DESIGN OF VITRIFICATION MACHINE

BY

Todd William McDonald

Submitted to the graduate degree program in Mechanical Engineering and the Graduate Faculty of the University of Kansas In partial fulfillment of the requirements for the degree of Master's of Science.

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Chairperson

Committee members ______________________

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Date defended: ______________________

The Thesis Committee for Todd William McDonald certifies
that this is the approved version of the following thesis:

DESIGN OF VITRIFICATION MACHINE

Committee:

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Chairperson

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Date approved: __________________________

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Abstract

Cryopreservation of living cells and biological material by vitrification requires the expertise of a skilled lab technician and a large amount of time. Vitrification must be performed one sample at a time on tiny subjects, which makes for a tedious and unreliable process. Moreover, there is a lack of standardization in the methods for preparing cells and biological material for the vitrification process.

The purpose of the Vitrification Machine is to greatly simplify the process by making it faster, more efficient, cheaper and more reliable. The machine will be capable of handling several subjects at a time and will completely automate the most tedious portions of the vitrification process. This ease of use will allow researchers to experiment with new vitrification preparation methods on a larger number of samples more quickly and reliably.

The applications of the Vitrification Machine are wide, but it is specifically being developed to automate the vitrification of human oocytes and eventually embryos. Female patients diagnosed with diseases whose treatments are detrimental to the reproductive process (such as chemotherapy), can have their oocytes preserved for use after their treatment is complete. The automation of vitrification by the Vitrification Machine will make this process faster, more reliable, more affordable, and therefore more available to patients.

There currently is no known product on the market that fills all these needs or has the potential to drive down the cost of this portion of the fertility preservation process. Since the Vitrification

Machine will be useful to clinical In-Vitro Fertilization (IVF) laboratories, animal science research and fertility specialists in both research and clinical settings, the market potential of the product is very large.

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Acknowledgments

I would like to thank Dr. Sarah Kieweg, my faculty advisor, for her guidance and support, Dr. Terry Faddis and Dr. Lisa Friis, my graduate studies committee, for their help and insight during this design project and Mr. Charles Gable and the employees of the University of Kansas Mechanical Engineering (KUME) Machine shop for their advice and prompt production of the machine detailed within this document. Last, but not least, I must thank Dr. Samuel S. Kim of the University of Kansas Medical Center (KUMC). Dr. Kim served as medical advisor to this project and supported it with his research funds.

Specific Aims

The overall objective of this machine design project is to design and construct a microprocessor-controlled machine prototype to automate testing and research methods for vitrification of biological materials. The machine should be functional, but will serve as a proof-of-concept prototype for evaluating and improving the design for future generations of the device. The knowledge and experience gained in this project will be applied to designing a second-generation device capable of being manufactured on a large scale for use in research facilities, fertility clinics, and wherever quick, inexpensive cryopreservation of biological material is needed.

Much research is being conducted in the field of vitrification, but current methods require the expertise of a skilled lab technician and a large time commitment. The vitrification process is a repetitive process, lending itself well to automation. By introducing a machine that is capable of automating the process, research results will become more reliable, comparable, and repeatable, providing a solution to the need for a better vitrification process at a small cost. This machine has the promise of greatly reducing the time required for testing while increasing the reliability of results. By reducing the time and improving the reliability, advancements in the field of vitrification will become more frequent and significant.

Specific Aim 1 – Design a Machine to Automate the Vitrification Process

Vitrification of cells and biological material is a very repetitive process that requires the expertise of a skilled lab technician and a large amount of time. Technicians must move each sample to be vitrified one-by-one through the process. This limits the sample size when testing new vitrification protocols, and introduces a large amount of human error into the timing of each protocol. With the Vitrification Machine, researchers will be able to quickly and accurately test a variety of vitrification protocols with a large number of samples, greatly accelerating the evaluation of new protocols.

The Vitrification Machine prototype produced in this machine design project will be capable of quickly receiving input data from a user for vitrification and thawing protocols of up to four different stages and performing the protocol on up to four specimens at once. Since all protocols provided by this project's medical advisor make use of four or fewer cryo-protectants, this is deemed to be an appropriate number of fluids for the machine to support. Parameters for the protocols will be input using a three-button and LCD interface. These features will allow lab technicians to vitrify or thaw at least four times as many specimens in the same amount of time as by-hand procedures, greatly increasing the sample sizes in the testing of vitrification and thawing procedures. The larger sample size afforded by the Vitrification Machine means more accurate evaluation of these procedures, and the reduction in the time required means more (and more complex) procedures can be developed.

Specific Aim 2 – Create a Prototype of the Designed Machine

Upon the completion of Specific Aim 1, a prototype of the machine will be constructed. The prototype will be constructed with commonly available materials and will take advantage of the in-house capabilities of the University of Kansas' Mechanical Engineering (KUME) department. All parts that will be machined and fabricated, such as the metal case, will be done in the KUME

Machine Shop while all plastic parts will be produced in the KUME rapid prototyper. Should any of these options become unavailable, an external contractor will produce the parts.

Specific Aim 3 – Evaluate the Prototype and Plan Design Refinements

Once the machine has been constructed, its capabilities will be evaluated. Tests will be conducted to determine how accurately the machine deforms protocols (exposure times), and if it is capable of reliably and repeatedly controlling the Petri Dish Tray. These tests will serve to evaluate both the machine's physical design as well as its software design.

Along with the machine's actual function, its overall design will be evaluated in order to find possible areas for improvement. Possible areas of improvement include, but are not limited to human interface, ergonomics, specimen handling, intermediate steps between cryo-protectant exposures (such as dabbing away excess fluid), larger sample sizes, capability of using more cryo-protectants, ease of construction and cost.

Any design refinements developed in this segment of the project will be considered for use in future work to design a second-generation device. This second-generation device will be easier to use and manufacture, and will be capable of being licensed to a medical equipment manufacturer for large-scale production. The second-generation device's target market will be expanded as well, providing quick and inexpensive cryopreservation of biological material for not only research laboratories, but fertility clinics and hospitals as well.

Background and Significance

Cryopreservation and Vitrification

Vitrification of cells and living tissue is a process by which a biological sample is rapidly cooled to cryogenic temperatures to facilitate long-term storage and preservation. Cooling to cryogenic temperatures suspends nearly all cellular activity, allowing for long-term storage of samples without damage. This rapid cooling, which is the unique facet of vitrification (as opposed to slow-cooling methods), produces a glass-like solid devoid of ice crystals, which can form during the cooling process. The formation of ice crystals in a cell, colloquially known as "frostbite," can rupture the cell membrane and destroy the sample.

The technique of vitrification provides several advantages over another prevalent cooling method, controlled-rate slow cooling. As the name implies, controlled-rate slow cooling is a slow process, requiring hours to freeze a sample. In contrast, vitrification requires only a few minutes for the cryo-protectant exposures to be performed, and then the actual vitrification is nearly instantaneous. This means that many more samples can be frozen by vitrification than can be by controlled-rate slow cooling in the same amount of time. Another advantage of vitrification is its low cost in comparison to slow cooling methods. Vitrification requires only inexpensive, common laboratory supplies, cryo-protectant fluids and liquid nitrogen. Slow cooling methods require dedicated machinery that can cost well over $7000[1].

A typical vitrification protocol for an oocyte consists of exposing the sample to several cryo-protectant fluids for various lengths of time (dependent upon the protocol) before they are quenched in liquid nitrogen for freezing. The cryo-protectant fluids are used to help displace some of the water within the cell and protect it from the stresses associated with the freezing process. Typical fluids include sucrose solutions, dimethyl sulfoxide (DMSO), ethylene glycol (EG) and human serum albumin (HSA)[1], but newly developed protocols may make use of entirely different solutions.

Potential Beneficiaries of Vitrification Techniques

Preservation of oocytes through vitrification is an attractive process as it avoids many of the ethical dilemmas of embryo storage and expands the number of population segments that can make use of fertility preservation techniques. Women who would like to preserve their fertility for personal or professional reasons and those who do not have a reproductive partner or find embryo preservation objectionable could find vitrification of oocytes a viable option. While helping women of the general populace preserve their reproductive options has merit, the main focus of the research associated with this document is to help cancer patients preserve their fertility.

Chemotherapy and radiotherapy are typical treatments for cancer, but they can also be detrimental to the reproductive process. A young woman diagnosed with cancer could face a loss of her fertility with these common treatments[2, 3]. By harvesting immature oocytes before cancer treatment is started and preserving them through vitrification, a cancer survivor can maintain their reproductive options. The cancer survivor could make use of their preserved oocytes in the future, once they have completed their treatments. Preserving fertility after cancer is a major concern for reproductive age cancer patients[2, 4], and vitrification of immature oocytes is a process that is poised to address this concern.

Need for Automated Vitrification Machine

Vitrification as a process for oocyte cryopreservation still needs improvement. There is no standard protocol for vitrification; new and more successful protocols are always being developed. While vitrification is a quick process, it is labor intensive. A typical protocol is performed as follows: A lab technician pipettes each sample to be vitrified, one-by-one, onto an EM (electron microscopy) Grid or a small slice of plastic mesh. These grids are used for their ready availability and their copper construction, which gives them a high heat conduction rate. The mesh or grid containing the specimen is then moved through of series of Petri dishes containing cryo-protectant fluids, generally up to four (personal interview, Medical Advisor, Dr. Kim), using tweezers (alternatively, a pipette system may be employed). The technician must monitor the time each specimen has been in each fluid using a timer in order to accurately perform the protocol. When the allotted time has expired, the technician must recover the grids with tweezers and quickly move them to the next fluid. As this description shows, performing a vitrification protocol, even a simple one, is not a trivial matter. The precision and dexterity required along with the repetitive nature of this task lends itself well to automation.

Since no standard protocol exists and a large amount of repetitive testing will be required in the research of new protocols, a machine that can help automate the research and execution of protocols is desired. The machine described in this thesis will facilitate testing of new vitrification protocols with complexity, accuracy, repeatability and speed not achievable with current lab technician methods. As with any successful scientific experiment, the results must be verifiable and repeatable, and any data set supporting the results must be large enough to be statistically relevant. These requirements present a problem for vitrification protocol development since there are inherent limitations on accuracy, repeatability and sample size when a human performs the protocols. By introducing a machine that can automate the procedure and perform it on multiple samples at a time, all of these limitations are removed. Any protocol programmed into the machine will be performed exactly the same every time on multiple samples, enhancing the accuracy and repeatability, and multiplying the number of samples that can be vitrified at once. Once research is complete or acceptable vitrification protocols are developed, the machine can then be used to perform the protocols for actual patient use.

In the near future, vitrification could be used to preserve tissue samples (e.g. ovarian tissue) much larger than single cell specimens (e.g. gametes). The development of a machine capable of automating the vitrification process for both laboratory and practice use could provide a huge jump-start to this promising application of the technology. The machine described in this thesis could be easily adapted for use with larger samples. This added application furthers the justification for the development of such a machine.

Research Design and Methods

The specific aims described earlier were accomplished through the following methods:

Specific Aim 1 – Design a Machine to Automate the Vitrification Process

The design process began with the identification of the customer requirements, followed by concept generation and concept evaluation. The concept generation process included brainstorming sessions and sketching of possible machines. The three most plausible concepts from the concept generation phase were chosen for further development in the concept evaluation process. The concepts were evaluated using a decision matrix to impartially quantify how well each concept satisfied the customer requirements.

The decision matrix provided a clear choice for the concept to be developed into the final machine. This concept (labeled in the Detail Design section as the "Twist Dishes") then underwent an iterative design development using a full 3D model. The model of the machine was developed in SolidWorks 2009 (a 3D CAD software package produced by Dassault Systèmes SolidWorks Corp), which eliminated much of the need for building and testing parts before the final proof-of-concept prototype was built. It also facilitated quick and easy incremental changes to the design, which would not have been possible otherwise. The 3D model also made the creation of production drawings very easy, leading to a greatly reduced production schedule.

Specific Aim 2 – Create a Prototype of the Designed Machine

The Vitrification Machine prototype consists of a variety of custom manufactured parts of various materials (plastic, PTFE, aluminum, steel, etc.). All parts to be machined and fabricated were first detailed in a production drawing (available in Appendix 1). These parts were then produced in the KUME Machine shop.

The complex plastic parts used in the Vitrification Machine (such as the rotation gears) were produced using the KUME Rapid Prototyping machine. The parts were first designed in full 3D in SolidWorks 2009, and saved as stereo lithography (.stl) files. These files were then imported into the rapid prototyper's software, where the models were sliced into layers, and tool paths were programmed. The result of this process was very accurately produced parts with material properties similar to PVC.

During the production process, the KUME rapid prototyper became non-functional, so not all of the parts that were planned to be rapid-prototyped in-house could be produced. This required some of the remaining plastic parts to be made at a local area rapid prototyping facility. These parts, made on commercial and more modern rapid prototypers, are of a much higher quality than the plastic parts produced in-house.

One major design revision to the Vitrification Machine required the addition of some very small metal parts. These parts are very small and intricate, resembling a small set of forceps for handling the EM Grids on which the specimens for vitrification reside. In order for these parts to be made accurately, affordably and in a timely manner they had to be laser cut. The KUME

Department did not have any facilities to produce these parts, so an external contractor produced them as well.

Specific Aim 3 – Evaluate the Prototype and Plan Design Refinements

Once the prototype was constructed, its human interface, software, and overall design were evaluated, as well as its actual function.

Timing and Positional Accuracy

In order to evaluate the machine's timing and positional accuracy, exposure times ranging from 5 seconds to 2 minutes at 5 second increments were provided as input parameters for the machine. When the protocols were performed, the actual length of each exposure was found by using a stopwatch. The stopwatch was started when the machine stopped raising the Petri Dish Tray for each exposure, and was stopped when the machine began lowering the arm. During each protocol, notes were kept regarding the positioning of the Petri Dish Tray (in a pass/fail fashion). If the machine placed the forceps in the correct dish for each protocol (dishes 1, 2, 3 and 4, in that order), it was awarded a "pass" for each and vice-versa. These data were used to evaluate the machine's positioning capabilities. The actual exposure times were compared against the input parameters, the results of which (and the results of the positioning data) are available in the "Results and Discussion" section.

Evaluating the machine for incremental design changes can be considered an extension of the detail design portion, or the very beginnings of the design for manufacturing portion of the design. Any problems identified in this portion of the design process can be corrected, improved upon and incorporated into the design of the second-generation device. The questions in the following paragraphs, among others, were asked during the evaluation. The answers to these questions lead to the development of design refinements. These refinements are discussed in the “Conclusions and Future Work” section provided later in this document.

Human Interface

Are the buttons placed to allow comfortable operation? Are the buttons’ functions clear or intuitive to the user? Do the buttons provide tactile feedback that is pleasing to the user? Is the LCD readout at a comfortable viewing angle? Is the LCD bright enough and is the contrast high enough for it to be easily read? Does the LCD readout provide adequate feedback during operation (also a programming issue)? Are the dishes easy to insert and remove from the Petri Dish Tray? Is the beaker easy to insert and remove from the Petri Dish Tray? Is the beaker easy to fill with liquid nitrogen when it is in place?

Software Design

Does the machine allow for easy input of parameters? Is the program algorithm intuitive to the user? Does the machine pause for user input when it should, and should it pause for user input at any other time? Should the program structure be re-evaluated?

Overall Design

Does the machine operate smoothly? Are there any ways to make the machine operate more smoothly? Should the dimensions of any parts be changed to enhance the machine’s function? Should the Petri Dish Tray be counter-balanced? Are the electronics adequately cooled during operation? Do the motors have enough holding torque? Does the motor controller provide accurate enough control of the motors? Does the case of the machine help to prevent damage from spills? Is the machine easy to clean?

Design

Design Overview

The Vitrification Machine is a three degree of freedom, microprocessor-controlled, fully automated device. The machine is capable of performing any proposed vitrification protocol that uses up to four cryo-protectant fluids, with any exposure times the user desires (up to 9 minutes and 59 seconds each). The machine was designed for up to four cryo-protectants since all the vitrification protocols provided by this project's medical advisor use four fluids or less. The machine is designed to accept standard, inexpensive, disposable 35mm Petri dishes as its containers for cryo-protectant fluids, allowing the machine to remain clean and minimizing clean-up time. It also accepts standard 50mL glass beakers and cryo-vials for use in the final step where the specimens are exposed to liquid nitrogen.

Two stepper motors and one DC gear head motor control the machine's motion. The stepper motors are controlled by a BS0710USB BiStep Motor Controller (Peter NorBerg Consulting, Inc.), which receives instructions serially from an SX28 Microcontroller (Parallax Inc.). Single-pull single-throw (SPST) micro-switches are also employed to provide position limits and feedback directly to the motor controller. The stepper motors control the rotation and elevation of the Petri Dish Tray, while the DC motor controls the articulation of the forceps, which hold the specimens.

Customer Requirements

The design of any product is heavily driven by the identity of the customer and how the customer will be using the device. In this case, the Vitrification Machine would need to be designed for use in a laboratory setting. The users would be either medical doctors or laboratory technicians. To help acquire a robust array of customer requirements, a laboratory visit to Dr. Samuel Kim's research facilities at the University of Kansas Medical Center was arranged. Both this researcher and Dr. Sarah Kieweg, graduate advisor, attended the visit. Dr. Kim provided details about the vitrification process, his laboratory and his future research goals. As a result of this visit, the following main customer requirements were determined and separated into the categories of expecters, spokens, unspokens and exciters[5]:

Expecters: These are features that the customer naturally expects the machine to have.

1) The machine must be capable of exactly automating the currently used vitrification protocols. This will allow researchers to better evaluate the effectiveness of current vitrification protocols.
2) The machine must be capable of performing new vitrification protocols. Beyond evaluating current vitrification protocols, it will be integral in developing and evaluating new protocols.

Spokens: These are features of the machine that the customer has explicitly stated they would like it to have,

1) The machine must easily fit on a desktop or lab workbench.

2) The machine must be capable of stand-alone operation, i.e. it must function without being hooked up to a PC. This feature would make the machine much more portable, but will likely make software development and control of the machine more difficult.
3) The machine must minimize the possibility of the cells (specimens) being lost during the protocol (the cells floating away during the protocol is a common problem in current manual methods).

Unspokens: These are attributes of the machine that are important to the customer, but they have not spoken about them.

1) Must be capable of performing protocols that are more complex than a human is capable of. This will allow the development of protocols that are possibly less harmful to the specimens being vitrified, and therefore more successful.
2) Must be more accurate and repeatable than current manual methods. Since current methods are conducted by hand, the accuracy of the protocol is limited to the accuracy of the technician. This requirement will allow protocols to be more accurately compared, since they will be performed exactly the same every time.
3) Must be capable of operating on more than one sample at once. This will greatly speed up the evaluation of protocols, since the sample sizes required to make accurate comparisons can be developed more quickly.
4) Must allow for quick set-up for each trial.
5) Must allow for quick clean up after each trial.

6) Machine uses a minimum amount of cryo-protectant fluids. Since cryo-protectant fluids can be expensive or require special preparation, this customer requirement will help keep operating costs to a minimum.

Exciters: These are features of the machine that set it apart from the competition. These features can make a customer very happy, but will not be missed if they are absent.

1) The machine should be capable of completely new methods of vitrification.

Concept Generation

Once a satisfactory group of customer requirements was acquired (the fulfillment of which would produce a machine the customer would be very pleased with), numerous concepts for machines capable of satisfying these criteria were generated. From this list of concepts, three were selected for further development. Some features of other concepts were absorbed into the selected three, and some were completely discarded. The three concepts selected for further development were as follows:

Concept I – Throttle Valves

The throttle valves concept focused on smoothly changing the concentration of the cryo-protectant fluids that the specimens were to be exposed to. The machine would have an exposure chamber for the specimens through which the cryo-protectant fluids would flow. The concentration of the exposing fluid would be changed by using throttle valves on the ends of large reservoirs of various concentrations of cryo-protectant fluids. The fluids would flow through the valves, into a mixing chamber, then into the exposure chamber where they would flow over the specimens.

This concept would not be capable of exactly replicating current vitrification protocols, would use a large volume of (potentially expensive) cryo-protectant fluids, and would have a high probability of losing specimens since a large volume of fluid would flow over them.

Concept II – Robotic Arm

The robotic arm concept was designed to use a robotic arm to pick up specimens and move them through multiple static dishes of cryo-protectant fluids. This would allow the exact replication of current vitrification methods. The arm would only be able of picking up one specimen at a time, unless a multiple specimen holder was designed, but this would complicate individual freezing and storage. Moving the specimens multiple times would increase the likelihood of them being lost during a protocol.

Concept III – Twist Dishes

The twist dishes concept focused on keeping the specimens stationary and therefore minimizing the chance of them being lost in the cryo-protectant fluids during a protocol. The machine would pick up the specimens and keep them stationary while moving dishes of cryo-protectant fluid underneath them. The machine would be capable of picking up multiple specimens at a time, greatly reducing the time required to test protocols.

Concept Evaluation

As can be seen in Table 1 (next page), a decision matrix was used to evaluate the three concepts. The three concepts were compared against a datum, the currently used manual methods. Each concept was rated by how well it fulfilled the Customer/Engineering Requirements listed in the left column. The concept was given a plus (+) if it fulfilled the requirement better than the datum, an S if it fulfilled it in the same manner, or a minus (-) if it did not fulfill the criteria as well as the datum. The criteria listed in the left column were developed specifically to address the customer requirements discussed earlier. Each criterion was also assigned a weighting factor (the sum of which is 100) proportional to its importance in the customers' view. The weighting factors were not arbitrarily assigned; the customer (Dr. Kim) was given an opportunity to provide input for the weighting factors. The weighted totals were then computed and recorded at the bottom of the table.

The results of the decision matrix were quite clear. The Twist Dishes concept far outperformed the two other concepts and was therefore selected as the concept for further development. The Twist Dishes' greatest advantages were its low probability of losing a subject during a protocol and its ability to exactly replicate current vitrification methods. The Robotic Arm concept suffered from its likelihood of losing subjects while the Throttle Valves concept lagged behind in multiple criteria.

Table 1: Decision Matrix

A (+) indicates a concept satisfies the criterion better than the datum, an (S) indicates a concept satisfies the criterion the same as the datum, and a (-) indicates a concept does not satisfy a criterion as well as the datum.

Customer / Engineering Requirements	Wt	I - Throttle Valves	II - Robot Arm	III - Twist Dishes	Datum - Manual
Capable of Exactly Automating Current Vitrification Methods	17	-	+	+	D
Capable of Vitrification Protocols	7	+	+	+	A
Capable of Completely New Methods of Vitrification	3	+	S	S	
Quick Set-Up for each trial	5	+	+	+	T
Quick Clean-Up After Trial	5	S	S	S	U
Minimum Number of Parts Needed to be Cleaned	4	+	S	S	M
Uses Minimum Amount of Fluid	4	-	S	S	
Fits on Desktop	5	S	S	S	
Stand-Alone Operation	5	-	+	+	
Customizable Exposure Times	12	+	+	+	
Automates N2 Exposure	5	S	+	+	
Cost	6	-	-	-	
Low Probability of Losing Subject	15	S	S	+	
Easy to Use Interface	7	+	+	+	
Total +		7	7	8	0
Total -		4	1	1	0
Overall Total		3	6	7	0
Weighted Total	100	21	52	67	0

Detail Design

Upon selection the Twist Dishes concept for further development, the embodiment and detail design phase could begin. This phase began with roughing out the dimensions of the machine, deciding upon major components (embodiment design) and progressed to determining all dimensions and parameters (detail design).

The design produced in this portion of the project incorporated two stepper motors (one for raising the dishes of fluid and one for rotating them) an electromagnet for holding onto small, metal electron microscopy grids (EM Grids) which supported the specimens during the vitrification process, and an SX Microprocessor for controlling the machine. When the prototype was constructed, it was found that the electromagnet could not provide as much force as was calculated, it consumed a large amount of power, and produced a great deal of waste heat. These setbacks forced the electromagnet to be cut from the design, being replaced by four forceps controlled by a DC gearhead motor.

All parts designed during this process were either fabricated in a machine shop, laser-cut, or rapid prototyped. Detail drawings of all fabricated and laser-cut parts are available in Appendix 1 and Appendix 3, respectively. Images of the 3D models of the rapid prototyped parts are available in Appendix 2.

Rotation Assembly

The main function of the Rotation Assembly (Fig. 1) is to control the position of the Petri Dish Tray (1, see labels in Fig. 1). The angular position is controlled by the Rotation Motor (2) (a 1.8 degree per step stepper motor), which is in turn controlled by the BiStep Motor Controller. As the Rotation Motor rotates, it turns a plastic Pinion Gear (3) mounted on its output shaft. The Pinion Gear mates with the larger Rotation Gear (4) with an approximate 8:1 ratio, reducing the angular velocity of the assembly and increasing its positional accuracy. The underside of the Rotation Gear contains a key that fits into a complementary keyway on the Rotation Shaft (5). This keyway constraint keeps the gear from rotating freely on the shaft, and is what provides for the positioning of the Petri Dish Tray. The Rotation Shaft is held in place and supported by two PTFE bushings, one at the top and one at the bottom (6). The PTFE bushings allow smooth rotation and hold up well to wear, allowing the machine to be used for a long time (perhaps its entire life) without replacing the bushings. The Petri Dish Tray is constrained to the Rotation Gear through the Elevation Motor and Acme Screw, which will be discussed in the Elevation Assembly section below.

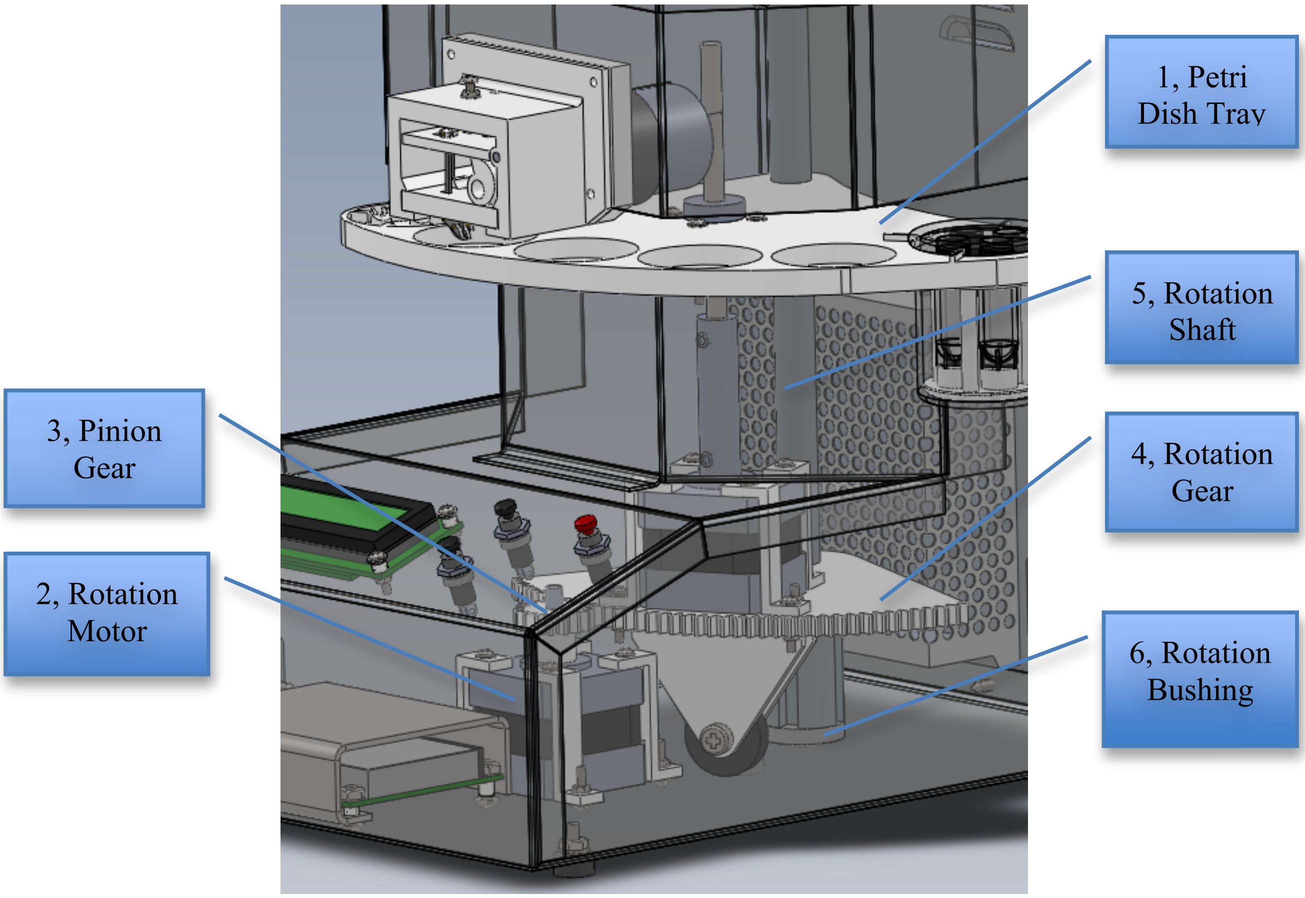


Figure 1: Detail of the Rotation Assembly

When the machine is powered on, the Rotation Assembly is automatically rotated until the Rotation Limit Switch is activated. This sets the zero value for the angular position of the Rotation Assembly for the BiStep Motor Controller. With the zero position set, the machine can theoretically control the angular position of the Petri Dish Tray to within 0.114°, which, at the five inch distance between the Petri dishes and the rotation shaft, is an accuracy of approximately 0.002 inches (see Appendix 7 for calculations).

Elevation Assembly

The function of the Elevation Assembly (Fig. 2) is to move the Petri Dish Tray up and down, dipping the specimens in the cryo-protectant fluids for the specific time required for the current protocol. The up and down motion is controlled by the Elevation Motor (7), a stepper motor identical to the Rotation Motor, which is also controlled by the Bi-Step Motor Controller. As the Rotation Motor rotates, an Acme Screw (8) coupled to its output shaft by the Rotation Coupler (9) is rotates as well. This ¼" Acme Screw (1/3" per revolution), with its helical threads, moves the complementary Acme Nut (10) up and down, depending the on the direction of its rotation. The Acme Nut is bolted to the Petri Dish tray, which allows the tray's vertical position to be accurately controlled.

When the machine is powered on, the Rotation Motor is automatically turned to lower the Petri Dish Tray. The tray is lowered until the Elevation Limit Switch is activated. This sets the zero value for the vertical position of the Elevation Assembly for the BiStep Motor Controller. Using a stepper motor and Acme screw allows the machine to theoretically control the vertical position of the Petri Dish Tray to within 1/1000th of an inch (Appendix 7).

