Integrating MALDI-MSI with LCM-MS: Advancing spatial multi-omics analysis in brain tissue

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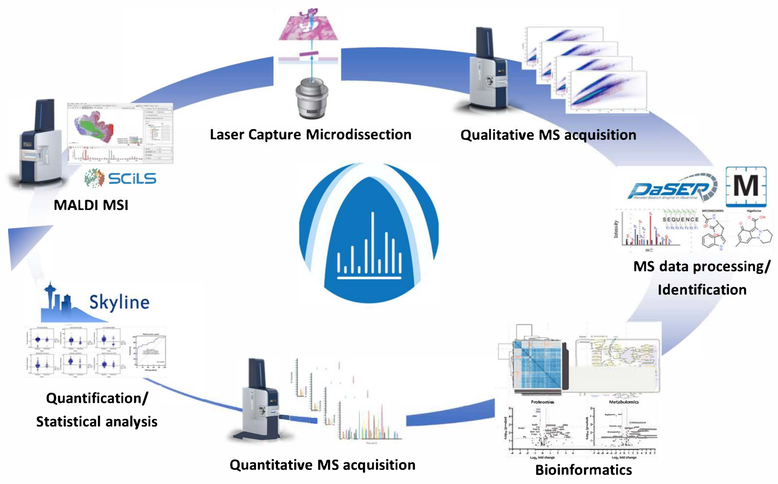
Introduction

Spatial omics has revolutionized biomolecular analysis by preserving molecular spatial context in tissues, cells, and diseases. This advancement has found applications in neuroscience, cancer biology, and drug discovery. To enhance current analytical techniques, we introduce MALDI-MSI-guided LCM-MS, combining mass spectrometry imaging and laser capture microdissection. Our approach enables comprehensive profiling and quantitative analysis of proteome, metabolome, and lipidome, providing new insights into complex biological systems and molecular distribution.

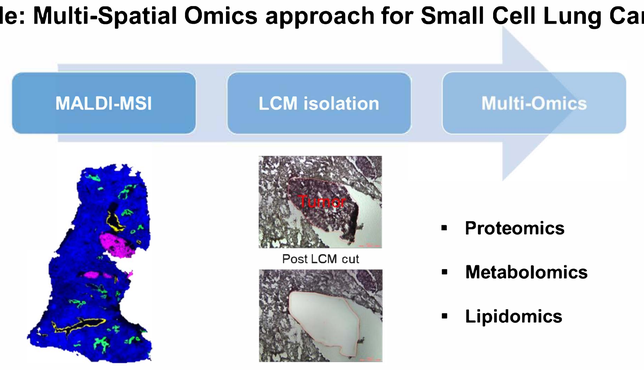
Advanced Workflow

MALDI-MSI guided Multi-Spatial Omics

Multi-Spatial Omics approach utilizing both MALDI-MSI and LCM-MS can uncover the spatial connections between the metabolome, lipidome, and proteome across different disease states.



Example: Multi-Spatial Omics approach for Small Cell Lung Cancer

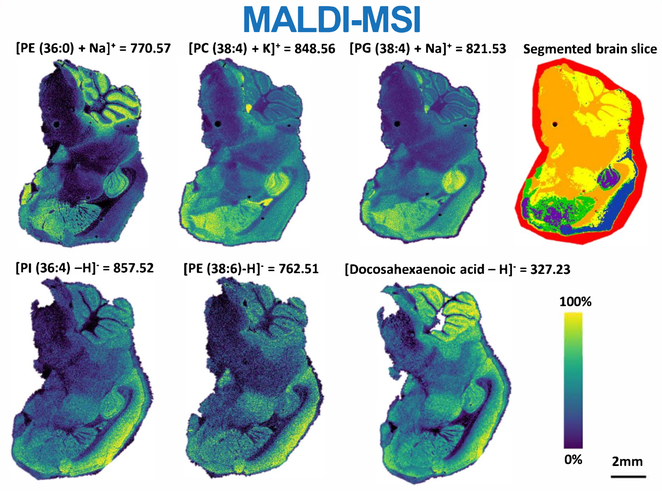


MALDI-MSI analysis revealed several distinct regions of interest within the tissue. The presence of [PC (32:1) +K]⁺ (denoted in blue) was primarily localized in healthy tissue, [PE (36:3) +K]⁺ (denoted in pink) was exclusive to tumor regions. [PC (40:6) +K]⁺ (denoted in yellow) was found to be predominantly in airways. [PC (36:4) +Na]⁺ (denoted in green) also has a very distinct ion distribution in suspected blood vessels. These regions were isolated with LCM for metabolomics and proteomics. Collectively, this approach further strengthens the use of spatially-aware and ultrasensitive MS platforms to enhance cancer research.

Results

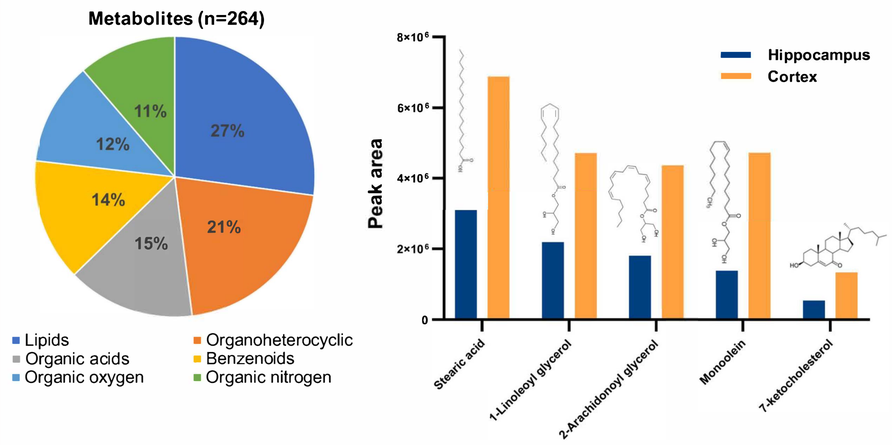
MALDI-MSI identified over 300 putative metabolite and lipid identifications in mouse brain, with high abundance of [PC (38:2) +K]+ and [PC (32:0) +Na]+ in the hippocampus and cortex, respectively. MALDI-MSI-guided LCM-MS identified 264 small molecules (Metabolomics) and 2982 proteins (Proteomics) from 1M cells. To validate our approach, we applied MALDI-MSI guided LCM-proteomics to a biological system involving different age groups of mice. By examining the distinct spatial distribution of molecules in the hippocampus and cortex using MALDI-MSI, we identified over 3900 proteins using LCM-proteomics. Among these, 359 and 86 proteins showed differential expression in the cortex and hippocampus, respectively, between the two age groups. Biological network analysis highlighted the involvement of differentially expressed proteins in mitochondrial dysfunction, glutamatergic synapse, and AMPK signaling pathways associated with aging.

MALDI-MSI



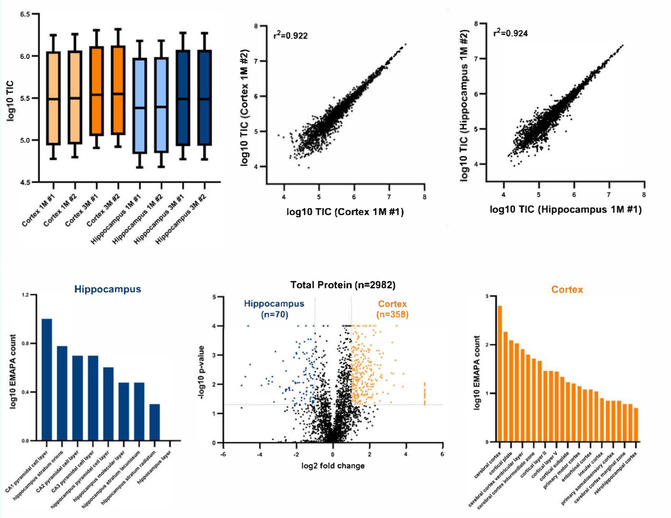
The unique spatial arrangement is evident either within the hippocampus (PE36:0, PC38:4, and PG38:4) and the cortex (PI36:4, PE38:6, and docosahexaenoic acid). Utilizing SCiLS for segmentation, these areas exhibited molecular distinctions, rendering them as the primary targets for LCM-MS.

LCM-Metabolomics



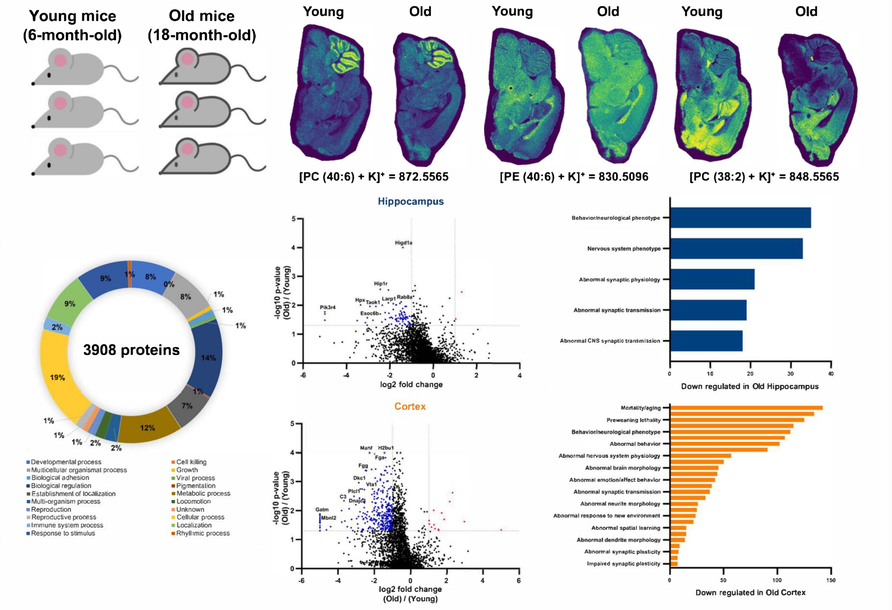
LCM-MS Metabolomics detected a total of 264 features encompassing small molecules and lipids. Notably, within the cortex region, 7-ketocholesterol, 2-arachidonoyl glycerol, and stearic acid exhibited noticeable upregulation, signifying their distinct molecular presence.

LCM-Proteomics



We optimized LCM-MS proteomics for hippocampus and cortex regions using two cell counts: 1M and 3M. We identified around 3000 proteins and achieved reliable quantitative data with 1M cells. The distinct biological functions of differentially expressed proteins in both regions exhibited strong correlation by EMAPA.

Application: MALDI-MSI guided LCM-Proteomics



Through our multi-spatial omics approach, we investigated a biological system encompassing two distinct age groups. Utilizing MALDI-MSI, we identified lipid variances. Additionally, employing LCM-Proteomics, we unveiled the presence of 3900 proteins within the hippocampus and cortex. Notably, our analysis revealed a significant downregulation of proteins in both brain regions among the older mice.

Conclusion

- MALDI-MSI guided LCM-MS approach enables comprehensive profiling and quantitative analysis of biomolecules in the cortex and hippocampus regions of mouse brain from < 1 million cells.
- Multi-Spatial Omics applications on two distinct mouse age groups revealed significant differences in protein expression within pathways associated with aging.
- Integration of MALDI-MSI and LCM-MS in spatial omics provides a comprehensive understanding of complex biological systems and holds potential for future neuroscience research.

Acknowledgments

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