Stage Top Platform for Stable and Long-Term Intravital Imaging of Mouse Mammary Tumor Models

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Preliminary Report

October 12, 2022

BME 200/300

Client: Dr. Suzanne Ponik – Assistant Professor, Cell and Regenerative Biology

Advisor: Dr. Kris Saha – Biomedical Engineering, UW-Madison

Team Members:

Joel Matthews, Team Leader

Hailey Kanter, BWIG

Abbylee Maeder, Communicator

Amara Monson, BSAC

Christy Li, BPAG

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Abstract

The metastatic dissemination of breast cancer tumors is one of the leading causes of death in breast cancer patients. Breast cancer cells can disseminate from the primary tumor and spread throughout the body and invade organs separate from the primary tumor. Intravital imaging has become a primary tool on analyzing the progression of breast cancer proliferation within the mammary glands. This tool allows for assessment of tumor cell intravasation as well as the behavior of the tumor cells within the microenvironment. Tumor cell migration and invasion is a process that takes place over several days; long-term intravital imaging is needed to capture the evolution of the disease. The BME team must design a stabilizing platform for the new PDMS lense which will allow for long-term observations of the tumor microenvironment. In order to do so, an evaluation of the current platform was conducted, and a novel manipulation of the plate, designed to fit the parameters of the new PDMS lens, will be fabricated. After fabrication, tests will be performed to compare the stability of the old platform to the new platform. Upon the finalization of tests, an analysis of the plate will be conducted to determine future work.

I. Introduction

Motivation

In 2019, 264,121 women were diagnosed with breast cancer, and 42,280 women died of it [1]. Improving the efficiency and accuracy of experiments using intravital imaging through the development of this product will further research aimed at understanding, treating, and even curing breast cancer, and will have an impact on millions of women across the world.

Competing and Current Designs

No market products are currently available for purchase in use with their current system. Even within the literature, stabilization devices were designed for use by individual research teams. These designs are not licensed and are not available for commercial use. [2] These custom designs are prototypes that are designed to fit upright microscopes. Unfortunately, without access to the design file, the team is unable to determine whether the prototype will work with the clients current system. The original device currently in use within the clients lab was designed by a prior PhD student that has since graduated.

Problem Statement

Due to the complex network of cells in the microenvironment of mouse mammary tumors, an intravital imaging system has been developed that can accurately image the microenvironment surrounding a tumor in the mammary gland of a living mouse over an extended period of time, as seen in Figure 1. The system includes accurate and clear imaging techniques, along with a safety system to ensure the well-being of the lab mouse while it is being imaged. Currently, the client is using a small circular lens made of metal and glass which is inflexible. The inflexibility of this lens allows for a maximum of 2-3 weeks of imaging of one mouse before the lens falls out due to activity of the mouse. A new flexible PDMS lens [2] has been created which is flexible and has allowed for imaging to occur for 6-8 weeks in one mouse. This allows for a better understanding of the long term effects in the microenvironment of a tumor as well as the opportunity to create multiple observation points of the tumor. There is

currently no stage top platform to allow imaging with this new lens. Creating a stage top platform that keeps a PDMS lens stable throughout imaging will allow researchers to use PDMS imaging lenses more often, and allow them to gain information and a better understanding of the tumor microenvironment. Additionally, pressure must be put on the mammary gland of the mouse to observe the tumor properly during imaging. To solve this, a stabilizing mechanism will be added to the platform for constant pressure on the mammary gland for clear imaging. The goal is to create a new stage top platform to accurately and clearly image the microenvironment of the mouse mammary tumor with the use of the new PDMS lens, so the imaging can be used to further mammary tumor treatment for human patients.

