REMIX, REUSE, and SHARE: APPLYING AMBIENT IONIZATION TO FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETERS

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Introduction

Ambient ionization embodies dozens of various techniques with distinct desorption and ionization mechanisms **(Fig.1)**. *These techniques require minimal to no preparation,* but largely have not been implemented on modern FTICR systems even though FTICR is the only mass analyzer which can routinely resolve various complexities within modern –omics applications.

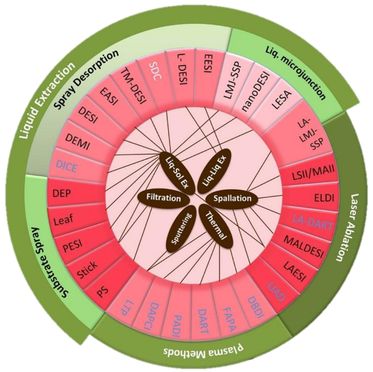

Figure 1. Classification of ambient ionization methods based upon extraction method. Reproduced from [1].

Of all the factors impeding routine ambient ionization by FTICR, the atmospheric pressure inlet **(Fig.2)** on modern instruments makes interfacing spray desorption based ambient ionization (among others) more challenging than for grounded inlet instruments.[2] Here we detail the prototyping and design of a modular 3D printable source for ambient FTICR-MS, together with a custom capillary extension **(Fig.2)** high sensitivity ambient analyses are possible. We highlight two unique applications of DESI-FTICR for MS/MSI, and demonstrate the utility of computer aided design (CAD) with 3D printing.

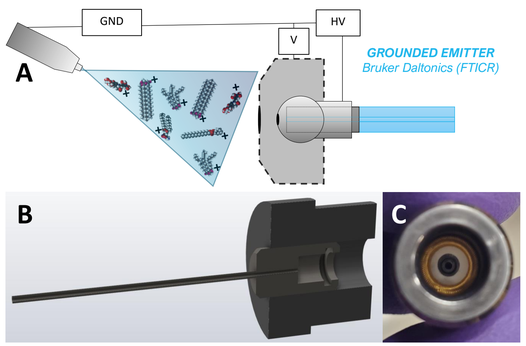

Figure 2. (A) Schematic of high-voltage application for ESI at the atmospheric pressure inlet is outlined with a grounded electrospray emitter. Where for DESI the standard capillary nub is replaced by **(B)** a machined (316L SS) capillary extension with a gas diverter (Torlon). **(C)** This extension uses an o-ring (Kalrez 7075) to form a vacuum seal with a ceramic spacer and gold spring to form an electrical connection.

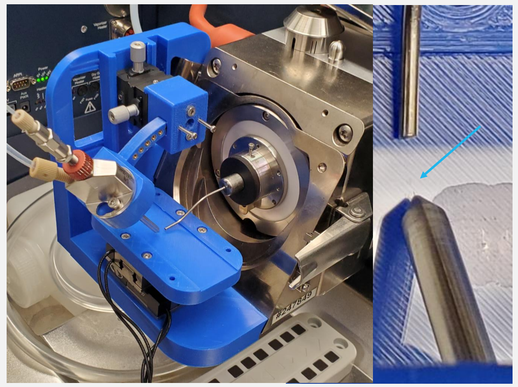

Figure 3. 3D printed source mounted on a commercial 12T FTICR spectrometer, with zoomed view of the DESI thin film formed from the standard electrospray emitter with 100/200 µm ID/OD fused silica pulled to 30 µm ID. (Sutter Instruments P-2000) The source uses an XY array of piezoelectric stages (Agilis, Newport Corporation) for motion control and was printed on a Creality Ender 3 in PLA. Reproduced from [3].

