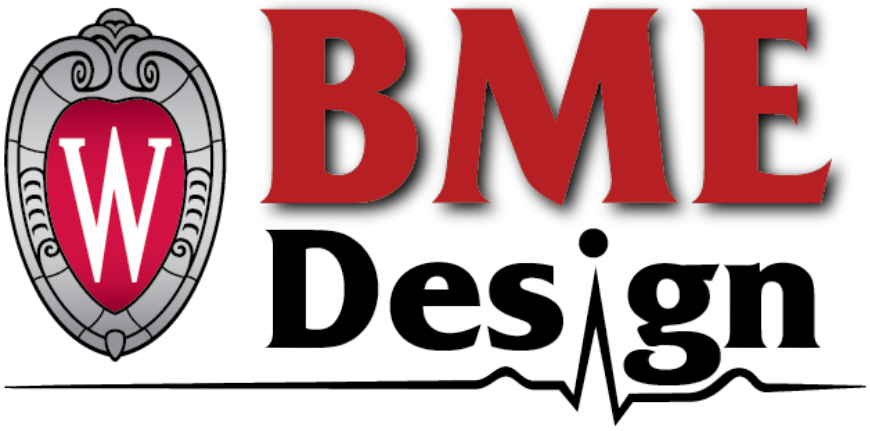
University of Wisconsin - Madison



Longitudinal Migration Tracking of Fluorescent Stem Cells in Vivo in the Mouse Brain

BME 200/300

10/20/2021

Client: Dr. Jayshree Samantha

Advisor: Dr. Sarah Gong

Team Members:

Dana Stumpfoll (Co-Team Leader), Lauren Heller (Co-Team Leader),

Rebekah Makonnen (BSAC) , Tyler Anderson (BPAG),

Alex He (BWIG) , Alexis Block (Communicator)

ABSTRACT

Studying how stem cells migrate can be beneficial to the research of regenerative medicine. The client's goal is to study the migration of stem cells post injury in mice brains. However, this could not be done using the limited field of view of an existing device. To design a device that can be used to track the migration of stem cells longitudinally, the team strived to find components that would enhance the field of view to meet the requirements of the client. After extensive research on existing devices and determining how GRIN lenses work the team came up with three designs that could potentially enhance the field of view to meet the needs of the client. The device had to be feasible and reliable, meaning it could be fabricated and used for the purposes of the clients research. The device was expected to contain three GRIN lenses, but after further evaluation of the team's designs by a Biomedical Engineering specialist in optics and imaging, it was found that this would not be feasible for this team. Instead the team has decided to pursue enhancing the field of view of an existing device that will be loaned to them by another Biomedical Engineering professor. Once fabrication and testing begin future changes will be considered.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Motivation / Global Impact

During the design process of developing a new device, it is important to consider the broader impact this device may have. This device specifically will be useful in studying the migration of stem cells in-vivo since most stem cell research has been conducted ex-vivo. This is important because stem cell research has become more prominent and can be used for understanding tissue and cellular regeneration [1]. To be able to understand how stem cells migrate in the brain of freely behaving mice after injury can contribute to the advancement of regenerative medicine.

New approaches for tracking stem cells have been beneficial to understanding how stem cells work. This device specifically would be beneficial for longitudinal tracking in the sub lateral ventricle of the mouse brain. Being able to track the migration of stem cells in mice brains would further allow researchers to understand how stem cells migrate in response to trauma in human brains in the future.

1.2 Existing Devices & Current Methods

Star Protocols Mini Endoscope

The device used in this protocol is used for tracking neural stem cells in freely behaving mice [2]. The cells are marked using a specific plasmid that will be expressed in the cells and cause them to fluoresce [2]. The calcium dynamics of the neural stem cells are also determined to see how the calcium cascade in the activation of the stem cells relates to their migration. Using this device allows for stem cells to be tracked in a short field of view. This protocol allows for examination of cells for long-term minimally invasive procedures. For gradient-index(GRIN) lenses the lens must be implanted in the brain first. This device does not utilize a flat-tip GRIN lens. A micro-prism was attached to the bottom of the GRIN lens to allow for imaging in a side-view orientation [2]. This design also caused less damage to the surrounding brain tissue due to its bevelled tip that fit perfectly into the sub lateral ventricular zone.

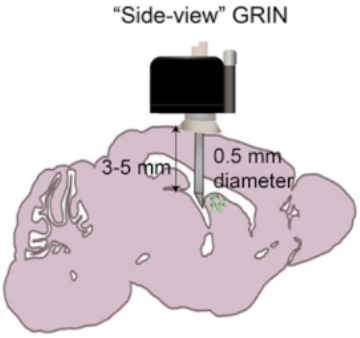


Figure 1: Side view GRIN lens in Star protocol. [2]

Visualizing the neural stem cells that are labeled during insertion was essential to know when to stop inserting the lens into the ventricle [2]. A spacer was used to put distance between the cannula and the skull. Dental cement was then used to secure the endoscope in place. Identifying the region and amount of fluorescent neural stem cells was useful in adjusting the coordinates for implantation of the endoscope. 10-14 days after the endoscope was implanted imaging was able to begin [2]. It was found that the entire migration of the cells was not able to be captured because the field of view of the singular GRIN lens was too small. From this

protocol, our client has decided they would like to upgrade the field of view of this design to account for migration of the cells through the entire ventricle.

