High-Throughput Lipid Profiling of Protein Classified Cells Using Optically Guided Single-Cell Mass Spectrometry

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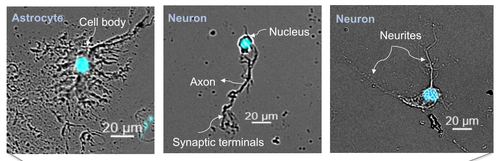
Introduction

Investigating the chemical content of a cell provides clues about the state of the cell and details on cell-to-cell heterogeneity. Lipids, as one of the most abundant classes of molecules in the brain, with over 20,000 distinct species, are essential for a wide range of processes within the nervous system. Lipid heterogeneity has been observed not only among various cell types but also within cells of the same type. Recent advancements in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) have enabled the characterization of lipids in thousands of single cells in an untargeted manner (1). When combined with immunocytochemistry (ICC) labeling, it can reveal chemical differences in SCs labeled by their canonical cell types. Here, we integrate high-throughput optically guided MALDI SCMS with photocleavable mass-tags (PCMTs), enabling multiplex cell classification beyond the traditional fluorophore-labeled ICC method. This allows us to **classify and subclassify cell types and correlate them to their unique lipid features**. By using rodent models of neurodegenerative disease, we hope to correlate the observed neurochemical changes with aging and neurodegenerative progression.

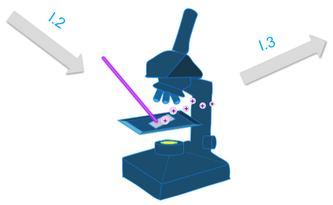
Method

Single-Cell MS (SCMS) Workflow

Round I - Untargeted SCMS:

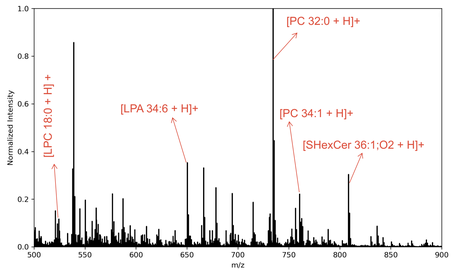


I.1 Dissociated heterogeneous rat hippocampal single cells with unique morphological features are plated on ITO-coated glass slide.

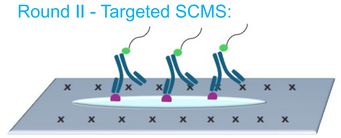


SCs are assayed through optically guided MS using microMS (2).

Average lipid spectrum of 1336 cells. Some peaks are labeled with their putative lipid classes.

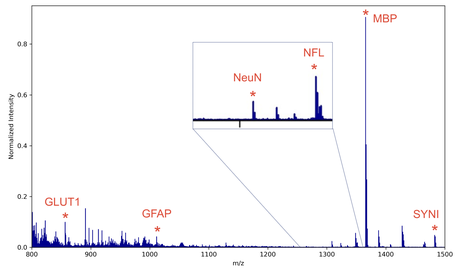


Round II - Targeted SCMS:



II.1 SCs are labeled with PCMTs through ICC.

Average mass-tag ion spectrum of the same cells labeled with antibodies.



GLUT1 (Glucose Transporter 1)	Mostly associated with blood-brain barrier endothelial cells and astrocytes.
GFAP (Glial Fibrillary Acidic Protein)	Supports the cytoskeleton of astrocytes.
NeuN (Neuronal Nuclei)	Labels the nucleus of neurons.
NFL (Neurofilament Light)	Associated with the axons of neurons.
MBP (Myelin Basic Protein)	Labels oligodendrocytes and myelinated neurons.
SYNI (Synapsin I)	Associated with the synaptic vesicles and synaptic terminals of neurons.

Results

Spatial Ion Distribution of Photocleaved Mass-Tags in MSI

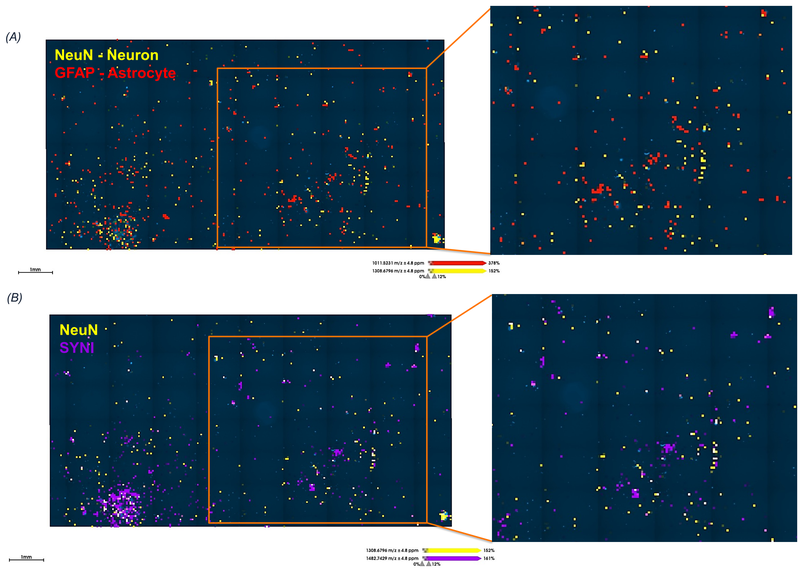


Figure 1 Overlay of the mass-tag ion image with the DAPI fluorescence image. *(A)* NeuN, a neuron-specific cell marker, and GFAP, an astrocyte-specific cell marker, show unique spatial ion distribution within the cell region. *(B)* While SYNI, a synaptic vesicle surface protein marker, is highly expressed, clustered cell regions are avoided for SCMS analysis..

