Rodent Rotation and Translation Stage

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Abstract

Millions of Americans suffer from blinding diseases such as macular degeneration and glaucoma. In an effort to treat such diseases, extensive research is conducted, often involving the imaging of retinal tissue. In the case of Dr. Rogers' research, this imaging leads to conclusions relevant to conditions in the human eye. The process of imaging the retinas of rodent eyes involves precision alignment of the specimen eye within the field of view of the imaging device and requires the ability to adjust the angular position of the eye to image across the spherical retina. Current products used to hold and position the imaged specimen provide rotational and translational degrees of freedom, but fail to provide alignment of the specimen within the rotating elements. This makes adjustment throughout the imaging process cumbersome due to the fact that the eye changes translational position as it is rotated because it is not aligned at the intersection of the rotational axes. Therefore, the aim of this design project is to develop a device providing pitch and yaw rotational freedom as well as a mechanism for the alignment of the pupil of the specimen at the intersection of these rotational axes. Testing was conducted to find that the specimen eye can be easily aligned, yet it somewhat deviates in position during rotation and the stage bends under load. Therefore, improvements can be made but this device takes significant strides towards facilitating the ocular imaging process that will streamline related research.

Table of Contents

I. Introduction

Motivation

The team's goal is to create a device that will aid the client in the imaging of photoreceptors in the eye of a rodent. Rodents are commonly used as imaging subjects because of their frequent reproduction, genetic purity, and biological similarity to humans [1]. Ocular imaging of rodents is used to study glaucoma, macular degeneration, cell replacement therapy and gene therapy in humans. Glaucoma is the leading cause of blindness in people over the age of 60 [2]. Macular degeneration is the leading cause of vision loss affecting more than 10 million Americans [3]. By studying these diseases, researchers in the ocular imaging field hope to better understand the cause of these diseases and develop treatments for humans. In the client's research, the device that currently supports the rodents requires him to adjust the rodent frequently to image the entire eye. The goal is to create a design that provides 5 degrees of freedom, 3 translational (x, y, z) and 2 rotational (pitch and yaw), that will allow the client to image the entire eye without the need for repositioning. To do this, the eye is placed and held at the center of rotation of the device.

Existing Devices & Current Method

Bioptigen RAS: Device features an alignment stage with two concentric tubes for holding the specimen. It has two degrees of rotation, roll and yaw in the tube. Roll is achieved by rolling the inner tube and yaw is achieved by rotating the tubes side to side. There is one degree of translation within the rotational axes which is the inner tube sliding forwards and backwards. Outside of the rotational axes there are 3 degrees of translational freedom by the whole stage sliding in all directions. This solution was expensive and is no longer on the market (Figure 1).

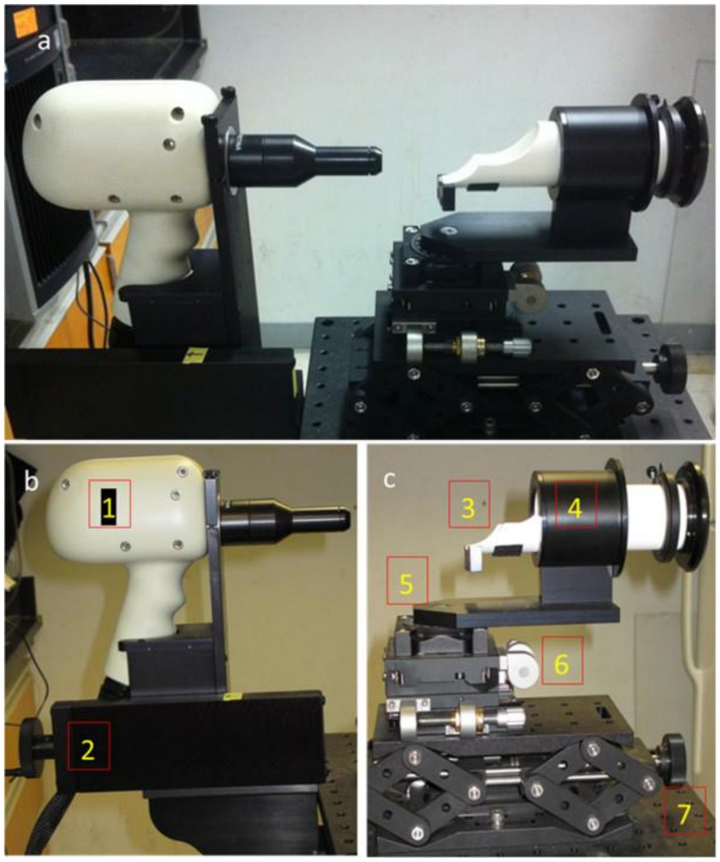


Figure 1: Bioptigen's rodent alignment system (RAS) composed of concentric cylinders presenting pitch and a pivoting joint providing yaw; no element allows for centering of subject pupil [4]

Rodent Rotation and Translation Stage: Past design team utilized a 3D printed solution to hold a specimen for imaging of the eye. Device provided 5 degrees of freedom and was built on a gear system for rotation. Device provided an adequate solution to the problem of a device needing 5 degrees of freedom, but failed to keep an eye at the center of rotational axes which is needed to image the eye continuously (Figure 2).

Figure 2: Past UW Madison BME design team's solution; presents 3 degrees of rotational freedom but limited internal translational freedom; extensive gearing prevents facile sterilizability; prototype 3D printed [5]

Problem Statement

Research of mammalian retinal photoreceptors, conducted via the imaging of rodent model organisms, requires precise alignment of the specimen. A device providing facile alignment of rodent eyes within the imaging system's field of view as well as rotational freedom for accessibility to a holistic view is called for. This device must provide at least 2 rotational degrees of freedom, pitch and yaw, as well as 3 translational degrees of freedom in the x, y and z directions for the positioning of the eye at the intersection of the rotational axes.

II. Background

Biology & Physiology

According to the client, the rodents that will be used most often in the lab will be a rat. Rats typically have a mass between 250-500 grams with a length of 17-21 centimeters [6]. The current way the client's stage positions the rodent does not allow for rotational and translational freedom with the eye at the center of rotation. The main priority of the design is the rotational freedom of yaw (side-to-side) and pitch (up-and-down) of the eye while keeping the eye of the rodent at the center of rotation of the device.

The client's lab performs research on the retina of rodents. The retina is home to photoreceptors which are responsible for converting light into neural signals, and sending those neural signals to the brain. The central portion of the retina is called the macula, and is responsible for focusing central vision. Macular degeneration is caused by deterioration of the central portion of the eye, and is currently an incurable disease [3]. There are two types of macular degeneration, dry (85-90%) and wet (10-15%) [3]. Dry is caused by the thinning of the retina, leading to growth of tiny clumps of protein. Wet is caused by new abnormal blood vessels growing under the retina [3]. Specific factors causing macular degeneration are not conclusively known. Research is limited by insufficient funding, but some causes are thought to be both hereditary and due to the environment [3].

Glaucoma is a disease due to the optic nerve. Fluid builds up in the front of the eye causing an increase in pressure that can damage the nerve. There are two major types of glaucoma, primary open-angle and angle-closure [2]. Primary open-angle is the most common type of glaucoma. It happens gradually when the eye does not effectively drain fluid [2]. This is painless and causes no vision change at first. Some people have optic nerves that are sensitive to normal eye pressure, which is why it is important to regularly get examined for nerve damage. Angle-closure glaucoma is when a person's iris is very close to the drainage angle of the eye and the iris ends up blocking the drainage [2]. The eye pressure increases very quickly, which is called an acute attack. Some people develop this type of glaucoma slowly and it can result in severe eye pain. This can cause blindness if not immediately treated [2].

