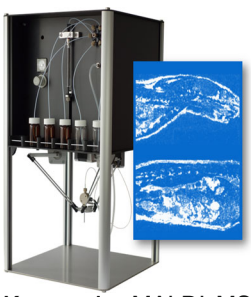
J. Am. Soc. Mass Spectrom. (2015) 26:911–914
DOI: 10.1007/s13361-015-1099-9

FOCUS: IMAGING MASS SPECTROMETRY: RESEARCH ARTICLE

Reproducible Matrix Deposition for MALDI MSI Based on Open-Source Software and Hardware

Markus Stoeckli, Dieter Staab

Novartis Institutes for BioMedical Research, WSJ-155.2.27, Novartis Campus, 4056, Basel, Switzerland



Abstract. The new open-source software and hardware matrix deposition device named iMatrixSpray was optimized and specified for homogeneity, reproducibility, and sensitivity in MS imaging experiments. The results confirm the design claims, with the device delivering uniform coatings with a constant quality from experiment to experiment. The robustness in combination with the open design allows developing and sharing of matrix deposition and sample preparation protocols between labs. This tool therefore enables researchers to enter the field of MALDI MSI without previous experience in matrix coating.

Keywords: MALDI MSI, Imaging, Matrix, Spray, iMatrixSpray, Open-source

Received: 8 January 2015/Revised: 10 February 2015/Accepted: 10 February 2015/Published Online: 21 March 2015

Introduction

If there is one single step in a MALDI MS imaging (MSI) experiment to be named, which proved to be the most difficult to optimize and reproduce, then matrix deposition leads this list. In contrast to a traditional MALDI MS experiment, imaging requires for the matrix to be evenly and reproducibly distributed over the area to be imaged. That matrix application requires analyte extraction and matrix incorporation while maintaining spatial fidelity imparts additional complexity to this step. It is for this challenge that the search for the optimal matrix deposition is still ongoing, despite the many published solutions.

Early reports on MALDI MSI describe manual matrix deposition procedures [1, 2], which turned out to be extremely difficult to be reproduced by other labs, and often just a single person in the originating lab would have the experience to replicate the procedure. It is for this reason that frequently the only way to gain matrix deposition skills was to learn from an experienced scientist. This scientific knowledge exchange was fostered by initiatives including the foundation of the National Research Resource for Imaging Mass Spectrometry (http://www.mc.vanderbilt.edu/root/vumc.php?site=ims) and the COST Action BM1104 on Imaging Mass Spectrometry (http://www.cost.eu/COST_Actions/bmbs/Actions/BM1104).

While these initiatives proved successful in spreading the knowledge and skills on matrix deposition, academic labs and instrument manufacturers started to develop devices which would automate this process. Examples are drop deposition devices [3–5], sprayers [6–8], nebulizers [9], sublimators [10–12], or combination thereof [13]. We decided to take the knowledge sharing concept one step further by designing a matrix deposition device named iMatrixSpray and making the design publicly available [14]. This device is published under the free and open source software and hardware concept and aims at providing a platform that could be freely built and modified by anyone at low cost. In parallel, the device is being distributed as a prebuilt kit to provide access to labs that do not have the capability to build the device (http://imatrixspray.com). The aim of this project is to have a standardized and wide-spread device that can be used to run published protocols without manual interactions. This lowers the barrier for new users to enter the MALDI MSI field, and supports the global effort to move MALDI MSI forward.

In this article, we report test results of the latest version of the iMatrixSpray (Figure 1), evaluating the two most critical parameters: homogeneity and reproducibility. An optical evaluation of surfaces sprayed with dye was selected for this experiment to separate artifacts that might be introduced by the MS analysis.

While the test with ink can provide data on the robustness of the device, it is the resulting MS images that are the most relevant readout for a matrix coating device. For example, a dry deposition of the matrix can result in a homogeneous matrix layer, but the resulting MS data might only contain signals from lipid. An ideal matrix deposition device with an optimized protocol will result in a sample that exhibits high

Correspondence to: Markus Stoeckli; *e-mail:* markus.stoeckli@novartis.com

Figure 1. Open-source software and hardware device for matrix deposition. The design was optimized to allow a fully automated process, including priming and cleaning. Protocols can be downloaded to the device from any source, fostering co-development between labs

signal intensities in MS, while maintaining the spatial distribution of the analytes matching to the imaging resolution. An optimized protocol was tested using dosed whole-rat sections.

Experimental

Materials

SERVA Blue G dye, α-cyano-4-hydroxycinnamic acid (CHCA), and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Dye Coating

SERVA Blue G dye was dissolved in acetonitrile/water 50:50 (v/v) to a concentration of 10 mg/mL and used as spray solution. Generic copier paper of 80 g/m^2 was coated using these parameters on the spray: 1 to 5 spray cycles, density 1 µL/cm^2, speed 180 mm/s, 1 mm pitch between lines, 60 mm distance of spray to surface. The sprayer was placed in a chemistry hood to protect the operator from fumes. Each experiment was run in triplicate, resulting in 15 samples, which were scanned with a HP Scanjet 5590 flatbed scanner at 254 dpi and a color depth of 24 bits. The images were imported into BioMap and evaluated using the "Pixel Statistics" function by defining a region of interest with the dimensions of a SBS microtiter plate (128×86 mm).

For the reproducibility evaluation, 15 paper sheets were sprayed in sequence with the protocol listed above (five spray cycles each) and evaluated by scanning and importing the data into BioMap according to the procedure described above. The intensity of the whole plate area was averaged and the mean intensity value was compared between the samples.

MALDI MSI

With a focus on low molecular weight pharmacological lead compounds and peptides, the following spray coating procedure was optimized and applied to a 40 µm thick whole-rat section: 10 mg/mL CHCA in acetonitrile/water 50:50 (v/v), four cycles, density of 3 µL/cm^2 each, 180 mm/s speed, 1 mm line pitch, 60 mm spray height. The rat was orally dosed with 30 mg/kg of an active substance and sectioned as described here [14]. The section was subsequently measured with a FlashQuant System (AB SCIEX, Framingham, MA, USA) in SRM mode and 500 µm spatial resolutions.

Results and Discussion

The visual inspection of the dye-coated sheets of paper confirmed a uniform layer over the spray area, with the surface not being completely covered after a single spray cycle. This results for the measured coefficient of variance (CV) of the pixel intensities to be 15% for these samples. Increasing the number of cycles reduces the CV to 7% after four coats, which is a typical number for a matrix coating protocol (Figure 2, top). An inspection for systematical artifacts by integrating the pixels along both axes of the images did not reveal any systematic inhomogeneity. This result confirms the validity of the design concept, with a relatively broad spray cone of 1 cm in diameter and defining the spray path to cover an area which is 2 cm wider on each sided than the actual sample plate. While this results in a waste of spray reagent of 43% outside of the sample, it guarantees a homogeneous coating from border to border. It is for this reason that we always coat the full area, also when smaller samples are to be processed.

The outcome of the coating to coating reproducibility experiment is shown in the bottom graph on Figure 2. The values stay close together, with the largest deviance from the mean value of 3%. This high reproducibility is a product of the fully automated operation that includes priming and cleaning of the spray. Beside the spray parameters, there are other parameters that have an influence on the crystallization of the deposited matrix, including temperature, humidity, and air flow. The

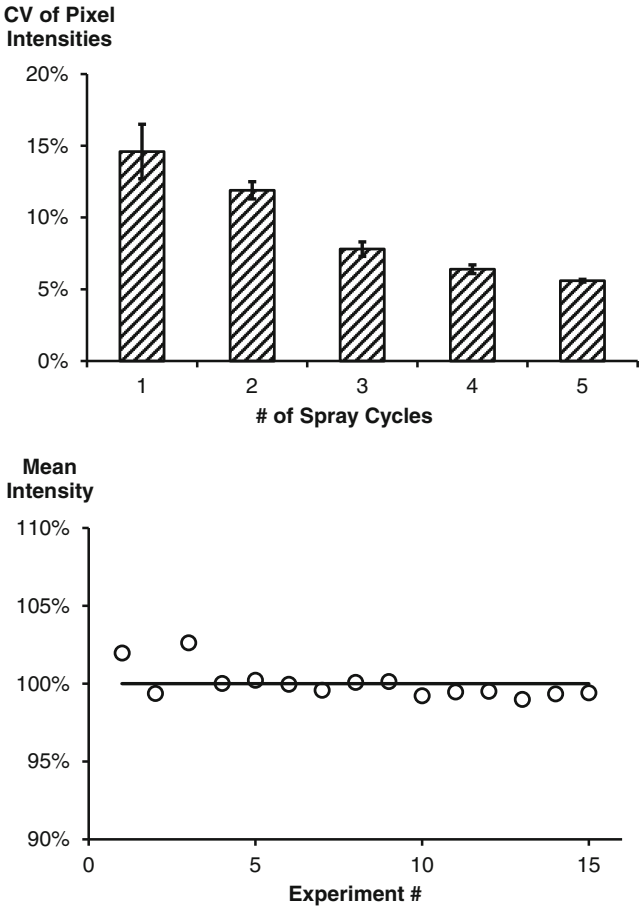


Figure 2. Effect of the number of spray cycled to the pixel to pixel CV (top). A spray suitable for matrix deposition consists of at least four cycles and results in a coefficient of variance of 7%. This deposition can be accurately reproduced by applying a fully automated design (bottom)

effect of these factors was not part of this study, since they can be controlled by keeping the device in a chemistry hood in a temperature-controlled lab.

The MS image acquired from a section of a compound dosed rat is shown in Figure 3, with the intensity scale covering the full range of 0 to 800 counts. A broad range of signal intensities was measured in the section, with tissues of high intensity (e.g., brown fat and skin) being well separated from the surrounding areas of lower intensities. These well-defined contours are an indication of limited analyte spreading during matrix deposition, matching to the image resolution. This is critical, as MSI studies often aim at the evaluation of smaller structure including intestinal wall or retina.

As with any spray-based method, the need for analyte extraction (from the tissue) means there is a balance between sensitivity and spatial resolution. The protocol discussed here proved to be suitable for image resolutions down to 50 µm only, because of the relatively large drop size of the spray. By changing the spray parameters to result in smaller drop size, one can achieve higher spatial resolution down to at least 10 µm with some sacrifice in sensitivity. The spray can be tuned to specific requirements, and the device can be programmed to facilitate additional steps like derivatization or on-tissue digest. This is supported by providing three reservoirs and full flexibility in programming. The significant aspect, which we like to highlight, is the ability to reproduce and share a coating procedure once it has been developed. This is a substantial improvement compared with the original situation, where manual spay procedures were described in literature and which could often not be reproduced. With the coating process only requiring the spray solution to be supplied in one of the reservoirs and for the protocol or parameters to be uploaded on to device, this becomes a simple step.

Eight devices have been built to this date and deployed to different labs. Spray performance was tested for each device before shipment, confirming consistency based on the optimized design. Out of these devices, we tested three in MSI experiments and they uniformly resulted in quality MSI data right from the first coating. A dedicated space on the website http://iMatrixSpray.com supports sharing of protocols and discussion of new ideas related to the device.

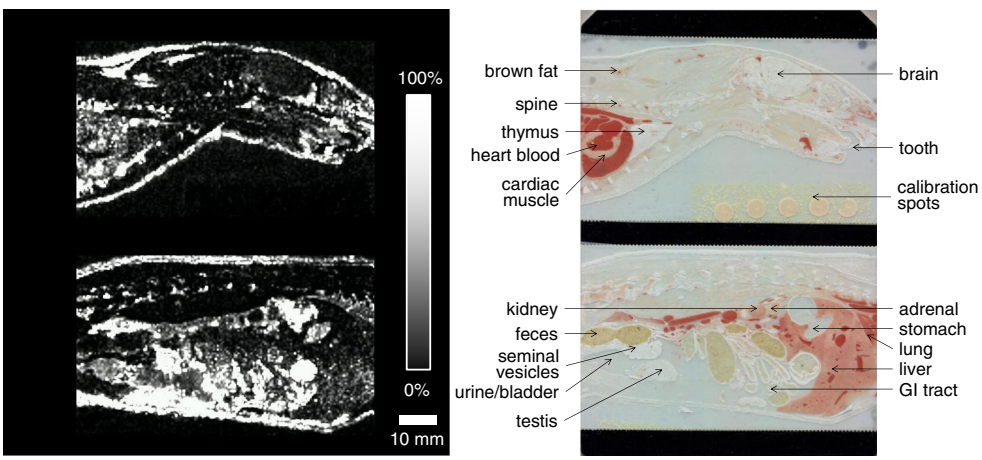


Figure 3. MS image of a compound on a rat section orally dosed at 30 mg/kg. The section was automatically coated using the iMatrixSpray

Conclusions

By developing and specifying protocols for the open source spray device, we can remove the "magic" previously required to produce a matrix deposition that would result in quality MALDI MS images. The data shown in this article demonstrate the robustness of the protocols derived from the simple design concept of the device. Pixel to pixel variance was measured to be better than 7% (five cycles), and the variance between repeated coatings is better than 3%. The MS images acquired after matrix coating with an optimized spray protocol show high signal intensities and well defined structures.

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