Progress Report 10: April 13 to April 19, 2007

MALDI-MSI Tissue Coating Device

Client: Dr. Amy Harms, Ph.D., Biotechnology Center
Advisor: Professor Brenda Ogle, Biomedical Engineering
Team Members: Laura Piechura (Leader)
Kellen Sheedy (Communicator)
Holly Liske (BWIG)
Jenna Spaeth (BSAC)

Problem Statement

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is an imaging method that allows for label-free spatial analysis of biological tissue samples. This technology can be used to identify and quantify proteins, monitor protein biomarkers, and sequence polypeptide chains, techniques that can be applied to proteomic analysis of disease formation. However, sample preparation methods, especially with regard to the application of the matrix tissue coating, are difficult to control but require accuracy and precision. A device must be developed to apply a fine, uniform coating of light-absorbing compounds in order to simplify the sample preparation process. The goal is to provide a reliable tool to enhance the MALDI-MSI technique in order to speed and simplify potentially life-saving research.

Last Week's Goals

- On Friday, April 13, we will use our class time to make decisions about the quantity of plastic we need to order for our polypropylene encasing, the length and specifications of the belts we'll need for the conveyor, and send this information to our client.
- In addition, we'll conduct additional testing with the air compressor or air tank if it has arrived, create a more detailed timeline as to when we'd like each individual component of our prototype fabricated by, perhaps divide into specialized teams to complete these tasks, and begin construction with the parts that we have and those that arrive throughout the week.
- If enough of the parts arrive within the week for us to create a rough mock-up of the prototype, we may take a trip to the hardware store to begin purchasing the smaller hardware required to assemble the final design.

Summary of Accomplishments

- On April 13, we met with Professor Ogle and watched our recorded midsemester presentation. The exercise proved very helpful as group members critiqued their own performance, as well as received input from Dr. Ogle and the rest of the group. Hopefully this activity will result in an improved final presentation. Also, we met as a group to decide the ideal size of the enclosure for our prototype, and as we experienced difficulty locating a ¼″ sheet of clear polypropylene, we instead decided to order polyester, which was readily available without color, and devise a way to coat the plastic with a substance to increase the chemical compatibility of the enclosure. With these decisions, we formulated an order for a 48″ x 48″ sheet of polyester plastic and the bands needed to assemble the conveyor.
- In addition, on Sunday, April 15, Kellen, Holly, and Laura took a trip to Home Depot and found screws for assembling the enclosure, hinges for the doors of the enclosure, and plastic adhesive to fuse the polyester together.
- On Monday, April 16, the order was placed by our client, and we were given notification on Tuesday, April 17 that our plastic had arrived from McMaster-Carr.

This Week's Goals

- On Friday, April 20, we will meet during class time to begin construction of our prototype. Specifically, we will begin cutting the plastic to the specific sized required, and plan our progress for the remaining two weeks. However, most of the final decisions will be reserved for when the bands arrived and we can better map out the components of the conveyor.

Project Difficulties

Our main difficulty with the project this week was finding a supplier of clear sheets of polypropylene plastic. To solve this problem, we opted to purchase polyethylene plastic, which is compatible with most of the chemicals of the matrix, and we hope to devise a method of coating the plastic interior to increase its durability.

Activities

Team Member	Activities	Time for Week	Total Time
Holly	Research of conveyor design and components, mounting alternatives, group meetings	6 hours	60.5 hours
Jenna	Research of mounting alternatives, group meetings	6 hours	59 hours
Kellen	Research of mounting alternatives, group meetings	6 hours	59 hours
Laura	Research of mounting alternatives, group meetings, Progress Report 9	6 hours	61.5 hours

Project Timeline

	2/2	2/9	2/16	2/23	3/2	3/9	3/16	3/23	3/30	4/6	4/13	4/20	4/27	5/4	5/9	5/11
Client Meeting	Δ									SPRING BREAK						
Research Project	Δ	◆														
Write PDS		Δ														
Brainstorm Design Ideas		Δ		◆												
Choose 3 Designs to Enhance					Δ											
Chose 1 Design to Pursue					Δ											
Work on Midsemester Presentation					Δ	◆										
Work on Midsemester Paper					Δ		◆									
Finalize Design					Δ											
Order Materials					Δ											
Build and Test Prototype							Δ						◆			
Work on Poster Presentation												Δ		◆		
Work on Written Report													Δ		◆	
Final Meeting with Advisor																Δ

Tissue Coating System for MALDI Mass Spectrometric Imaging Applications

Biomedical Engineering Design 201
9 May 2006

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Abstract

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is a technology that allows for label-free spatial analysis of biological tissue samples. This technique can be used to identify and quantify proteins, locate and monitor biomarkers, and sequence polypeptide chains, data that can be applied to proteomic analysis of disease formation[1]. However, sample preparation methods, especially with regard to the application of the matrix tissue coating, are difficult to control but require accuracy and precision. The goal of this project is to design a device to apply a fine, uniform coating of light-absorbing compounds to simplify the sample preparation process and facilitate the MALDI-MSI technique. Designs involving pneumatic sprayers, pressure-valve systems, and single-action airbrushes have been explored and considered. The final design implements a polyester enclosure that supports an automatic sprayer which administers a misting solution onto a motor-driven conveyor carrying the tissue sample. Future development of the prototype may include incorporating time-delay relays into the motor circuitry to make the device more independent, as well as clinical testing in our client's laboratory

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Problem Statement

The goal of this project is to design and construct a device to apply a fine, uniform coating of matrix compounds to a tissue sample in a replicable manner so as to simplify the sample preparation process of MALDI-MSI and in doing so, facilitate potentially life-saving biological research.

Introduction

MALDI Imaging Process

Matrix-assisted laser desorption/ionization mass spectrometric imaging is a technology, developed in the 1990's by Dr. Richard Caprioli of Vanderbilt University, that allows for the study of the spatial arrangement of molecules, most notably proteins and peptides, within biological tissues[3]. The technique involves coating a tissue sample with a UV-absorbing matrix, or mixture of organic compounds, shooting the sample surface with a laser to ionize the crystals formed by the matrix, and collecting these ions with a time-of-flight analyzer. Computer algorithms process the mass to charge (m/z) values obtained from the analyzer, and mass spectrums, along with two-dimensional images, are created detailing the location of molecules within the tissue based upon the weight of the molecules (Figure 1). Further analysis through protein extraction, HPLC fractionation,

