Mouse Positioning Device for Longitudinal Cancer Research

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Abstract

The Small Animals Imaging Lab at the Wisconsin Institutes for Medical Research provides state-of-the-art, noninvasive imaging techniques to the University of Wisconsin Carbone Cancer Center to monitor the development of cancerous growths in mouse models [8]. The imaging lab uses a Siemens Inveon micro PET/CT scanner, which combines Positron Emission Tomography and Computerized Tomography to identify the locations of cancerous growths within the body. Mouse models are scanned repeatedly over two to three week periods to monitor any changes in cancerous growths. During scans, mice must be secured to the scanner bed with their limbs restrained and their noses secured in the nose-cone, which delivers isofluorane gas to the animals. Lab personnel currently restrain mice by taping them to a rectangular cardboard bed, which is then taped to the carbon fiber scanner bed (Figures 1 and 2). This method of restraint is highly imprecise in repositioning animal models for serial scans over the two to three week monitoring period and lab personnel spend too much time aligning serial images. The Small Animals Imaging Lab would like a more precise, hassle-free device that would allow lab personnel to reposition mice for serial scans [3]. To address this problem our group has built a device that will precisely position a mouse's limbs and body using a peg positioning system. The device attaches easily to the existing carbon fiber scanner bed, attenuates minimally in scans, and will help the lab reposition animals quickly and precisely for scans.

Figure 1: The animal's limbs are secured with tape during a scan. This method makes it difficult to reposition the mouse for consecutive scans.

Figure 2: The cardboard bed attaches to this carbon fiber bed with tape. The carbon fiber bed automatically slides into the scanner during a scan.

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Introduction

Evaluating New Cancer Treatments

The Small Animals Imaging Lab located in the Wisconsin Institutes for Medical Research (WIMR) needs a solid, adjustable device to position mice for Positron Emission Tomography and Computerized Tomography (PET/CT) scans. These scans are used in cancer research to pinpoint and monitor cancerous growths within the animal. The device must restrain the animal’s extremities in case anesthesia fails and must include a quantitative analysis device to replicate the animal's anatomical position. The device should improve efficiency for animal restraint compared to the current cardboard restraint device.

Reasons for a New Device

The Small Animals Imaging Lab at WIMR is a prestigious institution that provides high quality PET/CT images to major imaging companies, UW cancer research facilities, and imaging development research [8]. Jamey Weichert, PhD, is currently working on PET/CT imaging techniques using mouse models for cancer research for the UW Carbone Cancer Center. Lab directors and graduate students depend on a mouse positioning device to ensure the quality of these images and to ensure that time will not be wasted aligning images from serial scans. Unfortunately, the current method for positioning mice during scans does neither. Lab personnel are looking for a device that will effectively restrain the animal subject during a scan, will not interfere significantly with the imaging, and can be used to effectively reposition a mouse for serial scans over a two to three week period [3]. By designing a more effective mouse positioning device, our group can help ensure the integrity of data acquired from these scans, reduce the amount of time wasted restraining mice and aligning images, and reduce distortion in registered images.

Devices Used by Other Labs

Though many devices currently exist for restraining mice during PET/CT scans, these devices tend to be complicated and expensive. For instance, Numira produces a ‘multimodality imaging chamber’ designed to ensure precise repositioning for serial scans (Figure 3). This device uses a disposable foam bed to ensure precise repositioning and provides easy attachment points for tubing during the scan. However, a mouse model of Numira's imaging chamber costs $1650, which is far outside what the lab is willing to spend on an animal imaging chamber [6].

In a study of the methodology of image registration for small animal multimodality imaging, Patrick L. Chow, David B. Stout, Evangelia Komisopolou, and Arion F. Chatziioannou created a custom chamber for holding mice during scans (Figure 4). The device consists of a cylindrical Lucite chamber with removable 'alignment posts for the mouse's limbs'. Though the chamber will not attenuate considerably in PET/CT images, lab attendants must tie down each limb individually to ensure reproducible positioning [1].

In a third type of imaging chamber produced by m2m, mice are secured in an adjustable tube undetectable in PET/CT scans (Figure 5). This device includes a heating mat and is compatible with mounting platforms on the Inveon micro PET/CT scanner. However, this device is priced at $3100, far outside the budget of the lab [9].

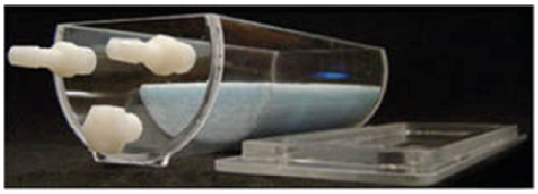

Figure 3: The Numira multimodality imaging chamber with disposable foam bed costs $1650 [6].

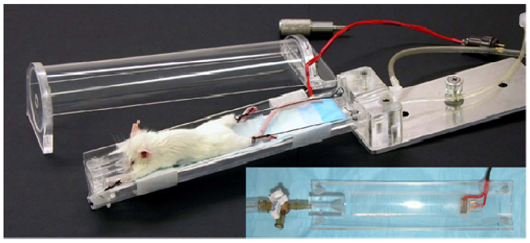

Figure 4: This custom Lucite imaging chamber has removable alignment posts for a mouse's limbs [1].

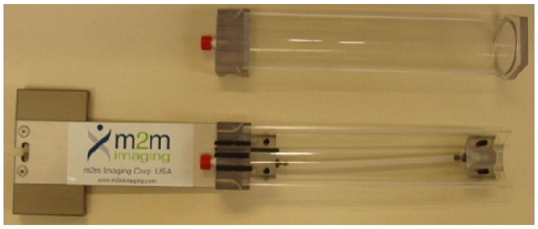

Figure 5: This imaging device from m2m has a heating mat and costs $3100 [9].

Design Criteria

Our design must be compatible with the Siemens Inveon micro PET/CT scanner and equipment currently used by the client. In order to achieve this, our device must be 5’’ to 6” long, no more than 3 1/2’’ wide, and no more than 1/2’’ thick. The device must attach securely to the existing carbon fiber scanner bed and must not interfere with the nose cone attached to the bed. It must be able to accommodate mice ranging from 20-50 grams in weight. The device should be adjustable and include a method of measurement so lab personnel can replicate the anatomical position of the mouse within 1 mm for future scans. The device should be durable enough to withstand multiple scans of 3-10 mice over multiple 2-3 week periods. The material used to make the device must attenuate less than soft tissue so it will not interfere with the image. Carbon fiber would be the preferred material because it is the same material as the scanner bed. The device must restrain each of the mouse’s extremities to prevent the animal from leaving the bed in the event that it wakes up during a scan, and it must conform to RARC and lab protocols for animal safety. It should take minimal effort and no more than 5-10 minutes to restrain the mouse. Since hygiene is important when dealing with animals, the device must be easy to clean between uses and cannot be made of cloth or absorbent material. The target cost for this product is $100 or less.

Overview of Design Alternatives

All of our design alternatives have two things in common: the materials they are made of and how they are attached to the existing carbon fiber bed. The boards for all three designs will be fabricated from the thermoplastic acrylonitrile butadiene styrene (ABS). This material was chosen after we scanned various materials in the lab’s CT scanner. We tested a LEGO piece in the scanner and it had an acceptable density and attenuated less than other tested materials. We determined that LEGOS are made of ABS plastic and decided that ABS would be the best material for our boards [4]. In order to allow for exact repositioning of the device on the bed, two short rods will be attached to the underside of the device where the mouse’s head and tail will be positioned. One rod will be 1/2” from the tail side edge and the other will be 1/2” from the head side edge. Both will be placed 1 3/4” from both the left and right edges. These rods will be glued to the bottom of the device and will fit into two female fittings that have been glued to the scanner bed.

Sliding Velcro Slot Design

The sliding Velcro slot design incorporates an adjustable restraint into a simple board design (Figure 6). The board measures 5 1/2’’ long, 3 1/2” wide, and 1/8’’ in thick. Two 1 1/2’’ long slits separated by 1/4’’ will be cut in each of the four corners of the board. Pairs of slits will be separated 1 1/2’’ widthwise and 1’’ lengthwise. They will be 1’’ from the long edge of the board and 3/4’’ from the short edge of the board. A ruler with English System measurements will be etched on the outside edge of each pair of slits.

Prior to placing a mouse on the board, a 1/4’’ thick and 1’’ long double-sided strip of Velcro with the loops on one side and the hooks on the reverse side will be threaded through each pair of slits, making four Velcro strips in total. Additional Velcro strips will also be provided to replace dirty, misplaced, or worn strips. After the mouse is positioned, the Velcro strips will be tightened around the animal’s wrists and ankles until the mouse is secure. The Velcro strips will slide lengthwise along the body of the mouse to adjust for different sized mice. A ruler along the outside edge of the slits is used to record the location of each Velcro strip to ensure that the mouse will be precisely repositioned on the board for each successive scan.

