Resolving the tissue topology of the Alzheimer's brain

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Research Question

Alzheimer's disease (AD) is known to affect neural cells, yet previous research done by Dr. Naomi Habib and others, have shown that crucial changes occur to other nearby cells, such as astrocytes and microglia, as well (Habib N., et al., 2017; Strooper, B.D. and Karran, E.H., 2016). We hypothesize that these changes can play an active role having a pathological effect on the brain and surrounding cells, and should be looked at as part of AD progression. Single cell/nucleus RNA-seq that can profile the state and type of cells in the brain is being used to profile cellular populations altered in Alzheimer's, however the position of these cellular populations and the interactions between them are lost due to the dissociation of the tissue.

The goal of the project is to chart the topology of specific brain regions in Alzheimer's brains, specifically the position of the different cellular populations within the tissue and the cellular "neighborhoods". Combining image processing of single molecule RNA-FISH with previous single cell RNA-seq data and analysis, I plan to build a spatial-transcriptional map, for AD and WT. This may illuminate a path to further understand the connectivity within the tissue of an AD affected brain.

Background

Alzheimer's disease (AD) is an age-related neurodegenerative disease affecting cognitive and behavioral practice. AD's causes aren't fully known, and much research is underway to better understand the disease (Alzheimer's Association, 2019).
An important undefined impact is the effect that different cell types within the brain and their cell-cell interactions have on AD progression (Moor A.E., et al. 2017).

Previous research on single-cell (or nucleus) has been trying to allocate which cells and their gene expressions are altered, thus taking an active role in the disease throughout its different stages (Stuart T. and Satija R. 2019). This has allowed accumulated data on cellular populations revealing a difference between the cells of WT and AD brains, based on their gene expression pattern. These different populations can be split into two categories: frequency in cell types and differences in cell states (Habib N., et al. 2016). Analysis of such data allows defining different cell's functionally and retrieving specific target genes suspected to have an impact on AD progression.
However, single cell/nucleus RNA-seq data is missing the spatial aspect of the cells, i.e. their position within the tissue and their cellular neighbors, seeing that it is retrieved from tissues which dissociated to single cells.

Single molecule mRNA Fluorescent in Situ Hybridization (smFISH) is a method of super resolution microscopy to enable visualization of gene expression amongst cells (Frickmann H., 2017), usually allowing the staining of up to 3 different colored probes and as a result marking up to 3 different genes. smFISH was used to resolve more complex tissue topologies by integrating data from larger number of genes quantified separately on different tissues and the data was computationally "merged" together. The Iterative Single Molecule FISH enables quantification and visualization of a larger number of genes within the same tissue. This is done by doing multiple rounds of hybridization as above, where at the end of each iteration the fluorescent probes are "stripped" and then the probes are re-hybridized while being coupled to different fluorophores, allowing over 100 genes to be shown within the same tissue (Lubeck E. et al., 2014). This method can provide us with quantification and imaging of multiple genes in their original position within both WT and AD brains.

The imaging data can be combined with the single nucleus RNA-seq data to connect to known cellular populations, analyze cellular neighborhoods within the brain and specific populations of interests and their neighboring cells. This will hopefully advance our understanding of the cell-cell interactions and their effect on each cell's functionality and disease progression. A spatial map of such neighborhoods can hopefully shed light on much more of the causes and aspects of AD.

Work Plan

1. Building a pipeline for processing smFISH data.
    a. Background correction: noise cleaning, merge and alignment of RGB channels when necessary.
    b. Regional quantification - part 1: defining tissue **regions** and quantifying gene expressions amongst each region's population. To be done on both WT and AD data.
    c. Regional quantification - part 2: segmentation of single cells, identifying cell **positions** and classifying by quantification of gene expressions per cell/position. To be done on both WT and AD data.
2. Combining with single nucleus RNA-seq data to connect the spatial information to previously identified cellular population, to build a spatial-transcriptional map of the brain in WT and AD.
3. Predicting cell-cell interactions and signaling molecules that drive such interactions.
4. Combining all analysis stages to build a modular and robust pipeline to be applied on several such data sets for continuous AD and intercellular communication research.

Data & **Tools**

1. Initial Mouse Single Molecule FISH images of WT and AD mouse model (5xFAD) produced by 2 iterations with ~10 genes on multiple cuts of tissue. Acquired from mice experiments at Dr. Habib's lab.
2. Single cell RNA-seq data acquired from same mice experiments as of FISH data.

3. More imagery data coming soon with additional targeted genes.
4. Cell Profiler – cell image processing and analysis software. Their Python code can be found on their GitHub repository.

References

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