Final Report

BME 200/300

December 12, 2018

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Abstract

Ophthalmology research often requires the viewing of photoreceptor cells in the back of the eye. In order to successfully image all of the subject's photoreceptors, the eye of the subject must be rotated about two axes. Traditional stages for microscopic viewing provide easy solutions for translational movement, but lack the features necessary to rotate while keeping the focus on the pupil. The client desires a device which can provide rotational movements around two axes and translational movements in all three dimensions. Different sized subjects will need to be viewed on the device without compromising its movements or accuracy. The final device shall concentrate on increasing the possible motion and accuracy while keeping the center of all rotations focused on the pupil of the subject.

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I. Introduction

According to the World Health Organization as of October 11, 2017, there were 253 million people living with vision impairment, 36 million of whom were completely blind while the other 217 million had moderate to severe blindness. It is estimated that by 2050 there could be upwards of 115 million people who are blind. This is mostly attributed to a growing population and also an increasing age of the existing population [1].

Scientists are researching the sources of visual impairments by using microscopy and other optical techniques. Imaging the eye is a procedure that requires the subject to remain stationary while photos are being taken to account for the uneven topography of the eye surface [2]. Both lateral and rotational movement with high precision are required to effectively visualize individual photoreceptors.

Competing Designs

Figure 1. *The figure above depicts a Posi drive from Deltron. It is a modular microscope attachment for stage translation (XYZ).*

Posi drives (Figure 1) are devices that allow for XYZ positioning, 12” of travel, and “provide[s] both accuracy and excellent positional repeatability” [3]. This device is, “fitted with lead screw and anti backlash nut and [is] supplied with a motor adaptor and coupling” [3]. Some of the design elements for the translational movement of this device could be implemented into the team’s final design. Stand alone, this device does not account for rotational movement.

Figure 2*. The figure above depicts a Micrometer Positioning Stage from Deltron. It is a modular microscope stage capable of fine XYZ translation.*

Similar to the Posi Drives, The Micrometer Positioning Stage (Figure 2) allows for movement in the XYZ direction, but only allows 0.25” to 2.0” of travel [3]. Because of the small limit of travel, the device is extremely accurate and moves efficiently. However, the device cannot move a subject any further than 2.0” which could be a disadvantage if the user needs to move the stage any further.

Figure 3*. The figure above is a rotary stage from the company Standa. It allows for fine radial translation (rotation about the z-axis).*

The Rotary Stage of Big Platform 7R170-190 (Figure 3), provides 360-degree rotation about the z-axis; something that could be very useful when it comes to turning the subject while keeping the eye centered. The device can rotate to an “accuracy of 1 degree, and [so] finely to adjust them within 10 degree by micrometer” [4]. This precision is important to a user so that the device is accurate and easy to position. Another interesting aspect of this device is that it has preset holes that could be used for different modular stage components.

Problem Statement

While doing research on photoreceptors in the retina of an eye, images are frequently viewed through a stationary device. In order to view all of the photoreceptor cells, the eye needs to rotate with at least five degrees of freedom. With six degrees of freedom, the device can accommodate different viewing angles of the microscope. A device must include translational and rotational movements while keeping the center of focus on the pupil.

II. Background

Client Information

Dr. Jeremy Rogers is an assistant professor in the department of biomedical engineering at the University of Wisconsin-Madison and is the principal investigator of a biomedical optics and biophotonics laboratory at the Wisconsin Institute for Medical Research. He has extensive training and experience in optical sciences and imaging. Throughout his research, Dr. Rogers images photoreceptors in the eyes of many different specimens.

Dr. Rogers lab currently uses a half round PVC pipe with an attached bite bar to stabilize image subjects including mice, thirteen-lined ground squirrels, and rats. The device is placed on a TMC vibrationless table to compensate for the vibrations in the room and ground. Each animal image subject is anesthetized and placed on top of a warming pad to maintain body temperature while under. Software paired with an AOSLO microscope is used to compensate for varying degrees of deformities in each eye. Each component used in the Roger's lab setup is detailed below.

<u>TMC Vibration Control CleanBench</u>

This bench isolates the microscopes and other lab equipment from vibration for more accurate results. The table top (stainless steel) has a honeycomb design such that equipment with screws on the base can thread into the table. It uses Gimbal pistons to maintain vibrational equilibrium [5].

<u>Kent Scientific Infrared Warming Pads</u>

While imaging, living specimens are heavily sedated so they require warming pads to maintain constant body temperature. The current pads measure 15.2 cm x 20.3 cm x 0.64 cm and have a removable sleeve and a rechargeable battery. The pads generate infrared waves (animal bodies absorb 90% of these infrared rays) to heat the specimens. The temperature in these pads range from 20 to 40 degrees Celsius [6].

<u>AOSLO Microscope</u>

Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO) can capture high-resolution images of the retina. It can image individual photoreceptor cells, retinal pigment epithelium cells, microscopic capillary vessels, and the nerve fiber layer. The process is quite complex, but it is important for the design that a prism on the microscope must be placed within 1 mm of the eye [7].

Eye Anatomy

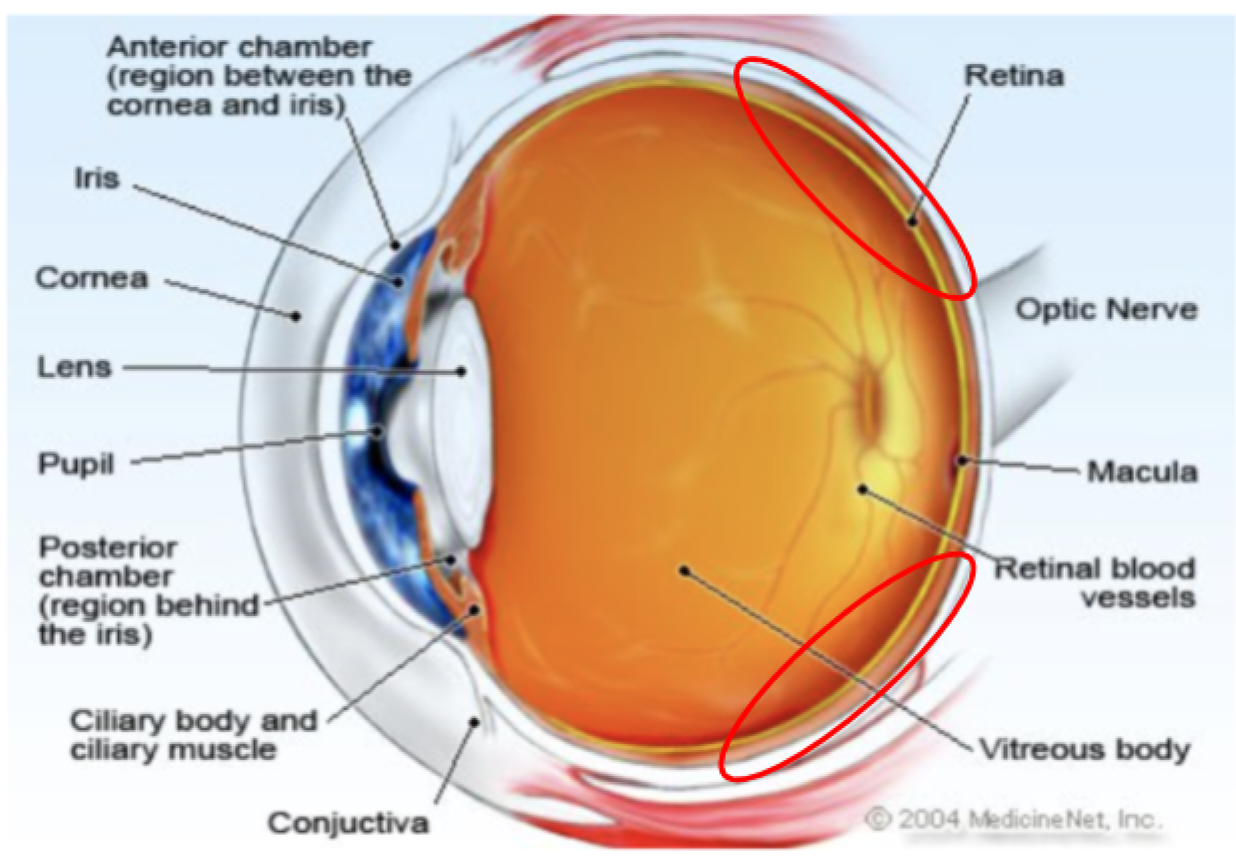


Figure 4. *The figure above is a detailed diagram of the human eye.*

The goal of the design is to image the retina, which, as shown in figure 4, is in the very back of the eye in relation to the pupil. Microscopes are required to be perpendicular to the image subject of interest throughout photo capture. Since the eye is curved, it is necessary to move the microscope rotationally and keep the subject fixed. By putting the pupil at different angles in relation to the imaging device, it is possible to see photoreceptors in all locations of the retina, specifically the parts marked with the red circles [8].