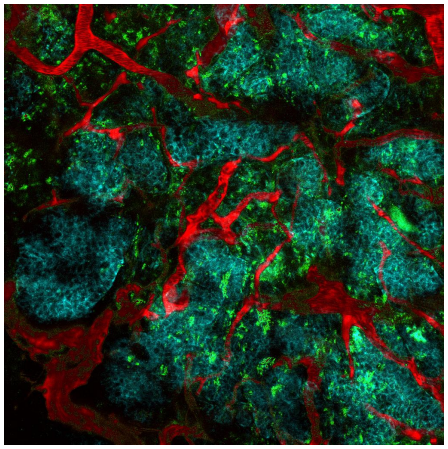


Figure 1: Tumor Microenvironment. *A collagen dense tumor microenvironment captured by the lab members at Ponik Lab [3].*

II. Background

Physiology and Biology

The process of intravital imaging is a very important tool for cancer research. Surgically implanting an imaging window provides researchers with a way to directly observe and image

the living cells in and around a tumor as it develops over many days or weeks. Research focuses aided by the use of an imaging window include the process of metastasis, immune responses to cancer cells, and tumor responses to cancer therapies, among others [4]. There are various imaging window designs that allow for placement in many places on a mouse's body, including, but not limited to, the brain, lungs, and mammary glands [2]. This report will focus on mammary imaging windows (MIWs).

MIWs are generally fabricated from metal and glass [5], as seen in Figure 2, or PDMS, a light, non-reactive material that is clear enough for optimal imaging [2], as seen in Figure 3. A rigid metal and glass imaging window is implanted into a mouse's abdomen by cutting a hole larger than the window in the skin above the mammary gland and then suturing the skin tightly to the window. A flexible PDMS window is implanted by cutting a hole slightly smaller than the window, bending the window to fit it into the hole, and allowing the tension caused by the stretching of the skin around the window to keep it in place. The skin will then heal around the PDMS imaging window, keeping it in place long-term [2].

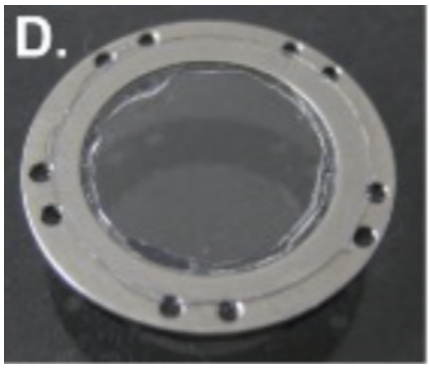


Figure 2: Metal and Glass Imaging Window. *A metal and glass imaging window, similar to the one originally used by our client [2].*

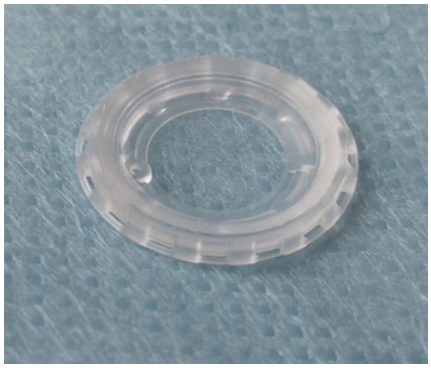


Figure 3: PDMS Imaging Window. *A PDMS imaging window, similar to the one currently used by our client [2].*

Client Information

Dr. Suzann Ponik has a Ph.D in cell physiology and biophysics, and Dr. Brian Burkel, our alternate client, has a Ph.D. in zoology. Dr. Ponik and her team at the Wisconsin Institute for Medical Research are using intravital imaging to research the signaling pathways created by the extracellular matrix in interactions with other cells, and how they are involved in the development of breast cancer tumors [3].

Product Design Specifications

The client is requesting one stage top platform for imaging that is compatible with the new PDMS lens, and that it be done with a budget of $1,500. The goal is to produce a clear image and for this aspect to be successful the stage top platform needs to keep the flexible PDMS lens stable throughout the entire 8 hour imaging process. The design will contain an additional stabilizing apparatus connected to the plate that applies constant pressure to the mammary gland to allow for a clear image.

The size requirements are crucial as the platform must fit into place on the microscope stand. The requirements are 2.75 inches wide by 4.0 inches long. The height of the current stage top platform is exactly 1.0 inch, but our client is requesting that we lower the height by 2-3 mm to allow the microscope more variability in the Z range. The movement restrictions of the microscope are currently 91mm in the X range, 67mm in the Y range, and 9.3mm in the Z range. These dimensions are limited by the current stage top platform.

This stage top platform must be strong enough to support the weight of a mouse and light enough to easily be removed and inserted onto the microscope stage. The only restrictions on materials for this device are that it must be able to withstand the temperature of the heating chamber and weight of a mouse. Lastly, this device must be reusable and able to stay in use for many years.

