3 Automated Protein Crystal Growth Facility

3.1 Summary

3.1.1 Design Objective

The goal of this design project is to develop an interactive protein crystal growth facility for Space Station Freedom. This protein crystal growth facility must include a sensing device to monitor crystal growth and an electronic control system for automatic growth. Our design objective also includes the design and fabrication of an end effector to be used with the current Zymate II system that allows for fully automated protein crystal growth in space.

3.1.2 Abstract

A customer for the protein crystal growth facility fills the specially designed chamber with the correct solutions, fills the syringes with their quenching solutions, and submits the data needed for the proper growth of their crystal. To make sure that the chambers and syringes are filled correctly, a NASA representative may assist the customer. The data needed is the approximate growth time, the growth temperature, and the desired crystal size, but this data can be changed anytime from the ground if needed. The chambers are gathered and placed into numbered slots in special drawers. Then, data is entered into a computer for each of the chambers. Technicians map out when each of the chamber's growth should be activated so that all of the chambers have enough time to grow. All of this data is up-linked to the space station when the previous growth session is over.

Anti-vibrational containers need to be constructed for the high forces encountered during the lift off and the landing of the space shuttle, and though our team has not designed these containers, we do not feel that there is any reason why a suitable one could not be made. When the shuttle reaches the space station, an astronaut removes a drawer of quenched chambers from the growth facility and inserts a drawer of new chambers. All twelve of the drawers can be replaced in this fashion. The optical disks can also be removed this way. The old drawers are stored for the trip back to earth.

Once inside the growth facility, a chamber is removed by the robot and placed in one of 144 active sites at a time previously picked by a technician. Growth begins when the chamber is inserted into an active site. Then, the sensing system starts to determine the size of the protein crystal. All during the crystal's growth, the customer can view the crystal and read all of the crystal's data, such as growth rate and crystal size. When the sensing system determines that the crystal has reached the predetermined size, the robot is told to pick up a syringe filled with the correct quenchant solution and inject it into the chamber to stop the crystal growth. The chamber is then removed from the active site and placed into its original storage slot. Another chamber is then placed into the active site and the process is repeated in all of the active sites until all of the chambers have completed their growth.

After ninety days (the scheduled time between shuttle visits), the crystal growth is completed, and the old drawers are replaced with new ones. Once the customer extracts the crystals, the chambers are retrieved for future customers.

3.2 Glossary

active sites	- locations within the experimental facility where growth occurs
cap (press fit)	- removable lid on top of chamber used when removing the crystal
CCD	- (charged coupling device) miniature camera used for viewing crystals
chamber	- self-contained capsule holding solutions necessary to grow protein crystals
chromex	- fibrous high molecular weight polyethylene material
CIN	- (code interface node) a specific function in Labview
CRT	- (cathode ray tube) computer monitor display
convolution filter	- a process used for enhancing and filtering images through the use of mathematical operations
Easy Lab controller	- computer that runs the Zymate II robot
experimental facility	- part of the ISPR that contains the robot, end effector, chambers, and syringes
FFT	- (Fast Fourier Transform)process converting image data into the frequency domain for processing and analysis
growth chamber	- site within chamber where actual crystal growth takes place
image conduit	- device used to transmit crystal image to the CCD
ISPR	- (International Standard Payload Rack) modular containment vessel used on Space Station Freedom
keyway tabs	- projections on the side of the chamber which secure the chamber within the experimental facility
Labview	- data acquisition program used to develop user-interface for the control system
MicroMo	- company which supplied the motors of the end effector
PCMCIA	- standard computer interface
pedestal	- platform where the protein solution rests
PET polymer	- Polyethylene Terestrate
pistons	- devices within the chamber used to extrude solutions into growing area of chamber
plungers	- cylindrical devices located in the active sites used to extrude solution in the chamber
precipitant solution	- solution used to instigate crystal growth
protein solution	- solution consisting of desired protein to be grown
quenchant solution	- diluted precipitant solution used to halt further crystal growth
storage sites	- locations where chambers are stored before and after protein crystal growth
Zyline	- informational source associated with Zymark
Zymark	- company which designed the robot used in our design
Zymate II	- the name of the robot

3.3 Background

3.3.1 Importance of Protein Crystals

The complete understanding of the properties of proteins will provide researchers with valuable insight into many areas which are an integral part of life. Proteins are used by all forms

of life to grow and resist disease. A better understanding of these proteins will enable medical breakthroughs (in the areas of cancer treatments and tissue transplants) to become a reality.

Protein crystals must be grown for study because individual protein molecules are too small for analysis. A protein crystal is simply a regular, repeated chain of protein molecules which is large enough to be analyzed. Certain growing conditions must be maintained to ensure the successful growth of a protein crystal. The optimum temperature range is between 4 and 25 degrees Celsius. Specific concentrations of growing and quenching solutions are required for crystal growth. These vary depending on the type of protein crystal. The length of time required for crystal growth also depends on the type of protein.

The growth of protein crystals on Earth is altered by convective effects. A two-dimensional crystal is formed by buoyancy-induced flows caused by density gradients within the growing solution. In microgravity, these convective effects are negligible and a three-dimensional crystal with an improved internal structure is produced. Computer analysis performed on crystals grown in space is superior to that performed on Earth grown crystals. Figure 3.1 compares space grown and Earth grown crystals.

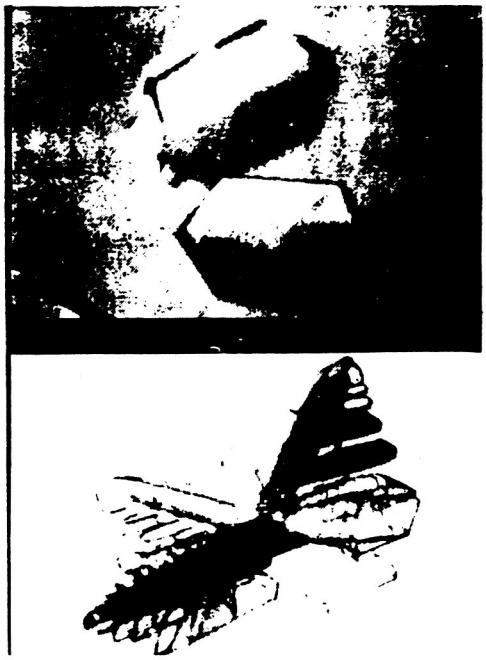

Figure 3.1 Comparison of protein crystals grown in space (top) and on Earth (bottom).

3.3.2 Vapor Diffusion Method

Vapor diffusion is a common method of growing protein crystals. Crystalline material and precipitating salts are combined with a drop of solvent and suspended by surface tension over a higher concentration of the same precipitating salts. Low vapor pressure is produced by the concentration gradient in the gas-tight chamber. This procedure causes the solvent to evaporate from the suspended drop. Crystal nucleation occurs as the volume of the drop decreases and the protein concentration in the drop approaches its saturation point. Crystalline material collects around the newly-formed nuclei and forms a crystal. This growth process continues until a quenchant solution is introduced into the chamber.

3.3.3 Sensing

The sensing system for the automated protein crystal growth facility is based on the facility designed by W. Z. Zuk and W. M. Rosenblum.[1,2] The sensing system is designed with the observer (scientist) in mind. The primary concern for the experimenter is the system's ability to automatically initiate, monitor, and stop specimen growth successfully for up to 90 days. To fully automate the facility, there is an obvious need for the on-board computer to automatically determine the size of the crystal. A sensing system is needed to transfer details of the crystal image to the on-board computer for analysis. The computer algorithm needs to be designed so that parameters (e.g. temperature and experiment duration) which control growth can be adjusted

automatically. A manual override capability by the terrestrial operator is a desirable feature. It is also necessary to design some level of robustness into the system.

3.3.4 Robot End Effector

One part of the group's design objective is to design and fabricate an end effector to be used with the current Zymate II system that allows for reliable transportation of chambers within the experimental facility. The Zymate II robot is on loan to Vanderbilt University from NASA and is the basis of our end effector design. The current end effector on this system does not operate with our rack and chamber designs. Therefore, a new end effector design is developed that coordinates well with the entire facility. As a result of the rack and chamber designs, two procedures are performed by the new end effector:

1. Transportation of the chambers
2. Plunging of the quenchant solution.

3.4 The Design Concept

3.4.1 Protein Crystal Chamber

3.4.1.1 Previous Chamber Designs

NASA designed a chamber for its previous protein crystal programs. We incorporated many of their ideas in our design. We are using the same vapor diffusion method for growing protein crystals. Their method of containing the precipitant solution by surface tension to chromex (a plastic wool material) is retained. Included in the design is a pedestal on which the protein drop adheres.

Last year's design teams conducted extensive research and design to create a feasible protein crystal growth chamber. We incorporated much of that research and design into our growth chamber design. The same PET polymer material is used for the construction of the thin chamber walls. Keyway tabs are located on the side of the chamber to secure the chamber within the drawers of the protein crystal growth facility. The protein crystal drop pedestal consists of a thermocouple (used to monitor the temperature of the crystal) and a heating wire designed by last year's team.

Protein and precipitant solutions are pre-loaded into the chamber. These solutions are separated from each other by a partition within the chamber. These liquids are dispensed onto the pedestal by annular plungers (see Figure 3.13 in the appendix). The outer annular region contains the precipitant solution. A membrane (a high molecular weight polyethylene) initially restrains the movement of the precipitant until it is extruded at which time the resulting pressure breaks the membrane and the precipitant is permitted to flow. The precipitant flows into the chromex and clings to the chromex by means of surface tension. Fritted glass keeps the precipitant from floating into the interior of the crystal chamber and disrupting the growth of the crystal. The porous nature of the fritted glass allows the vapor diffusion method for growing crystals to occur within the chamber.

3.4.1.2 New Chamber Design

The new chamber (Figure 3.11 in the appendix) has a diameter of 0.885 inches and a height of 1.45 inches. Two annular regions contain the protein solution (inner annular region) and the precipitant solution (outer annular region). When the chamber is placed into the active site,

these solutions are automatically extruded by means of concentric cylinders located in the active site trays (see Figure 3.2).

The inner annular region contains the protein which is extruded onto the pedestal. This solution is contained in two ducts on opposite sides, each of which makes up 1/8 of the total ring (the other 6/8 of the ring is made of the same PET polymer). These ducts are 0.4 inches in length. The lower 0.5 inches of the ring is completely empty except for the annular pistons. A diagram of this configuration can be seen in Figure 3.12 in the appendix. This solid section provides a good place for the chamber to accept the force of the spring that is exerted during the insertion and during the entire cycle in the active sites. The piston configuration for this ring has a portion 1/16 inches by 1/8 inches that makes a full circle. The pistons extend 0.3 inches into the ducts during the extrusion process. The tip of the projections leaves a space of 0.05 inches between it and the top of the ring section. This correlates to 13 µl, which must be added to the desired drop size in order to extrude the right amount onto the pedestal. Like last year's design, the protein solution is extruded through six 1/64 inch diameter holes which are obliquely machined within the mixing ring. The resulting vortex type motion and turbulent flow assure adequate mixing of the protein and the precipitant within the protein solution.

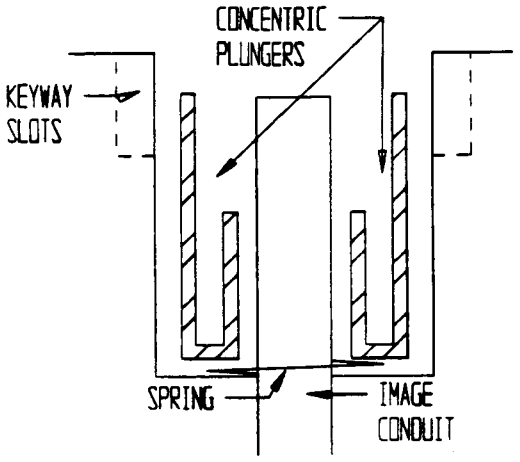

Figure 3.2 Concentric Plungers in the Active Sites

The quenching process is accomplished through the use of an external syringe (see Syringe section 3.4.1.3) interfacing with a rubber septum in the top of the chamber. The syringe also fills the growth chamber with the appropriate amount of quenchant solution. After the growth is halted, the chamber is removed from the active site and returned to the storage trays. The quenchant solution provides a safe medium for the transport of the crystal back to earth. When the protein crystal is ready to be removed from the chamber, the cap is removed and the solutions are poured from the chamber.

The 0.26 inch tunnel which runs through the center of chamber allows for the image conduit which is used to monitor crystal growth (see section 3.4.3). The wires for the thermocouple and heating element run up this conduit and hit contacts under the pedestal. The light needed to view the crystal is provided by interior lighting contained within the experimental facility (see section 3.4.2). The cap is made of a transparent hard plastic so that light can illuminate the protein crystal.

The chamber has specially designed grooves which allow for a simple and secure interface with the end effector gripper arms. Due to repeatability problems with the robot, the rings on the chamber's bottom are tapered.

3.4.1.3 Syringes

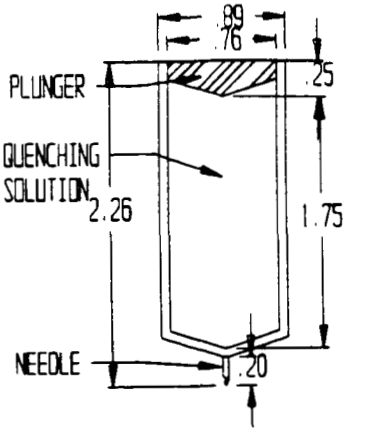

Figure 3.3 Quenching Syringe

The design of our protein crystal growth chamber necessitates the use of a syringe to quench the crystal. A diagram of the syringe and its dimensions can be seen in Figure 3.3. An entire tray in the experimental facility is used for the storage of these quenching syringes (see section 3.4.2.2). These syringes are designed so that the dispensing of the quenchant is accomplished through the use of the plunger mechanism on the end effector (see section 3.4.5). The needle of the syringe interfaces with the rubber septum of the chamber. This connection is air-tight and secure so that the quenchant does not leak out and float throughout the interior of the ISPR. The needle is designed to prevent the liquid flow from destroying the protein crystal. Again, the syringe must have specially designed grooves which allow for a simple and secure interface with the end effector gripper arms.

3.4.2 Experimental Facility

3.4.2.1 Geometry

The ISPR has specific limits on its spatial dimensions. It is approximately 75 inches by 41 inches with an arc on the back which gives a maximum depth of 36 inches. For our design purposes, a certain amount of space within the ISPR is allotted for the data acquisition equipment, HVAC (heating, ventilation, air-conditioning) units, lighting and sensing equipment.

Robot accessibility to all of the chambers and syringes is a high priority in deciding on the geometry of the experimental facility. The experimental facility containing all of the chambers, syringes, and the robot approximates a circular configuration (see Figure 3.4). The radius of this circle is 16.25 inches with the chambers protruding inward 0.5 inches to allow the end effector to grip the chamber. The Zymate II robot is mounted to the back wall with its base embedded in that wall, which enables the robot to extend the entire depth of the facility. Since the robot arm rotates in a circular direction, this circular configuration is best for robot accessibility. This configuration allows for a workable robot arm length which enables the robot arm to reach all chambers efficiently and not to cause interference with chambers when the robot arm is rotating.

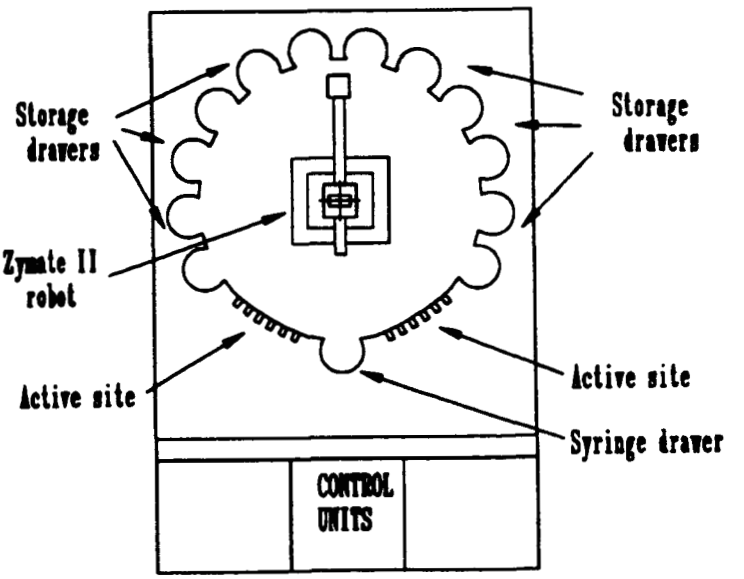

Figure 3.4 Circular Rack Configuration

To facilitate the configuration of the experimental facility and the mobility of the robot, each of the drawers of the rack is angled towards the center

axis of the Zymate II robot. Furthermore, all of the drawers are designed with the same radius of curvature which allows for secure compatibility between the chambers and the end effector. This consistent radius of curvature also aids in the mass production of the drawers. These drawers are inserted into the sleeve located in the facility and turned 180 degrees to place the drawer in the reach of the robot. The opposite action removes the drawer. A simple spring loaded lock keeps the sleeve at the proper orientation and a unique geometry keeps the drawer from moving in the sleeve after the rotation. The sleeve rotates in a cylinder cut from the rack and is kept in place by the door. Only space for the drawer is allotted in the face of the door. The drawers are designed to be air-tight. The door is 2 in. in depth and covers the front of the experimental facility. The top of the robot interfaces with the door to hold it in place securely. Also, the touch screen display for the control system is located on the door.

3.4.2.2 Active Sites, Storage Site, and Drawers

The design consists of twelve storage drawers (54 chambers per drawer), two active sites (72 slots per site), and a syringe drawer (57 syringes). Each of the twelve drawers is 3 slots across with 1.36 in. spacing and 18 slots deep with 1.23 in. spacing. The total number of storage sites is 684, which satisfies the given requirement of 500. A spring is used to keep the chamber locked in place. The drawer is based on a 5 inch diameter and 27.25 in. length cylinder with portions cut off above and below the axis (Figure 3.5 (top)). The sleeve surrounds the drawer except on the side of the chambers (Figure 3.5 (bottom)). It has a 5.5 in. diameter and a 28 in. length.

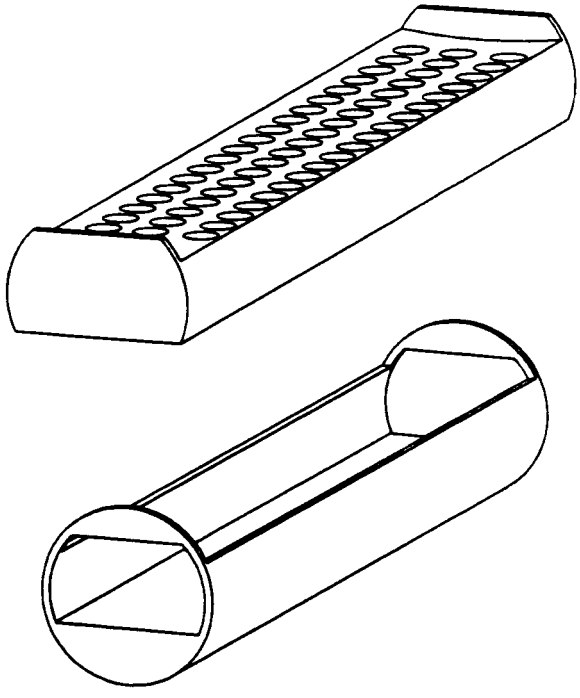

Figure 3.5 Storage Drawer (top) and Sleeve (bottom)

The total number of active sites is 144 (72 active sites in each shelf-Figure 3.6). Each slot is spaced 1.9 in. in each direction. The bottom of each site has two concentric cylinders for the purpose of extruding the solutions onto the pedestal and into the chromex. These cylinders are connected to a common base by a spring which is strong enough to extrude the liquid but flexible enough not to cause undo stress on the chamber (Figure 3.2). As mentioned earlier, all of the load is carried by the inner cylinder. The cylinders are not connected to the imaging conduit and the lead wires, so the contacts for the thermocouple and heating element must have a spring like quality.

The syringe drawer is located at the bottom of the circular configuration, and two active site drawers are located on each side. The syringe drawer and sleeve have a configuration very similar to the storage drawers and sleeves. The differences are that the slots are 3 by 19 and the spacing is 1.36 in. and 1.16 in. in the two directions, respectively. The base cylinder also has a larger diameter to account for the fact that the syringes are longer. Also, the spring bottom which holds the syringe in place must be formed to the syringe's dimensions and have a hole in the center to allow for the needle.

3.4.3 Sensing Device

3.4.3.1 Light Diffraction Method

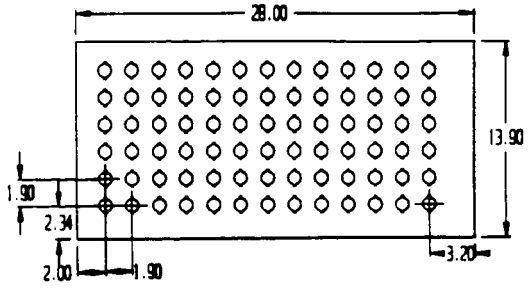

Figure 3.6 Active Site Shelf

To satisfy all of the investigator's requirements, it was necessary to consider sensing mechanisms other than the light diffraction method. The problem with the light diffraction method is that a true visual image of the crystal could not be obtained during the growth process. The light diffraction method also requires the use of pyrometric equipment which would not realistically fit within the physical constraints of the rack. Another problem with the light diffraction method is the number of media light needs to pass through prior to and after crystal penetration. Basic refraction theory explains that as the concentration of median changes, the angle of refraction differs accordingly. This is the basic principal of the diffraction method used in determining crystal size. Interference caused by the number of media involved (including the surface of the bubble and transparent shielding) will hinder the accurate determination of crystal size.

3.4.3.2 Two Possible Sensing Methods

A CCD camera was provided by NASA for use in the crystal sensing design. An experiment was conducted using the CCD to determine whether or not the camera has a good picture quality. The optimum position of the light source relative to the camera also needed to be determined. Since it is desirable to see the start of crystal formation for crystals as small as 50μm, it is necessary to use magnification along with the CCD camera. Various lenses were considered and analyzed for accomplishing this task. Of the lenses available in an "Edmund Scientific" magazine, none were capable of accomplishing the magnification requirements needed to produce a large crystal image. However, a CCD microscope adapter was found which can produce magnification up to 180X. The two possible set-ups which were analyzed for use of the CCD and the microscope adapter are explained below:

1. Without Image Conduit--This configuration consists of the CCD camera connected to a microscope which is focused directly onto the crystal droplet through the in the bottom of the protein chamber.

2. With Image Conduit--This configuration consists of the CCD camera connected to a microscope which is focused onto the end of a piece of image conduit. Image conduit is a flexible, glass-like tube through which an image may be transported. The image conduit runs from a given location through the porthole in the chamber to a point just beneath the protein droplet.

According to Professor Richard F. Haglund of the Vanderbilt University Physics Department, either configuration will work. The set-up without an image conduit is best if cost is a major problem. Cost was not the only factor to be considered. Due to the large size of the CCD microscope adapter (approximately 1.5 ft long), the cameras could not be placed directly beneath the active chamber sites. Therefore, the image must be sent to a location where the microscopes can be placed. This image piping is accomplished through the use of image conduit. It has not yet

been determined where the microscopes will be placed. They will probably be located in the empty spaces around the inactive sites. Several simplifying assumptions are made:

1. Since the droplet is located in a plane parallel to the end of the image conduit, there is minimal or no refraction to distort the image.
2. There will be plenty of room around the inactive sites for the microscopes.
3. The robot will not interfere with the light inside the rack.