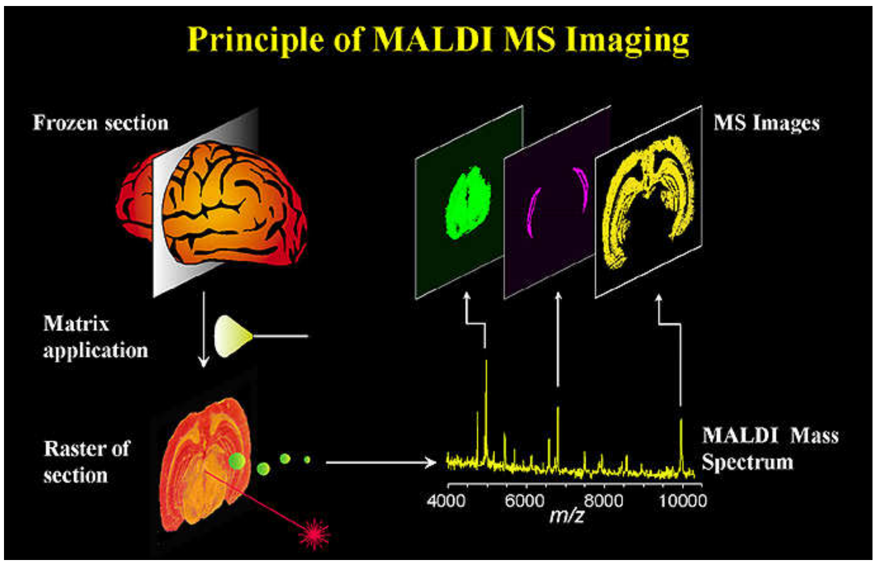

Figure 1: Essential steps in MALDI-MS imaging process[3].

proteolysis, and protein database searching can then determine which proteins correspond to these weights, giving researchers a detailed view of the molecular composition of their samples[1].

Relevance of MALDI-MSI

MALDI-MSI is a revolutionary technology with applications that span all areas of biological research, from cataloguing tissue compositions to investigating tumor formations. Central to the universality of MALDI-MSI is the fact that it allows for the analysis of the entire composition of a tissue sample in one reading, as opposed to individual scans for each molecule of interest[1]. In addition, biological processes can be investigated without the need for understanding the nature of the sample in advance, as is necessary with other techniques like immunohistochemistry or fluorescent labeling. Also, MALDI is supremely applicable to the study of diseases, as it can provide data for the molecular composition of a tissue prior to disease formation, as the abnormality progresses, and can even monitor the effects of potential treatments[7] (Figure 2).

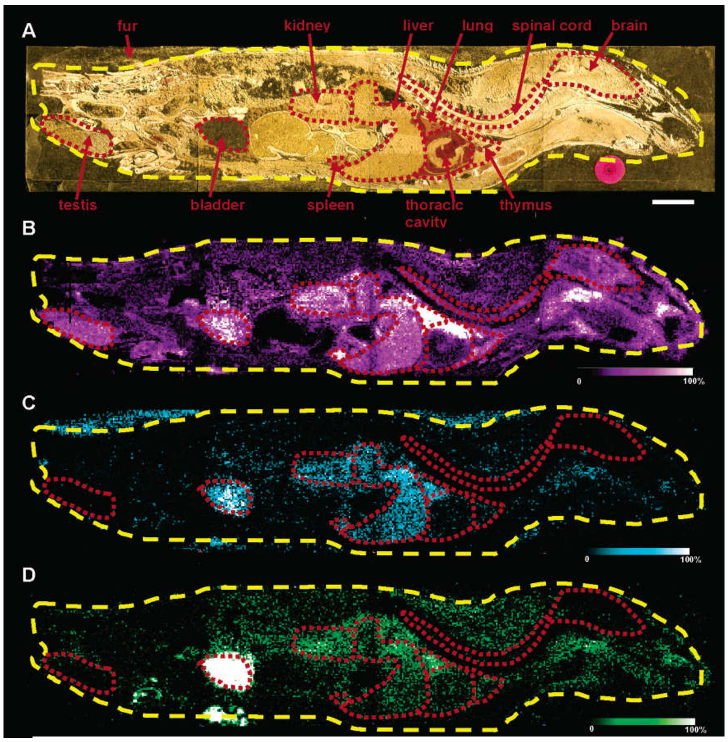

Figure 2: MALDI-MS images of the sagittal plane of a rat, evaluated as part of a pharmaceutical study. Within hours, researchers had determined that their drug had affected all target organs before concentrating in the bladder[7].

Matrix Application for MALDI-MSI

Vital to producing quality images with MALDI imaging is the application of the UV-absorbent matrix. This solution is usually composed of organic solvents that are reactive, volatile, and can absorb light energy[9]. The matrix must be applied to the tissue as a very fine mist, so that upon contact it is will not stream across the sample, but dry in place to form crystals that are ionized by the laser to produce the mass to charge readings[6]. The laser can be programmed to ionize samples within 100 micrometers of each other, and so the matrix must form a uniform crystallization on the tissue surface to ensure the integrity of the readings.

At present, our client and her team apply the matrix, composed of 50% methanol, 2,5-dihydroxybenzoic acid, and 0.1% formic acid, using an airbrush commonly employed for painting applications[2]. They position the sample, sliced to a thickness of only a few microns, on a stainless steel plate held vertically within a frame arranged in a fume hood. The matrix is sprayed horizontally at the sample with the discretion of the user determining the correct distance for optimal application, usually approximately fourteen inches[6].

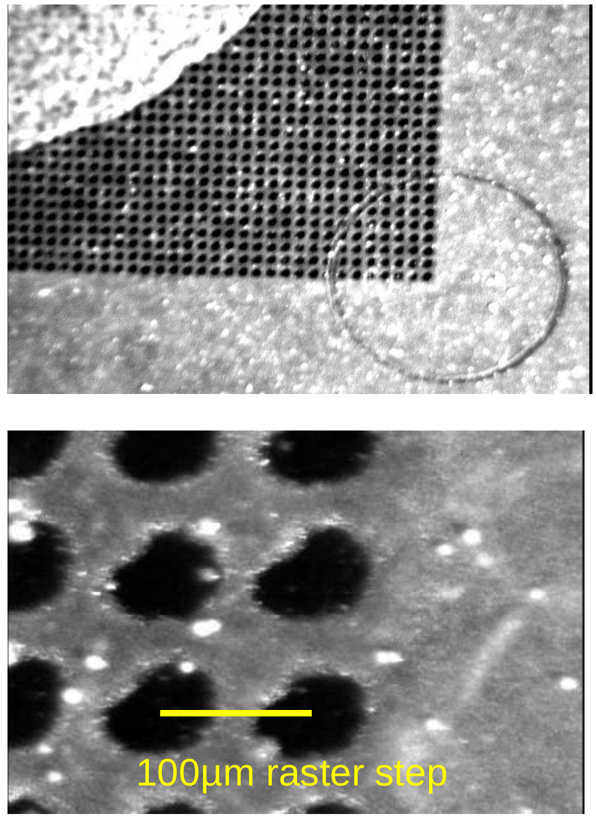

Figure 3: Microscopic images of laser-raster pattern on tissue sample. Metallic flecks on tissue are crystals formed by matrix[2].