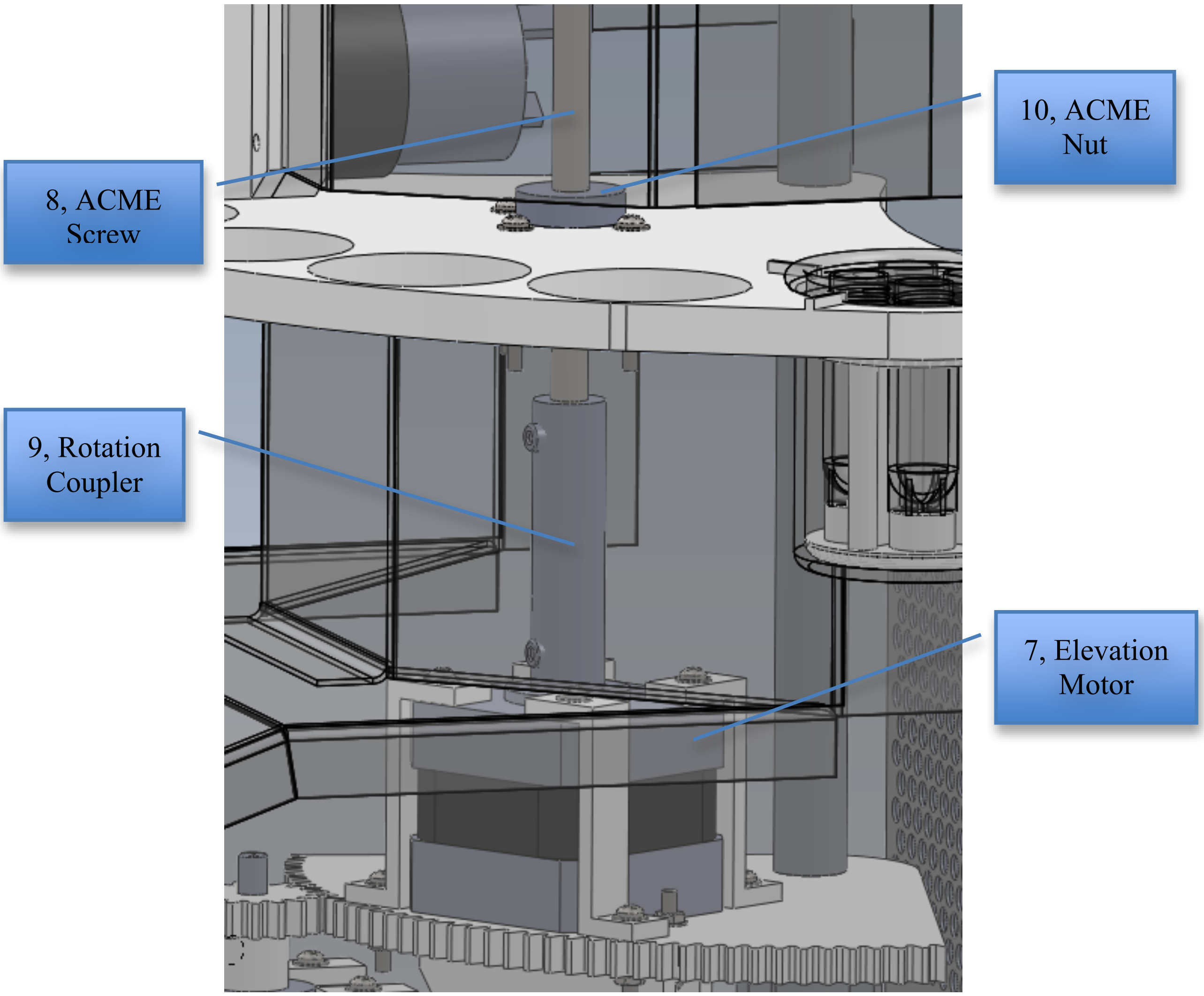


Figure 2: Detail of the Elevation Assembly

Specimen Handling Assembly

The Specimen Handling Assembly (Fig. 3) picks up the specimens to be vitrified, holds them as they are exposed to the cryo-protectant fluids and drops them into cryo-vials filled with liquid nitrogen for vitrification.

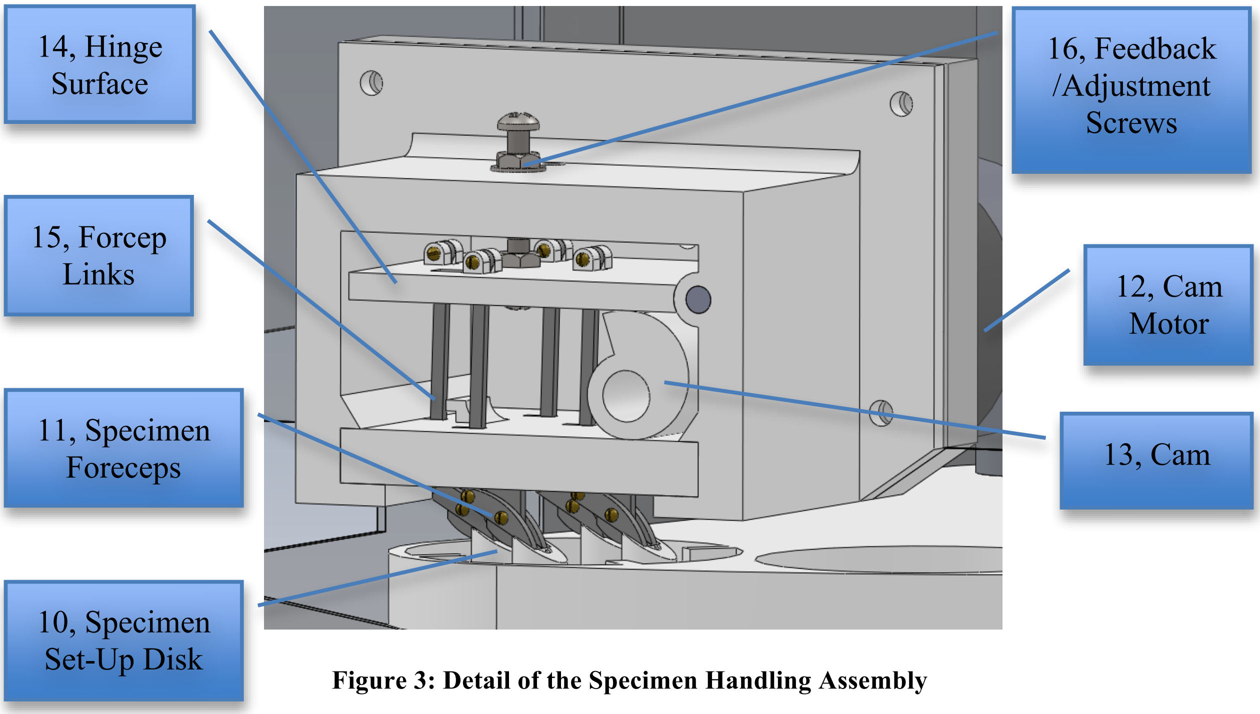


Figure 3: Detail of the Specimen Handling Assembly

The specimens are placed on EM grids, which are then placed on the Specimen Set-Up Disk (10). This dish uses angled protrusions to hold the EM grids at a 30° angle from horizontal. The Rotation and Elevation Motors are used to position the Petri Dish Tray, and therefore the EM Grids, into the jaws of the Specimen Forceps (11, drawings of which are available in Appendix 3). When a procedure is started, the Cam Motor (12, shown in the background in Figure 3) is activated which turns the Cam (13). As the Cam turns, the Hinge Surface (14) rotates about its axel and pulls the Forceps Links (15) upward until the Feedback/Adjustment Screws (16) connect and grounds the circuit. This movement closes the forceps, securing the EM grids for the rest of the procedure. The Cam Motor is controlled using an 8-pin DC Motor Controller integrated circuit (see Appendix 5).

Human Interface Assembly

The Human Interface Assembly (Fig. 4) of the Vitrification Machine is composed of an LCD Readout (17) and three buttons. The buttons are context sensitive to the machine's software, but are generally designed to be an Increment Button (18), Decrement Button (19) and an OK Button (20). This interface is used to get all input data about the protocol to be performed from the user. The program asks the user to provide the number of cryo-protectants (0-4) that will be used in the protocol and the time of exposure for each cyro-protectant. The user selects the desired values by using the Increment and Decrement Buttons and presses the OK Buttons to confirm their selection.

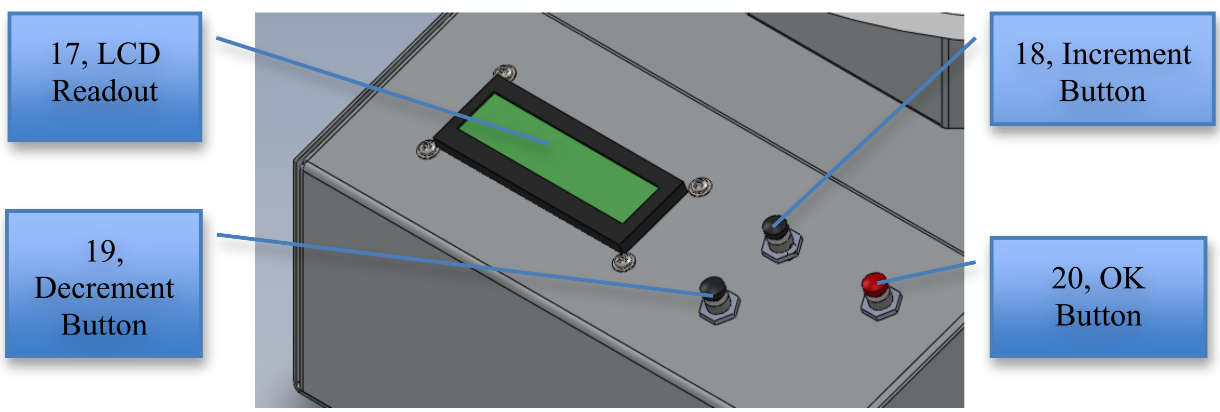


Figure 4: Detail of the Human Interface Assembly

Electronics Assembly

The Electronics Assembly (Fig. 5) controls all functions of the machine. It interfaces with the user, runs the program, controls the motors and makes all necessary calculations. The assembly consists of the SX Microprocessor (21), LCD Readout, BiStep Motor Controller (22), and Power

Supply (23). The SX Microprocessor is the central controller of the machine. It is connected to both the LCD Readout and the BiStep Motor Controller through a serial interface, which it uses to send all instructions (Appendix 6). The use of separate controllers for the motors and LCD Readout (instead of directly controlling them from the SX Microprocessor) greatly reduces the program complexity and allows the SX Microprocessor to perform other tasks while the BiStep Motor Controller and LCD Readout are busy. Detailed wiring diagrams for the LCD Readout, BiStep Motor Controller, SX Microprocessor and all other electronic components are available in Appendix 5.

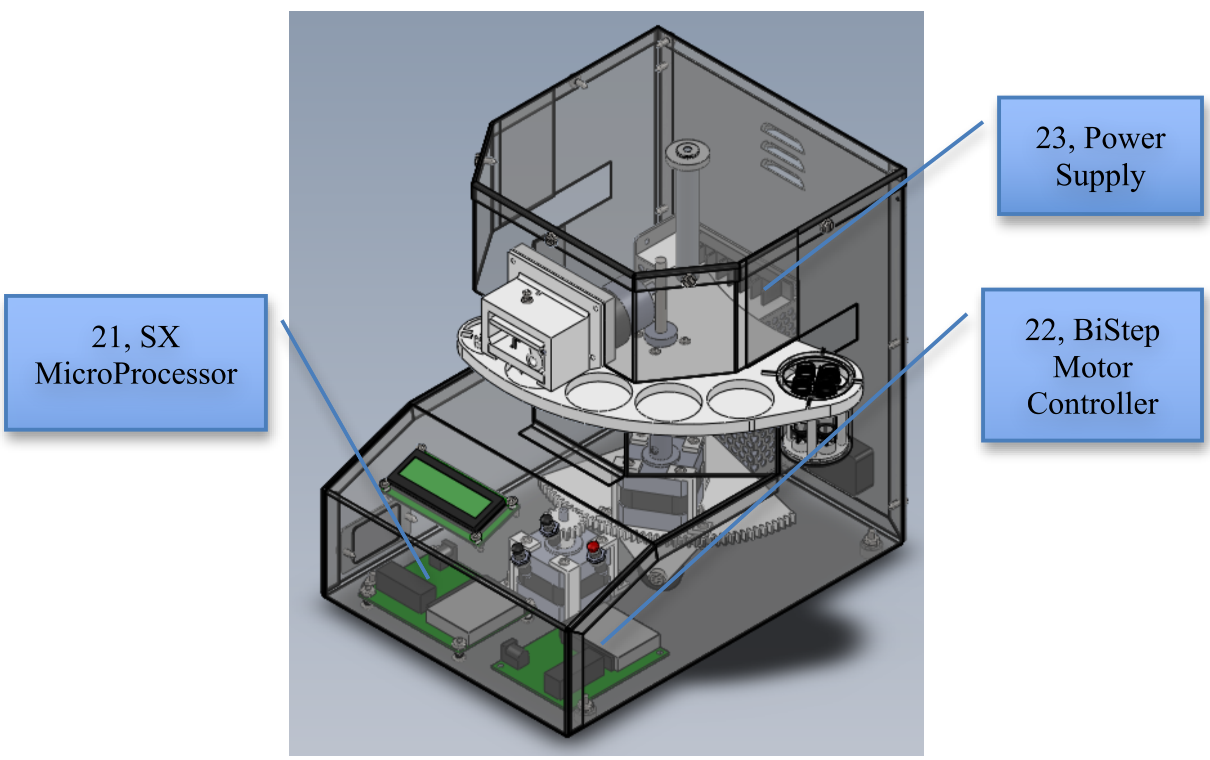


Figure 5: Transparent View of Overall Machine

Software Development

The software controls the Vitrification Machine is coded in BASIC for use with the freely available SXB (SX Basic) compiler. The program code is quite large (in comparison with the SX's onboard memory capabilities, see Appendix 4), but is based on a simple algorithm:

1) Boot Up
2) Turn motors in the negative direction to zero their position
3) Ask user for the number of exposures desired
4) Loop to get the times associated with the exposures
5) Pick up the specimens
6) Loop to run the protocol
7) Ask user to provide Liquid Nitrogen
8) Drop specimens in Liquid Nitrogen
9) Go to 1

This is the underlying algorithm for the entire Vitrification Machine's control software. When code is assembled in the SXB complier, every line of code produces multiple lines of assembly language code. A call to a subroutine produces only a jump table and the code for the subroutine, whereas copying and pasting the same code over and over produces new lines of code for each time it is used. Because of this, much of the Vitrification Machine's code has been nested in subroutines and functions in order to minimize the size of the assembled code. The machine's control program code is available in Appendix 4.

Overall Machine Function

For the convenience of the reader, and to further elucidate the function of the machine, a flowchart of the machine's actions is provided in the following figure, Figure 6.

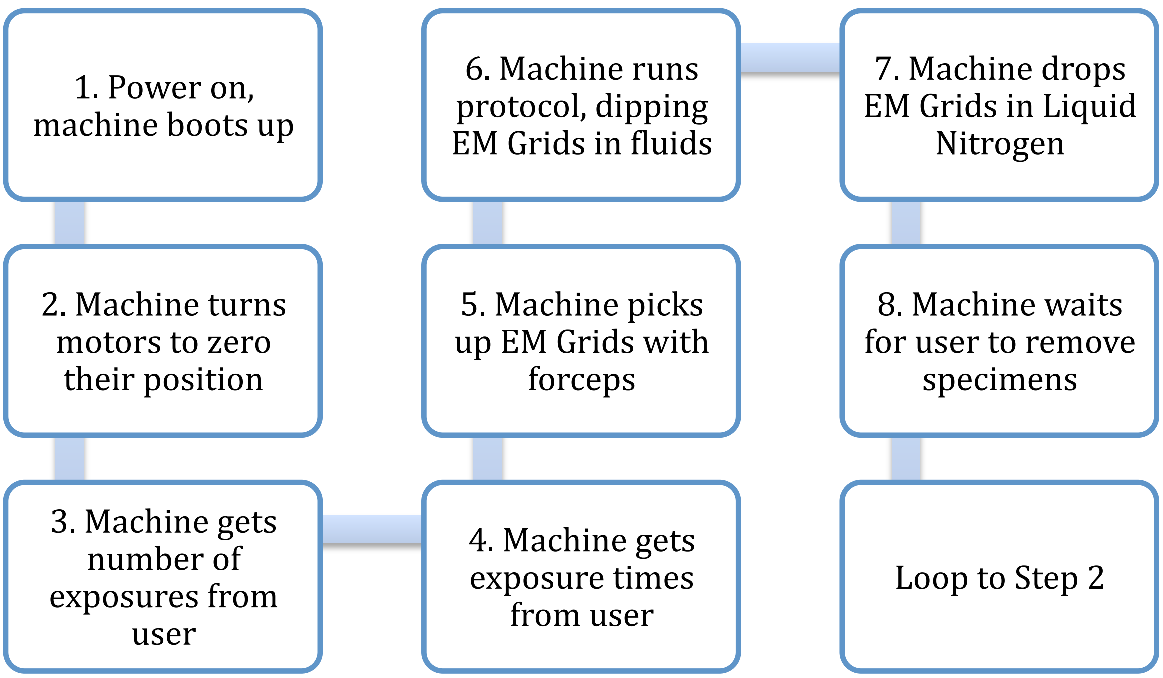


Figure 6: Flowchart of Machine Function

Results and Discussion

Specific Aim 1

The first specific aim of this project was to design a machine capable of automating the vitrification process. As a result, a design was produced that can hold up to four specimens at a time and can expose them to up to four cryo-protectant fluids during any given protocol. Each exposure can have a unique duration, after which the machine drops the specimens into cryo-vials filled with liquid nitrogen for freezing and long-term storage.

Using stepper motors capable of precise rotational positioning and a BiStep Motor Controller capable of $1/16^{th}$ micro-steps, the machine is capable of extremely accurate control of the Petri Dish Tray. The machine can theoretically control the angular position of the Petri Dish Tray to within 0.114° and the elevation of the tray to within $1/1000^{th}$ of an inch.

The incorporation of forceps for handling the specimens means that there are no special material requirements for the specimen medium. The initial design used an electromagnet to attract the small metal grids that the specimens were placed on. With the introduction of the forceps, any material can be used (though a material with a high thermal conductivity and low specific heat is preferred). This added flexibility should make the incorporation of added functionality (see Conclusions and Future Work) into the second-generation device much easier.

Specific Aim 2

The second specific aim was to produce a prototype of the design that could be used to evaluate the design's feasibility for being developed into a production model in the future.

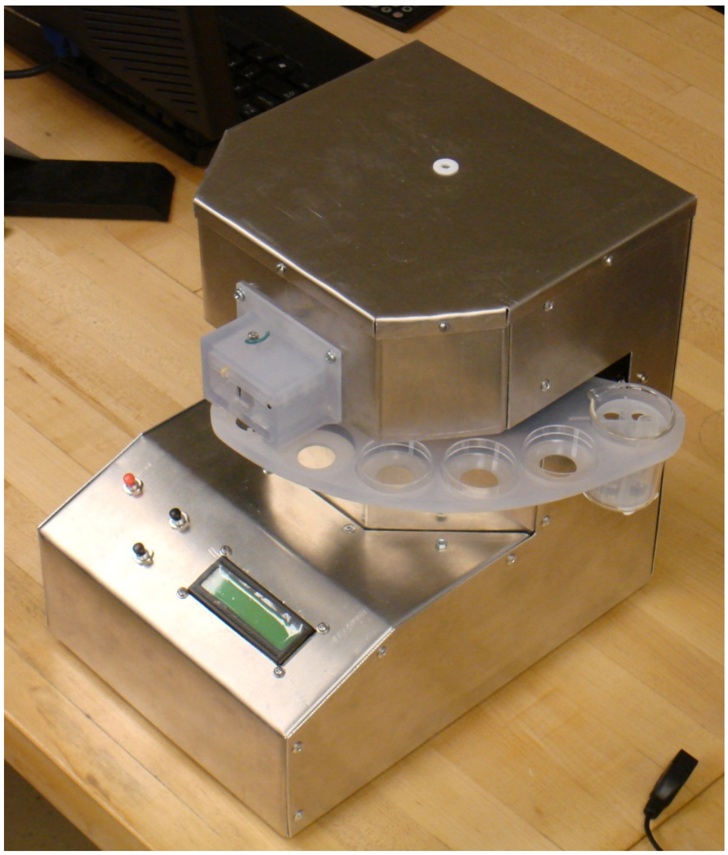


Figure 7: The Vitrification Machine Prototype

The produced prototype (Fig. 7) is nearly identical to the design, but differs in a few ways. The front panel that contains the LCD and the human interface buttons is reversed, so the buttons are on the left, and the machine's case was riveted together instead of welded. The case was riveted together at the suggestion of Mr. Charles Gable, Research Technologist and head of the KUME

Machine Shop. The difficulties inherent in producing a very accurate case out of sheet metal are apparent, as some filing and grinding had to be done to get the machine to fit together properly. The prototype does, however, demonstrate the design quite well and shows that it is capable of being produced, though revisions will likely lead to an easier-produced, more accurate machine.

Specific Aim 3

The third and final specific aim was to evaluate the constructed prototype and develop recommendations for revisions to its design. The prototype built as a result of Specific Aim 2 is not a perfectly functioning machine, but it does attain much of the functionality it was designed for and proves that this design can be refined into a fully functioning production model in the future. The machine is capable of getting all input parameters from the user and running the protocol with up to four exposures with each exposure lasting between one second and nine minutes and 59 seconds.

Table 2 provides the data taken during the evaluation of the Vitrification Machine's functional capabilities. As is shown in the table, the machine is capable of performing any protocol the user desires very accurately, and is capable of accurately controlling the Petri Dish Tray every time a protocol is performed. These initial tests included protocol parameters from 5 seconds to two minutes input into the machine for exposure 1, exposure 2, exposure 3, and exposure 4 in a cyclical pattern. This was deemed appropriate for initial testing of the machine since the vitrification protocols provided by the medical advisor to this project had parameters in that range.

The results of this testing show the machine is generally within 1% of the desired time, and always accurately controls the placement of the Petri Dish Tray. The increase in error at very short exposure times could be due to human error in data collection, since the rest of the times have a very small, predictable error. Further increases in timing accuracy could be achieved through editing of the machine's code at the assembly level, and more accurate methods of data acquisition. These methods might prove fruitless, however, since there will be some natural exposure time variance during the machine's operation due to the variance in the level of fluid in each Petri dish.

Table 2: Timing and Placement Accuracy Data

Input Time (seconds)	Trial 1 (seconds)	Trial 2 (seconds)	Trial 3 (seconds)	Average (seconds)	Relative % Error	Placement Accurate?
5	5.2	5.1	5.1	5.13	2.67	Yes
10	10.1	10.1	10.0	10.07	0.67	Yes
15	15.1	15.0	15.1	15.07	0.44	Yes
20	20.0	20.1	20.1	20.07	0.33	Yes
25	25.2	25.1	25.1	25.13	0.53	Yes
30	29.9	30.0	30.0	29.97	0.11	Yes
35	34.9	35.0	34.8	34.90	0.29	Yes
40	40.0	40.0	39.9	39.97	0.08	Yes
45	44.8	45.1	44.9	44.93	0.15	Yes
50	49.9	50.0	49.9	49.93	0.13	Yes
55	54.9	54.8	54.8	54.83	0.30	Yes
60	59.8	59.9	59.7	59.80	0.33	Yes
65	64.8	64.7	65.1	64.87	0.21	Yes
70	69.8	69.9	69.8	69.83	0.24	Yes
75	74.7	74.8	74.7	74.73	0.36	Yes
80	79.9	79.7	79.8	79.80	0.25	Yes
85	84.6	84.8	84.8	84.73	0.31	Yes
90	89.8	89.6	89.7	89.70	0.33	Yes
95	94.7	94.7	94.6	94.67	0.35	Yes
100	99.7	99.6	99.6	99.63	0.37	Yes
105	104.7	105.2	104.7	104.87	0.13	Yes
110	109.5	109.6	109.7	109.60	0.36	Yes
115	114.6	114.7	114.7	114.67	0.29	Yes
120	119.7	120.1	119.4	119.73	0.22	Yes

At the time of the writing of this document, the articulation of the forceps is not as smooth as was hoped, so a few design revisions are the likely course of action (see the Conclusions and Future Work section), though the motion may become smoother as the mechanism is "broken in" and more adequately lubricated. An integrated circuit (IC) specifically designed for driving DC motors should be implemented (see Appendix 5.) Modifications to the case of the device are also likely since fabrication from sheet metal makes precise production and alignment quite difficult.

For the convenience of the reader, a summary of the machine's function is provided in the following table, Table 3. See the "Conclusions and Future Work" for specific design revision suggestions.

Table 3: Summary of Machine Function

Function	Detail	Result
Rotation Accuracy	100% success rate of placing forceps in desired location	Complete
Elevation Accuracy	Machine is capable of raising Petri Dish Tray to the correct height	Complete
Timing Accuracy	Timing is within 1% of desired time (Table 2)	Complete
Forceps	Assembly suffers from excessive friction	Needs Refinement
DC Lift Motor	Functions, but needs a dedicated controller	Needs Refinement
Software	Algorithm works well, needs user testing	Acceptable
Microprocessor	Fast and works well, but is discontinued and should be changed soon	Acceptable
Case	Functional, but different materials and construction should be considered	Needs Refinement
LCD Readout	LCD is easy to read and is mounted at an appropriate angle	Complete
Buttons	Buttons function well, but do not provide positive feedback	Acceptable

As can be seen from the table above (Table 3), the Vitrification Machine Prototype has achieved many of its functional goals. It has also provided insights into design revisions that should be implemented when a second-generation device is designed and built (see "Conclusions and Future work" section). For these reasons, Specific Aim 3 can be considered a success.

Conclusions and Future Work

As a result of this design project, all three specific aims were achieved; a prototype was designed, built and evaluated for future design revisions. The prototype proves that a production version of this design is feasible with current technology and methods, but requires further revision. The design recommendations developed in the final stage of this project will be incorporated into a second-generation device that is capable of added functionality, easier production and licensing to a medical device manufacturer.

The Vitrification Machine has gained IAMI (Institute for Advancing Medical Innovation) funding; so the result of this project is not an end, but an intermediate step toward producing a machine capable of an expanded set of functional and production goals. This future work will include modifying the current prototype to get it to function perfectly so it may be used in vitrification protocol evaluation, incorporating the design recommendations outlined in this document, and producing a second-generation machine. The second-generation machine will have expanded functionality; it will be capable of vitrifying much larger biological samples (such as biopsy and tissue samples) and performing more complex vitrification protocols.

Specific recommendations for design revisions include (i) changing the case structure from sheet metal to a more solid material, (ii) expanding the vertical dimension of the gap the Petri Dish Tray fits through, (iii) changing the buttons in the Human Interface Assembly, (iv) including a spiral spring in the Specimen Handling Assembly, and (v) changing to a different microprocessor. The need for these design revisions and possible solutions are outlined in the following paragraphs.

First, the sheet metal construction of the Vitrification Machine's case has made alignment and production quite difficult. Changing to a machine with a solid metal skeleton should provide better accuracy and simpler bolt-together construction. This design will also be more rigid and will allow for a non-load bearing, aesthetically pleasing exterior to be added.

Second, the slot that the Petri Dish Tray fits through is unnecessarily thin. This slot should be expanded to allow for more vertical movement of the Petri Dish Tray. In the original design that incorporated an electromagnet, the dimension of this slot was not an issue, and was kept small to increase the rigidity of the case. With a change to a metal skeleton, this slot can be expanded without fear of the case becoming too flexible.

Third, the buttons currently used in the Vitrification Machine Prototype are adequate and function as they should, but they do not provide the tactile feedback many users of electronics have come to expect. The buttons should be changed to a type that "click" when activated, providing feedback to the user and making them feel more confident in using the device.

Fourth, the Specimen Handling Assembly currently uses rubber bands to pull the Hinge Surface (that controls the articulation of the forceps) down to keep it in constant contact with the Cam (Fig. 3). The rubber bands are adequate for a well-lubricated and smooth working mechanism, but the accumulation of dirt and wear-and-tear from use could easily provide more resistance than the rubber bands can overcome. Not only that, but rubber bands deteriorate over time, and replacing the small rubber band in the small Specimen Handling Assembly is a task best not left to the end-user. Instead, a conical compression spring should be incorporated between the Hinge Surface and the inside top of the Specimen Handling Housing. The conical shape will allow the spring to compress to a very small size and be retained by the feedback screws in the Specimen Handling Assembly.

Finally, the final design recommendation is to change from the SX line of microprocessors for two reasons: memory limitations and future problems with availability. Any expansion of the control code of this machine (provided in Appendix 4) causes an overflow of the SX's memory capabilities when the code is compiled. Since added functionality is one of the goals of the future work on this machine, a microprocessor with added memory is desired.

On August 3, 2009, Ubicom, the owner of the SX design, gave notice of the End of Life (EOL) of the SX microprocessor line[6]. Parallax, the supplier of the SX chips, has stated that their supply of SX chips might last years, but with a number of alternatives available, the time to change microprocessors is now. A microprocessor with at least eight input/output ports, a large amount of onboard memory and a BASIC or C++ compiler is desired.

By following these design revision recommendations, a second-generation device capable of achieving all its function and production goals is possible.

Summary of Appendices Contents

Appendix 1: Drawings of Fabricated Parts – Contains all detailed production drawings of all fabricated and machined parts contained in the Vitrification Machine.

Appendix 2: Rapid Prototyped Parts – Contains images of all 3D models of parts in the Vitrification Machine that were produced by rapid prototyping.

Appendix 3: Laser-Cut Parts – Contains detailed production drawings of all parts in the Vitrification Machine that were produced by laser cutting.

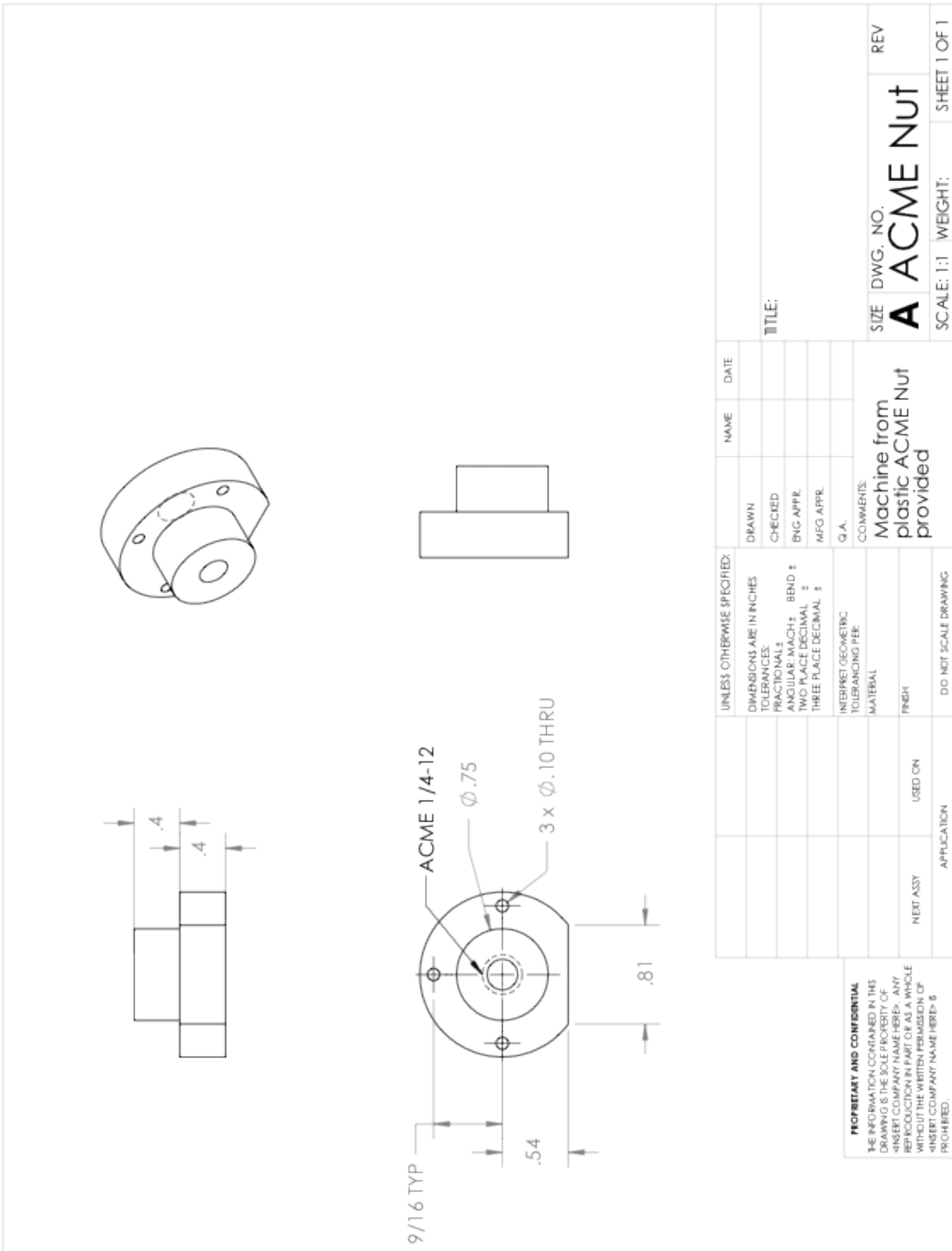
Appendix 4: Program Code – Contains the actual SXB language program code that is used to control the Vitrification Machine.