3.4.3.3 Selected Sensing System

The selected sensing system (see Figure 3.7) is based on the use of a CCD (Charged Coupled Device) camera which is attached to each active site. The visual image captured from each camera is digitized and processed to obtain the dimensional information which is the key factor in determining the success or failure of the sample. The primary advantage of using a CCD camera is the ability to present the actual visual image to the scientist as well as providing the crystal information to the on-board computer for automatic analysis.

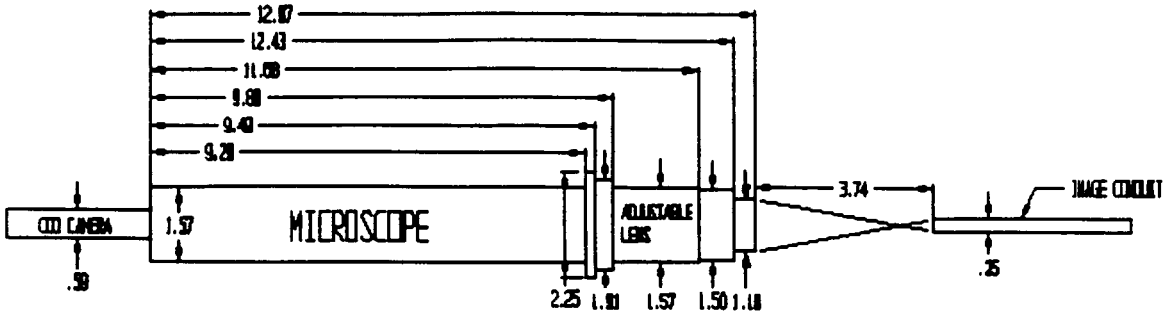

Figure 3.7 Selected Sensing Device

The first step in automatically determining the size of the protein crystal involves digitizing the crystal's image. After the digitization, the image is processed using a convolution filter, FFT (Fast Fourier Transform), and a geometric operation. The convolution filter is used to implement Laplacian filters primarily to separate the crystal edges from the background. The FFT converts the digital image from the time domain to the frequency domain which removes noise from the image. Geometric operations are performed to magnify the image to obtain an optimal result from the image analysis. The validity of using a CCD camera and digital filters for image processing is shown in various papers.[1,2]

A simulation was conducted using the CCD camera so that we could test the imaging process and camera resolution. In order to simplify the simulation, several assumptions are made:

1. A 15 mm lens attached to the CCD camera is the only optical magnification lens used.
2. Surface of the drop is simulated using a thin plastic film.
3. Light source is positioned directly below the surface.
4. Image is digitized from an image on a VCR tape.
5. Color image is used instead of black and white.
6. Image conduit is not used in the simulation.

Note: These assumptions were made because of limited resources as well as the need to simplify the process.

3.4.3.4 Lighting within the Chamber

Accurate detection of the micron-size crystal within the growing bubble is a challenge. The image of the small crystal must be separated from the background clutter by properly contrasting the image. Light must therefore illuminate the crystal so that an image can be obtained with the CCD camera.

Several options are available for the positioning of the light source relative to the crystal and camera.:

1. The crystal growth site could be illuminated from the direction of the camera
2. The crystal could be placed between the camera and the light source (the selected method).
3. The crystal could be illuminated from the sides.

The second method is chosen for simplicity and because of spatial limitations. If the light comes from the direction of the camera, then it would be necessary to slightly enlarge the diameter of the image path to allow light to pass on the outside of the image conduit. Similarly, lighting from the sides would be achieved by "piping" light through optic fibers to the growth site. Both of the above methods, however, require the reduction of space allocated for the radial plungers and for the necessary wall thickness. Lighting the crystal from behind using the background lighting in the rack, simplifies the chamber design and provides for the best image. The chamber lid is transparent so light can pass from the light sources mounted on the walls of the rack to the crystal. Polarized light is used in order to deliver optimum contrast between the crystal edges and the background. It is assumed that background clutter does not interfere with crystal detection because only the crystal growing area lies within the focal length of the sensing system.

3.4.4 Electronic Control System

The control system is designed to have the following functions:

1. Initiate the experiment
2. Obtain and store visual information
3. Control and monitor the heating units
4. Analyze information
5. Control the mechanical manipulator
6. Supply the principal investigator (PI) with necessary information
7. Allow investigator to have control over the experiment with minimal training
8. Operate with minimal power requirement
9. Easy to maintain the components by modular design
10. Easy to re-task depending on the sample
11. Allow a multiple-user environment

The key element of this design is a strong emphasis on the use of a computer to automate this facility. The Intel 486 DX4 based laptop computer was selected to obtain maximum performance at a relatively low power consumption. Also, the computer will use Windows NT from Microsoft Corporation for its preemptive multitasking capability which allows sharing of the processor time necessary to finish the task efficiently. The computer is equipped with an image processing board, a data acquisition board, a PCMCIA removable 1.8 inch hard drive, maximum memory size, and a writable optical drive unit (instead of a floppy disk unit).

The computer is equipped with a DT 2867 integrated image processing board from Data Translation Inc. The DT 2867 was selected over other available boards because of its ability to perform two functions. This board grabs the frame (frame capture) from the analog image and processes the image with the necessary filter with minimal reliance on the main computer system. This on board processing capability is necessary since the computer needs to handle numerous tasks other than image processing. The DT 2867 is capable of handling a math-intensive convolution filter which is processed by three dedicated math processors on the board.

The user interface of this system is designed based on the need to automate the protein crystal growth facility for extended periods of unmanned operation. The user is not concerned with the computer algorithm which creates the user-friendly menus. This system allows multiple users to share the facility. This system also allows the user the ability to alter the experimental parameters on each sample. There are two main screens available in the user interface. The automated mode, which only displays information, is shown in Figure 3.14 in the Appendix. The manual mode allows the change of the growth temperature and the estimated time remaining in the growth process. This screen is shown in Figure 3.14 in the Appendix. Each mode utilizes a touch screen interface with a stylus pen and a ten-key keyboard. Each scientist receives a security code which prevents the parameters of the experiment from being accidentally changed by someone else. The flow charts for the automatic and manual operation of the control system are seen in Figures 3.8 and 3.9. An overall layout of the control system is shown in Figure 3.15 in the Appendix. The system designed is based on the Labview 3.01 control system for Windows NT from National Instruments. To control each component, the code interface node (CIN) of Labview is used. CIN's are the interfaces between Labview and Pascal, C, or C++ based code. This CIN is used to control the DT 2867 image processing board, heating elements, and Zymate robot.

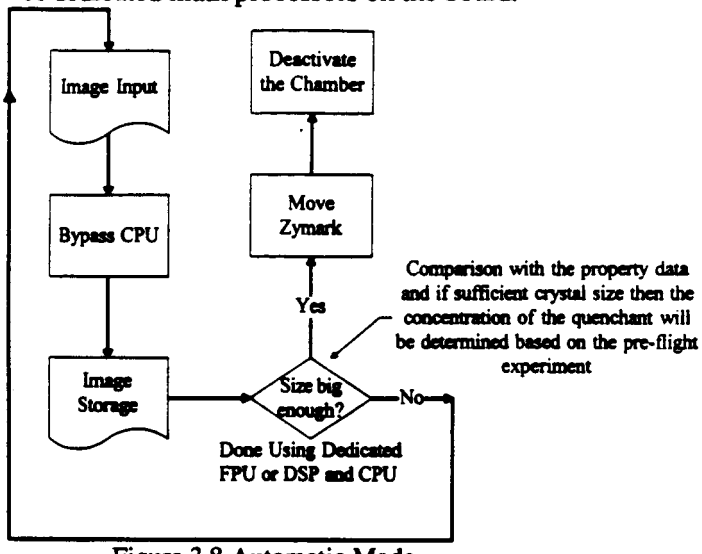

Figure 3.8 Automatic Mode

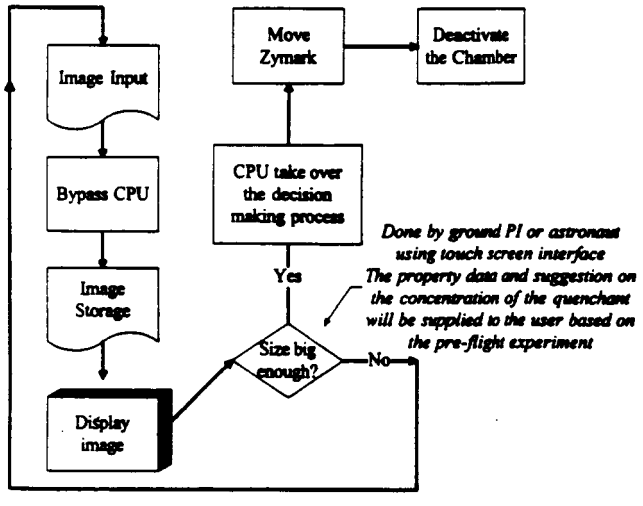

Figure 3.9 Manual Mode

The graphical user interface is the same on ground and on board. The user interface is based on two different displays. These displays are the active matrix technology on board Space Station Freedom and the regular CRT on ground with the same capability. One of the displays is used to supply the user with a real time image from the CCD camera. The other display is designed as an input console using Labview.

This system is based on the use of a black and white micro charged coupled device (CCD). Images are directly deposited onto the optical storage device for further analysis. Most of the image processing, including filtering and magnification of the image, are done within the DT 2867 board. The board supplies Labview with the necessary information to control the experimental parameters. Labview determines the experimental parameters based on the pre-determined information, such as duration of experiment, maximum size of crystal and acceptable temperature range. Pre-determined information is retrieved from the PCMCIA based hard drive, which stores the operating system, as well as the experimental parameters of each sample in ASCII format. If the conditions are satisfied, then Labview initiates the CIN command to begin the deactivation of the chamber. The chamber is deactivated with a syringe containing the proper concentration of quenchant solution. Then, the Zymark robot places the chamber in a non active site for storage. To make this facility successful, it is imperative that extensive ground based simulation is done to obtain the information necessary from preflight experiment and the simulations.

Several assumptions are made to make this preliminary stage of design possible:

1. The switching system for heating, and imaging is neglected to simplify the design
2. Detailed programming of Labview is neglected
3. The Zymark robot can be controlled by using C++, C, or Pascal
4. The Programming of the DT 2867 image processing board using C++ is neglected
5. The selection of an image storage device is neglected
6. Communication with the space station and ground is done through a parallel port
7. A high capacity PCMCIA hard disk drive is available (approximately 240 MB to 320 MB)

The advantages of the current design are:

1. All the data is stored in the optical drive before any analysis is done.
2. Minimal training is necessary to operate the system.
3. It is a sufficiently fail safe system to allow multiple user environment.
4. Low power consumption is used by the laptop computer components.
5. It is relatively easy to re-task and update the system by use of PCMCIA storage device.
6. It is easy to maintain in case of component failure since it is modular.

The disadvantages of the current design are:

1. It need extensive modifications of the stock components.
2. Each component of the system must be integrated.
3. There is a high cost associated with employing the CCD cameras on each active site.
4. Extensive programming for the image processing board, the Zymark, and the switching devices for the heating and imaging elements must completed.

Recognizing the fact that this is a preliminary stage of design it is necessary to determine the validity of each component for the next stage of the design.

3.4.5 Robot End Effector

3.4.5.1 Zymate Robot

The Zymate II robot has six degrees of freedom and is operated by the EasyLab Controller. EasyLab uses seven primary commands to move the robot:

Rotary -This controls the rotation motion of the base and has a range from 0 to 360.
Vertical -This controls the height of the arm and has a range from 0 to 30.
Reach -This controls the extension on the arm and has a range from 0 to 30.
Wrist -This controls the rotation motion of the arm and has a range from 0 to 360.
Grip -This controls the gripping motion of the gripper arms and has a range from 0 to 30.
Syringe -This controls the syringe on the end effector and has a range from 0 to 30.
Home -This command sends the robot back to the home position.

