

Acoustic Droplet Ejection Enables Precise Timing and Positioning for Deposition of Matrix to Optimize MALDI Tissue Imaging

Siobhan Pickett, Pierre Chaurand*, Royal Huang, Richard Stearns, Brent Browning, Stephen Hinkson, Richard Ellson, Richard M. Caprioli*

Labcyte Inc., Sunnyvale, CA, U.S.A.

*Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN, U.S.A.

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INTRODUCTION

MALDI tissue imaging reveals the distribution of hundreds of small molecule and/or protein signals ranging in size from 200-200,000 daltons. Automated, precise, high-resolution deposition of MALDI matrix is critical to realize the full potential of imaging mass spectrometry. Conventional methods of applying matrix to tissue include cumbersome manual capillary deposition, and sub-optimal tools designed for other purposes such as depositing matrix onto 2D-blot. We have developed the Labcyte® Portrait™ 630 reagent multi-spotter using acoustic droplet ejection (ADE; see sidebar below) to deposit sub-nanoliter volumes of matrix in a precise array with user-defined timing in a fully automated platform that provides superior positional data, mass spectra, and experimental reproducibility.¹

ACOUSTIC DROPLET EJECTION: MOVING LIQUIDS WITH SOUND

In Acoustic Droplet Ejection (ADE), focused ultrasonic energy is used to eject small droplets from the free surface of a liquid (Figure 1). The technology can eject droplets smaller than one picoliter and as large as 10 µL. Larger volumes can be transferred as multiple drops. There is no contact between the ejection mechanism and the ejected sample. This technology is especially well suited to biological applications in which volume and/or positional precision are critical, and in which cross-contamination of samples can interfere with accurate interpretation of the results. The acoustic mechanism completely eliminates the need for pipette tips, pin tools or nozzles.



Figure 1: Stroboscopic image of ADE.

PORTRAIT™ 630 REAGENT MULTI-SPOTTER

The Portrait 630 system uses proven ADE technology.² An acoustic transducer is tuned to eject 170 pL droplets of 20 mg/mL sinapinic acid in 1:1 acetonitrile: 0.1% TFA. The droplets are ejected to the target sample from a COC membrane reservoir (800 µL fill volume.) The target holder accommodates a range of metal and glass MALDI targets. A full-color flat-field CCD imaging system captures an image of the target sample, upon which the user specifies the locations of the spotting grids. The images are stored as TIFF files. The matrix deposition spot coordinates can be transferred to a mass spectrometer as CSV files.

TISSUE PREPARATION

We investigated a variety of matrix deposition conditions on 12-micron-thick rat brain coronal sections prepared as previously described.³ Sequential sections were mounted either onto glass microscope slides and H&E stained, or onto indium tin oxide-coated glass MALDI target plates for spotting.

SPOTTER CONTROL PROTOCOLS

We used two different spotter control protocols (Figure 2) to evaluate spot morphology, spot positioning, and tissue wetting behavior.

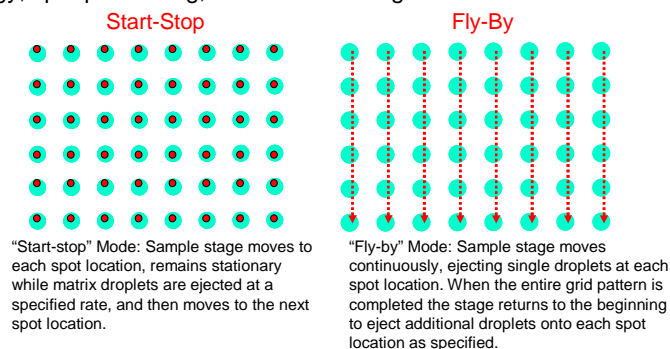


Figure 2: Spotter control protocols.

RESULTS

A. Benchmark

To benchmark the Portrait 630 system, one tissue section was spotted using the same conditions that are currently used on a prototype instrument.¹ Sinapinic acid matrix was deposited in four cycles. Each cycle deposited 13 170 pL droplets of sinapinic acid matrix at a rate of 8 Hz and 300 µm center-to-center spot spacing (Figure 3.) The spot diameters do not increase significantly with subsequent cycles (i.e., increasing reagent volume), indicating that this cycle timing allows sufficient solvent evaporation for high-quality crystal formation, and that the Portrait 630 provides excellent drop-on-drop precision.

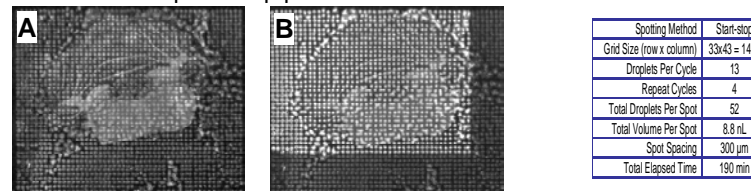


Figure 3: Rat brain section, spotted in four cycles of 13 droplets each, reveals excellent spot morphology and drop-on-drop positioning. A) First spotting cycle. B) Fourth spotting cycle (smaller grid).

