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Ambient Ionization and FAIMS Mass Spectrometry for Enhanced Imaging of Multiply Charged Molecular Ions in Biological Tissues

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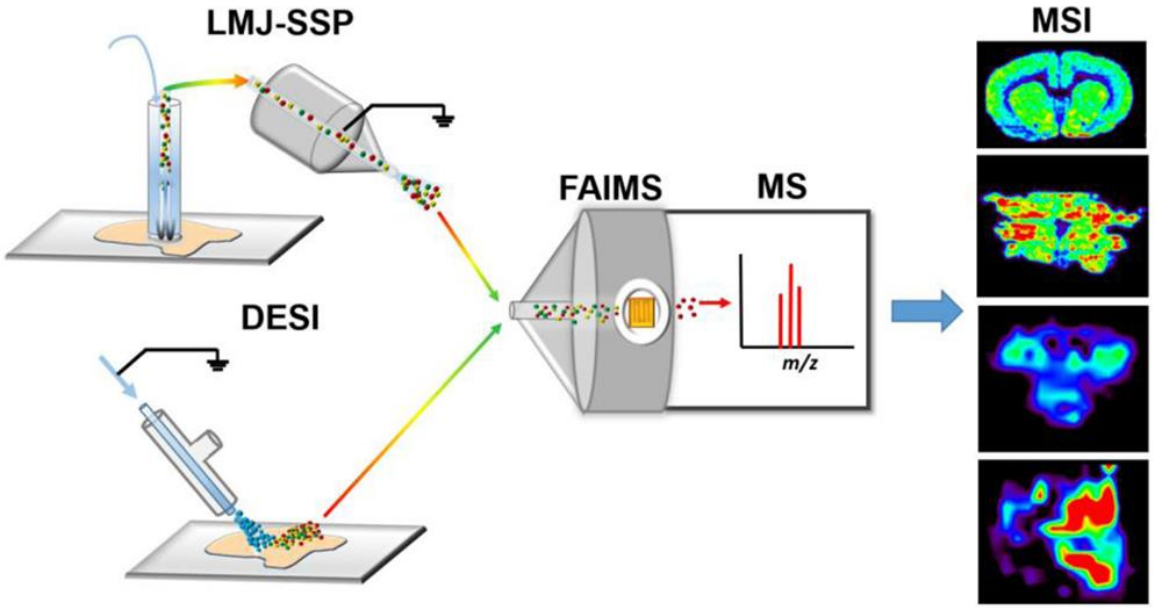
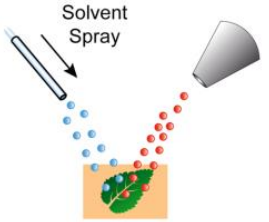


A. Suganya, 25th March 2017

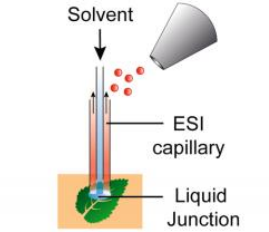
Introduction

- Mass spectrometry imaging (MSI) provides the outstanding ability of probing the spatial distribution of molecules in a sample surface with high specificity and sensitivity.

- In particular, ambient ionization MSI techniques have revolutionized the means by which spatial and molecular information is obtained from biological samples by enabling in situ, real time analysis of tissue samples with minimal pretreatment.



DESI

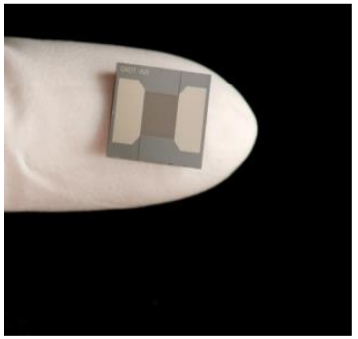


LMJSSP

Challenges

- Inherent challenges of sample complexity.
- Naturally occurring lipids, for example, present enormous diversity of molecular structures and are observed over a relatively narrow mass-to-charge (m/z) range as molecular ions.
- Protein imaging directly from biological samples is also an ongoing analytical challenge for ambient ionization techniques, although recent progress has been made.