The above ranges are displacement values used by the EasyLab Controller; they are not distances. The use of the commands is quite simple. To extend the arm to its maximum distance the operator would type the following in the direct robot control mode or into a program; reach=30. In general, the method for using the commands is: command name=value. Two commands, grip and syringe, control the motions of the end effector.

Zyline provided information on the internal workings of the robot and end effector. There are two 6 volt DC servo motors inside the end effector housing. A circuit board is also contained in the housing that controls the electronic components of the end effector. The robot arm outputs 6 volts to the end effector in order to operate the two motors and the sensing mechanisms. By examining the end effector and its movements, it is determined that the gripper arms and syringe operate using a rack and pinion system.

The gripper arms travel at 1.07 cm/sec and the syringe travels at 1.36 cm/sec. This information is used to help determine the gear ratios for the motors in our end effector. The end effector and arm connect electrically by means of 12 pin connectors with the female receptors being on the end effector

3.4.5.2 Overall End Effector Design

The end effector is composed of two primary components: the gripper arms and the syringe plunger. The gripper arms can move linearly from an open to a closed position, and vise versa. The mechanism to provide this motion is a double rack and pinion configuration. The syringe plunger also operates in a linear manner which is accomplished through a single rack and pinion configuration (see Figure 3.10). The gripper hands are located on the center line of the robot arm. Similarly, the syringe plunger is centered between the hands with the linear travel occurring along the axis of the robot arm. The rack and pinion systems are each powered by a 6 volt motor from MicroMo. Attached to each motor is a gearhead from MicroMo to provide the necessary step down ratios needed for the system. A circuit board to control the motors and force sensors is also included in the design. Each gripper hand has a strain gauge to serve as a force sensor to control the motors. The above components are all encased in a plastic housing which will fit the arm on the Zymate II robot. The two motions can still be achieved using grip and syringe commands in the EasyLab controller.

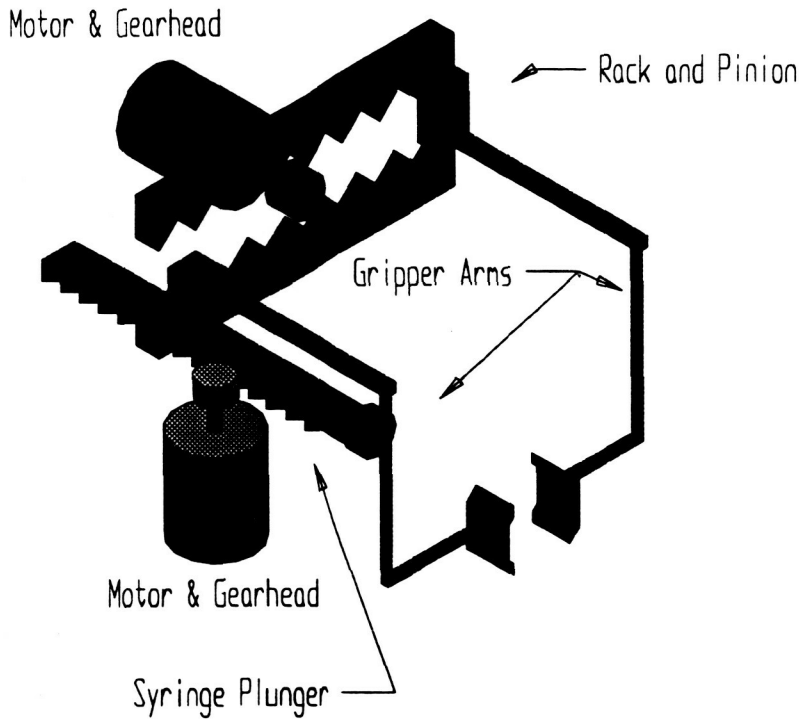

Figure 3.10 End Effector Design

3.4.5.3 Gripper Arm Design

The gripper arms serve two primary functions in the automated protein growth facility. First, the arms grip the chambers so that they may be locked and unlocked into the active and storage sites. Secondly, the arms grip the chambers and syringes so that they can be moved about the facility.

The chambers, when transported about the facility, must be locked and unlocked into the active and storage sites. This procedure requires a pushing and twisting motion to engage the chamber into the keyway (see also chamber design). The wrist and arm on the robot provide rotating and pushing motions, respectively. To utilize these movements, it is necessary to locate the center of the gripper hands along the axis of the robot arm. With this configuration the gripper hands rotate in an on-center fashion when the wrist of the robot rotates. When used with the extending movement of the robot arm, chambers can easily be inserted or removed from the sites.

The gripper arms must also be able to hold the syringes and chambers. To accomplish this task, a simple rubberized male-female notch system is used to ensure a secure fit between the hands and chamber or syringe. It should be noted that while this is an important part of the design, it is not included in the drawings. Located on each gripper hand is a strain gauge that serves as a sensor for the motion of the arms.

The motion of gripping the chambers and syringes is performed using a simple double rack and pinion design (see Figure 3.10). This mechanism is housed in Segment 1 of the end effector (see Figures 3.16 -3.18 in the Appendix). The gripper arms are attached to each of the two racks to provide the linear gripping motion of the arms.

Refer to Section 3.8.2 in the Appendix for a detailed list of the specifications on the racks and pinion used in the end effector.

3.4.5.4 Syringe Plunger Design

The plunger on the end effector is used to dispense the quenchant solution from the syringe into the chambers. The syringe plunger design closely relates to the gripper arm design. Due to the gripper arms location on the center line of the robot arm, it is also necessary to orient the plunger in a similar fashion. This enables the syringes to be plunged easily while being held by the gripper arms. The linear motion of the syringe plunger is achieved using a single rack and pinion configuration (see Figure 3.10). The mechanical components of this mechanism are housed in Segment 2 of the end effector (see Figures 3.19 - 3.21 in the Appendix).

The reader is referred to Section 3.8.2 for a detailed list of the specifications on the rack and pinions used in the end effector.

3.4.5.5 Motors and Gearheads

The movement of the gripper arms and syringe plunger is accomplished by using small DC servo motors and gearheads. Two identical motors were selected from MicroMo use in the end effector. The motors selected are used by Zymark in their end effectors and were chosen because of their small size. To achieve the necessary linear speeds for both the gripper arms and syringe plunger, two gearheads were selected from MicroMo. These were chosen because of their small size and easy compatibility with the motors. The gripper arms have a linear travel speed of 1.07 cm/sec. A gearhead with a step-down ratio of 548:1 was chosen to provide the needed rotational speed for the gripper arms. The syringe plunger has a linear travel speed of 1.36 cm/sec. A gearhead with a step-down ratio of 308:1 was chosen to provide the needed rotational speed for the syringe plunger.

A motor mounting plate was designed to enable the connection of the motor and gearhead system to the housing (see Figure 3.22).

3.4.5.6 Fabrication

Part of the original design goal was to fabricate a working end effector. Due to several unforeseen obstacles, the fabrication of our end effector design was unable to be completed. The majority of the work has been finished on the end effector. There are only a few small details that need to be completed before the fabrication can begin. First, the rest of the parts needed for the end effector would have to be ordered. Secondly, detailed calculations of the required torque for the gripper arms and plunger would need to be performed. Finally, consideration would have to be given to tolerances for the gearing components.

Several parts have already been ordered from Zymark:

2 MicroMo DC Motors
1 Wire Harness
1 Circuit Board
1 Hand Connector Housing

Refer to Section 3.8.2 for a detailed listing of the parts used in the end effector.

The determination of the step-down ratios for the gearheads was done as an approximation. It is not to say that the values calculated should be ignored, but rather reexamined before fabrication. The selection of the gearheads was done by trying to match velocities. Required torque for the gripper arms and syringe plunger was not considered.

A detailed set of machine drawings was generated for Segment 1, Segment 2, and the Motor Mounting Plate. It should be noted that these drawings do not include any tolerances for the gearing mechanisms.

Before fabrication can begin, the tolerances and spacing between the two rack and pinion systems should be considered. This is needed to prevent backlash during operation. If consideration is not given to the tolerances and spacing, binding or skipping can occur in the gear systems. For the two rack and pinion systems in the end effector, tolerances should be not greater than 0.002 of an inch for the center distance between the gears. The Precision Industrial Components Design catalog has a very complete technical section on gear tolerances and spacing. This should be referenced for finalizing the end effector machine drawings.

3.4.6 Robot Simulation

A parametric design software program was used in the design of the growth chamber and rack. I-DEAS CAD and simulation software uses solid modeling as an effective way of designing a system in 3-D. The design process can be substantially accelerated because of the increased visualization capability. I-DEAS is also used in the kinematic simulation of the overall system. Interference and motion limitations are identified at an early stage in the design and can, therefore, be taken into account.

3.5 Conclusions

3.5.1 Overall Progress

The objective of this design project was to develop an interactive, automated protein crystal growth facility. Our focus was to continue the overall design efforts of last year's design teams. Our design involved the integration and development of the protein crystal chambers, the experimental facility, the robot and the end effector, the sensing system, and the control system.

3.5.2 Chamber and Experimental Facility

The design of the chamber and facility was not a primary design goal for this year's team. However, the design of the end effector, sensing system, and control system warranted changes in the previous design. Furthermore, several details (dimensions, feasibility, manufacturability, etc.) of last year's design were not considered. This year's design tried to include these details and integrate them with the design of the other systems.

3.5.3 Sensing System

Part of the design objective was to finalize and integrate a feasible sensing system which would be able to detect protein crystal growth within the experimental facility. A visual image is required by the experimenter for evaluating the structure of the crystal. It was also necessary to produce an image that the computer could dimension (so the control system would know when to terminate growth) and store on disk for future reference. The components of the sensing system

had to be sized and arranged in such a way as to fit within the growth facility. The system which was developed integrated the use of a CCD camera, image conduit, and the digitizing part of the control system for monitoring the crystal growth. Although it hasn't been tested, we feel that the chosen design will work, or least provide a strong basis for further research in this area.

3.5.4 Control System

Another aspect of our design included the selection and integration of a computer control system which would provide a user-friendly interface between the growth facility and the multiple number of principal investigators. The control system was designed with strong emphasis on full automation of the facility while still maintaining the ability to alter the experiment. To make this design functional with minimal interference of one experiment with another, extreme care was taken when considering the security and power management system. It is also true that this control system will require more extensive study to make it reliable and realistic. This is because of the rigorous requirements placed on the system due to the sensitivity of the experiment to any variables introduced.

3.5.5 End Effector

The objective to design and fabricate an end effector to be used with the current Zymate II system that will allow for fully automated protein crystal growth in space has been met for the most part. The end effector perforns two primary functions. Firstly, the end effector serves to grip the chambers and syringes. Secondly, the end effector must plunge the syringes that contain the quenchant solution. The gripping movements are achieved through a double rack and pinion mechanism. There are two gripping arms which attach to the racks and provide the means for clamping. The plunging motion is performed with a single rack and pinion system. When operating the plunger extends and dispenses the quenchant from the syringe.

There are several advantages to this design. The end effector design was arrived at because of its simple integration into the Zymate II EasyLab controller. The same two commands will still be able to be used when operating the end effector. Also, no modifications will have to be made to the robot in order for it to work with the end effector. This was an important factor when selecting a design. Limiting the number of functions that the end effector perforns reduces the power requirement of the robot. This is important because of the power restrictions in space.

One part of the design objective was not satisfied. The group was not able to have the end effector fabricated. This was the result of several unforeseen obstacles. The material included in this section of the report is complete enough to have the end effector fabricated with minimal additional work.

3.6 Recommendations

3.6.1 Chamber and Experimental Facility

An optimal range of diffusion area and chamber volume that NASA had determined was mentioned last year, but our team was unable to find that information. By changing the height of the chamber or by moving the fritted glass inward or out ward new ratios can be attained. These alterations would not cause serious disturbances to the chambers, the drawers, or the facility as a whole. If the chamber is lengthened, it must be remembered that the ring section must be elongated because there will be more liquid to fill the chromex space, and more syringes may be needed to account for volume change in the growth chamber.

As opposed to last year, the solutions are extruded immediately when inserted into the active sites, so the pedestals temperature will take time to attain the optimal temperature. This time lag should not cause a problem, but investigation into the heating time and the affects of the low temperature on the growth should be carried out.

The time it takes for the robot and end effector to insert the chamber into the active site has an affect on the speed which the solution is extruded into the chamber. A high velocity may not be conducive to a drop being properly placed on the pedestal. Also, the velocity of the quenching fluid may harm the crystal even if the stream is diverted. These subjects would need to be tested on actual prototypes in order to give good results.

3.6.2 Sensing

Due to the large number of cameras and other sensing equipment required, a detailed cost analysis needs to be conducted on the sensing system. A test of the system needs to be conducted to determine the reliability of the sensing equipment and its integration within the experimental facility.

3.6.3 Control System

For the future, it is necessary to implement the humidity control as well as a temperature control. The humidity control system could be learned in detail from the paper presented by L.J. Wilson of the Georgia Institute of Technology[4]. Also, as of this preliminary design of the control system, it is necessary to determine the viability of each selection based on the necessary physical and electrical requirements specified by NASA.

3.6.4 End Effector

There are a few areas that need further research and development before fabrication can occur. A detailed analysis of the speed and torque requirements for the gripper arms and syringe plunger needs to be performed. Preliminary calculations were made and appear in this report. This data should be used as a starting point for further analysis. The spacing and tolerances for the gear systems have to be calculated and integrated into the machine drawings. Also, the electrical connections of the system need to be determined. We have the circuit board that Zymark uses on their end effectors. This board should be examined so that connections for the motors and sensors can be determined. Finally, the entire end effector system needs to be qualified for use in space before it can be used in Space Station Freedom.

3.7 References

(1) W. M. Rosenblum, J. P. Kennedy, and Brad Bishop, "Examination of protein crystals in the hanging drop using digital image capture techniques," J. Crystal Growth, 110, 171-176, (1991).

(2) W. Z. Zuk, and K. B. Ward, "Methods of analysis of protein crystal images," J. Crystal Growth, 110, 148-155, (1991).

(3) 1992-1993 NASA/USRA Advanced Design Program, Vanderbilt University, Chs 6-9.

(4) L. J. Wilson, T. L. Bray, and F. L. Suddath, "Crystallization of proteins by dynamic control of evaporation," J. Crystal Growth, 110, 142-147, (1991).

(5) Gary Walker, Electronic Technician, Mechanical Engineering Department, Vanderbilt University

(6) MicroMo Electronic, Inc., 742 2nd Avenue South, St. Petersburg, FL 33701, (813) 822-2529.

(7) Precision Industrial Components

(8) Vivian Marchand, Zymark Corporation

(9) Glennys A. Mensing, Free Electron Laser Lab, Physics Department, Vanderbilt University

(10) Wayne Anderson, Professor, Biochemistry Department, Vanderbilt University

(11) Craig W. Morton, Material Sciences & Engineering Group, Applied & Engineering Sciences Department, Vanderbilt University

(12) Anthony B. Hmelo, Research Assistant Professor, Center of Microgravity Research & Application, Applied & Engineering Sciences Department, Vanderbilt University

(13) William H. Hofmeister, Research Associate Professor, Material Sciences & Engineering Group, Applied & Engineering Sciences Department, Vanderbilt University

(14) Richard F. Haglund, Professor, Physics & Astronomy Department, Vanderbilt University

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(16) Joel Barnett, Mechanical Engineering Department, Vanderbilt University

(17) Robert L. Galloway, Associate Professor, Biomedical Engineering, Vanderbilt University

(18) Bill Gentry, Supervisor of Machine shop, Mechanical Engineering Department, Vanderbilt University

(19) Steven W. Peterson, Assistant Professor, Mechanical Engineering Department, Vanderbilt University

