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

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Final Report

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BME 200/300

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Abstract

The metastatic dissemination of breast cancer tumors is one of the leading causes of death in breast cancer patients [1]. Breast cancer cells can disseminate from the primary tumor and spread throughout the body and invade organs separate from the primary tumor such as the bone, liver, lung, and brain [1]. 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 polydimethylsiloxane (PDMS) lens which will allow for long-term observations of the tumor microenvironment [2]. 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, was fabricated. After fabrication, tests were conducted to compare the stability of the old platform to the new platform. Upon the finalization of tests, an analysis of the plate was conducted to determine the success of the prototype for future use along with any additions that need to be made.

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Introduction

Motivation

In 2019, 264,121 women were diagnosed with breast cancer, and 42,280 women died of it [3]. 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

During intravital imaging, the specimen under observation will be anesthetized and placed directly over the objective lens. The stage-top riser must completely stabilize the PDMS lens during the entire duration of the imaging process. In addition, there must be an adjustable clamping mechanism that will shift the body cavity away from the resection site as well as applying direct pressure to the mammary tumor on top of the PDMS lens. With that, there are no commercially available stage-top risers that are able to accommodate the new PDMS lens. This is not surprising as the PDMS lens was only released for commercial use within the last 2 years [2]. The current design that the client is using within her lab was designed by a prior PhD student that has since graduated. This design was designed to align with the outer dimensions of the old metal lens. Within the literature, there are experimental designs utilized for intravital imaging, however, the difference lies within the type of lens used as well as the type of microscope [4]. However, this design will not be able to accommodate the clients needs as the experimental design utilizes a top-down objective microscope while the clients has a microscope where the objective is on the bottom [4]. The focus of this project was on designing a stage-top riser as well

as a clamping mechanism that will stabilize the implanted outer dimensions of the PDMS lens. In addition to designing an adjustable clamping mechanism that is effectively able to isolate the mammary tumor directly above the implantation site of the PDMS lens.

Problem Statement

Due to the complex network of cells in the microenvironment of mouse mammary tumors, a 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 [5]. This is called in vivo because it is the imaging of a live tumor as the tumor is able to continue to develop [6]. 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. The imaging technique used is intravital imaging, or intravital microscopy (IVM), the imaging of live animals through a microscope. The IVM method used by the client is the implantation of an imaging window in the skin of a mouse to allow a view of the living cells underneath [7]. Currently, the client is using a small inflexible, circular imaging window made of metal and glass. The inflexibility of this window 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 PDMS window [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 window. Creating a stage top platform that keeps a PDMS window 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 window, so the imaging can be used to further mammary tumor treatment for human patients.

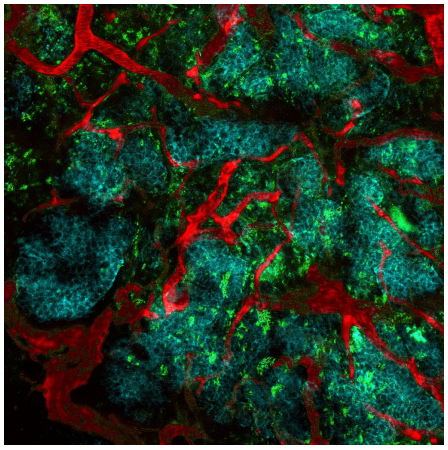


Figure 1: The Tumor Microenvironment. *A collagen dense tumor microenvironment captured by the lab members at Ponik Lab [5].*

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 [8], immune responses to cancer cells [9], and tumor responses to cancer therapies [10], among others [11]. 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 [12], 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].

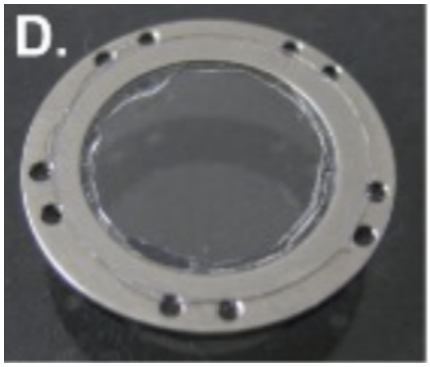


Figure 2: Metal and Glass Imaging Window. *A metal and glass imaging window, similar to the one currently used by our client [2].*

