BME Design-Fall 2021 - LAUREN HELLER
Complete Notebook

PDF Version generated by

LAUREN HELLER

on

Dec 13, 2021 @08:22 PM CST

Table of Contents

Team contact Information

DANA STUMPFOLL - Sep 14, 2021, 6:37 PM CDT

Last Name	First Name	Role	E-mail	Phone	Office Room/Building
Gong	Sarah	Advisor	shaoqingong@wisc.edu		
		Client			
Stumpfoll	Dana	Co-Team Leader	dstumpfoll@wisc.edu	262-745-9893	
Heller	Lauren	Co-Team Leader	lnheller@wisc.edu	414-731-8249	
Block	Alexis	Communicator	atblock2@wisc.edu	612-559-7860	
Anderson	Tyler	BPAG	tanderson47@wisc.edu	763-77-1237	
He	Alex	BWIG	ahe7@wisc.edu	630-290-8020	
Makonnen	Rebekah	BSAC	rmakonnen@wisc.edu	763-445-0305	

Project description

Rebekah Makonnen - Sep 17, 2021, 10:45 AM CDT

Course Number:

BME 200- 27695

BME 300- 27706

Project Name:

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

Short Name:

Stem Cell Trackers

Project description/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.

About the client:

Motivation Statement

LAUREN HELLER - Oct 19, 2021, 11:15 AM CDT

Title: Project Motivation Statement

Date: 10/19/2021

Content by: Team

Present: N/A

Goals: Establish an understanding of motivation and possible project impact.

Content:

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. 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, as well as in a healthy individual's brain in the future.

Currently, there are no viable ways to track stem cells longitudinally to the extent that our team aims to track. By creating a wider field of view, stem cells will not only be able to be observed, but new conclusions can be drawn based on the migration patterns and fluorescence observed.

Conclusions/action items:

N/A

Initial Client Meeting

Rebekah Makonnen - Sep 17, 2021, 12:27 PM CDT

Title: Initial Client Meeting

Date: 9/16/2021

Content by: Dana, Lauren, Alex, and Lexi

Present: Dana, Lauren, Alex, and Lexi

Goals: Gain a comprehensive understanding of the clients wants for this project

Content:

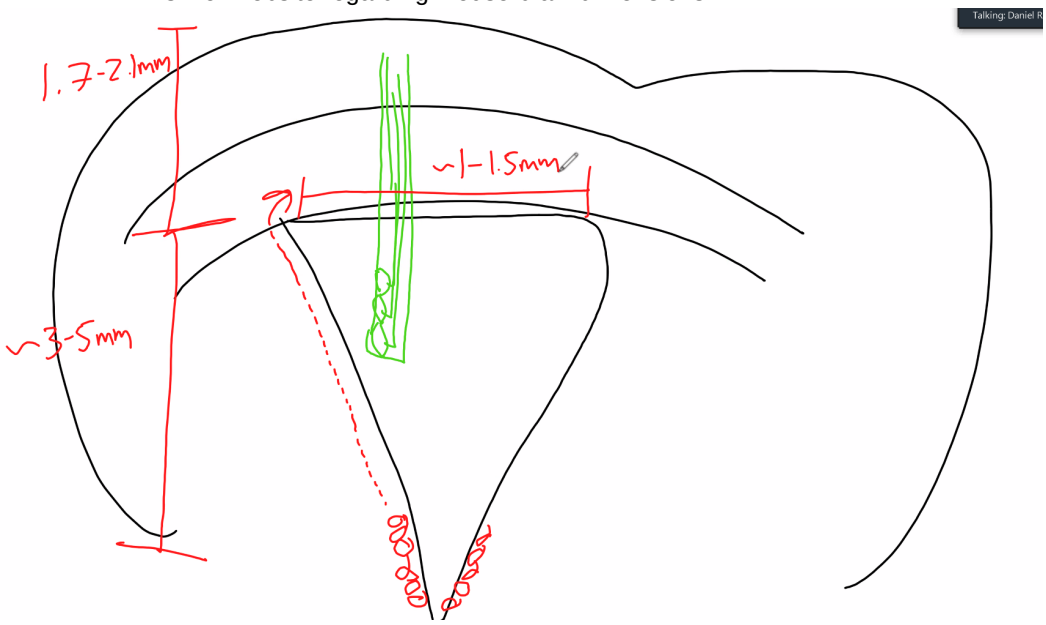
Questions for the client

- Will multiple lenses be provided to us? Yes.
- What is the size of the environment the mice will be housed in?
    - Standard mouse cage. Find dimensions.
- How is the endoscope secured into the brain of the mouse?
    - Cement is a standard practice.
    - Can attach a platform.
- Specific size specifications?
    - Will be sent to us
- What type of materials/information should we expect to be provided by you? What should be provided by us instead?
    - Lenses provided, rest on us

Meeting Notes:

- How neural cells migrate in the healthy brain and how they migrate after injury?
- Induce loss of myelin in the brain
- Currently hard to observe process in vivo, usually done after euthanization or in a dish
- Preproposal written by Dan and Abby
    - Biocompatible implantable device
- Survive in wet environment for prolonged periods of time
- South north directional tracking, tail is south, want it facing east west to make a 90 degree turn
- On the scope of mm, maybe ½ cm,
- **Stack grin lenses** and have them output individually or as an overlapping image
- **Array of lenses - prioritize this**
- Budget of $750 minimum - lenses are covered?
- Email Dan about mouse brain CAD or software that mimics the mouse brain
- Looking for software for binary monochrome imaging, fiber optics coming out to some unit
- Way to excite cells with a laser and observe the cell population.
- Embedded laser that shines into the cavity.
- Carries light into the cells where they can fluoresce.
- Need cannula part as well.
- Look into paper on one lens and learn how to add two or three
- Implanting a laser is standard now
- Priority is something implantable with lens array
- Need an acquisition light path to make a 90 degree turn.
- East to west visualization of cell tracking south to north

- Design thoughts:
    - Lens on side of cannula
- The lenses don’t have very many properties.
- Ask for CSF Properties
- Would need to determine how much refraction the csf will cause from the light.
- Front view cross section of mouse brain
    - Ask for website regarding mouse brain dimensions



Conclusions/action items:

Begin working on the product design specifications based on information from the clients and meet with our advisor.

Client Meeting 2: 10/01/2021

LAUREN HELLER - Oct 01, 2021, 11:49 AM CDT

Title: Secondary Client Meeting

Date: 10/01/2021

Content by: Lauren Heller

Present: All group members present

Goals: Further discuss details of the project and gain a deeper understanding of what goals are to be accomplished.

Content:

The team met with Dr. Samanta and Daniel to further discuss the project. Meeting notes detailed below.

Meeting Notes:

-The product should be able to be reused for 2-3 years ideally. It should also have a little bit of flexibility to it, as that makes it less likely to cause injuries in the mounting and removing procedures.

- There should be an opening in the center of the endoscope to allow light to reach each lens.

-Can use multiple GRIN lenses or could engineer an elongated lens

-Ideally we should incorporate a coating to minimize damage, it would need to be an inert material that does not mount an immune response, otherwise these cells will also be visible, compromising the study. We will be able to test the design in cerebrospinal fluid to make sure that it is still functional after being submerged. In a healthy individual, CSF has a similar viscosity to water

- Need to avoid phototoxicity when designing, too bright of a light causes cell death, which would be detrimental to the research results

- Be conscious of autofluorescence, if another cell emits at the same wavelength, this will cause them to appear as stem cells, when they are not. Aim for the red spectrum, not the green.

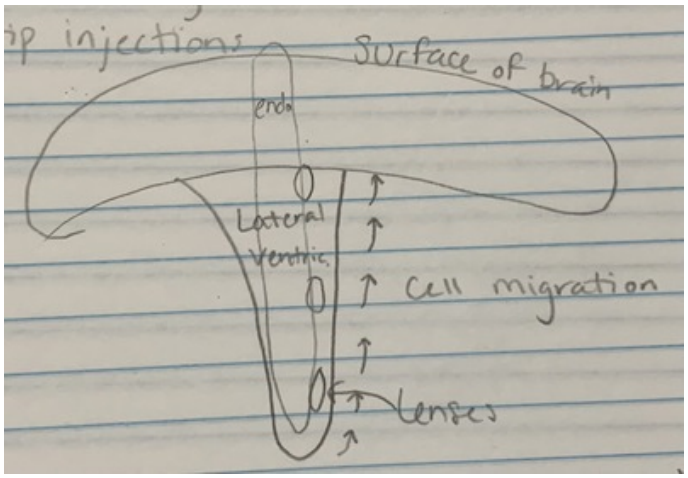
-All lenses need to be on one side.

-Look into "Teenie tomato excitation and emission"?

Conclusions/action items:

Conduct further research with updated information, rule out designs that do not meet needed components, modify design ideas to better incorporate knew knowledge.

LAUREN HELLER - Oct 01, 2021, 11:41 AM CDT



IMG_8259.jpg(283.3 KB) - download

10/29/2021- Progress and Upcoming Plans

LAUREN HELLER - Oct 29, 2021, 12:32 PM CDT

Title: Project Progress and Upcoming Plans Meeting

Date: 10/29/2021

Content by: Lauren Heller

Present: All team members were present.

Goals: Communicate with client regarding project plans for the duration of the semester.

Content:

The team met with our client this morning in order to update them after the meeting with Dr. Eliceiri. They agreed that it will be a good start to focus on gathering data using the miniscope from Dr. Trevathan, as they currently do not have data to go off of. The phantom gel matrix will be used to avoid the use of a mouse this semester. The client needs to be able to track the cells in a field of view between 1mm and 2mm, and needs to be able to see single cells. From the data that we are able to obtain, either another BME team or group of engineers can further the project.

Conclusions/action items:

Research potential ways to make a phantom gel matrix in order to plan for testing. Develop a testing protocol. Prepare for Show and Tell.

Initial Advisor Meeting

Rebekah Makonnen - Sep 17, 2021, 2:28 PM CDT

Title: First Advisor Meeting

Date: 9/17/2021

Content by: Rebekah

Present: Team

Goals: Introduce the team to our advisor and gain a general understanding of the speed and course dynamics.

Content:

- Went over individual introductions about each team member
- Given expectations for this semester
- Discussed where to find the course schedule and other important resources
- Talked about making mentor-mentee relationship between each 200 and 300 on the team
- Got information about lab information since it is likely that this project require lab access this semester
- Discussed PDS specifics and how the client meeting went

Conclusions/action items:

- Work on PDS
- Complete more research as necessary

10/1/21- Advisor Meeting

Rebekah Makonnen - Oct 01, 2021, 1:55 PM CDT

Title: Advisor Meeting

Date: 10/1/21

Content by: Rebekah

Present: Team

Goals: Meet with advisor and presented brainstormed design ideas

Content:

- Discussed and explained design matrix criteria
- Explained the individual team member designs
- Discussed the need to go back and revise original brainstormed ideas in order to meet their requirements after meeting with the clients
- It might be helpful to the project to consult with a professor that has a specialty with microimaging- Kevin W Eliceiri
- Discussed possible ways to have an adequate light source- most feasible idea is to use micro prism to reflect the light source by 90 degrees.

Conclusions/action items:

Revise original designs to what the client wants. Finish up design matrix, evaluate designs and determine final design that we will be fabricating. Begin working on preliminary deliverables.

10/08/2021-Advisor Meeting

LAUREN HELLER - Oct 12, 2021, 7:46 PM CDT

Title: Advisor Meeting 3 Notes

Date: 10/08/2021

Content by: Lauren Heller

Present: All group members were present.

Goals: Discuss plan of action for preliminary deliverables and share design matrix.

Content:

In the meeting, we shared our three designs and sketches, as well as our design matrix. In our design matrix, we evaluated the designs to ultimately decide on a design that implements three GRIN lenses inside the canula. This will be the design we plan to continue for the rest of the semester. Dr. Gong recommended that we meet with Dr. Eliceri for consulting regarding the feasibility of our design and how to go about designing our product.

Conclusions/action items:

Schedule a meeting with Dr. Eliceri and prepare the Preliminary presentation and report.

Meeting 1: 9/12/2021

LAUREN HELLER - Sep 12, 2021, 12:27 PM CDT

Title: Team Meeting 1

Date: 9/12/2021

Content by: Lauren Heller

Present: All group members were present.

Goals: Establish a plan of action for first week tasks.

Content:

All members of the group met on zoom to discuss tasks that needed to be completed in the first week. We discussed strategies to find research articles, as well as how to prepare for a client meeting. All group members will complete some initial individual research, and findings will be shared with the group on Tuesday. From this, we will collaborate on a problem statement, and fill out the rest of the progress report. The group figured out time slots to suggest to our client, and we will arrange a meeting sometime during the upcoming week.

Conclusions/action items:

The group needs to complete some initial research, brainstorm questions for the client meeting, and complete the week's progress report.

Meeting 2: 9/14/2021

LAUREN HELLER - Sep 14, 2021, 6:45 PM CDT

Title: Team Meeting 2

Date: 9/14/2021

Content by: Lauren Heller

Present: All group members were present.

Goals: Share individual research findings, work on progress report, and brainstorm questions for client meeting.

Content:

All members of the team met on zoom to share our research. From this research, we were able to come up with an initial problem statement (that may be modified after the client meeting, as needed). Additionally, we worked on setting up and completing the first week's progress report so that it can be finalized and submitted. As our client meeting approaches, we decided it would be a good idea to put some thought into questions that we would like to ask our client in our Thursday meeting. Each team member will spend some time brainstorming questions which will be compiled into a prepared list for our meeting.

Conclusions/action items:

Continue research, attend and document client meeting, and adjust problem statement as needed.

Meeting 3: 9/24/2021

LAUREN HELLER - Sep 24, 2021, 1:21 PM CDT

Title: Team Meeting 3 - PDS Draft Finalization

Date: 9/24/2021

Content by: Lauren Heller

Present: All group members present

Goals: Meet as a group to work on the PDS and take a team photo.

Content:

The team met at Union South to wrap up the first PDS draft. Each team member worked individually on designated parts over the week, and we met to finalize the content and prepare it for submission. The team will have an advisor meeting at 1:30 to discuss the week's plans. The team also took a team photo which will be uploaded to our website.

Conclusions/action items:

Come up with criteria for the design matrix, and rank our design ideas using the design matrix.

Meeting 4: 9/29/2021

LAUREN HELLER - Sep 29, 2021, 10:25 PM CDT

Title: Team Meeting 4 - Design Ideas and Design Matrix Criteria

Date: 9/29/2021

Content by: Lauren Heller

Present: All group members present.

Goals: Discuss brainstormed design ideas, decide on decision matrix criteria.

Content:

The team met over zoom to discuss the design ideas that we each brainstormed individually. Each group member used their research and knowledge of the design's problem statement to come up with potential ways in which we can solve the problem at hand. After sharing our ideas, we all discussed how certain designs could be mixed with others, and what we liked and disliked about each design. Following this, we came up with a set of criteria that we would like to judge our top design ideas on in order to narrow it down to one design to pursue for the rest of the semester. We also worked on this week's progress report, and made sure to include our design criteria and potential design ideas.

Conclusions/action items:

Meet with the client on Friday to gain more knowledge and find out if they have any preference on criteria (i.e. reliability is most important, would rather increase the budget to account for that if need be, etc).

Meeting 5: 10/05/2021

LAUREN HELLER - Oct 07, 2021, 11:00 PM CDT

Title: Design Ideas and Matrix Meeting

Date: 10/05/2021

Content by: Lauren Heller

Present: All team members were present.

Goals: Discuss new modified design ideas and evaluate on a decided set of criteria.

Content:

The team met to share new ideas after having our second client meeting. From this, we picked three different designs to evaluate using the design matrix. After evaluation, we were able to have one final preliminary design to further for the duration of the semester. Additionally, we worked on the progress report to prepare it for submission. The team will meet again on friday to work on getting things in order for the preliminary presentation and report.

Conclusions/action items:

Work on preliminary deliverables for next week, work on better drawings or solidworks files for the presentation.

Meeting 6: 10/08/2021

LAUREN HELLER - Oct 12, 2021, 1:03 PM CDT

Title: Meeting 6 Notes

Date: 10/08/2021

Content by: Lauren Heller

Present: All team members were present

Goals: Finalize design matrix and organize preliminary deliverables

Content:

The team met on Friday prior to the advisor meeting in order to sort out portions of the project. We began by creating better sketches of our three preliminary designs. We also finalized our design matrix to show in our advisor meeting. We also divided up portions of the presentation and report.

Conclusions/action items:

Work on designated preliminary deliverable sections and meet again to collaborate and practice for Friday Presentations.

Meeting with Dr. Eliceri

DANA STUMPFOLL - Oct 12, 2021, 10:11 PM CDT

Title: Discuss design ideas with Dr. Kevin Eliceiri

Date: 10/11/2021

Content by: Dana

Present: Dana, Alexis, Alex, Beki

Goals: Get advice and suggestions from Dr. Eliceiri on our designs

Content:

The team met with Dr. Eliceri to discuss our design and see if he had any advice. From this meeting Dr. Eliceri told us that our three lens design was not feasible and is a PhD level design. He said it would not be feasible to create in one semester and suggested we build off of an endoscope that has already been created. He suggested we take a mechanical approach where we design a canula in which the lens could move inside so that the cells could be tracked. He also suggested that we modify the prism idea to increase the field of view. From this we determined we need to reevaluate our designs and come up with new ones.

Conclusions/action items:

Redesign the designs after preliminary presentations. Contact the client and ask if they would prefer a mechanical moving design or a stationary design with a wider field of view. Work on the report with the new design and discuss why our previous design is too complex to be created in one semester. Keep in contact with Dr. Eliceiri about the mini endoscope we might use from his colleague.

Meeting 7: 10/12/2021

LAUREN HELLER - Oct 12, 2021, 7:27 PM CDT

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items:

Meeting 8: 10/18/2021

LAUREN HELLER - Oct 19, 2021, 11:03 AM CDT

Title: Preliminary Deliverables Meeting

Date: 10/18/2021

Content by: Lauren Heller

Present: All team members were present.

Goals: Establish tasks that need to be completed this week as a team.

Content:

Team To-Do:

-Finish Preliminary Report editing for submission by 10/19

-Finish Notebook edits by 8pm on 10/19 to allow for downloading time

-Set up meeting with James and Kevin, potentially client as well (or update email)

-Research possible materials for modifications

The team met briefly to establish a list of tasks that need to be completed this week. From there, we branched off to work on editing the report individually so that it can be touched up and prepared for submission.

Conclusions/action items:

Complete tasks and submit preliminary deliverables.

Meeting with Dr. Eliceiri and Dr. Trevathan

LAUREN HELLER - Oct 26, 2021, 5:45 PM CDT

Title: Meeting with Dr. Eliceiri and Dr. Trevathan

Date: 10/26/2021

Content by: Lauren Heller

Present: Lauren Heller, Dana Stumpfoll, Alex He

Goals: Meet with Dr. Kevin Eliceiri and Dr. James Trevathan to discuss the plan of action for the duration of the design process.

Content:

Part of the team met with Dr. Kevin Eliceiri and Dr. James Trevathan to discuss our upcoming goals for the duration of the semester. Going forward, we aim to use an existing endoscope from within the BME department in order to gather data on current range for field of view measurements. The endoscope will be tested in a phantom gel matrix with the addition of fluorescent beads in order to simulate an environment that the endoscope would ultimately be placed in. By testing in a phantom matrix, we will be able to test the field of view and image quality without having to involve live mice and worry about potentially harming a mouse in the process. From there, we will have some data to work off of, as the client does not currently have a working endoscope in their possession. With this data, either another BME design group, or a professional engineering company, will have some data in which a design can be based off of. The team will be meeting with the client again in the upcoming days to establish the plan from this point moving forwards, and will also ask additional questions that can help us further our testing apparatus.

Links provided in meeting:

Inscopix - https://www.inscopix.com/

http://miniscope.org/index.php/Miniscope_V4

UCLA miniscope - http://miniscope.org/index.php/Main_Page

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342183/

Conclusions/action items:

The team will review the important links provided in the meeting. Additionally, a meeting with the client will be arranged within the next few days in order to clarify expectations and a plan of action. The information that comes out of the client meeting will be sent to Dr. Kevin Eliceiri and Dr. James Trevathan in order to keep them updated. Additionally, we will arrange a time to meet with James Trevathan in order to test his endoscope using the phantom matrix we design.

LAUREN HELLER - Oct 26, 2021, 5:49 PM CDT

Embedded private docs from Google Drive cannot be displayed in PDF

Meeting 10: 11/10/2021

Rebekah Makonnen - Nov 12, 2021, 1:01 PM CST

Title: Gel Phantom and Testing Meeting

Date: 11/10/2021

Content by: Rebekah

Present: Rebekah, Dana, Lauren, and Alex

Goals: Determine a starting point for gel phantom recipe, order materials needed from amazon, contact Dr. Trevathan to determine when the team will be able to start performing tests

Content:

- the team discussed the idea of using a recipe that Rebekah already has for the gel phantom as a starting point, then adjusting the ratios from there based on if a more or less stiff recipe is needed
- determined that the best vessel to test the FOV of miniscope is by having the gel phantom in the tall elongated shot glasses, since it will allow a greater longitudinal distance to track and the team is able to control the amount of light allowed to reach the phantom
    - Dana ordered these and they are expected to arrive on Saturday (11/13)
- emailed Dr. Trevathan, to determine a date when we would be able to use the miniscope and fluorescent beads to begin testing

Conclusions/action items:

Rebekah- determine final recipe for gel phantom over the weekend

As a team, wait to hear back from Dr. Trevathan and begin testing the FOV of the miniscope.

Meeting 11: 11/19/2021

Rebekah Makonnen - Nov 21, 2021, 3:17 PM CST

Title: Testing Plan and Material Orders

Date: 11/19/2021

Content by: Rebekah

Present: Rebekah, Lauren, Alex, Tyler, Alexis

Goals: To create a plan for testing, discuss the ideal type of fluorescent beads for this project and order them

Content:

- Rebekah discussed the outcome of her recipe testing and the team was able to decide on a final recipe for the gel phantom
- created a rough outline for a testing plan, the team plans to revisit this and change as necessary
- discussed what type and size of fluorescent microbeads are ideal for this project
- ordered fluorescent microbeads for gel phantom fabrication, the beads should arrive by Wednesday, 11/24
- emailed Dr. James Trevathan to set up a testing time for after break

Conclusions/action items:

Once the beads arrive, begin fabrication of gel phantom for testing. Begin testing the FOV of the miniscope

Elongated Lens Design

LAUREN HELLER - Oct 18, 2021, 8:16 PM CDT

Title: Elongated Lens Design

Date: 10/18/2021

Content by: Team

Present: All members

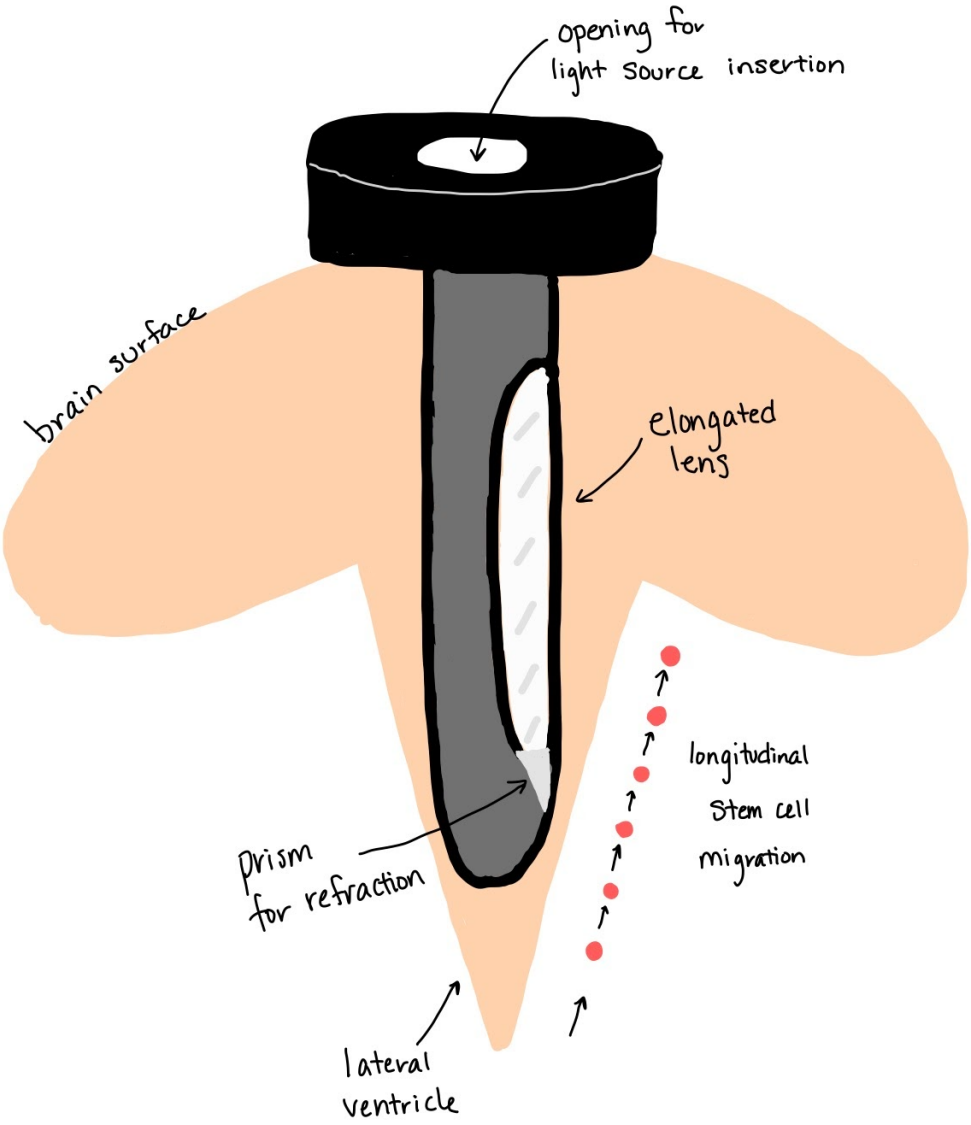
Goals: Collaborate on preliminary designs for preliminary deliverables.

Content:

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 X, light can initially enter through the top of the cannula in which the elongated lens would send the light out through the micro-prism 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.

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.



Conclusions/action items:

Research potential materials for endoscope and evaluate preliminary designs on various criteria.

Three Lenses Outside the Brain Design

LAUREN HELLER - Oct 18, 2021, 8:18 PM CDT

Title: Three Lenses Outside Brain Design

Date: 10/18/2021

Content by: Team

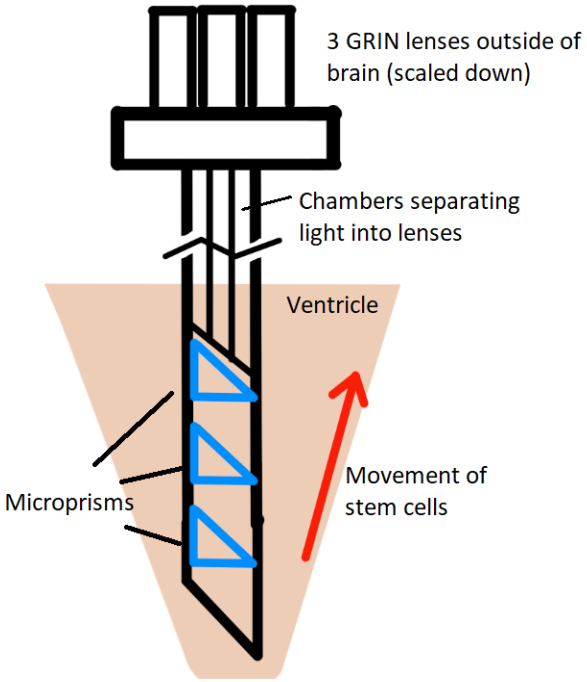
Present: All team members present.

Goals: Collaborate on design ideas for preliminary deliverables.

Content:

This design involves 3 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 Y, the cannula instead hosts 3 micro-prisms 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 3 chambers that separate light as they travel back into the GRIN lenses to decrease light interference between lenses.

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.



Conclusions/action items:

Evaluate preliminary designs using a design matrix and research materials that are needed/can be used.

Three Lenses at Different Depths Design

LAUREN HELLER - Oct 18, 2021, 8:21 PM CDT

Title: Three Lenses at Varying Depths Design

Date: 10/18/2021

Content by: Team

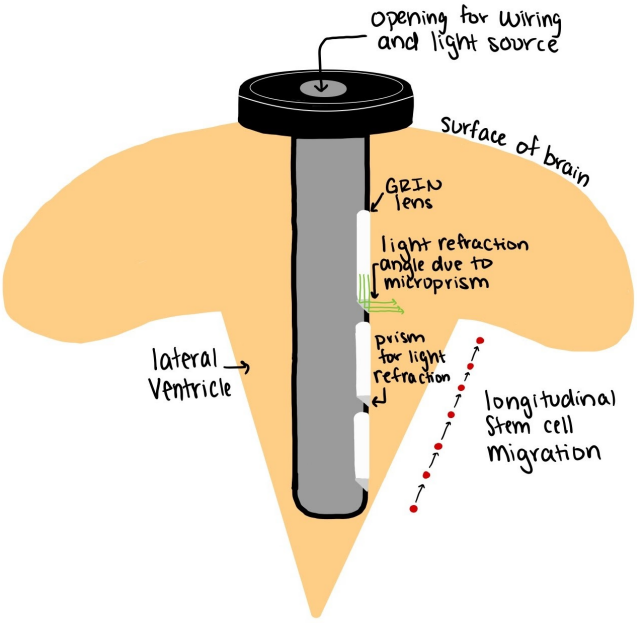
Present: All team members were present.

Goals: Communicate design ideas and narrow down designs to ultimately decide on one design to proceed with.

Content:

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



Conclusions/action items:

Evaluate designs using the design matrix and report in preliminary deliverables submission.

Design Matrix for Preliminary Report

LAUREN HELLER - Oct 18, 2021, 8:04 PM CDT

		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

11/10/2021- Rough BOM and Materials List

Rebekah Makonnen - Dec 13, 2021, 3:14 PM CST

Title: Rough BOM

Date: 11/10/2021

Content by: Rebekah

Present: Rebekah

Goals: To create a rough bill of materials list for this project

Content:

1. Mini Plastic Cups
    1. https://www.amazon.com/dp/B01LVZU0EN?psc=1&ref=ppx_yo2_dt_b_product_details
        1. 1.75 ounces
        2. **25 for $11.95**
2. Unflavored Gelatin
    1. Find at any grocery store
        1. 1 ounce, split into 4 packets containing 0.25oz
        2. **$2.49 for one box**
3. Fluorescent Microspheres
    1. https://www.cospheric.com/UVPMSBG_fluorescent_green_spheres_density101.htm
        1. 27-32 microns in diameter
        2. **1g for $215**

Conclusions/action items:

This is just a preliminary BOM of what was discussed at the team meeting today. The team also discussed testing in mini petri dishes as a type of preliminary test before we test using the mini plastic cups. So mini petri dishes could also be added in the future.

11/14/2021- Gel Phantom Recipe Test

Rebekah Makonnen - Nov 19, 2021, 12:30 PM CST

Title: Creating the Test Samples of Gel Phantom

Date: 11/14/2021

Content by: Rebekah

Present: Rebekah

Goals: To create the test samples of the gel phantom and determine if it is needed to adjust the recipe

Content:

To begin the fabrication of the gel phantom, I heated water on the stove until it began to boil. In order to test multiple consistencies I used 3 different amounts of agar powder, 0.25oz, and 0.125oz. The procedure I followed for the fabrication of the gel phantom is:

1. Heat water to a rolling boil on the stove
2. Remove the water from heat and measure out 19oz of water into a heat proof bowl
3. Add 0.5oz of agar powder to the boiling water and whisk until its completely dissolved
4. Pour the agar-water mixture into the vessel of your choice and place in the fridge for 4 hours (or until the mixture is set)
5. Repeat the steps above twice, but instead using 0.25oz and 0.125oz of agar

I have also attached my calculations for the concentration percentage for each of the ratios below.

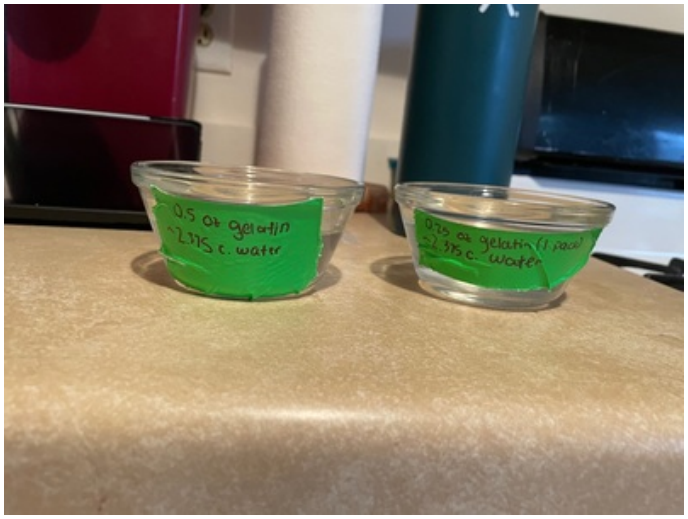
Conclusions/action items:

Let the test phantoms set in the fridge. Consult the team on ideal consistencies and begin testing.

Rebekah Makonnen - Nov 14, 2021, 4:03 PM CST

Agar_Recipe_Calculations-2.jpg(307.9 KB) - download

Rebekah Makonnen - Nov 14, 2021, 3:58 PM CST



0E009A2B-9735-4CE8-A73E-09EC5478EE9D.jpg(265 KB) - download

12/2/2021 - Creation of Phantom Samples for Testing

LAUREN HELLER - Dec 06, 2021, 4:44 PM CST

Title: Phantom Matrix Creation for Testing with Dr. Trevathan

Date: 12/2/2021

Content by: Lauren Heller

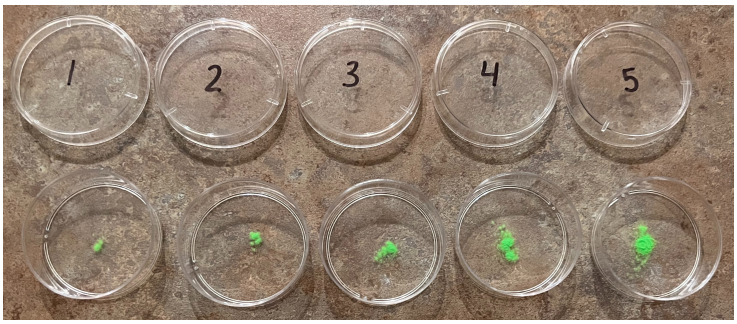
Present: Lauren, Dana, Beki

Goals: Create the phantom matrix samples to prepare for testing at WIMR.

Content:

Following the gel phantom recipe detailed in the entry above this, we created sample phantoms in small petri dishes to bring in for imaging.

The mixture was poured into petri dishes containing various amounts of the fluorescent beads. There was an additional control petri dish (not pictured) containing only the agar gel solution without the fluorescent beads. The petri dishes were placed in the refrigerator overnight, and were taken to the lab for testing the next morning. We decided to make the matrix samples close to testing to ensure quality and no dramatic changes in consistency.



Conclusions/action items:

Conduct testing to gather data and analyze findings.

Phantom Gel Matrix Fabrication Protocol

LAUREN HELLER - Dec 09, 2021, 9:53 PM CST

Title: Phantom Gel Matrix Fabrication Protocol

Date: 12/09/2021

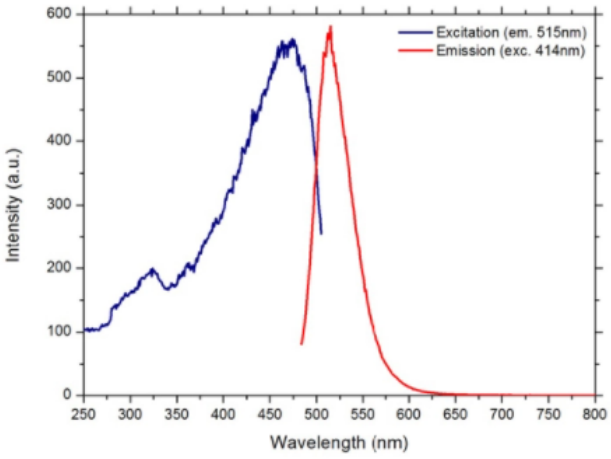
Content by: Lauren Heller

Present: Rebekah, Lauren, Dana

Goals: Create a phantom gel matrix that is able to be replicated for future use.

