Overview of scRNA-seq technology

**scRNA-seq Technologies**

- **Droplet**
  - 10X Chromium
  - DropSeq
  - InDrop
- **Well based**
  - sci-RNA-seq
  - SmartSeq
- **Nanowell**
  - SeqWell
- **Spatial*** (Not always single cell)
  - Nanostring
  - 10X Visium

**Analysis Approaches & Tools**

- **General scRNA-seq**
  - Seurat (R)
  - Monocle (R)
  - Scanpy (Python)
- **Many specialized tools**
  - Ligand & receptor interactions
  - Trajectory Inference (pseudotime)
  - Transcription Factor Enrichment
  - Geneset Enrichment

Slides prepared by Nick Calistri
Will focus on 10X + Seurat due to popularity

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10X Chromium scRNA-seq experimental design

- 5k-20k cells recovered per library
  - Typically want ~10x target as input

- 20k reads per cell

- 1-8 conditions/samples per experiment
  - >1 requires multiplexing

- ~$4000 cost with sequencing

Hwang et al.
Typical Seurat Workflow

- ~37,000 Features (genes)
- 2,000-5,000 Variable Features
- 10-50 PCs
- UMAP
- Clustering
- Differential Expression
Typical Seurat Workflow

- 37,000 Features (genes)
- 2,000-5,000 Variable Features
- n Cells
- 10-50 PCs
- Clustering
- UMAP
- Differential Expression

2700 cells
58 seconds elapsed time (standard desktop)

*Disclaimer: Parameters should be optimized for the specific data set*
Seurat default plots can quickly visualize results

**UMAP**

**Heatmap**

**Violin Plot**

```r
DimPlot(so, 
  label = TRUE, 
  label.size = 6) +
coord_equal()+
theme(legend.position = 'none')

goi <- c('CD3E', 'CCR7', 'CD4', 'CD8A', 'CD14', 'CD74')
DoHeatmap(so, 
  features = goi)

VlnPlot(so, 
  features = 'GZMB')
```
Additional computational methods

Integration

**Liger**

INMF approach for learning across:
- Sequencing technologies
- -omic modalities
- Cross species

Welch et al, cell 2019
Kriebel and Welch, Nat.comm 2022

Ligand & Receptor Interactions

**NicheNet**

Imputes ligand-receptor interactions by:
- Evaluating ligand and receptor expression
- Cross checking with PPI network and downstream DEG

Browaeys et al, Nat.Methods 2020

Trajectory Inference

**DynVerse**

Unified interface for TI:
- 50+ dockerized TI method
- GUI interface to help select appropriate algorithms/methods

Cannoodt, Saelens et al, Nat.Biotech 2019
Links and resources

• **General scRNA-seq:**
  • Seurat: [https://satijalab.org/seurat/](https://satijalab.org/seurat/)
  • Monocle: [https://cole-trapnell-lab.github.io/monocle3/](https://cole-trapnell-lab.github.io/monocle3/)

• **Integration:**
  • Seurat: [https://satijalab.org/seurat/articles/integration_introduction.html](https://satijalab.org/seurat/articles/integration_introduction.html)
  • Harmony: [https://portals.broadinstitute.org/harmony/](https://portals.broadinstitute.org/harmony/)
  • Liger: [https://github.com/welch-lab/liger](https://github.com/welch-lab/liger)

• **Interaction analysis:**
  • NicheNetR: [https://github.com/saeyslab/nichenetr](https://github.com/saeyslab/nichenetr)
  • Natmi: [https://github.com/forrest-lab/NATMI](https://github.com/forrest-lab/NATMI)
  • Remi: [https://github.com/plevritis-lab/REMI](https://github.com/plevritis-lab/REMI)

• **Trajectory Inference**
  • Monocle: [https://cole-trapnell-lab.github.io/monocle3/docs/trajectories/](https://cole-trapnell-lab.github.io/monocle3/docs/trajectories/)
  • Velocyto: [http://velocyto.org/](http://velocyto.org/)
  • DynVerse: [https://dynverse.org/](https://dynverse.org/)