
3000788 Intro to Comp Molec Biol

Lecture 14: Single-cell transcriptomics

October 2, 2023



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- Research Affairs
- Center of Excellence in Computational Molecular Biology (CMB)
- Center for Artificial Intelligence in Medicine (CU-AIM)



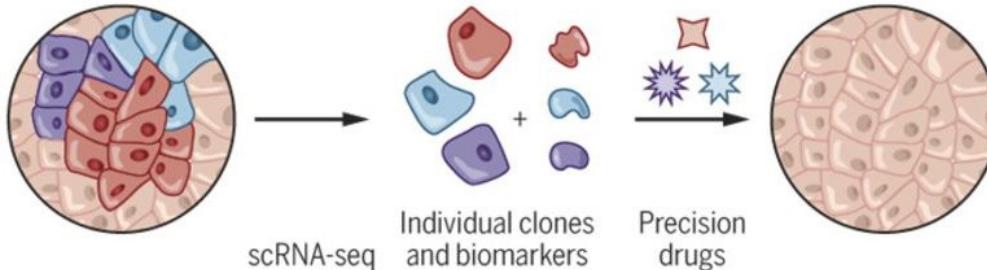
Why single-cell?

Tissue consists of multiple cell types

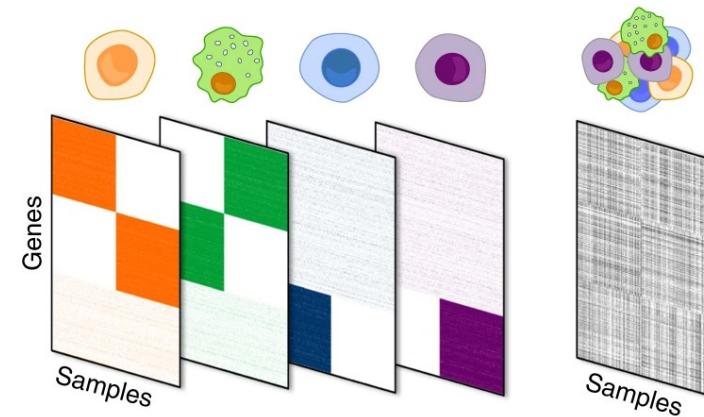
Bulk analysis



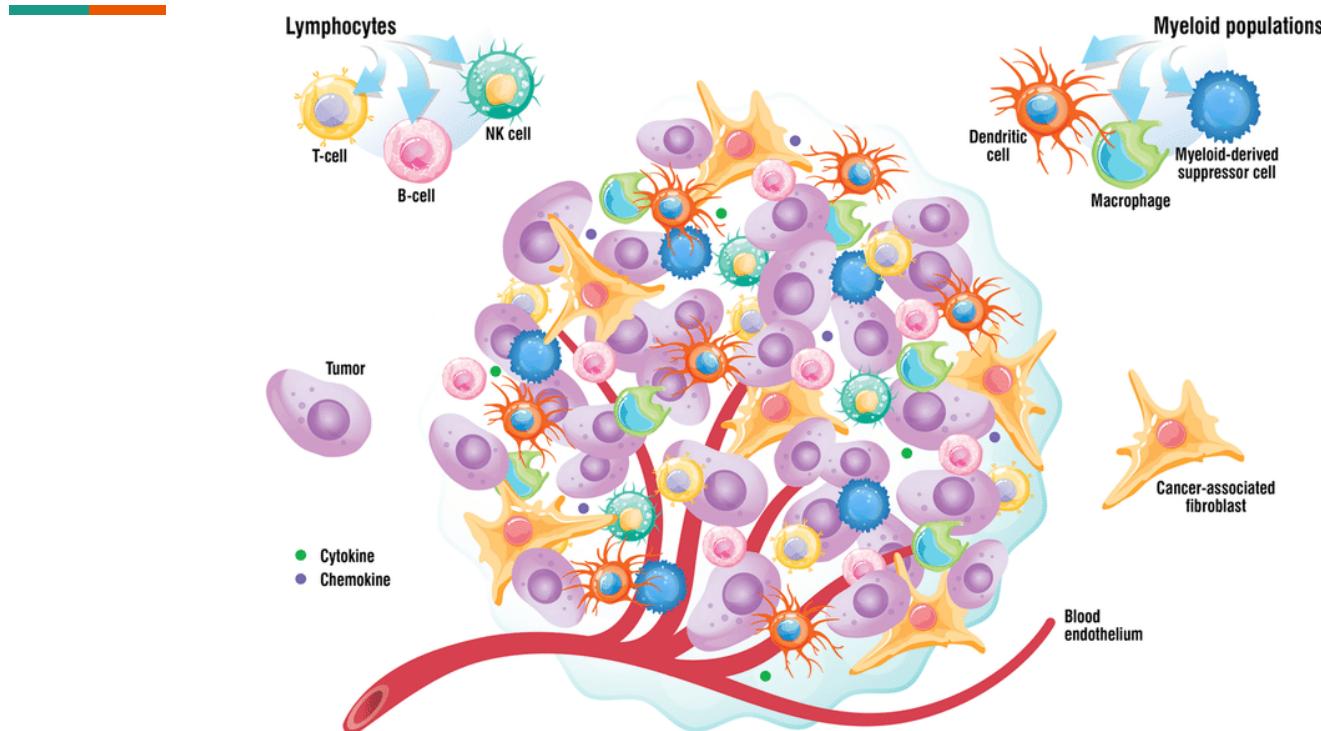
scRNA analysis



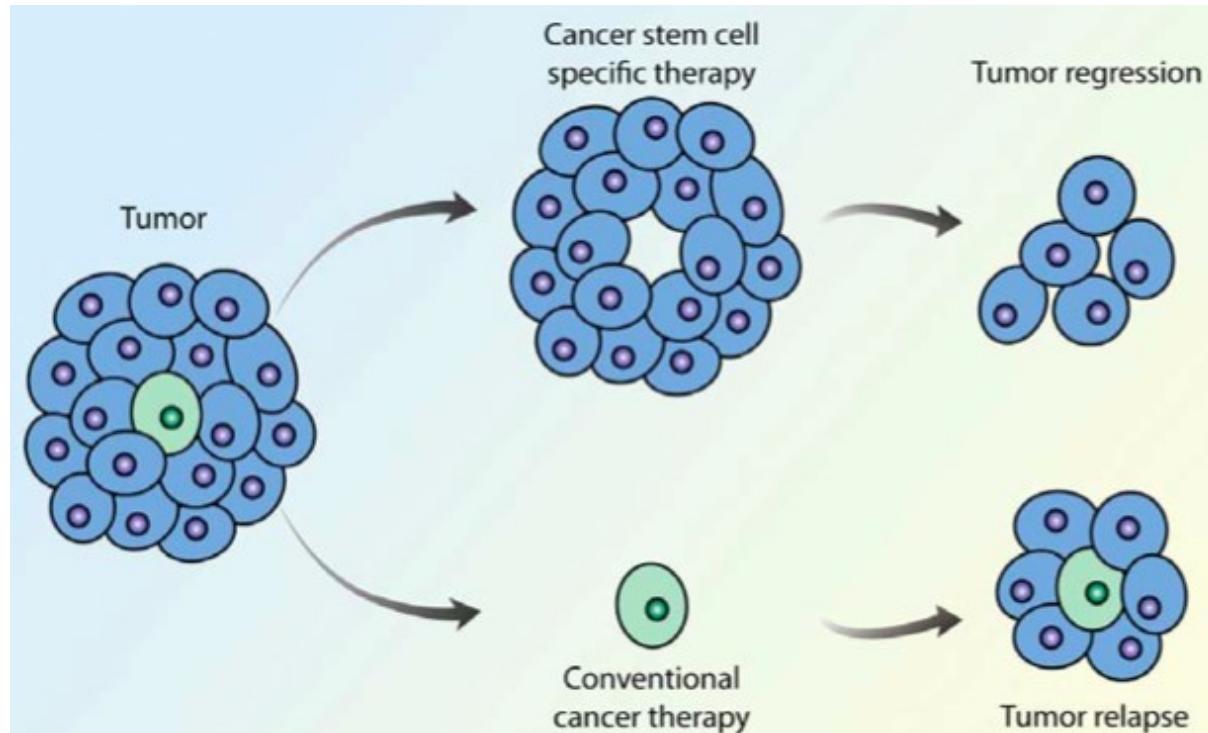
Each biological sample is a mixture of many cell types



