



## Module 4 Resource List: Datasets and Tutorials

*The datasets and tutorials below were selected by Madeline Lancaster, Aparna Bhaduri, and Madeline Andrews, faculty from Module 4 of Stem Cells and Reprogramming Methods for Neuroscience: An SfN Training Series.*

### **Selected Summary of Currently Available Single-Cell Organoid Datasets**

#### **[Human Cerebral Organoids Recapitulate Gene Expression Programs of Fetal Neocortex](#)**

##### **Development**

The authors use Fluidigm C1 and SmartSeq single-cell sequencing to compare organoids generated with the Lancaster whole brain organoid protocol and by sub-dissecting cortical rosettes for detailed analysis to developing human cortex.

#### **[Cell Diversity and Network Dynamics in Photosensitive Human Brain Organoids](#)**

The authors use 10X sequencing to profile long-term organoid cultures generated with the Lancaster whole brain organoid protocol. They discover a set of cells within retinal parts of the organoid have photoreceptive properties.

#### **[Human Astrocyte Maturation Captured in 3D Cerebral Cortical Spheroids Derived From Pluripotent Stem Cells](#)**

The authors use the Pasca lab protocol and long-term culture to observe the emergence of astrocytes in cortical spheroids.

#### **[Transcriptome and Epigenome Landscape of Human Cortical Development Modeled in Organoids](#)**

The authors seek to use organoids to study neurodevelopment and features of Autism. As part of this analysis they compare organoids to primary development prior to 16 weeks, using a unique protocol with limited time in aggregate culture.

#### **[Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution](#)**

The authors compare organoids generated using the Sasai lab differentiation protocol to primary tissue in order to utilize organoids to explore evolutionary differences in cortical development. They observe an up-regulation of stress programs in organoid single-cell analysis compared to primary cells.



### **Cerebral Organoids at the Air–Liquid Interface Generate Diverse Nerve Tracts With Functional Output**

The authors innovate a new strategy to improve the health of organoid cultures resulting in better axon growth and electrophysiological activity. After a period of time in aggregate culture, the organoids are sectioned and grown at the air-liquid interface

### **Differentiation and Maturation of Oligodendrocytes in Human Three-Dimensional Neural Cultures**

The authors generate a new protocol for organoid differentiation that enhances oligodendrogenesis, producing oligodendrocytes that are rarely observed using other organoid generation protocols. They characterize these cells as similar to primary oligodendrocytes using marker gene analysis from their single-cell sequencing.

### **hESC-Derived Thalamic Organoids Form Reciprocal Projections When Fused With Cortical Organoids**

As an example of other regional brain structures that can be generated in organoids, the authors create thalamic organoids and cortical organoids and aggregate together. The neurons in each respective organoid project to each other and were profiled using single-cell sequencing.

### **Links to Genome Browser Datasets**

<https://cortex-dev.cells.ucsc.edu/>

Primary data from [Nowakowski et. al., 2017](#).

<https://organoids.cells.ucsc.edu/>

Organoid data from [Pollen et al, 2019](#).

<https://organoidreportcard.cells.ucsc.edu/>

Primary data from the Module 4 video, “Using Organoids and Single Cell RNA Sequencing Approaches to Study Human Cortical Development”

<https://organoidreportcard.cells.ucsc.edu/>

Organoid data from the Module 4 video, “Using Organoids and Single Cell RNA Sequencing Approaches to Study Human Cortical Development”



## **References to Key Single-Cell Computational Tutorials and Methods**

Here we present a few algorithms and strategies, starting with alignment, clustering, and expanding to lineage trajectory analysis. However, this list is simply the tip of the iceberg for single-cell analysis as the field continues to innovate and iterate over the suite of algorithms, and many problems in single-cell analysis remain unsolved. Use this list as a broad overview, but by no means can it be a comprehensive list of available tools.

### **CellRanger**

For 10X data, an all-in-one pipeline for barcode to cell assignment, alignment to reference genome (mouse and human downloadable, others easily constructable), and gene count generation. It also includes the option of visualizing data in Loupe browser format.

### **Seurat**

This includes tutorials and a variety of functionalities, including processing data from 10X output or other matrix format, combining datasets with a “batch effect”, treatment condition, or species difference. It also integrates with many other single-cell tools.

### **Scanpy**

This is a Python implementation of single-cell toolkit. It includes many functionalities similar to Seurat, as well as integration with a number of other single-cell analysis, batch correction, and lineage algorithms.

### **Benchmarking Methods**

This article gives an overview of many different strategies to analyze single-cell data, including clustering, imputation, normalization, and lineage analysis, among many other applications.

### **Cell Browser Based Visualization**

This is a visualization tool to explore datasets which also allows you to make your own. It includes a variety of visualizations, such as tSNE, UMAP, and others. It also enables search features for genes of interest, metadata coloration, and generation of violin plots.