Content:

Phantom Gel Matrix Fabrication:



Fluorescent Green Response: Peak emission of 515nm when excited at 414nm

Materials:

- 0.125oz Unflavored Gelatin
- 19oz Distilled Water
- Cospheric Fluorescent Green Polyethylene Microspheres (27-32 micron diameter)
- Small Petri Dishes

Procedure:

1. Prepare petri dishes with desired amount of fluorescent microspheres, as well as one without for a control
2. Bring 19oz of water to a boil
3. Remove from heat
4. Add 0.125oz of gelatin to the hot water
5. Whisk the mixture until the powder is completely dissolved
6. Add gelatin mixture to prepared petri dishes
7. Combine mixture and break up any clumping of fluorescent microspheres
8. Cover petri dishes and place in the fridge for 4 hours or until the mixture is set

Conclusions/action items:

Add testing protocol, which goes along with the fabrication protocol, as the gel matrix will be used in testing. It is important to have a clear testing protocol so that it can be replicated by the team, or another team, as needed.

Phantom Gel Matrix Testing Protocol

LAUREN HELLER - Dec 09, 2021, 10:00 PM CST

Title: Phantom Gel Matrix Testing Protocol

Date: 12/9/2021

Content by: Lauren Heller

Present: Lauren, Dana, Tyler, Alexis, Rebekah

Goals: Document the testing protocol so that it can be reproduced, replicated, or critiqued by others.

Content:

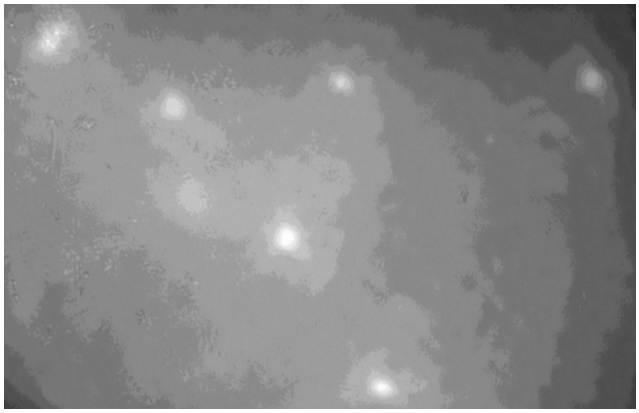
Phantom Matrix Testing:

Materials:

- Prepared Phantom Gel Matrix
- Inscopix nVoke Miniscope

Procedure:

1. Obtain prepared gel matrix and place on testing apparatus
2. Set up the miniscope by adjusting the height, gain, EX-LED Power, and lens focus settings to obtain appropriate image quality
3. Take a video of the matrix 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.



Conclusions/action items:

Work on final deliverables for end-of-semester submission.

12/03/2021 - Testing the matrix and field of view of endoscope

DANA STUMPFOLL - Dec 13, 2021, 12:08 PM CST

Title: Testing the Field view

Date: 12/03/2021

Content by: Dana

Present: Lauren, Beki, Dana, Lexi, Alex

Goals: Test the field of view of the microscope using the fabricated matrix.

Content:

- Materials:
  - Built matrix from gel matrix fabrication protocol
  - 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 matrix 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.

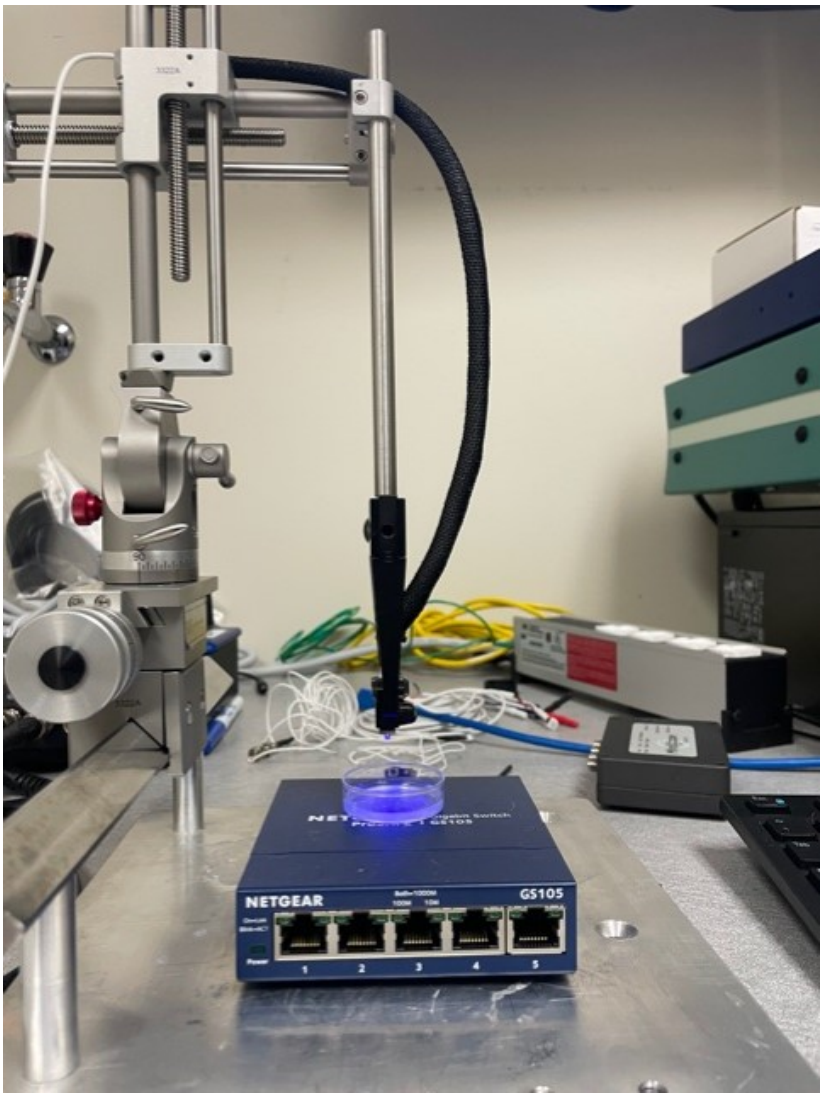


Figure 1: This image shows the setup as testing was running. The nVoke miniscope was used. It emits a blue light and records a green fluorescence.

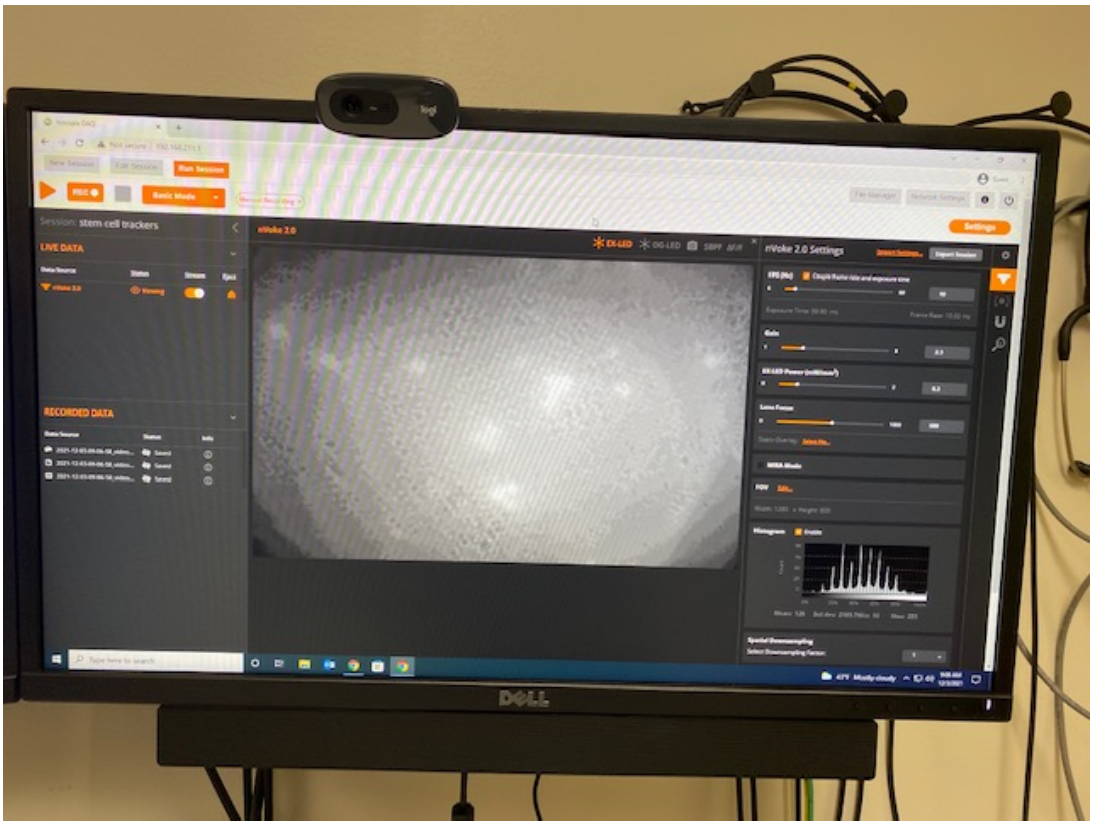


Figure 3: Pictured is an example of the software settings used to take videos of the beads over the gel.

Overall testing went very well and Dr. Trevathan was very helpful in explaining the aspects of the endoscope and how to use it. He was able to send us two videos to analyze and obtain the field of view from to show the client. He explained that this miniscope is currently used to attach to mice already to study brain activity in mice and how it affects their behavior. The testing took about two and a half hours just to obtain the two images because the beads clumped up a lot and dominated the field of view with a giant white fluorescent spot. There was not enough time to test the field of view by attaching a GRIN lens but this would be beneficial data to show our client to determine if the field of view would suite the purposes of their research. The maximum field of view is 1280 x 800 pixels, or 1050 x 650 µm [1].

Conclusions/action items:

Overall, the testing took about two and a half hours and the images turned out to be pretty clear. Dr. Trevathan was a tremendous help in the testing of the endoscope.

Action items: Analyze the data using ImageJ software and do a statistical analysis.

Citations:[1] *Inscopix.com*. [Online]. Available: https://iq.inscopix.com/user-manuals.

12/06/2021 - Statistical analysis of the field of view

DANA STUMPFOLL - Dec 13, 2021, 6:11 PM C

Title: Data Analysis

Date: 12/06/2021

Content by: Dana

Present: Dana

Goals: Test the fluorescence intensity and how it relates to the field of view of the miniscope.

Content:

- **Measuring Fluorescence Intensity:**
  This analysis was used to determine the total fluorescence of each bead by subtracting out the background fluorescence signal. [1]
  - 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) [1].
  - By measuring the Fluorescence intensity the field of view can be analyzed based on if some of the cells would be lost toward the edges of the field of view.

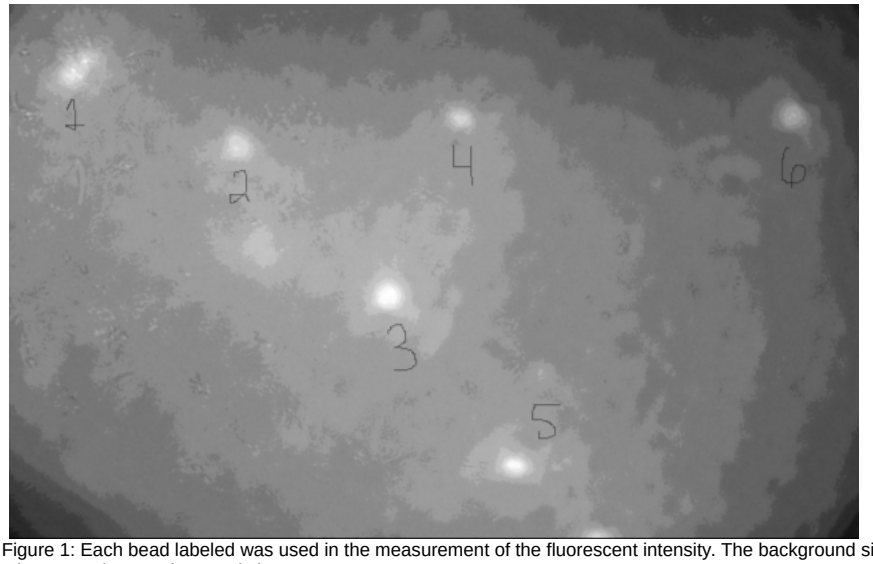


Figure 1: Each bead labeled was used in the measurement of the fluorescent intensity. The background signal can overpower the intensity of the beads. Overall the resolution of microscope is very clean and clear.

- Using the ImageJ setting for measuring mean grey value, area, and integrated density, the fluorescence activity was able to be calculated by subtracting out the mean background signal from the signals produced by each of the beads.

Bead Number	Fluorescent Intensity
1	1098660
2	1081530
3	1469190
4	620672
5	1203240
6	224386

Table 1: Each beads fluorescent intensity was calculated. Bead three was found to have the greatest fluorescent intensity.

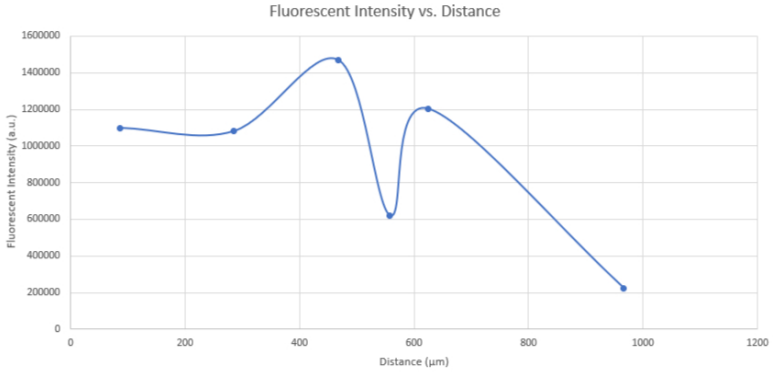


Figure 2: This graph shows the fluorescent intensity versus the distance from the left edge of the field of view. It is clear that at the center of the field of view (around 500 µm) th fluorescent intensity is the greatest. The intensity varies away from the center of the field of view with an outlier at 557 µm.

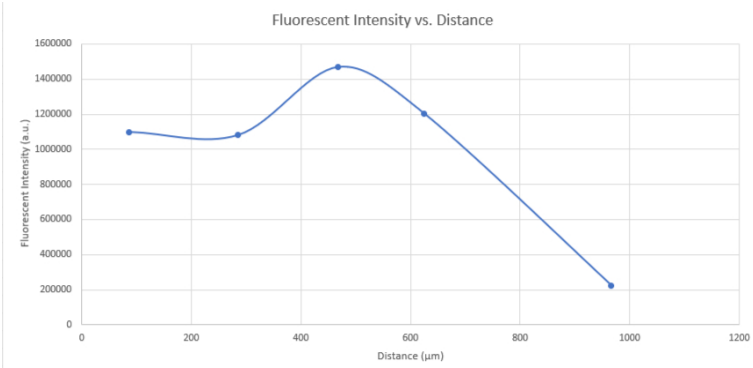


Figure 3: This graph shows the fluorescent intensity versus the distance from the left edge of the field of view excluding the data point at 557 µm that does not follow the trend. These graphs prove that the fluorescent intensity of the beads decreases as the beads move away from the center of the field of view. For tracking of stem cells this is important because the fluorescent intensity of the cells may not be as bright and it will be harder to track the cells through the entire field of view.

Conclusions/action items:

Overall, the testing took about two and a half hours and the images turned out to be pretty clear. The field of view was 1280 x 800 pixels, or 1050 x 650 µm [2]. More testing woul need to be conducted in the mouse brain to determine if the cells would show up on the miniscope. Since the beads mimic the cells, it is very likely this microscope could be used the purposes of our clients research. Future works includes testing a bigger field of view and seeing if the fluorescence intensity is still lost toward the edges of the frame. Attachir different GRIN lenses would also be useful since that is what would be implanted into the mouse brain. The timeline of this course did not allow for this extra testing, but the base data we found is useful for the client in deciding if they would like to invest in this miniscope.

Citations: [1]*Umbc.edu*. [Online]. Available: https://kpif.umbc.edu/image-processing-resources/imagej-fiji/determining-fluorescence-intensity-and-positive-signal/.

[2] Inscopix.com. [Online]. Available: https://ig.inscopix.com/user-manuals.

Preliminary Design Presentation

LAUREN HELLER - Oct 18, 2021, 8:22 PM CDT

Stem_Cell_Trackers_Prelim_Presentation.pdf(1.7 MB) - download

12/6/2021- Testing Data and Data Files

Rebekah Makonnen - Dec 13, 2021, 3:23 PM CST

Title: Testing Data and Files

Date: 12/6/2021

Content by: Rebekah

Present: Team

Goals: To get images and possibly video footage of the fluorescent beads and data for the project

Content:

I have attached the video files (.tiff file) the team obtained from testing with Dr. Trevathan below

Conclusions/action items:

Dana will analyze these images using ImageJ and perform a statistical analysis to determine the relative intensity of each bead.

Rebekah Makonnen - Dec 13, 2021, 3:23 PM CST

second_video.tiff(737.1 MB LA S3) - download

Rebekah Makonnen - Dec 13, 2021, 3:22 PM CST

first_video.tiff(373.5 MB LA S3) - download

09/26/2021- Neural Stem Cell activity

DANA STUMPFOLL - Sep 26, 2021, 3:05 PM CDT

Title: Adult neural stem cell activation regulated by day and night cycle

Date: 09/26/2021

Content by: Dana Stumpfoll

Present: Individual Work

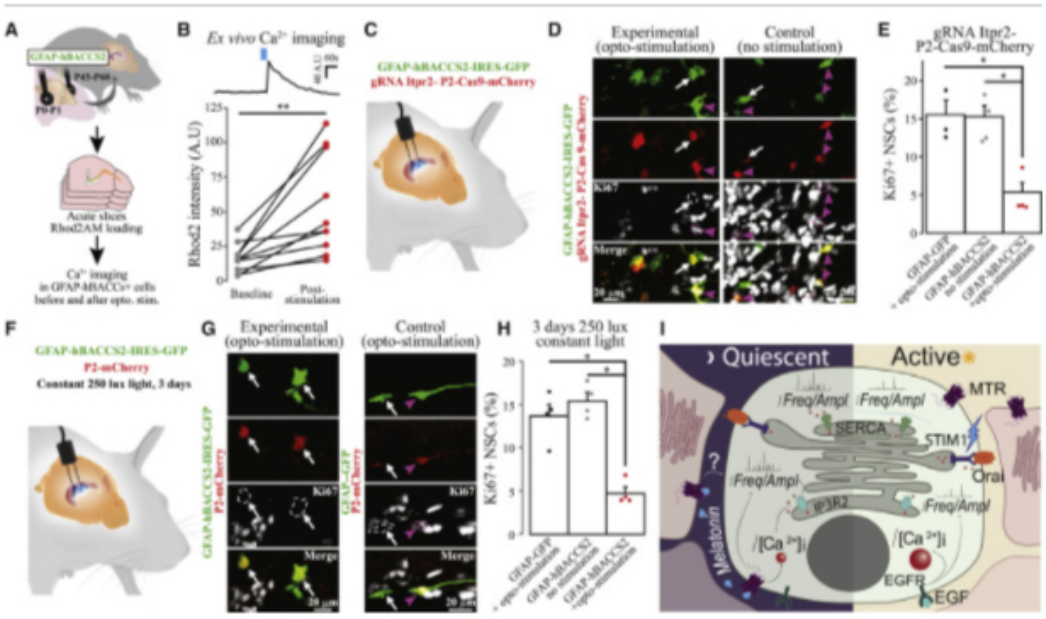
Goals: Understand how neural stem cells are activated in mice.

Content:

Link: https://www.sciencedirect.com/science/article/pii/S0092867420317487?casa_token=VcpctgwRbw8AAAAA:Hw_iT9PcceTN9bqv4k-9W5ue_hWo1cC7XwruxobpeLPH3Ojy80ZhN_znUpt1HsrPnv2frGR3Aw

- Study done to image activation kinetics in freely behaving mice.
- It was found that NSC dividing is more frequent during the daylight compared to the nighttime darkness.
- Most studies on NSCs are conducted on mice under anesthesia and this causes the neurons to not be active and good data cannot be contrived from these studies.
- Mini-endoscopes were implanted into the lateral ventricle for about 2 months.
- The lens were side view lenses used in the lateral ventricle to image the sub ventricular zone.
- The sub ventricular zone contains the largest amount of NSCs in the brain.
- Melatonin and exposure to daylight impacted the amount of NSCs that divided.
- Calcium ions were also studied and how they are an essential integrator of signals for NSC proliferation.
- A GRIN lens with a 45 degree prism glued to it was used to image inside of the brains of the mice and the implantation of the endoscopes had no effect on the proliferation of the NSCs.
- Successful division of NSCs was determined by one cell splitting into two cells and each daughter cell containing fluorescence.
- Two-color imaging was used to clearly image the mitosis of the NSCs.
- It was found that the NSCs do not migrate out of the sub ventricular zone.
- The imaging in live mice made it possible to change the environment for the mice and see how that effected NSC proliferation.
- 70% of the division of the NSC happened during the day.
- Consistent darkness lead to a significant decrease in the division of the NSCs.
- Consistent light lead to the production of NSCs to increase by 2.5-4 times.
- Luzindole was used to block the production of melatonin and it increased the number of proliferative NSCs.
- Day and night regulation of NSC division is regulated by melatonin.
- The transition from quiescent to proliferative NSCs is regulated by Calcium ion levels.
- Luzindole is linked to the modification of calcium ion levels in the brain.
- Epidermal growth factor was also shown to increase the proliferation of the NSCs.

- Epidermal growth factors regulate calcium ions in a similar way that luzindole was used to regulate melatonin production.



Download : Download high-res image (1MB) Download : Download full-size image

- This image shows how the device is implanted in the brain and graphs of data that these researchers found from analyzing calcium levels and adjusting whether the mice are in the dark or light. =

Conclusions/action items:

- NSCs proliferation can be mediated by a lot of different aspects depending on the environment the mice are in.
- The way the lenses were used in monitoring this experiment is useful for determining the orientation and best imaging techniques for our product design.
- Citation: A. Gengatharan *et al.*, “Adult neural stem cell activation in mice is regulated by the day/night cycle and intracellular calcium dynamics,” *Cell*, vol. 184, no. 3, pp. 709-722.e13, 2021.

09/26/2021- PDS research

DANA STUMPFOLL - Oct 19, 2021, 3:41 PM CDT

Title: Guidelines for using mammals in neuroscience research

Date: 09/26/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand the requirements of using mammals for research.

Content:

Link: https://grants.nih.gov/grants/olaw/national_academies_guidelines_for_use_and_care.pdf

- When using animals in research there are a lot of guidelines that come into play due to the fact that the animals should not be harmed and taken care of during the experiments.
- One important aspect is that anything implanted into the animals should be free of contaminants.
- This was used for the materials portion of the PDS.
    - **j. Materials:** As a requirement of the client, GRIN lenses will be used. The materials implanted into the brain should be free of contaminants [1].

Conclusions/action items:

- These guidelines should be analyzed in depth to ensure that they are being followed when animals are used in experiments.
- The researchers who are using our endoscope will have to follow these guidelines when they implant the endoscope.
- Citation: [1] *Nih.gov*. [Online]. Available: https://grants.nih.gov/grants/olaw/national_academies_guidelines_for_use_and_care.pdf. [Accessed: 21-Sep-2021].

10/18/2021- Materials and immune response

DANA STUMPFOLL - Oct 31, 2021, 7:33 PM CDT

Title: Cannula material used in animal research

Date: 10/18/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Start of research on materials.

Content:

Links: file:///C:/Users/dstum/Documents/BME%20300/materials-12-03166.pdf and https://elifesciences.org/articles/40805

- 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 [1].
- 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 [2].
- However, long term use showed great biocompatibility when the material was used as a bioelectrode [1].
- The use of polyimide biomaterials in animal research has been successful but more research is needed to determine the long term cytotoxicity[2].

Conclusions/action items:

- This information on polyimide can be useful if our team ends up having to design a cannula for the microscope we use.
- More research on materials needs to be conducted.
- Citation: [1] 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.
  [2] 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.

09/13/2021- STEM Cell Tracking Technologies

DANA STUMPFOLL - Sep 26, 2021, 3:08 PM CDT

Title: STEM Cell Tracking Technologies

Date: 09/13/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand technologies used in STEM cell tracking in the brain.

Content:

Link: https://www.hindawi.com/journals/sci/2017/2934149/

- MRI, nuclear resonance imaging, and optical imaging are all technologies used as cell tracking technologies.
- Neural STEM cells are self-renewing, proliferating and differentiating and are used to renew tissues in the brain.
- It is important to track the migration and survival of the STEM cells in the brain.
- Most studies the animals have to be killed for the tracking of the cells.
- MRI has been used for its good tissue contrast and noninvasiveness.
- Optical imaging is not ideal for its low resolution and its inability to penetrate deep into tissue.

MRI

- Provides excellent image quality and spatial 3D resolution.
- Cells can be labeled in their anatomical context.
- Additional information about the surrounding environment.
- Nontoxic and noninvasive.
- Gadolinium provides a great contrast in the tissue.
- Manganese also provides great contrast in the brain for MRI.
- Iron oxides have also been used.
- Drawbacks: labeling will be diluted due to STEM cell proliferation, false signals, it cannot reflect the survival of STEM cells and the changes in their environment.

MPI

- Extremely sensitive with high-signal to noise ratio.
- Has potential to detect a single stem cell.
- 19F can be used in concentration to track STEM cells.

Nuclear resonance imaging

- The cells are labeled using C, O, N, or F and can be recognized using PET or SPECT scanners.
- Two high energy photons will be released and can be tracked by the PET camera.
- PET has high sensitivity and temporal resolution.
- Image reported genes can also be injected into the cells genome ex vivo.
- This way only viable cells will be detected since only live cells can translate this gene.

Optical Imaging

- Low cost, no radiation toxicity, and high sensitivity.
- Fluorescent techniques are used.
- Bioluminescence is also used using a reported gene.

- Light is directly related to the number of cells.
- BIL is limited to small animals because it can only penetrate a few centimeters of tissue.

Multimodality

- Combination of multiple imaging techniques.
- Fusion proteins need to be used and can cause loss of bioactivity.

Conclusions/action items:

- Their are many different ways to track STEM cells but no particular method is perfect.
- Some of these imaging techniques can be used as a reference for the endoscope we are designing.
- Citation: Y. Zheng *et al.*, “Stem cell tracking technologies for neurological regenerative medicine purposes,” *Stem Cells Int.*, vol. 2017, pp. 1–9, 2017.

09/13/2021- GRIN lens probe

DANA STUMPFOLL - Sep 26, 2021, 3:08 PM CDT

Title: GRIN lens probe for endoscopic tomography with fast dynamic focus tracking

Date: 09/13/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand GRIN lenses and how they work.

Content:

Link: https://www-osapublishing-org.ezproxy.library.wisc.edu/oe/fulltext.cfm?uri=oe-14-8-3238&id=89308

- GRIN can be used to access difficult tissue areas.
    - Contact or no contact
- Imaging speed of 8 frames per second.
- Fixed focal plane from the distal end of the probe.
- A high numerical aperture lens must be used to get a focused image.
- Signal to noise ratio will decrease with increasing depth from the focal lens.
- Bulk in lens size has limited the amount of focal view from the lens.
- Dynamic focusing is used by the light entering the surface of the lens is the same as the light on the distal end of the lens.
- In this study the lens was used inside rabbits that had been euthanized.
- The sample was placed on a stage that moved to be in focus of the lens.

Conclusions/action items:

- The GRIN lens was used in a different way in this experiment.
- Some of these techniques used to adjust the lens and focal plane for the purpose of our endoscope.
- Citation: T. Xie, S. Guo, Z. Chen, D. Mukai, and M. Brenner, “GRIN lens rod based probe for endoscopic spectral domain optical coherence tomography with fast dynamic focus tracking,” *Opt. Express*, vol. 14, no. 8, pp. 3238–3246, 2006.

GRIN lens rod based probe for endoscopic spectral domain optical coherence tomography with fast dynamic focus tracking

Tinjing Xie, Shuguang Guo, Zhongping Chen
Beckman Laser Institute, Department of Biomedical Engineering
University of California, Irvine, Irvine, CA 92617

David Mukai, Matthew Brenner
Beckman Laser Institute, University of California Irvine
Pulmonary and Critical Care Division
UC Irvine Medical Center, Orange, CA 92868

Abstract: In this manuscript, a GRIN (gradient index) lens rod based probe for endoscopic spectral domain optical coherence tomography (OCT) with dynamic focus tracking is presented. Current endoscopic OCT systems have a fixed focal plane or working distance. In contrast, the focus of this endoscopic OCT probe can dynamically be adjusted at a high speed (500 mm/s) without changing reference arm length to obtain high quality OCT images for contact or non-contact tissue applications, or for areas of difficult access for probes. The dynamic focusing range of the probe can be from 0 to 7.5 mm without moving the probe itself. The imaging depth is 2.8 mm and the lateral scanning range is up to 2.7 mm or 4.5 mm (determined by the diameter of different GRIN lens rods). These dimensional imaging can be performed using this system over an area of tissue corresponding to the GRIN lens surface. The experimental results demonstrate that this GRIN lens rod based OCT systems can perform a high quality non-contact *in vivo* imaging. This rigid OCT probe is solid and can be adapted to safely access internal organs, to perform front or side view imaging with an imaging speed of 8 frames per second, with all moving parts proximal to the GRIN lens, and fast axial potential for use in endoscopic compact OCT endoscopes for *in vivo* imaging in both biological research and clinical applications.

© 2006 Optical Society of America

OCIS codes: (110.4500) Optical coherence tomography; (110.3700) GRIN lens; Dynamic focusing; (170.3880) medical and biological imaging; (170.5120) Imaging system; (170.2150) Endoscopic imaging

References and links

1. H. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, "Optical coherence tomography," Science **254**, 1178-1181 (1991).
2. G. J. Tearney, M. E. Brezinski, B. E. Bouma, S. A. Boppart, C. Pitris, J. F. Southern, J. G. Fujimoto, "*In vivo* endoscopic optical biopsy with optical coherence tomography," Science **276**, 2037-2039 (1997).
3. B. A. Standish, J. A. Lee, M. K. Ho, D. Huang, C. P. Lin, J. S. Schuman, C. A. Puliafito, and J. G. Fujimoto, "*In vivo* endoscopic imaging by optical coherence tomography," Opt. Lett. **28**, 1864-1866 (2003).
4. A. Rollins, R. Ung-arunyawee, A. Chak, C. Wong, K. Kobayashi, M. Sivak, Jr., and J. Izatt, "Real-time *in vivo* imaging of human gastrointestinal ultrastructure by use of endoscopic optical coherence tomography with a novel efficient interferometer design," Opt. Lett. **24**, 1358-1360 (1999).

#8847 - $15.00 USD Received 17 January 2006; revised 24 March 2006; accepted 3 April 2006
(C) 2006 OSA 17 April 2006 / Vol. 14, No. 8 / OPTICS EXPRESS 3236

GRIN_lens_rod_based_probe.pdf(974.6 KB) - download

09/19/2021- Implantation protocol

DANA STUMPFOLL - Sep 26, 2021, 3:06 PM CDT

Title: Implantation protocol for using endoscopes in live imaging of mice

Date: 09/19/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand how the lenses are implanted into the mice.

Content:

Link: https://starprotocols.hivebench.com/protocols/723

- This protocol allows for examination of cells for long-term minimally invasive procedures.
- It allows for live imaging of NSCs in mice.
- Different plasmids can be used for activation of the NSCs.
- For GRIN lenses the lens must be implanted in the brain first.
- The appropriate size of the lens has to be decided on before implantation because the diameter and length of the lens can vary.
- A 0.5mm diameter was used to cause the least amount of damage on the brain tissue.
- Flat-tip lenses allow for imaging from the top while side-view lenses cause less damage to the brain tissue.
- The side view lenses are prism shaped and can be inserted directly into the lateral ventricle.
- Visualizing NSCs that are labeled during insertion is essential to know when to stop inserting the lens into the ventricle.
- A spacer is used to put distance between the canula and the skull.
- Dental cement is used to secure the endoscope in place.
- Identifying the region and amount of fluorescent NSCs can be useful in adjusting the coordinates for implantation of the endoscope.
- 10-14 days after the endoscope is implanted imaging of the cells can begin.

Conclusions/action items:

- This protocol goes into great detail on how to implant the endoscope and how it is used to monitor the cells.
- Citation: *Hivebench.com*. [Online]. Available: https://starprotocols.hivebench.com/protocols/723. [Accessed: 20-Sep-2021].

09/19/2021 - ThorLabs GRIN lens

DANA STUMPFOLL - Sep 26, 2021, 3:08 PM CDT

Title: GRIN lenses for imaging

Date: 09/19/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand GRIN lenses from article from client.

Content:

Link: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=11167

- Used in areas where normal microscopes cannot access.
- GRIN lenses can be placed into tissue with minimal invasiveness.
- AR-coated lenses.
- GRIN lenses vary the index of refraction within the lens itself.
- Coated features a working distance of 0.25mm from the sample side, and a 0.19mm distance from the objective side.
- The coating changes the changes the working distance on the lens.

Conclusions/action items:

- The GRIN lens is the lens that will be used for the project.
- Understanding how the GRIN lens works is essential to doing the project.
- Citation: *Thorlabs.com*. [Online]. Available: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=11167. [Accessed: 20-Sep-2021].

SCIENTIFIC REPORTS

OPEN

Time course images of cellular injury and recovery in murine brain with high-resolution GRIN lens system

Chelsea D. Pernici, Benjamin S. Kemp & Teresa A. Murray

GRIN_lenses.pdf(2 MB) - download

09/26/2021- Implanted reconnectable fiber bundles

DANA STUMPFOLL - Sep 26, 2021, 7:17 PM CDT

Title: Deep Brain imaging in freely moving mice using implanted reconnectable fiber bundles

Date: 09/26/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand how this device was used for imaging inside mouse brains.

Content:

Link: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jbio.202000081

- A fiber bundle was used to image fluorescent cells in different parts of the brain.
- The fiber bundle contains a gradient-index fiber lens.
- This devices intention was to image cells in different areas of the brain.
- The device is cemented in place with dental cement and cyanoacrylate glue.
- Each probe is a different length to account for them being placed in different depths of the brain.
- The diameter of the lens is 70 micro meters 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.
- The system sends an inverted image from the GRIN lens where it is then analyzed to determine if it is cell fluorescence or reflected or scattered laser radiation.
- Fluorescence reached a maximum and clear images of the cells were able to be taken.
- Removing and reconnecting the fibers allows for the experiments to be stop and restarted from weeks to months time.
- Movement of the mice did not destabilize the scope or ruin any images taken.
- Suppression of spontaneous cortical activity has shown to lower calcium signaling activity.
- The small size of the endoscope has been useful in implanting the device for mice who are still free moving.

Conclusions/action items:

- This endoscope utilized a similar idea to what our client wants.
- Using multiple lens in different areas helped the researchers be able to image two different parts of the brain at the same time.
- Citation: 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.

10/04/2021 - CHendoscope

DANA STUMPFOLL - Oct 04, 2021, 5:43 PM CDT

Title: A compact head-mounted endoscope for imaging in freely behaving mice

Date: 10/04/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand how this device is used for calcium imaging inside mouse brains.