Tumor microenvironment

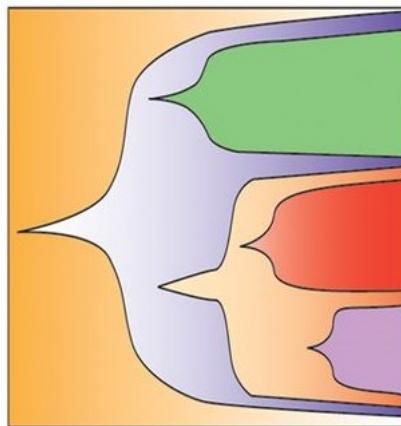


Cancer stem cell

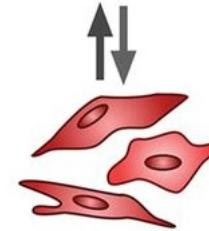
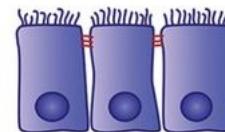
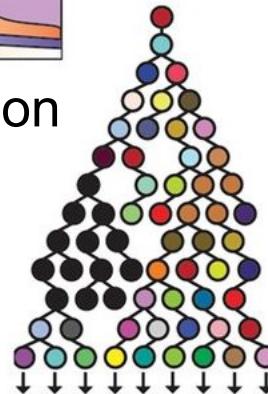


