1. Introduction
Neuroinflammation is increasingly linked to the etiology and progression of Alzheimer’s disease (AD). Microglia, the critical stewards of the brain’s immune microenvironment, display phenotypic and state diversity in AD as shown in single cell transcriptomic and proteomic analyses on tissue homogenates. These approaches lack spatial information, which is crucial to interpreting microglial involvement in neuroinflammation. We thus set out to develop a workflow that provides both, deep biomarker readouts and excellent spatial resolution. To this end, we developed a PhenoCycler®-Fusion (PCF) workflow that is compatible with formalin-fixed human brain tissue. The PCF is a novel spatial biology platform that generates ultrahigh-pixel spatial data for millions of cells on a single tissue section within a matter of hours. Using this technology, we characterized the immune microenvironment – with emphasis on microglia – in normal and AD brain tissues. Our findings suggest an enrichment of certain microglial subtypes in AD brain tissues and in proximity to Aβ plaques. In its sum, this study provides (1) a method for ultrahigh-pixel immunofluorescence analyses of AD pathology and new insights into the inflammatory microenvironment of AD.

2. PhenoCycler-Fusion Workflow

The PhenoCycler-Fusion workflow is compatible with a wide range of commercially available antibodies. Antibodies can be customized via conjugation to activated oligonucleotide barcodes that are complementary to existing antibody panels (e.g. Neuron Panel to the left). Following conjugation, antibodies are titrated and tested for appropriate target recognition, and then added to a panel. We routinely deploy panels with 500 antibodies.

3. Comprehensive Mapping of Alzheimer’s Pathology in Human Brain Tissue

(A) Whole-side image of an AD prefrontal cortex stained with 32 antibodies. The inset shows the high Aβ plaque load in the gray matter of this patient. (B-E) High-resolution mapping of pathology throughout the whole slide dataset. (B) Differential expression of Aβ, Aβ1-42 and Aβ40 in plaques with different maturation levels. (C) Astroglia engage mainly mature Aβ plaques, i.e. those stained with Aβ and Aβ40 antibodies. (D-E) Microglia express inflammatory biomarkers relative to their distance from mature and immature plaques. (F) Mapping resident vs. systemic immune cells is key to understanding the AD-inflammation. We have combined markers for the vasculature with immune cell antibodies to distinguish these systems.

4. Stardist Nuclear Segmentation for Neuronal Cell Classification

(A) Fluorescent image showing major cell-type defining markers. (B) Spatial map showing cell classification results for 110,088 DAPI-based segmentation masks, obtained using Stardist. GM: Grey Matter, WM: White Matter (WM).

5. OTSM (‘Awesome’) Segmentation Accurately Registers Microglia

(A) Image showing microglia and Aβ plaque staining. (B) DAPI-based Neuron markers; arrows show processes of one cell that extend to the St reached layer of an adjacent one. (C) OTSM Microglial cell masks and (D) OTSM-predicted Aβ plaque. (E) Combined masks used for unsupervised analysis (F) OTSM plots showing 11 microglial clusters obtained using unsupervised (mean Aβ) clustering of 46,146 cells. Clusters are colored by cluster number (left), specimen (middle) and sample type (right). (G) Heatmap showing mean expression of 10 microglial markers used for unsupervised clustering. (H) OTSM enabled identification of microglia based on markers expressed in their ramifications.

6. Inflammatory State of Microglia Depends on Proximity to Aβ Plaques

A) Fluorescent image of Aβ staining (left) and a spatial map showing microglial cells grouped by distance to their nearest Aβ plaque in mm (right panel; cortex). Note that microglia furtthest from the Aβ plaque are located in the WM. (B) In GM distance analysis reveals a high expression of CD11c and CD163 in the microglial associated with Aβ plaques. (D) Representative fluorescent images showing specific microglial populations in contact with an Aβ plaque.

7. Conclusions and Outlook

• More than five million Americans are living with Alzheimer’s Disease, but our understanding of the etiology and progression of this disease remains limited.
• We developed a comprehensive Spatial Biology workflow aimed at uncovering the inflammatory biology of AD in human patient samples in situ.
• Our work encompasses the development of a custom antibody panel, an imaging workflow, as well as a novel bioinformatic analysis method.
• Deployment of this workflow on post-mortem AD tissues allowed us to study different microglial cell populations, according to biomarker profiles and spatial distribution; in doing so, we identified different area and disease-associated microglial subpopulations in the cortical brain.
• Our workflow will allow large-scale unsupervised analyses of the AD inflammatory microenvironment that is needed to better characterize neurodegenerative disease.

References: