table 2.4** below.

Table 2.4: Parameters for DBS treatments based on the target region of the brain

Region of the Brain	**Frequency**	**Voltage**
ATN	≥100 Hz	1-10 V
Hippocampal and STN	≥130 Hz	1-5 V
CMTN	≥200 Hz	1-10 V
Cerebellum (low)	10 Hz	1-10 V
Cerebellum (high)	200 Hz	1-10 V

2.2 Current Experimental DBS Studies

DBS has been used as a treatment for neurological disorders for many years and is still used today. However, there are many limitations of these procedures and much is still unknown about the mechanisms of action. Therefore, the limitations of the current research will be

discussed in the following section. As well as the studies which are being conducted on model organisms to try and approach these limitations.

2.2.1 Limitations of Current Research

While DBS has been a groundbreaking treatment that has helped over 160,000 patients as of 2019, there is much that needs to be improved in the implementation and utilization of DBS [Lozano, Lipsman, Bergman, et a 2019]. Alongside gaps in treatment, research into both the underlying pathology and DBS itself will need to be done to further understand and better treat patients. With this in mind, the purpose of this study is to use a model system (*C. elegans*) to help further the understanding of why DBS works, and how improvements can be made with a clear standard of dosing for each unique patient.

Some of the current studies being conducted on *C. elegans* are similar to the proposed study in that they use microfluidics, electrical stimulation and Parkinson's models of *C. elegans*. One study in particular conducted by Youssef and colleagues [Youssef et. al., 2020] used a similar experimental set up. This experiment was focused on movement in the *C. elegans* when electrical current was applied. The strength of the electrical current applied to the animals was 3.7 V/cm and the polymodal (Amphid neurons) ASH sensory neurons were activated. This electric field strength was proven through previous experimentation to be within the minimum viable field strength of 2-4 V/cm [Rezai, Siddiqui, Selvaganapathy & Gupta, 2009] required for electro taxis on young *C. elegans*. Although this study has a similar set up the focus is on the movement and behavior of the animals rather than the neural changes going on during the electrical stimulation. *C. elegans* can specifically detect e-fields and their orientation with specific sensory neurons, however, the goal for our research is to alter neural excitability to other input signals, rather than directly activate neurons. This highlights the gap in current research focusing on understanding and monitoring neural activity during DBS.

Understanding how DBS operates is essential to both understanding how to improve it as a treatment option and understanding the underlying pathology of the treatments themselves. It is suspected that it may affect resting membrane potentials and thereby alter neural excitability thereby ceasing the symptoms [Deep Brain Stimulation for Movement Disorders, n.d]. Despite this, there still is not a definite answer to how exactly it works which is why our study will develop an experimental method to begin the analysis of what is happening on a neurological level during DBS.

2.2.2 Non-Human Studies

As the years have gone by the need for more information on the study of DBS has increased. To acquire this information humans as in most scientific studies began to use animals and other animal like models for their testing in hopes of understanding more about it. Some of these animals include mice and monkeys, however, although important breakthroughs have been achieved in what we know so far, there were always problems remaining unsolved. Specifically, the mechanism by which DBS acts and the long-term effects that may be a result of the

emergence of new or a strengthening of existing neuronal circuits that compensate for the absence of dopamine in the brains of young rats [Badstuebner et. al., 2017]. Today, model organisms are being used in place for ethical and practical reasons, and particularly for DBS *C. elegans* prove to be advantageous for multiple reasons, some of which are explained further below.

2.3 *C. elegans* as a Model System

As previously discussed, many animal systems can be used to model treatment methods for diseases and disorders. One of these models is the *C. elegans* specimen which is particularly useful when studying neurological disorders. Their small size and well-defined nervous system make them an ideal system for neural imaging. They can also be genetically modified to create models of neurological disorders such as PD allowing for specific studies of disorders to be completed. The *C. elegans* specimen also has a striking similarity to human neurological physiology, making it a good model for human disorders.

2.3.1 *C. elegans* Background

The nematode worms *Caenorhabditis elegans* (*C. elegans*) are miniscule organisms that can grow to be 1 millimeter long as adults. Mature *C. elegans* consists of roughly 1000 somatic cells and 2000 germ cells and 302 neurons, with connectome mapped. The fact that there are only 302 neurons is important for understanding and drawing conclusions from the changes in neural activity and specifically understanding what areas and neurons are being affected. Despite their relatively simple anatomy, it has a relatively complex genome comprising roughly 18,000 genes, which makes it ideal for the correlations to human neurons [Cooper & Van Raamsdonk, n.d]. The majority of *C. e*legans worms are hermaphrodites with 1 in 1000 being male worms.

The life cycle of *C. elegans* consists of an embryonic stage, four distinct larval stages, and adulthood. The embryo stage can last for roughly 13 hours. The fast maturity of the animals makes them ideal for running tests, as there doesn't need to be long periods of time waiting for them to be ready for experimentation. The process begins with the proliferation of the cells after fertilization. Proliferation can take roughly three hours and consists of the cells dividing until the beginning of the second phase, organogenesis. Once organogenesis begins, the cells begin to differentiate into the respective organs they will become. Once fully developed, the *C. elegans* hatches as a fully formed worm coated with a cuticle which is a hardened exoskeleton-like material. The four larva stages (L1-L4) are characterized by the *C. elegans* molting their cuticle as it grows in size. At the end of its final larva stage, hermaphrodites may begin laying eggs continuing the cycle. The typical lifespan for a worm is 2-3 weeks. Because of the well documented life cycle of *C. elegans*, researchers are able to isolate the exact life cycle which they want to conduct studies into. For this study, *C. elegans* in L4 would be ideal in order to observe a mature brain whilst avoiding behavioral changes due to pregnancy. In order to achieve

this result, experiments should be conducted on *C. elegans* between 56-65 hours after hatching at 20° Celsius [Hermaphrodite Introduction, n.d].

Due to having a short lifespan and being a self-fertilizing hermaphrodite [WormBook, n.d], they are excellent for uses in eukaryotic genetic studies. In the field of neuroscience, they are especially useful because they are the first species whose whole connectomes have been discovered by researchers [Cook et. al., 2019]. The following is significant because, while *C. elegans* are genetically different from humans, the same genes that control neural connections are the same genes that malfunction causing neurological disorders such as PD.

C. elegans have been used as model organisms for discoveries of human disease genes and pathways as well as for engineering human genes into *C. elegans* to develop models of human disease. These models have been to facilitate discovery of other genes that modulate that same human disease. The use of these models began over 50 years ago when it was first proposed to use *C. elegans* to investigate developmental biology and neurobiology. There were three important discoveries that were made, one relating to development and cell death machinery, one was the discovery of RNA interference (RNAi) and lastly the discovery of Green Fluorescence Protein (GFP) and the suggestion of it as a useful tool in other organisms including *C. elegans*. Overall, the small size, life cycle, and ideal genetic and genomic tools that are involved have made *C. elegans* a great model organism for many purposes in studies in biology, one which will be used for this project as well [Apfeld & Alper, 2018].

2.3.2 Neural Imaging of the *C. elegans*

C. elegans are an excellent model for neural imaging in vivo due their transparent bodies as well as their easily defined nervous system. Due to their transparency, this neural imaging is typically carried out using fluorescence via calcium indication. There are multiple different options for detecting a calcium response in the neuron. Most commonly genetically encoded calcium indicators are introduced into the specimen through polymerase chain reaction (PCR) amplification. Some of the most commonly used indicators in the application of *C. elegans* are cameleon, and GCaMP. Cameleon is what is referred to as a FRET-based indicator, meaning that it is comprised of two different fluorescent proteins. Indicators such as this typically have greater accuracy and are less reactive to minimal movement in the animal however, special equipment is required in order to complete this imaging. GCaMP is a non-FRET-based indicator, and only consists of one fluorescent protein. While the application of this indicator can be detected on a traditional imaging set-up, it is more likely to pick up on movement artifacts of the animal [Rothman & Singson, 2012].

For the imaging process, the animals are typically immobilized in order to reduce the potential for movement artifacts in the reading. They are then stimulated in some manner, whether it be via chemical stimulus, light stimulus, or for the sake of this study, electrical stimulus. Microscope and imaging technology is then used to measure the fluorescence caused by the calcium indication method chosen. The levels of fluorescence would then directly

correspond to the level of neural response. This is because when the action potential of the neuron is reached, calcium ions are released as a result [Chung et. al., 2013].
The greatest issue with the imaging of this specimen is effectively immobilizing them in order to record an accurate reading. In many cases, a chemical immobilizer can be utilized however, this can have adverse effects on the specimen and may not be an option when studying physiological effects. Another option is physically securing the specimen down to the surface which can be done using a particular type of glue. [Rothman & Singson, 2012]

2.3.3 *C. elegans* Parkinson's Model

In this study, *C. elegans* will be used as a model system for monitoring neural response and understanding the mechanisms of action. Although there are five different disorders that DBS usually targets, all of these disorders are not readily available to simulate in *C. elegans*. The two disorders which have easily obtainable *C. elegans* strains are PD (α-synuclein or LRRK2) and epilepsy (cca-1 ortholog), although PD will be the focus for this study due to the abundance of resources available. Different genes in *C. elegans* have been linked to the genes in humans causing the disorder. To better analyze the DBS treatment being applied to the *C. elegans* it can be applied to a control and a test group where mutant *C. elegans* with PD. Some of the most commonly used options include the NL5901 which expresses genetic modifications in some of the muscle cells which have been tagged with yellow fluorescent protein. These transgenic *C. elegans* express the human synaptic protein α-synuclein in body wall muscle showing inclusions of aggregated protein, which affect similar genetic pathways as in humans. Besides providing better neurological insight into the physiology of DBS it is also possible to get behavioral observations from these experiments as well using *C. elegans* models that have behavioral features [Parkinson's disease (disease), n.d].

2.3.4 *C. elegans* and Correlation to Human Model

Another one of the reasons that *C. elegans* are such a good model system is due to the fact that its connectome is fully mapped. In combination with its connectome being fully mapped, its genome has also been completely sequenced [1998: Genome of Roundworm C. elegans Sequenced, n.d.]. With the combination of the field of genetics and neuroscience, neurological disorders can be modeled in these animals which allow researchers to better understand how these disorders occur. Because of the completed genome of *C. elegans*, biologists can introduce the genetic mistakes that cause the specific disorder to occur into the DNA of the worms. Once introduced, the worms will proliferate creating a population of worms with the intended disorder. Because the genes are the same that direct neuron connections as in humans, observation of *C. elegans* neural circuits can be related to human neural circuits. As well, there have been a number of genes and pathways identified in the *C. elegans* model that are the same in the human model. These neural similarities allow researchers to draw conclusions based on the *C. elegans* research that correlate to predicted effects in a human model. While the

connection may not be perfect analogs, the behaviors can still be used to make assumptions that can predict human response.

In order to effectively study these organisms and use them as human models, neuroimaging is performed to acquire large-scale recordings of neuronal activity. This can be achieved using in awake and unrestrained animals to provide information of how these populations of neurons generate this animal behavior. In our project, the software ImageJ is used to record intracellular calcium transients in the head of a freely behaving *C. elegans* with cellular resolution and also records the animal's position and orientation. By using this a whole-brain image of the behaving animal is provided in cellular resolution. Although there may be noise encountered in the system due to animal motion, neuroimaging shows that across worms, multiple neurons show significant correlations with modes of behavior with respect to the amount of stimulation they endure [Parker & Furman, 1975].

3 Project Strategy

3.1 Project Goals and Approach

The goal of this project is to design an automated signal generator that can distribute electrical stimulations to model organisms (*C. elegans)*. This design will be used to complete further experiments and study the effects that Deep Brain Stimulation has on patients with Parkinson's disease and other neurological disorders. In this process there were several steps and stages involved, the first of which was finding a signal generator, designing and adjusting it appropriately by adding serial controls and creating a user interface. Apart from this was finalizing the method by which the stimulation tests would be done. Various parameters and aspects of the project were dependent on the approaches taken and a final design was decided upon after comparisons and testing.

3.2 Initial Client Statement

To establish the client statement, the final goals of the project were determined with the assistance of our advisor who serves as the client for this project. The overall goal is to improve the process of DBS in humans by studying the results of applying DBS parameters to *C. elegans* models. Taking these goals into account the client statement developed is to: create an experimental system to measure the neural effects of DBS stimulation parameters. This will include creating a physical device to administer a patterned electrical stimulus to the model system of *C. elegans*.

3.3 Technical Design Requirements - Stimulus System

One of the first steps in this design process was establishing the baseline design requirements to meet the client statement and fulfil the goals of this project. For each of these design requirements, parameters were set to narrow down design fulfillment options.

3.3.1 Objectives

1. *Stimulation of the C. elegans AWA (head) neuron*: The system needs to provide sufficient current to the head neuron of the *C. elegans* specimen, ideally in a multi-specimen arena setup.
2. *User- Friendly*: The device needs to be easy to use and be easily adjusted by the user.

3.3.2 Specifications

1. *DBS Stimulation parameters*: The device needs to be able to provide both low and high frequency stimulation to the specimen. The device also must have the capability to provide enough voltage to stimulate the neuron of the animal.

3.3.3 Constraints

1. *Cost*: The total cost of the device should not exceed $200 in order to be comparable to existing stimulators on the market.
2. *Size*: The full device needs to fit into the lab space, approximately 2 square feet.
3. *Integration into system*: The device will need to be able to connect to the existing microscope and microfluidic setup in the lab. This means the electrode connection and device itself cannot impede the use of the microscope and the camera utilized to complete the imaging of the specimen.

3.3.4 Functions

1. *Adjustment of Parameters*: The device needs to not only meet the previously stated parameters but have the ability to change or adjust them to create different testing situations.
2. *Neural Stimulation of the Specimen*: The device needs to provide sufficient voltage/current to directly stimulate the neurons of the *C. elegans* specimen.

After establishing each of the design requirements, they were compared in a pairwise analysis (See **Appendix A**). This analysis allowed the team to establish the most important design considerations and prioritize. The results of this analysis ranked the ability to provide voltage, the ability to stimulate the neurons, and the ability to control signals as the top-ranking design requirements, all with equivalent rankings. Being user-friendly and customizable ranked second in this analysis, meaning that these requirements should be considered but should not take top priority. Size should also be considered, but again only after meeting higher ranking requirements. At the same time, the cost was one of the lowest ranking requirements, and due to the budget associated with this project, it still needs to be considered. Aesthetics received the lowest rank and will only be considered if all other design requirements are met.

3.4 Technical Design Requirements - System Automation

In order for the system to effectively handle all of the design requirements, a software protocol will need to be created in order to handle the serial control of the device. The protocol will need to meet the following requirements.

3.4.1 Objectives

1. *Automated System*: the code for the control of the stimulus system needs to run mostly on its own based on input from the user.
2. *User Friendly*: the user interface should be easy to use.
3. *Efficient*: the system automation should run with limited delays in order to ensure accuracy of the tests being run and should control all of the variable parameters in one program.

3.4.2 Specifications

1. *Serial Control*: The system must use serial commands to communicate with the device.
2. *Graphical User Interface*: The system must allow the user to input the experiment parameters via a graphical user interface (GUI) that would relay these parameters to the device.

3.4.3 Constraints

1. *Bean Shell Language*: the protocol must be written in Bean Shell to conform to the current standard lab protocol.
2. *Micromanager 1.4*: The protocol must use currently existing code to run the microscopy experiment which currently utilizes the open-source software known as Micromanager.
3. *Integration into the system*: The protocol must integrate the current code into the timing mechanisms set forth by the microscopy code (i.e., the microscopy code will be the clock and the stimulation must happen in sync with it).

3.4.4 Functions

1. *Bidirectional communication*: The system should be able to send/receive input from the device in order to verify sent commands are received.
2. *Parameter Variation*: Changing of experiment parameters such as (voltage, current limit, frequency, duty cycle)
3. *Timing*: the system must accurately control the timing of stimulation duration
4. *Pulse Trains*: the system must have the capability to produce accurate pulse trains of specified length and duration.

3.5 Design Requirements - Standards

When creating a design, certain standards must be considered in order to ensure the design meets any regulatory needs. For this design there are two primary aspects that must be considered: a) *C. elegans* testing and b) serial control of the signal generator.

Since *C. elegans* are classified as invertebrate, the standards that relate to human care do not need to be considered. However, to ensure the safety and maintenance of these microorganisms, certain parameters should be considered. There are specific guidelines, published on the website: wormbook.org, for example, the culture and creation of *C. elegans.* These guidelines include how to design appropriate experimental methods which take into consideration the health of the worm and maximum life span. Worm health can be established through maintenance of temperature, humidity and similar aspects, along with ensuring age synchronizing. During testing, it will be important to keep the age consistent among all of the worms for more accurate results. Included in these considerations are also the solutions and gels used in the testing experiments which are further explained and taken into account from the "American Society for Testing of Materials (ASTM)". To begin monitoring the neural response of these animals, the International Standard Organization (ISO) specifies certain standards related to microscopy, which can be seen in section: ISO 8036:2015. This standard is

particularly relevant as it describes the characteristics of immersion liquids used in microscopy. The *C. elegans* being utilized in tested will be immersed in a liquid solution to ensure their ability to be transferred into and out of the microfluidics device. ISO classifies immersion liquids according to their field of application and specifies requirements and test methods for each type. Various equipment will be used for microbiology research, such as microscopes, microfluidic devices and more. The principle of this standard may also be applied to test similar products or applicators with modification of the procedure as appropriate. In order to ensure product quality and safety for consumers, an appropriate microbiological risk analysis will be performed to determine what International Standard would be applicable. [ISO 8036:2015, 2020]

For the automation of the system, which will be part of the design of the device for testing the animals, there are other standards which need to be adhered to. These are explained further in section ISO 9001:2015, in which the functional safety features to prevent failure and harm to the user, are explained further. In computing, a programming language specification is necessary so that users and implementors can agree on what programs in that language mean. In this project, two or more devices will interface, which is called RS-232 and is the interchange of serial binary data between two devices. [ISO 6951:1986, 2018]

3.6 Revised Client Statement

After further evaluation of the requirements necessary for the design, a more specific client statement was required. To incorporate all of these requirements, the revised client statement is to: create an experimental system to measure the neural effects of DBS stimulation parameters of voltage, frequency, and pulse width, which includes creating a physical device to administer a patterned electrical stimulus to *C. elegans*. The system should provide automated control of electrical stimulation, delivered to an existing apparatus used to assess neural & behavioral responses in the nematode model.

3.7 Project Approach

The timeline for this project was broken up based on what needed to be determined for each term as well as what resources were available under the given circumstances. The general approach followed was: 1) Background/Literature Review, 2) Microfluidics Testing & Signal Generator Determination, 3) Preliminary Testing & Serial Control Creation and lastly 4) Power Supply & Final Parameter Determination. To better display this information a Gantt chart (**Figure 3.1**) and experimental timeline (**Figure 3.2**) can be seen below.

Gantt Chart

	A Term						
	Reach Goal: Begin working in the lab with stimulation device						
	Realistic Goal: Have stimulation device picked out and purchased						
Task	W1(8/31-9/6)	W2 (9/7-9/13)	W3 (9/14-9/20)	W4 (9/21-9/27)	W5 (9/28-10/4)	W6 (10/5-10/11)	W7 (10/12-10/16)
Client Statement		**9/8**					
Literature Review				**9/27**			
Design Section (Objectives, Functions, Means, Design Alternatives, and revised Client Statement)						**10/11**	
Methods Section							**10/16**

	B Term							
	Reach Goal: Beginning testing on C. elegans							
	Realistic Goal: Have system prepared to begining tetsing on C. elegans							
Task	W1(10/21-10/25)	W2 (10/26-11/1)	W3 (11/2-11/8)	W4 (11/9-11/15)	W5 (11/16-11/22	W6 (11/23-11/29	W7 (11/30-12/6)	W8 (12/7-12/11)
Test/figure out generator		**11/1**						
Test fluid for microfluidic device (S. Basal)			**11/8**					
Figure out how to measure current being applied				**11/15**				
Test device on microscope and observe neural response								**12/11**
Experimental methods								**12/11**
Revised preliminary design and final design					**11/22**			
Revise sections from A-Term w/ advisor feedback			**11/8**					

	Winter Break			
	Reach Goal: Complete all COMSOL models and coding			
	Realistic Goal: Get a large portion of the coding and COMSOL stimulation done			
Task	W1 (1/4 - 1/10)	W2 (1/11 - 1/17)	W3 (1/18 - 1/24)	W4 (1/24 - 1/28)
Create a preliminary COMSOL Stimulation		**1/17**		
Start to make the COMSOL anlysis more complex				**1/28**
Edit the paper based on comments				**1/28**
Work on the code for the Raspberry Pi			**1/24**	

	C Term						
	Reach Goal: Conduct experiments on specifc strains of C. elegans						
	Realistic Goal: Conduct mutiple experiments on C. elegans						
Task	W1 (1/28-2/4)	W2 (2/5-2/10)	W3 (2/11-2/17)	W4 (2/18-2/24)	W5 (2/25-3/3)	W6 (3/4-3/10)	W7 (3/11-3/18)
Conduct a preliminary experiment on C. elegans	**2/4**						
Perform frequency experiments on C. elegans			**2/17**				
Perform pulse width experiments on C. elegans					**3/3**		
Test out different pulse trains on C. elegans							**3/18**
Complete methods section					**3/3**		
Results section							**3/18**

	D Term					
	Reach Goal: Conduct a dual-channel experiment					
	Realistic Goal: Finalize paper and presetation as well as complete all DBS experiemnts on C. elegans					
Task	W1(3/24-3/28)	W2 (3/29-4/4)	W3 (4/5-4/11)	W4 (4/12-4/18)	W5 (4/19-4/25)	W6 (4/26-4/30)
Finish serial control	**3/28**					
Run experiment using serial control		**4/4**				
Run dual-channel experiment			**4/11**			
Discussion Section		**4/4**				
Conclusions/Recomendations Section		**4/4**				
Introduction		**4/4**				
Final Report First Full Draft			**4/11**			
Final Report Second Full Draft					**4/25**	
Final Report Final Draft!						**5/3** **eCDR due 5/6**
Executive Summary					**4/23**	
First Draft of Presentation		**4/4**				
Final Draft of Presentation						**4/26**
Presentation Video						**4/26**

Figure 3.1: Gantt Charts

Experiment Name	Objectives	Date Ran	Stimulation Device Use	Microfluidic Device Used
Pilot Experient	Determine minumum viable voltage	12/09	Grass SD9	Exisitng multi-animal (R5)
Trap Device Experiments	Determine role of frequency, duty cycle/pulse width and voltage	2/17	WHDTS Stuimulator	Single animal trap device
Optiomizing Pin Orientation	Determine accets of horizontal electrode palcemnt	2/23	WHDTS Stimulator	Exisitng multi-animal (O4) with horizontal electrode placement
Deep Brain Stimulation Experiement	Determine the if the device can supply suffient electrical impusle to cause change in response	3/2	WHDTS Stimulator	Existing multi-animal (O4) device with vertical electrode placemnt
Deep Brain Stimulation Experiements Round 2	Determine the effect of leaving the stimulation on during the second round of chemical stimulaiton	3/10	WHDTS Stimulator	Existing multi-animal (O4) device with vertical electrode placemnt
Final Verification Experients	Determine that the device is working properly, determine the effects of DBS when using optogenetic stimulation, determine the differences nerual changes between a control and experiemntal group at the saeme time	4/22	WHDTS Stimulator with Serial Control	DBS1 dual-channel device

Figure 3.2: Experimental Timeline

4 Design Process

4.1 Needs Analysis

The quantitative values for these specifications were based on the current values used in DBS of human patients with Parkinson's disease. While these may not be the same for the model system of *C. elegans*, this will be a baseline starting point. The three parameters that the device will be focused on are frequency, pulse width and voltage, these device specifications are available in **Table 4.1** below.

Table 4.1: Quantitative parameters for the device to meet in terms of voltage, pulse width, and frequency

Generate Frequency	Output at least 150 Hz with a resolution of 0.1 Hz
Pulse Width	Output a minimum pulse width of 60 μs
Output Voltage	Output able to stimulate neuron

Voltage will define the amplitude of the waves and thus, controls how strong the stimulus will be. As seen in an analysis of current DBS treatments many regions of the brain typically received 1-10V of stimulus [Deep Brain Stimulation for Movement Disorders, n.d.]. This voltage parameter does not define the upper limit of the device but rather the necessary strength which is required at the neural level. A higher voltage may be necessary in order to achieve this amplitude at the animal's neuron due to impedance caused by the devices and the microfluidics design.

As previously stated, the frequency is another necessary parameter of the design. Frequency is the rate at which the stimulus is applied and is defined by the number of pulses per second. In DBS there are two different breakdowns, low and high frequency, although high frequency is much more common. The most common range of high frequency stimulation is from 130-180 Hz although some areas of the brain and specific disorders require frequencies outside this range [Groiss, Wojtecki, Südmeyer & Schnitzler, 2009].

The final parameter is the pulse modulation which can also be referred to as pulse width or duty cycle. Pulse width defines how long each pulse occurs while the duty cycle refers to a percentage of how long the stimulus is on for in ratio to the total period. There is not as much literature focused on pulse modulation, but the available publications indicate that based on the disorder being treated the pulse modulation can change dramatically. The parameters for the device for pulse modulation are based on the studies for Parkinson's disease as this is focused on the most and maintains the most research for DBS procedures. The pulse modulation for Parkinson's is 60-90 μs so the lower limit of the device has been set to 60 μs with the ability to

increase the pulse modulation by increments of 1 µs [Groiss, Wojtecki, Südmeyer & Schnitzler, 2009].

4.2 Brainstorming and Concept Mapping

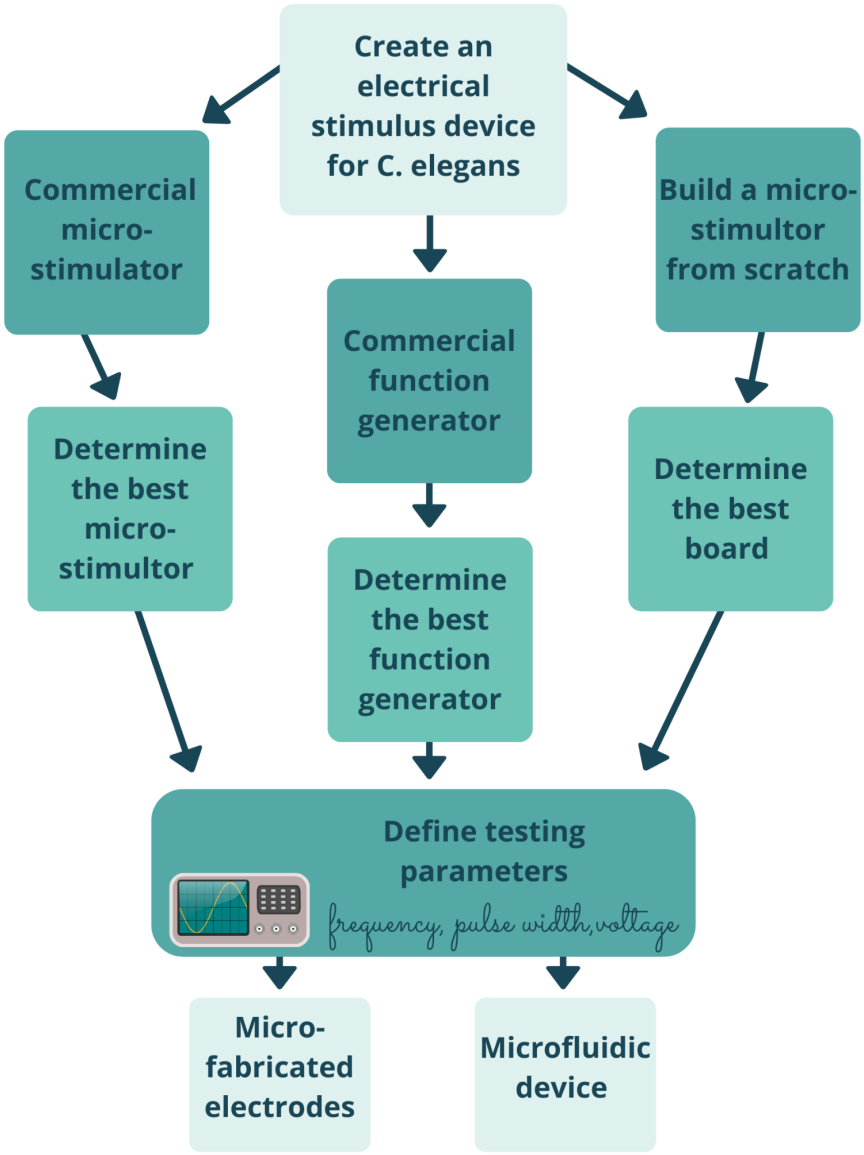


Figure 4.1: Concept map of thought process the team went through to decide on design concepts

The preliminary brainstorming the team went through led to the development of three different conceptual ideas. The first was the use of magnetic nanoparticles to deliver targeted therapy that can be monitored using fluorescence. A second idea was to use an electrode array to allow for more area to be stimulated at the same time. Lastly is the idea of using a patterned electrode placement that allows for the amplification and minimization of neural activity in

different areas which would be dependent on the symptoms presented. These were only a few preliminary ideas which the team has started to identify, until our problem was better defined.

To organize and better understand the design of the device a concept map was developed. The concept map can be seen in **Fig. 4.1** above to analyze the best way to approach our problem of creating an electrical stimulus device for *C. elegans*. When looking at our problem we can break it up into three different sections. These include the controlling device, the testing parameters and the electrodes. First is the controlling device. Our team has developed 3 different approaches to the controlling device. These include using a commercial micro-stimulator, a commercial function generator and building a microstimulator from scratch. From here the team had to determine the best option for each of these categories by conducting research on the three different products and what options are available. Once the best options were decided, the team then had to analyze and decide on the testing parameters we would be using. The testing parameters include the frequency, pulse width and velocity applied to the model system. Since one of the design requirements is to have an easily customizable device it is likely that the team will have a range of testing parameters and can easily test a number of different combinations of parameters. Finally, the team has to decide what electrodes we will be using. Both of the options for electrodes are applicable and capable of being used with any of the devices. The electrode options available are using a microfluidic device where the current is applied across the entire device. The second option is using microfabricated electrodes on the microfluidic device.

4.3 Concept Evaluation

Before analysis of the design was done, model systems needed to be chosen to be used in the selection. Using the quantitative qualities above, the team filtered through different products in each category until the optimal products were found. For the signal generator due to the simplicity of the circuit, the team decided that finding a product that was low cost was paramount for this category.

There were many signal generators available for less than 20 dollars that fit the requirements, but the WHDTS Signal Generator (**Fig. 4.2**) was chosen due to the customization option in terms of the choice of either a knob or button user interface and the 4 leads that allowed for it to be a part of a larger circuit. Additionally, the signal generator can be attached to a USB to TTL serial cable allowing for serial control of the generator from a computer. For the neurostimulator category, the S88X Dual Output Square Pulse Stimulator by Grass-Astro (**Fig. 4.3**) was chosen because it was the most cost-effective option in the neurostimulator category. Many of the machines in this category were easily over $1,000, but the Square Pulse Stimulator is roughly $1,000, and it far exceeds the parameters needed for this project. Lastly for the microcontroller design, the Arduino board (**Fig. 4.4**) was chosen. The Arduino was chosen as the microcontroller mostly due to the flexibility of the product. It had a very user-friendly environment which allowed for easy serial control of the board. Likewise, it had very clear documentation and commands which allowed for easy customization.

Figure 4.2: WHDTS Signal Generator

Figure 4.3: S88 Grass Instruments neurostimulator

Figure 4.4: Arduino Uno

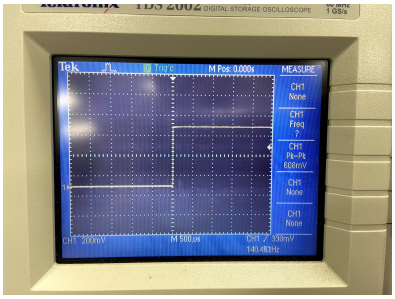
After the model products were chosen, the requirements were weighted per the pairwise analysis, and the products were compared amongst themselves. The team decided this was the best course of action because there isn't a gold standard for this field given its novelty. As seen in **Table 4.2,** the signal generator design was chosen. The neurostimulator design lost points in size, cost, and customization. If the device cannot fit in the space, it would perform poorly and require a redesign of the entire system which gives rise to another problem. The cost is too excessive for this project. The project budget is at most $1,000, and even if it could be found used, it would cost most of the budget. The Arduino option lost points in being aesthetically pleasing and user-friendly. In this case, being aesthetically pleasing means being compact and organized, however, this design would lend itself to potentially being wiry and hard to manage with moving parts. It also lost points on being user-friendly because of how disorganized it would potentially be making it hard to easily fix or change out parts.

Table 4.2: Analysis of three different designs compared against one another

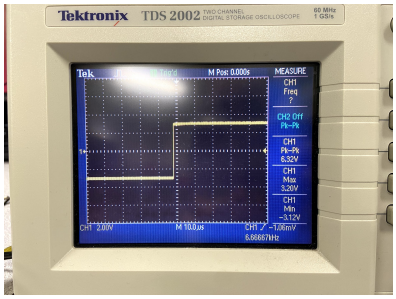
Design Requirement	Weight	Design 1: WHDTS Signal Generator	Design 2: Square Pulse Stimulator	Design 3: Arduino
Ability to provide voltage	6	1	1	1
Stimulate the neurons	6	1	1	1
Ability to control the signal	6	1	1	1
Cost	1	1	-1	0
Aesthetically pleasing	0	0	1	-1
User-Friendly	3.5	0	1	-1
Customizable	3.5	1	-1	1
Size	2	1	-1	1
Total	-	**24.5**	**15**	**18**

4.4 Alternate Stimulator Design

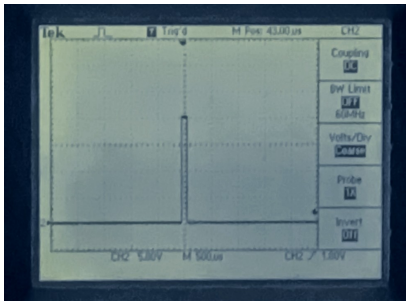
Along with the previously discussed signal generator, two other possible options for devices were identified and tested. These include an Agilent 33220A waveform generator and a Grass Instruments S88 Stimulator. Baseline tests were conducted on each of the alternatives with a Tektronix TDS 2002 oscilloscope to compare the signal noise and amplitude accuracy.



Signal Generator



Waveform Generator



Grass S88 stimulator

Figure 4.5: A comparison of the signal output from the three designs

The results of the baseline tests are shown in **Fig. 4.5**. Both the wavelength generator and Grass stimulator had similar signal output and results compared to the signal generator as expected. The device overall had to be capable of stimulating tissues, meaning it needed to have the optimal output for stimulating neurons in *C. elegans.* Since multiple adequate designs were identified, a table was created to compare the different aspects of the devices as shown in **Table 4.3**.

Table 4.3: Comparison of the different devices that can be used and the parameters which they are able to meet

Stimulator	Waveform Generator	Signal Generator	GRASS Stimulator (SD9 or S88)
Ability to Produce Pulse Trains	Needs to connect to external device		Can produce Pulse Trains
Power Information	Can be plugged into wall	Needs a power supply	Can be plugged into wall
Voltage Limit	Rated for: 25 V	Rated for:30 V	Rated for: 150V
Wave form	Square wave signal		
Noise in Signal	Slightly noisy signal	Clear signal	Clear signal
User Interface	Easy UI	Tedious UI	Tedious UI
Cost	> $300	~ $14	~ $200
Control Options	Manual and serial control	Manual and serial control	Manual control and analog control
Size	Larger in size	Small in size	Larger in size
Image			

4.4.1 Alternate Electrode Design

With the primary requirement of this design being to stimulate the head neuron of the specimen, electrodes were an important design consideration. Since the exact external voltage necessary was unknown, various electrode designs needed to be considered in order to optimize the amount of current reaching the neuron. While there are abundant options for the application of electrical stimulus, the two that were considered for this application were microfabricated electrodes and microfluidic inlet/outlet electrodes.

The electrodes fitted to the microfluidic design includes hollow or solid metal ‘pins’ that can be inserted into the PDMS block that houses the specimen(s). Alligator clips can then be attached to these pins for the application of electrical stimulus. Since the microfluidics utilize a buffer solution to load the animals into the arena, the electrical stimulus can be applied through this liquid to transport the current to the head neuron of the animal. While this option does not allow for specific stimulation to a desired neuron, it can effectively apply the stimulus to the entire arena.

The other option is microfabricated electrodes. Microfabricated electrodes are millimeter to micrometer sized electrodes that are fabricated on a substrate which can be fitted to microfluidic devices. Microfabricated arrays can be fabricated in a variety of different ways, one of which is using indium tin oxide glass slides utilized in a photolithographic process in order to create the arrays.

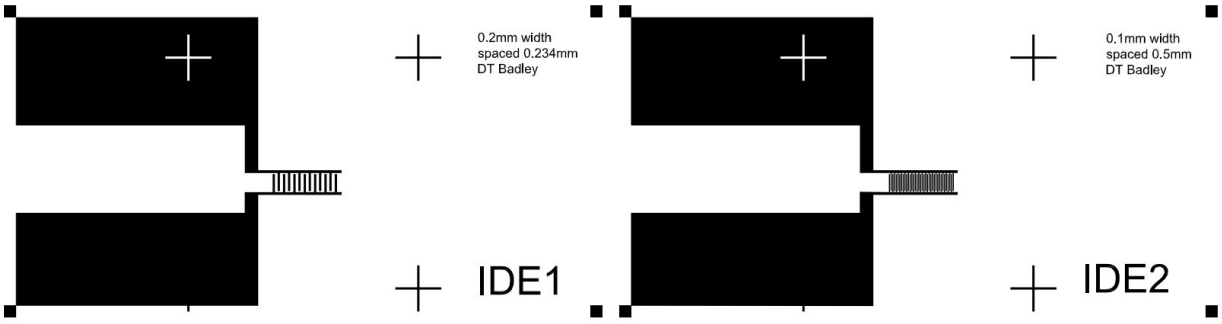


Figure 4.6: CAD design for microfabricated electrodes

The design shown in **Fig. 4.6** shows two different microfabricated electrode arrays. In order to stimulate the arena evenly, the electrodes are interdigitated. Interdigitated electrodes would allow current to be uniformly applied to the entire arena instead of having certain areas having a more condensed electric field. By varying the number of electrodes and the spacing between the electrodes, the electric field strength can be customized. In **Fig. 4.6**, IDE1 shows 0.2mm width electrodes spaced 0.234mm apart, and IDE2 shows 0.1mm spaced 0.05mm apart from each other. The designed arrays should test one condition with a nearly continuous electric

field (IDE2) and another with more space allowing for the electric field to grow weaker in the center areas. The large rectangular areas shown are contact pads designed to minimize resistance.

For this project we chose to utilize electrodes fitted to the microfluidic design. While they do not allow for targeting of specific neurons, they provide the ability to apply a consistent current. They also do not have any issues with durability which is especially relevant when the design needs to be able to run many tests without having to replace or maintain specific parts of the design.

4.5 Final Conceptual Design

Through the process of determining the different requirements for this device, a final conceptual design was created. This design takes into account the best options to meet each requirement and allow for the device to encompass all necessary parameters. Each element of this design can be seen in **Fig. 4.7**.

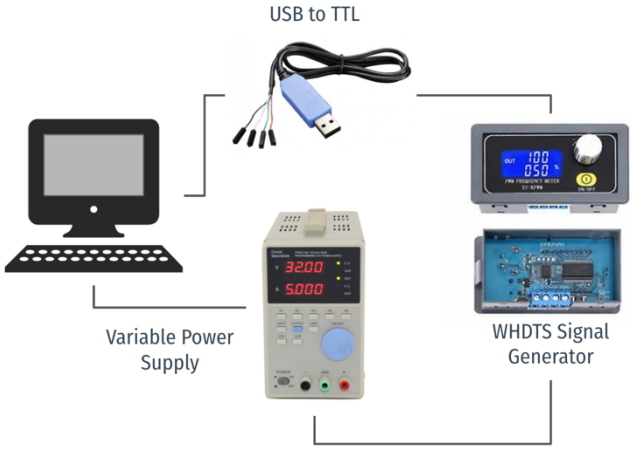


Figure 4.7: Diagram of the final design concept

The functional generator that was decided upon was the WHDTS Signal Generator as it proved to be the best overall functionally and had the best design. It includes rotary switch options and is 79 x 43 x 27 mm in size. It is cost-efficient at $13 and was purchased on Amazon to do further testing and come to a more detailed consensus. Furthermore, the parameters that are included in the final design are the pulse width, which is duration of each stimulus, the amplitude, which is the intensity of the stimulus, and the frequency which is the average number of pulses per second. For the sake of our study, we plan to test different combinations of the parameters to determine the most effective parameter ranges for DBS testing to increase and decrease neural response.

This signal generator provides direct control of both the pulse width and frequency parameters; however, an external device is required in order to control the voltage being applied. As previously stated in **Table 4.1**, the voltage at the neuron of the specimen needs to be at least 10 V, meaning traditional battery options would not provide sufficient voltage. This device also has a limitation on the voltage that can be handled without frying the board. Keeping this limitation in mind, a power supply was chosen to fulfill the voltage requirement as it can provide a controlled voltage as well as commonly having an upper limit of 30 V, the upper limit of the signal generator.

A smaller trap design (**Fig. 4.8**) that contained one specimen was initially used to complete analysis on the required current and voltage to receive a response. The information collected from these tests was then used to optimize the placement of the electrodes on a microfluidic design with a multi-specimen arena (**Fig. 4.9**). The multi-specimen arena was necessary in order to complete the final DBS tests as well as to observe potential movement responses to the stimulation.

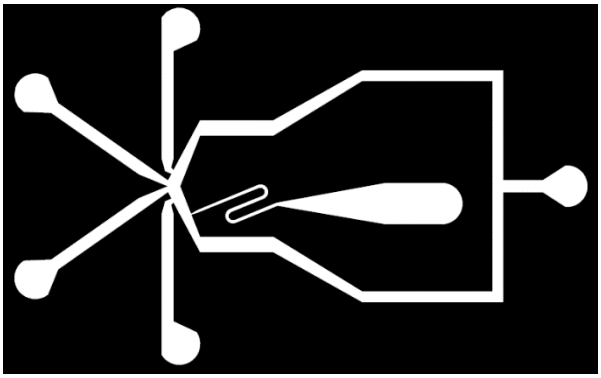


Figure 4.8: An example of the single specimen trap design

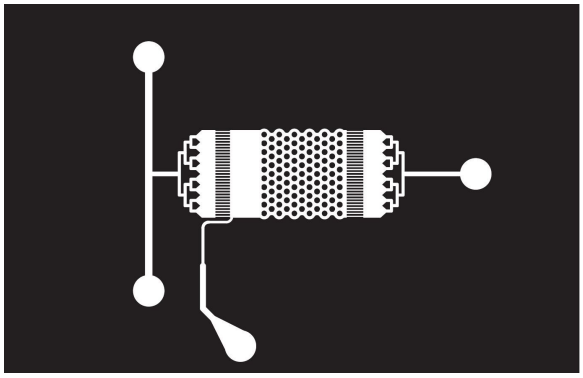


Figure 4.9: An example of the multi-specimen arena

As part of the initial client statement, the device needed to be automated to more efficiently run multiple tests. The signal generator previously discussed possesses the functionality for serial control through three built-in pins. These pins allow for the device to be controlled by the existing microscope software implemented in the lab. By modifying existing coding, the electrical stimulus can be integrated into the current system that controls chemical and optogenetic stimuli.

All of the functions needed in our final design, as well as the means by which we are planning on achieving them, can be found in **Table 4.4**, including time scale requirements and electrical stimuli abilities.

Table 4.4: Functions and means table with different design functions necessary and means of accomplishing these functions

Design Functions	Means
Easily controllable/changed	Knobs, dials, switches, touch screen, keypad
Able to handle time scale	Microcontroller, microstimulator, computer, phone
Able to produce electrical stimulus	Battery, outlet, solar power
Apply electrical stimulus	Microfabricated electrodes, microfluidic device
Able to fit within the physical constraints	Microcontroller, microstimulator
Ability to monitor neural response/activity	Microscope, Microelectrode Arrays

4.6 Final Design Prototype

For the final design, the WHDTS signal generator will be the primary modulation device of the circuit. In order to ensure control over the voltage, a power supply will be used to complete the circuit, fulfilling the voltage requirement.

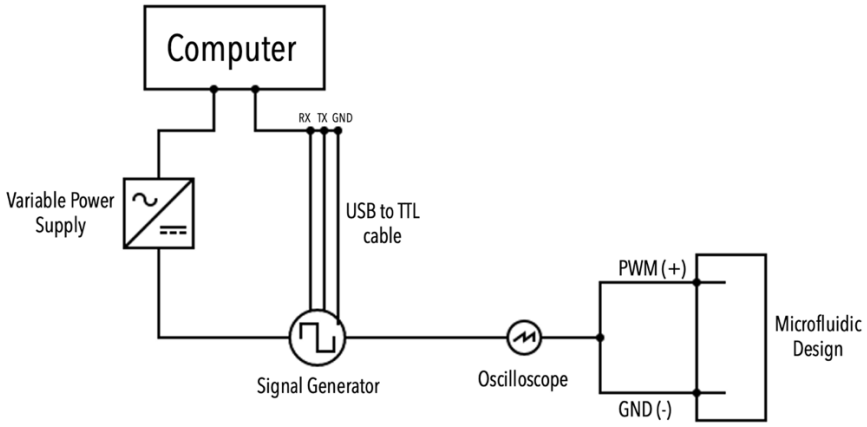


Figure 4.10: The circuit diagram for the final design

As seen in **Fig. 4.10** above, a positive and negative lead will provide output from the power supply, connecting to the corresponding positive and negative inputs of the WHDTS Signal Generator. The signal generator outputs a square wave pulse which can be modulated using the PC which contains the micromanager program. Micromanager is an open-source program which allows for acquisition of images from different scientific cameras and microscopes. The program allows for serial control of external devices whilst managing several devices simultaneously. Using the program, the duty cycle and frequency are modulated via serial control pins built-in to the signal generator. The input is received from the PC which also controls the timing of the chemical stimulation and the microscope. By using this system, more precise control of changing frequencies and duty cycles was possible as well as automation of the device.

In order to ensure the current and voltage of the device are sufficient, the team decided to utilize COMSOL simulation to approximate the electric field and current density by modeling the system in a 2-D system. Given the relatively narrow proportions of the microfluidic devices, it was determined that 2-D simulations would be sufficient for estimations. Based on the results of the COMSOL analysis (see section 5.2.2), the microfluidic device that was ultimately chosen was the 3mmx3mm arena with vertical pin orientation.

4.7 Final Design Selection

Of the three designs, the signal generator approach shows the greatest promise as the final design for this project. It has the potential to meet the design requirements necessary for an optimal system. As an alternate choice, the Grass Stimulator could also prove useful in case the signal generator is ineffective in neural stimulation. While the Grass S88 shows promise due to its high voltage rating, it isn't ideal due to the fact that it could not inherently produce pulse

trains, and it did not have a method of serial control. The waveform generator would be the last choice due to its low voltage rating which would most likely be insufficient to stimulate neurons.

As previously discussed in **Table 4.1**, the signal generator meets essential design requirements such as the ability to stimulate the neurons, customize the signal, handle frequencies of at least 150 Hz with a resolution of 0.01 Hz, but is capable of achieving 60 µs pulse width. The device has a minimum duty cycle of 0.1% and a maximum frequency of 150kHz. Therefore, a pulse width of 6.66 ns is the minimum pulse width. The minimum viable frequency for the device to be usable is 17Hz which would have a pulse width of 58.8µs. Therefore, the functional operating range of the device would be from 17Hz to 150kHz. It will be the primary system used in this project to stimulate *C. elegans.* The signal generator is better than the waveform generator in all aspects other than from the user interface.

Figure 4.11: The Circuit Specialists 32 Volt, 5.0 Amp DC Power Supply

The signal generator also required an external power supply in order to apply a specified voltage to the specimen. In order to meet the specification of this device being automated a variable power supply needed to be utilized. The upper voltage limit of 30 volts on the signal generator also provided added limitations to the device that could be selected. After research and consideration, the Circuit Specialists 32 Volt, 5.0 Amp DC Power Supply (**Fig. 4.11**) was chosen to fulfil this element of the final design. It was chosen due to the detail in documentation for serial control of the device as well as its ability to meet the voltage constraints previously discussed for this design.

To complete automation of the system, a USB to TTL cable will allow for serial control of the signal generator. An external device was not necessary for the power supply as it already included a cable to allow for direct connection and control via a computer.

In addition to the completed electrical stimulus device, microfluidics will be utilized in the physical stimulation of the model *C. elegans* system. This device encapsulates a certain number of the organisms inside channels etched into PDMS on a glass slide. The electrical stimulus device will connect to the microfluidics via pins placed into the PDMS (**Figure 4.12**).

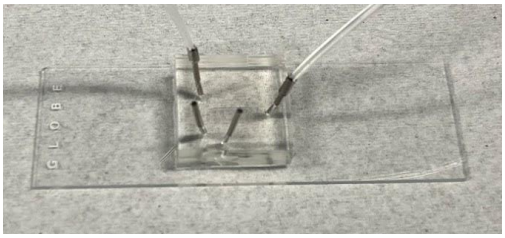


Figure 4.12: The microfluidics slide with pins inserted

4.8 Automation of the System

To enhance the current system it was deemed important that automation features be implemented. These features allow the device to accurately and efficiently run multiple tests with

little oversight, including those utilizing pulse trains. To accomplish this goal an external device was necessary to control the output of the signal generator as well as the power supply.

4.8.1 Automation of the Signal Generator

The signal generator utilized in this design has built-in functionality for serial control through three pins that can be soldered onto the main board that correspond to RX (receiving data), TX (transmitting data), and GND (ground). Once connected via a USB to TTL cable**,** the signal generator can be adjusted by the user through sets of commands on a computer. These serial commands allow for the device to be turned on and off, the frequency to be set, and the duty cycle to be set (**Table 4.5**). Upper and lower limit values for the duty cycle can also be set through the serial control. Each of these parameters can be set manually by the user, however, in order to achieve the design goal of automation of the system and to effectively run pulse trains, serial control is necessary.

Table 4.5: Serial commands for the signal generator [Amazon.com: WHDTS Signal Generator 1-Channel 1Hz-150KHz PWM Pulse Frequency Duty Cycle Adjustable Module LCD Display: Industrial & Scientific, n.d]

UART communication and parameter settings		
No.	Parameter	Value
1	Baud rate	9600bps
2	Data bits	8bit
3	Stop bit	1bit
4	Check bit	none
5	Flow control	none
No.	UART Command	Function
1	READ	Read Set Parameters
2	ON	Enabled PWM Output
3	OFF	Disabled PWM Output
4	DNxxx	Set the Lower Limit Value for Duty Cycle:
		DNxxx:Used for Normal mode."xxx"can be 000~100
		DNxx.x:Used for Precise mode."xx.x"can be 0.00~100
5	UPxxx	Set the Upper Limit Value for Duty Cycle:
		UPxxx:Used for Normal mode."xxx"can be 000~100
		UPxx.x:Used for Precise mode."xx.x"can be 0.00~100
6	MODEx	Set work mode:
		MODE0:Normal mode
		MODE1:Precise mode
		"x" can be 0 or 1
7	Fxxx	Set frequency:
		"F101":Set frequency 101Hz."101"can by replace by 001~999
		"F1.05":Set frequency 1.05KHz."1.05"can by replace by 1.00~9.99
		"F10.5":Set frequency 10.5KHz."10.5"can by replace by 10.0~99.9
		"F1.0.5":Set frequency 105KHz."1.0.5"can by replace by 1.0.0~1.5.0
8	Dxxx	Set duty cycle:Set duty cycle is XXX.
		Normal mode:"xxx"can be 000-100. E.g."D051" means PWN duty cycle is 51%
		Precise mode:"xx.x"can be 00.0-100. E.g."D20.8" means PWN duty cycle is 20.8%
9	Return	Set successfully Return:DOWN
		Set failed Return:FALL

In order to test the functionality of these commands, the USB to TTL cable was utilized alongside the Serial Monitor in Arduino. Through these tests it was determined that there needed to be a delay between commands as the signal generator could not accept multiple commands at once. In order to combat this issue, the frequency and duty cycle were set before the experiment

and for pulse train functionality the ‘ON’ and ‘OFF” commands were used to control the stimulus.

4.8.2 Automation of the Power Supply

The design also required the incorporation of a variable power supply in order to achieve complete automation of all parameters. The power supply that was chosen was the 32 Volt DC 5.0 Amp Programmable Linear Power Supply, shown in **Fig. 4.13**. In order to automate the system, the control communications protocol for USB control attached in the power supply website was used (**Table 4.6**). The commands were used in Arduino to test for effective functionality. The specific commands tested include how to turn on and off the signal generator and the set the amplitude and voltage. The commands for turning on and off the signal generator from the table provided are o1 and oo respectively. The commands for adjusting the voltage on the signal generator is suXXXX, where XXXX would be the voltage for Channel 1 (only Ch1 was used for the device). Similarly, to adjust the amplitude, siXXXX was used in the same manner with the appropriate value. These commands were then used in the main GUI code to control the power supply during the set up for the experimental tests. Thus, the parameter switch was no longer manual and could be used in the main code of the serial control while running the test.

Figure 4.13: Variable, programmable, power supply [32 Volt DC Programmable Linear Power Supply 5.0 Amps, n.d.]

Table 4.6: Serial commands for the power supply [32 Volt DC Programmable Linear Power Supply 5.0 Amps, n.d.]

Send Command Word	Perform Operation	Read-back Command Word	Read-back Command Analysis
a + line break (Hereafter, every command must take 0x0a as the line break to over, ignore the following)	Back to device model	Like “3203”	Device model
		N	Communication fail
suXXXX	CH1 preset output voltage, units V; e.g. 1200 stands for 12.00V	OK	Preset success
		N	Communication fail
siXXXX	CH1 preset output current, units A; e.g. 2500 stands for 2.500A	OK	Preset success
		N	Communication fail
saXXXX	CH2 preset output voltage, units V; e.g. 1200 stands for 12.00V	OK	Command Accepted
		N	Communication fail
sdXXXX	CH2 preset output current, units A; e.g. 2500 stands for 2.500A	OK	Command Accepted
		N	Communication fail
O0	Output indicator light switch-off	OK	Command Accepted
		N	Communication fail
O1	Output indicator light switch-on	OK	Command Accepted
		N	Communication fail
O2	Parallel, series, trace, output indicator light switch-off	OK	Command Accepted
		N	Communication fail
O3	Series, trace, output indicator switch-	OK	Command Accepted

4.8.3 System Integration

The current experimental setup that this system needed to be integrated into is based in a program called Micro-manager. Micro-manager is an open-source microscopy program capable of interfacing multiple devices whilst handling serial control. Micro-manager runs using a programming language called Bean Shell which is a variation on the Java programming language. The existing system had two main programs, the Graphical User Interface (GUI) and a run file that our program needed to either run or be integrated within. The existing GUI was modified, in order to maintain existing functionality while incorporating the new commands to modify the DBS parameters previously discussed. This code needed to run the entire test from beginning to end while maintaining proper timing. This began with displaying a window to adjust the parameters for the tests in the beginning (**Fig. 4.14**) and then running these tests to the specified parameters and timing.

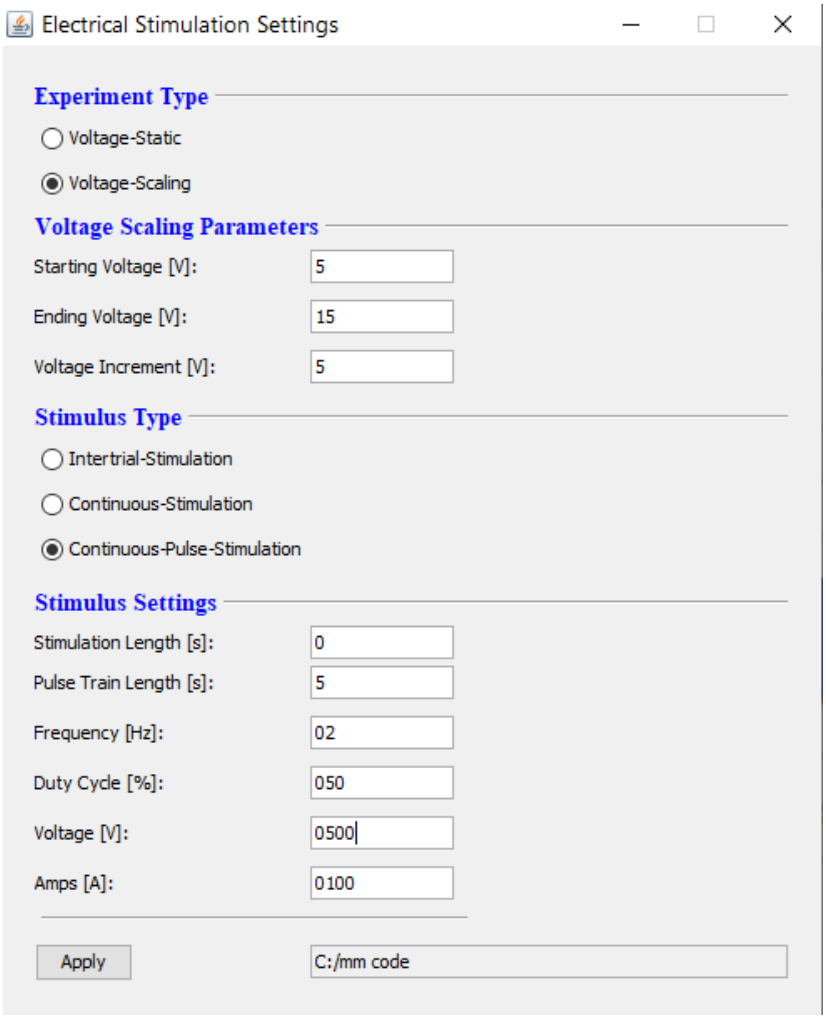


Figure 4.14: User-interface for experimental parameters set-up

The main GUI code consisted of a few main sections: “Set-up GUI: component definitions”, “JLABELS”, “JRADIOBUTTONS”, “GUI SECTIONS 1-3: Initial Startup” and

then ultimately applying by writing to a text file. The first 3 sections are commands to set up the rest of the code, which included definitions of certain components of the lab's device set up, as well as creating labels and buttons for selection of the parameters. From there, starting with "Action Listeners" and following to the "GUI Code 1-3", the code was split into three sub sections. The first GUI section was the "experiment type", then second was "experiment settings" and third was "save information and apply settings". Lastly, on the GUI code file the final step is applying all the settings by writing a text file to be called by run acquisition.
The GUI Code is integrated into a main code called "RUNEXP", which runs the entire experiment. This code consists of the GUI code that sets up the experiments and prompts the user to adjust the parameters, shown in **Fig. 4.14**. In this script the use of if statements and while loops are also used to automatically increase the voltage at consistent increments during the experiment, so it doesn't need to be done manually. The full documentation for the automation can be seen in **Appendix B**.

5 Chapter 5: Design Assembly & Verification

The effectiveness of the final design was validated through modeling analysis as well as experimental analysis. COMSOL finite element analysis software was used to validate the current requirement as well as optimal placement of electrodes for stimulation. Experimental tests were run in order to realistically determine the optimal parameters for the final DBS experiments to draw final conclusions. Once experiments were completed, data analysis methods were utilized in order to uniformly analyze multiple data points from each collected set of data. These data analysis methods included using ImageJ image processing software and MATLAB coding to create cohesive plots.

5.1 COMSOL Analysis

While designing the device, estimations of the necessary input voltage, input current, and microfluidic device dimensions were needed to acquire the necessary components that suited the parameters of the experiment. Given the complexity of the problem due to the number of unknowns, running a simulation of the system was the best method to accurately test whether a setup would be sufficient for neural activation, as well as modulation, of *C. elegans.* COMSOL was selected as the program that was utilized for these tests. COMSOL is a finite element analysis, Multiphysics simulation software that allows for electrical, mechanical, fluid, sound, and chemical simulations. Using this software, the optimal experimental setup was explored for analysis of deep brain stimulation in *C. elegans.*

Due to the number of unknown variables, it was crucial to set assumptions that will help to simplify the problem. The main assumption was that a 2D analysis could be used to represent the problem because the height of the microfluidic device is negligible in comparison to the other dimensions. Turning this originally 3D problem into a 2D problem made the associated calculations and modeling easier. From these simulations it was determined that the intended results were determining the current density and longitudinal electric field in a given microfluidic device design. In the four COMSOL analyses conducted, a previously designed microfluidic device was imported into the program to be used as a baseline. A pilot experiment was conducted with the device (R5), which gave crucial insights into the meaning of the results of the analysis. As well, a trap device was used in more of the pilot experiments to determine other parameters of the stimulation being applied. In the trap device only one *C. elegans* can be used and stimulated at a time. Since the trap device had this constraint of only a single animal, a multi-animal device (O4) was used for the DBS experiments. The final device which had to be modeled in COMSOL was based on an existing device (P10) and was a dual-channel design specific to this project, called the DBS1 which was used in the final experiments and recommended for use in future research.

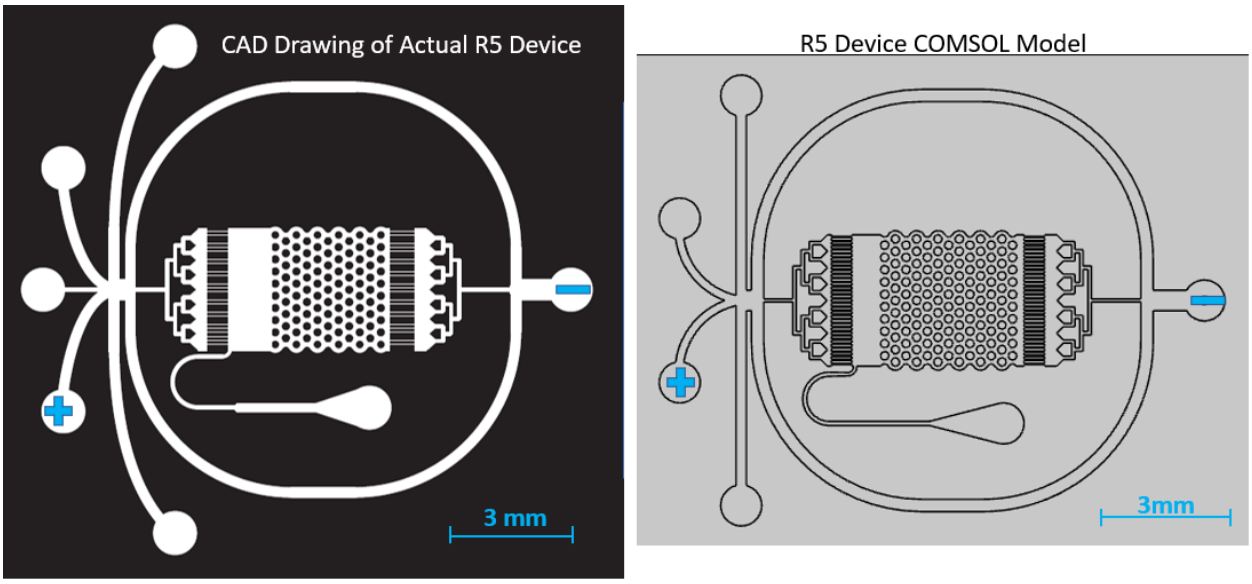


Figure 5.1: The full R5 device that was input into COMSOL

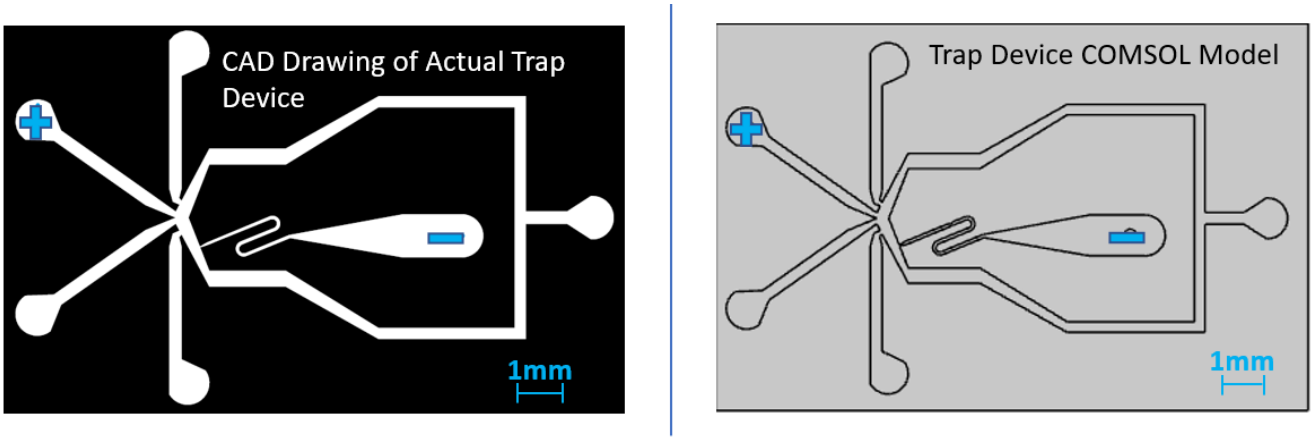


Figure 5.2: The full trap design that was input into COMSOL

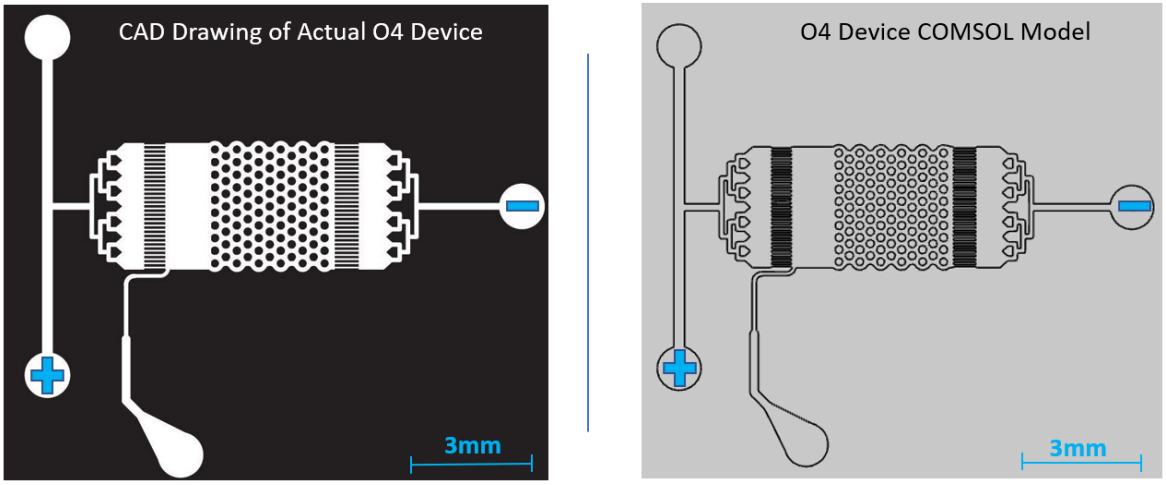


Figure 5.3:The full O4 device that was put into COMSOL

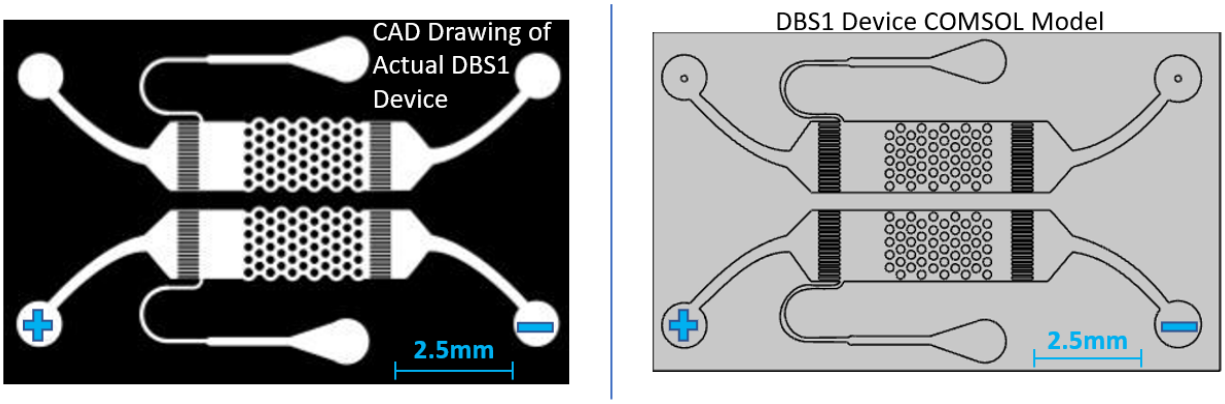


Figure 5.4: The DBS1 device

Each figure displays the original CAD file on the left and the COMSOL drawing on the right. All of the drawings include the location of the positive and negative electrode as well as a scale bar.

The above figures depict the four designs that were made using the COMSOL tool as well as shows the actual designs of the four different microfluidic devices. **Fig. 5.1** shows the existing microfluidic R5 device used in preliminary testing. Figure 5.2 shows the trap device which is a single animal device that was used in the first experiments with live animals. The third image, **Fig. 5.3** shows the existing O4 device used in the majority of the experiments and has the capabilities to house multiple animals. The final figure, **Fig. 5.4** shows the final design created by the team, DBS1, which is a dual channel device able to house multiple animals and was used in the final experiment. The electrode pins were put in the inlet and the outlet channels for all devices as denoted by a blue + and - on the image. Each design used the same three materials for the insulator, conductor, and pins. The insulator was borosilicate (glass) which is an approximation of the slides. The conductor was H_2O with a conductivity of 1.6 siemens per meter similar to the environment the *C. elegans* reside which is in a solution of S. basal media,

and the electrode pins consisted of 99.1% copper. In each of the COMSOL simulations the input voltage was able to be changed based on the desired value. From here the current density, electric potential and electric field was able to be mapped out on the device so that the values within the arena where the worms are housed could be determined. The values obtained and information gained from these COMSOL analysis will be discussed in the results section.

5.2 Experimental Methods

5.2.1 Testing for Stimulation Device Selection

The first steps in setting up the experiment were analyzing the different options for stimulation equipment and then getting familiarized and understanding the hardware. Two different stimulation devices were tested including the signal generator and a Grass S88 stimulator. These devices were all connected to an oscilloscope to monitor the waveform output. Not only did this experiment allow for deciding which device was the best for the purpose of the experiment but it also allowed the team to compare the results from each device to identify any issues or discrepancies. The signal generator was tested first as this was the primary option for use as the stimulation device. A square wave pulse was observed although there was a slight slant seen on the oscilloscope getting lower as it moved more towards the right as can be seen in **Fig. 5.5** below.

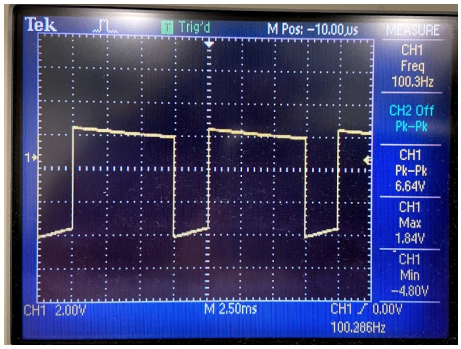


Figure 5.5: Square wave from the signal generator at 6V and a frequency of 100.3 Hz

The Grass S88 stimulator which was also tested had similar results as the signal generator in terms of signal output and this device had a much wider range of parameter settings. Due to the similarity in output and ease of access this device was used for the preliminary testing. However, this device is unable to be controlled using serial output, so it is not the best option for the final design which is why the signal generator was ultimately deemed the best option.

5.2.2 Device Setup

Another major component to the experiment besides the stimulation device is the microfluidic device used to house the *C. elegans*. There were four different microfluidic device options available and used by the team. In all of these devices the stimulation device is

connected by attaching the electrodes to the metal inlet and outlet pins. The microfluidic device options included an existing multi-animal device (O4) and R5 as well as a single animal trap device and finally a dual-channel device modeled by the team (DBS1). The O4 and R5 devices have a 3mm x 3mm arena where multiple *C. elegans* can be housed and stimulated at the same time. The difference between these two devices is the circular channel which surrounds the arena of the R5 device while this channel is absent in the O4 device. The trap device can only hold a singular animal in a curved 250 µm channel. To better visualize the O4 and trap device an image of both devices can be seen below in **Figs. 5.7 and 5.8** respectively with the distances and electrical inputs labeled.

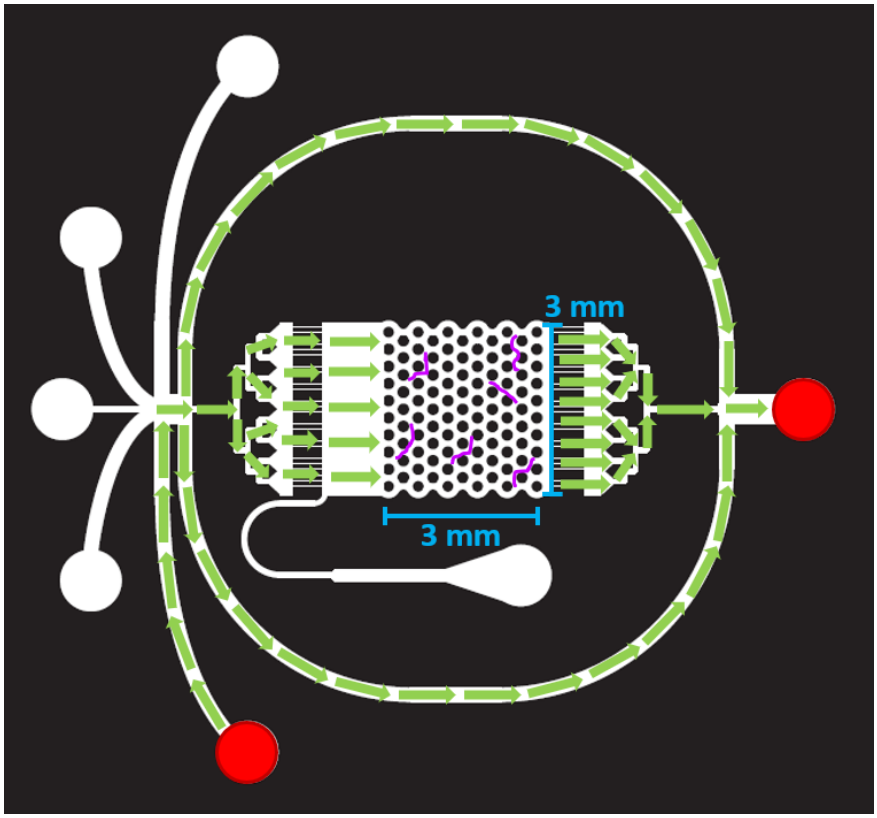


Figure 5.6: The existing microfluidic device, (R5) option can be seen above with the 3 mm by 3 mm arena labeled.