Reconnectable Fiber Bundles

For this device a fiber bundle was used to image fluorescent neurons in different parts of the brain. The fiber bundle contains three GRIN lenses all placed in different areas of the brain. This was used to image cells in different areas of the brain at different depths using different length GRIN lenses [3]. The device was cemented in place with dental cement and cyanoacrylate glue. The diameter of the lens is 70 μmeters and was thinned using an oxide etchant mixture. A 473 nm continuous wave - output from a Nd: YAG laser was used to excite the cells and activate their fluorescence [3].

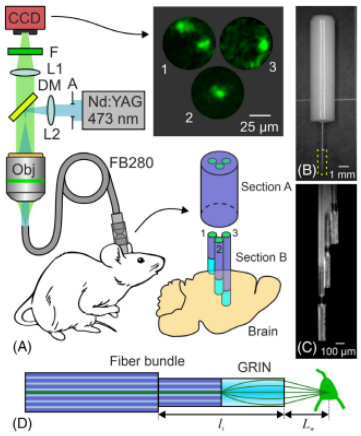


Figure 2: Reconnectable fiber bundles. [3]

The GRIN lens recognized the fluorescence from the cells and produced inverted images of the cells in the fiber bundles. The fiber bundles then transferred the image to a camera with an objective lens where the image could be analyzed using a computer. Having removable fibers that are able to be reconnected allowed for the experiments to be stopped and restarted from weeks to months time. Movement of the mice did not destabilize the scope or ruin any images taken [3]. The small size of the endoscope has been useful in implanting the device for mice that are freely behaving. From using this device it was found that suppression of spontaneous cortical activity has shown to lower calcium signaling activity [3]. This device is unique in that it utilizes three GRIN lenses to obtain images in different areas of the brain.

Existing Patents

One patent that exists on the market for a similar device is for the Inscopix systems and methods for optogenetic imaging [4]. This company has developed many devices which are on the market for in vivo imaging in mice brains. This patent claims all of the aspects of their designs from the illumination device to the field of view of the microscope. This patent applies to the devices owned by the company Inscopix. One device this patent applies to is the nVista microscope. This microscope consists of a flat top GRIN lens that was used to image the excitation of neurons in the brain. Similar to the other competing designs the base plate of the endoscope is cemented to the head of the mouse after the lens is inserted. A camera device is then attached to the baseplate to allow for imaging. By imaging calcium ions in the neurons of the brain this can be correlated to the behavior of mice [5]. This device would not be ideal for the client since it utilizes a flat top GRIN lens that does not incorporate a

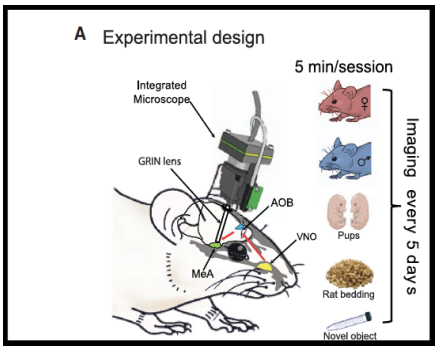


Figure 3: Inscopix nVista implant. [5]

longitudinal field of view. This is one of the leading devices on the market since it does allow for imaging in freely behaving mice. Overall, this patent does not seem to pose any issues with the development of a new design.

1.3 Problem Statement

Current setups for imaging intact neural networks only allow for imaging through a field of view in a single lens. This setup only allows for imaging in a restricted area of the tissue. An endoscope with multiple fields of view is required for accurate tracking of cell migration dynamics in mice that are awake and functioning normally. The design of this endoscope must be small in size and weight and must allow the mouse to maintain normal function and behavior. The endoscope must be able to function in the fluid region of the brain. The endoscope will be powered through a connection port and must be compatible with a Windows OS computer where images will be uploaded using basic software to communicate.

2 BACKGROUND

2.1 Anatomy and Physiology

The high demand for stem cell research in recent years has prompted the need for researchers to track not only the spatial arrangement of stem cells, but the migration pathway and the final distribution of these cells in mice. Moreover, adult neural stem cells (NSC's), the type of cell being tracked in our client's research, have the capability to regenerate, self-renew, proliferate, and finally differentiate into a number of different naturally occurring cell families. The imaging of NSC movement can be executed in two different manners, ex-vivo and in-vivo. Possible imaging modalities include MRI, PET, and CAT scans, along with optical imaging. Due to the nature of our clients work in vivo, the optical imaging technique was favored over all other techniques as these techniques relied on anesthetics. Additionally, optical imaging and endoscopy have the advantage of being able to track cells for a long time (1 week - 2 months) with no radiation damage [6]. The ability to track adult neural stem cells longitudinally will not only result in an influx of data and information, but will revolutionize regenerative medicine in the modern world.

