Multiplexed imaging as an innovative approach for single-cell spatial profiling of brain pathology

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The Lo & Zeng Labs will move to Syracuse University starting January 2025 (www.lo-zeng-labs.com)

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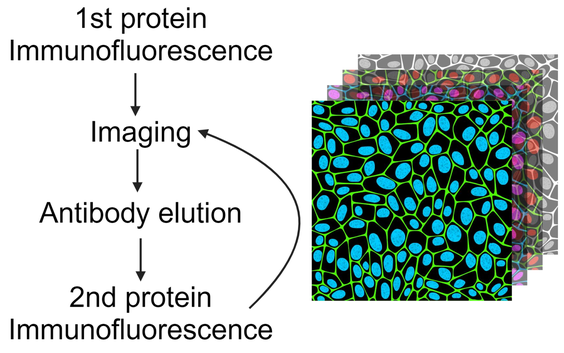
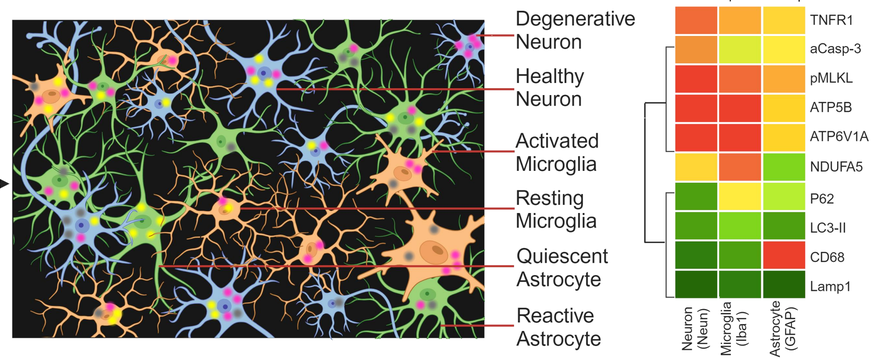
Background and Approach

- Single-cell spatial proteomic analysis maps protein interactions and localization at a single-cell resolution to understand various pathway correlation[1,2].
- The current proteomic system that studies brain abnormal pathologies averages across a group of cells which may hide certain lineages and spatial heterogeneity that is unique to each cell[3].
- In this study, we investigate the use of 4i staining and single-cell analysis techniques to uncover molecular events or pathways that determine precise protein abundance and interaction within a cell in the brain.

Multiplex imaging with 4i



Single-cell spatial proteomic analysis



Single-cell Proteomic Analysis

Image Acquisition	Pros	Cons
4i: Multiplex protein measurement using fluorescence (Gut et al.Science, 2018)	Visualize 80~ unique epitope; off-the-shelf 1° and 2° antibody; up to 40x magnification; commercial cost; time efficient; cellular localization	Light sensitive; limited to panel antibodies; high background fluorescence; decreasing tissue integrity; labor intensive
SWITCH: Synchronizing tissue preservation reaction (Murray et al. Cell, 2015)	Visualize 100~ unique epitope; off-the-shelf 1° and 2° antibody; commercial cost; thicker samples; ensures uniform staining	Requires more time for clearance sequence and gel formation; autofluorescence; gradual tissue weakening; does not preserve mRNA; 25x magnification
IMC: Imaging using mass-tags (metal) (Kuett et al. Nature Cancer, 2021)	Visualize 40~ unique antigens simultaneously; minimal background noise; no cross-talk between channels	Expensive antibodies; longer imaging time; 16x resolution; insensitive to low abundance proteins
MSI: Capture intensity distribution using ions (Shimma. Mass Spectrom, 2022)	Visualization of biodistribution or organic structures; non-antibody; simultaneous molecule detection; fast scanning speed	10x resolution; not feasible to cellular-level observation; limit separation capabilities; expensive equipment and maintenance
MALDI: Matrix crystallization ionization with laser beams (Wehder et al. J of HistoChem & Cytochem, 2010)	Big database for different peptide mass; identify 96 proteins simultaneously; commercial cost; pseudo-color for intensity	Protein database limitation; 10x resolution; limited to proteins <20kDa, high abundance and soluble polymer; long imaging time

