Benefits of the Tissue Preparation System TM-Sprayer™ for MALDI MSI applications

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Introduction

MALDI Mass Spectrometry Imaging (MSI) is an emerging technology that can simultaneously identify and localize molecules within biological samples. However, preparing samples for MSI can pose a challenge. Attempting to balance the positive effects of the matrix solution coating the tissue and co-crystallizing with the analytes, and the negative effects of analyte delocalisation can be problematic. Variations in matrix application rate, dry times and the amount of matrix being applied are all parameters that can affect the detection of compounds that are low in abundance[1].

The TM-Sprayer™ (HTX Imaging) is a new matrix depositor designed to deliver a high quality and uniform matrix coating as the unique combination of heat (ambient to 140ºC) and pressure contributes to several beneficial properties (Fig 1):

- An improved ability to extract the analytes from the superficial layers of the tissue.
- An increased rate of evaporation for minimal analyte delocalisation.
- A greater control in drying speed due to the combined effect of the nozzle velocity and sheath gas flow.

The two new dimensions of temperature control and nozzle velocity as variable parameters enable the end user to better optimize extraction and signal intensity.

Figure 1: Set up of the Tissue MALDI Sample Preparation System. Source: HTX Imaging[2] by HTX Technologies, LLC.

Spray deposition can be undertaken in a linear or serpentine mode. The loop holds 5 mL - 30 mL of matrix and can spray a standard glass slide in ~2 min per pass. The spray is 10mm in diameter and provides matrix droplets of < 20 µm. Off-setting the starting point of passes allows for greater coverage and homogeneity crucial for high-resolution imaging.

Aims

To prepare control rat liver tissue using the TM-Sprayer™ in order to detect low abundance compounds.

To assess and discuss the ease of method development in relation to improving signal intensity and extraction.

Method Development

The matrix deposition quality and quantity can be controlled using five fully adjustable parameters:

- Number of passes (NP)
- Matrix solution concentration (Cm)
- Matrix solution flow rate (FRm)
- Velocity (V)
- Track Spacing (TS)

Calculating the amount of matrix deposited in mg/mm^2, onto a sample can be calculated with the formula:

$$W_m = \frac{NP \; x \; Cm \; x \; FRm}{V \; x \; TS}$$

Parameters can be adjusted independently to create a wetter or dryer spray depending on the sample type.

		Nozzle Temp	Number of Passes	Matrix Conc.	Pump flow rate	Velocity	Track Spacing	Matrix density
	N2 Pressure	T	NP	Cm	FRm	V	TS	Wm
	psi	°C	Each	mg/mL	ml/min	mm/min	mm	mg/mm^2
Set 1	6.5	80	6	7	0.120	1200	1.5	0.00280
Set 2	6.5	80	10	7	0.080	1300	1.5	0.00287

Table 1: Parameters of two different methods. Set 1 provides a standard matrix application. Set 2 provides a "wetter" application. Matrix density in mg/mm^2 calculated per Set. Source: HTX Imaging[2] by HTX Technologies, LLC.

Experimental

Control rat liver tissue, sectioned at 30 µm, 16 µm and 8 µm were sprayed with 7 mg/mL α-Cyano-4-hydroxycinnamic acid (α-CHCA) with a solvent composition of 70% Methanol containing 0.2% Trifluoroacetic acid (TFA) using the TM-Sprayer™. The tissues were spotted with a known GSK compound and analysed for signal intensity. Figure 2 shows moderate signal from the 100 µg/µl spot however the 10 µg/µl and 1 µg/µl remain undetected.

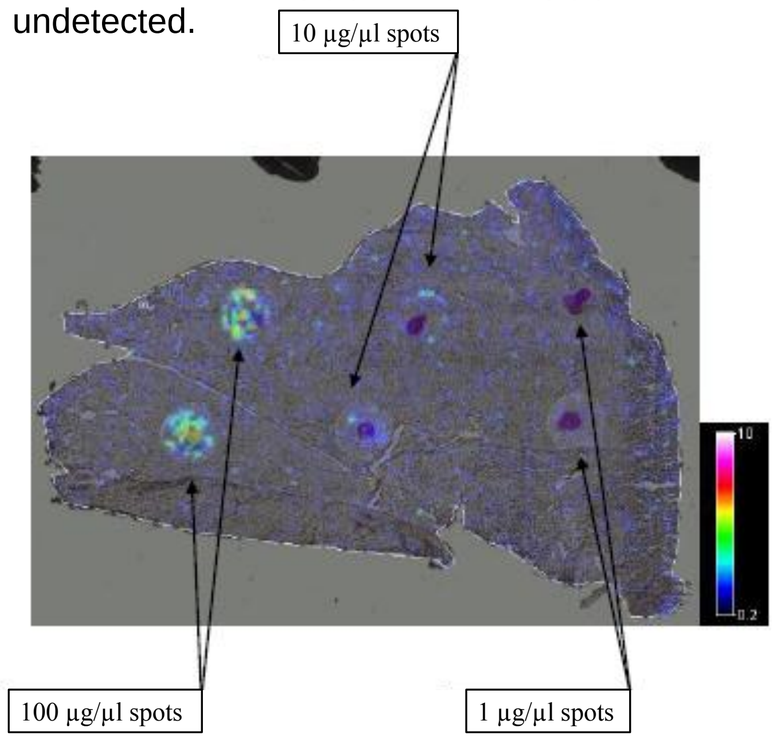

Figure 2: 8µm section prepared using method Set 2 and 7mg/mL CHCA. Source: Image courtesy of GlaxoSmithKline.

Discussion

Results from method set 1 and 2 were analysed and although set 1 produced a slightly stronger signal it is apparent that signal intensity from the lower concentration spots is minute (Fig 2).

Adjusting the spray parameters would increase chances of achieving better data, thus enabling the detection of the 10 µg/µl and 1 µg/µl compound spots.

It must be emphasised that the results from Figure 2 are only a preliminary test. Further method development would be required in order to obtain sufficient signal intensity. A good starting point would be to keep the amount of matrix (mg/mm^2) on the sample consistent but to vary the way in which the matrix is applied, for example, decrease the number of passes to 4 and increase the flow rate to 0.180 ml/min for method set 1.

Following this process would help isolate the parameters that are most effective in improving signal intensity to give an indication as to how much.

The ability to analyse the localisation of larger proteins may be aided by the use of in situ digestion to generate smaller peptide fragments. The result of such enzymatic cleavages are easier to detect and visualise by MALDI MSI.

Utilising the validated protocol for in situ trypsinization using the TM-Sprayer™ is another key characteristic of this sample preparation system.

References

[1] GOODWIN, R.J.A. (2012). Sample preparation for mass spectrometry imaging: Small mistakes can lead to big consequences. *Journal of Proteomics*. **75**: 4893-4911.

[2] HTX IMAGING. (2012). TM-Sprayer™ Tissue MALDI Sample Preparation System. [Online]. http://www.htximaging.com/. Last accessed 14th December 2012.

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