Project Motivation

The motivation of our client and her team in proposing this project is to obtain a tool to facilitate the use of MALDI technology by simplifying the matrix application process, and with this tool, centralize a reliable and revolutionary resource in MALDI-MSI at the University of Wisconsin-Madison Genetics - Biotechnology Center for biological researchers on campus and world-wide.

Design Specifications

According to criteria established by our client, the device must spray a uniform coating of matrix over a tissue sample mounted on an 81 centimeter by 123 centimeter stainless steel plate. To achieve this consistency, the matrix must strike the plate as a liquid but not in defined droplets, a characteristic to be controlled by the liquid to gas mixing ratio of the designed apparatus. The device must also include a means of altering the pressure of the air delivering the liquid matrix, an adjustable spray aperture, and variable positioning of the plate and sprayer.

As the applied matrix is a solution of organic solvents, safety is a vital factor in this design. In fact, spraying the matrix creates aerosols, which if allowed to concentrate, could harm the operator. To combat this health risk and isolate the spraying solvent, the application currently takes place in a fume hood. Therefore, the new device must be enclosed in a casing so that the excess matrix is contained and can be easily cleaned each time the device is used. However, this enclosure can not impede the escape of aerosols, due to danger of accumulated pressure, and thus, the designed device must still be operable within a fume hood.

The device will be used daily and maintain function through the current research project, corresponding to a life in service of multiple years. It will also be automated or require minimal manual force to operate. Finally, the device needs to be constructed of materials, costing in total less than $300, that will not dissolve when coated with the caustic matrix, composed of benzoic acid, formic acid, and a 50% methanol solution.

Existing Products

Multiple patent searches using the U.S. Patent and Trademark Office website revealed no patents detailing methods for spraying matrix on to plates for the MALDI-MSI process. Patent 7,095,018 was granted for a sample spot drop method, and the device for which patent 6,918,309 was issued involves an using electric fields to pull the matrix droplets to the correct area on the plate, but no patent directly related to our spray approach was found.

Further internet searches revealed that many MALDI researchers utilize industrial-grade pneumatic sprayers to apply the matrix to their samples, as well as designs employing airbrushes, much like the current method utilized by our client[8].

Design Alternatives

Component Matrix

As a design to address the presented problem involves the assembly of several independent variables, we elected to organize the possible design alternatives in a component matrix. Four components were recognized in this matrix: the spraying system, the method of application, the enclosure, and the orientation of the application (Figure 4).

Feature	**Ideas**				
Sprayer	Automatic Spray Gun	Nozzle-valve with pressure vessel	Nebulizer	Pneumatic sprayer	Produce irrigation system
Method of Application	Movable sprayer, Stationary plate	Stationary sprayer, Movable plate			
Enclosure	Fume hood	Integrated covering	Detached covering		
Orientation	Horizontal	Vertical			

Figure 4: Component Matrix organizing independent design alternatives.

Spraying Mechanisms

As the success of our design rests on the uniformity of the generated spray, we spent considerable time brainstorming potential spraying mechanisms. Five unique ideas were developed, each with their own advantages and disadvantages. The first of these was the produce irrigation system. Derived from ideas inspired by a grocery store produce department, this sprayer would consist of multiple nozzles placed in a horizontal pattern, all having the same aperture and subjected to the same pressure. The individual sprays would mix and interfere with one another prior to contacting the tissue, creating a turbulent cloud of mist that would settle in a more consistent coat than if the individual spray beams were allowed to concentrate in any one location. The disadvantages of this system were the possibility for uneven pressure caused by clogging in any of the multiple nozzles, and consequently, difficulty in maintaining the apparatus.

The second spraying component, the pneumatic sprayer, is the current technology used by other MALDI researchers[8]. The industrial pneumatic sprayer provides an even, homogenous coat of solution at the correct pressure and speed. However, the disadvantages of this system were the feasibility of cost and the location for purchasing the system. Companies who use pneumatic systems did not desire to disclose any information about their model's origin or expense. After much investigation, only one model was located and it cost around $2300, well outside our $300 budget.

A nebulizer, a component of an inhaler system used by patients with asthma, was another original idea generated during brainstorming. While the component could provide adequate pressure, dispense a substantial amount of fluid, is sufficiently maneuverable, and is small in size, it can not provide the required degree of accuracy with regard to the location of the applied spray. In addition, the range of pressure values compatible with the nebulizer is too narrow for the degree of variability we hope to achieve.

The fourth spray system, referred to as the nozzle-valve pressure vessel system, is a design that would involve constructing a custom sprayer system from primary materials. Proposed as an assembly of a pressure vessel, tubing, an air supply, and an appropriate nozzle, the design would meet the requirements of providing a variable pressure, and controllable spray. However, the pressure vessel design is disadvantageous in that it could take a significant amount of time to assemble the device, especially due to our lack of knowledge in constructing pressurized systems.

The final spraying system proposal is the air gun sprayer. The sprayer is an automatic system similar to the airbrush system currently utilized by the client. However, instead of having to manually press a button and subsequently pull it back to control the liquid to gas mixing ratio, the operator simply has to adjust the pressure and fluid levels prior to making a plate sample. At a reasonable cost and minimal construction, the automatic air gun sprayer is another advantageous proposed component of the design.

Method of Matrix Application

In addition, brainstorming was conducted to form designs for the exact manner in which the matrix would be applied by the system. General options include implementing a moving spray system and a stationary sprayer, as the client manually employs at present, or fixing the spray system in a stationary position while the plate containing the sample is made mobile. As the sanctity of the spray consistency is essential to a successful prototype, we decided that leaving the sprayer in one location may improve accuracy with regard to matrix application. Also, a moving conveyor system would be much easier to design to move the plate through the spraying matrix than a mechanism to reliably move the sprayer, as well as automate the application process.

Enclosure

Another aspect of the design, independent of the other components, is the encasement that will surround the sprayer and plate to contain the airborne matrix from coating the fume hood. Three possibilities were formulated for this design characteristic, the first of which involved covering the entire spraying apparatus with a completely independent, clear plastic covering with perhaps one, or even multiple hinged doors cut into the sides to allow for user-access. Another explored option involved a clear, plastic covering that would be integrated into the spraying mechanism, perhaps with the sprayer passing through the top of the plastic into the interior of the box and secured by an o-ring to allow for adjustment. Doors would again allow access to the boxes interior, and although this design might make cleaning the enclosure more difficult, it would also ease user control of the sprayer.

Finally, we also proposed simply leaving the process open to the interior of the fume hood, which would allow the operator complete access to the sprayer and plate, but would also do nothing to shorten the amount of cleaning and maintenance required between applications.

Orientation

The last component of the matrix is the orientation. The client currently sprays the matrix onto the plate in a horizontal orientation, but the idea to explore a vertical orientation was encouraged by her team. This option may benefit the effectiveness of the matrix spray, in accuracy and conservation, as it would utilize gravity to pull the matrix towards the tissue, instead of pulling it away from the desired flight pattern, as with the horizontal orientation.

Design Matrix

To assist in decision making, we elected to quantify the effectiveness of our design based on a set of criteria that modeled the design specifications put forth by our client. Specifically, each of the four components was ranked based on reliability, adjustability, user-interaction, cost, maintenance, and ease of manufacture. Figure 5 shows the breakdown of the weighing system. Reliability and adjustability were weighted most heavily, as they were stated as being the most important criteria for a successful design by our client. The next highest weight was given to user-interaction, which must be minimized in order to prevent human error in the process. Finally, the last three criteria, cost, maintenance, and ease of manufacture, are not as crucial to the success of the design, and therefore we given lower weights of 0.15, 0.10, and 0.05, respectively.

Criteria	Weight
Reliability	0.25
Adjustability	0.25
User-interaction	0.20
Cost	0.15
Maintenance	0.10
Ease of Manufacture	0.05
Total	1.00

Figure 5: Weighted criteria used to evaluate design matrix.

To simplify the presentation of the design matrix, each of the four components, namely spraying mechanism, method of application, enclosure, and orientation, was organized into four separate ranked matrices (Appendix A). The ranking system is based on a scale from 1 to 5, with a score of 5 signifying a component that best met the indicated criteria.

Final Design

The final design incorporates the components that best satisfy the specifications of the sprayer, method of application, enclosure, and orientation of the device, as determined by the rankings of the design matrix. Specifically, the automatic spray gun has been employed to satisfy the spraying component of the design, which remains stationary while the tissue sample transported on the steel plate passes below the misting matrix on a motor-driven conveyor. Thus, the matrix itself is applied vertically, with the spray gun and conveyor components integrated into the plastic enclosure. Following is a detailed description of the constructed prototype, with discussion of the contribution of each component to the end goal of satisfying our client’s design specifications.

Enclosure

The purpose of the enclosure is to contain the spraying method within a confined area to facilitate clean up after sample preparation has been completed, as well as to concentrate the acidic aerosols in a space away from the operator. In addition, the enclosure protects the motor casing from the caustic chemicals and the sparking mechanism within the motor from the flammable solvents. For these reasons, the enclosure is fabricated as a solid shell with two internal cavities from ¼” thick polyester, chosen for its resistance to exposure with ethanol, methanol, benzoic acid, formic acid, and acetonitrile, all chemicals incorporated into the matrix solution (citations). The larger cavity (20” by 16” by 12”) contains the sprayer and conveyor mechanisms, while the small cavity (4” by 2” by 12”) houses the motor.

Figure 6: Front view of the enclosure, supporting the sprayer through a $^{5}/_{8}$” hole in the top panel and the plate conveyor on a panel mounted 2” from the floor. The smaller rectangular box at right is the motor cavity, and the larger enclosed area houses the conveyor and spraying process.

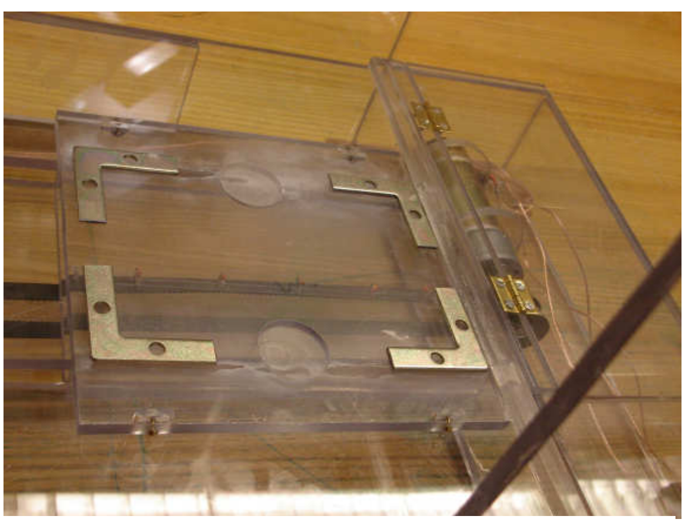

Figure 7: Polyester plate cart (5 $^{7}/_{8}$” wide by 5 ¾” long) that carries the tissue sample along the conveyor through the misting matrix. Three washers have been mounted on a ½” nail axle in each corner to reduce friction as the belt pulls the unit. Finger holes ($^{1}/_{2}$” diameter) are positioned to assist the user in loading and unloading the plate, and brass 1½” square corner brackets hold the plate in position.

At the top of the enclosure, the spray gun is inserted into a $^{5}/_{8}$” diameter hole, in which the nozzle fits snuggly. To maintain the vertical orientation of the spray gun, a ¾” by ¼” by 2$^{7}/_{8}$” stand with a ½” diameter semicircle removed from the top serves as a support for the additional weight of the pipe nipple and air hose. As the design allows for the gun to only be placed into the top plane of the enclosure, the sprayer is easily removable for cleaning and attachment of the air supply. The enclosure front includes a 8 ½" by 11" door hung with two ¾" square hinges along the top edge for easy insertion and removal of sample plates. This door also features a brass latch centered at the bottom edge to maintain the inclusiveness of the enclosure should any pressure build-up within the cavities push the door open, releasing matrix aerosols into the surrounding environment. Moving lower on the device, a polyester cart (5 7/8" by 5 3/4") holds the sample plate and travels on small wheels beneath the spray gun. The wheels travel along the conveyor top, which is easily removable if maintenance or cleaning is necessary. This conveyor top is cut to allow passage of a polyester box that is attached to the underside of the cart. The box serves as the point of attachment to the conveyor belt below while providing coverage of the conveyor belt and pulley from the matrix spray. The conveyor belt travels through a slot in a vertical wall of the enclosure that separates the spraying chamber from the motor. The motor chamber also houses the rocker switch that controls the motor direction, as well as the associated circuitry. A door on the motor chamber, modeled proportionally to the door in the front panel, allows for adjustment or maintenance if needed.