Content:

Link: https://madison.hosts.atlas-sys.com/shib/illiad.dll?Action=10&Form=75&Value=3599701

- Miniature fluorescence microscope used to image calcium transients in the brain.
- Low-cost compound head-mounted microscope.
- Uses gradient index lens and fluorescence to image the cells.
- GCaMP used to fluoresce the cells.
- Increased fluorescence when calcium ions are present due to increased neural activity.
- Fiber photometry does not provide cellular resolution while 2P imaging effects the free behaving nature of the mice.
- A miniature epifluorescence microscope with GRIN lenses is used.
    - Contains a light source, filters to focus light and excitation, and a detector for fluorescence.
- Assembly:
    - Base plate, filter box, and camera body.
    - Filter box includes the LED, filters, mirrors, and lenses to collect the data.
    - CMOS camera sensor to capture the images from the lenses.
- A detailed protocol on how to assemble the device is provided.
- A detailed procedure on how to install the device by two simple surgeries is also provided.
- Assess the field of view of the microscope before imaging.
- A troubleshooting guide for any issues that arise with the use of the microscope is also provided.
- Fear based conditioning is used in the experiment.
- Open environment monitoring is also used.
- An endoscope can be assembled in less than 6 hours and it is relatively low in cost.

Conclusions/action items:

- The protocol used to develop this device might be useful in coming up with our device.
- Multiple parts are going to need to be developed for the purpose and implementation of the project.
- Citation: A. D. Jacob *et al.*, “A compact head-mounted endoscope for in vivo calcium imaging in freely behaving mice,” *Current Protocols in Neuroscience*, vol. 84, no. 1, p. e51, 2018.

10/04/2021 - Doric lenses

DANA STUMPFOLL - Oct 04, 2021, 6:17 PM CDT

Title: Miniature endoscopes

Date: 10/04/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Understand how these lenses are already used by the company Doric.

Content:

Link: https://neuro.doriclenses.com/pages/miniatured-microscopy-solutions

- Used to record cell activity in a specific group of cells.
- Used to correlate cell behavior to animal behavior.
- Can be used to monitor the same area for months.
- Can combine imaging with other techniques such as injection.
- There is a limited sampling depth due to light scattering and absorption.
- This can be fixed by the twist on efocus microscopy system.
    - The GRIN relay lens is always perfectly aligned with the microscope.
    - 1mm lens for superficial surface monitoring of the brain, or 0.5mm lens for deeper imaging in the hypothalamus region of the brain.
- The twist on design allows for easy attachment and removal of the microscope from the mouse's head.

Conclusions/action items:

- The implementation of this device by Doric can be used to come up with design ideas for our device and how to implement them.
- The electrical system of this device and how it is attached can also be useful to come up with a solution to our problem.
- Citation: “Miniatured Microscopy solutions,” *Doriclenses.com*. [Online]. Available: https://neuro.doriclenses.com/pages/miniatured-microscopy-solutions.

10/11/2021 - Inscopix nVista

DANA STUMPFOLL - Oct 19, 2021, 3:10 PM CDT

Title: Inscopix nVista

Date: 10/11/2021

Content by: Dana Stumpfoll

Present: Individual Work

Goals: Find competing designs on the market.

Content:

Link: https://www.inscopix.com/nvista

- Large-scale brain circuit imaging.
- Used in freely behaving animals.
- Used to study sex-specific behavioral cues [1].
- Visualize same field of view longitudinally.
- One patent that exists on the market for a similar device is for the Inscopix systems and methods for optogenetic imaging [2].
- 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 [1].
- This device would not be ideal for the client since it utilizes a flat top GRIN lens that does not incorporate a longitudinal field of view.
- This is one of the leading devices on the market since it does allow for imaging in freely behaving mice.

Conclusions/action items:

- The Inscopix company has developed a lot of miniendoscopes that can be used in mouse brains.
- These designs can be used and our team could build off of them but they are too expensive.
- Citation: [1] 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.
- [2] Trulson et al, "Systems and methods for optogenetic imaging," US 20180303573 A1, Oct. 25, 2018.

10/31/2021 - Phantom gel matrix

DANA STUMPFOLL - Oct 31, 2021, 7:55 PM CDT

Title: Phantom gel matrix to model mouse brain

Date: 10/31/2021

Content by: Dana

Present: Individual work

Goals: Find a phantom gel matrix recipe to use for data collection.

Content:

- Most matrices have been used to absorb red or between 400 to 650 nm fluorescence.
- This recipe was used to model the oesophageal wall.
    - They used sodium azide and and a buffered saline solution.
    - Two grams of agarose is used as the base for the model.
    - Some fluorochromes or photosensitizers can be added while the mix is still liquid.
    - Ink and blood are also added as optical absorbers.
    - Bovine serum is added to add proteins.
        - Most of the fluorescence mechanisms attach to proteins to allow for fluorescence.
    - Penicillin and azide are added to ensure the gel will last long if stored in a 4 degree Celsius fridge.
    - Agarose is used for its mechanical properties.
- This experiment was used for cancer research purposes and there was a lot of autofluorescence which interfered with the image capture of the probe.
- The incorporation of silica powder is 10 times as important as the incorporation of Intralipid.
- One advantage of this gel is that it can be used simultaneously at different wavelengths.
- This gel incorporates a lot of different aspects that we would not necessarily need in our phantom matrix.

Conclusions/action items:

- Using agarose for a gel matrix would be good since it has very good mechanical characteristics and would be cheap to make.
- A bovine serum where we could also use fluorescence proteins would be interesting too and would produce better data than beads.
- Citation: G. Wagnières *et al.*, “An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy,” *Phys. Med. Biol.*, vol. 42, no. 7, pp. 1415–1426, 1997.

Physics in Medicine & Biology

IPEM

An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy

To cite this article: Georges Wagnières et al 1997 Phys. Med. Biol. 42 1415

View the article online for updates and enhancements.

You may also like

Recent citations

IPEM IOP

Your publishing choice in medical physics, biomedical engineering and related subjects.

Start exploring the collection–download the first chapter of every title for free.

Georges_Wagni_res_1997_Phys._Med._Biol._42_1415.pdf(541.1 KB) - download

10/31/2021 - Realistic Brain Gel model

DANA STUMPFOLL - Oct 31, 2021, 8:54 PM CDT

Title: Gel matrix to model mouse brain

Date: 10/31/2021

Content by: Dana

Present: Individual work

Goals: Find a phantom gel matrix recipe to use for data collection.

Content:

- An agarose gel with TBE was used for the purposes of this project.
- MRI imaging was used.
- The gel was made in a clear rectangular container.
- Probes were placed in the gel and the pig brains.
- Lower concentration gel better mimicked the pig brain pressure profile.
- The gel was more homogenous and isotropic than the brain but that is to be expected.
- The MR images were used to detect the flow of the infusate.
    - This would be similar to what we track except we would use beads.
- Hard to model the complex flow patterns of the brain with agarose gel.
- Agarose gel is a much simplified version of the human brain.
- The exlcusion volumes for the brain and the gel were very similar.
- Further studies on the gel along with infusion need to be conducted.

Conclusions/action items:

- In this study, agarose was used to model a human brain.
- Agarose has many similar mechanical properties to the brain and it would be easy to create for the purposes of our data collection.
- Citation: Chen, Zhi-Jian, George T. Gillies, William C. Broaddus, Sujit S. Prabhu, Helen Fillmore, Ryan M. Mitchell, Frank D. Corwin, and Panos P. Fatouros. " A realistic brain tissue phantom for intraparenchymal infusion studies". Journal of Neurosurgery 101.2 (2004): 314-322. < https://doi-org.ezproxy.library.wisc.edu/10.3171/jns.2004.101.2.0314>. Web. 1 Nov. 2021.

A realistic brain tissue phantom for intraparenchymal infusion studies

'

_Journal_of_Neurosurgery_A_realistic_brain_tissue_phantom_for_intraparenchymal_infusion_studies.pdf(505.9 KB) - download

11/08/2021 - Beads on agarose coated gel slide

DANA STUMPFOLL - Nov 08, 2021, 8:10 PM CST

Title: On-bead expression of recombinant proteins in an agarose gel matrix coated on a glass slide

Date: 11/08/2021

Content by: Dana

Present: Individual work

Goals: Find a phantom gel matrix recipe to use for data collection.

Content:

- Biotinylated PCR products were embedded in a gel matrix on a slide.
- Target proteins in the beads were expressed and captured on the same bead to correlate DNA and encoded proteins.
- Expression and display of target proteins are reliant on viable cells and this can be difficult to obtain.
- This method allows for cell-free expression and instant immobilization of recombinant proteins.
- The presence of the gel matrix restricts migration of the protein and prevents cross-contamination of the beads.
- Recipe:
  - 5 µL of microbeads
  - 65 µL of 3% low melting agarose from Bio-Rad Laboratories
  - Allowed to solidify at room temperature
- The fluorescence of the beads were analyzed using a plate reader.
- The EGFP fluorescence was uniform over the entire bead.
- The Cy3-labeled beads only had a thin layer existing on the surface of the bead.
- The proteins diffused into the gel matrix.
- It was found there was a lot of cross contamination between beads.
- This method is beneficial for expressing proteins from genetic templates.

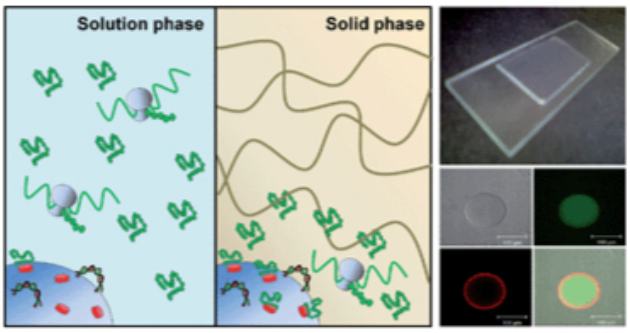


Figure 1: This image shows an example of the agar gel on the glass slide and the images produced from the microbeads.

Conclusions/action items:

- An agarose gel on a glass slide containing microbeads would be a beneficial first step for imaging.
- The agarose they used for their experiment may also be beneficial to look into for our recipe.
- Citation: Kyung-Ho Lee,a Ka-Young Lee,b Ju-Young Byun,b Byung-Gee Kimc and Dong-Myung Kim, “On-bead expression of recombinant proteins in an agarose gel matrix coated on a glass slide,” Rsc.org, Sep-2012. [Online]. Available: https://pubs.rsc.org/en/content/articlelanding/2012/lc/c2lc21239k.

11/18/2021 - Fluorescent Microspheres

DANA STUMPFOLL - Dec 08, 2021, 8:22 PM CST

Title: Fluorescent Microspheres for testing

Date: 11/18/2021

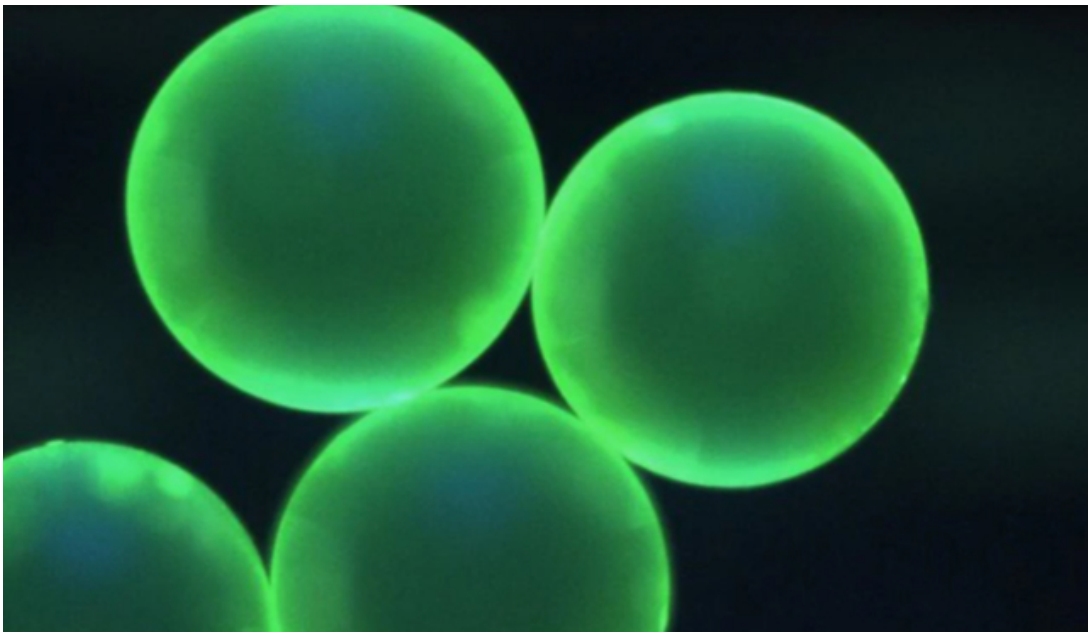
Content by: Dana

Present: Individual work

Goals: Research different fluorescent microspheres for testing.

Content:

- Dr. Trevathan has suggested we find microspheres greater than 10um since anything smaller would not show up due to the resolution of the miniscope.
- The filters for the miniscope are:
  - ET470/40x excitation filter
  - ET525/50m emission filter
  - T496lpxr dichroic mirror
- The miniscope emits a blue light and recognizes green fluorescence, so we need to find green microspheres greater than 10um.
- Using the thermofischer website from Dr. Elicieri I found two types of spheres.
- Fluospheres are used to measure bloodflow.
  - These beads could be used since they are 15um which would be visible by the miniscope.
  - Since we aren't doing any testing with flowing and this miniscope to detect blood flow, these beads are not ideal for our testing purposes.
  - The beads would need to be stored in a refrigerator and where there is no light.
- Another option from thermofischer is the Fluoro-Max Green Dry Fluorscent Particles.
  - This is what the microspheres look like under the microscope.



  - These spheres could be used since they are also 15um which would still be detected by the miniscope.
  - They have a density of 1.05g/cm^3.
  - They have a refractive index of 1.59 at 589nm.
- The final spheres the team decided on are fluorescent green polyethylene microspheres 1.00g/cc that range from 10um-1400um.
  - These spheres are from cospheric.com.
  - These were the cheapest spheres the team could find so they are the ones we decided to order.

- They are $215 and are between 27um-32um so they will be detected by the miniscope [2].
- 
- This is image of the spheres under a microscope.

Conclusions/action items:

Overall, finding the fluorescent spheres needed to be completed for testing. The beads we found will be used in our matrix and we will see how testing goes.

Citations: [1] “Microspheres,” *Thermofisher.com*. [Online]. Available: https://www.thermofisher.com/search/browse/category/us/en/90220126?filter=dimDiameterMetric_dim_ss%3A15%20%26mu%3Bm%C4%89dimColor_dim_ss%3AGreen.

[2] *Cospheric.com*. [Online]. Available: https://www.cospheric.com/UVPMSBG_fluorescent_green_spheres_density100.htm.

09/29/2021- Design ideas

DANA STUMPFOLL - Oct 19, 2021, 2:41 PM CDT

Title: Design ideas

Date: 09/29/2021

Content by: Dana

Present: Individual work

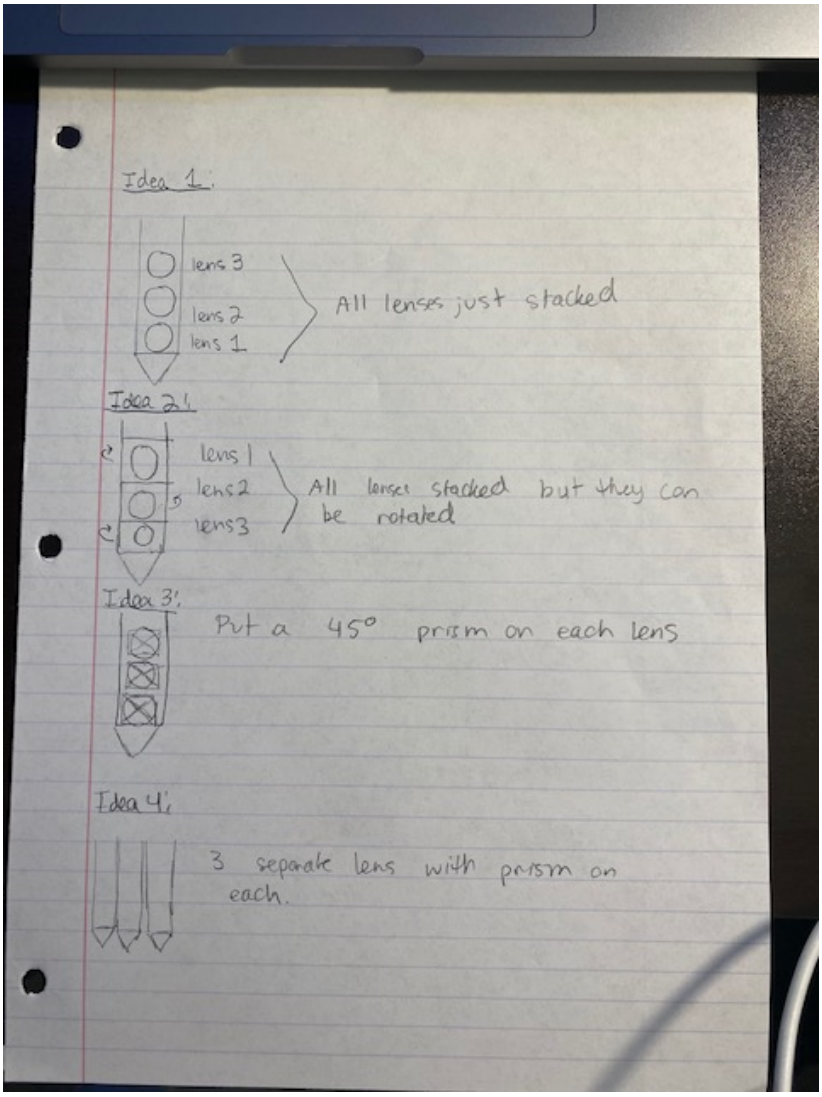
Goals: Come up with design ideas.

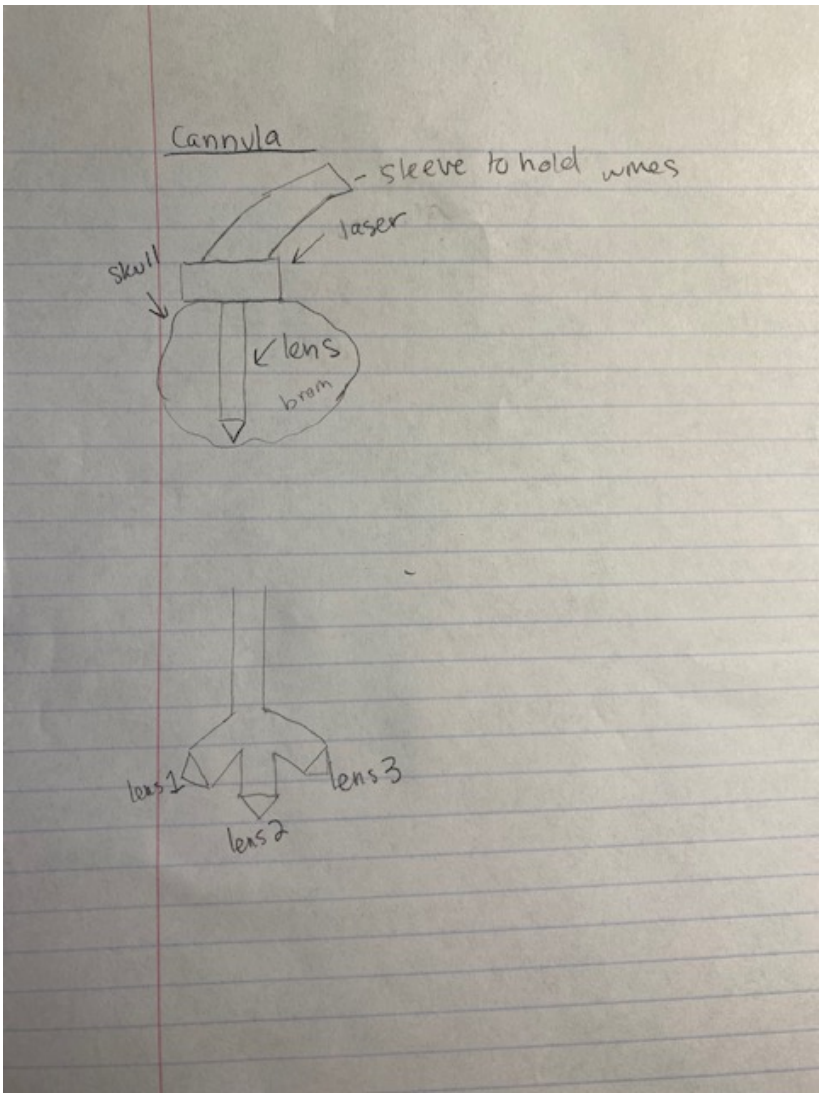
Content:

Ideas 1 and 2 below are similar in that the lenses would be stacked and pointing out of the cannula .

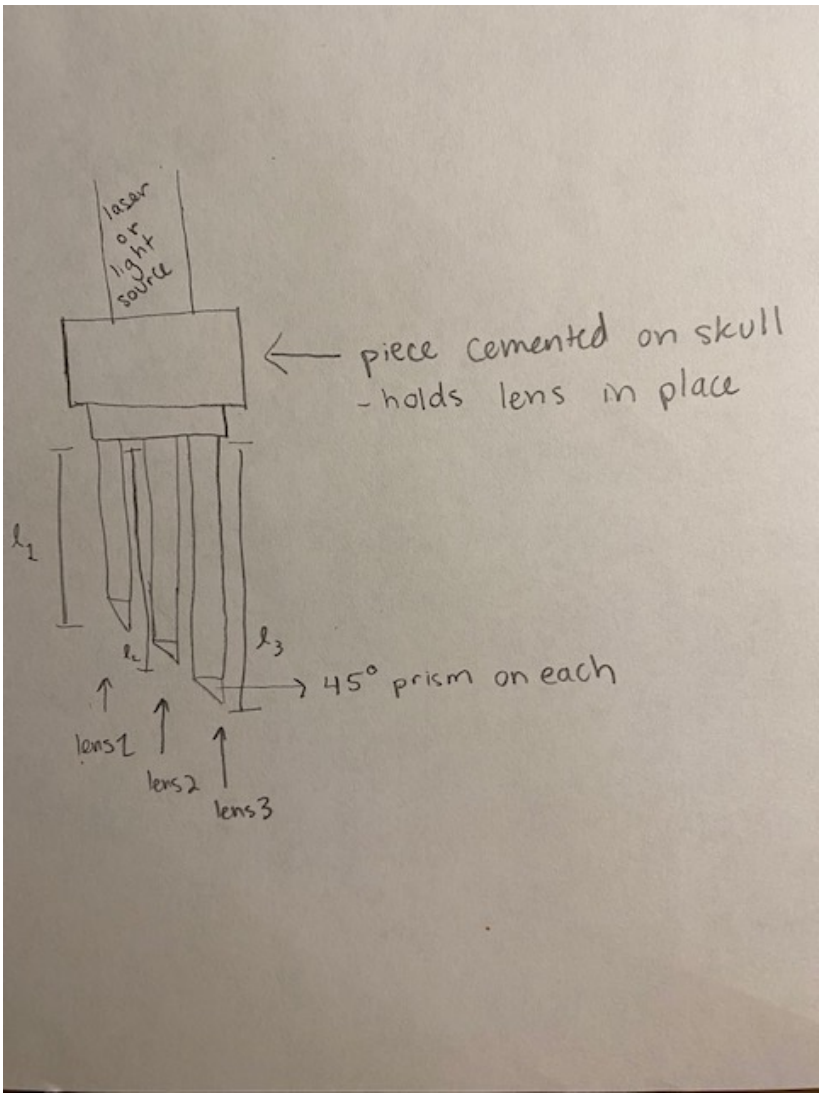
Idea 3 is similar in that the three lenses are stacked and there is a 45 degree prism coming out to change the field of view.

Idea 4 also utilizes the prisms to shift to a side view, but instead of stacking the lenses they are all separated.





The ideas above all consist of a different cannula. The first image provides a visualization of what the current design looks like. The second image provides a different design for the cannula consisting of three separate slots for three separate lenses. Each lens would have a beveled structure to ensure no damage was inflicted on the surrounding tissue.



The final design I came up with was to have three separate length GRIN lens all placed in the vetricle at different depths. Again these designs would incorporate the prism to turn the field of view 90 degrees for longitudinal tracking.

Conclusions/action items:

After doing research I found that none of my designs would would. Incorporating three GRIN lenses in one endoscope is beyond the abilities of our team and the one design I thought was feasible would not fit into the ventricle of the mouse brain. Overall, we chose another team members design for this project.

10/19/2021- Code of federal regulations for endoscope

DANA STUMPFOLL - Oct 19, 2021, 4:29 PM CDT

Title: Code of federal regulations for endoscope and accessories

Date: 10/19/2021

Content by: Dana

Present: Individual work

Goals: Find standards relevant to our project.

Content:

- This code regulates the use of endoscopes in the body that are used for imaging purposes.
- This is apart of the standard ISO 8600.
- This is specific to gastroenterology and urology devices, but can be used for the purpose of our design since it will also be implanted.
- This classifies the device as class 2 because it will contain an LED light source.

Conclusions/action items:

- This code is important for purposes later on if we were to commercialize our product.
- Continue more research on relevant standards.
- Citation: *Fda.gov*. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=876.1500.

12/02/2021 - Matrix Fabrication

DANA STUMPFOLL - Dec 08, 2021, 9:05 PM CST

Title: Matrix Fabrication

Date: 12/02/2021

Content by: Dana

Present: Lauren, Beki, Dana

Goals: Make the matrix for testing for testing on 12/03/2021.

Content:

- The matrix protocol was used for the creation of the matrix for testing.
- Materials:
    - 0.125oz of unflavored gelatin
    - 19oz of distilled water
- Procedure:
    1. Bring 19oz of water to a boil.
    2. Remove from heat.
    3. Add 0.125oz of gelatin to the hot water.
    4. Whisk the mixture until the powder is completely dissolved.
    5. Pour the mixture into the testing vessel of your choice (petri dish or shot glass).
    6. Add a spatula tip full of the spheres and mix into the gel.
    7. Place in the fridge for 4 hours or until the mixture is set.
- The matrix was poured into 5 petri dishes and 2 shot glass cups.
- The fluorescent microspheres were mixed thoroughly throughout the mixture.

Figure 1: Here pictured is Beki pouring the gel matrix and Lauren mixing in the beads.

Figure 2: Pictured here is an image of a gel that has set overnight. The little green dots are the microspheres.

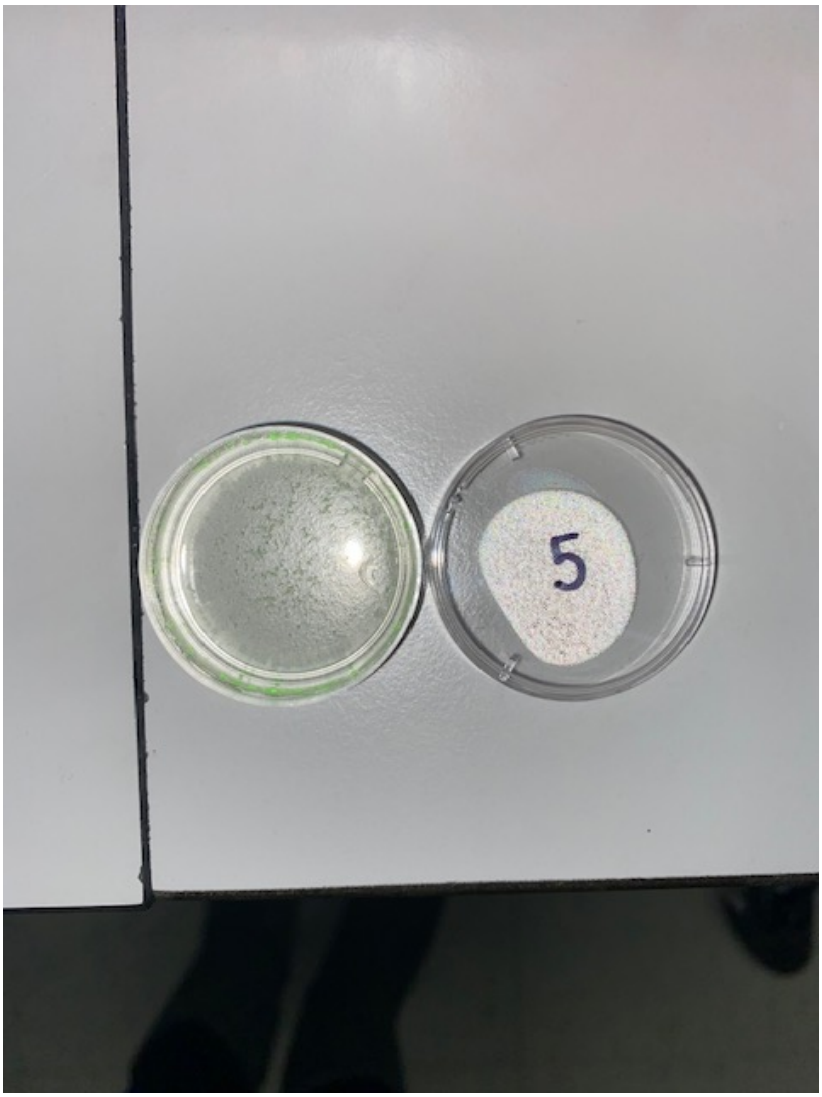

5

Figure 3: Pictured is gel 5 which contained the most amount of the spheres. This is apparent as more green dots can be seen without the use of a microscope.

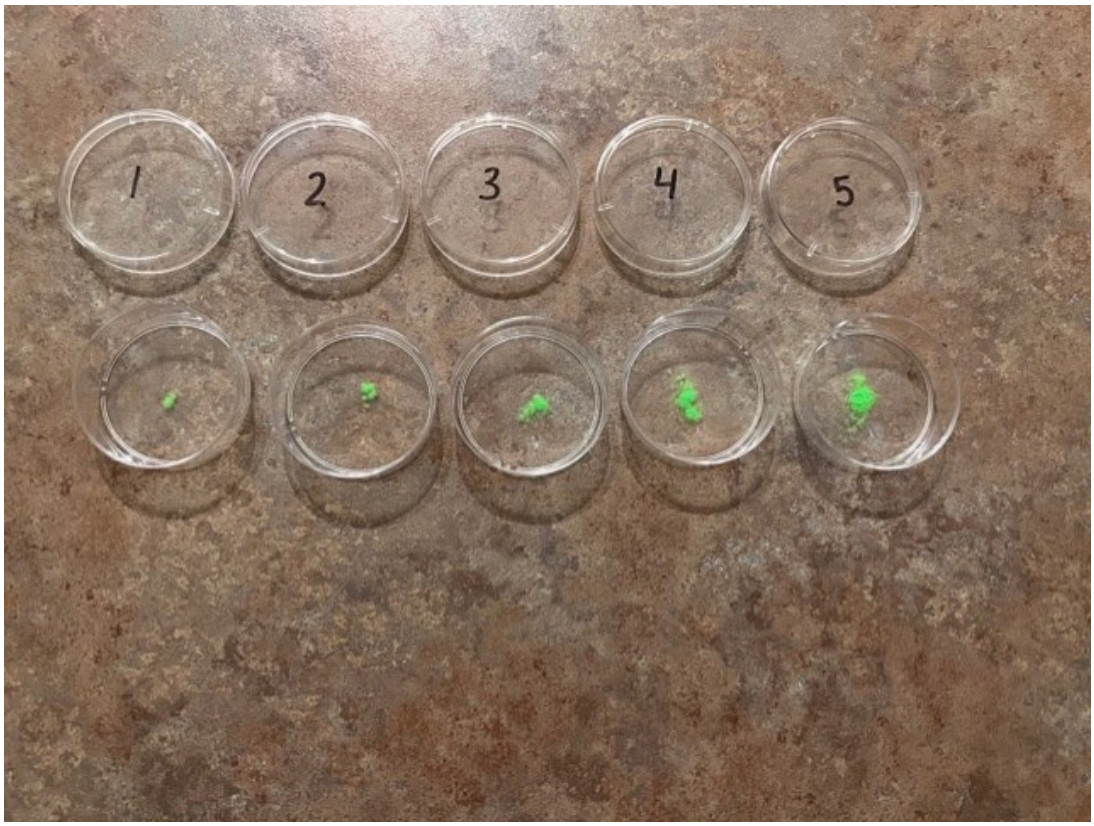


Figure 4: Pictured are the different concentrations of the microspheres used for the gels. Once the gel was poured the matrix was mixed so the spheres are evenly dispersed throughout the dish.

Conclusions/action items:

Overall, the creation of the matrix only took 1 hour and the gels were allowed to set overnight in the fridge.

Action items: Test the matrix using Dr. Trevathan's miniscope. Analyze the data after testing and do some sort of statistical analysis.

12/03/2021 - Testing the field view

DANA STUMPFOLL - Dec 08, 2021, 9:46 PM CST

Title: Testing the Field view

Date: 12/03/2021

Content by: Dana

Present: Lauren, Beki, Dana, Lexi, Alex

Goals: Test the field of view of the microscope using the fabricated matrix.

Content:

- Materials:
  - Built matrix from gel matrix fabrication protocol
  - 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 matrix 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.

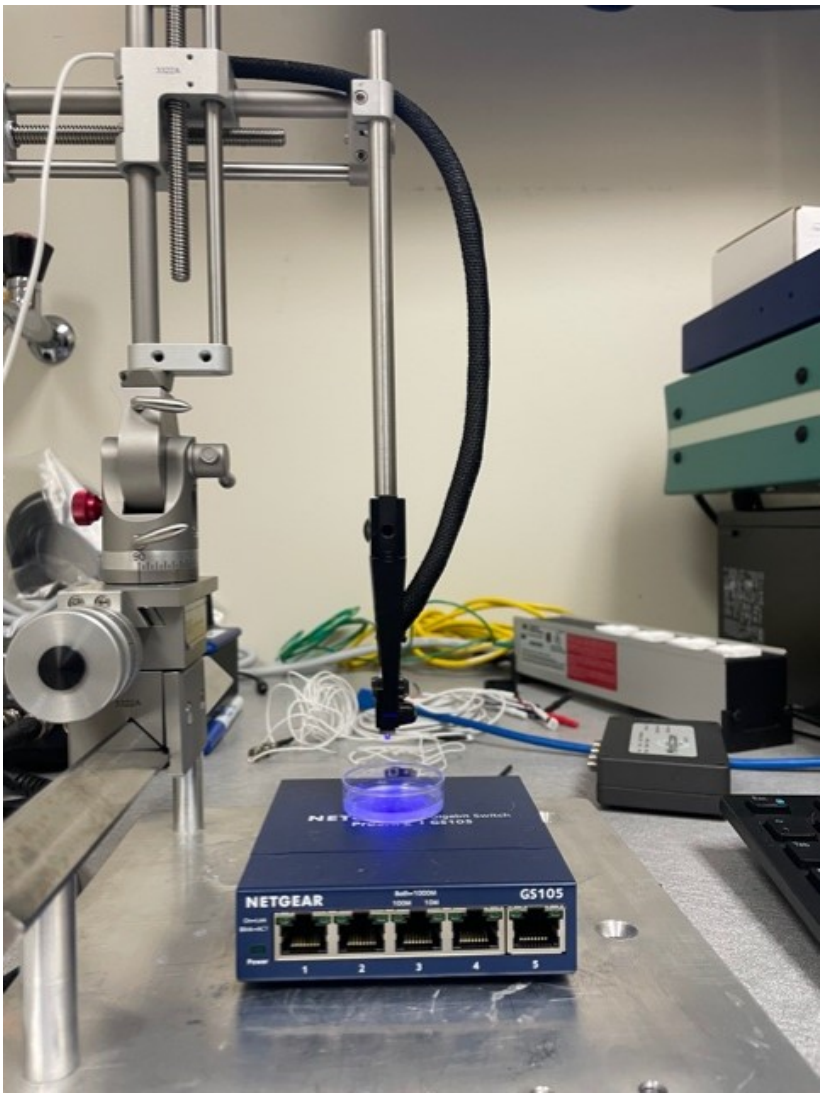


Figure 1: This image shows the setup as testing was running. The nVoke miniscope was used. It emits a blue light and records a green fluorescence.

