



Design of an Automated Device to Prepare *Drosophila melanogaster* for Image Analysis

Submitted By
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Abstract

The Tufts University Robotics Academy was approached by researchers at the New England Medical Center to automate a time and labor intensive procedure that quantifies food intake by *Drosophila Melanogaster* (fruit fly) larvae. The NEMC researchers must screen over 25,000 mutant *Drosophila* lines, and at the screening rate of 20 lines per day with the current manual assay, it would take 5-6 years of full-time labor to complete the screening [1]. However, a more reasonable completion time is now a reality as the Robotics Academy team successfully designed a semi-automated system enabling high throughput screening of 16 mutant fly lines in only 45 minutes.

The semi-automated screening system isolates and prepares fruit fly larvae for accurate identification of food intake via larval fluorescent emission. A plate of 16 separate vials of mutant fly lines in the third-instar larval stage are inserted into the stand-alone dispensing rotation mechanism, and over a time period of 45 minutes, the larvae are fed, cleansed, and imaged. In chronological detail, the automated device is calibrated to dispense a precise amount of food and fluorescein dye in each larval vial, allow the larvae to feed for a given amount of time, thoroughly remove the food, drain all excess liquid, and then optically quantify food consumption of each fly line. The system provides a controlled and adequate living environment for the larvae, and is transportable and reconfigurable for adaptation to other applications requiring precise positioning of living specimen under liquid dispensing stations.

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I. Introduction

In industry, it is common practice for manufacturing companies to implement time and labor efficient automated solutions within their assembly productions [15]. For example, by combining mechanical and electrical components through a computerized user-interface, a job that previously required 20 tedious labor hours by 20 individuals could be reduced to 4 monitoring hours by only 2 individuals. In addition to the reduction in time and labor, margin of error and cost are effectively reduced. However, a stand-alone automated assembly system is appropriate for applications beyond the industrial manufacturing environment. For example, the tedious genetic research procedures performed at the Molecular Pharmacology Research Center (MPRC) of T-NEMC required an automated solution. Consequently, the Tufts Robotics Academy team of 2004-05 designed and fabricated a semi-automated system to facilitate efficient genetic research processes.

While the ultimate purpose of research at MPRC is to identify functionally important homologous genes within mammals, the actual experiments are performed with the simplified model organisms of *Drosophila* larvae. The ability to genetically manipulate larvae to carry a precisely mapped single gene insertion facilitates identification of novel genes regulating food intake. It is predicted that through the screening of 25,000 available mutant fly lines, a subset of genes regulating feeding behavior as well as associated metabolic function will be discovered [11]. However, accurate screening of such a large number of lines would take years to complete, and thus an automated system to screen the fly lines in a timely manner is necessary.

II. Background

Drosophila larvae as model organisms in research of abnormal feeding behaviors

Drosophila in the third instar (L3) larval stage were specifically selected for genetic research because the two primary physiological processes during this life cycle stage include feeding and locomotor activity [2]. Furthermore, the molecular mechanisms controlling these serotonin mediated functions have been conserved from insects to humans, thus justifying the fruit fly as a tool for identifying additional genes with corresponding mammalian homologs that modulate feeding behavior and associated metabolic functions [4]. Initial research efforts to identify the role of serotonin receptors in the control of feeding behavior led to the development of a highly sensitive assay that accurately quantified food intake of L3 fly larvae [11].

Establishment of a colorimetric food intake assay

Food intake measurements from the assay are based on optical density readings of ingested fast green dye. This non-absorbable dye was selected as an indicator of food intake due to its sharp optical density peak and pH insensitivity. To develop the assay, researchers identified and standardized variables that could potentially influence food intake. These variables include genetic background, breeding conditions, density of larvae in feeding environment, composition of larval food, and temperature and time of day at which the assay was performed [11]. Consequently, the assay was always conducted in the morning at 25°C and only eight larvae of one mutant line were tested at one time. To begin the assay, L3 larvae were manually removed from their hatching vials and placed into a food/dye mixture. This mixture included *Drosophila* Instant Food

(Carolina Biological Supply Co.) base with 10% Brewer's inactive yeast (ICN Biomedical) and 0.2% fast green dye. After allowing the eight larvae to feed, fully submersed, in the food/dye mixture for 30 minutes, they were manually removed, cleansed, and lysed. Finally, an optical density (OD) measurement of the homogenate at the maximum emission wavelength of the dye (625nm) was taken. A higher OD reading indicated a greater quantity of food consumed by the corresponding mutant fly line, and abnormally high or low OD readings were relevant to the scope of the research [11].

Development of an alternant method for quantifying food intake

In the summer of 2004, the NEMC research team approached the Robotics Academy to develop an automated solution to expedite the current assay and produce comparable results. One of the team mechanical engineers immediately began research, and over the course of three months, optimized an alternative method to assess larvae food consumption. By replacing the fast green dye in the larval food with an equivalent quantity of fluorescein dye, food intake could be quantified without actually crushing the larvae, which was required by the original assay. The transparent bodies of larvae enabled live larvae visualization of food consumption after a 30 minute feeding period (*Figure 1*). Ultraviolet illumination of the larvae within an enclosed, dark environment facilitated image processing for quantifiable results. The brightness of each larva exhibited a linear relationship to food intake. The inexpensive and non-toxic fluorescein is ideal for image processing because it emits at a different wavelength than its excitation wavelength, thus allowing for the excitation light to be filtered, leaving only the dye's spectrum for analysis. Preliminary results from the fluorescein assay exhibited potential to mirror the results of the current colorimetric assay. Controlling variables such as light

emission, dye type, feeding time, and camera magnification would optimize the new assay to produce comparable optical density assay results.

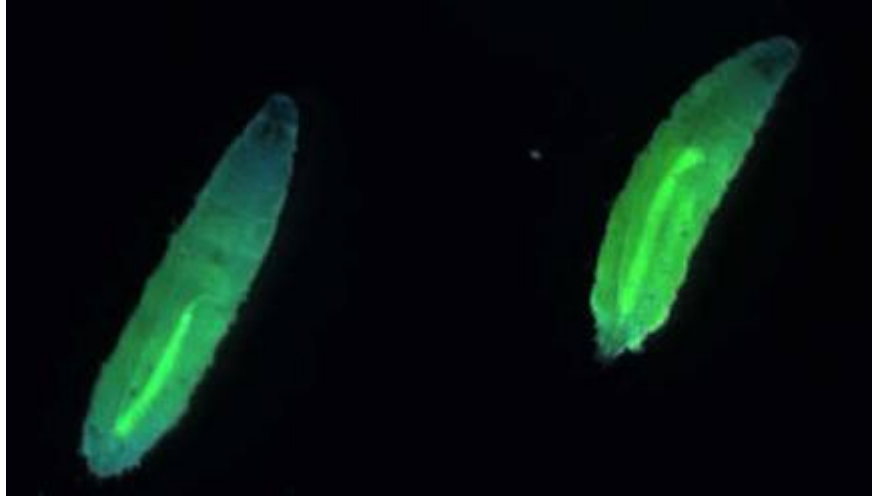


Figure 1: Fluorescent emission quantifies food intake of L3 larvae

Upon establishment of fluorescent quantification of food consumption in L3 larvae, the challenge of expediting larval preparation processes remained. To ensure accurate image processing, feeding and cleansing of a small number of isolated larvae is required, similar to the processes characteristic of the optical density assay.

Unfortunately these preparation processes are time consuming and labor intensive due to the miniscule size of larvae, which are approximately 3 mm in length. The eight step process is detailed in Appendix B, but in general each larva must be manually removed from the food/dye mixture with a fine tipped brush and thoroughly cleansed of external particles that could potentially contaminate results. A device to minimize the time and manual labor required to prepare each fly larvae line for image analysis would need to be designed in order for the new fluorescent emission assay to be relevant and useful for genetic research at the MPRC.

III. Project Overview

Project Goal

The Tufts Robotics Academy Team's goal was to design, prototype, and manufacture a robotic system to efficiently quantify the amount of food consumed by *Drosophila* larvae. The team, comprised of two mechanical engineers, an electrical engineer, and an engineering psychologist, integrated talent from three separate engineering fields to produce an operable system. The system consisted of two subsystems: (1) feeding, cleansing, and separation of larvae specimen, and (2) image processing and data analysis. Each mechanical engineer was responsible for the respective subsystem. The integration of the two subsystems required the expertise of the electrical and psychology engineers to design the system actuation and user-interface, respectively.

The scope of this document covers the design and fabrication of an automated subsystem to produce *Drosophila* larvae that are isolated, fed, and clean for accurate image analysis. This subsystem was then integrated with the image processing subsystem to complete a semi-automated machine that efficiently prepares and screens *Drosophila* larvae.

Project Specifications

A computerized mechanical apparatus was designed to accommodate the needs of the current NEMC genetic research project of quantifying food intake in mutant fly lines; however, the system was intended to be versatile enough to efficiently assist future research projects that involve general screening of *Drosophila* larvae. Initial system calibration prepared larvae for fluorescent quantification of food consumption. Upon the manual insertion of third instar larvae in a water-soluble food medium, an automated device (1) washed larvae from the initial food, (2) fed larvae with a fresh food and fluorescein dye mixture, (3) thoroughly washed larvae from the second food, and (4) captured high-resolution images of glowing larvae for data analysis.

Project Design Requirements

Due to the scope of the automated fruit fly analysis system, each subsystem and their respective components are graphically organized in *Chart 1*. The input subsystem of feeding and cleansing fly larvae was comprised of (1) specimen well-plate; (2) rotation mechanism; (3) feeding unit; (4) washing unit; (5) drainage system; and (6) PC control program. The output subsystem of data collection and analysis was comprised of (1) high resolution camera; (2) uniform UV light emitter; (3) stationary light fixture; and (4) PC control program.

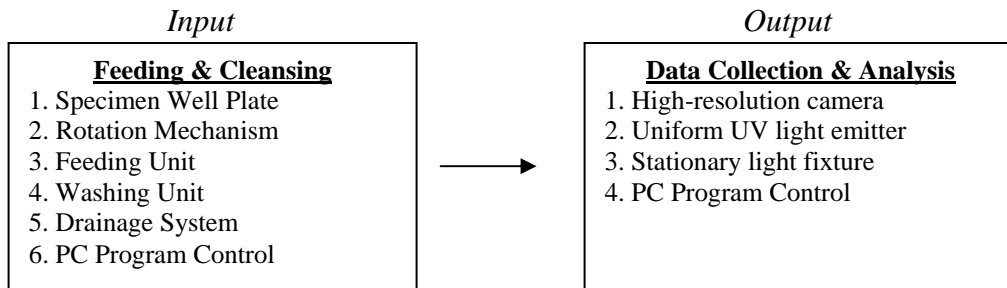


Chart 1: Organization of Automated Fruit Fly Analysis System

The design requirements for the entire automated system are

1. maintain an adequate, stress-free living environment for fruit fly larvae,
2. reconfigurable for application to any fruit fly research analysis,
3. reduce container transfer and handling,
4. reduce time required to feed and cleanse larvae,
5. exhibit reproducibility and reliability for daily operation and consistent results,
6. allow for setup on a single lab table, and be self-contained, and
7. controllable through a standard PC with a user-friendly computer interface.

IV. Input Subsystem Initial Design

Introduction

The input subsystem is required to autonomously perform the following three tasks:

- (1) Cleansing the larvae entirely from the initial food medium;
- (2) Depositing new food medium for controlled feeding time;
- (3) Cleansing the fed larvae entirely of the secondary food medium.

Brainstorming of the input subsystem mechanical design focused on the specimen output required for proper image analysis. The Robotics Academy team had already established fluorescent image analysis as a viable method of quantifying food consumption, and this method mandated the larvae to be fully fed, cleansed, and isolated. Isolation of clean larvae was one of the most challenging design constraints because of the existing research lab hatchery conditions. Hundreds of larvae, confined within a single 2.4 cm (1-in) diameter vial, fed on partially water-soluble food and subsequently developed to the third instar stage after an approximate thirty-six hour time period (*Figure 2*). Within the vial, however, it was often difficult to differentiate the L3 stage

larvae from slightly slower developing larvae. In addition, the thick consistency of the food made it difficult to easily obtain a single clean larva for imaging.

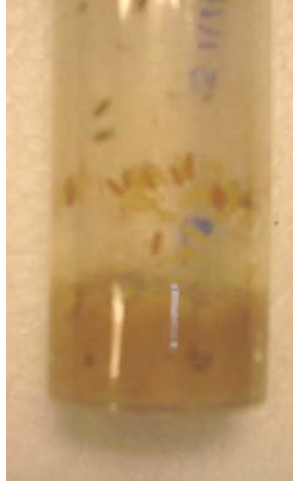


Figure 2: Initial hatchery conditions of third instar larvae

Hence the first goal of the input subsystem design was to identify a method of isolating a small number of the micro-sized larvae (*Figure 3*) at the third instar stage for proper image analysis, all the while maintaining an adequate living environment.



Figure 3: Drosophila larvae are approximately 3 mm long and 1.5 mm in diameter

Food Selection

After background research and discussion with NEMC biologists, the Robotics Academy team determined that the least stress inducing means to isolate clean fly larvae was through washing. This decision led to the need for a new water-soluble food medium. Fruit fly larvae generally feed on many different types of media, but the media

always contains yeast to prevent mold. Therefore, pure water-soluble yeast was selected and tested as a new food medium for two reasons: (1) Larvae like to feed on yeast, and (2) Elimination of non-soluble substances inherent to the previous medium. Brewer's inactive yeast, a dry powder in standard form, with the addition of water in a 2:1 mixing ratio, results in a soupy solution with viscosity similar to that of chocolate syrup. The biologists performed tests to verify that the fruit fly eggs and larvae would maintain standard development and feeding patterns with the selected yeast solution, and test results were favorable: larvae consumed a comparable amount of food in the 30-minute feeding period with the yeast-only solution as they did in the original fortified yeast solution. However, in line with maintaining an adequate living environment, the yeast-only solution should not deviate from the 2:1 ratio so as to avoid drowned larvae.

Specimen Container Selection

With a water-soluble media identified, the next step was to select an appropriately-sized mesh large enough for yeast particles to wash through, yet small enough to contain the larvae at their narrowest dimension or body diameter. After experimentation, stainless steel strainer grade woven wire cloth with 0.015 cm (0.006-in) diameter wire was selected as the optimum mesh type. The non-rusting and non-absorbing material properties of stainless steel allowed the mesh to be unaffected by the excessive exposure to water and fluorescein dye. Because the largest yeast particles were approximately the diameter of the third instar larvae, the dry yeast powder was sifted through a screen of the selected mesh before mixing the solution. The pre-sifting resulted in a more complete wash. *Figure 4* verifies the wire mesh size selection at the microscopic level.

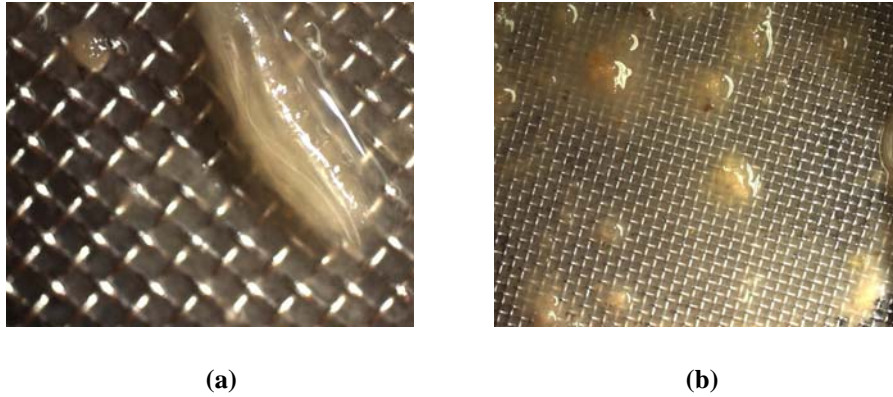


Figure 4: Microscopic image of mesh size as compared to (a) larva size, and (b) food particles

The 2:1 yeast solution exhibited unique properties of high surface tension and syrup-like viscosity, which allowed the solution to remain entirely suspended on the mesh until the addition of more water diluted the yeast completely and drained through the mesh apertures (*Figure 5*). As a result, the handling and transfer of larvae was eliminated as they could remain on the mesh through both feeding and washing.



Figure 5: Pure yeast solution exhibited appropriate viscosity to remain on top of wire mesh

Wash Method

The next step in the system design was identification of an optimal washing technique that (1) imposed the least amount of stress on the larvae, (2) required the least amount of time, and (3) diluted and drained the yeast solution entirely from the mesh,

leaving only clean and fully fed larvae. Two different washing techniques were tested: (1) dunking, and (2) flushing. The first washing prototype design, constructed out of Lego bricks (*Figure 6*), vertically submerged a mesh-bottomed container of larvae in a water bath, oscillated horizontally for a variable amount of time, and then emerged from the bath. Unfortunately, the dunking method did not impose enough wash pressure on larval vial to sufficiently cleanse the specimen in a timely manner, and thus the dunk wash method was discarded.

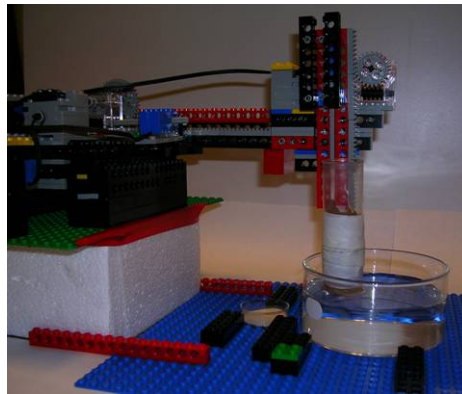


Figure 6: Prototype of dunking wash method

The second washing method of flushing was tested with a standard sink water faucet. The combination of faucet back pressure and a gravity driven water stream sufficiently washed the yeast media through the wire mesh cloth (*Figure 7*). Upon finalizing a water-soluble food media with a feasible washing method through wire mesh, automation of the feeding and washing processes ensued.



Figure 7: The flush wash method thoroughly cleansed wire mesh of yeast solution

Prototype I: Proof of Concept

The first task in designing the automated feeding and cleansing system involved identifying the moving components and selecting the optimal method of motion. Existing laboratory robotic dispensing systems use either linear or rotary motion of specimen and stationary or moving dispense arms. Evaluating the pros and cons of various designs, a central rotary system with stationary dispense arms was determined as the most compact, inexpensive, and time efficient design solution.

To test the central rotation and stationary dispense arm concept, a plywood prototype was constructed (*Figure 8*). The overall concept proved successful, with only a few minor changes of individual components to be made in the construction of the final system.

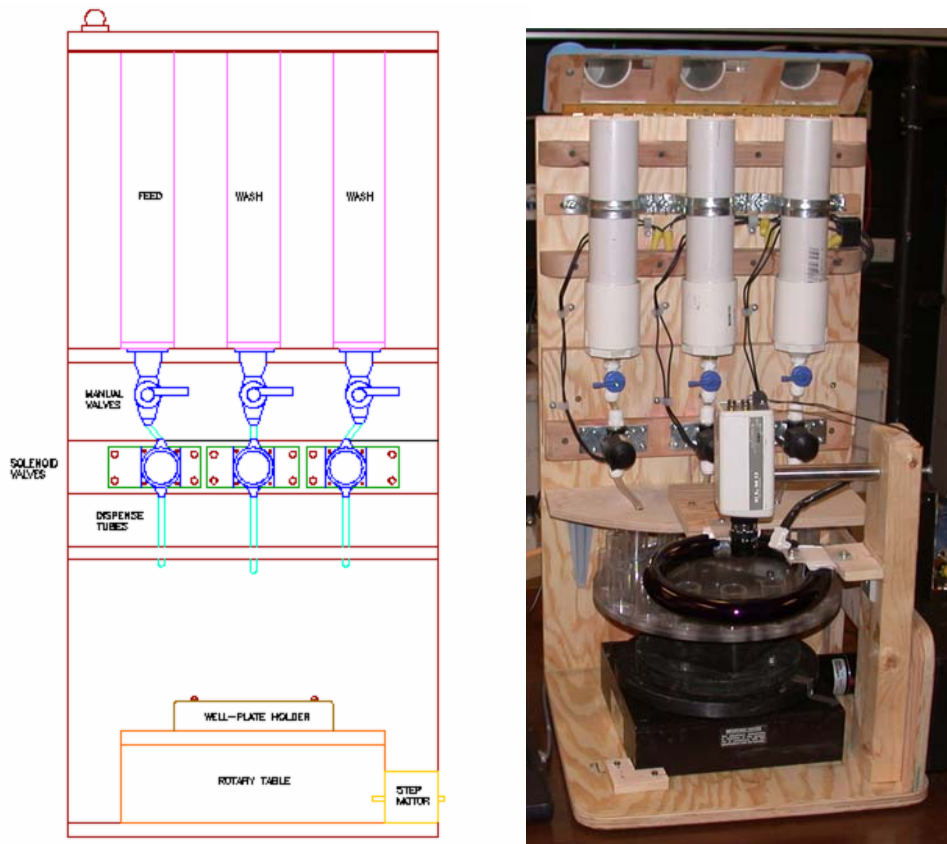


Figure 8: Prototype of the automated cleansing and feeding system

The layout of the final design was based on the prototype, with a central circular specimen well-plate rotating beneath four separate stations along the outer perimeter: two water washing, one yeast feeding, and an image capturing. The rotation transfer method allowed feeding, washing, and imaging to be executed simultaneously within one rotation of a well-plate, thus minimizing the total amount of time required to complete one automation sequence.

V. Final System Design

Overall Design

With the overall central motion and stationary dispensing concepts verified by the prototype, modeling and manufacturing of the final system began. The complete skeleton structure of the system was constructed of 80/20 aluminum frame units (*Figure 9*). This material, commonly referred to as the industrial erector set, exhibited favorable characteristics such as high strength-to-weight ratio, ease of assembly and disassembly, and affordability. By creating a frame-only system with minimal solid material, the weight was maintained at a transportable level.



Figure 9: 80/20 Aluminum framing structure used in the final design

The rotating specimen plate was designed central to the system, and the remaining components were arranged accordingly around the rotation unit (*Figure 10*). In determining the placement of these components, compactness and ease of attachment to the 80/20 frame were considered. A left compartment was constructed to house components not needed within immediate proximity to the rotating well plate. These components included the yeast solution container, wires, and control board. On the other hand, the wash and food dispensing stations needed to be directly above the rotating specimen, and such was the justification for their placement at the top back of the main compartment. The back wall dispense stations were positioned to avoid standard system operation interference, yet remain accessible.



Figure 10: Overall final design with separated compartments

To ensure accurate alignment and spacing of each component prior to fabrication in the machine shop, a 3-D model was constructed (*Figure 11*). While the wood prototype proved the feasibility of general system operations, the 3-D model provided the hardware alignment details for manufactured components.

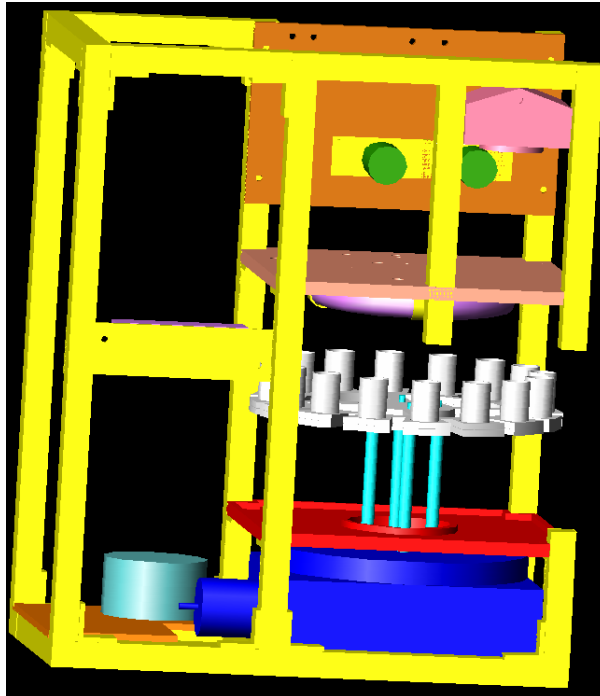


Figure 11: 3-D Model of Final Design

In tandem with developing the 3-D model was that of material selection. All mount and cover plates were constructed from only three different materials, including aluminum, Delrin, a smooth, highly wear-resistant plastic, and polycarbonate, a clear, high impact-resistant plastic. For convenience, the mounting method was consistent for all components, and utilized the 80/20 corner brackets with $\frac{1}{4}$ -20 UNC thread socket cap screws (*Figure 12*).

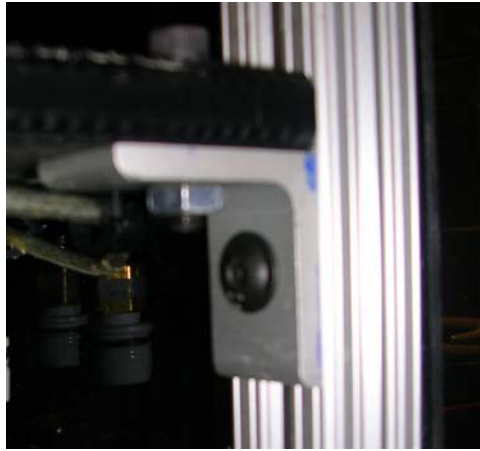


Figure 12: 80/20 mount brackets utilized throughout entire structure

The specification and design justification of each system component (*Figure 13*) is separately detailed under the following subheadings:

- A. Specimen Well Plate
- B. Rotation Mechanism
- C. Wash Dispense Unit
- D. Food Dispense Unit
- E. Drainage System
- F. Aesthetics & Usability

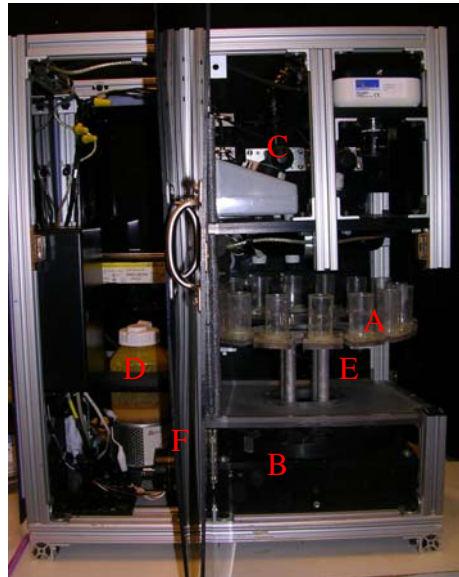


Figure 13: Location of each component in the entire automated system

A. Specimen Well Plate

In designing the specimen holding container for rotation, requirements of usability, maintainability, manufacturability, and adequate living conditions for the larvae were all considered. To meet high throughput requirements, a 30.48 cm (12-in) disk with 2.54 cm (1-in) diameter wells evenly spaced at 22.5° increments around the

circumference was fabricated. The well diameter was selected as a sufficiently sized environment in which ten fruit fly larvae could comfortably feed. The disk itself consisted of three layers: two 0.64 cm (1/4-in) clear polycarbonate plates, sandwiching the previously selected stainless steel mesh. Both the plastic and wire materials of the well-plate satisfied the non-absorbing, non-rusting, and non-warping requirements for repeated use. AutoCAD drawings of the two polycarbonate sheets were submitted to the CNC machine for fabrication (*Figure 14*).

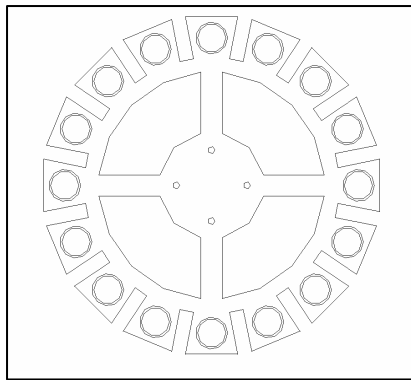


Figure 14: AutoCAD drawing of specimen well-plate for CNC fabrication

The spoke and gear-like shape of the well plate was designed after experimenting with a solid-surface prototype plate. Due to the drips from the dispense stations that occurred during system operation, all unnecessary plate material between wells was removed to allow drips to fall through the plate and into the drainage system. Likewise, material was removed from the plate center to eliminate the formation of surface liquid pools (*Figure 15*).



Figure 15: Removal of excess material from well plate to eliminate unwanted liquid pools

Unfortunately, the 1.27 cm (½-in) depth wells created by the sandwiched plates alone was not deep enough to contain the fruit fly larvae through the feeding and washing processes, and thus height needed to be added to each well. In experiments prior to adding height, the larvae floated out of each well. For convenience and cost reduction, the clear plastic 2.54 cm (1-in) diameter vials used in the NEMC research lab were modified and attached to the well-plate. The enclosed end of each vial was sliced, removed, and the resulting open cylinder, 5.08 cm (2-in) high, was glued to each well to provide the required additional height (*Figure 16*).

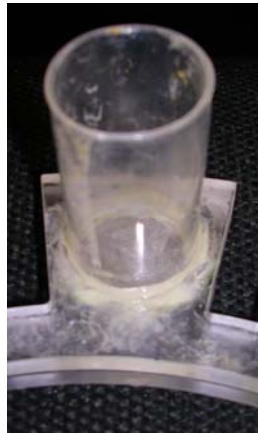


Figure 16: Utilization of existing NEMC vials with adequate height to contain the larvae

Four locating holes in the plate center allowed for quick and convenient mount and dismount to vertical aluminum rods on the rotary table (*Figure 17*). This characteristic was essential as the overall system was designed to enable the use of many well-plates in succession. After one plate of 16 different fly lines were fed, washed, and imaged by the automated system, an entire new set of 16 on an identical plate could immediately be inserted into the machine while the first plate was cleaned and prepared for another test set.

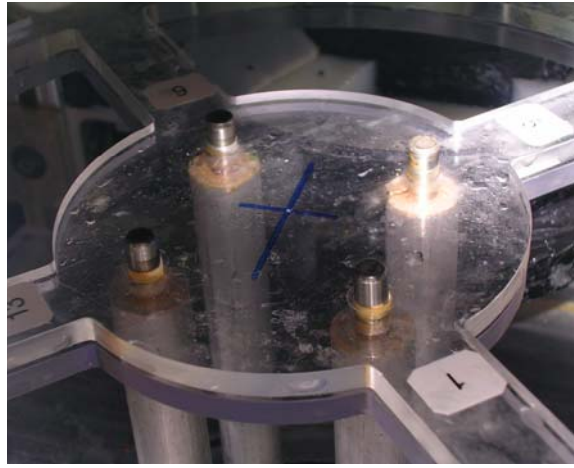


Figure 17: Mounting of well plate to rotary unit via four locating holes

Two-part epoxy was used to bind each vial and circular wire mesh cutout to the polycarbonate plates. The epoxy around the perimeter of each well also acted as a seal to prevent migrating specimen between wells (*Figure 18*). The 1.5 mm (0.06 in) diameter larvae could easily float through the 2.5 mm (0.10-in) gap created by the two sandwiched plates and potentially contaminate test results.



Figure 18: Sealed wells prevent migrating larvae within well plate gap

It should be noted that the well plate itself is light, stackable, easy to wash and reuse, and easy to mass produce with a CNC, laser cutter, or equivalent computer automated machine.

B. Rotation Mechanism

To ensure both accurate and precise positioning of the rotating well plate, a 25.4 cm (10-in) diameter precision worm gear rotary positioning table was selected (*Figure 19*). The 6.8 kg (15-lb) aluminum alloy table was not designed for high torque or high inertia applications, and thus remained within an affordable price range. The turntable maintained a low profile with a height of 8.89 cm (3.5-in) and a footprint of 645.16 cm² (100-in²), and conveniently mounted in four corners to the 80/20 30.48 cm (12-in) x 30.48 cm (12 in) base frame.



Figure 19: Rotary positioning table is the central system component

The center of the rotary table contained four 1/4-20 UNC thread mount holes for attachment of the well-plate aluminum support rods. The 12.7 cm (5-in) high, 1.59 cm (5/8-in) diameter rods were press-fitted with 0.635 cm (1/4-in) diameter steel dowel pins to which the well-plate was mounted (*Figure 20*). The steel pins, more durable than the aluminum rods, were particularly selected to endure repeated system use, involving mount and dismount of the well-plate. Finally, the slender, light-weight aluminum rods

raised the well-plate high enough above the rotary table to allow space for an adequate drainage system.

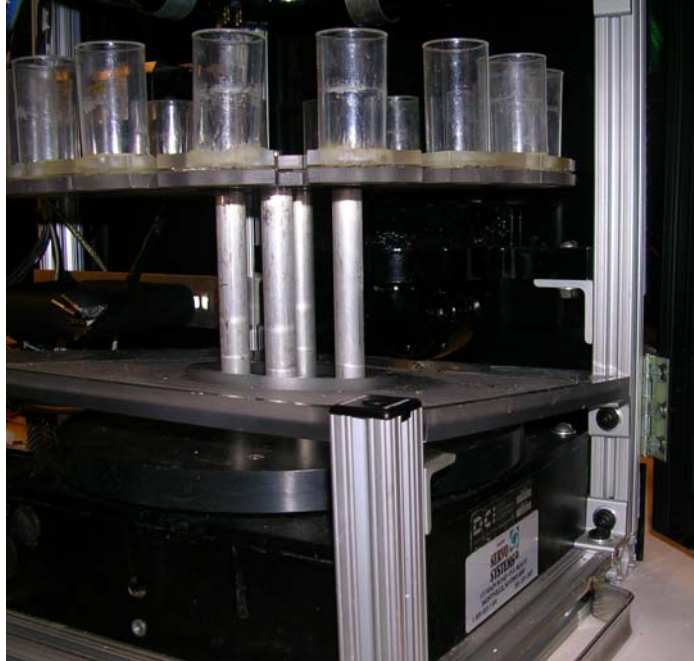


Figure 20: Aluminum rods threaded to rotary table facilitate convenient mount of well-plate

The turntable precision worm gear specification (90:1 ratio) ensured very little backlash during table operation, and could therefore be set into motion by a low-voltage stepper motor. Benefits of using a stepper motor as the turntable actuator are that no tuning was required, no encoder was necessary, and the highest torque existed at the lowest speed. In addition, the stepper motor's holding torque could hold the turntable's position in between rotation increments. This characteristic eliminated the need for a separate mechanical break. The stepper motor selected for the system was 2-phase, high resolution (0.9 degrees/phase) with an increased resolution from 200 steps/revolution of standard motors to 400 steps/revolution (*Figure 21*). The increase in resolution increased angle accuracy within the stepper motor, thus improving accuracy of turntable positioning. However, the limitation inherent to using a stepper motor is the assumption

that no steps would be skipped during operation and the actual rotary table position corresponded to the ideal position maintained by the controller.



Figure 21: Stepper motor selected for actuation of rotary table.

To control the stepper motor, the team electrical engineer constructed a micro controller board to connect to the serial port of the system PC (*Figure 22*). This controller board was multipurpose and also controlled the system dispensing components, (the wiring diagram is included in Appendix D). Sub VI's corresponding to each output of the controller board were individually programmed in LabVIEW, and then integrated into the final control program, which is discussed further in PC Program Control & Operation. LabVIEW was used as the main program because it was easy to program and design an effective user-interface.

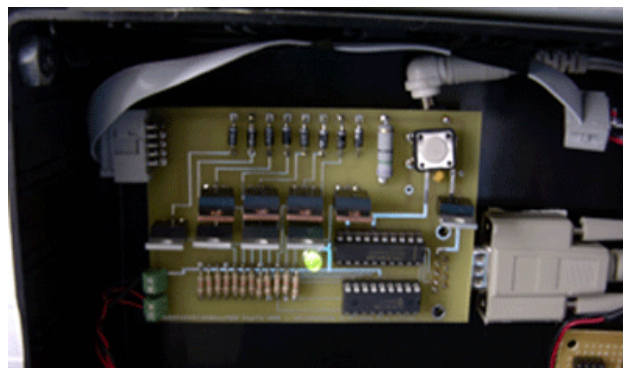


Figure 22: Multi-output controller board controls the stepper motor for well plate rotation.

C. Wash Dispense Unit

Before designing the wash dispense stations, an optimum volume flow rate and pressure corresponding to an adequate wash was researched. The definition of an adequate wash was such that all food particles were thoroughly flushed through the mesh-bottomed well, leaving only clean larvae within the vial. During testing, a sample vial was filled with 3.2 mL of yeast solution, and the amount of time required for a varied diameter water stream to thoroughly clean the vial of yeast particles was recorded. Overflow occurred if the faucet was turned on at full force because the yeast solution clotted from increased pressure. After several trials, the volume flow rate that maximized yeast flushing was calculated at 90 mL/sec for 5 seconds with a 1.59 cm (5/16-in) diameter water stream. Upon the establishment of washing requirements, the design of dispense method ensued.

Based on the water stream initially tested, 0.634 cm (1/4-in) dispense tubing was chosen for convenient purchasing and replacement. The next step in system design was determining the source of wash water, either from a liquid reservoir or directly from the faucet. The prototype system used a liquid reservoir design with shut off valves (*Figure 23*), but two problems resulted from this design. First, the size of the liquid reservoir would need to be unreasonably large, (14.4 Liters based on washing requirements), to accommodate the two washings of just one well-plate without refilling. The 2-liter PVC pipe reservoirs used in the prototype were certainly not sufficient and required refilling more than once during a single trial run. The prototype washing system required constant monitoring, and thus countered the original design goal of minimizing labor.



Figure 23: Prototype washing stations with refillable liquid reservoirs & shutoff valves

Secondly, without a pump, dispensing of the wash water with shutoff valves relied solely on hydrostatic pressure, which decreased during system operation, and thus caused a decrease in volume flow rate with the washing of each additional well. This decrease in volume flow rate resulted in partially washed wells in the desired 5-second time period. Adequate washing would require more time, thus countering the original design goal of minimizing time.

The liquid reservoirs were discarded in the final system design, and the shutoff valves were directly connected to a faucet. The faucet connection eliminated the two design flaws of the prototype. Now, with a continuous water source and back pressure, no refilling was necessary and the volume flow rate remained consistent to ensure system repeatability and efficiency.

For ease of control, reproducibility, and accuracy, Delrin direct-acting solenoid valves (12 VDC) were selected as the wash shutoff valves (*Figure 24*). Solenoid valves are simple in design and operation and thus ideal for the washing unit. The valve plug is

held in place by a spring, and when electric power is applied to the solenoid, the current generates an electromagnetic force opposing the spring, causing the plug to open and fluid to flow. When the valve solenoid is de-energized, the spring returns the plug to the normal closed position. Operation with high/low signals allowed compatibility with the existing computer control of the stepper motor and rotary table setup, and two additional outputs were integrated into the micro-controller board. As a fail-safe precaution, manual valves were incorporated between the faucet and software-reliant solenoid valves.

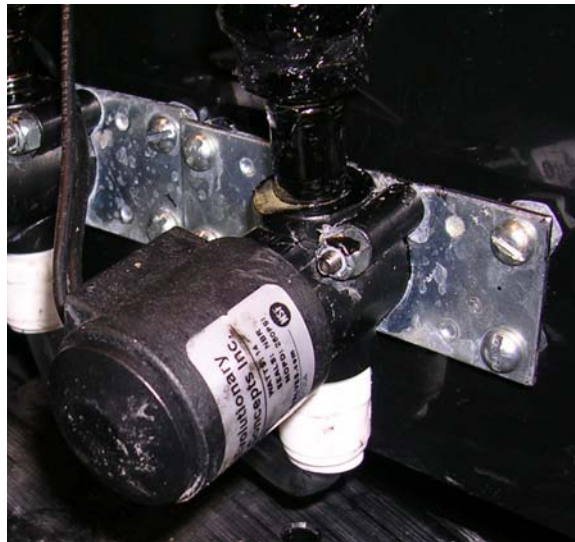


Figure 24: Mounted DC Solenoid valve to control dispensing of wash water

Two washing stations, (and therefore two solenoid and two manual valves), were utilized to minimize washing time. Because each well required 5 seconds of washing, it would take approximately 4 minutes for one wash rotation of 16 wells (including the rotary table speed of 0.75 rpm and computer communication delay). Abiding one of the main system design requirements of a time efficient process, the incorporation of two valves reduced the total washing time by half, yet maintained an adequate wash.