III. Preliminary Designs

Design 1: Extruded Cylinder

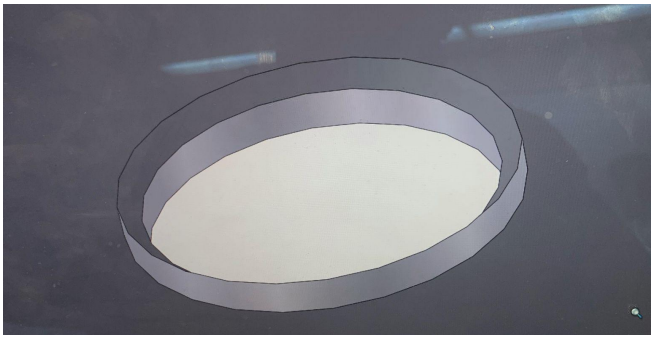


Figure 4 : Top-view of SolidWorks Extruded Cylinder Design.

The extruded cylinder design will have a piece extruded to the thickness of the PDMS lens which is approximately 0.075 inches. The outer diameter of the cylinder is 0.7 inches and the inner diameter is 0.6 inches to match the contour of the PDMS lens. Furthermore, there is a

0.25 inch chamfered edge to properly house the lens during imaging. The main advantage to this design is the mouse's body acting as a stabilizing agent. Because the lens will be surgically implanted in the mouse, there will not be enough material on the outside of the mouse to fully reach the depth of the extruded cylinder. Therefore, to reach the full depth of the cylinder the mouse's body will need to wrap around the extruded portion and in turn will further stabilize the viewing port. This design also has a very significant disadvantage. When a piece of metal is extruded to a height of 0.075 inches there is going to be an inherently sharp edge. When pressure is applied to the mouse and maintained over an imaging session (up to eight hours long), there is a risk of damage to the mouse's skin.

Design 2: Indented Cut Cylinder

Figure 5: *Top-View of Solidworks design of the Indented Cut Cylinder.*

The indented cut cylinder was designed with familiarity of use in mind. This design is closely modeled after the lens currently in use with the metal window within the clients lab. This design was modified in order to fit the specific dimensions of the new PDMS. This design is a modified countersink where the outer indent will wrap around the outer dimension of the PDMS lens while the inner indent will support the inner protrusion of the PDMS lens that sits outside of the mouse's body. In theory this design will support the PDMS lens, however, the protrusion of the

PDMS lens is minimal compared to the older metal lens so the design will need to be tested in order to adjust the dimensions.

Design 3: Gel Ring

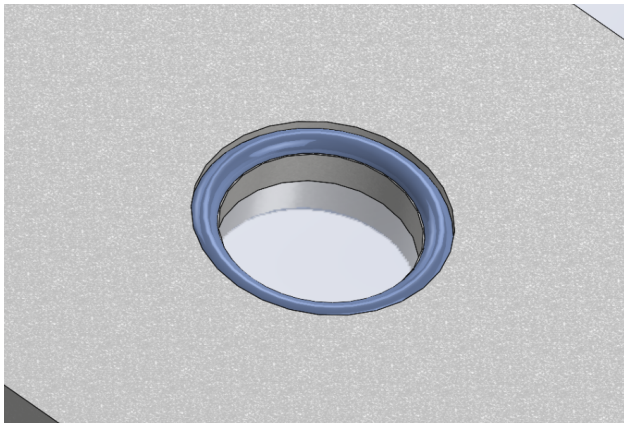


Figure 6: ***Top-view of SolidWorks Gel Ring Design.***

The gel ring will be placed in a cut hole with a diameter of 0.7 inches (the outer diameter of the PDMS lens). Along with being the diameter of the lens, the hole will be cut to a depth of 0.075 inches to fully house a non-implanted lens. The gel ring will be thick enough to have the top layer of gel flush with the top face of the riser plate. The major advantage to this design is the gel acting as a self-conforming mold. There should be almost a perfect fit around the lens so it holds snugly in place for imaging. Also, the gel will likely get in the mouse's fur and help to hold it steady. The gel will be composed of a non-Newtonian fluid like silly putty: acting more like a liquid when the lens is placed softly on the mold and acting more as a solid if any movement of the stand or mouse abruptly tries to remove the lens from the gel. The major downfall of this design is the need for maintenance. As the gel ring gets used it will slowly degrade and the fit will become less snug over time. The ring will need to be replaced every couple imaging sessions to avoid a permanent setting of the putty to the lens' shape. If the ring is left to break

down for long enough, it will affect the imaging results.