Plate Movement

The conveyor belt is driven by a 12 VDC, 300 mA gear motor from Buehler, Incorporated. The motor speed of 60 rpm was chosen to move the tissue sample the length of the conveyor in approximately three seconds. This corresponds to a spraying time that appropriately covers the tissue sample for one coat. The motor is reversible by alternating the polarity of connection to the power supply, a 120 VAC to 12VDC converter. Polarity reversal is controlled by a rocker switch that is easily accessible to the device operator. Attached to the

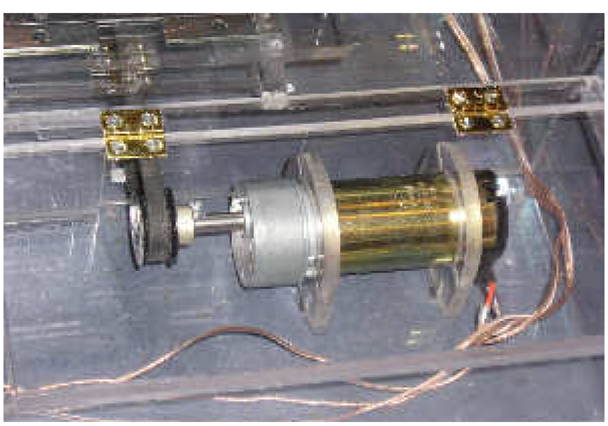

Figure 8: 12 VDC, 60 rpm, 300 mA gear motor from Buehler, Incorporated. As pictured, a 0.787" diameter pulley is attached to the motor shaft to drive the ¼" thick conveyor belt.

motor shaft is a 2.75 cm diameter, polycarbonate miniature timing pulley. A second pulley is mounted at the opposite end of the conveyor, and a 49 cm urethane timing belt serves as the conveyor belt. A polyester tension member preserves the conveyor belt length, which was chosen to span the length of the device. The pulleys and belt are ¼ inch width and share a pitch of 0.08 inches.

When the tissue sample has received one matrix coat and reaches one end of the conveyor, the sample cart flips a normally-closed microswitch that opens the circuit and stops the motor. This allows time for the matrix coat to dry, after which the operator may flip the rocker switch to drive the sample cart back for a second matrix coat.

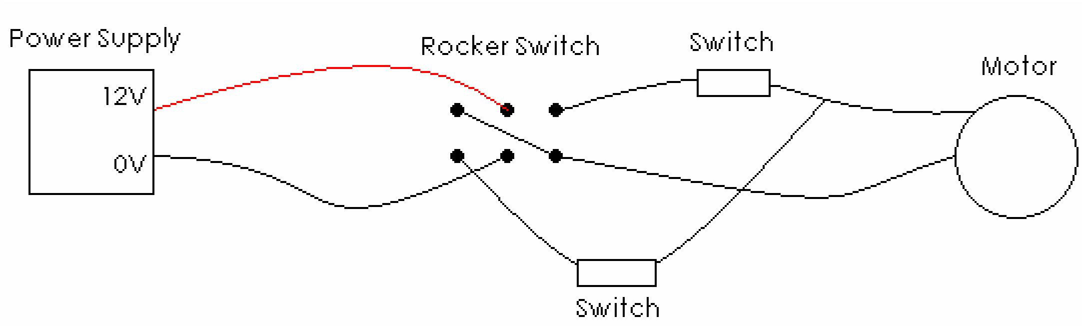

Figure 9: Circuitry that powers the moving conveyor. The operator can use the rocker switch to reverse the polarity of the motor, and in doing so, moves the plate cart towards one of the microswitches, positioned at either end of the conveyor. Upon contact with the switch, the circuit is broken and the plate remains at the respective end of the track to dry before being sent under the spray again by pressing the rocker switch in the opposite direction. This allows for drying time to be determined by the user.

Sprayer

The primary component of the device is a Paasche© A-AU Automatic Spray Gun that is mounted 16 inches above the conveyor top. Preliminary testing of the spray gun determined that this separation results in the fine, even coating desired by the client. The spray gun is equipped with a flat-spraying aperture that is interchangeable if desired. The flat spray spans the conveyor width and covers the tissue sample most

Figure 10: Paasche© A-AU automatic spray gun and corresponding flat spray nozzle operate at a minimum of 50 psi, supplied through the pipe nipple pictured in the background. Flow rate is determined by the black hand valve, also located on the pipe nipple. The attached matrix reservoir can hold up to three ounces of liquid to allow for multiple coats without refilling.

efficiently; however, apertures may be purchased from Paasche© that spray in other variations of the traditional cone shape. The volume of matrix dispensed is controlled by a valve on the spray gun, and a regulator adjusts the matrix-to-air mixing ratio. Matrix enters the spray gun from a 3 oz reservoir, which holds sufficient matrix for multiple coats.

Overall, the client's design specifications included multiple variables to be adjustable or fixed. The device components chosen have been effectively incorporated into the prototype to meet the criteria established and to provide a well-functioning device that improves upon the client's current process of matrix application.

Prototype Testing

Upon completing construction of the prototype, we conducted formalized testing to see if we had indeed met our design criteria, most notably whether the sprayer system could provide a consistent coating o the tissue sample.

To do this, we took ten glass microscope slides, a mixture of water, ethanol, and food coloring, and coated five of these slides with the sprayer system and five with our client's airbrush. Within the five slides for each apparatus, we subjected them to between one and five coats. Once the droplets had dried, we imaged these slides at 100 times magnification using phase contrast techniques with an Olympus Ix51 microscope and SimplePCI software.

We used the obtained images to conduct a qualitative drop size consistency comparison, and found that in general, the droplets sprayed with the sprayer system have a much more uniform shape, size, and spacing within the slide, as well as across all five passes, than do the slides sprayed with the airbrush, for which we observed much irregularity (Appendix ?).

Then, we conducted a quantitative comparison of the overall percent coverage of the slides sprayed with the respective devices. For this study, we printed copies of all of the fields of view that covered the slides, and measured the distance between approximately 100 droplets and the droplets in closest proximity. The number of measured distances less than or equal to 100 microns were recorded, along with the total number of measurements made per slide, and these data were used to produce percent coverage values. We defined 100% coverage as a state in which all of the droplets on the slide were within 100 microns of each other, as this is the step size that can achieved by the laser during imaging. Again, the conducted study showed that the sprayer system was able to provide a greater percent coverage with each pass than was the airbrush, achieving our defined "100%" coverage within the five coats, while the airbrush did not.

Based upon these results, we concluded that with our design sprayer system, we had indeed provided our client with a prototype that could achieve a more consistent coating of tissue samples than her original airbrush.

Future Work

The prototype provides an improved method of matrix application with a more even coating and greater percentage of tissue sample coverage. However, further testing conducted in the Mass Spectrometry Laboratory could result in identification of the ideal flow rate of matrix from the spray gun and the ideal speed of the conveyor. We expect that a decreased flow rate may further improve the performance of our prototype by applying more even coatings and minimizing excess matrix used.

Polyester was chosen for the device enclosure due to its translucence and chemical resistance to the matrix chemicals. Although polyester is resistant to the matrix used by our client, high concentrations or additional chemicals could degrade the device enclosure. Because the matrix composition may be changed, we have begun researching liquid coatings from 3M Corporation that would provide additional chemical resistance. Applying a coating inside the spraying chamber would further protect the device enclosure and components, and identifying an appropriate liquid coating would be our first priority of future work.

Ethical Considerations

The success of our project is contingent upon not only fabricating a functioning prototype, but also fulfilling all ethical considerations involved with our design. Namely, the main ethical consideration that we must consider in creating the prototype is safety. As the sample preparation methods for MALDI-MSI involve volatile chemicals forced out of a nozzle at high pressure in a manner that produces aerosols, we must design our prototype to protect the operator from the matrix, and ensure that the prototype can be operated in an environment, such as a fume hood, that provides further protection. In addition, the materials used to construct the device must be as resistant to the matrix as possible to ensure that over time the parts will not erode and eventually cease functioning, perhaps in a manner that could harm the operator. Finally, we must design the prototype to house the motor in a separate chamber away from the matrix, as any significant spark from the motor could ignite the chemicals of the matrix, potentially injuring the user. Therefore, to successfully engineer a prototype to meet the needs of our client, in addition to fulfilling all design criteria, we must evaluate the ethical consequences of our decisions and construct a design that is safe for all involved.

Conclusion

In summary, the tissue coating sprayer system satisfies all criteria set forth by our client, as corroborated by a series of conducted tests. The Paasche© A-AU spray gun and matrix reservoir can hold up to three ounces of liquid and deliver this solution in a fine, flat spray at a variable pressure at or above 50 pounds per square inch. The tissue sample, carried by the conveyor driven by a 12 VDC reversible gear motor, can be coated multiple times at the operator's discretion. With the polyester enclosure, the spraying process can be conducted within a fume hood without coating the entire area with the chemical compounds while maintaining the operator's separation the misting aerosols. In addition, the enclosure separates the moving conveyor parts from the matrix compounds to extend the working life expectancy of the prototype. While additional improvements to the prototype may be required after additional testing in a clinical laboratory setting with the client present, we believe that our final design meets the specified criteria, and hope that our client may utilize the device to assist biological researchers in reliable and timely data acquisition, potentially to study and cure human disease.

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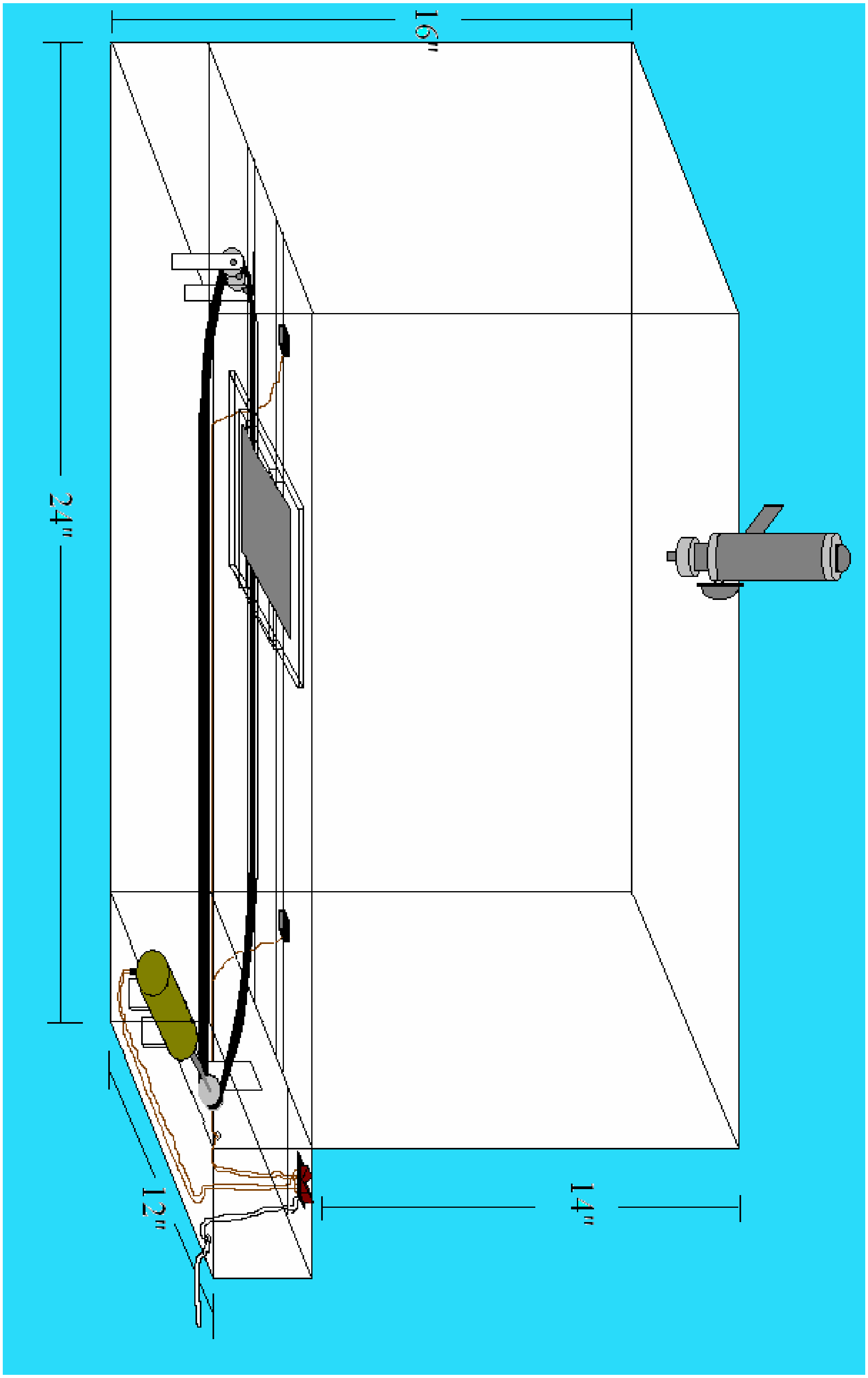
[8] MALDI-MSI, the Site for MALDI Mass Spectrometric Imaging. <http://www.maldi-msi.org>

[9] “Polyester – Chemical Resistance.” The Engineering Tool Box. <http://www.engineeringtoolbox.com/chemical-resistance-polyester-d_784.html>

[10] Sugiura, Yuki. “Two-Step Matrix Application Technique to Improve Ionization Efficiency for MALDI-MSI.” *Analytical Chemistry*. 2006, 78(24) 8227-8235.