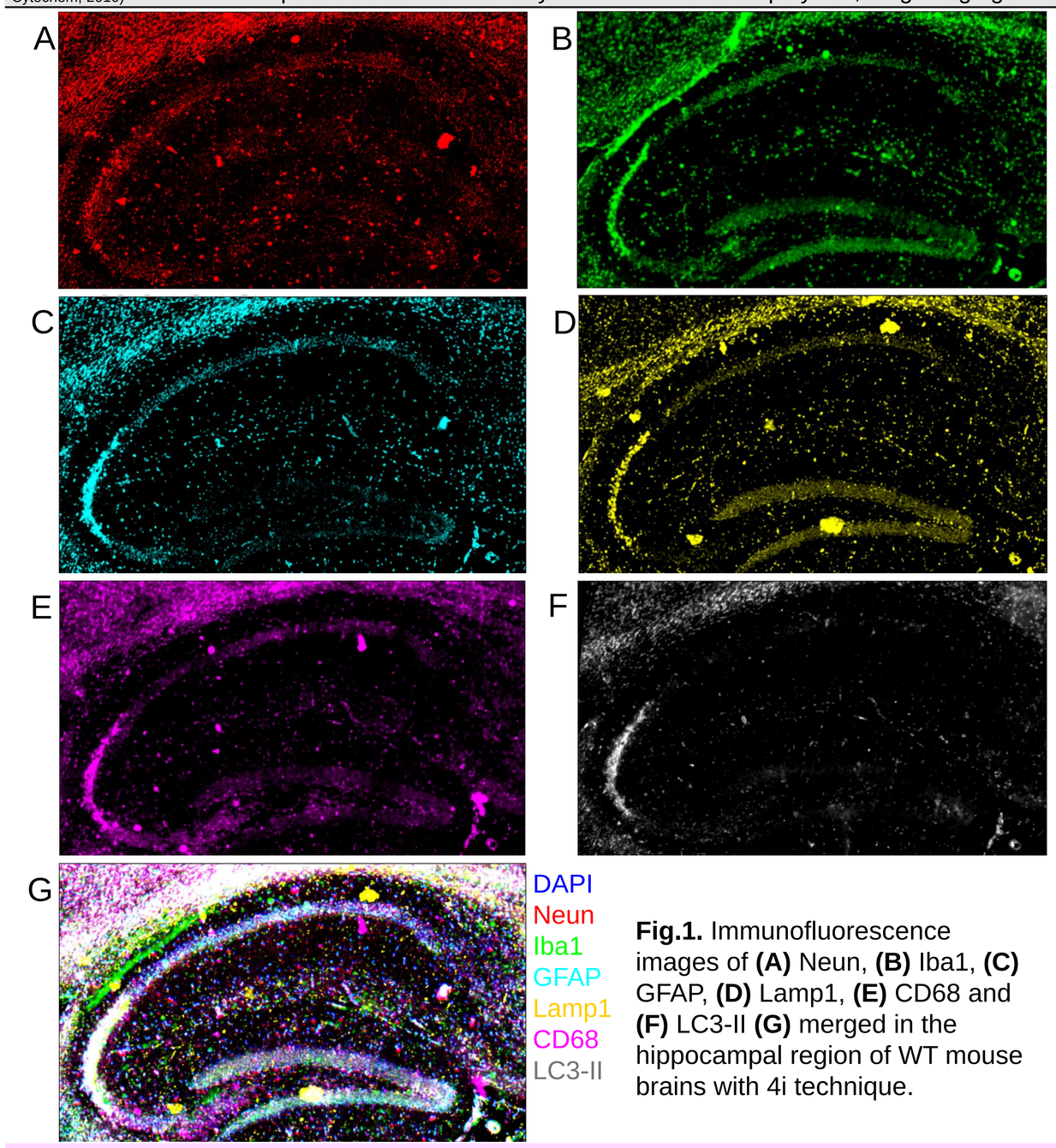


Fig.1. Immunofluorescence images of **(A)** Neun, **(B)** Iba1, **(C)** GFAP, **(D)** Lamp1, **(E)** CD68 and **(F)** LC3-II **(G)** merged in the hippocampal region of WT mouse brains with 4i technique.

Comparison of Single-Cell Proteome Analysis Techniques

Image Processing	Pros	Cons
FIJI (Descarpentrie et al. NIH, 2024)	Compatible to different imaging modalities; extensive plugins; multichannel data; adjustable threshold	Only able to open files up to 50MB; open-source tools have limited official technical support
PENGUIN (Sequeira et al. Comput Struct Biotechnol J, 2024)	Preprocessing tools increase efficiency; decreases background noise; percentile normalization; user-friendly interface	Significant variability due to sensitive 'P' and 'T' tailoring
SpatialDE (Svensson et al. Nat Methods, 2018)	Rapid and efficient image preprocessing; enhanced normalization and denoising capabilities	Requires programming knowledge for customization; primarily for transcriptomics
Steinbock (Windhager et al. Nat Protoc, 2023)	Multiplex capability on a single section; utilizes pre-learnt machine models; image pre-processing tools; segmentation accuracy; feature extraction	Limited to certain imaging modalities; large computational power; limited technical support; sensitive parameters
Watershed (Meyer et al. Visual Comm and Image Rep J, 1990)	Compatible for images with complex and overlapping structure; minimal computation time; can isolate subcellular structures	Sensitive to noise (over-segmentation); user parameter and edge selection sensitivity

Phenotyping	Pros	Cons
histoCAT: (Schapiro et al. Nat Methods, 2017)	Characterizes cellular morphological and functional phenotypes; analyze up to cellular social networks; spatial organization and interaction	Prior knowledge in programming languages; memory and processing power intensive for large datasets
CellProfiler (Bray et al. Nat Protoc, 2016)	Available official tutorials; adjustable modular designs; deep learning integration; supports 3D images	Limited built-in features; memory intensive; incapable to handle multichannel data
Ilastik (Berg et al. Nat Methods, 2019)	Amateur-friendly; supports various classifiers; customizable pixel features to increase accuracy; modifiable segmentation system	Requires powerful CPU/GPU; incapable to handle multichannel data; limited training representation
CytoMAP (Caleb R et al. Cell Reports, 2020)	Advanced interface with positional correlation and dimensionality reduction; comprehensive cellular morphological characterization	Significant computational resources; data overlap; normalization/clustering challenges
CellSeg (Lee et al. BMC Bioinfo, 2022)	Pre-trained nucleus segmentation; high accuracy; diverse image input; automated; visualization tools	Limited to pre-trained models; limited customization; python knowledge

Computational	Pros	Cons
Pseudotime (Hou et al. Nat Com, 2023)	Identify genes that drive biological process; quantitative; versatile process identification; identify cellular progression through a developmental trajectory	Inaccurate smooth trajectory; significant computational skills and time needed
SVCA (Arnol et al. Cell Reports, 2019)	Yield robust spatial variance signature; reveal key molecular pathways; identify effects of cell-cell interactions on gene expressions	High expertise in computational biology and statistics; sensitive to noise or artifacts
Giotto (Dries et al. Genome Biol, 2021)	Supports various imaging datasets; interactive modules; identify tissue composition and spatial expression pattern; quantitative	Specifically targeted for spatial transcriptomic use (algorithms); sensitive to noise and artifacts; interpretation challenges
ImaCytE (Somarakis et al. IEEE, 2021)	Amateur-friendly; identify cells phenotypes and microenvironments	Limited to data from IMC; no quantification feature
PHATE (Moon et al. Nat Biotechnol, 2019)	High resolution mapping; versatile to various cellular composition and gene expression patterns; preserve distance	Advanced computational and statistical skills; sensitive to noise/artifacts; no quantification

Outcome

Deciphering cell-cell interactions to uncover disease mechanisms and drive the development of more effective therapeutics

Publications from the Labs

1. Lo et al. (2021) Astrocyte Heterogeneity in Multiple Sclerosis: Current Understanding and Technical Challenges. *Front Cell Neuroscience.* Vol 15.
2. O'Connor et al. (2023) Integrative multi-omics and systems bioinformatics in translational neuroscience: A data mining perspective. *Journal of Pharmaceutical Analysis.* 13(8), 836-850.
3. O'Connor et al. (2023) Data Mining of Microarray Datasets in Translational Neuroscience. *Brain Science.*19(9):13(9), 1318.

Summary & Acknowledgements

- In summary, the use of 4i for image acquisition and further processing with FIJI and histoCAT will provide an accurately segmented and characterized multiplex image.
- Imaging data can be analyzed for spatial interaction, trajectory progress and more in brain samples from various neurodegenerative models.

References: (1) Rosenberger et al. (2023). Spatial single-cell mass spectrometry defines zonation of the hepatocyte proteome. *Nature Methods*. 20,1530–153… Gut et al. (2018) Multiplexed protein maps link subcellular organization to cellular states. *Science*. Vol 361, Issue 6401. (3) Vandereyken et al. (2023), Methods and applications… single-cell and spatial multi-omics. *Nature Reviews Genetics.* 24, 494–515. We thank Assistant Professor Anna Barron for hosting Dr Chih Hung Lo as a Dean's Postdoctoral Fellow in her lab.