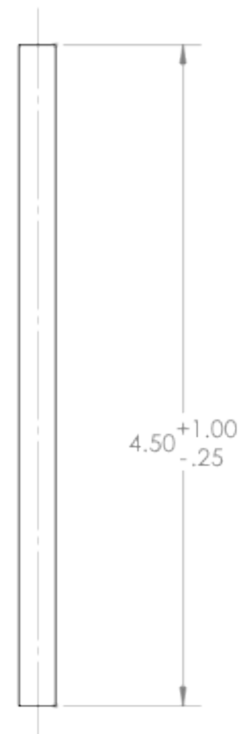
Appendix 5: Wiring Diagrams – Contains diagrams detailing all the wiring between different components in the Vitrification Machine.

Appendix 6: Calculations of Motor Controller Instructions – Contains a scanned document of a hand calculation that determines the step instructions for the SX Microprocessor to transmit to the BiStep Motor Controller for each protocol step.

Appendix 7: Calculation of Rotation and Elevation Resolution – Contains a scanned document of a hand calculation that determines the theoretical minimum movement of the Petri Dish Tray per BiStep Motor Controller step.

Appendix 1: Drawings of Fabricated Parts





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NEXT ASSY	USED ON	DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±		NAME	DATE
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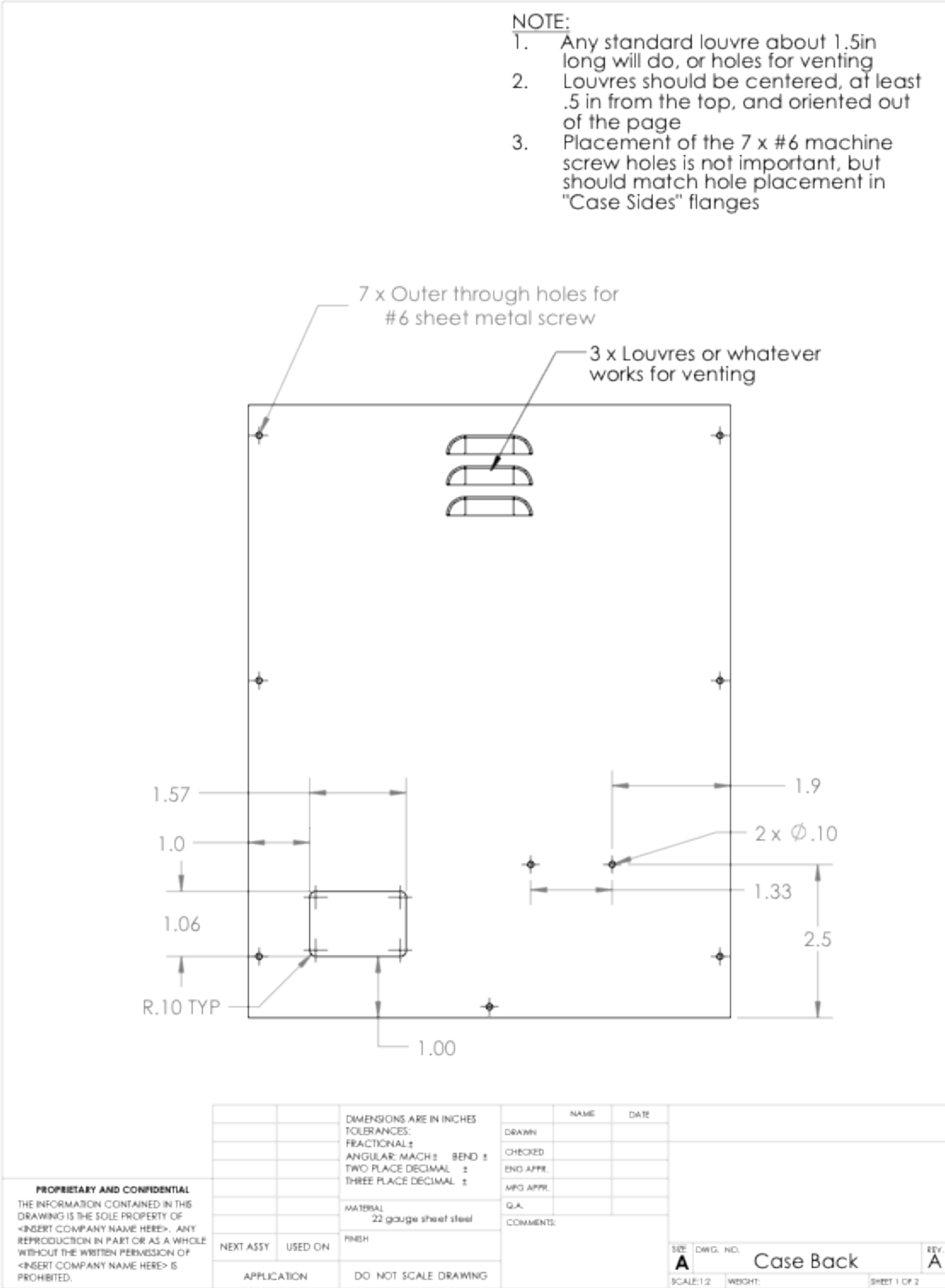
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7 x Outer through holes for #6 sheet metal screw
3 x Louvres or whatever works for venting
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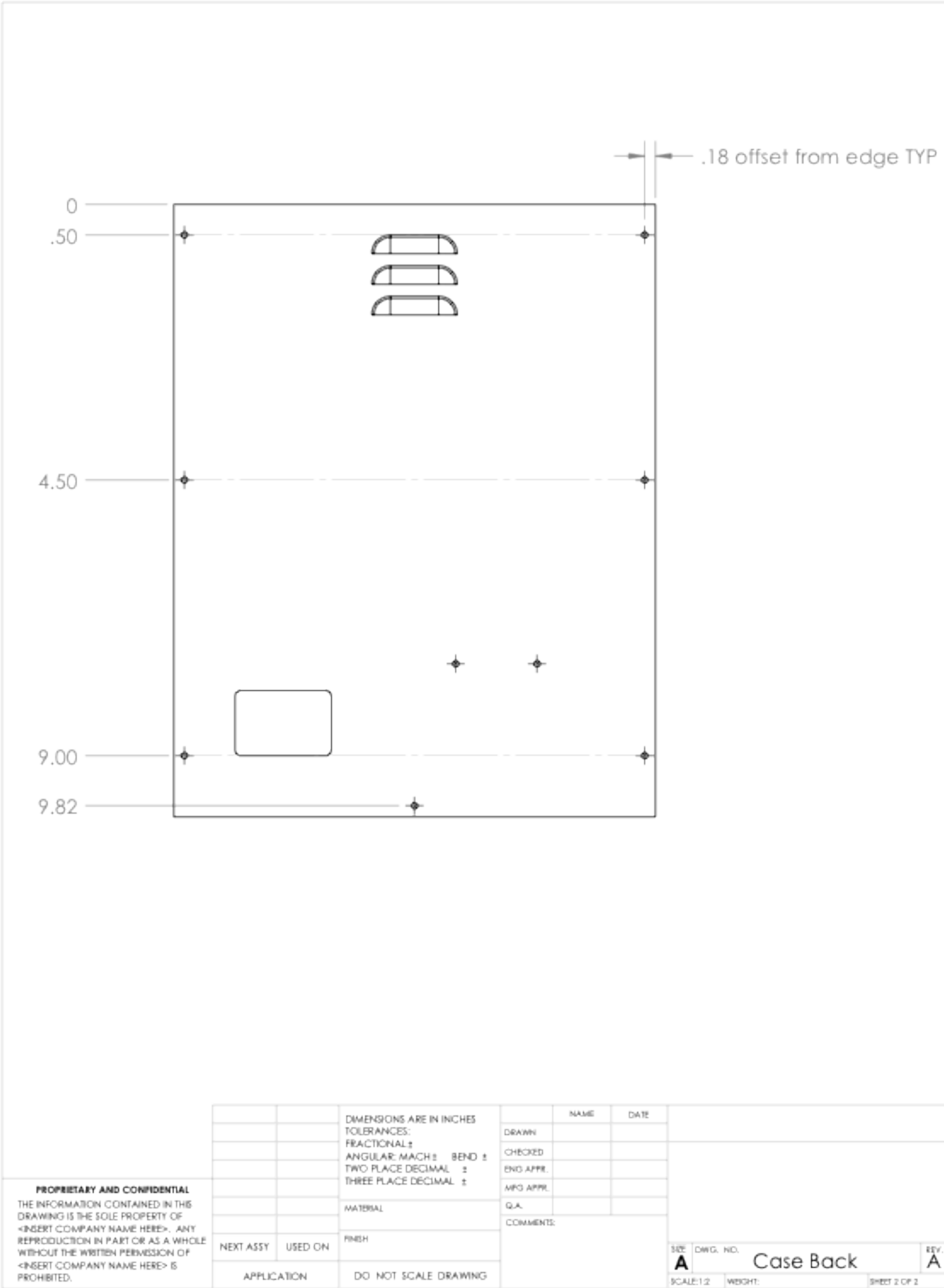

.18 offset from edge TYP
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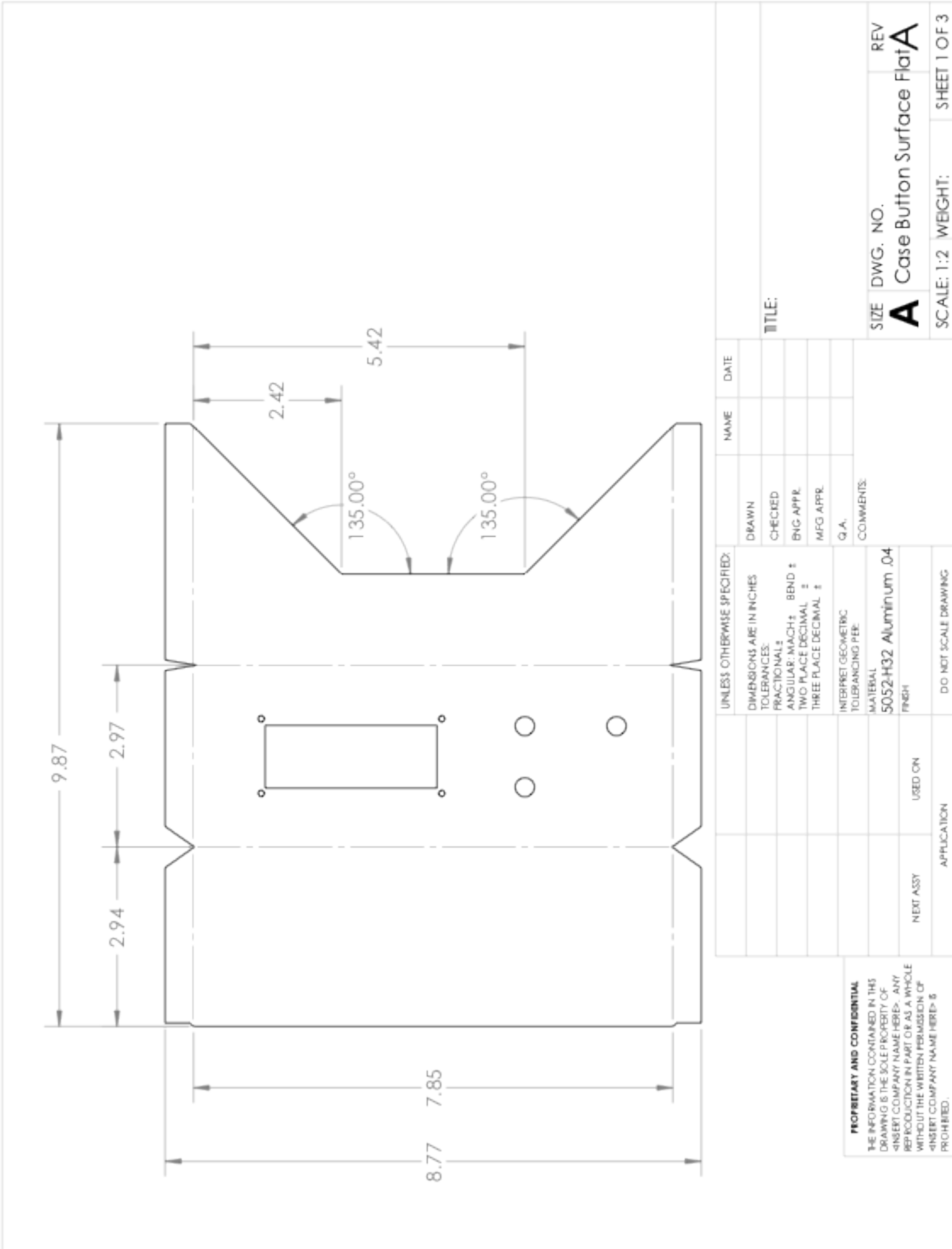

5.42
2.42
135.00°
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2.97
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A
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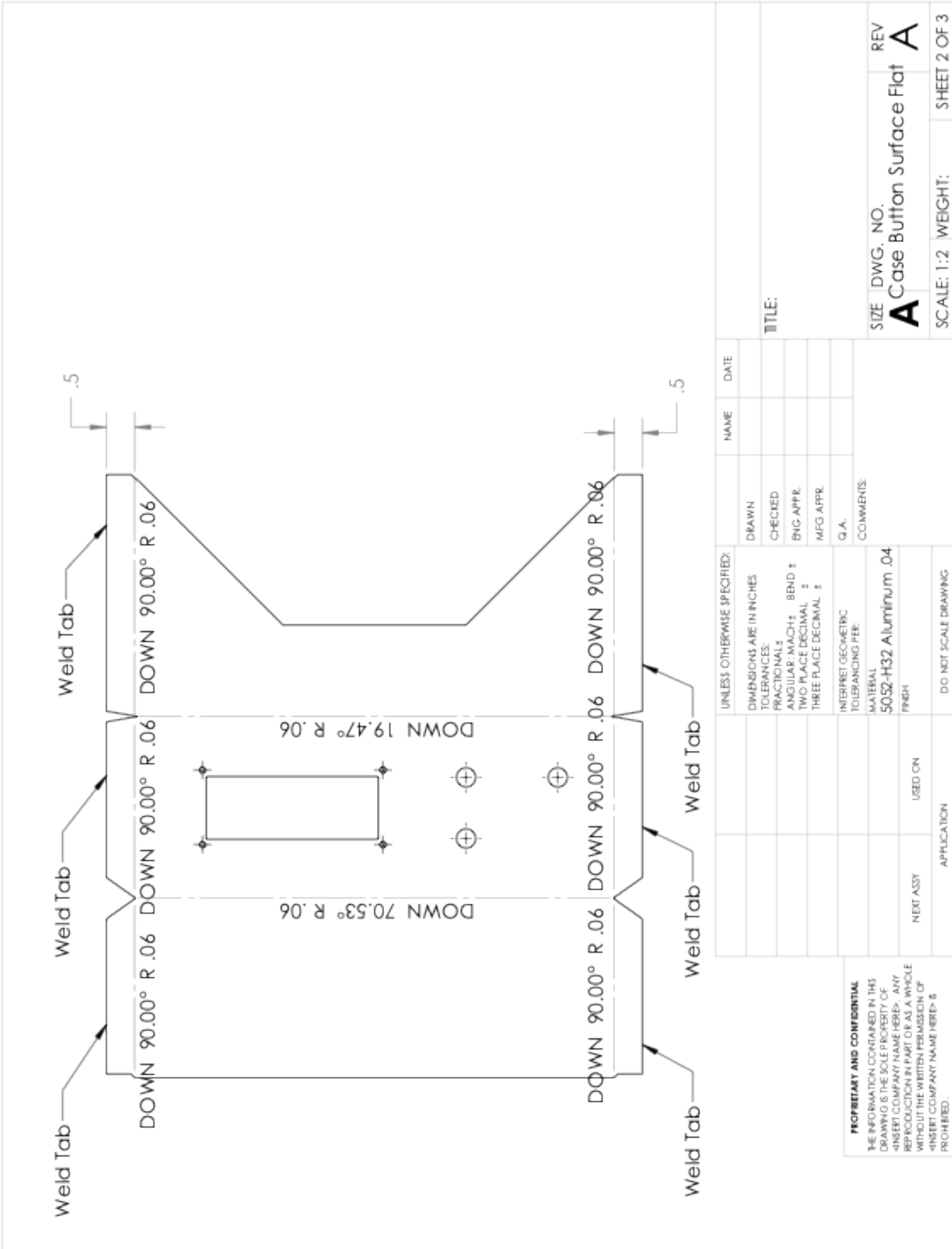

Weld Tab
Weld Tab
Weld Tab
Weld Tab
Weld Tab
Weld Tab
.5
.5
DOWN 90.00° R .06
DOWN 90.00° R .06
DOWN 90.00° R .06
DOWN 90.00° R .06
DOWN 90.00° R .06
DOWN 90.00° R .06
DOWN 70.53° R .06
DOWN 19.47° R .06
UNLESS OTHERWISE SPECIFIED:
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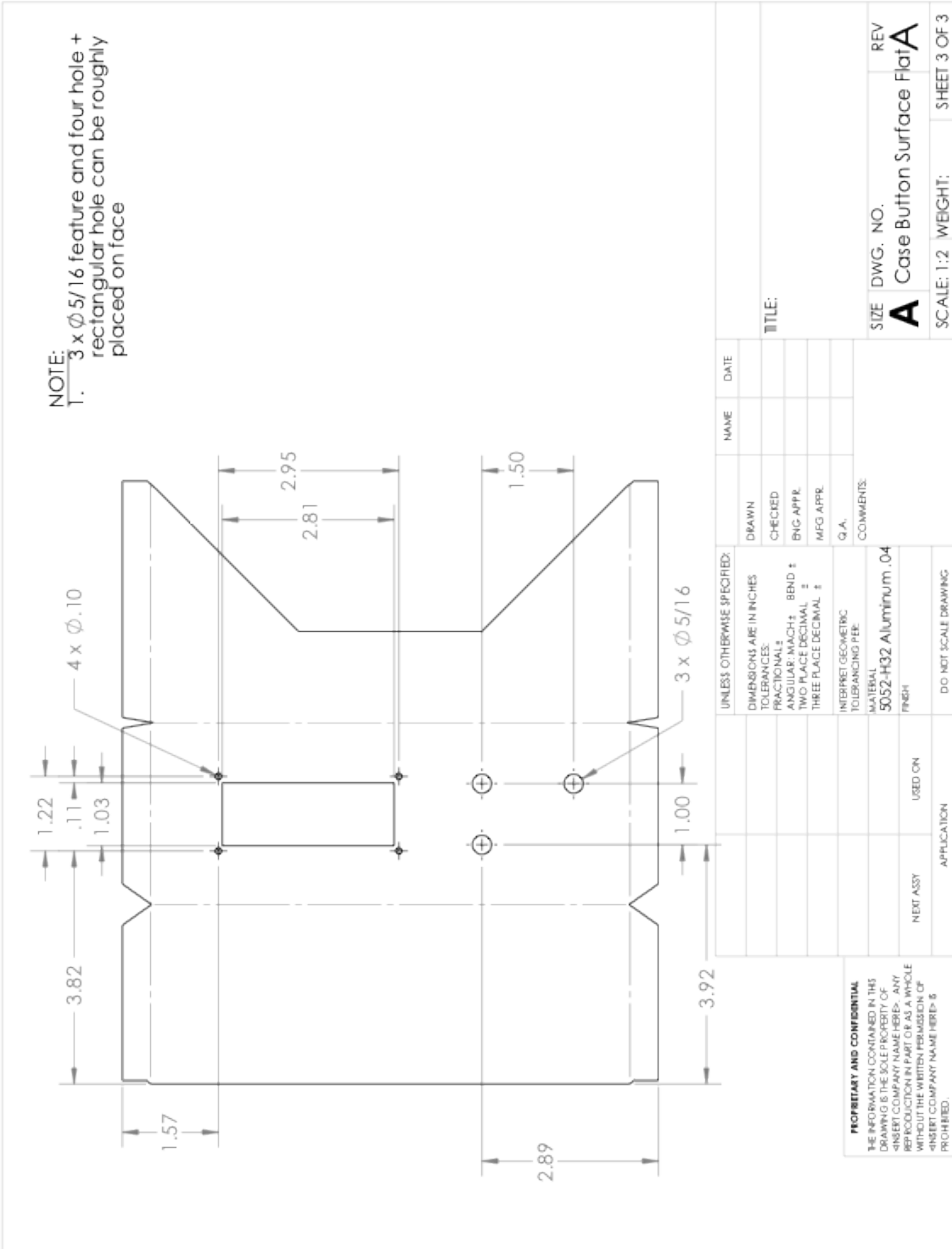

NOTE:
1. 3 x Ø5/16 feature and four hole + rectangular hole can be roughly placed on face
4 x Ø.10
3 x Ø5/16
2.95
2.81
1.50
1.22
.11
1.03
1.00
3.82
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MATERIAL
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ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
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SIZE
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DWG. NO.
Case Button Surface Flat
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SCALE: 1:2
WEIGHT:
SHEET 3 OF 3
NEXT ASSY
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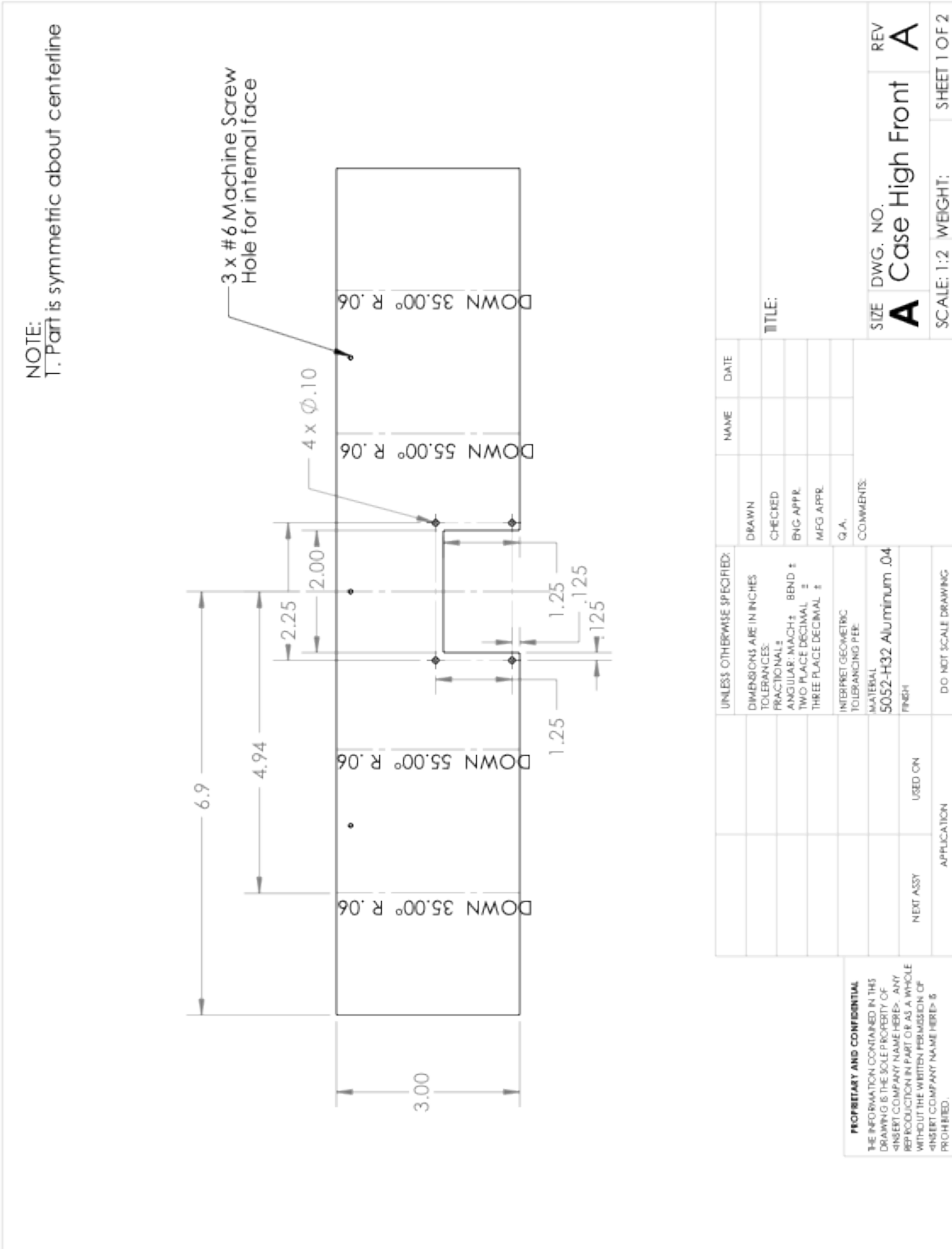
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3 x #6 Machine Screw
Hole for internal face
4 x Ø.10
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DOWN 55.00° R .06
DOWN 55.00° R .06
DOWN 35.00° R .06
6.9
4.94
2.25
2.00
1.25
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COMMENTS:
TITLE:
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DWG. NO.
Case High Front
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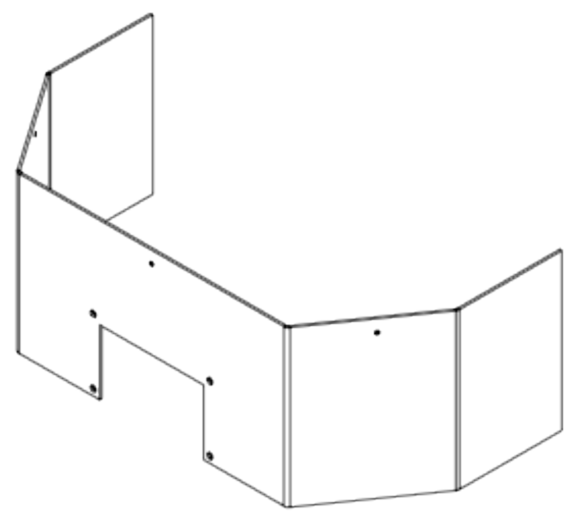

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TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
5052-H32 Aluminum .04

FINISH

DO NOT SCALE DRAWING

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ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

TITLE:

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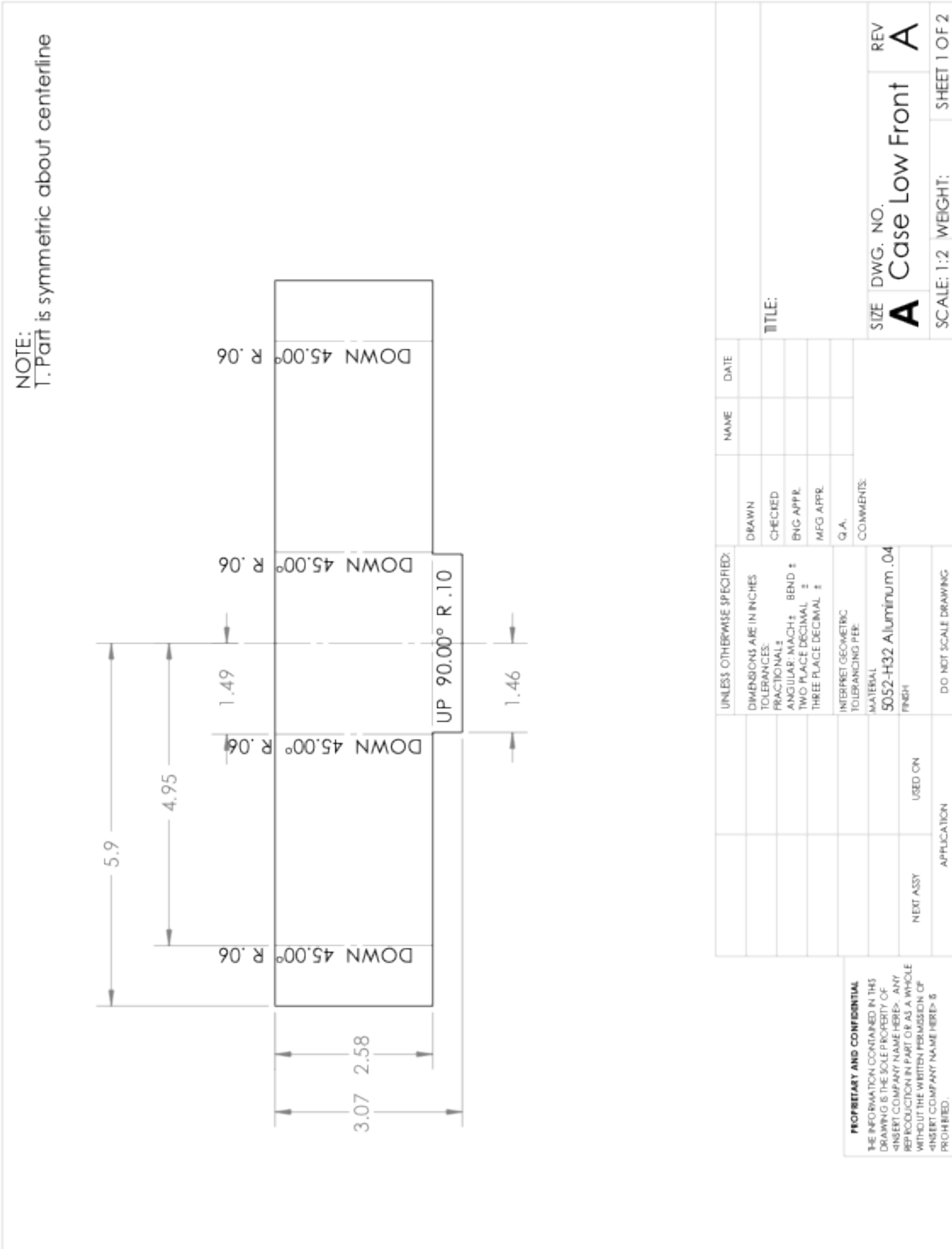
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5.9
4.95
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1.46
3.07
2.58
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DOWN 45.00° R .06
DOWN 45.00° R .06
DOWN 45.00° R .06
UP 90.00° R .10
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REV
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SCALE: 1:2
WEIGHT:
SHEET 1 OF 2
NEXT ASSY
USED ON
APPLICATION
PROPRIETARY AND CONFIDENTIAL
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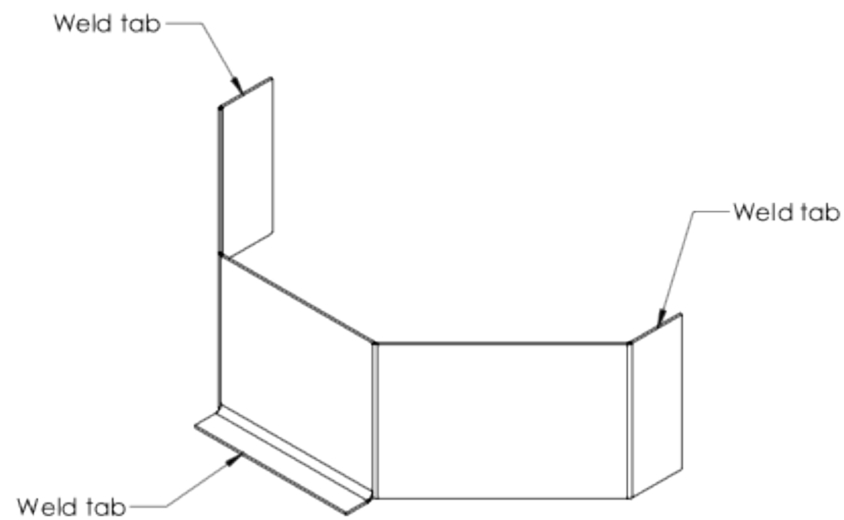
5
4
3
2
1