Research Required to Design Prototype

To create the prototype and design, research needed to be done on ways to translate and rotate something while keeping the object in the center of the axes. The best way to accomplish the rotation is through friction. Friction allows for something to be translated without the use of gears and other similar methods. Friction also takes away the need for lubricants. A large frictional force will also allow for precise positioning. First, for friction to occur, two surfaces must be in contact. Using the equation $F = \mu N$, it is apparent that in order to increase friction, normal force (N) and/or coefficient of friction (μ) must be increased [7]. These requirements mean surfaces must be fitted together precisely, are pushed together by some force equal and opposite to the normal force and are rough enough to produce significant friction. Normal force can be obtained through screws or anything with threads, gravity and elasticity of material. The largest concern with static friction which is the frictional force between the surfaces of two substances when they are not in motion with respect to each other [7]. The static friction should be enough to keep the device motionless and in the correct position after rotation. One negative effect of using friction as a driving force in rotation is that it requires materials that are heavily wear resistant which would require more expensive materials to be used in assembly [8].

To accomplish the translational requirements, the best options in the x, y, and z components involve a linear translation stage design. Linear stages allow for sliding back in forth in one degree of freedom while constraining the other 5 degrees of freedom in translation and rotation. The linear stage can be translated by using friction, roller bearings, air bearings, belts and pulleys, or by wheels [9]. The translation of a linear stage along the length of a translational rod would create a bending moment about the rod. This bending moment would cause the rods to deflect downward under any force applied to the stage [10]. The linear stage requires a feedback system to be precise and find the exact location desired. The client has an existing feedback system that could be incorporated into the linear translation stage.

Client Information

The clients are Prof. Jeremy Rogers and Dr. Ben Sadjak from the University of Wisconsin-Madison. They currently perform research and imaging on rodents in the Wisconsin Institute of Medical Research and require a new stage for the rodents. Their research involves the imaging of the retina, which focuses on quantification of lipofuscin autofluorescence spectral changes in age-related macular degeneration, imaging metabolic activity in differentiating retinal stem cells in vitro, optical metrology of scattering properties of tissue, and Mueller Matrix Enhanced Backscattering Spectroscopy for detecting polarization dependent scattering properties in glaucoma [11]. Dr. Rogers conducts his imaging research on the eyes of rodents to gain insight about the eye and the retina in order to provide information that could lead to the treatment of diseases alike age-related macular degeneration and glaucoma.

Design Specifications

The purpose of this stage is to employ five degrees of freedom, 3 translational and 2 rotational, while still keeping the rodent's eye in the intersection of the axes. There should be 100 microns of precision in the translational axis and 2° of precision in the rotational axis. The device should be able to support up to a 500g organism and be less than 10kg to allow for easy transport around the lab. The stage as a whole should last for 5 years in normal lab conditions while the replaceable sample holders should have a life of service for at least 1 year. The device should be sterilizable and securely hold an anesthetized rodent. There is a flexible budget of $350 for this design project. *See Appendix A for complete list of product design specifications.*

III. Preliminary Designs

Design 1: Bowls

The Bowls design, shown in Figure 3, provides six degrees of freedom, including three degrees of rotational freedom and three degrees of internal translational freedom. Pitch and roll are achieved via the concentric, partial spheres ("bowls") that slide/pivot across one another. The adjustment dials corresponding to each of these rotational axes are friction based such that the rotational position of the specimen may be adjusted but will hold position afterward. Yaw is available via the pivoting disk that the entire design sits upon. This disk component, again, operates based upon friction. The three degrees of internal translational freedom are achieved via perpendicular tracks along which the rectangular stage within the rotational components may be shifted. This track system is also friction based, with a thumb screw at the base of the vertical rail for extra support of the weight of the specimen. The translating stage is fitted with threaded holes providing extensive flexibility for attachment of a variety of sample or subject holders.

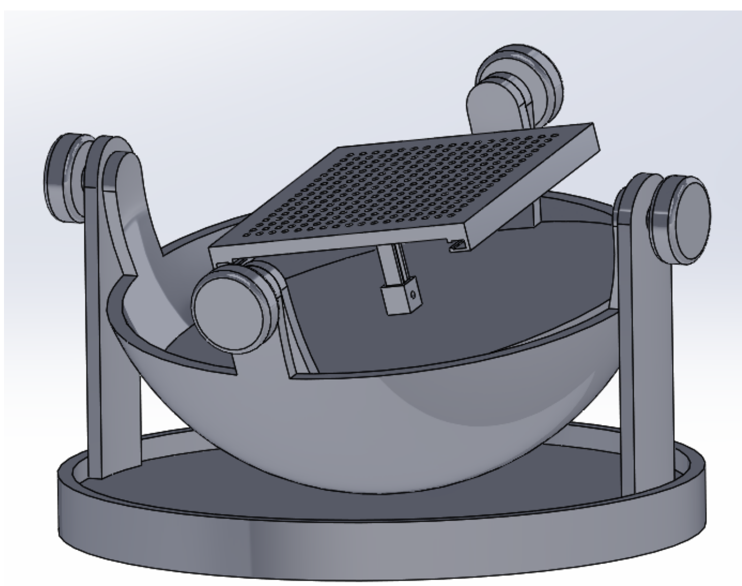


Figure 3: Bowls alternative design; utilizes concentric hemispheres to provide pitch and roll, base spins to provide roll; internal translation provided by stage travelling along rails; all movements are friction based

Design 2: The Pizza

The Pizza design, depicted in Figure 4, provides only the five required degrees of freedom - three of internal translation and two of rotation - which eliminates some of the complexity in adjustment that comes with the Bowls design. This design builds on the ideas implemented by Bioptigen's RAS design, specifically relating to the rotational adjustment methods [12]. Yaw is available via a pivot across the triangular base while pitch is manipulated through turning of the large cylinder. The Pizza design improves upon the precision of the Bowls design by including dials for the adjustment of the specimen stage along the three internal degrees of freedom. The translating stage is fitted with threaded holes providing extensive flexibility for attachment of a variety of sample or subject holders. The dials will turn threaded rods to manipulate the position of this stage along the x, y, and z axes such that the pupil of the imaged specimen may be accurately positioned at the intersection of the perpendicular rotational axes. Also of note, unlike the bowls design, this intersection is positioned towards the front of the design, providing more room for the body of potential rodent specimens.