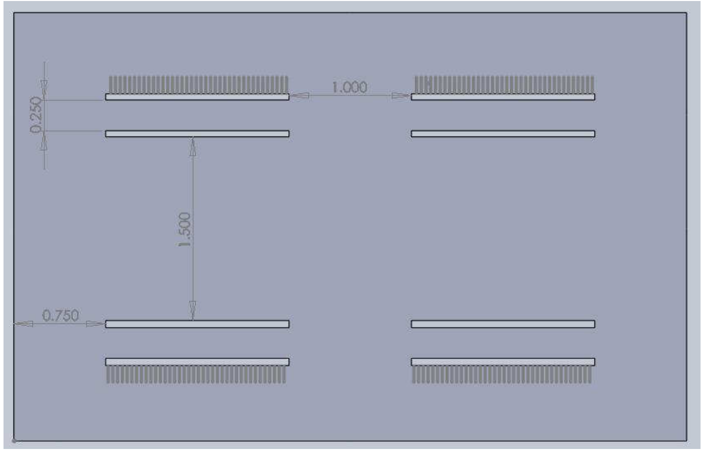

Figure 6: The Velcro slot design has two slits in each corner. A piece of Velcro is threaded through these slits and fastened around each of the mouse’s limbs.

Since the board is flat, the mouse’s body can be outlined with an EXPO marker on the board to enable repositioning of the body in future scans. If the mouse’s body is traced, multiple devices would have to be fabricated for the lab because each mouse would need its own board.

After the mouse is no longer a part of the study, however, the board can be reused. Since the lab does serial scans of 3-10 mice at a time, a maximum of ten boards would have to be fabricated.

LEGO Board Design

The LEGO board design would use a LEGO board, fabricated by the LEGO group, with a length of 5 1/2”, a width of 3 1/2”, and a height of 1/8” (Figure 7). On the top of the board are right circular cylinders that extend 1/16” above the board and have a diameter of 3/16”. The cylinders are evenly spaced in rows and columns with 1/8” between each cylinder. The cylinders on the outer quarter inch of each side will be sanded down to allow a coordinate system of numbers and letters to be placed on the edge of the board. The remainder of the board will have 16 rows and 9 columns of cylinders for a total of 144 cylinders. The coordinate system will consist of the numbers 1-16 along the rows and the letters A-I along the columns.

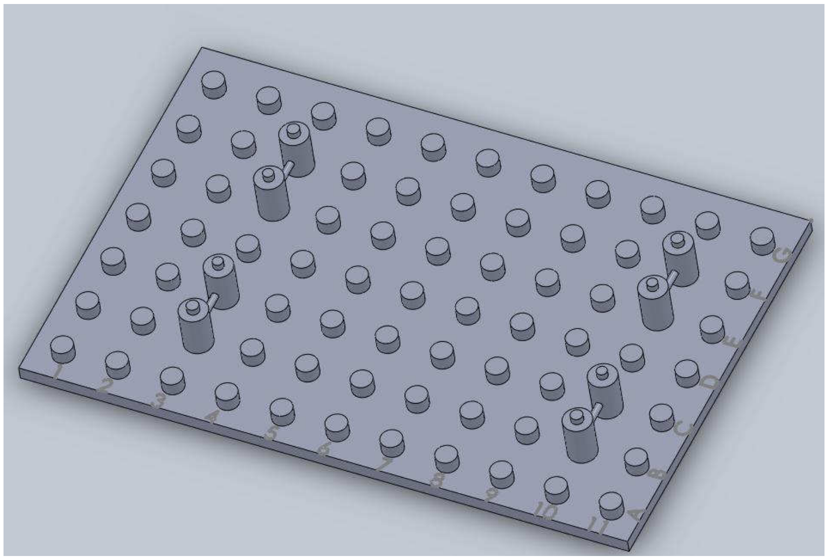

Figure 7: The LEGO board design uses two LEGO pegs connected by rubber bands to restrain each of the animal’s limbs. These LEGO pegs snap into the LEGO board.

The mice will be positioned on the device and LEGO pegs will be placed around the body to prevent it from shifting. The LEGO pegs would have female parts that fit tightly around the raised cylinders on the board. To restrain the mouse’s limbs, pegs made from two small LEGO pieces connected by rubber bands will be snapped in over the animal’s arms and legs. The bands connecting the small LEGO pieces will be made of a rubber material to prevent discomfort for the mouse and guarantee that the device can be cleaned if exposed to radioactive materials.

After the pegs are snapped onto the cylinders, the coordinates of the pegs can be recorded so that the mouse’s position can be replicated in future scans.

Peg Board Design

The third design alternative follows the general idea of the LEGO design, but could be thought of as the inverse of that design (Figure 6). The device has a length of 5 7/16”, a width of 3 9/16”, and a height of 1/8”. 1/16” holes will be drilled through the ABS sheet 1/8” apart. There will be an edge 1/4” wide surrounding the holes. This allows for 27 rows and 17 columns of holes for a total of 459 holes. Because there are so many holes, a coordinate system will be added to the tail side edge and the left edge. Numbers 1-27 will represent the rows and letters A-Q will represent the columns.

After being positioned on the device, the mouse’s limbs will be restrained with bands that have a peg on each end. The pegs on the end of each band will fit precisely into any hole on the peg board. The band will be made of a rubber material so that it is not too uncomfortable for the mice and so that it can be cleaned if exposed to radioactive materials. Only four of these bands will be necessary at any given time, but several different sets will be made to accommodate different sized mice. After the pegs are placed in the holes, the exact hole can be noted for the correct positioning of the mouse in future scans. Since the board is flat, the mouse’s body can be outlined with an EXPO marker on the board to enable repositioning of the body in future scans. If the mouse’s body is traced, multiple devices would have to be fabricated for the lab because each mouse would need its own board. After the mouse is no longer a part of the study, however, the board can be reused. Since the lab does serial scans of 3-10 mice at a time, a maximum of ten boards would have to be fabricated.

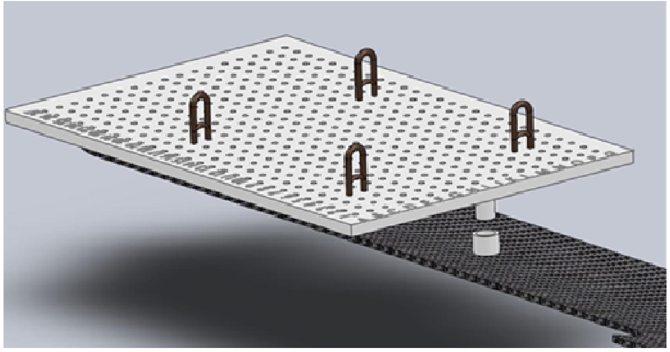

Figure 8: The peg board design consists of a board with many holes. Limb pegs fit around each of the mouse’s limbs and the ends of the pegs then fit into the holes of the board to secure the mouse.

Evaluation of Design Alternatives

In order to choose the final design, a design matrix was created that rated each design alternative on six criteria: accuracy, ease of use/speed of attachment, animal safety, feasibility, sterility, and cost. More weight was given to more important criteria (Table 1).

The accuracy rating reflects the predicted ability of the device to replicate the position of the mouse during subsequent scans. This category was weighted the most because precise anatomical replication is the primary goal of the device. All three designs would be secured to the scanner bed using rods glued to the underside of the device that would fit into slots on the existing carbon fiber bed. This would ensure that the device has the same position relative to the bed every time. However, the three designs differ in repositioning accuracy. The Velcro slot design would not replicate the mouse's position as precisely as the other designs, despite the measurement system alongside the slots. This is because the Velcro strips would not necessarily be secured around the animal’s limbs in the same location for each scan and the Velcro straps could possibly shift after being secured. The LEGO board design would be more precise because the placement of the LEGO pegs could be replicated exactly each time. However, the male ends of the LEGO board are not close enough together to allow for precise placement of the pegs around the shape of the body. The peg board design would be the most precise option because the pegs could be placed in the exact same position every time and the holes in the board would be close enough together to allow for precise placement of pegs around the body. The peg board would also be flat, allowing lab personnel to trace the body of the mouse. This would make it easier to replicate the position of the mouse’s body and limbs.

The ease of use/speed of attachment rating reflects the predicted efficiency of attaching the mouse to the device and attaching the device to the bed. This category was given more weight because efficiency is another important goal for the device. A shorter attachment time will reduce the overall duration of the scanning process and lower the chances of the mouse dying due to loss of body heat. The Velcro slot design would have a difficult attachment process because each Velcro strap would have to be woven down through one slot, up through the other, and then connected around the mouse's leg or arm. This would require two hands and careful manipulation of the straps. The LEGO board attachment process would be much more efficient. LEGO pegs could be placed with one hand, and the attachment locations would be easy to record. The peg board design would be slightly more difficult to use because the holes would be closer together than the LEGO attachments and would also be small, requiring more concentration to place the pegs.

The animal safety rating reflects the predicted safety of the mouse during attachment and scanning. The two issues considered were the ability of the device to restrain the mouse if it were to wake up during a scan and the comfort level of the straps on mouse’s limbs. While animal safety is an important part of the design, it was not given a lot of weight because all three designs would be sufficient to restrain the mouse and would conform to RARC protocol. The mouse would have little ability to escape the Velcro slot design because the Velcro straps would be wrapped tightly around its arms and legs. However, the Velcro material is rough and could cause some discomfort. Both the LEGO and peg board designs would use a rubber strap that would be more comfortable than the Velcro strap, and still secure the mouse’s limbs. One concern with the peg board design is that if the pegs do not fit tightly into the holes, it would be easier for the mouse to escape if anesthesia failed.

The feasibility rating reflects the team's predicted ability to fabricate a prototype of the design before the end of the semester. All three designs would be possible to fabricate so feasibility was not as heavily weighted in our final design selection. The Velcro slot design would be the most difficult to fabricate because eight thin slots would have to be cut in the board. The LEGO board design would be the easiest to fabricate because the LEGO board and LEGO pegs are existing products. Only fabrication of the strap would be necessary. The peg board would be slightly more difficult to fabricate because holes would have to be drilled in the board in addition to the fabrication of the straps.

The sterility rating reflects how easy it would be to clean the device. While the ability to clean the device is a requirement for the device, it is not one of the main goals of the project and was not given a lot of weight. The Velcro slot design would be hard to keep clean because Velcro is not smooth and would be hard to wipe off. The LEGO board would be able to be wiped off but the raised and indented parts on the board would be hard to wipe completely. The peg board would be easier to clean because it would be a completely flat surface.

The cost of the device was not an important factor in our decision because the materials that will be used are inexpensive and well within the $100 budget. The Velcro slot design would be more costly because the Velcro straps would have to be replaced if they became too dirty.

Table 1: After completing a design matrix, the peg board design and LEGO board design had similar point values.

Criteria	Velcro Slot	LEGO Board	Peg Board
Accuracy (35)	20	25	33
Ease of Use/Speed of Attachment (20)	12	18	16
Animal Safety (15)	12	12	10
Feasibility (15)	10	14	12
Sterility (10)	8	9	10
Cost (5)	3	5	5
TOTAL (100)	**65**	**83**	**86**

Rationalization for Final Design Choice

We chose the peg board design as our final design because it meets our client's needs, is the most precise, and will be the easiest to clean. The accuracy of the peg board design will be sufficient because the position of the mouse will be easily replicated from scan to scan. If the user records the coordinates of each arm and leg peg after positioning the mouse on the device for the first scan, the user will know exactly where to put the arms and legs on the device for every proceeding scan. After the arm and leg coordinates have been recorded, the user will use an EXPO marker to outline the body of the mouse. This will ensure the mouse's body position is within 1 mm of preceding scans. The device will be precisely positioned on the bed of the scanner via the two rods on the device and the two female fittings on the bed of the scanner. When the device is positioned on the bed, it will be in the exact same spot relative to the scanner bed. Then the user will use the zeroing lasers on the scanner to put the bed of the scanner in the correct location. This method will ensure the mouse is positioned within 1 mm of its original position each time it is scanned. The peg board design is more precise than the LEGO board design because the increments between coordinates are smaller. It is more precise than the Velcro slot design because the Velcro slot design is prone to user error when attaching the mouse to the device.

The speed of positioning the mouse in the scanner will be greatly increased with the peg board design in comparison with the current method. One of the main problems with the current method is the use of tape to restrain the animal on a cardboard sheet and to connect the cardboard sheet to the scanner bed. The tape often sticks to the wrong part of the bed, the animal, or itself while the user is trying to attach it to the animal. With the peg board, the pegs will be easy to push into the correct positions in a timely manner. The user will know exactly where the mouse has to go so it will not take as long trying to repeatedly reposition the mouse to get it to line up with the preceding scans. The user will also be able to detach the mouse quickly by pulling out the pegs rather than removing tape from the mouse and the bed. It will also take less time to attach the peg board to the bed than the current method. The peg board design will simply sit into the two female fittings on the bed. The current method requires taping the cardboard sheet with the attached mouse onto the scanner bed, which takes too much time to precisely reposition the cardboard sheet. The LEGO board design would take less time to attach the mouse because the increments are bigger and it is easier to line up the pegs. The Velcro slot design would take longer because it would be hard to hold the mouse limb in the correct position and put the Velcro around it at the same time.

The peg board device will effectively keep the mice safe from injury. The restraint pegs will not allow mice to fall into the scanner if they wake up during the scans. The restraint bands will be made of rubber so that the mice's limbs will not be crushed when the pegs are pushed into the peg board.

The peg board device will be feasible to fabricate because it does not involve complex machining or materials. ABS is a readily available plastic that is easily machinable. The only machining necessary is the drilling of the holes in a grid pattern. This can be done with a CNC mill available to the team.

The device will also be easy to clean because it is smooth and easy to wipe down. It will be able to withstand continual cleaning with the cleaning solution Lift-Away. The device is smooth and will be easy to wipe off. The Velcro design would be hard to clean because the Velcro would retain small particles that cannot be wiped away. The LEGO board design would also be harder to clean because it has many ridges on the top surface and many craters on the under surface. These obstructions would make it hard to wipe the LEGO board clean.

The cost of all three designs will be well within the $100 budget. Each design involves the use of ABS plastic which is inexpensive. A 12”× 12”×1/8” sheet of ABS plastic can be bought for a price as low as $7.76 from McMaster-Car [7]. This is enough material to make six peg board devices not including pegs and bed connectors. Production costs for the LEGO Board and the peg board designs will be almost identical. The Velcro slot design will be more expensive to maintain because it would require the continual purchase of Velcro strips to replace the old ones when they become dirty or worn.

Final Positioning Device Design

Since the mid-semester presentation, the final design has acquired some changes. The final design consists of a 5 1/2”×3”×1/8” ABS plastic board, four limb pegs, and three body pegs (Figure 9). The width of the board was changed from 3 1/2’’ to 3’’ to allow for more room on either side of the board so the device will easily clear the sides of the scanner tube. There is a grid system of 1/16” diameter holes on the board consisting of 27 rows and 14 columns, making a total of 378 holes. We decreased the number of columns after we performed a preliminary test scan with our board. The team realized it would be easier to reposition the pegs if the letters were drawn on both sides of the board and if the numbers were drawn on both the top and bottom of the board. To have space on the board to do this, three columns of holes were removed. The holes are 3/16” away from each other center to center. The outside holes are centered 9/32” away from each side, which allows for 1/4” of solid material between the edge and the edges of the holes, where a coordinate system is written. Numbers 1-27 are written on the two long edges of the board to represent the rows and letters A-N are written on the other two edges to represent the columns. In between columns F and G a line is etched into the peg board. This was also added after the preliminary test scan. The line on the peg board can be used to ensure the body of the mouse is lined up with a laser that is attached to the scanner. This allows for the mouse to be located in the center of the scanner for each scan and thus allows for better consistency of scans. Two ABS rods with a diameter of 1/4’’ and a length of 0.215’’ are located 1 1/2’’ from the long edge of the board and 0.656’’ from the short edge of the board. These rods fit into two 0.14’’ long ABS tubes with a 1/4’’ hole bored in the centers which are attached to the carbon fiber scanner bed. This ensures that the board will be located at the same position on the scanner bed each time.

The mouse is restrained by four limb pegs and three body pegs. The limb pegs consist of 1 1/2’’ long, 1/16’’ diameter natural Halar miniature cord sections that have been bent in half and sanded at the ends so they fit into the peg board holes. An orthodontic rubber band is located 3/16” above the bottom of the pegs and fits snugly around the mouse’s limbs to restrain the mouse without being too uncomfortable. The body pegs consist of the same material as the limb pegs. The body pegs are about 3’’ long and are also sanded at the ends. After our preliminary test scan, we decided that two or three body pegs might help to keep the animal’s body from shifting throughout scans. After the pegs are placed in the holes, the exact location of the pegs can be noted using the coordinate system for precise repositioning in future scans.

The ABS plastic is easy to clean and allows lab personnel to be able to outline the mouse’s body with an EXPO marker to speed up the attachment process. We fabricated one initial prototype and after doing an initial test scan with that prototype, we made changes which led to our final design. Five prototypes (including the necessary limb and body pegs) of our final design were fabricated. Some extra limb and body pegs were also fabricated for the lab in case some are lost. This allows each mouse in a study to have its own bed so the outline of each mouse can be traced on the respective board. The outline can then be erased at the end of the study and the board can be reused.

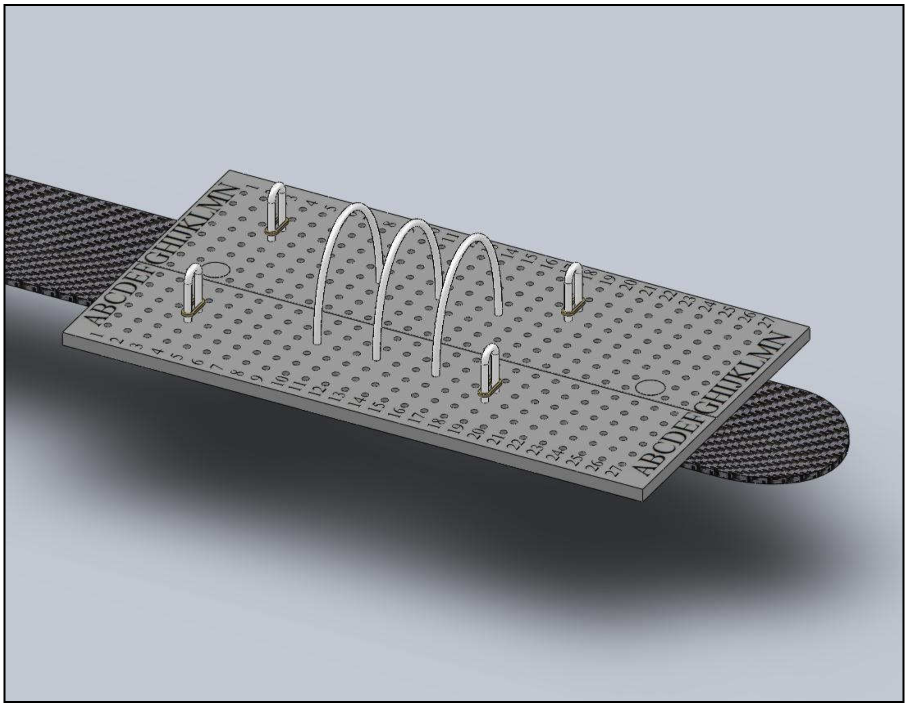

Figure 9: Our final prototype is compatible with the lab’s existing carbon fiber bed and consists of a peg board with a grid system on it, four limb pegs, and three body pegs.