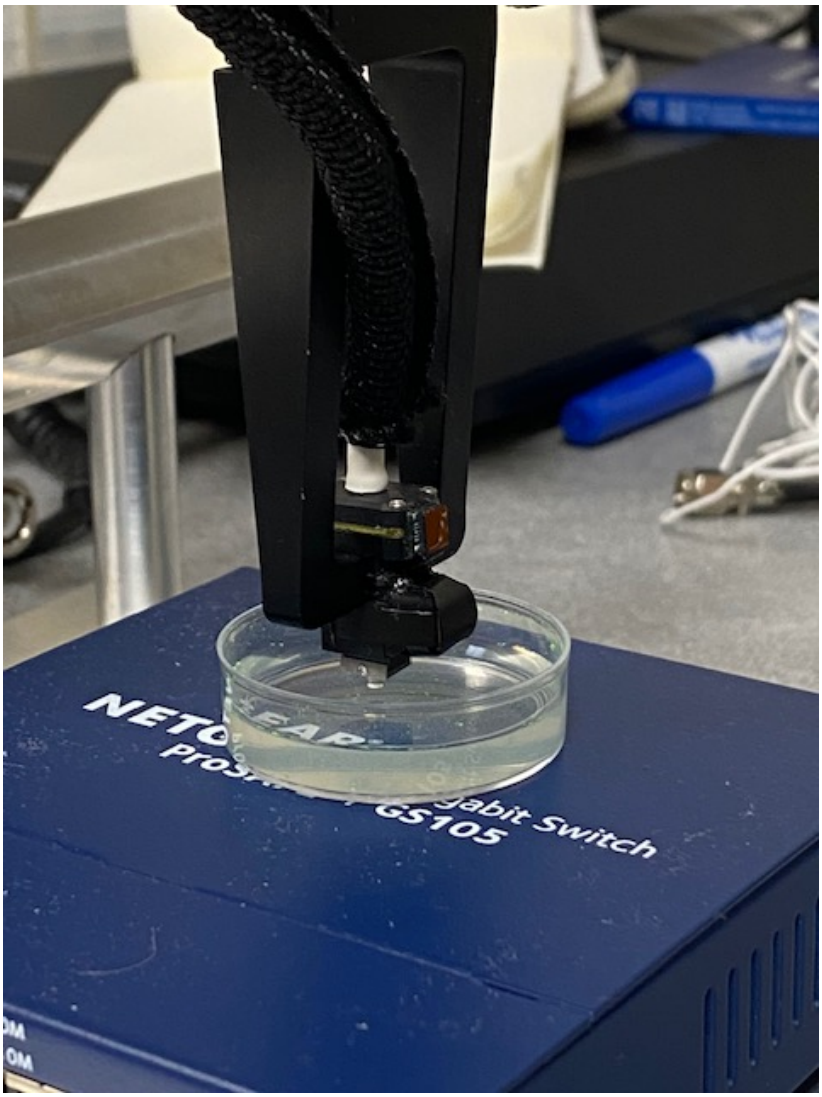


Figure 2: A close up of the miniscope over the top of the matrix. The petri dish is 35x10mm and the miniscope looks small next to it.

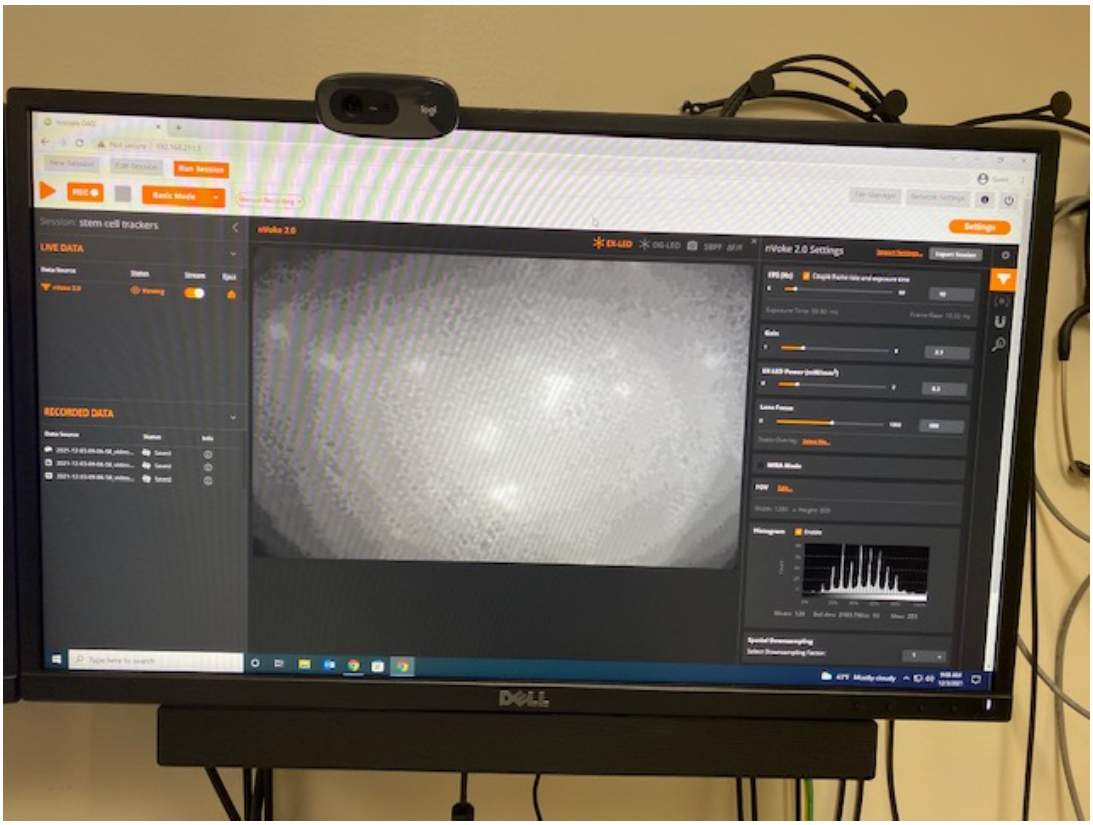


Figure 3: Pictured is an example of the software settings used to take videos of the beads over the gel.

Conclusions/action items:

Overall, the testing took about two and a half hours and the images turned out to be pretty clear. The field of view was 1280 x 800 pixels, or 1050 x 650 µm [1].

Action items: Analyze the data using ImageJ software. Use a statistical analysis to analyze the image quality.

Citations:[1] *Inscopix.com*. [Online]. Available: https://iq.inscopix.com/user-manuals.

12/06/2021 - Data Analysis

DANA STUMPFOLL - Dec 12, 2021, 7:28 PM C

Title: Data Analysis

Date: 12/06/2021

Content by: Dana

Present: Individual work

Goals: Test the fluorescence intensity and how it relates to the field of view of the miniscope.

Content:

- **Measuring Fluorescence Intensity:**
  This analysis was used to determine the total fluorescence of each bead by subtracting out the background fluorescence signal. [1]
  - 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) [1].
  - By measuring the Fluorescence intensity the field of view can be analyzed based on if some of the cells would be lost toward the edges of the field of view.

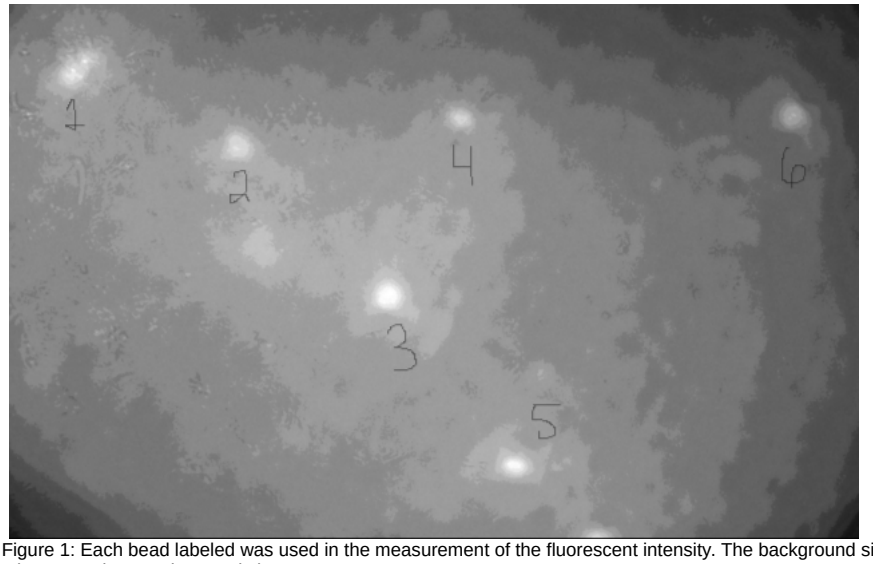


Figure 1: Each bead labeled was used in the measurement of the fluorescent intensity. The background signal can overpower the intensity of the beads. Overall the resolution of microscope is very clean and clear.

- Using the ImageJ setting for measuring mean grey value, area, and integrated density, the fluorescence activity was able to be calculated by subtracting out the mean background signal from the signals produced by each of the beads.

	A	B	C	D	E	F	G	H	I	J
1	Cell	Area	Mean	IntDen			CTCF	corrected total cell fluorescence		
2	1	1025.519	3268.771	3352186			1098664			
3	2	1028.21	3249.302	3340967			1081531			
4	3	979.761	3696.985	3622161			1469189			
5	4	707.904	3074.22	2176253			620671.8			
6	5	1020.135	3376.937	3444933			1203242			
7	6	1268.44	2374.344	3011714			224385.7			
8										
9	Backgrour	Area	Mean	IntDen						
10	1	1028.21	1830.682	1882326						
11	2	1119.727	1535.019	1718802						
12	3	1071.277	2110.367	2260787						
13	4	1270.459	2241.949	2848304						
14	5	1170.868	2615.226	3062084						
15	6	1173.56	2851.432	3346325						
16										
17		Mean fluc	2197.446							

Figure 2: The data collected from imageJ. The corrected total cell fluorescence was calculated using the equation above. (Total Fluorescence = Integrated Density - (Area of Bead*Average Background Fluorescence)) It is apparent that bead three had the highest fluorescence intensity and that the intensity was lost for the beads towards the edge like bead six.

Bead Number	Fluorescent Intensity

1	1098660
2	1081530
3	1469190
4	620672
5	1203240
6	224386

Table 1: Each beads fluorescent intensity was calculated. Bead three was found to have the greatest fluorescent intensity.

Conclusions/action items:

Overall, the testing took about two and a half hours and the images turned out to be pretty clear. The field of view was 1280 x 800 pixels, or 1050 x 650 µm [2]. More testing woul need to be conducted in the mouse brain to determine if the cells would show up on the miniscope. Since the beads mimic the cells, it is very likely this microscope could be used the purposes of our clients research. Future works includes testing a bigger field of view and seeing if the fluorescence intensity is still lost toward the edges of the frame. Attachir different GRIN lenses would also be useful since that is what would be implanted into the mouse brain. The timeline of this course did not allow for this extra testing, but the base data we found is useful for the client in deciding if they would like to invest in this miniscope.

Citations: [1]*Umbc.edu*. [Online]. Available: https://kpif.umbc.edu/image-processing-resources/imagej-fiji/determining-fluorescence-intensity-and-positive-signal/.

[2] Inscopix.com. [Online]. Available: https://ig.inscopix.com/user-manuals.

9/14/21-Adult Neural Stem Cell Tracking in Mice

LAUREN HELLER - Sep 14, 2021, 12:18 PM CDT

Title: Live Imaging of Adult Neural Stem Cells in Freely Behaving Mice Using Mini-Endoscopes

Date: 9/14/2021

Content by: Lauren Heller

Present: N/A

Goals: Research an article provided by our client to get a better understanding of the project prior to the client meeting.

Content:

Typically, adult neural stem cells (NSC's) are studied in vivo when the animal is anesthetized, or ex vivo where post-mortem tissues are studied. This protocol differs in the sense that it observes freely behaving animals, and can be done continuously for up to several months. The protocol looks at live imaging of NSC division and Ca2+ activity.

The procedure goes as follows:

-Plasmid Preparation for Postnatal Electroporation (10min): In this step, preparation of DNA, Solutions, and glass pipets is completed. Specific dilutions and recommended concentrations are all detailed.

-Mini-endoscope preparation (10min): Proper GRIN lens is selected, depending on what the goal of observation is. The lens will likely be the flat-tip or side-view lens, as the prism-coupled lens is much more challenging to install in addition to the flat-tip. There are critical notes at the end of this section that may be useful when we know more about the project goals.

-Postnatal Electroporation (1-3hr): Animal preparation, glass pipet loading, and plasmid injection steps are all detailed. The transfer of current should be done immediately after DNA injection. The mouse pups are then reunited with their mothers.

-Electroporation Efficiency Checking (24hr): 1-2 pups are sacrificed to collect and study their brains, checking for fluorescently labeled NSCs in the subventricular zone (SVZ).

-Mini-endoscope Installation and Fixation (2-4hr): Anesthetize animal and shave the hair, inject non-inflammatory. Specimen is then cut open, and endoscope is installed before a gel glue is applied to seal the opening. The GRIN lens is implanted in the brain. The skin is sewn back together in front and behind the implant. Care needs to be taken when doing this, as too much tension in the skin causes discomfort to the animal, and it may try to scratch out the implant.

After this surgery is complete, the imaging can begin about 2 weeks after if it is a side-view lens, and 3-4 weeks after if it is a flat lens.

-In vivo Imaging of Adult NSC Activation and Physiology (3hr-3 days): Imaging times are progressively increased, to allow the mouse to get accustomed to it.

Following the study, the endoscope is removed.

Limitations: Ability to monitor the activity of the same cells may be compromised over long periods of time. Daughter cells may migrate out of the field of view. Once the GRIN lens is installed, the technique was unable to adjust the focus to better see NSCs in the field of view. Finding a way to adjust the focal plan and track cells in the z direction may resolve these issues.

Conclusions/action items:

Continue research and revisit this protocol further into the project. While there are still a lot of unknowns now, this may prove to have useful information later in the project's development.

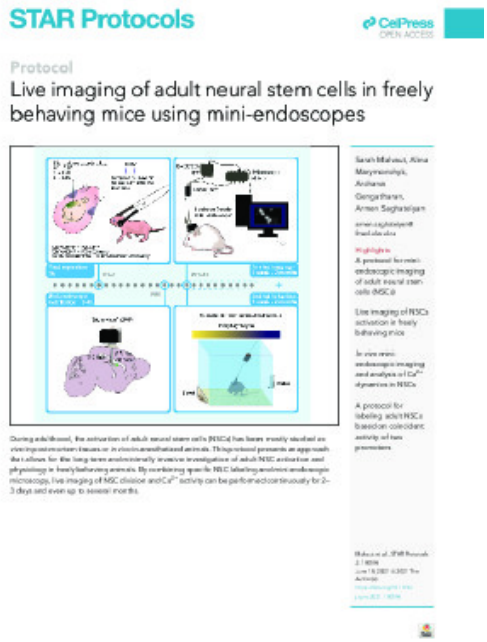
LAUREN HELLER - Sep 14, 2021, 9:24 AM CDT

DOI: https://doi.org/10.1016/j.xpro.2021.100596

Citation:

S. Malvaut, A. Marymonchyk, A. Gengatharan, and A. Saghatelyan, “Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes,” *STAR Protocols*, 12-Jun-2021. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S2666166721003038?via%3Dihub. [Accessed: 14-Sep-2021].

LAUREN HELLER - Sep 14, 2021, 12:19 PM CDT



1-s2.0-S2666166721003038-main.pdf(3.5 MB) - download PDF of Protocol

9/14/21-Long-Term in vivo single-cell tracking reveals the switch of migration patterns

LAUREN HELLER - Sep 14, 2021, 5:50 PM CDT

Title: Long-Term in Vivo Single-Cell Tracking Reveals the Switch of Migration Patterns in Adult-Born Juxtaglomerular Cells of the Mouse Olfactory Bulb

Date: 9/14/2021

Content by: Lauren Heller

Present: N/A

Goals: Research long-term in vivo stem cell tracking to better understand the goals and scope of this project.

Content:

Studying longitudinal adult-born stem cells in vivo is a big challenge in science currently. Most studies on migratory behavior of these cells provide static views of a dynamic process. Scientists believe that migration of cells along the periglomerular layer may occur, but that the hypothesis could not be tested due to lack of ability to track single cells. This study also states that the observation time was four hours, and that they were unable to perform long term single cell tracking in vivo. The two main challenges with single cell tracking are that it is hard to mark individual cells with unique tags, and that there is a lack of a landmark to visualize repetitively.

This study uses an approach called the "optical cell positioning system" (oCPS), which allows for many cells to be accurately measured over the course of days or weeks. Typically, blood vessels carry the fluorescent dyes via intravenous injection, however this needs to be repeated on a daily basis, which is inefficient for long-term tracking. From testing, it was found that 85.8% of the cases precisely tracked individual cells.

Conclusions/action items:

Continue research and revisit this journal article as the project proceeds. Share findings of initial research with the group at the Tuesday meeting.

LAUREN HELLER - Sep 14, 2021, 4:45 PM CDT

DOI: 10.1038/cr.2016.55

Citation:

Y. Liang, K. Li, K. Riecken, A. Maslyukov, D. Gomez-Nicola, Y. Kovalchuk, B. Fehse, and O. Garaschuk, “Long-term in vivo single-cell tracking reveals the switch of migration patterns in adult-born juxtaglomerular cells of the mouse olfactory bulb,” *Cell Research*, vol. 26, no. 7, pp. 805–821, 2016.

10/05/21-Macrophage Response to Hydrophilic Biomaterials Regulates MSC Recruitment and T-Helper Cell Populations

LAUREN HELLER - Oct 05, 2021, 7:19 PM CDT

Title: Macrophage Response to Hydrophilic Biomaterials Regulates MSC Recruitment and T-Helper Cell Populations

Date: 10/05/2021

Content by: Lauren Heller

Present: N/A

Goals: Research potential materials that can be incorporated into our endoscope design

Content:

Abstract: "Successful biomaterial implantation can be achieved by controlling the activation of the immune system. The innate immune system is typically the focus on synthetic material compatibility, but this study shows an effect of surface properties in the innate as well as the adaptive systems. These studies look at how macrophages respond to the implanted materials by releasing factors to regulate the microenvironment and recruit additional cells. Our research demonstrates how macrophage response to material surface properties can create changes in the adaptive immune response by altering T-helper cell populations and stem cell recruitment. Titanium (Ti) implants of varying wettability (rough, and rough-hydrophilic) were placed in the femur of 10-week-old male C57Bl/6, or macrophage ablated clodronate liposome injected and transgenic MaFIA (C57BL/6-Tg(Csf1r-EGFP-NGFR/FKBP1A/TNFRSF6)2Bck/J) mice. The microenvironment surrounding Ti implants was assessed using custom PCR arrays at 3 and 7 days following implantation. Changes in specific T-helper, macrophage and stem cell populations were evaluated locally at the implant surface and systemically in the contralateral leg bone marrow and spleen by flow cytometry at 1, 3 and 7 days. Macrophage importance in T-helper and stem cell population changes with metallic surfaces was examined in both in vitro and in vivo with macrophage ablation models. We demonstrate that surface modifications applied to titanium implants to increase surface roughness and wettability can polarize the adaptive immune response towards a Th2, pro-wound healing phenotype, leading to faster resolution of inflammation and increased stem cell recruitment around rough hydrophilic implants with macrophages present."

-Following macrophage ablation, there are two different parts of the immune system that respond. The innate system consists of macrophages and dendritic cells (main effector cells) and the adaptive immune system (B and T lymphocytes). The innate responds first, followed by the adaptive immune system. For the purpose of our project, we need to avoid both of these responses from happening.

-The study found that macrophages respond differently based on properties, in this case titanium, and characteristics including roughness, wettability, and chemistry could control the polarization of macrophages to either be pro-inflammatory or anti-inflammatory. Anti-inflammatory macrophage activation was found when rough-hydrophilic implants were used, and correlated with high success rates and lower healing times.

-For this study, smooth, rough, and rough-hydrophilic disks were used and were degreased in acetone, and then processed in a solution of 2% ammonium fluoride/2% hydrofluoric acid/10% nitric acid solution at 55 degrees C for 30 seconds. Some were then microblasted. The implants for the in vivo studies were made from grade 4 titanium rods, and they were all sterilized by gamma radiation.

-The implants were placed in the right femoral medullary canal or mice. The mice were anesthetized.

-Flow cytometry was used to quantify changes in cell populations. At the three day mark, similar levels of macrophages were on each implant, and at day seven, a higher percentage was on rough titanium compared to the hydrophilic implant. All surgical procedures resulted in T-helper cell changes. Reduced pro-inflammatory Th17 cells were found on the Ti implants. Th2 cells were found in the greatest quantity on the rough-hydrophilic implants.

Conclusions/action items:

Research more on how to avoid innate responses to materials. Look for research articles pertinent to the brain, as there will be differences between the brain's healing response and that of the femoral medullary cavity.

9/20/21-Devices, methods, and systems for fluorescence-based endoscopic imaging and collection of data

LAUREN HELLER - Sep 29, 2021, 6:25 PM CDT

Title: Devices, Methods, and Systems for Fluorescence-based Endoscopic Imaging and Collection of Data

Date: 9/20/21

Content by: Lauren Heller

Present: N/A

Goals: Research similar products on the market and patents that pertain to them.

Content:

Abstract: "A portable, handheld device for fluorescence-based imaging is provided. The device comprises a wireless communication device having a sensor configured to detect optical signals. The device further comprises an assembly configured to receive and secure the wireless communication device therein. The assembly includes a housing, at least one light source coupled to the housing, a power supply, and an optical filter holder coupled to the housing and configured to receive one or more optical filters. An endoscope portion of the device is positioned relative to the sensor to visualize at least a portion of a confined anatomical space and to receive optical signals from a visualized, illuminated portion of a target positioned within the confined anatomical space. A processor of the device includes image analysis software and is configured to produce a composite representation of the illuminated portion of the target positioned within the confined anatomical space."

Possible patent to be aware of.

Conclusions/action items:

Continue researching competing designs and information regarding the device. It does not appear that implantable endoscopes are very common, and there does not seem to be any protocols regarding a multilens implantable endoscope.

LAUREN HELLER - Sep 20, 2021, 1:12 PM CDT

Link: https://patents.google.com/patent/US20180242848A1/en?q=stem+cell+endoscope&oq=stem+cell+endoscope

IEEE Citation:

R. Dacosta, B. C. Wilson, and K. Zhang, “Devices, methods, and systems for fluorescence-based endoscopic imaging and collection of data.”

9/30/21-In Vivo Photoacoustic Tracking of Mesenchymal Stem Cell Viability

LAUREN HELLER - Sep 30, 2021, 5:10 PM CDT

Title: In Vivo Photoacoustic Tracking of Mesenchymal Stem Cell Viability

Date: 9/30/2021

Content by: Lauren Heller

Present: N/A

Goals: Research competing designs and methods for stem cell tracking in vivo.

Content:

The goal of tracking the stem cells in this study is to gain information on stem cell engraftment, the role of MSCs in vascular repair, and mechanisms of regeneration. This study also states that using MRI for tracking allows for high spatial resolution and long term tracking ability, however the high costs make it a bad choice for quick and continuous analysis. This makes sense why we would not use MRI tracking, as it needs to be in use for up to 4 weeks continuously.

Tracking Stem Cell Viability in vivo:

US/PA imaging was performed right after injection of a PEG-fibrin and MSC mixture, as well as on days 1,3,5,7 and 10 following the injection. There were living cells on day one, which confirmed that the MSCs were alive during implantation. To visualize the stem cells viability, they used a ratiometric heatmap of the photoacoustic signal to visualize the location of the MSCs. They found more living populations and less dead populations. They observed that there is a large reduction in the cell population within the first day, but then the death rate slows through day 10. They noted that the nanoprobe was limited in the sense that it can only detect the viability of stem cells that are loaded with the nanoprobe, and cannot track proliferation, leading to reduced sensitivity over time. All materials and methods are detailed after the conclusion of the journal article if we need to reference it.

Conclusions/action items:

There is a common theme seen in all of these reports in which there is a lack of ability to track stem cell function longitudinally (aka where our design aims to differ).

LAUREN HELLER - Sep 30, 2021, 4:58 PM CDT

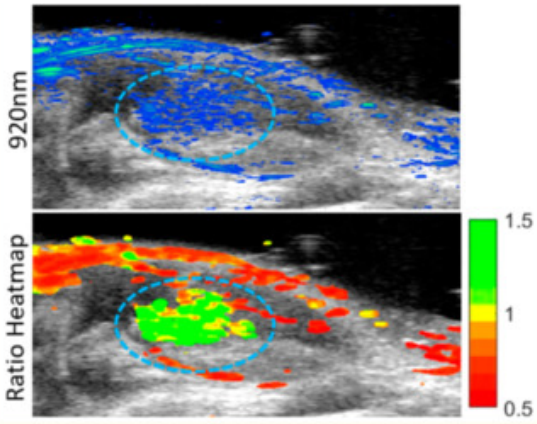


Figure 6.

Imaging stem cells *in vivo*. The US/PA image of transplanted stem cells on day 0 is visualized at 795 and 920 nm excitation, correlating with the IR775c dye and AuNR peak. The labeled stem cells are circled in blue. The last image is a heatmap using the ratio of 795 nm/920 nm. The stem cells display a high ratio as expected due to them being alive directly after transplantation.

Screenshot_139_.png(508.1 KB) - download

LAUREN HELLER - Sep 30, 2021, 4:58 PM CDT

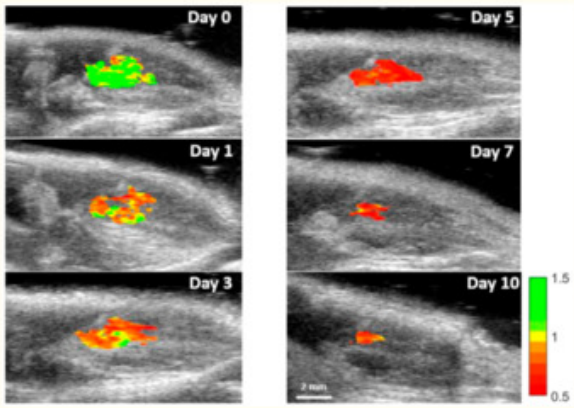


Figure 7.

Ratiometric imaging of stem cell viability *in vivo*. PA images of day 0, 1, 3, 5, 7, and 10 at 795 and 920 nm were obtained, and the ratiometric images were created. The surrounding tissue signal was subtracted to better visualize the change in stem cell viability during the study. Reduction in ratio and signal is visible.

Screenshot_140_.png(362.8 KB) - download

LAUREN HELLER - Sep 30, 2021, 4:57 PM CDT

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7155740/

IEEE Citation:

K. S. Dhada, D. S. Hernandez, and L. J. Suggs, “in vivo photoacoustic tracking of mesenchymal stem cell viability,” *ACS nano*, 23-Jul-2019. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7155740/. [Accessed: 30-Sep-2021].

9/13/21-Gradient-Index (GRIN) Lenses for Imaging

LAUREN HELLER - Sep 14, 2021, 9:16 AM CDT

Title: Gradient-Index (GRIN) Lenses for Imaging

Date: 9/13/2021

Content by: Lauren Heller

Present: N/A

Goals: Research articles and websites recommended by client.

Content:

The GRIN Lens is designed to be used as an implantable lens element for in vivo imaging applications. It is ideal for widefield, confocal, or multiphoton microscopy processes. This lens is intended for regions of interest that are inaccessible with a microscope. These lenses are implanted into a specimen (in our case, mice) and are done so by a minimally invasive procedure. For specific specs, see "features" and/or "specs" on webpage. The GRIN lens varies the index of refraction within the lens, which makes it different from traditional, spherical, and aspheric lenses. The website has three different types of GRIN lenses, each with their own ideal applications. As far as lens care goes, the lenses need to be handled with stainless steel tweezers, methyl alcohol or pure acetone can be used as cleaning solvents. When storing, the lenses need to be placed in a dry container.

Conclusions/action items:

Our project description states that, "So far, endoscopes accomplishing this purpose consist of a head-mounted miniscope and a GRIN, gradient index lens. However, current set ups only allow for imaging in a restricted tissue area, defined by the field of view (FOV) of a single lens. Therefore, we are looking to develop an endoscope combining multiple side-view lenses to create a vertically extended FOV to track single cell migration dynamics in awake, behaving mice. This could potentially be accomplished via a moveable GRIN lens, or an endoscope with multiple stacked GRIN lenses that have overlapping fields of view." Further research needs to be done on the importance of GRIN lenses, as well as how they can be implemented over a traditional FOV lens.

LAUREN HELLER - Sep 14, 2021, 9:03 AM CDT

Link: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=11167

IEEE Citation:

“Gradient-Index (GRIN) Lenses for Imaging,” *Gradient-index (grin) lenses for imaging*. [Online]. Available: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=11167. [Accessed: 14-Sep-2021].

10/27/21-In vivo imaging of unstained tissues using long gradient index lens multiphoton endoscopic systems

LAUREN HELLER - Oct 28, 2021, 4:39 PM CDT

Title: In Vivo Imaging of Unstained Tissues Using Long Gradient Index Lens Multiphoton Endoscopic Systems

Date: 10/27/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Research provided articles to further knowledge.

Content:

-Use of long GRIN lens (up to 285 mm) for multiphoton imaging.

- In most devices, the maximum imaging depth is limited to ~1mm. The strategy for this study is to overcome the depth limitation by developing a two-photon fluorescence miniscope that can be used as an endoscope.

- Studies are showing great promise for GRIN endoscopy to be used as a replacement or guide for traditional surgical biopsies. However, these studies have been limited to short GRIN systems (<4cm).

-A low NA relay lens avoids a tight focus of the excitation light within the glass, while the higher NA objective lens allows for higher resolution two-photon imaging.

-The GRIN lens systems were protected by a 0.1 mm thick stainless steel tube.

-Table 1 shows various GRIN systems, as well as their size and the diameter of the field of view (FOV). There are fields of views ranging from 199 microns to 370 microns, however the lenses with a field of view over 200 microns have a diameter that is too wide for the diameter of the lateral ventricle.

-Increasing the length of the relay lens results in some decrease in the lateral resolution quality. Axial resolution quality decreases more rapidly.

Conclusions/action items:

Report new findings and plans to client.

LAUREN HELLER - Oct 27, 2021, 8:44 PM CDT

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342183/

IEEE Citation:

D. M. Huland, C. M. Brown, S. S. Howard, D. G. Ouzounov, I. Pavlova, K. Wang, D. R. Rivera, W. W. Webb, and C. Xu, “In vivo imaging of unstained tissues using long gradient index lens multiphoton Endoscopic Systems,” *Biomedical optics express*, 01-May-2012. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342183/. [Accessed: 28-Oct-2021].

10/28/2021-Miniscope V4

LAUREN HELLER - Oct 28, 2021, 5:01 PM CDT

Title: Miniscope V4 Specs

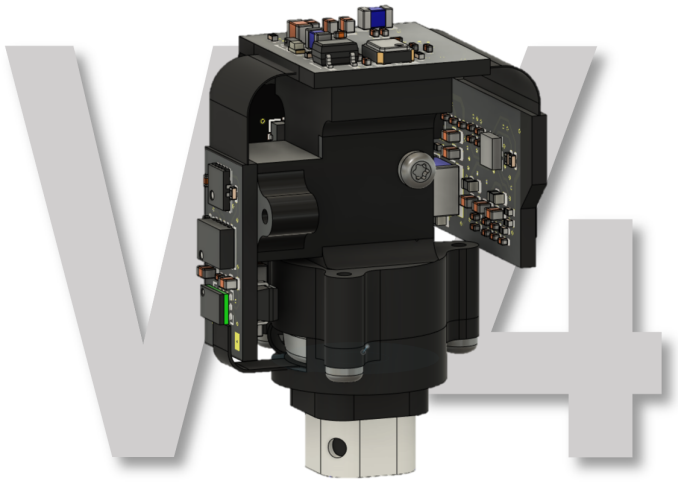
Date: 10/28/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Research the endoscope we plan to use for testing purposes.

Content:



-This miniscope is meant for neuroscience labs to build, use, and modify the miniscope itself for desired use.

Specifications:

-1mm diameter FOV

- approximately 1 mm working distance

- +/- 200 micron adjustable electronic focal range

- weighs 2.6 grams

- 22 mm tall

-Uses a single coaxial cable for communication, power, and data.

Conclusions/action items:

Discuss the specs of the Miniscope V4 with our client and share our plans for testing.

Link: https://github.com/Aharoni-Lab/Miniscope-v4/wiki

IEEE Citation:

Aharoni-Lab, “Home · Aharoni-Lab/Miniscope-V4 Wiki,” *GitHub*. [Online]. Available: https://github.com/Aharoni-Lab/Miniscope-v4/wiki. [Accessed: 28-Oct-2021].

9/20/21-FDA Neurological Endoscope

LAUREN HELLER - Sep 20, 2021, 12:18 PM CDT

Title: Neurological Endoscope FDA Classification

Date: 9/20/2021

Content by: Lauren Heller

Present: N/A

Goals: Find standards and specifications to adhere to in the design process

Content:

The neurological endoscope listed on the FDA website is classified as a Class II device, and requires a premarket notification 510(k). There are no implantable endoscopes currently listed on the FDA website, however the similar devices are all classified as Class II with the same premarket notification. More research on standards will need to be done to fully assess.

Class II (General and Special Controls):

"

> General controls are regulatory requirements authorized by the FD&C Act, under sections 501, 502, 510, 516, 518, 519, and 520. General controls apply to all medical devices, unless exempted by regulations. If a device is exempted from one of the general controls, such exemption is stated in the classification regulation for that device.
>
> For example, the classification regulation for manual tooth brush, 21 CFR 872.6855, states the general controls from which tooth brushes are exempted and certain limitations on the exemptions.
>
> General controls are described in the following sections of the FD&C Act:
>
> - 501: Adulterated devices
> - 502: Misbranded devices
> - 510: Registration of producers of devices
>   - Establishment registration and device listing
>   - Premarket Notification (510k)
>   - Reprocessed single-use devices
> - 516: Banned devices
> - 518: Notifications and other remedies
>   - Notification
>   - Repair
>   - Replacement
>   - Refund
>   - Reimbursement
>   - Mandatory recall
> - 519: Records and reports on devices
>   - Adverse event report
>   - Device tracking
>   - Unique device identification system
>   - Reports of removals and corrections
> - 520: General provisions respecting control of devices intended for human use
>   - Custom device
>   - Restricted device

- Good manufacturing practice requirements
- Exemptions for devices for investigational use
- Transitional provisions for devices considered as new drugs
- Humanitarian device exemption

Special controls are regulatory requirements for class II devices. FDA classifies into class II devices for which general controls alone are insufficient to provide reasonable assurance of the safety and effectiveness of the device, and for which there is sufficient information to establish special controls to provide such assurance.
Special controls are usually device-specific and include:

- Performance standards
- Postmarket surveillance
- Patient registries
- Special labeling requirements
- Premarket data requirements
- Guidelines

"

Conclusions/action items: We would like to adhere to the classification requirements of the endoscopes listed on the FDA website. Continue research on standards and specifications.

LAUREN HELLER - Sep 20, 2021, 11:58 AM CDT

Link: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=3771

IEEE Citation:

“Product classification-Neurological Endoscope,” *accessdata.fda.gov*. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=3771. [Accessed: 20-Sep-2021].