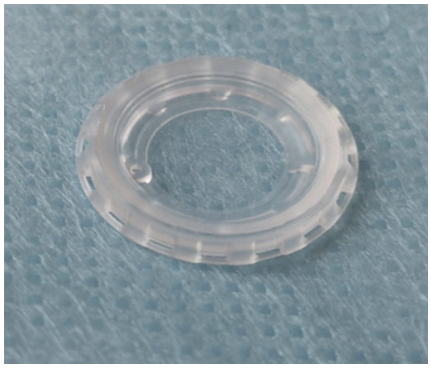


Figure 3: PDMS Imaging Window. *A PDMS imaging window, similar to the one our client wishes to use [2].*

Client Information

Dr. Suzanne Marie Ponik has a Ph.D in cell physiology and biophysics, and Dr. Brian Burkel, a partner in her lab, 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 [5].

Product Design Specifications

The client is requesting one stage top platform for intravital 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, stable image. The final image produced using the stage top platform created must have a maximum drift of 10 microns throughout the entire imaging process. For this aspect to be successful, the stage top platform needs to keep both the flexible PDMS lens and the tumor that is being imaged stable throughout the entire imaging session. To help stabilize the image the

design will contain an additional clamping mechanism connected to the plate that applies constant pressure to the mammary gland while still allowing adequate blood flow to the tumor.

Our client requested that we lower the height of the platform by 2-3 mm because the microscope is currently at a maximum height for focus, so lowering the platform will give the microscope more variability in the Z range. The current movement restrictions of the microscope are currently 91 mm in the X range, 67 mm in the Y range, and 9.3 mm in the Z range.

The platform is placed in a heating chamber, as shown in Figure 4, at 31 degrees Celsius for the entirety of the imaging session which is typically 4 to 8 hours long. Therefore, the materials must be able to withstand being at 31 degrees Celsius for an extended period of time. The materials chosen must be strong enough to hold the weight of the mouse and the clamping mechanism combined. The heating chamber is a small box that will surround the platform and the clamping mechanism. It is crucial that the entire design fits within this heating chamber to keep the mouse at a stable temperature during imaging. Lastly, this device must be reusable and able to stay in use for many years.

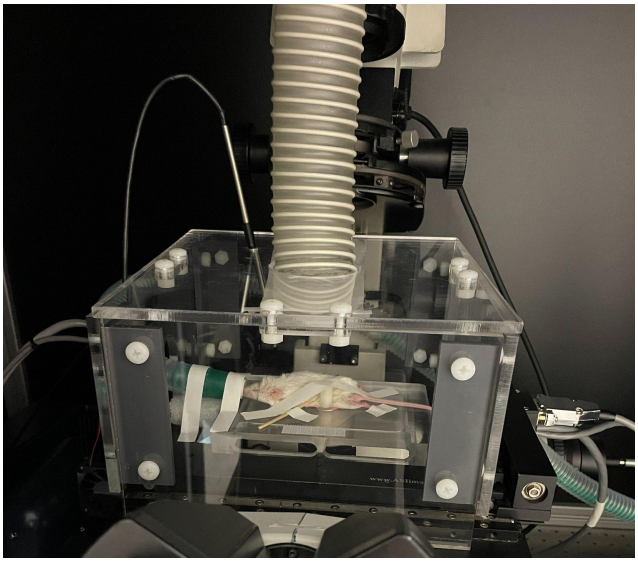


Figure 4: Heating Chamber. *The heating chamber is the small box placed over the platform, there is a thermocouple inserted into the chamber to the left of the white tube that is maintaining a temperature of 31 ℃, and the white tube is bringing heat from the heating device according to readings from the thermocouple.*

Preliminary Designs

Design 1: Extruded Cylinder

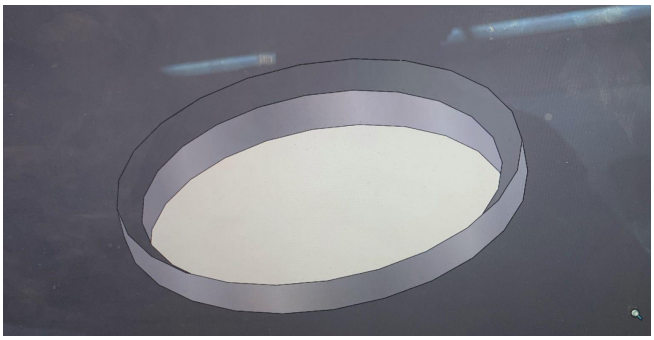


Figure 5: *Top-view of SolidWorks Extruded Cylinder Design.*

The extruded cylinder design will have a piece extruded to the thickness of the PDMS lens which is approximately 0.19 cm. The outer diameter of the cylinder is 1.78 cm and the inner diameter is 1.52 cm to match the contour of the PDMS lens. Furthermore, there is a 0.64 cm 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 0.19 cm there is going to be an inherently sharp edge. When pressure is applied to the mouse and maintained over an imaging session (up to 8 hours long), there is a risk of damage to the mouse's skin.

Design 2: Indented Cut Cylinder

Figure 6: *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

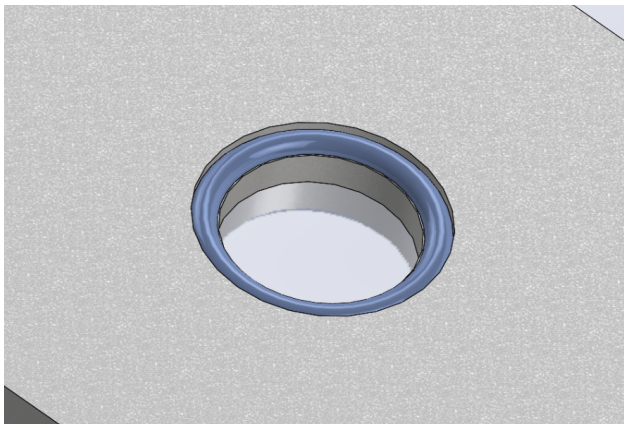


Figure 7: *Top-view of SolidWorks Gel Ring Design.*

The gel ring will be placed in a cut hole with a diameter of 1.78 cm (the outer diameter of the PDMS lens). Along with being the diameter of the lens, the hole will be cut to a depth of 0.19 cm 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.

Preliminary Design Evaluation

Design Matrix

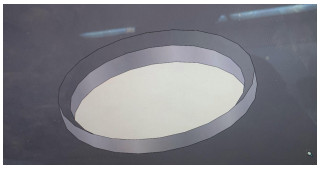
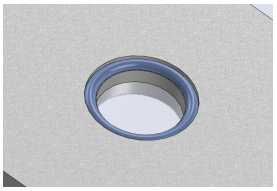
Design Criteria	Extruded Cylinder 		Indented Cut Cylinder 		Gel Ring 	
	Score out of 5	Weighted Score	Score out of 5	Weighted Score	Score out of 5	Weighted Score
Precision (Lack of Movement) (25)	**4/5**	20	**4/5**	20	**5/5**	25
Accuracy (Quality of Image) (25)	**4/5**	20	**4/5**	20	**5/5**	25
Ease of 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 8: Design Matrix. *The teams design matrix which outlines our 3 chosen designs as well as scoring to decide which design to move forward with. The green indicates the best score of the three designs in that category.*

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 mouse'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.

Fabrication/ Development Process

Materials

For the fabrication of the platform, an acrylic piece with dimensions 15.24 x 15.24 x 2.54 cm was obtained from the TeamLab. Acrylic was used because of its low thermal conductivity and the fact that it will not injure or harm the mouse's skin when in the heating chamber[12]. Plastic tables were bought along with a 1.27 cm bolt for the clamping mechanism and rubber shrink wrap. Rubber shrink wrap was again used due to its low thermal conductivity [13]. Velcro was used to attach the clamping mechanism to the stage top platform.

