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

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.

Experimental Methods
NTs were prepared at a ratio of 1 AuNP:10³ analyte molecules for target plate experiments and were performed on a Kratos Axima MALDI-TOF MS (Shimadzu Instruments, Columbus, MD). Zebrafish embryos up to 5 days post-fertilization (dpf) were sacrificed using 600 mg/L MS222. Embryos were frozen in a cryomold and then embedded in M1 cryo medium (Thermo Scientific). Tissues were 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 Rapid (Bruker Daltonics, Billerica, MA). Comparisons to traditional organic acids matrices were done for both types of experiments.

Results: Target Plate
Figure 3 shows a positive ion LDI mass spectra of glutamate using (a) organic acid CHCA matrix (b) 2 nm AuNPs. The intact molecule appears at m/z 147, and fragmentations are indicated by asterisks. CHCA results in high chemical noise while AuNPs show less.

Results: Neuroblastoma Imaging
Figure 4 shows a 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 shows MSI of sagittal 5 dpf zebrafish embryos tissue section imaged at 5 nm lateral spatial resolution, where (a) is the optical image showing eye, forebrain, midbrain, and hindbrain, (b) is anatomic, (c) is histrionic, (d) is ACh, (e) is GABA, (f) is DA/OT, (g) is NE, and (h) is 5-HT.

Results: Rabbit Brain Imaging
Figure 6 shows MSI of coronal rabbit brain tissue section imaged at 20 nm lateral spatial resolution; (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 anatomic regions.

Results: Neuroblastoma Imaging
Figure 7 shows MSI of coronal rabbit brain tissue section sprayed with CHCA imaged at 20 nm lateral spatial resolution; (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 advantages of using AuNPs in terms of delocalization.

References
McLaughlin, Nolan; Bielinski, Tyler; Treiber, Caleb; Barton, Eric; Claudi, Kristine; Stumpo, Katherine A. “AuNPs for neurotransmitter analysis in LDI-TOF-MS: JAMS 2020 submitted.

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

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.

Figure 3. Positive ion LDI mass spectra of glutamate using (a) organic acid CHCA matrix (b) 2 nm AuNPs. The intact molecule appears at m/z 147, and fragmentations are indicated by asterisks. CHCA results in high chemical noise while AuNPs show less.

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.

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

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