The endoscope, specifically the imaging cannula, will be implanted into the sub lateral ventricle of the mouse brain. The distance from the base of the skull to the base of the sub lateral ventricle covers a depth of 3-5 mm. To prevent unnecessary tissue damage, the diameter of the endoscope cannot exceed 0.5 mm [2]. The mice will be generally anesthetized during the implantation procedure. Once the imaging cannula is installed, it will be fixed to the skull. The weight of the device will be equilibrated across the skull. The mice, aged p60 - p90 will be held

in a closed box-like enclosure allowing them to freely behave during the imaging process [2]. After the device is carefully installed and the imaging software is connected to the endoscope, research may commence. Two prominent complications arose during the process. Researchers must be aware of the innate physiological response from the body to a foreign object, including bacterium growth and rapid scar tissue formation. To combat this, the endoscope should be removed in a timely manner (< 2 months) [7]. A more eminent complication is in regard to the field of view of imaging. For this reason, our team has plans to alter the standard field of view to accommodate for this problem.

Endoscopes currently on the market have fields of view ranging from 90 degrees to 170 degrees with the most common being around 140 degrees [8]. This limited field of view poses many problems in regard to precision of imaging. In our model, we will utilize a device that stacks three different lenses to diminish this problem. Our device will utilize three specific components: GRadient INdex (GRIN) lens, a laser system, and the naturally occurring labelization (fluorescence) of NSC. The GRIN system works similarly to a conventional lens, but this lens depends on a continuous change of the refractive index [9]. The purpose of the lens is to refract the fluorescence labeled NSC's and allow for the computer system to receive the image. The second component (laser system) works hand in hand with the third component (labeling of NSC's). The laser should be set at a specific wavelength that will optimize excitation. Drawing from previous research, the wavelength should be ≈ 405 nm [10]. When this wavelength comes in contact with the stem cells, the cells will become excited and appear fluorescent [6]. The imaging of these cells can continue while the mice continue to freely behave.

2.2 Client Information

The client for this project is Doctor Jayshree Samanta, a scientist in the Department of Comparative Biosciences here at the University of Wisconsin - Madison. Doctor Samanta and her lab members primarily focus on how adult neural stem cells generate myelin in the brain during development as well as during recovery. This project will use mice in vivo to further investigate how these stem cells migrate longitudinally. She has tasked the team with developing a low cost, highly effective implantable endoscope that will increase the field of view during imaging.

2.3 Product Design Specification

Since the target area for the endoscope placement is in the lateral ventricle of the mouse brain, there are some size and weight restrictions that must be placed on the design. The diameter of the lateral ventricle tapers down, however at the point of observation, the ventricle diameter will be roughly 1.19 mm [11]. The implanted endoscope diameter must therefore be smaller than this diameter. Additionally, it should weigh less than 7 grams [12]. The endoscope and corresponding cannula will be fastened onto the brain of the mouse, which is being observed *in vivo*. Therefore, adding too much weight may render the mouse unable to support its head, or interfere with the natural actions of the mouse's movement. Our client emphasized that our

design should not cause an innate immune response upon placement, as the cells that migrate to the site of implantation can interfere with the desired tracking of the stem cells, as additional cells will be visible in the same location that is being observed. For this reason, our design must use materials that do not harness an immune response. Additionally, the shape of the endoscope should minimize trauma to the mouse brain to reduce potential harm to the mouse, as well as discourage an immune response. The lateral ventricle is filled with cerebrospinal fluid (CSF), and therefore the endoscope must be submersible. The ability to submerge the device includes the ability to maintain clear imaging and visibility, as well as no harm to the device's technology itself. The device should have the ability to be continuously used for up to four weeks, and should be able to be removed and re-implanted multiple times to be reused for at least two years, as specified by the client. Our team's budget has been initially set at $750, however it has been stated that this budget is flexible, and can be adjusted accordingly. For a full breakdown of the product design specifications, see Appendix 7.1.

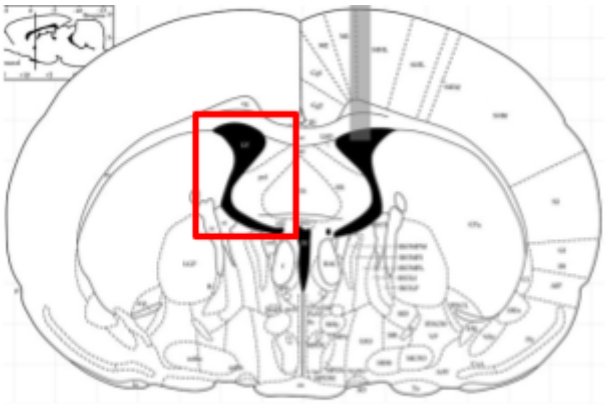


Figure 4: Lateral Ventricle of Mouse Brain [13]

3 PRELIMINARY DESIGNS

3.1 Design 1 - Elongated Lens Design

One method to solve the problem of increased field of view is to incorporate a lens that can refract the input light to more area along the ventricle. Using an elongated lens was a potential solution that the team came up with. As shown in Figure 5, light can initially enter through the top of the cannula in which the elongated lens would send the light out through the microprism and a wider angle. The light would excite more stem cells travelling along the lateral ventricle wall, in which more light will return back into the lens, forming an image with a larger lateral field of view.