Methods

Stage Top Platform

Using the obtained sheet of acrylic, a mill with an end mill drill piece was used to get the necessary dimensions of 10.9 x 15.75 x 2.15 cm. Turning the piece over to the underside, the mill and end mill was once again used to make multiple passes to mill down .76 cm. This inner rectangle has dimensions of 7.9 x 11.9 cm which is centered on the plastic. An additional .9 cm was milled down with multiple passes for an inner 5.7 x 9.9 cm. The piece was then turned over again to the top side to make the holes for the PDMS lens. A hole with a diameter of 1.52 cm was drilled through the rest of the plastic. Another hole of size 1.78 cm was drilled .17 cm down. This is due to the lens being .17 cm thick so that the lens could sit flush with the top of the platform. The larger radius is the outer radius of the lens (Fig. 9) and the smaller radius is the inner radius of the lens (Fig. 9). An end mill was used to drill the holes because the drills were cutting the plastic too harshly and would crack the plastic. The piece was then turned on its side to have 2 viewing windows of size 5 cm. They were both 1 cm off center. The edges were then rounded using a belt sander.

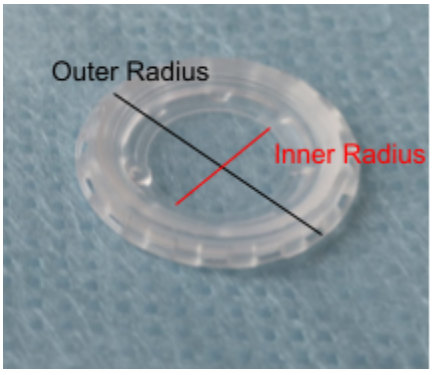


Figure 9: *Labeled diagram of the inner and outer radius of the PDMS window.*

Figure 10*: An image of the underside of the stage top platform that was created using a mill.*

Clamping Mechanism

A 1.27 cm hole was drilled through the plastic table. Velcro was attached to the bottom of the plastic table and the top of the stage top platform. The rubber shrink wrap was then added to the top of the bolt and the bolt was put through the 1.27 cm hole and tightened. The bolt can be tightened at different heights to add different amounts of pressure to the mouse.

Final Prototype

Figure 11*: An image of the final prototype. The clamping mechanism is attached via velcro and then the bolt is put through the hole.*

Testing

Mimic pressure

When a mouse is anesthetized, the breathing changes from short and choppy, to long and deep breaths that will have approximately equal pressures: that pressure can be calculated to less than 0.002 grams/breath (exact measurement: 0.001293 grams/breath) [15]. By slowly increasing weight applied to the lens through the objective hole, our team can test the force required to push the PDMS out of the selected gel. If the force required to remove the PDMS lens is greater than the pressure of a mouse's breath, the design will be functionally adequate.

Platform

A specific microscope and camera is used for intravital imaging. The design needs to fit snugly onto the microscope stand without allowing movement of the riser. To test the fit, the platform will be measured with a level, while the riser is on the platform. The objective will also be moved to apply pressure to the riser, to replicate an imaging session, while using the level.

Imaging

The goal of the team's design is to build a riser that, when used with a PDMS lens, allows for constant and clear imaging of a mammary tumor's extracellular matrix. To test whether or not the goal is accomplished, the team's design will need to undergo an imaging session, or sessions if multiple prototypes are made. The session will be multiple movies of ten minute cycles to get images and the average quality across multiple imaging movies. Those images will be reviewed with Dr. Brian Burkel to determine adequacy and precision of the stage top riser.

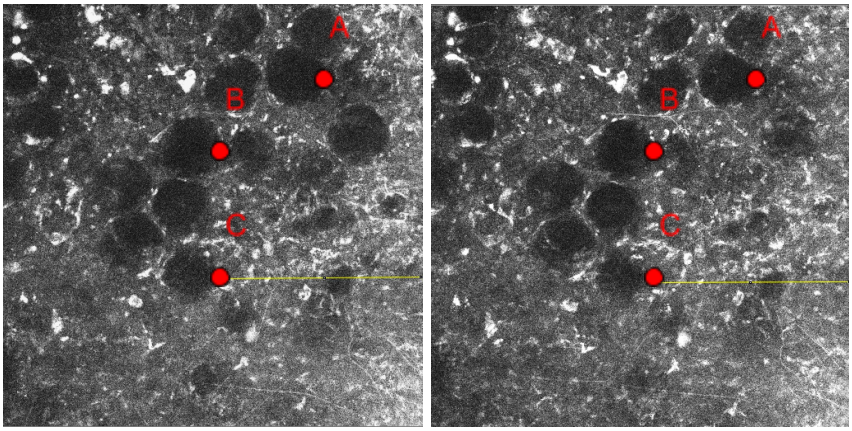
Results

The team was able to test the prototype at three different points during the design process. The first session was to test the fit of the stage top riser in the frame of the microscope stand. Due to the square corners of the team's initial design it did not sit properly in the microscope. After alterations were made to the corners, the second stage of testing to confirm the riser snugly fit, and to get intravital images of a living mouse using the team's design.