(20) Barry J. Dunn, Smart Structures Lab, Mechanical Engineering Department

3.8 Appendix

3.8.1 Figures

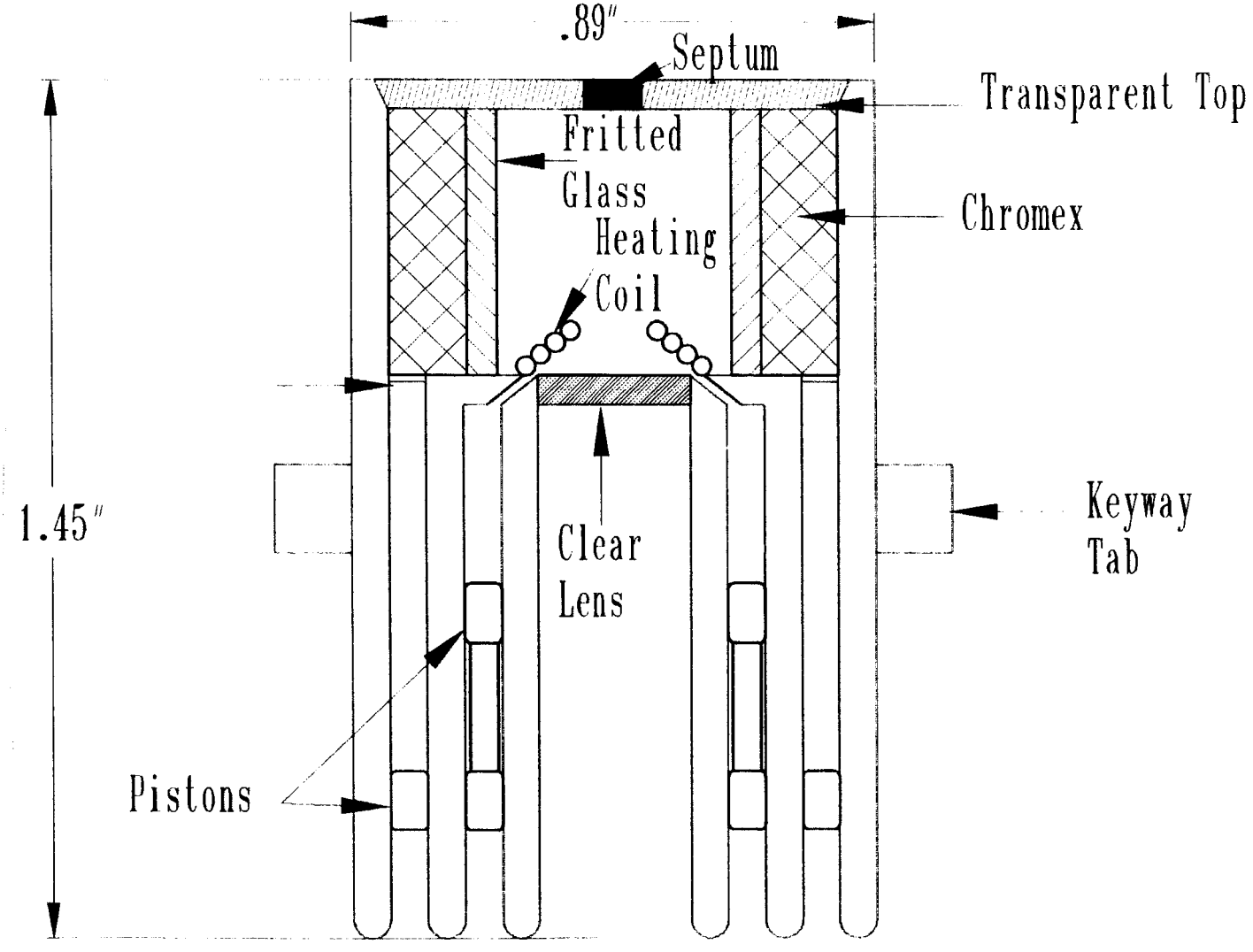

Figure 3.11 Protein Crystal Growth Chamber

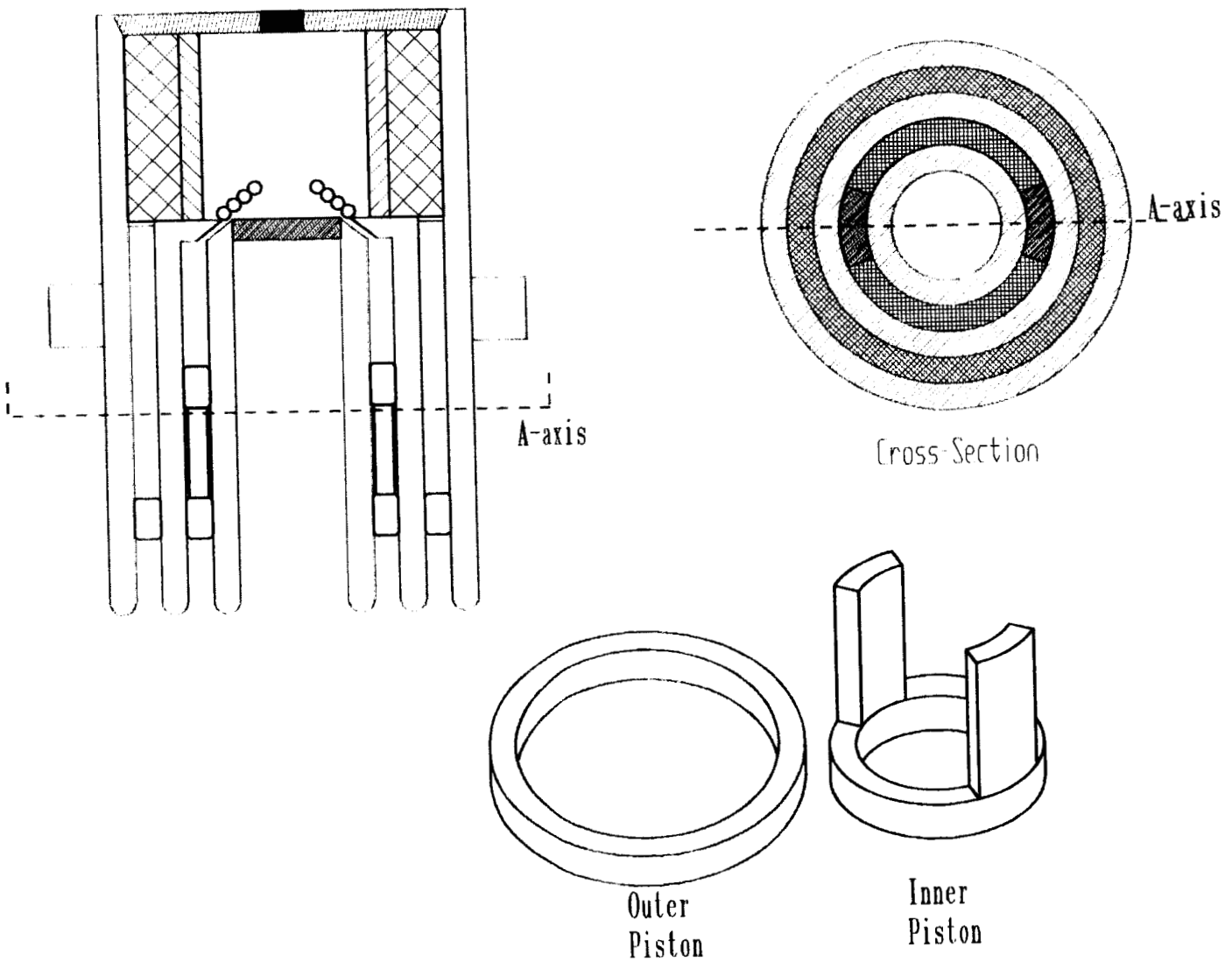

Figure 3.12 Plunger Configuration

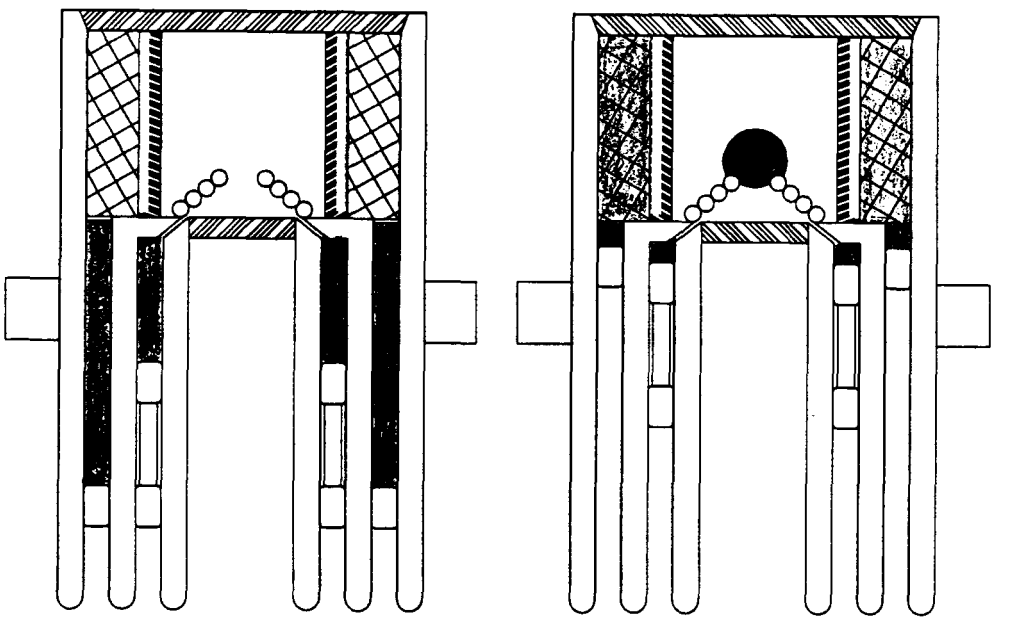

Figure 3.13 Before (left) and After (right) Plunging

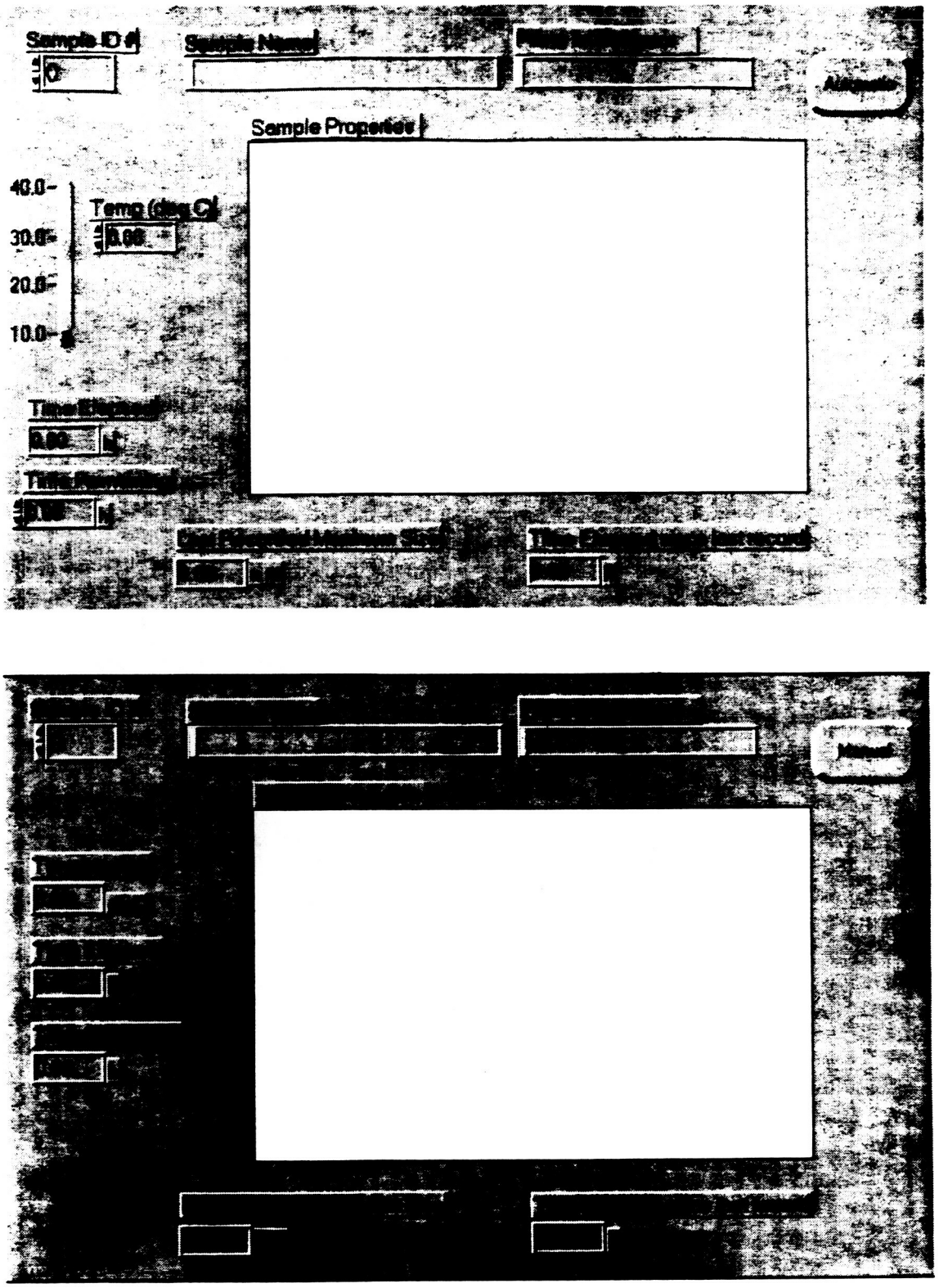

Figure 3.14 Manual (top) and Automated (bottom) Modes

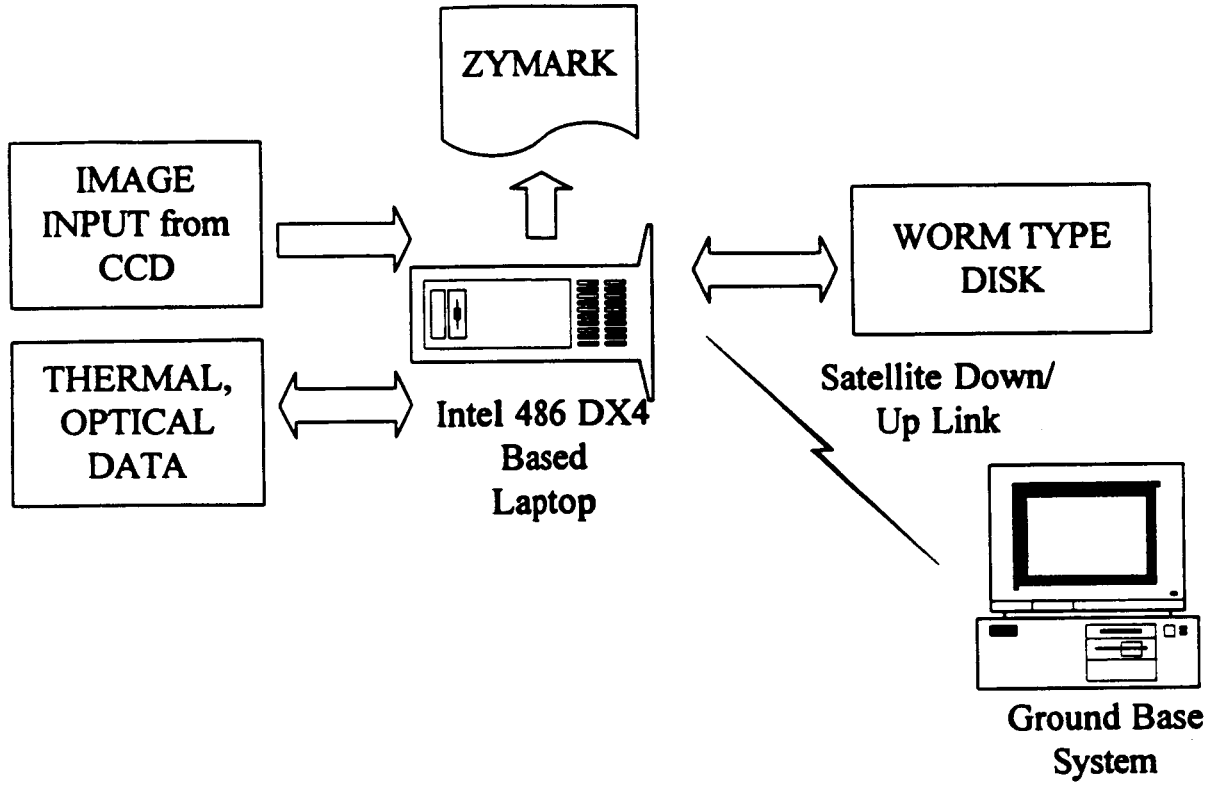

Figure 3.15 Control System Layout

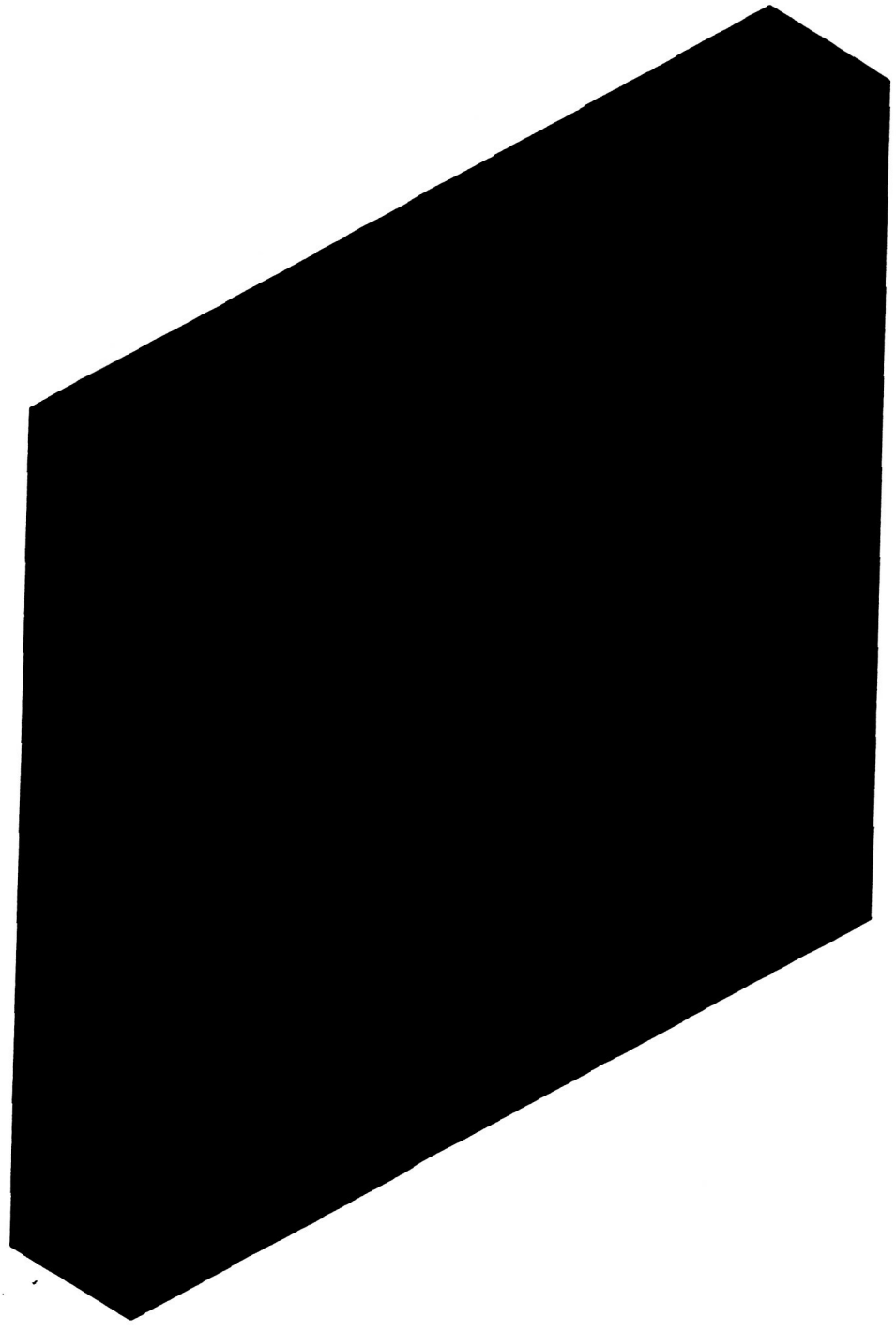

Figure 3.16 Segment 1 - Solid Rendering

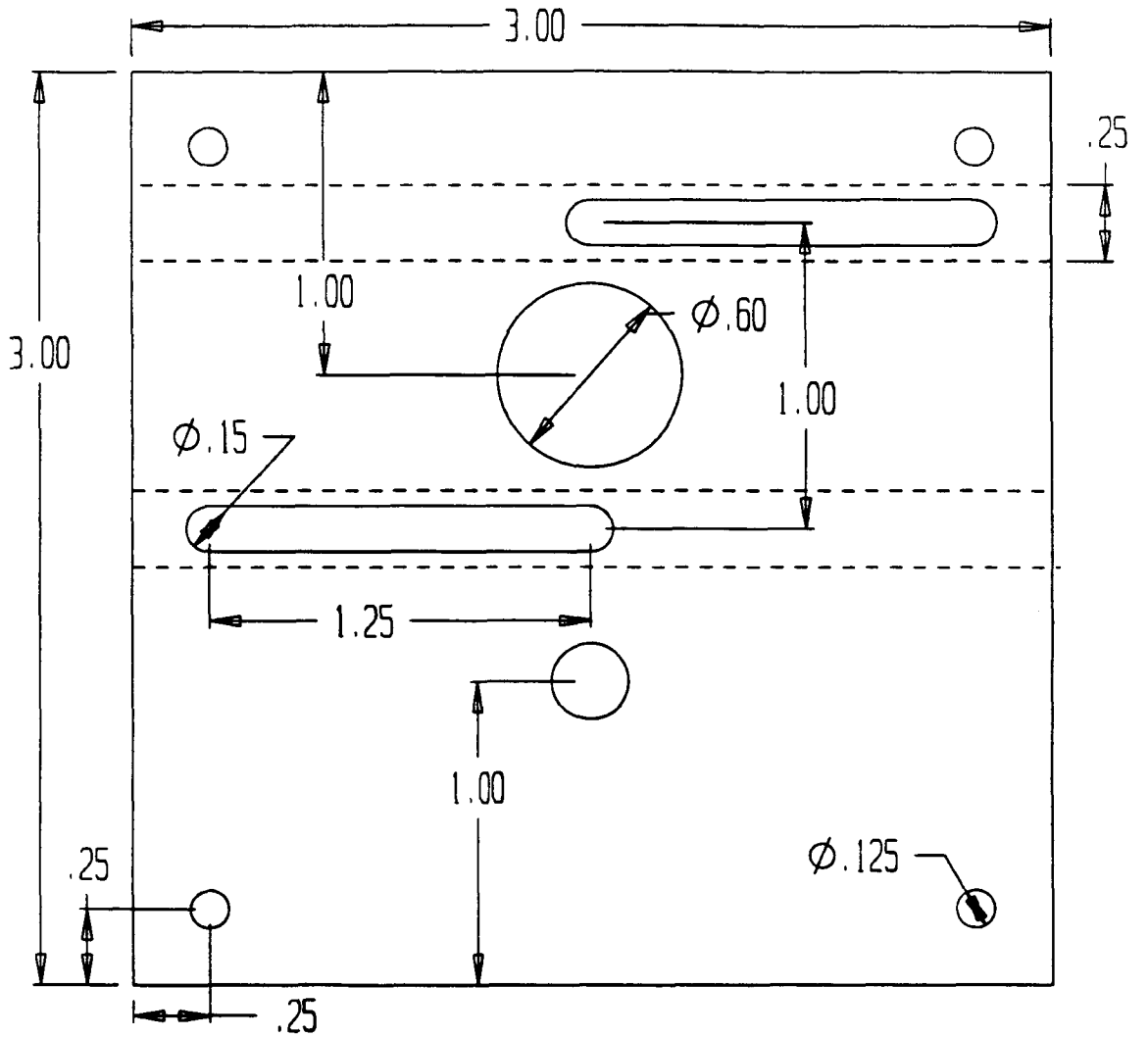

Figure 3.17 Segment 1 - Machine Drawing - Front View

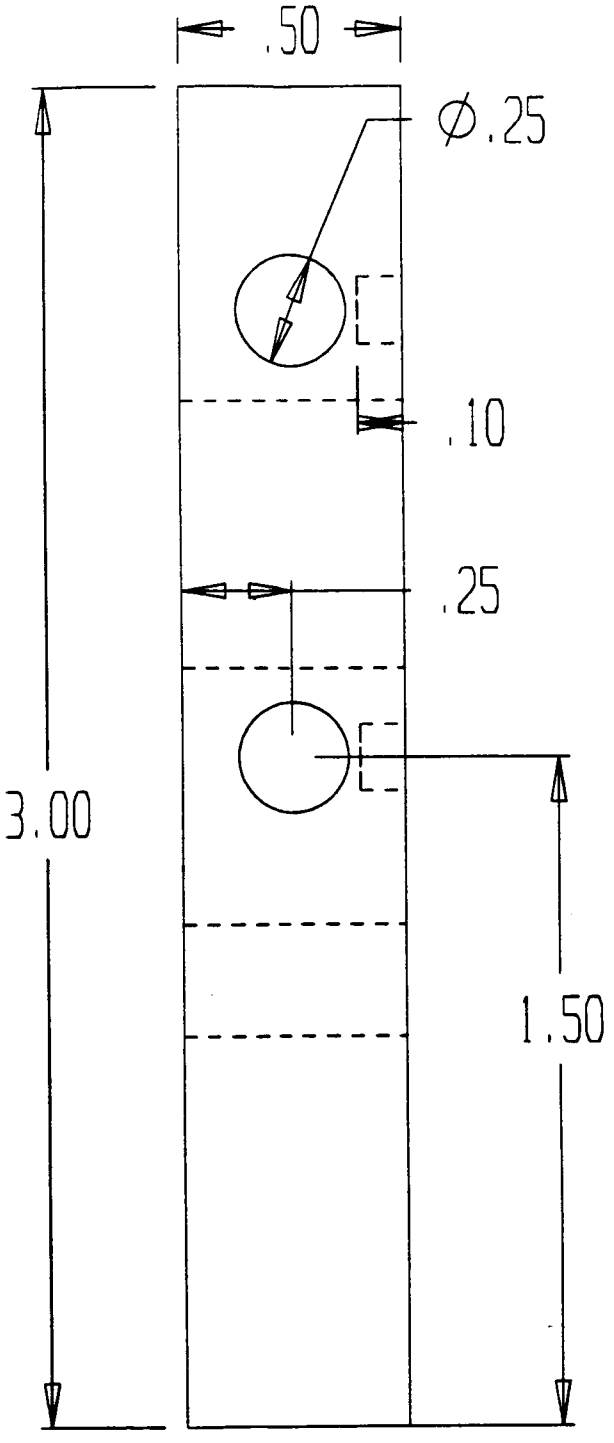

Figure 3.18 Segment 1 - Machine Drawing - Side View

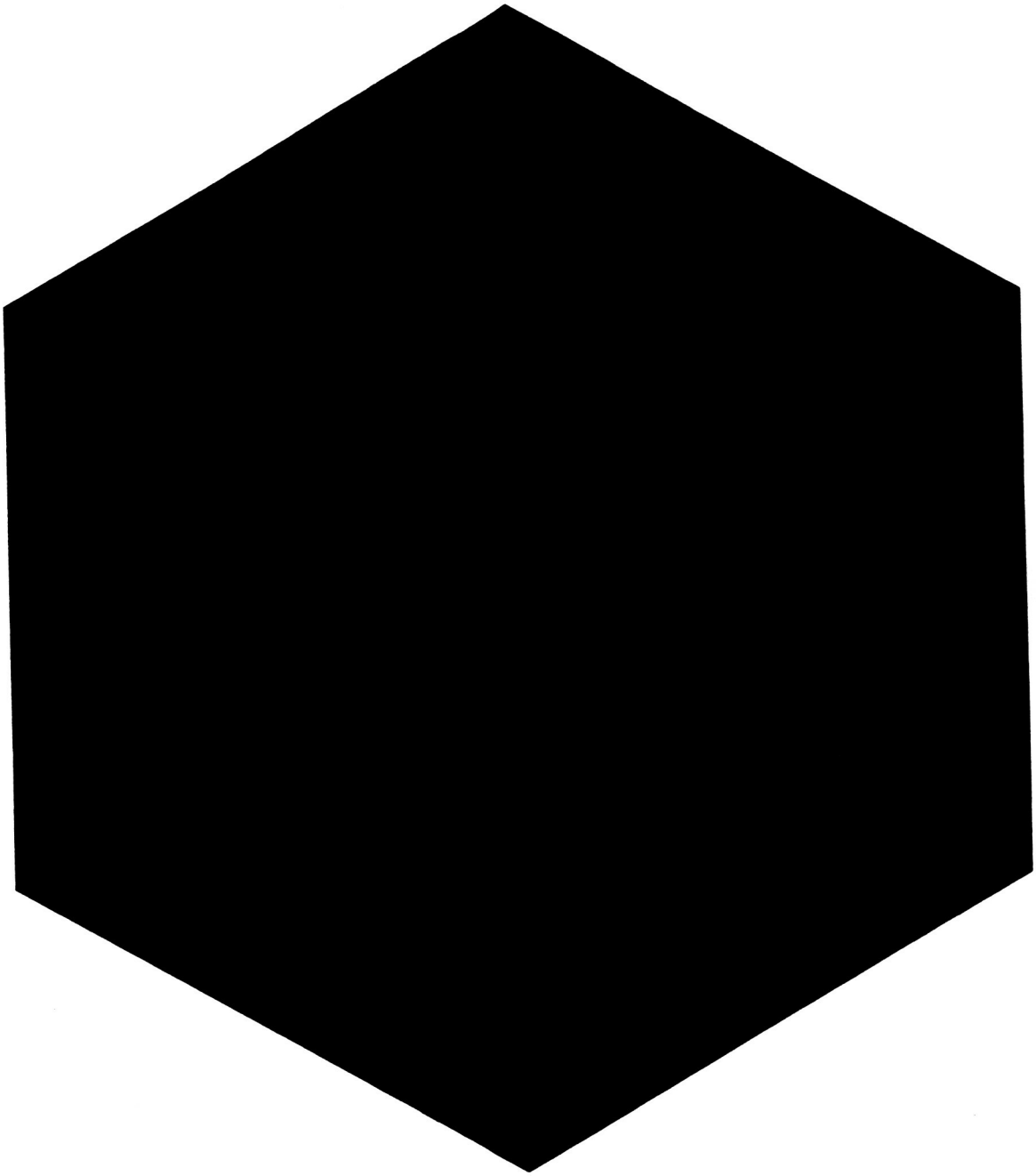

Figure 3.19 Segment 2 - Solid Rendering