IV. Preliminary Design Evaluation

Design Matrix

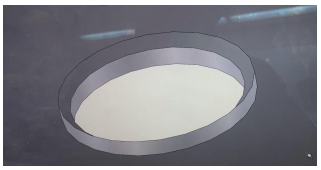
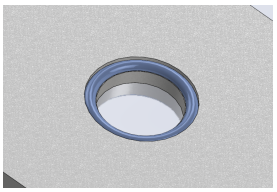
Design Criteria	Extruded Cylinder 		Indented Cut Cylinder 		Gel Ring 	
	Score out of 5	Weighted Score	Score out of 5	Weighted Score	Score out of 5	Weighted Score
Precision (Lack of Movement) (25)	**4/5**	20	**4/5**	20	**5/5**	25
Accuracy (Quality of Image) (25)	**4/5**	20	**4/5**	20	**5/5**	25
Ease of Use (15)	**5/5**	15	**5/5**	15	**4/5**	12
Ease of Fabrication (15)	**3/5**	9	**5/5**	15	**4/5**	12
Cost (10)	**5/5**	10	**5/5**	10	**3/5**	6
Safety (10)	**4/5**	8	**5/5**	10	**5/5**	10
Total (100)	**82**		**90**		**90**	

Figure 7 : *The teams design matrix which outlines our 3 chosen designs as well as scoring to decide which design to move forward with.*

The Gel Ring and Indented Cut Cylinder were the designs that scored the best with our design criteria compared to the Extruded Cut Cylinder. Below will be a more detailed description of how we chose to score these designs and why.

Precision (Lack of Movement)

The precision of our device is one of the most important criteria in our design matrix. It is a requirement that the design causes a lack of movement of the PDMS lens. In this category, the Gel Ring design received the highest score because of the friction that would happen between the mouse's skin and the gel ring in the design.

Accuracy (Quality of Image)

The accuracy and quality of image that our device produces is heavily important in the design process. The quality of image leads to better research for the team's client. The team decided that the Gel Ring design would lead to a better quality image due to the stability that it causes for the lens and how it is held in place.

Ease of Use

Ease of use refers to the familiarity of the design compared to what they have been using in the past. Our clients have been using a plate that allows the steel lens to click into place. Therefore, the Extruded Cylinder and Indented Cut Cylinder had the best scores for ease of use. Ease of use did not have a high weight due to the fact that we thought all of the designs would be simple to use but we thought that complexity mattered.

Ease of Fabrication

The team decided that how easy it was to fabricate the design must be taken into account when analyzing which design to move forward with. The Indented Cut Cylinder scored the best in this category. We found that it would be easier to cut a small amount of a cylinder out in place for the lens rather than trying to extrude a piece of that metal. Additionally, the Gel Ring did not score well because we have to take into account the fabrication of adding the gel ring to the metal. Overall, ease of fabrication did not have a high weight in the design matrix due to the fact that they all do not require a lot of materials or steps for fabrication but we thought that complexity mattered.

Cost

The two most cost effective designs were the Extruded Cylinder and the Indented Cut Cylinder. This is because these two designs do not involve the gel ring in the design. The budget for the project is a large enough amount where the team is not worried about if the project will go over. Therefore, it does not have a high weight in the design matrix.

Safety

When working with live animals or subjects, it is important to consider the safety of the animal. Overall, these proposed designs are safe, which is the reason that safety is not weighted very high. The team is choosing a metal material that will not react with the mice's skin. As for the Extruded Cylinder, the team thought the design should score the lowest due to a sharp edge cutting into the mouse's skin. For a long period of imaging time, this may be uncomfortable and harmful to the mouse.

Proposed Final Design

After analyzing the design matrix and the three proposed designs, the team made a decision of which designs to start prototyping. Since the Gel Ring design and the Indented Cut Cylinder received the highest scores for all the design criteria, the team decided to move forward with a combination of the Indented Cut Cylinder and the Gel Ring. The lower score for safety of the Extruded Cylinder design meant that we could not continue with the design as we are working with live subjects. With the Indented Cut Cylinder getting the highest score in half of the design criteria and the Gel Ring design scoring the best in the other half, a unanimous decision was made by the team to continue moving forward with a combination. An indented cut would be made to our metal material and the gel ring would be placed so that it is not flush with the surface of the metal platform. When pressure is applied to the top of the mouse (which is on top of the mammary gland and lens), the indented cut and gel ring will both ensure the lack of movement and therefore, a better quality image. The combination of these two designs would fill the product requirements the most.