The purple objects within the arena are a representation of how the worms would be in the device as they need to navigate around the black posts. The red dots represent the electrode inputs which have the ability for the orientation of the negative vs positive electrode to be changed. The green represents the electrical path with the arrows indicating that the path is continued throughout the arena. Due to the complex geometry a more sophisticated model is needed to represent the electrical field distribution within the arena.

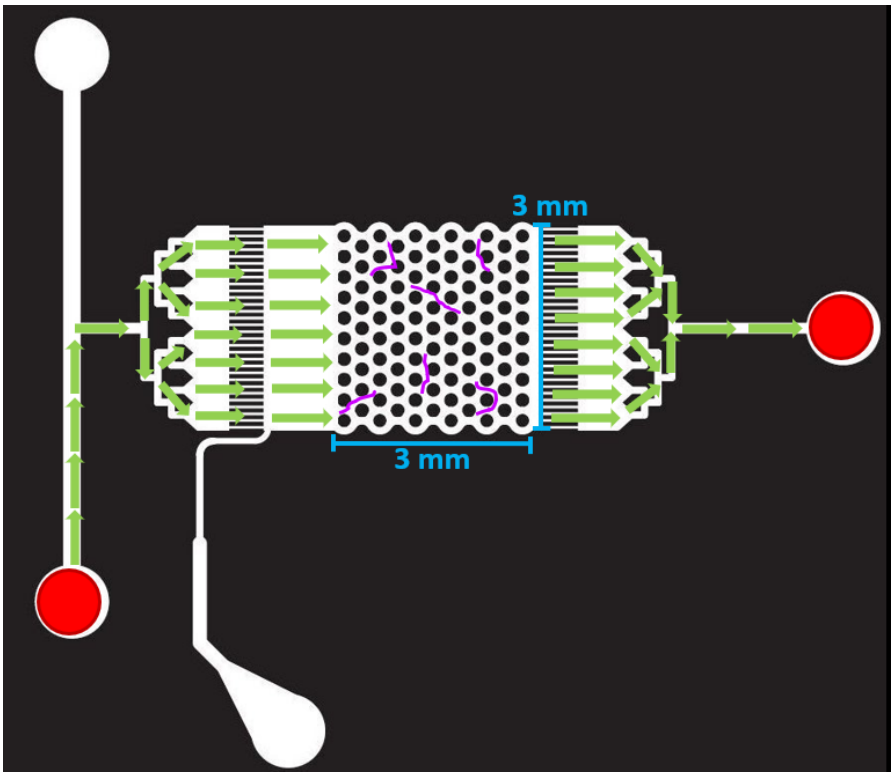


Figure 5.7: The existing microfluidic device, (O4) option can be seen above with the 3 mm by 3 mm arena labeled.

The purple objects within the arena are a representation of how the worms would be in the device as they need to navigate around the black posts. The red dots represent the electrode inputs which have the ability for the orientation of the negative vs positive electrode to be changed. The green represents the electrical path with the arrows indicating that the path is continued throughout the arena. Due to the complex geometry a more sophisticated model is needed to represent the electrical field distribution within the arena.

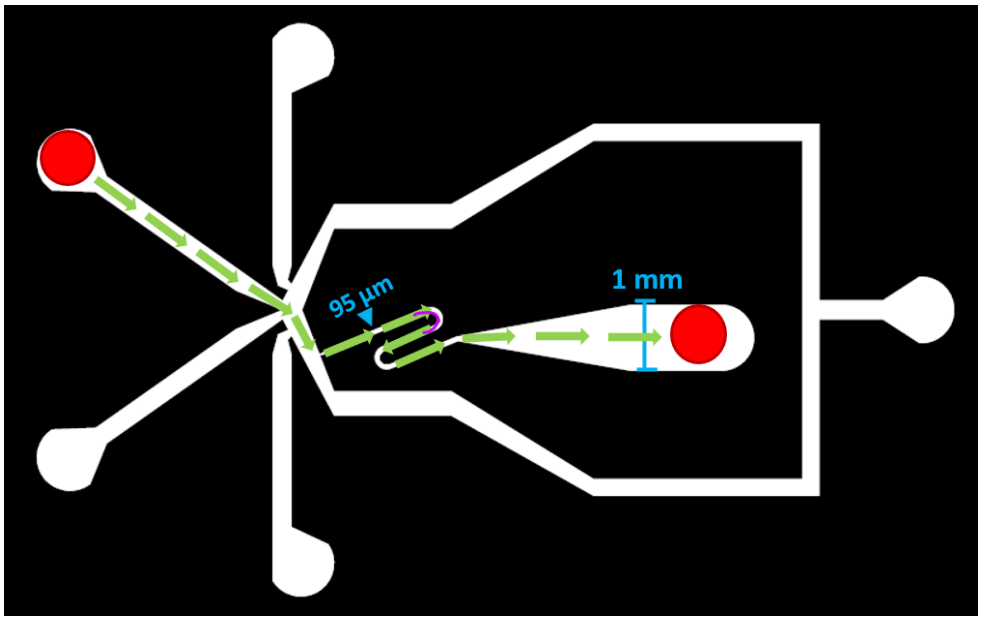


Figure 5.8: The trap microfluidic device option,

the inlet channel is 1 mm in diameter while the trap channel is ~95 µm in diameter. The green represents the electrical path, and the red dots indicate the pins where the electrodes are connected. The positive and negative electrode can be switched between either pin.

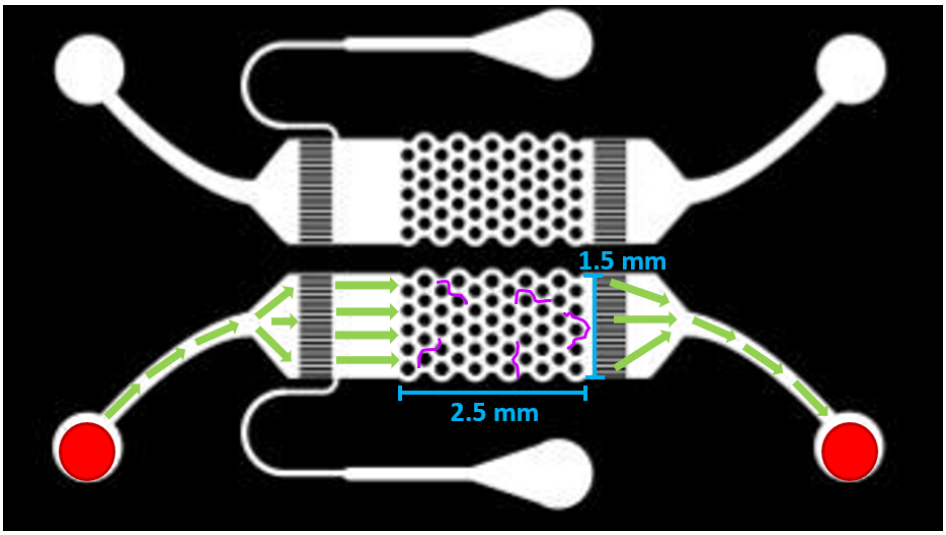


Figure 5.9: The DBS1dual-channel microfluidic device option,

the arenas are both 1.5 mm by 2.5 mm. The purple objects within the arena are a representation of how the worms would be in the device as they need to navigate around the black posts. The green represents the electrical path with the arrows indicating that the path is continued throughout the arena. Due to the complex geometry a more sophisticated model is needed to represent the electrical field distribution within the arena. The red dots indicate the pins where the electrodes are connected. The positive and negative electrode can be switched between either pin.

5.2.3 Preliminary Testing

The preliminary experiments the team conducted were focused on whether or not there was a neural response at a given set of parameters. There are three important parameters involved in the stimulation experiments which include voltage, frequency and duty cycle. The voltage defines how strong the stimulus is and is seen to affect the amplitude of the wave form. Next the frequency defines the rate of the pulses therefore representing the number of pulses per second. Finally, the duty cycle defines the duration of each stimulus and can also be referred to as pulse width. The three different parameters can be seen depicted for better understanding in **Fig. 5.10** below.

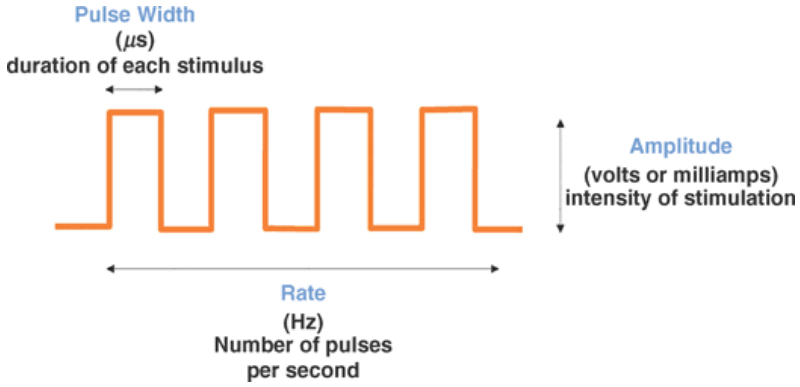


Figure 5.10: Visual representation and explanation of the three main parameters: voltage, frequency, and duty cycle

It was determined that of the three parameters the voltage was the most important in determining first as this was believed to have the biggest impact on the neural response. Therefore, first experiments were focused on defining the voltage limits for testing. This was conducted using the Grass S88 stimulator due to ease of accessibility and limitations on acquiring the signal generator. This proved to be useful however as the voltage range for the Grass S88 stimulator is 0-100V while for the signal generator was 0-30 V. The voltage experiments ran testing starting at 100 mV and increasing all the way to 100 V. The frequency and duty cycle were held constant during these experiments at 5 Hz and 50% respectively. This test was run in the general O4 multi-animal microfluidic device. From these experiments the minimum viable voltage was determined.

Following this experiment all other experiments were conducted using the signal generator, as well the preliminary tests were run using a single worm trap device rather than the multi-animal arena. A number of experiments were run to determine the effects of frequency, duty cycle and pulse trains. The voltage was also tested again since a different microfluidic device was being used than in the previous experiments. The specific parameters for these experiments can be seen in the **Table 5.1** below. As well as having the three different parameters and pulse trains, the orientation of which electrode was negative, and which was positive was also tested. The negative head orientation means the negative/ground electrode was connected to

the pin closest to the head while the negative tail means that the negative/ground was connected to the electrode closest to the tail.

Table 5.1: Preliminary Testing Conditions Conducted on Single Worm Device with Signal Generator

Parameter	**Voltage**	**Frequency**	**Duty Cycle**	**Stimulus Orientation**	**Pulse Trains**
Frequency	30 V	5-100 Hz	50%	Neg Tail	No
Frequency	30 V	5-100 Hz	50%	Neg Head	No
Duty Cycle	30 V	20 Hz	10-75%	Neg Tail	No
Voltage	5-30 V	20 Hz	50%	Neg Head	No
Pulse Train	30 V	20 Hz	50%	Neg Head	Once per min

Table 5.1. Shows the parameters for the five different tests run with the signal generator on the single worm trap device. The different parameters tested include the voltage, frequency, duty cycle and pulse trains. For the frequency the value was increased by increments of 5 Hz until 30 Hz was reached then the value was increased by increments of 10 Hz. The duty cycle and voltage trials had an increase in value by 5% and 5V respectively.

5.3 Data Analysis Methods

5.3.1 Image J Analysis

TIF files were obtained for each experimental setup from the Zeiss AxioObserver microscope following the experiments on the *C. elegans* with the stimulation device. To analyze the neural data the Neuro Tracker plugin on ImageJ was used. This feature allowed the head neurons to be identified and tracked throughout the duration of the experiment. To identify the head neurons the brightness and threshold features needed to be manipulated. This is because the gut and neurons located in the tail of the *C. elegans* also appeared illuminated. The head neuron was distinguishable as a singular or sometimes two neurons near one end that were illuminated and not closely connected to other highlighted areas. This was sometimes difficult and required the images to be zoomed in on to identify the appropriate point to track. An example of an annotated ImageJ of the *C. elegans* with the head neurons identified can be seen in **Fig. 5.11** below. One the image 6 random animals have been selected to discuss whether or not they would serve as suitable test subjects.

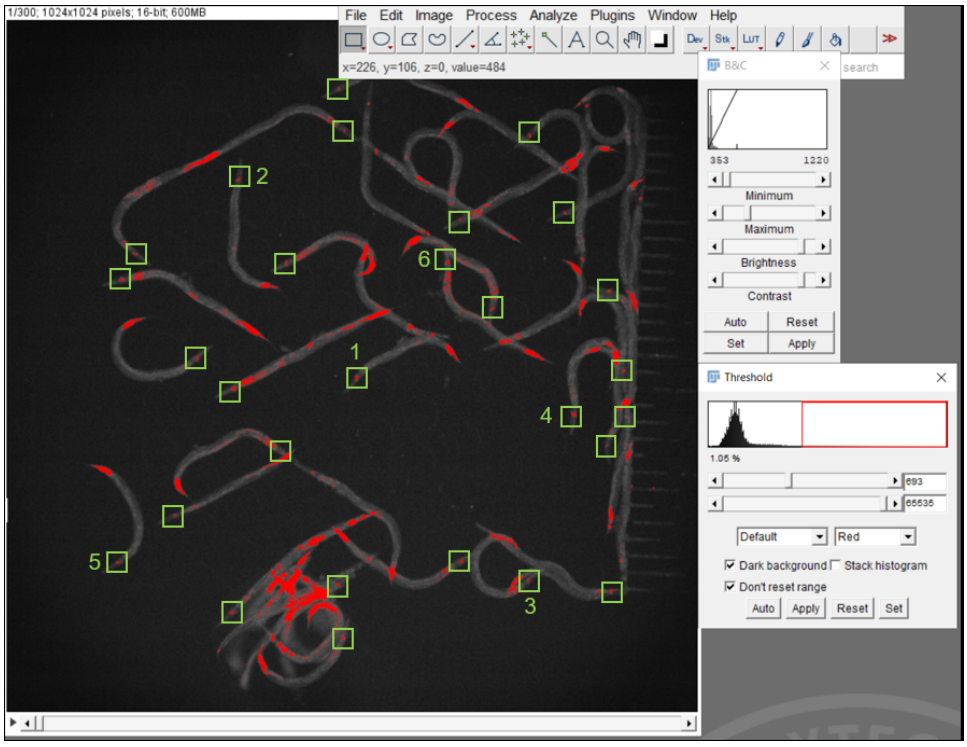


Figure 5.11: ImageJ software with an example of C. elegans being analyzed.

The bar in the top right corner is the general ImageJ toolbar. The interface below this is the brightness and contrast adjustment. Below that is the interface to adjust the threshold. As can be seen on the image the head neurons of a few different C. elegans are being indicated with the green boxes. A few random animals have also been numbered to discuss which ones would be good to pick as neurons to track. Number 1, 2, 4 and 5 are all good animals to track while number 3 and 6 would not be good subjects as they are too close to other animals. As well, when tracking neurons, it is important to watch the tracking and make sure the animal does not move too much as this can skew the data.

Once the neurons had been identified and selected the analysis ran and text files were then created for each experimental set up. There was one text file per neuron analyzed. These text files were uploaded to the MATLAB code **(example of the code is seen in Appendix C)**. The MATLAB code then analyzed the files and output three different displays.

5.3.2 MATLAB Analysis

These three displays include 2 graphs and a heat map of the neuron activity. The two graphs show similar information, although the second one allowed for more changes and customizability to occur. The first of the two graphs, shown below in **Fig. 5.12**, shows each individual neural output, graphing each one on a different line so all animals can be observed at the same time.

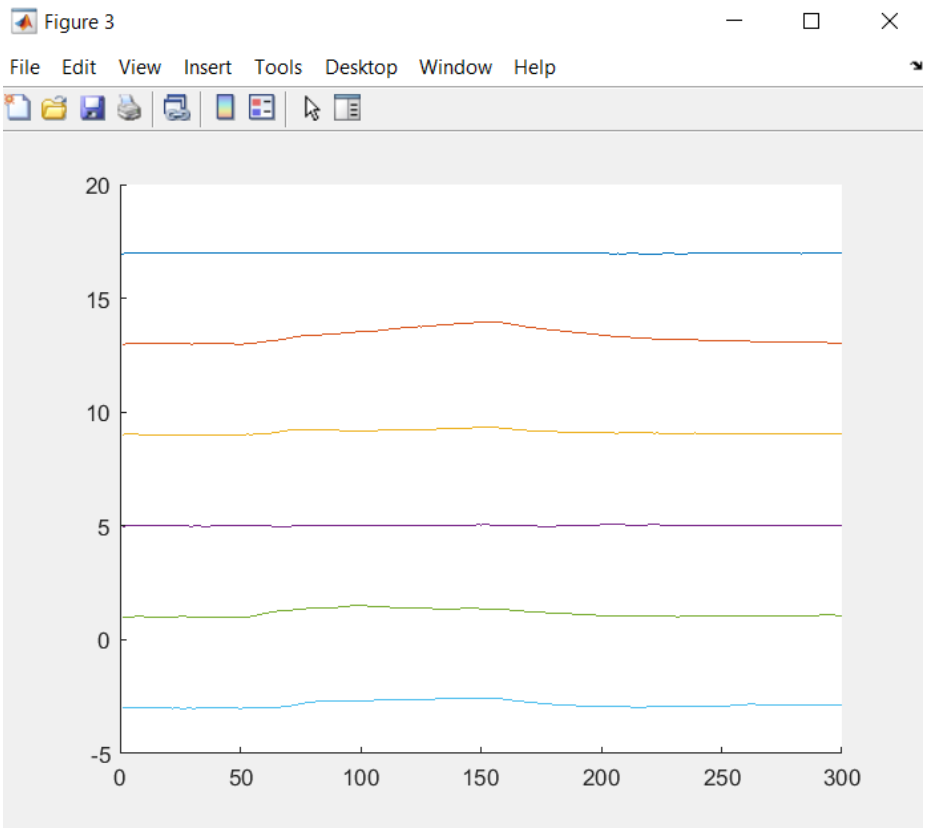


Figure 5.12: The first output graph of the MATLAB code. Each line represents a different animal. The x-axis is time in seconds while the y-axis is the individual animal activity with the values being the change in calcium level over the baseline calcium level and are separated to clearly visualize each animal's activity into its own individual line. In this case the graph represents 6 different animals and their neural responses.

The second graph allows for many different things to be graphed such as the individual neural output, the mean of the outputs, the SEM, the standard deviation, the lines to be filled and stats. The neural activity in both of these plots is represented on the y axis while the x axis displays the time. The final MATLAB output is a heatmap display. An example of both the heat map and

graph which was previously discussed can be seen below in **Fig. 5.13.** The heat map shows the neural activity over time with a color code that represents the strength of the response.

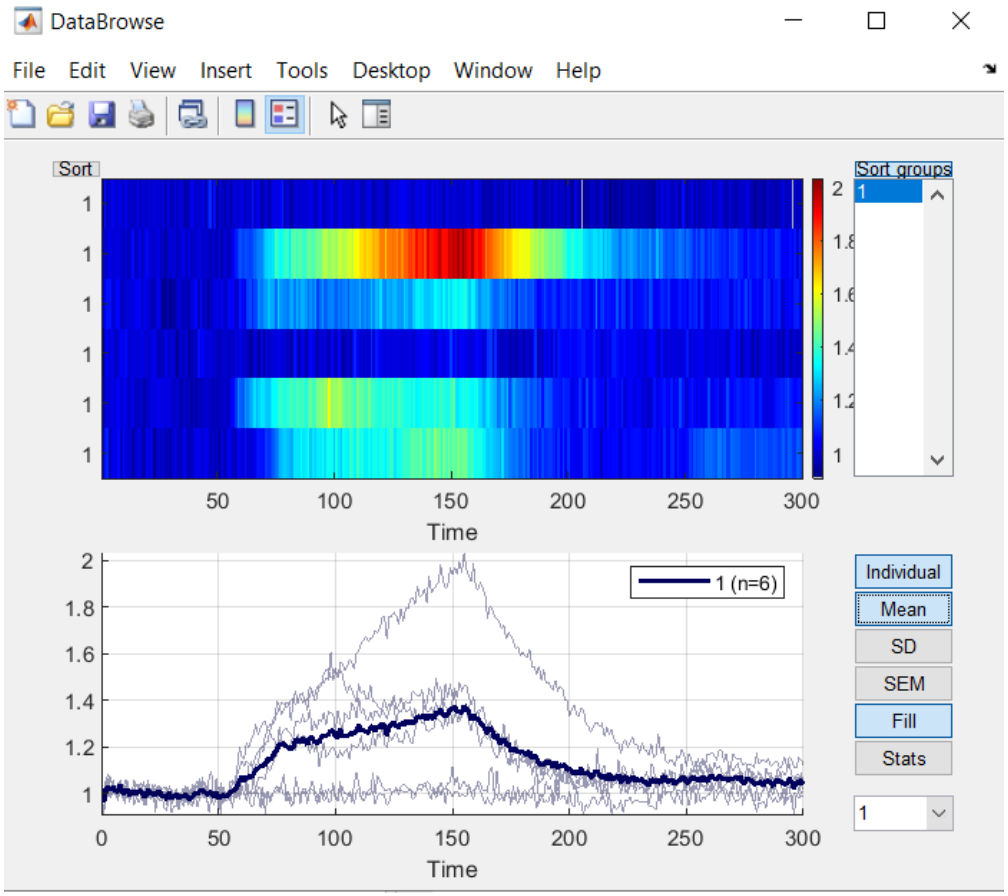


Figure 5.13: The second graph (bottom image) and heat map (top image) can be seen above. The heat map shows each individual animal on a separate line with the colors representing the strength of the response. The magnitude of each color can be found using the key to the right of the image. The bottom graph is the second neural output graph. This graph can be customized using the buttons seen on the right of the image. The current display shows both the individual animals (represented by the thin lines) and the mean of the six animals (represented by the bolded line). In this graph the x-axis is the time in seconds and the y-axis is the magnitude of the neural response normalized to 1 as the baseline value.

The MATLAB code works by monitoring and recording the calcium response at the AWA neuron. The AWA neuron is a sensory neuron that responds to changes in calcium levels by affecting the fluorescence level of the neuron. Therefore, a stronger fluorescence means an increase in intracellular calcium levels [Larsch, Ventimiglia, Bargmann & Albrecht, 2013]. In the MATLAB program a strong response is considered to be a value of 2 or higher, with a value of 2 originally representing a 100% increase in intracellular calcium levels at that neuron. When graphing/calculating the neural activity the MATLAB code uses the first four data points as the normal. This means that anything higher or lower than this will be an increase or decrease respectively. In order to provide clear understanding of the graphs a baseline of 0 was set. This means that a value of 1 would mean a 100% increase has occurred to the neuron. The values

were seen as going negative in some cases because it is possible for the neuron to be lighter/less activated than it was at the initial time point. Originally, the default baseline of the MATLAB code was set to a value of 1 therefore the function 'databrowseS' was used to normalize the values to 0 rather than 1. This function not only allows for the data to be normalized to 0 but it also allows for the data to be grouped and named. Therefore, the experiments were organized based on such features as experiment number or animal number. In addition, specific aspects of the results were grouped together such as orientation of the animal or voltage strength. This ability to group the results allowed for conclusions to be drawn much more easily and helped in comparing the results.

5.4 System Verification Methods

Once all the systems that will be used were determined and set up, it was necessary to run some verification tests, to obtain data that proves the system works. This will verify for future experiments that the entire system that was designed for this project is effective. Thus, the results of future experiments can be valid with the knowledge that the system is working properly. A data logger was used to capture the timing and actual values of the set parameters. A data logger is an electronic device that records data over time or in relation to location either with a built-in instrument or sensor or via external instruments and sensors. The data logger used in these experiments was the LabQuest Mini. Four main parameters were validated in these experiments including: frequency, voltage, pulse train length, and LED timing.

The entire experimental setup was assembled including the Zeiss microscope with LEDs for this experiment. As a representation of previous experiments, the parameters chosen for these trials were 20Hz at 50% duty cycle with 5 second pulse trains. The experiment would be voltage scaling to test the system at 5V, 10V, and 15V. Due to the limitation of the LabQuest mini's voltage sensors only being capable of reading +/-10V, a secondary experiment was run to validate voltage magnitude.

To ensure that the data would properly be recorded, a voltage divider circuit was constructed to half the voltage output of the system which would allow for the datalogger to receive the 15V signals. In order to capture the LED timing, a light sensor was placed near the stimulation LED of the microscope, then it was connected to the LabVIEW quest mini sensor as shown in **Figs. 5.14 and 5.15** below.

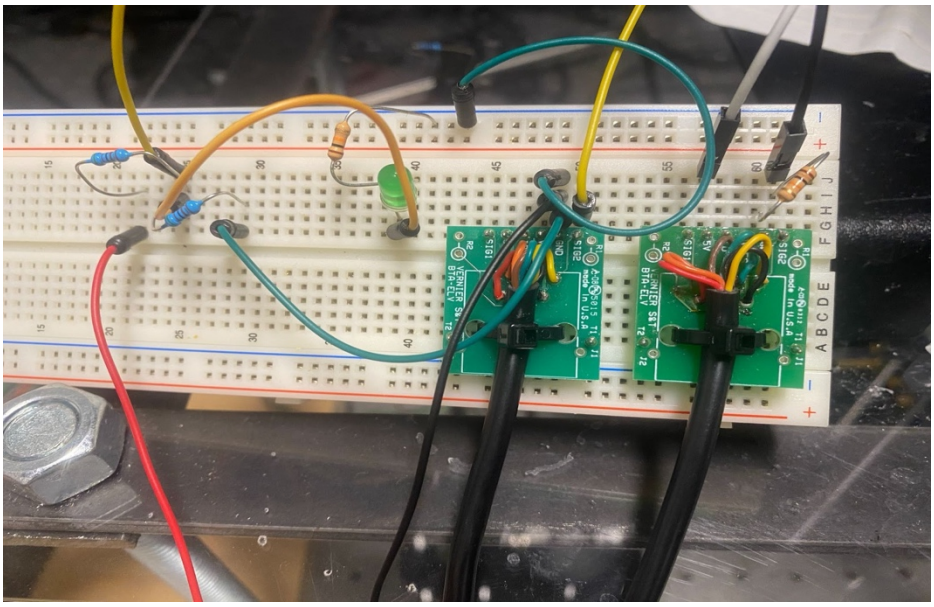


Figure 5.14: Data Logger setup for the verification experiments

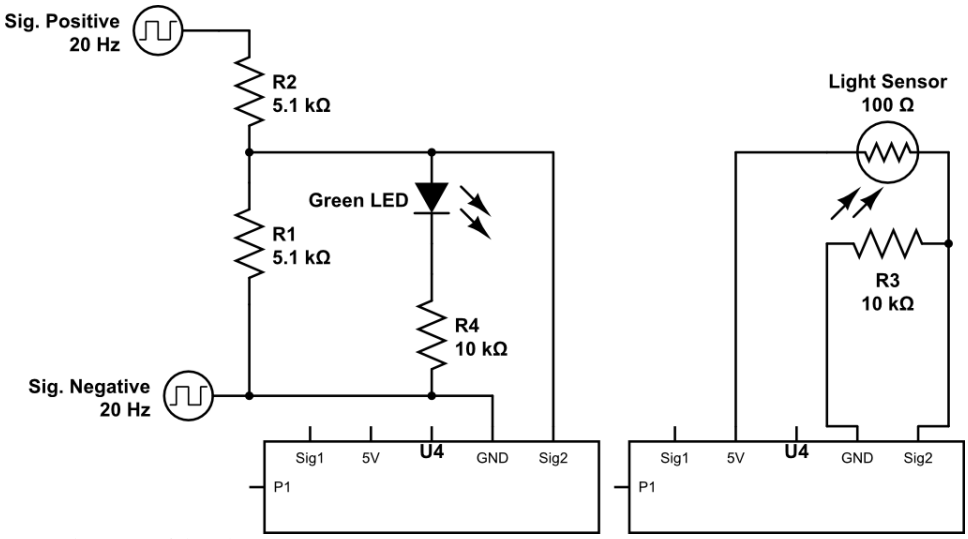


Figure 5.15: Circuit diagram of data logger set-up.

The above images show the voltage divider built to capture the electrical data for the signal generator on the left, and the circuit to capture the LED data on the right.

6 Results and Design Validation

To validate the design, a variety of tests were run to determine that it could meet the desired parameters. First, pilot tests were run using an established device that had a much higher range of parameters than necessary. From these pilot data experiments, the data can be analyzed to modify or validate the design created to meet the prespecified design requirements. Once it was determined that the device could meet the requirements, DBS imitation tests can be run to further validate the use of the design as a testing device for DBS.

6.1 Pilot Data

Using a GRASS SD9 Stimulator and the O4 multi-specimen microfluidic design, initial tests were done as proof of concept for the electrical stimulus of the *C. elegans* specimen. Before these experiments very little was known on the effect of electrical stimulus on *C. elegans* neural response. The GRASS Stimulator was used to run these pilot data tests as a benchmark was needed to compare the response gained from the signal generator design.

6.1.1 Voltage Effects on Response

The first priority was to determine the voltage and in turn, the current, necessary to obtain a neural response from the specimen. Multiple tests were done under the parameters of a frequency of 5 Hz, duty cycle of 50% and varied voltages. The voltage range was from 100 mV - 100V. The minimum voltage in which a response was observed was 40 V and the magnitude of response increased with the voltage (**Fig. 6.1**). However, it can also be observed that while the stimulus begins at 5 seconds, the neural response is delayed. This can be due to the setup of the multi-species microfluidic design in which the current must travel through many small channels prior to reaching the arena in which the specimens are housed. However, a delay in calcium response is common regardless of stimulation modality, usually <1s. This neural activity reporter indicates free intracellular calcium, as released by voltage-gated calcium channels (VGCC) upon depolarization. In addition, the effect of the orientation of the housed specimens were also analyzed on the role it played in the effectiveness of voltage application. The vertical pin placement was determined to be the one that showed the greatest field strength in response, which is why following all tests were completed in that set up. This orientation therefore is implemented into the future experiments on the O4 device including the second two DBS trials.

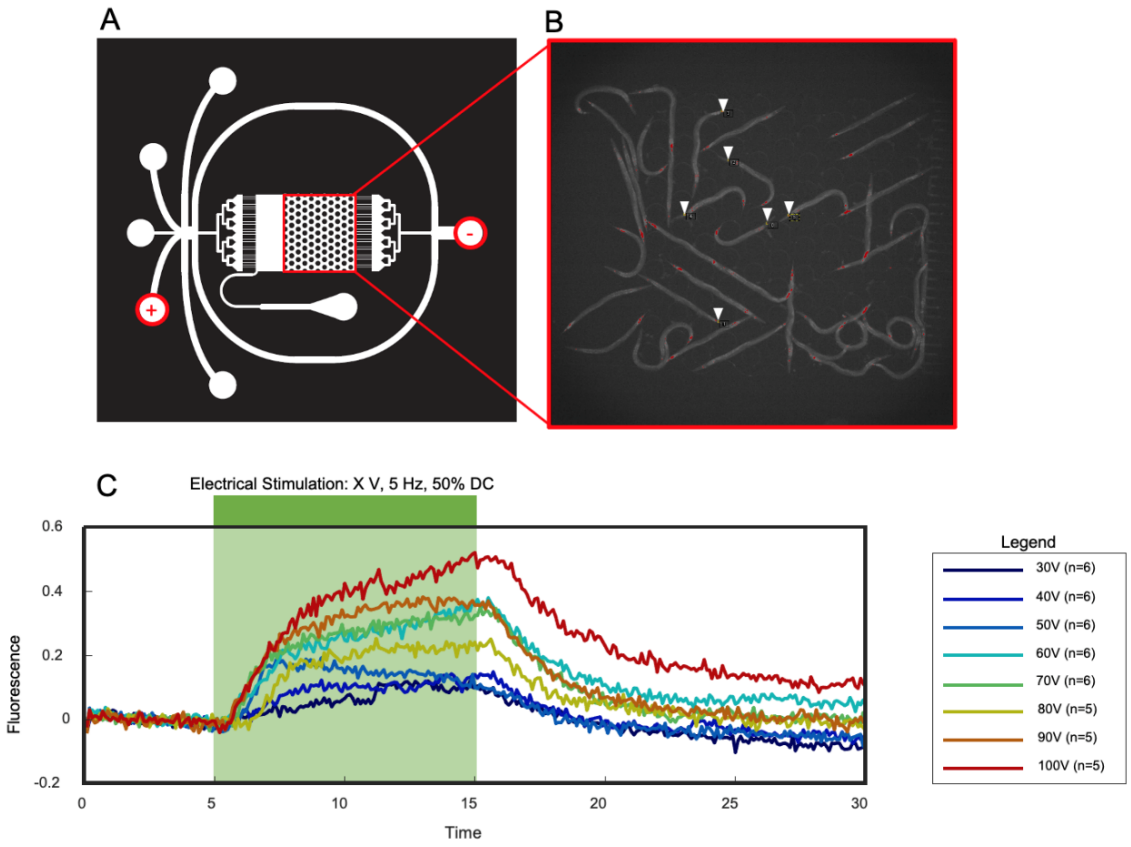


Figure 6.1: Voltage Response from 40 to 100 V

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in image of the arena with the head neurons that were tracked indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the 7 animals (n = 6) chosen from the tests run at the various voltages (indicated in the legend)

Utilizing COMSOL finite element analysis, the results from this test can be analyzed in the context of the microfluidic design. The theoretical voltage at each point in the design can be determined as well as other key factors such as the electric field at each point and the current density.

6.1.2 Electric Field Required for Neural Activation (COMSOL)

When using the COMSOL software a number of parameters can be determined and analyzed. For the purpose of these experiments the two factors which were deemed important were the current density and the electric field strength at the neuron of the animal. Both of these values were found for the existing R5 microfluidic device at voltages 30-100V. The associated values from these computations can be seen in the **Fig 6.2** below, where the electric field is plotted on the y-axis against the current density on the x-axis to analyze their relationship.

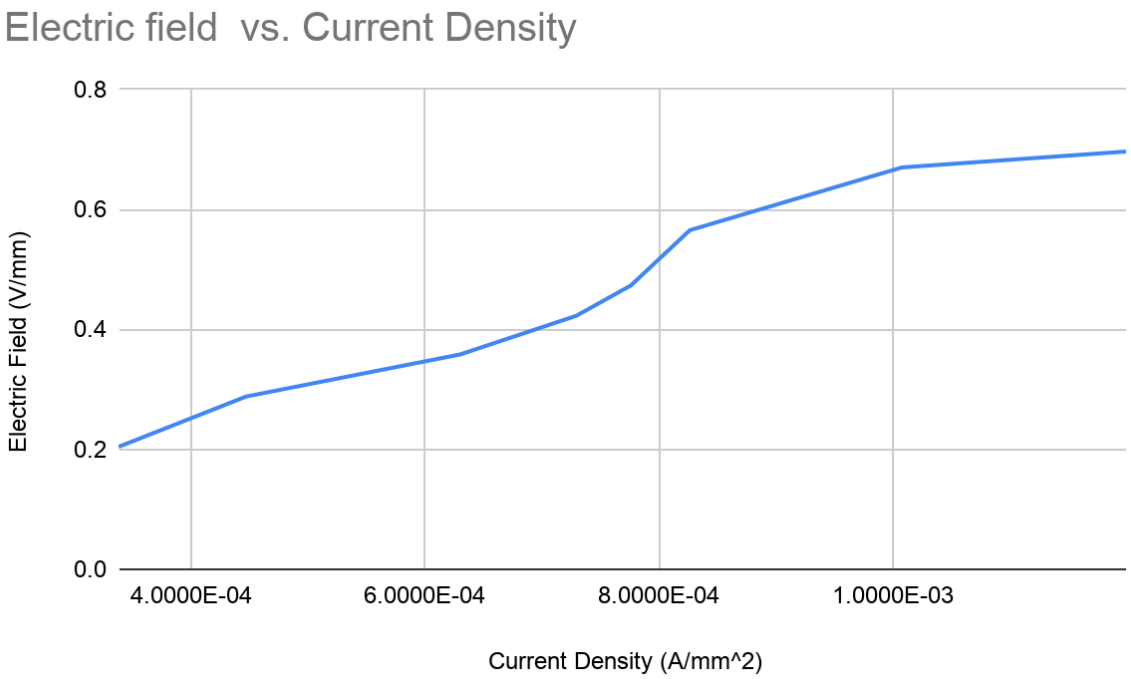


Figure 6.2: The associated current density and electric field at voltages 30 - 100 V. Each of these values was obtained using a COMSOL simulation of the O4 device.

From the above **Fig. 6.2** it is obvious that the current density and electric field strength have a linear relationship. This was supported by the COMSOL maps of the values on the device itself which can be found in **Appendix D**. Since these two factors have a linear relationship and follow the same trends only one of the features was mapped on the further COMSOL analysis. Since the values for electric field strength are larger it makes them easier to work with, so the electric field strength was determined to be the important factor to analyze and compare between devices going forward.