UNLESS OTHERWISE SPECIFIED:		NAME	DATE		
DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN			TITLE:	Case Low Front Finished Part
	CHECKED				
	ENG APPR.				
	MFG APPR.				
INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.				
MATERIAL 5052-H32 Aluminum .04	COMMENTS:			SIZE A	DWG. NO. Case Low Front — REV A
FINISH					
DO NOT SCALE DRAWING				SCALE: 1:2	WEIGHT: — SHEET 2 OF 2

NEXT ASSY	USED ON
APPLICATION	

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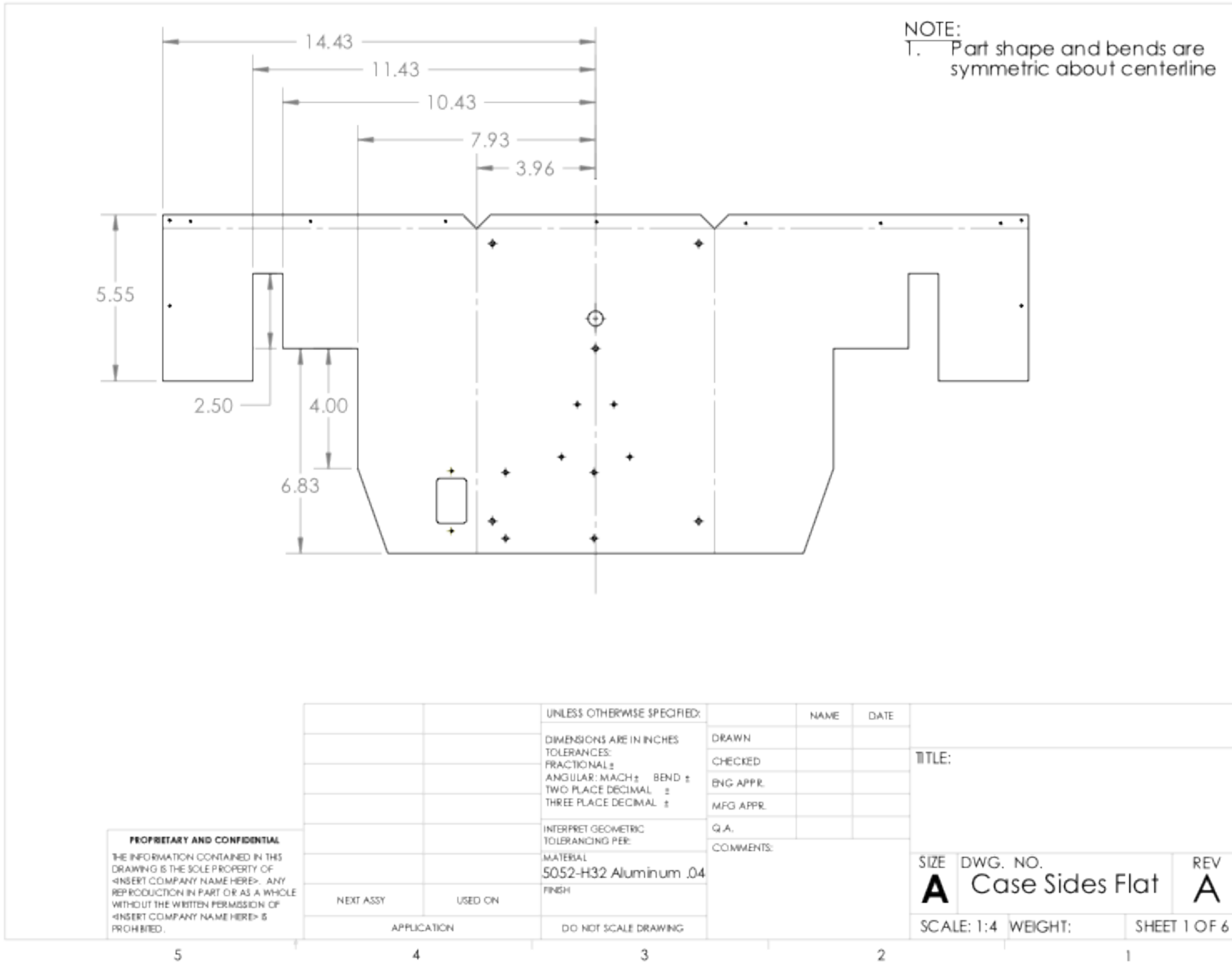
5 4 3 2 1



NOTE:
1. Part shape and bends are symmetric about centerline
14.43
11.43
10.43
7.93
3.96
5.55
2.50
4.00
6.83
PROPRIETARY AND CONFIDENTIAL
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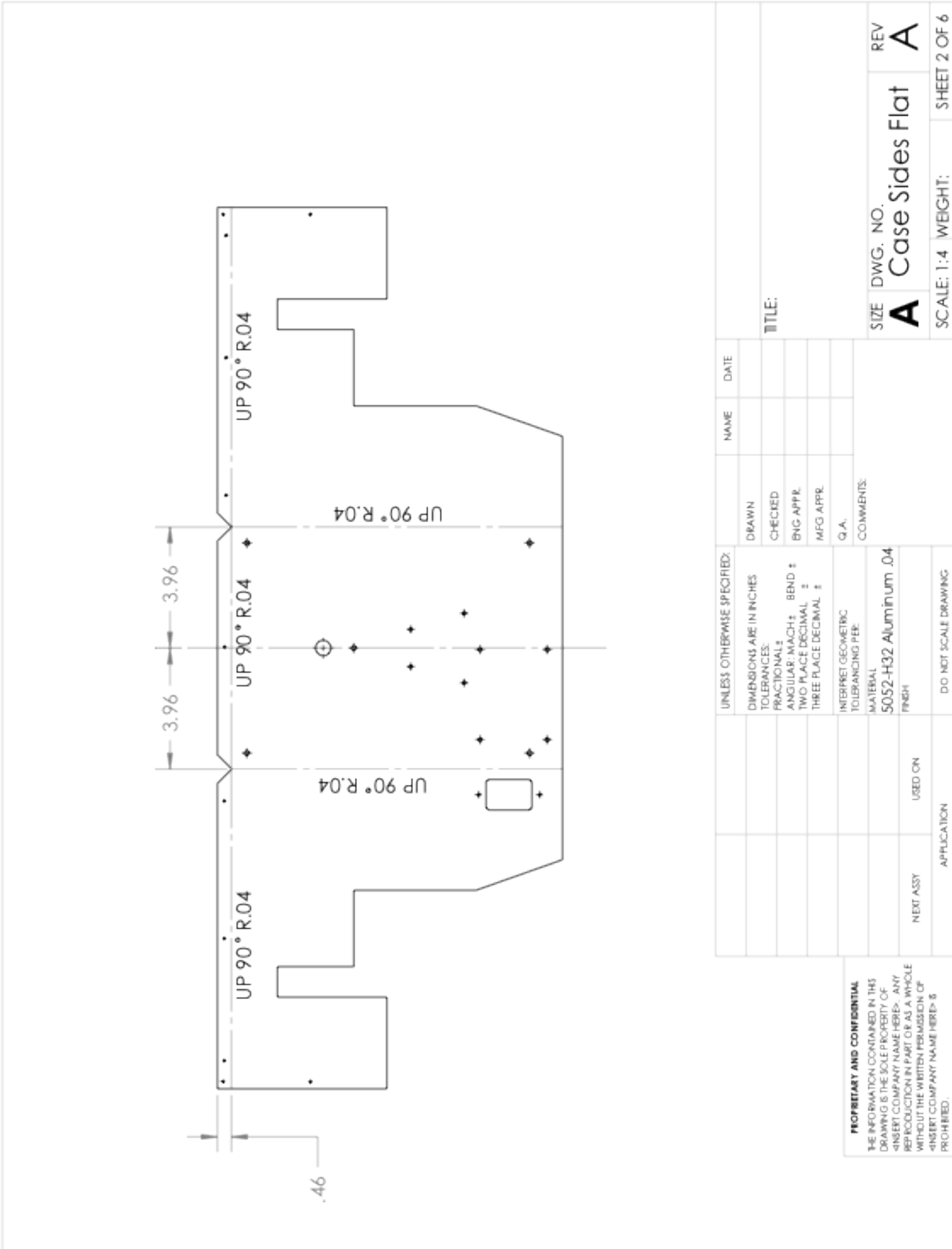
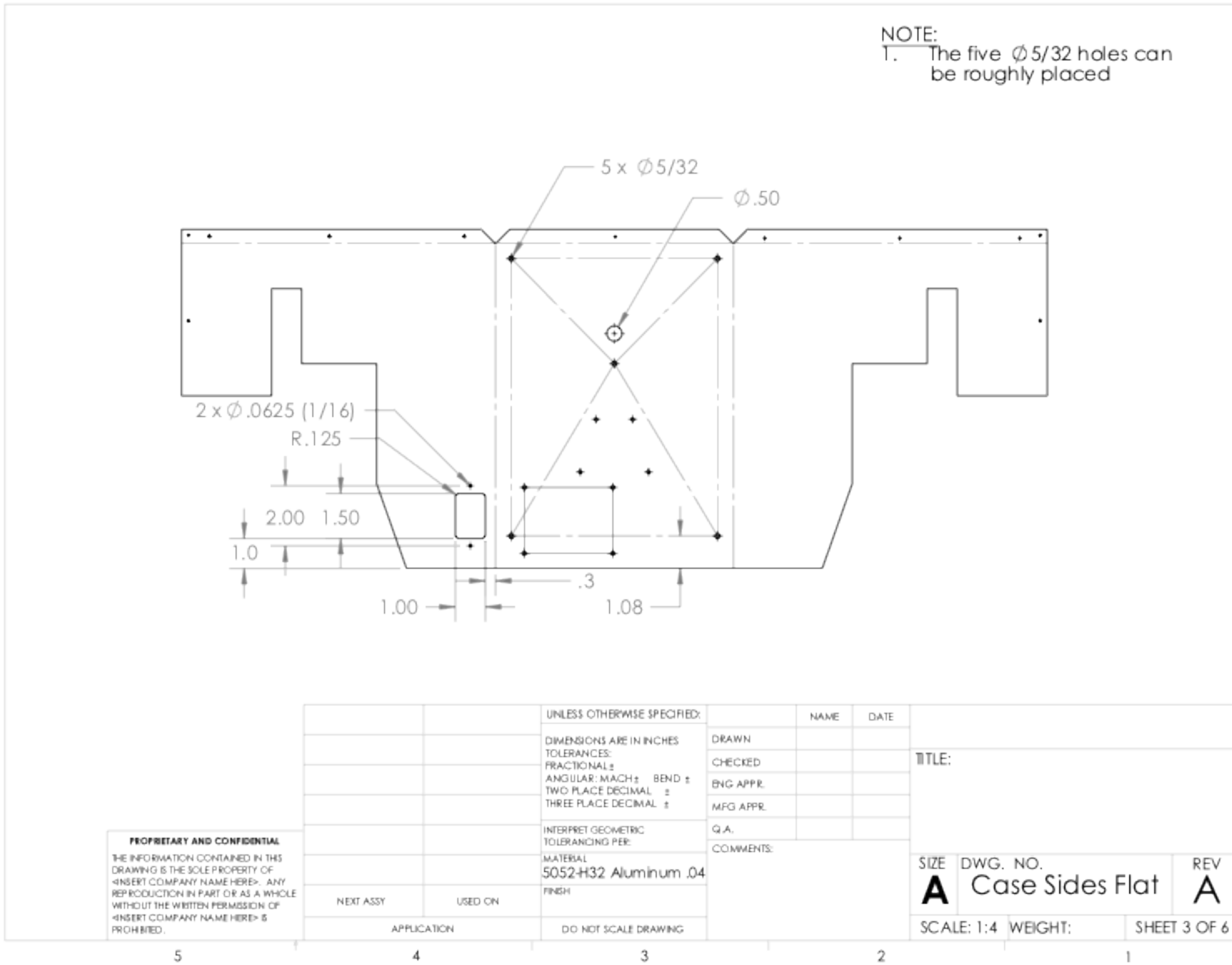
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 1 OF 6
5
4
3
2
1


UP 90° R.04
UP 90° R.04
UP 90° R.04
UP 90° R.04
UP 90° R.04
3.96
3.96
.46
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NEXT ASSY
USED ON
APPLICATION
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
NAME
DATE
TITLE:
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 2 OF 6
PROPRIETARY AND CONFIDENTIAL
1
2
3
4
5



NOTE:
1. The five Ø5/32 holes can be roughly placed
5 x Ø5/32
Ø.50
2 x Ø.0625 (1/16)
R.125
2.00 1.50
1.0
.3
1.00
1.08
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NEXT ASSY
USED ON
APPLICATION
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 3 OF 6
PROPRIETARY AND CONFIDENTIAL
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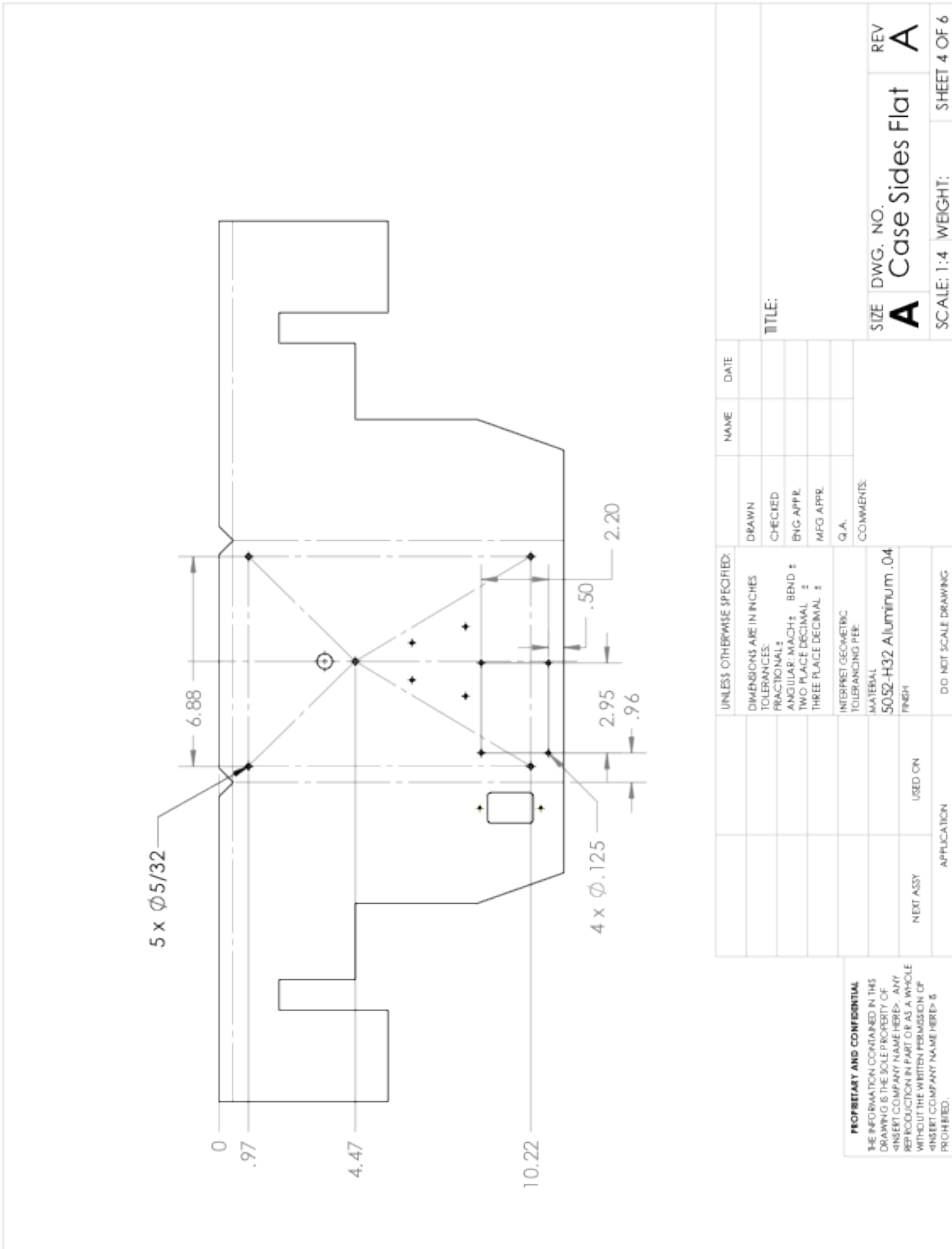
5
4
3
2
1


5 x Ø5/32
6.88
2.20
.50
2.95
.96
4 x Ø.125
0
.97
4.47
10.22
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NEXT ASSY
USED ON
APPLICATION
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 4 OF 6
PROPRIETARY AND CONFIDENTIAL
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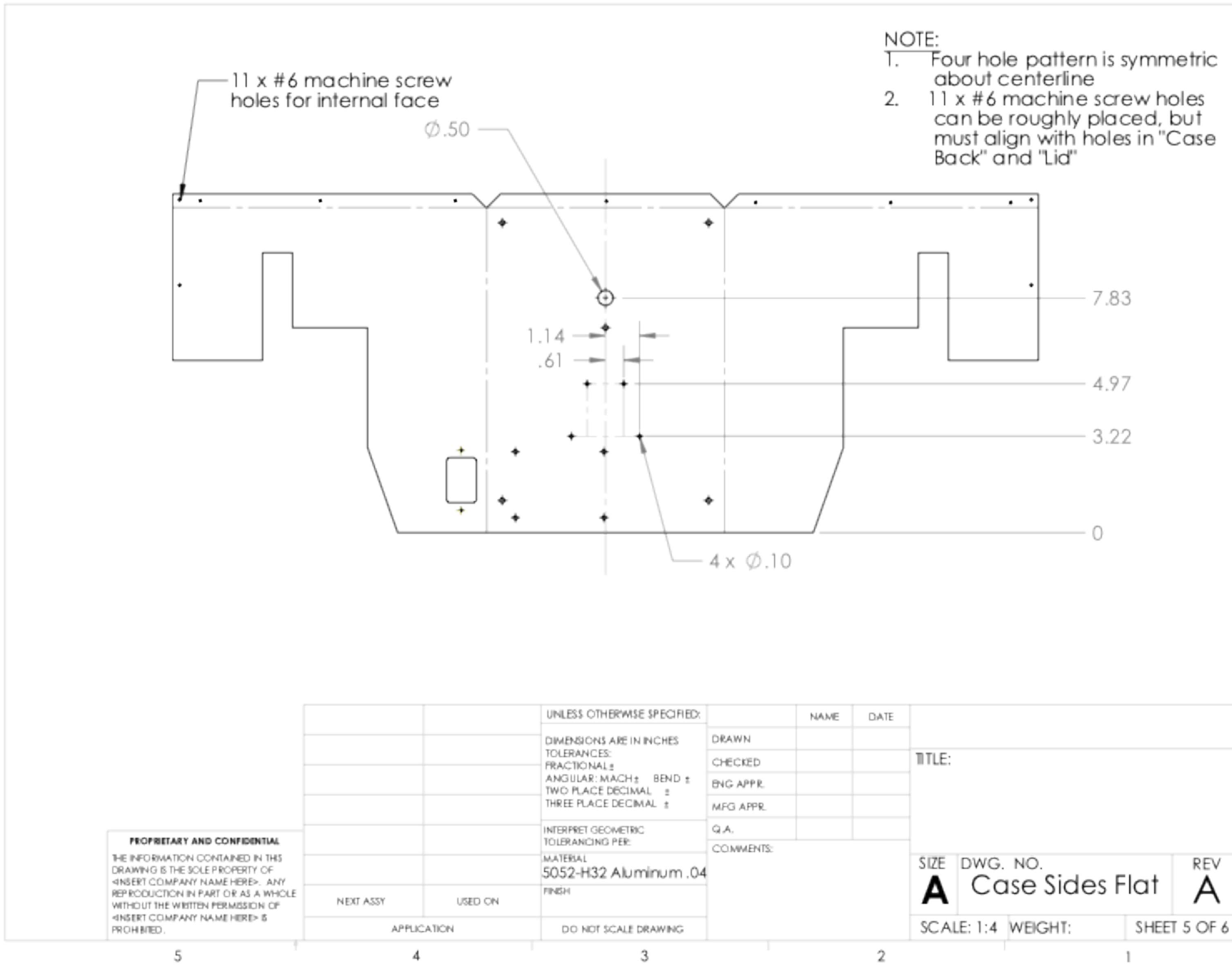
NOTE:
1. Four hole pattern is symmetric about centerline
2. 11 x #6 machine screw holes can be roughly placed, but must align with holes in "Case Back" and "Lid"
11 x #6 machine screw holes for internal face
∅.50
1.14
.61
7.83
4.97
3.22
0
4 x ∅.10
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 5 OF 6
NEXT ASSY
USED ON
APPLICATION
PROPRIETARY AND CONFIDENTIAL
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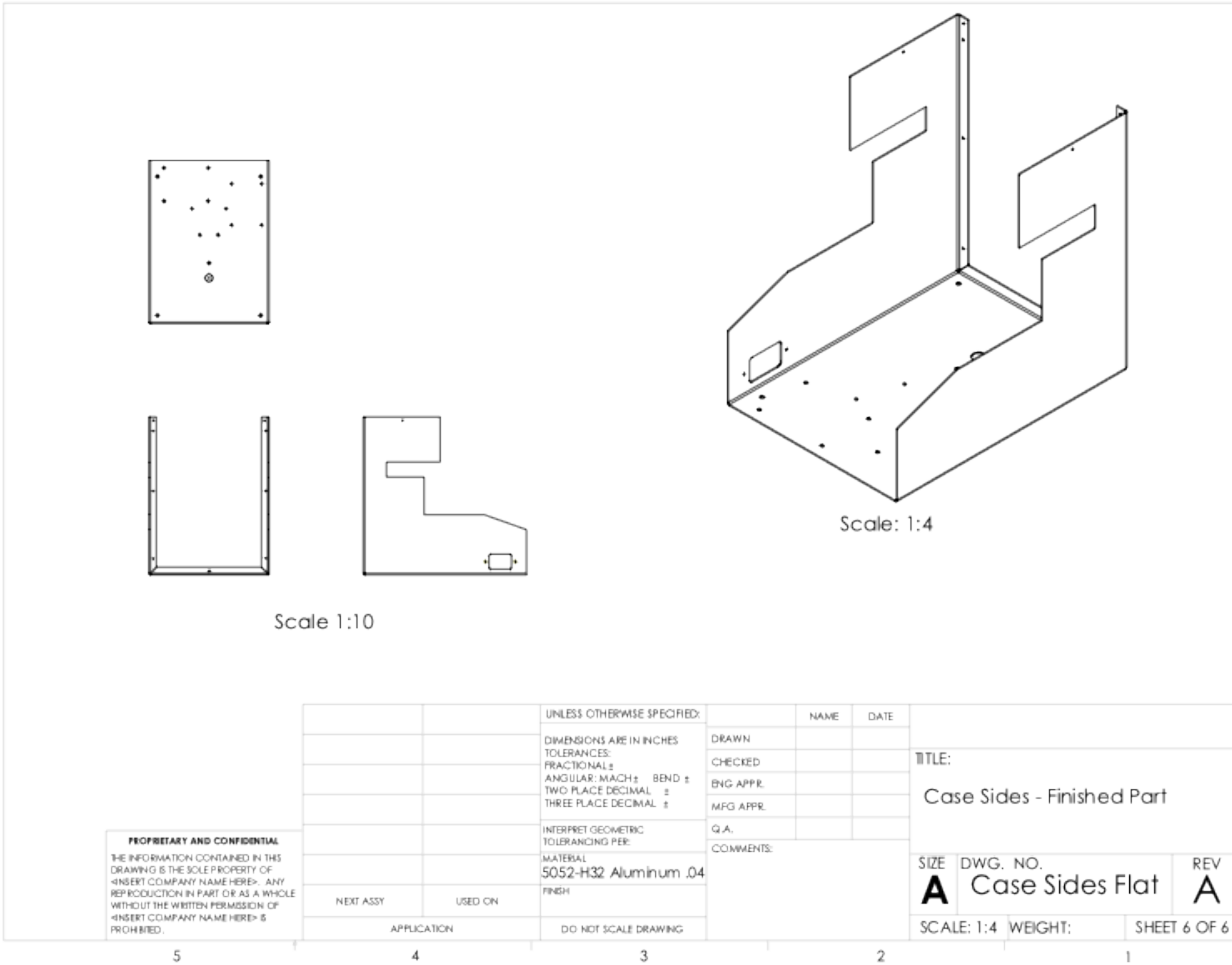
5
4
3
2
1


Scale: 1:4
Scale 1:10
PROPRIETARY AND CONFIDENTIAL
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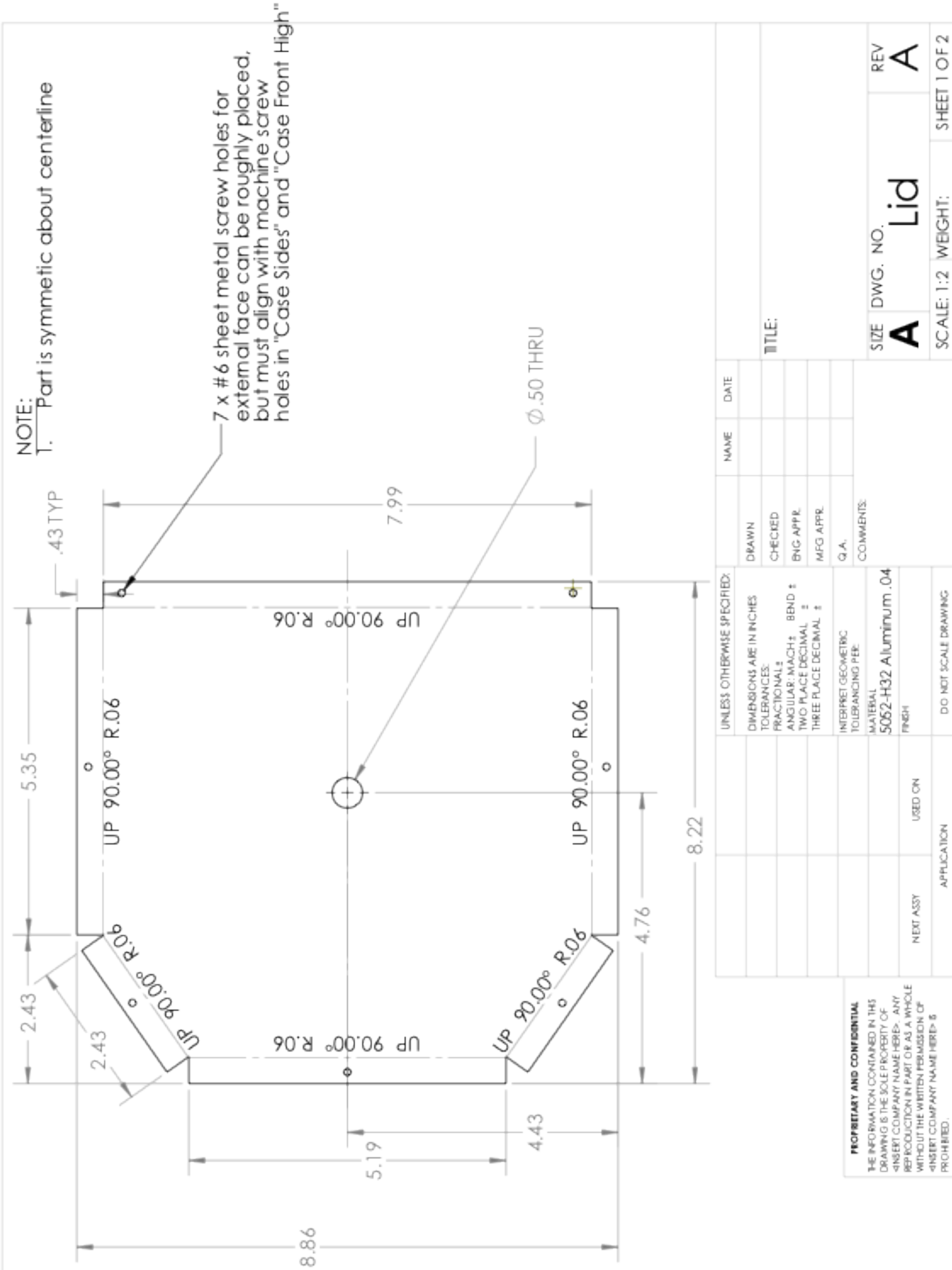
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
Case Sides - Finished Part
SIZE
A
DWG. NO.
Case Sides Flat
REV
A
SCALE: 1:4
WEIGHT:
SHEET 6 OF 6
5
4
3
2
1


