The new gold standard for Mass Spectrometry Imaging

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Objectives

- Determine the breadth of compounds that can be detected using AuNPs for Laser Desorption Ionization Mass Spectrometry
- Optimize ionization conditions using AuNPs
- Explore advanced computational techniques for streamlined data analysis

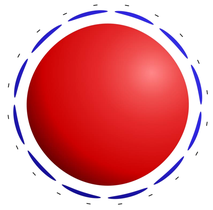


Figure 1. Cartoon of citrate-capped gold nanoparticles that facilitate ionization of small molecules in LDI-MS.

Background

The detection of endogenous metabolites, such as neurotransmitters (NTs), is becoming increasingly important in biological systems. Tracking NT spatial location and concentration will significantly impact the understanding and treatment of disease. The use of novel citrate-capped gold nanoparticles (AuNPs) for laser desorption ionization (LDI) mass spectrometry (MS) has several advantages over previous methods. Specifically, low chemical noise background and high ionization efficiency are observed for NTs. Mass Spectrometry Imaging (MSI) is a powerful label-free technique that can determine the lateral spatial distribution of hundreds of compounds in one experiment. Figure 2 shows the method for the MSI process; briefly, tissue sections are pneumatically sprayed with AuNP solution, imaged at each spatial location via a raster pattern, and can be histologically stained after the MSI experiment. Each mass spectral peak of interest can be interrogated to give an image that shows the spatial location of that molecule of interest.

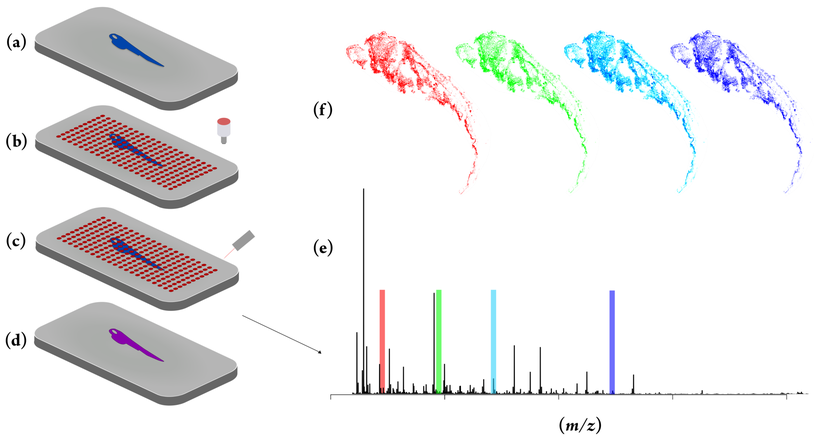


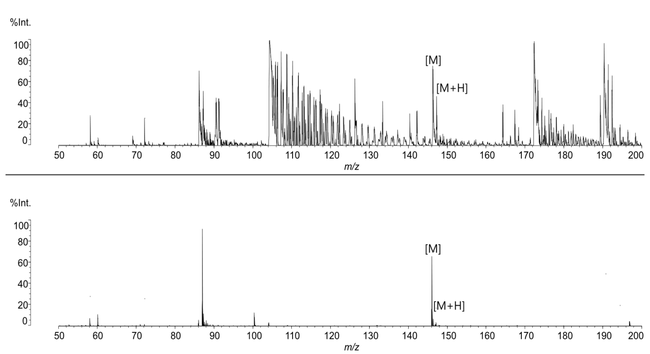
Figure 2. MSI Workflow showing sample preparation steps and resulting data. Where (a) is tissue sectioning, (b) is pneumatically applying AuNPs, (c) is analysis using a raster pattern, (d) is histological staining, (e) is the resulting mass spectrum from which the images in (f) can be generated for unique m/z ratios.

Experimental Methods

NTs were prepared at a ratio of 1 AuNP: 10^5 analyte molecules for target plate experiments and were performed on a Kratos Axima MALDI-TOF MS (Shimadzu Instruments, Columbia, MD). Zebrafish embryos up to 5 days post-fertilization (dpf) were sacrificed using 600 mg/L MS-222. Embryos were frozen in a cryomold and then embedded in M1 cryomedium (Thermo Scientific). Tissue was sectioned using a Leica cryostat at 10 mm thickness, then sprayed with AuNP solution using an HTX TM sprayer (Chapel Hill, NC) and imaged using a Bruker Rapiflex (Bruker Daltonics, Billerica, MA). Comparisons to traditional organic acids matrices were done for both types of experiments.

Results: Target Plate

Figure 3. Positive ion LDI mass spectra of glutamate using (a) organic acid CHCA as matrix (b) 2 nm AuNPs. The intact molecule appears at *m/z* 147, and fragment ions are indicated by asterisks. CHCA results in high chemical noise while AuNPs does not.



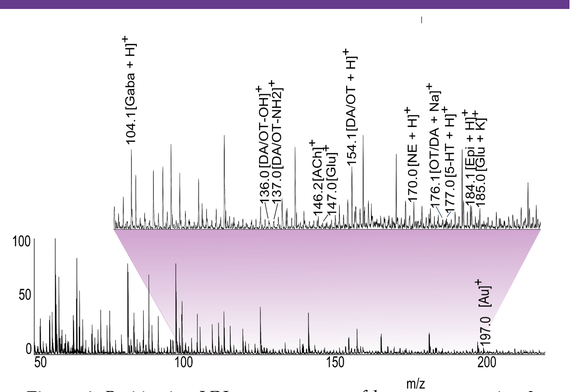


Figure 4. Positive ion LDI mass spectrum of human serum using 2 nm AuNPs. Various NTs are observed and are indicated. Comparison with organic acid (not shown) results in no NT identification.