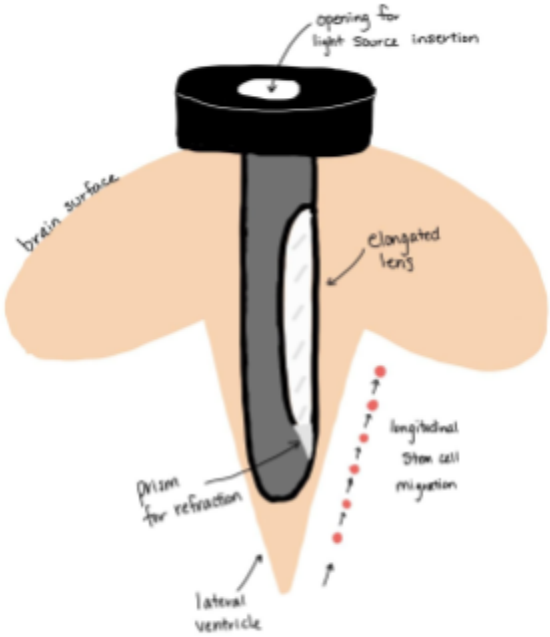


Figure 5: Elongated Lens in Cannula

One advantage of this design is that the elongated lens would create a continuous image as opposed to separate overlapping images. This design is also relatively simple, reducing the amount of moving parts and assembly time.

However, the major disadvantage of this design is the necessity for a custom lens, which would not only be costly in terms of manufacturing, but also would need an extensive understanding of optics. This makes the design difficult to create within the given time frame, as well as difficult for undergraduate students who are not specialized in optics.

3.2 Design 2 - Three GRIN Lenses Outside Brain

This design involves three separate GRIN lenses outside of the mouse's brain. The major constraint the team found was the size of the lateral ventricle being 1.19 mm at the location of study. Commercially available GRIN lenses were found to have diameters of at least 500 µm, making it impossible for these GRIN lenses to exist inside the ventricle side by side. Therefore, by keeping the GRIN lenses outside of the ventricle, this design can bypass that major size restriction. As shown in Figure 6, the cannula instead hosts three microprisms at different latitudes, each associated with their individual lens. This accomplishes the goal of obtaining more lateral imaging of the stem cells travelling up the ventricle. The cannula also contains three chambers that separate light as they travel back into the GRIN lenses to decrease light interference between lenses.

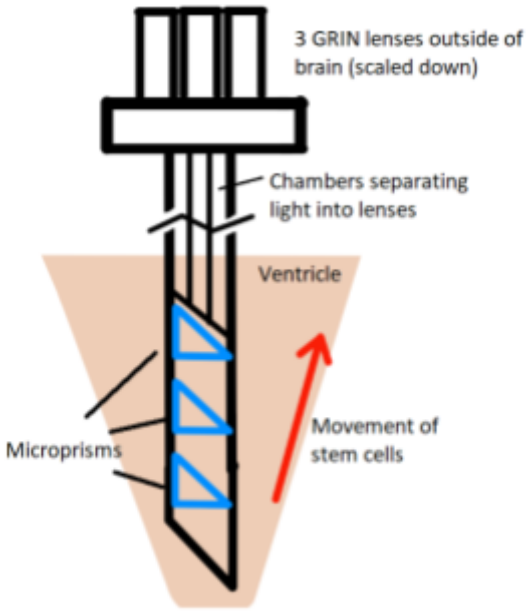


Figure 6: Three GRIN lenses with microprisms in the cannula

The biggest disadvantage this design faces compared to the others is the sheer complexity of the device by virtue of the large number of moving parts. Going forward with this design would require much more research and knowledge with optics which may be unfeasible given our time constraints.

3.3 Design 3 - Three Stacked GRIN Lenses Inside Cannula

The team's third design alternative will utilize three stacked GRIN lenses, each with a microprism attached to the bottom of the lens in order to allow the longitudinal tracking of stem cell movement. The three stacked lenses will allow a field of view three times that of a singular lens.

As shown in Figure 7, the light source will be allowed to enter through the port on the surface of the brain. The light will then be refracted through the lens and prism to excite the stem cells. The now excited stem cells will give off fluorescence which will be detected by the

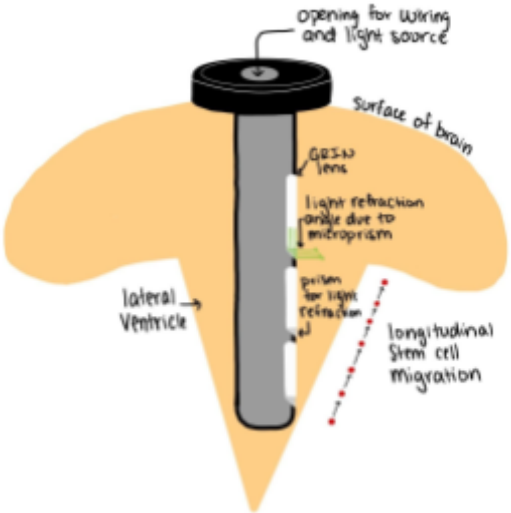


Figure 7: Three Stacked GRIN Lenses Inside Cannula Design

GRIN lenses and that information will be relayed to the computer and put into an image processing system.

One major disadvantage that this design faces is its size. The lateral ventricle has a diameter of about 1.19mm [11], this poses a major issue in that this design would require that not only three GRIN lenses to fit inside of the ventricle but the entire cannula which would be larger than the lenses since the cannula also includes the fiber optic wires and the laser as a light source.

4 PRELIMINARY DESIGN EVALUATION

4.1 Design Matrix

Regarding the three different designs the team came up with, the model which included the three lenses at different depths scored the best. In terms of the criteria, the team decided that feasibility, or in other words, the realisticness that the team could finish this project in a single semester was assumed to be the most vital for this project. Reliability and accuracy was then equally the next most important criteria. The team defined reliability as how the device would be able to withstand use - meaning does it work inside the mouse brain and can it be functional for many years. In terms of accuracy/precision, this criteria is about whether the device can provide enough data for the client to come to conclusions in their study. For safety, the client emphasized how they want this device to be as minimally invasive as possible. The client does not want the stem cells to react to the device because this would impact the migration of the stem cells. Regarding the ease of use category, this device should be fairly simple to grasp what it does and then utilize its features. Finally, in regards to the cost criteria, the client assured us that they were quite flexible when it came to financing the project.

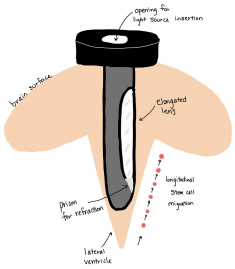
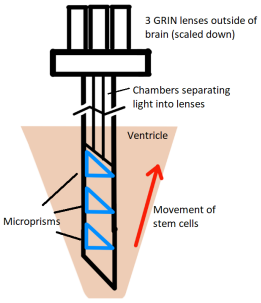
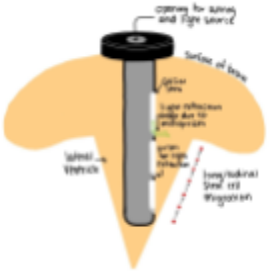
		 **Outside the Brain Lenses**		 **Elongated Lens**		 **Three Lenses at Different Depths**	
Criteria	**Weight**	**Score (10 max)**	**Weighted Score**	**Score (10 max)**	**Weighted Score**	**Score (10 max)**	**Weighted Score**
Feasibility	25	4	10	3	7.5	5	12.5
Reliability	20	7	14	7	14	8	16
Accuracy/Precision	20	5	10	3	6	6	12
Safety	15	8	12	8	12	8	12
Ease of Use	15	5	7.5	8	12	5	7.5
Cost	5	5	2.5	3	1.5	9	4.5
Sum	100	**Sum**	56	**Sum**	53	**Sum**	64.5

Figure 8: Preliminary Design Matrix.

Design 1 - Elongated Lens Design

This design is most similar to the third design in the fact that the GRIN lenses will be inside the brain. However, this design is just a singular, elongated lens which would ultimately maximize the field of view. Seeing that this design only contained one lens, this design scored lower than the third design in regards to the accuracy/precision category. The safety for this design, and similar to the other design's as well, was ranked quite high as there have been many implantable endoscopes which have been utilized in other studies. Therefore, this design will minimize the invasiveness. Additionally, ease of use was ranked the same as the third design as they both are single cannulas with similar dimensions. However, they differ in the make-up of what is inside the cannula.

Design 2 - Three GRIN Lenses Outside Brain Design

This design was pretty similar to the first one in the fact that the feasibility and reliability criteria were scored similarly. However, the accuracy was scored lower because having the GRIN lenses on the outside of the brain, meaning only the light and the prisms are inside the brain, would distort the images and a clear image might not be possible. For ease of use, this design scored the best because fitting the three GRIN lenses inside the very tiny ventricle would not be an issue as they will be outside the brain. This design would be a little more expensive

because it would be quite difficult to have the GRIN lenses on the outside of the brain and only contain the prisms inside the cannula.

Design 3 - Three GRIN Lenses Inside Cannula Design

This design was the most feasible, when compared to the other models. None of the designs would be easy to fabricate and nothing close to any of these designs is currently on the market. Nevertheless, this design was the most feasible for us to complete in the allotted time. This model also scored best when it came to the cost for the individual GRIN lenses which are not too expensive, at around $130. Additionally, this model would be the best for obtaining the results and truly getting the best longitudinal view of the ventricle by utilizing three stacked and staggered lenses.

4.2 Proposed Final Design

The model which scored the best on the design matrix was the third design with the three GRIN lenses inside the cannula, each at different depths. All the designs scored pretty similar but the third design was a step ahead of the competing designs in multiple categories. One of the deciding factors was that the third design scored the highest in feasibility. However, after speaking with optics specialist, Doctor Kevin Eliceiri, he cautioned us not to follow through with this design as this and the other designs simply are not feasible for undergraduate students with the time restriction of the course.