With the wash water source established and the valve dispense method finalized, the next design challenge was to integrate the faucet end to the two ¼-in NPT thread solenoid valves in minimal space. Two 45-degree elbow arrangements of threaded brass adapters were used to connect the Y-hose end connector to the two adjacent vertically mounted solenoid valves (*Figure 25*). In assembling the brass couplings, Teflon tape was wrapped around all threads to prevent leakage. External silicon sealant was avoided for an overall cleaner and more reliable plumbing connection. The plumbing arrangement was designed for the faucet hose to enter through the back top of the system so as not to interfere with the central operation area, yet still allow for accessibility to the manual shutoff valves on the Y-connector.



Figure 25: Threaded brass adapters connecting the faucet to Solenoid valves

The valve mount back plate (*Figure 26*) was secured to the 80/20 frame so that the solenoid valve outputs were positioned six inches above the well-plate vials. Clear nylon FDA approved 0.635 cm (1/4-in) inside diameter tubes were connected with threaded tube adapters to the valve outputs. To appropriately align the wash dispense

tubes to the center of two adjacent wells on the well plate, the tubes were routed through offset holes in a top 1.27 cm (½-in) thick Delrin plate.



Figure 26: Final valve mount plate secured on the system back frame.

Initial testing of the wash station resulted in an inadequate washing (*Figure 27*). A single vial was filled with 3.2 mL of yeast solution, and only 30% of the well was washed. This partial wash was caused by (1) a small diameter dispense tube, and (2) lack of water pressure.

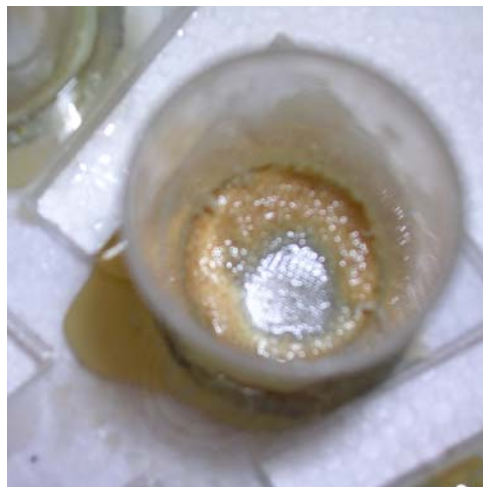


Figure 27: Initial tube-only dispense and resulting partial wash

To slightly increase the wash pressure, 0.95 cm (3/8-in) diameter aerators and 0.635 cm (1/4-in) threaded tube adapters were connected to the original tube ends beneath the top plate (*Figure 28*). The addition of aerators resulted in an increased dispense water pressure, and thus a 70% clean well (*Figure 29*).

Volume flow rate measurements were taken with the final aerator dispense system and the results met the initial washing requirements as determined from the first manual trials under the faucet. Each valve exhibited a volume flow rate of 80 mL/sec, which was within acceptable range of the target 90 mL/sec.



Figure 28: Aerators attached to the dispense tube ends for a desired well wash



Figure 29: Result of aerator wash

D. Food Dispense Unit

Just as important as a reliable method of washing was that of dispensing a consistent volume of yeast media into each well. Preliminary tests were performed to determine if the yeast solution could be easily pipetted into the wells. The tests proved that the yeast mixture contained enough water to exhibit similar properties as pure water. This discovery was the basis for the yeast station in the prototype design, which utilized a PVC pipe liquid reservoir and shutoff valve configuration like the washing stations. While the yeast successfully deposited into the wells, the same problem with inconsistent volume flow rate occurred. Unlike the wash water, however, only 3.2 mL of yeast was required in each well and thus a large liquid reservoir or refilling was not necessary.

In the final design, a clear plastic 500 mL container of yeast solution was used in combination with a peristaltic pump. The peristaltic pump, a type of metering pump specifically for low-flow and low-pressure applications, draws a precise amount of yeast solution on one stroke and discharges it on the next (*Figure 30*). This sequence was accomplished with rollers that squeeze fluid filled silicon tubing against the pump housing to force forward fluid movement. The pump is self-priming, can be run dry, easy to clean, and lacks seals that could potentially leak or contaminate the yeast solution. To control the pump, a transistor was incorporated on the control board and used as a switch to supply the required voltage from a separate 12 VDC power adapter.



Figure 30: Peristaltic pump to dispense 3.2 mL of yeast solution in each well

Four holes matching the pattern of the pump's rubber foot pads were milled into the top plate for a secure, yet easily removable pump mount. The top plate location next to the washing valves was appropriate, maintaining compactness of the system (*Figure 31*). Clear nylon FDA approved 0.635 cm (1/4-in) tubing was used to transport the yeast from the holding container, around the pump rollers, and through an alignment hole in the top plate next to the wash tube holes.



Figure 31: Pump location on top plate is in proximity to washing stations

The clear graduated 7.62 cm (3-in) x 7.62 (3-in) x 10.16 cm (4-in) yeast holding container was placed in the side compartment to allow the user to easily identify when refill was necessary, which should be infrequent as only 51.2 mL of yeast solution is

dispensed in each experiment run. One of the problems quickly identified of the yeast solution was its tendency to settle; therefore, a magnetic stirrer was placed beneath the container so the solution would remain homogeneous during operation (*Figure 32*). To ensure stability of the yeast container, a rubber-lined bracket was mounted to the 80/20 frame and the container fit snugly within this bracket, yet could still be easily removed.



Figure 32: Yeast holding container and magnetic stirrer to maintain a homogenous solution

E. Drainage System

The design of a low-profile and high capacity drainage system posed one of the greatest challenges of the overall system design. After the construction of the prototype, it was apparent that two types of drains were required. A main drain was necessary for the collection of flushed yeast solution and excess wash water directly beneath the three

dispensing stations. In addition, a drip plate between the entire well plate and rotary table was necessary to collect the few drips from the well plate underside.

To alleviate the drip problem, a 30.48 cm (12 in) x 30.48 cm (12 in) drip plate to fit the 80/20 frame was fabricated (*Figure 33*). The 1.27 cm (½-in) thick plate was milled with a 0.635 cm (¼-in) deep pocket to serve as a drip holding pool, and was tilted at a 10-degree angle so all liquid would flow off the plate through a 0.635 cm (¼-in) diameter hole in the back edge center to which a nylon tube was attached. The 0.635 cm (¼-in) tube size was experimentally verified as sufficient to handle all drips collected on the plate in a single test run.

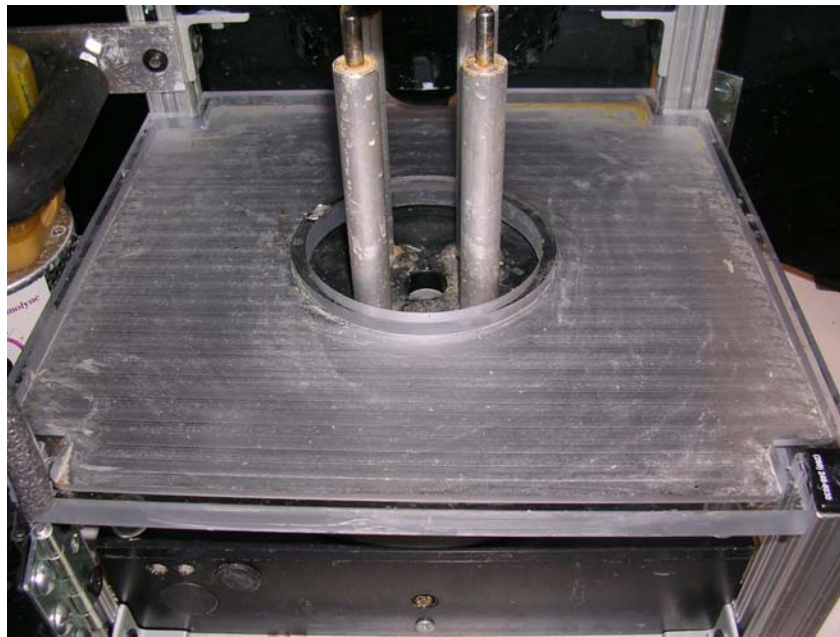


Figure 33: Drip plate to collect well plate drips that would otherwise strike the table

A separate main drain was constructed to collect all fluid from directly below the wash stations. A total of 14.4 liters of water would be flushed through the wells in one single experiment, and thus a high-capacity drain was necessary to collect and deposit the fluid into a larger waste bin separate from the system. An obvious design solution for the

main drain was a funnel, but due to space limitations, a unique funnel was required. The funnel opening had to be wide enough to collect liquid from three adjacent wells, yet converge quickly to a reasonably sized exit drain tube diameter. The 11.43 cm (4.5-in) vertical space between the base of the well plate and the top of the drip plate was the main constraint in the funnel design. Utilizing a 2-D CNC machine with a 45-degree mill tool and an inexpensive standard hard plastic oil drain funnel, a suitable drain was manufactured (*Figure 34*). The wide opening of the purchased funnel was used to collect liquid from the three adjacent dispense stations and guide the liquid through the CNC machined mount. The mount served two purposes: (1) attachment of the collecting funnel to the 80/20 structure via side flanges, and (2) funneling of the liquid into the 2-inch PVC elbow exit drain pipe.



Figure 34: Modified funnel drain to collect excess liquid directly below dispense stations

The elbow exit drain pipe was specifically selected so that all waste liquid would immediately exit the back of the system and remain outside of the work space (*Figure 35*). All components of the custom drain were glued with 2-part plastic epoxy and then sealed with silicon. Flexible nylon 5.08 cm (2-in) diameter tubing connected the exit

drain pipe to a waste bucket below the table. The waste bucket should be emptied after two complete experiments; otherwise, the nylon tube could be directly connected to a floor drain.



Figure 35: Drainage exit pipe and tube to deposit all excess wash fluid into a separate bucket

F. Aesthetics and Usability

The final considerations in overall system design included aesthetic and usability aspects. One of the first issues at hand was that of loose electrical cords. All wires from the valves, pump, magnetic stirrer, and controller board were routed through steel loop straps with 0.635 cm (1/4-in) clearance holes enabling them to be directly mounted along the 80/20 frame (*Figure 36*). All wires were then channeled through a single 6.35 cm (2.5-in) diameter hole in the lower back panel.



Figure 36: Organized routing of system wires

The image analysis camera required a dark environment for accurate UV light images; therefore, the entire system was encased in 0.318 cm (1/8-in) black Delrin sheets (*Figure 37*). The two front and right side panels were hinged with handles to enable quick and easy access to the well plate in the right compartment and yeast holding container in the left compartment. Magnetic latches were used to ensure that the hinged doors would remain closed, especially during system operation. The back, top, and left side panels were attached with Velcro to the 80/20 frame for convenient detachment on rare occasions. The fully enclosed system was only 50.8 cm (20-in) x 30.48 cm (12-in) x 60.96 cm (24 in) and thus could be manageably transported by two people, especially with the addition of raised handles mounted on the left and right sides of the 80/20 base frame (*Figure 38*).



Figure 37: Delrin sheets encase the system to create an appropriate environment for image analysis



Figure 38: Handles mounted on the right and left sides of 80/20 aluminum base frame

VI. Full System Integration

After designing a complete subsystem to produce clean, fed fruit fly larvae for image processing, integration with the data analysis subsystem was necessary.

Components of the data analysis subsystem were incorporated into the physical space of the washing and feeding system to produce an entirely automated analysis machine. The image processing system required the addition of a Q-Color 3 camera and 20.32 cm (8-in) diameter circular UV lamp (*Figure 39*). Appropriate space was reserved above the top plate for mounting the camera over a well for imaging the fed and washed larvae. In addition, the UV lamp was secured with padded U-brackets on the underside of the top plate, concentric to the well plate to emit uniform light relative to the wells.



Figure 39: UV light of image processing system mounted to underside of top plate

Integration with the electrical and user-interface systems also required additions within the washing and feeding device. A black electrical box was incorporated in the

left compartment of the system to house the custom controller board. This location was appropriately in proximity to the back panel cord outlet hole (*Figure 40*).



Figure 40: Electrical box mounted in left compartment to house the controller board.

One of the requirements of the computer user interface was to include a real-time camera for continuous feedback. A USB webcam was mounted on an 80/20 corner frame and focused on the wells directly under the three dispensing stations. The webcam updated continuously on the front interface panel and facilitated user-awareness of problems that could occur during washing and feeding operations. The team engineering psychologist performed a usability analysis and designed an appropriate user interface panel layout for the system PC (*Figure 41*). The panel allowed the user to manually enter the genetic fly line corresponding to each labeled well. A high resolution image of the fully washed fluorescent specimen from the Q-Color 3 camera was also displayed on the front panel for real-time qualitative observation of test results.

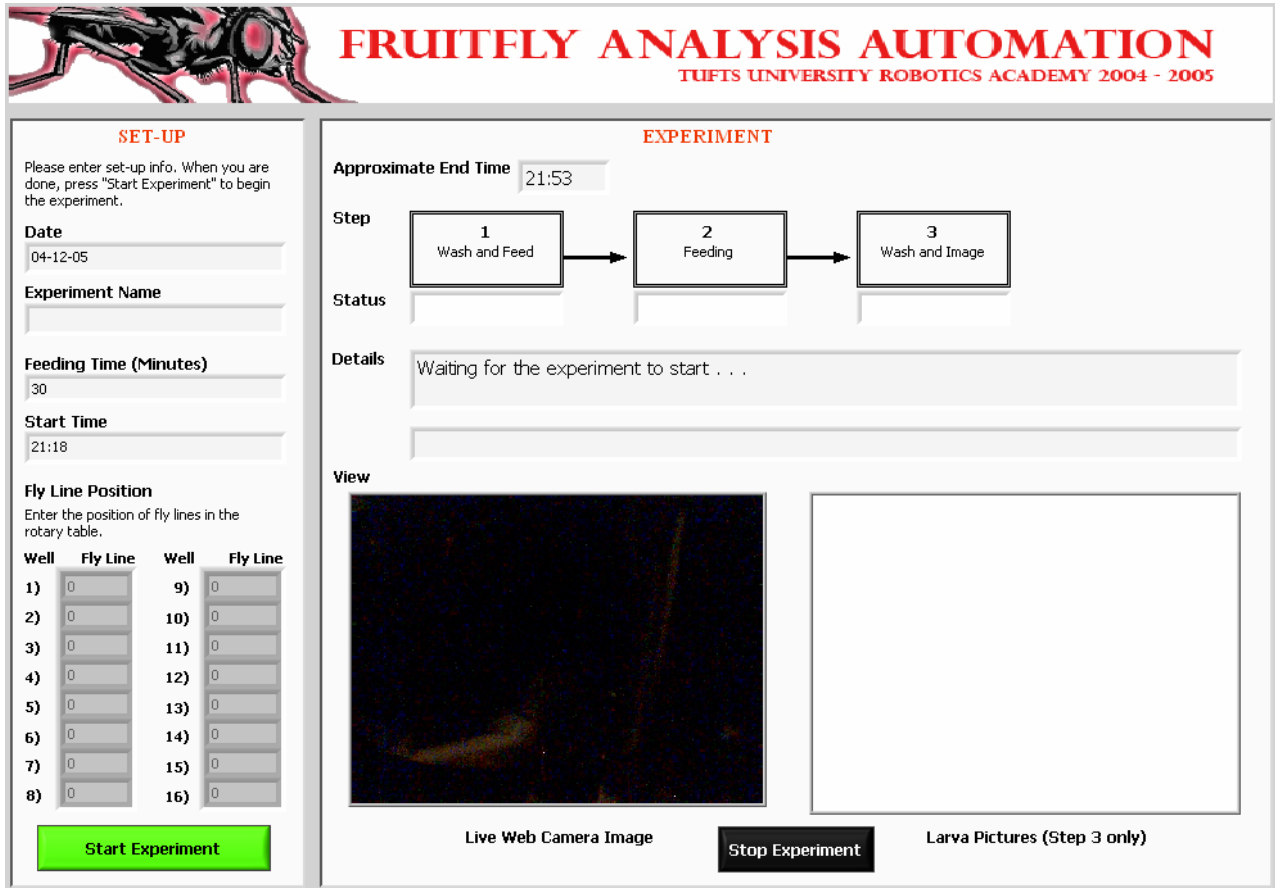


Figure 41: Front panel user-interface of PC control program

System Operation

Upon completion of manufacturing the washing and feeding subsystem and integrating it with the other subsystems, programming and testing began. The following outline illustrates the separate manual and automatic processes of the final system:

Manual:

1. Fill well plate of fruit fly eggs in pure yeast medium, approximately 10 larvae per well. Store plate for thirty-six hours to allow development to third instar stage. Place plate on rotation unit.
2. Enter genetic line type to corresponding location on PC control program. Press ‘Start Experiment’ to begin automation.

Automatic:

1. Plate rotates one full rotation and each well undergoes a wash, wash, and feed sequence.
2. Rotation stops for 30-minute feeding period.
3. Plate rotates one full rotation and each well undergoes a wash, wash, and snap image sequence.
4. Images are saved under their corresponding experiment name, date, time and fly line, and data analysis is performed at a later date.

Programming

Prior to programming the above automatic sequences in LabVIEW, a timing chart was constructed to gain an understanding of well plate rotation and position relative to the washing, feeding, and imaging stations at any given time (Appendix C). Tests were performed to determine the optimal feed and wash times, and the optimal step number and speed to rotate through the 22.5° spacing between adjacent well centers. With a primed pump, a 1-second pump time would fill a well with 3.2 mL of yeast solution. It is evident that in minimizing the time of the wash, wash, and feed sequence, the pump time was not the limiting factor. Rather, the limiting factor was the wash time, which was optimized at 5 seconds for each well. At the end of the wash, wash, and feed sequence, the well plate was rotated back through half the well separation distance to allow stray yeast drips to fall directly into the drain below instead of filling the last well with excess food. The total time for the wash, wash, and feed sequence was 6 minutes. To ensure smooth execution of the dispensing process, the webcam, which was focused on the three dispensing stations, provided continuous feedback on the front panel interface. Any system operation could be aborted at any time by clicking the red STOP panel button.

After the wash, wash, and feed sequence, the larvae were required to feed for 30 minutes. Therefore, a 30-minute wait time was incorporated into the program sequence, thus allowing the user to walk away and perform other tasks without worrying about manually timing. After 30-minutes had passed, the program automatically began the wash, wash, and image sequence, provided that the program was not aborted at any point during the feeding period, which would cause the timer to stop. If for some reason the program was stopped due to a power outage or other uncontrollable factor, the experiment would need to be restarted from the very beginning with a new set of fly lines to ensure accurate experiment results. Otherwise, assuming an ideal experiment free of emergency stops, the UV lamp would turn on at the beginning of the washing and imaging sequence and all system covers would need to be in place at that time.

A second timing chart was constructed to determine the rotation increment number to begin snapping images of the washed larvae. Due to the position of the camera, which was halfway between adjacent well centers relative to the three dispenser locations, (*Figure 42*) the well plate was half stepped during the washing and imaging sequence. System space and camera size constraints forced the camera to be mounted at such an inconvenient location. The inclusion of half-step rotations increased the total time to fully wash and image one well plate, but this time was still faster than two separate full plate rotations of only washing and only imaging, respectively.

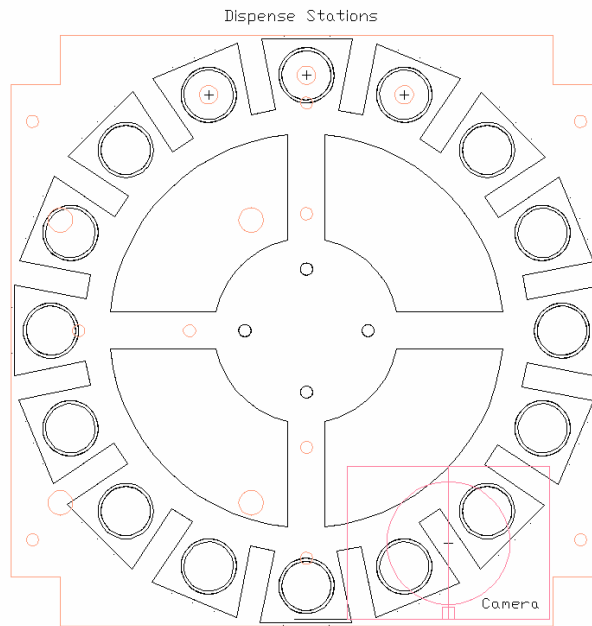


Figure 42: Camera mount position is exactly halfway between adjacent well centers

VII. Testing

Upon completion of the programming sequences customized to the physical system design, repeated tests were performed to obtain optimal wash times. Accurate data analysis was directly reliant on fully cleansed larvae, and so it was imperative to achieve the best possible wash with the system. One of the interesting discoveries that resulted from testing was the decreased flow rate (and therefore wash pressure) with increased valve open times. The optimal flow rate of 90mL/sec was achieved with a 1 second wash time, but as the open valve duration increased, the pressure difference that developed while the valve was closed diminished. To obtain a more consistent flow rate, pulsed washing was introduced to the system. The valves were turned on and off over the course of a varied number of seconds to obtain an overall higher pressure wash than would be achieved with a continuous equivalent time wash. Chart 2 compares the near-constant flow rate of 1-second pulsed washes with the decreasing flow rate of continuous washes for the corresponding open valve time.

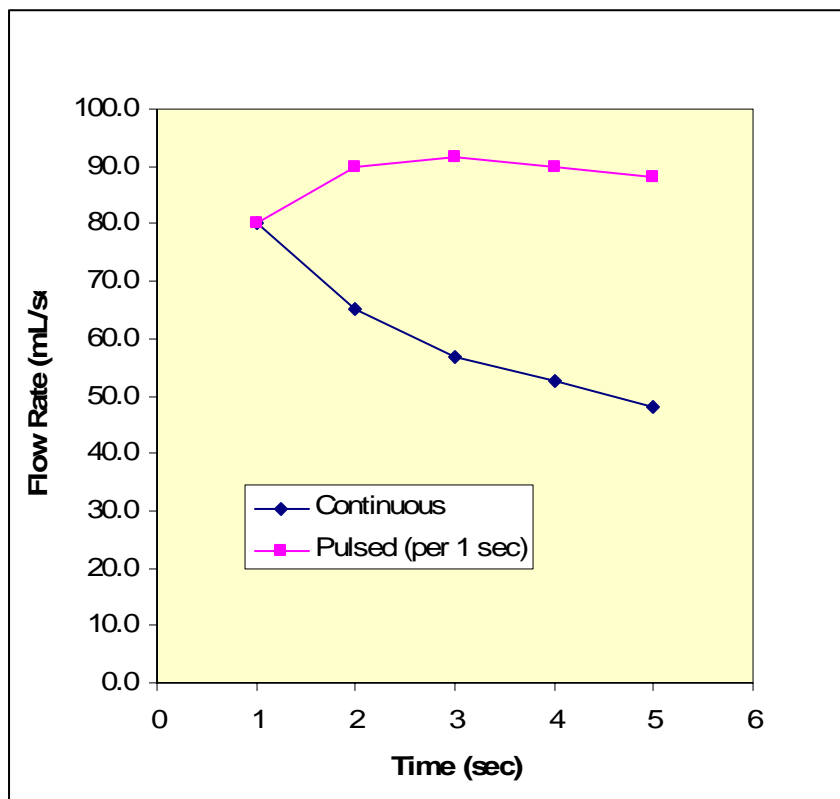


Chart 2: Pulsed washing vs. continuous washing

Extensive testing with the actual fly larvae submersed in yeast-filled wells should be done to optimize the pulsed-wash time. However, preliminary tests of pulse-washing wells with 3.2 mL of yeast solution indicated that 4 1-second pulse washings, with each pulse over a different portion of the well, would produce a fully washed well (*Figure 43*).



Figure 43: Before (left) and after (right) of pulse-washing

VIII. Redesign

Many of the initial design weaknesses were identified in the wood prototype system and subsequently eliminated in the construction of the final system. Even so, it was difficult to compensate for every design flaw, and if time allowed, reconstruction of a more streamlined design would ensue. While the 3-D model made it possible to avoid some errors, fabrication the physical system unveiled additional design faults.

The system as a whole was as compact as possible, and the use of an 80/20 frame with plastic and aluminum mount plates resulted in a lightweight, transportable structure. Nevertheless, spacing of components within the structure itself could be altered for a more user-friendly design. The positioning of the dispensing stations at the back of the system would remain unchanged, as these stations were appropriately offset from the main access area. However, the vertical distance between the well plate and rotary table would be increased to allow for easier well plate mount and dismount. On the current system, the top of the well plate vials often hit the base of the wash dispense tubes during plate removal. In addition, it was difficult to easily slide the well plate onto the steel pins of the aluminum mount rods (*Figure 44*). The well plate clearance hole diameters would be slightly increased, and the steel pins would be shortened by 0.953 cm (3/8-in).

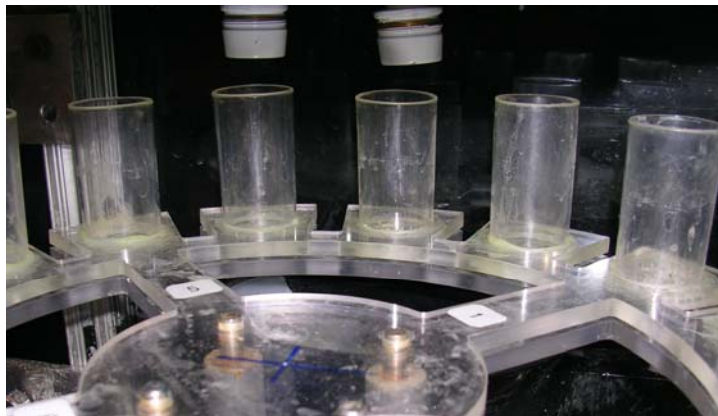


Figure 44: Space constraint of mounting well-plate on rotation unit

Another component rearrangement is that of the webcam. In its current position, the webcam slightly protrudes into the space required for inserting the well plate onto the rotation unit (*Figure 45*). In the redesign, the webcam would be mounted in the left compartment to permit an appropriate well plate clearance envelope above the rotary table, but remain in view of the dispensing stations.



Figure 45: Webcam protrusion into well plate clearance envelope

The Q-Color 3 camera used for image analysis should also be repositioned such that it aligns with the wells at the same instant as the dispense stations. The repositioning is necessary to avoid half-stepping the rotation table in the wash, wash, and image program sequence and thus result in a faster execution time. The integration of the camera subsequent to the completion of the washing and feeding stations caused this design flaw, and it should be noted that in designing future subsystems, integration should be considered at the beginning design stages.

The last major design modifications entail the valve station and drainage system. The valve to faucet connection was laborious to construct, destruct, and then reconstruct, all in a vain effort to eliminate leaks characteristic of threaded connections. There inevitably remained a small leak on the left valve, which resulted in a maximum 10.16

cm (4-in) diameter water pool on the top plate after one experiment run (*Figure 46*). The brass adapter arrangement was also cumbersome and heavy, neither of which meet the general streamlined and lightweight system requirements. In a redesigned system, sweltered copper water pipes should be used between the faucet and dispense valves for an externally clean, lightweight, and leak proof wash station.