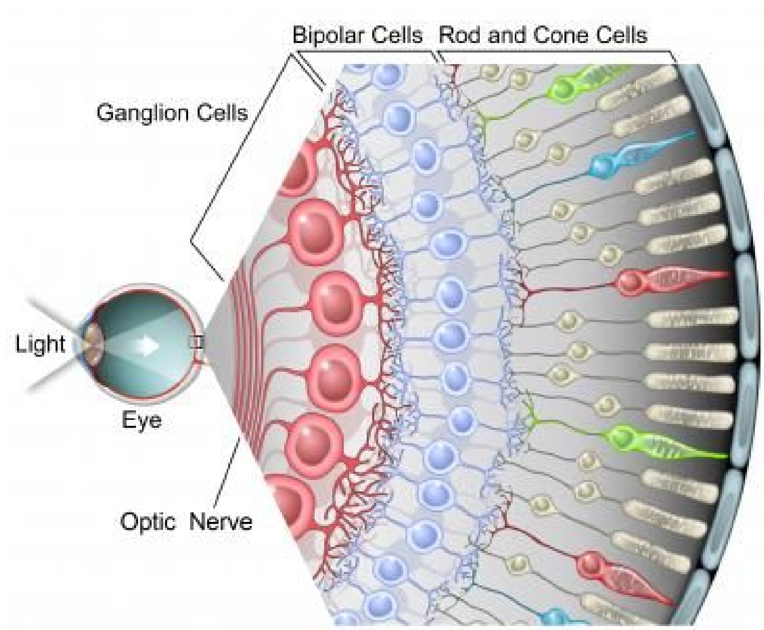


Figure 5. *Diagram of detailed anatomy of the eye and retina. Light enters the eye and interacts with multiple cell types that make up the retina.*

The most important thing to notice about Figure 5 is the number of photoreceptors situated on the retina. The photoreceptors span across the entire surface of the retina, forming an arc on the back of the eye. Due to this configuration, a stationary stage could only allow imaging of a portion of the photoreceptors. A stage that could be rotated or translated would allow for precision imaging all photoreceptors in the retina. This device would greatly improve the ergonomics of the imaging process and allow researchers to construct detailed maps of certain photoreceptors location in respect to others [9].

The goal of Dr. Rogers' research is to study the photoreceptors to develop a deeper understanding of the causes behind blindness or visual impairment. Higher precision translation and rotation for viewing photoreceptors in the eye would aid researchers in viewing all photoreceptors. Images of which would provide an overall map of existing photoreceptors and ultimately underlying sources of blindness or related diseases.

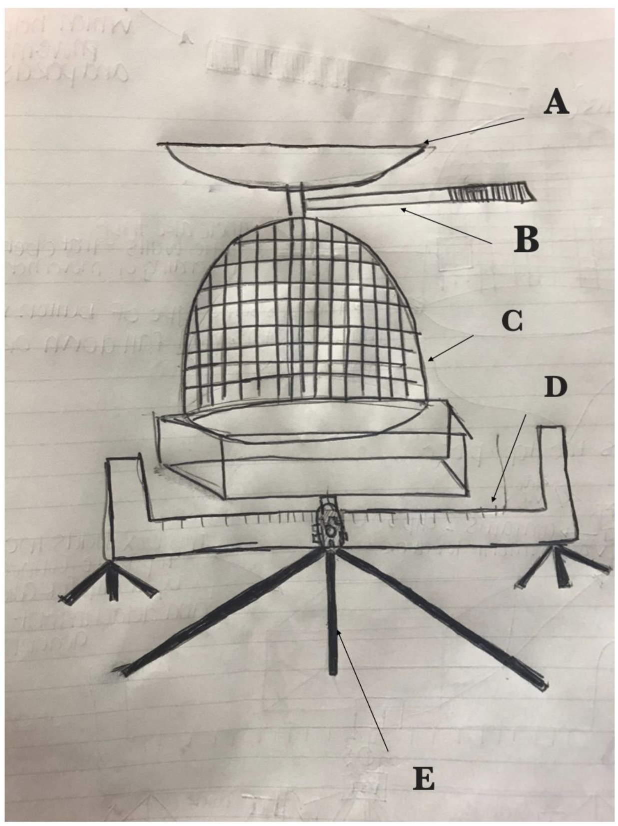
Design Specifications

The stage must have 5 degrees of freedom (i.e. if the x-axis is in the direction orthogonal to the pupil, then rotation about the y and the z is required along with translation on all three axes) and must translate with 100-micron precision. Scientists need to rotate the specimens to focus on photoreceptors which are approximately 25-microns in length. It must include interchangeable stage mounts for different viewing subjects (detached human eye (2.4 cm diameter, 7.5g), a thirteen lined ground squirrel (33 cm long and 227g), and a white mouse (12.5 - 20 cm long and 12 - 30g) [10][11][12]. The device shall contain non-absorbing surfaces and must be assembled in a way that limits the number of small creases so that it is sterilizable between uses via alcohol wipes.

Because this is a custom device being used in a research setting that does not store human patient data, there are no international or national standards to meet. The device must operate optimally in normal room temperature, pressure, humidity, etc. for approximately 500 hours of use. The device shall weigh under 5 kg and stand less than 15 cm tall to fit conveniently on the lab bench. The device shall withstand drops of 1.0 meter without breaking into shards and satisfy a project budget of $250.

III. Preliminary Designs

Design 1: The Park



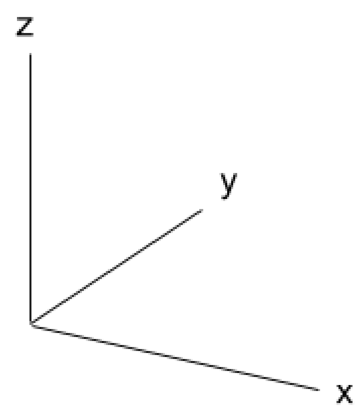


Figure 6. *A: Detachable bed with a small neck (2 cm). B: Handle which acts as a ratchet allowing precise movements (8 cm long). C: Dome base, with a grid system and individual walls which allows movement forward but not backward (7 cm diameter). D: Gear track which allows for movement in the XY direction (15 cm). E: Tripod attachment used for stability and translation in the Z- direction (varies).*

This design functions similarly to the stick shift of a car. It has a function that allows for the stick shift to go forward, lock-in, and then prevents any further movement unless prompted by the user. This function allows for the stick shift-like mechanism to remain fixed in place on the predetermined grid system.

The top piece consists of a dome, with a grid-like system, and a bed with a smaller neck and a handle that acts as a ratchet. This piece is then mounted onto a box, which is connected to two different clips. One clip is attached to gear track which swings left and right to allow for translation in the XY direction. There is a tripod connection which is used for stability and translation in the Z-direction.

Design 2: The Rigamortis

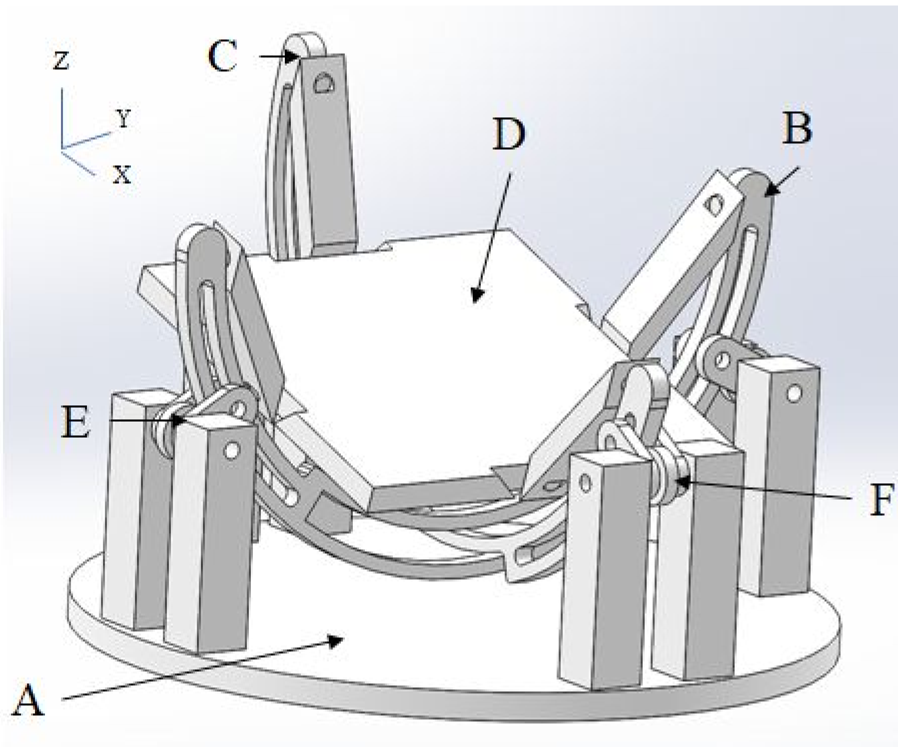


***Figure** 7. A: Circular base which can rotate around the z-axis. B: Curved arm which provides rotation around the x-axis. C: Curved arm which fits inside arm B and provides rotation about the y-axis. D: Table attached to arms B and C whose center sits 5 cm below the focal point for rotation about all three axes. E and F: Gears to move arms C and B respectively.*

The Rigamortis design places emphasis on keeping the center of rotation on a fixed focal point, while allowing maximum rotation. Specifically, the device allows 45 degrees of rotation around the x and y-axes and 360 degrees around the z-axis. The axes of all three motions intersect at a fixed point in space 5 cm above the center of the subject table. This makes it so the rotations will always be centered on that point.

Curved guide arms provide rotation around the x and y-axes in The Rigamortis design. The arms are semicircular with a 12 cm radius. The upper 45 degrees on each side will contain gear teeth which match up with gears attached to the base supports. These gears enable the user to rotate the device about the x and y-axes. The gear between the base support arms has 20 teeth, and if the curved arms were completely circular and continuous, it would be a 200 tooth gear. Thus, one rotation of the base gear gives 36 degrees of rotation for the table. This can be further reduced with additional gear combinations, which will become fine and coarse adjustment knobs. In the bottom of one guide arm is a slot which allows the other arm to slide within it. Due to this slot, the arms can have the same radius, but not hit each other at the bottom.

The 5 cm spacing between the table and focal point allows for the creation of individualized modular devices for a specific viewing subject. Different sized subjects can have their own specific holder to attach to the table in order to bring the pupil of the eye of that subject to the focal point of rotation. This holder will be attached manually by the user with velcro, magnets, adhesive, suction cups, or some other method which allows the user to move the device to a specific translational position on the table. Each holder shall also include a small mechanism for adjusting the height. With those mechanisms in place, the holder can be translationally adjusted in the x, y, and z directions, giving The Rigamortis 6 degrees of freedom.

Design 3: The Rocking Chair

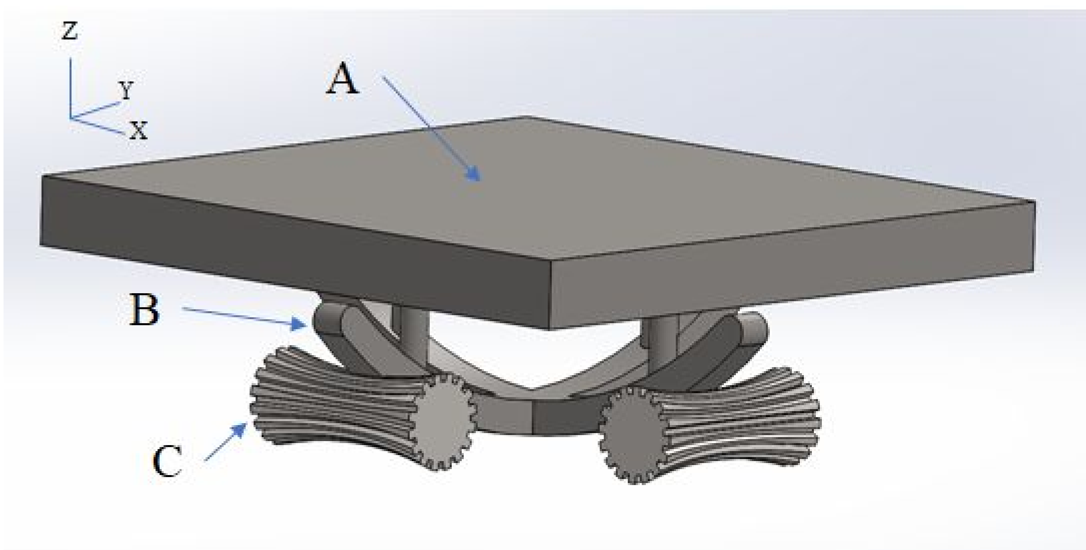


Figure 8. *A: Table for the subject holder. B: A curved gear track which transmits the rotation of a gear (C) to the table (A). C: A curved gear which can rotate to move the table (A) which also allows the gear track (B) to slide when rotating in the opposite direction.*

The rocking chair design features a large, wide, square stage that rests atop a curved gear rack. Two curved gears control the rotation about the x and y-axes. When one rotates (acting as a gear), the other allows the teeth on the curved gear rack to slide. Like the Rigamortis, this allows rotation around the x and y-axes while keeping the focal point 5 cm above the center of the table.

This design would have the same modular holders for different subjects to translationally position the pupil of the eye in the focal point of rotation.

The flaw in this device is that the pitch diameter of the gears changes between their center and outsides. Since the number of teeth does not change, the gear's module (diameter/teeth) is not constant, so it will no longer line up with the track below the table. In reality, this would allow only a few degrees of rotation before friction between gear teeth would lock the movement.

IV. Preliminary Design Evaluation

Design Matrix

Design Aspect	Weight	**Design 1: The Park Rank (1-5)**	Weighte d Rank	**Design 2: The Rigamortis Rank (1-5)**	Weighte d Rank	**Design 3: The Rocking Chair Rank (1-5)**	Weighted Rank
Precision	35	3	21	4	28	4	28
Usability	25	3	15	5	25	2	10
Height	15	4	12	2	6	2	6
Amount of Rotation	10	2	4	3	6	1	2
Ease of Build	5	3	3	3	3	3	3
Cost	5	4	4	5	5	5	5
Safety	5	5	5	5	5	5	5
Total:	**100**		**64**		**78**		**59**

Figure 9. *Design Matrix for prototype evaluation.*

The design matrix, shown above, was used to evaluate the three preliminary designs: The Park, The Rigamortis, and The Rocking Chair. Before fully grading each of the designs, the design specifications were ranked from highest to lowest importance. The design specifications were defined as follows:

Precision: Precision is defined as how accurately the device keeps the center of rotation during rotations. While this was originally defined in the product design specifications as a measurement in microns, an accurate reading would not fully be determined for each device until they were fabricated. The precision ranked for each device was thus determined based on the ability to keep the eye at the center of rotation.

Usability: Usability can be defined as how easily one can adjust the rotational and translational movements of the device. The rotational precision has to be less than one degree.

Height: Height is defined by the requirement that the device is less than 15 cm tall. Shorter devices will score higher in this category because they allow for more workable room under a microscope if image subjects are viewed from above.

Amount of Rotation: The amount of rotation indicates the degree a device can rotate around the focal point in comparison to two different axes (e.g. pitch and roll). This criterion was based upon rotational dimensions of each design provided.

Ease of build: Ease of build is defined as how easy it is to produce a prototype and how easy it would be for someone to fabricate and manufacture the device.

Cost: The cost is defined by a budget of $250. Devices which cost less to produce and test will score higher in this category. This price reflects the allowable budget for fabrication, testing, and product development.

Safety: Safety is defined as how well the device limits harm to the user(s). This criterion entails safety for both the subjects which are mounted within the holding device and the researchers who operate the anesthetic and imaging procedure.

As shown (from top to bottom), the aspects of the design that were of most importance were: precision, usability, and height (weights 35, 25, 15, respectively). These three were followed closely by amount of rotation, ease of build, cost, and safety (weighted 10, 5, 5, 5, respectively). The specifications were then distributed by weight, with the aspect of greatest importance having the highest weight and the one of least importance with the lowest weight. Then, each design was ranked from one to five by design aspect.

Designs 2 and 3 scored highest in the precision category because the focal point remains constant for all device rotations. Design 2 scored highest in the usability category because its gear design provides the highest and most user-friendly degree of rotational control and the best method of rotation. This is due to the fact that the gear ratios can be adjusted to allow for both fine and coarse adjustment of rotation. Design 1 scored highest in the height category because the tripod base can be adjusted to a lower height than the other designs. Design 2 scored highest in the amount of rotation category because it allows 45 degrees of rotation. Designs 2 and 3 scored highest in the cost category because they include all 3D printed parts, which keep them well below the $250 budget. All three designs scored five out of five for safety because all gears are contained, there are no sharp edges, the devices are inflammable, and there are no other significant sources of danger. Therefore, Design 2: The Rigamortis, finished with the highest score of 78 when ranked against the other two designs. Based on its score in the design matrix, and after discussing further with the team, it was decided that The Rigamortis was best suited for a final design.

V. Fabrication/Development Process

Final Design

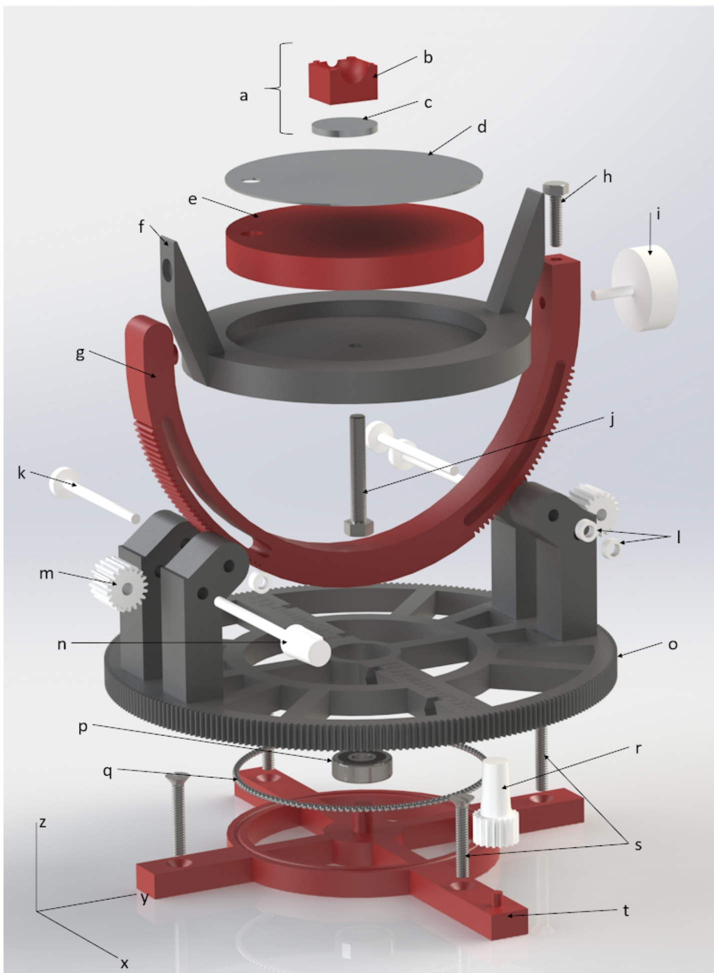


Figure 10. *Exploded view of the final design containing the following components:* ***(a)*** *A replaceable modular device for holding a detached human eyeball made from a PLA holder* ***(b)*** *and a magnet* ***(c)****. The magnet is attached to a metal plate* ***(d)*** *which allows the eyeball holder to be easily translated in the x and y directions. The metal plate is attached to a plastic base* ***(e)*** *that sits above the swinging table* ***(f)****. This table enables rotation about the y-axis by rotating about its attachments to the support guide* ***(g)****. It is secured by tightening the bolt* ***(h)*** *after turning the adjustment knob* ***(i)****. The height of the eyeball can be adjusted using another bolt* ***(j)*** *which screws through the swinging table and into the circular plastic base. Support rods* ***(k)*** *hold the support guide above the rest of the device and are secured by caps* ***(l)****. Two gears* ***(m)*** *allow the device to be rotated around the x-axis when the knob* ***(n)*** *is turned. The entire device is rotated around the z-axis via its gear teeth* ***(o)****. This rotation is assisted by a needle bearing* ***(p)*** *and ball bearings* ***(q)****. Knob and gear* ***(r)*** *are turned by the user to control the z-axis rotation. Lastly, four screws* ***(s)*** *are screwed through the base* ***(t)*** *to secure the device to the vibration-less lab table.*

The final design (figure 10) contained numerous changes from the initial Rigamortis design suggested. The most important modification was switching from two curved supports to one. When using two supports, the subject could only be rotated in one direction at a time. After being rotated in one direction, the axis of the other direction is rotated with it and it is no longer parallel to the ground. This prohibits it from turning in the other direction until the table is returned to the initial position. In the final design, x-axis rotation is provided by the gears running along the curved support. Y-axis rotation is controlled by a dial on the side of the curved support. Once the dial is adjusted to the correct location, the table is locked in place with a screw that pushes into the shaft of the dial, holding it to the inside of the curved support. With this system, the device can rotate about the x and y-axes at the same time.

The initial Rigamortis design also contained individual pieces connecting the curved support arms with the base gear. These would sink down slightly under the load of the table, which made turning the gears more difficult. To fix this issue, additional area was added to the towers on the base gear to connect directly to the curved support arm, providing more accuracy and stability.

In the final design, the entire device rotates around a cross shaped base, which contains spaces for two different types of ball bearings. The first is eighth inch ball bearings, which sit between the base and the rest of the device allowing for easier rotation. The second is a size 6200 deep-groove ball bearing which is used to secure the base to the device without prohibiting rotation. The base has four holes that fit quarter-inch screws and are spaced to fit into the TMC Vibration Control CleanBench.

In order to adjust the positioning of the subject to reach the focal point for rotations, the final design incorporated a circular table which could be raised and lowered by rotating about the threads of a bolt. On top of the circular table is a thin metal plate which allows different modular devices to be attached with magnets. By using magnets, the modular devices can be translated to position the subject in the center of the focal point, while allowing subjects of many different shapes and sizes.