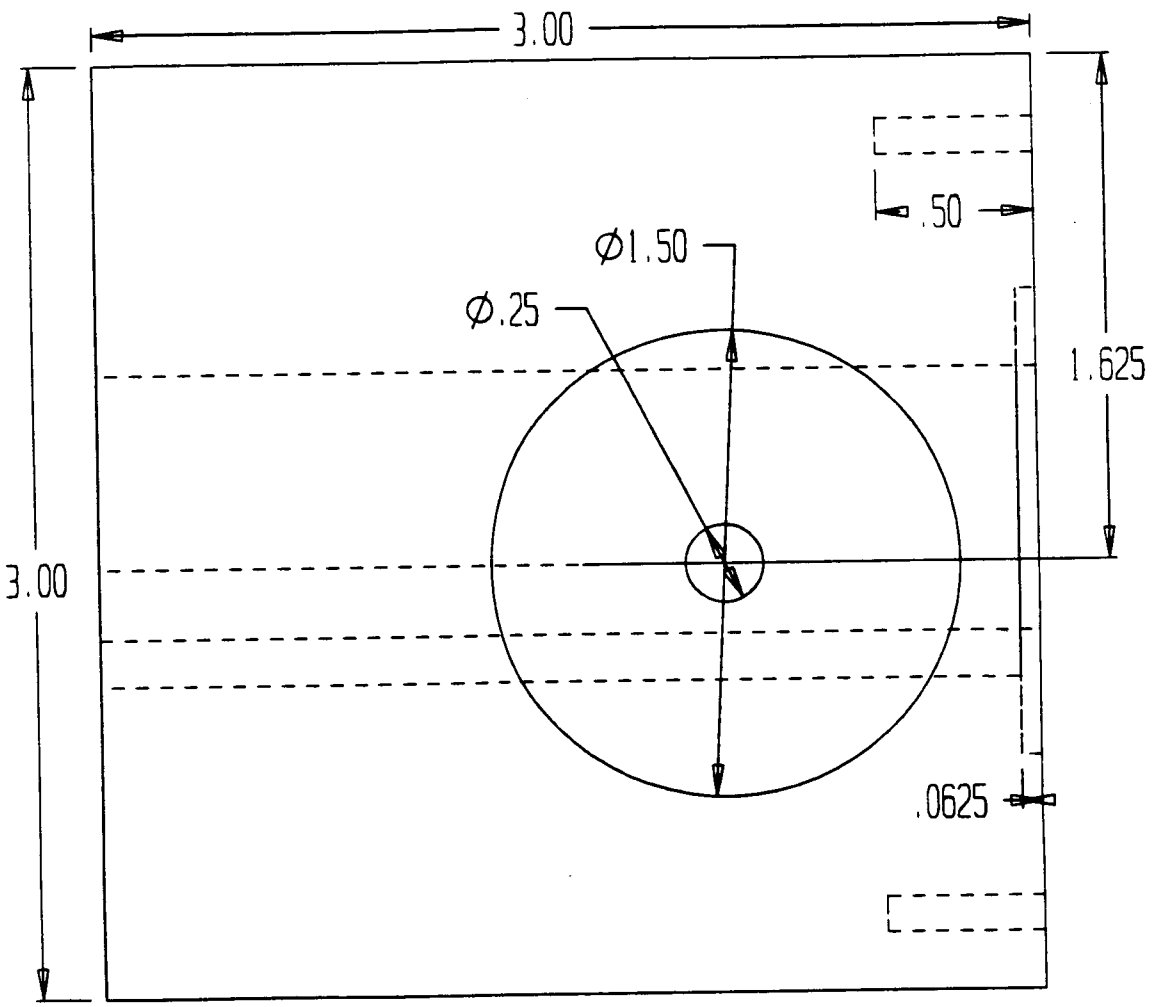

Figure 3.20 Segment 2 - Machine Drawing - Bottom View

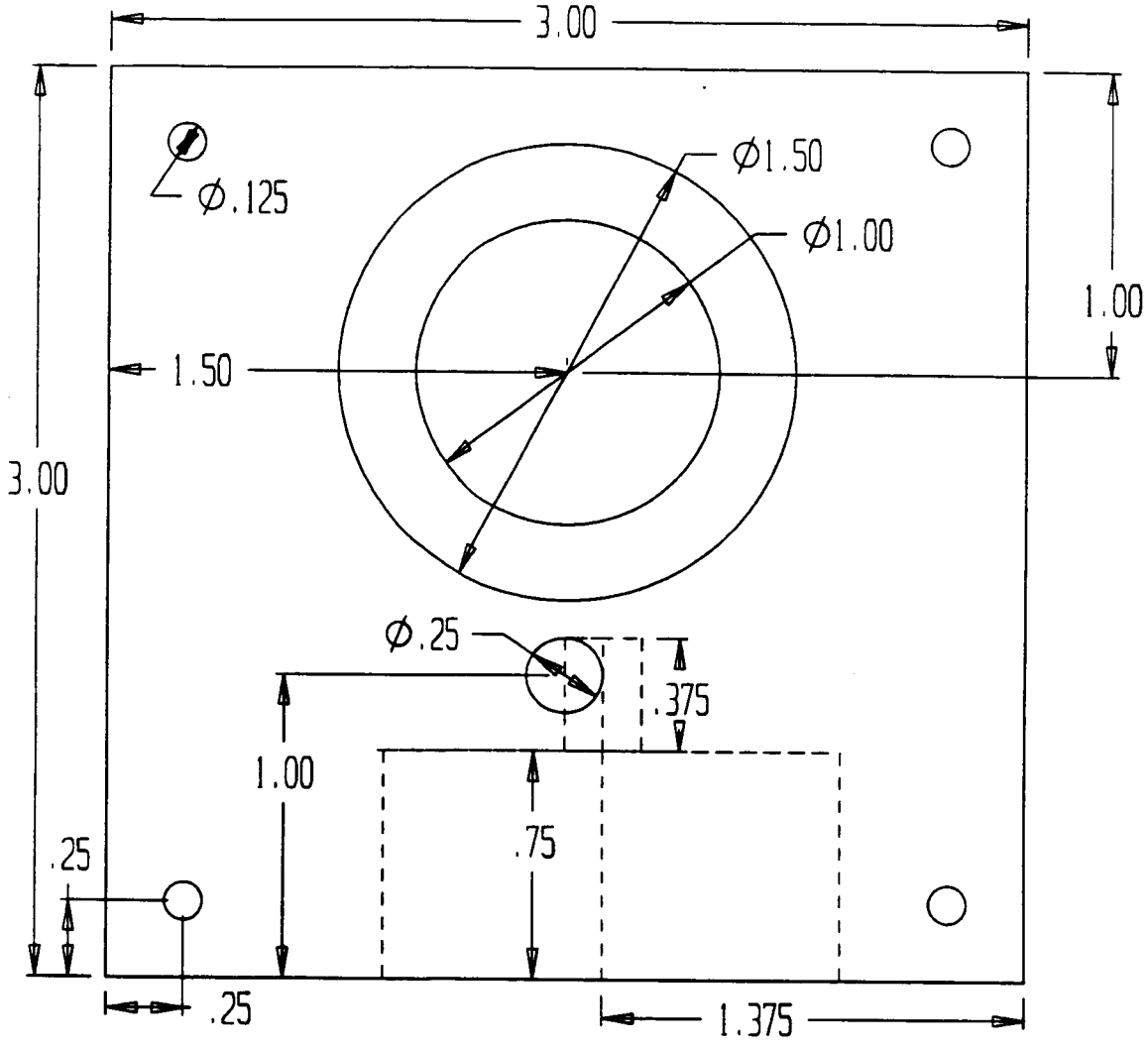

Figure 3.21 Segment 2 - Machine Drawing - Front View

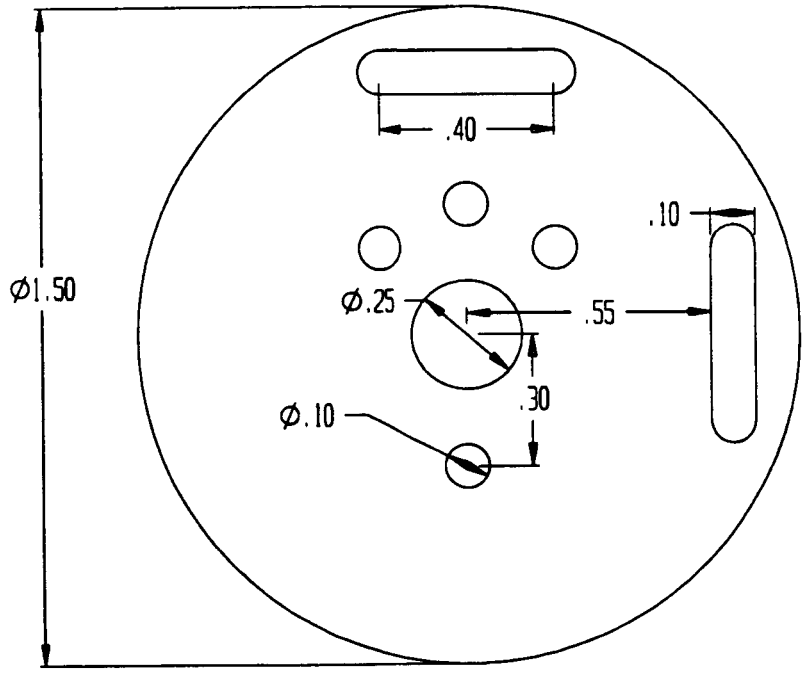

Figure 3.22 Motor Mounting Bracket - Machine Drawing

3.8.2 Bill of Materials

End Effector				
Part Number	**Quantity**	**Price Each ($)**	**Vendor**	**Description**
36792	1	63.00	Zymark	Hand Connector Housing
39591	1	80.00	Zymark	Wire Harness
36831	1	25.00	Zymark	Circuit Board
2225T006S	2	120.00	MicroMo	DC Servo Motor
22/2	1	65.60	MicroMo	308:1 Gearhead
22/2	1	69.00	MicroMo	548:1 Gearhead
G5-21	1	-	PIC Design	Pinion Gear
AG-19	18"	68.82	PIC Design	Fine Pitch Rack
OTS	3"x3"x4"	-	-	Plexiglass for Housing
OTS	12"	-	-	1/4" Al Stock for Arms
Sensing System				
Part Number	**Quantity**	**Price Each ($)**	**Vendor**	**Description**