B. Optimizing Matrix Volume

To assess the impact of matrix volume on spot morphology and spectral signal we used the fly-by printing mode to deposit matrix onto the tissue in four overlapping tiled sectors, each shifted by 10 columns (Figure 4). The excellent drop-on-drop positional repeatability is especially apparent in the droplets on the empty space to the left of the tissue, each of which has been 'revisited' up to 48 times with minimal change in spot diameter or roundness, indicating that the droplets land at the same location each time.

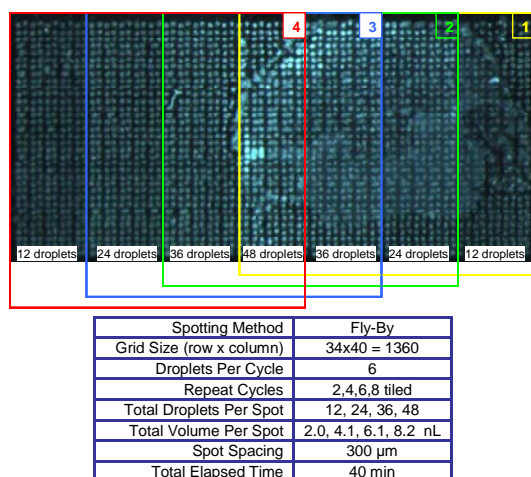


Figure 4: Rat brain spotted in four overlapping sectors in the order indicated. The right-most (yellow) sector received a total of 12 droplets on the tissue, the second (green) sector received an additional 12 droplets, the third (blue) sector received an additional 12 droplets, and finally the left-most (red) sector received an additional 12 droplets. The total number of droplets on each sector is indicated.

The MS signal intensities also vary over of this range of matrix volumes, with increasing matrix volume giving increasing overall spectral signal intensity, apparent as a visible gradient in Figure 5A and in the relative curve heights in Figure 5B.

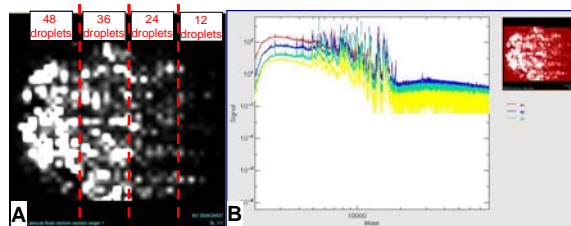


Figure 5: Intensity gradient corresponding to tiled matrix deposition. A) Spectral image ($m/z=3034$) with number of droplets per sector indicated. B) Spectra for each sector. Both axes are in log-scale to reveal intensity differences. Colors correspond to tiled regions on tissue in Fig. 4. Spectral mapping was done using BioMap software (www.maldi-msi.org).

C. Optimizing Fluid Deposition Rate

Our experiments revealed that the droplet deposition rate over a given area may impact the evaporation rate of the matrix solvent, the ability of the tissue to absorb the matrix, and the formation of discrete crystal spots. The range of spotting protocols on the Portrait 630 system enables the user to control the fluid deposition rate, thereby optimizing solvent penetration, spot morphology, and crystal formation. (Figure 6.)

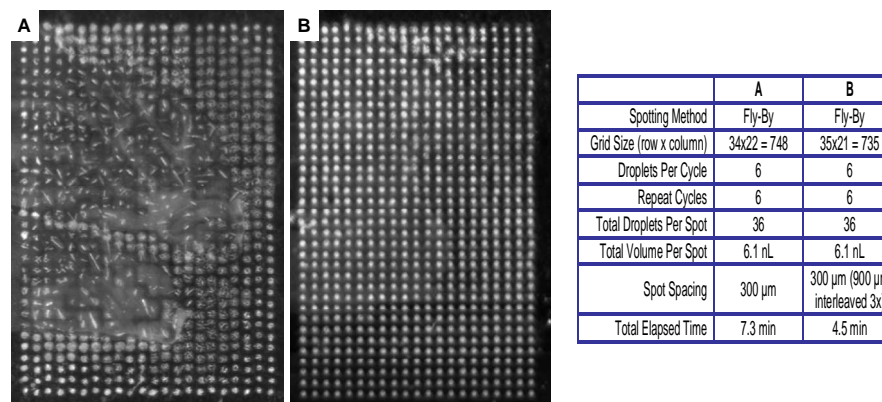


Figure 6: Rat brain tissue spotted with two different methods to control wetting rate for optimized spot and crystal formation. A) Depositing matrix too fast can cause matrix pooling on the surface; B) An interleaved spotting protocol enables adequate matrix absorption and solvent evaporation for discrete spot formation while maintaining the same spatial resolution (300 μm).

