

# Spatially Resolved Multiomic Correlative Image Analysis Software for MALDI-MSI of Lipids and Targeted Transcripts and Proteins on the Same Tissue Section



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## Introduction

MALDI-IHC and MALDI-ISH are top-down mass spectrometry imaging (MSI) approaches based on novel photocleavable mass-tags (PCMTs) conjugated to antibody and oligo probes [1,2]. They provide a basis for achieving high-plex MALDI-based workflows to image intact proteins and nucleic acids and enable multimodal and multiomic imaging of label-free, untargeted small molecules such as lipids, glycans and drugs on the same tissue section. Here, we demonstrate a novel computational workflow including a graphical user interface (GUI) to integrate multiomic/multimodal images of the same tissue section, scalable to an entire cohort of samples, and profile them through statistical and machine-learning analyses. This spatially resolved multiomic correlative analysis software, developed using Python/FIJI, is designed to rapidly profile entire cohorts from which multiomic/multimodal measurements were made.

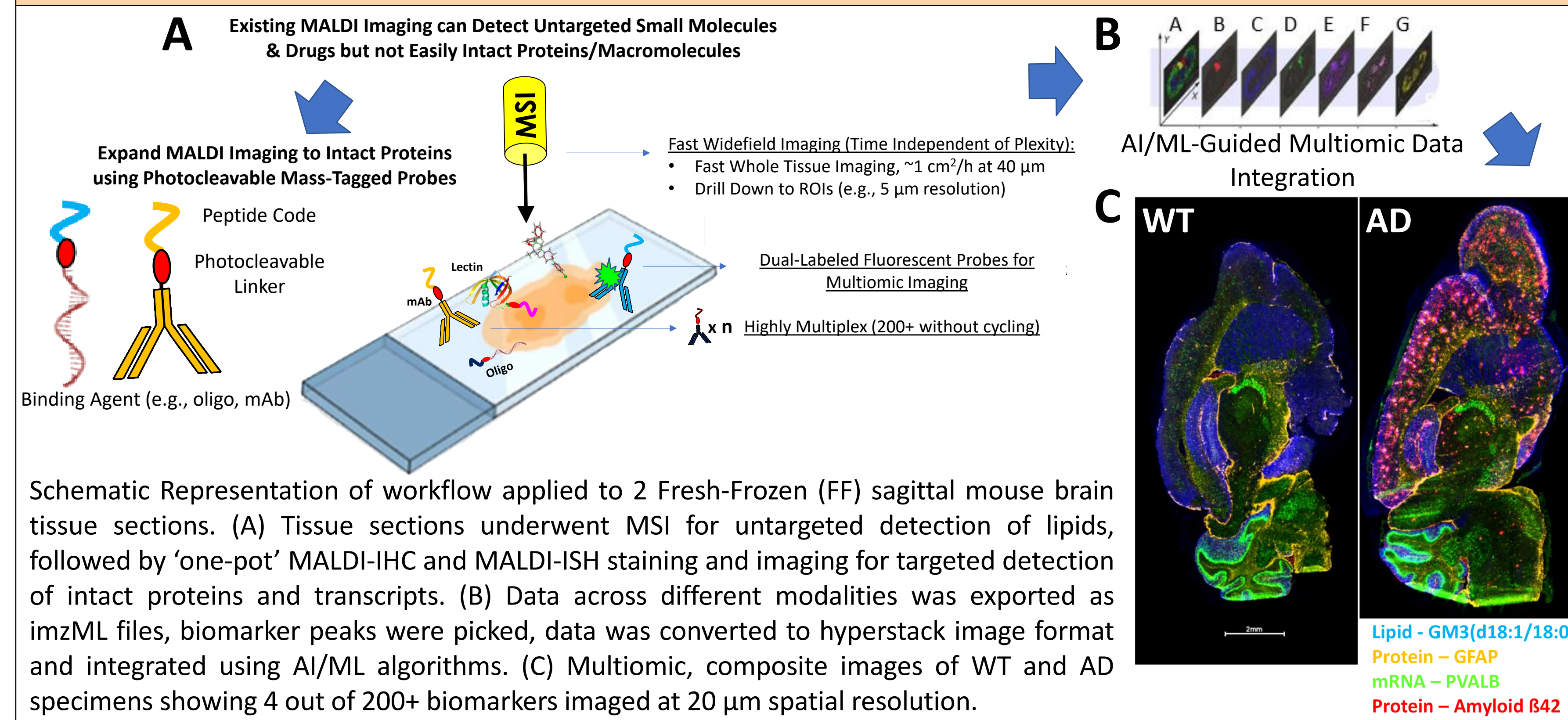
## Methods

Fresh-frozen, sagittal tissue sections from transgenic Alzheimer's Disease (AD) and Wild-Type (WT) mouse brains (strains hAbetaSAA and C57 from JAX and ZYAGEN, respectively) underwent untargeted MALDI-MSI scanning for lipids (180), followed by 'one-pot' MALDI-IHC/ISH staining and targeted MALDI-MSI scanning for proteins (25) and mRNA (9). All scans were done at 20 μm spatial resolution on the same samples and same instrument (Bruker timsTOF flex). Data was preprocessed in Bruker SciLS Lab and exported as imzML files. Our software was used to import imzML files for the entire cohort, integrated all multiomic layers of each sample into the same spatial coordinates, scaled each biomarker signal intensity according to entire cohort, and created multiomic Tiff/Hyperstack images for subsequent spatially resolved multiomic correlative analysis.

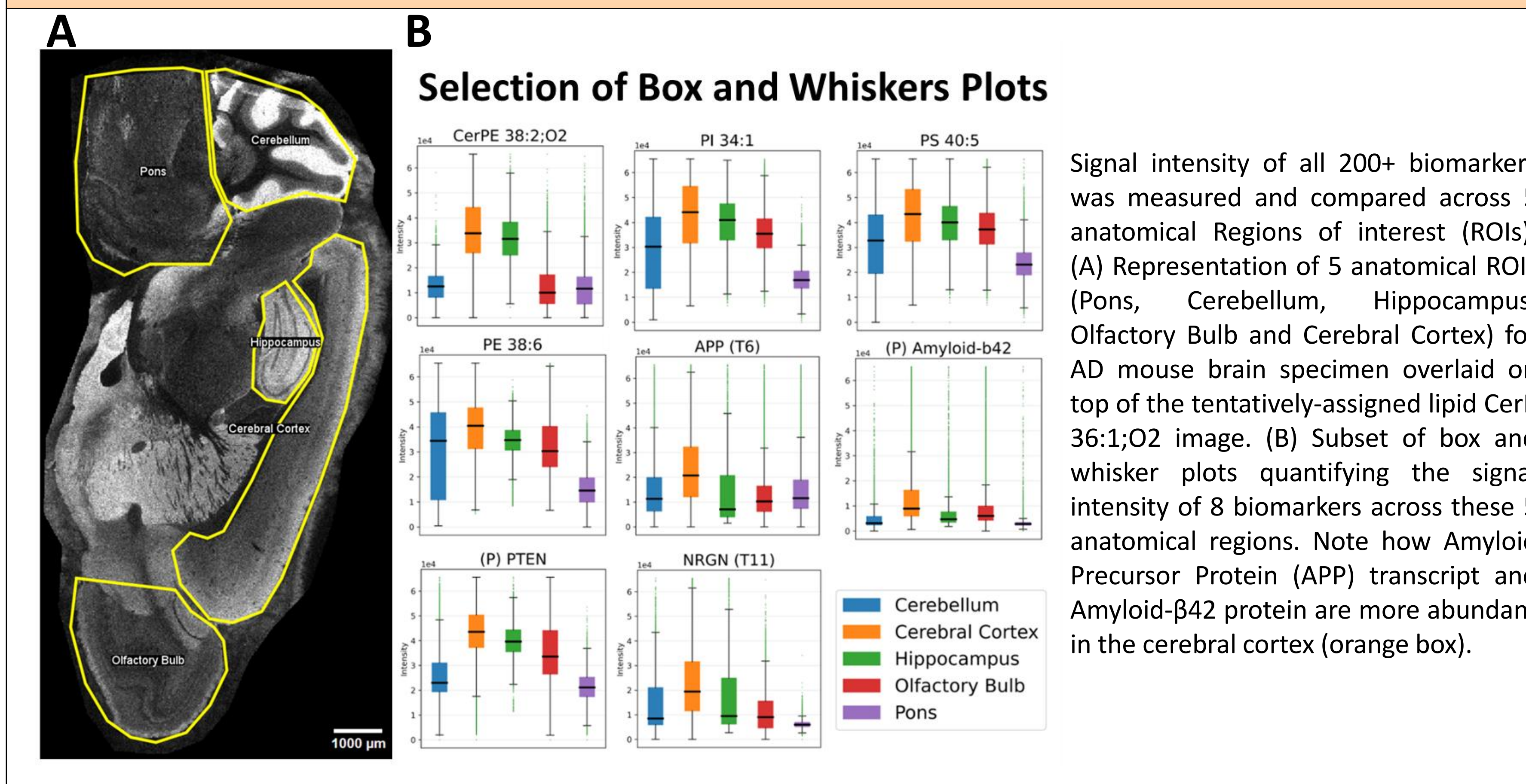
## Novel Aspects

**Multiomic correlative analysis software reveals biological insights for MALDI-MSI of label-free lipids and targeted proteins/transcripts from the same tissue section.**

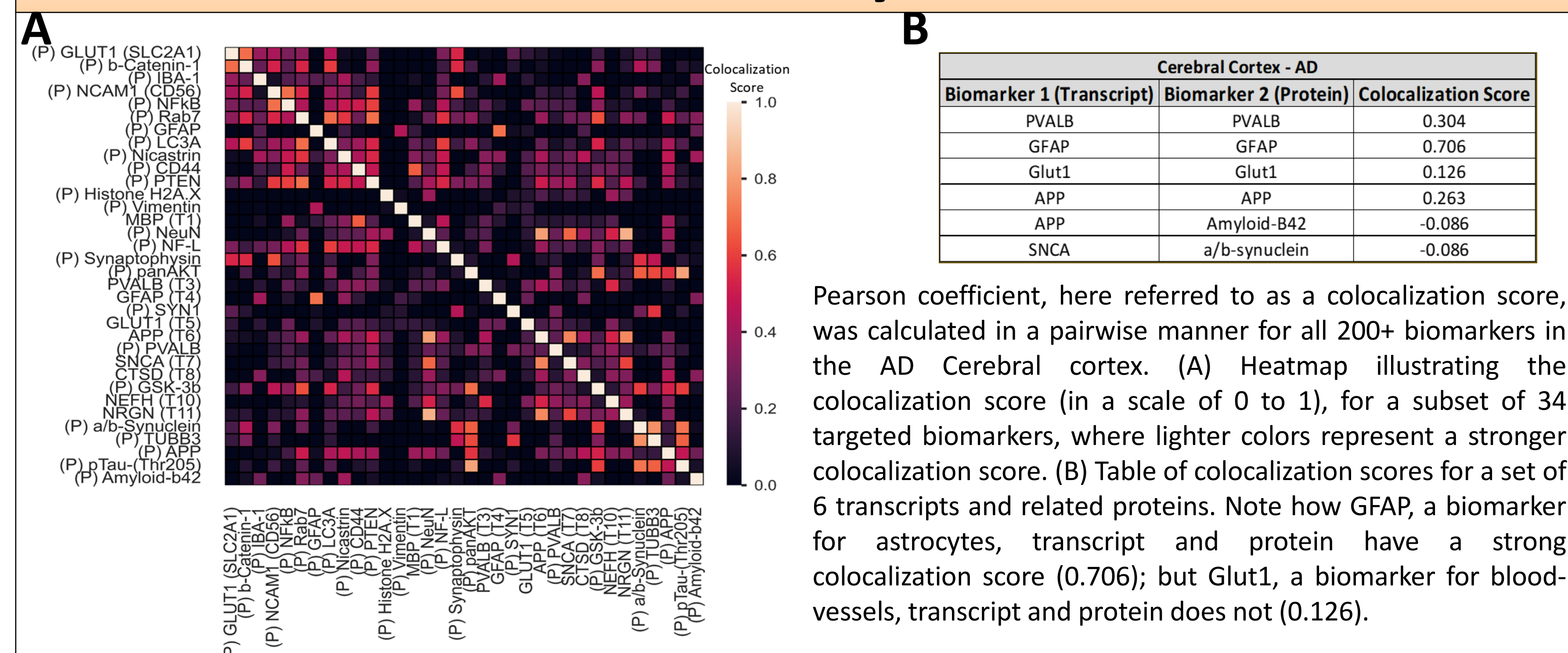
## Key Features of Lab Workflow and Data Integration



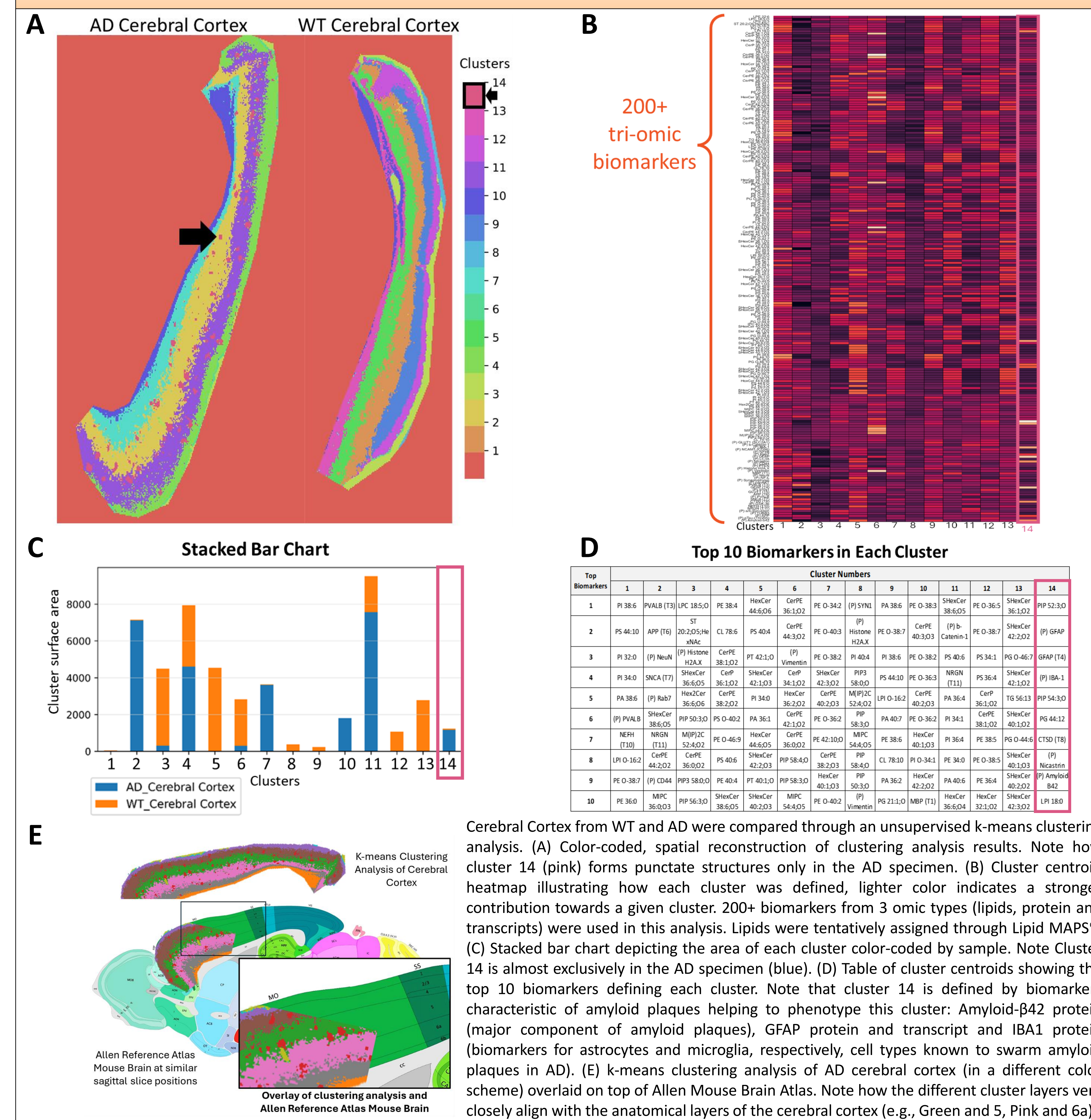
## Descriptive Statistics of Anatomical Regions for AD Mouse Brain



## Pairwise Colocalization Analysis for AD Mouse Brain



## AI/ML Clustering Analysis of WT and AD Cerebral Cortex



## Conclusions

This was an initial study utilizing a novel lab workflow and accompanying multiomic data analysis software, developed in Python and FIJI, demonstrating the comparison of AD and WT mouse brain tissue sections. This study demonstrated the following features of the workflow:

- Multiomic Data Collection:** MALDI-MSI for untargeted detection of small molecules can be combined with MALDI-IHC and MALDI-ISH for targeted detection of intact proteins and transcripts, respectively, for obtaining highly multiomic data across several tissue sections;
- Data Integration:** AI/ML algorithms can be used to seamlessly integrate different datasets from the same sample for subsequent analyses;
- Relative Quantification:** Signal intensity quantification of the same biomarker across different ROIs can reveal preferential localization to ROIs;
- Colocalization and Correlative Analyses:** Pearson score analysis can reveal if 2 biomarkers of interest colocalize with each other in a certain ROI, or over the whole sample; while unsupervised AI/ML clustering analyses including all detected biomarkers, or a subset, can identify clusters of interest that help to explain the complex biology of these systems and identify differences between samples (e.g., AD and WT).

### References:

- Yagnik et al. (2021) J Am Soc Mass Spectrom 32(4): 977-988 <https://doi.org/10.1021/jasms.0c00473>
- Lim, Yagnik et al. (2023) Front. Chem 11 <https://doi.org/10.3389/fchem.2023.1182404>

**Conflict of Interest:** Authors LGD, JB, GY, ZW, PC, ML and KR are employed by the company AmberGen, Inc. Photocleavable mass-tag imaging probes plus their high-plex, multiomic and multimodal workflows are patented by AmberGen which also sells photocleavable mass-tag imaging probes.

