High-resolution Tissue Imaging Using sub-Atmospheric and Atmospheric MALDI Sources

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Overview

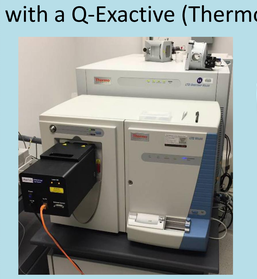
Novel Aspects: High-spatial resolution with **AP-MALDI(ng)** and **subAP-MALDI (ng)** MSI sources utilizing highly volatile (DHA) and standard matrices (CHCA).
Wavelength dependence of ion yields and S/N ratios in MALDI and Ag-nanoparticle (AgNPs) LDI processes.

Introduction

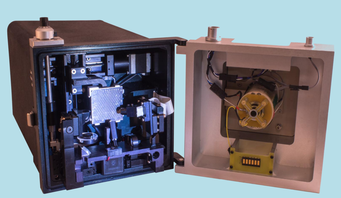
The AP-MALDI source from MassTech Inc was used for MS high-resolution tissue imaging (MSI). Contrary to vacuum and subAP-MALDI sources, this source is indispensible when working with highly volatile MALDI matrices.
2,6 dihydroxyacetophone (DHA) has proven to be an excellent matrix for MSI of lipids, especially gangliosides. A major problem with the use of DHA, as it applies to MSI, is that it sublimes under vacuum within a few hours. Utilizing the AP-MALDI source allows one to apply DHA for MS imaging of entire organs that takes typically over 12 hours.
CHCA, on the other hand, is the most efficient matrix for the analysis of a broad range of biomolecules in tissues. The subAP-MALDI source ensures significantly reduced overall ion loss and thus provides higher ion throughput. This diminishes 2-4 fold the analysis time in MSI studies.
In this study, we used AP and subAP MALDI sources to obtain high spatial resolution images of lipids in rat kidney and brain.

Materials

Rat brain and kidney were harvested after euthanasia and stored at -80C. Tissue sections (18 µm thick) were cut using a cryostat and placed on a flat stainless steel target. DHA was prepared at 10 mg/mL in 50% ethanol with 125 mM ammonium sulfate and 0.05% HFBA and sprayed onto the tissue section with a mist sprayer (HTX Imaging, LLC).
An Orbitrap Velos Pro mass spectrometer (ThermoFisher Scientific) was used with the AP-MALDI source (MassTech Inc). The SubAP-MALDI source (MassTech Inc) was used with a Q-Exactive (ThermoFisher Scientific)



AP-MALDI(ng) source installed into Orbitrap Velos



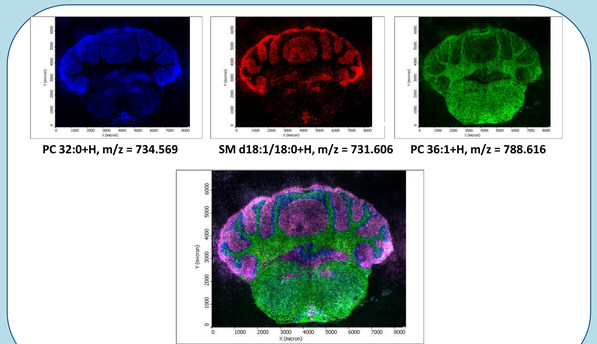
AP-MALDI(ng) source operates with the ion funnel at 2-10 Torr pressures

MS imaging studies were conducted in positive ion mode within a mass range of 200-1,000 Daltons with mass resolution up to 70K and spatial resolution down to 20 µm.

Results

Rat brain MS imaging in SubAP MALDI source

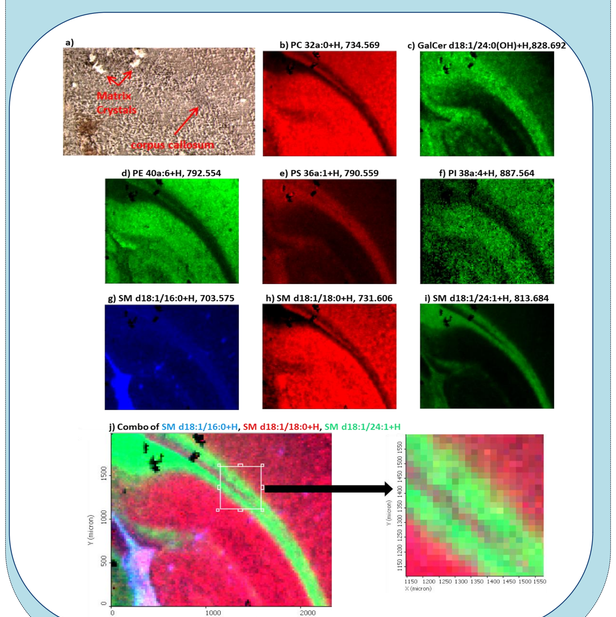
SubAP-MALDI MS imaging at 4 Torr was conducted, for the first time, on a rat brain **(CHCA)** in a positive mode. Spatial resolution was 30 µm, mass resolution was 60-70,000. The total run time for the entire brain MSI was ~16 hours; there was no significant change in ion counts from start to finish.



Approximately 100 lipid species were detected and imaged as [M+H]+ peaks. Combo images clearly reveal the major anatomical regions of the rat cerebellum.

AP-MALDI MSI with high spatial resolution

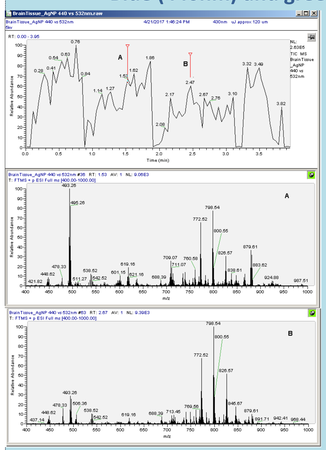
MSI at a 20 µm spatial resolution on a coronal rat brain section in Bregma -6.00mm. **Matrix: DHA.** Combo plots of white matter and grey matter lipid species (e.g., SMs) produced high spatial resolution image of the lateral ventricle in the *corpus callosum* down to 20 µm (single pixel).



Studies on LDI of tissue lipids with AgNPs as a matrix using a tunable laser and AP-MALDI source

Brain tissue sections were implanted with AgNPs using an **NPlanter (Ionwerks, Houston, TX)** which uses a magnetron sputtering source. AgNP 6 nm in size were selected using a quadrupole mass filter. The selected charged particle beam was accelerated to 500 eV. The automated system scanned the beam across the sample to ensure uniform deposition. The result is a thick 2–4 monolayer Ag-coated tissue sample.
At atmospheric pressure, AgNPs were proven to be a very effective matrix for the analysis of *cholesterol as a silver adduct, and phosphatidylcholines (PC) and sphingomyelins (SM)* producing +H, +Na, and +K mass peaks.
Opolette 355 tunable laser from **Opotek (Carlsbad, CA)** was used to generate laser radiation from 410 nm to 1400 nm.

Blue (440nm) and green (532 nm)

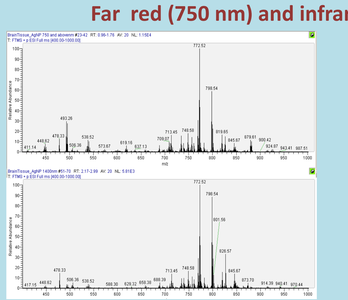


Total ion chromatogram for a single line MS scan across central section of the rat brain. First two scans - 440 nm and the third one at 532 nm.

A. Mass spectrum recorded at 440 nm wavelength (WL) corresponding to a maximum of UV absorbance of AgNPs (6-10 nm) in tissues.

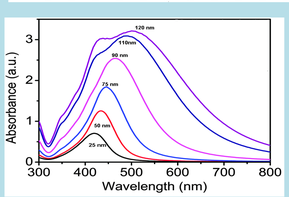
B. Mass spectrum at 532 nm, noticeable reduction in low MW peak heights.

Far red (750 nm) and infrared (1400 nm)



Mass spectrum at 750 nm. The trend in reduction of peak heights at low MW continues.

Mass spectrum at 1400 nm. Overall peak height dropped a factor of 6-10 with respect to 440 nm

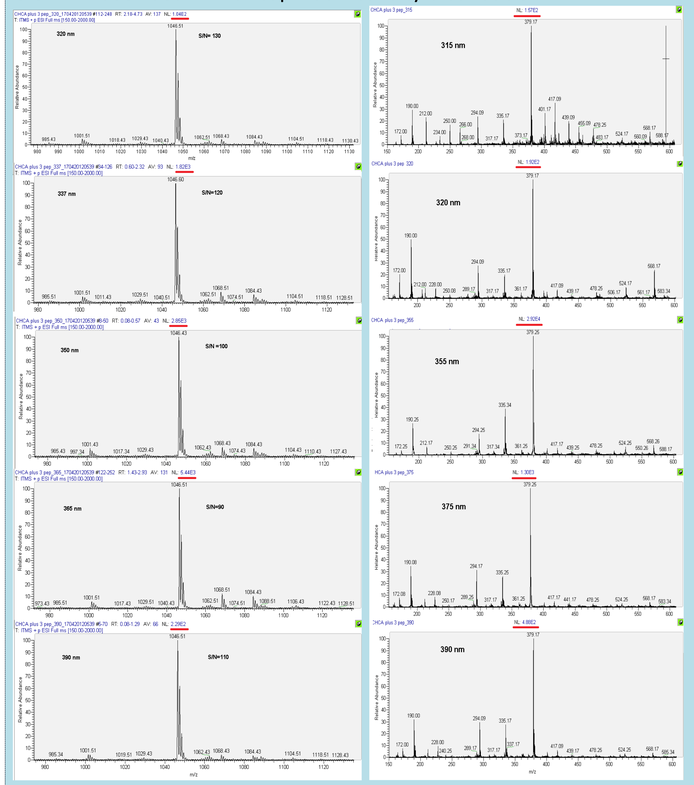


UV-VIS absorbance of AgNPs of different size in water. (R.Zong *et al*, PCCP, 2014,16: 4236)

The fact that AgNP-implanted tissues absorb photons over a very broad range of WLs indicates that in the process of implantation, AgNPs form aggregates of various size.

Studies on MALDI on peptide mix and CHCA with a tunable laser and AP-MALDI source

Samples: dried droplets (10 mg/mL, CHCA), 2 mm in diameter, containing three peptides (1 pmole/µL concentration), scanned with 0.3 mm laser beam with top-hat intensity distribution.



"Chemical noise" in the vicinity of Ang II peak at different WLs.

"Matrix-related" peaks (MW<600Da) at different WLs.

Conclusion

- **AP-MALDI** MSI source coupled with the Orbitrap allowed a use of highly volatile matrices (i.e., DHA) efficient for imaging of neutral lipids.
- **Sub APMALDI** MSI source coupled with the ion funnel produces larger ion signals at the same laser fluences. A use of the sub AP source reduces the time of MS imaging runs.
- **Laser WLs** has not very significant effect on the quality of LDI mass spectra of lipids in MSI obtained using AgNPs, though it effects ionization efficiency of low MW components (e.g., cholesterol).
- **Laser WLs** has a noticeable influence on MALDI analysis of peptides, effecting the ion yield. Yet, the S/N ratios stay practically the same. Also, the intensity of peaks corresponding to products of matrix/matrix clusters dissociation at low MW is lower at "red " WLs (>355 nm)

Acknowledgment

Authors greatly acknowledge Dr. Mark Little from Opotek for his aid in conducting tunable laser experiments