To identify the minimum viable electric field strength for *C. elegans* neuron activation both experimentation and COMSOL modeling was required. The threshold at which electrical stimulation caused the neurons of *C. elegans* to activate had to be identified, this was done using a COMSOL simulation based on results from the preliminary testing. In the preliminary experiment, a voltage of 40V had a distinct response in contrast to lower voltages. By utilizing the COMSOL analysis of the existing R5 microfluidic device, the approximate electric field could be determined at 40V for the points where the neurons were being stimulated. The theoretical threshold for electrical activation can be estimated to be ~0.28 V/mm as seen in **Fig. 6.3** below. This value for the electric field was found by averaging 12 points in random spots across the arena.

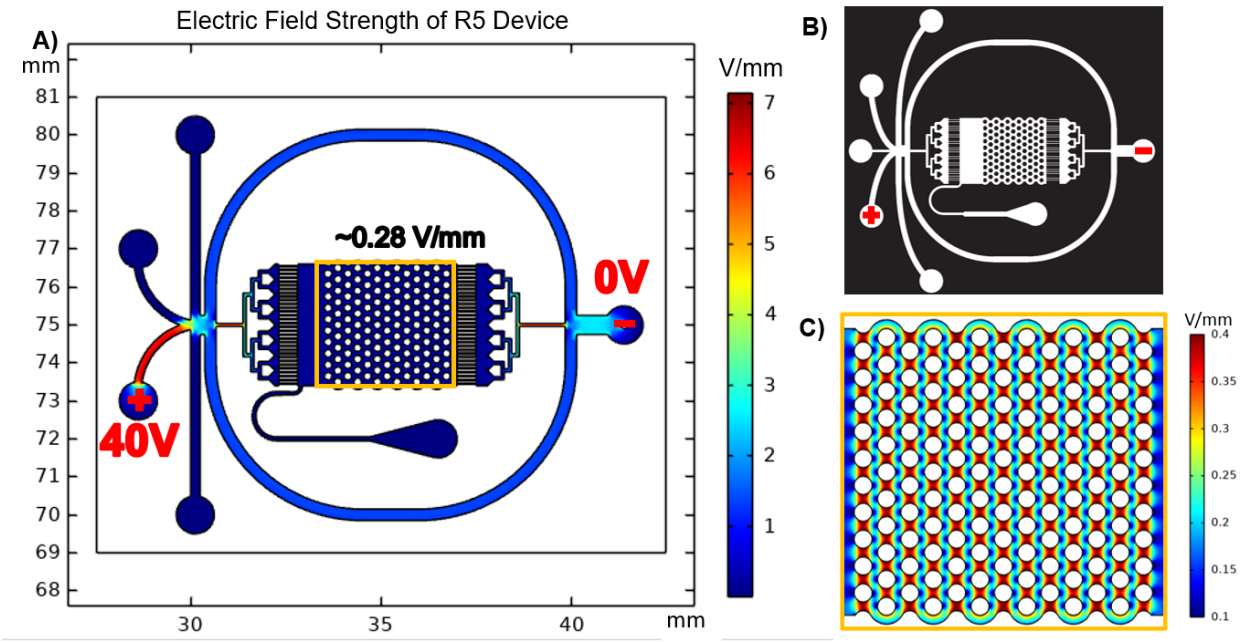


Figure 6.3: Results from the R5 microfluidic design COMSOL analysis.

Panel A shows the electric field (V/mm) at 40V of the microfluidic device. The majority of the current is seen being lost over the small inlet and outlet channels. Panel B shows the actual microfluidic device with the positive and negative electrodes indicated. Finally, panel C shows the electric field, and to allow for easier readability the scale of the values has been modified to only include ranges from 0.1-0.4 V/mm. The average electric field strength was determined by averaging the values from 12 random points across the arena.

6.2 Microfluidics Trap Design

Once it was determined that the minimum viable voltage for the multi-specimen microfluidics design was 40 V, a new design needed to be considered to accommodate the maximum voltage of the team's design of 30 V. The trap design encases a single specimen and has a shorter distance between the pins allowing for a lower voltage to provide sufficient current for electrical stimulation.

6.2.1 COMSOL Analysis of Trap Design to prove sufficiency

In order to verify whether or not the trap design could provide sufficient stimulation at 30V despite the pilot data indicating that stimulation would require 40V, a COMSOL analysis was run for the trap design to determine if the threshold electric field was suitable for stimulation. As shown in the **Fig. 6.4** below, the electric field is strongest within the narrow section of the design which is to be expected. After averaging the values near where the head/tail of the animal would be located, the estimated electric field would be 6.52 V/mm. This number exceed the established threshold of 0.28 V/mm, so it was determined it could be a suitable design.

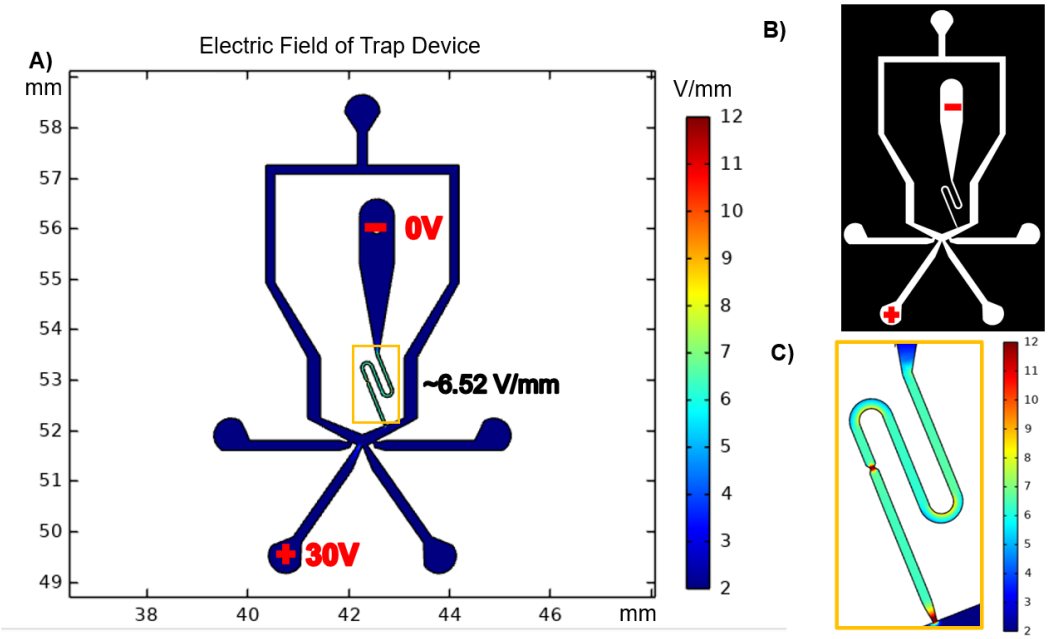


Figure 6.4: Electric field strength in the trap design where the worm is housed in a small channel.

This channel and shortened distance between pins increase the electric field greatly to approximately 6.52 V/mm. The full device is seen in panel A with the electric field being greatest over the narrow housing channel. For better visualization the narrow channel has been zoomed in on in panel C where the high voltage can be seen. Panel B shows the full CAD file of the microfluidic device.

6.2.2 Voltage Effects on Response

The minimum voltage necessary to cause a visible response was determined through running various voltage tests. Six trials were run with frequency set to 20 Hz and duty cycle set to 50%. The voltage was the independent variable in these trials and was changed accordingly. The first test run was at 25 V with each subsequent test decreasing the voltage by 5 volts and the final test being the maximum voltage of 30 V. Based on the results of these tests (**Fig. 6.5**) it can be observed that the minimum viable voltage is 10 V. However, based on the profile of the results, 10, 15, and 20 V may not provide sufficient current in order to stimulate the neuron effectively. This can be seen in the delay in the neural response with the peak of response being around 20 seconds when the stimulus began at 5 seconds.

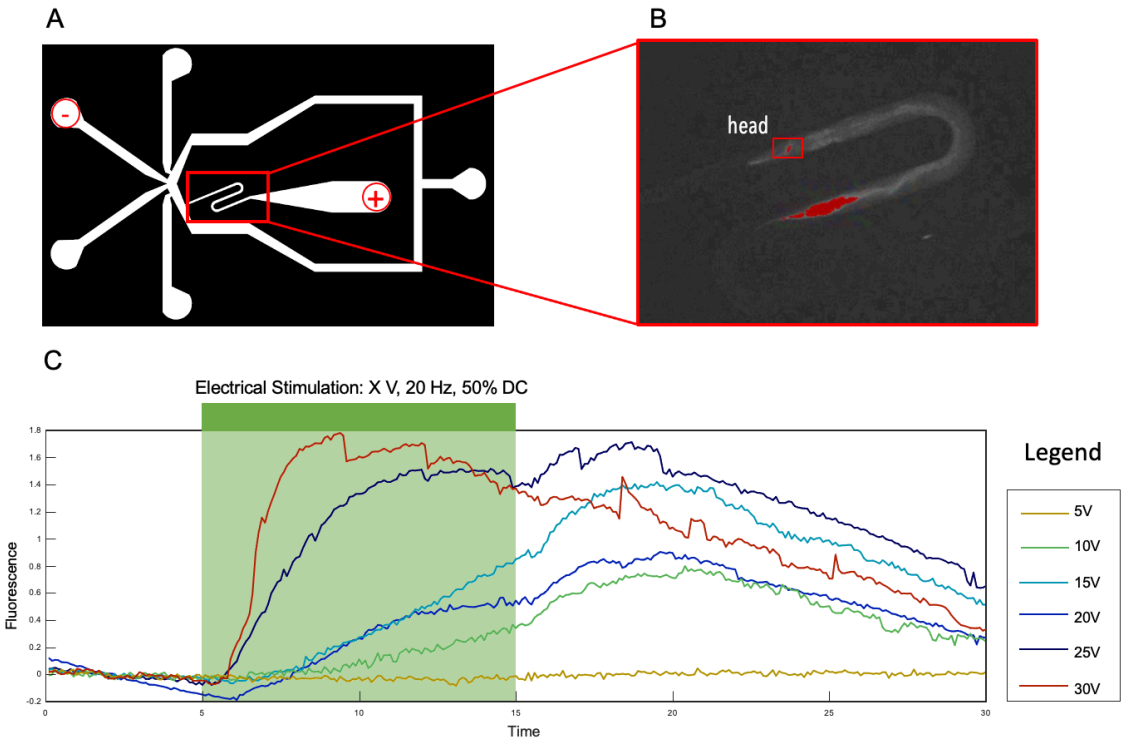


Figure 6.5: Results from varied voltage tests in the trap design.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in image of the specimen itself with the head neuron indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the tests run at the various voltages (indicated in the legend)

These results verify the ability of the design to meet the necessary voltage parameters for DBS testing.

6.2.3 Limitations of Microfluidic Trap Design

While the trap design was effective for providing sufficient current for stimulation, there were clear limitations to this design. When running consecutive tests at 30V and changing another parameter such as the frequency or duty cycle, it was observed that after the first test no other tests produced a response. While it cannot be concluded as to why this was the case, it can be hypothesized that this was the result of too high an electric field of ~6.52 V/mm. This value is much higher than the determined electric field of 0.28 V/mm necessary to elicit a neural response from the specimen. This pattern was only observed in the voltage tests using a parameter of 30 V, which was tested last.

As seen in **Fig 6.6**, only the first test caused a minimal response while all subsequent tests yielded no response. This first test had a negative tail orientation and was done at 20 Hz, 30 V, 50% duty cycle. The subsequent tests were then done at an increase of 10 Hz without changing the rest of the parameters and then once 100 Hz was reached, the frequencies were changed to 5, 15 and 25 Hz and tested.

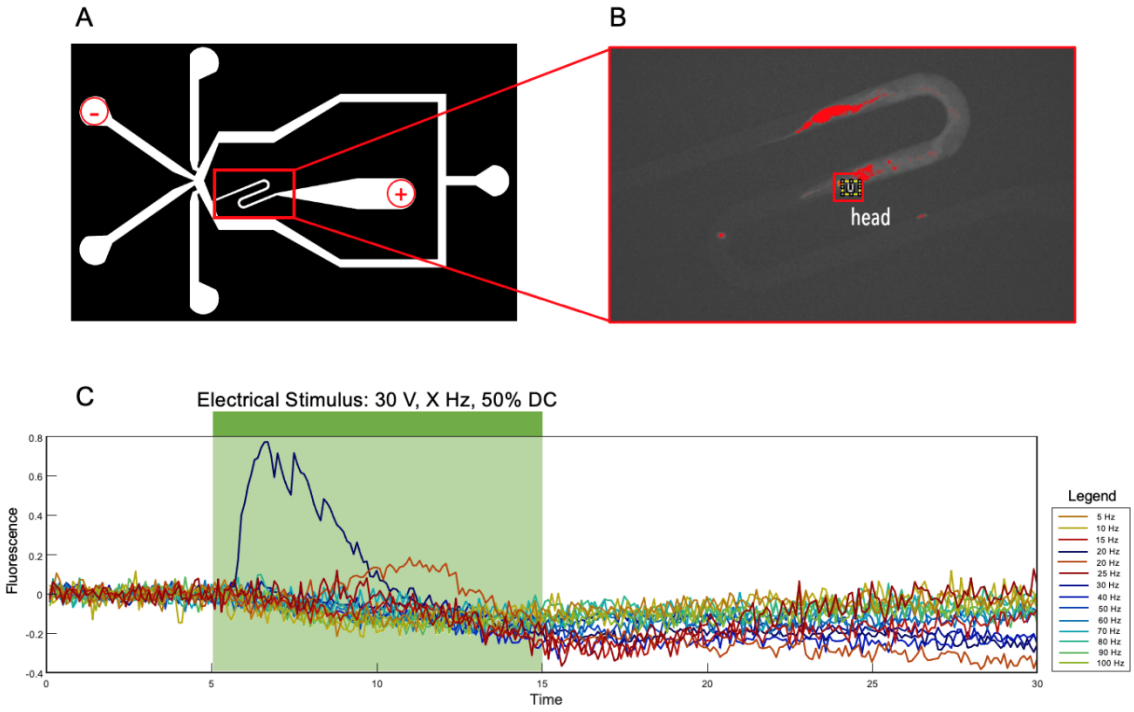


Figure 6.6: Frequency plots from 5 - 100 Hz at 30V and a 50% duty cycle with negative tail orientation.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in image of the specimen itself with the head neuron indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the tests run at the various frequencies (indicated in the legend).

To determine whether these results were a result of the orientation another set of tests were run on a different animal with the same parameters. In this test, however, the head was in the negative position rather than the tail, and the results of this test are shown in **Fig. 6.7**. This test involved the same parameters in the same order. Overall, they yielded all the same results, with the exception of the first test, where a stronger response was shown than the second test, which yielded a weaker response.

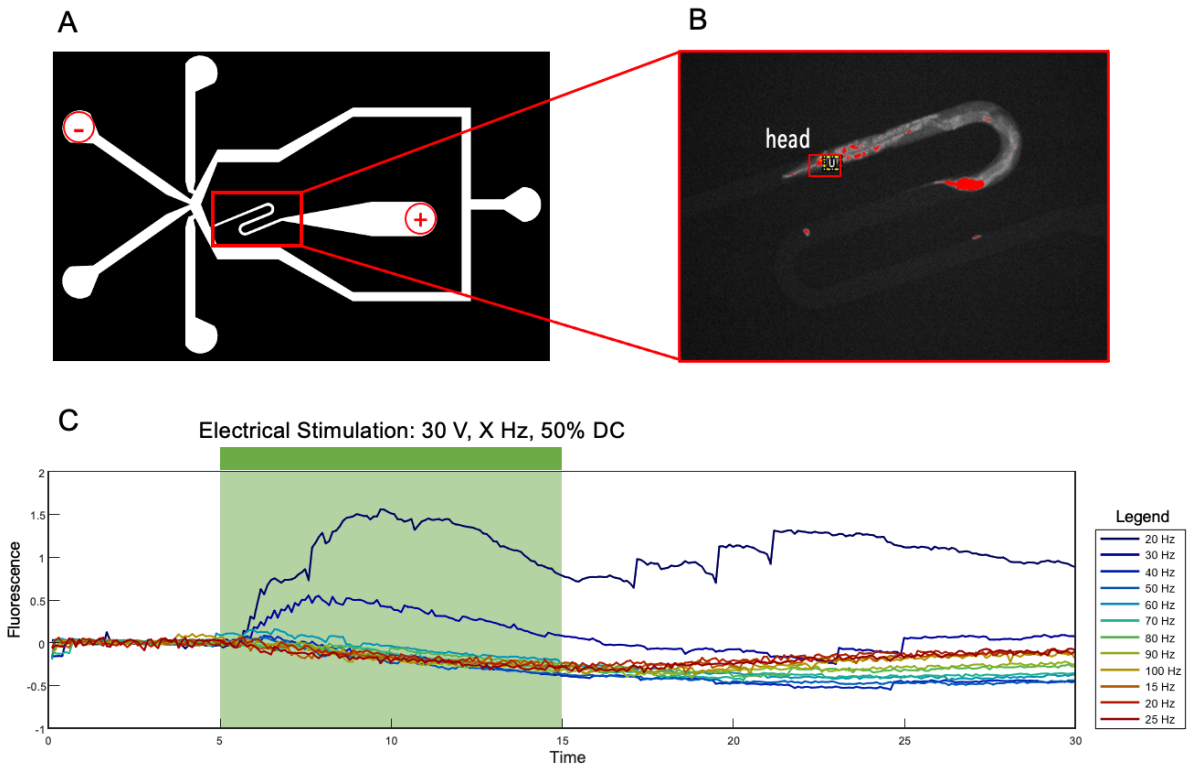


Figure 6.7: Frequency plots from 5 - 100 Hz at 30V and a 50% duty cycle with negative head orientation.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in image of the specimen itself with the head neuron indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the tests run at the various frequencies (indicated in the legend).

Similar tests were then run with a varied duty cycle with the same voltage maintained at 30V and frequency at 20 Hz. The results from this test are outlined in ***Fig. 6.8*** below. The legend on Panel C in this figure also outlines the duty cycle for each test in order, starting with a 10% duty cycle and increasing until 75%

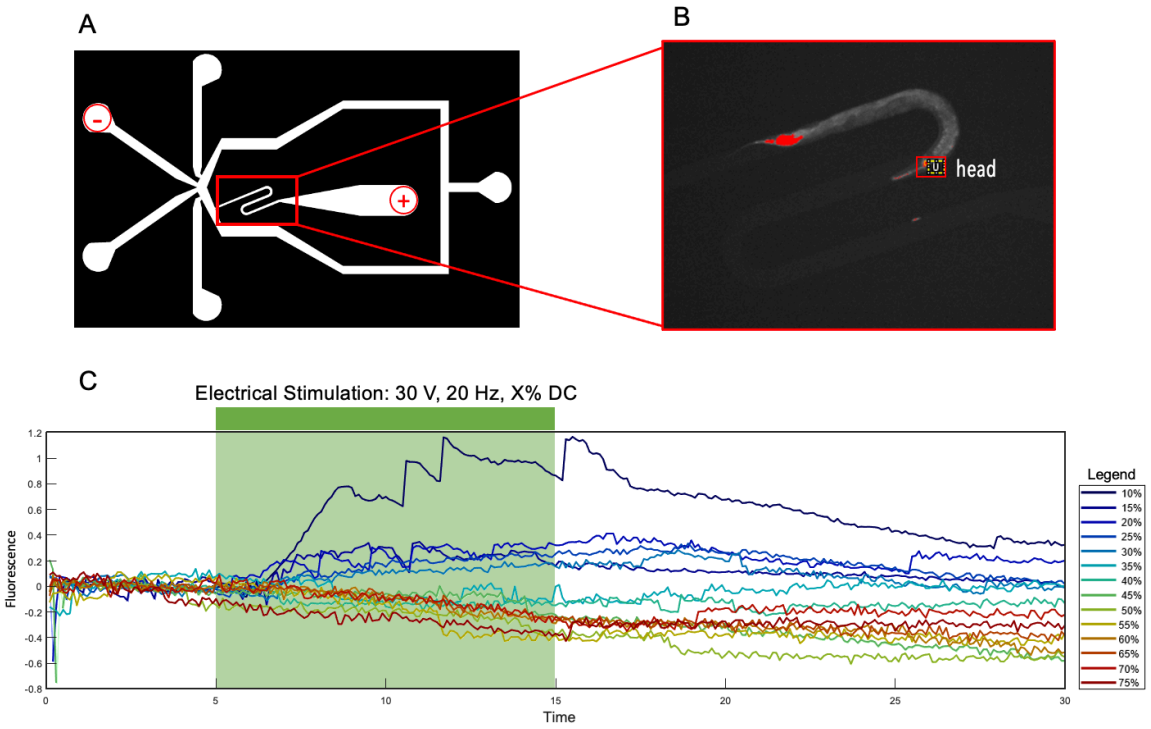


Figure 6.8: Duty cycle results from 10 - 75% at 30V and 20 Hz with a negative tail orientation.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in image of the specimen itself with the head neuron indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the tests run at the various duty cycles (indicated in the legend).

While it is clear from these results that stimulation done at 30V did indeed provide a neural response, a single test can be done on only the single specimen. The trap design allows for the smallest distance between pins, which provides the highest current, although this current hyperpolarizes the neuron after the initial response to the electrical stimulation. While the goal of these tests was to determine the ability of the device to produce different frequencies and duty cycles, this was unable to be determined. Due to the hyperpolarization after the first test, no conclusions can be drawn on the subsequent tests. Each of these tests were done within minutes of each other which proves that the neuron is being hyperpolarized after the first test and no longer yields a response after the initial pulse.

6.3 Pin Orientation Optimization

Once determining that the trap design would not be optimal for the testing due to the fact that only one animal could be tested at a time as well there were issues with being able to apply repeated stimulation. Since this design had these limitations other options for microfluidic designs had to be determined. It had been previously concluded that the traditional multi-specimen arena could not be used, as the minimum threshold for response was 40 V which is outside the range of the 30 V maximum in which the signal generator can function. This led to the use of a different existing multi-specimen arena called the O4 which lacked the channel

surrounding the device. Along with this modification of the existing multi-specimen arena was made, with altered electrode placement to achieve stronger electric field strength at the neuron with 30V as the maximum voltage.

6.3.1 COMSOL Analysis

COMSOL simulation software was used first to determine the electric field strength with original electrode placement in the O4 device to see if removing the surrounding channel would increase the electric field strength. This model can be seen below in **Fig. 6.9**. The approximate electric field strength obtained from this simulation will serve as the baseline to compare the results from the changes in pin orientation. The models were compared by looking at the 3 mm x 3 mm arena containing the *C. elegans* excluding the rest of the device from the images. The new pin orientations which were modeled included a diagonal, horizontal and vertical pin orientation. The results for the electric field strength in each of these examples can be seen in **Fig. 6.10** below.

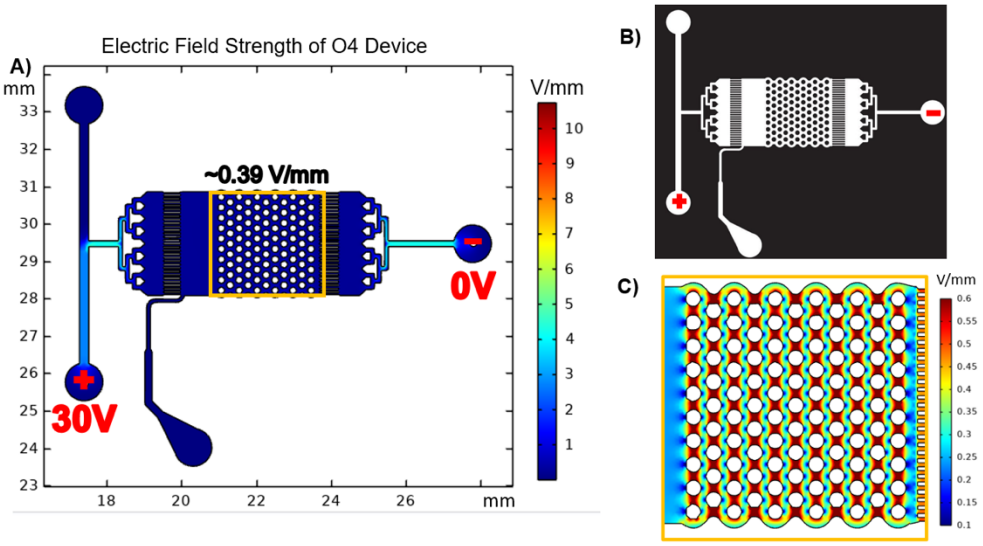


Figure 6.9: Results from the O4 microfluidic device COMSOL analysis.

(A) shows the electric field (V/mm) at 30V of the microfluidic device. The majority of the current is seen being lost over the small inlet and outlet channels. (B) shows the actual microfluidic device with the positive and negative electrodes indicated. Finally, (C) shows the electric field, and to allow for easier readability the scale of the values has been modified to only include ranges from 0.1-0.6 V/mm. The average electric field strength was determined by averaging the values from 12 random points across the arena.

The majority of the current was dissipated in the narrow channels leading up to the 3mm x 3mm arena with the *C. elegans* in the arena experiencing an electric field of ~0.39 V/mm. Although this value exceeds the minimum viable electric field strength of 0.28 V/mm it is still a small value, and a larger electric field strength was desired to determine the effects this would play on the *C. elegans* neural activity. This is why the different pin orientations were modeled in COMSOL to determine their effects on the electric field strength.

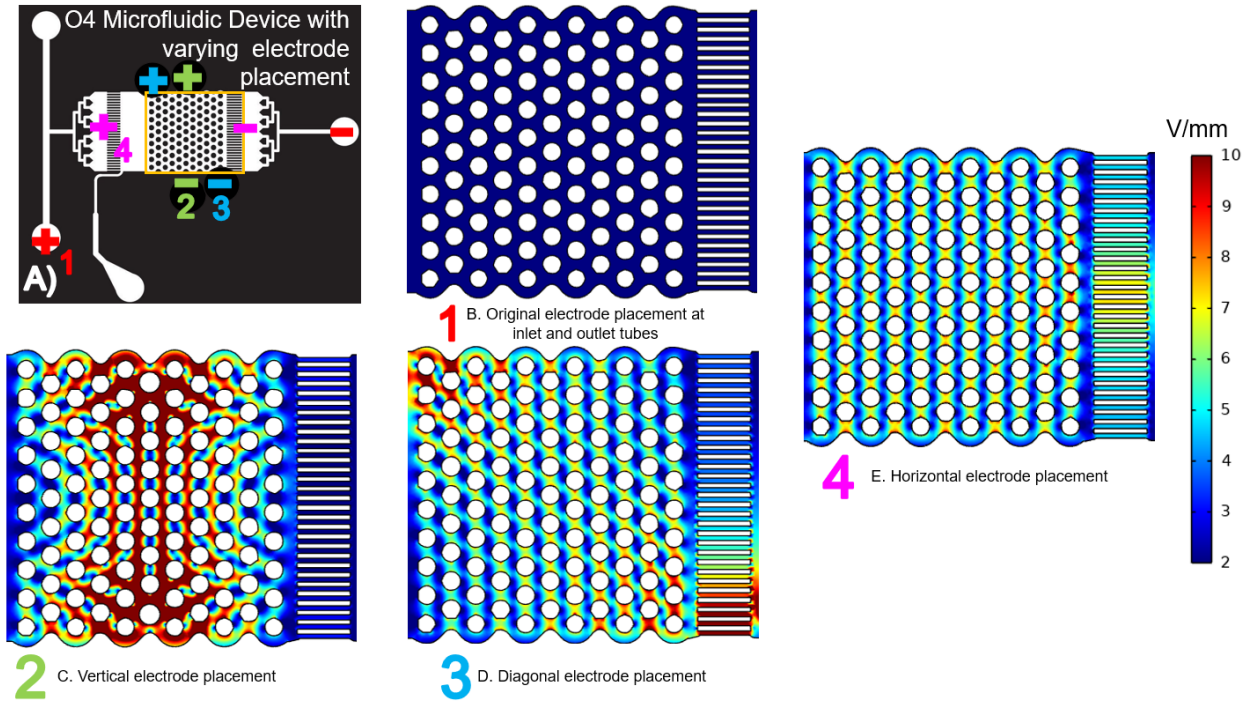


Figure 6.10: Results from the O4 varying electrode placement COMSOL analysis.

Panel A shows the full O4 device with the different pin placements indicated by number and color. Panel B (1) shows the arena of the original-baseline microfluidic device expressing the electric field in V/mm. The third Panel C (2) shows the results of the electric field when the pins were oriented in the vertical direction. Panel D (3) shows the results of the electric field when the pins were oriented in the corners of the device. Finally, Panel E (4) shows the electric field results when the pins were oriented in the horizontal direction. The range for the magnitude remained constant at 2-10 V.mm with the key for the magnitude of each color can be seen next to Panel E. The voltage applied to each device remained constant at 30V.

As expected, the baseline design had the lowest electric field strength when compared to the other three designs at a value of ~0.39 V/mm. The diagonal configuration has the *C. elegans* experience an average electric field of ~5.40 V/mm. The vertical configuration has the *C. elegans* experience an average electric field of ~ 9.05 V/mm. While the horizontal electrode placement had an average electric field of ~4.84 V/mm. **Table 6.1** that numerically compares the different features of the designs. It is obvious however that moving the pins inside or just outside the arena and not having the current go through the channels increases the electric field strength exponentially.

Table 6.1: Electric Field Comparison of Different Designs.

The numerical values for the different devices electric field strength can be seen in the table above. The values were obtained from within the arena around the pins from 12 random points which we averaged to obtain the magnitude.

Device	**Magnitude of Electric Field (V/mm)**
O4 with Regular electrode placement	0.3902
Diagonal Pin Placement	5.4003
Horizontal Pin Placement	4.8407
Vertical Pin Placement	9.0473

Based on the results from this analysis the vertical pin orientation was determined to be the optimum choice for pin configuration for future experimentation. Along with having the highest values for electric field strength this pin orientation was also deemed the easiest to implement in the devices. To implement this orientation the electrode posts were pushed into the PDMS within the arena and due to the size of the device visualizing accurate placement is difficult especially in some of the other configurations such as the diagonal placement. This orientation therefore is implemented into the future experiments on the O4 device including the second two DBS trials as well as the frequency tests which will be discussed in the following sections.

6.4 Frequency Tests

Two different tests were conducted to analyze the effects of high frequency on the neural responses. Both tests were run with the same parameters and compared to two other tests run with the same parameters and a low frequency. The parameters used for testing included a voltage of 30V, a duty cycle of 50%, with the negative electrode being placed on the upper pin and the electrical stimulus was started at the 5 second mark remaining on for 10 seconds. The high frequency trial was run at 100 Hz while the comparison trial was run at 20Hz. The comparison between the two trials can be seen in the **Figs. 6.11 and 6.12** below with the first figure showing the high frequency trials and the second showing the low frequency.

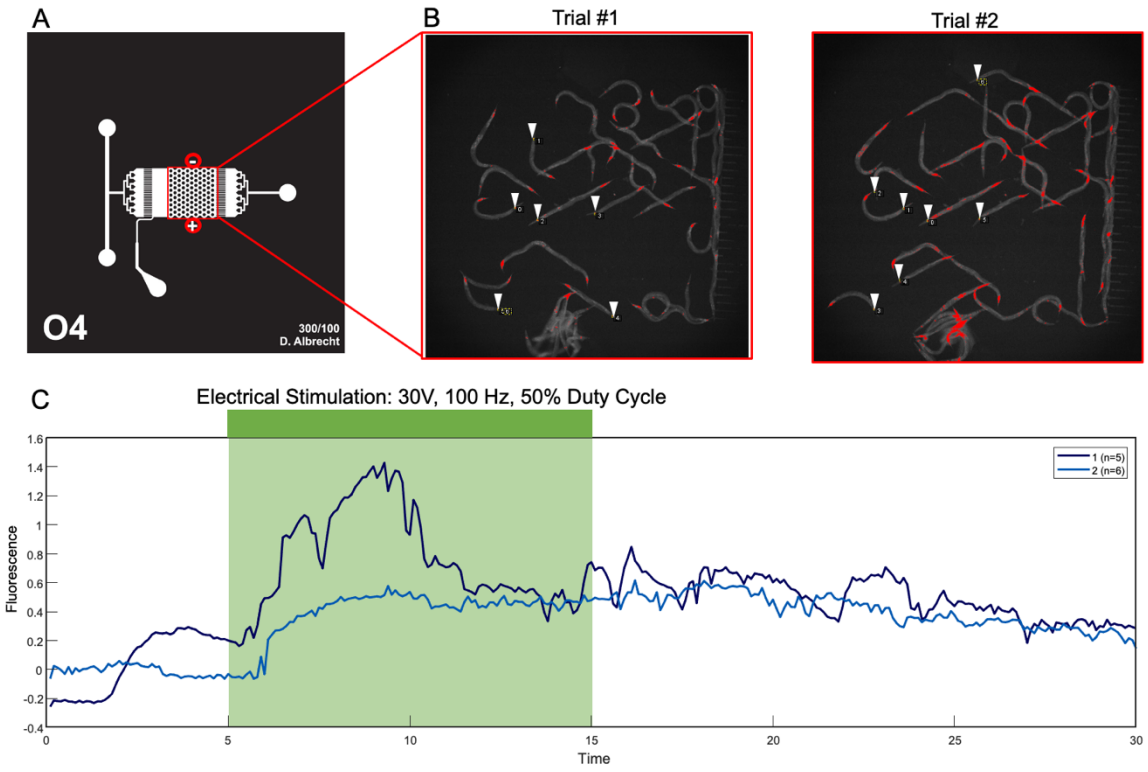


Figure 6.11: High Frequency trials done at 30V, 100 Hz and a 50% duty cycle.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in images for both trials 1 and 2 with the head neurons of interest indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the two different trials where 1 = trial 1 and 2 = trial 2(indicated in the legend). The peak magnitude for the two trials is 1.291 and 0.616 respectively.

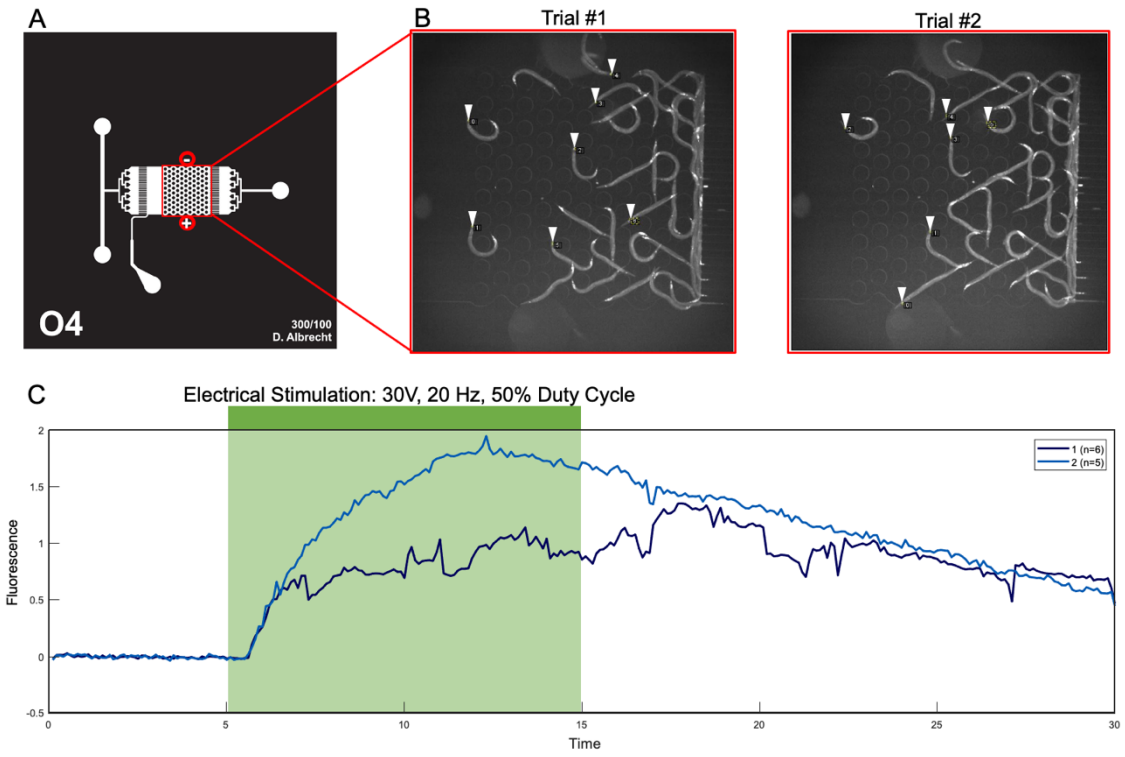


Figure 6.12: Low frequency trials done at 30V, 20 Hz, and a 50% duty cycle.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in images for both trials 1 and 2 with the head neurons of interest indicated. (C) shows the results of the neuro tracker and MATLAB analysis of the specimen from the two different trials where 1 = trial 1 and 2 = trial 2(indicated in the legend). The peak magnitude for the two trials is 1.352 and 1.863 respectively.

Based on the comparison between the two different trials it is evident that the lower frequency trials have a higher peak magnitude than the high frequency trials with values of 1.6075 and 0.9535 respectively. Although these results show that using the low frequency has a greater impact on the neural response level this does not give any information on how high frequency is actually affecting the neuron and will change response when conducting a deep brain stimulation experiment and therefore further testing and research must be conducted. The high frequency values used in this experimentation are similar to those used in DBS within human models whereas the low frequency values are much lower than anything used in human models. In a human model low frequency is referred to as 60-80 Hz while high frequency is 130-150 Hz [Groiss et. al., 2009]. It is understandable that these values would be different however due to how small the *C. elegans* are and the fact that the electrical stimulation system was external rather than internal.