Knowledge at single-cell resolution

Heterogeneity

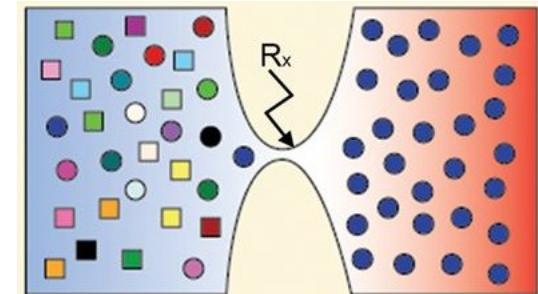


Clonal expansion

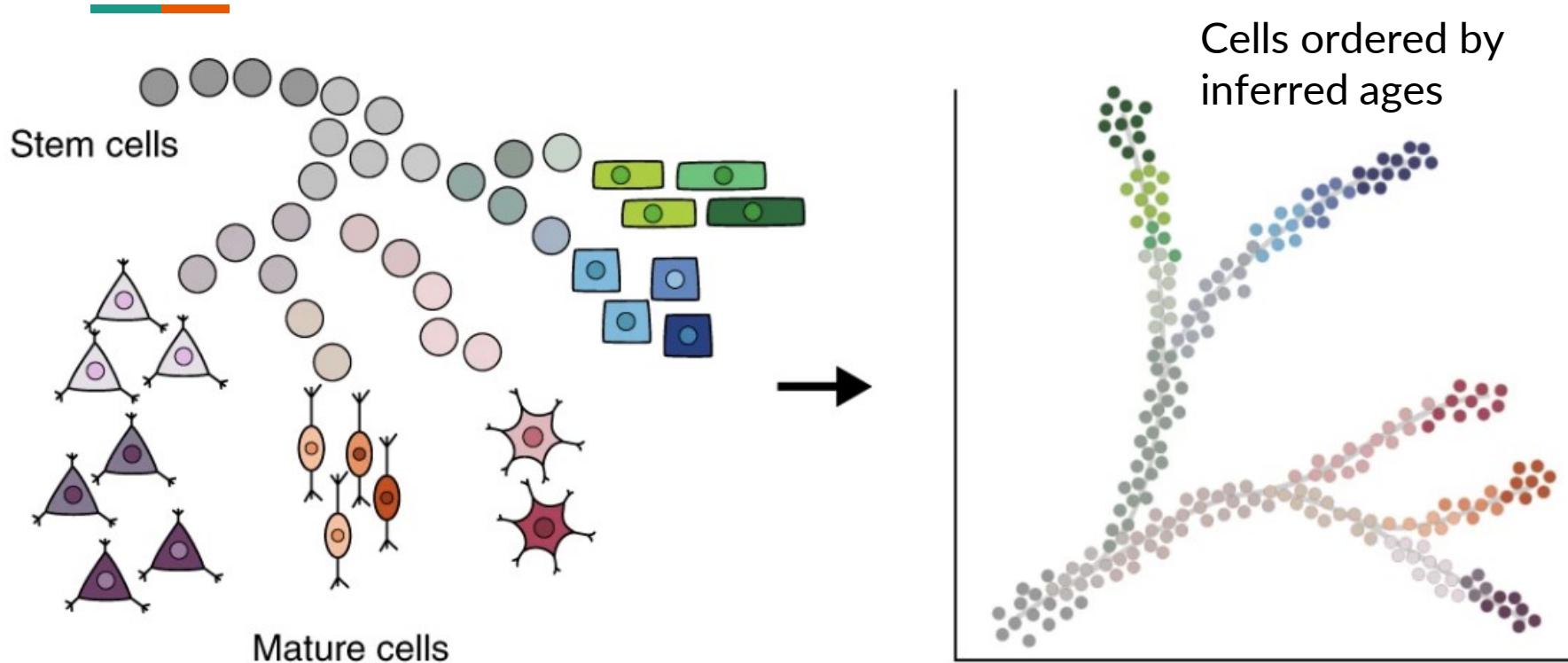


Differentiation

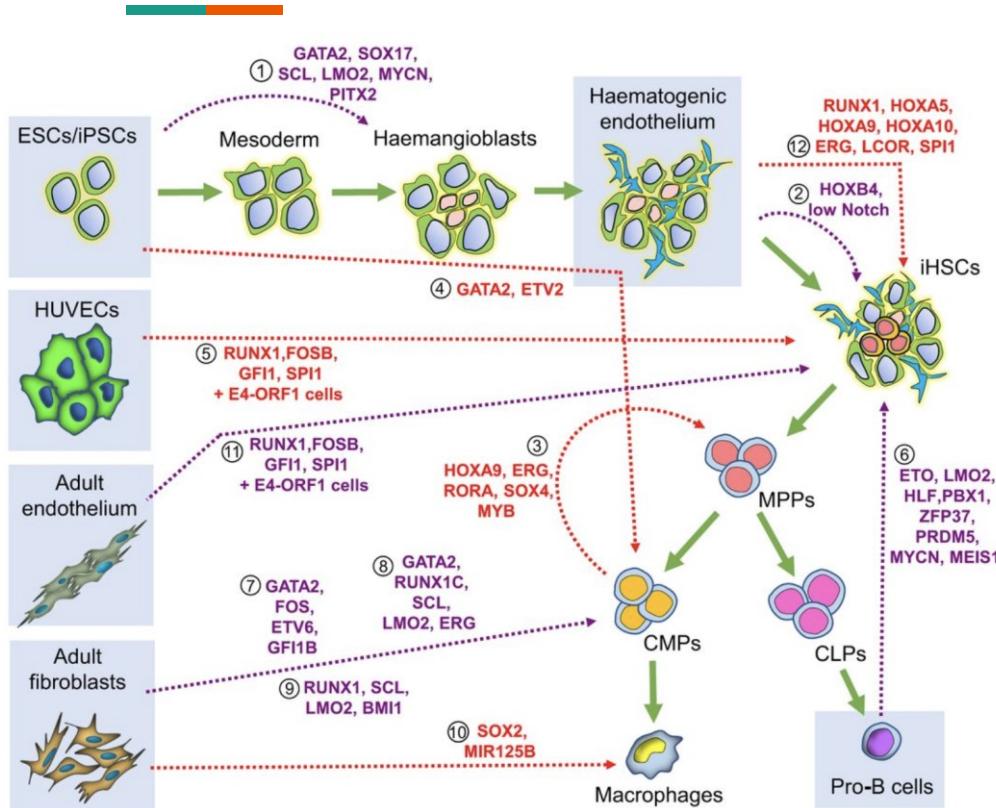
Treatment response



Cell development through single-cell data



Detecting gene switches



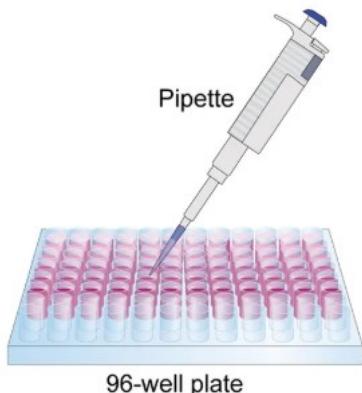
- Genes whose expressions:
- Change over inferred developmental time
- Diverge between two developmental branches
- Turn on/off across cell type