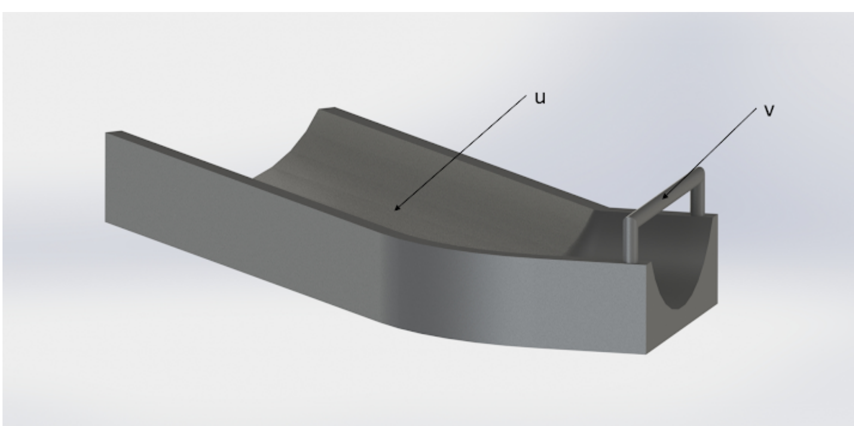


Figure 11. *In addition to the eyeball holder, there is another module device which is designed to hold a lab mouse or thirteen-lined ground squirrel. This holder features an angled trough* ***(u)*** *where the animal lays, and a cylindrical bite bar* ***(v)***. *The incisors of the rodent are laid over the bite bar to stabilize its head.*

Part of the final design was two different modular devices designed to hold two types of subjects to the rest of the device. The first holder (seen in figure 10) is used to contain an ex-vivo human eyeball. In the front of this device is a large semi-circular cutout which allows the microscope to see into the pupil. The back contains a smaller cutout which makes room for the optic nerve of the eye. The second holder (figure 11) is used to hold an anesthetized rodent.

As designed, it will fit a large lab mouse, or a small thirteen lined ground squirrel, but could easily be scaled up or down and reprinted to support different sized animals. The rodent holder features a raised bite-bar. The rodent's incisors are draped over the top of the bite bar, which stabilizes their head to provide a better picture of the eye.

Materials

The majority of the design was 3D printed so comparing plastics was an important part of the design process. 3D printing was the best construction option because it is cheap, easy, customizable, and compatible with SolidWorks. The Makerspace staff was an important reference to pick among three different types of materials: ABS, PLA, and Grey Pro. Some decisions were made on the spot, based on the availability of the 3D printers in the makerspace. The gears needed to be printed in Grey Pro on the form 2 printer as the material properties of Grey Pro could handle more stress and would allow for a more detailed surface finish for the small gear teeth. Grey Pro is well known for its ability to withstand repeated use [13] .

Most of the pieces in the device needed to be strong and durable therefore ABS and PLA were both the main materials used in the prototype. These materials are similar in their functionality and strength. The main difference between these two materials is that PLA is biodegradable and ABS is chemically stable. Compared to other available 3D printing plastics ABS is the best plastic to use because it is cheap, easy to print, durable, impact-resistant, and does not dissolve in alcohol or bleach [14][15]. Tough PLA is commonly used for parts that undergo more stress. The difference in cost in both materials is negligible. Therefore, the material that was used was determined by availability in the printers.

There were some components of the device that could not be 3D printed because of lack of strength and magnetic characteristics. A steel metal plate on top of the table was used to magnetically translate the subject holder in the x and y axes. Neodymium disk magnets and a standard ferric oxide magnet were used to fix the sample holder to the metal plate on the rotating table. These two types of magnets are both strong enough to keep the subject on the table and allow for easy translational movement for the user.

Screws that fit the 3D printed holes in the base of the device were used to connect the two halves. A hex head bolt was used to stop the rotation about the x-axis because it is easier for the user to turn and keeps the stage fixed. A 5-16" bolt with a 8mm x 1.25 mm thread count was used for center z-translation because of its strength and durability. A 3D printed part of the same dimensions would fracture more easily under high amounts of friction and consistent use.

A needle bearing was added to the base of the device for smoother rotation around the z-axis. This type of bearing was chosen over a ball bearing because it could have a smaller diameter. The ball bearings were necessary to counteract the shear force due to friction while rotating the device in the z-axis. Epoxy, a strong adhesive agent, was used to attach the metal to

PLA. This agent is long lasting and specifically used for attaching metal to plastic.

Methods

Designs were first drafted on paper and discussed with other team members. Collaborative troubleshooting was used to determine whether it was a credible enough design to draw up in SolidWorks. After passing initial inspections from the team, the designs were drawn up in SolidWorks and rendered together to ensure functionality. Measurements for the device were determined based upon the design specifications for angular and translational precision as well as staying within the confines of the height, width, and length. Gears were a particularly important part of the design, so the team did additional research on how to properly create them in SolidWorks. Series of renders were made to determine possible issues with the design.

The individual parts were transferred from a SolidWorks file to an .STL file and then sliced by the 3D printer software. For any pieces that contained holes for bolts, the wall thickness was increase in the slicing software, so that the piece would not crack or deform when it was threaded. For the materials printed in Grey Pro on the Formlabs Forms 2 printer, the team was careful to print all supports on flat surfaces. By avoiding edges and corners, especially on gears, the supports were easily removed and cured without bumps or divots. For the ABS and PLA parts printed on the Cura MakerBot printers, supports were generated for any section with an overhang angle greater than 45 degrees and were easily snapped off with a tweezers. Lastly, the main large gear for z-axis rotation, and the curved support were printed with skirt build plate adhesion instead of brim, since the brim was difficult to remove from the teeth of the resulting gears.

Once every part was printed, the device was assembled by hand. Epoxy was used to fuse together any plastic pieces that needed to be attached to metal parts. Likewise, plastic to plastic welds were made with superglue. All glued connections were given at least 1 hour to dry before being put under strain.

Testing

Rotation of 45 degrees is necessary to successfully capture images of all photoreceptors in the eye. The 3D printed device was tested using a digital inclinometer gauge. The gauge was placed in the center of the stage and then the device was manually rotated around the x-axis and y-axis. Once the measurements were taken, the numbers were evaluated using a calculator. The device can rotate around the z-axis 360 degrees which was determined by measurements on SolidWorks and by rotating the prototype. Further testing was done to determine the maximum rotation about the x and y axes.

A translation test was done by placing a sheet of paper over a metal plate and puncturing various holes with a needle. The needle was attached to the modular specimen eye holder perpendicular to the paper surface and on the edge of the eye holder. The sheet was punctured at a starting point, then the user was prompted to move the eye holder in the smallest increments of which they were capable. The distance between the punched holes in the paper were measured using a digital metric caliper.

A standard ruler with millimeter precision was used to measure the range of z- translation for the center rotation table. A line was drawn between the two swivel pins on an image editing software to determine the focal point of the structure. The focal point of the z-translation feature was measured at its highest point and its lowest point effectively returning the values for range of possible z-translation.

Post-doctoral researchers, graduate students, and undergraduate students at the Wisconsin Institute of Medical Research (WIMR) were given an introduction to the device and asked to take a qualitative survey on the functionality and ergonomics of specific features of the device. The survey prompted users to perform certain translations and rotations and gave five answer options for each question relating to ease of use or functionality.

The device's material must withstand environmental conditions of -10 to 50 degrees Celsius. The material properties of ABS and PLA have been previously tested under more extreme conditions than -10 to 50 degrees and that information was referenced when determining the stress each component would see under loading [13].

VI. Results

Angular Test

After completing the angular test about both axes, the mean, the t-confidence interval, and the standard deviation of the angular measurements were calculated(figure 14). A box-plot was made to analyze if the range of rotation about both axes fell into the predetermined design specification of less than one degree rotational precision.

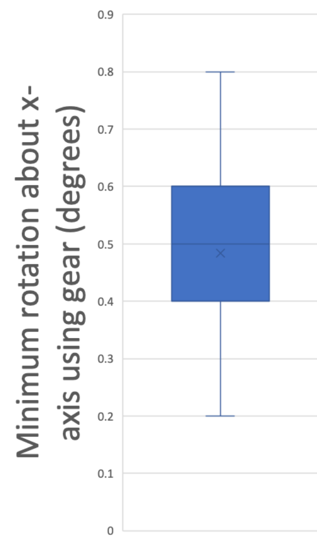


Figure 12. *X-Axis Rotation Box-Plot*

For rotation about the x-axis as shown in figure 12, it was found that the user's minimum rotation with the gear had a precision that satisfied the predetermined design specification.

