

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

Will focus on 10X + Seurat due to popularity

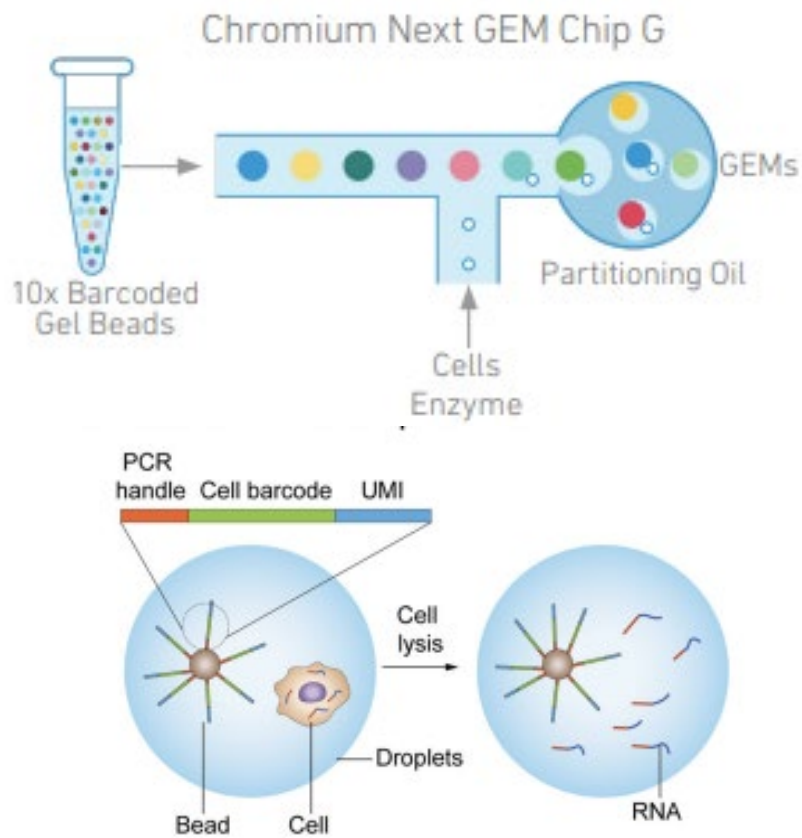
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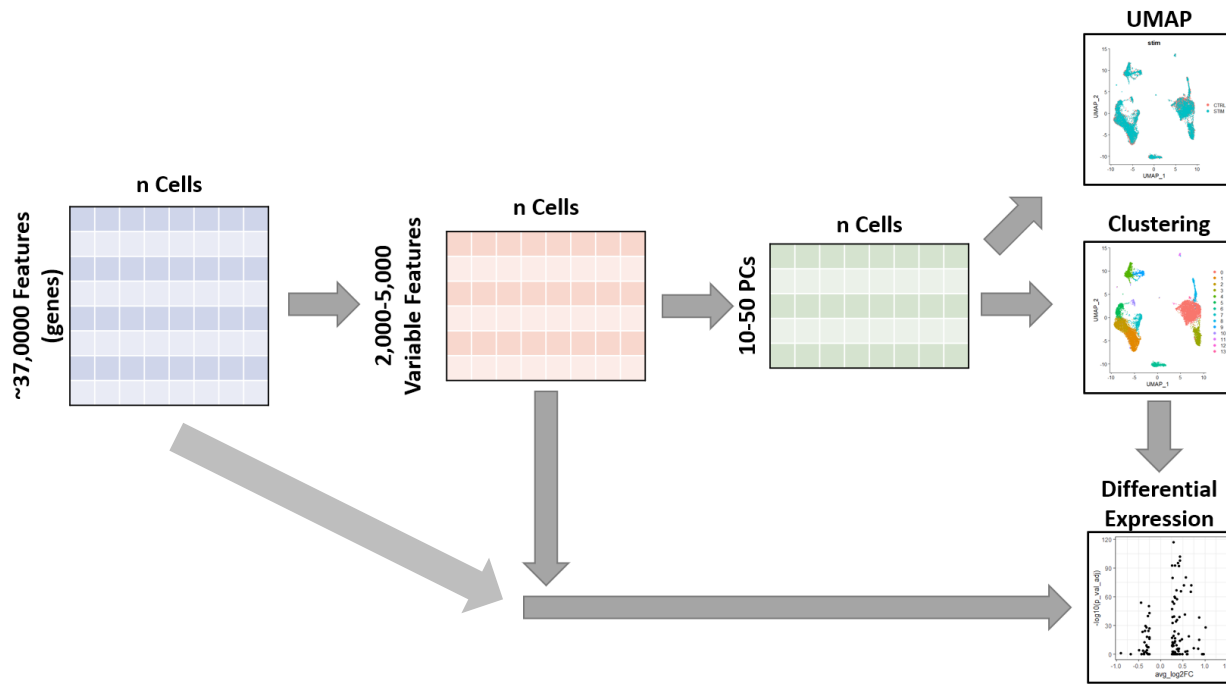
10X Chromium scRNA-seq experimental design



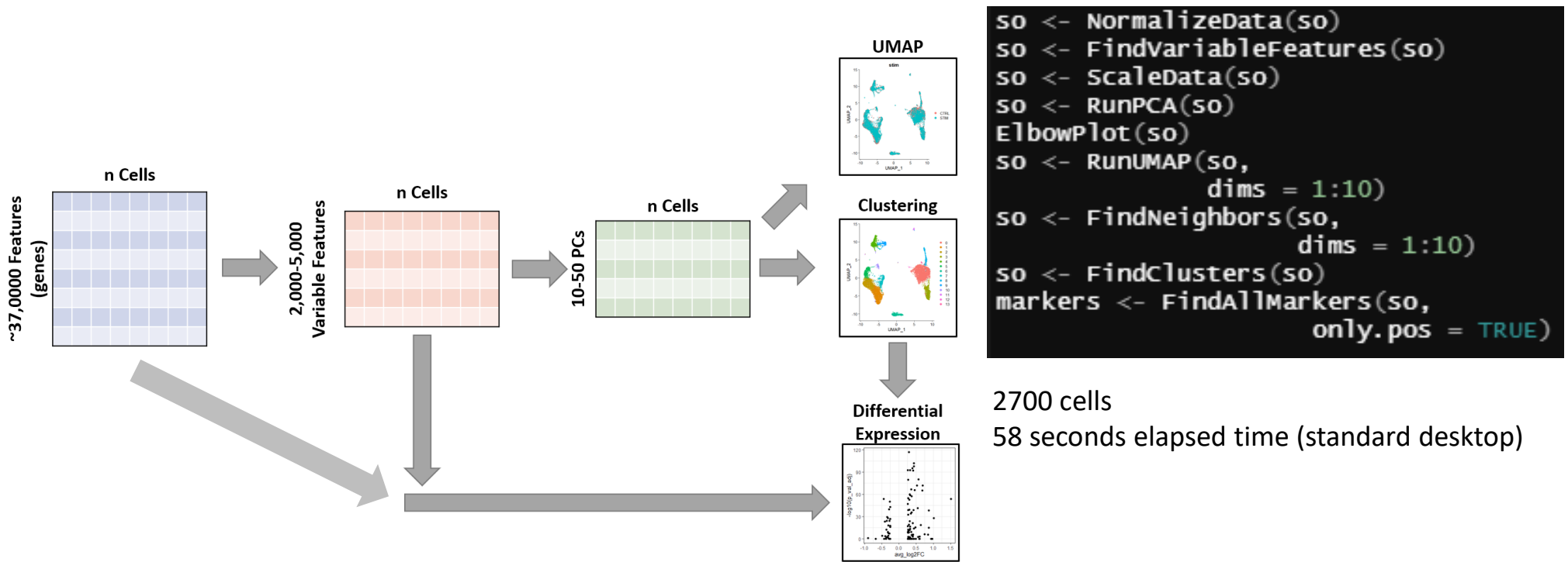
Hwang et al.

- 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

Typical Seurat Workflow



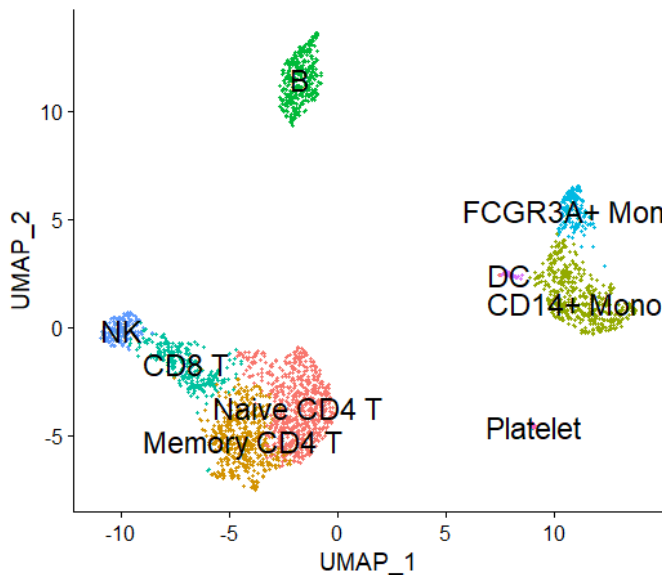
Typical Seurat Workflow



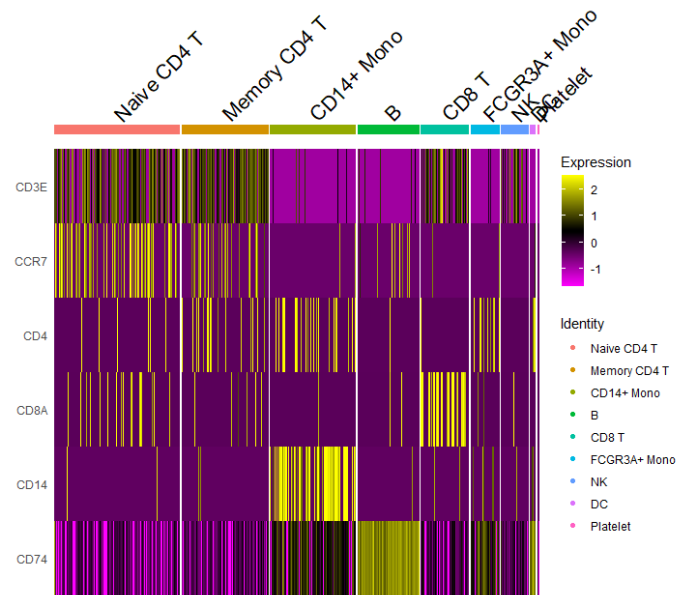
*Disclaimer: Parameters should be optimized for the specific data set

Seurat default plots can quickly visualize results

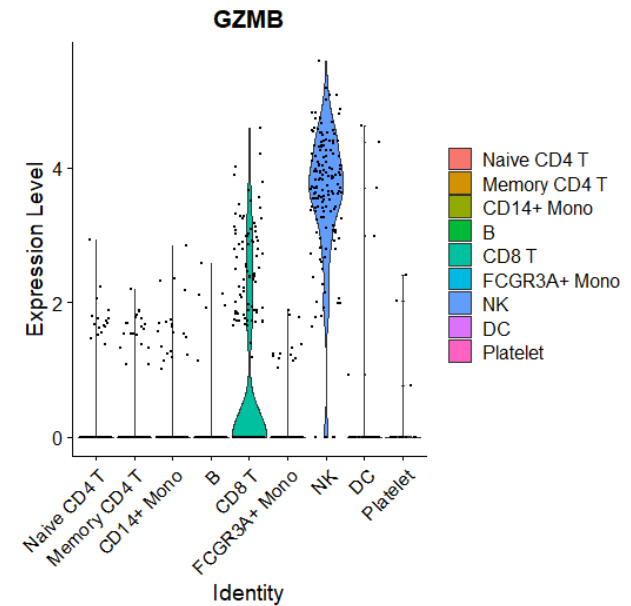
UMAP



Heatmap



Violin Plot



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

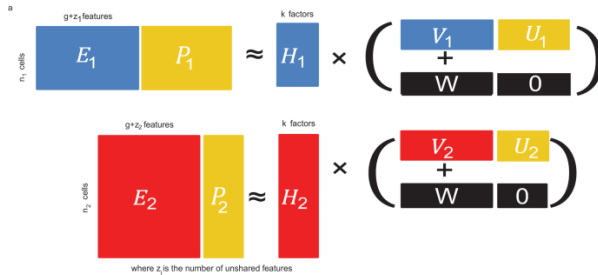
```
goi <- c('CD3E', 'CCR7', 'CD4', 'CD8A', 'CD14', 'CD74')  
DoHeatmap(so,  
  features = goi)
```

```
VlnPlot(so,  
  features = 'GZMB')
```

Additional computational methods

Integration

Liger



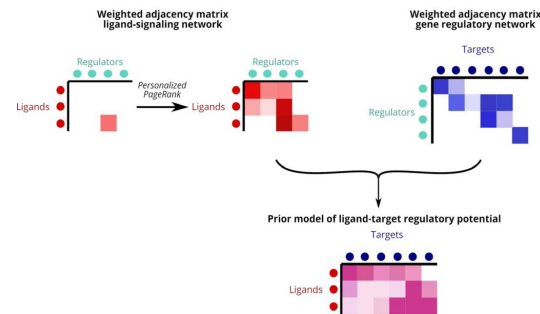
INMF approach for learning across:

- Sequencing technologies
- -omic modalities
- Cross species

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

Ligand & Receptor Interactions

NicheNet



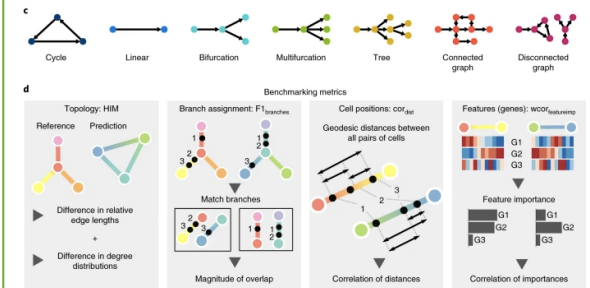
Imputes ligand-receptor interactions by:

- 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/>
 - Monocle: <https://cole-trapnell-lab.github.io/monocle3/>
 - Scanpy: <https://scanpy.readthedocs.io/en/stable/>
- **Integration:**
 - Seurat: https://satijalab.org/seurat/articles/integration_introduction.html
 - Harmony: <https://portals.broadinstitute.org/harmony/>
 - Liger: <https://github.com/welch-lab/liger>
- **Interaction analysis:**
 - NicheNetR: <https://github.com/saeyslab/nichenetr>
 - Natmi: <https://github.com/forrest-lab/NATMI>
 - Remi: <https://github.com/plevritis-lab/REMI>
- **Trajectory Inference**
 - Monocle: <https://cole-trapnell-lab.github.io/monocle3/docs/trajectories/>
 - Velocity: <http://velocity.org/>
 - DynVerse: <https://dynverse.org/>