5 DEVELOPMENT PROCESS

5.1 Materials

The materials used in the design will depend on the path that the team follows going forward. Since some unforeseen obstacles have arisen, the team is in the process of obtaining an existing endoscope that is already in use on campus. This device would allow the team to continue with the project and modify the field of view to meet the client's goals.The rigidity of the top of the endoscope will be assessed , as the implementation of a material with slight flexibility would be beneficial for the attachment to the skull of the mouse. A flexible material at the attachment site to the skull would minimize the risk of permanent injury to the mouse.

Competing devices have used GRIN lenses in the brains of the mice and this has posed no apparent issue. One material used in mouse brains to hold the GRIN lens in place was a polyimide cannula [14]. This cannula was used to hold the GRIN lens in place. Since polyimide becomes elastic and stretchy when submersed in liquid environments this could cause a problem with the use of our device [15]. However, long term use showed great biocompatibility when the material was used as a bioelectrode [14]. The use of polyimide biomaterials in animal research has been successful but more research is needed to determine the long term cytotoxicity[14]. Initially, the team planned to research materials that would be used from the team's constructed

endoscope. However, since the team will be using an existing endoscope, the team will compile a list of materials that are going to be used from the device. The materials will then be analyzed to determine if any immune response would come from implantation.

5.2 Methods

This device consists of the cannula which will be the part that is inside the mouse brain and a connection port outside of the brain. The cannula will house fiber-optic wires that connect the GRIN lenses and microprisms to the electronic components that are housed outside of the brain that will be attached via the connection port. The cannula will also house the laser which acts as the light source in order to excite the stem cells. The purpose of having the microprisms attached to the bottom of the GRIN lenses is to refract the laser beams at a 90° angle. The endoscope can then track the stem cells as they move from deeper inside the lateral ventricle out towards the surface of the brain. Once the laser hits the stem cells and they become excited, they fluoresce, giving off light. This fluorescence is then detected by the GRIN lens and relayed back up through the cannula to the computer and can be analyzed using an image analysis system.

5.3 Testing

Our design will be tested both to verify submersible capabilities and its ability to track stem cells longitudinally. In order to make sure that the device can withstand placement in CSF for a large duration of time, our team plans to directly submerse the implantable part of the design into CSF. This will be done without the involvement of a mouse first. Our client has informed us that they are easily able to obtain a large sample of cerebrospinal fluid, likely from a horse, in order to test in the same substance that the endoscope will be exposed to upon implantation. After the device has been submerged for at least several days, the capabilities of the endoscope will be compared to those from prior experiments in order to ensure there are no shortcomings in the design.

In order to test the accuracy of the field of view given by the GRIN lenses and to ensure that the field of view is wide enough, the team plans on testing the design in a phantom gel matrix using fluorescent beads. The fluorescent beads will act as stem cells and allow the team to test the accuracy of the design by ensuring that this design is able to track how stem cells migrate in response to trauma. By using this method to test the field of view, it allows the team to make adjustments to the spacing between each of the lenses which will then ensure that there are no overlapping images or blindspots that could possibly occur due to the lens spacing.

6 CONCLUSIONS

Overall, the third design including three stacked GRIN lenses seems to give the best and most feasible solution to the client's problem. However, after sharing our designs with Dr. Kevin Eliceiri, a BME professor with a focus in optics, the team determined that these designs will not be feasible due to a limited understanding of optics and time constraints. After discussing some other options with Dr. Eliceiri the team has decided on two possible options that would work to solve the client's problem. The first solution would take a mechanical approach to improving the field of view of an existing device. The team would take an existing lens setup and attach it to a mechanical rig that will physically move the lenses longitudinally to track the stem cell migration. The other solution would be to use an existing endoscope and test its existing field of view in a phantom gel matrix, using fluorescent beads in place of migrating stem cells, then use data from the initial field of view test to develop ways to improve the field of view. Ultimately, the final design choice will not be made until the team meets with the client to hear their input. Regardless of the final design choice, the data that the team plans on obtaining will help future teams to build a physical working prototype for the client.

7 REFERENCES

[1] C. Villa et al., “Stem cell tracking by nanotechnologies,” Int. J. Mol. Sci., vol. 11, no. 3, pp. 1070–1081, 2010.

[2] S. Malvaut, A. Marymonchyk, A. Gengatharan, and A. Saghatelyan, “Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes,” *STAR Protoc*, vol. 2, no. 2, p. 100596, 2021. Available: https://starprotocols.hivebench.com/protocols/723.

[3] M. S. Pochechuev, M. A. Solotenkov, I. V. Fedotov, O. I. Ivashkina, K. V. Anokhin, and A. M. Zheltikov, “Multisite cell- and neural-dynamics-resolving deep brain imaging in freely moving mice with implanted reconnectable fiber bundles,” J. Biophotonics, vol. 13, no. 11, p. e202000081, 2020.

[4] Trulson et al, "Systems and methods for optogenetic imaging," US 20180303573 A1, Oct. 25, 2018.

[5] Inscopix, Inc, “NVista miniature microscope A deep brain calcium imaging dynamics,” *Inscopix.com*. [Online]. Available: https://www.inscopix.com/nvista.

[6] Y. Zheng et al., “Stem cell tracking technologies for neurological regenerative medicine purposes,” Stem Cells Int., vol. 2017, p. 2934149, 2017.