Analysis of Expenses

Table 2: The total expenses for the team this semester was $23.32.

Item	Manufacturer	Cost
12”×12”×1/8’’ ABS Plastic Sheet	McMaster-Carr	$7.76
5’ ABS Plastic Rod 1/2” outer diameter 1/4” inner diameter	McMaster-Carr	$7.11
5’ ABS Plastic Rod 1/4” diameter	McMaster-Carr	$2.80
10’ Natural Halar Miniature Cord 1/16” diameter	McMaster-Carr	$3.66
Orthodontic Rubber Bands	Donated	$0.00
Super Glue	Ace Hardware	$1.99
		Total: $23.32 Added costs: material shipping

Our team purchased an ABS plastic sheet, two ABS plastic rods, and a plastic cord from McMaster-Carr for a total of $21.33 (Table 2). The orthodontic rubber bands used for the limb pegs were donated by Dr. Steven D. Peterson from Orthodontic Specialists of Madison. We purchased super glue at Ace Hardware for $1.99. The total expenses for were $23.32. The cost per prototype was calculated to be approximately $1.66 which was well within the budget specified by the client (Table 3).

Table 3: The total cost for each prototype is $1.66 and is within the team's budget.

Item/Material	Cost
5 1/2'' ×3''×1/8'' ABS Plastic Sheet	$0.97
1/2" ABS Plastic Rod 1/2" outer diameter 1/4" inner diameter	$0.059
1/2" ABS Plastic Rod 1/4" diameter	$0.023
20" Natural Halar Miniature Cord 1/16" diameter	$0.61
Orthodontic Rubber Bands	$0.00
Super Glue	Unknown per prototype
	Total per prototype: $1.66

Ergonomics

The device incorporates many aspects of universal design. The device will be symmetrical to encourage equal use from left-handed and right-handed users. The user should be able to attach and remove the pegs, the mouse, and the device with minimal effort. Early on, we realized that the small size of the pegs may hinder ease of use. We took this into consideration and made our limb pegs with two components. The main component of the limb peg is large enough so that it can be gripped easily, while the orthodontic rubber bands are glued lower down on the limb peg so that the peg will fit snugly across the animal's limb. Minimal moving parts will make the device intuitive to use and will avoid any user confusion. The numerical and letter coordinate system is written on all sides of the board to allow for easier repositioning of the pegs on either side of the animal. To accommodate users of all literacy abilities, there will be no writing on the device except numerical and letter coordinate indicators. It will also be simple enough that an instruction manual on how to use the device could consist of pictures with no words to demonstrate each step of use. The device will consist of simple parts that are easily taken apart and put back together for cleaning and repairing.

Fabrication Process

The first step to the fabrication of our device is to rough cut a piece of ABS plastic with a band saw to the dimensions 5 3/4”×3 1/4”×1/8” from a sheet of ABS plastic that is 1/8” thick, as shown in Figure 10.

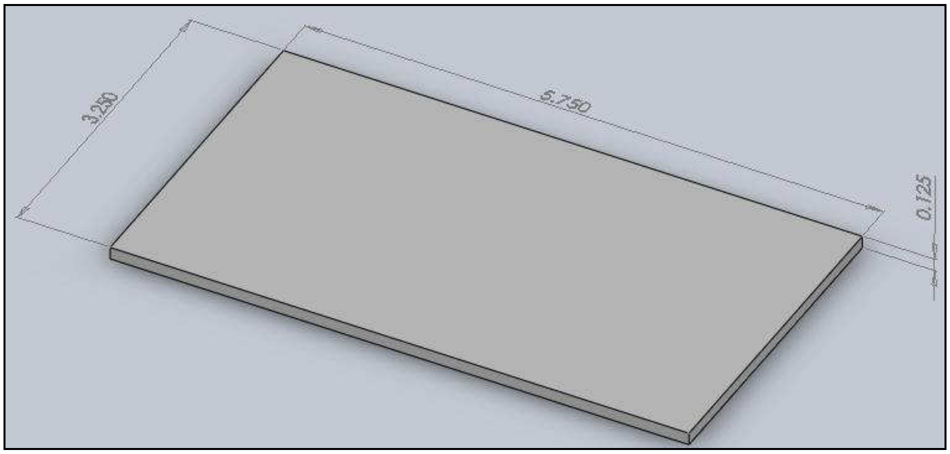

Figure 10: The ABS plastic sheet is first rough cut and has the dimensions 5 3/4"×3 1/4”×1/8”.

After the board is cut to the specified dimensions, it is finish cut with the mill. The piece is clamped into the mill vice and then using a 1/2” mill bit some material is taken off each side to make sure that all rough cuts are removed and replaced with a finished cut from the mill bit. Then the sides are taken down to the correct dimensions of 5 1/2”×3”×1/8” using the digital readouts on the mill. The final dimensions are shown in Figure 11.

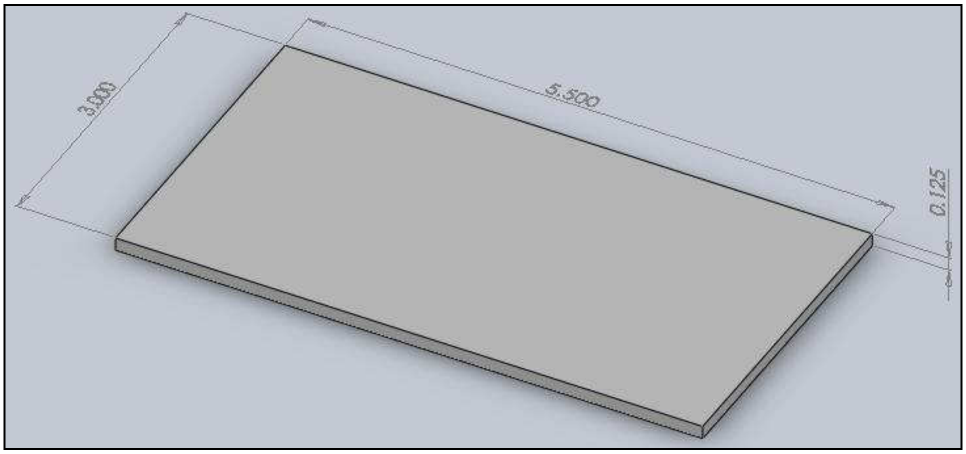

Figure 11: The final dimensions of the board are 5 1/2"×3”×1/8”.

Then the grid holes are drilled. To do this the piece is placed once again in the mill. The edges of the plastic are found with the mill edge finder. Once the edges are found in the X-Y plane, a program can be written to position the grid holes with the hole placement dimensions shown in Figure 12. The outside holes are centered 9/32” away from each side, which allows for 1/4” of solid material between the edge and the edges of the holes. The holes are centered 3/16” away from each other. Each hole has a diameter of 1/16”. Once the the program is written, the mill will place the drill bit over the location of the desired hole. Then the drill bit is manually lowered into the material and the hole is drilled. After each hole is drilled, the mill repositions the bit to the next hole location and this process continues until all of the holes are drilled as shown in Figure 13.

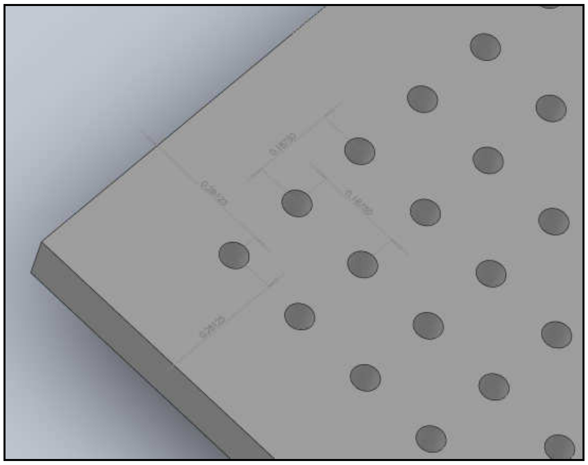

Figure 12: The grid holes have a diameter of 1/16” and are 3/16” away from each other center to center.