Appendix A: Schematic and Gross Dimensions of Final Design



Appendix B: Testing Results

Qualitative Drop Size Consistency Study:

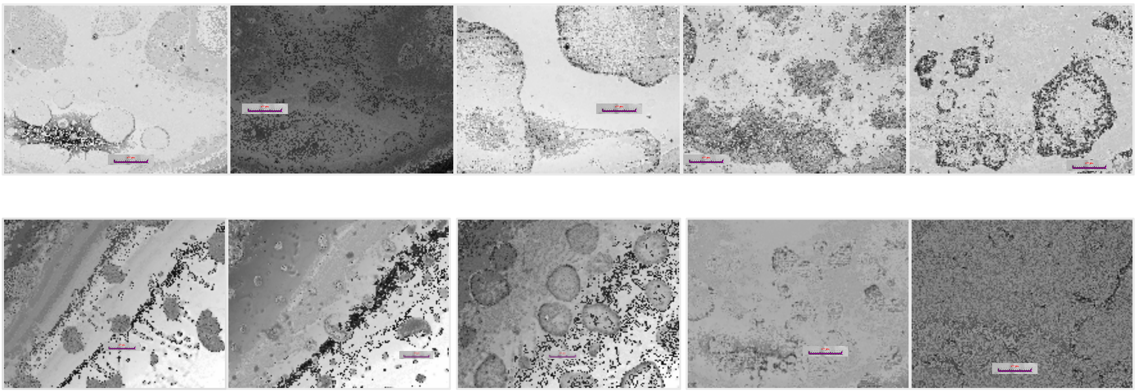


Figure 11: Images of dried water droplets taken with an Olympus IX51 microscope at 100x magnification with phase contrast microscopy. Purple scale bars indicate 100 μm. Pictures from left to right correspond from one to five passes made with the air brush (top) currently used by our client, and the spraying system (bottom), respectively. In general, drop size from spraying system appears more consistent than that of the airbrush with drops in each frame presenting very similar shapes until the fifth pass when drops were close enough together to pool. Drop size in the airbrush frames, however, varies greatly within each frame as well as across all five passes.

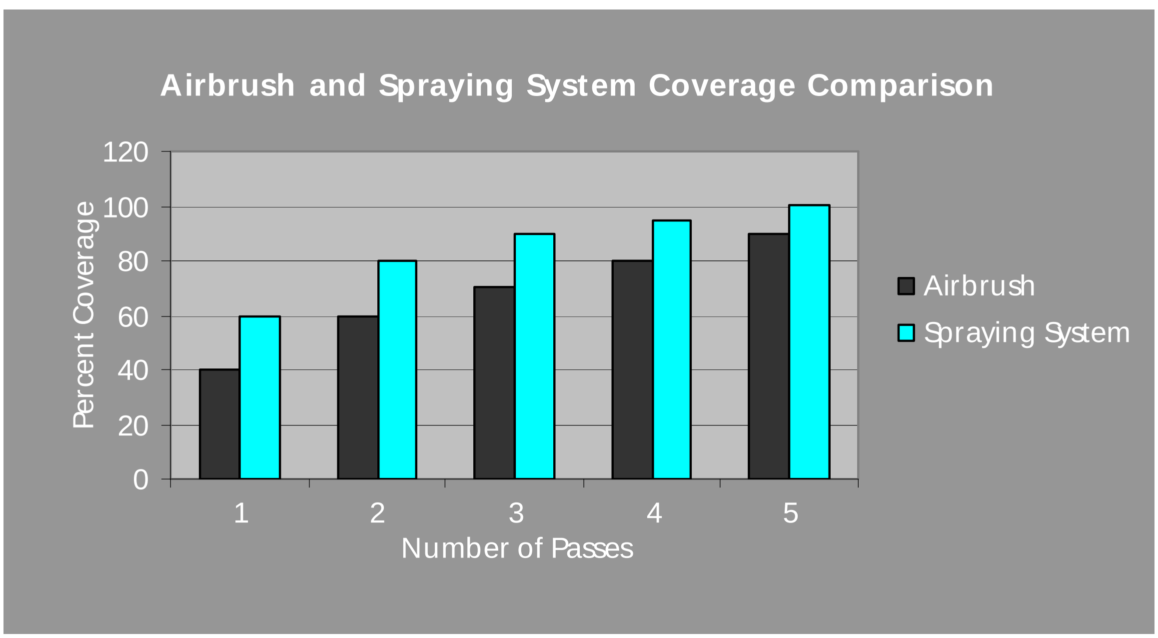


Figure 12: Using slides from the drop size comparison, percent coverage was estimated using a ruler to determine the distance between dried droplets. As the laser employed by MALDI-MSI can raster steps as close as 100 μm, total coverage was defined as a situation in which all of the droplets are within 100 μm those closest to it on the slide. In general, those slides sprayed with the spraying system had a higher percent coverage than those sprayed with the airbrush.

Appendix C: Cost Summary*

Description	Vendor	Price	Quantity	Total
Automatic Spray Gun	Paasche	$126	1	$126
¼” pipe nipple	Paasche	$34	1	$34
Flat spray nozzle	Paasche	$12	1	$12
Timing Belt	Sterling Instruments	$7	1	$7
0.787” diameter pulley	Sterling Instruments	$8	2	$16
12 VDC motor	MPJA, Inc.	$20	1	$20
Liquid Nails, Plastic Glue	Home Depot	$3.97	1	$3.97
4 pack of ¾” hinges	Home Depot	$1.96	1	$1.96
12 V 500 mA AC Adapter	Radio Shack	$16.99	1	$16.99
Spool of wire	Radio Shack	$4.99	1	$4.99
Microswitches	Radio Shack	$2.69	2	$5.38
3/8” Bolts	True Value	$0.06	4	$0.24
4 pack of flat 1 ½” corner brackets	True Value	$2.49	1	$2.49
2 pack of hooks/clasps	True Value	$2.79	1	$2.79
Screws	True Value	$0.05	16	$0.80
¼” washers	True Value	$0.05	12	$0.60

Grand Total: **$255.21**,
well within our $300 budget

*Due to the ordering process conducted by our client, the first six total values in this chart are estimations and have not taken into account shipping fees or taxes. Consequently, the grand total is subject to change, but should be relatively close to the displayed value.