NOTE:
1. Part is symmetic about centerline
7 x #6 sheet metal screw holes for external face can be roughly placed, but must align with machine screw holes in "Case Sides" and "Case Front High"
Ø.50 THRU
.43 TYP
7.99
5.35
2.43
2.43
8.22
4.76
4.43
5.19
8.86
UP 90.00° R.06
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
NAME
DATE
TITLE:
SIZE
A
DWG. NO.
Lid
REV
A
SCALE: 1:2
WEIGHT:
SHEET 1 OF 2
NEXT ASSY
USED ON
APPLICATION
PROPRIETARY AND CONFIDENTIAL
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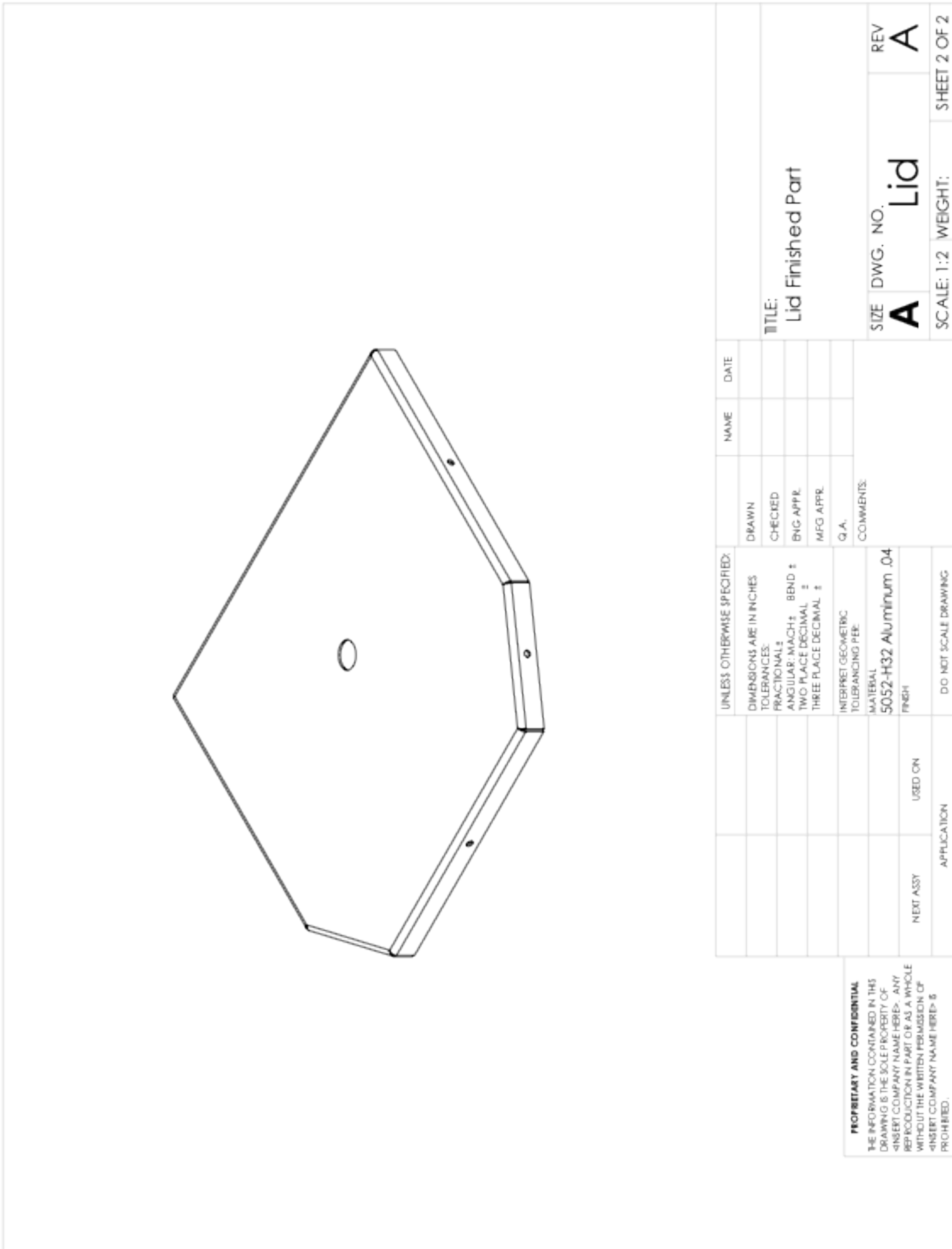
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC
TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
Lid Finished Part
SIZE
A
DWG. NO.
Lid
REV
A
SCALE: 1:2
WEIGHT:
SHEET 2 OF 2
NEXT ASSY
USED ON
APPLICATION
PROPRIETARY AND CONFIDENTIAL
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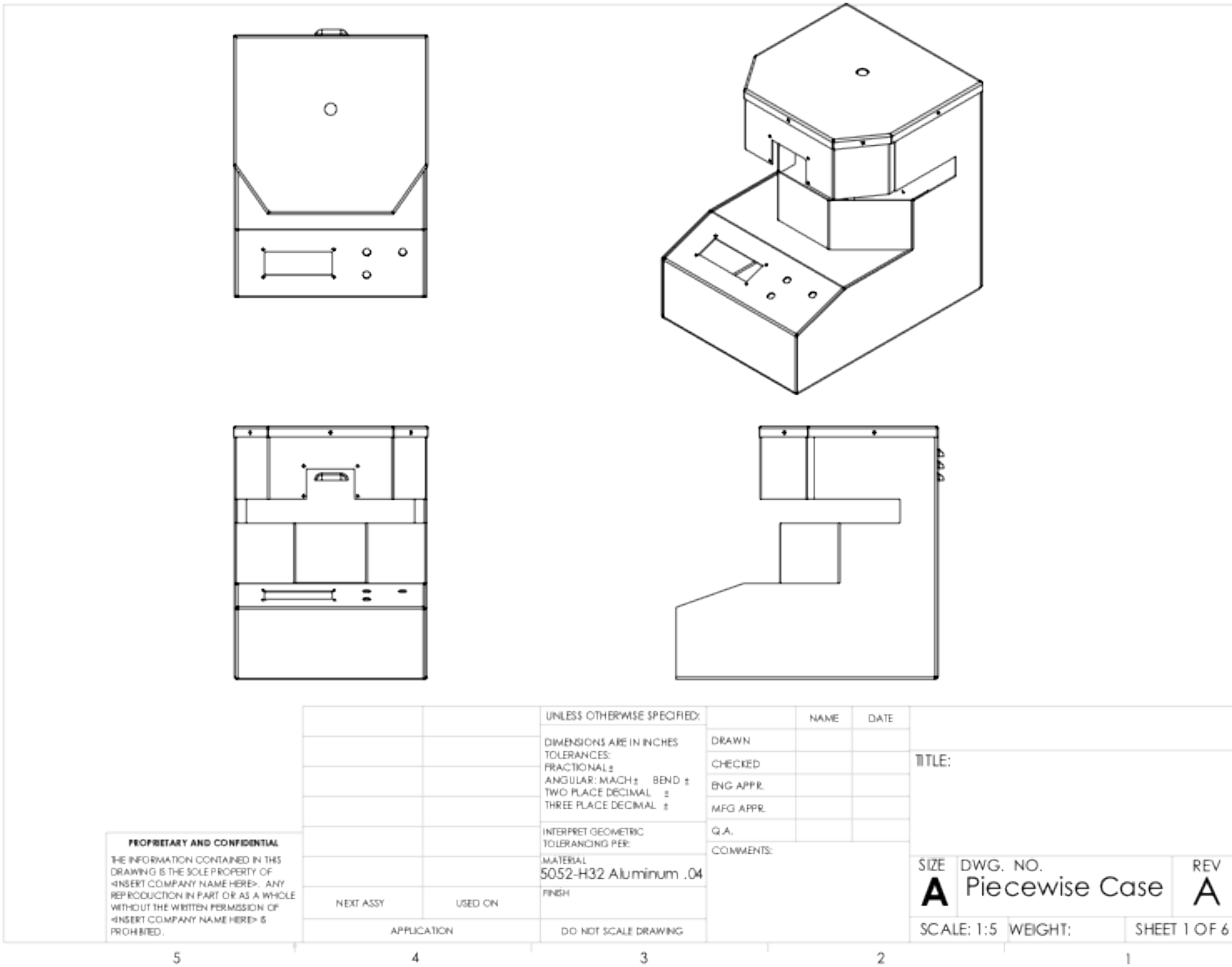
5
4
3
2
1


UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Piecewise Case
REV
A
SCALE: 1:5
WEIGHT:
SHEET 1 OF 6
NEXT ASSY
USED ON
APPLICATION
PROPRIETARY AND CONFIDENTIAL
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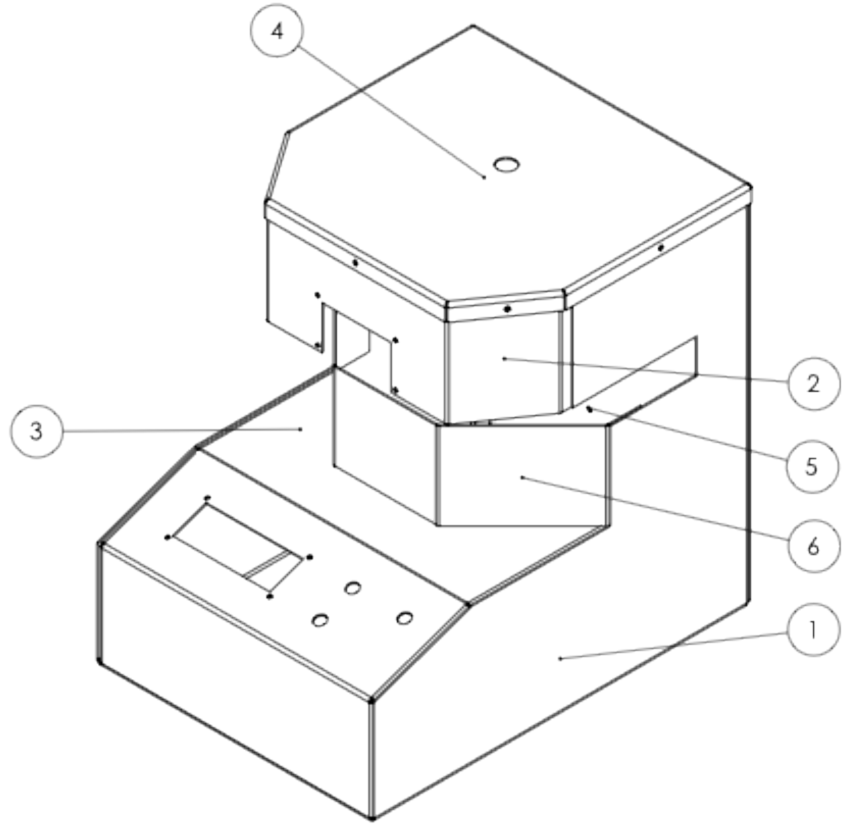
5
4
3
2
1



ITEM NO.	PART NUMBER	QTY.
1	Case Sides	1
2	Case High Front	1
3	Case Button Surface	1
4	Lid	1
5	Case Back	1
6	Case Low Front	1

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APPLICATION

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DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
5052-H32 Aluminum .04

FINISH

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN		
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

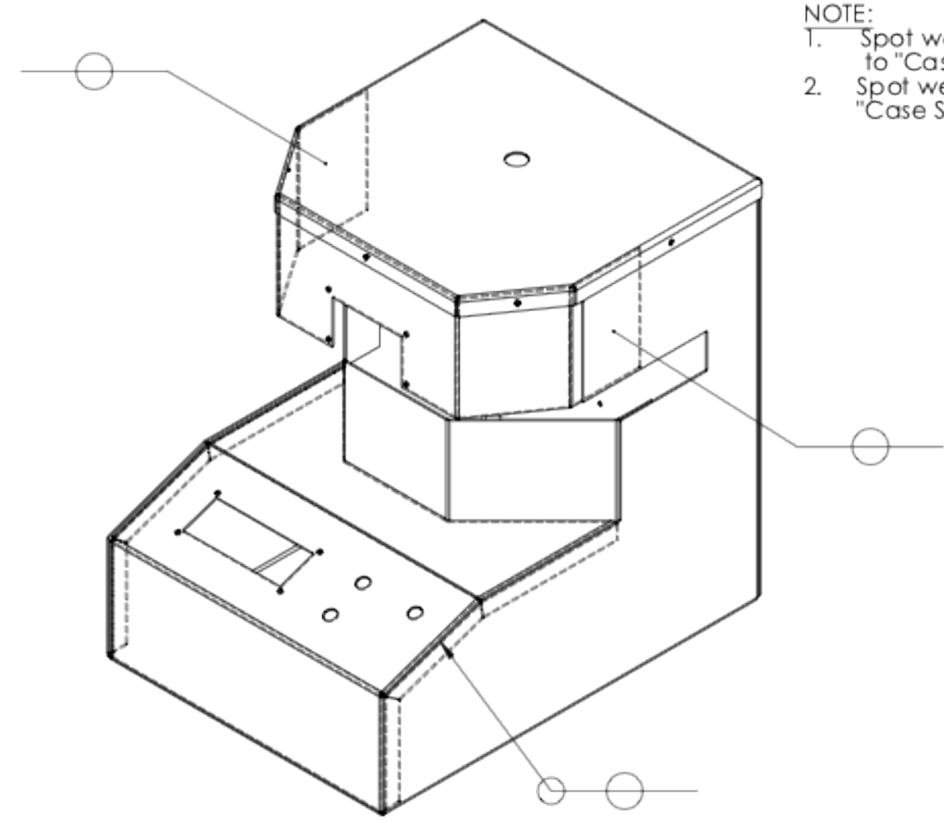
TITLE:

SIZE A | DWG. NO. Piecewise Case | REV A

SCALE: 1:3 | WEIGHT: | SHEET 2 OF 6

5 4 3 2 1

NOTE:
1. Spot weld tabs of "Case Button Surface" to "Case Sides"
2. Spot weld tabs of "Case Front High" to "Case Sides"



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NEXT ASSY	USED ON	
APPLICATION		

UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING

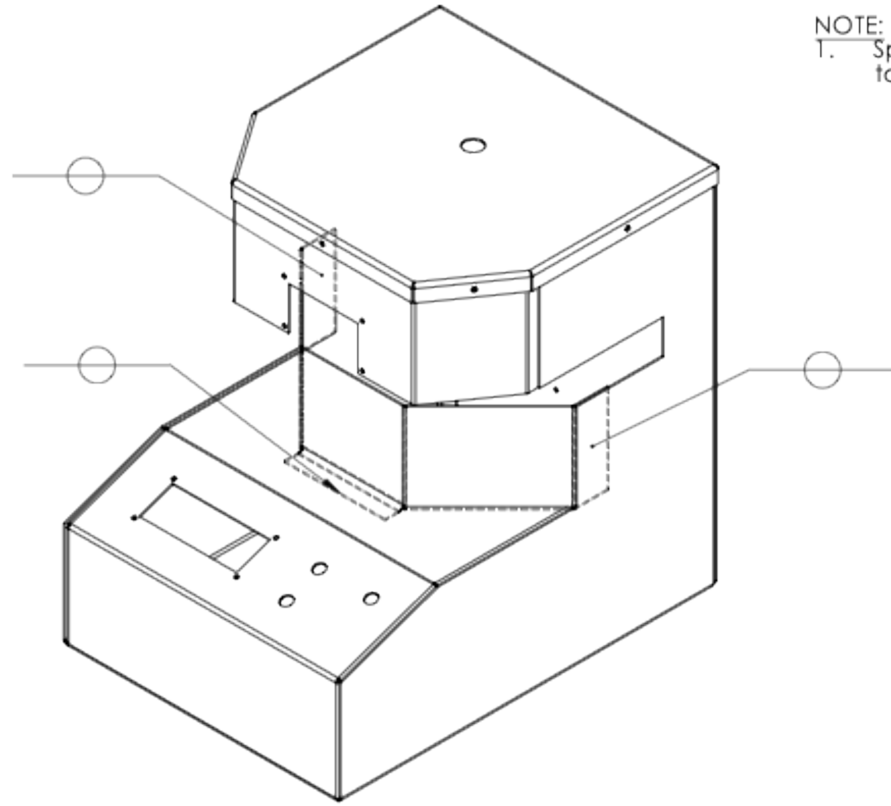
	NAME	DATE
DRAWN		
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:

SIZE	DWG. NO.	REV
A	Piecewise Case	A
SCALE: 1:3	WEIGHT:	SHEET 3 OF 6

5 4 3 2 1

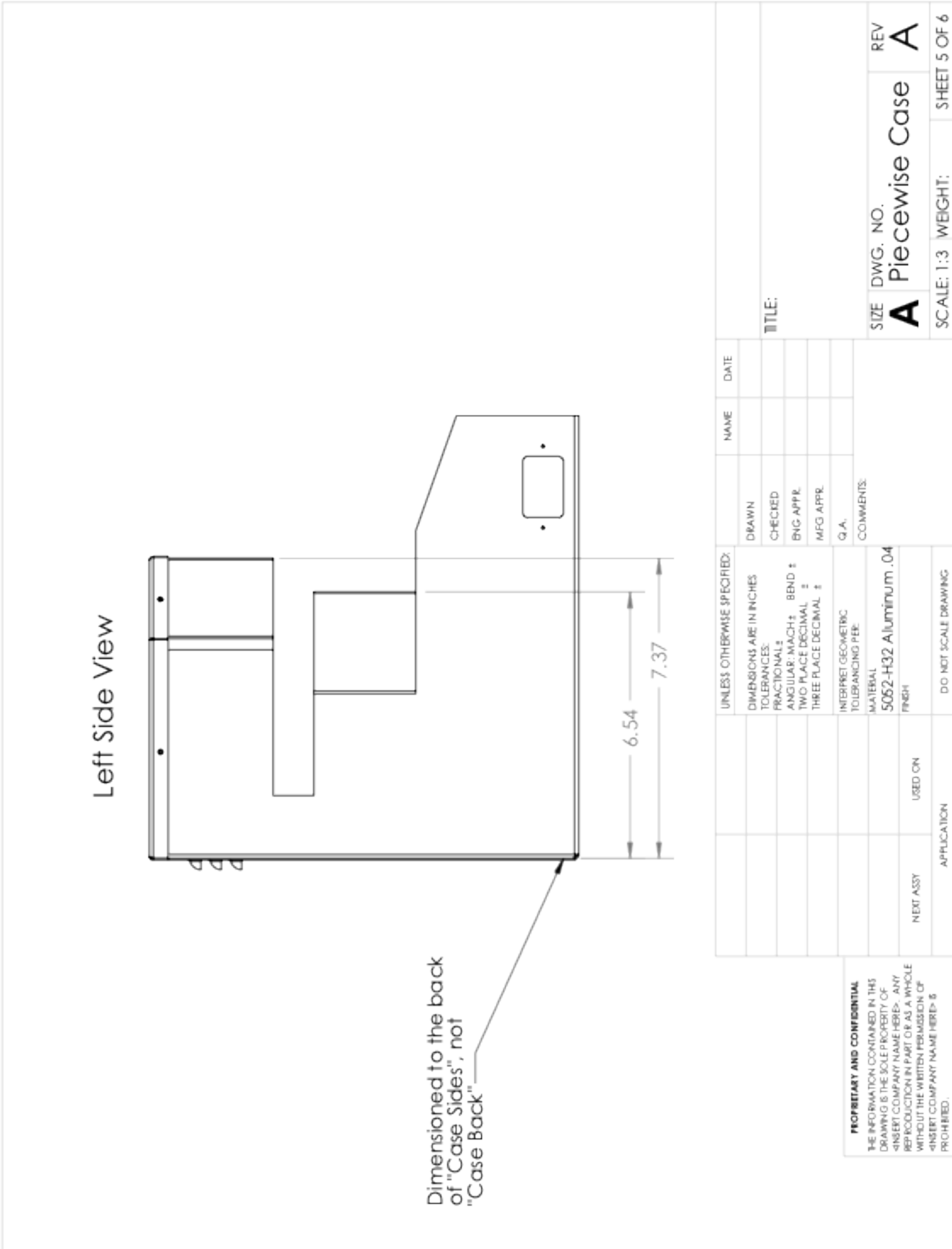
NOTE:
1. Spot Weld tabs of "Case Front Low" to "Case Sides"



UNLESS OTHERWISE SPECIFIED:		NAME	DATE		
DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±	DRAWN				
	CHECKED			TITLE:	
	ENG APPR.				
	MFG APPR.				
INTERPRET GEOMETRIC TOLERANCING PER:	Q.A.				
MATERIAL 5052-H32 Aluminum .04	COMMENTS:			SIZE A	DWG. NO. Piecewise Case · REV A
FINISH					
DO NOT SCALE DRAWING				SCALE: 1:3 · WEIGHT:	SHEET 4 OF 6

NEXT ASSY	USED ON
APPLICATION	

5 4 3 2 1


Left Side View
6.54
7.37
Dimensioned to the back of "Case Sides", not "Case Back"
PROPRIETARY AND CONFIDENTIAL
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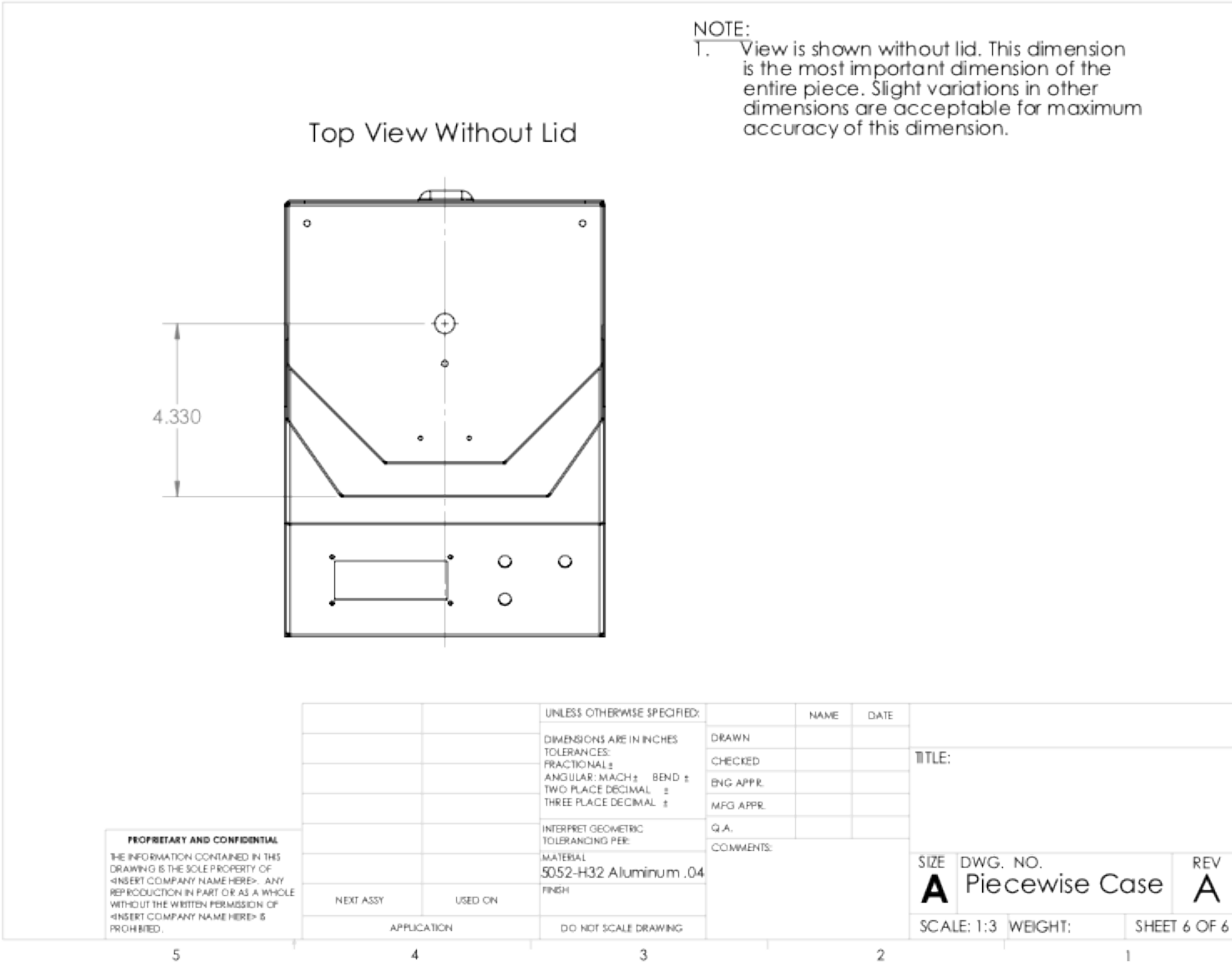
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Piecewise Case
REV
A
SCALE: 1:3
WEIGHT:
SHEET 5 OF 6
1
2
3
4
5


NOTE:
1. View is shown without lid. This dimension is the most important dimension of the entire piece. Slight variations in other dimensions are acceptable for maximum accuracy of this dimension.
Top View Without Lid
4.330
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Piecewise Case
REV
A
SCALE: 1:3
WEIGHT:
SHEET 6 OF 6
PROPRIETARY AND CONFIDENTIAL
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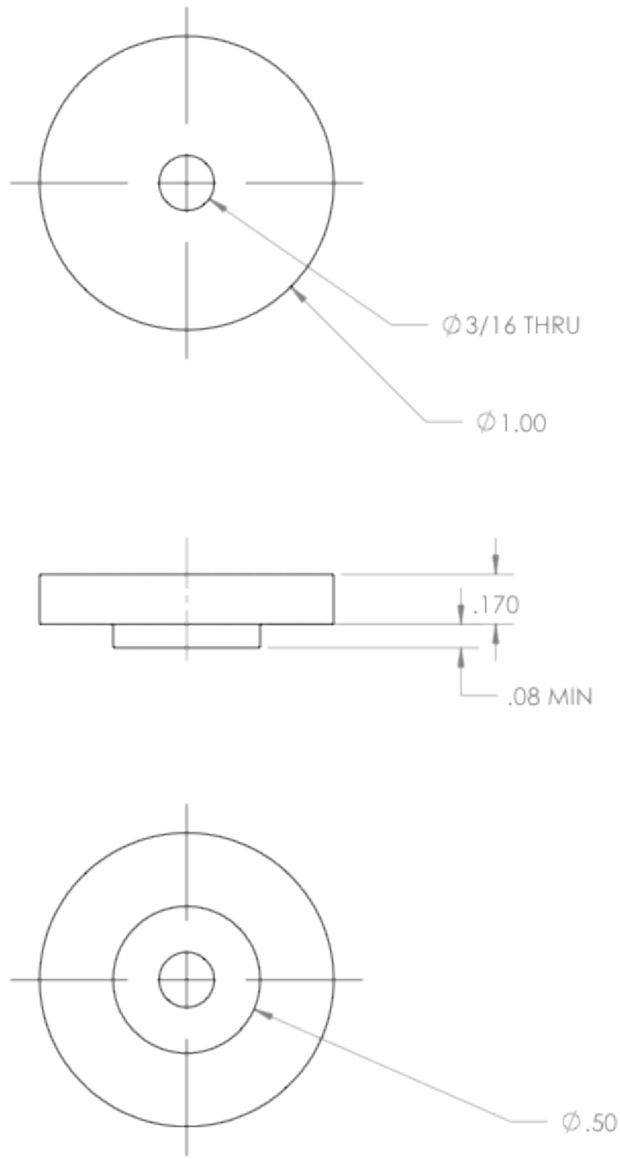
NEXT ASSY
USED ON
APPLICATION
5
4
3
2
1



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NEXT ASSY | USED ON

APPLICATION

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

MATERIAL

FINISH

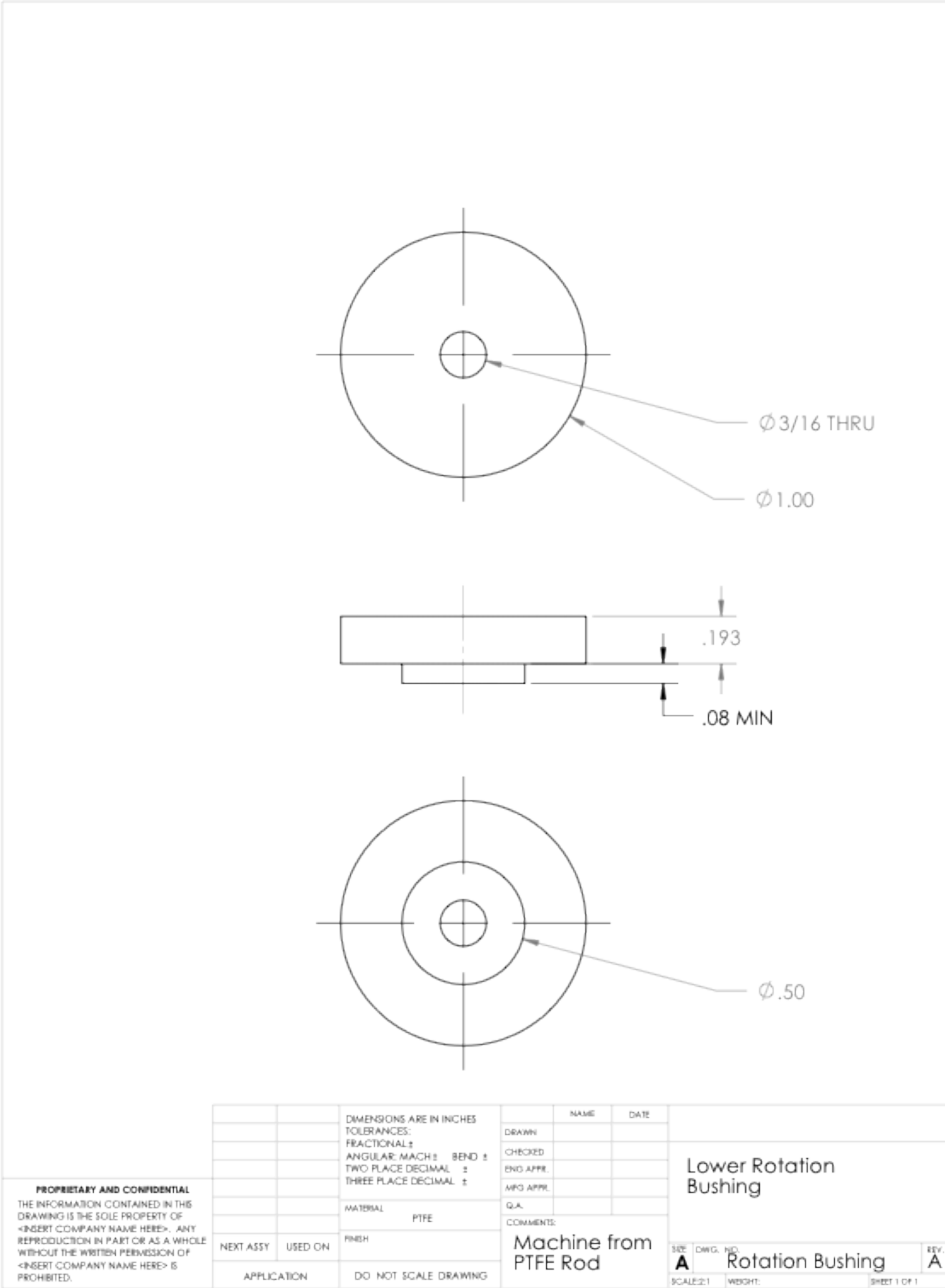
DO NOT SCALE DRAWING

	NAME	DATE
DRAWN		
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

SIZE A | DWG. NO. Rotation Bushing Upper | REV. A

SCALE:2:1 | WEIGHT: | SHEET 1 OF 1



∅3/16 THRU
∅1.00
.193
.08 MIN
∅.50
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
Lower Rotation Bushing
PROPRIETARY AND CONFIDENTIAL
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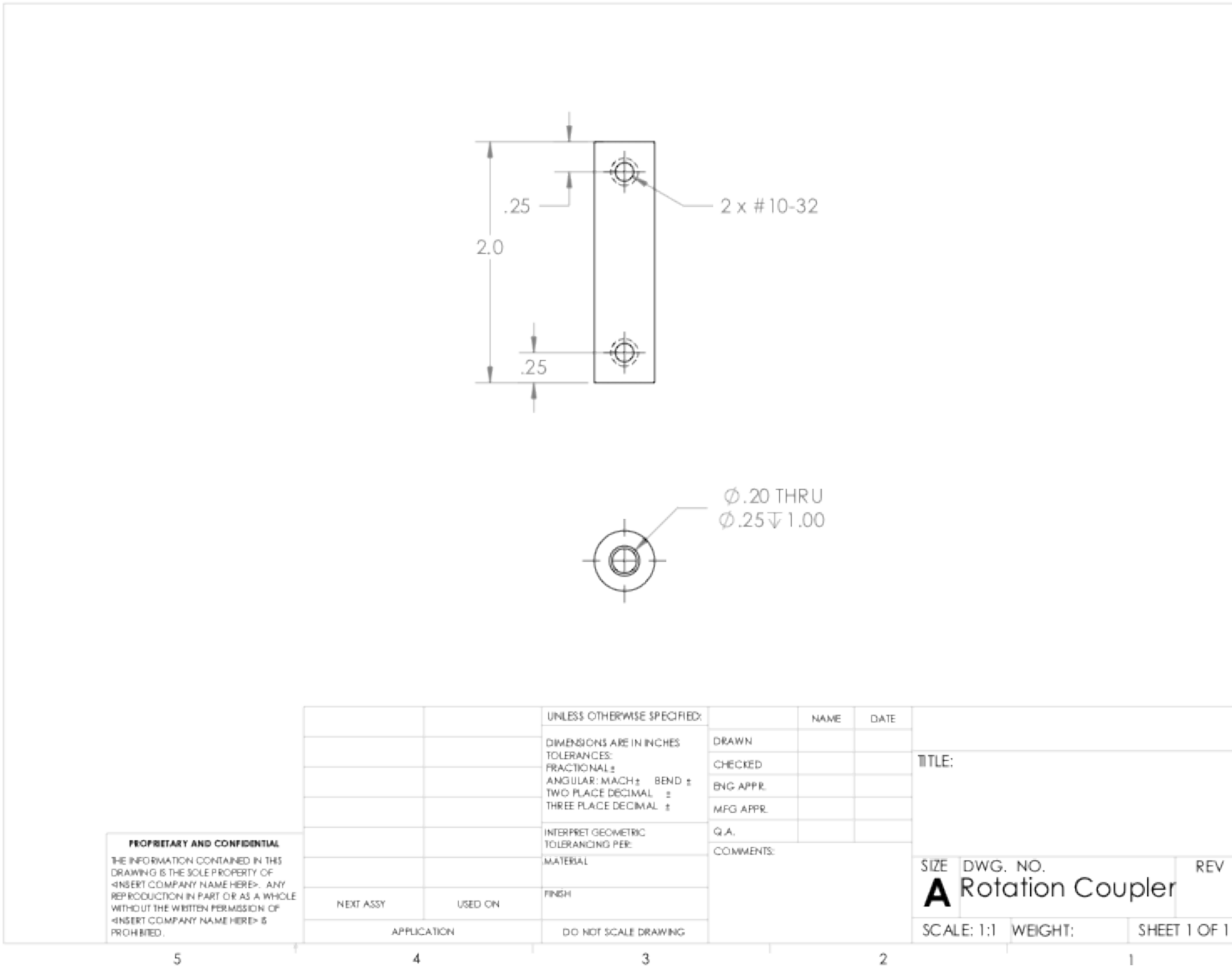
MATERIAL
PTFE
FINISH
NEXT ASSY
USED ON
APPLICATION
DO NOT SCALE DRAWING
Machine from PTFE Rod
SIZE
A
DWG. NO.
Rotation Bushing
REV.
A
SCALE:2:1
WEIGHT:
SHEET 1 OF 1