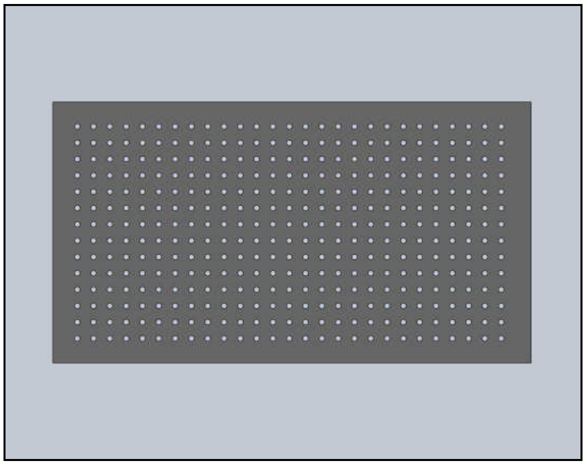

Figure 13: 378 grid holes are drilled in total.

Next, holes are drilled 1 1/2” from the long edge and 0.656” from the short edge as shown in Figures 14 and 15. Each hole is drilled with an E sized drill bit. This is done by marking the hole center on the plastic and using a drill press to drill the holes.

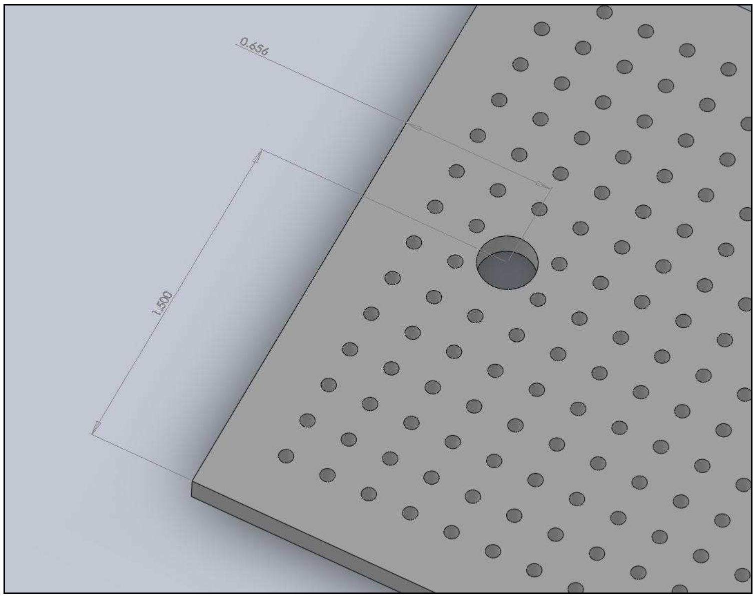

Figure 14: Two holes are drilled 1 1/2" from the long edge and 0.656” from the short edge of the board.

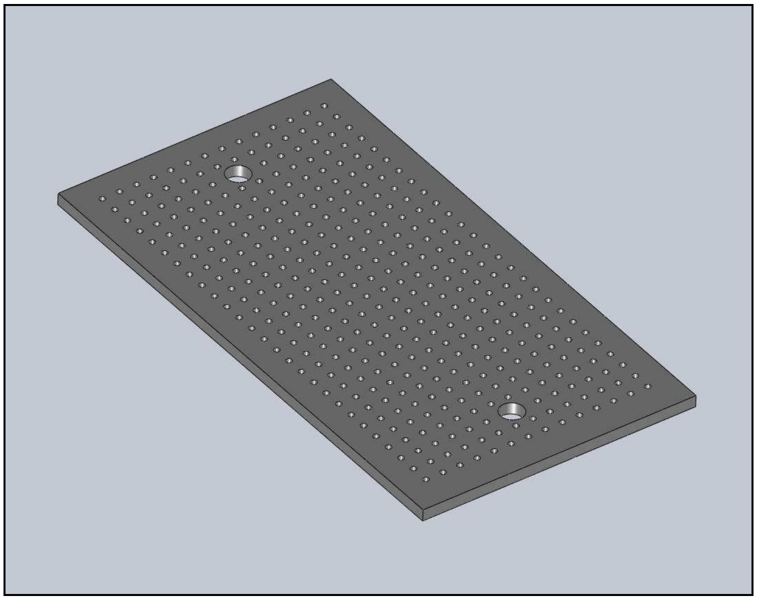

Figure 15: The ABS rods will be positioned in these two holes.

A 1/4”diameter, 1/4” length ABS rod is inserted in each of the holes previously drilled. Each rod is rough cut to a length slightly greater than 0.215” using a band saw. To cut the rods properly, the ABS rod is placed in a vice to make sure the rod does not start spinning while being cut. The vice is used to push the rod throught the saw. Then each rod is sanded down to the proper length of 0.215”. Length is determined by using digital calipers. Figure 16 shows one of the rods.

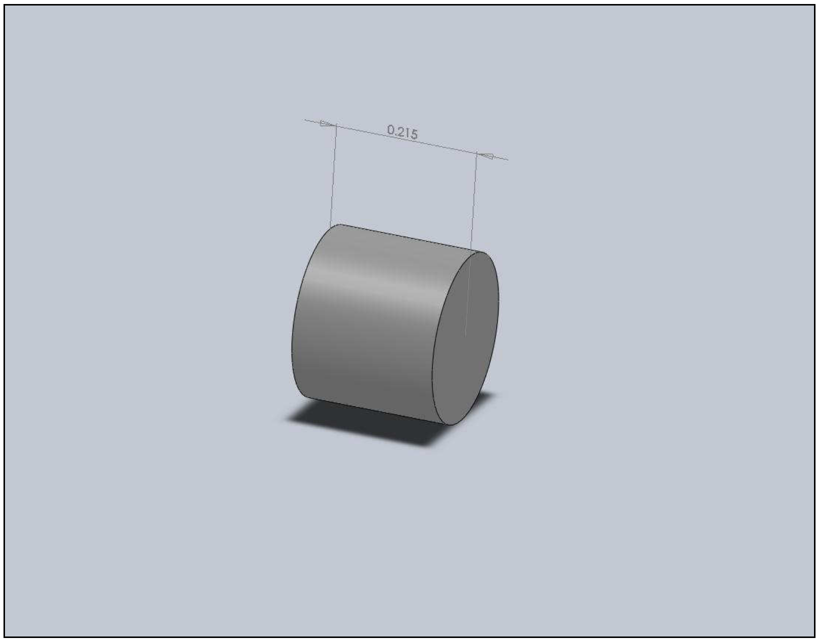

Figure 16: The ABS rods are 0.215” long.

The rods are pressure fit into the holes using a rubber mallet to pound them into place. Figure 17 is a view of this assembly from the top and Figure 18 shows the assembly from the bottom.

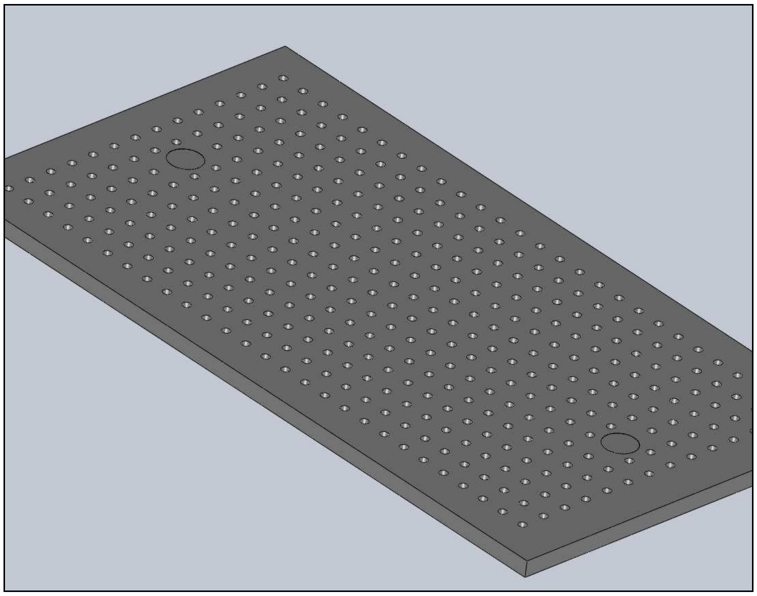

Figure 17: The ABS rods are flush with the top of the board.

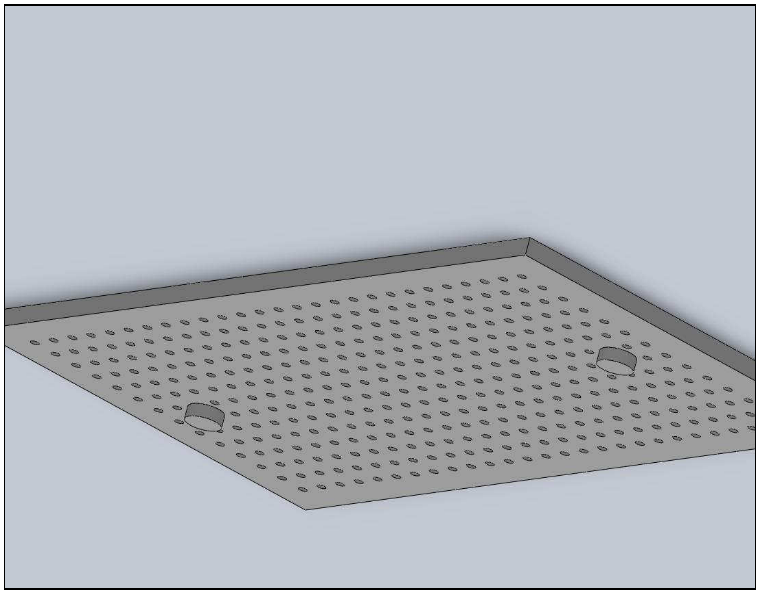

Figure 18: The ABS rods stick out from the bottom of the board so that they will fit into ABS tubes on the scanner bed.