Intravital imaging is taking pictures of objects that fit within the width of a hair which is 50 micrometers thick [11]. Very small things experience greater movement when low levels of force are applied. In the context of intravital imaging, the relatively low force produced by each breath of an anesthetized mouse has a massive impact on the movement seen in a 500

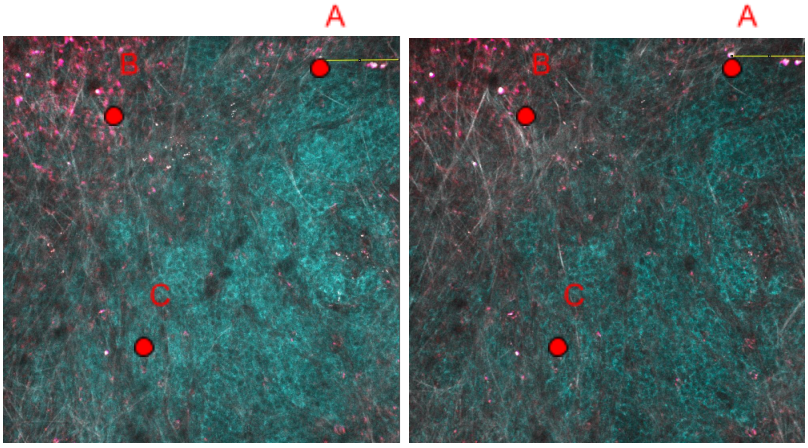
micrometer viewing frame shown by the microscope. The measurement of movement is quantified in one of two types of drift: constant or rebound drift. Constant drift is a steady amount of drift over a consistent period of time, whereas rebound drift involves the viewing frame jumping away and then back to the starting spot in a short burst. The client is able to eliminate constant drift using computer software to shift the microscope over the equivalent distance as the drift occurs.

After loading the team's prototype and anesthetized mouse into the microscope, with the assistance of the client's technical staff, the team successfully eliminated the question of rebound drift occurring. When checking for a focusable image manually on the microscope, the team noticed movement almost invisible to the naked eye; rebound drift is violent and very apparent, as stated by Dr. Brian Burkel during testing.



Figures 12 and 13: *The starting frame of the 10 minute imaging cycle using a wild-type mouse and the team's design is depicted in the left image and the right image is of the final frame of the imaging cycle using the same mouse. Points A, B, and C are labeled on both images and are the reference points used for data analysis, and the raw data measurements are in appendix A. The*

large black circles are adipocytes or fat pads. The long thing white lines are the protein structure of the mouse's extracellular matrix. The bright white circles are the mouse's immune cells.



Figures 14 and 15: *The starting frame of the 10 minute imaging cycle using a genetically altered and cancerous mouse and the current stage top design using a rigid metal lens is depicted in the left image. The right image is of the final frame of the imaging cycle using the same mouse. Points A, B, and C are labeled on both images and are the reference points used for data analysis, and the raw data measurements are in appendix A. The long thing white/gray lines are the protein structure of the mouse's extracellular matrix. The pink dots are the immune cells of the mouse and the cyan dots are the cancerous tumor cells.*

The results are shown in figures 11 and 12, where figure 11 is an image from the first frame of a 10 minute cycle of imaging and figure 12 is the final frame of that same movie captured. Those pictures were chosen to analyze the drift that occured over the course of ten minutes. Using ImageJ, the team determined the amount of drift by taking three reference points (labeled on both figure 11 and 12) and measuring the distance from the right edge of the viewing frame. The values were then averaged to get a single value. The measurements are shown in

appendix A and were compared to the measurements obtained, using the same methods, from a standard cycle of imaging the client captures using their current stage top riser design and rigid metal lens. The imaging compared against the team's results are shown in figure 13 and 14 (reference points are labeled on the figures). Using the current methods and rigid metal lens, the client's movies average consistent drift of 4.32 micrometers over a ten minute period whereas the movie obtained using the team's new design experienced consistent drift of 6.81 micrometers over a ten minute period. A t-test was conducted to determine if there was a significant difference between the measurements of drift obtained from both movies. The null hypothesis was no significant difference, and the alternate hypothesis was that the values were significantly different. The t-test resulted in a p-value of 2.102e-5 which is less than 0.05, meaning the team accepted the alternate hypothesis that the two different designs for imaging result in significantly different amounts of drift over ten minutes of imaging.