[7] M. Brückner, P. Lenz, T. M. Nowacki, F. Pott, D. Foell, and D. Bettenworth, “Murine endoscopy for in vivo multimodal imaging of carcinogenesis and assessment of intestinal wound healing and inflammation,” J. Vis. Exp., no. 90, 2014.

[8] Q. Wang, A. Khanicheh, D. Leiner, D. Shafer, and J. Zobel, “Endoscope field of view measurement,” Biomed. Opt. Express, vol. 8, no. 3, pp. 1441–1454, 2017.

[9] GRINTECH GmbH, “Gradient Index Optics,” Grintech.de. [Online]. Available: https://www.grintech.de/en/gradient-index-optics/. [Accessed: 18-Oct-2021].

[10] W. G. Telford, J. Bradford, W. Godfrey, R. W. Robey, and S. E. Bates, “Side population analysis using a violet-excited cell-permeable DNA binding dye,” Stem Cells, vol. 25, no. 4, pp. 1029–1036, 2007.

[11] “ISH data :: Allen Brain Atlas: Mouse brain,” ISH Data :: Allen Brain Atlas: Mouse Brain. [Online]. Available: https://mouse.brain-map.org/.

[12] Barbera, G., Liang, B., Zhang, L., Li, Y., & Lin, D.-T. (2019). A wireless miniScope for deep brain imaging in freely moving mice. Journal of Neuroscience Methods, 323, 56–60.

[13] B. Seyer, V. Pham, A. L. Albiston, and S. Y. Chai, “Cannula implantation into the lateral ventricle does not adversely affect recognition or spatial working memory,” Neuroscience Letters, 21-Jun-2016. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0304394016304438.

[14] G. Meng et al., “High-throughput synapse-resolving two-photon fluorescence microendoscopy for deep-brain volumetric imaging in vivo,” Elife, vol. 8, p. e40805, 2019.

[15] C. P. Constantin, M. Aflori, R. F. Damian, and R. D. Rusu, “Biocompatibility of polyimides: A mini-review,” Materials (Basel), vol. 12, no. 19, p. 3166, 2019.

8 APPENDIX

8.1 Product Design Specifications

Client Requirements:

- Design a biocompatible implantable device
- The device should incorporate three lenses
- Include a laser that shines into the cavity of the brain
- Design the cannula to hold the endoscope

Function:

Current setups for imaging intact neural networks only allow for imaging through a field of view in a single lens. This setup only allows for imaging in a restricted area of the tissue. An endoscope with multiple fields of view is required for accurate tracking of cell migration dynamics in mice that are awake and functioning normally. The design of this endoscope must be small in size and weight and must allow the mouse to maintain normal function and behavior. The endoscope must be able to function in the fluid region of the brain. The endoscope will be powered through a connection port and must be compatible with a Windows OS computer where images will be uploaded using basic software to communicate.

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements: This device must be able to run without interruption while attached to the mouse. Additionally, due to the nature of the test, the device must be fluid resistant as the lenses will be inserted into the brain where interstitial and cerebrospinal fluid is abundant. This implies that the lens will also need to compensate for any refractions in the fluid while maintaining accuracy [1]. This device must also resist wear and tear over the course of many experiments since it will be reused among different mice.

b. Safety: The device will be accompanied by a laser light source. The intention of this laser is to excite cells within the ventricle to obtain visible information. The strength of this laser may be strong enough to irritate sensitive tissues, so safety precautions must be taken when handling the laser outside of experimental use. All small parts will be encased within the tube of the device, leaving little to no harmful imperfections on the device's surface. Additionally, sterilizing between usages is recommended to prevent any unwanted contamination.

c. Accuracy and Reliability: The fourth ventricle of the mouse brain has a maximum width of around 1700 microns. Given this restriction, the device must be able to clearly record imaging of fluorescent stem cells within 1700 microns from the lens [2]. As requested by the client, the

device will also contain multiple lenses, increasing its field of view and depth. In order to maintain consistency between tests, the lenses must be set securely into the device to minimize any lens shifting.

d. Life in Service: This device must be durable enough to withstand months of use. The device must be able to operate on a continuous basis for periods of time extending from two days at the lowest, to three months at the highest. In order to reduce built up residue in between parts of the device, after use soak the implant for approximately twelve hours in a mixture of distilled water and soap.

e. Shelf Life: While not in use, the device will be stored at a standard laboratory environment. The temperature will be around 20°C or 68°F [3]. The device will be sterilized, reprocessed, and flushed with alcohol before being hung in storage. The standard shelf life should be no longer than approximately twelve weeks, any longer could result in an increase in microbial growth.

f. Operating Environment: The device will be used in a standard laboratory environment of about 20°C and be able to withstand 30% relative humidity. Before use, the device must go through extensive cleaning to ensure a sterile environment.

g. Ergonomics: The device must be easily installed, cement is the current standard for attachment. While a platform may be used for additional stability, it would be ideal to avoid this since it could have an impact on how the mice behave. The portion of the endoscope that will be outside the mouse brain should have an easily accessible connection port to export the images. The device as a whole should be as user friendly as possible and be practical for regular laboratory use.