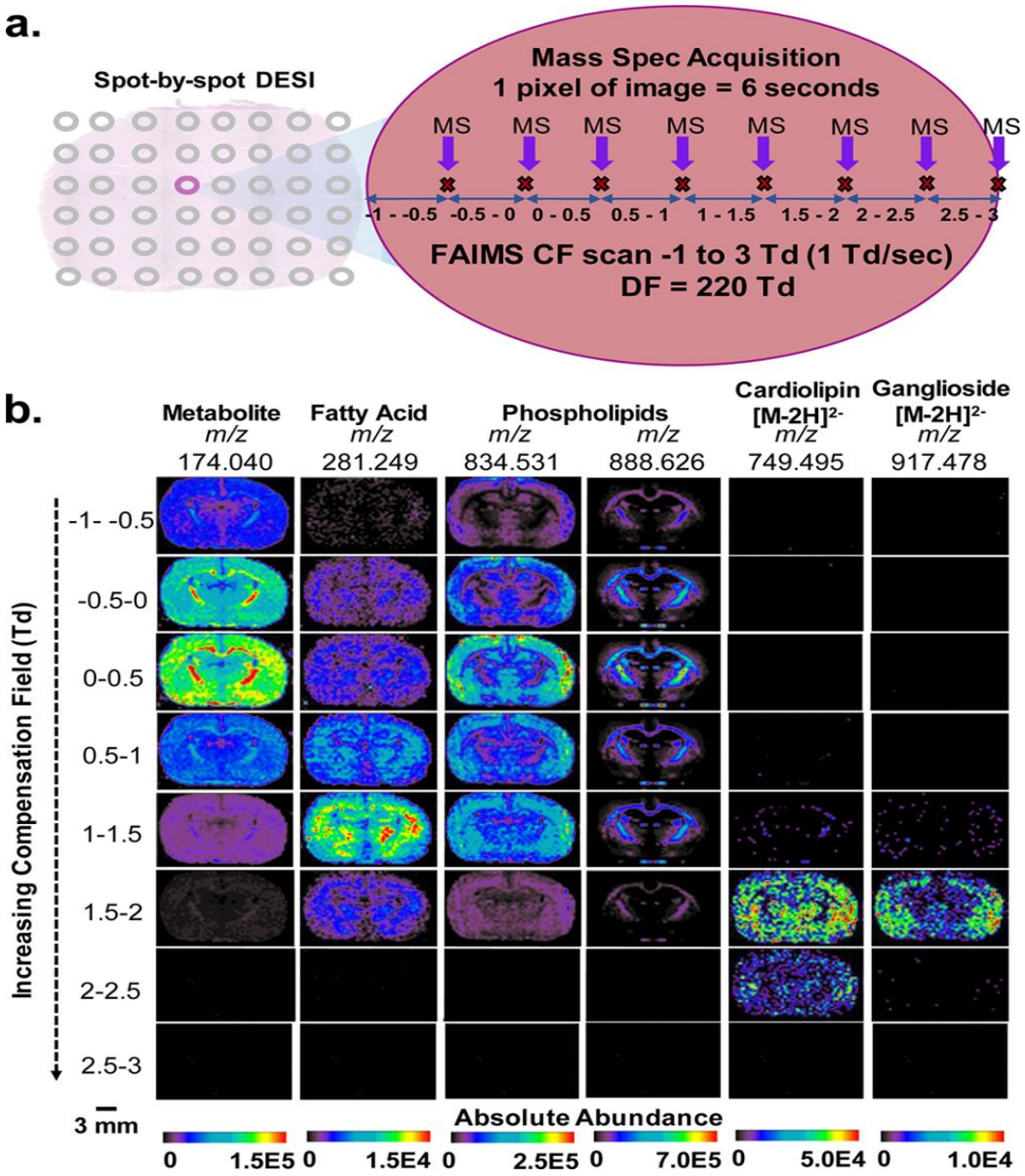
- Ion mobility has been increasingly applied to overcome issues in complex sample analysis by MS.
- In particular, high-field asymmetric waveform ion mobility spectrometry (FAIMS), or differential mobility separation, separates gas phase ions at atmospheric pressure on the basis of differences in their mobilities in electric fields prior to MS analysis.