.25
2.0
.25
2 x #10-32
Ø.20 THRU
Ø.25 ↧ 1.00
PROPRIETARY AND CONFIDENTIAL
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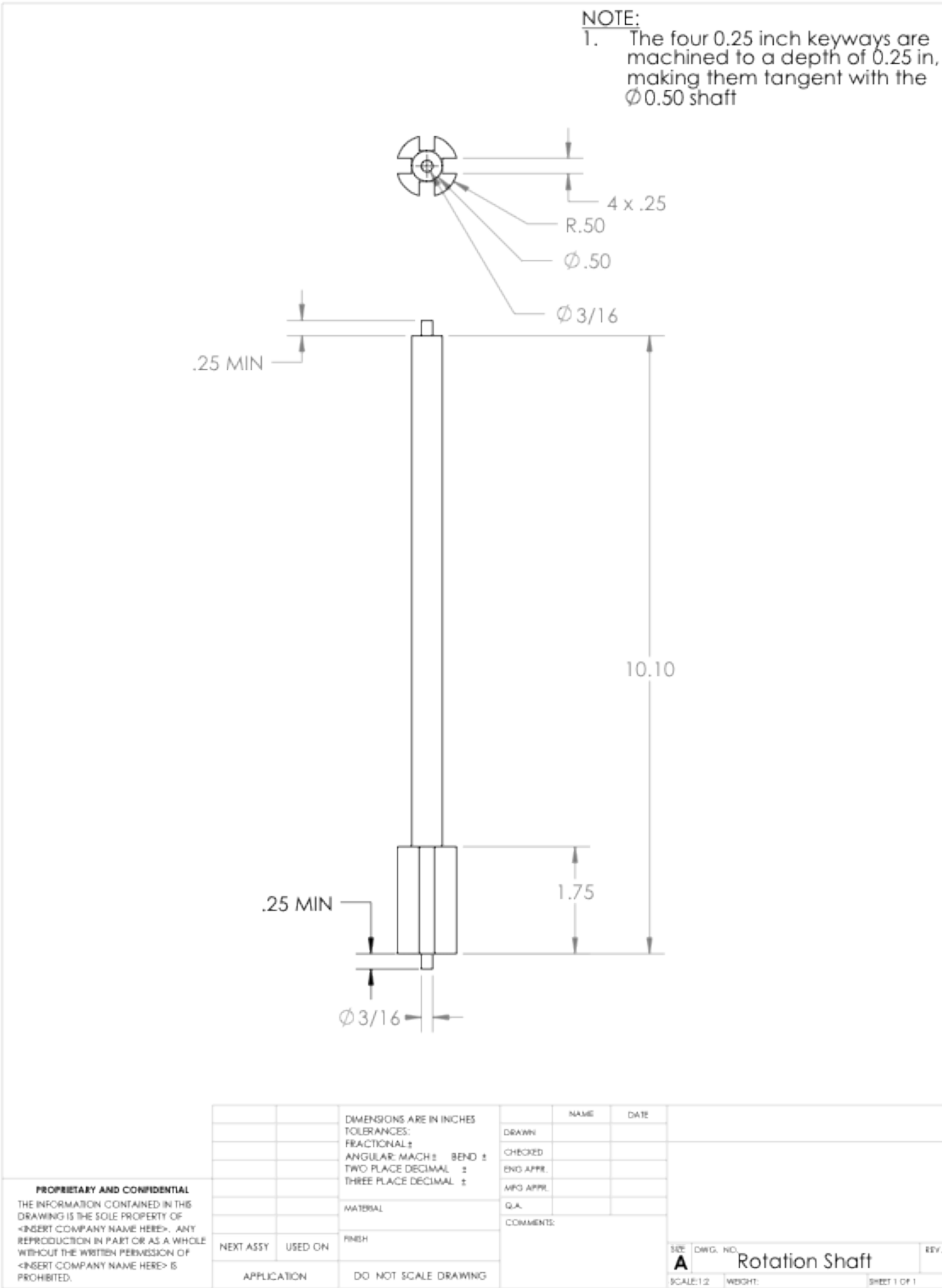
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
DWG. NO.
REV
A
Rotation Coupler
SCALE: 1:1
WEIGHT:
SHEET 1 OF 1
5
4
3
2
1


NOTE:
1. The four 0.25 inch keyways are machined to a depth of 0.25 in, making them tangent with the ∅0.50 shaft
4 x .25
R.50
∅.50
∅3/16
.25 MIN
10.10
1.75
.25 MIN
∅3/16
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
MATERIAL
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
PROPRIETARY AND CONFIDENTIAL
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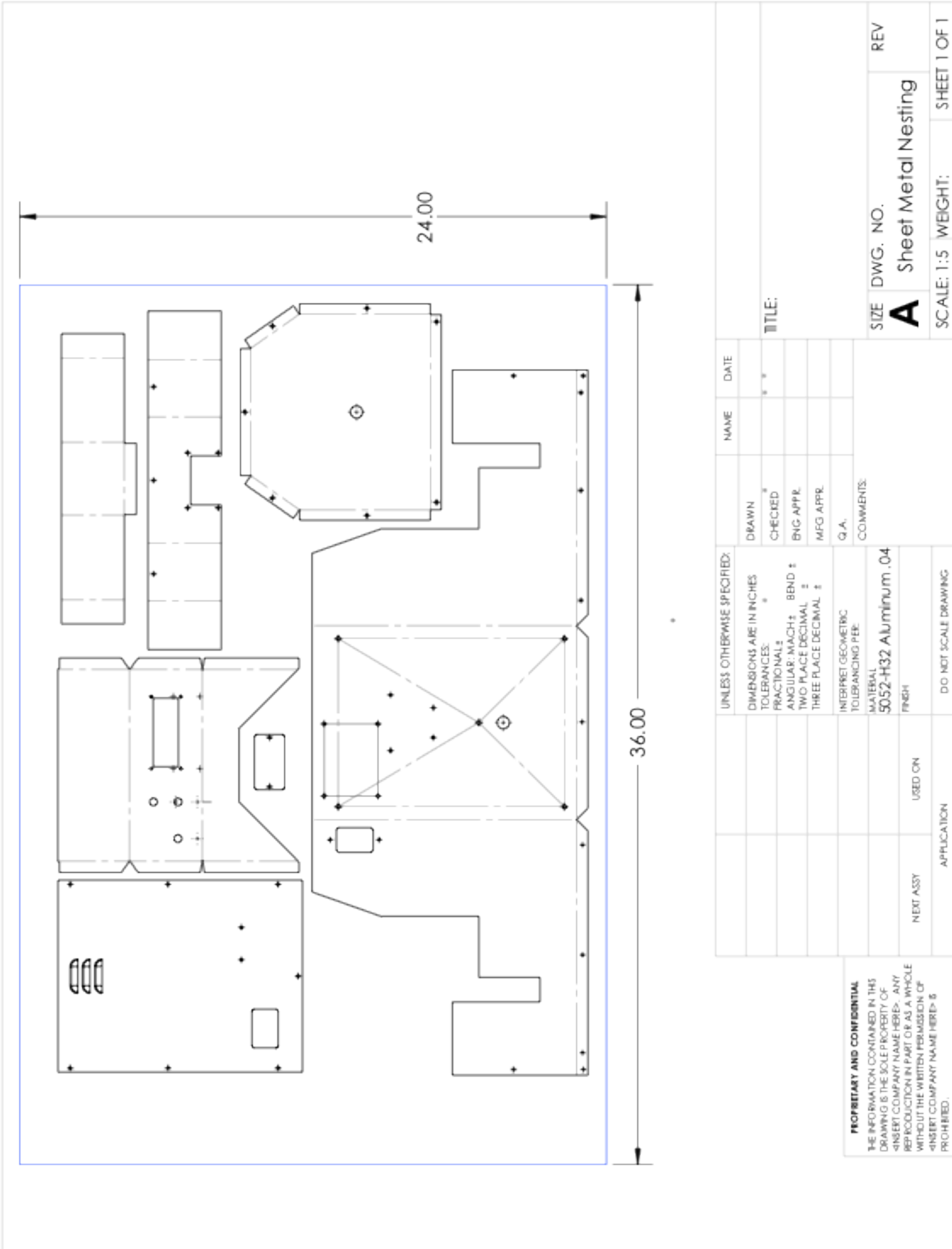
NEXT ASSY
USED ON
APPLICATION
SIZE
A
DWG. NO.
Rotation Shaft
REV.
SCALE: 1:2
WEIGHT:
SHEET 1 OF 1


24.00
36.00
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
SIZE
A
DWG. NO.
Sheet Metal Nesting
REV
SCALE: 1:5
WEIGHT:
SHEET 1 OF 1
NEXT ASSY
USED ON
APPLICATION
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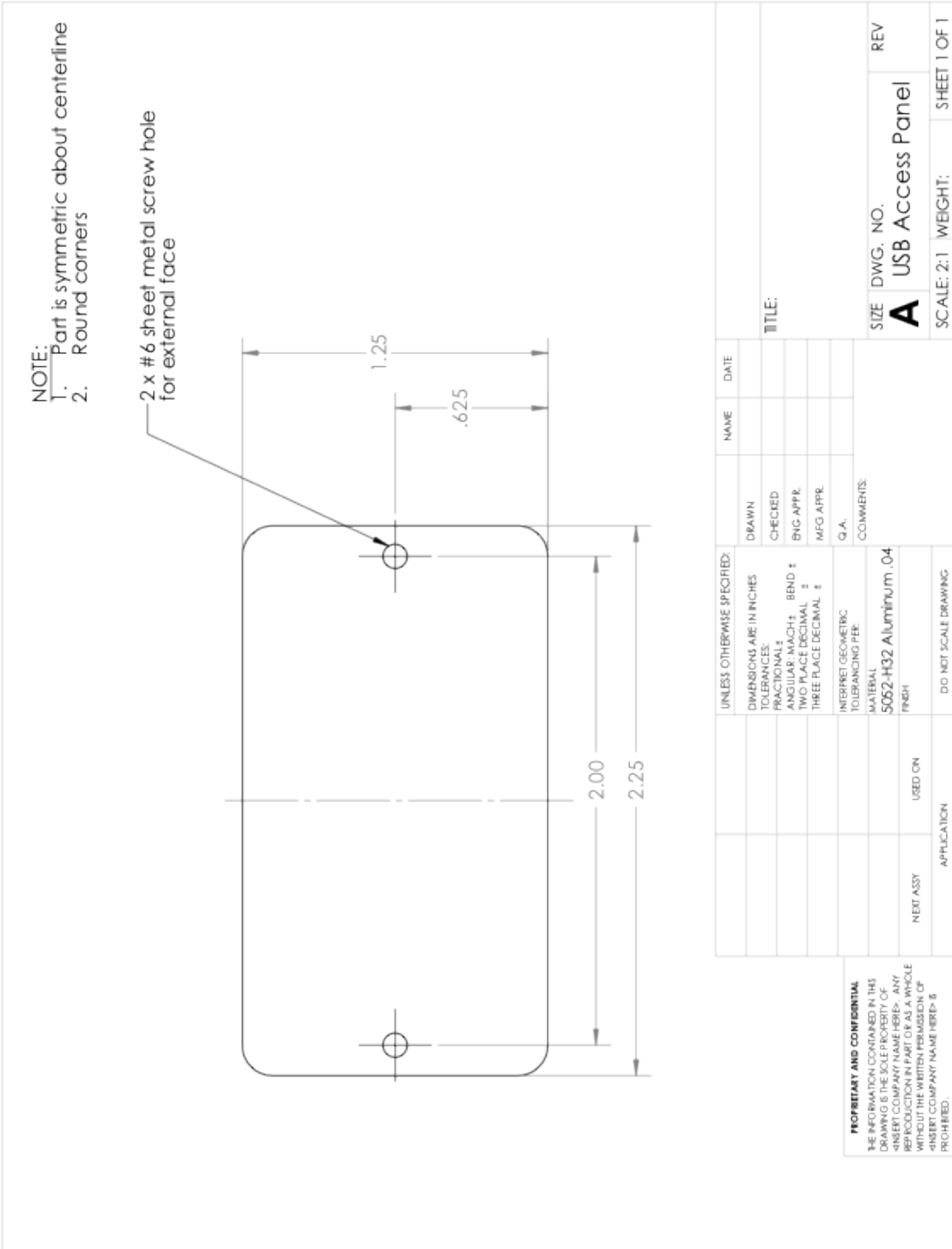
1
2
3
4
5


NOTE:
1. Part is symmetric about centerline
2. Round corners
2 x #6 sheet metal screw hole
for external face
1.25
.625
2.00
2.25
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC
TOLERANCING PER:
MATERIAL
5052-H32 Aluminum .04
FINISH
DO NOT SCALE DRAWING
NEXT ASSY
USED ON
APPLICATION
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
NAME
DATE
TITLE:
SIZE
A
DWG. NO.
USB Access Panel
REV
SCALE: 2:1
WEIGHT:
SHEET 1 OF 1
PROPRIETARY AND CONFIDENTIAL
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1
2
3
4
5