D. Optimizing Spot Density

Several brain sections were "pre-seeded" by manually dusting the mounted tissue sections with pulverized dry sinapinic acid prior to spotting with matrix solution as previously described.¹ Pre-seeding the tissue in this manner resulted in smaller matrix spots (~120 μm diameter), enabling us to create higher density grids (150 μm spacing) using both start-stop and fly-by spotting methods (Figure 7.)

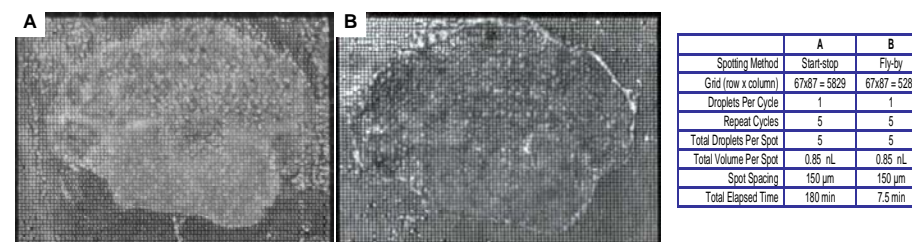


Figure 7: Pre-seeded tissue sections yield smaller spots, enabling higher density grids. A) Start-stop mode; B) fly-by mode.

These samples yielded excellent crystals (Figure 8), resulting in high quality mass spectra (Figure 9) and higher spatial resolution in the MALDI images (Figure 10.)

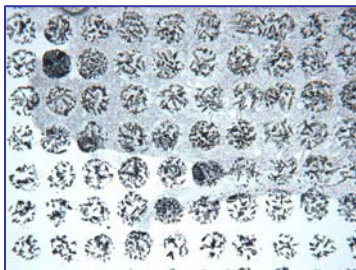


Figure 8: Light microscope image of sinapinic acid matrix crystals deposited onto seeded rat brain section. Border of tissue is visible—darker region is the tissue.

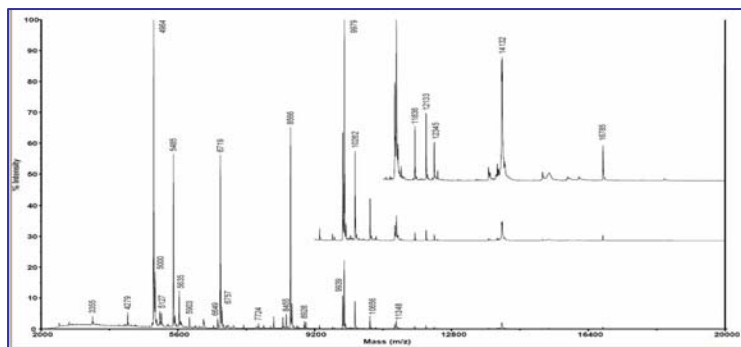


Figure 9: MALDI-MS sum spectrum of rat brain tissue section from Figure 7A. MALDI-MS was performed on a Bruker Daltonics autoflex™ MALDI-TOF mass spectrometer. Insets show scaled spectra of higher mass peaks.

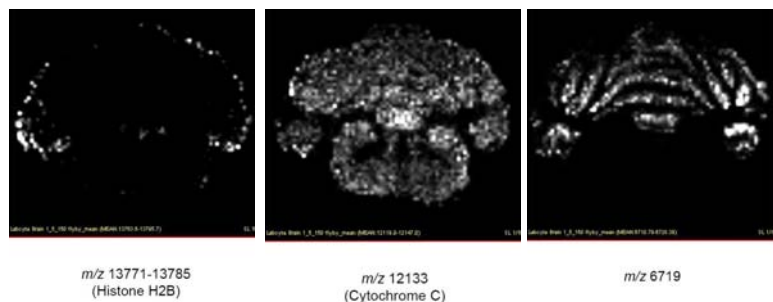


Figure 10: Representative MALDI tissue maps from pre-seeded rat brain section in Figure 7B. Sections were spotted at 150 μ m spacing. Each image represents the distribution of a single ion species in the tissue section as indicated.

CONCLUSIONS

The Labcyte Portrait 630 reagent multi-spotter uses automated precision acoustic droplet ejection to control MALDI matrix deposition on tissue. The system enables users to

- optimize matrix fluid volume
- optimize matrix deposition rate
- optimize spot density

Optimized crystallization parameters yield higher quality, high resolution MALDI tissue imaging mass spectrometry.

REFERENCES

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For more information contact:

Siobhan Pickett
siobhan.pickett@labcyte.com
 +1 408 747 2000 x191
www.labcyte.com

