Longitudinal Tracking of Fluorescent Stem Cells in the Mouse Brain

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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. 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 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 decided to pursue determining the field of view of an existing device that will be loaned to them by another Biomedical Engineering professor. Initial testing of the field of view of the device was completed and the results were analyzed using ImageJ software.

Background

Motivation:

- Current endoscopes on the market have a limited field of view.
- Be able to accurately track adult neural stem cells longitudinally in the mouse brain in-vivo, by increasing the field of view.

The 3 Components:

- GRadient INdex (GRIN) lens: similar to a conventional lens, with the additional capability to refract the image.
- Laser system: the laser will be set to a specific wavelength (≈455nm) [1] that should optimize excitation of the stem cells.
- Labeling of adult neural stem cells: the adult neural stem cells contain a protein (GFP and mCherry) that when in contact with the laser system, results in fluorescence.

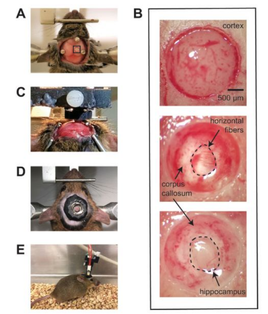

Figure 1: Procedure depicting endoscope attachment to the ventricle of the mouse [2].

Client:

- Dr. Jayshree Samanta is a scientist in the UW-Madison department of Comparative Biosciences. Dr. 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.

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's lateral ventricle.

Design Criteria

- Small in size; Lateral Ventricle Ø 1.19mm [3]
- Lightweight, ideally < 7 grams [4]
- Allow for up to four weeks of continuous acquisition
- Does not mount an innate immune response upon incorporation
- Withstand being submerged in cerebrospinal fluid
- Aim to adhere to a flexible $750 budget

Figure 2: A breakdown of design criteria, as well as respective weights.

Design Process

- Define: We met with our client and learned about their research and their need for a neurological endoscope with a wider field of view to track stem cell movement.
- Brainstorm: Devised three different ideas and moved forward with a three stacked GRIN lens design to optimize the field of view to track stem cells vertically.
- Challenge: After consulting with optics specialist Dr. Eliceiri, we realized that the project was not feasible within our time constraints due to our lack of knowledge and experience.
- Next Step: We shifted to gathering preliminary data from already existing tools on the market. We designed a protocol and recipe to create multiple gels with differing concentrations of microfluorescent beads to simulate a mouse brain ventricle. Dr. Trevathan let us gather data via the Inscopix microscope to study these samples.
- Improve: A field of view and effective distance can be found for future experiments to be further improved on.

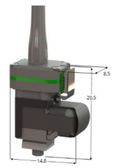

Figure 3: Inscopix system endoscope (nVoke miniscope)[1].

Results

Testing Procedure:

Materials:

- 0.125oz of unflavored gelatin
- 19oz of distilled water
- Fluorescent Green Polyethylene Microspheres 27-32 microns
- Inscopix nVoke miniscope

Procedure:

1. Create the matrix in the petri dish and let set overnight.
2. Set up the miniscope by adjusting the height, gain, EX-LED Power, and lens focus settings.
3. Take a video of the fluorescence of the beads moving over the surface of the matrix from left to right, ensuring that singular beads can be seen.
4. Export the video and analyze it using ImageJ software.

Testing Results:

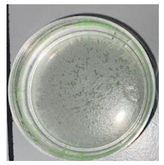

Figure 4: The matrix containing the green fluorescent beads used for testing.

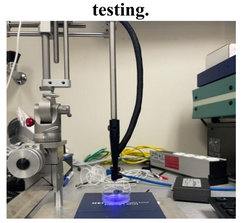

Figure 5: The setup used for testing. A microscope stand was used to lower and raise the microscope and move it left to right over the top of the matrix.

- The field of view of this endoscope is 1280 x 800 pixels, or 1050 x 650 µm [1].
- Different lens can be attached to increase the field of view of the microscope.
- The setting used was the smallest field of view.
- The corrected total cell fluorescence could be found by subtracting out the background noise [5].

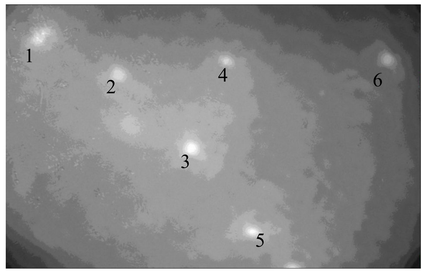

Figure 6: An image from the nVoke software. Singular beads can be as glowing white dots labeled 1 through 6 and are used to mimic stem cells.

Statistical Analysis

Measuring Fluorescence Intensity:

This analysis was used to determine the total fluorescence of each bead by subtracting out the background fluorescence signal.

- Using imageJ software the mean fluorescence of each bead and the mean fluorescence from spots with just background fluorescence were calculated.
- Using the equation: Total Fluorescence = Integrated Density - (Area of Bead*Average Background Fluorescence) [5].

Bead Number	Fluorescent Intensity (µm²)
1	1098664
2	1081531
3	1469189
4	620671.8
5	1203242
6	224385.7

Table 1: Each beads fluorescent activity was calculated. Bead 3 was found the have the greatest fluorescent intensity.

- From this, it was concluded that the beads in the center of the field of view had a greater fluorescence intensity.

Future Work

- Determine if adequate and improved field of view can be obtained without adding additional lenses
- Determine if the best way to improve field of view is by adding additional GRIN lenses
  - If so, determine the number of lens to obtain ideal field of view
- If design is adequate for the client, test design in the mouse brain

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- Dr. James Trevathan
- Dr. Daniel Radecki
- BME Department

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