Differences in the color and aesthetics of the movies shown in figures 11-14 are not a reference to image quality. The differences stem from what is being imaged in the movies. The mouse used for testing the image quality of the team's design was a wild type mouse whereas the mouse used for the client's imaging was genetically altered and cancerous. Dr. Brian Burkel described the breakdown of the images and what each color represented. Figures 11 and 12 depict the wild-type mouse and have three components of color where the black circles are adipocytes or fat pads, the white straight lines are protein structure making up the extracellular matrix (ECM) of the mouse's mammary gland, and the white dots are immune cells. In figures 13 and 14 the mouse is genetically altered and cancerous. The cancerous mouse depicts three different components of color: protein structure of the ECM in white/gray, immune cells in pink, and tumor cells in cyan.

Discussion

Implication of Results

The results of the first imaging session show that the final design, in use with the clamping mechanism, was able to successfully mitigate rebound drift. Through ImageJ we determined that the constant drift was around 8.62 micrometers. These results were comparable to the client's old design with the use of a metal lens. With the elimination of rebound drift, the client is able to factor in the constant drift of the imaging process and adjust the imaging program accordingly.

Ethical Considerations

With the finalization of the product, the ethical considerations include the comfort of the animal during imaging sessions. Any surface in contact with the mouse must be deburred in order to ensure the safety of the mouse in addition to the stability of the PDMS lens. Deburring any edges in contact with the mouse eliminates the possibility of hemorrhaging under the PDMS lens. Failure to adhere to these standards would result in additional duress on the mouse and ultimately lead to the death of the subject.

Evaluation of Testing

During the evaluation and testing sessions of the final design in addition to the clamping mechanism, the team noted modifications to improve future imaging sessions.

The main goal of the stage-top riser was to adhere to the outer dimensions of the PDMS lens. Although the team accounted for overage during the designing process, there was additional growth around the resection site where the PDMS lens was implanted. This phenomenon was

observed during the second imaging session where our client noticed inflammation of the surrounding tissue on the resection site. With the additional tissue growth, the PDMS lens was unable to sit within the stage-top riser. Specifically our client noted that due to the tissue growth surrounding the lens, manipulating the lens into the riser was unsuccessful. With the skewed placement of the lens, there was a diagonal break across the imaging window in which images were blurry or unfocused. Increasing the outer diameter of the stage-top window would account for additional tissue and allow the team a more accurate depiction on the stability of the stage-top riser. In addition, additional statistical analyses must be conducted in order for the team to have a standardized drift to report to the client.

The clamping mechanism was designed with the goal of allowing our client to manually adjust directly over the mammary tumor. The clamping device achieved this goal,however, the client noticed hemorrhaging under the lens, and although the reasons for the hemorrhage are unclear, the team should take additional precautions on the amount of pressure exerted by the clamping mechanism. Additional modifications to this device would be to increase the surface area of the clamping mechanism which would ideally push the body cavity away and isolate the mammary tumor. New testing plans should also include pressure testing to determine the exact pressure being exerted during imaging sessions.

Conclusions

The goal of this project was to design and develop a stage-top riser that was able to adhere to the outer dimension of the PDMS lens as well as an adjustable clamping mechanism able to hold the mammary tumor over the PDMS window during imaging sessions. This project

will remain of constant relevance in the future as there are no commercially available stage-top risers that are designed for the PDMS lens.

