DANISH SINGLE-CELL NETWORK NEWSLETTER

September - 2023

THIS MONTH'S HIGHLIGHTS:

Single-cell massively-parallel multiplexed microbial sequencing (M3-seq) identifies rare bacterial populations and profiles phage infection

Wang, B., Lin, A.E., Yuan, J. et al.

Single-cell brain organoid screening identifies developmental defects in autism

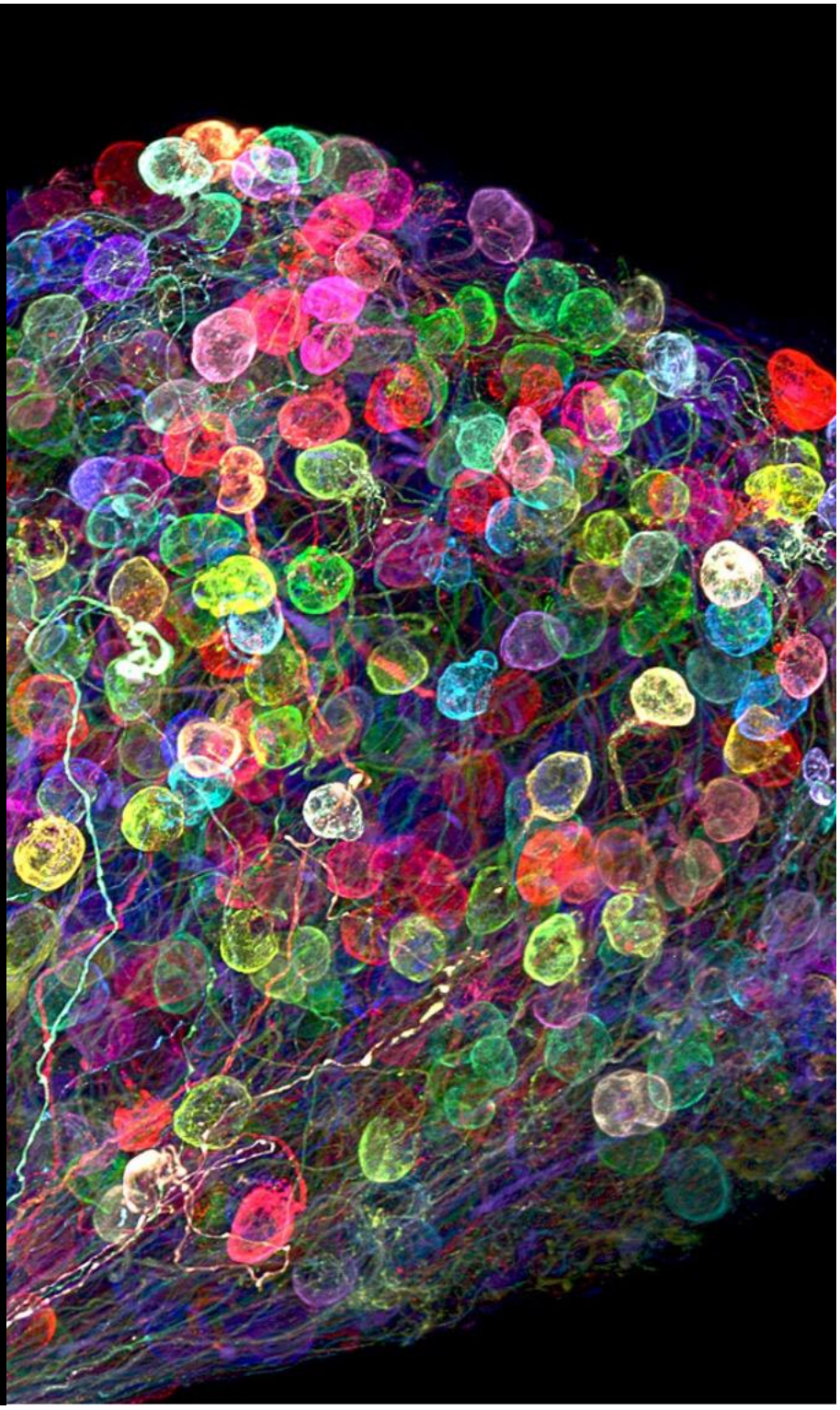
Li, C., Fleck, J.S., Martins-Costa, C. et al.