V. Fabrication/ Development Process

Materials

The combined Gel Ring and Indented Cut Cylinder Design will be composed of metal and a gel ring. More research is needed to decide what specific metal will be used, but cost, ease of fabrication, effectiveness, and safety will all be taken into consideration. Both the metal and gel ring will come into contact with the skin and fur of the mouse, so will need to be made out a

biocompatible, non-reactive material. Additionally, the gel ring will need to be malleable enough to form around the ring to keep it in place, but stiff enough to eliminate side-to-side movement due to the breathing of the mouse. The current design idea for the gel ring is a non-Newtonian fluid. This will allow easy infiltration of the PDMS lens into the gel when placing the mouse on the stage top riser due to the liquid nature of the fluid when under light equal pressure. Furthermore, the non-Newtonian fluid will act solid under fast unequal pressure changes caused by the mouse's breathing.

Methods

The construction of this design will call for the use of a mill and drop saw. The drop saw will first be used to cut the exact size plate needed to properly sit in the stage top riser. Next, the mill will be used to shave the corners of the plate to a 0.25 inch filet. Continuing to use the mill, a hole will be drilled entirely through the plate with a diameter of 0.6 inches. Next, using the mill again, another hole centered on the previous 0.6 inch hole with a diameter of 0.7 inches will be drilled to a depth of 0.075 inches (the full depth of the PDMS lens). Finally, the gel ring will be adhered to the plate using a water-soluble glue to allow for easy maintenance when required.

Testing

Due to the sensitive nature of the intravital imaging process, our final design will need to be tested with the exact set-up and procedure used by Dr. Ponik to ensure that the stage top platform works as intended. As the busy lab schedule will make it difficult to organize multiple long-term tests throughout the semester, more brainstorming is needed to come up with a way to

test our prototype outside of the lab as it is developed. The results for our testing should accurately show if the image created is clear or not. If the image is clear, then the stage top platform should work as intended.

VI. Results

Currently, no tests have been performed on this design. Multiple imaging sessions will need to be conducted while using this device to get a conclusive set of results. Once testing is completed, statistical analysis will be done to determine the average residual from zero, for the amount of movement in the lens over a period of time. This design will be considered functionally adequate if the average residual is less than five microns with a ninety-five percent confidence interval for that value.

VII. Discussion

Due to the complex nature of intravital imaging, very small amounts of error can cause drastic faults in the quality of image. Should this design average less than five microns of movement, it will generate repeatable results over long periods of time. If the device does not meet that threshold, changes will be made. A possible source of error will be maintaining the device. The gel ring will be a slow degrading material, but improper maintenance will affect results over time.

This device is going to be used in medical research labs with accompanied use of genetically altered lab mice. Because there is animal testing involved, there are some ethical concerns with this device over the abuse and safety of animals. The design process included an

evaluation of safety to the specimens being imaged, to avoid any possible harm or abuse to the animals.

VIII. Conclusions

With the fabrication of our final design, testing will commence after fabrication within the clients lab to ensure the slide is able to stabilize the specimen during observation. When testing is complete for the stabilizing slide, designing a clamping mechanism will be the next focus as the client is currently using a rubber piece fixed onto the platform with tape. With the current observation windows running approximately eight hours, the tape loosens over time and is inefficient at clamping the specimen onto the platform. If time allows, the client expressed desire to have a slide that can hold multiple specimens at once for observation. Overall, the clients priority is having a redesigned stabilizing slide that can be utilized with the current system while the clamping mechanism and multiple specimen slide follow behind in importance.

IX. References

[1] Centers for Disease Control and Prevention. “Cancer Statistics at a Glance.” *CDC.* Available: https://gis.cdc.gov/Cancer/USCS/#/AtAGlance/ [Accessed: 11-Oct-2022]

[2] G. Jacquemin, “Longitudinal high-resolution imaging through a flexible intravital imaging window,” *Science Advances*, Jun-2021. [Online]. Available: https://www-science-org.ezproxy.library.wisc.edu/doi/10.1126/sciadv.abg7663.

