INCREASED MOLECULAR INFORMATION FOR SPATIAL IMAGING USING DESI MS

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INTRODUCTION

Over the last 15 years, desorption electrospray ionisation (DESI) mass spectrometry (MS) has been increasingly used for a wide variety of applications such as MS imaging of biological tissue specimens. DESI is well suited for the spatial mapping of small molecules such as metabolites and lipids. However there are some limitations to get DESI adopted by a broader range of users, for instance the high degree of parameters to optimise the spray quality as well as the geometrics parameters. Furthermore improvements in reproducibility and signal intensities are aims to most scientists. It can bring access to low abundant molecules and/or it can speed up the analysis time which can be rather long for MS imaging experiment in general.

Here we describe the benefits of using a newly developed DESI sprayer as well as the use of a heated ion inlet tube (HTL) into the mass spectrometer for DESI MSI.

METHODS

DESI imaging experiments have been performed using porcine liver, mouse kidney and mouse brain tissue sections, produced using a cryotome and deposited on a standard microscope slide and preserved at -80°C until analysis by mass spectrometry.
Data were acquired using a DESI XS source (Waters, UK) which was mounted on Xevo G2-XS Q-Tof and SYNAPT XS (Waters, UK). Acquisitions were performed in positive and negative mode with a mass range of *m/z* 50-1,200. DESI spray conditions were set at 1-2 µl/min, 90 to 98% aq MeOH with nebulizing gas pressure of 8-15 psi and a capillary voltage of 0.7-1 kV. DESI imaging experiments were set-up and visualised using High Definition Imaging (HDI) 1.6. Data were mined using MassLynx V4.2.

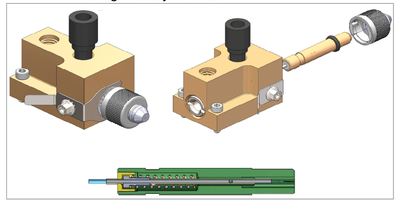

Figure 1. Diagram of the new sprayer assembly incorporating exploded view and a cross section of the new emitter assembly.

A new sprayer assembly, incorporating high precision, high tolerance parts has been developed. The sprayer incorporates a replaceable emitter cartridge. The metal emitter is based on tried and tested technology developed for the Waters ionKey source. Figure 1 shows a schematic of the sprayer assembly.

The new emitter cartridge allows for the precise and reproducible positioning of the emitter within the sprayer nozzle, removing the need for manual fine tuning of the emitter position. The new sprayer has also shown significant increases in sensitivity compared to the previous design as well as the production of a finely tuned spray dynamic.

A new heated transfer line (HTL) has also been developed and has been shown to increase the ionisation / transfer of wide range of molecules. The temperature can be tuned from ambient to 650°C. The inlet capillary is insulated with a protective sleeve, meaning the surface temperature does not exceed 120°C. Figure 2 shows an image of the heated transfer line as well as a thermal image.

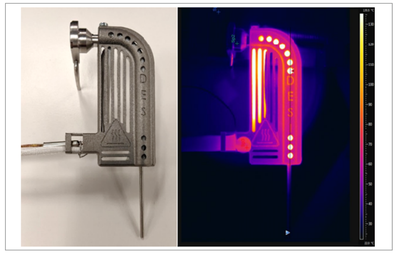

Figure 2. Newly developed heated transfer line and thermal distribution image

RESULTS/ DISUCUSSION

The fixed geometry of the new sprayer greatly simplifies the optimisation process. The main parameters are capillary voltage and gas flow, which are optimised in relation to the desired flow rate. Figure 3 shows the results of laser sheet experiments, where the gas flow and voltage have been optimised for different flow rates, in order to maintain a stable spray. The solvent stream was found to exit the nozzle with a diameter of approximately 20µm. The main effect of the optimisation was to change the distance for which this diameter was maintained.

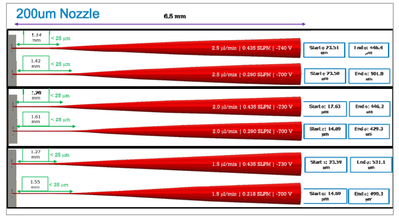

Figure 3. Results of laser sheet experiments investigating the effects gas flow and voltage at different solvent flow rates.

The new sprayer has been found to show excellent inter run reproducibility across. In figure 4, the results of 48 DESI imaging experiments are shown from consecutive liver tissue sections. The Total Ion Count (TIC) for a 50 pixel region from the centre of each tissue has been calculated. Across the 48 experiments run the RSD was found to be 10.5%.

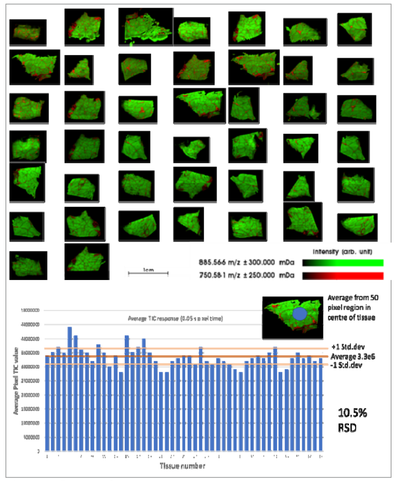

Figure 4. Results of experiments on porcine liver tissue showing the TIC for a 50 pixel region from the centre of each tissue. An RSD of 10.5% was observed across the 48 samples run.

The use of the new sprayer shows significant increases in sensitivity particularly in positive mode (data not shown).

In addition to the sensitivity increases afford by the new sprayer, the combination with the new heated transfer line has been shown to add further sensitivity increases. In this case whilst an increase is observed in positive mode (figure 5) the sensitivity increase is more prevalent for lipids in negative mode (figure 6).

The sensitivity gains from the combination of the new sprayer and heated inlet can also be utilised to improve other aspects of imaging performances . In figures 7 and 8, some of the sensitivity has been sacrificed to allow the use of the SYNAPT XS's higher mass resolution modes. It was found that despite the drop in sensitivity, good quality data could still be achieved use the High Resolution mode (FWHM 50,000). In addition, the sensitivity and tight spray focus could be used to both increase the speed and decrease the acquired pixel size (figure 9).

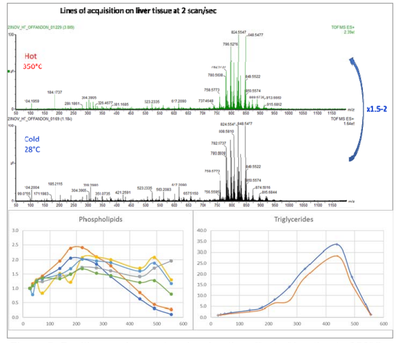

Figure 5. Positive mode spectra showing the effect of heating to 350°C on the main lipid cluster. Graphs showing temperature of the HTL vs. intensity for selected phospholipids and triglycerides. Phospholipids show a peak in intensity at ≈ 250°C. The triglycerides show a peak in intensity at ≈ 450°C. Excessive heating shows a decrease in intensity presumed to be due to fragmentation.

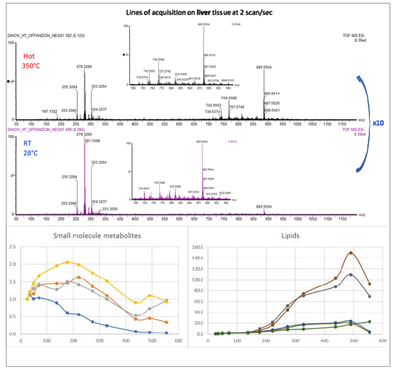

Figure 6. Negative mode spectra showing a significant increase (x10) with heating the transfer line to 350°C vs no heat (ambient). Graphs showing temperature of the HTL vs. intensity for selected lipids and selected small molecules / metabolites. The lipids from the main lipid cluster show a peak at ≈ 500°C. The small molecules / metabolites show a peak at ≈ 190°C. Excessive heating shows a decrease in intensity presumed to be due to fragmentation. The results show the ability to optimise the temperature of the HTL to maximise the intensity of selected molecular classes of interest.