Peripheral blood single-cell sequencing uncovers common and specific immune aberrations in fibrotic lung diseases

Zhao, A.Y., Unterman, A., Abu Hussein, N. et al.

Spatial atlas of the mouse central nervous system at molecular resolution

Shi, H., He, Y., Zhou, Y. et al.



COVER IMAGE
Credit: Dr. Ryo Egawa, Nagoya University, Japan
Title: Individually labeled axons in an embryonic chick ciliary ganglion (2017)

UNIVERSITY OF COPENHAGEN

Single-cell massively-parallel multiplexed microbial sequencing (M3-seq) identifies rare bacterial populations and profiles phage infection

Wang, B., Lin, A.E., Yuan, J. *et al. Nature microbiology* (2023), https://doi.org/10.1038/s41564-023-01462-3

This article introduces the development of a new technique, **massively-parallel, multiplexed, microbial sequencing (M3-seq)**, for single-cell RNA sequencing (scRNA-seq) in **bacteria**. Bacteria have the ability to adapt to changing environments by specializing individual cells, which can manifest as morphological changes or functionally distinct states. Existing bacterial scRNA-seq methods have **limitations, including the reliance on pre-designed probes and issues with ribosomal RNA (rRNA) interference**. M3-seq combines plate-based and droplet-based indexing with post hoc rRNA depletion, allowing for **massively parallel gene expression profiling of single bacterial cells across multiple samples**. The technique was applied to hundreds of thousands of cells, revealing independent phage induction programs in *Bacillus subtilis*, a bet-hedging subpopulation in *Escherichia coli*, and the detailed heterogeneity of phage infection. M3-seq significantly improved the capture of mRNA compared to earlier methods, and its performance was evaluated in experiments involving *B. subtilis*, *E. coli* MG1655, and *E. coli* Nissle under various conditions. The results showed high single-cell resolution and information capture across different conditions. M3-seq offers a promising tool for **studying gene expression at the single-cell level in bacteria, addressing some of the limitations of existing techniques and enabling researchers to gain insights into bacterial responses to environmental stressors and other biological phenomena**.

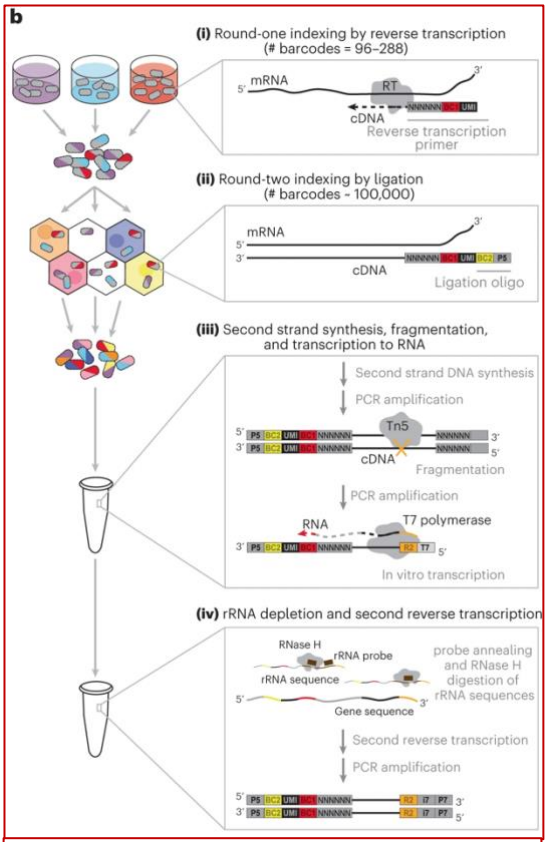


Figure 1. M3-seq workflow. RNA is reverse transcribed in-situ with indexed primers, marking cells with unique sequences. After collection, cells undergo a second indexing in droplets with barcoded oligos. Cells are then lysed to release cDNA, which is synthesized, fragmented by Tn5, PCR-amplified with a T7 promoter, and converted to RNA. Ribosomal sequences are removed by hybridizing the RNA to DNA probes and cleaving with RNase H. Sequences revert to DNA, adaptors are attached, and sequenced.