6.5 DBS Trials

The main function of the design is to run deep brain stimulation tests in order to understand the underlying functionality of DBS responses in humans. To test the ability of the device to recreate this phenomena, electrical stimulation was done between sets of chemical

stimuli. Two sets of chemical stimuli were run, and then electrical stimulus was applied. After the electrical stimulus two more rounds of chemical stimulus were run in order to evaluate the effect of the electrical stimulus on the neural response.

6.5.1 Trial #1

The first trial of DBS testing consisted of two rounds of chemical stimulus, labelled 1 and 2, followed by 10 minutes of electrical stimulus at 30 V, 20 Hz, and a 50% duty cycle. After this electrical stimulus two more rounds of chemical stimulus were run, labelled 3 and 4. This can be seen in **Fig. 6.13** below.

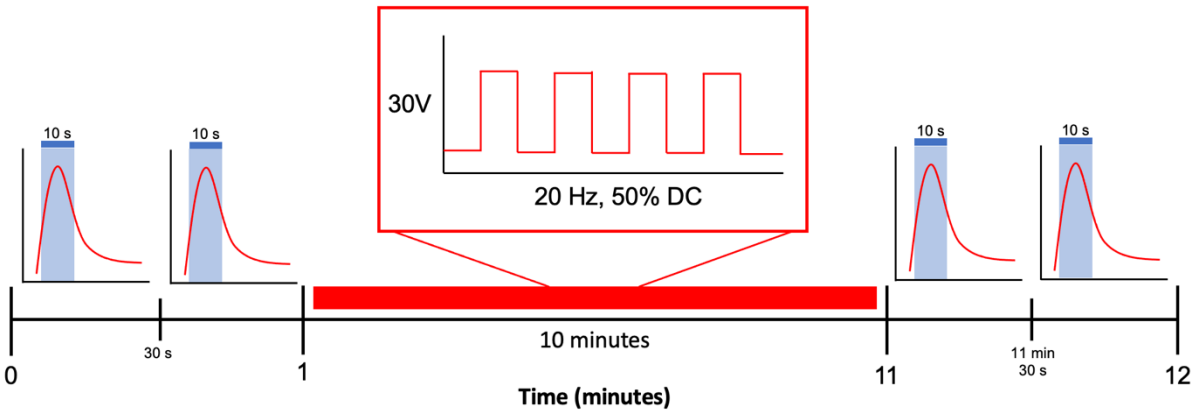


Figure 6.13: Timeline of stimulus for the first DBS trial experiment

This test was run in the multi-specimen arena with pins placed within the arena. This pin placement potentially skewed the responses as the pins used were solid and created holes within the arena that many of the animals got stuck in. This also caused movement among many of the animals which in turn affected the results of the tests. For these reasons, the results of the results of the test were inconclusive, however, new aspects of the design were determined. Through this test it was determined that the hollow pins must be used to attach the electrodes to, otherwise, bubbles can easily form underneath the pin and in many cases the user cannot tell which presents an issue to the efficacy of testing.

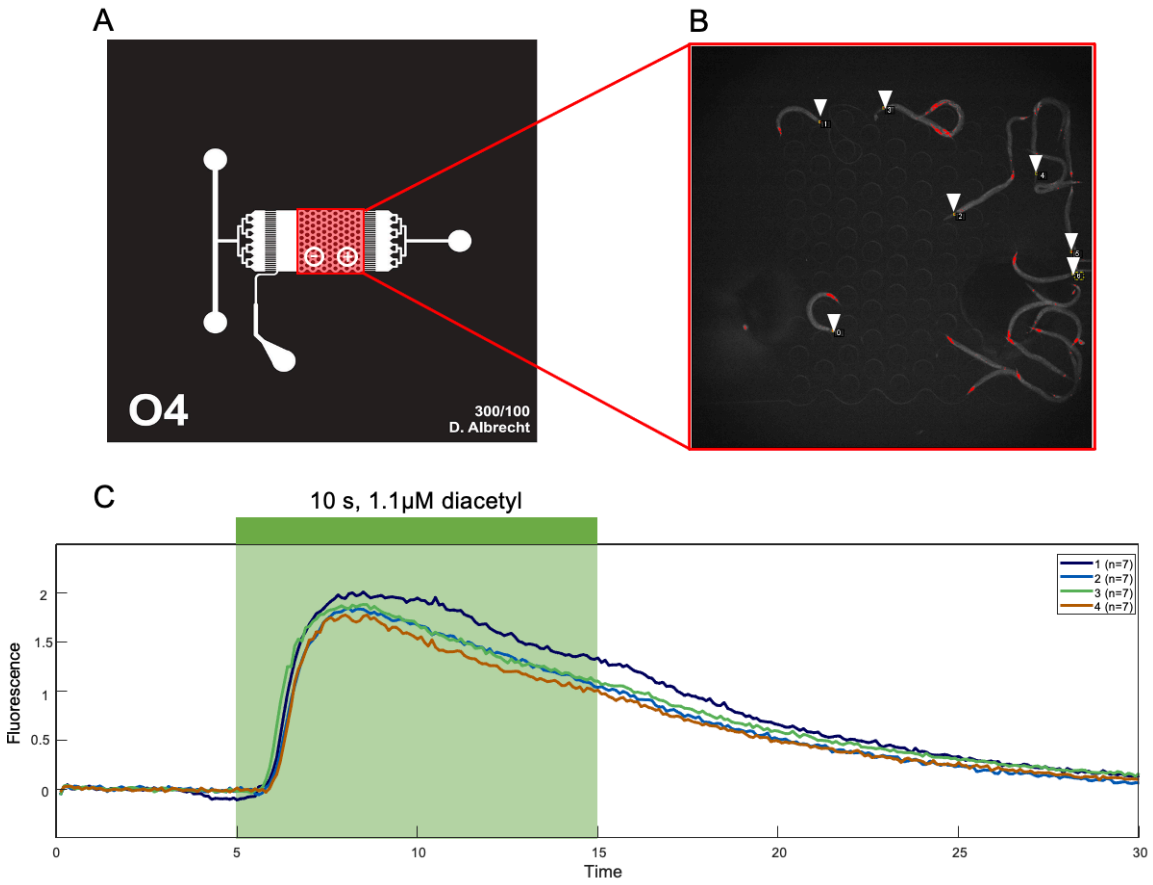


Figure 6.14: Results from the chemical stimulus.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in images with the head neurons studied indicated with the white arrows. (C) shows the results of the neuro tracker and MATLAB analysis with 1 & 2 being the baseline responses before electrical simulation and 3 & 4 being the responses to chemical stimulus after the electrical stimulus of 30V, 20 Hz, and a 50% duty cycle

As observed in **Fig. 6.14**, there does appear to be a lower neural response in the chemical stimulus tests run after the electrical stimulus. While conclusions cannot be drawn on this data set alone, it provides a baseline that the device may provide the sufficient parameters necessary to run Deep Brain Stimulation tests required by this design. This can help determine the optical stimulus, which requires no flow, and easier to adjust the light stimulus strength (intensity).

6.5.2 Trial #2

The second trial of DBS involved two pulses chemical stimulation, followed by electrical stimulation performed at 15 V, 20 Hz, and a 50% duty cycle for a 10-minute duration. This electrical stimulation was then followed by two more sets of chemical stimuli, labelled 3 and 4. The pin placement for these tests was in the vertical orientation, as analysis proved this was the most promising orientation. The initial chemical pulses, 1 and 2, as well as chemical pulse 3, showed promising results, as all the neurons selected appeared to respond at the 5 second time mark when the stimulation was introduced. The last chemical pulse had to be excluded because

an air bubble was unintentionally inserted into the microfluidics device 10 seconds into this pulse and therefore affected the rest of the stimulation.

To determine if the orientation of the animal had an effect on the magnitude of the response, the angles of the neuron in relation to the direction of the current was measured. Based on this analysis no clear correlation could be drawn between the orientation and the magnitude of response. It was later concluded that this analysis was flawed as it did not take into account the actual flow of current through the microfluidic design. This can be observed in **Fig. 6.10** with the vertical electrode placement design. The current flows in an hourglass shape making it difficult to approximate the location of the neuron in reference to this flow.

6.5.3 Trial #3

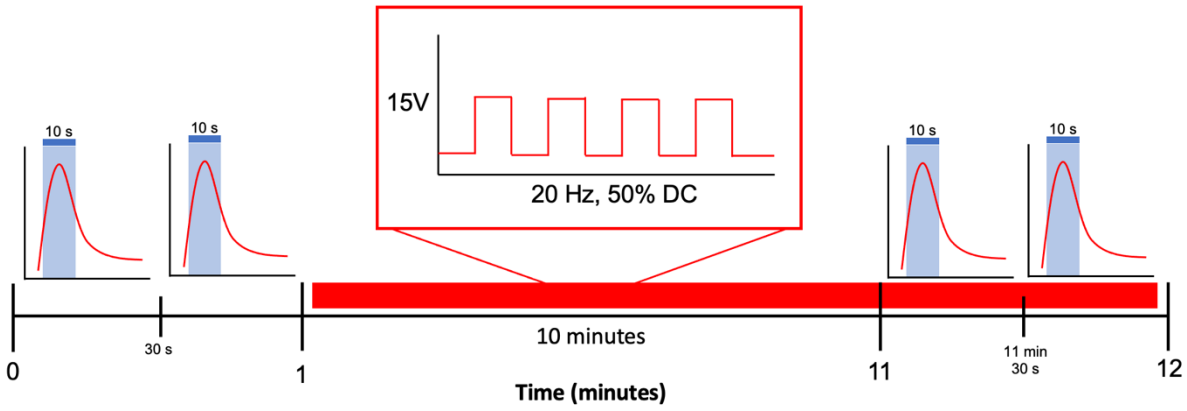


Figure 6.15: Timeline of stimulus for the third DBS trial

Similar to the second DBS test, this test was run with the parameters of 15V, 20Hz and 50% duty cycle. There were two rounds of chemical stimulus conducted on the *C. elegans* prior to any chemical stimulation. These can be seen as trial 1 and 2 below in **Fig. 6.15**. The electrical stimulus was turned on for 10 minutes and left on when the second round of chemical stimulus was conducted. These two second rounds of stimulus can be seen as 3 and 4 in the **Fig. 6.16** below.

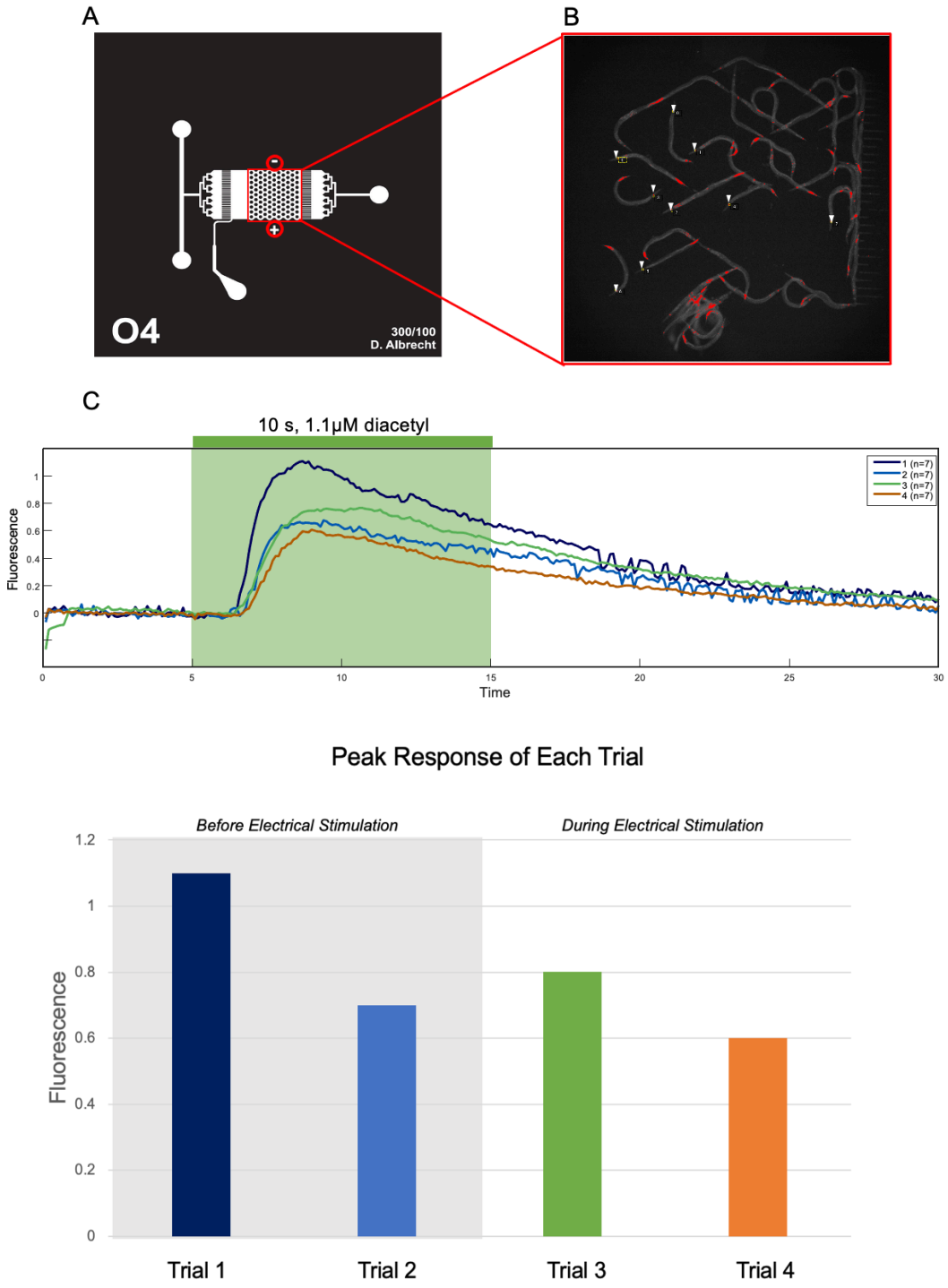


Figure 6.16: Results from the chemical stimulus.

(A) shows the full microfluidic design with the inlet and outlet of electrical stimulation noted. (B) shows the zoomed in images with the head neurons studied indicated with the white arrows. (C) shows the results of the neuro tracker and MATLAB analysis with 1 & 2 being the baseline responses before electrical simulation and 3 & 4 being the responses to chemical stimulus while electrical stimulus of 30V, 20 Hz, and a 50% duty cycle is still present. (D) breaks up the analysis even further into the response before electrical stimulus and response while the electrical stimulus was still present. The peak magnitude for trials 1 and 2 were 1.105 and 0.678 while the peak magnitude for the trials 3 and 4 were 0.767 and 0.611.

Based on the graph and numerical values obtained for the different trials the chemical stimulus before the electrical stimulus had an average peak value of 0.8915 starting at a maximum baseline value with an average at 0.055. The average for the peak of the second trials was lower than before at a value of 0.689 and a maximum baseline value with an average at 0.033. Therefore, not only was the peak value lower but the starting baseline value was lowered as well.

6.6 Two Channel Device COMSOL Analysis

The final design which the team had created was a dual-channel microfluidic device, DBS1. This device was modeled based on an existing dual channel device called the P10 although some modifications were made to the original design. The dual-channel design allows for a control and experimental group to be monitored at the same time enabling the experimentation to go faster as both groups can be monitored, tested and analyzed simultaneously within the same device. Along with being faster this dual channel feature is important for being able to assess the eclectic field during the same experiment where all of the environmental conditions are the same besides the electrical stimulation reducing the error from outside sources. As well, since much of the electric field was seen being lost over the small channels in the R5 and O4 devices these were removed in the DBS1 in hopes of increasing the electric field strength capabilities. To determine whether or not this device would provide sufficient electric field strength the device was modeled in COMSOL and electric field analysis was conducted. The results from this analysis can be seen in **Fig. 6.17** below.

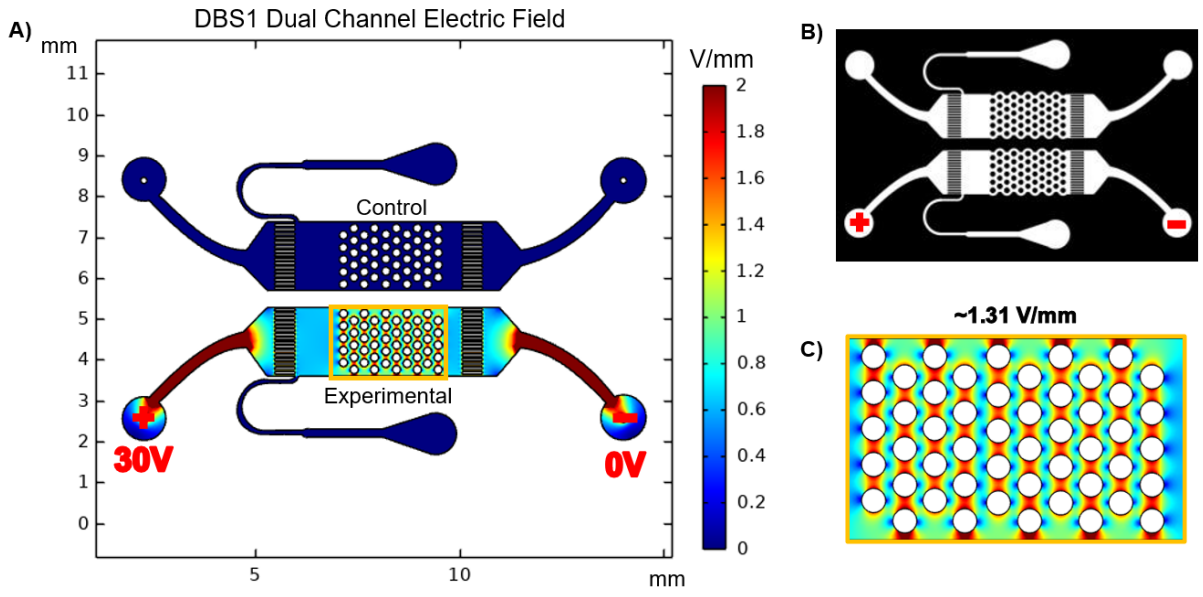


Figure 6.17: COMSOL analysis of the DBS1 device when an electric potential of 30V is applied to the device.

As can be seen on the device (A) the control and experimental channels are indicted and the control is receiving no electrical stimulation. (B) shows the original CAD file of the device with the electrode placement indicated. Finally,

(C) shows a zoomed in image of the arena to better observe how the electric field is displaced. The scaling for this is the same as for the original device and the electric field was averaged across the arena to obtain a value of ~1.31 V/mm.

Based on the COMSOL analysis it is obvious that the DBS1 device will provide sufficient eclectic field strength at a value of 1.31 V/mm which is greatly above the threshold for stimulation at 0.28 V/mm. Therefore, this device proves to be a sufficient and beneficial design for conducting the final experimentation. As well as future experiments on *C. elegans* should be conducted in this device as it is able to house multiple animals, house an experimental and control sample at the same time and provide sufficient electric field strength making for an ideal and successful experimentation system.

One of the other considerations and changes which was made to the design was the increased resistance over the barriers in the device. These barriers create multiple tiny channels to prevent the C. elegans from escaping but these channels also cause some of the electrical current to be lost. In **Fig. 6.18** below the barrier has been zoomed in on. It can be seen that the approximate electric field in the channels between the barriers is 1.49 V/mm. To prevent this electrical current from being lost a device was modeled and created without these barriers are well as without the worm important channel. The COMSOL model for this design can be seen below in **Fig. 6.19** (the worm important channel remained in the design in the COMSOL model since the current was flipped).

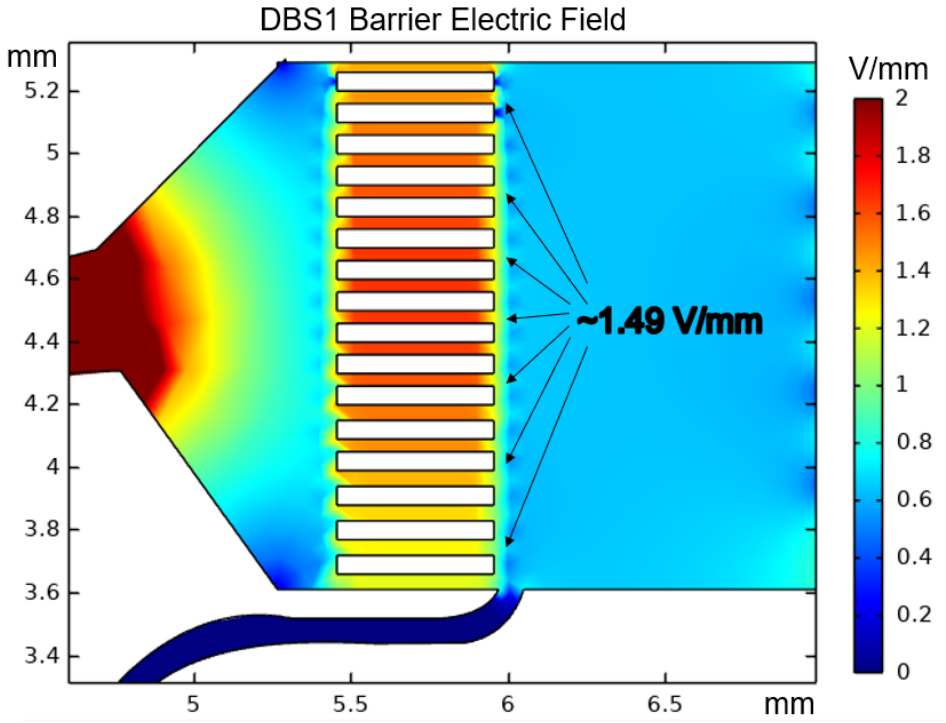


Figure 6.18: The electric field over the barrier channels which is approximately 1.49 V/mm.

As can be seen in the image the electric field lessens by the edges especially on the side with the worm inlet channel.

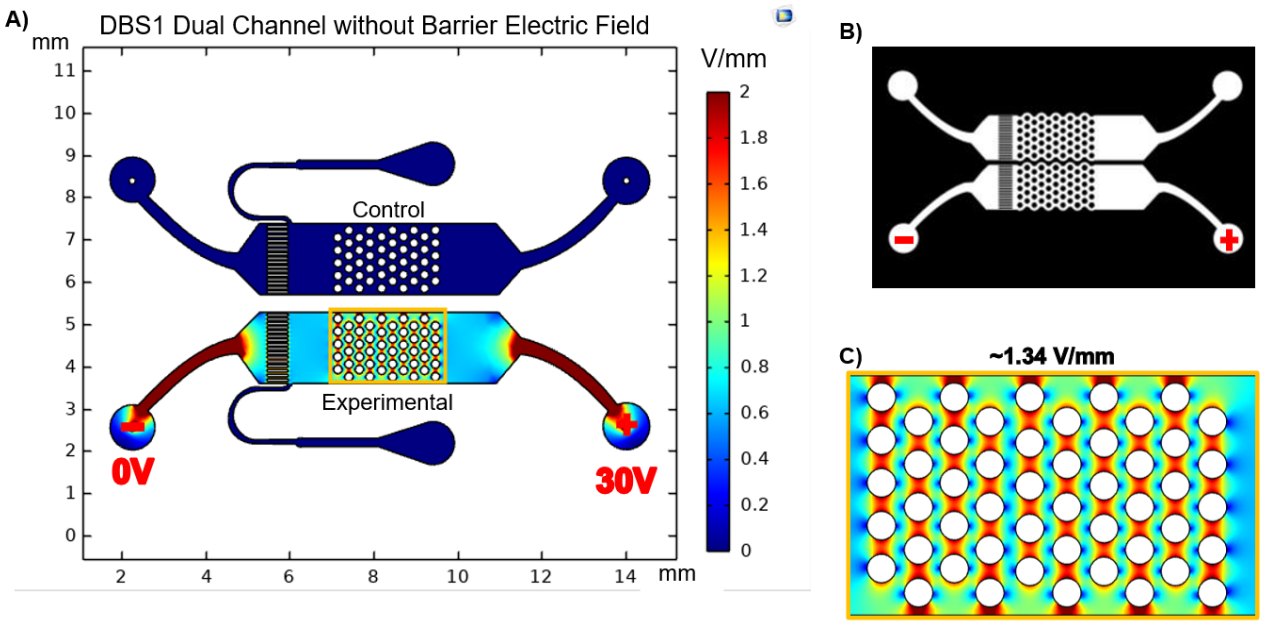


Figure 6.19: The newly modeled *DBS1 device with* the *barrier channel removed on the side of the* positive electrode.

The full COMSOL model is seen in panel A with a voltage of 30V being applied. The worm inlet channel was left on the model for simplicity and since the side of the positive electrode was flipped it did not impact the electric field at the arena in any way. Panel B shows the CAD file for this design. While panel C shows a zoomed in view of the arena with a slightly increase electric field strength of 1.34 V/mm.

From **Fig. 6.19** above it is obvious that the barrier channels do disperse some of the electric field strength, however when the arenas are compared the difference in this field strength is insignificant at only 0.03 V/mm. Therefore, using the DBS1 device with the barriers makes more sense as this makes experimentation easier. The barriers are in place so that the *C. elegans* remain in the arena and are not able to escape are the arena is what is in the field of view of the microscope and therefore captured making it critical that the animals remain in this area. For these reasons it is recommended from the team that future experiments be conducted on the DBS1 device with the barriers.

6.7 Final Design Cost Breakdown

The final design for the working device consisted of a number of parts. Those parts include: the WHDTS signal generator, USB to TTL cable, a serial controllable power supply and alligator clip connectors. The total cost breakdown for building a complete device can be seen in **Table 6.2** below.

Table 6.2: Cost breakdown to create the complete electrically stimulation unit used for this project

Component of Design	**Price**
WHDTS Signal Generator (Amazon.com: WHDTS Signal Generator...)	$12.99
USB to TTL Cable (Industries, n.d.)	$9.95
Serial Controllable Power Supply (*32 Volt DC Programmable Linear...*)	$129.14
Alligator Clips (x4) (*KAIWEETS 10PCS Electrical Alligator ...*)	$0 – theoretically $4.99 borrowed from lab
Total Cost	$152.08

As seen in **Table 6.2** above the total cost for creating a single electrical stimulus unit was just around $152. This is well beneath the budget of under $200 set forth by the team. In conjunction with the stimulus unit a number of other pieces of equipment were used which were available in the lab and therefore not included in the cost of the device. There was an oscilloscope connected to the system to ensure it was applying an electrical stimulus and that the input parameters were also being output. A computer was also used for both monitoring and recording in conjunction with the Zeiss AxioObserver microscope. An optogenetic stimulation unit was also used during experimentation which was connected to the microscope and computer. As well, a microfluidic system was connected to the microfluidic device used. This system supplied the S. basal media, chemical stimulus and was used to inject the C. elegans into the microfluidic device. A number of different microfluidic devices were used for experimentation as well. Majority of these devices were already prefabricated and therefore were not included in the cost analysis. The device which was designed (DBS1) was fabricated as part of a class. This device was created on a wafer using a photolithographic process. This process requires many steps and can cost around $95 to fabricate and get all of the necessary supplies such as tubing to run a microfluidics experiment.

Another consideration when it comes to cost that needs to be made is the cost of supplies used during iterations of the process. For the MQP project there was a budget of $1000 for all supplies and equipment used. The breakdown of the supplies used, and amount of the budget spent can be seen in **Table 6.3** below. Due to experimentation some supplies such as the WHDTS signal generator multiple backups were purchased. This was important as two of these backups were fried and unusable following some of the experiments.

Table 6.3: Cost breakdown of entire MQP project with all theoretical and acquired costs laid out

Device	**Quantity**	**Theoretical Cost**	**Actual Cost**
Signal Generator	4	$51.96	$51.96
USB to TTL Cable	1	$9.95	$9.95
Serial Controllable Power Supply	1	$129.14	$129.14
Alligator Clips	4	$4.99	$0 (Borrowed from Lab)
FTDI Friend (Industries, n.d.)	1	$14.75	$14.75
Arduino Uno Kit + Board (*Amazon.Com: ELEGOO...*)	1	$36.99	$0 (Borrowed and group member already had)
Data Logger (LabQuest Mini) (LabQuest Mini, n.d.)	1	$169.00	$0 (Borrowed from lab)
BB-BTA Cable Connector (*Breadboard Cable...*)	2	$24.00	$0 (Borrowed from lab)
Total	-	$440.78	$205.05

The theoretical cost in the above **Table 6.3** refers to how much the supplies cost when bought while the actual cost is what the team spent from the budget. Many of the supplies were borrowed from labs to save on cost, saving us a lot of our budget by $235.73. Many of the supplies which were borrowed were a part of the verification system including the Arduino uno kit, data logger and BB-BTA cables. These supplies were only used for these experiments to verify that the system was working and will not need to be used again in future experiments unless the device is modified, and the accuracy needs to be re-tested. As well, some of the supplies obtained were never actually used in the project due to shifts in design including the FTDI Friend and the Arduino uno board.

6.8 Final Validation Results

For the final validation tests, the two-channel DBS1 microfluidic design was utilized. These tests utilized optogenetic, or light stimulus, to verify the neural activity. There were three trials run for each of the eight different tests. Since the two-channel device was being utilized, the top channel was the ‘control group’ meaning that this arena did not receive any electrical stimulation while the bottom channel was the ‘experimental group’ which received both optogenetic and electrical stimulation (**Fig. 6.20**).

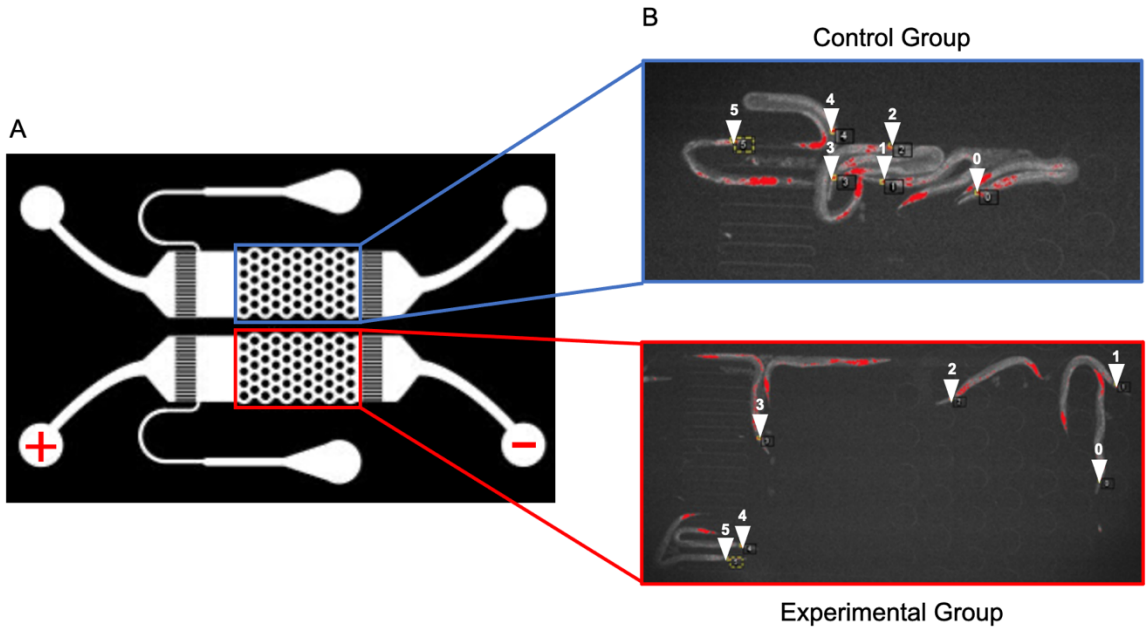


Figure 6.20: The experimental setup of the two-channel design.

(A) shows the full microfluidic design with inlet and outlet of electrical stimulus noted. (B) shows zoomed in images of both the control and experimental arenas with the neuron of interest both numbered and depicted with a white arrow.

6.8.1 Control vs Experimental Groups

The two-channel microfluidic design utilized in these tests allowed for the direct comparison of the electrical stimulation group and an unstimulated control group. Both arenas experienced optogenetic stimulation at the same times which allowed for this comparison.

Six animals in the control group arena were studied to monitor their neural response. Out of these six responses, all of which can be seen in **Appendix E,** only one animal (animal #5) had a unique response which is potentially a result of movement artifacts or interference with another animal. The other 5 animals (0-4) had responses consistent with the expected neural response, further validating the conclusions and observations of these experiments. A representative response from one of the animals (animal 4) in the control group can be seen below in **Fig. 6.21.** The first two optogenetic pulses elicited the highest neural response which then dropped by the third pulse. After the third pulse the neural response and baseline for the neural response remained relatively consistent (**Fig. 6.22**). They were consistent with the expected neural response, further validating the conclusions and observations of these experiments.

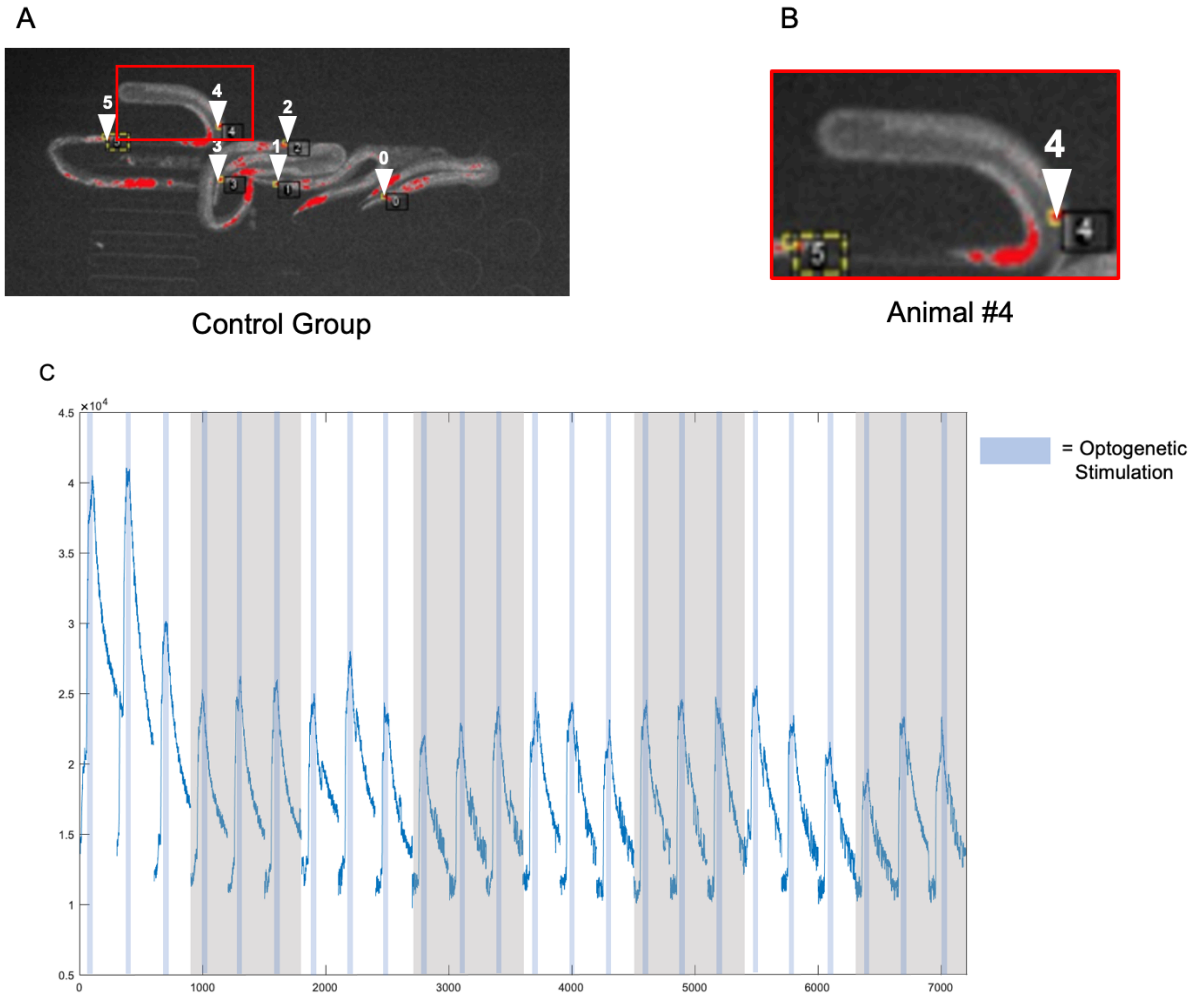


Figure 6.21: Final validation results for animal #4 of the control group.

(A) shows the full arena for the control group with the head neurons labelled. (B) shows the zoomed in image of the animal of interest with the head neuron labelled. (C) shows the final results for the neural response of animal #4 with the blue bars representing when the optogenetic stimulation is on (5 secs) and the grey bars representing the different trials of stimulation.