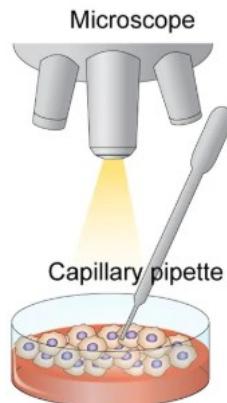
Single-cell vs bulk transcriptomics

Cell isolation techniques

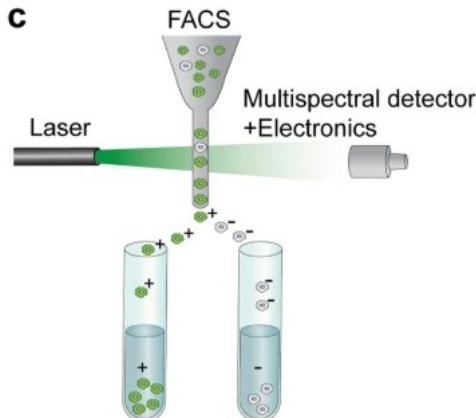
a



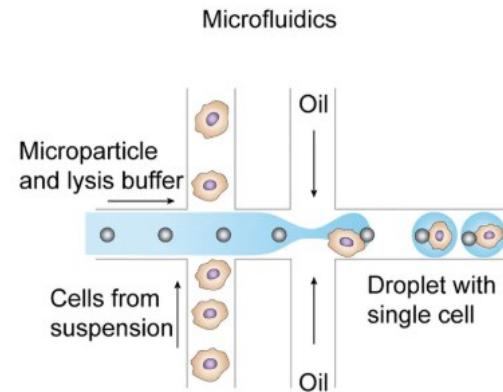
b



c



e



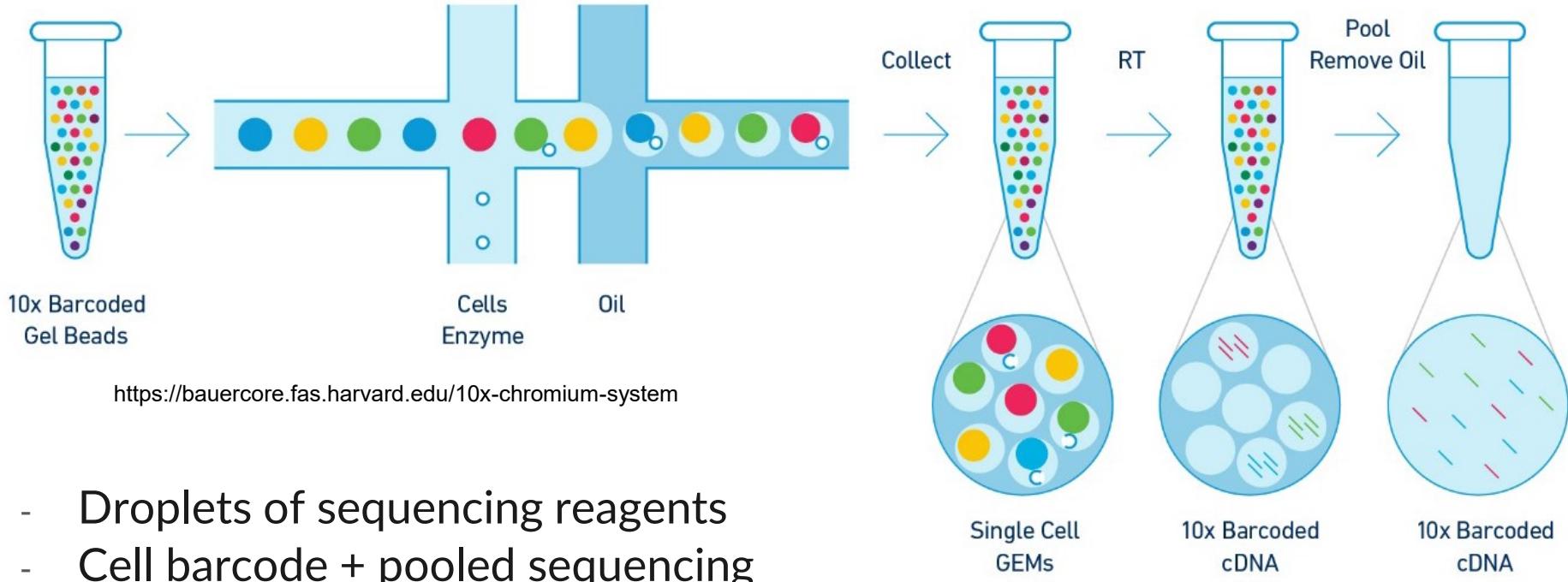
Diverse protocol choices

Methods	Transcript coverage	UMI possibility	Strand specific	References	CEL-seq	3'-only	Yes	Yes	Hashimshony et al., 2012
Tang method	Nearly full-length	No	No	Tang et al., 2009	CEL-seq2	3'-only	Yes	Yes	Hashimshony et al., 2016
	Full-length	No	No	Sasagawa et al., 2013	MARS-seq	3'-only	Yes	Yes	Jaitin et al., 2014
	Full-length	No	No	Fan X. et al., 2015	CytoSeq	3'-only	Yes	Yes	Fan H.C. et al., 2015
	Full-length	No	No	Ramskold et al., 2012	Drop-seq	3'-only	Yes	Yes	Macosko et al., 2015
	Full-length	No	No	Picelli et al., 2013	InDrop	3'-only	Yes	Yes	Klein et al., 2015
	Full-length	Yes	Yes	Sheng et al., 2017	Chromium	3'-only	Yes	Yes	Zheng et al., 2017
	5'-only	Yes	Yes	Islam et al., 2011, 2012	SPLiT-seq	3'-only	Yes	Yes	Rosenberg et al., 2018
					sci-RNA-seq	3'-only	Yes	Yes	Cao et al., 2017
					Seq-Well	3'-only	Yes	Yes	Gierahn et al., 2017
					DroNC-seq	3'-only	Yes	Yes	Habib et al., 2017
					Quartz-Seq2	3'-only	Yes	Yes	Sasagawa et al., 2018

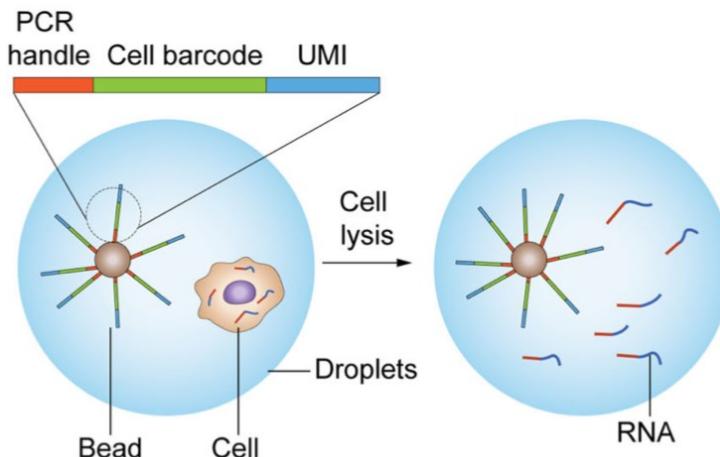
Chen *et al.* Front Genet. 10:317 (2019)

- Mostly sequence 3' ends of transcript
- Unique Molecular Identifier (UMI) = PCR barcode

10x Genomics' chromium technique



UMI and cell barcode



Hwang et al. Exp & Mol Med 50:96 (2018)

- All beads in each droplet have the same cell barcode
 - Reads with the same barcode came from the same cell
- Each PCR adapter contains different Unique Molecular Identifiers (UMI)
 - Reads with the same UMI came from the same original RNA molecule

Single-cell vs bulk data



Estimated Number of Cells

→ 5,593

Mean Reads per Cell

→ 26,716

Median Genes per Cell

2,880

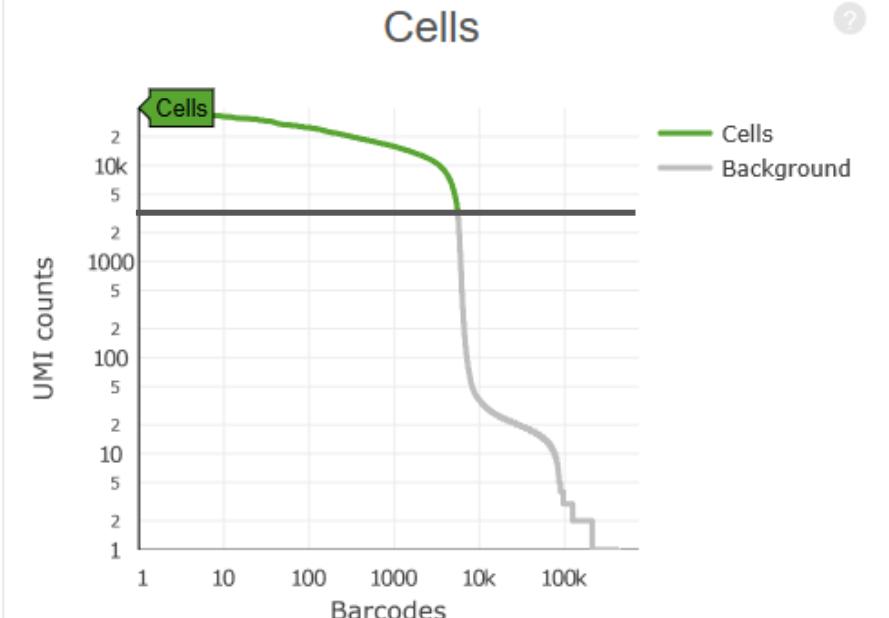
Sequencing

Number of Reads

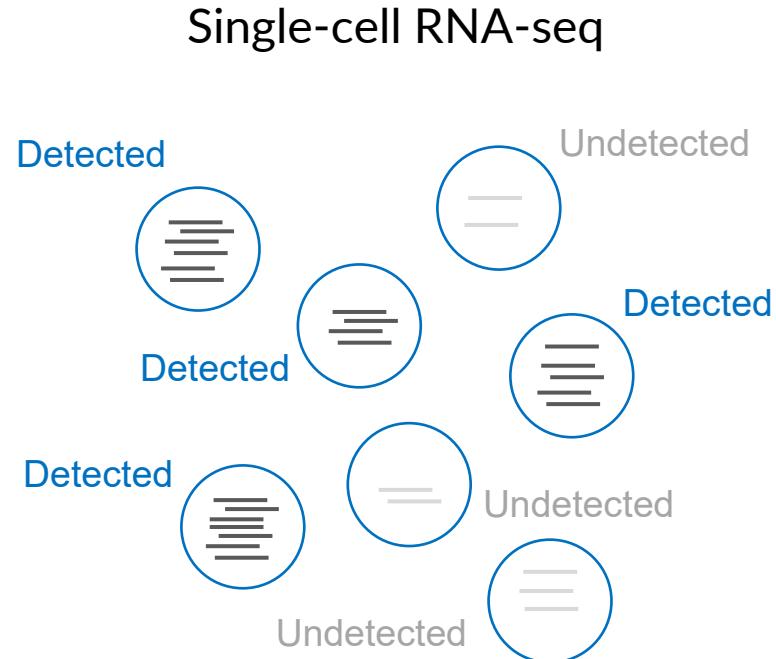
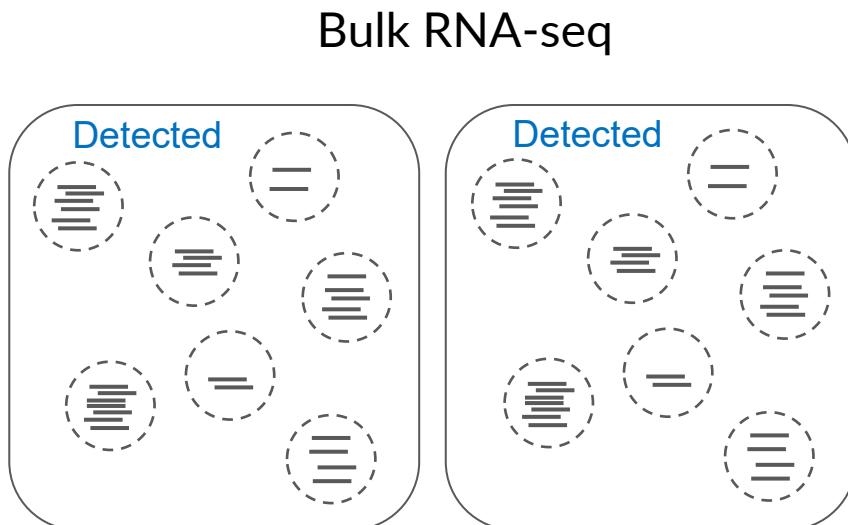
→ 149,425,634