In this Study

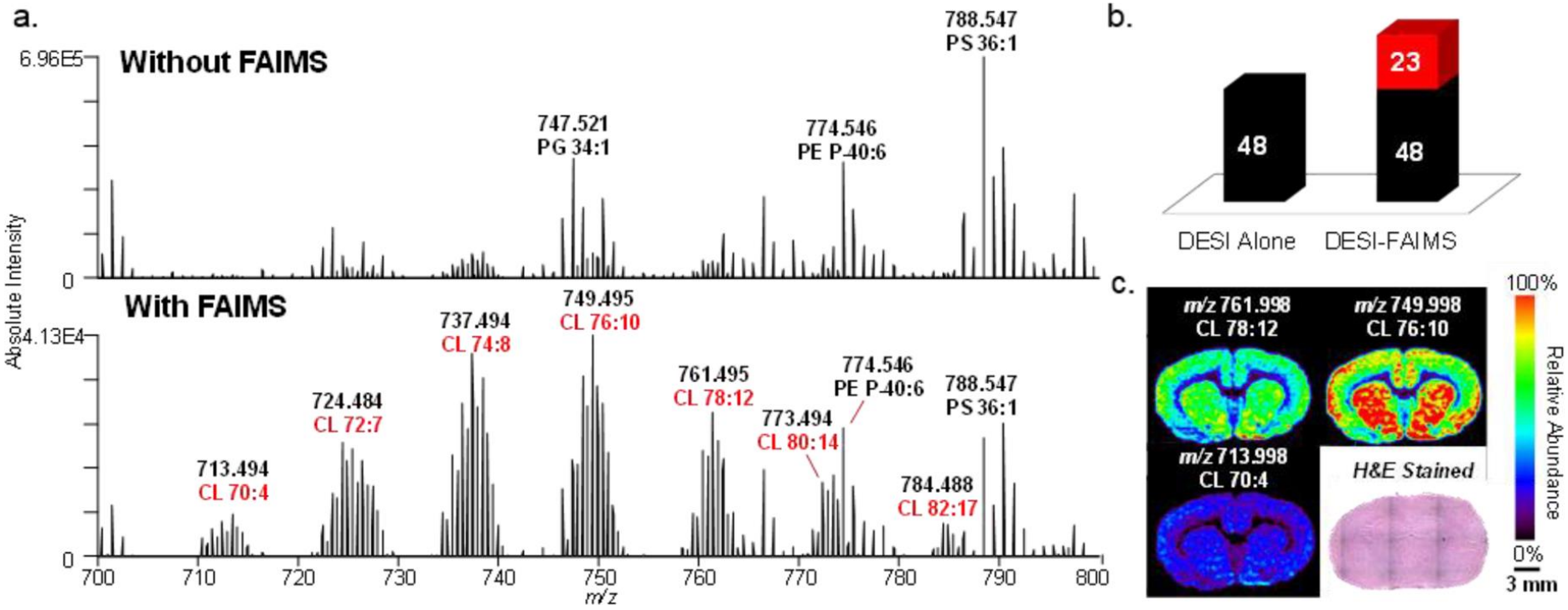
- Integrated and optimized a chip-based, highspeed ultraFAIMS device with DESI-MS/LMJ-SSP-MS and a high mass resolution mass spectrometer to image and characterize singly charged metabolites, singly- and doubly charged glycerophospholipids (GP) and glycosphingolipids, and multiply charged proteins in rat brain, human thyroid, and human ovarian cancer tissues.
- Indicate that integration of FAIMS with DESI or LMJ-SSP is valuable for imaging selected molecular classes in biological tissues.

Optimization of FAIMS for Separation of Lipids and Metabolites.



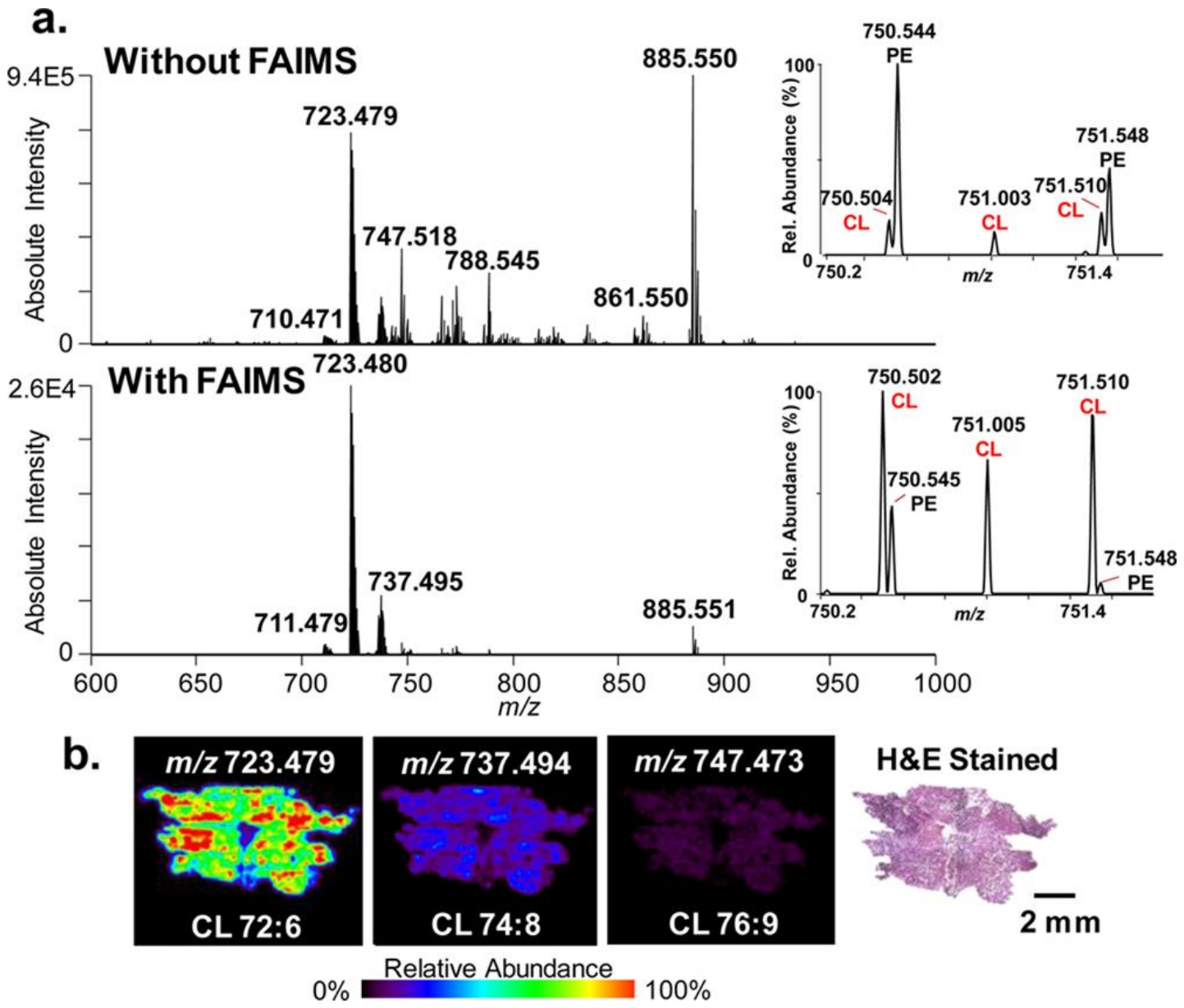
2D-FAIMS, spot-by-spot DESI-MS imaging of a rat brain tissue section. (a) Schematic of the 2D FAIMS sweep experiment used to create multiple sets of DESI-MS ion images at varied CF values in one tissue section.
(b) DESI FAIMS-MS ion images for a rat brain tissue section for 6 representative lipid and metabolite species, each acquired at DF = 220 Td and a different CF range, thus highlighting the increased transmission of different molecular species at each CF value.
Scale bar = 3 mm.

DESI-FAIMS Semiselective Imaging of Cardiolipins and Gangliosides in Rat Brain Sections.

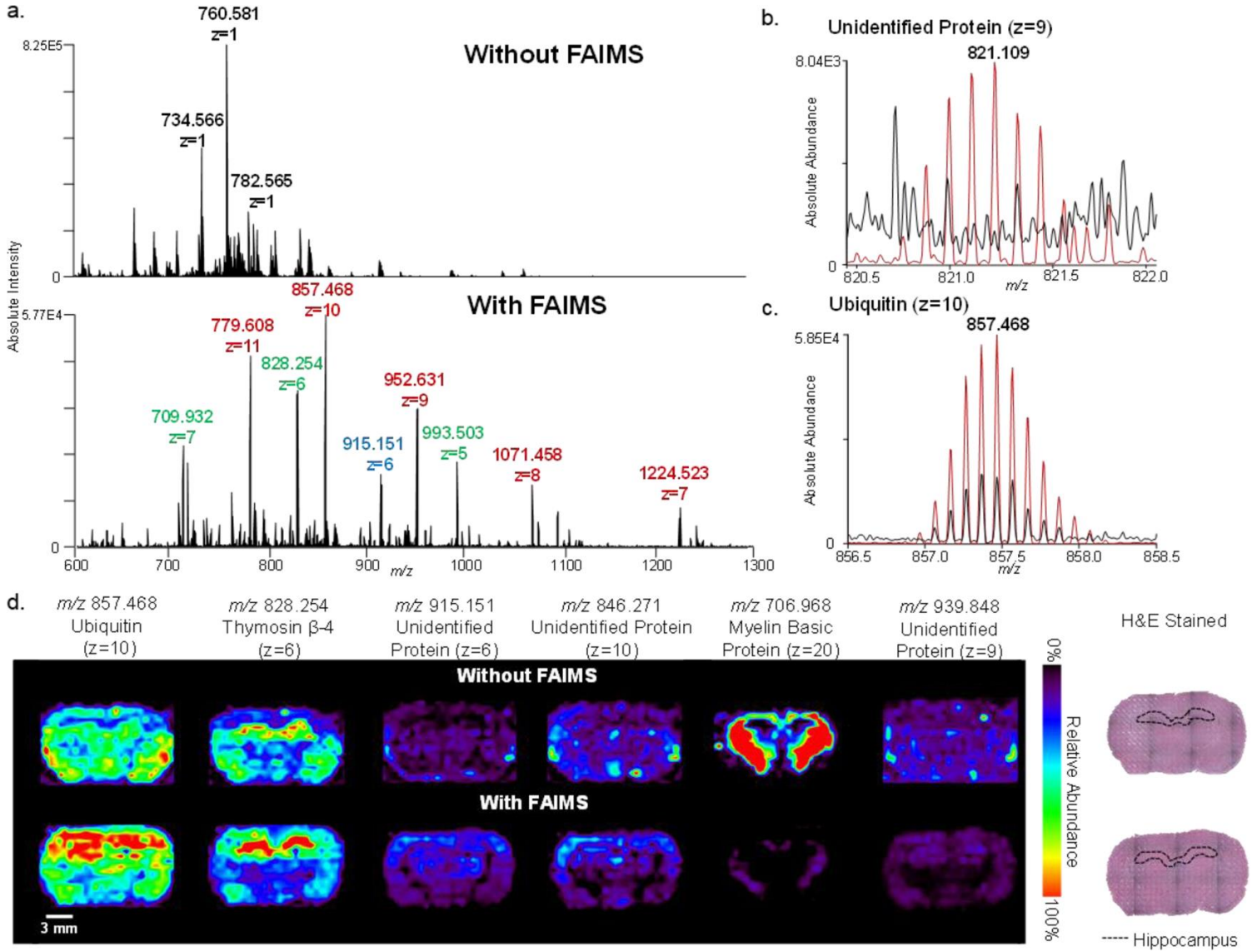


Static DESI-FAIMS-MSI of CL in rat brain tissue. (a) Representative negative ion mode DESI mass spectra acquired without and with FAIMS in the m/z 700−800 range at the optimized doubly charged CL parameters (DF = 220 Td, CF = 2.20 Td), showing a clear increase in the relative abundance of CL species (b) Chart of CL species detected with DESI alone and the DESI-FAIMS integrated approach. (c) 2D DESI-FAIMS ion images for selected CL species (spatial resolution of 200 μm).

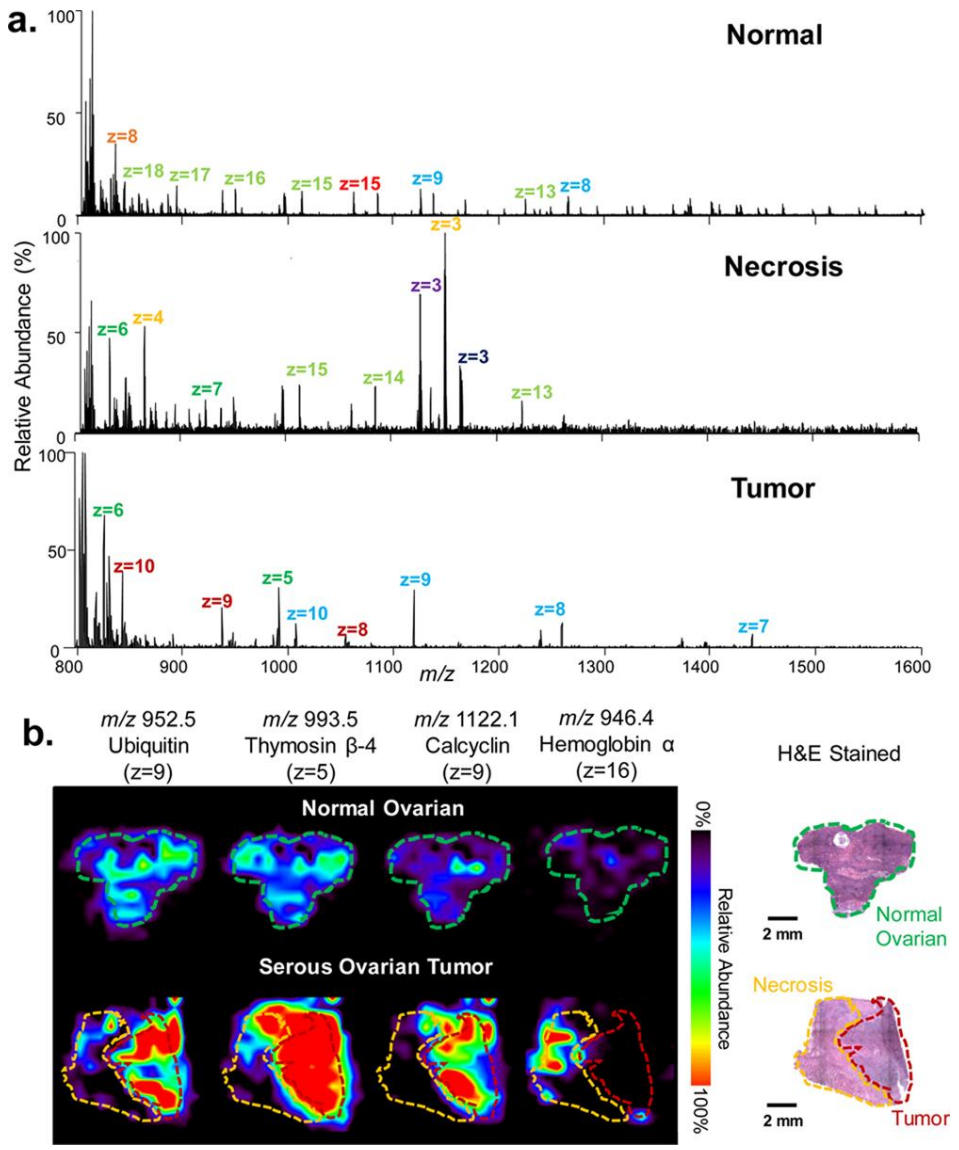
DESI-FAIMS-MSI of Cardiolipins in Human Oncocytic Thyroid Tumors.



LMJ-SSP-FAIMS-MSI of Proteins in Rat Brain Tissue Section.



LMJ-SSP-FAIMS-MSI of Proteins in Ovarian Cancer Tissue Sections.



Static LMJ-SSP-FAIMS-MS profiling and imaging of human normal and cancerous ovarian tissues.

(a) LMJ-SSP-FAIMS-MS spectra of normal ovarian, necrotic, and serous ovarian cancer samples in which different colored labels represent different charge states of same protein species.

(b) LMJ-SSP-FAIMS-MS ion images of ubiquitin, thymosin β-4, calcyclin, and hemoglobin α-subunit for a normal ovarian tissue sample compared with the high grade serous ovarian tumor sample, containing both necrotic and tumor regions (spatial resolution is ~630 μm). Optical images of H&E stained sections show regions of normal ovarian, necrotic, and high grade serous ovarian tumor.

Conclusions

- Optimized FAIMS parameters for proteins in the 4−12 kDa range allowed increase in the S/N, number detected, and visualization of the 2D spatial distribution of 84 protein species within rat brain, even with an overall decrease in total ion current.

- LMJ-SSP-FAIMS-MSI of human ovarian tissue samples enabled detection, identification, and correlation of spatial distribution of several proteins within the heterogeneous regions of the tissue samples, including regions of tumor, necrotic, and normal ovarian tissues. This is the first example of global protein imaging in human cancer tissue by ambient ionization MSI.

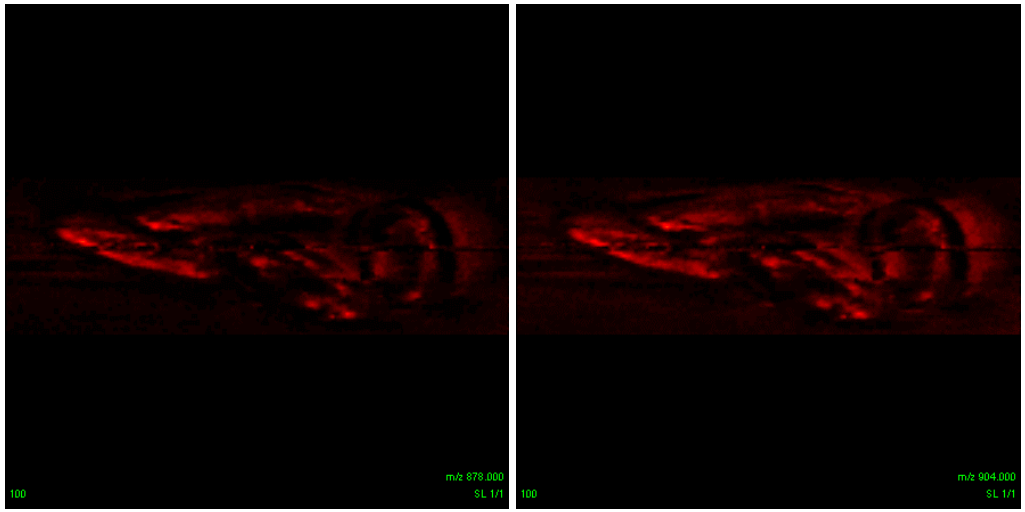
- Thus, addition of FAIMS to a DESI or LMJ-SSP MSI workflow at optimized conditions is valuable for the detection and spatial visualization of otherwise undetectable lipids and proteins of diagnostic importance in biological tissues.