h. Size: This design must be small enough that its size will not impact the normal behavior and movement of the mouse it is placed in. This device must not exceed the dimensions of 25mm x 25mm x 30mm. Staying within these specified dimensions will ensure the mice are not affected by the size. Since the lenses are the only portion of this device that will be installed into the mice these should be as small as possible without impacting the image quality. The diameter of the lens to be used is 1 mm with a length of 1.883 mm [4].

i. Weight: This device must be lightweight enough to be able to be supported by a mouse head, preferably this device will be as lightweight as possible. Ideally this mini endoscope will not weigh more than 7 grams, this weight is an estimate based on other devices currently available for lab use [5].

j. Materials: As a requirement of the client, GRIN lenses will be used. The materials implanted into the brain should be free of contaminants [6].

k. Aesthetics, Appearance, and Finish: The array of lenses should be prism shaped since this reduces the amount of damage caused to the brain tissue and fits well in the lateral ventricle [7].

2. Production Characteristics

a. Quantity: The client has requested one functional implantable endoscope utilizing three GRIN lenses.

b. Target Product Cost: The budget for this project is $750, however it is a flexible budget that can be moved accordingly. The GRIN lenses we will be using cost approximately $150. The final product will ideally cost less than $400 to allow for prototyping as well. Only one final implantable endoscope needs to be made.

3. Miscellaneous

a. Standards and Specifications: Currently, there is a neurological endoscope listed by the FDA, as well as many similar endoscopes, but none are specifically implantable endoscopes. We would like to adhere to the standards listed for these devices, and will need to meet Class II FDA standards that require a Premarket Notification 510(k) [8]. Class II FDA classification places this device under general controls and special controls.

b. Customer: The client would like for the final product to have three GRIN lenses incorporated, instead of just one. The client also requests that a cannula be created to attach to the implantable endoscope, and that software to analyze the findings is created as well. The main priority for the semester is to develop a working endoscope first in order to meet the client's primary request.

c. Patient-related concerns: Since this device will be made for mice, one will have to consider the experimental ethics that comes with working with animals. As we design the device, we will have to be conscious of the rules and limitations when it comes to what is safe and proper for animal experimentation. Since the device will be used on multiple mice it will have to be properly cleaned in between subject changes.

d. Competition: There have been studies conducted ex vivo in mice lung tissue that utilized two-photon microscopy in addition to GRIN lenses. Such experiments yielded conclusive results about how three-photon microscopy, which has its advantages, is possible [9]. In 2007, researcher Murayama and his team created a one-photon endoscope with a GRIN lense. Their probe was quite invasive but it did utilize a microprism which allowed for a 90 degree angle to film the dendrites they were studying [10]. Other researchers took simpler, less invasive approaches and were semi-successful in imaging the brain. Such studies, like done by Osanai, were able to capture cellular fluorescence images and the detection of calcium ions. But their

setup couldn't track the single cell activity of the calcium ions [11]. Essentially, many groups have attempted to capture brain activity in vivo but not many have been completely successful.

PDS Works Cited

[1] B. Bedussi *et al.*, "Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system," *Fluids Barriers CNS*, vol. 12, no. 1, p. 23, 2015.

[2] "Interactive Atlas Viewer :: Atlas Viewer," *Brain-map.org*. [Online]. Available: http://atlas.brain-map.org/atlas?atlas=1&plate=100960456.

[3] M. A. Funovics, H. Alencar, H. S. Su, K. Khazaie, R. Weissleder, and U. Mahmood, "Miniaturized multichannel near infrared endoscope for mouse imaging," *Mol. Imaging*, vol. 2, no. 4, p. 153535002003031, 2003.

[4] *Thorlabs.com*. [Online]. Available: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=11167.

[5] Barbera, G., Liang, B., Zhang, L., Li, Y., & Lin, D.-T. (2019). A wireless miniScope for deep brain imaging in freely moving mice. *Journal of Neuroscience Methods*, *323*, 56–60.

[6] *Nih.gov*. [Online]. Available: https://grants.nih.gov/grants/olaw/national_academies_guidelines_for_use_and_care.pdf.

[7] *Hivebench.com*. [Online]. Available: https://starprotocols.hivebench.com/protocols/723.

[8] "Product classification-Neurological Endoscope," *accessdata.fda.gov*. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=3771.

[9] D. M. Huland, K. Charan, D. G. Ouzounov, J. S. Jones, N. Nishimura, and C. Xu, "Three-photon excited fluorescence imaging of unstained tissue using a GRIN lens endoscope," *Biomed. Opt. Express*, vol. 4, no. 5, pp. 652–658, 2013.

[10] M. Murayama, E. Pérez-Garci, H.-R. Lüscher, and M. E. Larkum, "Fiberoptic system for recording dendritic calcium signals in layer 5 neocortical pyramidal cells in freely moving rats," *J. Neurophysiol.*, vol. 98, no. 3, pp. 1791–1805, 2007.

[11] M. Osanai *et al.*, "Development of a micro-imaging probe for functional brain imaging," *Neurosci. Res.*, vol. 75, no. 1, pp. 46–52, 2013.