Appendix D: Design Matrix Separated by Component

Criteria	Rank	Spraying Component: *Nebulizer*	*Nozzle-valve and pressure vessel*	*Automatic Spray Gun*	*Pneumatic sprayer*	*Produce irrigation system*
Reliability	0.25	1	3	4	5	2
Adjustability	0.25	1	4	5	5	2
User Interaction	0.20	5	4	4	5	3
Cost	0.15	4	3	5	1	3
Maintenance	0.10	1	3	3	4	2
Ease of Manufacture	0.05	1	3	4	1	2
Total	1.00	2.25	3.45	4.30	4.10	2.35

Criteria	Rank	Method of Application: *Movable Plate Stationary Sprayer*	*Stationary Plate Movable Sprayer*
Reliability	0.25	3	3
Adjustability	0.25	3	3
User Interaction	0.20	4	2
Cost	0.15	4	2
Maintenance	0.10	3	3
Ease of Manufacture	0.05	3	1
Total	1.00	3.35	2.55

		Enclosure		
Criteria	**Rank**	*Fume Hood*	*Integrated Cover*	*Detached Cover*
Reliability	0.25	-	-	-
Adjustability	0.25	1	5	2
User Interaction	0.20	1	4	4
Cost	0.15	5	2	3
Maintenance	0.10	1	4	5
Ease of Manufacture	0.05	5	3	4
Total	1.00	1.55	2.9	2.45

		Orientation	
Criteria	**Rank**	*Horizontal Spray*	*Vertical Spray*
Reliability	0.25	2	5
Adjustability	0.25	-	-
User Interaction	0.20	-	-
Cost	0.15	-	-
Maintenance	0.10	-	-
Ease of Manufacture	0.05	-	-
Total	1.00	0.5	1.25

Appendix E: Product Design Specification

MALDI-MSI Tissue Coating Device

Product Design Specification

Last Updated: March 12, 2007

Team Members:

- Holly Liske, BWIG
- Laura Piechura, Leader
- Kellen Sheedy, Communications
- Jenna Spaeth, BSAC

Function:

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is an imaging method that allows for label-free spatial analysis of biological tissue samples. This technology can be used to identify and quantify proteins, monitor protein biomarkers, and sequence polypeptide chains, techniques that can be applied to proteomic analysis of disease formation. However, sample preparation methods, especially with regard to the application of the matrix tissue coating, are difficult to control but require accuracy and precision. A device must be developed to apply a fine, uniform coating of light-absorbing compounds in order to simplify the sample preparation process. The goal is to provide a reliable tool to enhance the MALDI-MSI technique in order to speed and simplify potentially life-saving research.

Client Requirements

- The device must be able to spray an even coating of matrix over an 81 cm by 123 cm tissue sample to achieve uniform distribution of the compound.
- The device should be adjustable with regard to spray aperture, air pressure, and the positioning of the plate and sprayer to account for abnormalities in the application process.
- The device must be in an enclosed casing or must be operable within a fume hood as the spraying process involves the production of aerosols and utilized organic solvents.

Design Requirements:

I. Physical and Operational Characteristics

a. *Performance requirements:* This device will be used to apply a coating on tissue samples for MALDI imaging. The distance between the device and the tissue sample needs to be adjustable, and the device needs to be able to move so that it applies a fine, even layer of matrix onto the tissue sample. The pressure of the matrix needs to be adjustable, and precautions should be taken to contain excess matrix that misses the tissue sample. Finally, it should be easy to disassemble so that it can be cleaned after each use.

b. *Safety:* Since this experimentation involves aerosols, all testing should be done in a hood. Safety glasses and gloves should be worn at all times when dealing with the matrix to avoid getting it in the eyes or on skin. Breathing in the matrix should also be avoided.

c. *Accuracy and Reliability:* This device must evenly apply a layer of matrix onto the tissue sample. The layer must be fine, and strike the tissue sample wet, but water drops cannot form before hitting the tissue

d. *Life in Service:* This device must be able to endure concentrated periods of intense use of approximately four hours a day and also be able to endure up to two month periods when there is little or no usage. The life of the device should last at least five years.

e. *Shelf Life:* The matrix solution is an organic solvent capable of causing internal damage to device components. All components must therefore be easily disassembled to allow for regular cleaning. The design specifications include a device enclosure that will serve as protection from external conditions during and between uses.

f. *Operating Environment:* The device will operate at standard temperature and pressure, and low humidity will be maintained inside the device enclosure. All components must be able to withstand repeated coating with the organic solvent matrix.

g. *Ergonomics:* The well insert and matrix solution must be easily placed into the device enclosure, and the enclosure must be easily opened and closed between uses. Minimal user interaction is desired during operation.

h. *Size:* The device size is restricted to the working area of a standard laboratory fume hood, and minimizing the overall dimensions is desirable. The device height must allow for sufficient distance between the tissue sample and release of matrix but must not restrict the view of the device user. The device must accommodate a standard 384 well insert (123 by 81 millimeters) and hold 10 to 15 milliliters of matrix solution.

i. *Weight:* The weight limit of the unit may vary based on design proposals, however; a hand-held airbrush system should not exceed the weight of five pounds. On the contrary, a unit mounted on a base needing only to be moved when not under operation could have a weight limit of up to twenty pounds.

j. *Materials:* Any material that comes into contact with the matrix solution can not dissolve in organic compounds like methanol or acetonitrile. Inert materials like stainless steel, polypropylene, and Teflon are applicable.

k. *Aesthetics, Appearance, and Finish:* For this design, function far outweighs appearance. However, we will strive to give our client a professional, well-manufactured design.

II. Production Characteristics

a. *Quantity:* One unit is needed for this project

b. *Target Product Cost:* The target product cost is $300, the maximum funding allotted Dr. Harms. Significant expenses may include a stepper motor to power a conveyor that moves the plate and also a different spraying mechanism, should we decide to avoid the current air brush design.

III. Miscellaneous

a. *Standards and Specifications:* The device indeed must comply with all FDA standards related to the administration of aerosols and chemicals as well as the use of live tissue test subjects. Specific standards may be found at the Food and Drug Administration website.

b. *Customer:* Ideally, the client desires a chamber to enclose the matrix spray process in which the plate can be placed, a few buttons pushed, and the matrix is sprayed with perfect consistency. Also, the capability to adjust each of the variables in the current airbrush process is desired. However, our client is open to any solution that can be generated and has no prejudice against other design ideas.

c. *Patient-related concerns:* The "patient" of the MALDI-MSI process is a living tissue, let it be a cross-section or an entire animal. Therefore, for the health of any living animal that is scanned, and for the integrity of any results, the plate upon which the tissue rests should be cleaned and sterilized between uses. However, the matrix application device itself does not need to be sterilized after each use, but only cleaned regularly to ensure the purity of the tissue coating.

d. *Competition:* Multiple patent searches using the U.S. Patent and Trademark Office website revealed no patents detailing methods for spraying matrix onto plates for the MALDI-MSI process. Patent 7,095,018 was granted for a sample spot drop method, and patent 6,918,309 involved an invention using electric fields to pull the droplets to the correct area on the plate, but no patent directly related to our spray approach was found.