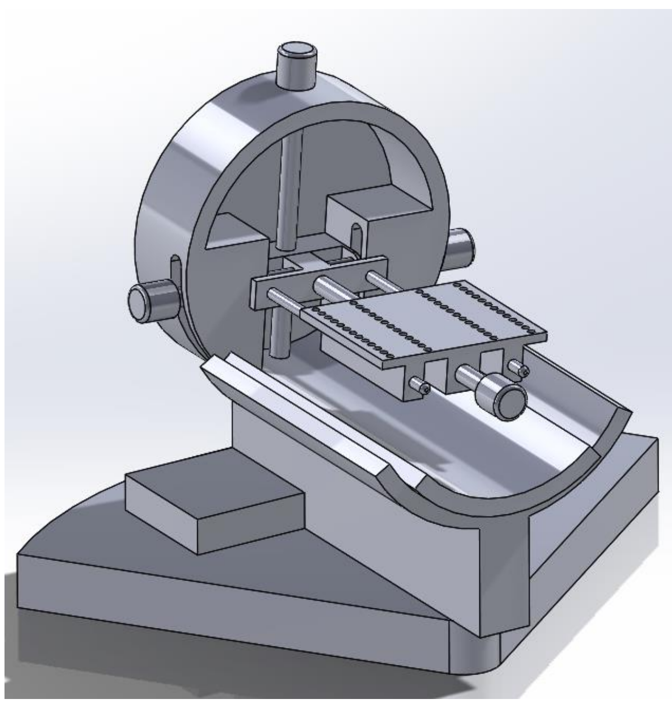


Figure 4: Pizza alternative design; features 5 degrees of freedom: pitch available via turning of the concentric cylinder while yaw via pivoting across triangular base; three degrees of internal translation due to travelling along threaded rods that are turned by dials

Design 3: The Field Goal

The Field Goal design, depicted in Figure 5, focuses on simplicity and cost efficiency. The base of the design holds the rest of the structure upright with an adjustable, telescoping post. This vertical adjustment along with the horizontal freedom gained from placing this design on a flat surface in front of the microscope, guarantees that the lens of the imaging device can be pointed directly at the center of the two axes of rotation. Above the base, there are two rotating arms which can be adjusted then tightened in place with thumb screws. These arms provide two orthogonal axes of rotation: pitch and yaw. Fixed on the inner of the two rotating arms is a structure that allows for a stage to be translated in three dimensions. Translation is achieved via a telescoping post and threaded knob system. While this knob system is more complicated than simply using more telescoping posts, it provides the user with additional ease of use and potentially finer adjustments. Lastly, the stage itself is covered in threaded holes allowing the user to fix a specimen holder in various positions prior to further adjustment.

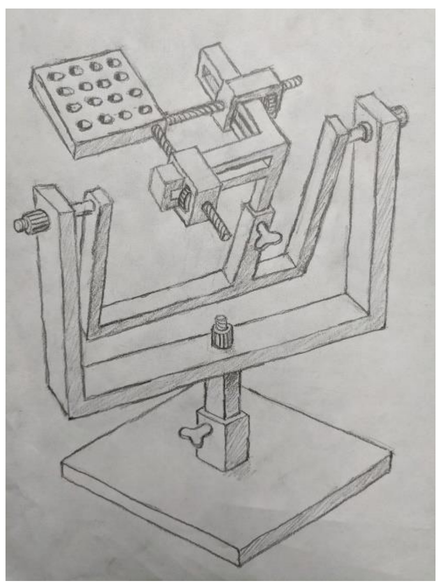


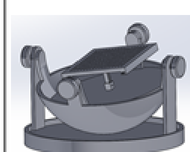
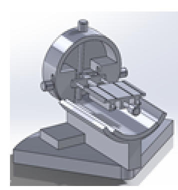
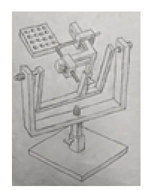
Figure 5: Field Goal alternative design; allows for pitch adjustment via swinging mechanism and yaw as the design spins; three degrees of internal translation via turning dials with threaded rods and vertical adjustments that move stage and whole design up and down

IV. Preliminary Design Evaluation

Criteria Weight

Table 1 presents the design matrix used to evaluate the three preliminary design alternatives. Each design was scored according to weighted criteria to determine an overall score out of 100. The most important criteria included, with a weight of 20, is the ease of adjustment. This is a key feature of the design due to the fact that the motivation behind the project is a need for a means to easily position and adjust the position of ocular specimens being imaged. The translational and rotational components of the stage must be easily manipulated by the researcher for the design to be effective. Other categories of high importance include rotational and translational freedom, with weights of 18 and 15, respectively. The stage to hold the specimen must be easily yet precisely rotated to quickly visualize a wide range of the specimen's retina. Additionally, the stage must be translated within the rotational elements such that the pupil of the imaged eye can be accurately positioned at the intersection of the rotational axes for a variety of specimens. Ease of fabrication and sterilizability received the next highest weight, at 12, due to the importance of developing a prototype to test and the client's wish for smooth surfaces that can easily be cleaned between imaging trials. Finally, the remaining criteria, including strength, safety, simplicity, and cost were considered during the evaluation of designs but held the least weight due to the lack of emphasis put on them by the client.

Table 1: Design matrix evaluating the Bowls, Pizza, Field Goal designs according to weighted criteria; each design scored on a scale from 0-5 for each criterion; total score based on weighted criteria scores and out of 100; Pizza design determined to be the most effective according to evaluation criteria

Criteria	Design 1: Bowls 	Design 2: Pizza 	Design 3: Field Goal 
Ease of Adjustment (20)	3	5	3
Rotational Freedom (18)	5	4	3
Translational Freedom (15)	3	4	2
Ease of Fabrication (12)	3	2	5
Sterilizability (12)	4	3	3
Strength (8)	2	4	2
Safety (5)	4	5	4
Simplicity (5)	3	2	5
Cost (5)	3	1	5
Total (100)	69	72.8	65.2

Evaluation Scores

Ease of Adjustment –

The most important characteristic when evaluating potential designs is the ease of adjustment that the stage design provides. For this reason, this criterion is given the highest weight. The purpose of this design project is to develop a product that provides accessible and adjustable alignment of specimens being imaged. In order for a product like this to be useful, it must be easy to use. Primarily, it must be easy to adjust the position of the rat within the rotational components, such that the eye to be imaged is directly at the intersection of the orthogonal rotational axes. This was a key concern of the client. Furthermore, it is clear that this design needs to be easily adjusted during the imaging process in order to access multiple angles and a large area of the retina. Due to its facile, dial-based translational adjustment and biaxial rotational freedom, the Pizza design excelled in this area, receiving a 5. This is compared to the Bowls design, adjusted manually against friction with an unnecessary rotational degree of freedom, and the Field Goal, requiring adjustment and tightening of several components for adjustment/fixation, both receiving a score of 3.

Rotational Freedom -

The second most important criteria for evaluation is how freely the device can rotate the eye. The eye must be able to rotate so the imaging device can image different parts of the retina on the rodent's eye. For accurate imaging, the eye must remain in the center of the two axes when rotation occurs. The two axes of rotation must intersect at the center and be perpendicular. The device should provide a wide range of degrees of rotation. Because the Bowls design presents three degrees of rotational freedom, it scored the highest with a 5. The Pizza design outscored the Field Goal (3) with a 4 based on the rotational precision that can be achieved through the friction-based design.

Translational Freedom -

Translational Freedom is important in order to locate the rodent's eye in the center of both rotation axes. It is very important that the user is able to achieve this in order to obtain accurate rotations. This translation needs to be adjustable but does not need to achieve as large a range of motion that rotation does. The Pizza design, again, bested the other design alternatives in this criterion due to the potential for a high degree of precision with the threaded internal translation components, scoring a 4. This design does not receive a perfect score due to the fact that improvements may still be made to help locate the intersection of the rotational axes and align the specimen at that point. On the other hand, Bowls scored a 3 because the translating stage can be positioned at many points around the center of the concentric spheres, while the Field Goal's translating stage is slightly offset from the rotating elements, scoring a 2.

Ease of Fabrication -

Ease of fabrication is important due to the semester long time constraint. Fabrication must be easily completed to allow time to tweak different aspects of the design according to the needs of the client. The Field Goal design scored the highest in this category due to its simplistic design and lack of complex geometry, with a 5. Due to its several moving mechanical components and cylindrical structure, the Pizza design scored the lowest with a 2 while Bowls received a 3.

Sterilizability -

Sterilizability is important because the easier the stage is to clean, the less time the client would need to spend on cleaning rather than doing the study. The stage needs to be able to be wiped down to prevent the spread of pathogens and other health hazards that a specimen could carry. The device does not need to be autoclavable, but would provide facile cleaning of the device. The easier that a design is to clean, the less likely the lab would need to purchase a new product overtime as well. Although not completely lacking of grooves or spaces, the Bowls design is largely composed of smooth surfaces that would be easily sterilized, earning it a score of 4. Due to their comparatively increased geometry complexity, the Pizza and the Field Goal were given scores of 3 for sterilizability.

Strength -

The strength of the stage is important because the stronger the design is, the more freedom researchers will be provided in imaging specimens of various weights and sizes. Furthermore, the more durable the product is, the longer it will maintain its integrity and be of use in a laboratory environment. Even though it is difficult to extrapolate strength performance at this stage, it seems that the base and structure of the Pizza design would lend to sturdy operation, even when holding large specimens. For this reason, the Pizza received a 4 for strength, while the seemingly less-supported Field Goal and Bowls each received a 2.

Safety -

Safety is not a primary concern for evaluation of potential designs, specifically because the main risk lies with the rodent specimens being imaged, rather than the humans simply adjusting the imaging stage. This is why the safety criteria has a weight of 5. It remains a concern, nonetheless, as consideration must be taken to ensure that the rats being imaged are not harmed in the process and are properly anesthetized. Furthermore, no components of this design must pose a risk of causing pinching or pain to researchers operating the product. Due to the combination of the sturdiness of the specimen stage and the ease of rotational adjustment, the Pizza design was deemed the safest for the living imaging subjects with a rating of 5. The other two designs both received a 4 in this area.

Simplicity -

Simplicity is not a significant concern for us, although the simpler the device is, the easier it will be to use during the imaging of the eyes. The device should be simple enough for the client to use and understand fully in order to achieve necessary images of the rodent's eyes. Specifically designed for simplicity, the Field Goal design exceeds in this category with a score of 5. On another hand, the Bowls design was considered to be the next simplest with a score of 3 due to its simplicity of the friction-based adjustment of rotation and translation. As it includes several dials that manipulate threaded components to translate the internal stage, the Pizza design received the lowest simplicity score at 2.

Cost -

The budget for this design is currently $350. A cheaper design would be preferred. However, the budget is flexible since the primary concern for the design is functionality. Due to its simplicity, it seems the Field Goal design will be the most cost effective, so it was awarded a score of 5 for this criterion. Beyond this, it seems that the threaded components required for the Pizza design will increase its price of production, so it was given a score of 1 for cost, while Bowls received a 3.

Proposed Final Design

Based on its high evaluation performance, specifically in the areas of adjustment and translational freedom, the Pizza rotation and translation stage design has been deemed most optimal. Therefore, this is the proposed design with which to move forward. Notably, this design implements pitch and yaw for the adjustment of the angular view of the specimen retina as well as three degrees of precisely-adjusted translational freedom of the internal stage. This stage will be movable via turning dials that manipulate threaded rods to move the position of the stage such that the center of the specimen pupil can be aligned. On the same note, moving forward, this design will be optimized for features that facilitate the pupil alignment at the rotational axes intersection by specifically locating this intersection. Furthermore, the selected Pizza design will ultimately be integrated with a cart and a height adjustment component to provide the crude, external degrees of translational freedom.

V. Fabrication/Development Process

Materials

The materials used in the design must be able to withstand direct imaging light and contact with any chemicals used to sterilize and anesthetize the specimen. Based on the use of friction to manipulate and preserve the rotation and translation of the stage, sufficient frictional force must be attained.

The base is fabricated out of acrylic and the carrier out of high-density polyethylene (HDPE). The large flat surfaces of these pieces provide a stable base, a smooth yaw rotation and create enough friction to stay in place upon release. The internal translation rods are made out of precision machined steel rods from Thorlabs. These steel rods create smooth translation, but allows for enough friction to be held in place following alignment. A thumbscrew is tightened up against the vertical rods to add extra friction in order to oppose the force of gravity. The concentric cylinders are composed of an 8” PVC pipe as well as a corresponding 8” coupling. Due to the concentric nature of the PVC cylinders, smooth rotation is permitted with enough friction to hold the stage following pitch rotation. The smooth surfaces of the cylinders additionally allow for facile sterilizability. The supports for the internal translation and custom bushing are composed of aluminum because of its availability, low cost, rigidity and machinability. Additionally, the aligner is 3D printed out of polylactic acid (PLA). This was chosen because of its low cost and because it is easily 3D printed. The aligner contains thin steel telescoping styli for the precise alignment of the eye at the center of rotation. Steel fasteners and threaded rod were chosen because of availability and strength.

Method

Fabrication of the Diamond RRaTS design prototype relied largely on machining equipment including a mill, lathe, drill press, bandsaw and drop saw. All of the plastic components, including the diamond shaped base and carrier components were cut on the bandsaw. To construct the carrier, sections of HDPE and the PVC coupling were fitted together using a combination of superglue and construction screws. Holes were then drilled into the carrier and base and a section of aluminum round stock was cut to act as an axle through those holes. This was to allow for rotation between the two parts. Then, a second hole was drilled into the carrier to line up exactly with the previous hole and the center of the PVC pipe. This was to ensure the rotational axes would line up. This precision was achieved through the use of a measuring tape and string. Next, the internal translation component was fabricated. Aluminum blocks, including the universal stage were cut on the drop saw and were precision milled, drilled and tapped on the mill. Here, precision was extremely vital to ensure the steel rods could be threaded on and line up exactly with other parts. These machined aluminum blocks, steel rods, set screws and thumbscrew were assembled by hand into the final translational component of the design. To attach the translational component to the carrier and allow for proper rotation, an axle needed to be fabricated. The first part of the axle fabricated was an aluminum bushing to protect wear on the plastic carrier. This bushing was made out of round aluminum stock, drilled on a lathe. The bushing was cut exactly to fit over a steel threaded rod allowing for the threaded rod to rotate freely inside the bushing. In order to keep the axle in the carrier, aluminum round stock was cut on the drop saw to make a knob. The threaded rod and bushing were inserted into the carrier and the threaded rod was fixed to the translational component on one side and the knob on the other. In order to ensure the knob and translational component did not come loose during rotation, a nut and star washer were used for each attachment. After measuring the design, the aligner was dimensioned to match the center of the rotational axes. It was 3D printed and the two styli were super glued onto the channels.

Some fabrication did not end up in the final prototype of the Diamond design. The PVC pipe that was intended to act as a concentric cylinder to the PVC coupling was cut on the bandsaw but was never added to the rest of the design.

Final Prototype

The final design prototype that was fabricated was the Diamond RRaTS (Figure 6). This design is the ultimate result of improvements made to the proposed final design, the Pizza Design, following preliminary design analysis. Notable improvements include a removable aligning device that houses extending probes meeting at the intersection of the two axes of rotation such that the eye of the imaged specimen can be aligned along the internal translation to the center of rotation as well as minimization of the concentric cylinder to promote access to the specimen and the adjustable components. Additional changes from the Pizza Design were made to accommodate the stock materials available for fabrication (Figure 6).