To fasten the peg board to the scanner bed, two 1/4" inside diameter, 1/2” outside diameter ABS tubes are used. Before they are cut to length, 3” sections of the tube are bored out on the drill press using 1/4” drill bit. This is done because the tolerance of the inside tube was large and the 1/4” diameter rod did not fit in it. Then they are cut to a length slightly greater than 0.14” using the band saw and the same method used for the ABS rods described earlier. After they are rough cut, the tubes are sanded and measured to the correct dimensions with the same method used on the ABS rods. A tube is shown in Figure 19.

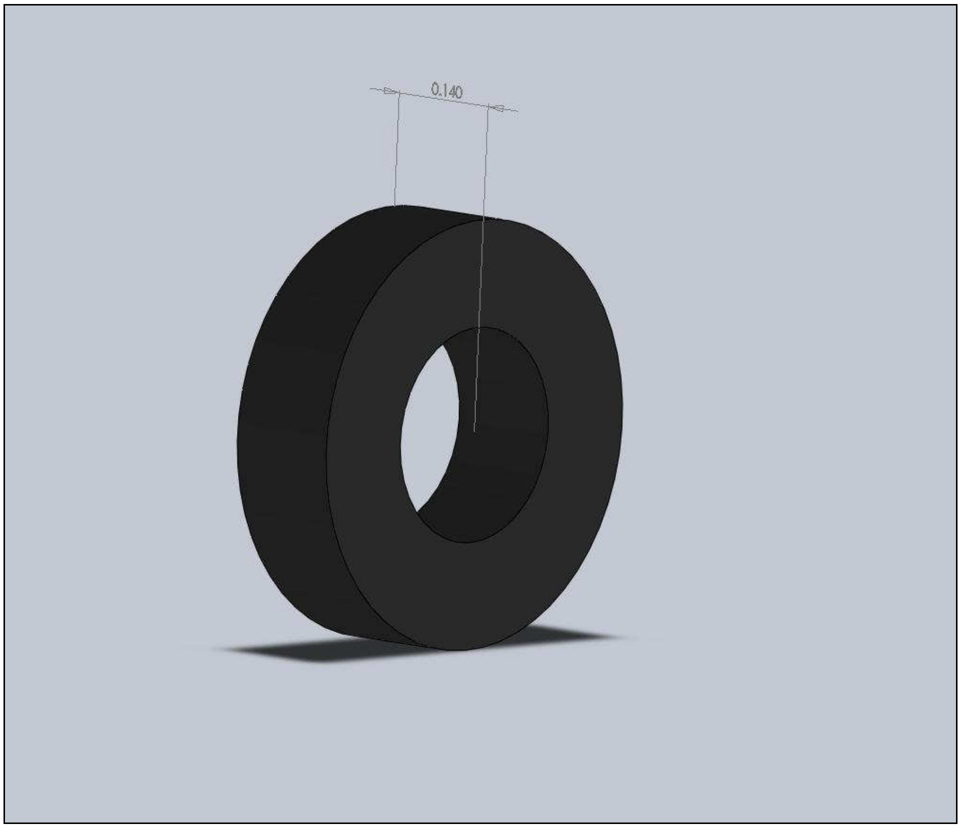

Figure 19: The ABS tubes are 0.14” thick and have an inner diameter of 1/4".

To fit the ABS tubes correctly to the carbon fiber scanner bed, they need to have the same curvature as the bed on the side of the tube that connects to the bed. To create this curvature on the ABS tubing, the curvature of the bed is lined with sandpaper and then each tube is sanded down inside the bed to ensure the curvatures are the same. Figure 20 shows where the tubes will be placed on the scanner bed.

Figure 20: The ABS tubes will be glued to the scanner bed.

Once the tubes have the correct curvature they are placed on the bottom of the peg board as shown in Figure 21. Then a small amount of super glue is applied around the bottom of each tube being careful not to get any on the inside of the tube. If this happens the peg board becomes glued to the scanner bed as well. Once the glue is applied to the tubes, the bed is carefully set down onto the scanner bed in the desired location marked out on the bed by the lab technician. The glue is allowed to set for fifteen minutes before removal of the bed.

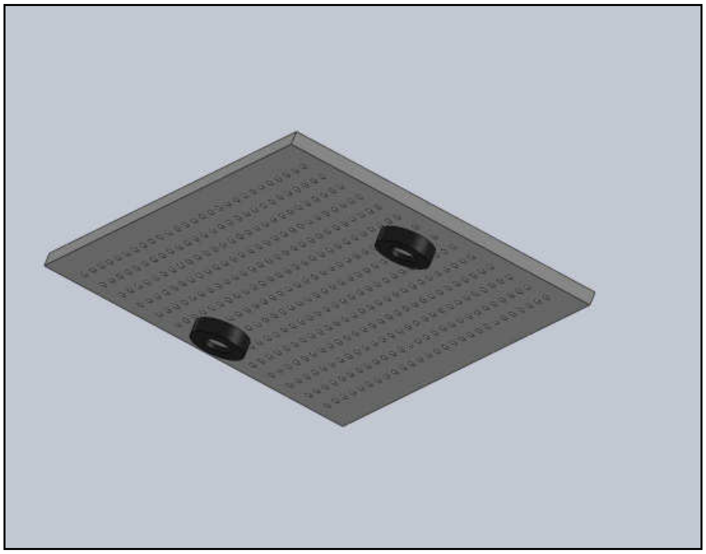

Figure 21: The ABS tubes are placed on the bottom of the board before being glued to the scanner bed so that they will be positioned correctly.

To make the limb and body pegs, 1/16" diameter natural Halar miniature cord is used. Sections of the cord are cut to 1 1/2" sections. Each section is bent in half as shown in Figure 22. Each end of the cord is lightly sanded with sandpaper to create a tapered end that will fit into the peg board holes. Then an orthodontic rubber band is glued 3/16" above the bottom of the pegs as shown in Figure 23. To make the body pegs shown in Figure 24, 3" sections of the cord are cut, and the tips sanded to a taper the same way as the limb pegs. The body pegs are bent slightly in order to roughly match the geometry shown in Figure 24. This is not exact because they are designed to be flexible to fit the positioning needs of the user.

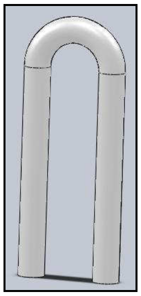

Figure 22: The edges of 1 1/2" sections of plastic cord are sanded and the cord is then bent in half to form the peg grip.

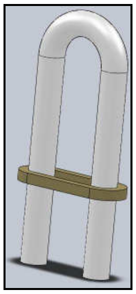

Figure 23: An orthodontic rubber band is glued 3/16" above the bottom of each peg.

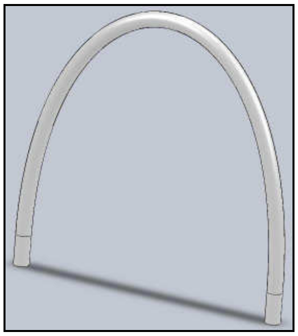

Figure 24: The edges of 3" sections of plastic cord are sanded and the cord is bent slightly to form the body pegs.

The positioning laser in each scanner differs. To ensure the body of the mouse is in the center of the scanner, the body is lined up with a laser marking the center of the scanner tube. To make sure the mouse will be lined up with the laser, a line where the laser will appear is etched into the peg board. Once the glue is set, the peg board can be attached to the scanner bed and the laser position can be marked. Then using an X-Acto knife and a straight edge, a thin line is etched into the peg board as shown in Figure 25.

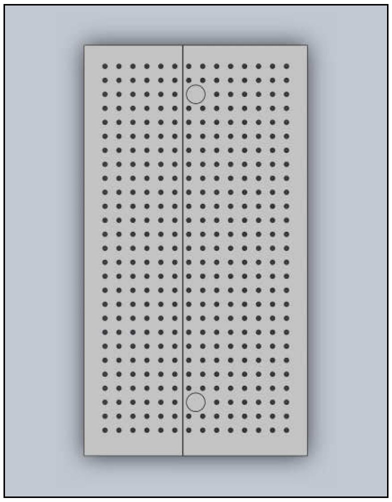

Figure 25: A line is etched into the peg board with an X-Acto knife.

The next step in the fabrication process is to assign letters and numbers to the rows and columns as shown in Figure 26. This is done with a steady hand and a fine tipped black permanent marker.

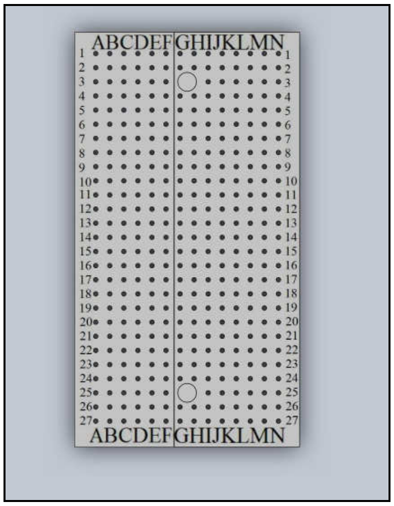

Figure 26: The numerical and letter coordinate system is written on all edges of the board in permanent marker.