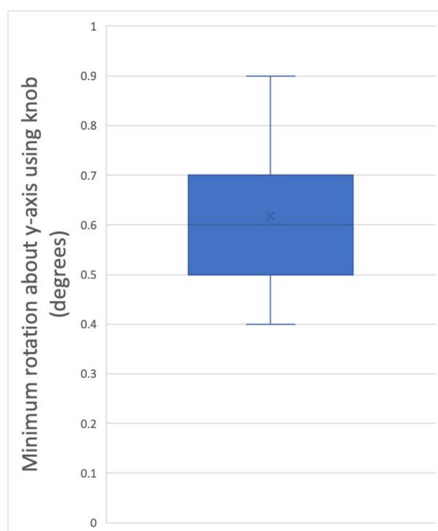


Figure 13. *Y-Axis Rotation Box-Plot*

The data analysis was repeated for the rotation about the y-axis as shown in figure 13. After analysis it was found that rotation about the y-axis also satisfied the preliminary design specification for precision.

Data Analysis	**X-axis Rotation (degrees)**	**Y-axis Rotation (degrees)**
Mean	0.4769	0.6182
T-confidence Interval	(0.3811, 0.5697)	(0.5025, 0.7338)

Standard Deviation	0.1536	0.1722

Figure 14. *Data Analysis for Angular Test*

In addition to the precision data collected, range values for each axis rotation was determined. Rotation in the x axis achieved a range of 43 degrees with 21.5 degrees range in each direction. Both the Y-axis and Z-axis rotation rotation satisfied a range of 360 degrees.

Translation Test

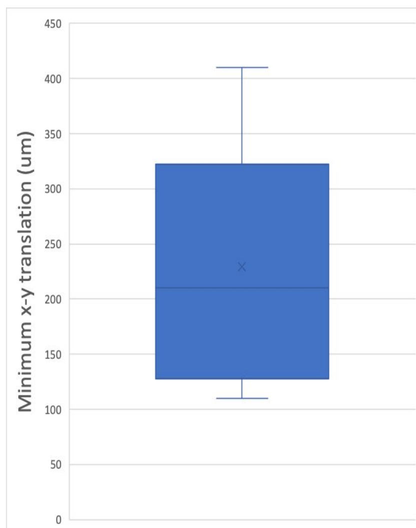


Figure 15. *X and Y Translation Box-Plot*

After completing the translation test for the x and y directions, the data was used to find the mean and the standard deviation of the measurements (figure 16). From the data a box-plot was made to analyze if the range of translation in the x and y directions fell into the predetermined design specification of 100 um precision. For translation as shown in figure 15, it was found that the user's minimum translation in the x and y directions with the subject holder did not satisfy the predetermined design specification.

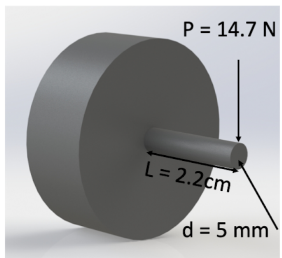
Data Analysis	**Measurement of X-Y Translation (um)**
Mean	239
Standard Deviation	106.2

Figure 16. *Data Analysis for Translation Test*

Stress Test

Mechanics of materials calculations were performed on the weakest element of the device with an applied load of 3.0 kg. A factor of safety of 2.31 was determined for the weakest weight supporting element in the device shown in figure 17. This factor of safety satisfies the initial design criteria of supporting a weight of 3.0 kg.

Mechanics of the Adjustment Knob (Max Stress Point)



Mechanical diagram of knob

$$I = \frac{\pi r^4}{4} = 3.067 \times 10^{-11}\ m^4$$

$$M = PL = 0.3234\ N \cdot m$$

$$Q = \frac{2r^3}{3} = 3.07 \times 10^{-11}\ m^3$$

$$t = 2\sqrt{r^2 + \left(\frac{4r}{3\pi}\right)^2} = 0.00453\ m$$

$$\sigma = \frac{Mr}{I} = 26.35\ MPa$$

$$\tau = \frac{PQ}{2rI} = 4.7\ MPa$$

Cured Grey Pro has an Ultimate Tensile Stress of 61 MPa [8]

$$\therefore f.o.s. = \frac{61\ MPa}{26.35\ MPa} = 2.31$$

Figure 17*. Max Stress Point and Safety consideration calculation*

WIMR Survey

After giving a survey to graduate and postdoctoral students at WIMR, graphs were created to view each of the answers in relation to each other. These results gave reliable feedback on the design and hinted at where it could improve. The results from the survey and their respective questions are shown in figures 18 to 21.

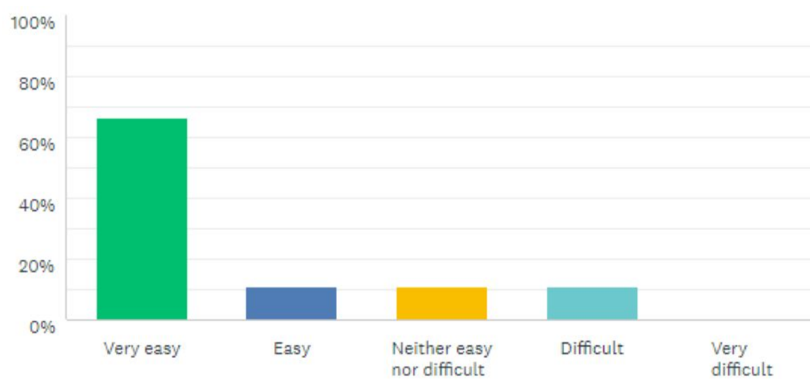


Figure 18*. Ease of z-axis translation*

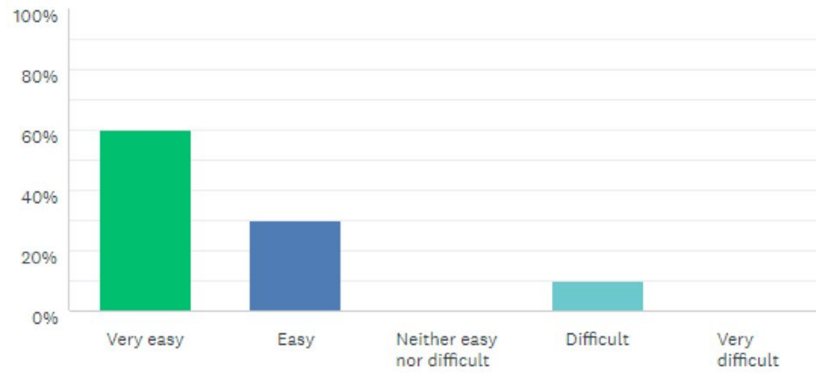


Figure 19*. Ease of y-axis rotation*

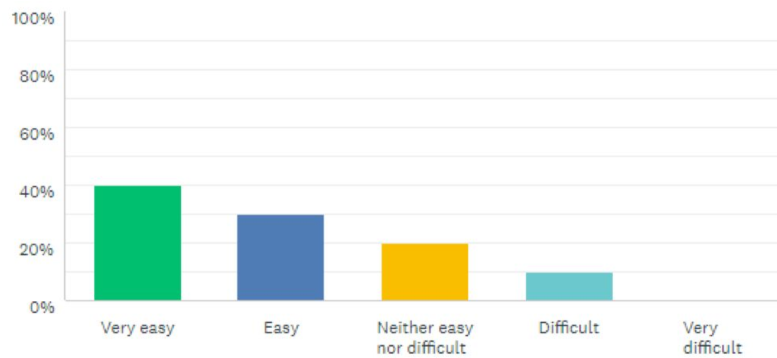


Figure 20*. Ease of z-axis rotation*

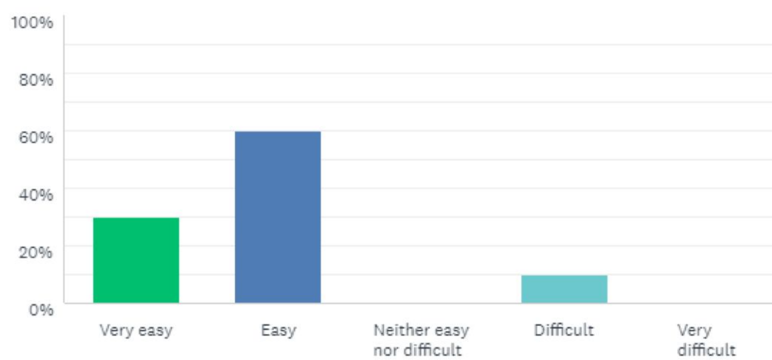


Figure 21*. Ease of x-axis rotation*

The relevant data received from the tests was the rotation and translation precision data. These data showed that the rotational precision movement was a success but there still needs to be some improvement in the translational movement precision strategy.