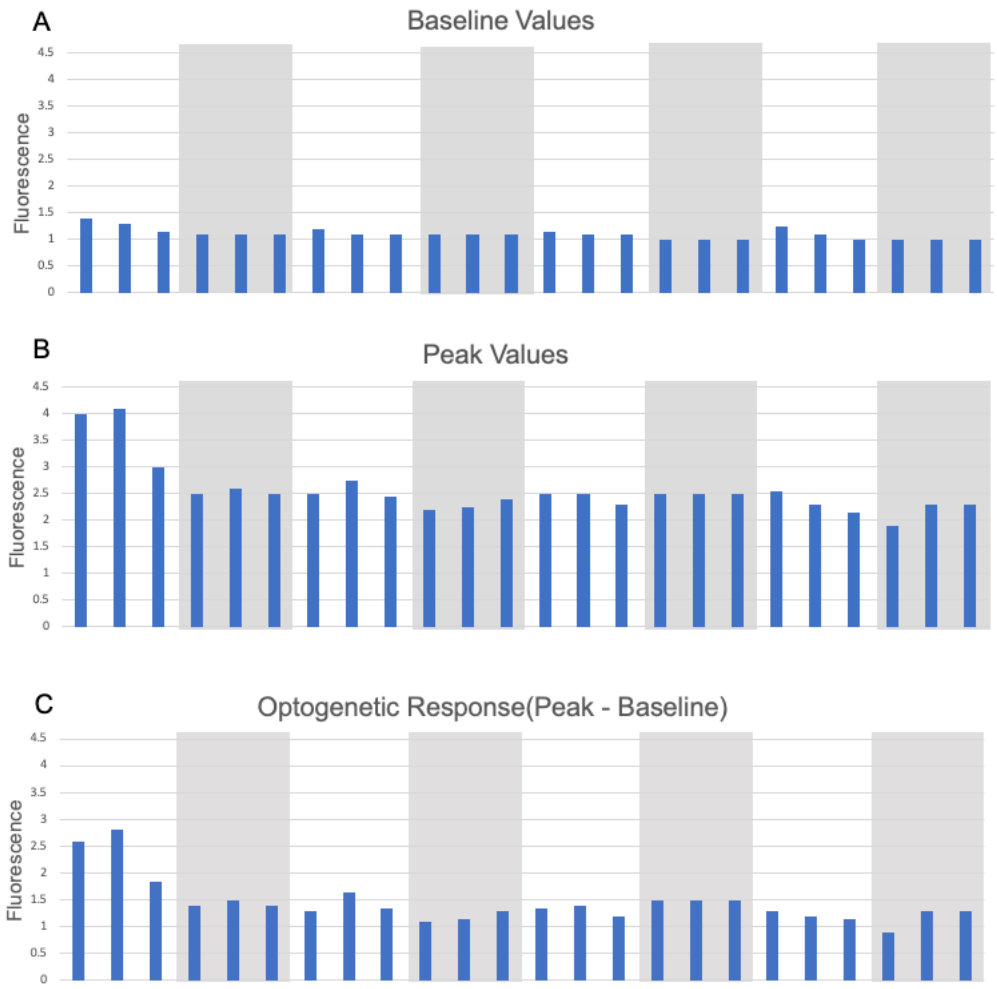


Figure 6.22: Breakdown of Control Group Animal #4 neural response.

Panel A depicts the baseline magnitude values for each of the trials, panel B shows the peak magnitude values for each of the trials and panel C depicts the peak value – the baseline value for each trial which equates to the magnitude of the response to the optogenetic stimulation.

In comparison to the control group, a representative response from one of the animals in the experimental group can be seen in **Figure 6.23** below. This animal received both the electrical and optogenetic stimulation. While the response to the first three optogenetic pulses is consistent with that of the control group, noticeable differences can be seen once electrical stimulation is introduced into the system. The most noticeable differences can be seen in the stimulation at higher voltages such as 20 and 30 volts.

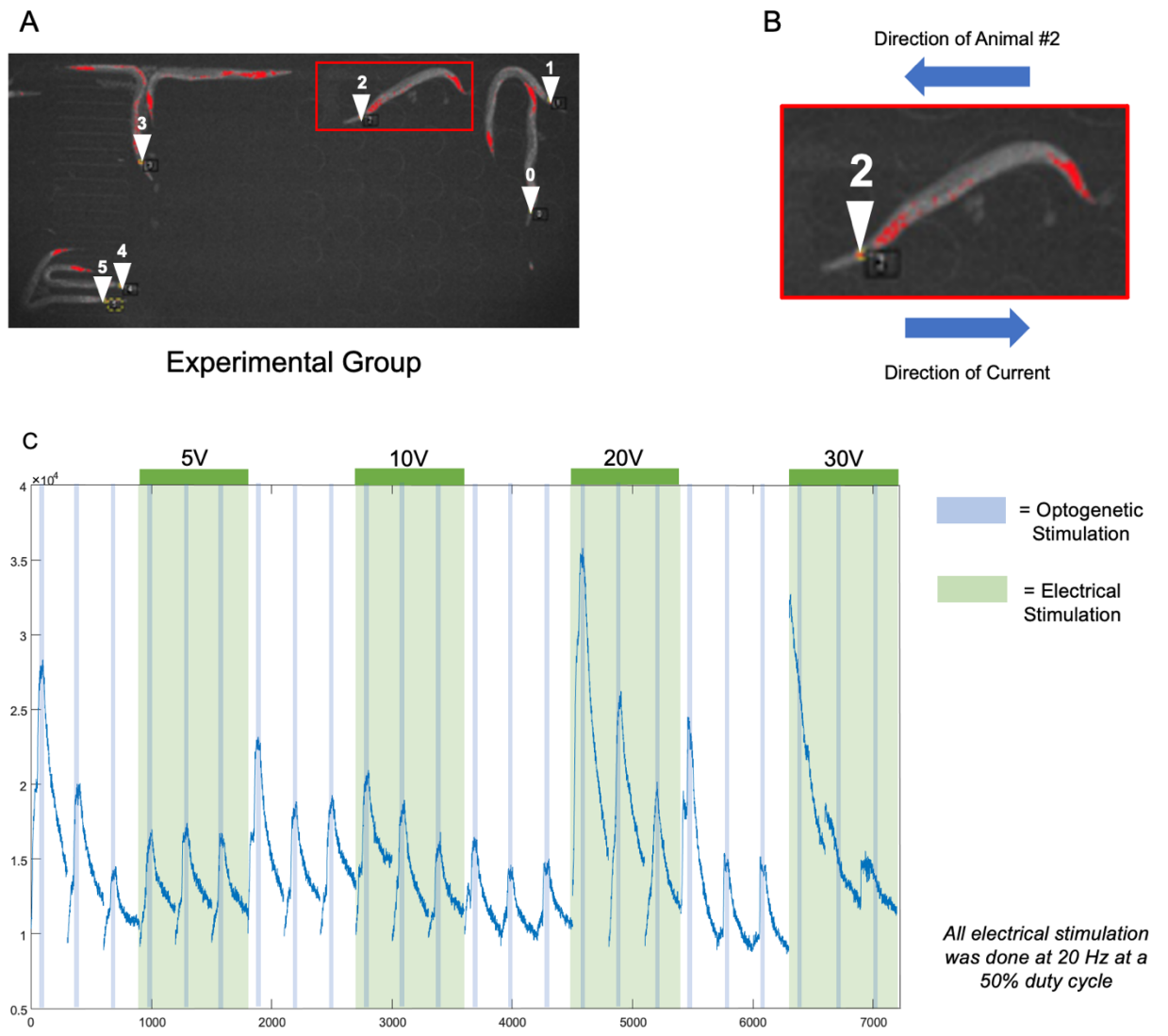


Figure 6.23: Final validation results for animal #2 of the experimental group.

(A) shows the full arena for the experimental group with the head neurons labelled. (B) shows the zoomed in image of the animal of interest with the head neuron labelled. (C) shows the final results for the neural response of animal 2 with the blue bars representing when the optogenetic stimulation is on (5 secs) and the green bars representing when the electrical stimulation is on at the respective voltage, 20 Hz, and a 50% duty cycle.

Unlike the control group, the neural responses observed from the experimental group varied greatly among the different animals observed.

6.8.2 Orientation of the Specimen

In contrast to optogenetic stimulation, electrical stimulation has directionality, meaning that orientation of the specimen plays a role in the resulting neural response. This can be seen when observing the neural response of animal 2 and animal 4 in this arena.

Animal 2 was oriented with the head neuron facing the direction of the current which resulted in the trace seen in **Fig. 6.23** above. For the stimulation done at the lower voltages of 5

and 10 volts, the response remained relatively consistent with what was observed in the control group above. However, at 20 V there was a 5x increase in the excitability of the neuron. At 30 volts the neuron was depolarized and directly stimulated by the electrical stimulation with no response associated with the optogenetic stimulation (**Fig. 6.24)**.

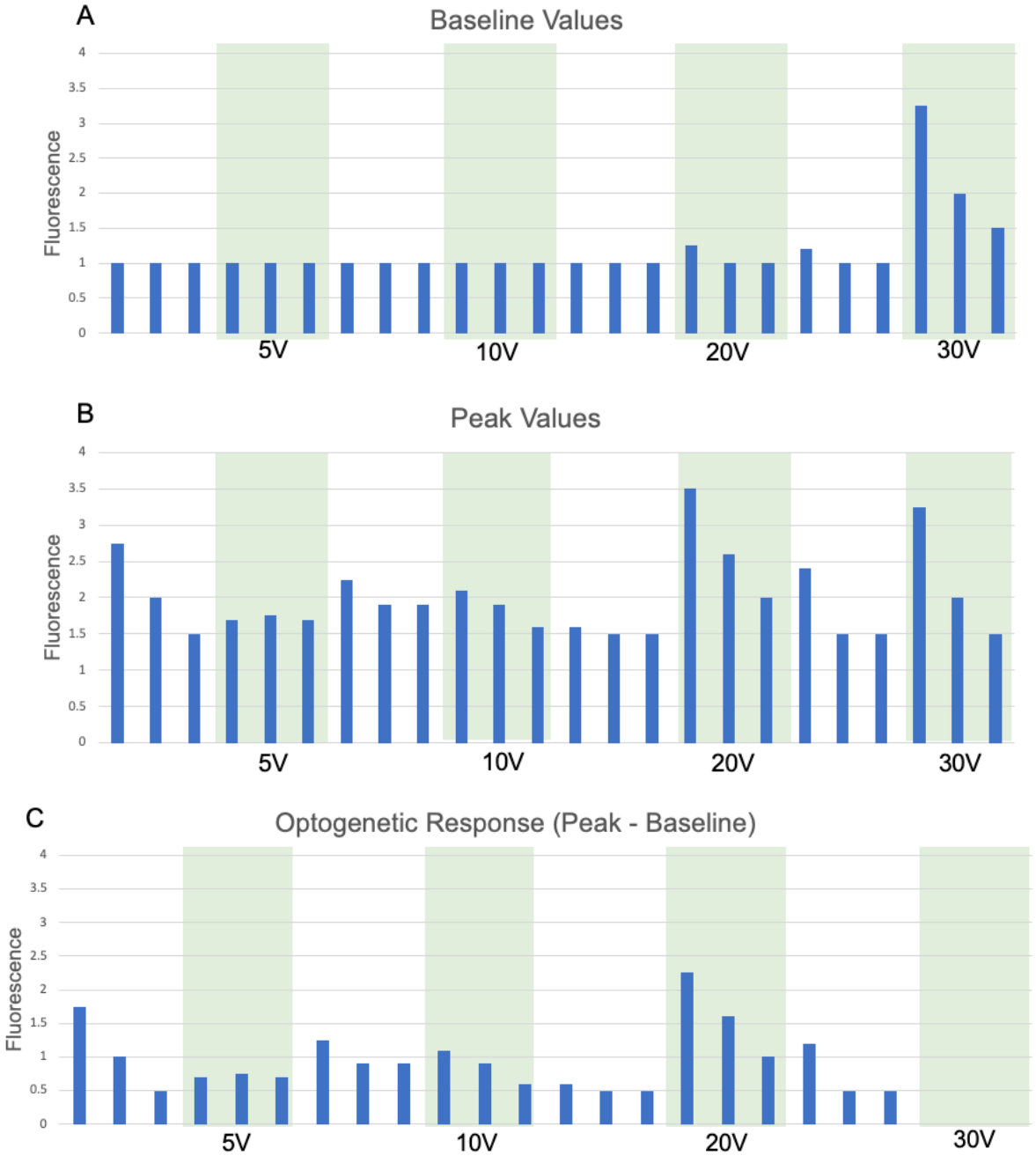


Figure 6.24: Breakdown of Animal #2 neural response.

Panel A depicts the baseline magnitude values for each of the trials, panel B shows the peak magnitude values for each of the trials and panel C depicts the peak value – the baseline value for each trial which equates to the magnitude of the response to the optogenetic stimulation.

Upon statistical analysis of the results from animal 2 it was found that both the optogenetic response at 20 V (p-value = 0.04) and the optogenetic response at 30 V (p-value = 0.03) had statistically significant differences in their response when compared to the previous optogenetic responses at 0 V. The increase in the baseline values at 30V in comparison with the prior baselines at 0V was also found to be statistically significant with a p-value of 0.04.

Animal 4 was oriented with the head neuron facing away from the direction of the current which resulted in a much different neural response as seen in **Fig. 6.25**.

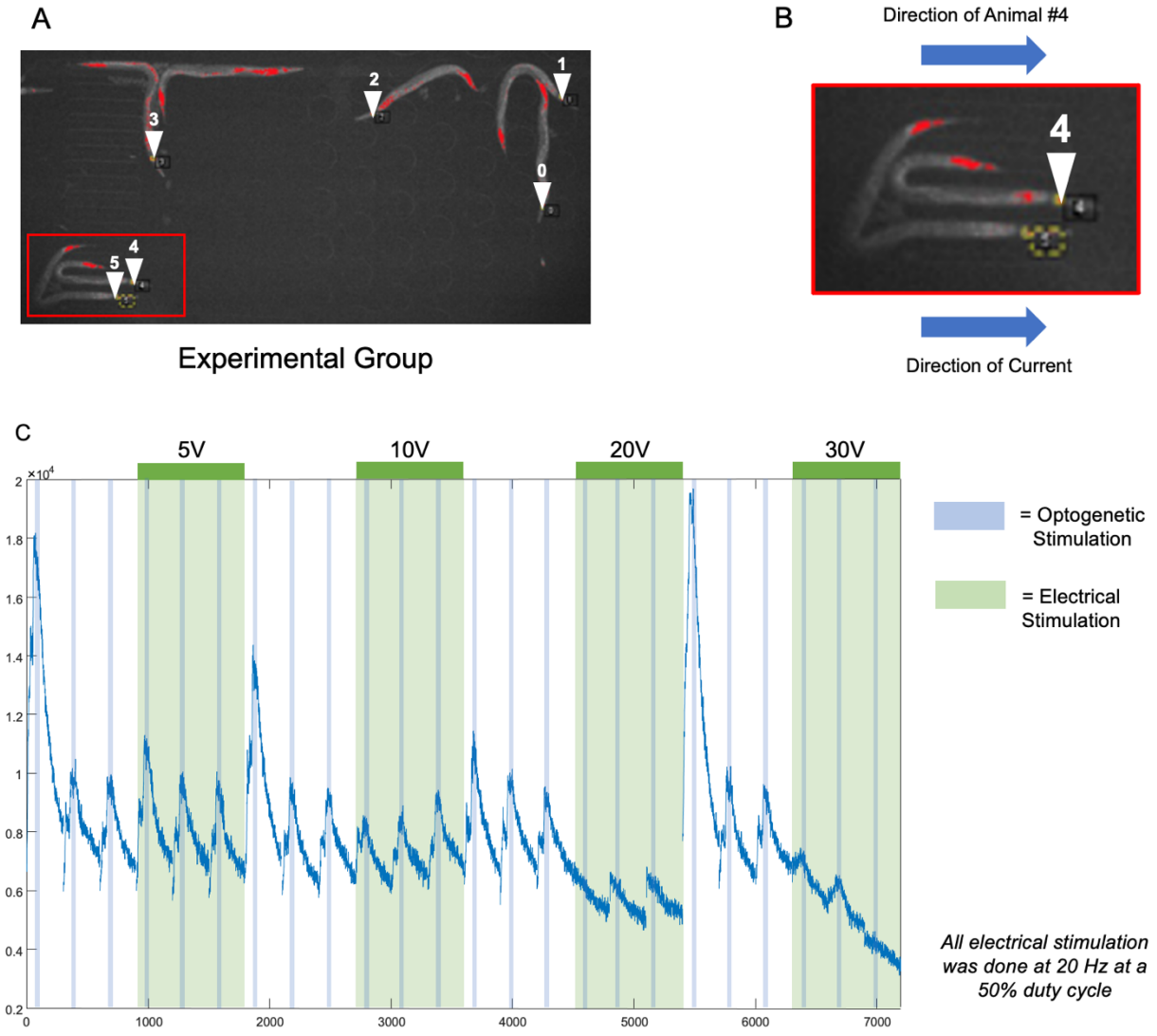


Figure 6.25: Final validation results for animal #4 of the experimental group.

Panel A shows the full arena for the experimental group with the head neurons labelled. (B) shows the zoomed in image of the animal of interest with the head neuron labelled. (C) shows the final results for the neural response of animal #4 with the blue bars representing when the optogenetic stimulation is on (5 secs) and the green bars representing when the electrical stimulation is on at the respective voltage, 20 Hz, and a 50% duty cycle

At the lower voltages the response was still consistent, however, at 20 and 30 volts the neural response was suppressed as a result of hyperpolarization of the neurons. There was also

rebound excitability of the neurons in the optogenetic stimulation after the suppression at 20 volts (**Fig. 6.26)**.

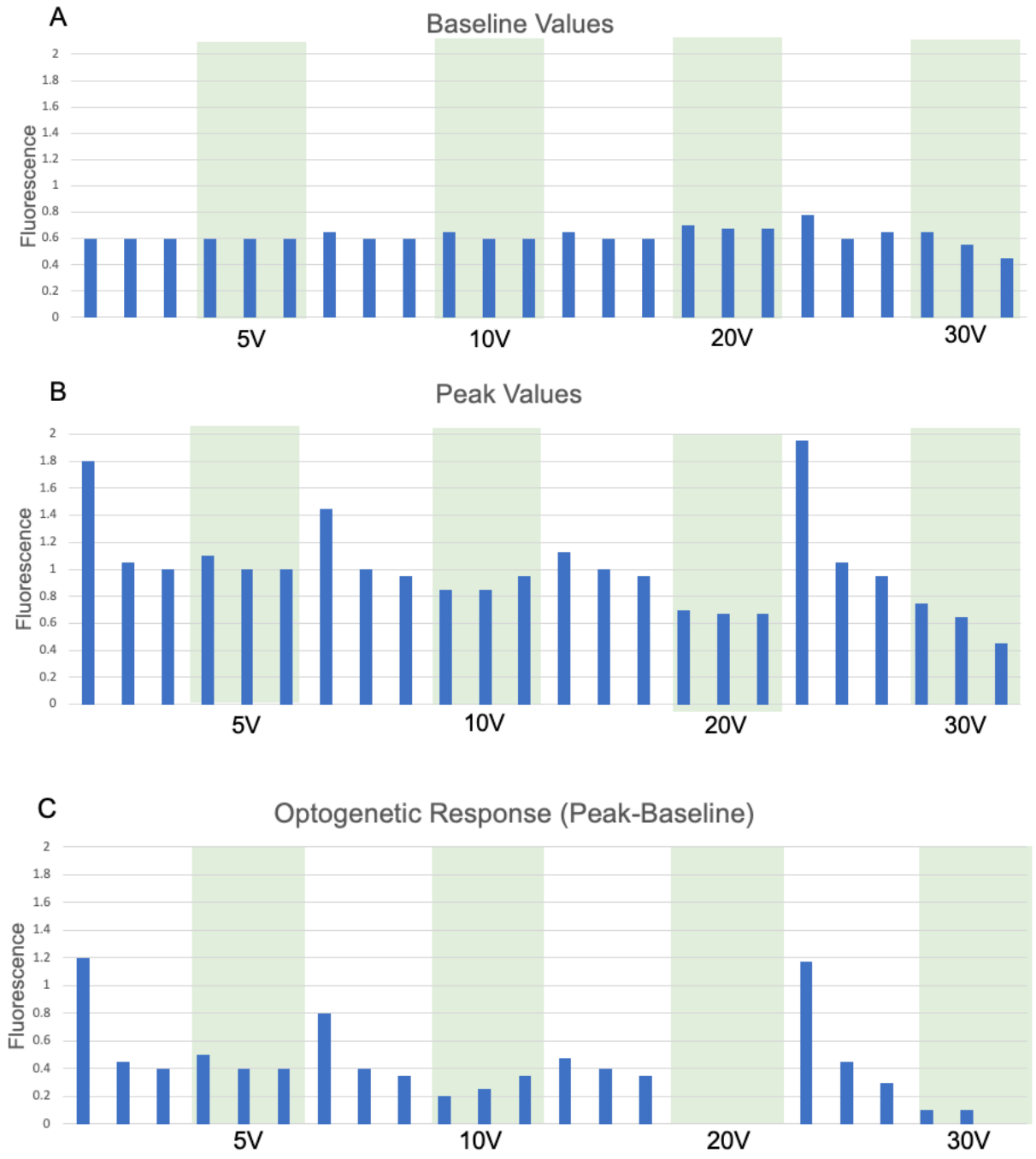


Figure 6.26: Breakdown of Animal #4 response

Panel A depicts the baseline magnitude values for each of the trials, panel B shows the peak magnitude values for each of the trials and panel C depicts the peak value – the baseline value for each trial which equates to the magnitude of the response to the optogenetic stimulation.

6.9 Final Verification Results

Two voltage scaling experiments were run in order to verify the system works as expected. The parameters of these two tests were: 20Hz at 50% DC with 5 second pulse trains. A third experiment was run to verify the magnitude of the voltage. The magnitude could not be verified in first two experiments as shown in **Fig. 6.27** due to an unintentional loading which hampered the results.

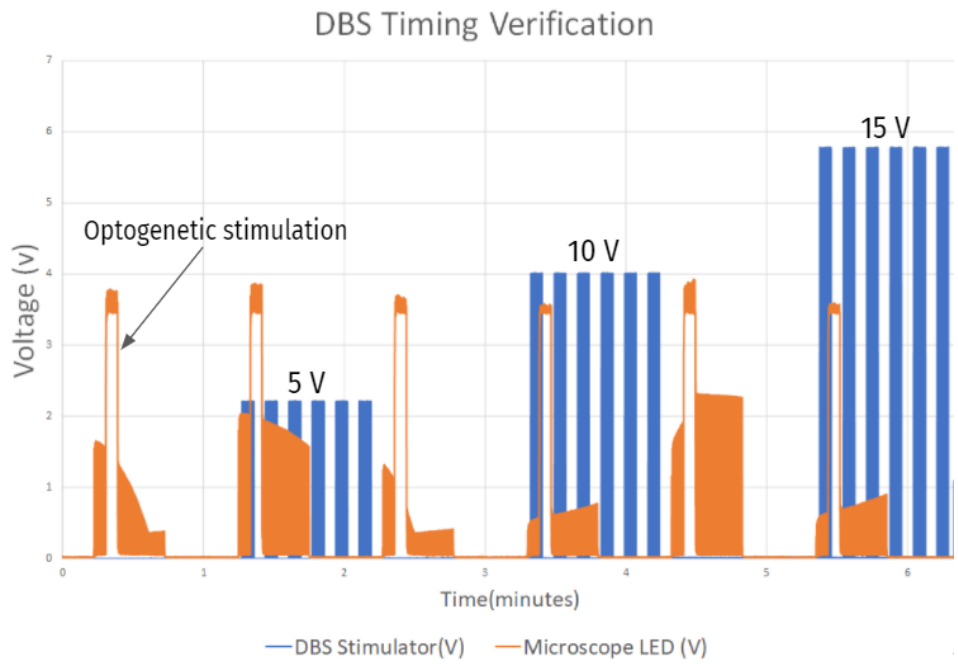


Figure 6.27: The above figure shows the timing for the various stimulation applied.

After running the experiments, the average pulse train, LED timing, and pulse width were obtained using a MATLAB program. The timing of the electrical stimulation was calculated by figuring out when the stimulus reached a certain threshold then recording whenever the signal crossed that threshold. By doing this, the timing of electrical stimulus could be shown as seen in **Fig. 6.28** below.

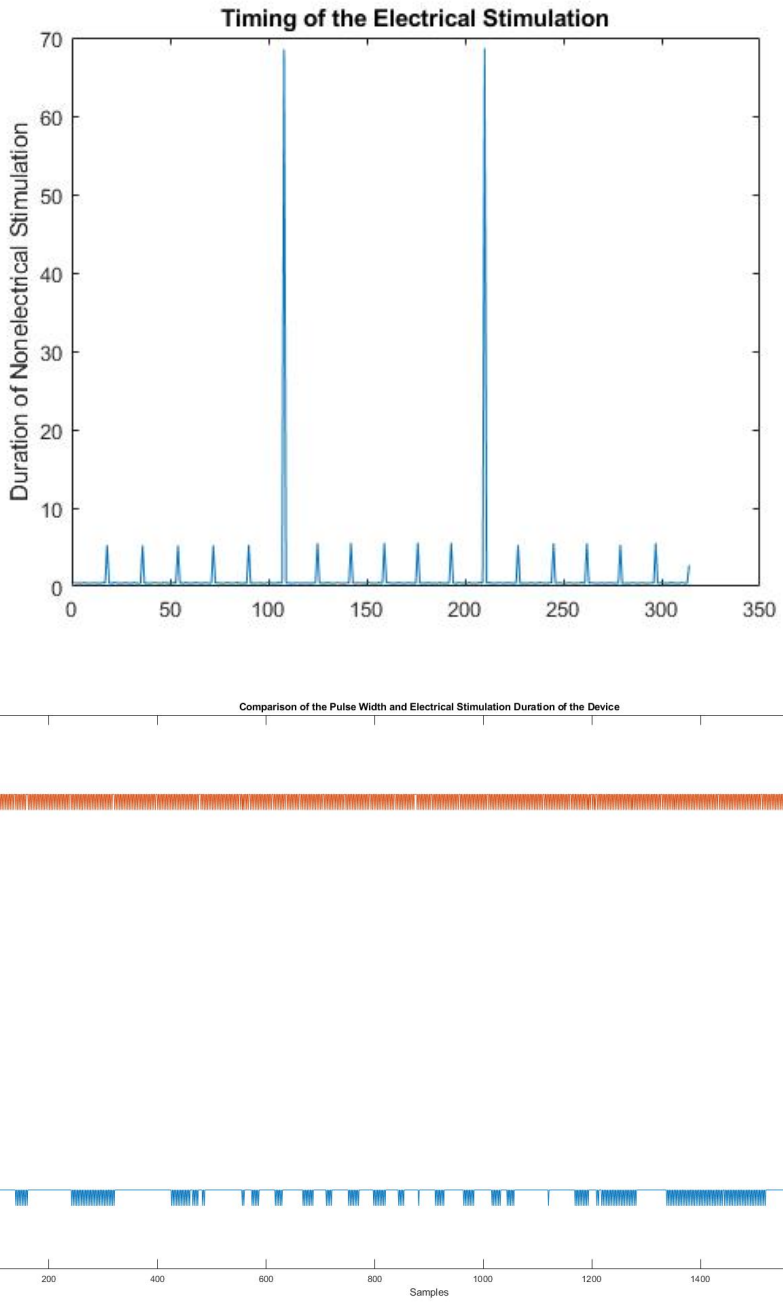


Figure 6.28: Timing results from the verification experiment.

The first graph shows the timing of the electrical stimulation for the entire experiment where the spikes represent when the system was in an "off" state. The smallest spikes (as can be seen more clearly on the graph on the right) represent the pulse widths, the medium sized spikes represent the pulse trains, and the largest spikes show the gap in between stimulus trials. The second graph compares the time the system was on to the pulse widths of the stimulation allowing for duty cycle to be shown visually.

Table 6.4: Verification results from the tests.
Each parameter such as pulse width, electrical stimulation duration, pulse train duration, voltage, and LED timing were calculated using MATLAB.

	Mean	Standard Dev.	Standard Error (mean)	95% Confidence Interval	Number of Samples
Pulse Width	0.0497	0.00047	0.000012	(0.0496, 0.0497)	1670
Electrical Stimulation Duration	0.0249	0.00035	0.0000086	(0.0248, 0.0249)	1683
Pulse Train Duration(sec)	5.25	0.031	0.0052	(5.244, 5.265)	34
Voltage(V)	9.8806	0.0091	0.00041	(9.8798, 9.8814)	576
LED timing	0.0949	0.0053	0.00038	(0.0942, 0.0957)	196

By filtering the data for the approximate magnitudes of the desired parameter, the average electrical pulse spacing, pulse width, pulse train, and LED pulse width could be calculated. From this data, as shown in **Table 6.4** the average electrical pulse spacing was found to be 0.0497 seconds ± 0.00047 seconds, the average pulse width duration was 0.0249 seconds ± 0.00035, the average pulse train was found to be 5.25 seconds ±0.031 seconds, and the average LED time was found to be 0.0949 seconds ± 0.0091 seconds. Likewise in the magnitude test, with 10V as the target voltage, 9. 8806 V ± 0.0091V was found to be the average voltage.

The average output voltage of the system was below the 10 V target, but the discrepancy can be explained due to internal resistances in the device and data logger. The average pulse train duration was 5.25 seconds which is higher than the 5 second target. The variability of the pulse train length may be due to system communication times as the command is sent from the computer and processed at the signal generator. Ideally, the pulse train duration would be more accurate, but the computation speed of the system may be variable, so the discrepancy would be unavoidable in this current system.

$$Duty\ Cycle = pulse\ width\ /\ T\ *\ 100\%$$

Equation 1

By utilizing **Eqn. 1**, where T = 1/frequency, the expected pulse width of the system at 20 Hz and 50% duty cycle is 0.025 seconds. As shown in **Table 6.4**, the system demonstrates the ability to consistently achieve this value. Certain outliers that were excluded because the average would have been artificially decreased due to stimulation abruptly ended due to the pulse train ending. For this reason, the conclusion can be made that the system outputs the correct frequency. With this noted, by using **Eqn.** 1, the experimental duty cycle of the system can be calculated as 50.1% which would give the system an accuracy of 0.1%

Likewise, the LED timing also excluded these outliers and can also be confirmed as the LED would also be abruptly turned off as the trial ended leading to a discrepancy in the target of

0.100 seconds. Overall, the system has shown to be able to function properly by timing the experiment as expected at the magnitudes expected.

6.10 Broader Impacts

Looking at this project on the small scale it appears to have no impact or relevance to society. However, through improvements and future research using the design created during this project a broader impact can be made. Allowing for the potential to have a large impact on a variety of different parts of society which will be discussed in the following sections including economic, environmental, societal, political, ethical, and health concerns. As well, the manufacturability and sustainability of the device will be discussed.

6.10.1 Economic Impact

Assuming the design outlined in this project continues to improve, it has the potential to have a large impact on the economy. Currently, DBS surgeries as well as the technology are expensive and can be unrealistic options for many of the patients suitable for this treatment. By creating this device there is now the possibility to study how DBS works in a relatively inexpensive model. Using results gained from model testing can help not only improve the current technology but open up opportunities to make the market more competitive and thus make treatments more affordable to patients.

6.10.2 Environmental Impact

This model system opens up the option for new materials to be investigated for use in DBS devices. Through this investigation, more eco-friendly materials can be looked into as well as materials that have a longer lifespan, creating less waste from potentially needing to replace parts of the system.

6.10.3 Societal Influence

As previously discussed in section 6.10.1, improvements on this design could allow for DBS treatments to be more accessible to the average person. Many people with neurological disorders could have the option to receive life-changing surgeries to permanently treat the disorder they suffer. It would also give the average person more options for treatment that are not just medication. Medication can have numerous potential side effects and could poorly interact with other medications or conditions.

6.10.4 Political Ramifications

Currently, DBS treatments are only an option to those in first-world countries due to both the cost and the complications of neural surgery. By making this technology more accessible to those in countries where this surgery is already accessible, will in turn open up opportunities for other countries.

6.10.5 Ethical Concerns

This design has the potential to improve the quality of life of many patients suffering various neurological disorders. By improving the technology and in turn, making it more accessible, a greater number of people will have the option to receive this treatment and potentially lead a more normal life. Many of the neurological disorders that DBS can be used to treat drastically impact the patient's ability to complete basic tasks. Introducing this treatment as an option to more people can increase their chances of being self-sufficient.

6.10.6 Health & Safety Concerns

This design has the potential to improve the current technology on the market. While the design cannot directly do this, it can be used to learn more about the mechanism, ideal parameters, and physiological effects of DBS treatments. By learning more about these aspects, DBS technology utilized in humans can be improved upon to both increase effectivity and understand any adverse effects the technology may have on the patient. This deeper understanding could allow for more people to receive this treatment and lessen the effects of their neurological disorder.

6.10.7 Manufacturability

The design outlined in this paper as well as the tests run could be recreated. All parts of the electrical stimulus device were purchased from consumer websites and were controlled using open-source software that could be downloaded onto any computer. The microscopic setup utilized for the imaging of the model system would be harder to come by, but realistically the average consumer would not be manufacturing this device. The microfluidic slides as well as the model system of *C. elegans* utilized in this design are also harder to come by but could still be acquired by one trying to recreate this design.

6.10.8 Sustainability

The production of this design should not have a negative impact on the environment; however, it also does not work to improve the state of the environment. Since the design is for electrical stimulus, it does have the potential to be powered by renewable energy rather than traditional electricity as a future improvement.

7 Discussion

The novelty of this project required creativity to design a functional device that met all of the necessary requirements previously discussed in Chapters 3 and 4. While the goal of this project was to design our own device, this process required comparison against existing devices with known functionality. As such, we utilized the Grass Instruments electrical stimulator to complete baseline testing and assess how the model system would react when varied stimulus was applied to it. This baseline testing also allowed us to determine the necessary voltage to stimulate the neuron of the *C. elegans* as this value was previously unknown. Through this testing we were able to determine that 40 V was the necessary voltage within the specific microfluidics design being utilized.

The COMSOL analysis provided a number of important calculations as well as information on the electric field of each device. As previously stated, the required electric field strength found from literature for *C. elegans* neural stimulation was 2-4 V/cm which is 0.2-0.4 V/mm [68]. The value obtained from the pilot experiments coupled with the COMSOL analysis determined the minimum field strength to be 0.28 V/mm which is consistent with the previously conducted research. This minimum viable eclectic field strength corresponded to 40V of electric potential which was above the threshold of the device driving the redesign of the microfluidic device. Some of the drawbacks and considerations that need to be made when taking into consideration these values however are the actual resistivity of the S. basal solution, the resistivity of the *C. elegans* themselves, assumptions made about being a 2D system and other material properties based on literature rather than obtained from the direction material samples being used. To combat these issues a number of steps can be made by future research such as testing the material properties and S. basal properties directly, modeling in 3D and considering the resistivity of *C. elegans* in the model when conducting the analysis. Even with these considerations though, the COMSOL system used was deemed appropriate and the adjustments which would occur due to these factors were considered to be negligible.

One of the biggest benefits of using the COMSOL model was the ability to rapidly manipulate the design and get assumptions on the electric field strength. One of the ways this can be seen is through the use of COMSOL models' multiple different orientations of electrode pin placement was able to be rapidly modeled. By modeling multiple different orientations in such a rapid manner, the electric field under different circumstances was modeled and the optimum redesign option was chosen. Through the analysis the vertical electrode pin orientation was deemed to be superior providing the maximum electric field at a value of 9.05 V/mm which is more than 30x the minimum viable strength necessary. Having such a high capability for electric fields allows for more options for voltage manipulation as even at 5V this value is still above the minimum viable threshold at 1.39 V/mm. Therefore, the full range of voltages can be manipulated and used for experimentation. One of the limitations of this device however was the inability to house an experimental and control sample at the same time requiring two separate experiments to be run.

To combat the issue of running two separate experiments a new microfluidic device was designed which had dual-channel capabilities. These dual-channel capabilities would allow a control sample and experimental sample to be run at the same time and therefore receive the same optogenetic stimulation. This device utilized the previous COMSOL models where it was observed that much of the electric field strength was being lost over the small channels. To combat this issue the channels on the new device called the DBS1 were much wider than the other exciting devices. This proved to be sufficient with the electrodes being placed at the inlet and outlet channels and the electric field strength was still above the minimum viable value at 1.71 V/mm. Although this device had a high enough electric field strength some of the future improvements and work which can be conducted include the use of microfabricated arrays. Interdigitated microfabricated electrodes (IME) would allow for a consistent or more controlled electric field in the lower arena of DBS1. In addition, with the added revelation of orientation of the worms being a major factor, IME would allow for different orientations to be tested based on the position of the *C. elegans* in relation to the positive and negative electrodes. By tracking which electrodes are positive and negative, different animals can experience difference polarities within the same experiment. As shown in **Fig. 7.1**, the IME arrays could potentially align itself with the DBS1 device to allow for stimulation of the bottom arena whilst allowing the top arena to remain as a control.