Valid Barcodes

97.5%



Detection limit of single-cell data



Challenges in single-cell data analysis

- Low read count per cell and gene
 - A lot of zeros in expression data
- Cells are biologically different
 - High variance across cells
- Cells are in continuous states of development
 - Not just control vs treatment
- Data is very large (256 GB of RAM for medium project)
 - 10,000 cells x 5,000 genes

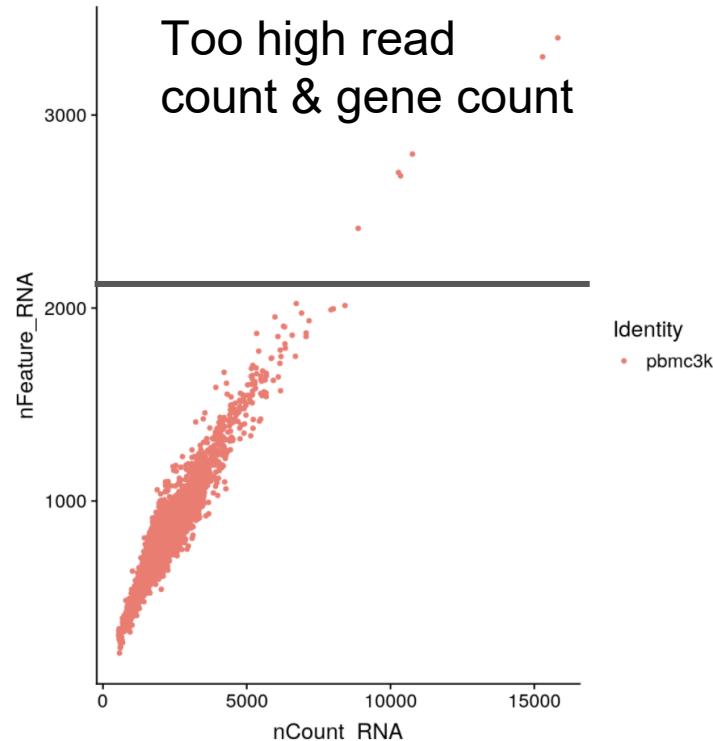
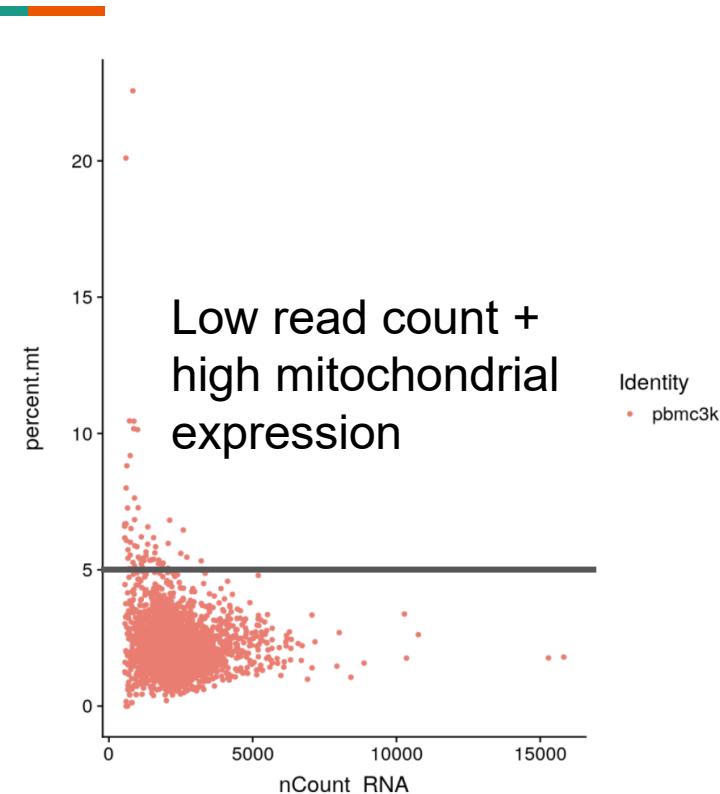


Single-cell data preprocessing

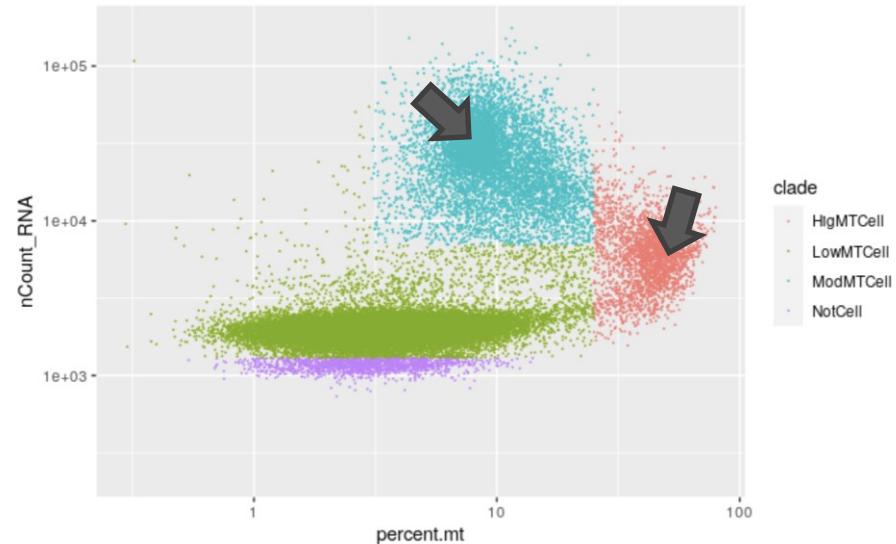
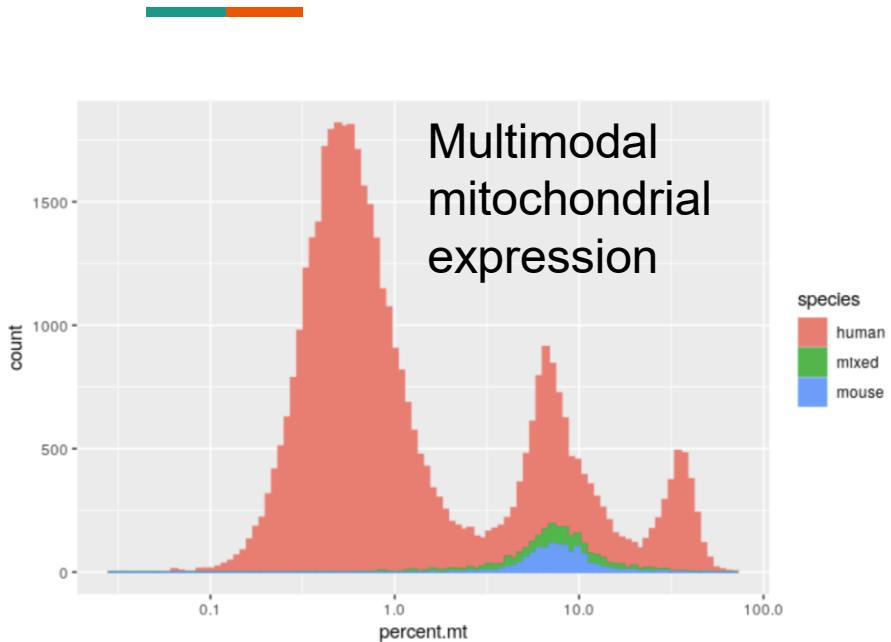
Key steps in single-cell data processing

