Comparative Analysis of Single Cell and Single Nucleus RNA Sequencing Reveals Different Cell Type Clusters in Mouse Brain Tissue

Single cell RNA sequencing (scRNA-seq) is the go-to method for uncovering cellular heterogeneity. However, it requires high-viability single-cell suspension as the starting material, which are challenging or sometimes impossible to obtain for complex tissues such as brain, heart, muscle, adipose tissues, or frozen samples.

Single nucleus RNA sequencing (snRNA-seq) overcomes this hurdle by isolating nuclei for library preparation, enabling single-cell insights from these difficult tissues.

Both approaches have strengths and limitations depending on tissue type and research goals. Here, we compare scRNA-seq and snRNA-seq using mouse brain samples, offering key insights to guide method selection.

Materials and Methods

Sample Preparation

Whole brain tissue from C57BL/6 mice was divided into two halves: One half was immediately dissociated using the sCelLiVE® Tissue Dissociation Kit for single cell analysis; The other half was snap-frozen for subsequent single nucleus analysis.

Library Preparation and Sequencing

The GEXSCOPE® Single Cell RNA Library Kit was used for scRNA-seq, and the GEXSCOPE Single Nucleus RNA Library Kit was used for nucleus extraction and snRNA-seq, following the manufacturer's instructions. Data analysis was then performed using the CeleSCOPE® analysis pipeline.

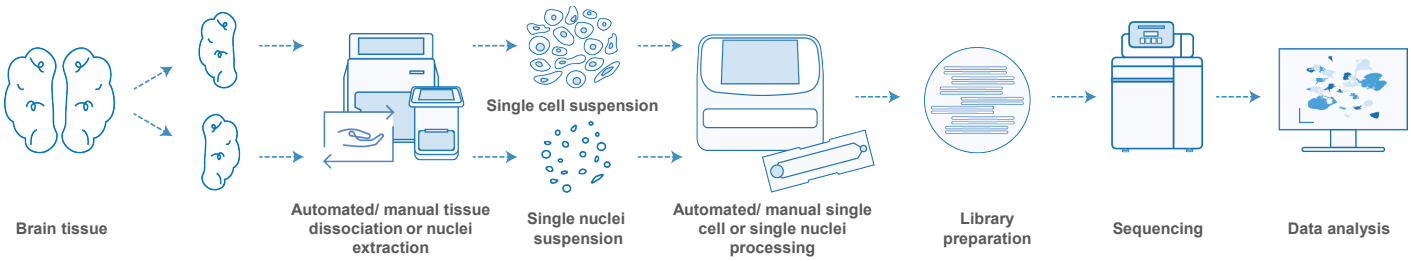


Figure 1: Experimental set up.

Step	Product(s) used
Tissue dissociation and nucleus extraction	• PythoN® Tissue Dissociation System • sCelLiVE® Tissue Dissociation Kit
scRNA-seq library preparation	• GEXSCOPE® Single Cell RNAseq Library Kit
snRNA-seq library preparation	• GEXSCOPE® Single Nucleus RNAseq Library Kit
Data analysis	• CeleSCOPE® analysis pipeline

Table 1: End-to-end single cell and single nucleus sequencing workflow. Singleron's kits and instruments were used for single cell/ single nucleus sample preparation, library preparation, and data analysis.

Results

Comparable Data Quality from Single Cell and Single Nucleus Analysis

The tissue dissociation for scRNA-seq yielded cell suspensions with a viability of 91%. After quality control and filtering, 4,532 single cells and 2,048 single nuclei were analyzed (Table 2).

Single cell sequencing yielded a higher median unique molecular identifiers (UMI) per cell (3,030) compared to single nucleus sequencing (1,655) (Table 2). The median number of genes detected per cell was higher in samples analyzed with single cell sequencing (1,519) compared to single nucleus sequencing (1,039) (Table 2). In addition, we observed a higher percentage of intronic reads in single nucleus sequencing (42%) compared to single cell sequencing (15%) (Table 2).

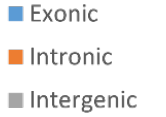
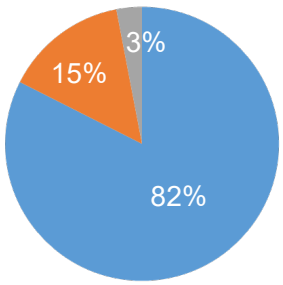
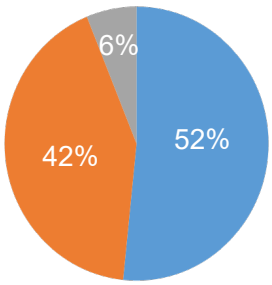
	Single cell sequencing	Single nucleus sequencing
Estimated number of cells	4,532	2,048
Fraction reads in cells	60.74%	50.97%
Mean reads per cell	12,685	32,640
Median UMI per cell	3,030	1,655
Total genes	23,901	22,106
Median genes per cell	1,519	1,039
Saturation	35.91%	74.69%
Exonic, intronic, and intergenic read percentages 		

Table 2. Quality control metrics for scRNA-seq and snRNA-seq of mouse brain samples.

Cell annotation

Both scRNA-seq and snRNA-seq revealed similar cell type diversity in the mouse brain tissue (Figure 3A). The identified cell types include microglial cells, astrocytes, excitatory neurons, oligodendrocytes, smooth muscle cells (SMCs), inhibitory neurons, B cells, T cells, endothelial cells (ECs), choroid plexus cells, oligodendrocyte progenitor cells (OPCs), macrophages, fibroblasts, neuroblasts, monocytes, pericytes, ependymal cells, and proliferating neural cells (Figure 2A).

While both methods identified similar cell populations, the proportions of each cell population differ significantly between scRNA-seq and snRNA-seq. Single cell analysis captured a larger proportion of immune cells, especially microglial cells; In contrast, single nucleus analysis captured a higher number of adherent cells such as excitatory neurons and inhibitory neurons (Figure 2B).

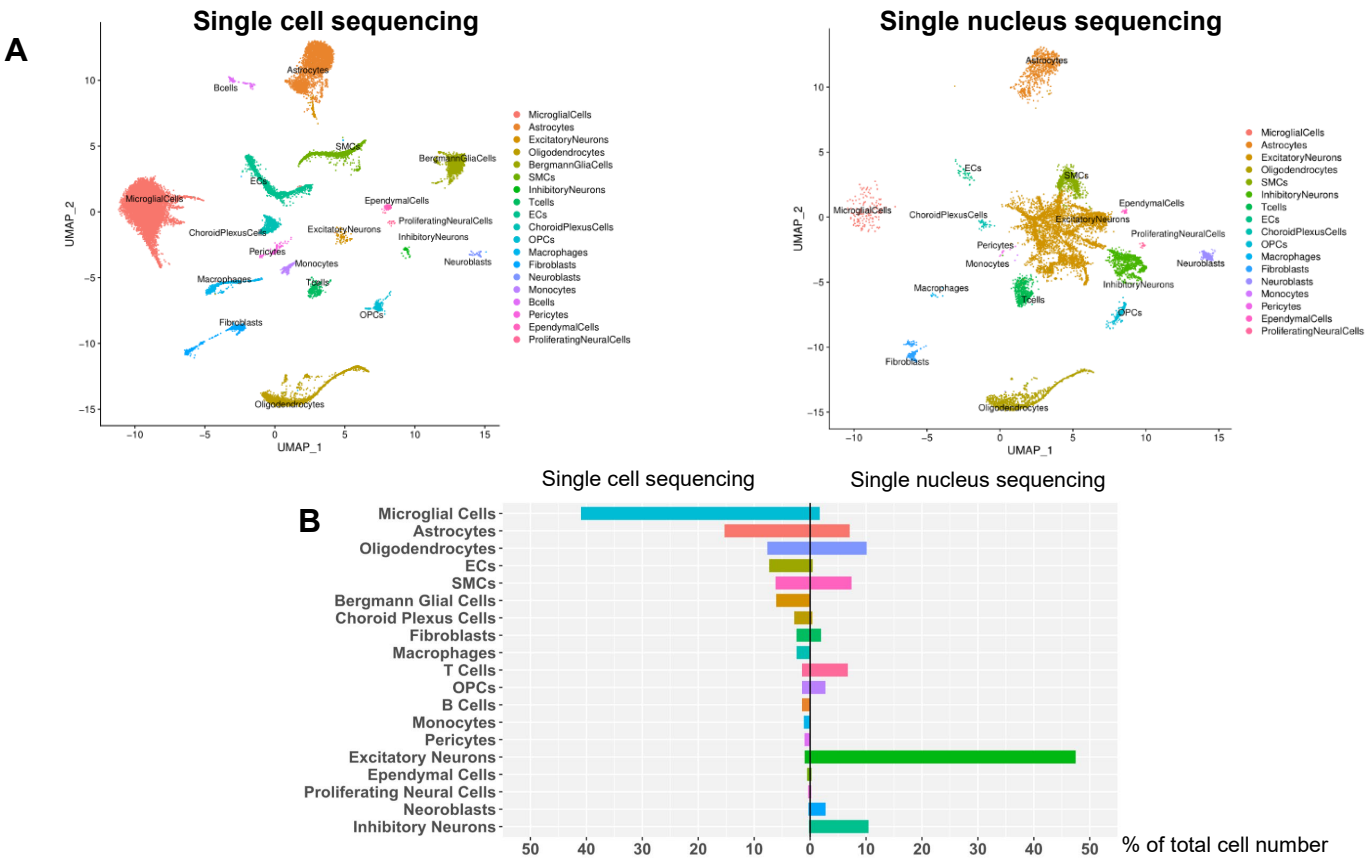


Figure 2: Cell type annotation of scRNA-seq and snRNA-seq data from mouse brain. (A) UMAP profiles (B) Proportions of each cell population detected using single cell and single nucleus sequencing. Left: Single cell sequencing. Right: Single nucleus sequencing. X-axis: the percentage of cell number detected in the total cell population.

Discussion

Compared to snRNA-seq, scRNA-seq yields a higher number of UMIs per cell, leading to the detection of more genes. This is due to scRNA-seq's ability to capture both cytoplasmic mRNA and nuclear mRNA [1, 2]. SnRNA-seq, on the other hand, captures only nuclear mRNA. Additionally, scRNA-seq detects a higher proportion of fully-spliced mRNAs, while snRNA-seq detects an abundance of pre-mRNA in the nucleus [1, 2]. This is reflected in the higher percentage of intronic reads from the snRNA-seq data.

However, snRNA-seq offers distinct advantages, especially for difficult-to-dissociate samples. ScRNA-seq tends to capture more immune cells, while underrepresenting cell types that are sensitive and easily damaged by the tissue dissociation process [3]. Single nucleus analysis, on the other hand, better captures adherent cell types, and therefore provides a more unbiased representation of the cell population distribution in tissue based on bulk sequencing and pathological analysis [3, 4, 5].

Finally, snRNA-seq allows the immediate snap-freezing of tissue samples, offering practical advantages in terms of sample preservation and reducing variations that may occur during storage and processing. This feature is particularly valuable for clinical samples or time-sensitive experiments.

Conclusion

Single cell and single nucleus RNA sequencing each have distinct advantages in capturing different cell populations. The choice between them depends on your research goals, target cell types, tissue characteristics, and sample handling needs. To assist your decision, we summarized the key considerations in the table below (Table 3).

	Single cell sequencing	Single nucleus sequencing
Source of mRNA	Mainly from the cytoplasm High proportion of fully spliced mRNAs	Mainly from the nucleus High proportion of pre-mRNA Nucleic mRNA = 30-40% of total mRNA Rare transcripts might be missed
Median genes/cell (@50K reads/cell)	1000-5000 depending on sample types	500-2000 depending on sample types
Advantages	Suitable for non-adherent cells or easy-to-dissociate tissue. High number of UMI/genes detected per cell.	Suitable for tissues that are hard to dissociate, such as brain, muscle, pancreas, liver, and tumor biopsy. Suitable for frozen tissues.
Disadvantages	Data may under-represent certain cell types (e.g. neurons) due to dissociation bias	Loss of cytoplasmic mRNAs. Nucleus extraction protocol requires additional steps
Tissue Storage	Fresh tissue can be stored for up to 3 days in sCelLiVE tissue preservation buffer at 4 °C.	Frozen tissue can be stored for up to 6 months.

Table 3: Key considerations for selecting between single cell and single nucleus sequencing.

References

[1] Bakken, Trygve E., et al. (2018). Single-nucleus and single-cell transcriptomes compared in matched cortical cell types. Single-nucleus and single-cell transcriptomes compared in matched cortical cell types. PLoS ONE, 13*(12)*: e0209648. https://doi.org/10.1371/journal.pone.0209648

[2] Lake, B. B., et al. (2018). Integrative single-cell analysis of transcriptional and epigenetic states in the human adult brain. Nature Biotechnology, 36*(1)*, 70-80. https://doi.org/10.1038/nbt.4038

[3] Santiago, C. P., et al. (2023). Comparative Analysis of Single-cell and Single-nucleus RNA-sequencing in a Rabbit Model of Retinal Detachment-related Proliferative Vitreoretinopathy. Ophthalmology Science. 3(4). https://doi.org/10.1016/j.xops.2023.100335

[4] Oh, J., et al. (2022). Comparison of cell type distribution between single-cell and single-nucleus RNA sequencing: enrichment of adherent cell types in single-nucleus RNA sequencing. Exp Mol Med. 54(12): 2128–2134. https://doi.org/10.1038/s12276-022-00892-z

[5] Melkebeke, L. V., et al. (2024). Comparison of the single-cell and single-nucleus hepatic myeloid landscape within decompensated cirrhosis patients. Front. Immunol.,15. https://doi.org/10.3389/fimmu.2024.1346520

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