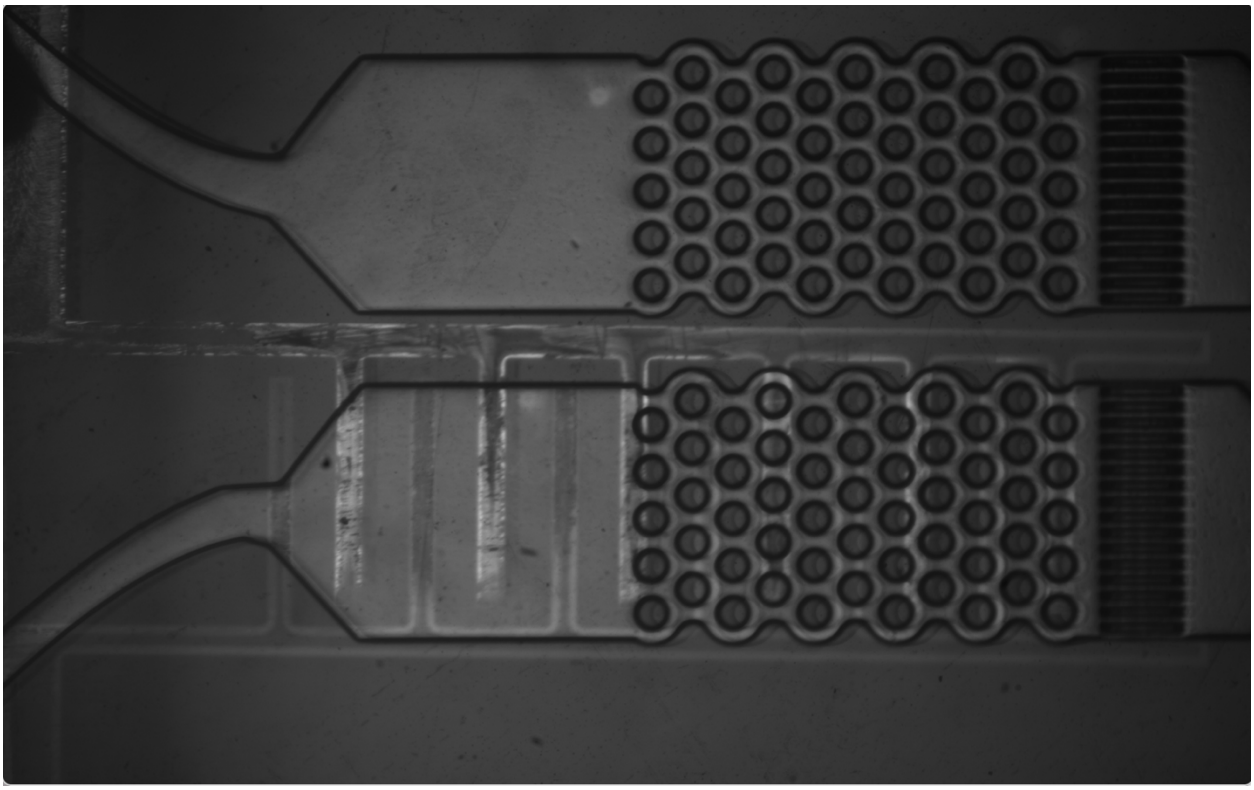


Figure 7.1: A picture showing how the IDE1 device aligns with the DBS1 microfluidic device

From this final experimentation in the DBS1 a number of things were learned including the importance of orientation. It was seen that depending on which way the animal was oriented the neural activity could be increased or decreased. This ability to directly manipulate the neural

response was an important discovery from these experiments. To better support these claims on orientation and neural response a number of other trials should be run where the results from animal facing toward and away from the direction of the electrical current is analyzed. From these experiments statistically analysis can be conducted to back up the claims and ensure that the results which were found were not due to error or outliers. An interesting experiment which could also be run to test this theory would be to conduct and experiment and using the same animals switch the electrode position and therefore the direction of the current and then run a second experiment to observe the differences in each animal when the current direction was changed. By modifying the existing system, it could be possible to put in place a relay type system where the electrodes are able to be switched from negative to positive through a code or automated system rather than manually. If this was implemented that would also allow for biphasic pulses to be applied. This may require the use of a different electrical stimulation system as well depending how the electrode switching property is implemented.

The signal generator chosen to be utilized was able to meet the needs of the team in that it was inexpensive and could be automated. For future research however there may be drawbacks to this system. The upper limit of the device is 30V (although this has not been tested) and the lower limit is 3.3 V. As well, the device is unable to supply short wave pulses if the frequency is too low. The device works on duty cycle which is expressed as a percentage of the pulse duration, duty cycle = pulse width/time period. Where the time period is determined by frequency where time period =1/frequency. Therefore, the duty cycle and resulting pulse width is dependent on the frequency. For example, at a low frequency such as 20 Hz the minimum obtainable pulse width would be 0.5 ms. However, this is obtained using a duty cycle of 0.01% which is not a practical value to be used during experimentation. Since the experimentation done during this project was not using such low pulse width the signal generator was suitable for the experiments run. Along with this, getting smaller pulse width may be an issue with the automation system being used as this has innate time delays which need to be accounted for.

The automation of this system provided the greatest number of limitations as it needed to fit into an existing system, run different parameters with high accuracy, and maintain precise timing. The automation system also needed to control the electrical stimulus, optogenetic stimulus, and imaging system. Despite these limitations, the system was proven to be able to set all of the desired parameters with accuracy and precision whilst controlling the imaging software as well. In order to improve the system, isolating the communication time between the signal generator and computer is necessary to ensure proper timing. Another flaw of the system is that the stimulation length parameter adds onto the intertrial duration instead of automatically starting after control trial. However, the system itself can alleviate this issue by setting that parameter to 0 causing the experiment to start directly after the allotted trial length has passed. As well, once this system was finalized there were a number of wires required for it to properly function. These wires were unorganized and could easily become unhooked causing issues to the system. One of the recommendations for the future researchers is to create a box or housing unit for the device so that this issue with the wiring is no longer a problem.

8 Conclusions and Recommendations

After analyzing all of the data that was obtained from our tests including our final design system, conclusions can be drawn on the effectiveness and reflection of our goals. As a final summary, an integrated, automated system was successfully designed that could electrically stimulate *C. elegans* and test a range of DBS parameters rapidly. It should be emphasized that our device demonstrated a clear increase and decrease in neural excitability in living C elegans, which is the first demonstration of its kind in this model organism. Specifically, the final validation tests using the two-channel DBS1 microfluidic design and optogenetic stimulus verified the changes in neural activity. Through analysis of the responses, it was hypothesized that the orientation of the specimen in the microfluidics design ultimately does matter in regard to the results, to prove this as accurate however further testing must be made to have experiments to compare. Furthermore, the local field threshold for activation was determined in the DBS1 device to be above the threshold for all voltages, allowing the voltage along with the other stimulus parameters to be manipulated. To rapidly test these different parameters the system was designed to be run on its own not only making it faster but also more efficient. Through the production of this device, it is evident that the team was successful in meeting the goals set out in the client statement.

To continue the research and expand upon the goals met by the team a number of recommendations have been made for future research. One of these recommendations is to conduct further tests on genetic mutants which express PD behavioral symptoms. These models can be used to determine whether DBS parameters can restore normal motor behavior. In addition, experimentation which manipulates certain pathways in the neural circuit can be conducted. Through these experiments the role that these pathways play in regard to the excitability of neurons can be determined and correlated to the effects on DBS. These types of experiments will help to close the knowledge gap that exists for the mechanism of DBS allowing for increased understanding of the physiological effects of the treatment. It is hoped that by implementing the knowledge acquired from this experimentation and from future projects that information from the model system can be relayed into human models. The ability to correlate this information to the human system will allow for improvement of DBS through optimization of parameters leading to increased success of DBS treatments.

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Appendices

Appendix A: Pairwise Analysis

Design Requirements	Ability to provide voltage	Stimulate the neurons	Ability to control signal	Cost	Aestetically Pleasing	User-Friendly	Customizable	Size	Total
Ability to provide voltage		0.5	0.5	1	1	1	1	1	**6**
Stimulate the neurons	0.5		0.5	1	1	1	1	1	**6**
Ability to control signal	0.5	0.5		1	1	1	1	1	**6**
Cost	0	0	0		1	0	0	0	**1**
Aestetically Pleasing	0	0	0	0		0	0	0	**0**
User-Friendly	0	0	0	1	1		0.5	1	**3.5**
Customizable	0	0	0	1	1	0.5		1	**3.5**
Size	0	0	0	1	1	0	0		**2**

Appendix B: Automation Code/ System/ Manual

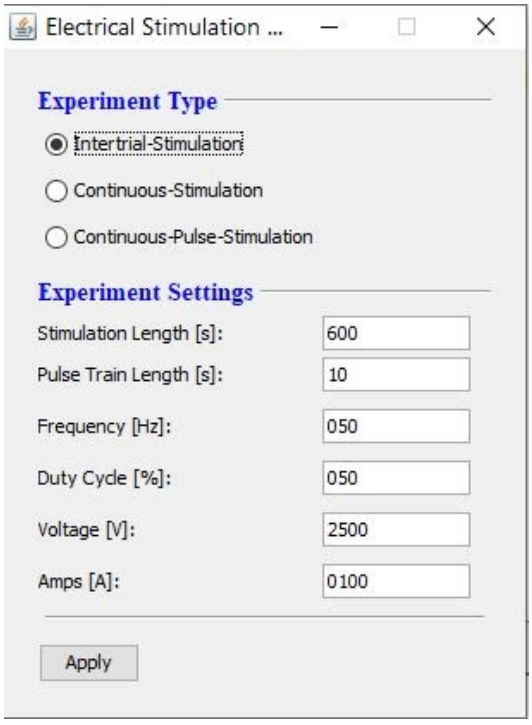
Product Name: DBS Square Wave Pulse Stimulator
Intended Use: The intended use of this device is to deliver controlled electrical stimulation to biological systems (such as *C. elegans*) with the intent of stimulating neurons. The stimulation is aimed to either increase or decrease neural activity based on the settings used.
Features: The system is capable of handling 0-32 volts and 0-5 amps. The stimulation can have square wave parameters such as 1Hz-150kHz of square waves and 100%-1% duty cycle. The system is designed to allow for pulse train of stimulation as well which can be specified during the user interface. Lastly, the system is controlled completely via serial control, but also has physical knobs/buttons that can allow for manual control.

Description of how to use the device:

In order to use the device, the user interface must be loaded into micromanager 1.4 (any other version of micromanager may result in an error). Once all of the files are added, the hardware must be properly configured in the hardware configuration wizard before the program can be run. By finding the correct ports the devices are connected to in the device manager, the devices can be found in the hardware manager using the "freeserialport" option. Once done, label the devices as necessary.

Once you have properly configured the external devices, the file "DBS_RUNEXP" can be executed. The program contains references to the GUI, experimental setup, and experiment programs, and the program will automatically run the experiment.

The device can be controlled via serial commands, and for the ease of the user, a simple GUI was created (As shown below).



The above image shows the electrical stimulation GUI for the device. The experiment type, stimulation length, pulse train length, frequency, duty cycle, voltage, and amperage can all be designated within this GUI.

The experiment type decides what type of stimulation will be given. Intertrial stimulation refers to pulsed stimulation that is given only during the break between experiments. (i.e two experiments are run, electrical stimulus occurs for a period then is stopped, then two experiments are run.).

Continuous stimulation refers to stimulation that begins at the end of the control experiment(s) and continues until the end of the rest of the experiments. As a note, the stimulation in this mode is NOT PULSED and will only be frequencies stimulation.

Lastly, Continuous Pulse Stimulation would function similarly to continuous stimulation with the exception of being pulsed.

While inputting the stimulation length and pulse length, input the time in seconds as an integer for the desired. The pulse train length must be smaller than or equal to the experiment to ensure no errors.

For the frequency input, input the desired frequency as shown in the chart below.

Mode	Format	Examples
1-99	Add a 0 before the integer	050 = 50 Hz 099 = 99Hz
100-999	Enter the integer normally	105 = 105 Hz 865 = 865 Hz
1000-9,990	Add a period after the first and before the second integer	1.23 = 1,230 Hz 5.21 = 5,210Hz
10k-99.9k	Add a period after the second and before the third integer	10.5 = 10,500 Hz 45.1 = 45,100 Hz
100k-150k	Add a period between each integer	1.2.3 = 123,000 1.5.0 = 150,000

The frequency MUST be between 1 Hz and 150kHz or an error will occur.

For the duty cycle input, input the desired duty cycle as shown in the chart below.

Mode	Format	Examples
1-10	Add two 0 before the integer	001 = 1% 005 = 5%
11-99	Add a single 0 before the integer	011= 11% 045= 45%
100	No 0 necessary	100 = 100%

The duty cycle MUST be an integer between 1 and 100 or an error will occur.

For the voltage input, input the desired voltage as shown in the chart below.

Mode	Format	Examples
1-9	Add a single 0 before the number	0753 = 7.53V 0510 = 5.10 V
10-32	Type in the number without a decimal	2500= 25.00V 3200= 32.00V

The range of the voltage is from 1V to 32 V for this system. Avoid using any periods or exceeding these bounds as this will result in an error.

For the amperage input, input the desired amperage as shown in the chart below.

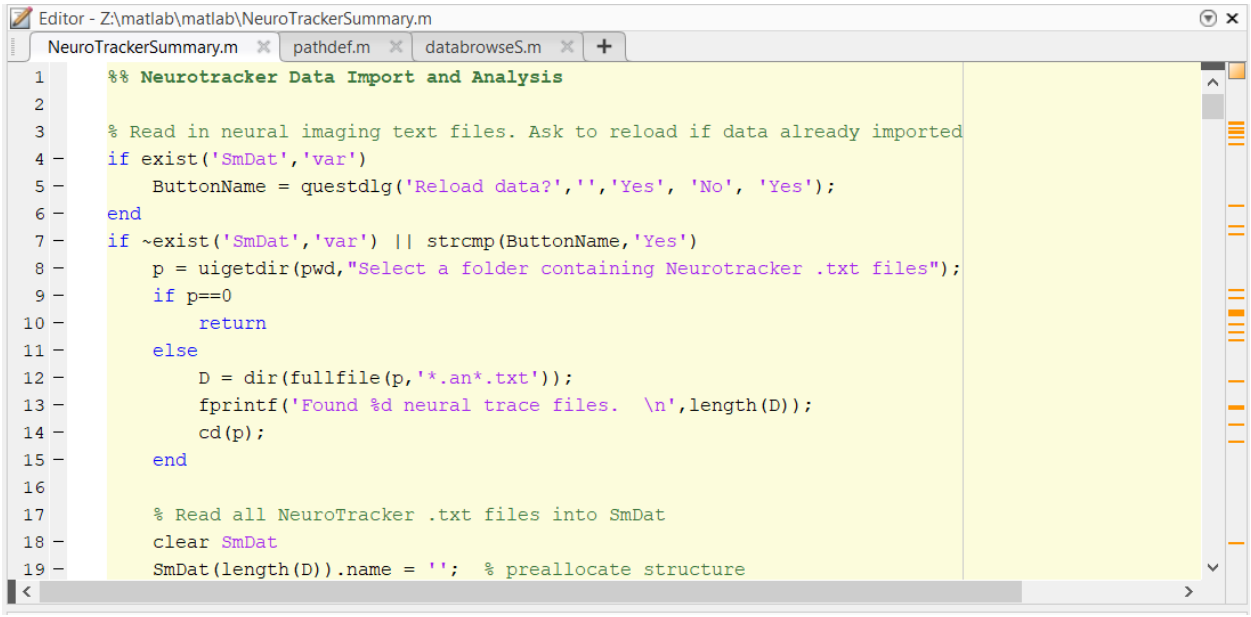
Mode	Format	Examples
0.1-0.999	Add a single 0 before the number	0123 = 0.123A 0421 = 0.421A
1-5	Type in the number without a decimal	3498= 3.498A 4999= 4.999A

The above commands will set the current limit for the device. It is advised to keep the limit lower to ensure the safety of the user. The range of the current limit varies from 0.100A to 5A. Do not exceed these boundaries or an error will occur.

Once the correct settings are input into the system, click the “apply” button. The settings can be saved using the prompt that comes up. For the purposes of the Deep Brain Stimulation MQP, the save the settings as C://mm_code/Electrical settings.txt as the run program will attempt to find the settings at this location. (NOTE: once the program is saved the experiment will immediately begin.)

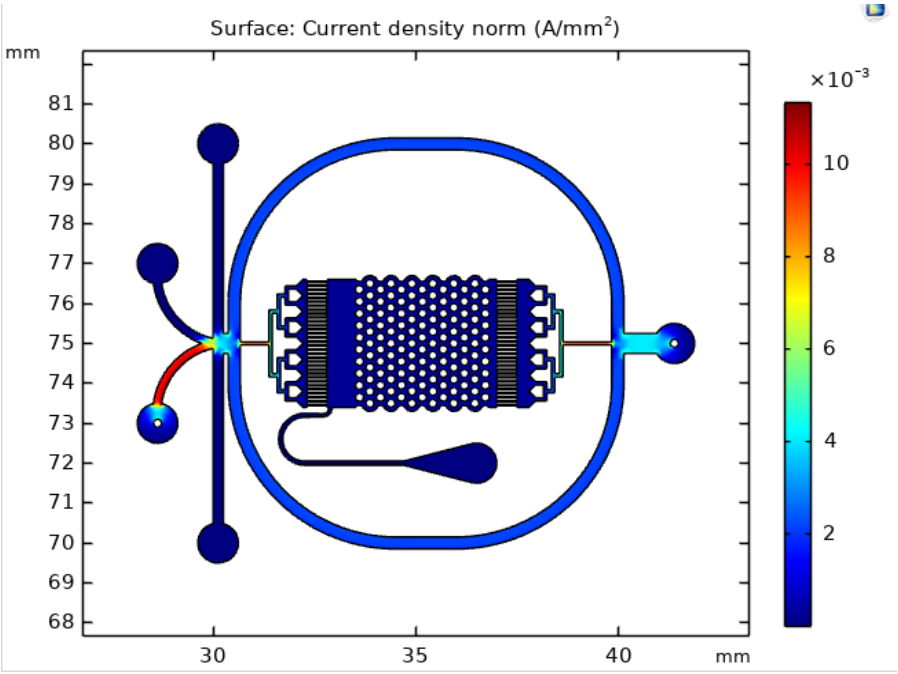
Appendix C: MATLAB Code

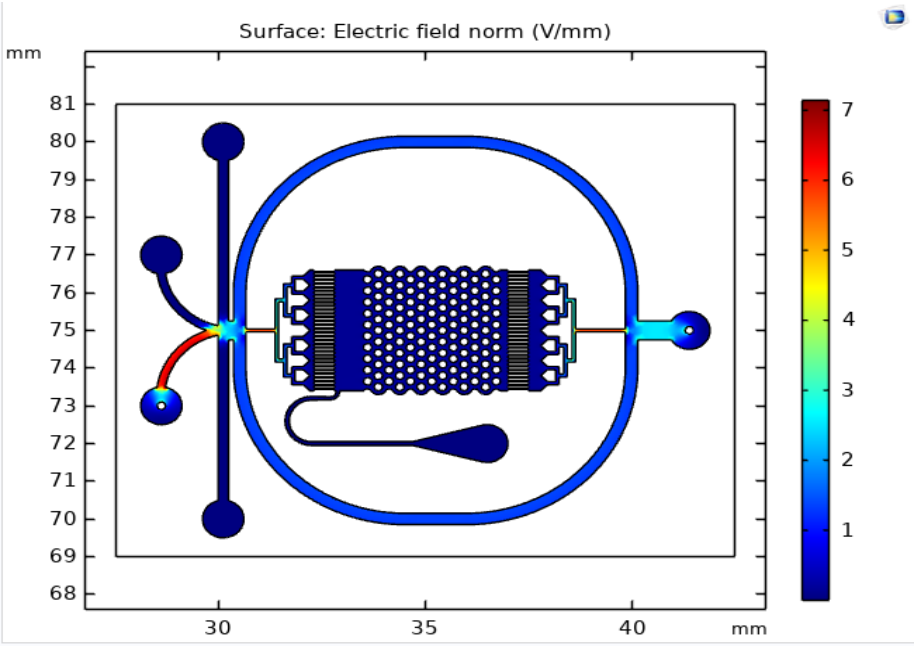
A screenshot showing the MATLAB code used for analyzing the data from the NeuroTracker program on ImageJ can be seen below. As is seen in the image there are three different code files. The NeuroTrackerSummary file is the one used to import the neural imaging data and create the three different graphical displays. The databrowseS file is used to sort the data and create new graphs based on the defined parameters such as animal or experiment number. Finally, the pathdef file is used to set the path which will be used in the code.



Appendix D: Full COMSOL Analysis of Existing R5 Device

The current density of the existing R5 device can be seen below in A/mm^2 while the values for electric field strength in V/mm can be seen below that. It is obvious when looking at the two figures side by side that the current density and electric field strength follow the same pattern across the device with the only difference being the scaling and units, making the assumption that these values will change uniformly a valid assumption.

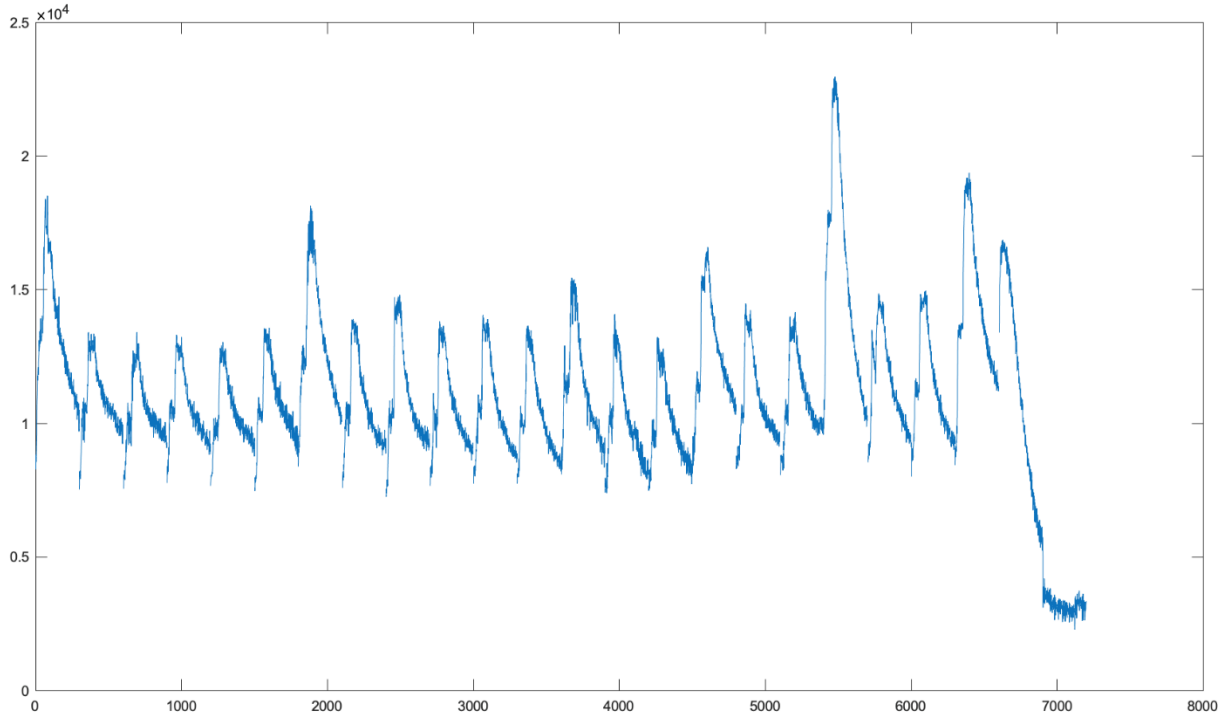




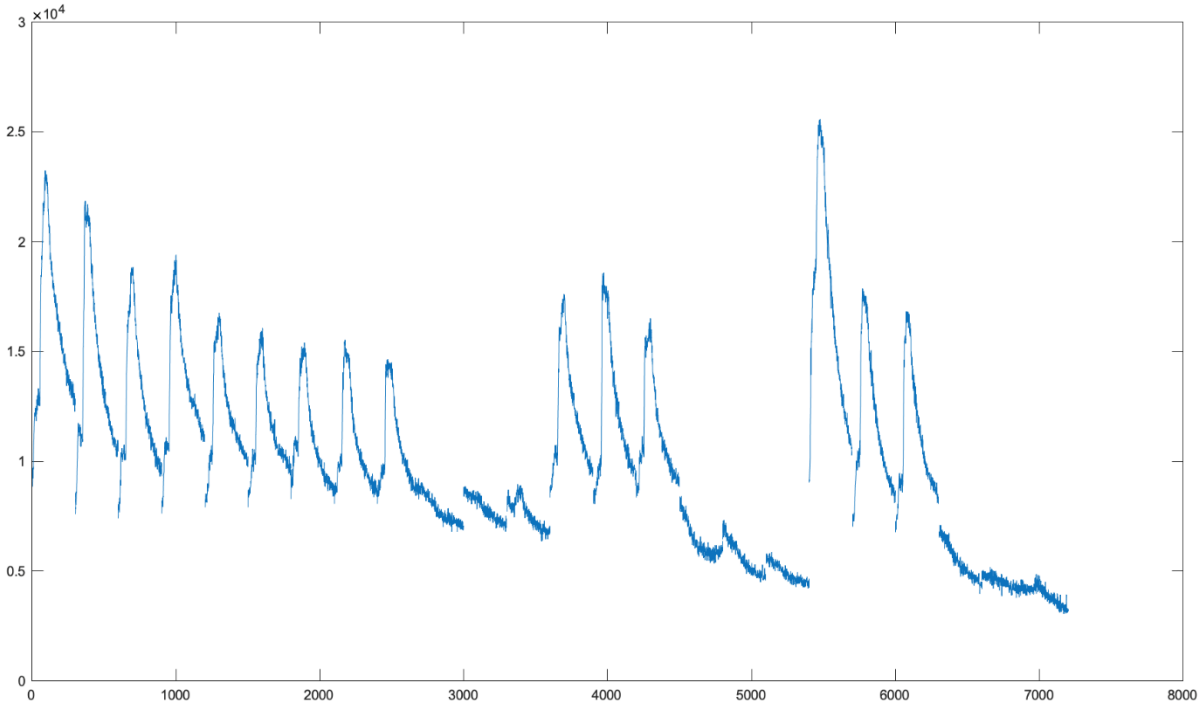
Appendix E: Final Validation Result Plots

Experimental Group

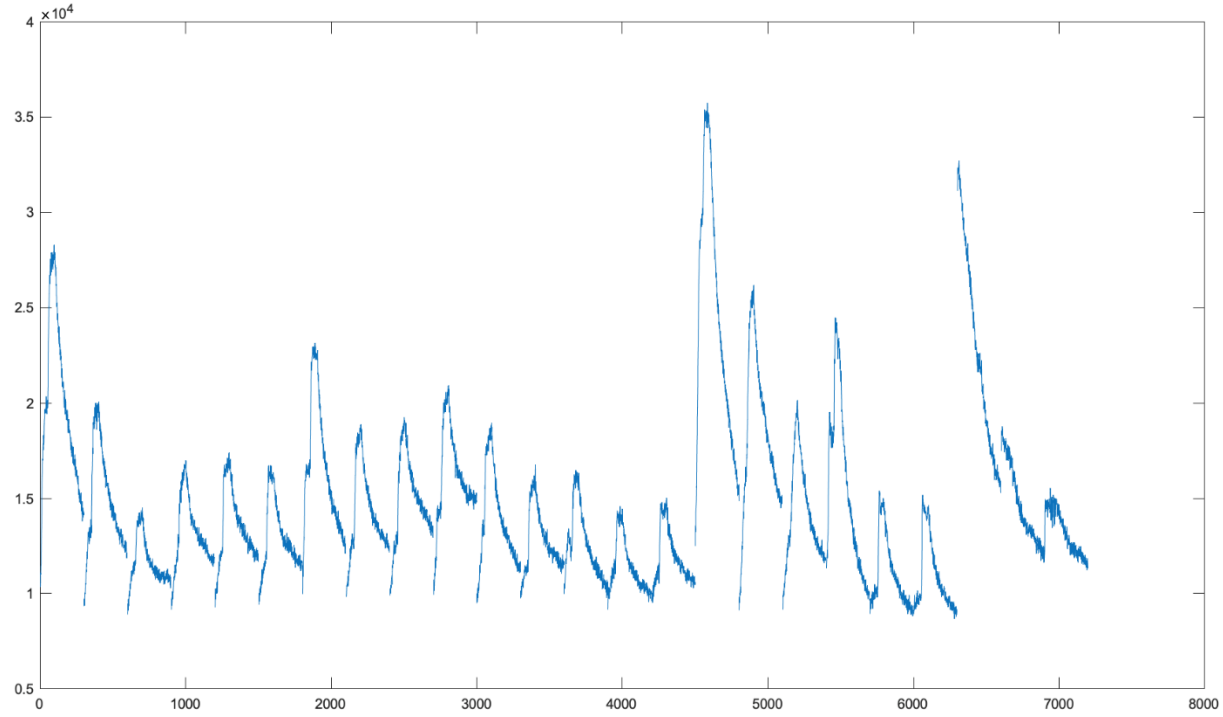
Animal 0:



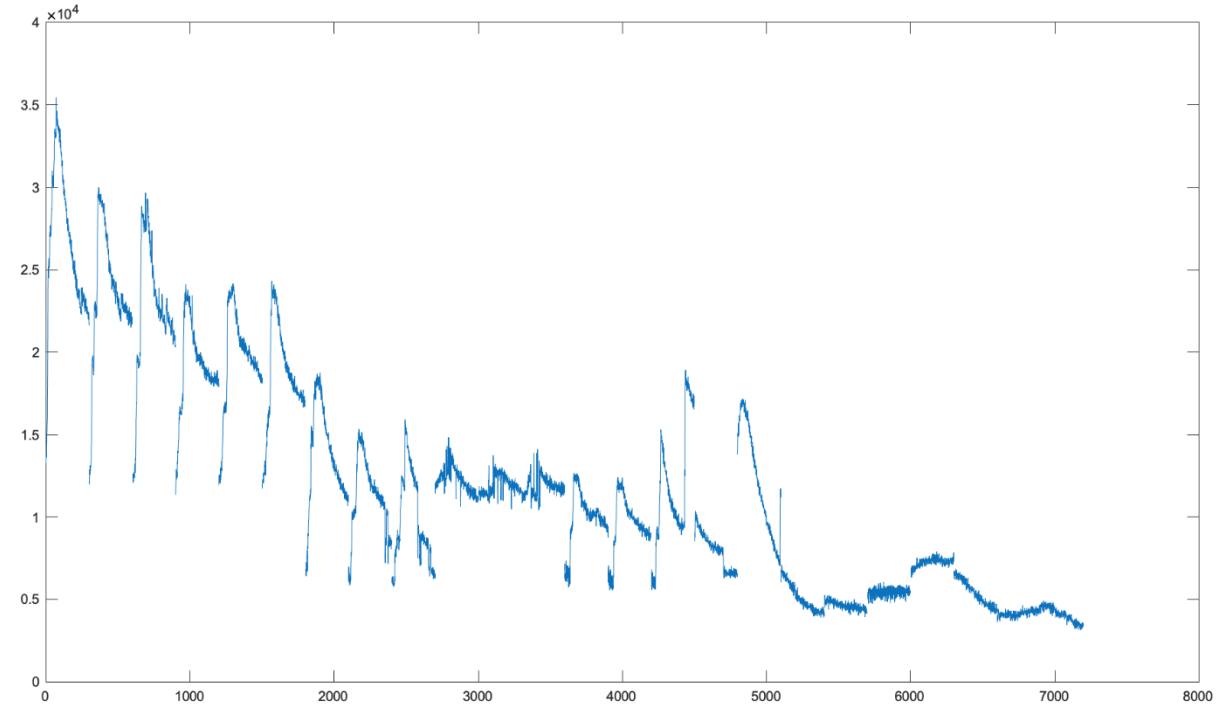
Animal 1:



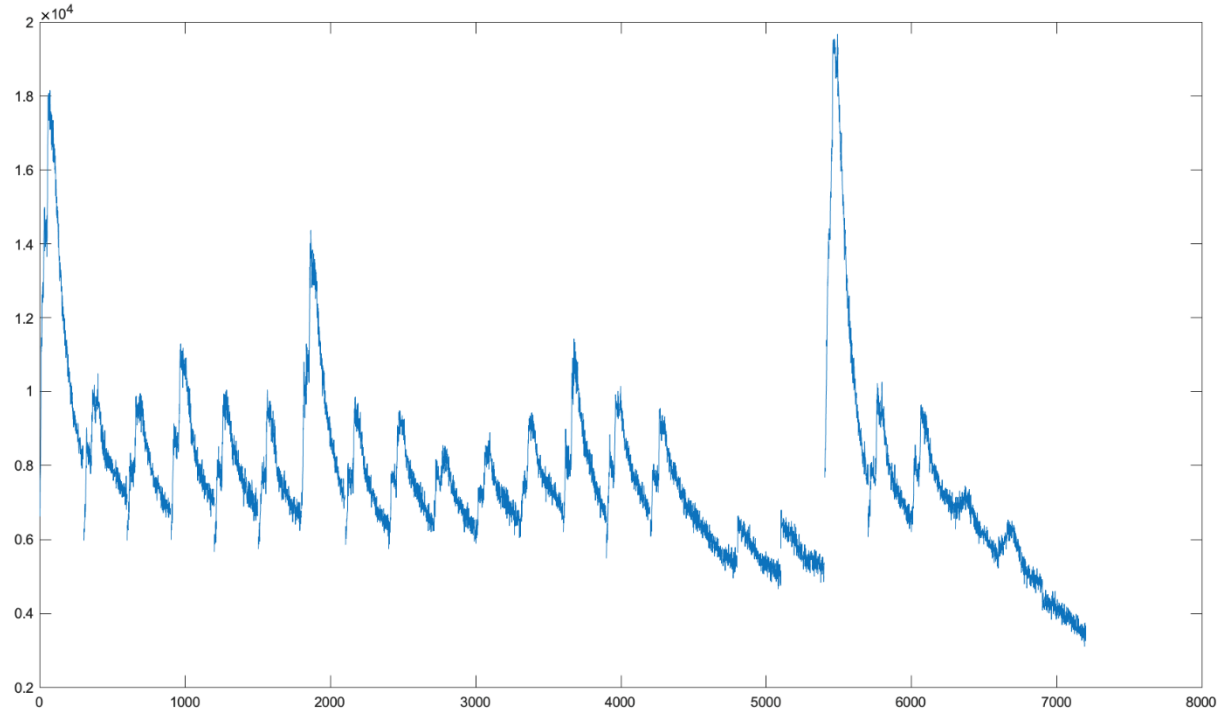
Animal 2:



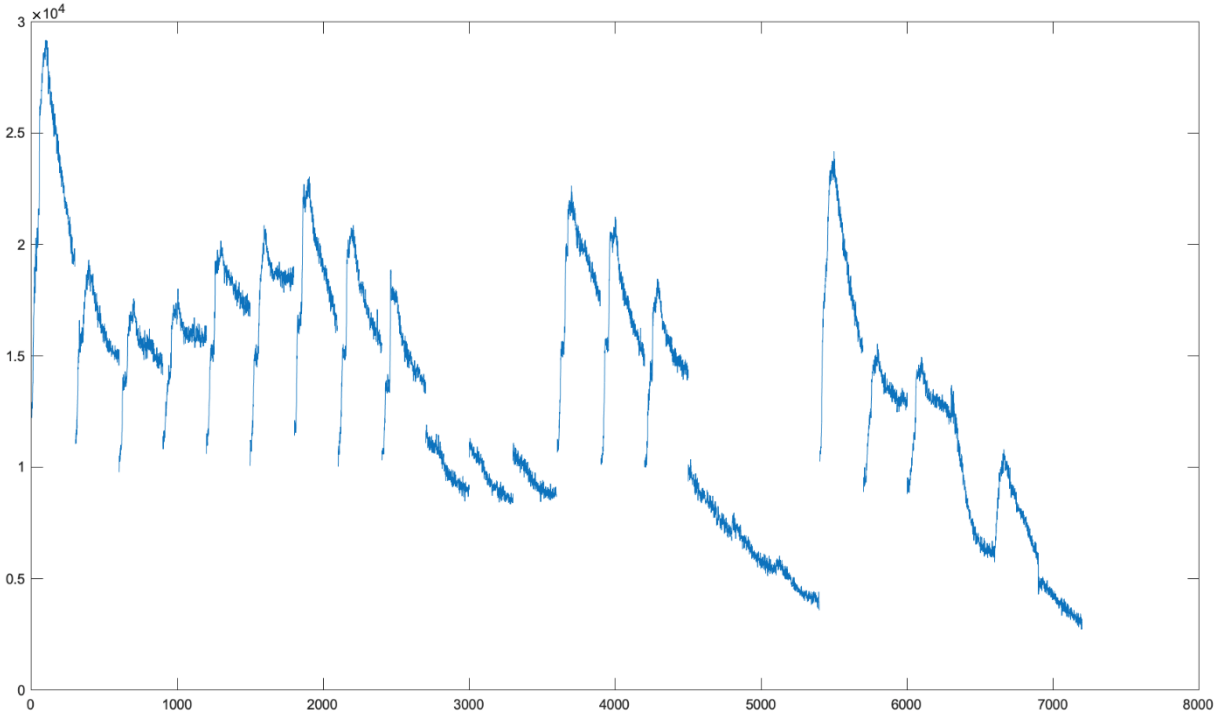
Animal 3:



Animal 4:

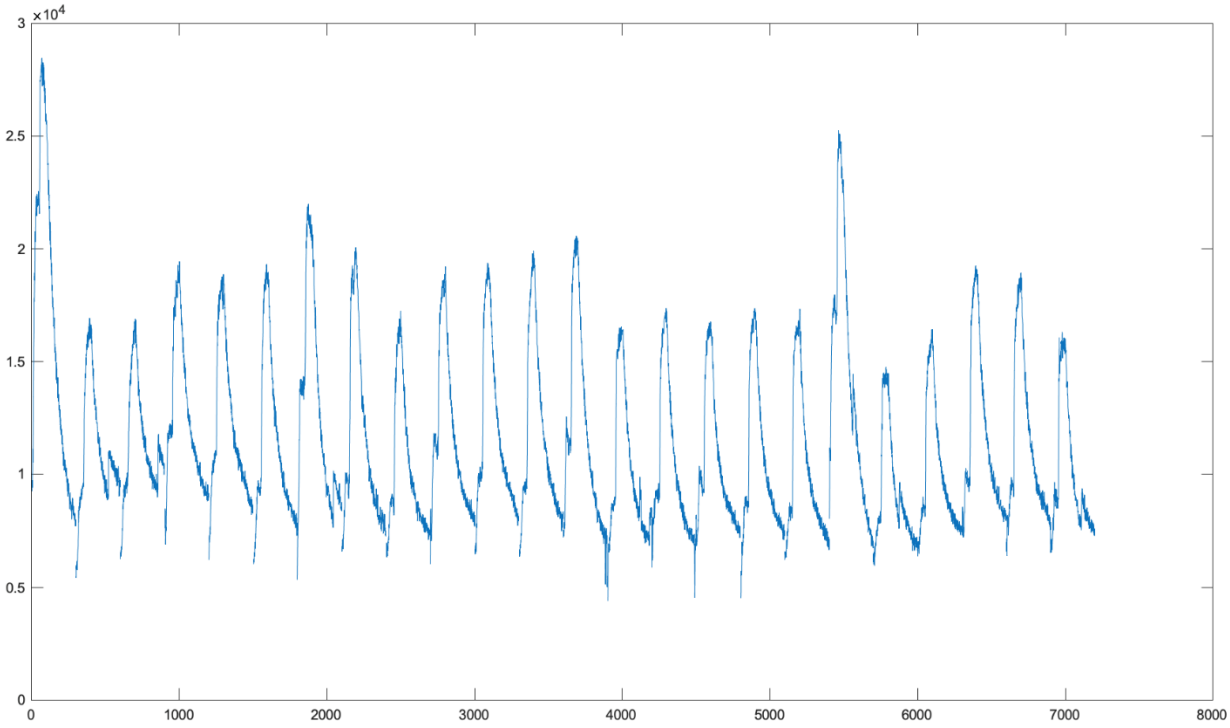


Animal 5:

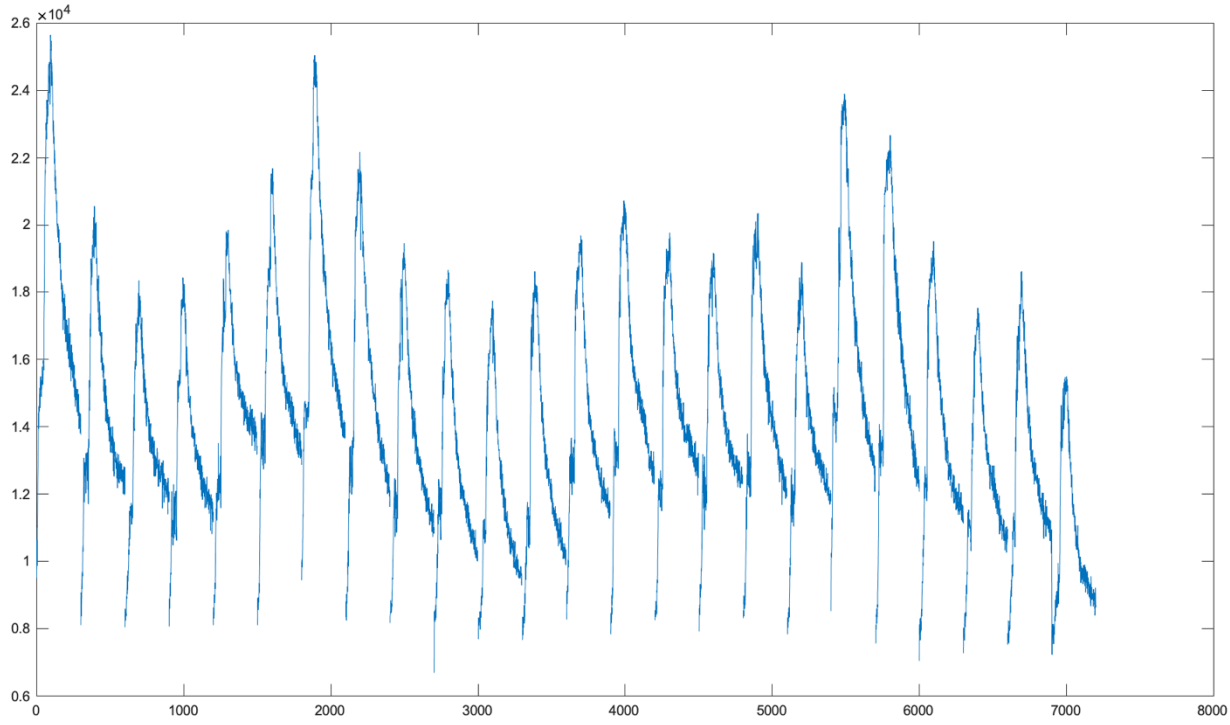


Control Group

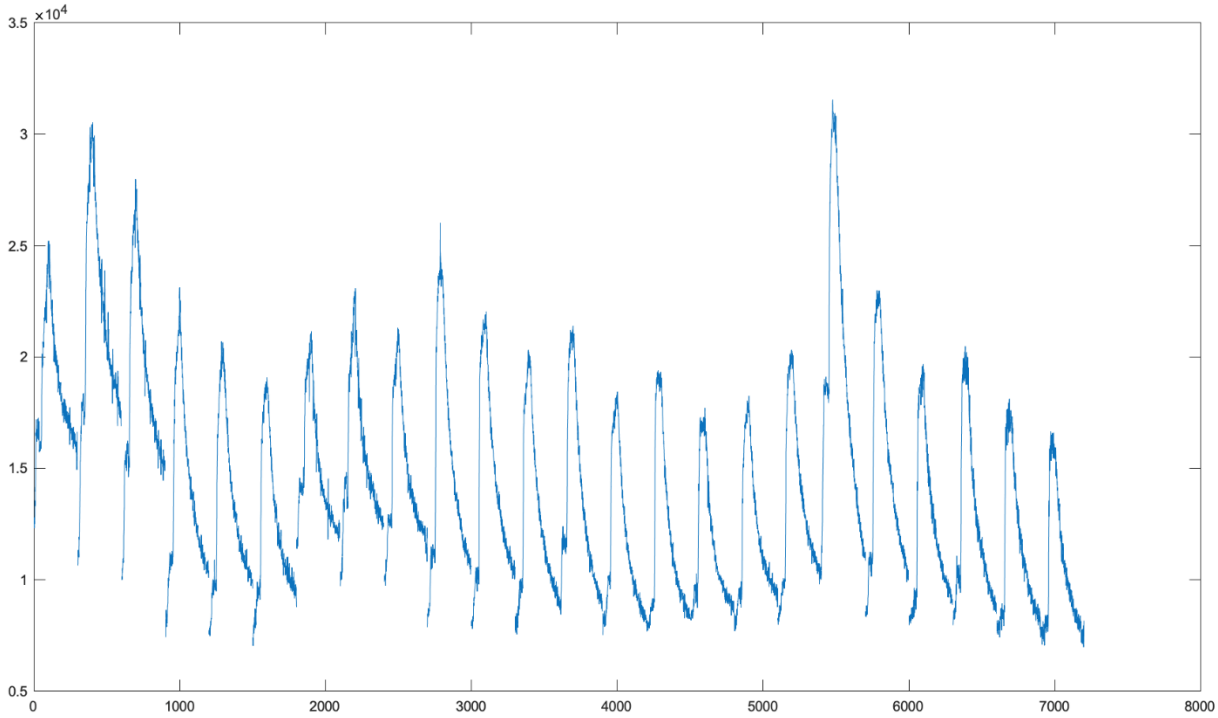
Animal 0:



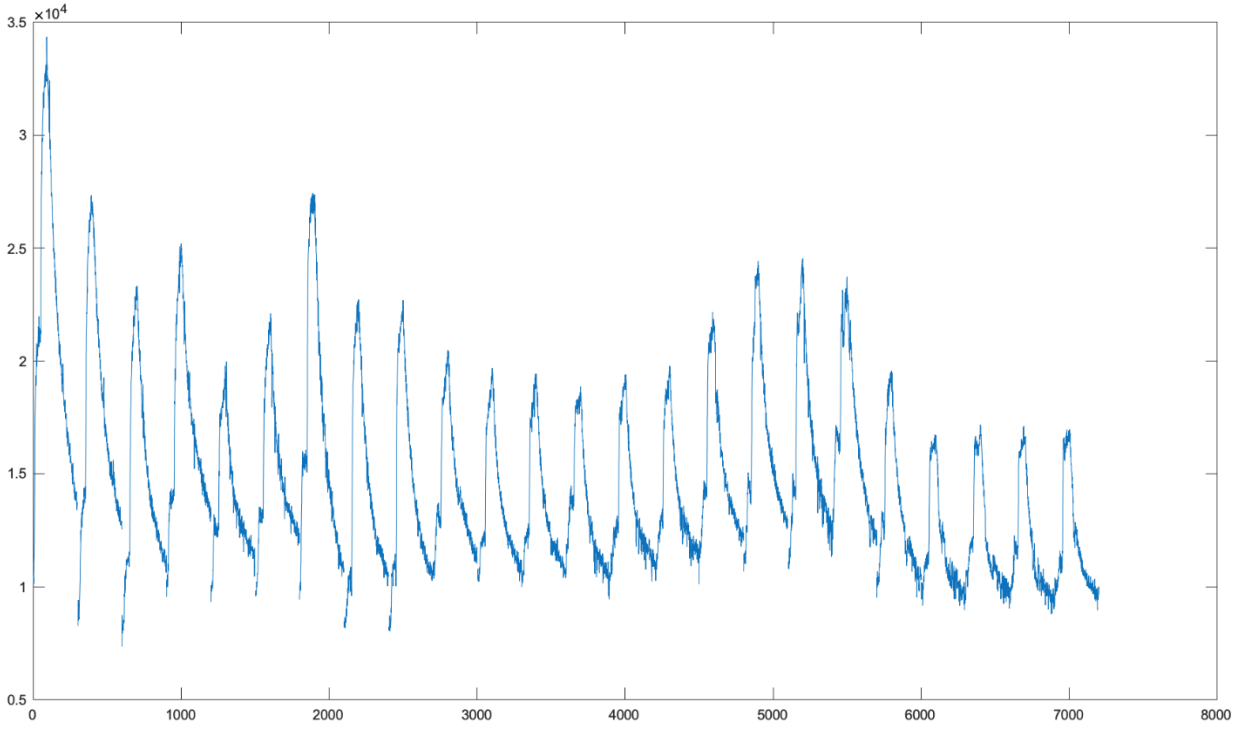
Animal 1:



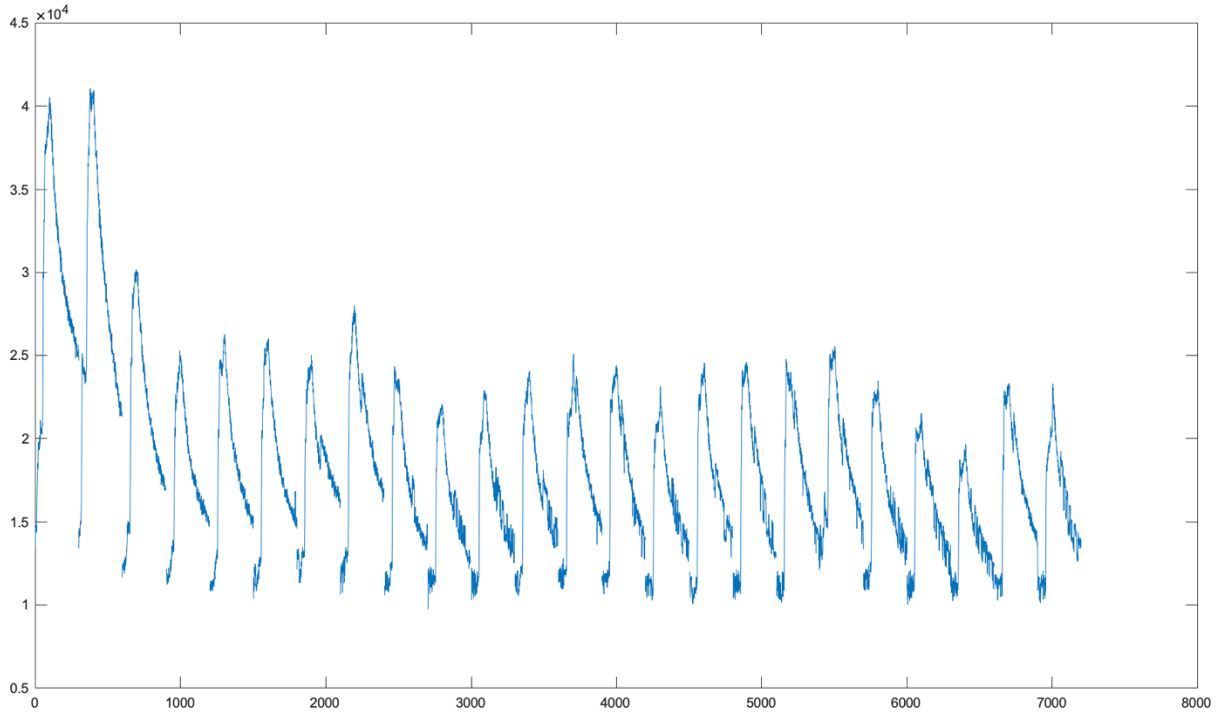
Animal 2:



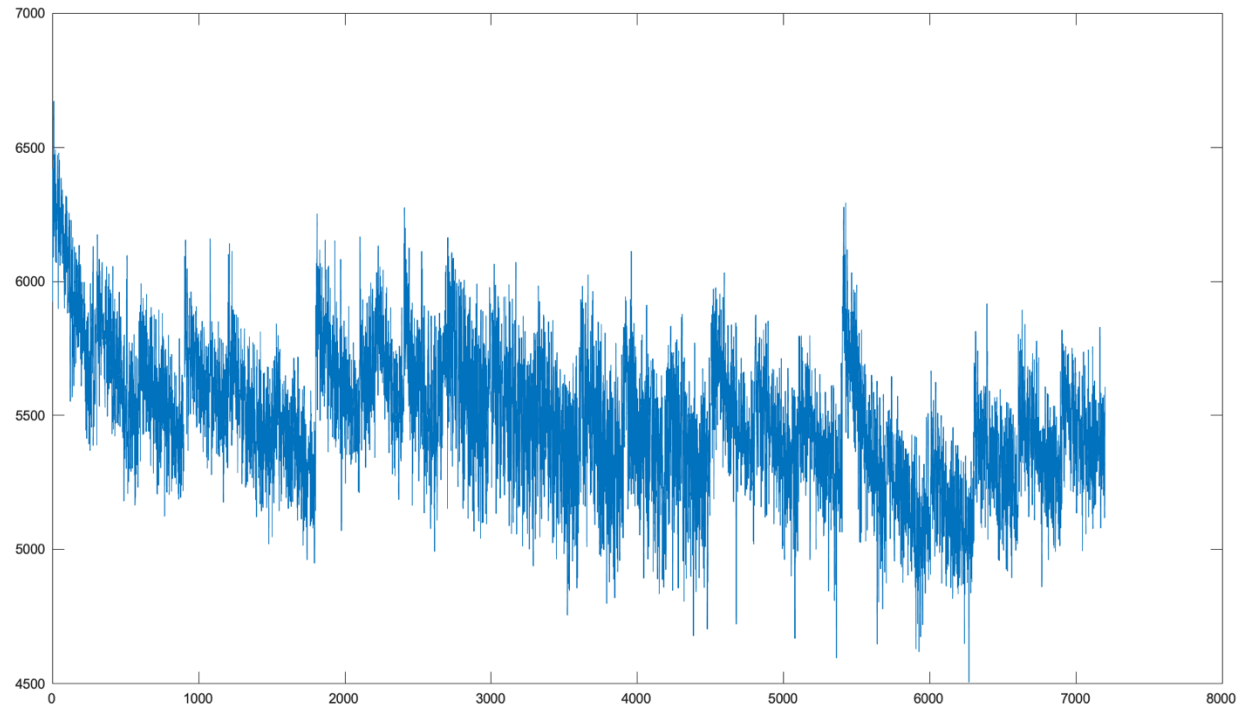
Animal 3:



Animal 4:



Animal 5:



Appendix F: Micromanager Code

ELECTRICAL GUI CODE:

```
import java.awt.*;
import java.awt.event.*;
import javax.swing.*;
import java.util.*;
import java.io.*;
import java.net.*;
import org.micromanager.api.AcquisitionOptions;
import net.miginfocom.swing.MigLayout;
import ij.process.*;
import ij.ImagePlus;
import ij.io.FileSaver;
import java.text.SimpleDateFormat;
import java.util.Calendar;
import java.util.Date;
import java.awt.EventQueue;

gui2Over=0;

int runGUI2() {
////////////////////////////////////////////////////////////////////////////////
//Set-up GUI, Component Definitions
////////////////////////////////////////////////////////////////////////////////
gui.closeAllAcquisitions();
gui.clearMessageWindow();
SERIAL_WAIT = 3000;
global.gotcommand = false;
global.stimchangevalid=true;
SAVE_TO_RAM = true;
global.otherload = 0;
f = new JFrame("Electrical Stimulation Settings");

f.setBounds(200,100,733,533);
f.setSize(500, 500);
f.setLocation(700, 300);
f.setResizable(false);
cp = f.getContentPane();
cp.setLayout(new MigLayout("ins 20", "[para]0[][1001p,fill][601p][951p,fill]"));

JButton apply = new JButton("Apply");
```

```
voltageStartInput = new JFormattedTextField("5");
voltageStartInput.setColumns(10);

voltageEndInput = new JFormattedTextField("30");
voltageEndInput.setColumns(10);

voltageIncInput = new JFormattedTextField("5");
voltageIncInput.setColumns(10);

pulseLengthInput = new JFormattedTextField("10");
pulseLengthInput.setColumns(10);

stimulationLengthInput = new JFormattedTextField("600");
stimulationLengthInput.setColumns(10);


frequencyInput = new JFormattedTextField("050");
frequencyInput.setColumns(10);

dutyCycleInput = new JFormattedTextField("050");
dutyCycleInput.setColumns(10);

voltageInput = new JFormattedTextField("2500");
voltageInput.setColumns(10);

ampInput = new JFormattedTextField("0100");
ampInput.setColumns(10);

directoryinput = new JFormattedTextField("C:/mm code");
directoryinput.setColumns(35);
directoryinput.setEditable(false);

////////////////////////////////////////////////////////////////////////////
//JLABELS
////////////////////////////////////////////////////////////////////////////
JLabel = experimenttypelbl = new JLabel("Experiment Type");
experimenttypelbl.setForeground(Color.BLUE);
experimenttypelbl.setFont(new Font("Serif",Font.BOLD,14));

JLabel = voltScalelbl = new JLabel("Voltage Scaling Parameters");
voltScalelbl.setForeground(Color.BLUE);
voltScalelbl.setFont(new Font("Serif",Font.BOLD,14));
JLabel = voltStartLbl = new JLabel("Starting Voltage [V]:");
JLabel = voltEndLbl = new JLabel("Ending Voltage [V]:");
```

```
JLabel = voltIncLbl = new JLabel("Voltage Increment [V]:");
JLabel = stimulustypelbl = new JLabel("Stimulus Type");
stimulustypelbl.setForeground(Color.BLUE);
stimulustypelbl.setFont(new Font("Serif",Font.BOLD,14));

JLabel = expsetlbl = new JLabel("Stimulus Settings");
expsetlbl.setForeground(Color.BLUE);
expsetlbl.setFont(new Font("Serif",Font.BOLD,14));
JLabel = stimulationLengthLbl = new JLabel("Stimulation Length [s]:");
JLabel = pulseLengthLbl = new JLabel("Pulse Train Length [s]:");
JLabel = frequencyLbl = new JLabel("Frequency [Hz]:");
JLabel = dutyCycleLbl = new JLabel("Duty Cycle [%]:");
JLabel = voltageLbl = new JLabel("Voltage [V]:");
JLabel = ampLbl = new JLabel("Amps [A]:");
JLabel = directorylbl = new JLabel("Select Dir: ");
JSeparator separator0 = new JSeparator();
JSeparator separator = new JSeparator();
JSeparator separator2 = new JSeparator();
JSeparator separator3 = new JSeparator();
////////////////////////////////////////////////////////////////
//JRADIOBUTTONS
////////////////////////////////////////////////////////////////

JRadioButton scale,norm,a,b,c;
ButtonGroup buttons = new ButtonGroup();
scale = new JRadioButton("Voltage-Scaling");
norm = new JRadioButton("Voltage-Static");
buttons.add(norm);
buttons.add(scale);
norm.setSelected(true);
ButtonGroup buttonGroup = new ButtonGroup();
a = new JRadioButton("Intertrial-Stimulation");
b = new JRadioButton("Continuous-Stimulation");
c = new JRadioButton("Continuous-Pulse-Stimulation");
buttonGroup.add(a);
buttonGroup.add(b);
buttonGroup.add(c);
a.setSelected(true);

////////////////////////////////////////////////////////////////
//ACTION LISTENERS
////////////////////////////////////////////////////////////////
apply.addActionListener(this);
pulseLengthInput.addActionListener(this);
```

```
a.addActionListener(this);
b.addActionListener(this);
c.addActionListener(this);

////////////////////////////////////////////////////////////////////
//ADD COMPONENTS TO THE JFRAME FOR INITIAL START UP
////////////////////////////////////////////////////////////////////

//GUI SECTION 1 (EXPERIMENT TYPE)


cp.add(experimenttypelbl, "gapbottom 1, span, split 2, aligny center");
cp.add(new JSeparator(),"gapleft rel,growx");
cp.add(norm,"wrap");
cp.add(scale,"wrap");

cp.add(voltScalelbl, "gapbottom 1, span, split 2, aligny center");
cp.add(new JSeparator(),"gapleft rel,growx");
cp.add(voltStartLbl);
cp.add(voltageStartInput,"wrap 10");
cp.add(voltEndLbl);
cp.add(voltageEndInput,"wrap 10");
cp.add(voltIncLbl);
cp.add(voltageIncInput,"wrap 10");


cp.add(stimulustypelbl, "gapbottom 1, span, split 2, aligny center");
cp.add(new JSeparator(),"gapleft rel,growx");
cp.add(a,"wrap");
cp.add(b,"wrap");

cp.add(c,"wrap 10");

//GUI SECTION 2 (EXPERIMENT SETTINGS)
cp.add(expsetlbl, "gapbottom 1, span, split 2, aligny center");
cp.add(new JSeparator(),"gapleft rel,growx");
cp.add(stimulationLengthLbl);
cp.add(stimulationLengthInput,"wrap");

cp.add(pulseLengthLbl);
cp.add(pulseLengthInput,"wrap 10");

cp.add(frequencyLbl);
cp.add(frequencyInput,"wrap 10");
```

```
cp.add(dutyCycleLbl);
cp.add(dutyCycleInput,"wrap 10");

cp.add(voltageLbl);
cp.add(voltageInput,"wrap 10");

cp.add(ampLbl);
cp.add(ampInput,"wrap 10");


//GUI SECTION 3 (SAVE INFORMATION AND APPLY SETTINGS)

cp.add(new JSeparator(),"gapleft rel,span 4,growx,wrap 10");
cp.add(apply,"al left, cell 0 20");
cp.add(directoryinput,"span 4");
f.pack();
f.show();
initializex = b.getText();
expTypeS = norm.getText();
//void actionPerformed(ActionEvent e) {


////////////////////////////////////////////////////////////////////////////////
//Apply all settings by writing to a text file to be called by run acquisition
////////////////////////////////////////////////////////////////////////////////
void actionPerformed(ActionEvent e) {
if (e.getActionCommand() == ("Apply")){
if(scale.isSelected()){
expTypeS = scale.getText();
}

if(norm.isSelected()){
expTypeS = norm.getText();
}

if(a.isSelected()){
initializex = a.getText();
}

if(b.isSelected()){
initializex = b.getText();
}
```

```
if(c.isSelected()){
initializex = c.getText();
}
String expType = expTypeS;
String stimType = initializex;
String stimLength = stimulationLengthInput.getText();
String pulseLength = pulseLengthInput.getText();
String freq = frequencyInput.getText();
String DC = dutyCycleInput.getText();
String volt = voltageInput.getText();
String amps = ampInput.getText();
String voltStartS = voltageStartInput.getText();
String voltEndS = voltageEndInput.getText();
String voltIncS =voltageIncInput.getText();

//=====================================================================
===
//Write to MicroManager Directory for setting data to be passed to second script
//=====================================================================
===
if(expTypeS.equals("Voltage-Static")){
   JFileChooser mySaveDialog = new JFileChooser(directoryinput.getText());
int saveChoice = mySaveDialog.showSaveDialog(null);

if (saveChoice == JFileChooser.APPROVE_OPTION){
sfilepath = (mySaveDialog.getSelectedFile().getAbsolutePath()+".txt");
FileWriter fw = new FileWriter(sfilepath);
PrintWriter pw = new PrintWriter(fw);
pw.println(stimType.trim());
pw.println(stimLength.trim());
pw.println(pulseLength);
pw.println("F"+freq);
pw.println("D"+DC);
pw.println("su"+volt);
pw.println("si"+amps);
pw.println(expType.trim());
pw.close();
}
print("SUMMARY");
print("Experiment Type: "+expType);
print("Stimulus Type: " +stimType);
print("Stimulation Length: " +stimLength);
print("Pulse Length: "+pulseLength);
print("Frequency: "+ "F"+freq);
```

```
print("Duty Cycle: " + "D"+DC);
print("Voltage: " + "su"+volt);
print("Current Limit: " +"si"+amps);
gui2Over = 1;
}
else{
   JFileChooser mySaveDialog = new JFileChooser(directoryinput.getText());
int saveChoice = mySaveDialog.showSaveDialog(null);

if (saveChoice == JFileChooser.APPROVE_OPTION){
sfilepath = (mySaveDialog.getSelectedFile().getAbsolutePath()+".txt");
FileWriter fw = new FileWriter(sfilepath);
PrintWriter pw = new PrintWriter(fw);
pw.println(stimType.trim());
pw.println(stimLength.trim());
pw.println(pulseLength);
pw.println("F"+freq);
pw.println("D"+DC);
pw.println("su"+volt);
pw.println("si"+amps);
pw.println(expType.trim());
pw.println(voltStartS.trim());
pw.println(voltEndS.trim());
pw.println(voltIncS.trim());
pw.close();
}
print("SUMMARY");
print("Experiment Type: "+expType);
print("Stimulus Type: " +stimType);
print("Stimulation Length: " +stimLength);
print("Pulse Length: "+pulseLength);
print("Frequency: "+ "F"+freq);
print("Duty Cycle: " + "D"+DC);
print("Starting V: " + voltStartS);
print("Incrementing by: " + voltIncS);
print("Ending V: " + voltEndS);
print("Current Limit: " +"si"+amps);
gui2Over = 1;
}

}
}
while(gui2Over!=1)
{
```

```
mmc.sleep(2);
}
return gui2Over;
}


runGUI2();
```

CONFIGURATION CODE:

```
import org.micromanager.api.*;
import java.lang.*;
import java.io.File;
import java.io.FileReader;
import java.io.IOException;
import ij.process.*;
import ij.ImagePlus;
import ij.io.FileSaver;
import java.text.SimpleDateFormat;
import java.util.Calendar;
import java.util.Date;
import java.util.Scanner;



configOver = 0;


//method to read in the text file
String[] readTextInfo( String filename)
{
File file = new File(filename);
FileReader reader = new FileReader(file);
char[] chars = new char[(int) file.length()];
reader.read(chars);
content = new String(chars);
reader.close();
return content.split("\n");
}

fromfile = readTextInfo("C:/mm_code/Electrical settings.txt"); //reads in electrical settings


double stimLength = 1000*Double.parseDouble(fromfile[1]);
double pulseLength =  1000*Double.parseDouble(fromfile[2]);
String freq= fromfile[3].trim();
String DC = fromfile[4].trim();
String voltage = fromfile[5].trim();
String amp = fromfile [6].trim();
//print(freq);
//print(DC);
//print(voltage);
//print(amp);
```

```
//turns on the device to set all of the settings
mmc.setSerialPortCommand("COM29", voltage,"\r");
mmc.sleep(500);
mmc.setSerialPortCommand("COM29", amp,"\r");
mmc.sleep(500);
mmc.setSerialPortCommand("COM29", "o1","\r");
mmc.sleep(500);
mmc.setSerialPortCommand("COM5", freq,"");
mmc.sleep(500);
mmc.setSerialPortCommand("COM5", DC,"");
mmc.sleep(500);


configOver = 1;
```

CONTINUOUS PULSE TRAIN CODE:

```
boolean stimulation = true;
double currentStart = System.currentTimeMillis();
double lastPulse = currentStart;

mmc.setSerialPortCommand("COM5", "OFF",""); //turns off the device

while (machine != 1)
{
if(machine == 1) //is used to externally end the loop that is otherwise infinite
{
break;
}
        double currentStart = System.currentTimeMillis();
          if(currentStart>=lastPulse+pulseLength){
             if(stimulation){
                mmc.setSerialPortCommand("COM5", "ON",""); // turns on the stimulation
                print("ON");
             }
             else{
                mmc.setSerialPortCommand("COM5", "OFF",""); // turns off stimulation
                print("OFF");
             }
             stimulation = !stimulation;
             lastPulse = currentStart;
          }
}

import org.micromanager.api.*;
import java.lang.*;
import java.io.File;
import java.io.FileReader;
import java.io.IOException;
import ij.process.*;
import ij.ImagePlus;
import ij.io.FileSaver;
import java.text.SimpleDateFormat;
import java.util.Calendar;
import java.util.Date;
import java.util.Scanner;

mmc.setSerialPortCommand("COM31", "OFF","");
mmc.sleep(500);
```

```
mmc.setSerialPortCommand("COM5", "o0","\r");

String[] readTextInfo( String filename)
{
	File file = new File(filename);
	FileReader reader = new FileReader(file);
	char[] chars = new char[(int) file.length()];
	reader.read(chars);
	content = new String(chars);
	reader.close();
	return content.split("\n");
}

pulseTrain(double stimLength, double  pulseLength) {
boolean stimulation = true;
double expStart = System.currentTimeMillis();
double currentStart = expStart;
double lastPulse = expStart;
mmc.setSerialPortCommand("COM31", "OFF","");

	while (currentStart < expStart+stimLength){

			double currentStart = System.currentTimeMillis();
				if(currentStart >= expStart+stimLength)
				{
					break;
				}
		if(currentStart>=lastPulse+pulseLength){
		   if(stimulation){
		      mmc.setSerialPortCommand("COM31", "ON","");
		      print("ON");
		   }
		   else{
		      mmc.setSerialPortCommand("COM31", "OFF","");
		      print("OFF");
		   }
		   stimulation = !stimulation;
		   lastPulse = currentStart;
		}
	}
		mmc.setSerialPortCommand("COM31", "OFF","");

}
gui.closeAllAcquisitions();
```

```
gui.clearMessageWindow();
gui2Over= 0;

//source("C:/MicroManager Control Scripts/DBS_ELECTRICAL_GUI(latest).bsh");  //is the
electrical settings gui



fromfile = readTextInfo("C:/mm_code/Electrical settings.txt"); //reads in experiment settings from
the gui
guiFile  = readTextInfo("C:/User Defined Acquisition Settings.txt");
double guitrialints_DBS = Double.parseDouble(guiFile[8]);
int inttrialinterval_DBS = guitrialints_DBS.intValue();

String electricalExperimentType = fromfile[0].trim();
double stimLength = 1000*Double.parseDouble(fromfile[1]); //gets intertrial stimulation length
double pulseLength =  1000*Double.parseDouble(fromfile[2]); // if it is pulse the pulse length


configOver =0;
source("C:/MicroManager Control Scripts/DBS_CONFIG.bsh");
while(configOver==0)
{
        mmc.sleep(5);
}
/*
guiOver=0;
source("C:/MicroManager Control Scripts/GUI_DA_DBS(latest).bsh");

while(guiOver==0)
{
        mmc.sleep(5);
}
*/
if(fromfile[7].trim().equals("Voltage-Static")){
        if(electricalExperimentType.equals("Intertrial-Stimulation"))
        {
                testOver=0;
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
                while(testOver==0)
                {
                        mmc.sleep(5);
                }
                pulseTrain(stimLength,pulseLength,false);
```

```
		source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
	}

	if(electricalExperimentType.equals("Continuous-Stimulation"))
	{
		testOver=0;
		source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
		while(testOver==0)
		{
			mmc.sleep(5);
		}
		mmc.setSerialPortCommand("COM31", "ON","");

	  print("ON");
		source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
	}

	if(electricalExperimentType.equals("Continuous-Pulse-Stimulation"))
	{
	testOver=0;
		source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
		while(testOver==0)
		{
			mmc.sleep(5);
		}
		machine = 0;
		bg("C:/MicroManager Control Scripts/PULSETRAIN_CONTINUOUS.bsh");
		source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
		machine = 1;
	}
}

if(fromfile[7].trim().equals("Voltage-Scaling")){
	double voltStart =  Double.parseDouble(fromfile[8]);
	double voltInc =   Double.parseDouble(fromfile[10]);
	double voltEnd =   Double.parseDouble(fromfile[9]);

	double voltCurrent = voltStart;
  while(voltCurrent <voltEnd)
  {
		if(voltCurrent<0.1)
		{
			int voltCurrentCommand = voltCurrent*100;
			String vCC= "su000";
```

```
                        String vCC = vCC+voltCurrentCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
        }
        if(voltCurrent<1 &&voltCurrent>=0.1)
        {
                        int voltCurrentCommand = voltCurrent*100;
                        String vCC = "su00";
                        String vCC =vCC +voltCurrentCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
        }
        if(voltCurrent<10 && voltCurrent>=1)
        {
                        int voltCurrentCommand = voltCurrent*100;
                        String vCC = "su0"+voltCurrentCommand;
                        String vCC = vCC+voltCurrentCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
        }
        if(voltCurrent>=10)
        {
                        int voltCurrentCommand = voltCurrent*100;
                        String vCC = "su";
                        String vCC = vCC+voltCurrentCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
  }

  if(electricalExperimentType.equals("Intertrial-Stimulation"))
{
        testOver=0;
        source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
        while(testOver==0)
        {
                mmc.sleep(5);
        }
        pulseTrain(stimLength,pulseLength,false);
        source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
}

if(electricalExperimentType.equals("Continuous-Stimulation"))
{
        testOver=0;
        source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
        while(testOver==0)
        {
                mmc.sleep(5);
```

```
            }
            mmc.setSerialPortCommand("COM31", "ON","");

      print("ON");
            source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
      }

      if(electricalExperimentType.equals("Continuous-Pulse-Stimulation"))
      {
      testOver=0;
            source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
            while(testOver==0)
            {
                  mmc.sleep(5);
            }
            machine = 0;
            bg("C:/MicroManager Control Scripts/PULSETRAIN_CONTINUOUS.bsh");
            source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
            machine = 1;
      }
      voltCurrent = voltCurrent+ voltInc;
      print(inttrialinterval_DBS);
      print("waiting test");
}

            if(voltEnd<0.1)
            {
                        int voltEndCommand = voltEnd*100;
                        String vCC = "su000";
                        String vCC = vCC+voltEndCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
            }
            if(voltEnd<1 &&voltEnd>=0.1)
            {
                        int voltEndCommand = voltEnd*100;
                        String vCC = "su00";
                        String vCC = vCC+voltEndCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
            }
            if(voltEnd<10 && voltEnd>=1)
            {
                        int voltEndCommand = voltEnd*100;
                        String vCC = "su0";
                        String vCC = vCC+voltEndCommand;
```

```
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
        }
        if(voltEnd>=10)
        {
                        int voltEndCommand = voltEnd*100;
                        String vCC = "su";
                        String vCC = vCC+voltEndCommand;
                        mmc.setSerialPortCommand("COM5", vCC,"\r");
  }
print("voltage final");

         if(electricalExperimentType.equals("Intertrial-Stimulation"))
        {
                testOver=0;
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
                while(testOver==0)
                {
                        mmc.sleep(5);
                }
                pulseTrain(stimLength,pulseLength,false);
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
        }

        if(electricalExperimentType.equals("Continuous-Stimulation"))
        {
                testOver=0;
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
                while(testOver==0)
                {
                        mmc.sleep(5);
                }
                mmc.setSerialPortCommand("COM31", "ON","");

           print("ON");
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
        }

        if(electricalExperimentType.equals("Continuous-Pulse-Stimulation"))
        {
testOver=0;
        source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
        while(testOver==0)
        {
                mmc.sleep(5);
```

```
                }
                machine = 0;
                bg("C:/MicroManager Control Scripts/PULSETRAIN_CONTINUOUS.bsh");
                source("C:/MicroManager Control Scripts/RUN_DA_DBS(latest).bsh");
                machine = 1;
        }

}


mmc.setSerialPortCommand("COM31", "OFF","");
mmc.sleep(500);
mmc.setSerialPortCommand("COM5", "o0","\r");
print("experiment ended");
```