The client has suggested further improvements to the final design to simplify the process and improve the output of each imaging session. The platform currently used by the client with metal imaging windows consists of a metal frame and interchangeable plates. Developing the final design into a plate and frame system matching the dimensions of the existing system would allow the client to use both a metal window and a PDMS window as needed and easily swap between them for imaging. Additionally, a lighting system could be added to the bottom side of the design to assist in the fine tuning of the microscope before imaging.To prevent outside light from interfering with the quality of the images, the microscope is set up in a dark box, and a temporary light is needed to focus the microscope. The current method of pointing a handheld flashlight at the objective is cumbersome and a built-in lighting system would streamline the process. During imaging, a mouse should be kept at approximately 37 ℃ [16]. As seen in Figure 16, the client currently uses a climate controlled box around the mouse and platform. Though this has been a functional mechanism, a heating element in the platform would allow for more precise control over the physiological conditions of the mouse during imaging. As the mouse is put under anesthesia during the imaging session, monitoring vital signs over the course of imaging is key to timely intervention. The client has suggested both an updated anesthesia delivery system and a system to easily monitor vital signs be added to the design. Finally, additional testing should be done to determine the correct amount of pressure to be applied to the tumor using the stabilizing mechanism. The pressure should stabilize the tumor against the imaging window, while still allowing blood flow for tumor growth.

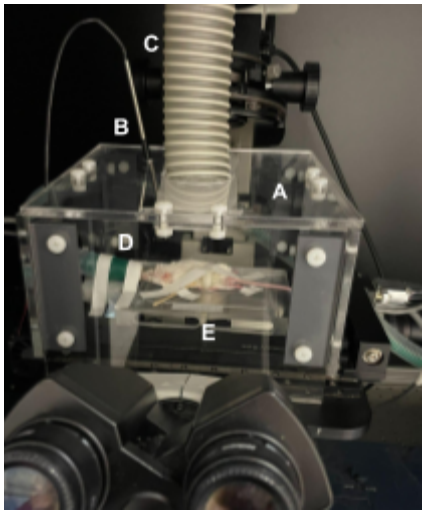


Figure 16: *The current imaging setup. [A] The heating chamber. [B] A thermocouple, maintaining a constant temperature in the chamber. [C] The white tube is bringing hot air to the chamber from a separate heating device. [D] The facemask supplying anesthesia. [E] The final platform design, without stabilizing device.*

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Appendix

Appendix A: Measurements for Data Analysis

Average Drift of Viewing Frame		
	PDMS Lens Stage	Metal Lens Stage
	6.92 um	4.45 um
	6.96 um	4.26 um
	6.82 um	4.25 um
Average	6.81 um	4.32 um

Appendix B: Expense Report

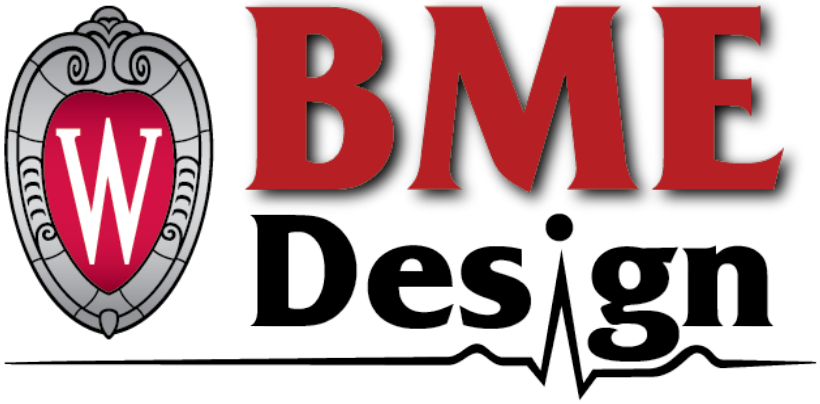
Materials	**Quantity**	**Place Purchased**	**Cost**
Acrylic Sheet 6x6x1	1	Team Lab	$10.35
Acrylic Table	2	Amazon: https://www.amazon.com/dp/B09NLW55ZN/ref=cm_sw_r_api_i_44G8NKGN58XYB3H0WQXV_0	$22.15
Bolts, Nuts, Velcro		Home Depot	$6.50
Shrink-wrap Tubing, Wing Nuts, Bolts		Home Depot	$6.17
Superglue, Shrink-wrap Tubing, Nuts, Rubber Pads		Home Depot	$18.67
Total			**$63.84**

Appendix C: Project Timeline

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

Final Notebook	12/14	All	100%	Completed

Appendix D: 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

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