Cell Classes with Distinct Lipid Profiles

Figure 2 Single cells from the rat hippocampus classified according to one or more of the six PCMT markers.

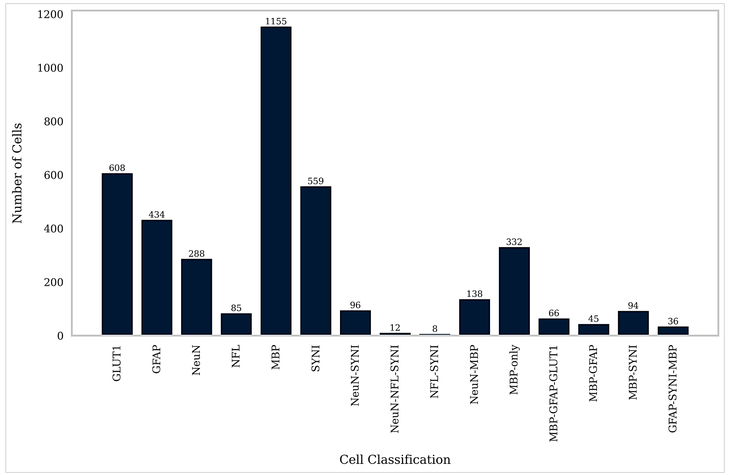
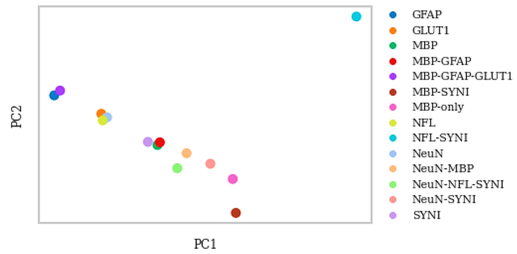


Figure 3 The principal component (PC) analysis of the average lipid spectrum for each cell class is shown in the 2D plot. PC1 accounts for 45% of the variance, while PC2 contributes to a cumulative variance of 72%. The plot demonstrates that GFAP, NeuN, SYNI, and MBP form well-separated clusters, indicating that these cell classes have unique lipid profiles.



Results

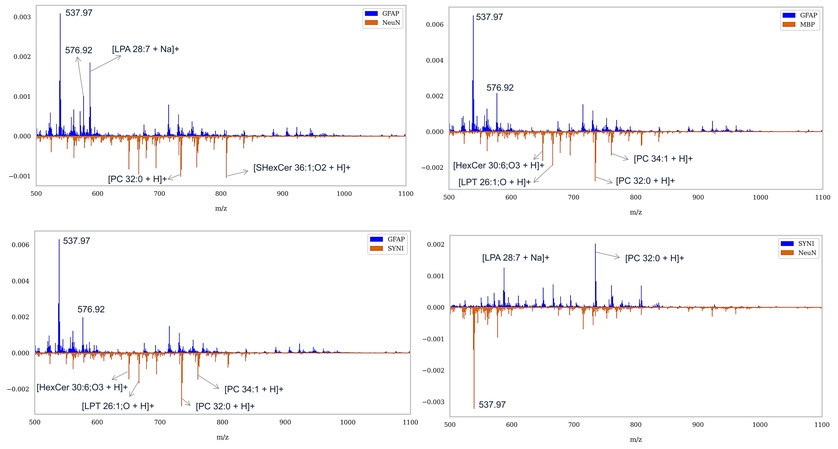


Figure 3 Mass spectrum subtraction highlights the ion intensity differences between classes of cells with distinct lipid profiles. The normalized ion peaks showing the greatest intensity differences are labeled with their m/z values or their putative lipid classes.

Ongoing Research: Expanding the Toolbox for Alzheimer's Disease (AD) Study

We are using the 5X familial mutation (5XFAD) transgenic mouse model to investigate the link between lipid alterations and amyloid plaque formation, a hallmark of AD.

Figure 4 *(A)* Spatial ion distribution of protein NCAM1-CD56 shows upregulation in the 5xFAD mouse model along with GFAP and Amyloid-β42 (Aβ42), colocalized in the cerebral cortex. *(B)* The average mass spectrum of single cells from the cerebral cortex show a similar trend, with Aβ42 association observed only in the 5XFAD model compared to the WT.

Conclusions

- A multimodal SCMS has been developed and demonstrated, capable of characterizing lipid species and selected proteins in single cells from the rodent brain. This approach has enabled us to identify lipid heterogeneity in individual brain cells, which have been classified based on their protein profiles.
- We identified overexpressed biomarkers in the 5XFAD brain tissue and dissociated single cells. This information, combined with cellular lipid content, allows us to examine changes in lipid metabolites of Aβ-associated cells.
- By extending our PCMT toolbox to target a variety of distinct brain cell types, we gain a better understanding of the biochemical changes underlying these neuropathological conditions.

References

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2. Comi, T. J.; Neumann, E. K.; Do, T. D.; Sweedler, J. V. MicroMS: A Python Platform for Image-Guided Mass Spectrometry Profiling. *J. Am. Soc. Mass Spectrom.* **2017**, *28* (9), 1919–1928.

Acknowledgements

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Conflict of interest disclosure: authors MJL, GY, and KJR are currently employed by AmberGen, Inc.