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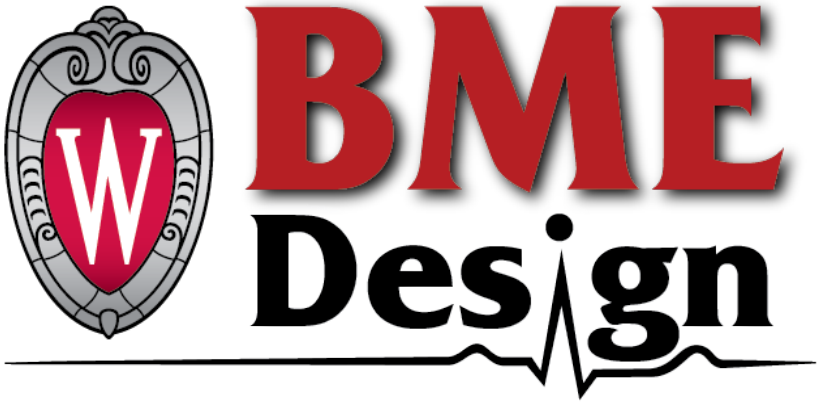
X. Appendix

Appendix A: Project Timeline

Project Goal	**Deadline**	**Team Assigned**	**Progress**	**Completed**
Research Components of Project	Ongoing	All	Ongoing	
Find Competing Designs	Ongoing	All	Ongoing	
Meet Client and Tour Facilities	9/16	All	100%	Completed
Develop Testing Plan	9/23	All	Ongoing	
Product Design Specifications	9/23	All	100%	Completed
Preliminary Presentation	10/7	All	100%	Completed
Preliminary Report	10/12	All	Ongoing	
Preliminary Notebook	10/12	All	Ongoing	
Develop Design Matrix	10/20	All	100%	Completed
Prototyping	10/20-12/1	All	0%	
Testing of Prototypes	10/20-12/1	All	0%	
Poster Presentation	12/8	All	0%	
Final Report	12/14	All	0%	

Final Notebook	12/14	All	0%	

Appendix B: Product Design Specifications



Stage Top Platform for Imaging of Mouse Mammary Tumor

Product Design Specifications

September 23rd, 2022

Lab Section 307

Joel Matthews (Team Leader), Hailey Kanter (Co-BWIG), Abbey Cohen (Co-BWIG), Abbylee Maeder (Communicator), Christy Li (BPAG), Amara Monson (BSAC)

Client: Dr. Suzanne Ponik and Dr. Brian Burkel

Advisor: Dr. Kris Saha

Function:

Dr. Suzanne Ponik and Dr. Brian Burkel have been using intravital imaging to analyze the microenvironment surrounding a tumor in the mammary gland of mice. Using a steel lens only allows for analysis of up to two weeks due to activity of the mice. Using a flexible PDMS lens [1] for intravital imaging has been shown to last in a mouse for up to 8 weeks, which allows for a better understanding of the long term effects in the microenvironment of a tumor in the mammary gland as well as multiple observation points. Creating a stage top platform that keeps a PDMS lens stable throughout imaging will allow researchers to use PDMS imaging lenses more and to gain more information about a tumor microenvironment. Additionally, pressure must be put on the mammary gland of the mouse to observe the tumor. To solve this, a clamp will be added to the platform for constant pressure on the specimen for clear imaging.

Client requirements:

- Create a stage top plate for a flexible PDMS lens for intravital imaging that allows the lens to remain stable for long periods of time.
- Add an additional apparatus to the plate that applies constant pressure on the mammary gland of the mouse to allow for clearer images.
- If there is time and money available, add an additional lens to the stage top platform so multiple mice can be imaged at once.
- The stage top platform must fit into the heating chamber of the microscope.