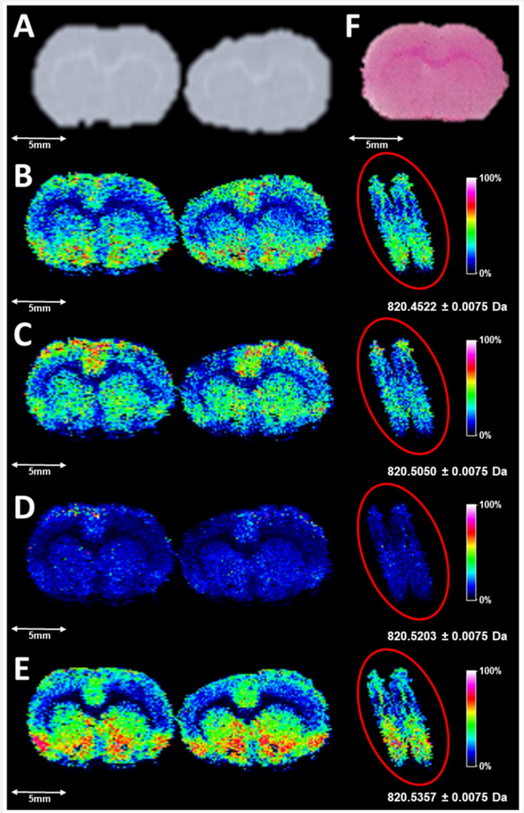

Figure 4. Ion images produced from DESI-FTICR-MSI within a continuous acquisition imaging run, here accumulation during detection (ADD; 1 s accumulation, 0.838 s transients) enabled 1 Hz acquisition on a 12T FTICR instrument. Imaging an area of 27 mm by 10.4 mm took 287 min with 150 µm spatial resolution. **(B-E)** show near isobaric phospholipid distributions, while acquisition during flyback at increased speed produced the circled artifacts with 50% ACN used as the solvent system in positive ion mode. Reproduced from [3].

Results

The first generation of the 3D printable DESI source **(Fig.3)** demonstrated the utility of the standard Bruker electrospray emitter for DESI-MSI with a pulled tip fused silica spray capillary. The pulled ID of the tip (between 10 to 50 µm) provides fine control over the sampled area and enabled high spatial resolution profiling without oversampling the surface. This was demonstrated with modest spatial resolution **(Fig.4)** detecting several near isobaric lipids within coronal sections of rat brain, which were readily resolved by a 12T instrument. Due to the stages, only a small area could be imaged (27 x 27 mm), however, CAD and 3D printing enabled design of a custom sample holders – which can be replaced by a well plate or other solutions to maximize the utility of the measurement region. Use of sliding dovetail components and a printed angular positioners created the robust and stable geometry needed for DESI, and all components as pictured in **(Fig.3)** and **(Fig.5)** are openly available as .STL or .STEP files. This source can be fit to any Bruker Daltonics MS with an Apollo II source.

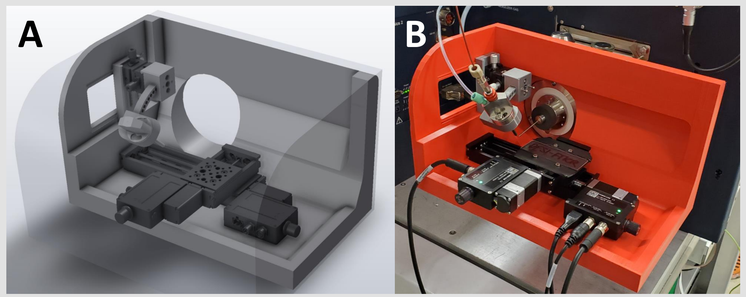

Figure 5. The current design of the printable source is highlighting in CAD **(A)** and mounted on a commercial 15T FTICR spectrometer **(B).** Here the modular design was fused and the entire source was printed on an Ultimaker3 Extended in PLA. The increased structure enabled a XY array of stages (X-LSM-25/100, Zaber Technologies).

Our current version source was printed as one fused part **(Fig.5)**, and additional support enables a larger array of positioners. We are currently working to integrate other ionization modes (e.g., LTP[4]) directly into the existing printable angular positioner. Here, we solely highlight results from DESI-FTICR-MS on a 15T instrument **(Fig.6)**, where SRFA III was drop cast for DESI and annotations were compared to ESI. Overall, the complementarity of detected species further highlights the potential of other ambient modes for high throughput organic matter characterization by DESI-FTICR-MS.

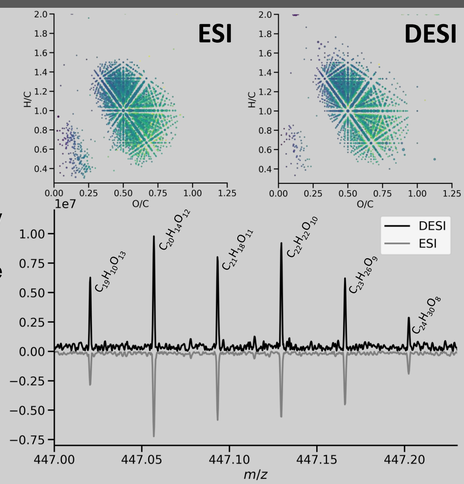

Figure 5. Preliminary evaluation of DESI-FTICR-MS (ADD; 1.75 s accumulation, 1.8874 s transient) versus ESI-FTICR-MS of SRFA III on the same commercial 15T Infinity cell instrument. DESI achieved a resolving power of 479k at *m/z* 447.05, and highlights similar coverage of $C_cH_hO_o$ species with 85% MeOH used as the solvent system in comparison to 100% MeOH ESI.

References

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[3] Zemaitis et al. *Metabolites*. 2021, 11(4), 253. [4] Martínez-Jarquín et al. *Anal. Chem.* **2016**, 88 (14), 6976-6980.

Acknowledgements

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