Integration of 3D Printing into Desorption Electrospray Ionization Mass Spectrometry

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Introduction

Adaptation of DESI to existing mass spectrometry platforms requires a laboratory-built solution manufactured with the capability of fine, stable adjustments of the electrospray emitter for liquid or solid spot profiling purposes. Herein described is an alternative, inexpensive approach utilizing fused deposition modeling (FDM) to assemble a stable source comprised of standard accessories from a electrospray mass spectrometry platform.

FDM is a readily available form of 3D layered printing, in which a thermoplastic filament is heated and then extruded through a nozzle into layers, building the model one layer at a time. Consumer grade 3D printers like the Creality Ender-3 can be purchased for less than two-hundred dollars, and readily print a large variety of polymers with high tolerances. FDM printed polylactic acid (PLA) filament was used for the strength and stiffness, which has been shown to be greater than injection molded parts to create a modifiable structure with positioning solutions for adapting commercial accessories to this source. Spot profiling can be completed for the cost of a few dollars' worth of plastic after roughly one days' worth of printing time, with inclusion of linear positioners to enable imaging for under three-thousand dollars.

Shown in Figure 1 is the DESI source attached to a Bruker 12T SolariX FT-ICR mass spectrometer, an example of a specialized high resolution platform without a commercial DESI available. The apparatus was designed utilizing printed solutions to minimize cost, with a printed angular positioner as well as sliding dovetail on the body allowing for the gross adjustment of the emitter. The sample holder can also be adapted to hold a mounted slide or a well plate.

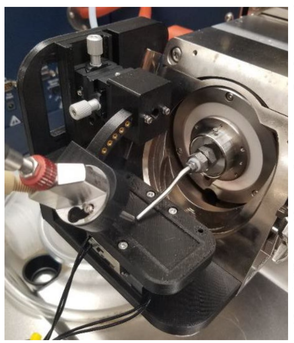

Figure 1: Digital photograph of a fully assembled FDM printed DESI-MSI source attached on the mass spectrometer.

Methods

DESI FT-ICR MS Spot Profiling:

A non-fixated and unembedded control Sprague Daly brain was harvested and sectioned using a cryotome at 15µm. Coronal sections were collected on cleaned microscope slides for analysis with spots profiled in the same stereotaxic coordinates in the somatosensory cortex of the rat brain in serial sections. Charge was placed either on the emitter or the capillary as shown in Table 2 with the source geometries stated in Table 1 with a 50:50 mixture of acetonitrile and water.

DESI FT-ICR MS Line Scan Acquisition:

Red Sharpie containing a principle dye of Rhodamine 6G was applied in parallel films to a clean microscope slide for these experiments, with a 2D array of Newport Agilis LS25-27 linear positioners with a calibrated speed of 295µm/s controlled by a Newport Agilis UGC-2 controller. The grounded emitter geometry and parameters were utilized for the line scans.

Derivatized MALDI FT-ICR Mass Spectrometry Imaging:

A chinchilla underwent a unilateral blast exposure one day prior to sacrifice. Non-fixated blast exposed and control brains were cryosectioned at 15µm onto ITO slides. A Bruker ImagePrep was used to spray a 2.0mg/mL of 2,4-diphenyl-6-methylpyrylium tetrafluoroborate (DPMP), followed by tissue acidification.[2,3] The tissue was sublimed with CHCA and imaged at 225µm with 1000 lasers shots and optimized settings in FlexImaging 5.0, with further processing in SCiLS Lab.

DESI Geometries	Charged	Grounded
Emitter Incidence Angle	55˚	55˚
Capillary Collection Angle	7˚	7˚
Emitter Distance to Capillary	3.0mm	2.0mm
Emitter Distance to Sample	3.0mm	2.0mm
Capillary Distance to Sample	>150µm	>150µm
Spray Capillary Protrusion	1.0mm	1.0mm

Table 1: Optimized DESI geometries for the two experimental setups with charge being applied at the emitter, or at the capillary with a grounded emitter.

Source Parameters	Charged	Grounded
HV at Electrospray Emitter	+4.5kV	GND
HV at Capillary Extension	GND	-6.0kV
Flow Rate	2µL/min	2µL/min
Nebulizing Pressure	8.0bar	4.0bar

Table 2: Optimized electrospray source parameters for a lipid fingerprint analysis, all parameters behind the source remained consistent for both experiments.

Results

DESI FT-ICR MS Spot Profiling:

Annotations of lipids profiled utilized two experimental setups varying the location of the applied high voltage potential are shown in Table 3 with a representative spectrum in Figure 2. A grounded emitter analysis exhibited enhanced lipid signal suggesting an additional point of optimization.