Figure 46: The left valve connection leak creates a small liquid pool on top plate

The drainage system should also be slightly modified. The conglomeration of a modified purchased funnel and machined plastic mount were functional, but excessive use of the system and exposure to liquid could cause the adhesive to weaken and components to disassemble. This design flaw became obvious through a drainage leak after initial system testing when the funnel slightly detached from the mount plate. The drain was repaired with a stronger 2-part epoxy, but a permanent solution would be to outsource the drain for 3-D fabrication from a single solid plastic block.

IX. Conclusion

Through an intensive design process and collaboration with the NEMC team, the Robotics Academy reached the desired goal of a functional semi-automated high throughput fly screening mechanism. The system was capable of screening 16 different fly lines in 45 minutes, which was a significant time reduction from the 90 minute manual screening process currently used in the Drosophila research laboratory. The automation of feeding, washing, and data collecting was expertly packaged in a transportable and reconfigurable sturdy structure. Each component within the structure was ergonomically designed for optimum operation efficiency and repeatability. The final overall system statistics may be viewed in Chart 3.

Sequence	Time	Accuracy	Repeatability
1st Wash & Feed	6 minutes	80%	100%
Feeding Period	30 minutes	100%	100%
2nd Wash & Image	8 minutes	80%	95%
Total	~45 minutes	86%	98%

Chart 3: Preliminary system operation results

While the semi-automated screening mechanism was initially developed to identify abnormal feeding behaviors in mutant fly lines, the general system design is adaptable for a multitude of studies that assess other Drosophila behavioral/physiological abnormalities. The potential of the automated device to detect and quantify significant abnormalities in mutant fly lines and thus reveal functionally important genes is largely relevant to the knowledge base of human genetics [11]. In summary, the collaboration of engineers from Tufts Robotics Academy and biologists from the Molecular Pharmacology Research Center at T-NEMC enabled the successful design, development, and operation of a timely automatic positioning and dispensing device to prepare molecular living specimen for relevant analyses.

X. Appendix A: Background Documentation

Background Documentation

Goal: Measure food intake by mutant fly lines to identify genes affecting metabolic functions.

Problem: Current fly screening process is time and labor intensive. Over 25,000 mutant fly lines of single gene insertions calls for the need of a high-throughput screening system.

Background:

A. Fruit Flies & Genetic Research

- small; easy to handle & store
- anesthetize with CO₂ or by temporarily freezing
- males & females are easy to differentiate
- short generation time (10-12 days); do well at room temperature
- care and culture requires little equipment; low cost; little space
- many types of media – should always contain yeast to prevent molding

B. Fruit Fly Life Cycle

- complete metamorphosis: egg, **larval**, pupa, flying adult
- time from egg to adult is temp. dependent (high temp = fast generation time)
- Females lay up to 100 eggs/day
- after eggs hatch, visible in media (white bodies)
- as feed, disrupt media with tunnels/burrows
- Larval Stage: 3 instars (molts); study the 3rd instar
- after 3rd instar, migrate out of media to top of vial to pupate

Life cycle by day (temp: 21-23 degrees C)

Day 0: Female lays eggs

Day 1: Eggs hatch

Day 2: First instar

Day 3: Second instar

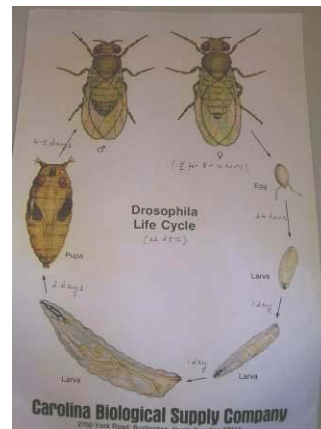
Day 5: Third and final instar

Day 7: Larvae begin roaming stage.

Pupariation (pupal formation)

occurs 120 hours after egg laying.

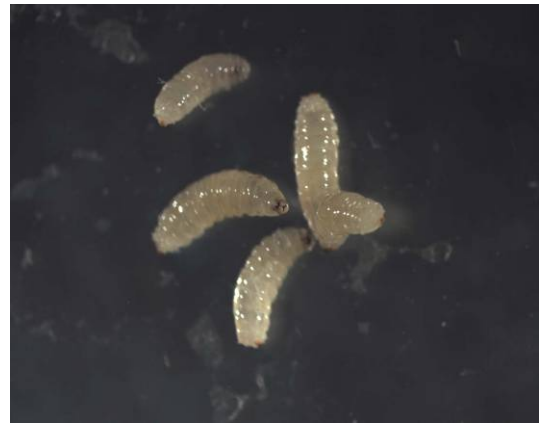
Day 11/12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.



Fruit Fly Life Cycle

C. Size of Third Instar Larvae

- Approximately the size of ball point pen tip



Pictures of larvae through microscope



Size of larvae: tick marks on ruler are in millimeters

XI. Appendix B: Hand Separation Process

Step 1: Eggs hatch & grow to 3rd instar larvae stage in vials w/ media.



Step 2: Fill Petri dish with new media



Step 3: Add Fluorescein dye to new media & mix.



Step 4: Remove larvae from old media in vial.



Step 5: Set larvae in new media for 30 min. to allow feeding.



Step 6: Remove fed larvae from media.



Step 7: Carefully clean each larvae of exterior dye & place in new dish.



Step 8: Place dish of cleaned, fed larvae in black box for analysis.



Rotation #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Well-Vial #																
1	F															
2	W	F														
3	W	W	F													
4		W	W	F												
5			W	W	F											
6				W	W	F										
7					W	W	F									
8						W	W	F								
9							W	W	F							
10								W	W	F						
11									W	W	F					
12										W	W	F				
13											W	W	F			
14												W	W	F		
15													W	W	F	
16														W	W	F
FILL TIME (s)	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	43
ROT TIME (s)	0	5.1	10	15	21	26	31	36	41	46	51	57	62	67	72	77
TOTAL TIME (s)																120

Timing chart indicating position and time of 16 vial well plate during steps 2 and 3

	Material	Description	Distributor	Part #	Unit Price	Quantity	Total
Plumbing							
	PVC Pipe	2" Inside Dia, 10" length	Ace Hardware	na	0.62	3	1.86
	Coupling	2" x 1/2" fpt	Ace Hardware	na	1.99	3	5.97
	Bushing	1/2" x 3/8" male to fip hex	Home Depot	A-828	1.49	3	4.47
	Manual Valve	3/8" x 3/8" male nptf straight valve	Home Depot	PL-3042	5.98	3	17.94
	Connector	3/8" x 1/4" ploypro nptf male connect	Home Depot	PL-3025	2.39	6	14.34
	Tubing	1/4" ID Nylon 11, drinking water	McMasterCarr	5548K53	0.51	2	1.02
	Solenoid Valve	Delrin Direct-Act, 1/4" nptf, 12 VDC	McMasterCarr	7876K58	34.38	3	103.14
	Sealer/Cement	PVC Cement, 1/4 pint	Ace Hardware	na	2.49	1	2.49
						Sub Total	\$151.23
Rotation							
	Rotary Table	Worm Gear Precision (90:1), 10" Dia	Servo Systems	10R90	2000.00	1	0
	Coupling	1/8" to 1/4" flex coupling	Servo Systems	na	10.00	1	10.00
	Stepper Motor	5.4VDC, 1.5 mA, 8 lead, 0.9°/phase	Servo Systems	PH268ME1.5B	75.00	1	75.00
						Sub Total	\$85.00
Electrical							
(Rotation)	Driver Board	PIC-STEP Motor Controller	JR Kerr	KAE-T3V1-BDV1	160.00	1	160.00
	Converter Board	Z232-485 Serial Port Converter	JR Kerr	KAE-Z232485-BDV2	60.00	1	60.00
	Com Cable	DB9 male/DB9 female straight cable	Radio Shack	26-117	13.49	1	13.49
	Ribbon Cable	10 pin flat ribbon, IDC connectors, 6"	DigiKey	A3AAG-1006M-ND	2.35	1	2.35
	Motor Connector	15-Position HD Male Solder D-Sub	Radio Shack	276-1501	1.89	1	1.89
	Heat Sink	Anodized aluminum, T0-220	Radio Shack	276-1368	1.69	1	1.69
	Motor Power	13.8 VDC, 3 A Power Supply	Radio Shack	22-507	41.99	1	41.99
	Logic Power	9 VDC, 500mA Power Adapter	Radio Shack	273-1612	15.99	1	15.99
(Valve)	Transistor	NPN Transistor	Radio Shack	276-2058	0.69	3	2.07

	Relay	5VDC/1A SPDT Micro Relay	Radio Shack	275-240	4.29	3	12.87
	Resistor	1 K	Radio Shack	271-1321	0.99	3	2.97
	Wire	22 Gage, 75' spool	Radio Shack	278-1218	4.99	1	4.99
	Circuit Power	9 VDC, 1200mA Power Adapter	Radio Shack	273-1612	15.99	1	15.99
	Valve Power	12 VDC, 5 A Switching Power Supply	All Electronics	PS-125	21.00	1	21.00
	Parallel Port Cable	1284 IEEE Printer Cable	Radio Shack	26-626	21.99	1	21.99
						Sub Total	\$379.28
Structure							
	Plywood	3/4", 1/2 full sheet	Ace Hardware	na			20.00
	Pipe Holder	Aluminum, 2" dia	Ace Hardware	na	0.29	3	0.87
	Valve Mount Plate	Mending Brace, 3.5" x 1-3/8"	Ace Hardware	na	3.29	3	9.87
	Project Box	8"x6"x3" Project Enclosure	Radio Shack	270-1809	6.99	2	13.98
	Bracket Mounts	L-Bracket	Ace Hardware	na	0.49	6	2.94
	Hinge	brass door hinge, 17" length	Ace Hardware	na	1.36	1	1.36
	Assorted Hardware	mounting screws, wing nuts, etc	Ace Hardware	na			5.00
						Sub Total	\$54.02
Cameras							
	Initial Prototype	Fire-I FireWire Digital Camera	Unibrain	2035	99.00	1	99.00
	Final Prototype	ELMO CCD TV Camera (TUFTL)	ELMO Man. Grp.	Model SE420	209.00	1	209.00
	Microscope Imager	Microscope camera (owned by lab)	Zeiss	AxioCam MRc	0.00	1	0.00
						Sub Total	\$308.00
Lighting							
	Ultra Violet LED	5mm 15deg 400nm	LED Supply	L3-0-U5TH15-1	1.35	10	13.50
	Handheld UV Lamp	Spectroline 365nm UV Bulb (TUFTL)	Spectroline	na	0.00	1	0.00
	Fluorescent Tube	Blacklite FC8 T9 8inch 22Watt	GE	C425BL	36.95	2	73.90
	Ballast	Rapid Start Ballast for FC8 T9	A+ Electrical Supply	ERFSC-122-TPK	11.05	1	11.05
						Sub Total	\$98.45
Chemicals							

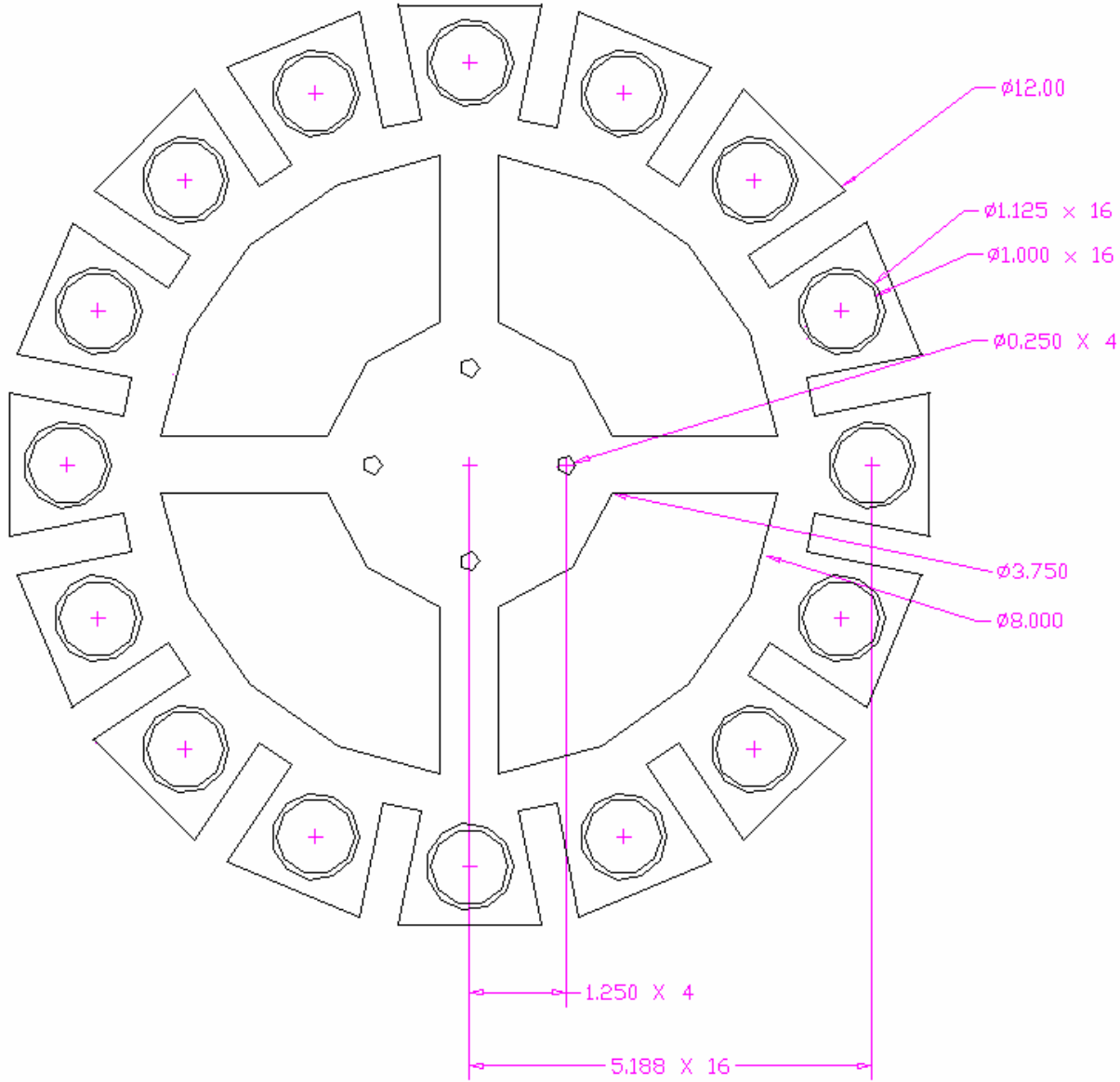
	Fluorescent Dye	Fluorescein (Uranine; Acid Yellow 73) C ₂₀ H ₁₀ O ₅ Na ₂ 100 G	Spectrum Chemicals	FL116-06	21.35	1	21.35
						Sub Total	\$21.35
						Grand Total	\$1,097.33

	Component	Description	Distributor	Part #	Unit \$	Quantity	Total
Dispense Stations							
	Brass Elbow	Threaded connector	Tags Hardware	na	2.49	2	4.98
	Brass Couplings	Threaded connectors	Tags Hardware	na	2.49	6	14.94
	Y Manual Valves	Plastic 3/4" nozzles	Tags Hardware	na	3.59	1	3.59
	Elbow Hose Connector	Plastic 3/4" nozzle	Tags Hardware	na	1.99	1	1.99
	Back Mount Plate	1/4" Black Delrin	McMasterCarr	8575K115	19.35	1	19.35
	Steel U-Bolts	3/8" OD	McMasterCarr	8896T118	2.69	3	8.07
	Valve Mount Plate	Mending Brace, 3.5" x 1-3/8"	Ace Hardware	na	3.29	3	9.87
	Aerator Nozzle	3/8" Nozzle End	Tags Hardware	na	3.99	2	7.98
	Nozzle-Tube adapter	3/8" to 1/4" tube adapter	Tags Hardware	na	2.99	2	5.98
	Connector	3/8" x 1/4" ploypro nptf male connect	Home Depot	PL-3025	2.39	4	9.56
	Tubing	1/4" ID Nylon 11, drinking water , (ft)	McMasterCarr	5548K53	0.51	3	1.53
	Solenoid Valve	Delrin Direct-Act, 1/4" nptf, 12 VDC	McMasterCarr	7876K58	34.38	3	103.14
	Top Plate	1/2" Black Delrin	McMasterCarr	8575K117	38.32	1	38.32
	Side Base Plate	1/4" Black Delrin	McMasterCarr	8575K115	19.35	1	19.35
	Magnetic Stirrer	120 V Magnetic Stirrer Circular Plate	Barnstead/Thermolyne	S17415	194.00	1	194.00
	Yeast Holding Container	Clear plastic graduated, 500 mL	McMasterCarr	4169T51	1.92	1	1.92
	Container Bracket	Al mount, threaded square bracket	McMasterCarr	3060T75	8.61	1	8.61
	Peristaltic Pump	0.0136-2.89 oz minimum flow	McMasterCarr	4049K33	175.39	1	175.39
	Teflon Tape	roll	Tags Hardware	na	0.89	2	1.78
	Black Enamel	rust prevention	Tags Hardware	na	4.99	1	4.99
	Sealer/Cement	PVC Cement, 1/4 pint	Ace Hardware	na	2.49	1	2.49
						Sub Total	\$637.83
Rotation							
	Rotary Table	Worm Gear Precision (90:1), 10" Dia	Tufts University	10R90	2000.00	1	0.00

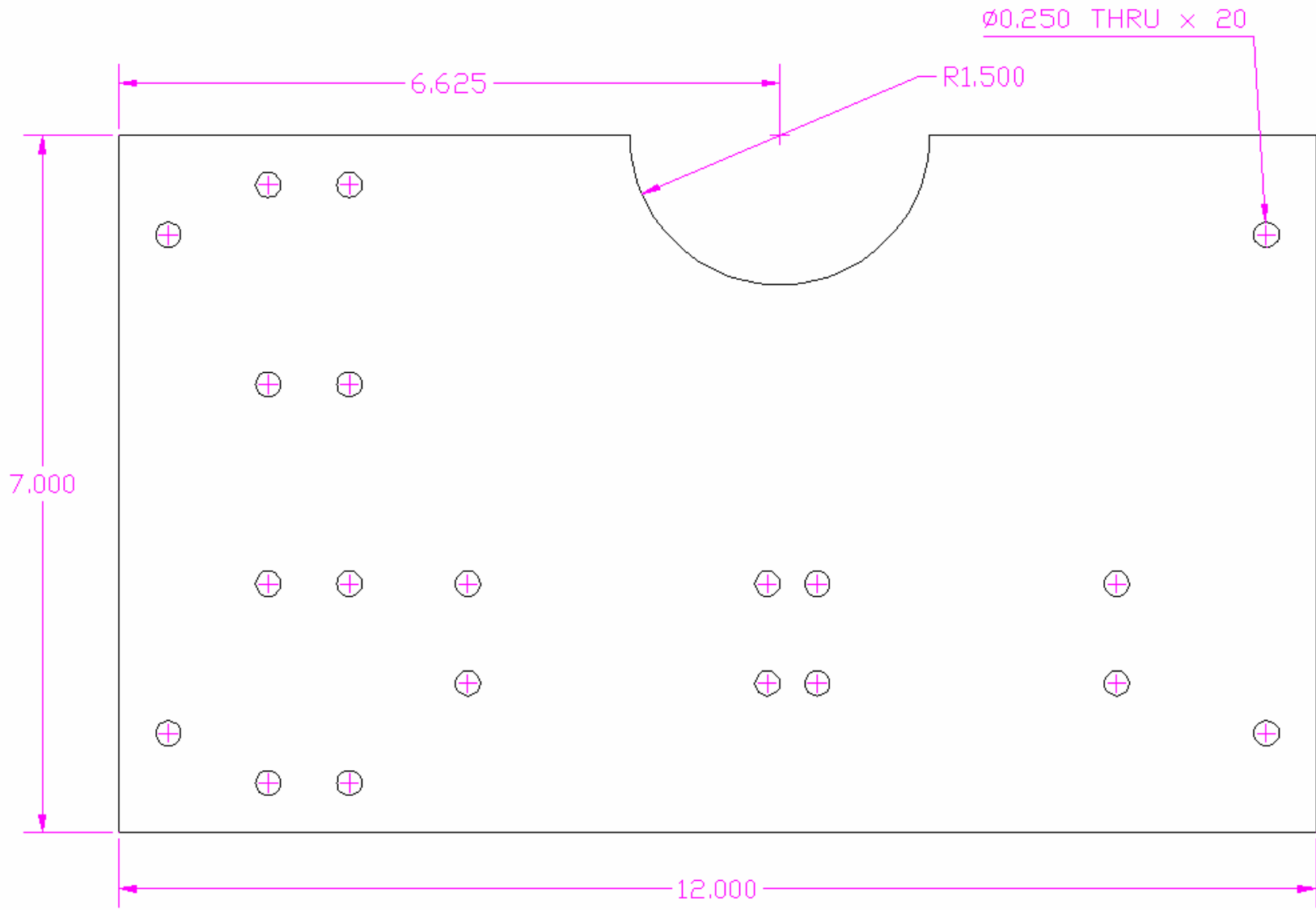
	Coupling	1/8" to 1/4" flex coupling	Servo Systems	na	10.00	1	10.00
	Stepper Motor	5.4VDC, 1.5 mA, 8 lead, 0.9°/phase	Servo Systems	PH268ME1.5B	75.00	1	75.00
	Aluminum Rod	5/8" dia machined	Tufts University	na	0.00	1	0.00
						Sub Total	\$85.00
Electrical							
	PCB Board	Custom mult-controller board	ExpressPCB	na	60.00	1	60.00
	Board Parts	Wires, LED's, etc	Radio Shack	na	20.00	1	20.00
	Project Box	8"x6"x3" Project Enclosure	Radio Shack	270-1809	6.99	1	6.99
						Sub Total	\$86.99
Drainage							
	Tubing	2" OD (ft)	Home Depot	na	1.00	2	2.00
	Drain Bucket	5-gallons	Home Depot	na	3.98	1	3.98
	PVC Elbow & Connector	2" OD	Home Depot	na	1.69	1	1.69
	Funnel Mount	3/4" machined plastic	Tufts University	na	0.00	1	0.00
	Plastic Funnel	6" top diameter	Tags Hardware	na	1.29	1	1.29
	Black Paint	Spray paint for plastics	Tags Hardware	na	4.99	1	4.99
	Plastic Welder	2-part epoxy	Machine Shop	na	0.00	1	0.00
	Drip Plate	1/2" Lexan	McMasterCarr	8574K28	22.44	1	22.44
	Tubing	1/4" ID Nylon 11, drinking water , (ft)	McMasterCarr	5548K53	0.51	3	1.53
						Sub Total	\$37.92
Structure							
	Delrin Panels	1/8" 24"x24" black Delrin sheet	McMasterCarr	8575K413	39.55	7	276.85
	Magnetic Latch	plastic/al, 2-hole mount	Tags Hardware	na	1.09	3	3.27
	Door Handles	Al, 4-hole mount	Tags Hardware	na	2.99	3	8.97
	Door Hinges	Al, 3-hole mount	Tags Hardware	na	2.99	6	17.94
	Velcro	1" wide, stick-on (ft)	Home Depot	na	0.59	15	8.85
	Side Handles	SS Pull Handle, 5-3/4"	McMasterCarr	5185A1	4.27	2	8.54
	Cord Loop Straps	galvanized steel, 3/8" OD (pkg 50)	McMasterCarr	8863T2	9.73	1	9.73
	80/20 Corner Brackets	1/4" mount hole	Action	4108	1.00	35	35.00
	80/20 Frame	1"x1" (ft)	Tufts University	1515	4.00	23	0.00

	Assorted Hardware	mounting screws, wing nuts, etc	Tags Hardware	na	30.00	1	30.00
						Sub Total	\$399.15
Imaging							
	High-Resolution Camera	Q-Color 3 FireWire Digital Camera	Olympus America	32-0045C-353	3000.00	1	3900.00
	Fluorescent Tube	Blacklite FC8 T9 8inch 22Watt	GE	C425BL	36.95	2	73.90
	Ballast	Rapid Start Ballast for FC8 T9	A+ Electrical Supply	ERFSC-122-TPK	11.05	1	11.05
	WebCam	Logitech USB Camera	Tufts University	na	0.00	1	0.00
						Sub Total	\$3,084.95
Chemicals							
	Fluorescent Dye	Fluorescein (Uranine; Acid Yellow 73) C ₂₀ H ₁₀ O ₅ Na ₂ 100 G	Spectrum Chemicals	FL116-06	21.35	1	21.35
	Yeast	Brewer's Inactive	Sigma-Aldrich	YBD-1KG	43.9	1	43.90
						Sub Total	\$65.25
						Grand Total	\$5,297.09

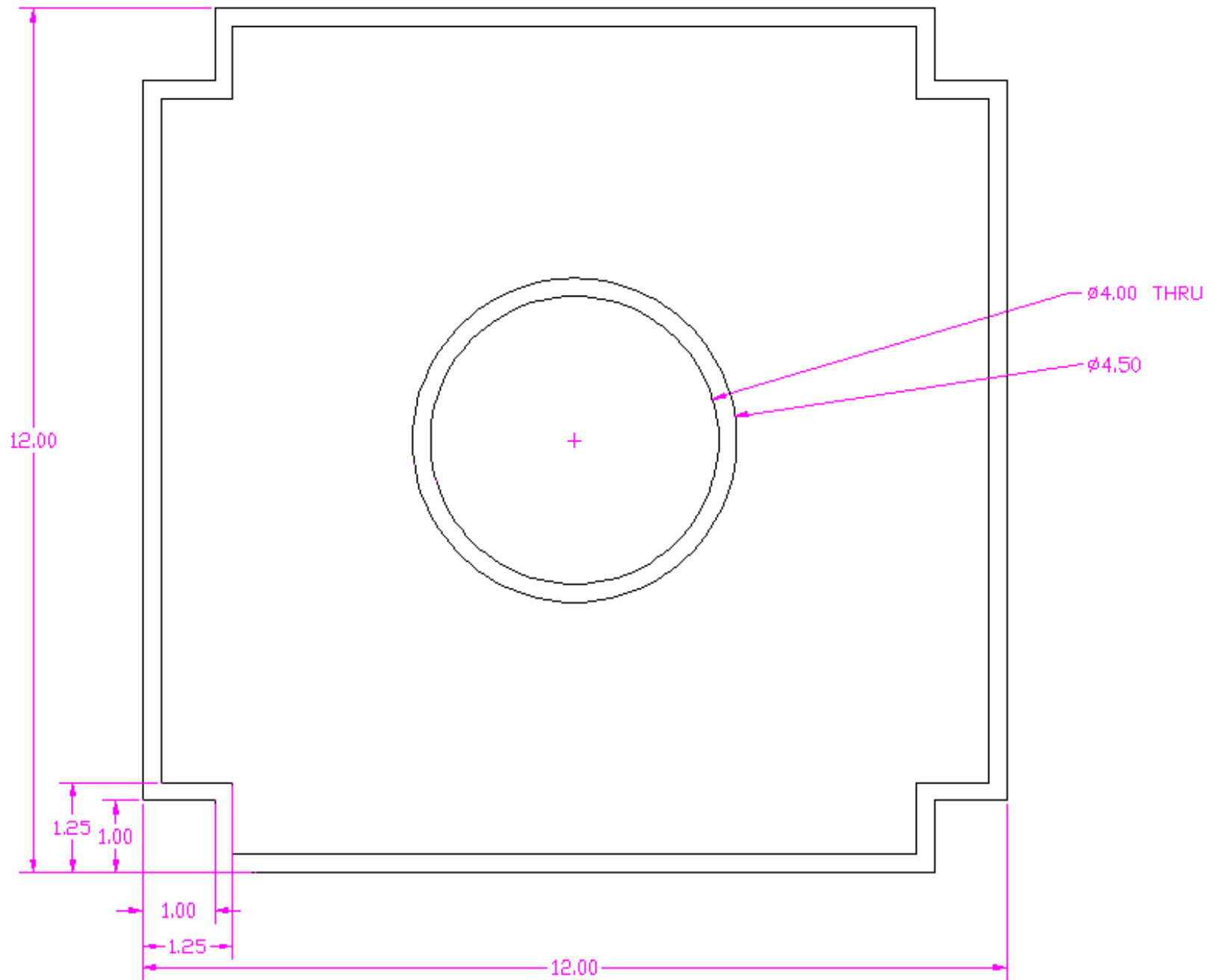
WELL PLATE
1/4" LEXAN

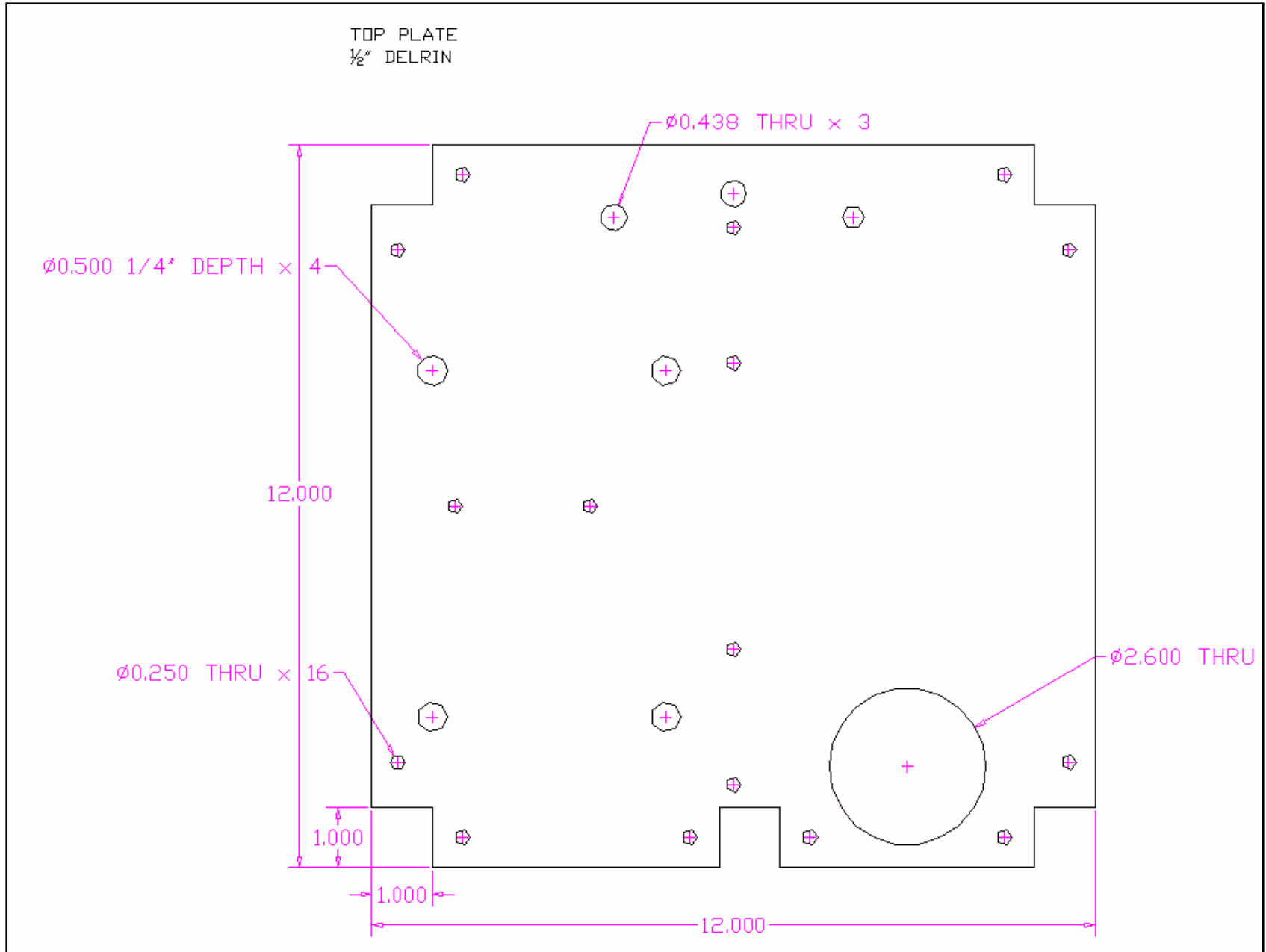


BACK VALVE PLATE
1/4" DELRIN

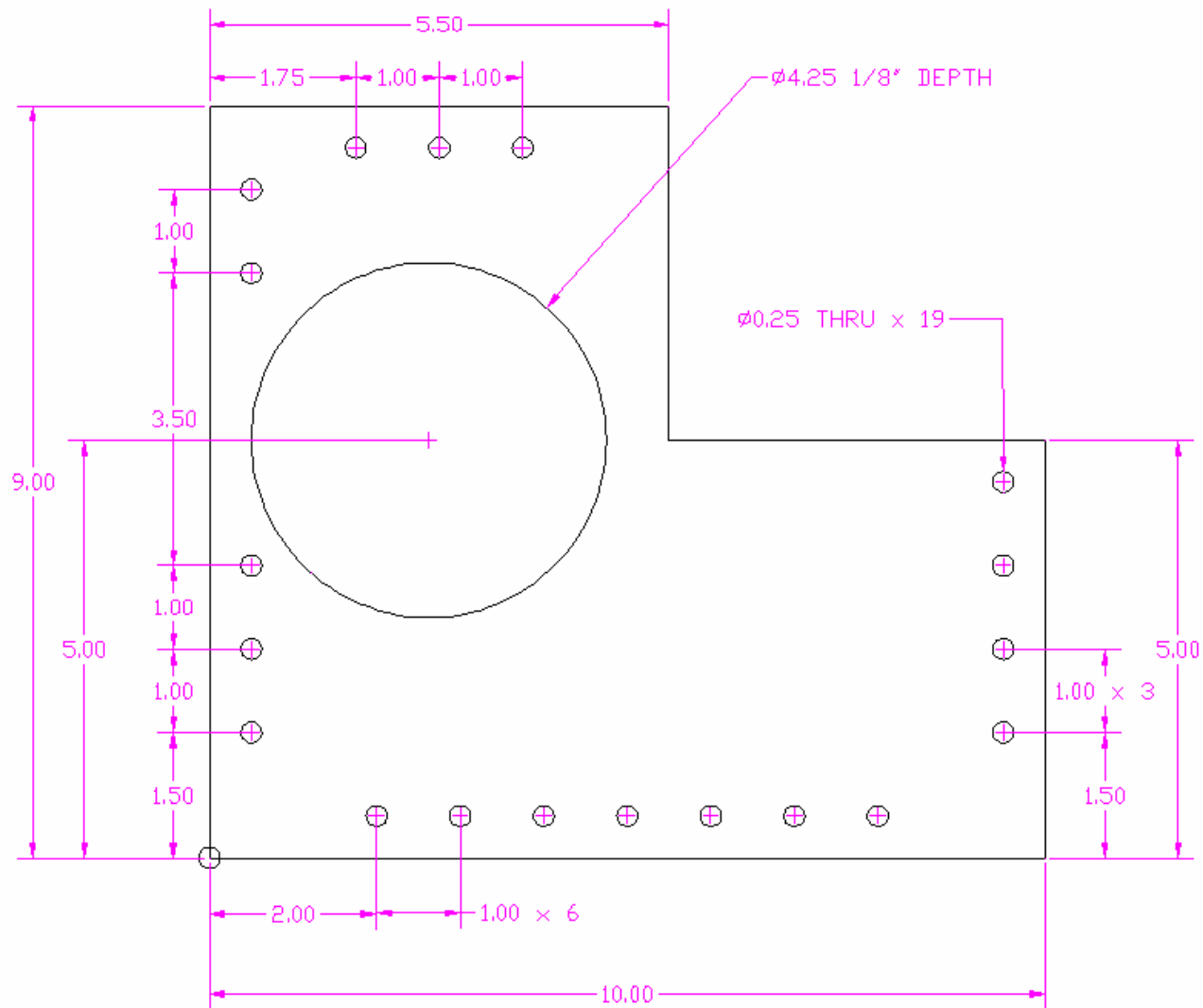


DRIP PLATE
½" LEXAN

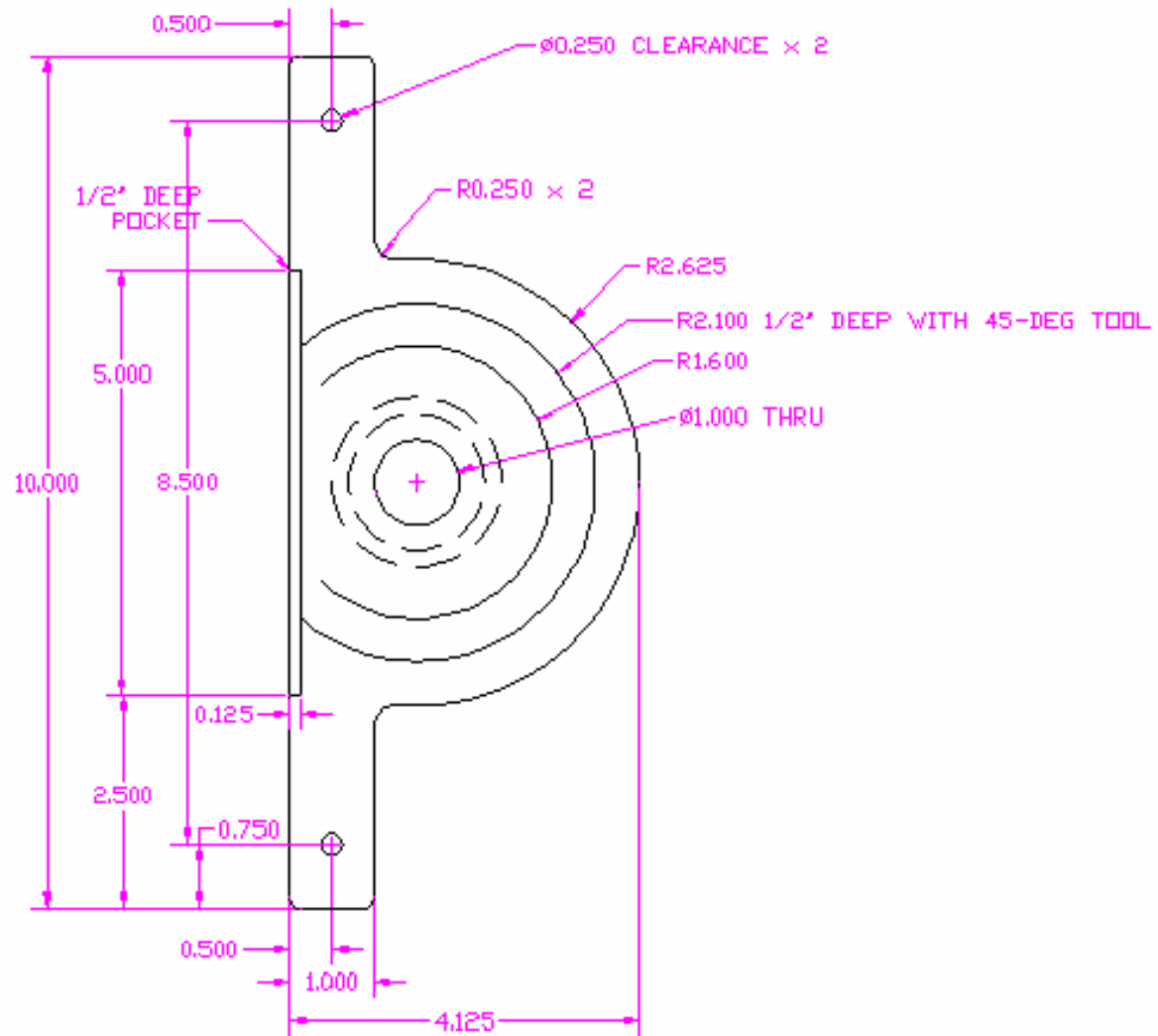




LEFT BASE PLATE
1/4" DELRIN



FUNNEL PLATE FRONT SIDE
 MACHINED ON 2 SIDES
 3/4" PLASTIC MATERIAL



XVII. References

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