The most salient feature from test results was the prototype feedback from the graduate and postdoctoral students from WIMR. These results were important because they provided quality feedback with constructive criticism from potential device users. Getting their feedback was useful to determine which aspects of the design still need to be improved while also figuring out which aspects are satisfactory.

VII. Discussion

Based on the research that was conducted prior to 3D printing, it was determined that ABS, PLA, and Grey Pro will be able to withstand the environment in the laboratory (i.e. temperature, humidity, alcohol sterilization, etc.) as outlined by the product design specifications [16].

The tests illustrated that the device was able to produce the rotational precision, though did not meet the translational precision. Specifically, the average minimum x-axis rotation was 0.5 degrees, and the average minimum y-axis rotation was 0.6 degrees, both of which fit within the PDS requirement of 1 degree of rotational precision. However, the average minimum xy translation was 239 micron precision, which is over the PDS limit of 100 micron precision. Although the translation design specification was not met, 239 micron precision could still be considered satisfactory for imaging. The mechanics of material calculation reveals that the weakest structure of the device is strong enough to hold the predicted weight of subjects on the table. The results of the ergonomics tests were positive. It was determined that device rotation was easy, although more work must be done to make the xy translation smoother, which could be accomplished by decreasing the magnet strength.

The device satisfies ethical treatment standards for laboratory animals. Modular holding devices could be modified to additionally hold a warming pad, which maintains the animal's body temperature during anesthesia.

Since 3D printers were the main source of fabrication for the device, the devices precision is largely dependent on the precision of the 3D printers. Therefore, inaccuracies could have arisen while 3D printing the device. Specifically, the gear interactions were slightly compromised, so the rotation along the x and z axes was not as smooth as anticipated. Therefore, some sources of error could arise during the testing of rotation and translation. This error can be reduced by printing on more accurate printers or using a different material that would account for the needed precision.

While the device satisfies most of the PDS requirements, more work must be done to optimize the design. First of all, improving the materials of the prototype would improve its safety, precision, and ergonomics. The stress analysis of the dial discussed earlier indicates a factor of safety of 2.31. A stronger material than Grey Pro should be used to better accommodate the stress on the object and increase the factor of safety. Currently, the threaded hole in table portion of the device responsible for z-translation is made of plastic, which causes the device to table to wobble as it rotates about the bolt. To improve the precision of the z-translation, the threaded portion of the table should be made of metal. To make rotation about the z-axis more precise and ergonomic, the needle bearing should be replaced by a size 6200 ball bearing and larger ball bearings are required for the gap between the two base components. Currently the ball bearings are too small, therefore they can fall out of the device while rotating it about the z-axis.

Additionally, the spacing between parts of the device - particularly at the gear interfaces, should be improved to enhance the precision and ergonomics of the device. Eliminating small gaps between gears would require precisely machined parts.

The rodent specimen holder could be improved by adding a securing device (e.g. an arm or strap) to ensure that the rodent does not fall out of the holder when the table tilts. Also, the sample holder dimensions would be adjusted to better accommodate the 15.2 cm x 20.3 cm x 0.64 cm warming pad while allowing the rodents' mouth to remain on the bite bar.

Finally, the xy-translation test indicates that one can, on average, translate the specimen holder a minimum of 239 micron increments, which does not fit the PDS requirement of 100 microns. Applying lubrication, using weaker magnets, or including electromagnets with adjustable strengths for coarse and fine adjustment could make xy translation smoother such that the minimum translation would be under 100 microns.

VIII. Conclusions

The use of a modular stage is important to all microscopy practices. Microscopy is used to visually analyze many different cellular structures and mechanisms including those of the photoreceptors in the eye. The team has been tasked with creating a microscope stage that can operate with five degrees of freedom about the human eye. The five degrees of freedom would include all three translational movements and two rotational degrees of freedom. Three distinct designs were developed to satisfy the client's need; The Rigamortis design was chosen as it scored highest in the design criteria matrix. The Rigamortis features movement capability with six degrees of freedom using a combination of curved racks with sliding tracks fitted to rotating gears. The team has designed and created a device that satisfies initial precision requirements in the rotational movements and and achieved satisfactory translational precision. PLA, ABS and Grey Pro were the main materials used via 3D printer to fabricate and assemble the working parts of the device. Through testing it can be concluded that this device is a great replacement for the client's current setup and has potential to replace other rotational and translational devices on market by undercutting the cost. The device was able to produce the level of accuracy that is necessary for a user. In the future, the device would need to be worked on to improve the z-axis rotation by making a better connection between the bolt and table to reduce unwanted movements. The dial strength could be modified in order to improve the factor of safety. With these improvements, the device would be optimized for the client's requirements, as outlined in the PDS.

IX. References

1. World Health Organization. (2018). Blindness and visual impairment. [online] Available at: http://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment [Accessed 10 Oct. 2018].
2. Courses.lumenlearning.com. (2018). The Human Eye | Boundless Physics. [online] Available at: https://courses.lumenlearning.com/boundless-physics/chapter/the-human-eye/ [Accessed 9 Oct. 2018].
3. Deltron Precision, Inc. *Product Selection Guide* [Online]. Available: https://www.deltron.com/Product_Selection_Guide.html?keyword_session_id=vt~adwords%7Ckt~translation%20stage%7Cmt~p%7Cta~264520901441&_vsrefdom=wordstream&gclid=CjwKCAjwio3dBRAqEiwAHWsNVVMzb3YttaikR92QcxCNJr1CHlirRaBjW20mXAZot3tvQ-7qVRzPNRoCqNEQAvD_BwE [Accessed: 12-Sep-2018].
4. Standa. *Translation & Rotation Stages* [Online]. Available: http://www.standa.lt/products/catalog/translation_rotation?item=243 [Accessed: 12-Sep-2018].
5. Techmfg.com. (2018). CleanBench Laboratory Table. [online] Available at: https://www.techmfg.com/products/labtables/cleanbench63series [Accessed 1 Oct. 2018].
6. Kentscientific.com. (2018). Far Infrared Warming Pads | Mouse & Rat Warming | Kent Scientific. [online] Available at: https://www.kentscientific.com/products/far-infrared-warming-pads/ [Accessed 1 Oct. 2018].
7. Merino, D. and Loza-Alvarez, P. (2018). Adaptive optics scanning laser ophthalmoscope imaging: technology update
8. MedicineNet, Inc. *Picture of Eye Anatomy Detail* [Online]. Available: https://www.medicinenet.com/image-collection/eye_anatomy_detail_picture/picture.htm [Accessed: 12-Sep-2018].
9. Salk Institute. *From eye to brain: Researchers map functional connections between retinal neurons at single-cell resolution* [Online]. Available: https://medicalxpress.com/news/2010-10-eye-brain-functional-retinal-neurons.html [Accessed: 21-Sep-2018].
10. E. S. Perkins and H. Davson, “Human eye,” Encyclopedia Britannica, 22-Jun-2018. [Online]. Available: https://www.britannica.com/science/human-eye. [Accessed: 21-Sep-2018].
11. E. C. Cleary and S. R. Craven, “Thirteen-lined Ground Squirrels and Their Control,” Internet Center for Wildlife Damage Management. [Online]. Available: http://icwdm.org/handbook/rodents/13linedgroundsquirrel.asp. [Accessed: 21-Sep-2018].