- Quality filter
 - Low read count & gene count = non-cells
 - Very high read count & gene count = multi-cells
 - High mitochondrial expression = dead cells
- Within-sample normalization
 - Dealing with missing expression values
- Multi-sample integration
 - Single-cell data have strong batch effects

Basic quality filters

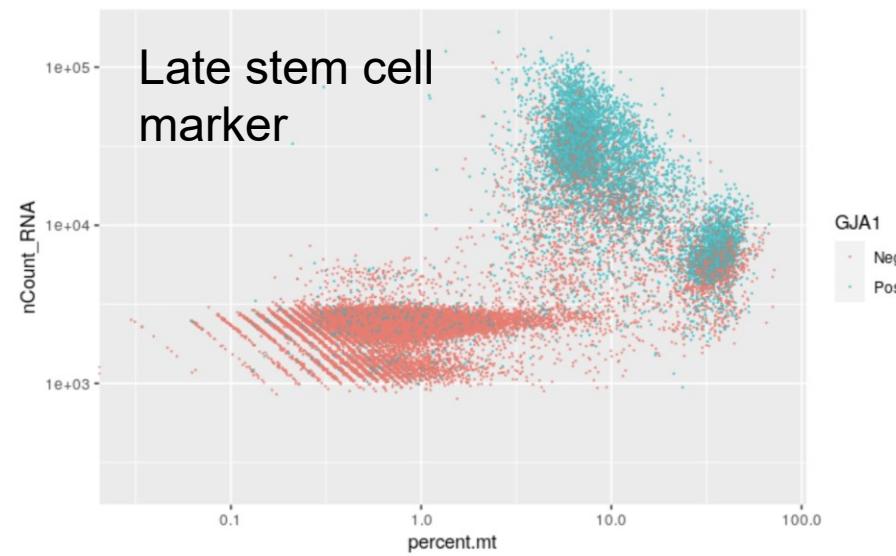
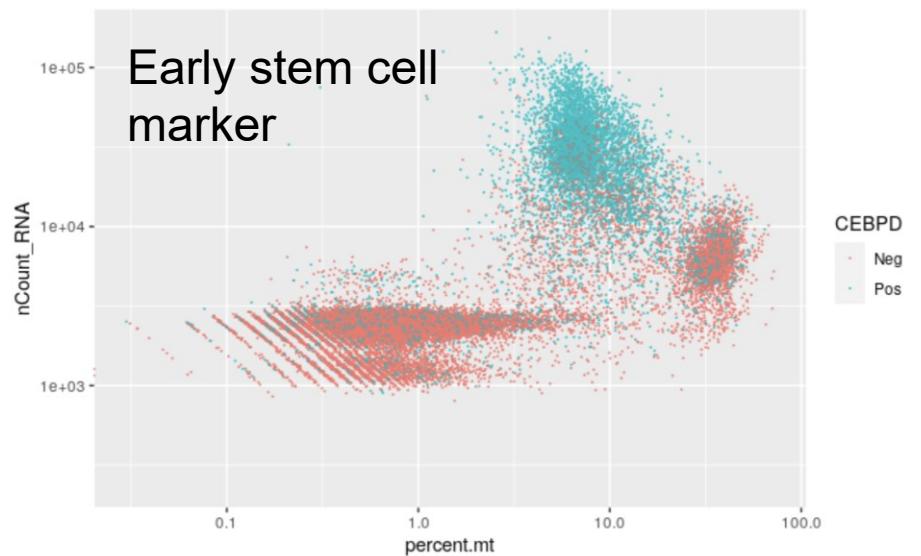


An exception

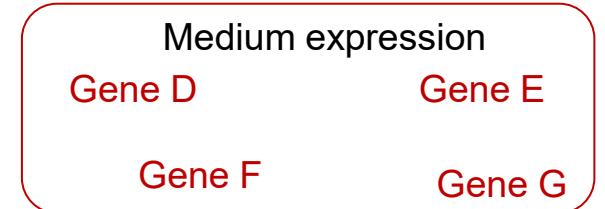
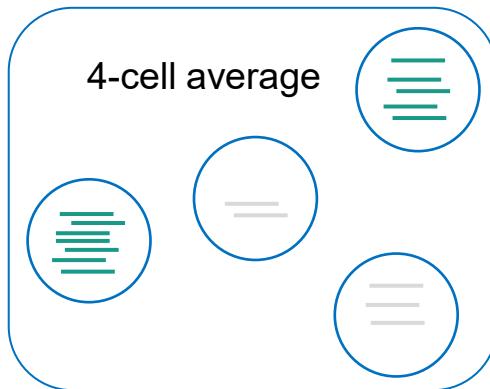
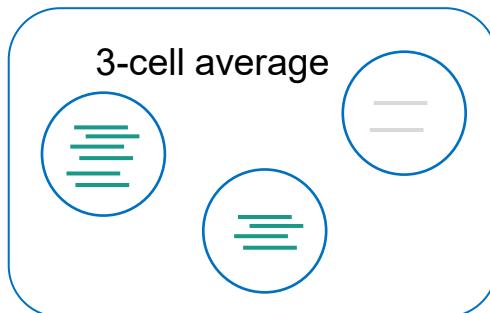