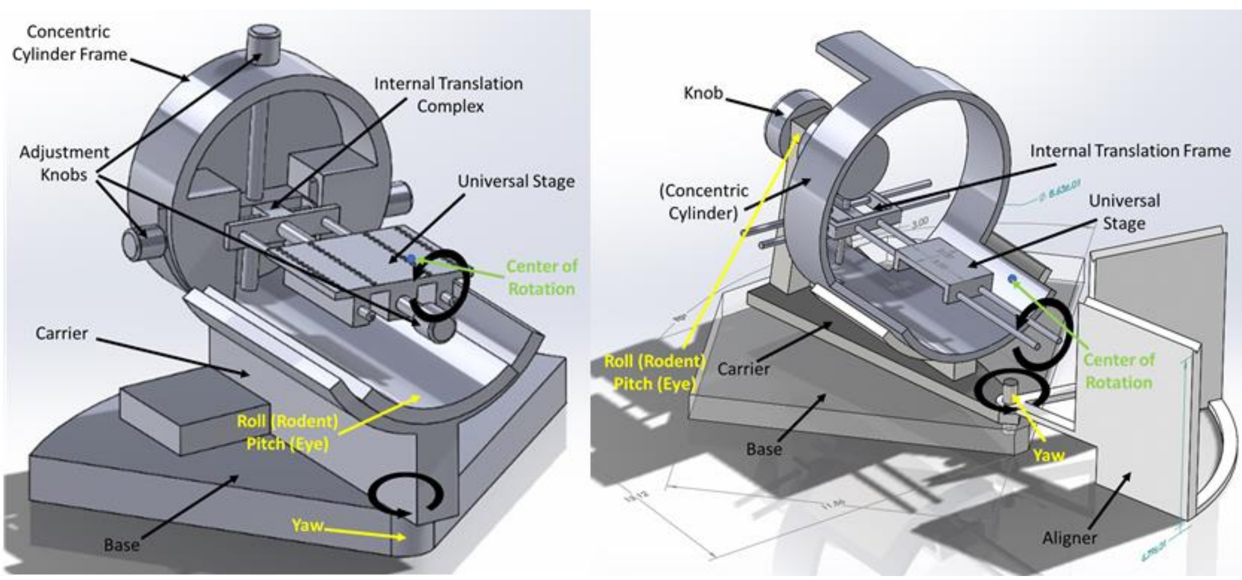


Figure 6: Dimensioned SolidWorks drawing of final prototype: Diamond RRaTS (right); design based on the selected Pizza Design (left); features 5 degrees of freedom: pitch of eye (roll of rodent) available via turning of the knob while yaw via pivoting carrier across base; three degrees of internal translation due to travelling along threaded rods of internal translation frame, held by friction; aligner allows user to position eye (via internal translation) at the intersection of two rotational axes

This Diamond RRaTS design, as fabricated, is presented in Figure 7. One notable difference between the physical prototype and the design drawing is the lack of the concentric cylinder component. The inclusion of this part is intended to add stability of the rodent roll rotation via frictional contact with the partial-cylinder of the aligner. However, due to the fact that the larger of the cylinders was fabricated from a PVC coupling, implementation of the internal concentric cylinder put strain on the internal translation rods, so it was not included. Friction between the knob and the back of the carrier provides sufficient friction to hold the universal stage in place following rotation (Figure 6).

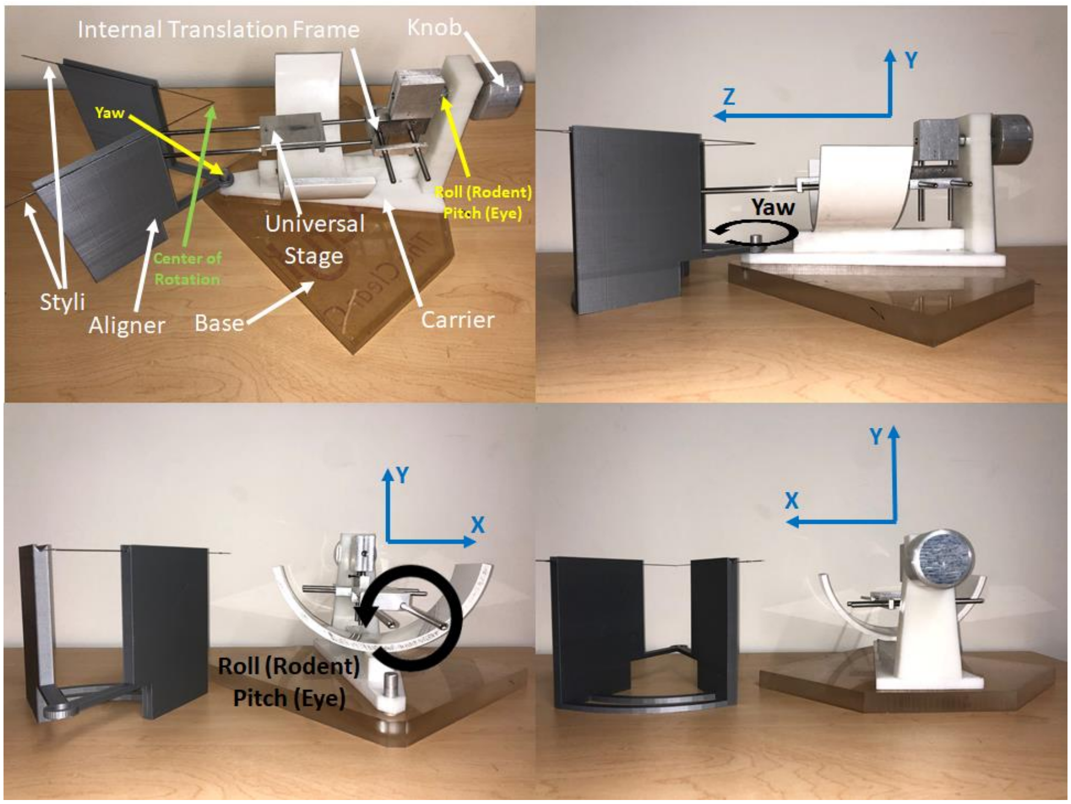


Figure 7: Images of final, full-sized, fabricated prototype of Diamond RRaTS design; labeled overview of design with removable aligned attached and points of rotation designated (top left); side view with yaw rotation and y/z axes orientation (top right); front view with roll rotation and x/y axes orientation (bottom left); rear view with x/y axes orientation (bottom right)

Testing

Test 1: Deviation from Intersection

The device was tested to see if the eye was kept at the intersection of the axes for various amounts of rotation in both the yaw and pitch directions. This is one of the client's main requests for imaging of the eye. It would be undesirable if the eye moved out of the center of rotation, because the client would need to use the internal translation elements after each rotation to position the eye back in the center. To conduct the testing, a stuffed animal badger was placed inside a makeshift sample holder made out of a PVC pipe and placed on the universal stage. The subject's left eye was positioned to the center of rotation using the internal translation and the alignment styli. At this point, the stage was rotated in the yaw direction at 15 degree increments with help from a protractor until the carrier was rotated 45 degrees from the starting point. At each turn, a fixed camera captured an image of the subject and stage (Figure 8). An image processing software named *ImageJ* was then used to calculate the deviation of the subject's eye from the center of rotation. A similar process was then carried out in the pitch direction where the stage was rotated in 7.5 degree increments until the universal stage was rotated 30 degrees from the origin. Again, captured images were uploaded into *ImageJ* and analyzed to calculate the deviation from the center of rotation.