- Further optimization of the integrated system is being pursued to increase sensitivity and ion transmission. As with other MSI techniques, the extent of molecular information obtained is significantly less than that achieved with standard HPLC-MS approaches.

- However, as ambient ionization MSI provides molecular and spatial information at a fraction of the time, without extensive sample preparation, we expect the integrated approach described here to be valuable in biomedical applications targeted at imaging specific diagnostic lipids and proteins which are otherwise difficult to detect in biological tissues.

DESI MS Imaging of Rice Grain Section

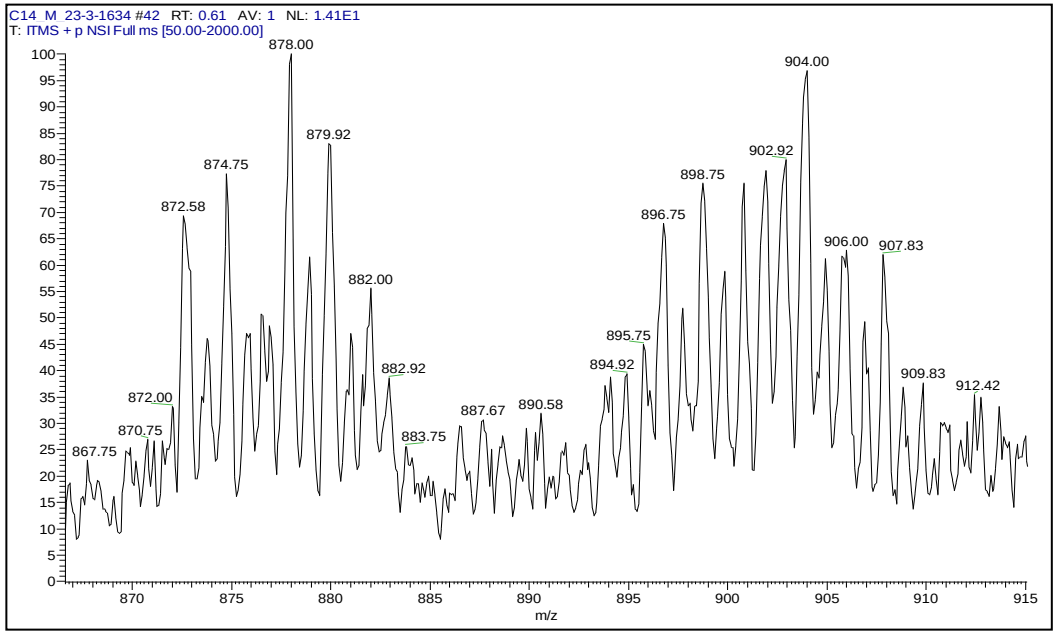
DESI MSI of hand sectioned multiple rice grains of C14 variety



m/z – 878

m/z – 904

Interfacing ion filters of this kind to DESI MS can aid in imaging of plant samples in high mass regions.



Thank you