Project Design Requirements:

1. Physical and Operational Characteristics

a. Performance requirements: The stage top platform must keep the flexible PDMS lens stable throughout the imaging process. This includes an apparatus attached to the platform applying constant pressure on the mammary gland for clear imaging to fully analyze the microenvironment of the tumor. The device must be reusable and work for imaging of multiple mice.

b. *Safety*: The mouse and the platform are in a heating chamber during imaging. Since the platform comes into contact with the mouse's skin and fur, the material cannot be thermally conductive and cannot cause burns to the skin. This also goes for the chemical makeup of the surface of the material and no damage should be done to the skin and fur of the mice.

c. *Accuracy and Reliability*: The device needs to allow for the accurate and precise range of motion of the objective lens. It also needs to maintain the same accuracy and precision when using different objectives. The stage that the platform will rest on moves relative to the fixed lens. The range in the Z-direction is about 9.3mm. The X range is about 91mm and the Y range is about 67mm. These dimensions are limited by the riser dimensions and lens diameter of the microscope.

d. *Life in Service*: The platform needs to be able to lay in the microscope for up to 8 hours without moving at all. It will need to allow the operator the microscope to move the lens without disturbing the platform

e. *Shelf Life*: The stage top platform should be able to stay in use for many years. It will be in a lab at room temperature. The only external factor in the shelf life is the mice that will be laying on the platform. The device needs to be strong enough to hold up a mouse, but that is the only environmental condition.

f. *Operating Environment*: The device will be in a room temperature lab and may need to be placed in storage for long periods of time. It will come in contact with a sedated mouse. There are no changes in pressure or other environmental factors that need to be considered.

g. *Ergonomics*: This device will be designed for optimal use while laying flat on a surface, though it will remain functional while being moved to and from the microscope while being carried in a flat position. It will be mobile, with no restrictions based on height or reach. The device must be strong enough and stable enough to support the weight of a mouse.

h. *Size*: The device will be fit into a riser in order to be secured to the microscope. Our client suggested that we work with existing risers, which creates a size restriction of 2.75 inches by 4.0 inches. Additionally, the current design of the imaging tray and riser system is one inch tall, but has the microscope at the very top of its vertical limits, so it would be advantageous to reduce the height of the tray when in the riser by 2-3 millimeters.

i. *Weight*: As this device will be moved to and from the microscope often, it needs to be light enough to allow for easy removal from and insertion into the system. This is the only weight restriction.

j. *Materials*: Currently there are two stage top platforms in use for imaging. The first is made purely of metal, and the second is 3D printed and made purely of plastic. The materials being used are to be placed in a heating chamber while imaging is occurring. It is critical that the materials chosen for the final design do not overheat while in the heating chamber over an extended period of time. In addition to this, the type of material must be strong enough to support the full weight of a mouse.

k. *Aesthetics, Appearance, and Finish*: There are no requirements regarding aesthetics, appearance, or finish.

2. Production Characteristics

a. *Quantity*: The client is requesting one stage top platform for imaging that is compatible with the new PDMS lens. The client is requesting that the one stage top platform fulfills the need for constant pressure on the mammary gland to allow for clear imaging. If given the time and resources, the client is requesting a second stage top platform with an additional lens allowing two mice to be imaged synchronously.

b. *Target Product Cost*: When asked, our client provided a budget of $1,500. Dave Inman will be the contact for all budget related questions as he is in charge of the budget for this project. Our client suggested using the cheapest materials available when prototyping. Contact: drinman@wisc.edu

3. Miscellaneous

a. *Standards and Specifications*: Due to the intended use of our device being for research involving animals, there is no pre-approval required from the FDA. For the registration of a patent the design will be required to be registered with the FDA. This device classification would fall under the hematology sector of the FDAs classification panel, part 864. [2]

b. *Customer*: The design is to be built for any member of the client's team, along with other microbiologists, that will be working in direct contact with the high-power microscope. The client has not provided any materials, practices, or techniques that are unwanted in the design. The main and only preference the client has is to minimize cost.

c. *Patient-related concerns*: With the design's intended use, the product will not come into contact with any patients. The device will not contain nor store any patient data, all data obtained will be recorded by the researcher. The product should be able to hold the anesthetized mouse without movement for the duration of the examination period. As the device will not be exposed to bodily fluids, sanitization will not be a concern.

d. *Competition*: From the literature, there is one stage top holder that is designed specifically for the PDMS viewing window. It's patented under the number EP3656349A1 by Institut Curie. [1] This project is focused on developing a plate that can be used with the client's current system hence, while existing products are useful, it's not pertinent in this case and cannot be utilized by our client

References:

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