After all the pieces are fabricated, they can be assembled as shown in Figures 27, 28, and 29.

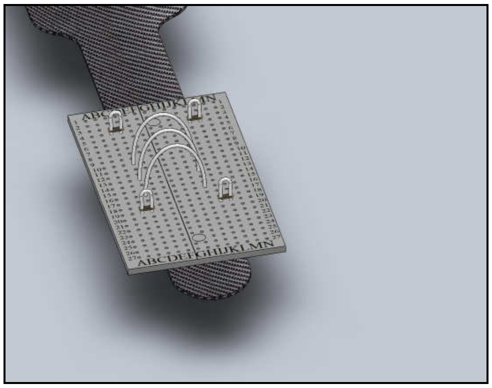

Figure 27: This is a front view of the finished peg board attached to the scanner bed.

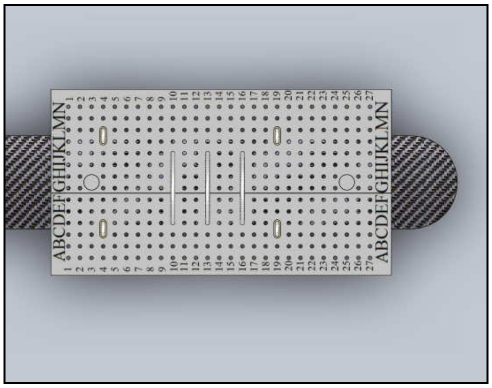

Figure 28: This is a top view of the finished peg board attached to the scanner bed.

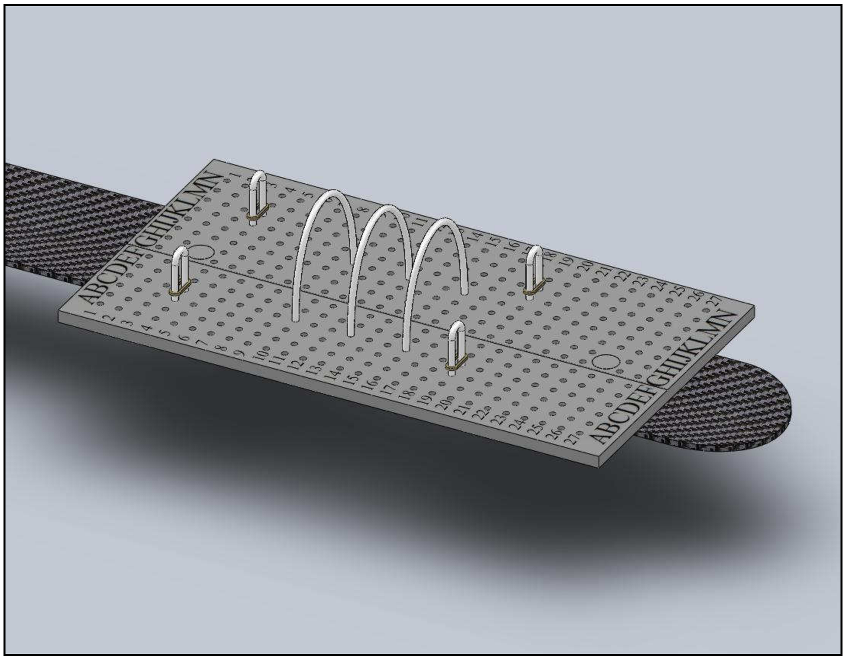

Figure 29: This is an isometric view of the finished peg board attached to the scanner bed.

Testing

The interference of the materials with the imaging, efficiency of the attachment process, and ability of the device to replicate the position of the mouse over multiple scans were tested. To test the materials' attenuation, the board and pegs were scanned. Using a feature of the Inveon imaging software, the attenuation coefficients were obtained for the ABS board and the plastic limb pegs. These values are in Hounsfield units and are listed with the attenuation coefficients of several other materials in Table 4. A lower attenuation coefficient means that the material interferes less with the imaging and an attenuation coefficient less than that of soft tissue is desired. The -800 HU value for the ABS plastic is low and means that the ABS board will not affect the quality of the image. The 40 HU value for the plastic pegs is higher than desired but is acceptable because the pegs are such a small part of the image. The interference of the limb pegs on the image is not a concern because the lab's studies focus on the mouse's body and the limbs are positioned in front of and behind the body. The body pegs can easily be removed from the image using the imaging software because the attenuation coefficient of the plastic is much different than that of the surrounding soft tissue.

Table 4: Using the lab's Amira imaging software program and a test scan of a mouse on our peg board, we found the Hounsfield units our ABS plastic peg board and our plastic limb and body pegs.

Material	Attenuation Coefficient (HU)
Air	-1024
Carbon Fiber	-825
ABS Plastic	-800
Soft Tissue	-200
Water	0
Plastic Pegs	40
Bone	>400

To test the efficiency of the attachment process and the precision of the positioning system, the device was used to scan the same mouse eight times (Figure 30). The mouse was attached to the device, scanned, and then removed from the device each time. This simulated the mouse being scanned on different days and being repositioned each time. For each scan, the amount of time needed to place the mouse on the device, attach the pegs, and prepare the mouse for a scan was recorded. The average time was 2 minutes and 49 seconds. Each individual time can be found in Table 5 of Appendix B. This attachment time was well under the desired 5-10 minutes.

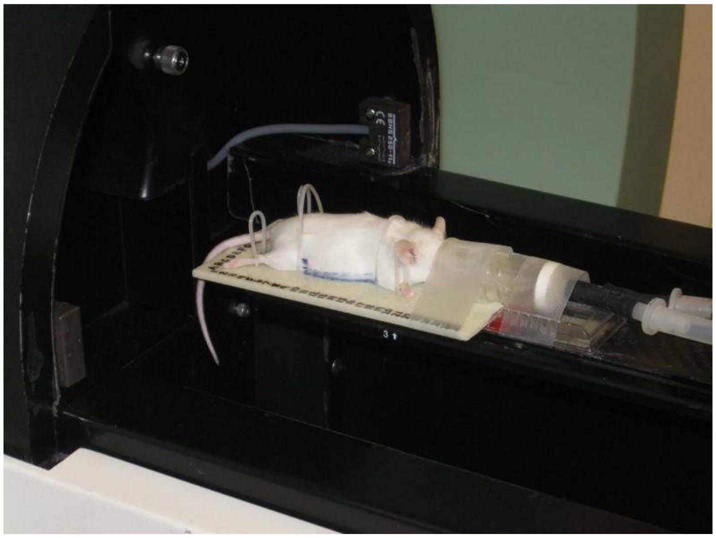

Figure 30: In order to test our device, a mouse was positioned on our board and a CT scan was performed. This process was repeated with the same mouse eight times.

The image from each scan was opened in Amira, an imaging software program in the lab. The affine registration feature was used to compare the position of the mouse in each image to its original position. Registration attempts to align one image with another by translating and rotating the image (Figure 31). After registration, the software displays how far the image had to be translated and rotated in each direction. With these results (shown in Table 6 of Appendix B), we were able to measure the difference in the mouse's position from the original scan.

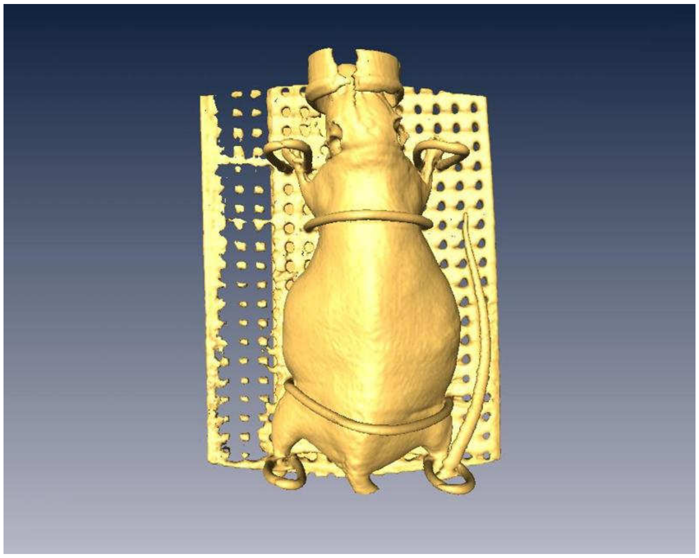

Figure 31: The registration of images from eight test scans of the same mouse was performed using the lab's Amira imaging software program. This is a picture of what one CT scan looks like in Amira.

The x-axis pointed from the mouse's tail to head, the z-axis from the mouse's left side to right side and the y-axis from the mouse's belly to its back. The translation in the x and z directions were used to find the total distance that each image had to be translated. The y direction was not considered because the vertical position of the mouse during the scan is controlled by the scanner rather than our device. The mouse's position can only change in two dimensions on our device. The data from the first and eighth scans were not used because the x-axis laser on the scanner was not positioned correctly and this affected registration data significantly. For the other scans, the laser was at the same number position on the board. The third through seventh scans were then each compared to the second scan to obtain the positioning data. This created a total of five data sets that were then averaged.

Using our device, the images had to be translated an average of 0.93 ± 1.49 mm in the x-z plane and rotated an average of 0.07 ± 0.11 degrees about the x-axis, 0.15 ± 0.30 degrees about the y-axis, and 0.25 ± 0.25 degrees about the z-axis.

To see if our device improved the replication of the mouse's position, we registered a series of scans from a past study that used the current method of attachment and positioning. The data obtained from this registration can be found in Table 7 of Appendix B. Twelve images were compared to an original image for our analysis of the current method. We found that on average the images had to be translated 3.88 ± 4.01 mm in the x-z plane and rotated 0.90 ± 1.50 degrees about the x-axis, 2.62 ± 2.66 degrees about the y-axis, and 1.44 ± 2.20 degrees about the z-axis.

These results show that our device significantly improved the precision of the mouse positioning. Also, on average, our replication of position was within the desired 1 mm.

Ethical Considerations

The device will be used for medical research on live animals, and therefore must conform to RARC and lab protocols for animal safety [3]. This will ensure that the device is ethically allowed for research. Any ethical concerns with the device would stem from restraint protocol. According to RARC protocol, physical restraint is defined as “the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination.” Primary concerns with restraint occur when the animal is subjected to prolonged restraint lasting longer than 10-15 minutes, or when the animal has a chance of harming itself. When our device is used, mice will be anesthetized and the device will not be restraining any movement. If the animal wakes up, the animal will not be restrained by the device for longer than 10-15 minutes, and constant monitoring will ensure that the animal will not harm itself [2].

Future Work

Further tests could be implemented to evaluate our device. Ideally, it would be necessary to complete at least 29 scans of the same mouse to obtain repositioning data with 95% power. This could not be completed due to lack of time. These 29 scans would need to be completed over at least a month time period as the mouse cannot withstand intense amounts of radiation exposure or many doses of anesthesia. Several other tests that could be completed include testing the durability of the pegs, testing if the device restrains the mouse effectively, and further testing of the attachment time. Because the placement pegs will be removed so often, wear and tear will ensue. It would be beneficial to determine how many times the pegs can be inserted and removed from the peg board before their properties have been compromised and they can no longer be used. For example, the orthodontic bands might break or the pegs themselves might break with extensive use. In the past, the lab has had problems with the anesthesia failing and the mouse waking up in the scanner. To see if our device adequately restrains the mouse, a mouse could be restrained on a bed without anesthesia and we could determine how long it would take the mouse to escape. Furthermore, it would be best to time animal attachment in an actual lab study. This time would be more accurate than the times we obtained because during our test scans the attachment method was cruder than what would take place in an actual study.

The lab would like to see a heating mechanism incorporated into the peg board design. Sometimes mice die during scans because the lab is too cold and the anesthesia causes the body temperature to decrease. Fans within the scanner are needed to cool down the scanner, which gets hot during scans, but the cool air these fans blow also affects the mouse's body temperature. Several features the lab would like to see include a half cylinder dome that would be compatible with any peg board. Perhaps it could have a hinged peg that would fit into the holes of the boards. A thermoregulation system with an inflow and outtake would be necessary to monitor the air temperature. This new apparatus would need to include the anesthesia tubes which slide into the scanner with the bed.

Another small item that could help the lab would be a small box that would attach directly to the scanner and hold the pegs. Because the pegs are so small, it will be easy for them to get lost. If the pegs were attached to the scanner, they would be within an arm's reach when restraining the mouse.

The lab sometimes does studies that involve rats. If rats were used in a study, a larger peg board would be needed. In the future, larger boards with a similar design could be fabricated.

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Appendix A

Product Design Specifications: Mouse Restraint Device

October 8, 2009
Eamon Bernardoni, Vanessa Grosskopf, Jim Mott, Samantha Paulsen, Brooke Sampone

Problem Statement:

The Small Animals Imaging Lab located in the Wisconsin Institutes for Medical Research is in need of a solid, adjustable device that will restrain mice during Positron Emission Tomography and Computerized Tomography (PET/CT) scans. These scans are used to pinpoint cancerous growths within the animal prior to treatment. The device must restrain the animal's extremities to prevent movement and must include a quantitative analysis device to replicate the animal's exact anatomical position. The device should improve efficiency for animal restraint compared to the current cardboard restraint device.

Client Requirements:

- Adjustable device to fit mice and accommodate 20-50 grams
- Device should be 5"- 6" in length to fit a mouse
- Device should not interfere with nose cone
- Device should include measurement device to replicate exact position of mouse
- Material should not interfere with imaging from PET/CT scanner and should not include cloth
- Method of restraining animal should take no longer than 10 minutes
- Device should prevent the animal from leaving the bed in the event that it wakes up
- Must attach to carbon fiber bed

Design Restraints:

1. Physical and Operational Requirements

a. Performance requirements: The device should accommodate mice ranging in size from 5"-6" in length and 20-50 grams. The mouse should be restrained in less than 10 minutes. 3-10 mice will be scanned several times over a period of roughly two weeks. 100 animals per year are scanned by the PET/CT machines. The device should be securely attached to the bed.

b. Safety: The device should conform to RARC and lab protocol. No animals should be harmed by the device. Absorbent material should not be used as to prevent retention of radioactive substances.

c. Accuracy and Reliability: The device should allow for the exact alignment of the anatomical position of each mouse over the duration of the study within 1 mm of the original position of the animal. Lasers are used to help align the position of the animal once it is attached to the bed.

d. Life in Service: The device will be used for approximately 400 hours each year.

e. Shelf life: If sliders are incorporated in the design, lubrication of the device may be necessary. Depending on the type of attachment, attachment material may need to be replaced once worn.

f. Operating Environment: The device will be used in the research laboratory. There may be corrosion of materials due to lubrication of the device and radioactive liquids.

g. Ergonomics: The device should be attached with minimal effort in a matter of 5-10 minutes. The size settings should not cause eye strain. Animal position should be easy to replicate.

h. Size: The device should be no more the 0.5” thick or 3” wide. The length should not exceed 12”.

i. Weight: The device weight should be less than one pound.

j. Materials: The materials used in the device should not interfere with the imaging procedures. Cloth should not be used. Carbon fiber would be the preferred material.

k. Aesthetics, Appearance, and Finish: The device should be neutral in color, smooth, and have no sharp edges.

2. Product Characteristics

a. Quantity: The client requires one device.
b. Target Product Cost: $100

3. Miscellaneous

a. Standards and Specifications: The device must comply with RARC and lab protocol for animal safety.

b. Customer: The device will be used in the Small Animal Imaging Lab at UW-Madison for PET/CT scans.

c. Patient (animal)-related concerns: The device must be wiped down between animals.

d. Competition: Due to the fact that the device is custom to this specific research lab, there is no foreseen competition.

Appendix B

Testing Data

Table 5: The time it took to attach the mouse to our peg board was recorded for each test scan.

Scan	Time
1	4:35
2	2:37
3	2:40
4	2:08
5	3:16
6	2:40
7	2:26
8	2:06

Table 6: The test images were registered using the lab's Amira imaging software program, and the translation and rotation about each axis was found.

	Translation				Rotation		
Scan	X	Y	Z	Distance	X	Y	Z
3	0.13450	3.67798	0.49051	0.50862	0.00073	0.03193	0.02335
4	0.08897	7.39117	0.02552	0.09255	0.12740	0.03873	0.03245
5	0.07708	0.59742	3.57277	3.57360	0.24422	0.67865	0.52589
6	0.36804	0.85928	0.00000	0.36804	0.00010	0.00037	0.50358
7	0.09190	1.01096	0.00000	0.09190	0.00025	0.00042	0.14265
Average	0.15210	2.70736	0.81776	0.92694	0.07454	0.15002	0.24558
Standard Deviation	0.12266	2.89936	1.55421	1.49044	0.10965	0.29604	0.25027

Table 7: Images from one of the lab's previous studies were registered using the lab's Amira imaging software program, and the translation and rotation about each axis was found.

	Translation				**Rotation**		
Scan	X	Y	Z	Distance	X	Y	Z
1	2.90230	1.36914	15.91370	16.17619	5.47070	1.50230	2.04920
2	0.25500	0.54370	0.22864	0.34249	0.04309	3.76470	0.20687
3	1.40421	8.36798	3.47385	3.74692	0.41230	5.70340	8.26277
4	2.01203	0.38940	3.70372	4.21495	1.02550	7.22140	1.00980
5	2.18691	0.23949	0.91879	2.37208	0.52140	7.03780	0.71716
6	1.07003	0.43753	1.43570	1.79059	0.08160	0.88928	0.22916
7	1.44025	0.24238	0.28547	1.46827	2.25590	1.29199	0.23241
8	2.69332	0.01568	5.46210	6.09003	0.25662	0.27718	2.78870
9	1.29461	0.47443	2.70115	2.99537	0.85587	0.01763	0.31617
10	0.46954	0.67661	2.04219	2.09547	0.12452	0.13287	0.82443
11	1.26432	0.49008	0.84037	1.51813	0.09124	2.05140	1.35158
12	2.05811	0.70921	1.95195	2.83653	0.00060	0.08903	0.11146
Average	1.66766	1.27417	3.30983	3.88403	0.89849	2.61672	1.44294
Standard Deviation	0.82927	2.23192	4.10324	4.00777	1.50388	2.65681	2.19694