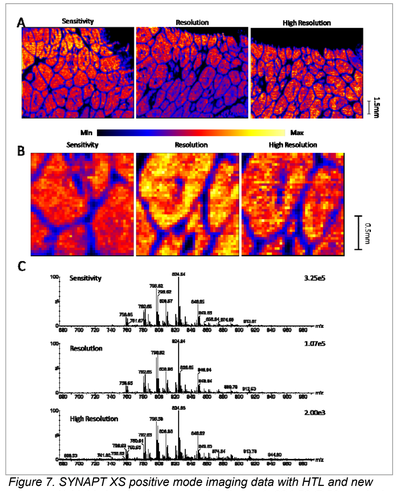

Figure 7. SYNAPT XS positive mode imaging data with HTL and new sprayer. A) Lipid visualisation on porcine liver m/z 824.56 @50µm pixel size in different mass resolution modes of analysis. B) Zoomed 4 mm² region showing detailed tissue morphology. C) Example mass spectra from a single pixel acquisition.

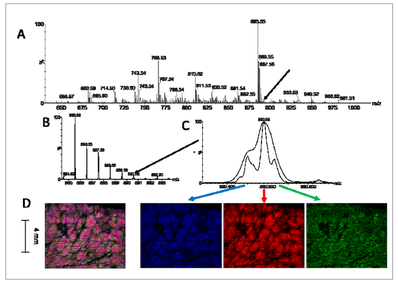

Figure 8. Negative ion mode High Resolution data. A) MS spectra from a 4mm² region (1,600 pixels) of tissue from D with peak of interest indicated. B) Expansion of the m/z 885.5 peak and its isotopic distribution showing peak overlap. C) Expansion of the m/z 890.6 peak in High Resolution mode (FWHM 50,000) with Sensitivity mode (FWHM 20,000) data overlaid. Multiple peaks are apparent in the High Resolution mode data that are not separated in in Sensitivity mode. D) RBG overlay of three separated peaks showing differences in distribution (Blue: m/z 890.50; Red: m/z 890.58; green 890.60.

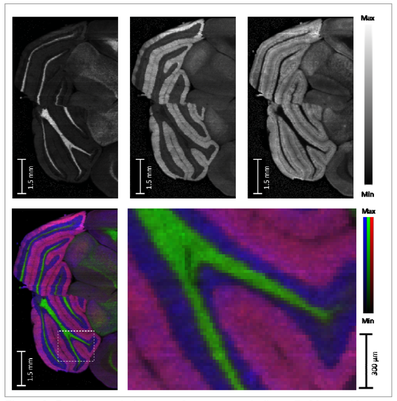

Figure 9. Positive mode images of mouse cerebellum, @ 20 µm, 10 scans per second.

Top left: m/z 788.7; Top middle: m/z 834.7; Top right: m/z 845.6.

Bottom left: three colour overlay; Red - m/z 834.7; Green - m/z 788.7; Blue - m/z 845.6.

Bottom right: expansion of area indicated in three colour overlay.

CONCLUSION

- **The newly designed sprayer offers increased ease of use whilst providing robust, reliable, and reproducible performances.**
- **The combination of the sprayer with the heated inlet capillary (HTL) shows significant increases in sensitivity. The use of the heated inlet is especially significant in negative lipid mode.**
- **Distinct molecular classes have been shown to respond differently to the effect of heating the inlet capillary allowing for the possibility of tuning the heated in let to maximise the intensity of molecular classes of interest.**
- **The increase in sensitivity has been shown to allow experimental optimisation in terms of mass resolution mode, pixel acquisition size and acquisition speed.**

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