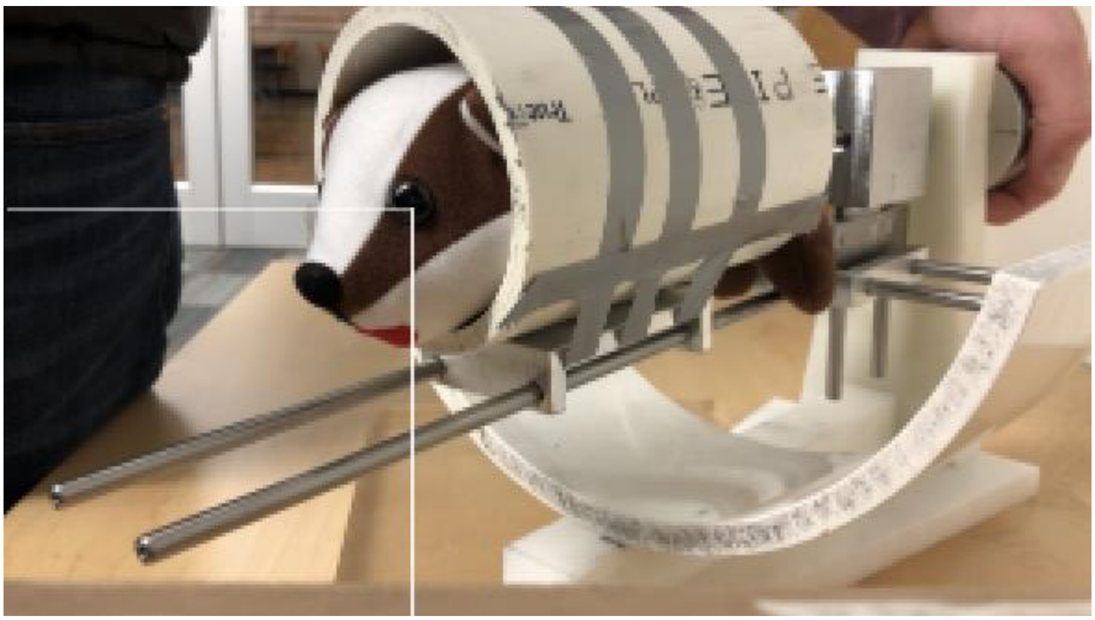


Figure 8: Example image as analyzed in ImageJ during testing of deviation of eye position upon rotation of the specimen; white grid lines meet at the center of the eye used to quantify the eye position; model specimen (stuffed Bucky Badger) fixed in an example sample holder taped to the universal stage during adjustment

<u>Test 2: Beam Deflection</u>

The device was tested for the amount of beam deflection that would occur under various loads. This was done because the rods deflecting could impact the ability of the eye to remain in the center of rotational axes. Too much deflection could cause the eye to become unaligned with the center of rotation during rotation. To test this, varying masses were placed on the two z-axis translational rods and the deflection was measured using a digital caliper. The center of mass of the masses were placed at the center of the rods, or the 5-inch (12.7 cm) mark and the deflection was measured at the 10-inch (25.4 cm) mark. This was to simulate various weights of rodents being placed in the middle of the rods and the location of its eye deflecting. The rods under no load was used as a zero for the deflection. Increasing masses were added after each deflection was measured.

Test 3: Alignment Time

A test was also conducted to test the time it took for one person to align the pupil of a specimen in the center of rotation with the aligner. Seven trials were conducted with seven different participants using the device to align the pupil in the center of axes of rotation. A stuffed animal was used in place of a live subject. The stuffed animal was then fixed to the universal stage using the makeshift sample holder. Before each trial, the eye was shifted from the center of rotation in a randomized manner. To simulate actual usage of the device, the center of rotation was defined as the point where the aligning styli met. A time measurement was taken for each trial with stop watch starting when the person touched the device to start aligning and ending when the person took his/her hands off the device after successfully aligning the eye at the center of rotation. An average time taken to align the eye was calculated based on the 7 trials.

VI. Results

Test 1: Deviation from Intersection

Upon analyzing the location of the eye based on different rotations, the device kept the eye in the center of rotation besides in the y roll case. The y deviation followed a negative correlation with increase in roll rotation. The largest deviation in y roll was .29 inches (.7336cm). The largest of the other three deviations from the center of rotation was .125 inches (.3175 cm) in the y yaw rotation, which was significantly less than the deviations seen in y roll (Figure 9). *See Appendix B Table 2 for raw data.*

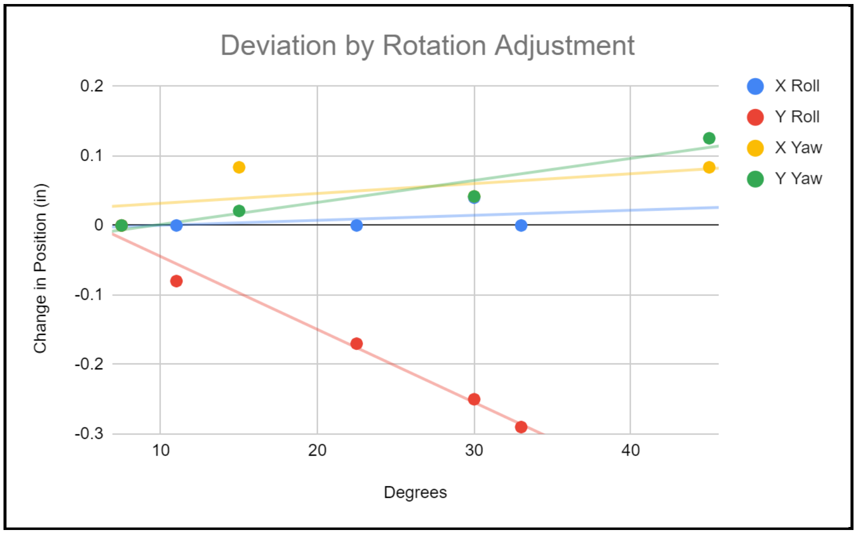


Figure 9: Deviation of eye along the X and Y axes with various rotations; change in position in inches plotted against degree of rotation from 0° to 45° in yaw and 0° to 30° in pitch.

