Histology Directed Liquid Surface Extractions Coupled Directly to Liquid Chromatography for Improving Identification Strategies in Advanced IMS Applications

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Overview

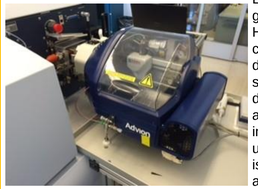
- The unambiguous identification of proteins detected in a matrix-assisted laser desorption/ionization mass imaging mass spectrometry (IMS) experiment is a challenge.
- Identifications are most commonly made with an offline experiment following the image.
- Spatially directed liquid surface extractions using a glass capillary (LESA© plus LC) coupled directly to high performance liquid chromatography (HPLC) allow the user to target discrete regions of for nanoESI.
- In this work bottom-up and top-down MS were used to investigate the efficiency of a LESA extraction as well as to define the robustness and reproducibility of the extraction.
- The combination of online and offline HPLC using liquid surface extractions allows for a new approach to generate robust, high throughput, reproducible extractions for the identification of proteins through top-down and bottom-up approaches.

Background

The ability to identify proteins in an IMS experiment is challenging, limiting the ability to frame protein imaging results in biological context. Due to the limited fragmentation efficiency of MALDI generated proteins ions, a number of approaches have been established leveraging bottom-up or top-down LC-MS/MS. These include tissue homogenizations and liquid surface extraction methodologies from serial sections of the tissue of interest.

The most commonly employed method of making offline protein identifications in an IMS workflow is completed by homogenizing a serial section of tissue followed by enzymatic digestion; subjecting the peptides to bottom-up methodologies. Although efficient, many proteins are still left unidentified in an image due to the inability to thoroughly match peptides to intact proteins from the loss of labile PTM's in the CID process. Other techniques have been introduced, such as peptide mass fingerprinting from digested tissue surfaces and trypsin-loaded hydrogels, but they still suffer from the same difficulty in matching identifications.

Liquid extraction surface analysis (LESA©) uses small volumes of solvent dispensed from a robotic mandrel to generate small, liquid micro-junctions between the tissue, liquid, and mandrel; allowing for the diffusion of analytes into the solvent. Top-down LC-MS/MS of LESA extractions from tissue sections has been shown to detect approximately 50-100 proteins with minimal sample preparation from manually pipetted extracts. This methodology is unique in that it allows the user to retain the spatial information gained during an IMS experiment.[1]



Herein we describe the use of an enhanced LESA© plus LC extraction with the use of a glass capillary that is coupled directly to HPLC. Through the use of a 150 µm i.d. capillary we are able to generate an increased droplet resolution on tissue using various solvent compositions, as well as generate data that is both robust and reproducible across a given experiment; showing a great improvement from our previous methods using manual pipetting. Lastly, the extraction is injected online to LC-MS in order to provide a new means to generate a higher-throughput means to gather spatially relevant protein identifications.

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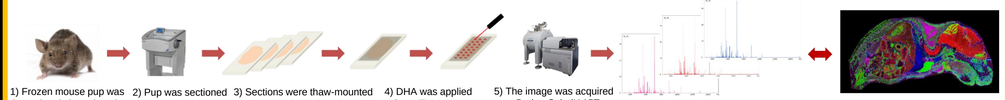
MALDI IMS



1) Frozen mouse pup was thawed and shaved on dry ice

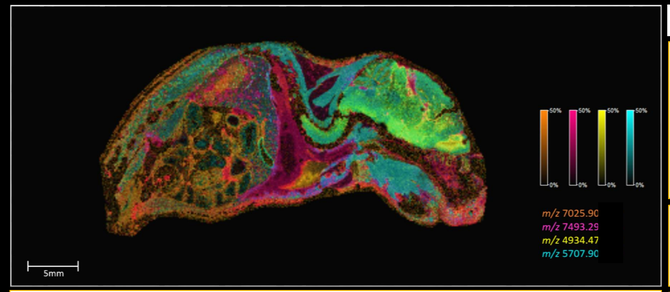
2) Pup was sectioned at 12 µm, at -20 °C using a Thermo Cryostat NX70

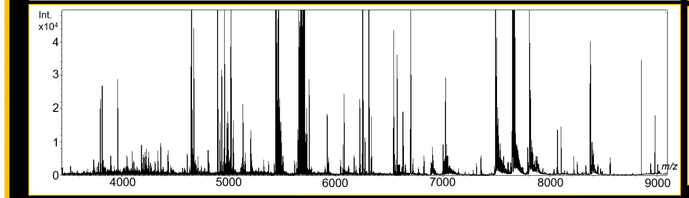
3) Sections were thaw-mounted onto conductive indium tin oxide slides and were either imaged or used for extractions

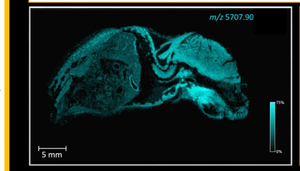
4) DHA was applied using a TM sprayer at a concentration of 15 mg/mL in 90:10, ACN:H_2O

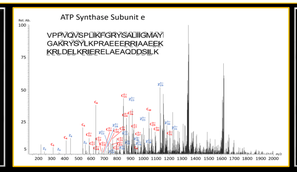
5) The image was acquired on a Bruker SolariX 15T FTICR at 125 µm spatial resolution

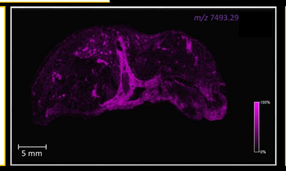
6) Data was analyzed and visualized on FlexImaging software. Isolation windows were 5 Da wide in FlexImaging for visualizing ion images.

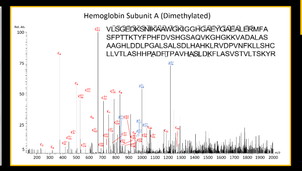


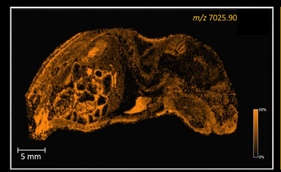


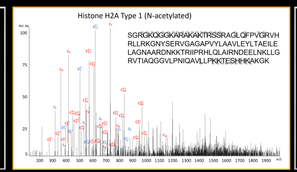


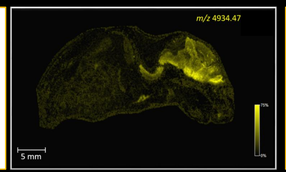


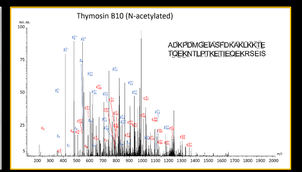






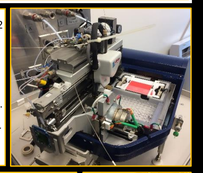


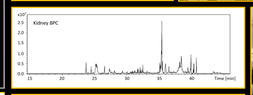


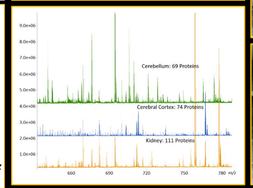


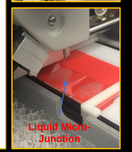
Online LC-MS

LC Methods: RP HPLC using 2 µL extract injected directly on column: 21 cm long, 100 µm i.d., packed with 20 cm of C_4 (3.4 µm, 400 Å). Solvent A: H_2O, B: ACN (0.1% FA). Proteins eluted at 0.400 µl/min. Gradient: 2-30% B in 23 min, 30-95% B in 10 min, 95% B for 5 min, 95-1% B in 3 min.



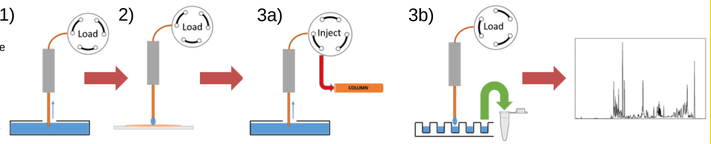


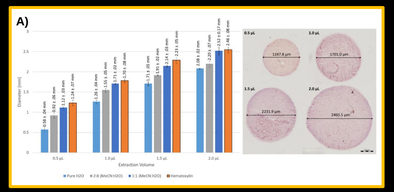


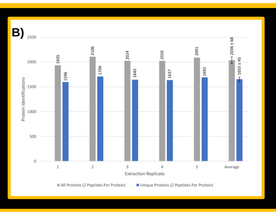


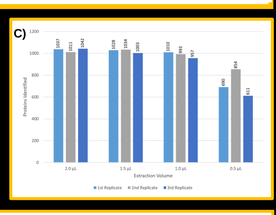
LESA Extraction and Offline HPLC

1) Extraction solvent (varying ACN:H2O mixtures) is aspirated into capillary from reservoir
2) Solvent is dispensed onto tissue surface and dispensed/re-aspirated repeatedly
3a) More solvent is aspirated to bring sample onto loop for LC analysis
Or
3b) Extraction is deposited into 96-well plate and is dried down for offline analysis







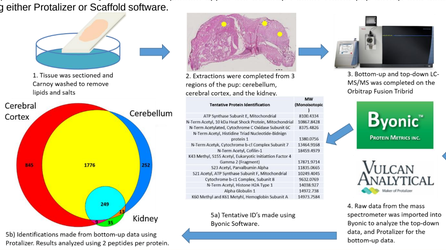


A) Various solvent compositions are measured from water soluble paper to illustrate the droplet resolution capable with the new glass capillary extraction. Droplet size on paper was able to be measured < 600 µm

B) Droplet reproducibility measured across 5 extractions. 2 µL of 1:1, ACN:H_2O, was dispensed on digested rat cerebellum and extracts were subjected to bottom-up LC-MS/MS workflows. Extractions proved to be reproducible with an RSD < 3.5% across the runs.

C) Total protein identifications vs. LESA droplet volume. The extractions were completed in triplicates and showed little variability across the 1.0-2.0 µL range. The variability among the 0.5 µL volume was expected due to the difficulty in manipulating that small of a volumes.

- Extractions were completed using 2 µL (unless otherwise noted) volumes of 1:1, ACN:H_2O (0.5% FA)
- Digested extracts were collected in 100 µL of ddH$_2$O (0.1% FA), dried down and re-suspended in 10 µL of the same solvent
- Intact protein extracts were collected immediately before analyses and dispensed in 15 µL of ddH$_2$O (0.1% FA)
- Samples were ran on the Q Exactive Plus (bottom-up) or the Orbitrap Fusion Tribrid (top-down), and data was analyzed using either Protalizer or Scaffold software.



CONCLUSION

- Liquid surface extractions were successfully completed from digested rat brain cerebellum
- Droplet spot size on water soluble paper was found to be below 600 µm and ca. 1.2 mm from tissue
- The extraction was determined to be reproducible across a given tissue type
- Extractions were completed from digested and non-digested mouse pup, in order to determine the total number of protein identifications from three distinct regions
- Top-down mass spectrometry was completed on intact protein extractions, and tentative identifications were made using Byonic Software; selected spectra where then manually interpreted.
- Online LC-MS was coupled directly to a liquid surface extraction from mouse pup.
- Future work entails the use of top-down fragmentation techniques such as ETD and CID to generate fragmentation data from online LC-MS/MS.

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