Explorations of spatial transcriptomics on brain samples for biological understanding

Tianfang Wang, Henning Hvid, Sarah Bau, Sofia Lundh, Lingling Jiang
Novo Nordisk R&D

Keyword: spatial transcriptomics, Xenium, Visium HD, brain

Spatial transcriptomics is an emerging technology to study gene expression and cell type interactions at spatial resolution. To assess which spatial transcriptomics platform is more suitable for studying brain samples, we performed a comparison between Visium HD and Xenium (including 5k and brain panel) on a human hypothalamus sample. Xenium brain panel and 5k datasets showed better sensitivity for transcript detection compared to Visium HD, similar to the observations from other studies. Xenium data also showed higher expression level for cell type markers and key genes involved in appetite regulation in the hypothalamus. In addition, we observed strong correlation in cell type expression between the Xenium brain panel and 5k data. Further benchmarking against relevant snRNA-seq data showed moderate correlation for cell type expression. Surprisingly, we found comparable specificity for known cell type markers for both neuronal and non-neuronal cell types relative to snRNA-seq data, albeit substantial challenges in cell segmentation based on Xenium data. However, discrepancies were observed between cell type label transfer from relevant snRNA-seq data and manual annotation.

In a pilot study, we utilized Xenium platform to profile spatial transcriptomics on mouse hypothalamus and hindbrain, in exploration of sex and age associated gene expression differences. In total 247 genes were assayed from the 10x brain panel, supplemented by 100 custom genes including known cell type markers and genes of interest. We applied cellpose on 18s rRNA and DAPI staining for cell segmentation, which yielded 80,000 and 200,000 cells from hindbrain and hypothalamus, respectively, across 8 mice after quality control. With scRNA-seq approaches for downstream clustering and annotation, we were able to identify major cell types including neurons, glial cells and vascular cells. Cell type compositional and neighbourhood analysis highlighted differences in cell type abundance and neighbourhood enrichment between young mice and old mice. Differential gene expression analysis uncovered more pronounced differences associated with age as opposed to sex. In summary, our analysis demonstrated that spatial transcriptomics is a promising tool to decipher biology in the brain.