Results: Zebrafish Embryo Imaging

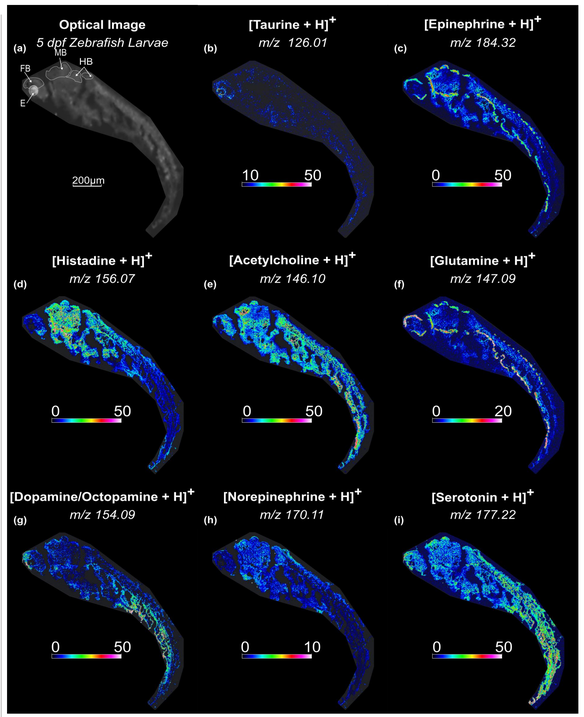


Figure 5. MSI of sagittal 5 dpf zebrafish embryo tissue section imaged at 5 µm lateral spatial resolution, where (a) is the optical image showing eye, forebrain, midbrain, and hindbrain, (b) is taurine, (c) is EP, (d) is histidine, (e) is ACh, (f) is GLU, (g) is DA/OT, (h) is NE, and (i) is 5-HT.

Results: Neuroblastoma Imaging

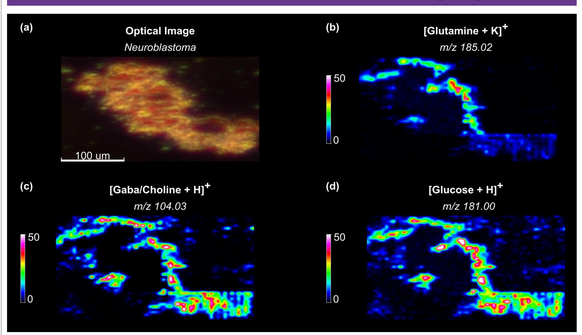


Figure 8. MSI of neuroblastoma cells imaged at 5 µm lateral spatial resolution where (a) is the optical image, (b) is the GLU + K adduct, (c) is GABA/Choline, and (d) is glucose.

Results: Rabbit Brain Imaging

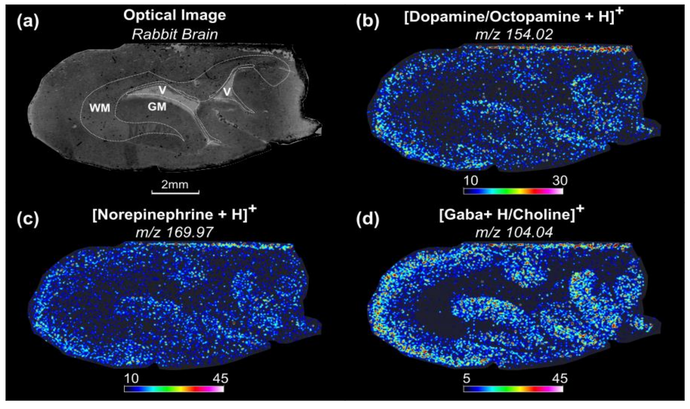


Figure 6. MSI of coronal rabbit brain tissue section imaged at 20 µm lateral spatial resolution where (a) is the optical image, (b) is DA/OT, (c) is NE, and (d) is GABA/Choline. Images generated from 1 spray of AuNPs. Distribution of NTs is appropriate for the anatomical regions.

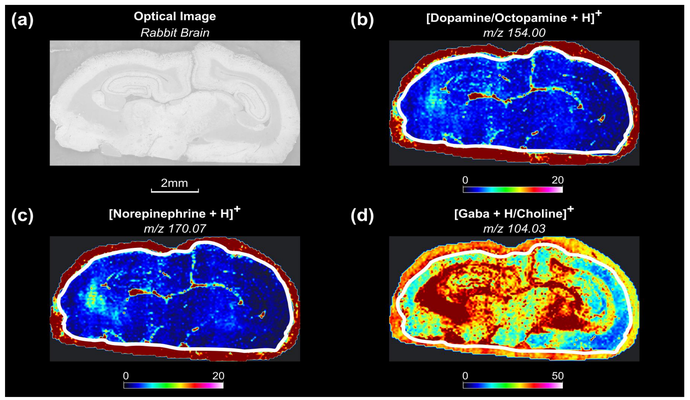


Figure 7. MSI of coronal rabbit brain tissues section sprayed with CHCA imaged at 20 µm lateral spatial resolution where (a) is the optical image, (b) is DA/OT, and (c) is NE, (d) is GABA/Choline. The superimposed bold white lines in (b) – (d) is the tissue border, showing the extent of delocalization from CHCA.

Conclusion

We have successfully shown that pneumatically sprayed AuNPs can be used to detect small molecules. We have shown detection of many neurotransmitters and their distributions, in situ. Finally, we have shown one of the major advantage of using AuNPs in terms of delocalization.

References

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