Spatial distribution of isobaric lipids using high-resolution ion mobility with the DESI XS

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INTRODUCTION

- Desorption electrospray ionization (DESI) imaging mass spectrometry (MS) is an ambient ionization technique that performs direct pixel-by-pixel imaging of tissue section.
- Without a chromatography separation, detected ions in DESI may represent several lipid molecules due to large diversity of lipid species present in a complex biological matrix.
- Ion mobility separation can separate lipid ions with the same or close *m/z* values, but have a difference in size or structure.
- In this study, our objective was to utilize multi-pass high-resolution ion mobility separation to improve the specificity of lipid imaging by DESI imaging MS.

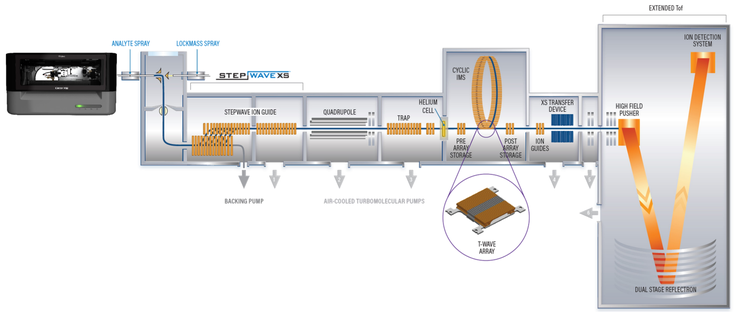

Figure 1. *The Select Series Cyclic IMS mass spectrometer geometry is illustrated here. The instrument incorporates novel cyclic ion mobility separation with high-resolution time-of-flight (ToF) capability of up to 100,000 mass resolution irrespective of the sampling frequency or scan time. Multi-function cyclic ion mobility device consists of a 100 cm path length RF ion guide comprising over 600 electrodes around which T-Waves circulate to provide mobility separation and includes pre and post storage arrays. The circular path minimizes instrument footprint while providing a longer higher mobility resolution separation path, a multi-pass capability for even higher IMS resolution, and additional functionality such as selectively selecting or ejecting ions within a range of mobilities, IMS^o capabilities etc.*

METHODS

- **DESI.** DESI- XS source (Waters) coupled with a quadrupole time-of-flight mass spectrometer with cyclic IMS (Waters) Tissue sections were thaw mounted on a glass slides, vacuum dried, and analyzed without any other sample preparation. DESI solvent (MeOH: water, 98:2, 0.1% formic acid) running at 2 µl/min was electrosprayed by holding capillary at 0.65 kV with nebulizing gas pressure set at 0.7 bar.
- **Data Processing.** Imaging MS data were mined using MassLynx V4.2, DriftScope V2.9, and HDI 1.6 for image visualization.
- **Ion mobility.** Survey image was collected using single pass DESI imaging with m/z 50-1,200, and multi-pass (7 passes) imaging was optimized on the lipid regions.

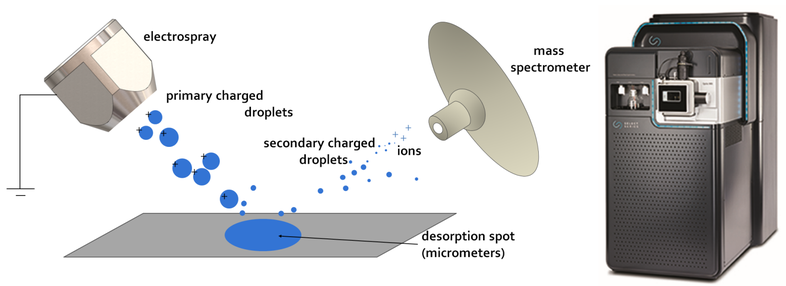

Figure 2. *Desorption electrospray ionization (DESI) is performed by extracting and ionizing sample on surface. The primary droplets from the sprayer forms a thin film of solvent at the surface extracting analyte molecules off tissue, the subsequent primary droplets colliding with the film generate secondary droplets that are ejected, desorbed, ionized, and analyzed by a mass spectrometer.*

RESULTS & DISCUSSIONS

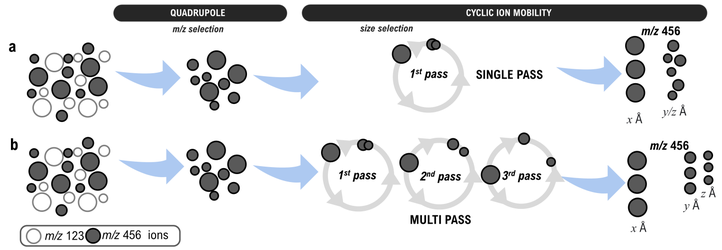

Figure 3. *A diagram showing separation of two sets of hypothetical ions at m/z 123 and 456. Quadrupole can select ions based on m/z value. (a) m/z 456 is selected by quad and single pass cyclic IMS was able separate a largest size ions from two smaller ones, but failed to separate two other ions similar in size. (b) the multi-pass cyclic IMS was able to separate all of three ions with separate Å values.*

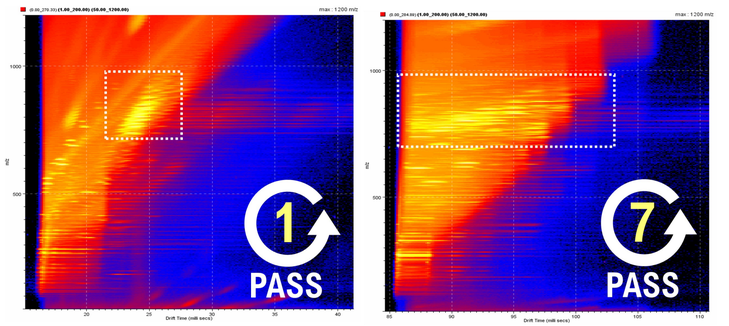

Figure 4. *2D mobility plot (m/z vs. drift time) a single pass cyclic IMS has partial separation of the complex phospholipid region. The separation was improved by cycling the phospholipid region for seven passes approximately increasing resolution to 150 (omega/delta omega) .*

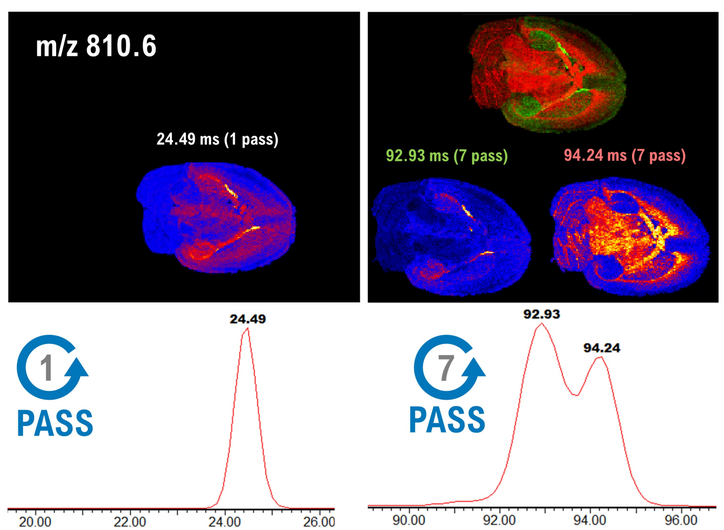

Figure 5. *(a) In single pass DESI imaging, for m/z 810.6, a single peak was observed with a drift time of 24.49 ms showing the ubiquitous localization throughout the brain tissue section. (b) Two ion peaks were observed for same m/z value after the seven passes, at drift time 92.93 and 94.24 ms showing distinct distribution of two molecules within the mouse brain.*

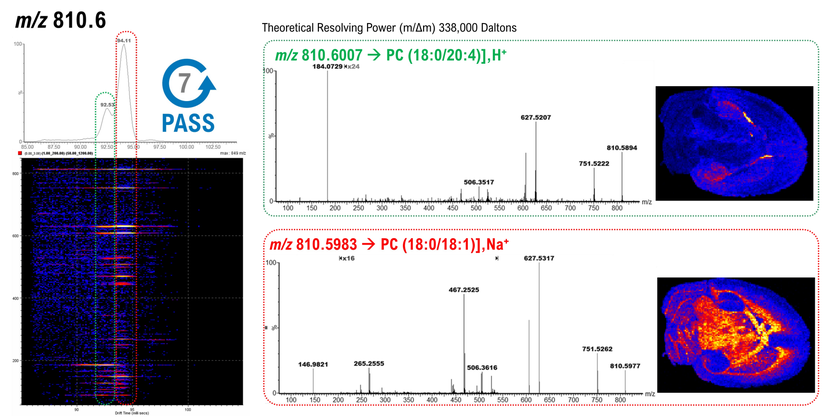

Figure 6. *MS/MS of m/z 810.6 after seven passes in cyclic IMS can be aligned to two detected peaks. Based on fragmentation, the first peak can be assigned to a protonated PC (18:0_20:4) with the accurate mass of 810.6007 with chemical formula of $C_{46}H_{84}NO_8P$. The second one is identified as a sodiated PC (18:0_18:1) with the accurate mass of 810.5983 and chemical formula of $C_{44}H_{86}NO_8P$. The mass difference between the two isobaric species are 2.4 mDa apart and the theoretical mass resolving power needed to discriminate them is 338,000 Da.*

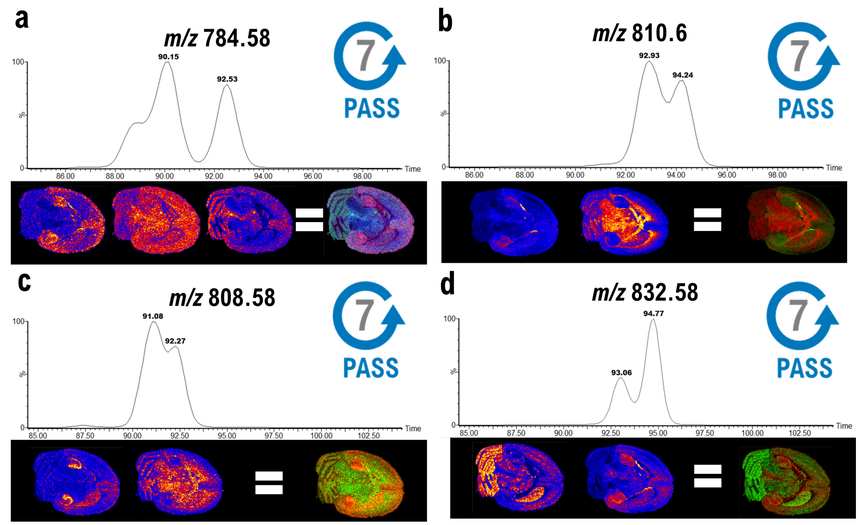

Figure 7. *Selected-m/z mobility of four m/z window after seven passes are shown. The m/z species could be separated into two or three different ions or molecules. The respective images of each of ions are shown in the bottom of the each mobility plot, which also displays as overlaid image of all different species overlaid as green, red, and blue (if needed) ions to illustrate difference in spatial distribution.*

CONCLUSIONS

- DESI imaging workflow is compatible with cyclic ion mobility for single pass and multipass image acquisitions.
- Multi-pass ion mobility separation in Cyclic MS was capable of high-resolution ion mobility separation of multiple ions with the same *m/z* values but different size.
- Tandem MS performed after multi-pass ion mobility separation was able to provide characteristic fragmentation pattern for separated ions leading to more accurate and confident identification of the molecule.

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