Appendix 2: Rapid Prototyped Parts

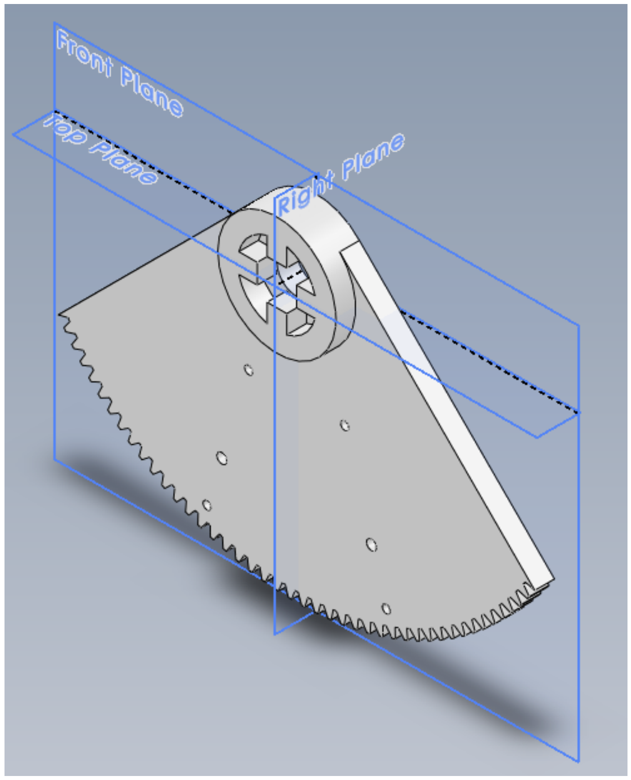


Figure 8: Large gear for Rotation Assembly

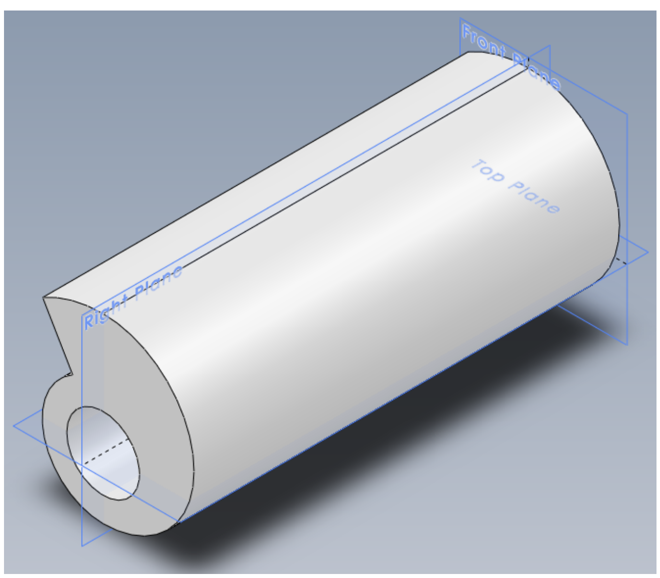


Figure 9: Cam for Specimen Handling Assembly

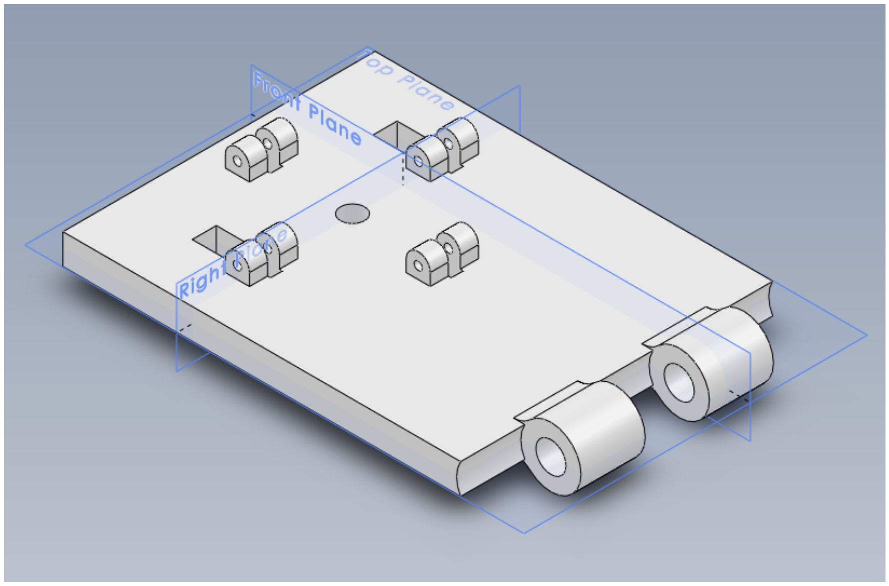


Figure 10: Hinge Surface for Specimen Handling Assembly

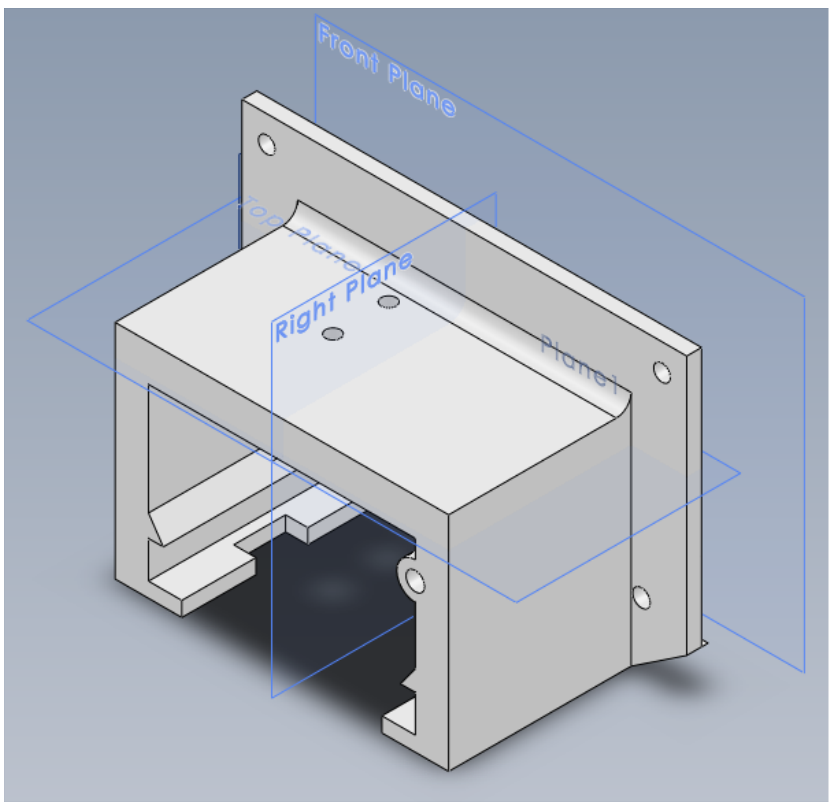


Figure 11: Housing for Specimen Handling Assembly

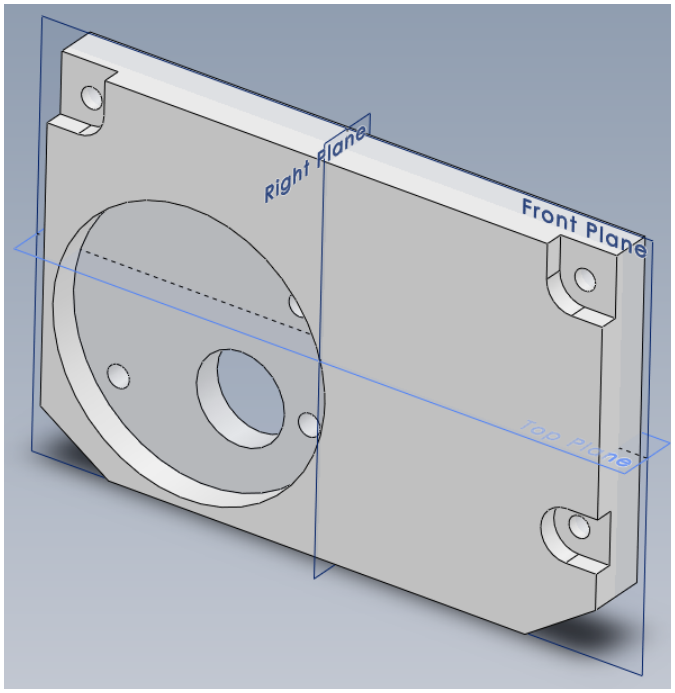


Figure 12: DC Motor Mount for Specimen Handling Assembly

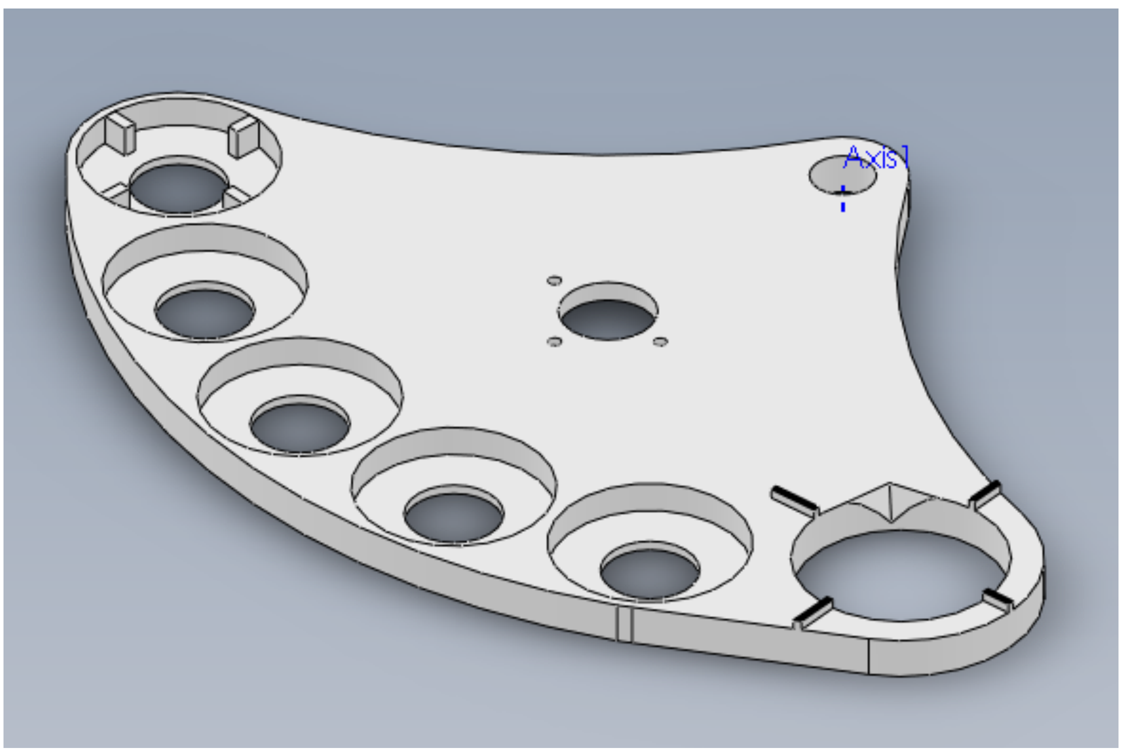


Figure 13: Petri Dish Tray for Elevation Assembly

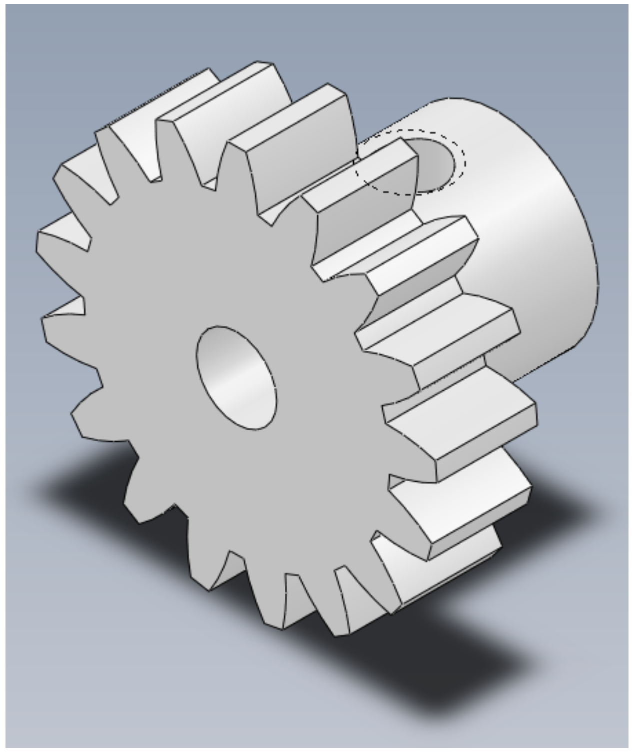


Figure 14: Pinion Gear for Rotation Assembly

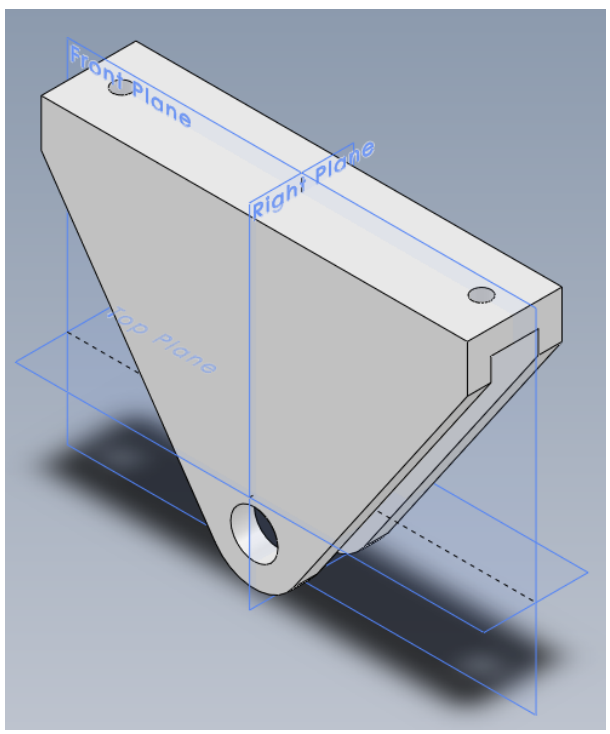


Figure 15: Roller Mount for Rotation Assembly

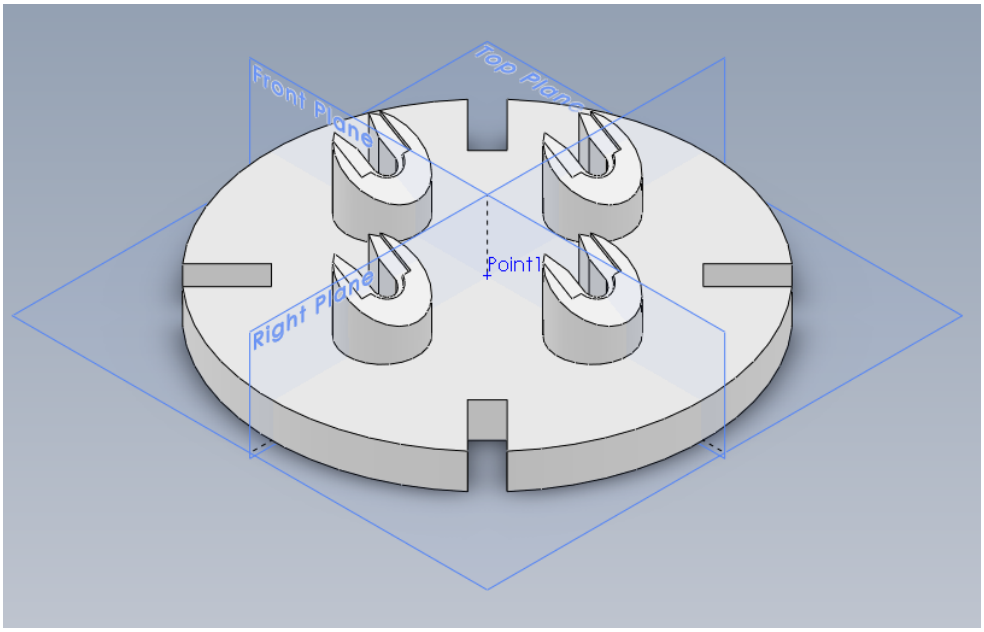


Figure 16: Specimen Set-Up Disk for Rotation Assembly

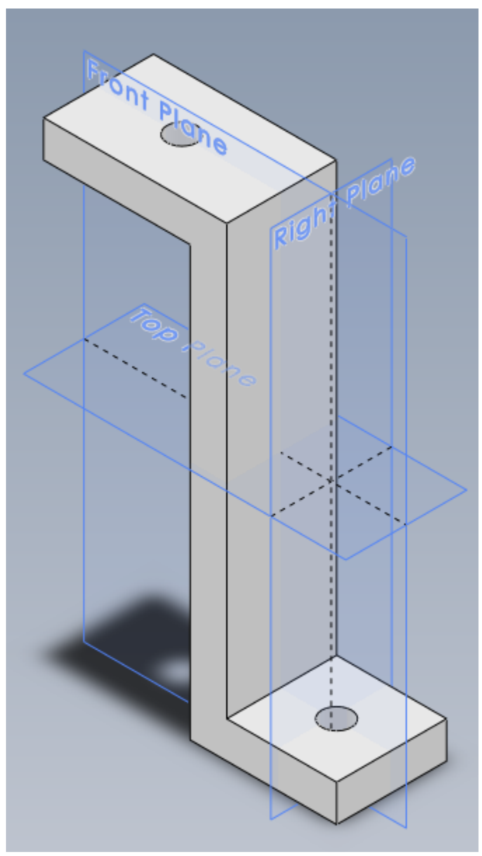


Figure 17: Stepper Motor Mount for both Rotation and Elevation Assemblies

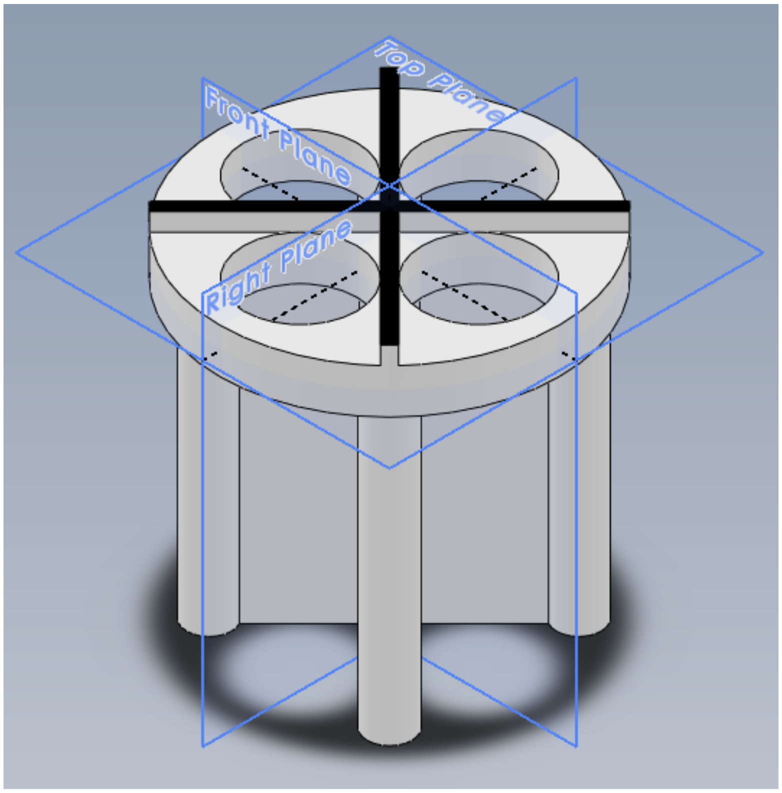


Figure 18: Cryo-Vial Holder

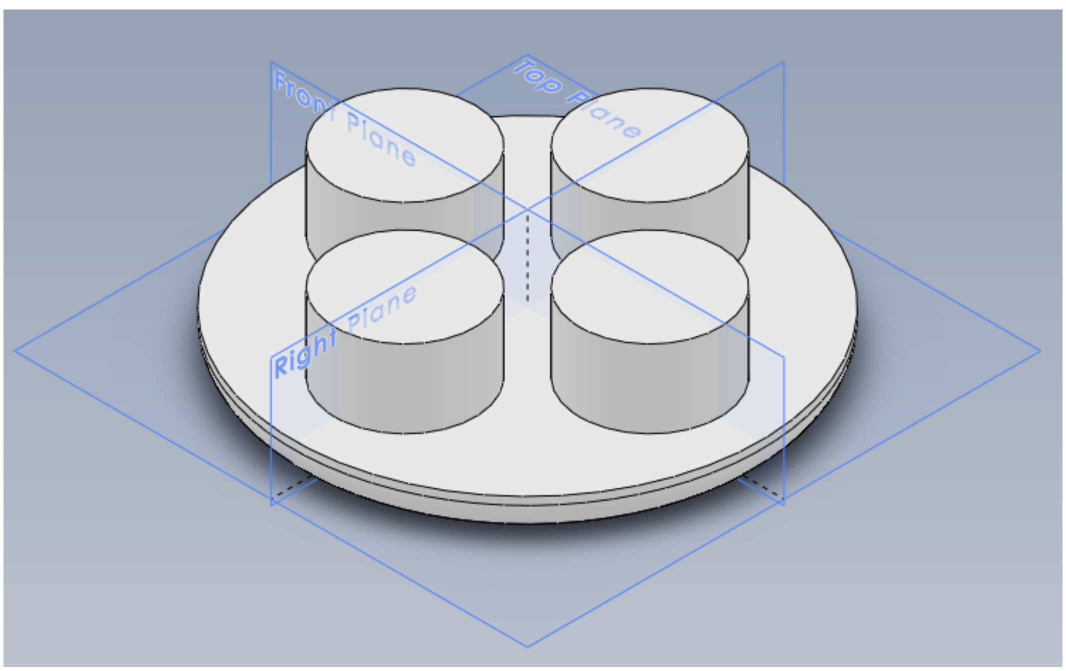
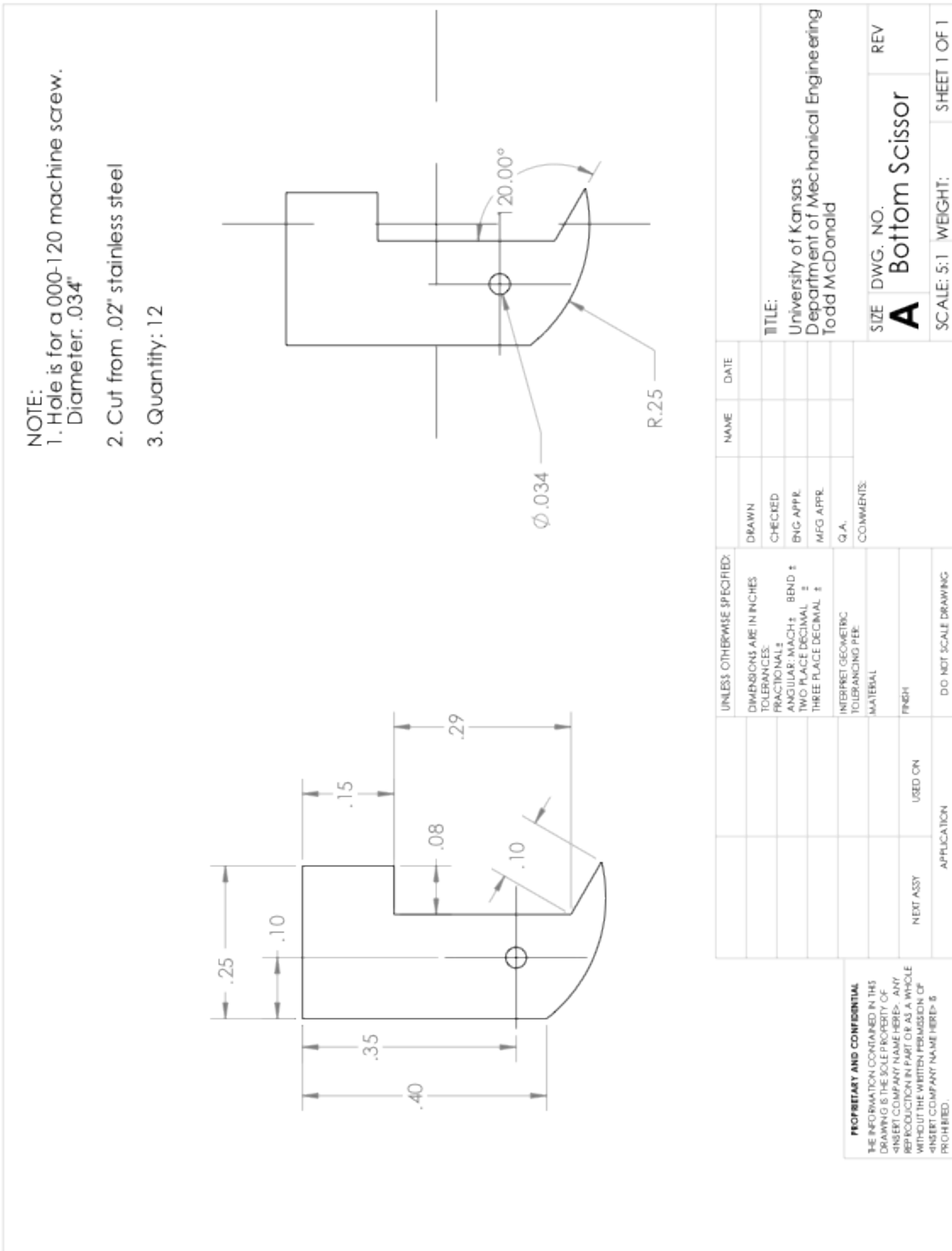
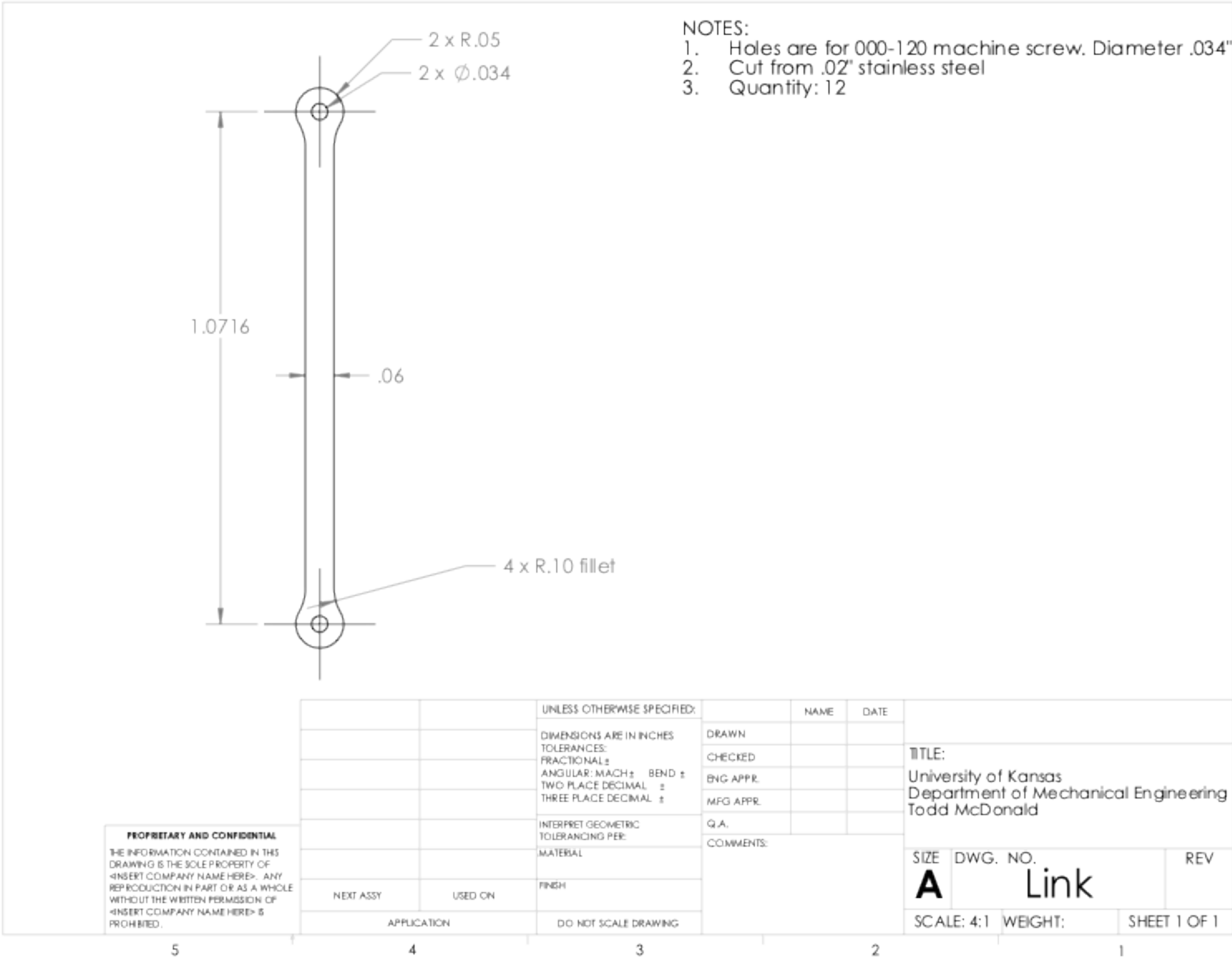


Figure 19: Cryo-Vial Holder Bottom

Appendix 3: Laser-Cut Parts




2 x R.05
2 x ∅.034
1.0716
.06
4 x R.10 fillet
NOTES:
1. Holes are for 000-120 machine screw. Diameter .034"
2. Cut from .02" stainless steel
3. Quantity: 12
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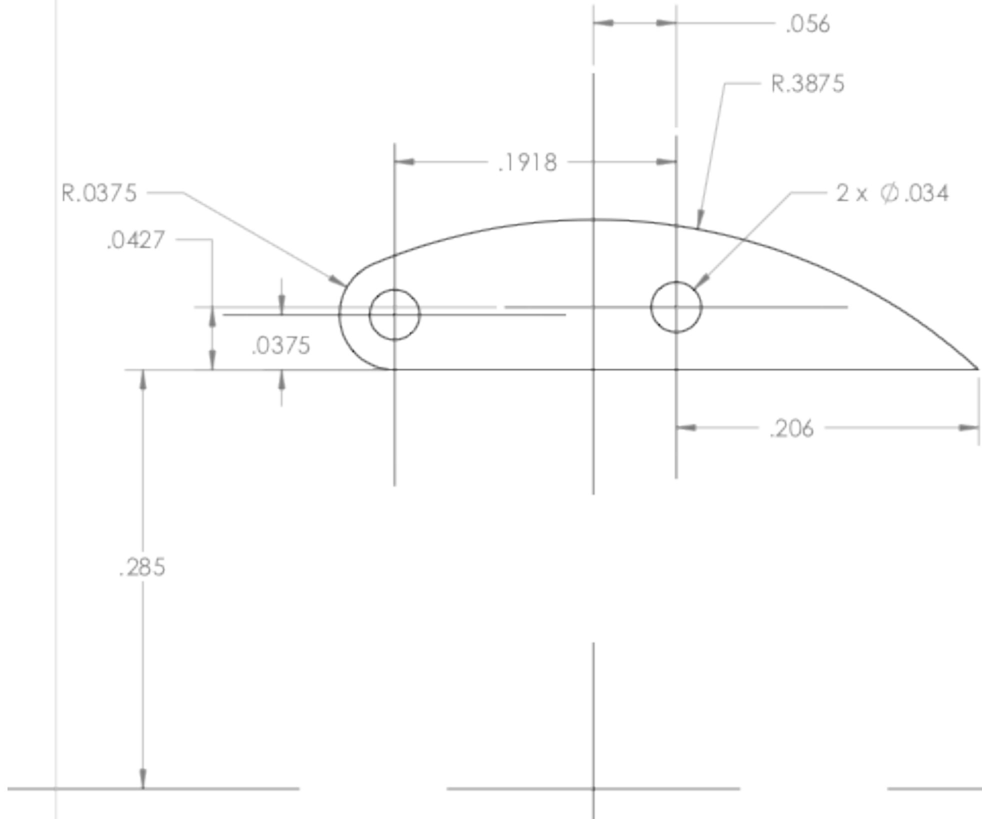
NEXT ASSY
USED ON
APPLICATION
UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
TITLE:
University of Kansas
Department of Mechanical Engineering
Todd McDonald
SIZE
A
DWG. NO.
Link
REV
SCALE: 4:1
WEIGHT:
SHEET 1 OF 1
5
4
3
2
1



NOTE:
1. Holes are for a 000-120 machine screw. Diameter .034"
2. Top edge geometry is not important. Drawn as a smooth curve.
3. Cut from .02" stainless steel.
4. Quantity: 12

UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
INTERPRET GEOMETRIC TOLERANCING PER:
MATERIAL
FINISH
DO NOT SCALE DRAWING

	NAME	DATE
DRAWN		
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		
COMMENTS:		

TITLE:
University of Kansas
Department of Mechanical Engineering
Todd McDonald

SIZE A DWG. NO. Top Scissor REV
SCALE: 10:1 WEIGHT: SHEET 1 OF 1

NEXT ASSY | USED ON
APPLICATION

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5 4 3 2 1

Appendix 4: Program Code

```
' =========================================================================
'
'   File...... VitFirmWare.SXB
'   Purpose... Controls the KVit Vitrification Machine
'   Author.... Todd McDonald
'   E-mail.... Todd.W.McDonald@gmail.com
'   Started... November 17, 2009
'   Updated...
'
'   This code contains portions of code freely available from Parallax for
'   use with their LCD screen. Portions of this code may have been
'   modified. No statement of ownership of said code is made.
'==========================================================================


' -------------------------------------------------------------------------
' Program Description
' -------------------------------------------------------------------------
' This program controls the Vitrification Machine as designed by Todd
' McDonald.
' The program initializes then activates the motors in order to find the
' "home" position. The motors stop when they engage the physical limit
' switches
' incorporated into the design of the machine. The machine then gets input
' parameters from the user. These parameters include the number of
' exposures and the length of each exposure.
' Y motor is rotation, X motor is Elevation

' -------------------------------------------------------------------------
' Device Settings
' -------------------------------------------------------------------------

DEVICE          SX28, OSCXT2, TURBO, STACKX, OPTIONX
FREQ            4_000_000

' -------------------------------------------------------------------------
' IO Pins
' -------------------------------------------------------------------------

LcdTx          VAR     RA.0                   ' LCD serial connection

IncreaseButton  PIN     RB.0   INPUT      ' Make Pin RB.0 an input pin
DecreaseButton  PIN     RB.1   INPUT      ' Make Pin RB.1 an input pin
OKButton        PIN     RB.2   INPUT      ' Make Pin RB.2 an input pin
LiftSense       PIN     RB.3   INPUT      ' Make Pin RB.3 an input pin
                                          ' senses if the forceps are
                                          ' open (0) or closed (1)

MotorCommand    VAR     RC.0          ' Motor Controller serial connection
MotorBusyLine   PIN     RC.1   INPUT  ' 1 if Motor Controller is idle, 0
                                      ' if busy
LiftMotor1      PIN     RC.2   INPUT  ' 1st control pin for DC Lift Motor
```

```
LiftMotor2      PIN     RC.3   INPUT  ' 2nd control pin for DC Lift Motor

' -------------------------------------------------------------------------
' Constants
' -------------------------------------------------------------------------

LcdBaud          CON   "T9600"          ' or T2400, or T9600 or T19200
MotorBaud        CON   "T9600"          ' or T2400, or T9600 or T19200

LcdBkSpc         CON   $08              ' move cursor left
LcdRt            CON   $09              ' move cursor right
LcdBLoff         CON   $12              ' backlight off
LcdCls           CON   $0C              ' clear LCD (need 5 ms delay)
LcdOn1           CON   $16              ' LCD on; no crsr, no blink
LcdOn2           CON   $17              ' LCD on; no crsr, blink on
LcdOn3           CON   $18              ' LCD on; crsr on, no blink
LcdOn4           CON   $19              ' LCD on; crsr on, blink on
LcdLine1         CON   $80              ' move to line 1, column 0
LcdLine2         CON   $94              ' move to line 2, column 0

IsPressed        CON   1
IsNotPressed     CON   0

' -------------------------------------------------------------------------
' Variables
' -------------------------------------------------------------------------

ExposureNumber   VAR     Byte   ' Number of Exposures
ExpLengthMin     VAR     Byte   ' Length of Exposure in Minutes
ExpLengthTens    VAR     Byte   ' Length of Exposure in seconds Tens Place
ExpLengthOnes    VAR     Byte   ' Length of Exposure in seconds Ones Place

Exposure1        VAR     Byte   ' Individual exposure lengths
Exposure2        VAR     Byte
Exposure3        VAR     Byte
Exposure4        VAR     Byte

Char             VAR     Byte
newChar          VAR     Byte
pos              VAR     Byte   ' position

temp1            VAR     Byte   ' subroutine work variables
temp2            VAR     Byte
temp3            VAR     Byte
temp4            VAR     Byte
temp5            VAR     Byte
temp6            VAR     Byte
temp             VAR     Byte

counter          VAR     Byte

' =========================================================================
  PROGRAM Start
' =========================================================================
```

```
' ------------------------------------------------------------------------
' Subroutine Declarations
' ------------------------------------------------------------------------

WAIT_MS          SUB   1, 2              ' delay in milliseconds
LCD_OUT          SUB   1, 2              ' send byte {+ count} to LCD
LCD_STR          SUB   2                 ' send string to LCD
MOTOR_OUT        SUB   1, 2              ' send byte {+ count} to Motor
MOTOR_STR        SUB   2                 ' send string to Motor
WAIT_READY       SUB   0           ' Wait for Motor Controller to be idle

WAIT_OK          SUB   0           ' Waits for Ok button to be released


' ------------------------------------------------------------------------
' Function Declarations
' ------------------------------------------------------------------------

num2char         FUNC        1, 1        ' converts number into character
GET_TIME         FUNC        1           ' Gets exposure time

' ------------------------------------------------------------------------
' Program Code
' ------------------------------------------------------------------------
Start:

HIGH LcdTx
WAIT_MS 100                        ' let LCD initialize

Main:
LCD_OUT LcdBLoff                   ' back light off
LCD_OUT LcdOn2                     ' LCD on, no cursor, blink on
LCD_OUT LcdCls                     ' clear the LCD
LCD_OUT LcdLine1                   ' Go to LCD line 1

' Code restarts here if RESET button pressed

WAIT_MS 1000                       ' Wait for stepper power on cycle

' Display Version Information

counter = 1                        ' Set counter to 1

DO
    LCD_OUT LcdCls                 ' clear the LCD
    WAIT_MS 250                    ' Off portion of LCD Blink
    LCD_OUT LcdLine1               ' Go to LCD line 1
    LCD_STR "Vitrification"        ' Output to LCD
    LCD_OUT LcdLine2               ' Go to LCD line 2
    LCD_STR "Machine V1.0"         ' Output Version Information
    WAIT_MS 750                    ' On potion of LCD Blink
    INC counter                    ' Increment counter
LOOP WHILE counter < 5

LCD_OUT LcdCls                     ' clear the LCD
```

```
LCD_OUT LcdLine1                  ' Go to LCD line 1

' Slew X Motor in Positive direction to find Elevation hardware limit
' ------------------------------------------------------------------------

MOTOR_STR "x+s"                   ' slew motor x in + direction
LCD_STR "Finding X Home"          ' Tell user what is happening
WAIT_READY                        ' Wait until Motor Controller is Ready
MOTOR_STR "x0="                   ' Set X Motor position to zero


' Slew Y Motor in Negative direction to find Rotation hardware limit
' ------------------------------------------------------------------------

MOTOR_STR "y-s"                   ' slew motor y in - direction
LCD_OUT LcdLine1                  ' Go to LCD Line 1
LCD_STR "Finding Y Home"          ' Tell user what is happening
WAIT_READY                        ' Wait until Motor Controller is Ready
MOTOR_STR "y0="                   ' Set Y Motor position to zero

' Loop to Get Number of Exposures
' ------------------------------------------------------------------------

LCD_OUT LcdLine1                  ' Go to LCD Line 1
LCD_STR "# of Exposures:"         ' Send string to LCD
LCD_OUT LcdBkSpc                  ' Put blinking cursor on input space

ExposureNumber = 1                ' Set exposure number to the minimum

DO
  temp = num2char ExposureNumber        ' call function, put in temp
  LCD_OUT LcdLine2                      ' move cursor to second line
  LCD_OUT temp                          ' send temp to LCD
  LCD_OUT LcdBkSpc                      ' Move Cursor back one space
  IF IncreaseButton = IsPressed THEN    ' If increase button is pressed
    INC ExposureNumber                  ' increase Exposure Number
    IF ExposureNumber = 5 THEN          ' if Exposure Number is out of
                                        ' range, make it 1
      ExposureNumber = 1
    ENDIF
  ENDIF

  IF DecreaseButton = IsPressed THEN    ' If Decrease Button is pressed
     DEC ExposureNumber                 ' Decrease Exposure Number
     IF ExposureNumber = 0 THEN         ' If Exposure Number is out of
                                        ' range, make it 4
         ExposureNumber = 4
     ENDIF
  ENDIF
  WAIT_MS 150                           ' Minimum Display Time
LOOP WHILE OKButton = IsNotPressed      ' Loop until the user presses the
                                        ' OK Button

' Force OK Button to be released
WAIT_OK
```

```
counter = ExposureNumber                ' Put Exposure Number in counter

' Loop to Get Length of Exposure1
' --------------------------------------------------------------------------

LCD_OUT LcdCls                          ' Clear the LCD
LCD_OUT LcdLine1                        ' Go to LCD Line 1
LCD_STR "Exp 1 Length"                  ' Ask user for Exposure 1 Length


' Get Exposure Length 1
' --------------------------------------------------------------------------

Exposure1 = GET_TIME                    ' Call function to get Exposure 1
                                        ' Length

DEC counter                             ' Decrease counter

IF counter > 0 THEN                     ' Use counter to decide where to
                                        ' go in program
   GOTO Able                            ' If user wants more than 1
                                        ' exposure, go to get Exposure 2
                                        ' Length
ELSE
   GOTO Dog                             ' If user wants only 1 exposure,
                                        ' go to protocol start
ENDIF


' Get Exposure Length 2
' --------------------------------------------------------------------------

Able:                             ' Label for getting Exposure 2 Length

LCD_OUT LcdCls                    ' Clear LCD
LCD_OUT LcdLine1                  ' Go to LCD Line 1
LCD_STR "Exp 2 Length"            ' Ask user for Exposure 2 Length

Exposure2 = GET_TIME              ' Call function to get Exposure 2
                                  ' Length

DEC counter                       ' Decrease counter

IF counter > 0 THEN               ' Use counter to decide where to go in
program
   GOTO Baker                     ' If user wants more than 2 exposures,
                                  ' go to get Exposure 3 Length
ELSE
   GOTO Dog                       ' If user wants only 2 exposures, go to
                                  ' protocol start
ENDIF

' Get Exposure Length 3
' --------------------------------------------------------------------------
```

```
Baker:                              ' Label for getting Exposure 3 Length

LCD_OUT LcdCls                      ' Clear LCD
LCD_OUT LcdLine1                    ' Go to LCD Line 1
LCD_STR "Exp 3 Length"              ' Ask user for Exposure 3 Length

Exposure3 = GET_TIME                ' Call function to get Exposure 3
                                    ' Length

DEC counter                         ' Decrease counter

IF counter > 0 THEN                 ' Use counter to decide where to go in
                                    ' program
   GOTO Charlie                     ' If user wants more than 3 exposures,
                                    ' go to get Exposure 4 Length
ELSE
   GOTO Dog                         ' If user wants only 3 exposures, go to
                                    ' protocol start
ENDIF

' Get Exposure Length 4
' -------------------------------------------------------------------------

Charlie:                            ' Label for getting Exposure 4 Length

LCD_OUT LcdCls                      ' Clear LCD
LCD_OUT LcdLine1                    ' Go to LCD Line 1
LCD_STR "Exp 4 Length"              ' Ask user for Exposure 4 Length

Exposure4 = GET_TIME                ' Call function to get Exposure 4
                                    ' Length

' Protocol Start
' -------------------------------------------------------------------------

Dog:                                ' Label for starting protocol

' Tell user to load cyro-protectant fluids

'LCD_OUT LcdCls                     ' Clear LCD
'LCD_OUT LcdLine1                   ' Go to LCD Line 1
'LCD_STR "Load Cryo-Fluids"         ' Tell user to load cryo-fluids
'LCD_OUT LcdLine2                   ' Go to LCD Line 2
'LCD_STR "Then Press OK"            ' Tell user to press OK

' Wait for OK to start

'DO
'WAIT_MS 250
'LOOP UNTIL OKButton = IsPressed

' Rotate to let user load specimens
' Tell user to load specimens
```

```
'LCD_OUT LcdCls                   ' Clear LCD
'LCD_OUT LcdLine1                 ' Go to LCD Line 1
'LCD_STR "Load Specimens"         ' Tell User to load specimens
'LCD_OUT LcdLine2                 ' Go to LCD Line 2


'LCD_STR "Then Press OK"          ' Ask user to press OK

' Wait for OK to start
DO
WAIT_MS 250
LOOP UNTIL OKButton = IsPressed

' Force OK Button Release
WAIT_OK

' Run Protocol
' --------------------------------------------------------------------------

' Pick Up Specimens

LCD_OUT LcdCls                          ' Clear LCD
LCD_OUT LcdLine1                        ' Go to LCD Line 1
LCD_STR "Picking Up      Specimens"     ' Tell User what is happening

MOTOR_STR "x-1824g"               '     ' Elevate to specimen height
WAIT_READY                              ' Wait until motor is ready

''Open Forceps

'DO
  '  LiftMotor1 = 1
  '  LiftMotor2 = 0
'LOOP WHILE LiftSense = 0    ' Run motor until forceps are closed

''Brake Lift Motor

' LiftMotor1 = 0             ' Forceps are now zeroed and closed

''Open forceps

' LiftMotor2 = 1             ' Run Lift Motor Backwards for .25 seconds
' WAIT_MS 250

''Brake Lift Motor
' LiftMotor2 = 0             ' Forceps are now open

MOTOR_STR "y141g"            ' Rotate tray to forceps
WAIT_READY                   ' Wait until motor is ready

''Close Forceps

'DO
  '  LiftMotor1 = 1
  '  LiftMotor2 = 0
```

```
'LOOP WHILE LiftSense = 0    ' Run motor until forceps are closed

''Brake Lift Motor

' LiftMotor1 = 0

MOTOR_STR "x0g"              ' Lower Tray
WAIT_READY                   ' Wait until motor is ready


'Rotate to First Exposure
' ------------------------------------------------------------------------

DEC ExposureNumber                        ' Decrease Exposure Number

MOTOR_STR "y1479g"                        ' Rotate to first exposure
WAIT_READY                                ' Wait until ready

LCD_OUT LcdCls                            ' Clear LCD
LCD_OUT LcdLine1                          ' Go to LCD Line 1
LCD_STR "Exposure 1"                      ' Display for first exposure

MOTOR_STR "x-3552g"                       ' Raise tray to expose
WAIT_READY

DO                                        ' Wait until exposure 1 is done
  WAIT_MS 1000
  WAIT_MS 866
  DEC Exposure1
LOOP WHILE Exposure1 > 0

MOTOR_STR "x0g"                           ' Lower Tray
WAIT_READY

IF ExposureNumber > 0 THEN                ' Determine if there are any more
                                          ' steps in protocol
   GOTO Easy
ELSE
   GOTO How
ENDIF

' Go to Second Exposure
'-------------------------------------------------------------------------

Easy:                                     ' Label for second exposure

DEC ExposureNumber                        ' Decrease Exposure Number

LCD_OUT LcdCls                            ' Clear LCD
LCD_OUT LcdLine1                          ' Go to LCD Line 1
LCD_STR "Exposure 2"                      ' Display for second exposure

MOTOR_STR "y2816g"                        ' Rotate to Second Exposure
WAIT_READY
MOTOR_STR "x-3552g"                       ' Raise tray for exposure
```

```
WAIT_READY

DO                                      ' Wait until exposure 2 is done
  WAIT_MS 1000
  WAIT_MS 866
  DEC Exposure2
LOOP WHILE Exposure2 > 0

MOTOR_STR "x0g"                         ' Lower Tray
WAIT_READY

IF ExposureNumber > 0 THEN              ' Determine if there are any more
                                        ' steps in protocol
   GOTO Fox
ELSE
   GOTO How
ENDIF

' Go to Third Exposure
'---------------------------------------------------------------------------

Fox:                                    ' Label for Third Exposure

DEC ExposureNumber                      ' Decrement Exposure Number

LCD_OUT LcdCls                          ' Clear LCD
LCD_OUT LcdLine1                        ' Go to LCD Line 1
LCD_STR "Exposure 3"                    ' Display for third exposure

MOTOR_STR "y4154g"                      ' Rotate to third Exposure
WAIT_READY
MOTOR_STR "x-3552g"                     ' Raise tray for exposure
WAIT_READY

DO                                      ' Wait until exposure 3 is done
  WAIT_MS 1000
  WAIT_MS 866
  DEC Exposure3
LOOP WHILE Exposure3 > 0

MOTOR_STR "x0g"                         ' Lower Tray
WAIT_READY

IF ExposureNumber > 0 THEN   '         Determine if there are any more
                                        ' steps in protocol
   GOTO George
ELSE
   GOTO How
ENDIF

' Go to Fourth Exposure
'---------------------------------------------------------------------------

George:                                 ' Label for Fourth Exposure
```

```
LCD_OUT LcdCls                          ' Clear LCD
LCD_OUT LcdLine1                        ' Go to LCD Line 1
LCD_STR "Exposure 4"                    ' Display for fourth exposure

MOTOR_STR "y5492g"                      ' Rotate to fourth Exposure
WAIT_READY
MOTOR_STR "x-3552g"                     ' Raise tray for exposure
WAIT_READY

DO                                      ' Wait until exposure 4 is done
  WAIT_MS 1000
  WAIT_MS 866
  DEC Exposure4
LOOP WHILE Exposure4 > 0

MOTOR_STR "x0g"                         ' Lower Tray
WAIT_READY

' Go to Liquid Nitrogen
'-----------------------------------------------------------------------

How:                                    ' Label for Liquid Nitrogen
                                        ' Exposure

LCD_OUT LcdCls                          ' Clear LCD
LCD_OUT LcdLine1                        ' Go to LCD Line 1
LCD_STR "Press OK when   LN2 is ready"  ' Display for LN2 confirmation

DO                                      ' Wait for OK
  WAIT_MS 250
LOOP WHILE OKButton = IsNotPressed

' Force OK Button Release
WAIT_OK

' Clear LCD
LCD_OUT LcdCls             ' Clear LCD
LCD_OUT LcdLine1           ' Go to LCD Line 1

MOTOR_STR "y7036g"              ' Rotate to Liquid Nitrogen
WAIT_READY
MOTOR_STR "x-1152g"             ' Raise Tray for LN2 drop
WAIT_READY

''USE DC MOTOR TO DROP SPECIMENS
''Open forceps

' LiftMotor2 = 1           ' Run Lift Motor Backwards for .25 seconds
' WAIT_MS 250

' Brake Lift Motor
' LiftMotor2 = 0           ' Forceps are now open

''Rotate away so user can remove LN2 Vials
```

```
MOTOR_STR "x0g"              ' Lower Tray
WAIT_READY                   ' Wait for motor to be ready
MOTOR_STR "y2816g"           ' Rotate to convenient position for user
WAIT_READY                   ' Wait for motor to be ready

' Program Complete
'--------------------------------------------------------------------------

counter = 1                              ' Set counter to 1

DO
  LCD_STR "Protocol        Complete"     ' Display for LN2 confirmation
  WAIT_MS 750
  LCD_OUT LcdCls
  LCD_OUT LcdLine1
  WAIT_MS 250
  INC counter
LOOP WHILE counter <10

' Wait for user to Remove Vials

LCD_OUT LcdCls                           ' Clear LCD
LCD_OUT LcdLine1                         ' Go to LCD Line 1
LCD_STR "Specimens       Removed?"       ' Ask user to remove specimens

DO                                       ' Wait for OK
  WAIT_MS 250
LOOP WHILE OKButton = IsNotPressed

' Force OK Button Release
WAIT_OK                                  ' Wait for OK release

GOTO Main                                ' Go back to beginning of program


' -------------------------------------------------------------------------
' Subroutine Code
' -------------------------------------------------------------------------

WAIT_READY:
 WAIT_MS 100               ' Wait 0.1 seconds for prior character to be
processed
DO WHILE MotorBusyLine = 0
 WAIT_MS 100
LOOP
RETURN                      'Wait till not busy return

'--------------------------------------------------------------------------

' Use: WAIT_MS milliseconds {, multiplier }
' -- multiplier is optional

WAIT_MS:
  temp1 = __PARAM1                               ' get milliseconds
```

```
  IF __PARAMCNT = 1 THEN                            ' if no multiplier
    temp2 = 1                                       ' set to 1
  ELSE                                              ' else
    temp2 = __PARAM2                                ' get multiplier
  ENDIF
  IF temp1 > 0 THEN                                 ' no delay if either 0
    IF temp2 > 0 THEN
      PAUSE temp1 * temp2                           ' do the delay
    ENDIF
  ENDIF
  RETURN

'-------------------------------------------------------------------------

' Use: LCD_OUT theByte {, count }
' -- sends "theByte" to LCD [optional] "count" times
' -- "count" defaults to 1 if not specified

LCD_OUT:
  temp1 = __PARAM1                                ' save the byte
  IF __PARAMCNT = 2 THEN                          ' "count" specified?
    temp2 = __PARAM2                              ' yes, save
  ELSE
    temp2 = 1                                     ' no, set to 1
  ENDIF
  DO WHILE temp2 > 0
    SEROUT LcdTx, LcdBaud, temp1                  ' transmit to LCD
    DEC temp2
  LOOP
  RETURN

'-------------------------------------------------------------------------

' Use: LCD_STR [ string | label ]
' -- "string" is an embedded literal string
' -- "label" is DATA statement label for stored z-String

LCD_STR:
  temp3 = __PARAM1                                ' get string offset
  temp4 = __PARAM2                                ' get string base
  DO
    READ temp4 + temp3, temp5                     ' read a character
    IF temp5 = 0 THEN EXIT                        ' if 0, string complete
    LCD_OUT temp5                                 ' send the byte
    INC temp3                                     ' point to next character
    temp4 = temp4 + Z                             ' update base on overflow
  LOOP
  RETURN

'-------------------------------------------------------------------------

' Use: MOTOR_OUT theByte {, count }
' -- sends "theByte" to LCD [optional] "count" times
' -- "count" defaults to 1 if not specified
```