G38,303	144	199.00	Edmund Scientific	Image Conduit
G52,347	144	570.00	Edmund Scientific	Microscope-180X
G52,171	144	2,975.00	Edmund Scientific	CCD Micro Camera
Control System				
Part Number	**Quantity**	**Price Each ($)**	**Vendor**	**Description**
DT2687-60Hz	1	5,495.00	Data Translation	Image Processor
Versa 486/75 C	1	5,000.00	NEC	Laptop Computer (requires modification)
OTS	1	1,296.00	National Instrument	Labview 3.01 Software
OTS	1	1,000.00	Microsoft Co.	Visual C++ Development Kit
OTS	1	298.00	Microsoft Co.	Windows NT
SP0580-CL	1	3,495.00	Data Translation	Global Lab Image Development
PC-PA2014U	1	1,349.00	L.A. Trade	Toshiba 16MB RAM Upgrade
6M40U	1	1,200.00	Sharp	6 in. NTSC Actrive Matrix Monitor
OTS	1	1,379.00	IBM	15 in. Touch Screen CRT
OTS	1	N/A	Sharp	16 in. NTSC Monitor
Pantera 90	1	5,512.00	Zeos	Pentium 90 MHz Desktop computer (32 MB RAM, 2 GB HD)
N/A	2	N/A		Optical Storage Device