Annotation	Adduct	*Observed m/z*	Grounded Emitter	*Observed m/z*	Charged Emitter	Peak Area Fold Change
PC (34:1)	[M+Na]	782.56715	-0.16 ppm	782.56802	-1.27 ppm	5.9
PC (34:1)	[M+K]	798.54090	0.08 ppm	ND	ND	NA
PC (32:0)	[M+Na]	756.55136	0.09 ppm	756.55124	0.18 ppm	4.4
PC (32:0)	[M+K]	772.52526	0.07 ppm	ND	ND	NA
PC (36:4)	[M+Na]	804.55132	0.07 ppm	ND	ND	NA
PC (36:4)	[M+K]	820.52493	0.47 ppm	ND	ND	NA
PC (38:6)	[M+Na]	828.55090	0.58 ppm	ND	ND	NA
PC (38:6)	[M+K]	844.52509	0.27 ppm	ND	ND	NA
PC (36:1)	[M+Na]	810.59878	-0.56 ppm	ND	ND	NA
PC (36:1)	[M+K]	826.57230	-0.04 ppm	ND	ND	NA
PE (40:6)	[M+Na]	814.53680	-1.32 ppm	ND	ND	NA
PE (40:6)	[M+K]	830.50911	0.66 ppm	ND	ND	NA
SM (36:1)	[M+K]	769.56255	-0.67 ppm	ND	ND	NA

Table 3: Annotations of lipid adducts detected from coronal rat brain sections in the somatosensory cortex utilizing both the charged and grounded emitter DESI setups. ND— not detected, and NA— not applicable

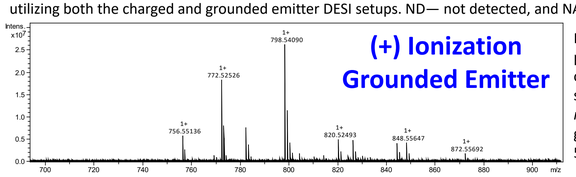

Figure 2: Example of a positive ion spectrum obtained from a coronal section of rat brain from *m/z* 700 to 900 using a grounded emitter with 50:50 acetonitrile: water.

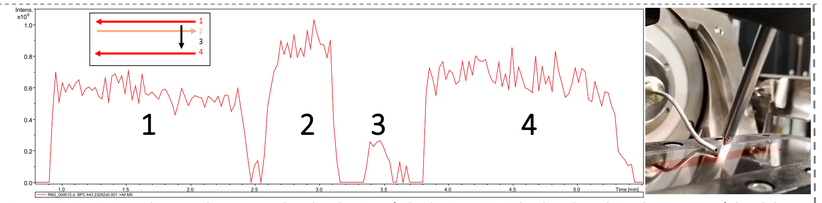

Figure 3: Acquisition showing the extracted molecular ion of Rhodamine 6G with a key describing the motion of the slide (top left) and photograph of a replicate experiment (right). (1) highlights a line scan of across a film of dye at 295 µm/s, (2) corresponds to the jog back to the initial start at maximum speed, (3) shows the jogging from the sampled film to a new film on the same slide, and (4) highlights a second line scan of across a film of dye at 295 µm/s.

DESI FT-ICR MS Line Scan Acquisition:

Line commands were sent during acquisition to move the sample slide in the pattern shown in the key in Figure 3. The average relative standard deviation during the line scans was ~15% which can be attributed to a heterogeneous film produced through the application method. For MSI purposes these line scans can be exported as .mzml from DataAnalysis 5.0 into imaging workflows.[4]

Derivatized MALDI FT-ICR MSI:

After derivatization by DPMP, the auditory pathway was profiled. The primary auditory cortex had a relative depletion of GABA in the left hemisphere of the blast exposed brain, as well as in the cochlear nuclei in the cerebellum shown in Figure 4. Other sections including the inferior colliculus were profiled with other differential amounts of lipids, glutamate and GABA.

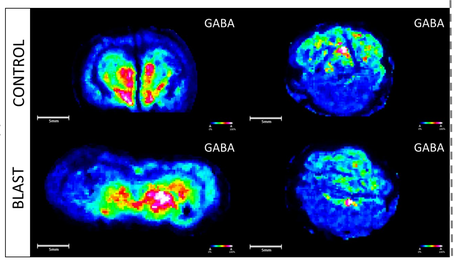

Figure 4: Images from SCiLS Lab of the coronal sections (left) containing the primary auditory complex, and cerebral sections (right) containing the cochlear nuclei of the blast exposed and control brains. Derivative ion of GABA was confirmed by MS/MS.

Conclusion

- ✓ Using FDM printing to rapidly construct and modify a DESI-MS source is feasible in a days worth of printing time, with MSI capable at the cost of few commercially purchased linear positioners with standard accessories
- ✓ Utilization of pyrylium salts for the derivatization of primary amine containing compounds has utility for analysis of neurotransmitters by MALDI-MSI for further verification of future DESI-MSI of disease states such as tinnitus models in the chinchilla brains shown.

Future Work

- ✓ Integration of ASC II line commands and triggering of acquisition into an automated DESI FT-ICR MSI sampling platform.
- ✓ Tandem detection of neurotransmitters and other species by DESI-MSI and MALDI-MSI on the same instrument.
- ✓ Utilizing DESI as a lipid washing step with histologically compatible solvents prior to MALDI imaging for neuropeptides..

References

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Acknowledgements

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