- High mitochondrial activity in stem cells and some cell types

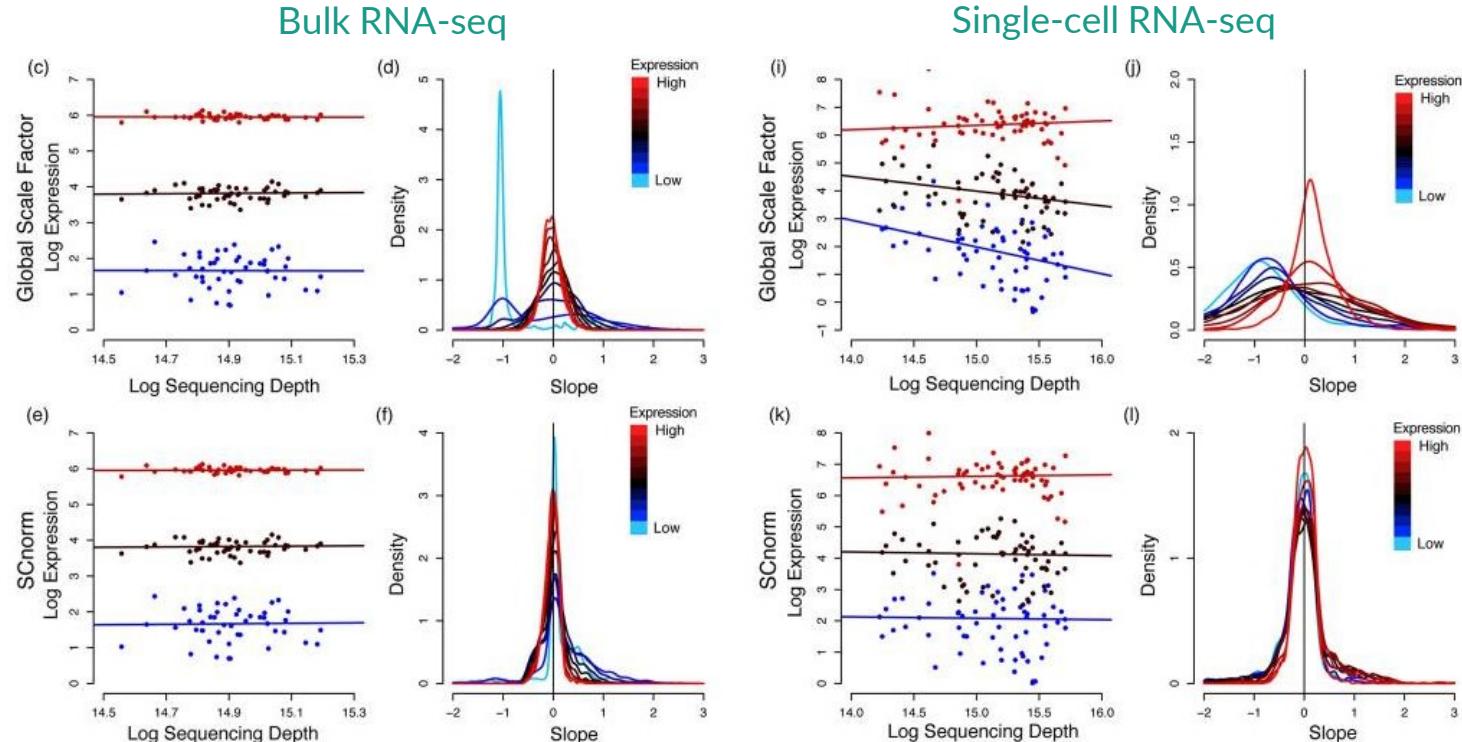
Stem cell markers in high-mitochondrial cells



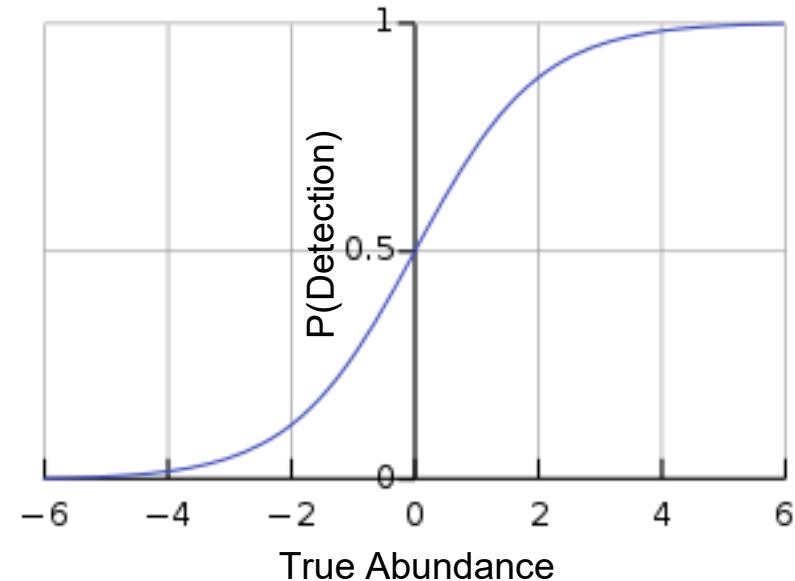
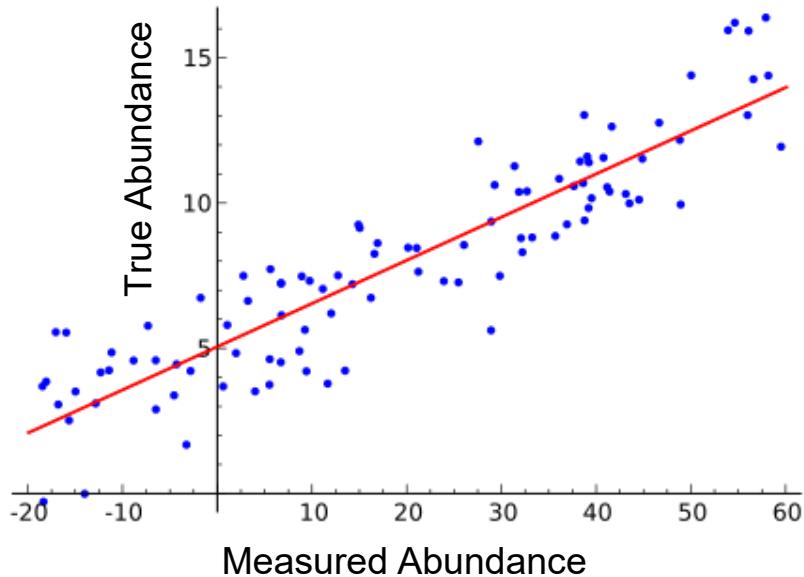
Normalization with pooling