Test 2: Beam Deflection

5 masses were added to the center of the translational rods to see how much they deflected. The deflection showed a linear relationship with weight with a slope of .000245 inches (.000622 cm) per gram of weight added. The maximum weight measured was 739.9 grams and this caused a deflection of .225 inches (.572 cm). This weight is above the average weight of a rat (250-500 grams). In the research lab, the rods should not deflect this much when a live rodent is placed on the stage (Figure 10). *See Appendix B Table 3 for raw data.*

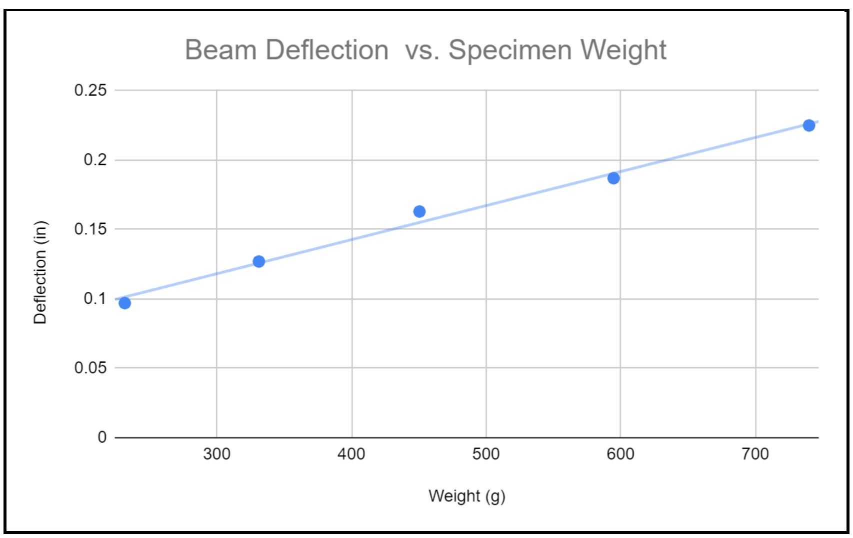


Figure 10: 10 inch (25.4 cm) translational rod deflection under various loads; deflection in inches plotted against weight loading the universal stage; universal stage positioned 5 inches from the base of the rods.

Test 3: Alignment Time

The average time taken to align the device over the 7 trials was 59.32 ± 18.96 seconds. The standard deviation shows that there was little consistency in the time it takes for alignment. The data provides insight to the time it may take for the first time a person aligns the rodent using the device. As the same person continues to use the device, this time should decrease due to an increase in experience. Since the device keeps the eye in the center of rotation, the alignment time is not significant because alignment should only occur once for each rodent. *See Appendix B Table 4 for raw data.*

VII. Discussion

The client, Dr. Rogers, conducts his imaging research on the eyes of rodents to gain insight about the eye and the retina in order to provide information that could lead to the treatment of diseases such as macular degeneration and glaucoma [11]. Based on the information provided by the McPherson Eye Research Institute, there is a lot of research being conducted by the University of Wisconsin in this area [13]. This research is in high demand, as macular degeneration currently has no cure. For these reasons, creating an adjustable stage to facilitate the imaging of specimen eyes by both Dr. Rogers and other researchers that may use the design would indirectly contribute to the furthering of research that will ultimately lead to effective treatments of these diseases. As no device currently exists to adjust rotation of a specimen following alignment at the center of rotation, there is no preceding performance data against which to compare the aforementioned results. However, according to the results that suggest the eye deviates somewhat during rotational adjustment, the device needs to have increased precision of the internal translation for the successful imaging of the retina of the eye. According to the tests, there was a deviation in the y roll of the device. This means that the rotation components in the device needs to be more precise. The bending of the translational components contributes to the unalignment of the eye when rotating which can be improved upon by increasing the stability of the rods. The rods deflecting when a load is placed on them is directly correlated to the higher deviation of the eye under roll rotation in the y direction as shown in test 1. When rotating, the bending of the rods causes the eye to become unaligned the further the device is rotated. This may result in the client needing to use the internal translational elements if their rotation extends beyond 30° in roll rotation. Ethical considerations concerning the rodent and researchers had to be considered. The specimen to be imaged must not be harmed in any way, and should be comfortable during the duration of the imaging. Also, the researcher should not be under any risk of harm while using the device. Based on the current resources available to the design team, the team was unable to test the device under live specimens but ethically this device would be tested with live subjects before being used to image to make sure it is not harmful to the rodent or researcher. A possible source of error for the testing would be how the design team used a makeshift sample holder to carry out the tests. The team did not have time to create sample stages because of the main focus on the alignment of the eye in the center of rotation, so a stage was created specifically to carry out the tests. The stage was not accurately machined or attached, so this could be responsible for some of the deviations in the tests. Another source of error could be due to the digital caliper used to measure beam deflection. The rod was round, not a flat surface, so measuring to a round source by eye-balling where to measure on the digital caliper provides some deviation to the actual deflection.

VIII. Conclusions

The team was asked to develop a solution for the alignment of a rodent's eye for Prof. Jeremy Rogers and Dr. Ben Sajdak. In their research lab, rodent's eyes are imaged in order to better understand the retina and make advances in the treatment of ocular pathology. The client requires the center of the imaged pupils to be in the center of the rotational axes so the eye does not move out of the microscope's view when the stage is rotated to view various portions of the retina. The team updated and improved its design throughout the design process and ultimately finalized the Diamond RRaTS design. This final design provides yaw and pitch of the eye that may be aligned at the intersection of these two rotational axes. The three degrees of internal translational freedom eliminate the need for repositioning the stage within the imaging device's field of view, ultimately streamlining the imaging process.

Following fabrication of the full-sized prototype Diamond RRaTS design, testing was conducted to evaluate ease of use, strength, and movement of the aligned eye during rotation. Primarily, it was found that untrained individuals spent only, on average, 59.32 ± 18.96 seconds aligning the specimen's eye to the center of rotation, meaning the alignment process does not add significantly to the time spent conducting the overall imaging process, while, following the alignment process, the imaging will be more efficient. On another hand, the internal translation complex on which the stage/specimen sits was found to deflect downward under increasing loads. This suggests that, although stable, the protruding rods lack the necessary strength to hold large specimens perfectly straight. Finally, movement of the specimen eye during rotations in both the pitch and yaw (roll of the rodent) directions was quantified, finding the most significant deviation to be in the y direction during roll of the rodent specimen. This points to the fact that increase in precision of the rotating components is required to completely immobilize the eye, but reduction in the deflection of the rods (as previously stated) will likely decrease this deviation as well.

Moving forward, a few improvements may be made to the Diamond RRaTS design to improve its performance and improve its use in practice. For one, the implementation of the internal concentric cylinder component, present in the design drawing but not in the final physical prototype, would contribute to the stability of the internal translation, decreasing the downward deflection under load and decreasing deviation of the aligned eye during rotation. Furthermore, adding this component would add friction that would hold the stage in place to a greater degree than is currently possible during rotational adjustment. In order to accomplish this, however, the cylindrical components - both internal and external - must be custom fabricated to perfectly match diameters and eliminate any tapering surface to ensure perfect contact and prevent the addition of strain on the remainder of the design. The next most pressing addition to be made to this design includes a variety of sample holders that can be attached to the universal stage to hold specific specimens to be imaged. The client images rodents of various sizes, from rats to mice, as well as individual eyes, such that sample holders to hold each must be constructed and made able to attach to the Diamond RRaTS stage. Furthermore, the design must be implemented with the full imaging system, specifically on a wheeled cart that could provide 3 additional, external degrees of translation freedom. This cart will likely hold the design such that it can be moved into the field of view of the imaging device for imaging procedures and stored away afterwards. This addition would also allow for validation of the specimen stage with the imaging device itself to determine its success in practice. Finally, there are a few improvements that could be made that, although unnecessary, would add to the efficiency of the stage. For one, a motor could be added to precisely adjust the rotation alignment robotically, eliminating the need for manual adjustment. Furthermore, the design could be refabricated with materials deemed autoclave-safe. Although more expensive, use of such materials would make sterilization between imaging processes much faster and easier.

Even so, it is clear that the Diamond RRaTS design successfully increases the efficiency with which the client is able to conduct imaging of rodent specimens. This design succeeded in developing a system that allows for the facile alignment of the imaged eye at the center of rotation such that the stage, as a whole, need not be moved with each rotational adjustment. Although improvements can be made, it is clear that the design is a significant step towards streamlining the imaging process that will enhance the research into the treatment of life-altering ocular pathology.

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X. Appendix

A. Product Design Specifications

Rodent Rotation and Translation Stage

Client	Prof. Jeremy Rogers	jdrogers5@wisc.edu	
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Date: September 20, 2019

Function

Research of mammalian retinal photoreceptors, conducted via the imaging of rodent model organisms, requires precise alignment of the specimen. A device providing facile alignment of rodent eyes within the imaging system's field of view as well as rotational freedom for accessibility to a holistic view is called for. This device must provide at least 2 rotational degrees of freedom, pitch and yaw, as well as 3 translational degrees of freedom for the positioning of the eye at the intersection of the rotational axes.

Client requirements

- Design must provide at least 5 degrees of freedom: pitch, yaw, and triaxial translation.
- Pupil of animal must adjustable to the intersection of the rotational axes.
- Area near head of rodent must be open and accessible for imaging and anesthesiology.
- Degree of translational precision should be within 100 microns.
- Smooth-finished surfaces for facile sterilization between imaging procedures.
- Removable sample holders of different sizes for different specimens.

Design requirements

1. **Physical and Operational Characteristics**

    a. **Performance requirements:** The device should keep the center of the axis at the pupil of the rodent's eye, despite the 5 degrees of freedom it will be available to move in. The five degrees of freedom include 3 translational degrees in the x, y, and z directions, and 2 rotational degrees in the pitch and yaw directions. This device will be used whenever the rat's photoreceptors are being imaged. The top of the device should be open to allow easy access for imaging and loading of the animal as well as anesthesia.

b. **Safety:** This device should be equipped to securely hold an anesthetized rodent model organism in accordance with any animal research treatment guidelines applicable to studies in which this device is used.

c. **Accuracy and Reliability:** The device must provide movement in 5 degrees of freedom, while keeping the middle of the pupil at the intersection of the rotational axes. Internal translational precision must allow positioning of the pupil within 100 microns of this point. Additionally, external position must be within 500 microns while rotational prevision must be within 2°.

d. **Life in Service:** The device must be able to support a specimen of up to 1 pound, keeping it stationary for up to an hour at a time. In addition, the device must remain functional following exposure to direct, imaging light for up to one hour at a time. Furthermore, it must not degrade with cleaning/sterilization after use with each specimen. The body of the device should maintain functionality according to these conditions for 5 years, whereas the easily-replaceable sample holders should have a life in service of at least 1 year.

e. **Shelf Life:** This device should be storable with humidity between 35% - 70% and temperature between 10°C - 30°C and continue to function properly. If electronic components are implemented, the power-source/battery should be functional after at least one year.

f. **Operating Environment:** During operation, the design may be exposed to lighting, necessary for the imaging procedures, that could increase temperature to 35°C for the extent of the imaging process. Furthermore, anesthetized rodents will be held within the design, so the material and ergonomics of the rodent specimen holder must be carefully considered.

g. **Ergonomics:** The device should allow for easy rotation and translation of the specimen on the stage.

h. **Size:** The device should be no bigger than one foot cubed and should be easily portable. The device should implement an open design concept.

i. **Weight:** The device should not exceed 10kg to allow for easy transport and movement around the lab.

j. **Materials:** The device should remain within the budget of $350, so the material cost should not exceed this value. The device should be made of materials that are easy to clean and contain little to no crevices where dirt and other things in a lab can fall into.

k. **Aesthetics, Appearance, and Finish:** Aesthetic appearance of the device is not a primary concern, as functionality takes precedence. The design must have a smooth finish that lends itself to facile cleaning and sterilization.

2. Production Characteristics

a. **Quantity:** 1 unit will be required for use with each imaging system. Requirement for interchangeable specimen holders would limit a requirement for multiple stages as the imaged specimen is changed.

b. **Target Product Cost:** Initial production budget set at $350.

3. **Miscellaneous**

a. **Standards and Specifications:** There are no international or national standards and this project does not require FDA approval because it will be used in a research setting.

b. **Customer:** The client would like the device to have swappable holders for different sizes of specimens and that the holder should be symmetrical. There should be a cutaway area for a warming blanket for the animals. The design should have an open concept to allow for easy access to the specimen such that eye drops and anesthesia may be administered.

c. **Patient-related concerns:** As the design is intended as a research tool for the study of rodent model subjects and tissue specimens, patients are unrelated.

d. **Competition:** The RAS system, created by Bioptigen (now owned by Leica Microsystems), is the primary competitor in this area. This device utilizes concentric cylinders, as well as a pivoting element, to provide rotational degrees of freedom.

B. Testing Tables

Table 2: Raw data from testing of deviation of eye position, quantified via ImageJ, upon rotational adjustment; roll (of the rodent) and yaw adjusted independently; deviation in X and Y directions quantified independently

	Deviation by Rotation Adjustment			
	Deviation (inches)			
Rotation (˚)	X Roll	Y Roll	X Yaw	Y Yaw
0.0	0.000	0.000	0.000	0.000
7.5	0.000	-0.080		
11.0			0.084	0.021
15.0	0.000	-0.170		
22.5	0.040	-0.250	0.042	0.042
30.0	0.000	-0.290		
33.0			0.084	0.125
45.0			0.042	-0.042

Table 3: Raw data from testing of the beam deflection upon loading of increasing weights

Beam Deflection vs Weight	
Weight (g)	Deflection (in)
0	0
231.6	0.097
331.2	0.127
450.5	0.163
594.7	0.187
739.9	0.225
Linear Fit	.000245*x +.0446

Table 4: Raw data from measuring the time for inexperienced individuals to align the eye of the specimen at the center of rotation using the aligner component of the Diamond RRaTS design

Alignment Time	
Trial	Time (seconds)
1	76.8
2	43.02
3	82.2
4	37.98
5	66.6
6	70.2
7	38.46
Average	59.32
Standard Deviation	18.96

C. Spreadsheet of Expenses

Table 5: Materials ordered for fabrication and prototyping procedures; total cost of all materials procured included; all materials presented were purchased, but not all were used; not all materials composing the final prototype were purchased, so these are not included in this table.

Item	**Description**	**Manufacturer**	**Part Number**	**Date**	**QTY**	**Cost Each**	**Total**	**Link**
Component 1								
Half-scale model	3D Printing Material for Scale Model	Makerspace	NA	10/28	1	$40	$40	
Component 2								
Cylinder part (inner)	8” PVC Pipe in 5-foot section	McMaster Carr	48925K26	11/22	1	$82.59	$82.59	
Component 3								
Cylinder part (outer)	8” coupling individually	McMaster Carr	4880K132	11/22	1	$25.30	$25.30	
Component 4								
Internal translation	3” rods come individually	Thorlabs	ER3-P4	11/22	2	$6.80	$13.60	
Component 5								
Internal translation	4 pack of 3” rods	Thorlabs	ER3-P4	11/22	1	$25.83	$25.83	
Component 6								
Internal Translation	10’ rods comes individually	Thorlabs	ER10	11/22	2	$13.08	$26.16	
Component 7								
Set Screw	4-40 ⅜”	Fastenal Company	25028	11/22	15	$.08	$1.20	
Component 8								
Set Screw	4-40 ½” comes individually	Fastenal Company	25030	11/22	10	$.19	$1.90	
Component 9								
Alignment tool	3D Printing alignment tool	Makerspace	NA	12/04	1	$20.94	$20.94	
TOTAL:								**$237.52**