12. L. Ballenger, “Mus musculus (house mouse),” Animal Diversity Web, 1999. [Online]. Available: http://animaldiversity.org/accounts/Mus_musculus/. [Accessed: 21-Sep-2018].
13. “ Choosing the Right material ,” *Formlabs Support*. [Online]. Available: https://support.formlabs.com/s/article/Choosing-the-Right-Material?language=en_US. [Accessed: 10-Dec-2018].
14. Plastics.ulprospector.com. (2018). Acrylonitrile Butadiene Styrene (ABS) Typical Properties Generic ABS | UL Prospector. [online] Available at: https://plastics.ulprospector.com/generics/1/c/t/acrylonitrile-butadiene-styrene-abs-properties-processing [Accessed 10 Oct. 2018].
15. Plastics.ulprospector.com. (2018). Polylactic Acid (PLA) Typical Properties | UL Prospector. [online] Available at: https://plastics.ulprospector.com/generics/34/c/t/polylactic-acid-pla-properties-processing [Accessed 10 Oct. 2018].
16. 3D Hubs. *Get Started with 3D printing: SOLIDWORKS tips from 3D Hubs* [Online]. Available: https://blogs.SolidWorks.com/tech/2015/07/get-started-3d-printing-SolidWorks-tips-3d-hubs-2.html [Accessed: 9-Oct-2018].

X. Appendix

Appendix A: Product Design Specification

Rodent Rotation and Translation Stage Device

20-Sep-2018

Jamison Miller, Cory Van Beek, Alexus Edwards, Aaron Patterson, Kevin Koesser

Function:

A stage to more precisely position a subject's eye at the center of rotation for easier capture of photoreceptors via microscope.

Client requirements:

- 5 degrees of freedom: If the x-axis is in the direction orthogonal to the pupil, then rotation about the y and the z is required along with translation on all three axes.
- Minimum stage adjustment with 100-micron precision.
- Modular design that allows interchangeable stages for different viewing subjects.
    - Subjects include a detached human eye (2.4 cm diameter, 7.5g) [1], a thirteen lined ground squirrel (33 cm long and 227g) [2], and a white mouse (12.5 - 20 cm long and (12 - 30g) [3].
- Sterilizable between uses via alcohol wipes

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements:

- The device should operate with 5 degrees of freedom keeping the center of rotation about the eye.
    - Degrees of freedom: If the x-axis is in the direction orthogonal to the pupil, then rotation about the y and the z is required along with translation on all three axes.
- The device shall be able to support a weight of 3.0 kg.
- The device will be used on average for 1.0 hour per day in a sterile environment by operating technicians.
- The device shall allow for interchangeable stages that accommodate different sized subjects.
- The device shall allow a warming device to keep the viewing subject at body temperature.

b. Safety:

- The device shall be sterile and protect the operating technician from animal contamination.
- The device shall withstand drops of 1.0 meter without breaking into shards.

c. Accuracy and Reliability:
- The device will be manipulated by hand such that a microscope can focus on a photoreceptor as small as one micron.
- The device shall support a minimum translational motion of 100 microns.

d. Life in Service:
- The device shall maintain optimal function through 500.0 hours of use.

e. Shelf Life:
- The device shall maintain optimal function through 10.0 years in storage at room temperature.

f. Operating Environment:
- The device shall operate between -10.0 and 50.0 degrees celsius.
- The device shall operate between 20.0% and 90.0% humidity.
- The device shall be non-absorbable for water and bodily fluids.
- The device shall be non-photosensitive.

g. Ergonomics:
- The device shall provide simple rotational and translational movements of the stage.

h. Size:
- The device shall not have crevices or open gaps that inhibit cleaning and maintenance.
- The device shall not exceed the following dimensions: 30.0 cm x 30.0 cm x 15.0 cm.

i. Weight:
- The weight shall not exceed 5.0 kg, to provide easy accessibility and movement throughout the lab space.

j. Materials:
- The device shall contain non-absorbing surfaces and must be assembled in a way that limits the number of small creases.
- The materials cost must be within a budget of $250.

k. Aesthetics, Appearance, and Finish:

- Aesthetics, appearance, and finish are not important in this device as it will be used and operated in a research setting.

2. Production Characteristics

a. Quantity:

- 1 unit is needed

b. Target Product Cost:

- The project budget is $250, which includes the costs of manufacturing and testing a prototype. Similar existing products cost over $4,500 [4].

3. Miscellaneous

a. Standards and Specifications:

- This is a custom device being used in a research setting; there are no international or national standards by which to abide.

b. Customer:

- The customer would prefer this device be modular for the use of interchangeable stages to account for varying imaging subjects.
- The customer wants the device to connect to the stabilization table.

c. Patient-related concerns:

- The device must be sterilizable by alcohol wipes between uses.
- There is no storage of patient data involved in this device.

d. Competition:

- Narishige Mag-2 head holding device with angle adjuster for mice [5].
- US Patent #5337178: Tiltable Optic Microscope Stage [6].
    - This stage gives three degrees of freedom. The patent mentions that translational adjustments may be required after rotational movement.

PDS References:

1. E. S. Perkins and H. Davson, “Human eye,” Encyclopedia Britannica, 22-Jun-2018. [Online]. Available: https://www.britannica.com/science/human-eye. [Accessed: 21-Sep-2018].
2. E. C. Cleary and S. R. Craven, “Thirteen-lined Ground Squirrels and Their Control,” Internet Center for Wildlife Damage Management. [Online]. Available: http://icwdm.org/handbook/rodents/13linedgroundsquirrel.asp. [Accessed: 21-Sep-2018].

3. L. Ballenger, “Mus musculus (house mouse),” Animal Diversity Web, 1999. [Online]. Available: http://animaldiversity.org/accounts/Mus_musculus/. [Accessed: 21-Sep-2018].
4. Narishige Pricelist - Tritech Research, Inc.[Online]. Available: http://www.tritechresearch.com/pricelist_nar.html. [Accessed: 21-Sep-2018].
5. “MAG-2 Head Holding Device (with Angle Adjuster / for Mice),” Narishige. [Online]. Available: http://products.narishige-group.com/group1/MAG-2/stereotaxic/english.html. [Accessed: 21-Sep-2018].
6. P. J. Kung and D. E. Sloman, “Tiltable Optical Microscope Stage,” 09-Aug-1994.

Appendix B: Raw Testing Data

Angular Test

Movement	X-axis rotation measurement (in degrees)	Y-axis rotation measurement (in degrees)
1	0.5	0.7
2	0.3	0.6
3	0.2	0.6
4	0.4	0.7
5	0.6	0.6
6	0.6	0.5
7	0.8	0.9
8	0.6	0.5
9	0.4	0.4
10	0.5	0.9
11	0.5	0.4
12	0.4	

Translation Test

Movement	X and Y translation movement measurement (um)

1	110
2	120
3	130
4	160
5	210
6	210
7	290
8	320
9	330
10	410

Appendix C: Materials List

Materials					
Description	**Supplier**	**Part/Model #**	**Link to Part**	**Qty**	**Total**
Aobbomok Chrome Bearing Steel Round Balls	Amazon	CSbb-3-1000	https://www.amazon.com/Aobbmok-1000pcs-Chrome-Bearing-Balls/dp/B079HPKSJ9/ref=sr_1_18?ie=UTF8&qid=1540575840&sr=8-18&keywords=small+ball+bearing#feature-bullets-btf	1	$10.99
Needle Roller Bearing	VXB.com	KIT7891	https://www.vxb.com/ProductDetails.asp?ProductCode=Kit7891	1	$7.70

DIYMAG powerful Neodymium Disc Magnets	Amazon	HLMAG03	https://www.amazon.com/DIYMAG-Powerful-Neodymium-Permanent-Scientific/dp/B06XD2X45M#feature-bullets-btf	1	$9.49
3D Printed Device	Makerspace	N/A	N/A	1	$76.03
Super Glue	Makerspace	N/A	N/A	2	$2.03
Magnet	Makerspace	N/A	N/A	1	$2.40
				Total:	$108.03
				Budget:	$250.00