LAUREN HELLER - Sep 20, 2021, 12:01 PM CDT


New Search | Back to Search Results

Device	Endoscope, Neurological
Regulation Description	Neurological endoscope.
Definition	If the device is reusable, validated reprocessing instructions and reprocessing validation data for this device type must be included in a 510(k) submission (82 FR 26807, available at https://www.gpo.gov/fdsys/pkg/FR-2017-06-09/pdf/2017-12007.pdf).
Regulation Medical Specialty	Neurology
Review Panel	Neurology
Product Code	GWG
Premarket Review	Neurosurgical, Neurointerventional and Neurodiagnostic Devices (DHT5A) Neurosurgical, Neurointerventional and Neurodiagnostic Devices (DHT5A)
Submission Type	510(k)
Regulation Number	882.1480
Device Class	2
Total Product Life Cycle (TPLC)	TPLC Product Code Report
GMP Exempt?	No
Summary Malfunction Reporting	Ineligible
Implanted Device?	No
Life-Sustain/Support Device?	No
Third Party Review	Not Third Party Eligible

'

Screenshot_100_.png(53.5 KB) - download

9/20/21-ISO 8600-3:2019

LAUREN HELLER - Sep 20, 2021, 12:47 PM CDT

Title: Endoscopes — Medical endoscopes and endotherapy devices — Part 3: Determination of field of view and direction of view of endoscopes with optics

Date: 9/20/2021

Content by: Lauren Heller

Present: N/A

Goals: Research standards and specifications regarding implantable endoscopes

Content:

Abstract: "This document applies to endoscopes designed for use in the practice of medicine. It specifies measurement requirements and describes two test methods for measuring the field of view and direction of view of endoscopes. Method A uses the distance from the distal window to calculate the field of view. Method B uses the distance from the entrance pupil. Other test methods can be used if they obtain equivalent results. "

This standard applies to testing field of view, which is likely going to be an important part of prototyping and our final design. When tracking stem cells, the field of view will be an important measurement to know, as the stem cells migrate naturally. This standard specifies measurements as well as the two methods to test the field of view. This standard costs $62.51 to purchase.

Conclusions/action items:

LAUREN HELLER - Sep 20, 2021, 12:51 PM CDT

Link: https://www.iso.org/standard/65018.html

IEEE Citation:

“ISO 8600-3:2019,” *ISO*, 29-Jul-2019. [Online]. Available: https://www.iso.org/standard/65018.html. [Accessed: 20-Sep-2021].

11/01/21-Low-Cost Fabrication of Optical Tissue Phantoms for Use in Biomedical Imaging

LAUREN HELLER - Nov 01, 2021, 1:45 PM CDT

Title: Low-Cost Fabrication of Optical Tissue Phantoms for Use in Biomedical Imaging

Date: 11/01/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Research potential ways to formulate a phantom gel matrix for testing.

Content:

This article describes the fabrication process of an agar gel phantom that is meant to simulate the optical properties of the brain, bladder wall, and lungs at 532 and 630nm wavelengths. The bulk matrix consists of agar powders dissolved in water, and then they are loaded with India Ink and aluminum oxide particles for optical absorption and scattering targets. They used polymeric Agar, comprising of seaweed polysaccharides, as they have similar optical and biochemical properties similar to those of biological tissue.

-Most fluorochromes used in cancer photodynamic therapy and photo-detection have an affinity for proteins, so bovine serum was used in order to introduce protein into the phantom matrix.

-The mold used in this was a softball display case, used for curing. Phantoms of various thicknesses were placed in an aluminum disk PMDS mold and then placed in a vacuum chamber for an hour to extract any air bubbles that were created. They cured at room temperature for 1-2 days.

Low-Cost Phantom Fabrication:

-Tryptone soy and Bacteriological Agar (Sigma Aldrich) were base materials used.

-For the Brain tissue sample using Bacteriological Powder for a 532nm wavelength laser, the recipe consisted of 2.0g agar powder, 0.3g Al2O3, and 0.3 ml India Ink

-For the Brain tissue sample using Soy powder for 532nm a mixture of 6.0g Agar powder, 0.3g Al2O3, and 0.3 g india ink were used.

-The solution was stirred and heated on a hot plate to ~94 degrees Celsius, then allowed to cool to 20 degrees, and wrapped with aluminum foil. It was then stored for 24 hours in a refrigerator to solidify.

-The absorption and scattering coefficients were determined by Beer-Lambert law relationship, under the assumption that there is not multiple scattering, and the scattering comes from the Al2O3 particles.

-Tables of power intensities through different thicknesses of the phantoms are given in table 2 and table 5 of the article.

Conclusions/action items:

Continue looking into phantom gel matrix options, share findings at team meeting, begin formulating a testing plan.

LAUREN HELLER - Nov 01, 2021, 1:49 PM CDT

DOI: https://doi.org/10.1016/j.heliyon.2020.e03602

IEEE Citation:

L. Ntombela, B. Adeleye, and N. Chetty, “Low-cost fabrication of optical tissue phantoms for use in biomedical imaging,” *Heliyon*, vol. 6, no. 3, Mar. 2020.

11/01/21-Decellularized Porcine Brain Matrix for Cell Culture and Tissue Engineering Scaffolds

LAUREN HELLER - Nov 01, 2021, 2:53 PM CDT

Title: Decellularized Porcine Brain Matrix for Cell Culture and Tissue Engineering Scaffolds

Date: 11/01/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Research methods for developing a phantom gel matrix.

Content:

- The extracellular matrix (ECM) is composed of a complex variety of proteins and polysaccharides. Many studies use purified proteins for cell culture coatings despite the ECM's complexity in vivo. Decellularized matrices allow for us to mimic native ECM.

- Brains were removed from female Yorkshire pigs. These brains were cut into halves, a sample was removed for later comparison studies, and then the brains were decellularized by adding 400ml 1% sodium dodecyl sulfate (SDS) in phosphate buffered saline (PBS) and 1% penicillin. The mixture was decanted each day, and then refilled until the tissue was fully decellularized. The mixture was separated into tubes and centrifuged. This was repeated up to 12 times to remove residual SDS before freezing for analysis. The ECM was then stored until needed.

- The ECM was sliced into 10micron slices and then stained with hematoxylin and eosin to make sure it was decellularized.

-To test using the brain matrix as a cell culture coating, the decellularized matrix was turned into a liquid form. From there, it was diluted and then incubated.

Conclusions/action items:

While this method seems to work well to create a matrix, it does not seem that it will fit the needs of our project. In addition to this, it is much more complex than the gel matrix that was researched above this, and would be much more costly. Due to time and cost restraints on the project, this would not be a good choice to use as reference when designing our testing apparatus.

LAUREN HELLER - Nov 01, 2021, 1:53 PM CDT

DOI: 10.1089/ten.tea.2010.0724

IEEE Citation:

J. A. DeQuach, S. H. Yuan, L. S. B. Goldstein, and K. L. Christman, “Decellularized porcine brain matrix for cell culture and tissue engineering scaffolds,” *Tissue Engineering Part A*, vol. 17, no. 21-22, pp. 2583–2592, Oct. 2011.

11/18/21-Stable Phantom Materials for Ultrasound and Optical Imaging

LAUREN HELLER - Nov 18, 2021, 4:36 PM CST

Title: Stable Phantom Materials for Ultrasound and Optical Imaging

Date: 11/18/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Work to create a better phantom matrix that will work for imaging. Work to find better transparency in the gel

Content:

- Water based phantoms tend to have easier degradation and lower temporal stability

- Water based phantoms tend to be cheaper, available, and easy to prepare. The issue of degradation here will likely not be an issue for our project, as we are not planning on needing to preserve them for a long time. If we planned to need them for a longer duration, there may be issues with bacterial invasion and/or dehydration.

- styrene-ethylene/butylene-styrene (SEBS) copolymers show good potential for producing translucent phantoms, and are also stable long-term.

- Tests for temporal stability were monitored on day 0, 16, 72, and 219. The SEBS phantom remained in the best condition over this period of time.

Conclusions/action items:

While the methods used here for SEBS gels are likely not feasible for our team given the amount of time left this semester, it certainly is good information to keep in mind for potential use in the future, or if this project is continued into another semester. The SEBS matrix may be a good idea for testing an implantable endoscope prior to implantation into a mouse brain.

LAUREN HELLER - Nov 18, 2021, 4:02 PM CST

Link: https://iopscience.iop.org/article/10.1088/1361-6560/62/2/432

IEEE Citation:

“Stable phantom materials for ... - iopscience.iop.org.” [Online]. Available: https://iopscience.iop.org/article/10.1088/1361-6560/62/2/432.

11/18/2021-Agarose-Based Tissue Mimicking Optical Phantoms for Diffuse Reflectance Spectroscopy

LAUREN HELLER - Nov 18, 2021, 5:30 PM CST

Title: Agarose-Based Tissue Mimicking Optical Phantoms for Diffuse Reflectance Spectroscopy

Date: 11/18/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Find a way to make an optimal phantom gel matrix for imaging testing.

Content:

- Acrylic molds were used (two rectangles and a U-shaped piece) to create an epidermal phantom with whole blood.

- Inverse Monte Carlo simulation was used to determine the absorption coefficient spectrum by measuring diffuse reflectance and total transmittance spectra with a spectrometer and integrating sphere

- Optical phantoms are used in place of the actual tissue, so they must mimic optical properties (light scattering and absorption coefficients) of those of living human and animal tissues.

- Polymer microspheres, titanium oxide powder, and lipid emulsions (like milk) are used as light scattering agents

- Black ink and molecular dyes are used as light absorbers.

Preparation of Base Material:

- 500mL standard saline, 0.9% NaCl are placed into a pod. 5g of agarose powder is added slowly while stirring the mixture

- Mixture is heated for 5 min with 1000W. Once mixture boils, mixture is heated on low heat for three more minutes

-Cool mixture to 70 degrees C, then pour into a container and keep in a constant temp bath at 60 deg C for 30 mins before making the phantom

-To mimic skin, a coffee solution can be used to mimic the absorption spectrum of melanin, as the coffee contains a brown pigment called melanoidin. (For this protocol, see article. Not applicable to our design process)

- Testing and data collection showed that the dermal phantom had a significantly higher absorption coefficient that was much more linear than those of the dermal phantom with oxygenated blood and the dermal phantom with deoxygenated blood.

Conclusions/action items:

Continue working to create a more optimized gel phantom for testing. Work with James and Kevin to purchase beads for testing.

LAUREN HELLER - Nov 18, 2021, 4:39 PM CST

Link: https://www.jove.com/t/57578/agarose-based-tissue-mimicking-optical-phantoms-for-diffuse

IEEE Citation:

A. Mustari, I. Nishidate, M. A. Wares, T. Maeda, S. Kawauchi, S. Sato, M. Sato, and Y. Aizu, “Agarose-based tissue mimicking optical phantoms for diffuse reflectance spectroscopy: Protocol,” *JoVE (Journal of Visualized Experiments)*, 22-Aug-2018. [Online]. Available: https://www.jove.com/t/57578/agarose-based-tissue-mimicking-optical-phantoms-for-diffuse. [Accessed: 18-Nov-2021].

9/29/21-Modified Traditional Endoscope

LAUREN HELLER - Sep 30, 2021, 12:12 PM CDT

Title: Modified Traditional Endoscope Design Idea

Date: 9/29/2021

Content by: Lauren Heller

Present: N/A

Goals: Brainstorm design ideas and criteria for the design matrix to ultimately narrow down to one final design idea

Content:

This model illustrates a traditional endoscope that is used commonly in medical practices. With this endoscope, there are light sources on both ends of the endoscope, with two lenses between the light sources. When the sources emit the light, they bounce back off the surface and back through the lenses for viewing. By modifying these concepts and making it both mini and implantable, it would be fairly feasible to design.

Some potential criteria:

-Feasibility

-Cost

-Ease of Use

Conclusions/action items:

Continue brainstorming design ideas and criteria, share these ideas with the group.

10/05/21-Elongated Lens Endoscope

LAUREN HELLER - Oct 05, 2021, 5:23 PM CDT

Title: Elongated Lens Endoscope Design Idea

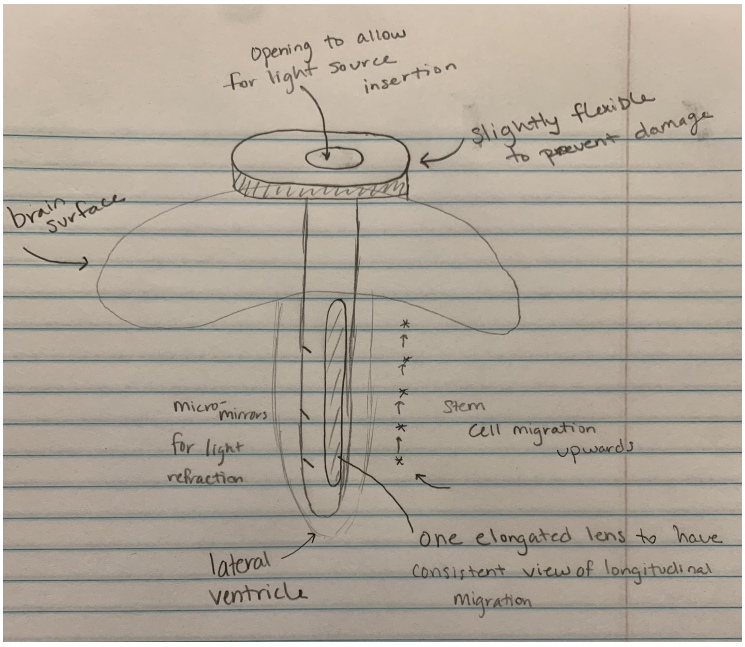
Date: 10/5/2021

Content by: Lauren Heller

Present: N/A

Goals: Brainstorm new design ideas after the secondary client meeting where concepts were clarified

Content:



This design features an elongated lens design rather than a lens array. There is also an opening in the top of the endoscope to place a light source into the device. The top of the endoscope is wider so that it can be secured to the surface of the brain, and then it would be made out of a material that has some slight flexibility to it. It was found that the endoscopes that were completely rigid were more likely to cause damage to the mouse brain, whereas those that had a slight give to them did not cause injury.

Conclusions/action items:

While this would make it much easier to track than a multi-lens design, these lenses are not something that is accessible to us (whereas the GRIN lenses are). In addition, these lenses would likely have to be created instead of purchased, which would make it less feasible than the multi-lens array. On the other hand, when it comes to looking at images, with one lens we would not have to take into account potential overlap or gaps in the longitudinal migration, since it would all be visualized through one lens.

11/09/2021-Testing Apparatus Ideas

LAUREN HELLER - Nov 10, 2021, 7:17 PM CST

Title: Testing Apparatus Brainstorm

Date: 11/09/2021

Content by: Lauren Heller

Present: Individual Work

Goals: Brainstorm ideas to create the gel phantom matrix for testing.

Content:

- The miniscope V4 is 22mm tall. We need to find an apparatus that will have more than enough depth to house the miniscope.

- I propose the use of a tall plastic shot glass. The plastic shot glass is 4.064 cm in diameter, and 10.41 cm tall.

- Plastic shot glasses are easy to obtain, inexpensive, and would be something that we can use given the remaining time of the semester.

- We need to decide on if the shot glass should be transparent or more opaque. If we do want to make it more opaque, we could paint the outside of the shot glass with either a paint or mod-podge of some sort.

Conclusions/action items:

By having an inexpensive and easy-to-find apparatus, we could easily experiment with the agar gel consistency through trial and error, rather than needing to hope that certain calculations are correct for our needs. By having the ability to test the consistency, we can more finely-tune our testing design and protocol.

Lauren Heller - Training Documentation

LAUREN HELLER - Dec 13, 2021, 8:21 PM CST

This certifies that Lauren Heller has completed training for the following course(s):

Course	Assignment	Completion	Expiration
Biosafety Required Training	Biosafety Required Training Quiz	2/15/2021	
Chemical Safety: The OSHA Lab Standard	Final Quiz	3/23/2021	

9/12/2021- Optimized Longitudinal Stem Cell Tracking

Title: Optimized Longitudinal Monitoring of Stem Cell Grafts in Mouse Brain Using a Novel Bioluminescent/Near Infrared Fluorescent Fusion Reporter

Date: 9/12/2021

Content by: Rebekah

Present: Rebekah

Goals: To gain information on longitudinal monitoring of stem cells in mouse brain

Link:
https://www.researchgate.net/publication/322893860_Optimized_Longitudinal_Monitoring_of_Stem_Cell_Grafts_in_Mouse_Brain_Using_a_Novel_Bioluminr

Citation:

Optimized Longitudinal Monitoring of Stem Cell Grafts in Mouse Brain Using a Novel Bioluminescent Near Infrared Fluorescent Fusion Reporter. Research
https://www.researchgate.net/publication/322893860_Optimized_Longitudinal_Monitoring_of_Stem_Cell_Grafts_in_Mouse_Brain_Using_a_Novel_Bioluminr
[Accessed: 13-Sep-2021].

Content:

There are many challenges to have sensitive longitudinal imaging of transplanted stem cells due to many factors, such as low light penetration in deep tiss now, stem cells have been imaged successfully using bioluminescence imaging(BLI). Another more recent but also promising way of imaging called sensitiv in vivo imaging. In addition to the effect of the reported expression on stem cell viability and differentiation potentials being evaluated, the sensitivity of stem in a longitudinal study. This article was focused on a comparison between BLI and FLI imaging, so although it could be helpful sometime in the future of this to giving me a background on the issue.

Conclusions/action items:

Complete more research on fluorescent imaging of stem cells in the brain and come back to this article in the future when I have more of a background knc

Rebekah Makonnen - Sep 12, 2021, 12:28 PM CDT

Optimized Longitudinal Monitoring of Stem Cell Grafts in Mouse Brain Using a Novel Bioluminescent/Near Infrared Fluorescent Fusion Reporter

Laura Mezzanotte[1], Jovita Delancy Ilas[2,3], Ivo Que[4], Alan Chan[1], Eric Kaijzel[4], Rob Hoeben[2], and Clemens Löwik[1]

Abstract

Introduction

Optimized_Longitudinal_Monitoring_of_Stem_Cell_Gra.pdf(963 KB) - download

9/13/2021- Protocol for live imaging adult neural stem cells in free moving mice

Rebekah Makonnen - Sep 16, 2021, 10:40 AM CDT

Title: Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes

Date: 9/13/2021

Content by: Rebekah

Present: Rebekah

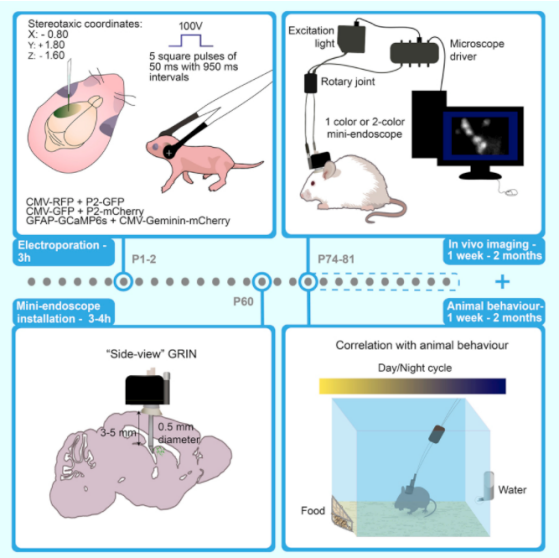
Goals: To understand what live imaging of adult neural stem cells in freely behaving mice looks like

Link: https://starprotocols.hivebench.com/protocols/723

Citation: Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes. *Hivebench.com*. [Online]. Available: https://starprotocols.hivebench.com/protocols/723. [Accessed: 13-Sep-2021].

Content:

The protocol in this article gives an approach that allows for long-term and minimally invasive imaging of adult neural stem cell (NSC) activation and physiology.



The image above is a picture representation for adult NSC imaging. The top left picture represents placing the endoscope in the mouse brain. The bottom left image is a side view representation of the endoscope that will be placed for imaging. The two images on the right are representative of data collection and information that can be obtained once the endoscope is places. The top right image shows the imaging that can be obtained from the endoscope. While the bottom right image shows animal behavior being tracked and how it can be linked to NSC imaging.

This protocol stated that NSC imaging can begin 2-4 weeks after the endoscope installation. It also stated that the imaging can last anywhere from 2-3 days up to several months.

Conclusions/action items:

This article was extremely helpful to visualize this project and have an understanding of the timeline of implantation to usable images. Next I plan on looking into the GRIN lenses more and look for ways that current designs can be improved.

9/15/2021- GRIN Lens

Rebekah Makonnen - Sep 15, 2021, 11:09 AM CDT

Title: GRIN lens rod based probe for endoscopic spectral domain optical coherence tomography with fast dynamic focus tracking

Date: 9/15/2021

Content by: Rebekah

Present: Rebekah

Goals: To learn about the GRIN lens, along with some of its drawbacks

Link: https://www-osapublishing-org.ezproxy.library.wisc.edu/DirectPDFAccess/3170103F-2ED2-4C95-8E39DD6C50AB5B56_89308/oe-14-8-3238.pdf?da=1&id=89308&seq=0&mobile=no

Citation: T. Xie, S. Guo, Z. Chen, D. Mukai, and M. Brenner, "GRIN lens rod based probe for endoscopic spectral domain optical coherence tomography with fast dynamic focus tracking," *Opt. Express*, vol. 14, no. 8, pp. 3238–3246, 2006.

Content:

A GRIN (gradient index) lens can capture three dimensional imaging using a system over an area of tissue that corresponds to the lens surface. Many current optical coherence tomography (OCT) systems have a fixed plane that is attached to the distal end of the probe. In an effort to improve focus on the images, a high numerical aperture (NA) lens is used and it is extremely important that the whole probe is located precisely where the focal spot scans the tissue at half the imaging depth under the tissue surface.

Dynamic focusing ahs also been used, along with zone focusing and image fusing, to maintain high transverse resolution over the entire depth scan. Many different dynamic focusing methods have been reported in order to perform different depth imaging scans that are required to improve lateral resolution. However, these methods are not adequate for endoscopic use due to their bulkiness.

In this study, a GRIN lens rod is able to dynamically adjust focus from the proximal end of the probe (outside the body) in order to improve lateral resolution and obtain high quality images. This probe is able compact, doesn't require any moving components on the distal end, and can image frontal or side viewing if it is connected to a time-domain OCT.

An in vivo study was performed on anesthetized rabbits, where lung tumors were induced. It is possible to increase the lateral resolution by decreasing the light focal spot size of the objective lens. When samples were being imaged, they were placed on a stage then imaged.

Conclusions/action items:

Overall this article was a little different from what I was expecting, the GRIN lenses were used differently than how they would be used in our current project. However, some of the techniques used to adjust these lenses could be helpful for our work on this project.

9/29/21- Deep Brain Imaging in Freely Moving Mice using Implanted Reconnectable Fiber Bundles

Rebekah Makonnen - Sep 29, 2021, 12:24 PM CDT

Title: Deep Brain Imaging in Freely Moving Mice using Implanted Reconnectable Fiber Bundles

Date: 9/29/2021

Content by: Rebekah

Present: Rebekah

Goals: To understand how this is used to take deep brain images in mice.

Link: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jbio.202000081

Citation: 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.

Content:

- This approach is not intended for a single-cell activation but it helps to simulate and monitor the activity of bulk cell populations
- Fiber endoscopes are minimally invasive and give high resolution one- and two-photon imaging in the deep brain
    - there is an issue, the fibers are prone to bend, which is nearly inevitable with freely moving mice, causing image distortions
- Issue with excessive brain volume displacement and using multiple GRIN lenses- keeping the displacement within 1% of the total brain volume
- This mini endoscope will have reconnectable implantable fiber-optic microendoscope that can integrate a branching fiber bundle(BFB) with GRIN lenses
    - this enables simultaneous imaging of individual cells in distinct brain regions, including the neocortex and hippocampus
- BFB microendoscope has two reconnectable sections
    - the bottom section (Section B), which will be implanted in the mouse brain, consists of 3 extremely short identical fiber bundles that are each spliced to a GRIN lens. one end of the fiber bundle is fixed in a triangular-lattice arrangement connected to a ceramic ferrule, this allows a connection to be made with the other part of the device that is removable. this section is also secured to the head using cement
    - the top section (Section A) is a stretch of bundle of identical optical fibers, this section is what makes a connection to software in order to process and analyze the images
- in order to minimize the brain displacement that is caused by implanting this device, extra effort was made to reduce the diameter of the fiber bundles and GRIN lenses to be implanted
    - many other studies show that brain-tissue alterations caused by fiber implantation does not have a significant impact on the microglia density
- GRIN fibers are able to pick up fluorescence from fluorescent markers, then they produce inverted images of brain cells in the distal ends of the Section B bundles

Conclusions/action items:

Complete more research for possible design ideas and brainstorm possible designs for design matrix

10/6/21- Acceptable light wavelengths for fluorescent protein

Rebekah Makonnen - Oct 06, 2021, 10:00 AM CDT

Title: Acceptable excitation values for tdTomato fluorescent protein

Date: 10/6/21

Content by: Rebekah

Present: Rebekah

Goals: Get excitation values for tdTomato protien

Link: https://catalog.takara-bio.co.jp/PDFS/200812_15.pdf

Content:

- excitation max: 554 nm
- emission max: 581 nm

Conclusions/action items:

Look into more lenses and continue research

Citation:

Takara-bio.co.jp. [Online]. Available: https://catalog.takara-bio.co.jp/PDFS/200812_15.pdf. [Accessed: 06-Oct-2021].

10/26/2021- In vivo imaging of unstained tissues using long gradient index lens multiphoton endoscopic systems

Rebekah Makonnen - Oct 27, 2021, 1:07 PM CDT

Title: In vivo imaging of unstained tissues using long gradient index lens multiphoton endoscopic systems

Date: 10/26/2021

Content by: Rebekah

Present: Rebekah

Goals: To understand how an in vivo test would be using a GRIN setup

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342183/

Content:

- GRIN lenses provide a great minimally invasive option in order to image deep (> 1mm) tissue areas due to the fact that they have shown to be biocompatible and their small size
- this study looks at how the length of the GRIN imaging system impacts the imaging performance
- most GRIN two-photon endoscope systems use a combo of relay and objective lenses in either a doublet (relay/objective) or triplet (objective/relay/objective) system
- although the triplet system offers higher resolution, there are many advantages that the doublet system offers such as:
  - similar FOV at reduced cost
  - greater image magnification
  - lower NA for coupling the laser
  - lower autofluorescence at endoscope surface
- this study used a doublet systems of 1 mm and 2 mm diameter, all of the systems were designed for an 800nm excitation light and a working distance of 100-140 microns of air on either side

Table 1

Summary of Optical Characterization Results[a]

GRIN System	Part Number	Diameter (mm)	Length (mm)	Relay Lens Pitch	FWHM (μm)		Diameter of FOV (μm)
Lateral	Axial						
1A	GT-ERLS-100-075-11-50-NC	1.00	35.9	0.75	0.94	10.8	199
1B	GT-ERLS-100-175-11-50-NC	1.00	79.9	1.75	0.97	12.0	195
2A	GT-ERLS-200-075-11-50-NC	2.00	81.4	0.75	0.99	12.6	370
2B	GT-ERLS-200-125-11-50-NC	2.00	132.6	1.25	1.05	15.6	365
2C	GT-ERLS-200-275-11-50-NC	2.00	285.0	2.75	1.17	25.0	359

Table 1: Summary of 5 different GRIN doublet system charecteristics

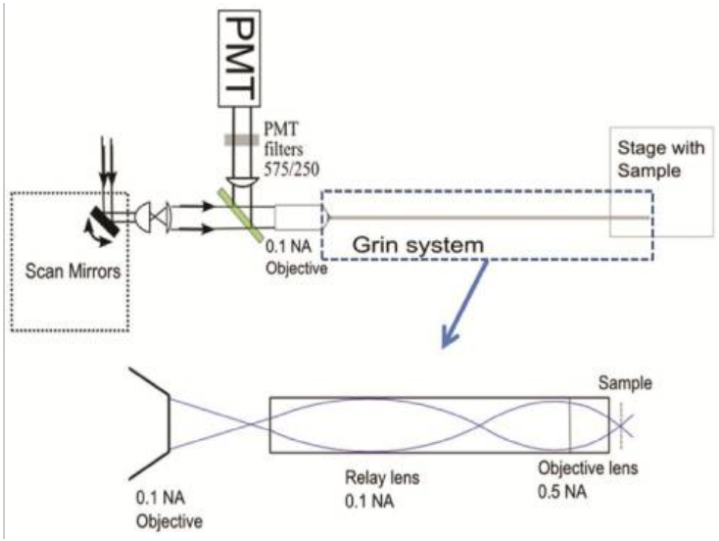


Image 1: Experimental setup used to get data in Table 1

- it was found that increasing the length of relay lens only causes a small decrease of the lateral resolution
- while there is a larger decrease for resolution in the axial direction for longer systems
- it was determined that the FOV for all systems examined in this study was 195-370 microns (varying depending on GRIN length)

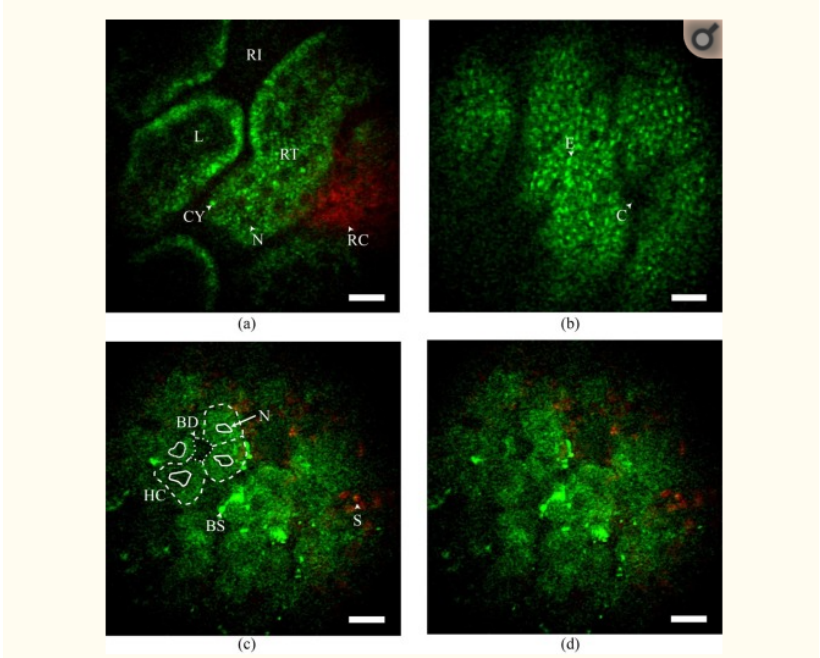


Fig. 6

Unaveraged *in vivo* images of unstained rat tissue. The pseudo-color images show red SHG signal (<405 nm) and green intrinsic fluorescent emission (405-700 nm). (a) Image of the superficial kidney renal cortex shows dark renal interstitium (RI), dark cellular nuclei (N) and bright intrinsic fluorescent cytoplasm (CY) that form the epithelial cells in the renal tubules (RT), SHG signal from the tough fibrous layer that forms the renal capsule (RC), and the dark blood filled lumen (L) inside the renal tubules. (b) Image of the inner colon wall shows bright intrinsic fluorescent signal from entrocytes (E) surrounding dark circular crypts (C). (c) Image of the rat liver showing ~20 μm diameter hapatocytes (coarse dashed line) with dark nuclei (N, solid line) chained together to form hepatic chords (HC), a dark bile duct (BD, fine dashed line) and bright intrinsically fluorescent bile salts (BS), as well as SHG emission from the septa (S) a fibrous tissue bands that separates hapatocyte nodules. (d) Image of the rat liver without labels shown for clarity. In these images, scale bars are

Image 2: Liver and Kidney images of a sedated adult male rat

- Most artifacts in image 2 are due to respiratory motion and increased the closer the organ was to the diaphragm
    - >90% of the colon images are of comparable quality to those of Image 2
    - ~70% of the kidney images are comparable quality to those of Image 2

Conclusions/action items:

Meet with the client in the coming days to clarify expectations and come up with a plan of action. Meet with Dr. Trevathan to use his existing setup to create tests to determine the FOV.

Citation: D. M. Huland *et al.*, “In vivo imaging of unstained tissues using long gradient index lens multiphoton endoscopic systems,” *Biomed. Opt. Express*, vol. 3, no. 5, pp. 1077–1085, 2012.

9/20/2021- UCLA Miniscope

Rebekah Makonnen - Sep 20, 2021, 10:22 PM CDT

Title: UCLA Miniscope Details

Date: 9/20/2021

Content by: Rebekah

Present: Rebekah

Goals: To understand designs currently in use

Link: http://miniscope.org/index.php/Main_Page

Content:

The most recent version of the miniscope is called the Miniscope V4. It has many updated features such as:

- >1 mm diameter FOV
- +/- 200 um electronic focal adjustment
- 2.6 gram weight
- 22mm height
- only requires ~1/5 of the excitation power of preceding systems

This site contains the most up-to-date public versions of the system and is frequently updated with any improvements and new features. There is also useful software and firmware specifications that will be helpful in the future when creating the product.

Conclusions/action items:

Use some of this information to create the product design specifications. Continue to do more research to finish the PDS.

9/22/2021- A wireless miniScope for deep brain imaging in freely moving mice

Rebekah Makonnen - Sep 22, 2021, 10:52 AM CDT

Title: A wireless miniScope for deep brain imaging in freely moving mice

Date: 9/22/2021

Content by: Rebekah

Present: Rebekah

Goals: Gain an understanding of other competing designs being used

Link: https://doi.org/10.1016/j.jneumeth.2019.05.008

IEEE Citation: 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

Content:

The wireless miniscope that is being used has onboard field-programmable gate array (FPGA), micro SD card storage, and it battery powered in a battery pack. The wireless miniscope allows for simultaneous recording of more than one mice in a group. This new wireless design broadens the types of behavioral experiments that can be done in the future since there are no wires that would possibly interfere with normal behavior.

Conclusions/action items:

Continue to do research on implantable endoscopes and finish up working on the PDS.

10/14/2021- Miniscope FOV Information

Rebekah Makonnen - Oct 15, 2021, 11:47 AM CDT

Title: Miniscope FOV Information

Date: 10/14/2021

Content by: Rebekah

Present: Rebekah

Goals: To gain an idea of the FOV of current miniscopes being used

Link: https://www.frontiersin.org/articles/10.3389/fncel.2019.00141/full

Content:

UCLA Miniscope:

- weighs ~3g
- has a field of view of 700 by 450 microns
    - 752 by 480 pixels (6 micron pixel size)
- allows single channel fluorescence imaging up to a max rate of 60Hz

FinchScope:

- weighs ~1.8g for the wired design and ~2g for the wireless
- has a field of view of 800 by 600 microns
    - 640 by 480 pixels
- allows acquisition at 30 Hz

miniScope:

- weighs ~2.4g
- largest field of view at ~1.1 by 1.1 mm
- allows acquisition at 10 Hz

CHEndoscope:

- weights ~4.5g
- field of view ~500 microns across
    - 648 by 486 pixels
- allows 20Hz sampling

Conclusions/action items:

Work with team to determine final design and develop testing process to determine FOV of endoscope and novel ways to improve the FOV of this project.

Citation: D. Aharoni and T. M. Hoogland, “Circuit investigations with open-source miniaturized microscopes: Past, present and future,” *Front. Cell. Neurosci.*, vol. 13, p. 141, 2019.

10/27/2021- Inscopix Miniscope

Rebekah Makonnen - Oct 27, 2021, 1:31 PM CDT

Title: Inscopix Miniscope

Date: 10/27/2021

Content by: Rebekah

Present: Rebekah

Goals: To learn about other miniscopes on the market

Link: https://www.inscopix.com/

Content:

Inscopix currently has three miniscope imaging systems the nVue, nVoke, and nVista systems.

Conclusions/action items:

Meet with client to clarify expectations and get some clarifying questions answered so the team can begin working with Dr. Eliceiri and Dr. Trevethan to test the current FOV of endoscopes to give future teams good place to pickup the project.

11/1/2021- Gel Phantom Materials

Rebekah Makonnen - Nov 01, 2021, 3:00 PM CDT

Title: An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy

Date: 11/1/2021

Content by: Rebekah

Present: Rebekah

Goals: To gain an understanding of what materials are needed to create a phantom matrix

Content:

- optical properties of the phantoms were between 400-650 nm
- these are low-cost phantoms made of agarose dissolved in water as the transparent mixture, other materials such as silicon dioxide, intralipid, ink, blood, azide, penicillin, and fluorochromes
    - agarose mimics the mechanical properties needed
    - silicon dioxide and intralipid particles were added in order to induced light scattering
    - ink and blood were added to act as absorbers
    - penicillin and azide were added to ensure the phantom is able to be stored
    - the fluorochromes were added to allow for autofluorescence similar to that of human tissue
- an advantage to using this method is that is allows for simultaneous use at differing wavelengths

Conclusions/action items:

Look more into agarose, as it looks like a promising material for phantom matrix. Also look into bovine serum as an alternate way to get fluorescence, it could be more accurate than using fluorescent beads.

Citation:

G. Wagnières *et al.*, “An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy,” *Phys. Med. Biol.*, vol. 42, no. 7, pp. 1415–1426, 1997.

Rebekah Makonnen - Nov 01, 2021, 2:52 PM CDT


Physics in Medicine & Biology

IPEM

An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy

You may also like

Recent citations

Georges_Wagni_res_1997_Phys._Med._Biol._42_1415.pdf(541.1 KB) - download

11/2/2021- A solid tissue phantom for photon migration studies

Rebekah Makonnen - Nov 03, 2021, 2:56 PM CDT

Title: A Solid Tissue Phantom for Photon Migration Studies

Date: 11/2/2021

Content by: Rebekah

Present: Rebekah

Goals: To gain an understanding of what materials work best for our work with the gel phantom.

Content:

- By adding agar to an already successful aqueous phantom, consisting of a scattering fatty medium (intralipid) and a water soluble dye (india ink), the study is able to combine the ease of an intralipid and ink solution with the mechanical properties of a solid phantom
    - agar allows higher hardening of the sample
- phantom preparation:
    - highly purified agar powder (A-7049, Sigma, USA) is dissolved to a 1% concentration, unless otherwise specified, and heated to its melting point of 95°C
        - this should be heated in a microwave since a normal heater could possibly burn the agar, causing an increase in the absorption coefficient
    - since the absorption properties of agar by itself are pretty negligible, the desired optical properties can be achieved by adding appropriate amounts of intralipid (absorbing medium) and india ink (scattering medium)
- the phantoms that were each tested once, right after preparation, and then again more than two months later, there was no significant alteration of optical properties identified between the two tests
    - samples were stored at room temperature, in containers with screw caps
- this study also tested how different agar concentrations impacted optical properties and it was determined that the absorption coefficient is not greatly impacted by agar concentration, however the reduced scattering is greatly dependent on the agar concentration

Conclusions/action items:

Meet with team to finalize agar concentration for gel phantom composition and determine what will be used to mimic fluorescent stem cells. Meet with Dr. Trevathan to determine next steps. Create a mold for gel phantom.

Citation: R. Cubeddu, A. Pifferi, P. Taroni, A. Torricelli, and G. Valentini, “A solid tissue phantom for photon migration studies,” *Phys. Med. Biol.*, vol. 42, no. 10, pp. 1971–1979, 1997.

Rebekah Makonnen - Nov 03, 2021, 2:57 PM CDT


Physics in Medicine & Biology

IPEM

A solid tissue phantom for photon migration studies

You may also like

Recent citations

Rinaldo_Cubeddu_1997_Phys._Med._Biol._42_1971.pdf(524.5 KB) - download

11/12/2021- Gel Phantom Recipe

Rebekah Makonnen - Nov 12, 2021, 3:03 PM CST

Title: Gel Phantom Recipe

Date: 11/12/2021

Content by: Rebekah

Present: Rebekah

Goals: To determine a starting point to test different ratios for gel phantom

Content:

Currently I have a recipe that I plan on using as a starting point and adjusting agar ratio based on the needed consistency of the phantom. The recipe that I plan on using is:

- 0.5oz of unflavored gelatin
- 32oz (4 cups) of water
    1. Bring water to a rolling boil, then remove from heat and add gelatin powder
    2. Mix until powder is completely dissolved
    3. Pour mixture into mold and place in fridge to set for 3 hours or until the mixture is solidified

I also plan on researching other recipes in order to get a good idea if the above recipe is a good starting point. In previous research, one study used highly purified agar powder and created a mixture that had a 1% concentration. However, since this project will be using commercial grade agar powder, the team wants to ensure an adequate gel phantom consistency.

A study performed to determine what concentration would be best to mimic the mechanical properties of brain tissues was done. This study tested 5 different levels of agar concentrations, 0.5%, 0.6%, 0.65%, 0.7%, and 0.8%, to determine which of these most closely mimicked the mechanical properties of brain tissue. By comparing the storage modulus and complex modulus this study was able to determine that the 0.65% concentration best mimics the mechanical properties of the brain.

Conclusions/action items:

Perform calculations to determine adequate ratio of agar to water. Once I do those calculations I will attach an image of them here. I plan on creating a trial of the gel phantom over the weekend to ensure that everything runs smoothly and so I am able to make adjustments if needed.

Citation: R. Deepthi, R. Bhargavi, K. Jagadeesh, and M. S. Vijaya, “Rheometric studies on agarose gel- a brain mimic material,” Sastechjournal.com. [Online]. Available: http://www.sastechjournal.com/pdf/Journals/Sept2010/4.pdf. [Accessed: 12-Nov-2021].

Rebekah Makonnen - Nov 12, 2021, 3:05 PM CST

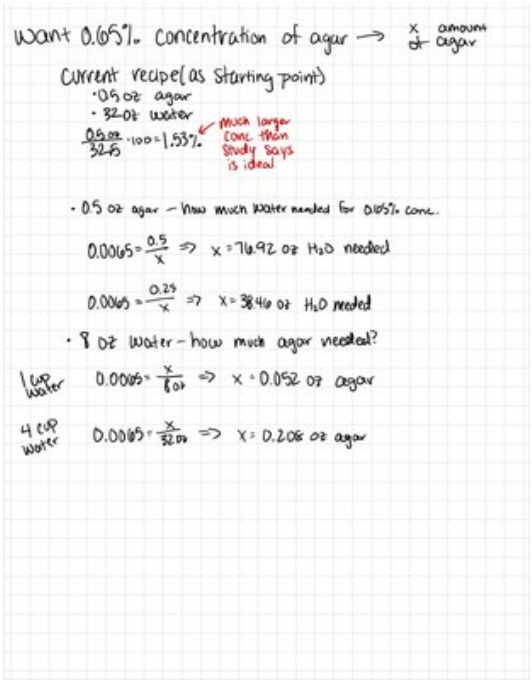


Image.jpeg(305.7 KB) - download

Rebekah Makonnen - Nov 12, 2021, 3:01 PM CST

RHEOMETRIC STUDIES ON AGAROSE GEL- A BRAIN MIMIC MATERIAL

B. Deepthi[1], R. Bhargavi[2], K. Jagadeesh[1], *M.S. Vijaya[1]

Biomedical Signal and Image Processing Research Group
M.S. Ramaiah School of Advanced Studies, Bangalore, 560 058
[2]Centre for Liquid Crystal Research, Bangalore, 560 013
*Corresponding Author, e-mail: vijaya@msrsas.org

Abstract

Drug delivery in post operative brain tumor therapy is a challenge to medical fraternity as the blood brain barrier prevents the drugs to reach the required site at the required rate. New techniques of drug delivery are being proposed by many research workers and are being tried as in-vitro experiments in the laboratories. These experiments require brain tissues. Most of the initial experiments are done with animal brain tissues (mouth porcine) or artificial brain tissue mimicking materials. The most commonly used brain tissue mimicking material is the agarose gel.

The aim of the present study is to standardize the preparation of brain mimic material using the commercially available agarose gel powder. Agarose gel samples of different concentrations in water are prepared and their mechanical parameters, (storage shear modulus, loss shear modulus and complex shear modulus) are studied using Rheological Measurements. The mechanical parameters are compared with those reported in the literature for porcine brain tissue and human brain tissue.

Keywords: Agar Gel, Brain Mimicking Material, Rheometric Studies, Complex Shear Modulus

Nomenclature

G' Storage modulus, Pa
G'' Loss modulus, Pa
G* Complex Shear modulus, Pa
γ Shear strain
ω Frequency, Hz

1. INTRODUCTION

Post operative treatment of brain tumor is a challenge to the medical fraternity, as the blood brain barrier prevents chemotherapeutic drug to reach the affected area easily. The blood brain barrier which is located at the blood capillaries is meant to protect the brain from viruses and bacteria in the blood and to shield the brain from hormones and neurotransmitters in the rest of the body. In this protective role, the blood brain barrier hinders the delivery of many important drugs to the brain, making the therapy ineffective. New drug delivery methods are required to make the drug reach the affected brain tissues.

One of the recent techniques of drug delivery in post-operative treatment of malignant brain tumor involves local drug delivery by implanting biodegradable drug wafers (e.g. Gliadel) in the post-operative resection cavity in the brain [1,2]. The wafers contain the drug commercially and they are designed to release this drug over a period of 2 to 3 weeks by normal diffusion process. This technique is more effective than the conventional chemotherapy, because the drug can reach the malignant cells directly bypassing the blood brain barrier. Clinical studies have shown that this technique enhances the survival rate after implantation by about 25%. Research work is under progress to find methods of enhancing the rate of drug delivery to the brain tissue to further increase the survival rate.

Two promising techniques under study for enhancing local drug delivery are:

- Convection enhanced drug delivery (CED)
- Ultrasonic fed enhanced drug delivery (UED)

In Convection enhanced delivery (CED) a catheter is guided into the brain parenchyma and the drug is pumped directly into the parenchyma [3, 4]. In Ultrasonic field enhanced technique (UED), a variable ultrasonic field is generated inside the resection cavity with the implanted drug wafers. The ultrasonic field in the cavity enhances the rate of drug diffusion into the parenchyma through the mechanical correlation effect which increases the permeability of the brain tissue to the drug molecules [5, 6, 7, 8]. The ultrasonic technique has been carried out mostly in-vitro, using animal (mostly porcine) brain tissues or brain tissue mimicking materials.

The most commonly used brain tissue mimicking material for these experiments is the Agarose gel. The gel is prepared by dissolving agar gel powder in distilled water and allowing it to set. The mechanical properties of the gel are quite sensitive to the concentration of the agar powder in water. The aim of the present work is to standardize the preparation of the agar gel which has mechanical properties closer to that of the brain tissue.

Previous investigators have studied the mechanical properties of agarose gel of different agarose concentrations in water. Qingchen Chen et al [9] have studied the complex shear modulus of agarose gel of concentrations 2, 3, 4 and 5% in the frequency range 1-20 Hz. Carlo Jolin et al [10] have reported their measurements on agarose gel using a Magnetic Resonance Elastography equipment and have shown that the agar phantom containing 2- 3% of agar in water and 0.9% NaCl, exhibits shear wave patterns consistent with those reported in the in-vivo studies of the brain. Luke Varkey [11] has studied the diffusivity of dye through agar gel brain phantoms with an aim to understand drug diffusion in brain tissue. He has used 0.5% agar gel concentration for the diffusion experiments. Lewis et al [5] have used 0.08% agar gel

RHEOMETRIC_STUDIES_ON_AGAROSE_GEL-_A_BRAIN.pdf(290.7 KB) - download

11/18/2021- Surfactant Research

Rebekah Makonnen - Nov 21, 2021, 2:55 PM CST

Title: Surfactant Research

Date: 11/18/2021

Content by: Rebekah

Present: Rebekah

Goals: To determine if a surfactant is needed to help disperse the fluorescent beads and if so, determine what type is ideal for the project

Content:

Product Page: https://www.cospheric.com/Tween80_Surfactant_density_gradients.htm

Bead Suspension Protocol: https://www.cospheric.com/tween_solutions_density_marker_beads.htm

- preparing the tween solution:
  - fill a heatproof container with distilled water
  - bring to a boil and leave boiling for 5 minutes
  - weigh 0.1g of tween for 100ml of solution
    - create a .1% concentration
  - add tween to boiling water and mix with immersion mixer for about 30 seconds
- suspending particles in tween solution:
  - place desired amount of particles into a container
  - pour prepared tween solution onto particles
  - cover tightly and place on centrifuge
    - spin on the highest setting for at least 5 minutes

Conclusions/action items:

After looking at this product in depth, I do not believe that this type of surfactant is needed for this project. This protocol is for suspending beads in an aqueous solution, the gel phantom is likely to have a higher density than the beads, so the beads are likely to stay suspended without the need for a surfactant. Even if one is needed, the team will be able to use dish soap as the surfactant.

Citations:

“Tween Biocompatible Surfactants - 2ml,” *Cospheric.com*. [Online]. Available: https://www.cospheric.com/Tween80_Surfactant_density_gradients.htm. [Accessed: 18-Nov-2021].

“Tween solutions for Suspension of Hydrophobic Particles in Water for Density Marker Beads in Percoll or other gradients or Flow Visualization,” *Cospheric.com*. [Online]. Available: https://www.cospheric.com/tween_solutions_density_marker_beads.htm. [Accessed: 18-Nov-2021].

9/29/2021- Device Brainstorming

Rebekah Makonnen - Sep 29, 2021, 12:35 PM CDT

Title: Mini endoscope Brainstorming

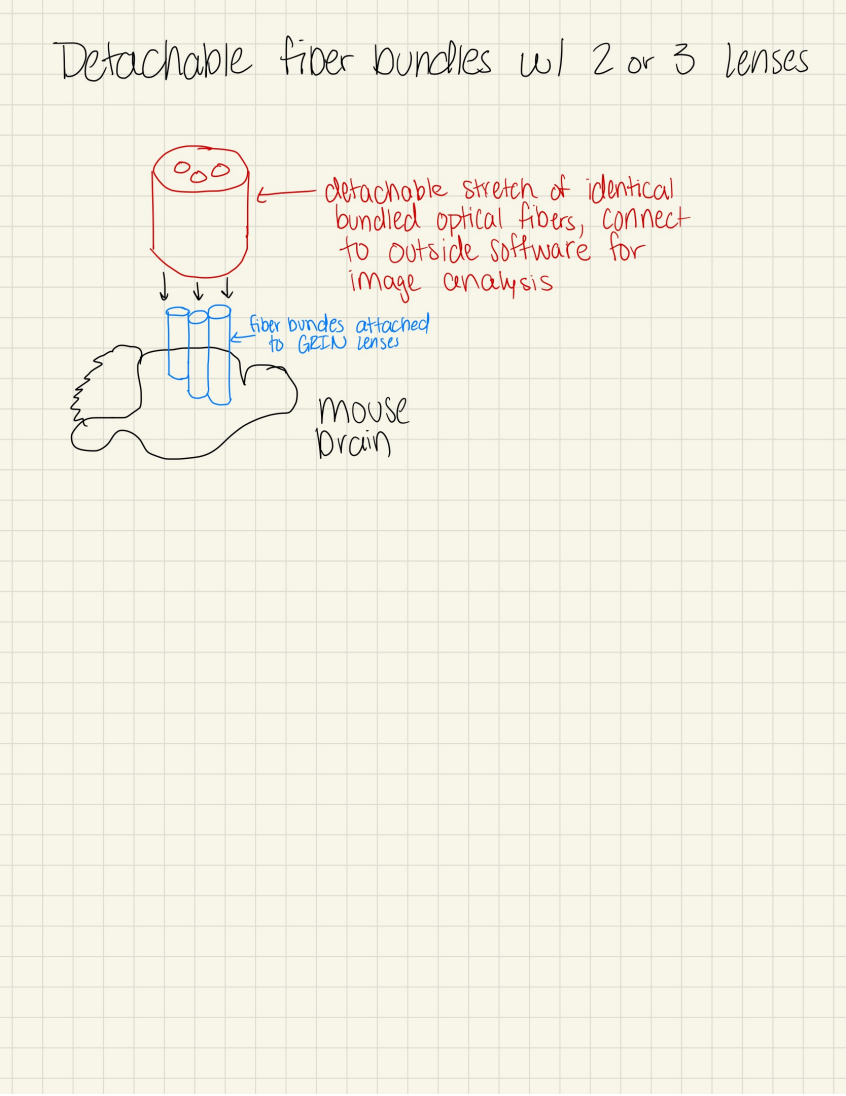
Date: 9/29/21

Content by: Rebekah

Present: Rebekah

Goals: Have 1-2 designs ready for team meeting and design matrix

Content:



Conclusions/action items:

Meet with the team to create a design matrix with the ideas that have been brainstormed and meet with advisor to finalize design.

10/5/2021 Revised Brainstorm Idea

Rebekah Makonnen - Oct 19, 2021, 8:12 PM CDT

Title: Revised and Cleaned Up Endoscope Design

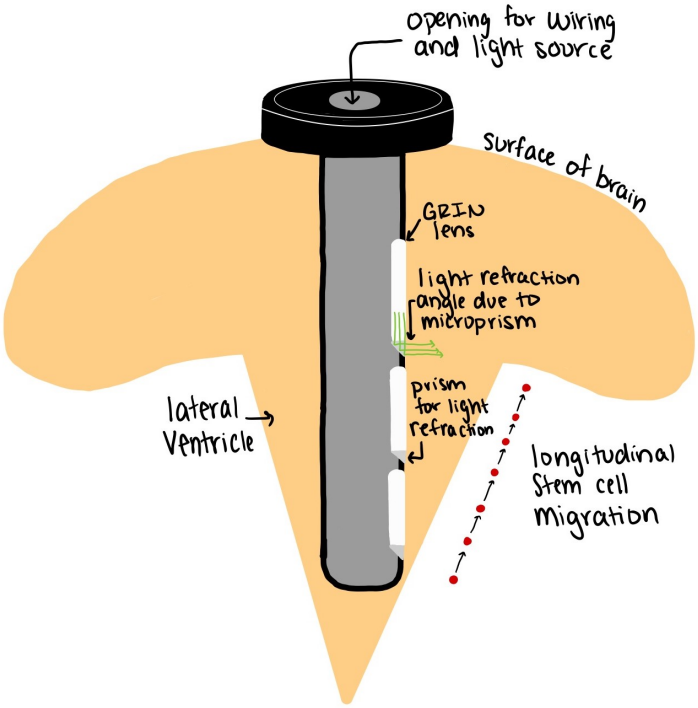
Date: 10/5/2021

Content by: Rebekah

Present: Rebekah

Goals: To create a cleaned up and revised design idea after the client meeting

Content:



Conclusions/action items:

Work with the team to meet with Dr. Kevin Eliceiri to talk about the feasibility of the designs and this project. Also begin to work on the preliminary presentation.

11/16/2021- Fluorescent Bead Research

Rebekah Makonnen - Nov 18, 2021, 10:34 AM CST

Title: Fluorescent Bead Research

Date: 11/16/2021

Content by: Rebekah

Present: Rebekah

Goals: To determine what is needed to accurately suspend fluorescent bead in the gel phantom and determine what type of beads will be used

Content:

- miniscope that the team plans on using for testing has a ET470/40x excitation filter and a ET525/50m emission filter
    - this means that the peak excitation wavelengths are 450nm to 488nm and the peak emission wavelengths are between 500nm and 547nm
- in order to get accurate results, using fluorescent beads that are within the excitation and emission ranges of the filters being used in the endoscope are important
- currently, the team is looking into these fluorescent green beads, these beads are a good option because they meet the requirements of the beads that are needed for this project
    - the excitation and emission maximums are within the ranges listed for the specific filters used in the miniscope
    - the size of the beads offered start at 10um and get larger, this is ideal since that is the smallest size that real cell bodies would be and it will give us an accurate image of what the cells would look like
- the page that has information on the fluorescent beads also recommends a surfactant if the beads will be suspended in an aqueous solution since they are hydrophobic, so more research will need to be done on surfactants

Conclusions/action items:

Order the fluorescent beads, complete research on surfactants to determine if they are needed and if so, determine they type that is ideal for this project and order it. Begin testing and gathering data.

Citations:

“Fluorescent Green Polyethylene Microspheres 1.00g/cc - 10um to 1400um (1.4mm),” *Cospheric.com*. [Online]. Available: https://www.cospheric.com/UVPMSBG_fluorescent_green_spheres_density100.htm. [Accessed: 18-Nov-2021].
“ET470/40x,” *Chroma.com*. [Online]. Available: https://www.chroma.com/products/parts/et470-40x. [Accessed: 18-Nov-2021].
“ET525/50m,” *Chroma.com*. [Online]. Available: https://www.chroma.com/products/parts/et525-50m. [Accessed: 18-Nov-2021].

Rebekah Makonnen - Nov 19, 2021, 12:58 PM CST

Cospheric

SAFETY DATA SHEET

POLYETHYLENE SPHERES, BEADS, MICROSPHERES, AND NANOSPHERES

1. IDENTIFICATION OF SUBSTANCE

1.1 PRODUCT NAME(S) Polyethylene Spheres, Beads, Microspheres, and Nanospheres

1.2 PRODUCT IDENTIFIER(S) DEFMS, BLPMS, CPS, CPMS, DPMS, GRYPMS, OMSPMS, PHYDPMS, PHSPMS, PHSPMS, RED PMS, RHSPMS, UVBDPMS, UVPMS-BS, UVPME-BG, UVPMS-BG, UVPMS-BB, UVPME-BV, UVPMS-BY, UVPME-BY2, UVYSPMS, VIOPMS, WPMS, YPMS, PEMS, UVSPMS

1.3 INTENDED USE Industrial and research applications

1.4 SUPPLIER'S DETAILS Cospheric LLC, PO box 6762, Santa Barbara, CA 93160

info@cospheric.com www.cospheric.com

1.5 EMERGENCY TELEPHONE +1-805-687-3347 Monday-Friday, 08:00-17:00 PST (UTC-8)

2. HAZARDS IDENTIFICATION

2.1 OSHA/HCS STATUS

This product is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200)

Classification of substance or mixture COMBUSTIBLE DUST

2.2 GHS LABEL ELEMENTS

Signal word Warning

Hazard statements May form combustible concentrations in air.

Precautionary statements Keep away from all ignition sources including heat, sparks and flame. Keep containers closed and grounded. Prevent dust accumulations to minimize explosion hazard.

2.3 HAZARDS NOT OTHERWISE CLASSIFIED Spilled material is extremely slippery. Dust may cause irritation.

3. COMPOSITION/INFORMATION ON INGREDIENTS

INGREDIENT	CAS NUMBER	% (W/W)
Polyethylene	9002-88-4	>70%
Proprietary additive	Trade secret	<30%

4. FIRST AID MEASURES

4.1 DESCRIPTION OF FIRST AID MEASURES

Eye contact Rinse with plenty of water. Seek medical advice if symptoms persist.

Skin contact If symptoms occur, wash with soap and water.

Inhalation If symptoms occur, move to fresh air. Seek medical advice if necessary.

Ingestion If symptoms occur, seek medical advice.

4.2 IMPORTANT SYMPTOMS/EFFECTS, ACUTE OR DELAYED

Inhalation of high concentrations of dust may cause respiratory irritation.

Page 1/5

Fluorescent_Beads_SDS.pdf(118.6 KB) - download

09/14/21 - High-fidelity Multimode Fibre-based Endoscopy for Deep Brain in Vivo Imaging

ALEX HE - Oct 19, 2021, 8:01 PM CDT

Title: High-fidelity Multimode Fibre-based Endoscopy for Deep Brain in Vivo Imaging

Date: 09/14/2021

Content by: Alex He

Present: Research

Goals: Looking for existing mice brain endoscopy procedures

Content:

This article offers many different technologies which help improve the efficiency of endoscopes.

A possible take for a more effective endoscopy revolves around using multimode fibres (MMF) as ultranarrow endoscopes. This allows for smaller size endoscopes without sacrificing attainable resolution. Digital micromirror devices (DMDs) are also a more recent form of technology that can greatly increase refresh rates leading to images that can almost approach video rates. They reveal their methodology in setting up the experiment and surgery. A calibration stage must take place in order to guarantee endoscope effectiveness.

Ultimately, with one MMF-based probe, they were able to obtain information covering over the entire dorsal-ventral section of the mouse brain.

DOI: https://doi.org/10.1038/s41377-018-0094-x

Conclusions/Action Items:

- There are many new technologies that can increase efficiency of our endoscope tests
- Calibration before testing actually begins is important to guarantee accurate data
- Regarding our circumstances, may not be the best course of action since we need the mice to be awake behaving normally with the apparatus
    - MMF does have slight problems with image quality degradation from temperature, movement, etc.
- Citation:
    - S. Turtaev, I. T. Leite, T. Altwegg-Boussac, J. M. P. Pakan, N. L. Rochefort, and T. Čižmár, “High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging,” *Light Sci. Appl.*, vol. 7, no. 1, p. 92, 2018.

09/21/21 - In vivo endoscopic multi-beam optical coherence tomography

ALEX HE - Oct 19, 2021, 8:03 PM CDT

Title: In vivo endoscopic multi-beam optical coherence tomography

Date: 09/21/2021

Content by: Alex He

Present: Research

Goals: Look for endoscopic references specifically regarding any lasers/multi lens methods

Content:

Article mentions specific technologies used in their endoscope design to help create better field of depth and accuracy. It looks like using multiple lenses results gives the possibility of mosaic images which in turn give a wider field of view. They also mention that they use a laser which can split into smaller "beamlets" which simply returned back and measured by the endoscope's photodiodes. Their results show that individual tissue layers could be easily identified. However, there were downfalls as discontinuities existed in their images, which was likely due to the light power sent through the optic fibers.

References:

DOI: http://dx.doi.org/10.1088/0031-9155/55/3/004

Conclusions/Action Items:

- The purpose of multi-lens endoscopy is definitely recognizable in its direct benefit of obtaining wider field of view/depth.
- Side facing lenses are a plausible option to fit multiple lenses.
- CItation:
    - B. A. Standish *et al.*, “In vivo endoscopic multi-beam optical coherence tomography,” *Phys. Med. Biol.*, vol. 55, no. 3, pp. 615–622, 2010.

09/21/21 - Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system.

ALEX HE - Oct 19, 2021, 8:16 PM CDT

Title: Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system.

Date: 09/21/2021

Content by: Alex He

Present: Research

Goals: Look for specific details regarding the environment inside the fourth ventricle of the mouse brain

Content:

The article mentions specific composition of interstitial fluids (ISF) within the mouse being derived from water and solutes that enter via the blood-brain barrier. In addition to this, there is blood plasma and also cerebrospinal fluid (CSF) which does not differ too much from interstitial fluid. Recent studies discovered that CSF and ISF have more complex interactions that involve them exchanging over the epithelium that covers the ventricular system. While this has many applications and raises questions of specific pathways the fluids could be flowing through, our specific case doesn't require incredible knowledge of any specific physiology.

References:

DOI: 10.1186/s12987-015-0019-5

Conclusions/Action Items:

- For the most part, we won't have to worry about any extreme degradation of endoscope materials.
- We must take note that out endoscopy needs to be watertight
- We must also take note that our light will be emitting through a fluid - refraction coefficients and the physics may have to be accounted for/researched further to guarantee accurate imaging.
- CItation:
    - 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.

09/29/21 - Autofluorescence mitigation for quantitative molecular imaging

ALEX HE - Oct 19, 2021, 7:08 PM CDT

Title: Target-to-background enhancement in multispectral endoscopy with background autofluorescence mitigation for quantitative molecular imaging

Date: 09/29/2021

Content by: Alex He

Present: Research

Goals: Look into imaging methods and ways of increasing accuracy without losing field of view.

Content:

This article discusses conventional imaging techniques used in endoscopy imaging and some of the major factors regarding probe sensitivity. While the main focus of the article was about cancer targeting in the GI tract, the information provided about probe sensitivity and fluorescent imaging may still prove useful for our applications in stem cell fluorescence. The main factors they addressed regarding sensitivity was limited under three major categories: characteristic of molecular probe, specialized performance for the molecular target, and any autofluorescence degradation of the image. Characteristics of the probe include things like probe-tissue binding affinity, nonspecific binding and diffusion coefficient, fluorophore characteristic, quantum efficiency, absorption coefficient, and fluorescence wavelength.

References:

DOI: https://doi.org/10.1117/1.JBO.19.7.076014

Conclusions/Action Items:

- Overall, all this article displays the involvement behind designing an endoscope
- Things we will need to worry about include autofluorescence degradation, probe-tissue binding, and fluorescence wavelength of light
- Citation
    - C. Yang, V. W. Hou, E. J. Girard, L. Y. Nelson, and E. J. Seibel, “Target-to-background enhancement in multispectral endoscopy with background autofluorescence mitigation for quantitative molecular imaging,” *J. Biomed. Opt.*, vol. 19, no. 7, p. 76014, 2014.

10/04/21 - Performance of the red-shifted fluorescent proteins in deep-tissue molecular imaging applications

ALEX HE - Oct 19, 2021, 7:45 PM CDT

Title: Performance of the red-shifted fluorescent proteins in deep-tissue molecular imaging applications

Date: 10/04/2021

Content by: Alex He

Present: Research

Goals: Look more into tdTomato fluorescence and understand how to interact with it to obtain imaging.

Content:

The article discusses not only tdTomato, but other fluorescent proteins like mCherry, GFP, mRaspb, etc. While originally GFP was the main protein of use for study, tdTomato and other red fluorescent proteins became much more valuable in terms of information. tdTomato was studied to have an excitation wavelength of 488 to 532 nm and release peak emissions of 581 nm. Compared to all the other proteins, it has a relative brightness at least 3 times higher than all the other proteins studied. This implies that excitation and emission of tdTomato is relatively exclusive, meaning autofluorescence of accidental of other proteins becomes less of a factor.

References:

DOI: http://doi.org/10.1117/1.2967184

Conclusions/Action Items:

- When studying stem cells with the tdTomato protein, we do not have to worry about overexcitation of additional fluorescent proteins
- We still must make sure our device minimizes damage since that can still release additional stem cells and overexcite the cells.
- We also must make sure we choose a correct wavelength light source to excite tdTomato exclusively.
- Citation
    - N. C. Deliolanis, R. Kasmieh, T. Wurdinger, B. A. Tannous, K. Shah, and V. Ntziachristos, “Performance of the red-shifted fluorescent proteins in deep-tissue molecular imaging applications,” *J. Biomed. Opt.*, vol. 13, no. 4, p. 044008, 2008.

10/04/21 - Pupil-segmentation-based adaptive optical correction of a high-numerical-aperture gradient refractive index lens for two-photon fluorescence endoscopy

ALEX HE - Oct 19, 2021, 8:01 PM CDT

Title: Pupil-segmentation-based adaptive optical correction of a high-numerical-aperture gradient refractive index lens for two-photon fluorescence endoscopy

Date: 10/04/2021

Content by: Alex He

Present: Research

Goals: Look into imaging techniques that reduce the limitations of GRIN lenses

Content:

The article discusses their study of using a pupil-segmentation based AO to correct aberrations in GRIN lens images. Essentially, their methodology was to check several illuminated segments of the objective for aberrations. If aberrations were detected, they would shift the image under full illumination of the pupil. After repeating this process across the entire pupil they would reconstruct the image using corrective techniques individually to create and overall brighter and more detailed image. This method is something our group could consider being that it accomplishes the goal of increased field of view while also correcting for chromatic aberration.

References:

DOI: http://doi.org/10.1364/OL.37.002001

Conclusions/Action Items:

- This is a more mechanical approach to imaging an entire area of study with GRIN lenses.
- The methodology is to image sections of the area of study separately and piece together a mosaic of the area of study
- Citation
    - C. Wang and N. Ji, “Pupil-segmentation-based adaptive optical correction of a high-numerical-aperture gradient refractive index lens for two-photon fluorescence endoscopy,” *Opt. Lett.*, vol. 37, no. 11, pp. 2001–2003, 2012.

09/13/21 - Aspherical Microlens for Fluorescence Microendoscopy

ALEX HE - Oct 19, 2021, 8:05 PM CDT

Title: Aspherical Microlens Assembly for Deep Brain Fluorescence Microendoscopy

Date: 09/13/2021

Content by: Alex He

Present: Competing Designs

Goals: Look for endoscopy examples and specific pros/cons

Content:

Article mentions the limitations of gradient refractive index (GRIN) lenses. While they are relatively reliable, the have intrinsic problems that lead to reduced field of view, poorer image quality, and limited experimental flexibility. Experimenters suggest an aspherical lens which changes radius of curvature, eliminating spherical aberrations. After testing their PAS probe (which used aspherical lenses) against a GRIN probe, they found less perimeter off-axis distortions in the PAS probe.

More importantly, using GRIN lenses results in chromatic aberration which is a fatal flaw especially when conducting multicolor imaging (such as fluorescence). The results of their testing with the PAS probe showed no axial and lateral chromatic aberrations.

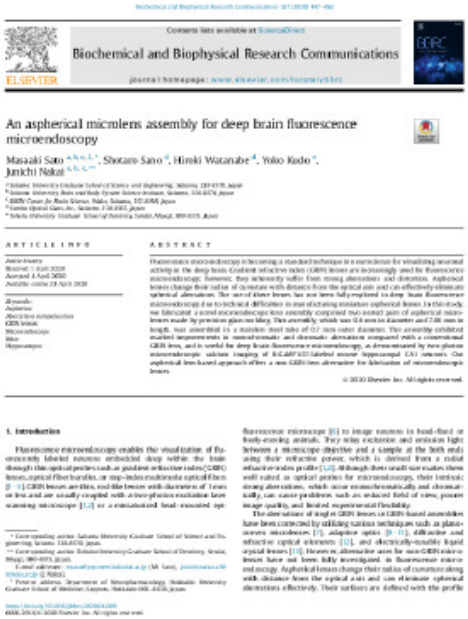
References:

DOI: https://doi.org/10.1016/j.bbrc.2020.04.009

Conclusions/Action Items:

- GRIN lenses relatively simple to make and effective, but they come with specific flaws detrimental to multicolor imaging.
- Aspherical Lens can bypass chromatic aberration allowing for more accurate color recognition - but might be more difficult to produce.
- CItation:
    - M. Sato, S. Sano, H. Watanabe, Y. Kudo, and J. Nakai, “An aspherical microlens assembly for deep brain fluorescence microendoscopy,” *Biochem. Biophys. Res. Commun.*, vol. 527, no. 2, pp. 447–452, 2020.

ALEX HE - Sep 14, 2021, 12:31 AM CDT



Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

An aspherical micmlens assembly for deep brain fluorescence microendoscopy

Masaaki Sato [a, b, c, 1, *], Shotaro Sano [d], Hiroki Watanabe [d], Yoko Kudo [e], Junichi Nakai [a, b, c, **]

ARTICLE INFO

Article history:
Received 1 April 2020
Accepted 3 April 2020
Available online 28 April 2020

Keywords:
Asphericity
Absorption compensation
GRIN lenses
Microendoscopy
Mirror
Hippocampus

ABSTRACT

Fluorescence microendoscopy is becoming a standard technique in neuroscience for visualizing neuronal activity in the deep brain. Gradient refractive index (GRIN) lenses are increasingly used for fluorescence microendoscopy; however, they inherently suffer from strong aberrations and distortion. Aspherical lenses change their radius of curvature with distance from the optical axis and can effectively eliminate spherical aberrations. The use of these lenses has not been fully explored in deep brain fluorescence microendoscopy due to technical difficulties in manufacturing miniature aspherical lenses. In this study, we fabricated a mini-microendoscope lens assembly comprised two contact pairs of aspherical microlenses made by precision glass molding. This assembly, which was 0.8 mm in diameter and 2.86 mm in length, was assembled in a stainless steel tube of 0.7 mm outer diameter. The assembly exhibited marked improvements in monochromatic and chromatic aberration compared with a conventional GRIN lens, and is useful for deep brain fluorescence microendoscopy, as demonstrated by two-photon microendoscopic calcium imaging of EGFP-expressed mouse hippocampal CA1 neurons. Our aspherical lens-based approach offers a non-GRIN lens alternative for fabrication of microendoscopic lenses.

© 2020 Elsevier Inc. All rights reserved.

1. Introduction

Fluorescence microendoscopy enables the visualization of fluorescently labeled neurons embedded deep within the brain through thin optical probes such as gradient refractive index (GRIN) lenses, optical fiber bundles, or step-index multimode optical fibers [1–3]. GRIN lenses are thin, rod-like lenses with diameters of 1 mm or less and are usually coupled with a two-photon excitation laser scanning microscope [1,2] or a miniaturized head-mounted epi-fluorescence microscope [4] to image neurons in head-fixed or freely-moving animals. They relay excitation and emission light between a microscope objective and a sample at the both ends using their refractive power, which is derived from a radial refractive-index profile [5,6]. Although their small size makes them well suited as optical probes for microendoscopy, their intrinsic strong aberrations, which occur monochromatically and chromatically, can cause problems such as reduced field of view, poorer image quality, and limited experimental flexibility.