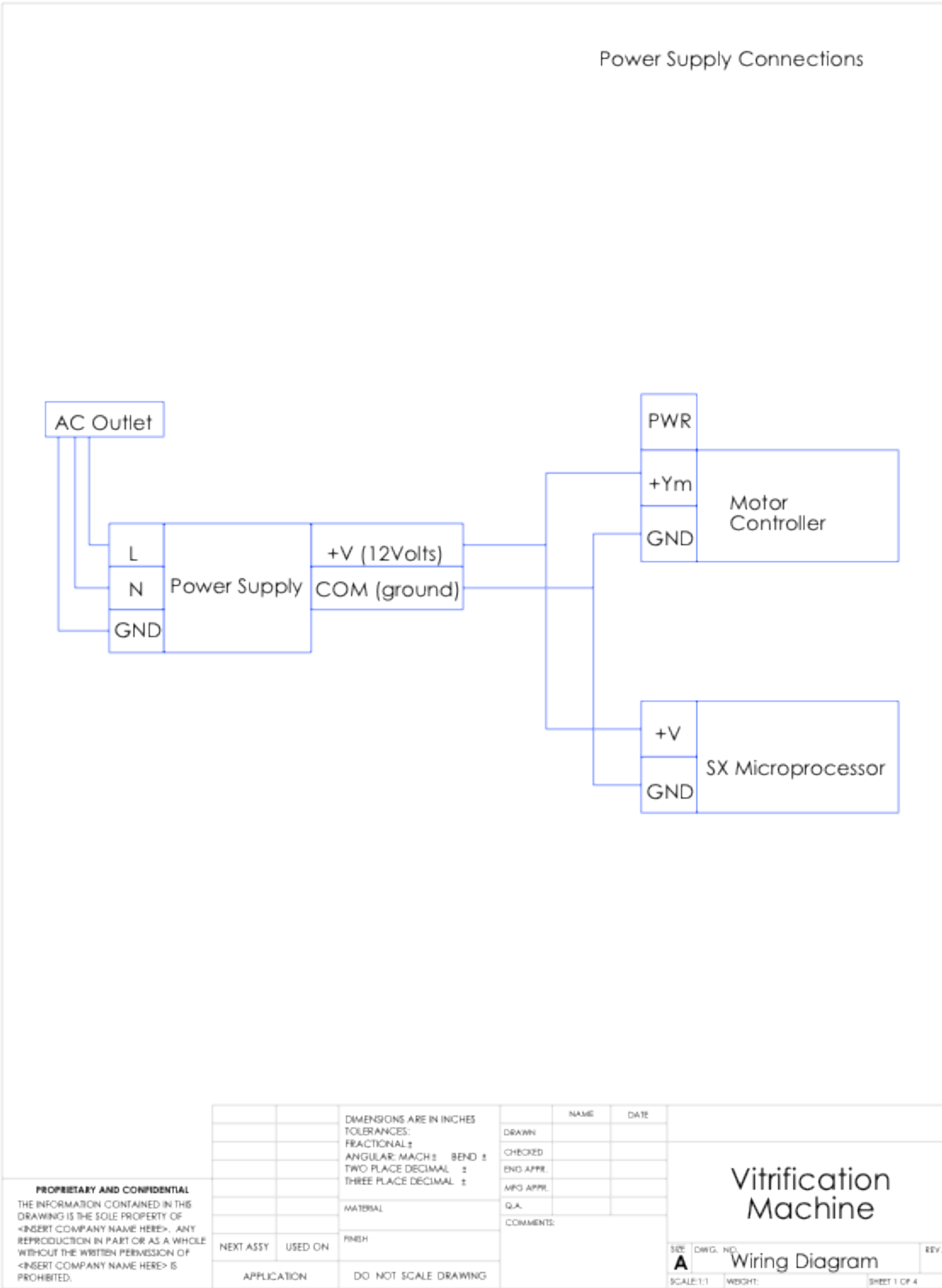
```
MOTOR_OUT:
  temp1 = __PARAM1                              ' save the byte
  IF __PARAMCNT = 2 THEN                        ' "count" specified?
    temp2 = __PARAM2                            ' yes, save
  ELSE
    temp2 = 1                                   ' no, set to 1
  ENDIF
  DO WHILE temp2 > 0
    SEROUT MotorCommand, MotorBaud, temp1       ' transmit to Motor
    DEC temp2
  LOOP
  RETURN

'-------------------------------------------------------------------------

' Use: MOTOR_STR [ string | label ]
' -- "string" is an embedded literal string
' -- "label" is DATA statement label for stored z-String

MOTOR_STR:
  temp3 = __PARAM1                              ' get string offset
  temp4 = __PARAM2                              ' get string base
  DO
    READ temp4 + temp3, temp5                   ' read a character
    IF temp5 = 0 THEN EXIT                      ' if 0, string complete
    MOTOR_OUT temp5                             ' send the byte
    INC temp3                                   ' point to next character
    temp4 = temp4 + Z                           ' update base on overflow
  LOOP
  RETURN

'-------------------------------------------------------------------------

' Waits for the Ok button to be released so the program does not jump
' forward

WAIT_OK:
' Force OK Button to be released
  DO
    WAIT_MS 50
  LOOP UNTIL OKButton = IsNotPressed
RETURN


'-------------------------------------------------------------------------
' FUNCTION CODE
' ------------------------------------------------------------------------

' num2char function
' converts a number into its ascii format
FUNC num2char
  temp6 = __PARAM1
  temp6 = temp6 + 48         ' Change number to ascii character code
  RETURN temp6
ENDFUNC
```

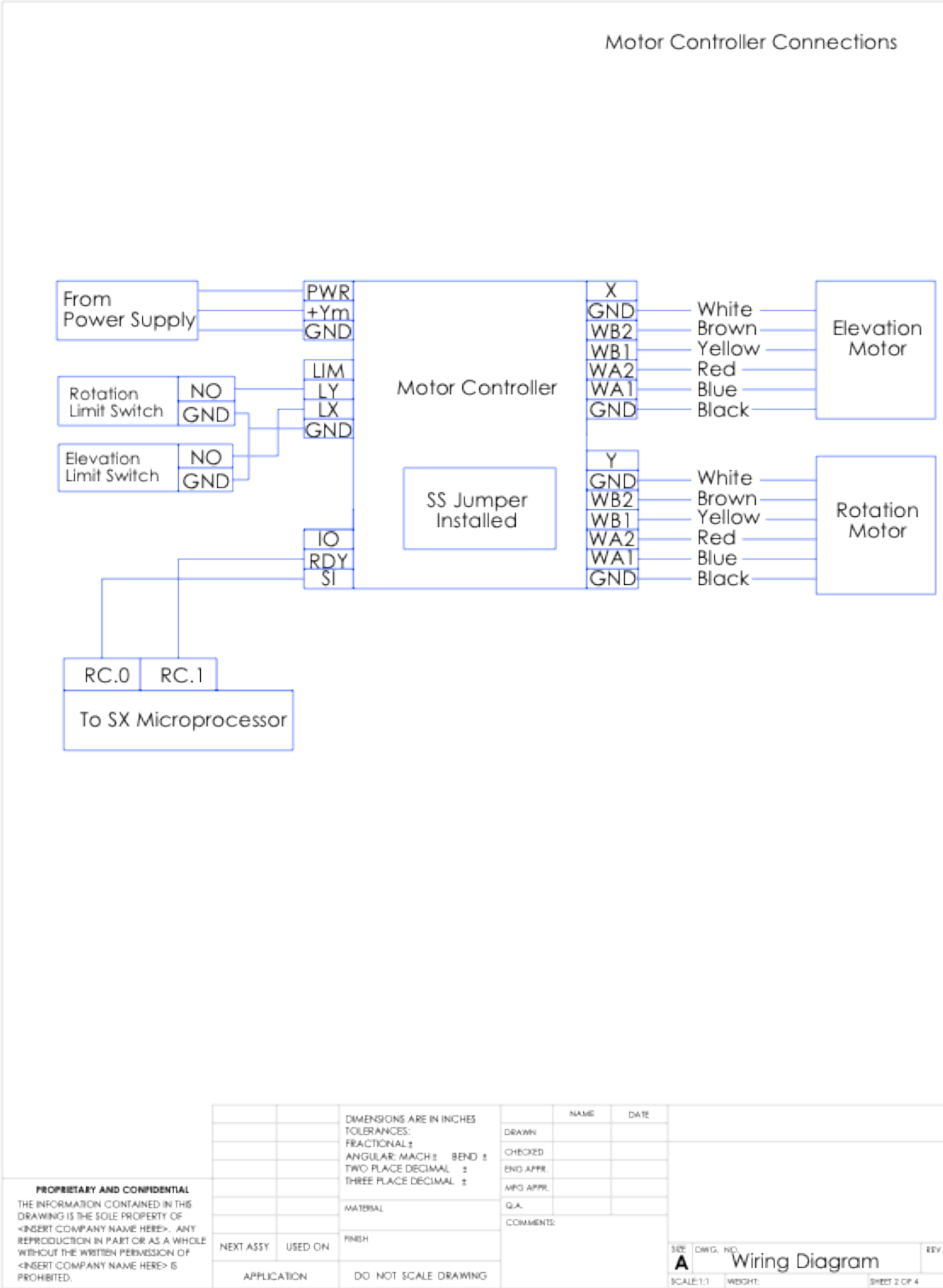
```
'---------------------------------------------------------------------------
'---------------------------------------------------------------------------

'Get Time function
'pass it nothing
'returns number of seconds for exposure

FUNC GET_TIME

' Initialize Variables
ExpLengthMin = 0
ExpLengthTens = 0
ExpLengthOnes = 0

' Get Exposure Length for minutes
'---------------------------------------------------------------------------
DO
   temp = num2char ExpLengthMin              ' call function, put in temp
   LCD_OUT LcdLine2                          ' move cursor to second line
   LCD_OUT temp                              ' send temp to LCD
   LCD_STR ":00"                             ' send to LCD
   LCD_OUT LcdLine2                          ' move cursor to second line

   IF IncreaseButton = IsPressed THEN
     IF ExpLengthMin = 9 THEN
        ExpLengthMin = 0
     ELSE
        INC ExpLengthMin
     ENDIF
   ENDIF

   IF DecreaseButton = IsPressed THEN
     IF ExpLengthMin = 0 THEN
        ExpLengthMin = 9
     ELSE
        DEC ExpLengthMin
     ENDIF
   ENDIF
   WAIT_MS 150                                    'Minimum Display Time
LOOP WHILE OkButton = IsNotPressed

' Force OK Button to be released
WAIT_OK

' Get Exposure Length 1 Tens Digit for seconds
'---------------------------------------------------------------------------

DO
   temp = num2char ExpLengthTens         ' call function, put in temp
   LCD_OUT LcdLine2                      ' move cursor to second line
   LCD_OUT LcdRt                         ' move cursor to second space
   LCD_OUT LcdRt                         ' move cursor to third space
   LCD_OUT temp                          ' send temp to LCD
   LCD_OUT "0"
   LCD_OUT LcdBkSpc                      ' Move cursor to tens place
```

```
   LCD_OUT LcdBkSpc

   IF IncreaseButton = IsPressed THEN
     INC ExpLengthTens

     IF ExpLengthTens = 6 THEN
        ExpLengthTens = 0
     ENDIF

   ENDIF

   IF DecreaseButton = IsPressed THEN
     IF ExpLengthTens = 0 THEN
        ExpLengthTens = 5
     ELSE
        DEC ExpLengthTens
     ENDIF
   ENDIF
   WAIT_MS 150                                'Minimum Display Time
LOOP WHILE OkButton = IsNotPressed

' Force OK Button to be released
WAIT_OK

' Get Exposure Length 1 Ones Digit for seconds
'-------------------------------------------------------------------------

DO
   temp = num2char ExpLengthOnes               ' call function, put in temp
   LCD_OUT LcdLine2                            ' move cursor to second line
   LCD_OUT LcdRt                               ' Move Cursor to ones place
   LCD_OUT LcdRt
   LCD_OUT LcdRt
   LCD_OUT temp                                ' send temp to LCD
   LCD_OUT LcdBkSpc                            ' Move cursor to ones place

   IF IncreaseButton = IsPressed THEN
     IF ExpLengthOnes = 9 THEN
        ExpLengthOnes = 0
     ELSE
        INC ExpLengthOnes
     ENDIF

   ENDIF

   IF DecreaseButton = IsPressed THEN
     IF ExpLengthOnes = 0 THEN
        ExpLengthOnes = 9
     ELSE
        DEC ExpLengthOnes
     ENDIF

   ENDIF
   WAIT_MS 150                                'Minimum Display Time
LOOP WHILE OkButton = IsNotPressed
```

```
' Force OK Button to be released
WAIT_OK

'Calculate Exposure Length and Put in temp
'---------------------------------------------------------------------------

temp1 = ExpLengthMin * 60
temp2 = 10 * ExpLengthTens
temp3 = temp1 + temp2
temp = temp3 + ExpLengthOnes
RETURN temp
ENDFUNC
'---------------------------------------------------------------------------
'---------------------------------------------------------------------------
```

Appendix 5: Wiring Diagrams





Motor Controller Connections
From Power Supply
PWR
+Ym
GND
LIM
LY
LX
GND
Rotation Limit Switch
NO
GND
Elevation Limit Switch
NO
GND
Motor Controller
SS Jumper Installed
IO
RDY
SI
RC.0
RC.1
To SX Microprocessor
X
GND
WB2
WB1
WA2
WA1
GND
White
Brown
Yellow
Red
Blue
Black
Elevation Motor
Y
GND
WB2
WB1
WA2
WA1
GND
White
Brown
Yellow
Red
Blue
Black
Rotation Motor
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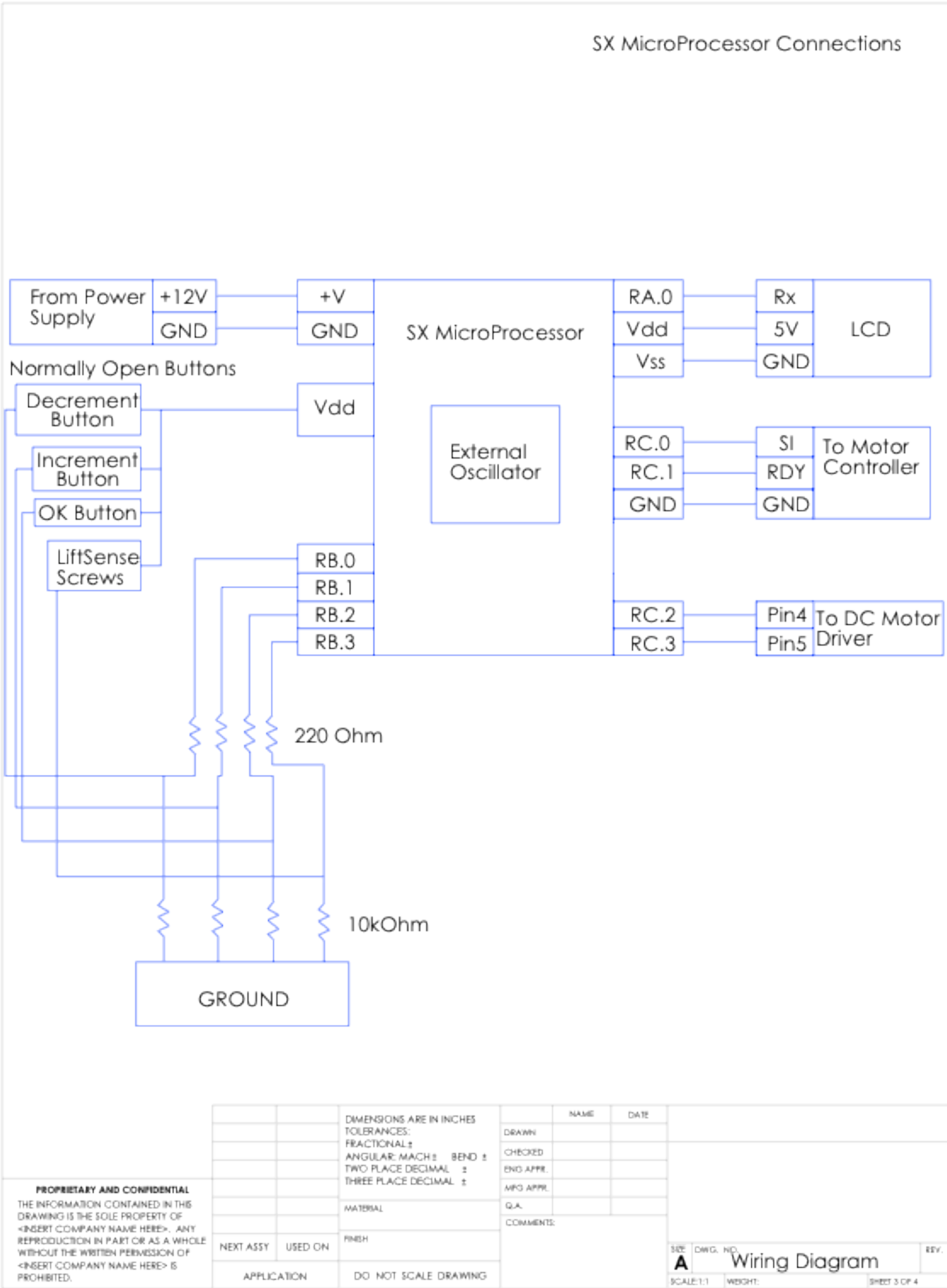
NEXT ASSY
USED ON
APPLICATION
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
MATERIAL
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
SIZE
A
DWG. NO.
Wiring Diagram
REV.
SCALE: 1:1
WEIGHT:
SHEET 2 OF 4


SX MicroProcessor Connections
From Power Supply
+12V
GND
+V
GND
SX MicroProcessor
RA.0
Vdd
Vss
Rx
5V
GND
LCD
Normally Open Buttons
Decrement Button
Increment Button
OK Button
LiftSense Screws
Vdd
External Oscillator
RC.0
RC.1
GND
SI
RDY
GND
To Motor Controller
RB.0
RB.1
RB.2
RB.3
RC.2
RC.3
Pin4
Pin5
To DC Motor Driver
220 Ohm
10kOhm
GROUND
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL±
ANGULAR: MACH± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
MATERIAL
FINISH
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
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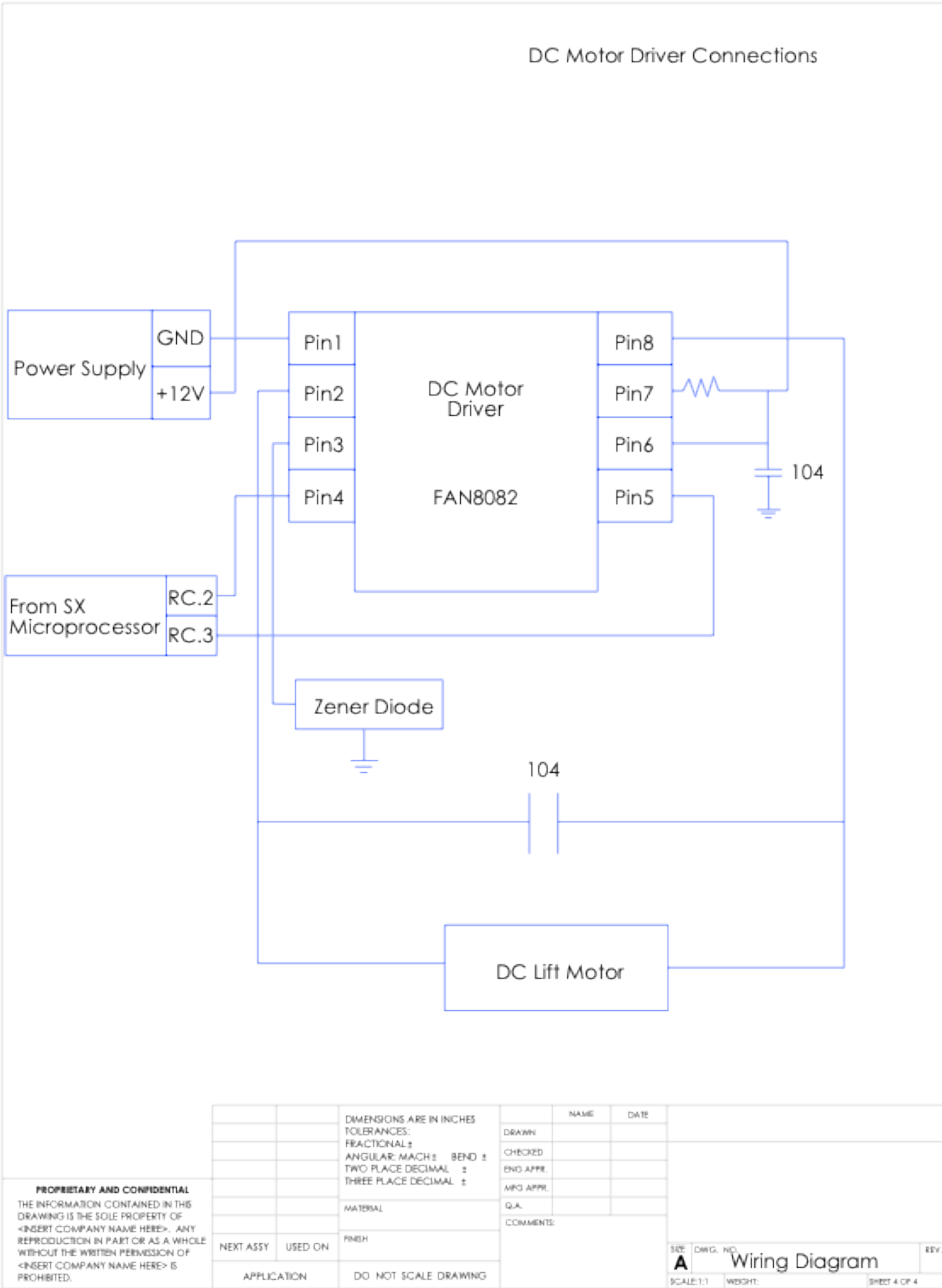
NEXT ASSY
USED ON
APPLICATION
DO NOT SCALE DRAWING
SIZE
DWG. NO.
REV.
A
Wiring Diagram
SCALE: 1:1
WEIGHT:
SHEET 3 OF 4


DC Motor Driver Connections
Power Supply
GND
+12V
Pin1
Pin2
Pin3
Pin4
DC Motor Driver
FAN8082
Pin8
Pin7
Pin6
Pin5
104
From SX Microprocessor
RC.2
RC.3
Zener Diode
104
DC Lift Motor
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NEXT ASSY
USED ON
APPLICATION
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±
MATERIAL
FINISH
DO NOT SCALE DRAWING
NAME
DATE
DRAWN
CHECKED
ENG APPR.
MFG APPR.
Q.A.
COMMENTS:
SIZE
A
DWG. NO.
Wiring Diagram
REV.
SCALE: 1:1
WEIGHT:
SHEET 4 OF 4

Appendix 6: Calculations of Motor Controller Instructions

1/2

This is a calculation to determine the "go to" instructions that the SX should send to the BiStep Motor Controller

Knowns: $1 \text{ Bistep Motor Controller Step} = \frac{1}{16} \text{ stepper motor step}$

$1 \text{ stepper motor step} = 1.8°$

Acme Screw: $\frac{1}{3}$" per rotation (360°)

Gears: $\frac{0.89473684\text{" pinion}}{7.10526316 \text{ gear}} \Longrightarrow 7.941176492 \frac{\text{gear}}{\text{pinion}}$

Elevation

$$\frac{1 \text{ Bistep Motor Controller Step}}{\frac{1}{16} \text{ stepper motor step}} \cdot \frac{1 \text{ stepper motor step}}{1.8°} \cdot \frac{360°}{1/3\text{" Acme Screw}} = 9600 \frac{\text{Bistep motor Controller Steps}}{\text{inch of Acme Screw Lift}}$$

Rotation

$$\frac{1 \text{ BiStep Motor Controller Step}}{1/16 \text{ stepper motor step}} \cdot \frac{1 \text{ stepper motor step}}{1.8°} \cdot \frac{360°}{1 \text{ rotation of Pinion}} \cdot \frac{7.941176492 \text{ rotation of pinion}}{1 \text{ rotation of gear}}$$

$$= 25411.76477 \frac{\text{Bistep motor controller Steps}}{\text{rotation of big gear}}$$

Petri Dish Arm

18.95° between dishes

$$\frac{25411.76477 \text{ Bistep Motor Controller Steps}}{360° \text{ Petri Dish Arm}} \cdot \frac{18.95° \text{ Petri Dish Arm}}{\text{Between Dishes}} = 1337.647062 \text{ steps between dishes}$$

21.88° between last dish and Liquid Nitrogen Beaker

$$\frac{25411.76477 \text{ Bistep motor Controller Steps}}{360° \text{ Petri Dish Arm}} \cdot \frac{21.88° \text{ Petri Dish Arm}}{\text{between last dish and LN2 Beaker}} = 1544.470592 \text{ steps}$$

2/2

Elevation

0.19" from home to picking up specimens (on model)

$$\frac{9600 \text{ Bistep Motor Controller Steps}}{1 \text{ inch of lift}} \Big/ 0.19 \text{ inches} = 1824 \text{ steps}$$

0.37" from home to expose specimens

$$\frac{9600}{1"} \Big/ 0.37" = 3552 \text{ steps} \quad \text{(units same as above)}$$

0.12" from home to drop specimens into LN2

$$\frac{9600}{1"} \Big/ 0.12" = 1152 \text{ steps} \quad \text{(units same as above)}$$

Results

Rotation	Steps (from zero)	Step Difference	Angle difference
Specimens	X		
		1337.647062	18.95°
Dish 1	X+1338		
		1337.647062	18.95°
Dish 2	X+2675		
		1337.647062	18.95°
Dish 3	X+4013		
		1337.647062	18.95°
Dish 4	X+5350		
		1544.47052	21.88°
LN2	X+6895		

where X is the step difference from the motor reset (limit switches) to the specimens

Elevation	Distance	Steps (from zero)
Specimen Pickup	0.19 inches	1824
Exposure	0.37 inches	3552
Liquid Nitrogen	0.12 inches	1152

Appendix 7: Calculation of Rotation and Elevation Resolution

Rotation and Elevation Resolution Calculation

Motor controller can do 1/64 step

so: 1 motor controller step = 1/64 motor step

1 motor step = 1.8°

ACME Screw: $\frac{1/3'' \text{ translation}}{360°}$

Gears: $\frac{0.89473684'' \text{ pinion}}{7.10526316'' \text{ gear}} = 7.941176492$

Elevation

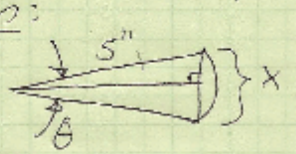
$$\frac{1/3'' \text{ translation}}{360°} \left| \frac{1.8°}{1 \text{ motor step}} \right| \frac{1/64 \text{ motor step}}{1 \text{ motor controller step}} = \boxed{\frac{2.60416 \times 10^{-5} \text{ inches of translation}}{\text{motor controller step}}}$$

Rotation

$$\frac{1/64 \text{ motor step}}{1 \text{ motor controller step}} \left| \frac{1.8° \text{ pinion}}{1 \text{ motor step}} \right| \frac{1 \text{ gear}}{7.941176492 \text{ pinion}} \left| \frac{1 \text{ Petri Dish Arm}}{1 \text{ gear}} \right.$$

$$= 0.0035416667 \frac{° \text{ Petri Dish Arm}}{\text{motor controller step}} = \theta$$

The petri dish arm is 5 inches from the point of rotation to the center of a petri dish. So, for every motor controller step:



$$\sin\left(\frac{\theta}{2}\right) = \frac{(x/2)}{5''}$$

$$5'' \sin\left(\frac{0.0035416667°}{2}\right) = \left(\frac{x}{2}\right)$$

$$\boxed{x = 3.090687208 \times 10^{-4} \text{ inches}}$$

per motor controller step

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