Single-cell brain organoid screening identifies developmental defects in autism

Li, C., Fleck, J.S., Martins-Costa, C. *et al.* *Nature* (2023), https://doi.org/10.1038/s41586-023-06473-y

The authors present a new system called **CHOOSE (CRISPR–human organoids–single-cell RNA sequencing)**, which combines **genetic perturbations with single-cell transcriptomic analysis in mosaic cerebral organoids**. The goal is to study the **impact of genetic mutations** associated with autism spectrum disorder (ASD) on human brain development. The study focuses on 36 high-risk ASD genes and employs a combination of CRISPR technology and single-cell RNA sequencing to analyze the effects of gene mutations on different cell types and molecular pathways in the developing brain. The results reveal specific cell type composition changes associated with mutations in the ARID1B gene, expanding ventral radial glial cells and increasing their transition to early oligodendrocyte precursor cells. The article also highlights the challenges of studying developmental defects in ASD, which often occur during fetal stages and are difficult to investigate. The CHOOSE system offers a promising approach to understanding the genetic basis of neurodevelopmental disorders and sheds light on the complex processes of human cortical development, providing insights into how these processes can go awry in conditions like ASD. The study emphasizes the importance of studying ASD in a human context and leveraging advanced techniques to **explore the role of genetic mutations in brain development**.

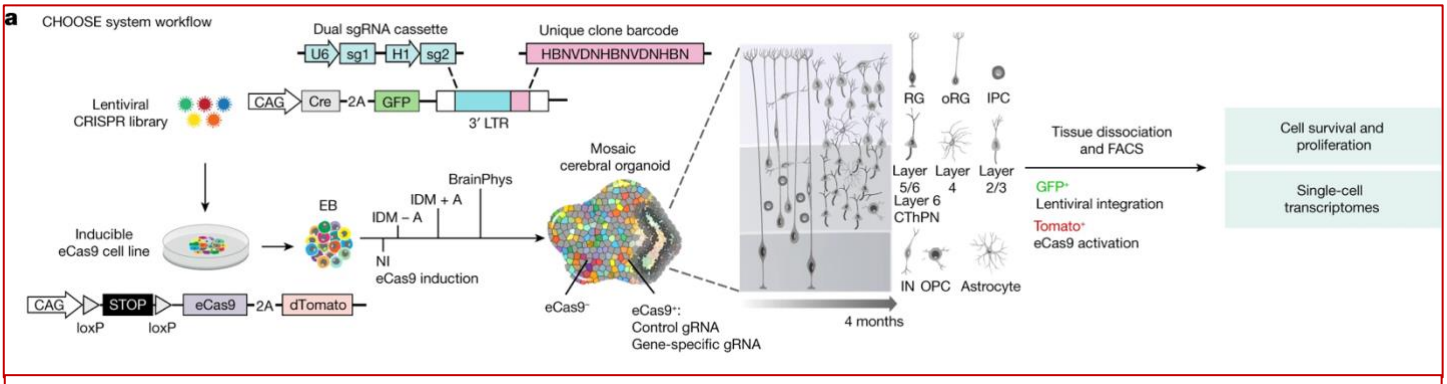


Figure 2. CHOOSE system overview. Barcoded dual sgRNA cassette located within the 3′ LTR of the lentivirus.

Peripheral blood single-cell sequencing uncovers common and specific immune aberrations in fibrotic lung diseases

Zhao, A.Y., Unterman, A., Abu Hussein, N. *et al. bioRxiv* (2023), https://doi.org/10.1101/2023.09.20.558301

In this study, researchers used cutting-edge single-cell profiling technologies to analyze **peripheral blood immune aberrations** in patients with **idiopathic pulmonary fibrosis (IPF)** and **fibrotic hypersensitivity pneumonitis (FHP)**. They performed single-cell RNA sequencing on peripheral blood and bronchoalveolar lavage samples from these patients and healthy controls. They identified **common immune mechanisms** shared by both IPF and FHP, including changes in monocytes and the presence of S100Ahi monocytes and SPP1hi macrophages in both diseases. However, **disease-specific immune aberrations** were also observed, with CD8+ GZMKhi T cells and activated B cells primarily enriched in FHP patients. The study also revealed unique T and B cell receptor compositions in FHP and significant IgA enrichment in IPF. Overall, these findings provide insights into the immune mechanisms underlying IPF and FHP, opening up new strategies for diagnosis and treatment. The research involved the analysis of 327,990 cells from 83 samples and included detailed investigations into transcription factor activity, cell trajectory analysis, RNA velocity analysis, cell-cell signaling, and receptor repertoire analysis. This comprehensive single-cell resolution analysis enhances our understanding of the distinct and shared immune mechanisms in these two fibrotic lung diseases, facilitating potential advances in their diagnosis and treatment.

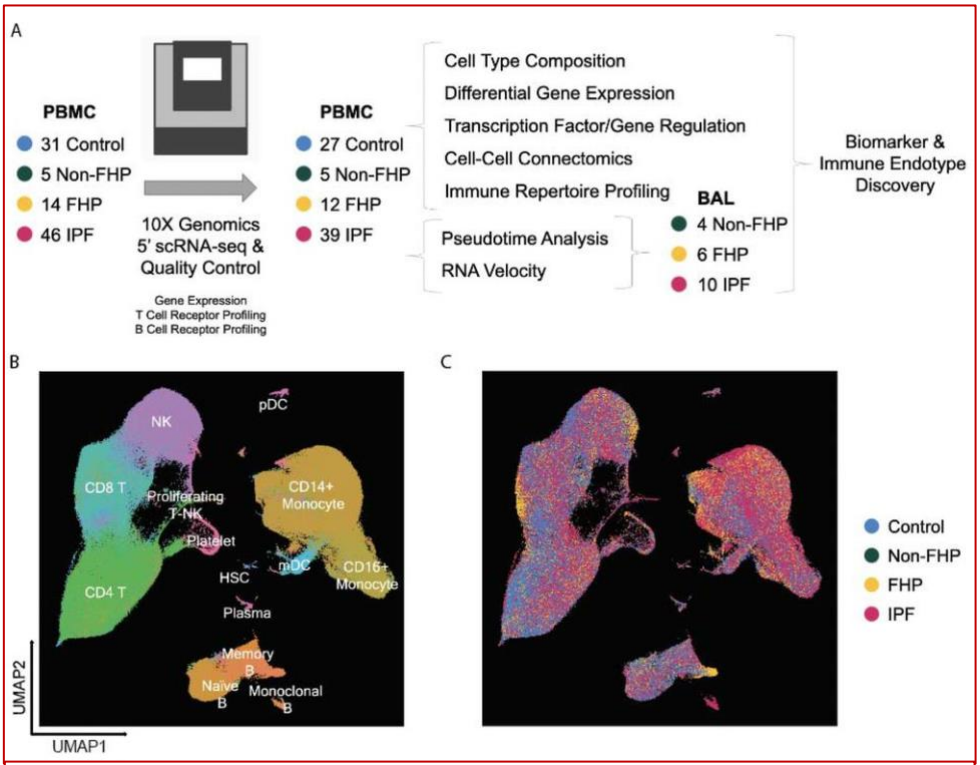


Figure 3. Study Design. Cryopreserved PBMCs from 96 individuals, including IPF, FHP, non-FHP patients, and controls, were sequenced. Post-filtering, 83 samples underwent in-depth analysis for cell type, gene expression, and more. BAL samples from 20 patients were also processed. BAL myeloid cells and PBMCs were used for pseudotime and RNA velocity studies. UMAP showcased 327,990 PBMCs post-quality control, labeled by major cell types and disease subtypes.

Spatial atlas of the mouse central nervous system at molecular resolution

Shi, H., He, Y., Zhou, Y. N. *et al. Nature* (2023), https://doi.org/10.1038/s41586-023-06569-5

The article presents a groundbreaking approach to understanding the molecular architecture of the nervous system by creating a **single-cell-resolved spatial atlas of the mouse central nervous system (CNS)**. This atlas was generated using a method called **STARmap PLUS**, which detected 1,022 endogenous genes in 20 CNS tissue slices with high spatial resolution. The data were integrated with a published single-cell RNA sequencing (scRNA-seq) atlas to create **molecular cell-type maps and molecular tissue region maps**. This comprehensive atlas comprises over one million cells with their transcriptome-wide gene expression profiles, spatial coordinates, molecular cell types, molecular tissue regions, and joint cell-type nomenclature. The study also applied this atlas to investigate the transduction landscapes of an engineered recombinant adeno-associated virus (rAAV) strain within the CNS. The generated atlas enables **precise annotation of molecular cell types based on their spatial distributions, revealing new insights into the organization of the nervous system**. The data further allow for the examination of **cell-cell adjacency patterns across the entire brain**, demonstrating that neuronal cell types tend to form near-range networks with the same cell type, while glial and immune cell types are more sparsely distributed among other cell types. Additionally, the study created **molecularly defined tissue region maps**, providing systematic and unbiased molecular definitions of CNS tissue domains. These maps were compared and annotated with anatomically defined tissue regions, offering a valuable resource for researchers studying the nervous system at the molecular and spatial levels.

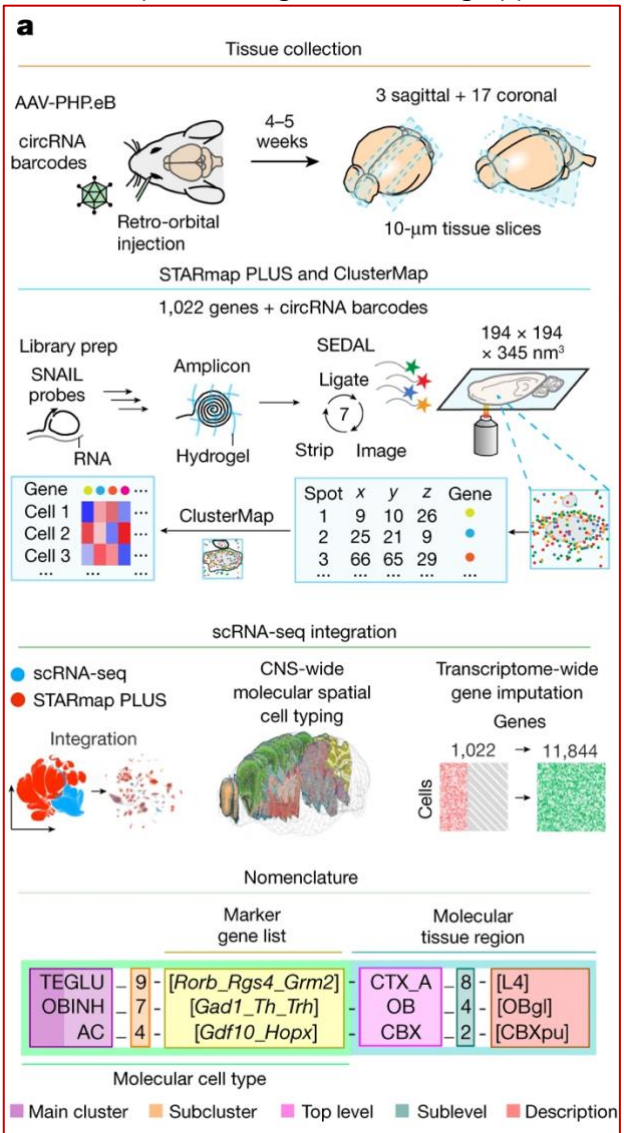


Figure 4. Overview of the study. After administering barcoded AAVs to mice, brain slices were collected 4-5 weeks later. STARmap PLUS3,4 detected RNA from 1,022 genes and AAV barcodes. RNA data was converted to cell-by-gene matrices using ClusterMap12. Integration with mouse CNS scRNA-seq data created a CNS spatial atlas, defining clusters by molecular cell types and regions. This atlas includes: AC (astrocytes), CB (cerebellum), CHOR (choroid plexus cells), OLG (oligodendrocytes), VSM (vascular smooth muscle cells), and more.

New papers from Danish researchers:

O'Rourke, Colm J., et al. "Molecular portraits of patients with intrahepatic cholangiocarcinoma who diverge as rapid progressors or long survivors on chemotherapy." *Gut* (2023). http://dx.doi.org/10.1136/gutjnl-2023-330748

Thorlacius-Ussing, Jeppe, et al. "The collagen landscape in cancer: profiling collagens in tumors and in circulation reveals novel markers of cancer-associated fibroblast subtypes." *The Journal of Pathology* (2023). https://doi.org/10.1002/path.6207

Dhumale, Pratibha, et al. "CD31 defines a subpopulation of human adipose-derived regenerative cells with potent angiogenic effects." *Scientific Reports* (2023). https://doi.org/10.1038/s41598-023-41535-1

Salachan, Paul Vinu, et al. "Spatial whole transcriptome profiling of primary tumor from patients with metastatic prostate cancer." *International Journal of Cancer* (2023). https://doi.org/10.1002/ijc.34708

Beydag-Tasöz, Belin Selcen, et al. "Integrating single-cell imaging and RNA sequencing datasets links differentiation and morphogenetic dynamics of human pancreatic endocrine progenitors." *Developmental Cell* (2023). https://doi.org/10.1016/j.devcel.2023.07.019

Ford, Shayne Lavondua, et al. "In vitro differentiated human CD4+ T cells produce hepatocyte growth factor." *Frontiers in Immunology* (2023). https://doi.org/10.3389/fimmu.2023.1210836

Farkas, Karin, and Elisabetta Ferretti. "Derivation of Human Extraembryonic Mesoderm-like Cells from Primitive Endoderm." *International Journal of Molecular Sciences* (2023). https://doi.org/10.3390/ijms241411366

Vieira, Ricardo, et al. "Young glial progenitor cells competitively replace aged and diseased human glia in the adult chimeric mouse brain." *Nature Biotechnology* (2023). https://doi.org/10.1038/s41587-023-01798-5

Tallaksen, Helene Bandsholm Leere, et al. "The multi-omic landscape of sex chromosome abnormalities: current status and future directions." *Endocrine Connections* (2023). https://doi.org/10.1530/EC-23-0011

Schmøkel, Sofie S., et al. "Improved protocol for single-nucleus RNA-sequencing of frozen human bladder tumor biopsies." Nucleus (2023). https://doi.org/10.1080/19491034.2023.2186686

Next Single Cell Seminar

Time and place: 13th October 2023, Mærsk Tower 15th floor, 7.15.92

9:00 – 9:45

Dorottya Ralbovszki, Vanessa Hall lab, Department of Veterinary and Animal Sciences, UCPH

Single cell mapping the evolution of the spatial processing centre in the brain

9:45 – 10:30

Albin Sandelin, BRIC, UCPH

A microfluidics workflow for spatial analysis of microenvironmental gradient impact on cancer cell phenotypes

If you would like to announce anything single cell related, e.g. job announcement, event, your published paper, technology development etc., please contact us.

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