The aberrations of single GRIN lenses or GRIN-based assemblies have been corrected by utilizing various techniques such as plasmonic metalenses [7], adaptive optics [8–11], diffractive and refractive optical elements [12], and electrically-tunable liquid crystal lenses [13]. However, alternative uses for non-GRIN microlenses have not been fully investigated in fluorescence microendoscopy. Aspherical lenses change their radius of curvature along with distance from the optical axis and can eliminate spherical aberrations effectively. Their surfaces are defined with the profile

* Corresponding author. Yokohama University Graduate School of Science and Engineering, Yokohama, 240-8501, Japan.
** Corresponding author. Yokohama University Graduate School of Chemistry, Yokohama, 240-8501, Japan.
E-mail addresses: masaaki@ynu.ac.jp (M. Sato), junichi@ynu.ac.jp (J. Nakai).
[1] Present address: Department of Biopharmacology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido 060-0812, Japan.

https://doi.org/10.1016/j.bbrc.2020.04.009
0006-291X/© 2020 Elsevier Inc. All rights reserved.

1-s2.0-S0006291X20307075-main.pdf(1.5 MB) - download

09/29/21 - Fast varifocal two-photon microendoscope for imaging neuronal activity in the deep brain

ALEX HE - Oct 19, 2021, 8:16 PM CDT

Title: Fast varifocal two-photon microendoscope for imaging neuronal activity in the deep brain

Date: 09/29/2021

Content by: Alex He

Present: Competing Designs

Goals: Look for any other designs which fulfill similar purpose to what we are designing.

Content:

The microendoscope used in this article had a focus or increasing field of view of the endoscope depth wise in a mouse brain. Instead of utilizing multiple lenses to view different depths, this device used 2 lens in series, one varifocal lens and one GRIN lens. Effectively, the varifocal lens was adjustable and rested right outside of the sight of study while the GRIN lens was in the mouse brain. Then, by adjusting the varifocal lens the combination of its depths with the GRIN lens could access images of different depths in the brain. As described in figures in their article, the varifocal lens effectively increased the focal depth of the GRIN lens, allowing for a larger field of view. While this differs from our goal of lateral tracking of cells, it is a method that could prove useful in later iterations of our design process.

References:

DOI: https://doi.org/10.1364/BOE.8.004049

Conclusions/Action Items:

- Micro endoscope utilizes a special lens that can change focal depth in real time.
- Design is mostly linear and not to the side like we need.
- Not particularly useful for our circumstance but could come in handy.
- Citation
    - M. Sato, Y. Motegi, S. Yagi, K. Gengyo-Ando, M. Ohkura, and J. Nakai, “Fast varifocal two-photon microendoscope for imaging neuronal activity in the deep brain,” *Biomed. Opt. Express*, vol. 8, no. 9, pp. 4049–4060, 2017.

10/28/21 - 3D physiological brain model culturing neural cells in hydrogels

ALEX HE - Dec 13, 2021, 2:43 PM CST

Title: 3D physiological brain model culturing neural cells in hydrogels

Date: 10/28/2021

Content by: Alex He

Present: Research

Goals: Look for existing scientific procedures that try to simulate brain tissues

Content:

Article mentions the struggles of studying 3d models of the brain using self organized tissue cultures from stem cells being "hard to grow, labor-intensive, and time-consuming". For this reason, turning to hydrogels as an alternative method looks promising. One of the most important things you must consider when modelling brain is the stiffness of the gel. For the applications of the research involved in the article, stiffness plays a key role in the regulation of neuronal cell shape, viability, expression, migration, and differentiation. For us, we would probably lean towards a softer gel since other studies have seen that softer variants promote neurite outgrowth and movement.

References:

DOI: https://doi.org/10.1177/2041731420963981

Conclusions/Action Items:

- It is reasonable for us to pursue hydrogels to mimic brain tissue
- We should opt for a softer gel when developing our recipe and procedure.
- Citation:
    - I. Raimondi, M. Tunesi, G. Forloni, D. Albani, and C. Giordano, “3D brain tissue physiological model with co-cultured primary neurons and glial cells in hydrogels,” *J. Tissue Eng.*, vol. 11, p. 2041731420963981, 2020.

10/28/21 - Controlled studies of 3D cell migration

ALEX HE - Dec 13, 2021, 3:41 PM CST

Title: Controlled studies of 3D cell migration

Date: 10/28/2021

Content by: Alex He

Present: Research

Goals: Look for research on how we may consider movement of micro-fluorescent beads in our phantom matrix

Content:

This article discusses concentration gradients of signaling molecules that cells use in processes like embryonic development and wound repairing. To study this effect, researchers use chemotactic assays like Boyden chamber, under-agarose assay, Zigmond chamber, Dunn chamber, and micropipette assay, which all help track cell migration. The only limitation is that these methods can only study 2D movement. For our research, this could still prove to be useful if we get to the phase where we are mimicking the movement of stem cells. However, if we cannot find a reliable method to move micro fluorescent beads through our matrix, we may not be able to move forward with this consideration.

References:

DOI: https://doi.org/10.1016/j.biomaterials.2010.12.019

Conclusions/Action Items:

- If we get far enough in our research to study cell migration in our matrices, we can look into these assays
- P. Tayalia, E. Mazur, and D. J. Mooney, “Controlled architectural and chemotactic studies of 3D cell migration,” *Biomaterials*, vol. 32, no. 10, pp. 2634–2641, 2011.

11/05/21 - Development of Tissue-Mimicking Phantom of the Brain

ALEX HE - Dec 13, 2021, 4:03 PM CST

Title: Development of Tissue-Mimicking Phantom of the Brain

Date: 11/05/2021

Content by: Alex He

Present: Research

Goals: Look for more methods of making tissue-mimicking phantom brain models

Content:

The brain model formed in this article was used for ultrasonic studies. The main issue they faced was that the low backscatter coefficient of brain tissue made ultrasonic studies difficult in matching backscatter and attenuation properties. In the end, they settled with polyvinyl alcohol as a base material since its properties could easily be manipulated via freeze-thaw cycling, variations in concentration, and the addition of scattering inclusions, allowing independent control backscatter and attenuation. Additionally, they used talc powder to optimize ultrasonic properties. Overall, they found that the ultra sonic properties of their phantom best matched brain tissue in the frequency range 1-3 MHz.

References:

DOI: https://doi.org/10.1016/j.ultrasmedbio.2018.08.012

Conclusions/Action Items:

- While the research is more extensive than what we need, we can still take inspiration from how they made their phantoms variable
- We could probably differ concentrations in many samples to see what quantitative effects it has on our data
- Citation
    - S. Taghizadeh, C. Labuda, and J. Mobley, “Development of a tissue-mimicking phantom of the brain for ultrasonic studies,” *Ultrasound Med. Biol.*, vol. 44, no. 12, pp. 2813–2820, 2018.

11/11/21 - Fabrication of Tissue-mimicking phantom and optimization in MRI

ALEX HE - Dec 13, 2021, 5:18 PM CST

Title: Fabrication of Tissue-mimicking phantom and optimization in MRI

Date: 11/11/2021

Content by: Alex He

Present: Research

Goals: Look for more methods of making tissue-mimicking phantom brain models

Content:

The goal of the research in the article was to form a phantom that mimicked tissue simulating the MR relaxation times of neonatal gray and white matter at 1.5 T. They used several agarose gel solutions, doped with paramagnetic Gadopentetic acid ions. To separately study T1 and T2 measurements, they underwent a Turbo-Inversion-Recovery Spin-Echo sequence and a Car-Purcell-Meiboom-Gill sequence respectively (2 different MRI methods). In the end, their phantom was accurate in mimicking the relaxation times of neonatal gray matter across different 1.5 T systems.

References:

DOI: https://doi.org/10.1016/j.ejmp.2018.10.022

Conclusions/Action Items:

- More extensive than our research, but additives were specially chosen for MRI purposes.
- We can probably stick with agarose as our choice
- Citation:
    - A. Kozana, T. Boursianis, G. Kalaitzakis, M. Raissaki, and T. G. Maris, “Neonatal brain: Fabrication of a tissue-mimicking phantom and optimization of clinical T1w and T2w MRI sequences at 1.5 T,” *Phys. Med.*, vol. 55, pp. 88–97, 2018.

11/11/21 - Tissue-mimicking phantom materials for narrowband and ultrawideband microwave applications

ALEX HE - Dec 13, 2021, 5:12 PM CST

Title: Tissue-mimicking phantom materials for narrowband and ultrawideband microwave applications

Date: 11/11/2021

Content by: Alex He

Present: Research

Goals: Look for more methods of forming a phantom matrix

Content:

This article proposes the idea of oil in gelatin to better approximate the dispersive dielectric properties of many soft tissues across the human body. Differing tissues can be modelled with different concentrations of oil. As a result, they suspect that these materials can form a phantom for narrowband and ultrawideband microwave technologies, such as imaging systems. Describing their methodology of creating the phantom, they say they used an aqueous gelatin solution and a solution of 50% kerosene and 50% sunflower oil and mix it with a solution of formaldehyde. Ultimately, they tested this phantom and found that it successfully simulates many biological tissues over a wide range of microwave frequencies.

References:

DOI: https://doi.org/10.1088/0031-9155/50/18/001

Conclusions/Action Items:

- Overall, another method of forming phantom matrix
- While the methodology is very simple, mixing oil in with the gel, we only need to visualize beads, so the oil will probably be superfluous for us.
- Citation
    - M. Lazebnik, E. L. Madsen, G. R. Frank, and S. C. Hagness, “Tissue-mimicking phantom materials for narrowband and ultrawideband microwave applications,” *Phys. Med. Biol.*, vol. 50, no. 18, pp. 4245–4258, 2005.

11/18/21 - Evaluating Optical Aberration Using Fluorescent Microspheres

ALEX HE - Dec 13, 2021, 6:08 PM CST

Title: Evaluating Optical Aberration Using Fluorescent Microspheres

Date: 11/18/2021

Content by: Alex He

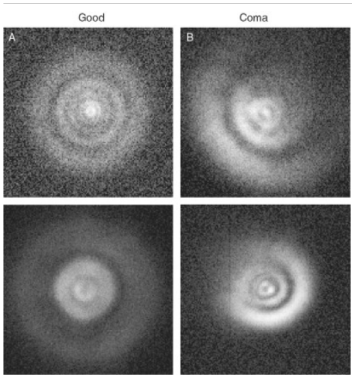
Present: Research

Goals: Understand what problems we may run into when observing fluorescent microspheres.

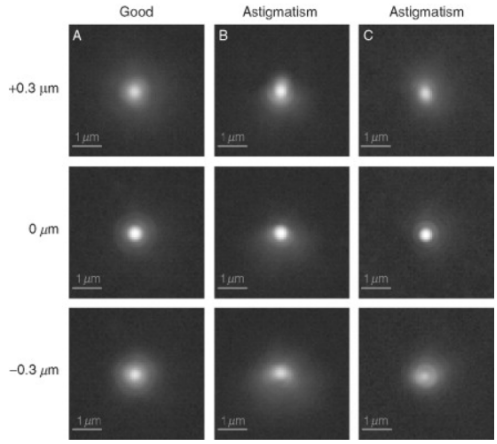
Content:

This article covers common methods, analyses, and corrective actions that is common nowadays when studying fluorescent microspheres under a microscope. Some of the main aberrations that are encountered when doing these type of studies are coma aberrations, astigmatic aberrations, and spherical aberrations. Coma aberrations are typically seen at the edges of the field of view. The fluorescent microspheres appear as asymmetrical due to rays of light that strike the lens elements in the optical path at oblique angles. In astigmatic aberrations, the microspheres blur due to misalignment of lens elements and typically will cause elongation of the image in some axis. Spherical aberrations are the most common aberrations seen and re marked by axial asymmetry. On one side of the plane of best focus, there are sharp diffraction rings. On the other side of focus, the object will appear to extend for a long distance and will not have distinct delineation.

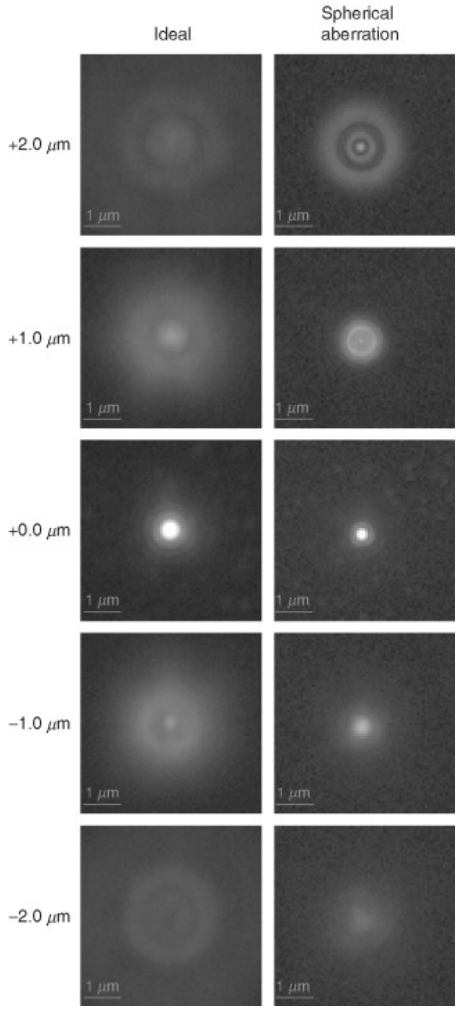
Coma aberration:



Astigmatic aberrations:



Spherical aberrations:



References:

DOI: https://doi.org/10.1016/S0091-679X(06)81018-6

Conclusions/Action Items:

- If we become familiar with these aberrations, we will be able to tell if our data is skewed
- This will prove to become useful since our data can become more accurate
- Citation:
    - P. C. Goodwin, “Evaluating optical aberration using fluorescent microspheres: methods, analysis, and corrective actions,” *Methods Cell Biol.*, vol. 81, pp. 397–413, 2007.

Three GRIN Lens Outside the Brain

ALEX HE - Oct 19, 2021, 8:15 PM CDT

Title: Three GRIN Lens Outside the Brain Design

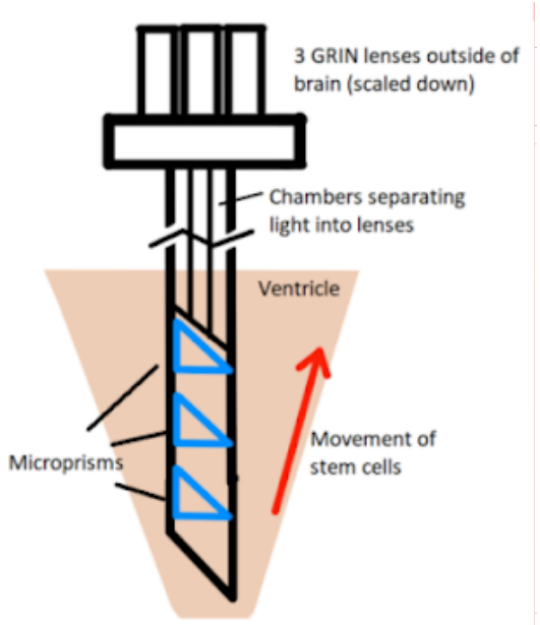
Date: 10/05/2021

Content by: Alex He

Present: Design Idea

Goals: Create a design that could accomplish the goal of increased field of view.

Content:



The idea of this design is that the GRIN lenses are outside of the brain while only the cannula with the micro prisms exist in the ventricle. The initial problem I found was that the ventricle diameter was too small to host all three GRIN lenses inside. With this design, we can bypass the size restriction of the GRIN lenses. Inside, the prisms all are at different levels, allowing them and their respective GRIN lenses to image a larger lateral field of view along the ventricle wall. The cannula also depicts 3 separate chambers for light to travel from and to the GRIN lenses individually to decrease light interference. The main drawback with this design is that there are many moving parts, making assembly time and the prototyping process much longer.

Conclusions/action items:

- Share ideas with group
- Evaluate in design matrix

09/14/21 - Monitoring of Implanted Stem Cell Migration in vivo: A Highly Resolved in vivo Magnetic Resonance Imaging Investigation of Experimental Stroke in Rat

ALEXIS BLOCK - Sep 14, 2021, 3:07 PM CDT

Title : Monitoring of Implanted Stem Cell Migration in vivo: A Highly Resolved in vivo Magnetic Resonance Imaging Investigation of Experimental Stroke in Rat

Date : 09/14/21

Content by : Alexis Block

Present : N/A

Goals : Research a similar project to ours to get a good idea of what it is we are actually working on and to observe how others in the past have gone about it.

Content:

- Studying stem cells in vivo is extremely beneficial due to the fact that you can observe the emigrational dynamics of the stem cells and all processes in which stem cells undergo throughout the body.

- Contrasts traditional methods such as invasive analysis of brain sections vs. utilizing the observations of cell dynamics within individual animals. The observations of cell dynamics were executed using an MRI contrast agent which was implanted into rat brains.

- MRI at "78 - um isotropic spatial resolution" allowed for researchers to observe the stem cells in contrast to the host tissue.

- Process : Rats were anesthetized, three rats used as control group, rats underwent implantation surgery, rats were observed over a three week period with an MRI, body temperature was held constant.

- Generally all higher level observations were made after just 3 weeks after the stem cells being implanted.

- Results: Compared to in vitro titrations of cells. Tested toxicity of cells which came back as only 2-3%. Findings were convincing in demonstrating the migration of stem cells across corpus callosum through the tissue.

Conclusion:

Continue to read other articles similar to this to gain general background information. Research this specific research paper's work.

ALEXIS BLOCK - Sep 14, 2021, 3:09 PM CDT

DOI: https://www-jstor-org.ezproxy.library.wisc.edu/stable/3073943?Search=yes&resultItemClick=true&searchText=longitudinal+migration+of+stem+cells+in+vivo&searchUri=%2Faction%2FdoBasicSearch%3FQuery%3Dlongitudinal%2Bmigration%2Bof%2Bstem%2Bcells%2Bin%2Bvivo&ab_segments=0%2Fbasic_search_gsv2%2Fcontrol&refreqid=fastly-default%3Ade422415434bf5e211e74b3fa9077ba8&seq=4#metadata_info_tab_contents

Citation:

(N.d.). Jstor.Org. Retrieved September 14, 2021, from https://www.jstor.org/stable/3073943?Search=yes&resultItemClick=true&searchText=longitudinal+migration+of+stem+cells+in+vivo&searchUri=%2Faction%2FdoBasicSearch%3FQuery%3Dlongitudinal%2Bmigration%2Bof%2Bstem%2Bcells%2Bin%2Bvivo&ab_segments=0%2Fbasic_search_gsv2%2Fcontrol&refreqid=fastly-default%3Ade422415434bf5e211e74b3fa9077ba8&seq=4#metadata_info_tab_contents

09/14/21 Seeing Stem Cells at Work In Vivo

ALEXIS BLOCK - Sep 14, 2021, 3:29 PM CDT

Title : Seeing Stem Cells at Work in Vivo

Date: 09/14/21

Content by : Alexis Block

Present : N/A

Goals : Gain general and background knowledge regarding our project.

Content:

- Stem cell transportation was introduced in 1945. Stem cell therapies are known to be a useful regenerative medicine due to their inherent biological characteristics that allow them to migrate and self generate.

- Traditionally, the transplantation of stem cells has relied on histological investigation, which is rather invasive.

- In vivo cell imaging techniques has created a rather noninvasive way of tracking transplanted cells. Researchers can now observe the migration and differentiation of these cells at whatever site of the body in a non invasive matter.

- In vivo relied on labeling of the cells. Labeling of cells allows for the tracking of specific cells. The first manner of labeling is the direct labeling method is generally the use of fluorescent semiconductor nanocrystals and observed on an MRI or PET. The second manner of labeling is the use of paramagnetic agents which utilize their seven unpaired electrons.

ALEXIS BLOCK - Sep 14, 2021, 3:29 PM CDT

DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946709/

Citation:

Srivastava, A. K., & Bulte, J. W. M. (2014). Seeing stem cells at work in vivo. Stem Cell Reviews, 10(1), 127–144.

09/20/21 Live Imaging Of Adult Neural Stem Cells in Freely Behaving Mice Using Mini-Endoscopes

ALEXIS BLOCK - Sep 21, 2021, 2:34 PM CDT

Title: Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes

Date: 9/21/2021

Content by: Alexis

Present: Alexis

Goals: To gain a more complete understanding of the mini endoscopes used in the mouse brain

Content:

- The endoscopes allow for a less invasive process while the animal is still living freely. Using the mini endoscopes is not only less invasive, but can be used for up to three days. The endoscope made of GRIN lenses must be implanted first, after deciphering the appropriate size and type (flat tip, side-view, prism view) of GRIN lenses.

-The protocol of using a mini endoscope works by combining specific adult neural stem cells (NSCs) labeling and mini-endoscopic microscopy, live imaging of cells division in addition to Ca2+ activity can be performed for 3 days up to a few months.

- Depending on depth and placement of implantation, a protrusion ring, or spacer, is selected to screw onto GRIN lens cannula. The ring is then used to alter the distance between the lens cannula and the skull while simultaneously holding everything in place. Right before implanting the lens, the camera must be prepared and ready to evaluate fluorescence changes in the tissue.

- Some limitations exist with this process. The ability to monitor the activity of identical cells over a long length of time may not be possible with this process. This process could lead to sparse labeling in the adult SVZ, dilution of the plasmid can decrease the intensity of fluorescence in the NSC's field of view, and finally the daughter cells may migrate and disappear from field of view.

Conclusions/action items:

The use of a mini endoscope in tracking adult neural stem cells has many benefits including the non invasive nature and the ability to track and image for up to three months, but it does also have some limitations. Continue to research limitations and other projects that have utilized the GRIN lenses and mini endoscope protocol.

DOI: https://reader.elsevier.com/reader/sd/pii/S2666166721003038?token=F2FCB3CD7D963EBC5037EB72483FAC02ED617DD4D626BBE5EF2A9CDC6D6EDE351A78F76BDE2B2DFE17EF1D6181DACAFE&originRegion=us-east-1&originCreation=20210921191033

Citation:

Malvaut, S., Marymonchyk, A., Gengatharan, A., & Saghatelyan, A. (2021). Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes. *STAR Protocols*, *2*(2), 100596.

09/21/21 Miniaturized Multichannel Near Infrared Endoscope for Mouse Imaging

ALEXIS BLOCK - Sep 21, 2021, 3:25 PM CDT

ALEXIS BLOCK - Sep 21, 2021, 2:34 PM CDT

Title: Miniaturized Multichannel Near Infrared Endoscope for Mouse Imaging

Date: 9/21/2021

Content by: Alexis

Present: Alexis

Goals: To gain a more complete understanding of the engineering behind the endoscope and to gain information regarding the shelf life, life in service, and operating environment.

Content:

- Near Infrared fluorescence (NIRF) has recently become a successful way of obtaining high level optics. The use of smart fluorescent probes with high target to background and activation ratios is one major distinction compared to other modalities. In addition, the use of NIR light allows for the penetration of biologic tissue to occur more easily than visible light.

-Need : simultaneous white light, two or more infrared channels, the availability of images in white light and real time which allows for anatomic orientation, image integration times which avoid motion artifacts, can miniaturize fiber bundles, and finally the use of CMIR image which allows for simultaneous display and storage of video frames streaming from all channels.

- For mouse studies, total diameter of 1.4 mm and 100 cm working length are utilized in the endoscope. Catheter consisted of an imaging fiber bundle of 15,000 fibers with a 0.5 mm diameter, of two illumination fiber bundles with 0.4 mm and of a working channel of 0.6 mm for irrigation, insufflation, and insertion.

-The use of the NIR fluorescence endoscope that combined the use of white light and two NIR channels, demonstrate the feasibility of imaging perfusion and activity of enzymes in the model. This method creates a rather nondestructive and nonintrusive way of allowing for repeated access of the same regions of the brain.

-Issues: In regard to illumination, the total photon flux is limited by the diameter and numeric aperture of the fiber bundles. A Xenon lamp was utilized to fix this issue. The concentration and quantum efficiency of the fluorochromes in region was also an issue. In order to fix this, less quenching fluorochromes should be used to allow greater perfusion imaging signal.

Conclusions/action items:

The use of NIRF endoscopes has many advantages. This article described all of the components of the endoscope and how to better the specific components.

DOI: https://journals.sagepub.com/doi/pdf/10.1162/15353500200303166

Citation:

Funovics, M. A., Alencar, H., Su, H. S., Khazaie, K., Weissleder, R., & Mahmood, U. (2003). Miniaturized multichannel near infrared endoscope for mouse imaging. *Molecular Imaging*, *2*(4), 153535002003031.

10/18/21 Stem Cell Tracking Technologies for Neurological Regenerative Medicine Purposes

ALEXIS BLOCK - Oct 18, 2021, 9:35 PM CDT

Title: Stem Cell Tracking Technologies for Neurological Regenerative Medicine Purposes

Date: 9/21/2021

Content by: Alexis

Present: Alexis

Goals: To gain a more complete understanding of the purpose for our research. Understand the reasoning behind why stem cell research is significant

Content:

- Stem cell therapy is becoming increasingly prominent in many realms of medicine. Specifically in regenerative medicine, stem cell therapy is being applied to a plethora of neurological and degenerative diseases. In order to execute this revolutionizing therapy, we must first understand stem cells. Moreover, how do stem cells migrate, how do they differentiate into neural lineage, etc.

- Neural stem cells (NSCs) have the ability of self renew, proliferate, and differentiate. In previous studies, NSCs have been proven to be some form of treatment for Parkinson's disease. PSC derived neurons were found to reestablish the damaged long range axonal projections and synaptic connections in the host brain. To fully comprehend what stem cells are capable of, understanding the cells survival rate, migration, and longitudinal movement are crucial to understanding their capabilities.

- Further explains the main modalities of tracking stem cells. These include MRI, PET, and optical imaging.

1. MRI: Noninvasive high resolution tracking technique. One benefit of MRI is it's high sensitivity and spatial 3D resolution. Nontoxic and noninvasive.

2. PET and SPECT: Prior to transplantation of stem cells into the host, a radiotracer will label the stem cells in order to detect the cells while in the PET/SPECT scanner. Both are highly sensitive. Can present the distribution of labeled stem cells in 3D.

3. Optical imaging: Lower cost, can obtain rapidly, no radiation, fairly high sensitivity. Fluorescence imaging is the main form in optical imaging. Somewhat limited due to short wavelength.

ALEXIS BLOCK - Oct 18, 2021, 9:37 PM CDT

Conclusion: The demand for a higher understanding of stem cells is crucial in understanding the basis of our research. MRI and PET supply extremely high detail images but cannot be done in vivo.

DOI: https://www.hindawi.com/journals/sci/2017/2934149/

Citation: Y. Zheng *et al.*, “Stem cell tracking technologies for neurological regenerative medicine purposes,” *Stem Cells Int.*, vol. 2017, p. 2934149, 2017.

10/18/21 Murine endoscopy for in vivo multimodal imaging of carcinogenesis and assessment of intestinal wound healing and inflammation

ALEXIS BLOCK - Oct 19, 2021, 10:09 AM CDT

Title: Murine endoscopy for in vivo multimodal imaging of carcinogenesis and assessment of intestinal wound healing and inflammation

Date: 10/18/21

Content by: Alexis Block

Present: Alexis Block

Goals: Gain a more complete understanding of how learning about mice can be translated to human data. Also understand how an endoscope can be used to gain this knowledge.

Content:

- While imaging of mouse models (ex-vivo) can be useful for gaining information, the use of an endoscope in imaging allows for the imaging to be done in-vivo. In vivo enables researchers to directly visualize certain live aspects of the mouse body that could never be done ex-vivo. Using this modality of imaging has extended diagnostic imaging modalities and may even have a significant impact on preclinical research in a variety of fields.

-The use of an endoscope allows for longitudinal observations, repetitive analysis, and a sophisticated image. In previous years, the technology of the endoscopes used has continued to develop and advance.

-In this study, researchers were interested in the gastrointestinal mucosa and the recovery of the mice after this surgery. With the help of an endoscope, they were able to conclude the specific time it took for the wound to heal, the percent of area the wound covered, and even percent weight loss. They also were able to collect information using the fluorescence technique. They used fluorescence to target colorectal tumors and were able to image these cells.

Conclusions/action items: The use of an endoscope was crucial in this study. It allowed them to lively track different cell groups, the recovery of an area, and even analyze peripheral blood. Also, the therapeutic impact of a topically applied drug was analyzed in vivo.

ALEXIS BLOCK - Oct 19, 2021, 10:10 AM CDT

DOI: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828016/

Citation: 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.

10/31/21 A realistic brain tissue phantom for intraparenchymal infusion studies

ALEXIS BLOCK - Oct 31, 2021, 11:20 PM CDT

Title: A Realistic Brain Tissue Phantom For Intraparenchymal Infusion Studies

Date: 10/31/2021

Content by: Alexis Block

Present: Alexis Block

Goals: Understand the use of a gel phantom matrix model in place of real tissue in the mouse brain.

Content:

- Agarose gel at a 0.6% concentration was found to closely resemble the mouse brain in vivo.

- The infusion pressure of the gel demonstrated similar characteristics in regard to the configuration and magnitude of the brain pressure (10-20 mm Hg).

-Agarose gels preparation:

1. Mixing agarose powder and 1 X TBE (89mM Tris; 89mM boric acid; 2 mM ethylenediamine tetra-acetic acid; and pH 8.4)
2. Heat mixture until powder is dissolved, boil for 5 minutes
3. Cool solution to 50 degrees C
4. Pour 500 ml into a rectangular (10 X 10 X 7 cm) container made of extremely thin plexiglass transparent walls
5. Let agarose gel solidify in room temperature.
6. Complete experiemental imagine on live pig and the agarose gel.
7. Compare results

Low concentration agarose gels are an effective alternate to in vivo insertion of an animal brain.

TABLE 1

*Summary of representative studies of infusions in gel models and porcine brain**

Authors & Year	Agent	Model	MW or Diameter	Microscale Structure	Results
Jilla, et al., 1999	lambda phage virus	0.2% gel	~150-nm tail	homogeneous	concentration 2.5×10^5 phage/ml; viruses did not infuse
Chen, et al., 2000	RT-2 GBM cells	agar gel	~10-μm radius	homogeneous	concentration 5×10^6 cells/ml; cells spread at ~1 μm/hr
Lee, et al., 2000	blue dextran dye	1.0% gel	MW ~2×10^6	homogeneous	macromolecular infusate; uniform V_d obtained
Chen, et al., 2002	BPB dye	0.2% gel	MW 690	homogeneous	test of gel poroelasticity; flows validated theory
Gillies, et al., 2002a	Y_2O_3:Eu^{+++} phosphor	0.6% gel	~8-nm particles	homogeneous	electrical charge +3/particle; infusions like those of low-MW dyes
Gillies, et al., 2002b	BPB dye	0.6% gel	MW 690	anisotropic	8-μm fibers placed in gel; axial flows observed
Holligan, et al., 2003	Fe_3O_4 magnetite	0.6% gel	~10-nm particles	homogeneous	external gradient (~3 teslas/m) moved particles in gel
present study	gadodiamide (Omniscan)	0.6% gel	MW 573	homogeneous	brain phantom material; infusions tracked
present study	gadodiamide (Omniscan)	porcine brain	MW 573	white matter	control experiments; pressure & V_d measured
present study	normal saline solution	0.6% gel	MW 58.5	homogeneous	control experiments; pressure sensors tested
work in progress	albumin–Gd-DTPA	porcine brain	MW 94,400	white matter	in progress; comparison to gadodiamide

* DTPA = diethylenetriamine pentaacetic acid; GBM = glioblastoma multiforme.

Summary of previous studies use of gel models.

Conclusions/action items:

Agarose gel, specifically at 0.6% concentration, is an effective way to model the mouse brain. We can follow the procedure described above.

ALEXIS BLOCK - Oct 31, 2021, 11:24 PM CDT

DOI:

https://thejns.org/view/journals/j-neurosurg/101/2/article-p314.xml

Citation:

Z.-J. Chen *et al.*, “A realistic brain tissue phantom for intraparenchymal infusion studies,” *J. Neurosurg.*, vol. 101, no. 2, pp. 314–322, 2004.

10/05/21 Multi-layer Cortical Ca2+ Imaging in Freely Moving Mice

ALEXIS BLOCK - Oct 05, 2021, 10:13 AM CDT

Title: Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes

Date: 10/05/21

Content by: Alexis

Present: Alexis

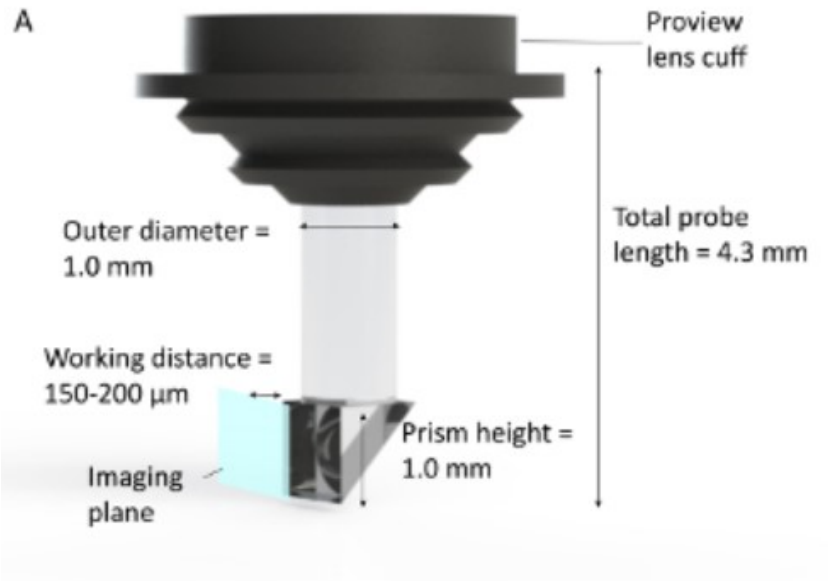
Goals: To understand a study currently utilizing mini endoscopes in freely moving mice.

Content:

- A procedure in which a miniature, two photon, microscope is utilized to track Ca2+ movement across multiple cortical layers. This specific microscope employs a GRIN lens and an implantable prism probe to simultaneously view the cortex and record the dynamics of cells. Moreover, the prism probe used here is composed of two GRIN lenses. The first GRIN lens is a is a prism which conjuncts with the light from the microscope which in turn excites the fluorescently labeled cells located in the field of view of the prism. The second GRIN lens is a cylindrical relay lens which collects the reflected light from the cells and later reaches the sensor of the microscope.

-The rest of the article goes into depth regarding the exact protocol of using the microscope in freely moving mice.

-The prism probe field of view specifications, allows for 90 degree imaging.



Conclusions/action items:

> This article provides a plethora of in depth images and diagrams explaining their mini endoscope. The use of a prism as the lens is something our group should consider.

ALEXIS BLOCK - Oct 05, 2021, 10:15 AM CDT

DOI: https://www.jove.com/t/55579/multi-layer-cortical-ca2-imaging-freely-moving-mice-with-prism-probes

Citation:

[1]	S. Gulati, V. Y. Cao, and S. Otte, “Multi-layer cortical ca^{2+} imaging in freely moving mice with prism probes and miniaturized fluorescence microscopy,” *J. Vis. Exp.*, no. 124, p. e55579, 2017.

10/05/21 Dual view capsule endoscopic lens design

ALEXIS BLOCK - Oct 05, 2021, 10:33 AM CDT

Title: Dual View Capsule Endoscopic Lens Design

Date: 10/05/21

Content by: Alexis

Present: Alexis

Goals: Understand the use of a different type of lens utilized to increase the field of view.

Content:

-This lens employs a dual view capsule like design in which the the front view and back view will function. The lens utilizes a catadioptric mirror and an aspherical surface in order to ensure both sides function. The front field of view would be 90 degrees. The back field of view would be approximately 260-290 degrees.

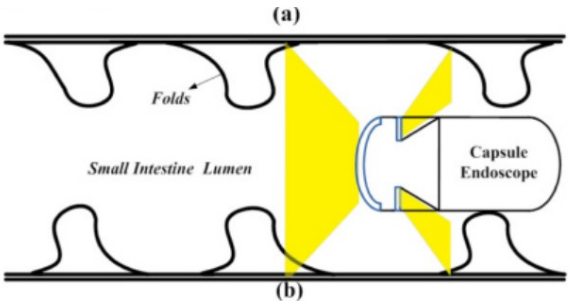
-The goal of this endoscope is to not only improve the quality of view, but to offer two completely separate fields of view from the front and back. A telocentric wide angle lens will be used for the front. The lens on the back will consist of an f-theta lens and a catadioptric mirror.

-This lens was designed in order for doctors to gain a better and more complete understanding of the intestinal tract. This way imaging could be done simultaneously between different folds of the tract.

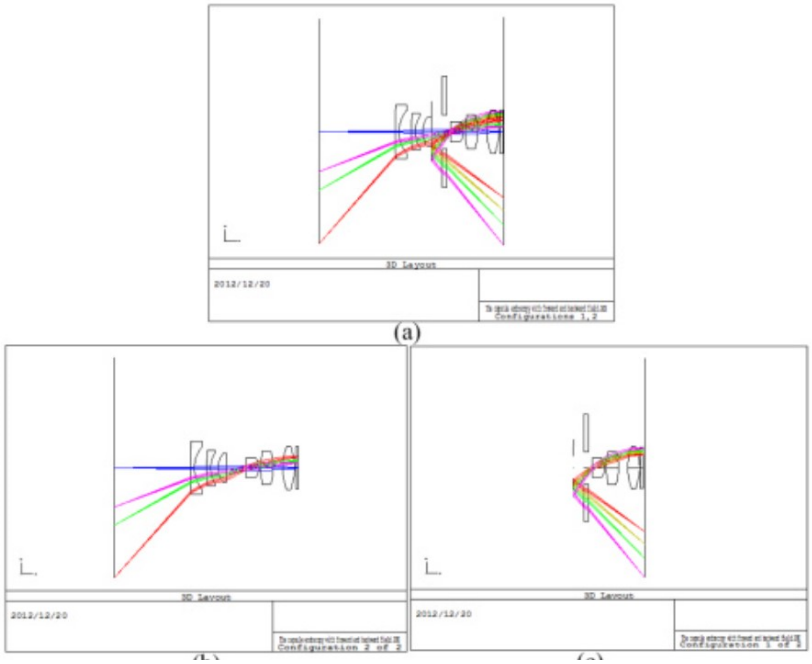
-Specifications:

1. front view: image height from 0 mm to 1.1 mm.

2. back view: 1.4 mm to 2.0 mm. Between 1.1 mm and 1.4mm is a blind spot with no image. This idea is shown in the image below.



This image demonstrates the use of the mirror to reflect the light to the back of the lens.



Conclusion:

This article provides an extremely interesting type of lens that could possibly be altered to fit our project. It could be used if the mirrors are adjusted to reflect the light to eliminate the blind spot, and in turn just create a much wider and taller field of view.

ALEXIS BLOCK - Oct 05, 2021, 10:33 AM CDT

DOI: https://www.osapublishing.org/oe/fulltext.cfm?uri=oe-23-7-8565&id=314178

Citation:

[2]	M.-J. Sheu, C.-W. Chiang, W.-S. Sun, J.-J. Wang, and J.-W. Pan, “Dual view capsule endoscopic lens design,” *Opt. Express*, vol. 23, no. 7, pp. 8565–8575, 2015.

10/19/21 Endoscope Field of View Measurement

ALEXIS BLOCK - Oct 19, 2021, 10:22 AM CDT

Title: Endoscope Field of View Measurement

Date: 10/19/21

Content by: Alexis Block

Present: Alexis Block

Goals: To gain a more complete understanding of the capabilities of an endoscope, specifically the maximum field of view of an endoscope

Content:

- The angular field of view (FOV) of an imaging device, specifically an endoscope, can be defined by the angle in object space over which objects are viewed on a sensor or film. The FOV can be expressed in terms of a one dimensional length or two dimensional area. Endoscopes are generally fixed at a certain distance from the object of interest, so defining the FOV is extremely helpful.

-Endoscopes generally have a wide FOV. This is a result of the restricted space and required range of movement during a procedure. If an endoscope has a smaller field of view, human interaction or machinery is required to reposition the endoscope to fully capture the image. To avoid human error, most endoscopes are being designed with a larger field of view.

- The field of view depends on the focal length of the lens in addition to the actual size of the imaging sensor. A correlation exists : an endoscope with a shorter lens focal length and a larger imaging sensor size will yield a larger FOV>.

-Flexible endoscopes usually have FOV of 90 degrees to 170 degrees with the average being around 140 degrees. Some other common numbers are 100 degrees, 120 degrees, and 170 degrees.

Conclusions/action items: Most manufacturers are working to build endoscopes with a larger field of view to reduce human error. The average FOV is around 140 degrees

ALEXIS BLOCK - Oct 19, 2021, 10:23 AM CDT

DOI:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5480555/#:~:text=According%20to%20manufacturers'%20labels%2C%20flexible,or%20170%C2%B0%20%5B7%5D.

Citation:

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.

10/19/21 Gradient Index Optics

ALEXIS BLOCK - Oct 19, 2021, 3:26 PM CDT

Title: Gradient Index Optics

Date: 10/19/21

Content by: Alexis Block

Present: Alexis Block

Goals: Fully understand the way a gradient index lens functions. Understand how light and the GRIN lens work hand in hand.

Content:

- An incoming light ray is refracted when it enters the shaped lens. This happens because there is an abrupt change in the refractive index from the air to a different material. The light ray passes the lens material directly until it reaches the exit surface of the lens. It is then refracted again due to the same reason spoken about earlier, an abrupt change from material back to the air. A GRIN lens depends on a continuous change of the refractive index within the lens material. Compared to a normal lens, instead of utilizing a shaped surface, a plane optical surface is utilized.

-The GRIN lens also defers from traditional lens' because the light rays are continuously bent within the lens until finally they are focused on a spot. The lens are fabricated down to 0.2 mm in thickness. The GRIN lens is also fairly cost-effective. Most Grin lenses are produces using silver and lithium ion exchange in special glasses. Moreover, in constrast to a spherical or aspherical lens, all optical path lengths are the same in a GRIN lens due to the radially differing refractive index.

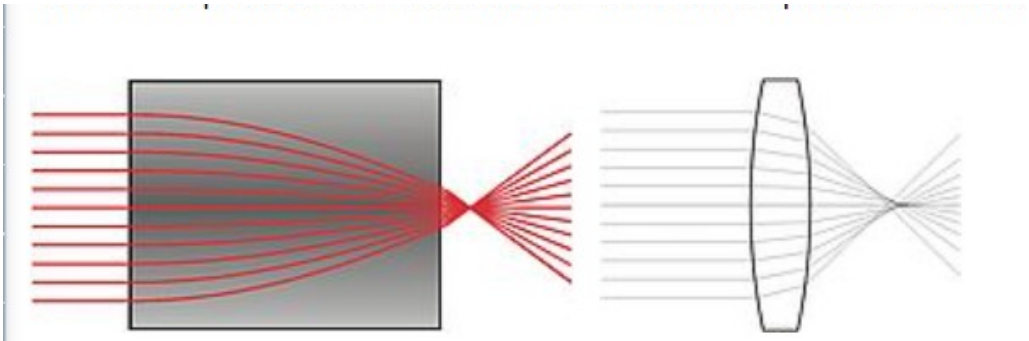


Fig. 1: GRIN lens *Conventional spherical lens*

Conclusions/action items:

Grin lenses affect the optical path within the lens itself. This is accomplished by varying the index of refraction.

ALEXIS BLOCK - Oct 19, 2021, 3:27 PM CDT

DOI: https://www.grintech.de/en/gradient-index-optics/.

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

11/19/21 Fluorescent Microrspheres

ALEXIS BLOCK - Dec 13, 2021, 12:33 PM CST

Title: Fluorescent Microspheres

Date: 11/19/21 - Updated 12/13/21

Content by: Alexis Block

Present: Alexis Block

Goals: Look into different fluorescent microspheres on the market for testing

Content:

- Fluorescent microspheres: round spherical particles that emit a bright fluorescent color when illuminated with UV light. The ability of the microspheres to emit a fluorescent light makes it easier for imaging. The spheres come in all different types, sizes, and colors and all have similar mechanical elements to that of a real life cell.

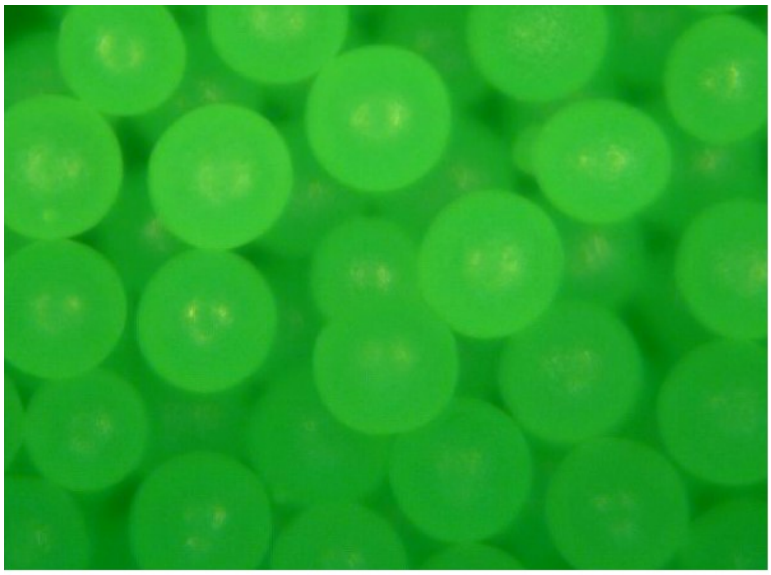
-The fluorescent beads are generally composed of a pure polyethylene substance that ensures that the beads are fully embedded into the polymer matrix while avoiding any separation.

-Some beads are polarized. They can be composed of a polarized outer shell that can be beneficial in certain cases of research.

-The general size bead for stem cell research is around 10 microns.

- A good choice for our research purposes would be the following beads, "Fluorescent Green Polyethylene Microspheres 1.00g/cc - 10um to 1400um (1.4mm)"

-The beads appear like this under UV light:



Fluorescent Green Polyethylene Microspheres Bright Green Polymer Beads Density 1.00g/cc - Neurtrally Buyoant for Aqueous Solutions

Conclusions/action items:

Continue researching different fluorescent beads to use for research. Possibly purchase the ones detailed above.

Citation:

“Fluorescent Green Polyethylene Microspheres 1.00g/cc - 10um to 1400um (1.4mm),” *Cospheric.com*. [Online]. Available: https://www.cospheric.com/UVPMSBG_fluorescent_green_spheres_density100.htm.

DOI:

https://www.cospheric.com/UVPMSBG_fluorescent_green_spheres_density100.htm

11/15/21 A Novel Method for Localizing Reporter Fluorescent Beads Near the Cell Culture Surface for Traction Force Microscopy

ALEXIS BLOCK - Dec 13, 2021, 12:11 PM CST

Title: A Novel Method for Localizing Reporter Fluorescent Beads Near the Cell Culture Surface for Traction Force Microscopy

Date: 11/15/21 - updated 12/12/21

Content by: Alexis Block

Present: Alexis Block

Goals: Comprehend how using a gel matrix and fluorescent beads can mimic a mouse brain

Content:

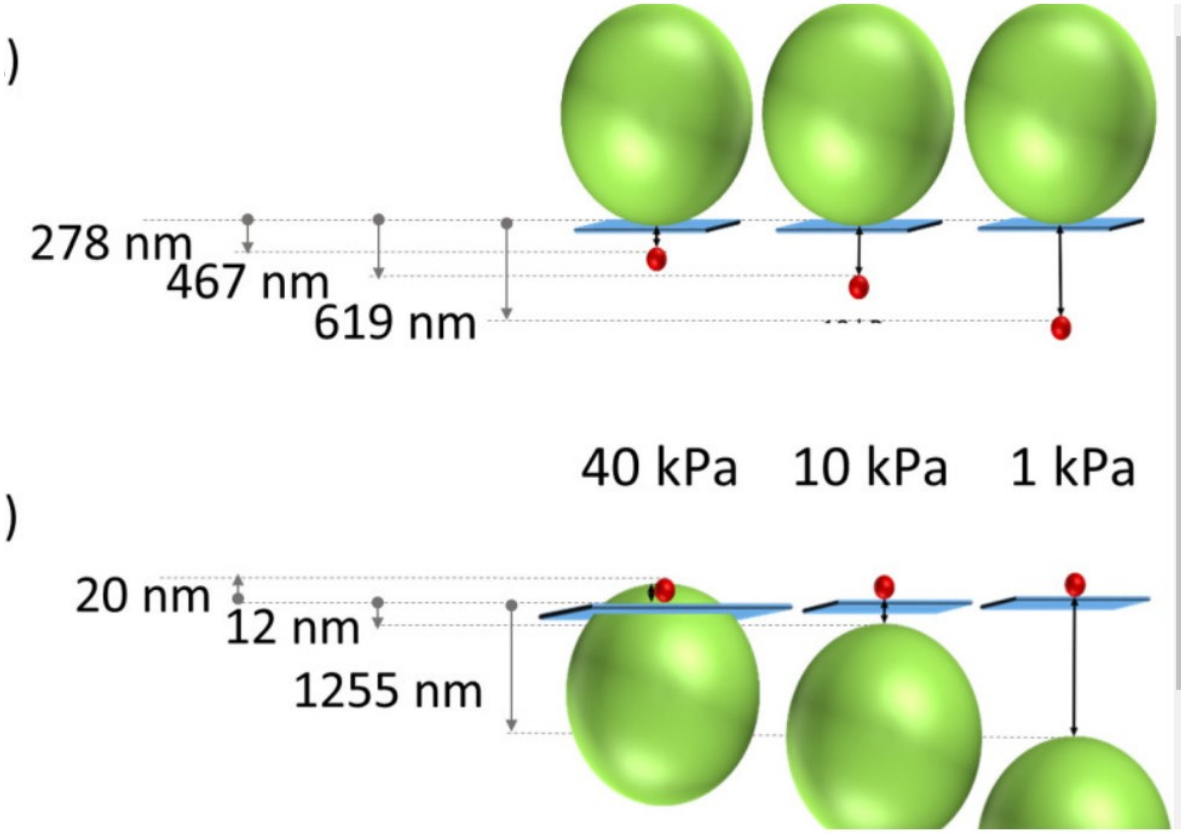
- Gel Microspheres are often used in placement of real life cells in vivo due to their ease of fabrication and their similar elastic properties.

- In most cases, the gel microspheres are intermixed throughout the PA gel in the liquid, unpolymerized state. As time progresses, the liquid will polymerize and contain the gel microspheres dispersed throughout.

- The beads often tend to migrate either towards the surface of the gel, or away from the surface of the gel, depending on the density.

- One way to combat this problem is by increasing the density of all beads which will result in a greater number of beads at the upper focal plane. Another way to combat this issue is polymerizing a thin layer of liquid with beads, and once that layer has polymerized, add another thin layer of liquid and repeat the process.

-Another thing to consider is the size of the individual beads chosen. Different sized beads will behave differently.



- This Image depicts the average distance of the bead relative to the gel matrix, given different sized beads. It also depicts the different stiffness' of beads as size of bead increases.

Conclusions/action items:

-Depending on what Dr. Trevathan prefers, we can refer back to this to decide what size fluorescent bead to use in our testing. We will most likely use a bead close to 10 um. Overall the use of a gel matrix in place of in vivo in the mouse brain is an appropriate way to gain rather precise preliminary data.

ALEXIS BLOCK - Dec 12, 2021, 11:16 PM CST

Citation:

[1] S. G. Knoll, M. Y. Ali, and M. T. A. Saif, “A novel method for localizing reporter fluorescent beads near the cell culture surface for traction force microscopy,” *J. Vis. Exp.*, no. 91, p. 51873, 2014.

DOI:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828080/

11/17/21 Reliable Preparation of Agarose Phantoms for Use in Quantitative Magnetic Resonance Elastography

ALEXIS BLOCK - Dec 13, 2021, 12:12 PM CST

Title: Reliable Preparation of Agarose Phantoms for Use in Quantitative Magnetic Resonance Elastography

Date: 11/17/2021 - updated 12/13/21

Content by: Alexis Block

Present: Alexis Block

Goals: Understand how to prepare an agar phantom for testing

Content:

- Agar solutions are usually inexpensive, easy to work with, store, and dispose of.

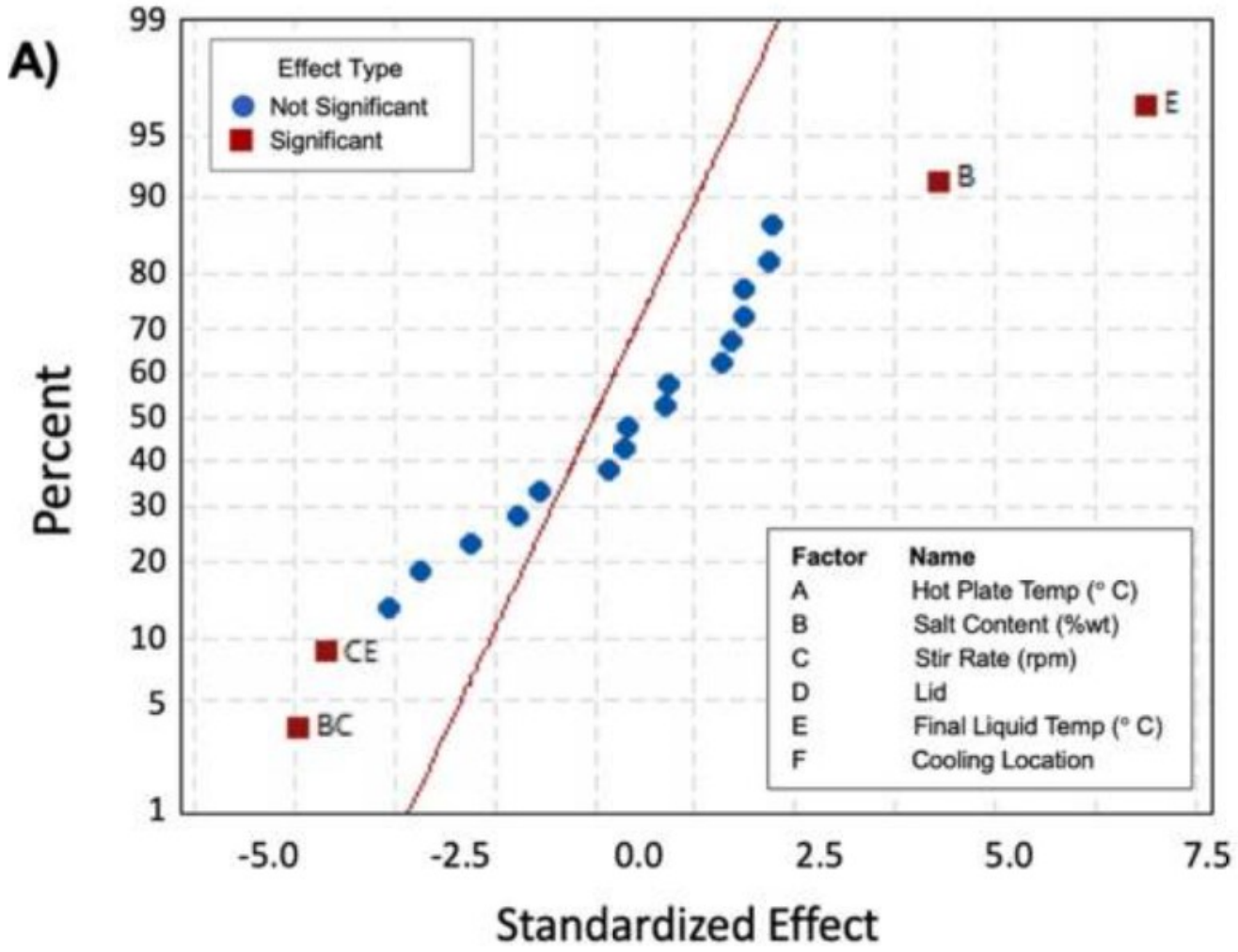
- Despite a lot of advantages, the preparation of an agar solution is still one that is not universally set. Because of this, it is easy to prepare a phantom that is inconsistent with it's expected mechanical properties.

-Cooling rate and temperature of liquid are two factors that affect the homogeneity of the phantom. Agar concentration affects the stiffness of the phantom. The interaction of salt with the phantom can also result in adverse affects.

-A study was done with different salt contents, stir rates, cooling location, and temperature and the stiffness was recorded. Here are the results:

	Hot Plate Temperature 300 °C – 550 °C	**Salt Content 0.3%wt – 0.9%wt**	**Stir Rate 100 rpm – 800 rpm**	**Lid with or without**	**Final Liquid Temperature 84 °C- 96 °C**	**Cooling Location Ice bath, refrigerator, or room temperature**
Stiffness, μ	$\eta^2 = 0.007$ $p = 0.387$	$\eta^2 = 0.106$ $p = 0.001$	$\eta^2 < 0.001$ $p = 0.936$	$\eta^2 = 0.010$ $p = 0.296$	$\eta^2 = 0.161$ $p < 0.001$	$\eta^2 = 0.018$ $p = 0.161$

Here is a graph of the data with correlation:



Conclusions/action items:

Looking at the graph, we can see that both salt content in phantom and final liquid temp are two factors that will ultimately affect the stiffness of the phantom.

ALEXIS BLOCK - Dec 12, 2021, 11:39 PM CST

Citation:

G. McIlvain, E. Ganji, C. Cooper, M. L. Killian, B. A. Ogunnaike, and C. L. Johnson, “Reliable preparation of agarose phantoms for use in quantitative magnetic resonance elastography,” *J. Mech. Behav. Biomed. Mater.*, vol. 97, pp. 65–73, 2019.

DOI:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6699912/

9-14-21 - Imaging of freely behaving mice

TYLER ANDERSON - Oct 18, 2021, 9:43 PM CDT

Title: Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes

Date: 9/14/21 (updated 10/18/21)

Content by: Tyler Anderson

Present: N/A

Goals: Gain more knowledge about the project before meeting with the client

Content:

- The GRIN lenses used in this experiment have a .5 mm diameter
- This study sought to image calcium ions in the mouse brain in vivo
- The process for attaching the microscope into the brain of the mouse is as follows [summarized]:
    - (With postnatal pups) Insert the mouse with anesthesia and wait 5 minutes to see the effects
    - After observing no movements, inject the plasmids into the brain
    - Sterilize the head then open up the skin, release the DNA into the brain -- these first few steps were for electroporation
    - Once mice are about 2 months old, we begin endoscope installations
    - Anesthetize the animals
    - Cut open the skin, drill hole in skull, lower GRIN lens into correct location
    - To solidify the lens in place, use dental cement to keep apparatus in same spot.
    - Close cuts and let mouse heal
- After animal has had time to heal, imaging of the brain begins
- Imaging will result in sufficient pictures of freely-moving mice

Conclusions/action items: This article was very thorough in explaining the process of how to take mice from birth and using them to image calcium ions in the brain in vivo. It was shown how the mice are prepared as pups with electroporation, to the endoscope installation, to the process of how the actual collection of data was obtained, and finally to the removal of the endoscope.

TYLER ANDERSON - Oct 18, 2021, 9:45 PM CDT

DOI:

https://doi.org/10.1016/j.xpro.2021.100596

Citation:

Malvaut, S., Marymonchyk, A., Gengatharan, A., & Saghatelyan, A. (2021). Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes. *STAR Protocols*, *2*(2), 100596.

9-16-21 - Murine Endoscopy for In Vivo Multimodal Imaging

TYLER ANDERSON - Oct 18, 2021, 11:02 PM CDT

Title: Murine Endoscopy for *In Vivo* Multimodal Imaging of Carcinogenesis and Assessment of Intestinal Wound Healing and Inflammation

Date: 9/16/21 (Updated 10/18/21)

Content by: Tyler Anderson

Present: N/A

Goals: Understand more about the scope of what we are trying to accomplish as a team

Content:

- There is only a limited amount of information one can gain by studying mice through non-invasive techniques, like looking at weight gain/weight loss, blood, urine, or feces analysis.
- Through the use of real time imaging, one will be able to see how endoluminal colonic disease pathologies such as wound healing and intestinal inflammation operate.
- Then one will take mice and introduce colorectal cancer and then put the animals on anesthesia.
- After the animals are non-responsive, introduce an endoscope up through the anus to observe the colon and the healing process.
- Images were taken to observe the intestinal wound healing over a few day range.
- Approximately 80 days after the induction of the tumor, the progression of the condition of the area could be observed very closely.

Conclusions/action items: Through non-invasive wellness checks, one can only obtain so much data about how an animal is truly doing. Through the use of murine endoscopy, there is a wider range of possibilities that will allow for qualitative and quantitative data to be collected that can lead to conclusions.

TYLER ANDERSON - Oct 18, 2021, 10:02 PM CDT

DOI:

10.3791/51875

Citation:

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.

10-8-21 - Strength of Mice

TYLER ANDERSON - Oct 19, 2021, 12:32 PM CDT

Title: Measuring the Strength of Mice

Date: 10/8/21 (Updated 10/19/21)

Content by: Tyler Anderson

Present: N/A

Goals: Understand relationship between Mice and Humans and how studying them is useful

Content:

- Mice can be used to model human motor functions.
- Studying the somatic and the central nervous system in mice, researchers can further study muscular dystrophy, myasthenia gravis, multiple sclerosis, spinal muscular atrophy, Parkinson's disease, Huntington's disease, among many others.
- One of the strength test has a researcher pick up a mouse by its tail and then lower the mouse and let it grab a bundle of tangled fine gauge stainless steel wire. Then attached to this bundle of wire - which is maximizing the grip a mouse can get - are varying amounts of block chains.
- There is also a test where there is an inverted screen of wire mesh and a mouse is placed holding it then it is turned upside down to test the mouse's strength of holding themselves up.
- After resuts were collected, a certain amount of mice could lift approximately 70g. Some mise which were inserted with certain conditions were also tested to see how they compared.
- Female knockout mice for the KATP Channel subunit Kir6.2 could lift more than the males with this condition.

Conclusions/action items: To say that muscles and brain functions are independent of each other is ignorant as muscles are activated by the brain. Therefore, studying strength can indicate brain functions and the level of certain functions.

TYLER ANDERSON - Oct 19, 2021, 12:33 PM CDT

DOI:

10.3791/2610

Citation:

R. M. J. Deacon, “Measuring the strength of mice,” *J. Vis. Exp.*, no. 76, 2013.

10-8-21 - Multimode Fibre-based Endoscopy

TYLER ANDERSON - Oct 19, 2021, 1:04 PM CDT

Title: High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging

Date: 10/8/21 (Updated 10/19/21)

Content by: Tyler Anderson

Present: N/A

Goals: Gain background knowledge on endoscopy

Content:

- The use of hair-thin multi-mode optical fibre as an imaging tool is super useful in yielding images for researchers to interpret.
- This type of endoscopy is different than the use of GRIN lenses and has better resolution and a reduced footprint.
- Through multi-mode fibres (MMFs), one does not have to worry about the size as these are very small compared to traditional GRIN lenses.
- Light transfers through MMFs to then create scrambling incident wavefronts which then turns into random speckle patterns.
- The foci behind MMFs can be scanned at several tens kHz, producing images at speeds that have rates similar to videos.
- The reduced footprint would allow for even deeper analysis of brain tissue. One would be able to see the neuronal connections even closer than before.

Conclusions/action items: MMFs offer many benefits in imaging and could very well be the future for imaging in vivo.

TYLER ANDERSON - Oct 19, 2021, 1:04 PM CDT

Citation:

S. Turtaev, I. T. Leite, T. Altwegg-Boussac, J. M. P. Pakan, N. L. Rochefort, and T. Čižmár, “High-fidelity multimode fibre-based endoscopy for deep brain in vivo imaging,” *Light Sci. Appl.*, vol. 7, no. 1, p. 92, 2018.

10-7-21 - GRIN Lens

TYLER ANDERSON - Oct 19, 2021, 11:35 AM CDT

Title: Gradient Index Optical Microsystems Visualize Living Cells in Deep Tissue

Date: 10/7/21 (Updated 10/19/21)

Content by: Tyler Anderson

Present: N/A

Goals: Learn about endoscopes and how they function and operate

Content:

- GRIN optics can range in diameters from .25 mm to 2.0 mm
- These GRIN lenses can be attached to flexible fiber optics (cables and wires)
- A GRIN lens works by a refractive index profile within the medium
- GRIN lenses are not like conventional lenses, they do not have curved surfaces which refract the light a certain way.
- The block functionality of the GRIN lens allows for the light to be continuously refracted until focusing on one final point at the end
- A typical endoscope consists of: a fiber optic, a spacer, the grin lens, and then some sort of refractive item. In this article, that item is a micro prism.
- In vivo imaging can be done using miniature gradient index optic systems (GRIN)

Conclusions/action items: Gradient Index Optical microscopy is a certain way one can view a freely moving animal. This article looks at how GRIN lenses operate and also some designs other studies have utilized for in vivo imaging.

TYLER ANDERSON - Oct 19, 2021, 11:37 AM CDT

Citation:

Bernhard Messerschmidt, Grintech GmbH, “Gradient index optical microsystems visualize living cells in deep tissue,” *Photonics.com*, 01-Sep-2007. [Online]. Available: https://www.photonics.com/Articles/Gradient_Index_Optical_Microsystems_Visualize/a38235. [Accessed: 7-Oct-2021].

10-7-21 - Nano-optic endoscopy

TYLER ANDERSON - Oct 19, 2021, 12:04 PM CDT

Title: Nano-optic endoscope for high-resolution optical coherence tomography in vivo

Date: 10/7/21 (Updated 10/19/21)

Content by: Tyler Anderson

Present: N/A

Goals: Understand different design ideas to help familiarize self with endoscopes

Content:

- Endoscopic optical coherence tomography (OCT) is providing closer looks at deep tissue enabling researchers to further study internal processes.
- Acquiring solid, clear images of the desired field of view in an animal is quite tricky however.
- In order to obtain a certain field of view in an animal, some studies use an angle polished ball lens, or some sort of prism configuration.
- Essentially, whatever refractory item that is used to obtain a field of view 90 degrees from the implanted endoscope, it is placed on the bottom of the endoscope, beneath the GRIN lens.
- Depending on the type of refractory item, the light will be refracted in a certain way, which will yield a certain field of view.
- If one is to use the polished ball, a fish eye field of view might be observed.
- If a micro prism is used, then a metalens would be needed to achieve near diffraction focusing.
- How the endoscope would work is that the light source would be driven through the fiber optic, the spacer, and the grin lens before it is then refracted off of the microprism. But this interaction with the prism would spread the light so a metalens would then refocus the light to a single spot (the field of view).

Conclusions/action items: Endoscopic OCT is very helpful in delivering images to researchers about certain tissues being studied. Through the use of refractor items such as a microprism, light sources would be refracted at a certain angle which will then yield a certain field of view for the researchers.

TYLER ANDERSON - Oct 19, 2021, 12:06 PM CDT

Citation:

H. Pahlevaninezhad *et al.*, “Nano-optic endoscope for high-resolution optical coherence tomography in vivo,” *Nat. Photonics*, vol. 12, no. 9, pp. 540–547, 2018.

11-2-21 - Polyacrylamide gel matrix

TYLER ANDERSON - Dec 13, 2021, 1:54 PM CST

Title: A mechanically strengthened polyacrylamide gel matrix fully compatible with electrophoresis of proteins and nucleic acids

Date: 11/2/21 (updated 12-13-21)

Content by: Tyler Anderson

Present: Individual work

Goals: Find a suitable gel matrix to simulate the v4 ventricle inside the mouse brain

Content:

- Polyacrylamide gel electrophoresis is a very integral tool that biochemists use in seperating nucleic acid and protein.
- Electrophoresis is utilized primarily just for analysis.
- The gel consists of acrylamide monomer and bisacyrlamide.
- This gel has excellent sieving properties but is unfortunately prone to tears as it is mechanically fragile.
- Through the addition of ethylene oxide, the gel matrix is made more durable.
- This more durable matrix is resistant to tears and could be utilized in other experiments.

Conclusions/action items: Polyacrylamide gel electrophoresis is a method that biochemists use to seperate nucleic acids and proteins for analysis. The gel that is initially used is more prone to tears and can tear easily. With the addition of ethylene oxide, the gel is more resistant to tears and is durable.

TYLER ANDERSON - Dec 13, 2021, 6:08 PM CST

Citation:

C. Pushparajan *et al.*, “A mechanically strengthened polyacrylamide gel matrix fully compatible with electrophoresis of proteins and nucleic acids,” *Electrophoresis*, vol. 39, no. 5–6, pp. 824–832, 2018.

11-2-21 - Collagen gel matrix

TYLER ANDERSON - Dec 13, 2021, 6:06 PM CST

Title: Collagen gel three-dimensionaal matrices combined with adhesive proteins stimulate neuronal differentiation of mesenchymal stem cells

Date: 11-2-21 (updated 12-13-21)

Content by: Tyler Anderson

Present: Individual work

Goals: Find a suitable gel matrix to simulate the v4 ventricle inside the mouse brain

Content:

- Three-dimensional gel matrices provide environments to simulate native tissue and allow for stem cells to grow and differentiate into specific cells.
- In this experiment, they utilized sacrificed rat bone marrow to extract mesenchymal stem cells (MSC).
- Then through the use of three different three-dimensional collagen based culture groups, the stem cells were mixed in.
- The MSC's were identified by co-immunostaining with stem cell markers, Oct4 and SSEA.
- The neuronal differentiation of MSC's cultured under two or three dimensional conditions were stained with anti-NeuN and anti-GFAP.
- In the three dimensional conditions, distinctions and differentiations could most certainly be observed.

Conclusions/action items: Through this experiment, it can be shown how collagen-based three dimensional gel matrices are certainly effective in inducing neuronal cell differentiation of MSC's. However, this project was done ex vivo and so the processes for animals, rats included, of stem cells would have to be taken into account.

TYLER ANDERSON - Dec 13, 2021, 6:07 PM CST

Citation:

J. H. Lee, H.-S. Yu, G.-S. Lee, A. Ji, J. K. Hyun, and H.-W. Kim, “Collagen gel three-dimensional matrices combined with adhesive proteins stimulate neuronal differentiation of mesenchymal stem cells,” *J. R. Soc. Interface*, vol. 8, no. 60, pp. 998–1010, 2011.

11-2-21 - Agar ultrasound phantoms for low-cost training without refrigeration

TYLER ANDERSON - Dec 13, 2021, 6:50 PM CST

Title: Agar ultrasound phantoms for low-cost training without refrigeration

Date: 11-2-21 (Updated 12-13-21)

Content by: Tyler Anderson

Present: Individual work

Goals: Find a suitable gel matrix to simulate the v4 ventricle inside the mouse brain

Content:

- Manufactured phantoms are particularly expensive. Easy to make ones at home are more accessible.
- Gelatin is usually a matrix used to suspend analogues and mimic tissue.
- Goal of experiment was to see if agar models would be superior to gelatin matrices.
- 5 models were constructed which consisted each of varying percentages of agar.
- The 5% by mass agar model with some suspended wheat flour conveyed that this was a viable model that could generate an ultrasound image that resembled a real tissue.
- Provided detail instructions on how to make the phantom matrix

Conclusions/action items: At the current moment, gelatin is the best option for homemade solutions to be used for ultrasound training. However, it isn't the best solution to use when it comes to last of use and pretty much anywhere that doesn't have a refrigerator. Therefore, this experiment sought to tackle that issue. From the images, one would be able to see how the agar solutions do in fact give a very good resemblence of native tissue.

TYLER ANDERSON - Dec 13, 2021, 6:51 PM CST

Citation:

M. Earle, G. D. Portu, and E. DeVos, “Agar ultrasound phantoms for low-cost training without refrigeration,” *Afr. J. Emerg. Med.*, vol. 6, no. 1, pp. 18–23, 2016.

2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

John Puccinelli - Nov 03, 2014, 3:20 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: