

Device to Apply a Matrix Coating to Tissue Samples for MALDI Mass Spectrometric Imaging

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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 technology can be used to identify and quantify proteins, locate and monitor 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. The goal of this project is to design a device to apply a fine, uniform coating of light-absorbing compounds in order to simplify the sample preparation process and facilitate the MALDI-MSI technique. Design involving pneumatic sprayers, pressure-valve systems, and single-action airbrushes have been explored and considered. The proposed final design implements a polyethylene enclosure that houses an automatic spray gun oriented to spray vertically-downward onto a motor-driven conveyor that carries the tissue sample through the misting solution. Future development on the prototype will include ordering materials, constructing the device, and testing the model in a laboratory setting to ensure fulfillment of the proposed design criteria.

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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 their

molecules weights (Figure 1). Further analysis though protein extraction, HPLC fractionation,

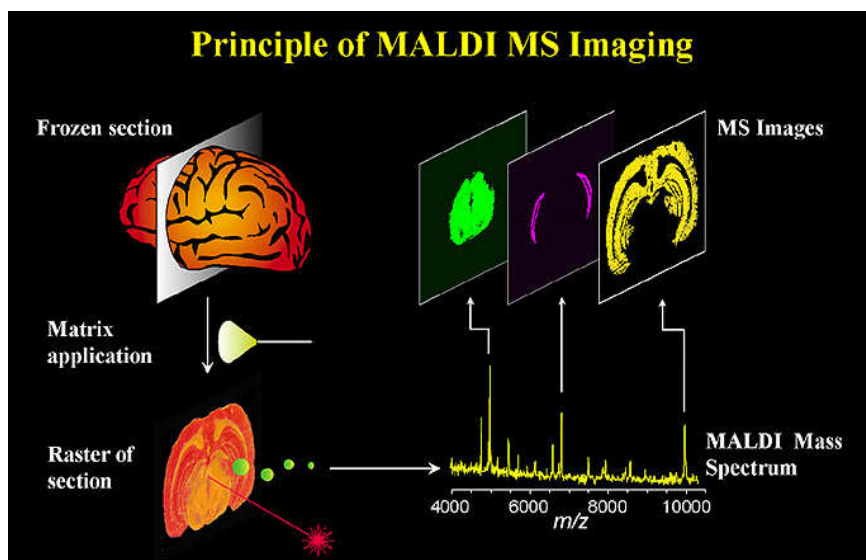


Figure 1: Essential steps in MALDI-MS imaging process [3].

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 that 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

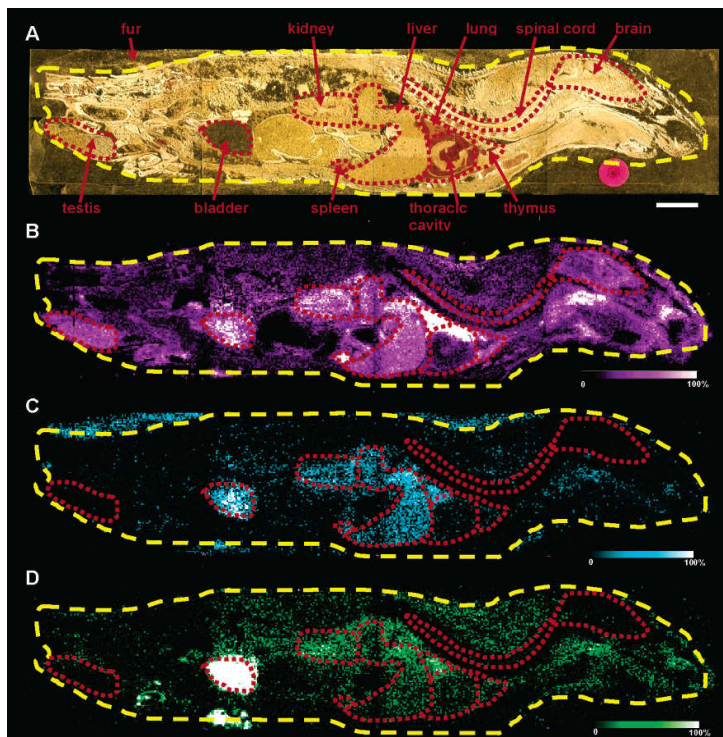


Figure 2: MALDI-MS images of the sagittal plane of a rat, evaluated as part of a pharmaceutical study. Within hours, researchers had determined that their drug had affected all target organs before concentrating in the bladder [7].

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 (Figure 2) [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 [8]. The matrix must be applied to the tissue as a very fine mist, so that upon contact it 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].

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

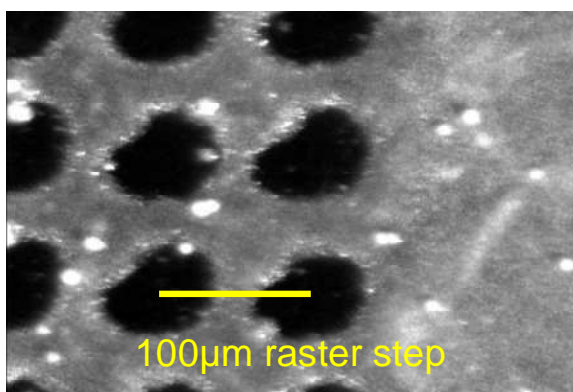
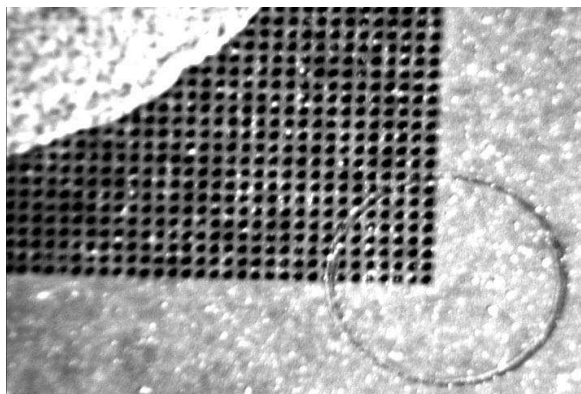


Figure 3: Microscopic images of laser-raster pattern on tissue sample. Metallic flecks on tissue are crystals formed by matrix [2].

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 researches 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.

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	Single-action airbrush	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. The industrial pneumatic sprayer provides an even, homogenous coat of solution at the correct pressure and speed. However, the disadvantage 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 pull 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 designs.

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. Following is a description of each component and its contribution to the overall device function.

Sprayer

A Paasche© A-AU Automatic Spray Gun will be utilized to spray the matrix (Figure 6) and will be held by an o-ring in the top face of the device enclosure (Figure 3c). The o-ring will allow for slight distance adjustment by allowing the sprayer to slide vertically, perpendicular to the plane of the top face of the enclosure. The spray gun is operated with compressed nitrogen, similar to the airbrush currently implemented by our client. However, the spray gun is controlled by an automatic, handheld device for greater accuracy in terms of air pressure and fluid volume adjustments than what is possible with a manual airbrush. Interchangeable spray gun apertures are also available that provide multiple options for optimizing the matrix distribution. We intend to purchase three to four apertures that produce both cone and flat sprays. Testing will determine the optimal aperture to satisfy the requirements of the sprayer. The spray gun is more expensive than a manual airbrush, but automation will minimize user-interaction and increase the reliability of matrix distribution.

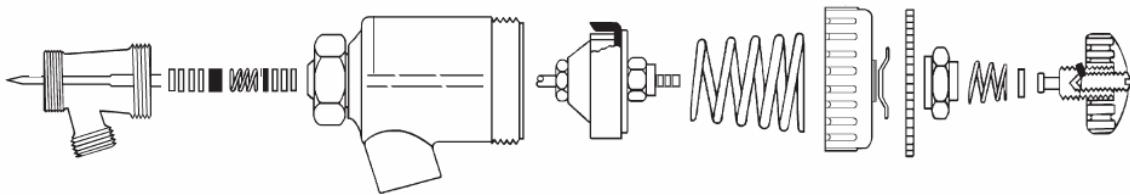


Figure 6: Expanded diagram of the Paasche© A-AU Automatic Spray Gun.

Method of Application

Based on the totals generated in the design matrix, the plate containing the tissue samples will move with respect to the spray gun, which will remain stationary. The tissue sample plate will be positioned on a platform connected to two timing belts. These timing belts, along with four pulleys and a small reversible motor, will drive the plate from where its initial position, through the matrix spray, to the opposite end of the device, out of the reach of the dispelling matrix (Figure 8b). The

spray gun will be turned off, and the matrix will be allowed to dry. After the coat has dried, the sprayer will again be initiated, the motor direction will be reversed by pushing the rocker switch (Figure 8c), and the next matrix coat will be applied.

The 60 rpm, 12 VDC reversible motor will turn the conveyor pulleys by an extended shaft or gear drive and will be housed outside the primary device enclosure (Figure 7). This is necessary because the motor could produce sparks that could ignite the matrix if direct contact occurs.



Figure 7: DC reversible motor that will drive the device conveyor (Buehler Motors).

Enclosure

A low density polyethylene (LDPE) encasement will be incorporated into the device to contain the matrix (Figure 8d). LDPE is relatively inexpensive and available in sheets of ¼ inch thickness, which makes it ideal for our application. The box will enclose the entire spraying process, and a small, hinged door will allow for positioning the tissue sample on the conveyor platform (Figure 8e). LDPE was chosen for its resistance to the matrix chemicals in order to increase the life in service of our design [4]. Also, LDPE is available as a colorless sheet, which is important for the operator's ability to monitor the spraying process. In addition, white opaque LDPE will be used as a secondary enclosure for the conveyor, motor, and circuitry of the device.

Orientation

The sprayer will be oriented vertically to maximize the consistency of the spray on the tissue sample. Gravity affects the direction of a horizontal spray, whereas the vertical orientation ensures that the matrix will fall directly towards the sample instead of along a hyperbolic path. Also, the orientation of a vertical sprayer can remain constant even for varying matrix densities, while a heavier matrix density could produce significant inconsistencies if applied horizontally.

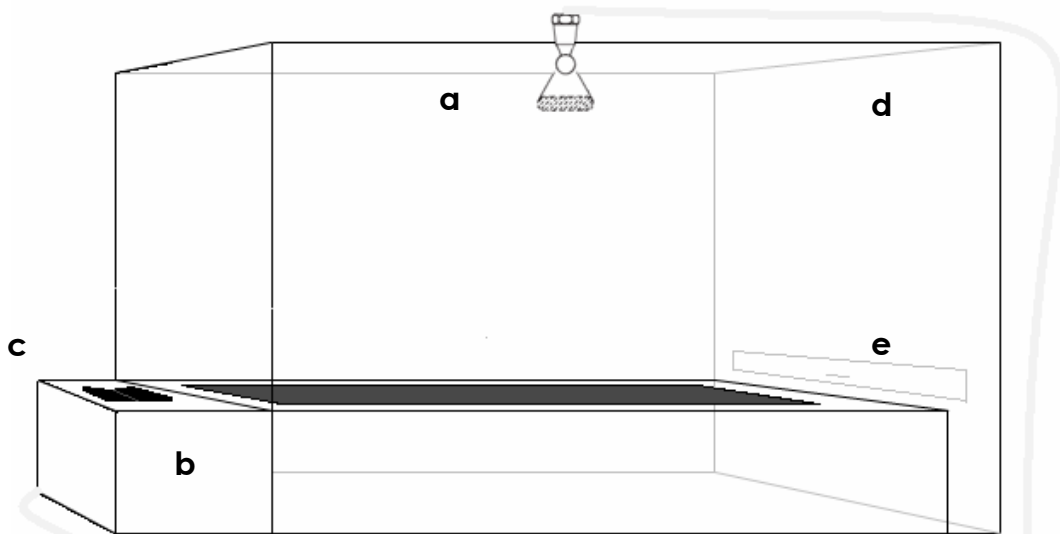


Figure 8: (a) Automatic Spray Gun. (b) Motor and conveyor. (c) Motor control switch. (d) Enclosure. (e) Opening to insert tissue sample.

Future Work

In the future, we need to confirm the details of our final design, such as how the sprayer is attached to the casing, and the dimensions of the device's enclosure. We must order the materials that we have selected for the final design, as well as determine if any other supplies are necessary for construction. We can then construct our prototype.

It is essential that we allow as much time as possible to test the device in our client's laboratory, as only there will we gain a true understanding of our prototype's clinical capabilities.

Initially, we will assess the device with the matrix solution employed by our client to learn how adjustments with the aperture, air pressure, and distance between the sprayer and plate can alter the uniformity of the matrix coating. In this way, we will be able to show the researchers that will be using the device how to adjust the settings to achieve the desired effects.

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

With the remaining weeks, we hope to utilize the final design created during the first half of the semester to construct a quality prototype for Dr. Amy Harms and her team of researchers at the University of Wisconsin-Madison Genetics-Biotechnology Center. With success in producing a device

to facilitate and simplify the matrix application process for MALDI-MSI sample preparation, we hope to provide our client with a reliable tool to revolutionize biological research both on campus and even throughout the global community.

References

- [1] Ashcroft, Alison. University of Leeds Astbury Centre for Structural Molecular Biology. “An Introduction to Mass Spectrometry.” <<http://www.astbury.leeds.ac.uk/>>
- [2] Barrett-Wilt, Greg. University of Wisconsin Biotechnology Center. Personal interviews.
- [3] Caprioli Research Laboratory Center. Vanderbilt Medical Center.
<<http://www.mcvanderbilt.edu>>
- [4] “Chemical Resistance of Polyethylenes.” SABIC EuroPetrochemicals 2005.
<http://polymers.sabic-europe.com/technical/_media/chemical_resistance_pe.pdf>
- [5] “Chemical Resistance of Polypropylene and Polyethylene.” Orion Fittings, Inc.
<http://www.orionpipe.com/docs/acidwaste/Orion-AW-Chem_Resistance.pdf>
- [6] Harms, Amy. University of Wisconsin Biotechnology Center. Personal interviews.
- [7] Khatib-Shahidi, Sheerin. “Direct Molecular Analysis of Whole-Body Animal Tissue Section by Imaging MALDI Mass Spectrometry.” *Analytical Chemistry*. 2006, 78(18) 6448-6456.
- [8] Sugiura, Yuki. “Two-Step Matrix Application Technique to Improve Ionization Efficiency for MALDI-MSI.” *Analytical Chemistry*. 2006, 78(24) 8227-8235.

Appendix A: Design Matrix Separated by Component

		Spraying Component				
Criteria	Rank	<i>Nebulizer</i>	<i>Nozzle-valve and pressure vessel</i>	<i>Single-action airbrush</i>	<i>Pneumatic sprayer</i>	<i>Produce irrigation system</i>
<i>Reliability</i>	0.25	1	3	4	5	2
<i>Adjustability</i>	0.25	1	4	5	5	2
<i>User Interaction</i>	0.20	5	4	4	5	3
<i>Cost</i>	0.15	4	3	5	1	3
<i>Maintenance</i>	0.10	1	3	3	4	2
<i>Ease of Manufacture</i>	0.05	1	3	4	1	2
Total	1.00	2.25	3.45	4.30	4.10	2.35

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

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

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

Appendix B: Product Design Specification

MALDI-MSI Tissue Coating Device

Product Design Specification

Last Updated: March 12, 2007

Team Members:

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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.