Expression-dependent scaling factor



Modeling of detection probability

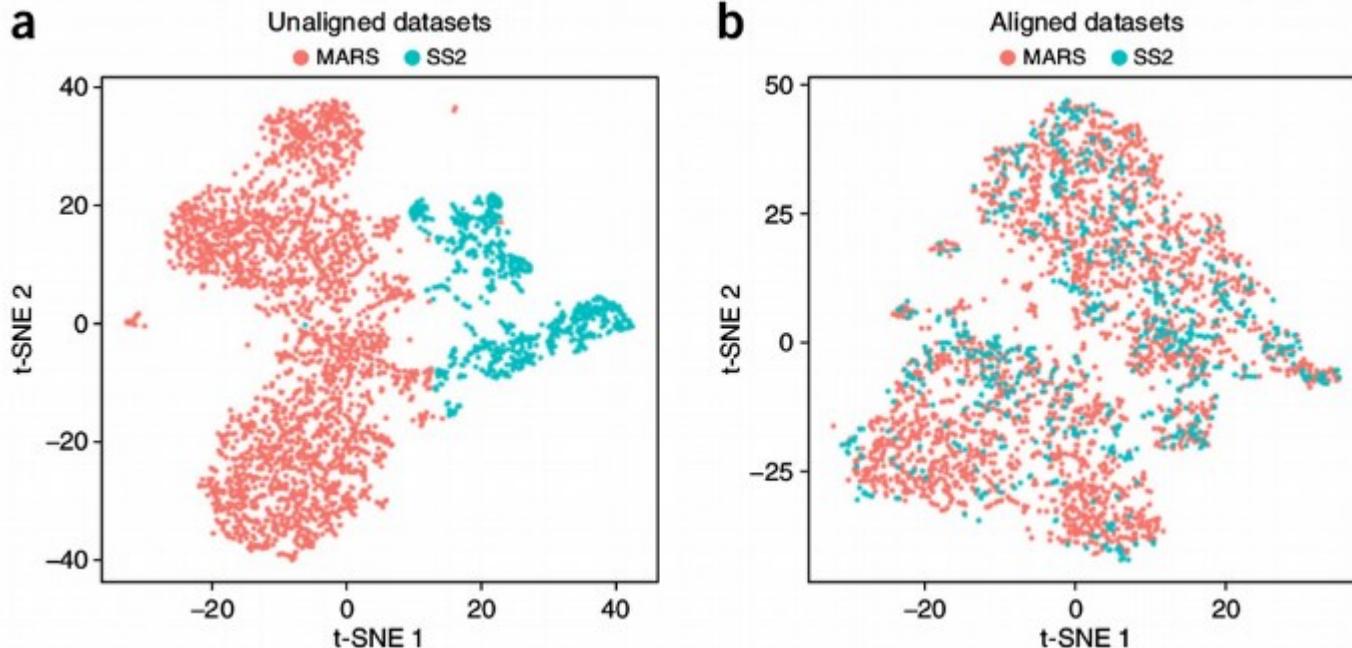


- Measured abundance = $f(\text{true abundance}) \times P(\text{detection} | \text{true abundance})$



Single-cell data integration

High bias across datasets



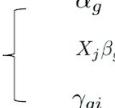
Linear effect removal (Combat-Seq)

Negative binomial regression models

Gene-wise model: for a certain gene g , count in sample j from batch i $y_{gij} \sim NB(\mu_{gij}, \phi_{gi})$

Explicit addition of batch parameter $\gamma_{g,i}$ 

$$\log \mu_{gij} = \alpha_g + X_j \beta_g + \gamma_{gi} + \log N_j$$
$$Var(y_{gij}) = \mu_{gij} + \phi_{gi} \mu_{gij}^2$$

Decompose scaled counts into 3 components 

α_g	Average level for gene g (in “negative” samples)
$X_j \beta_g$	Biological condition of sample j
γ_{gi}	Mean batch effect
ϕ_{gi}	Dispersion batch effect

$$N_j = \text{total read count for sample j}$$

Estimate batch effect parameters

Estimate parameters using established methods in edgeR

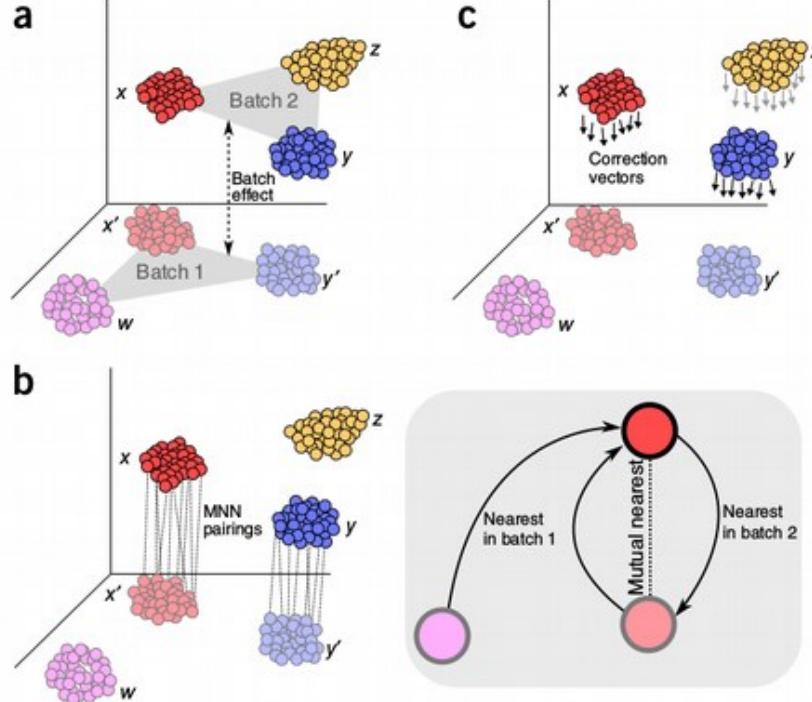
Calculate “batch-free” distributions

We assume the adjusted data also follow a negative binomial distribution: $y_{gj}^* \sim NB(\mu_{gj}^*, \phi_g^*)$

Subtract batch parameter $\gamma_{g,i}$ 

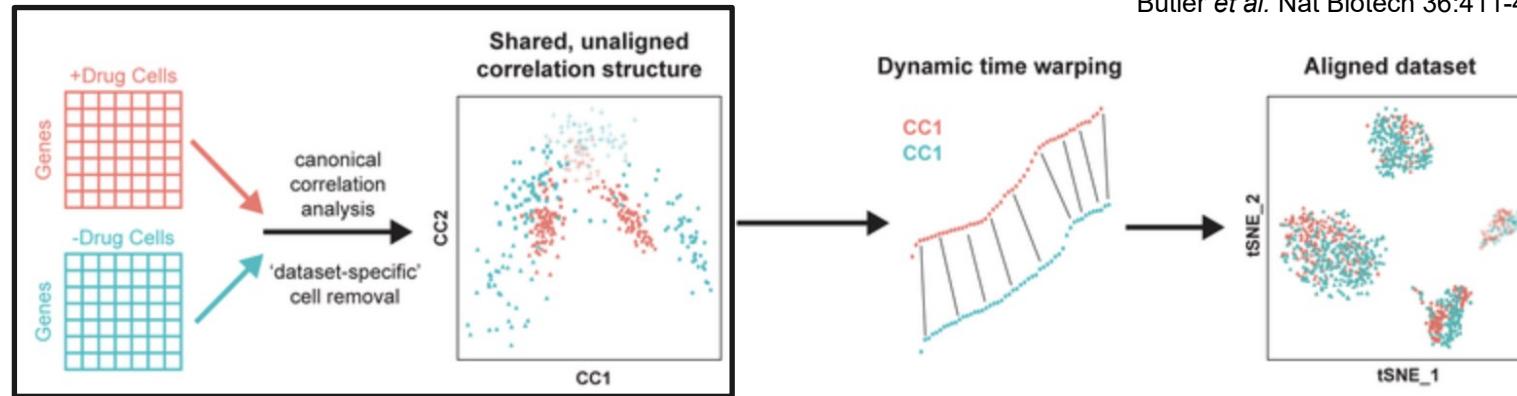
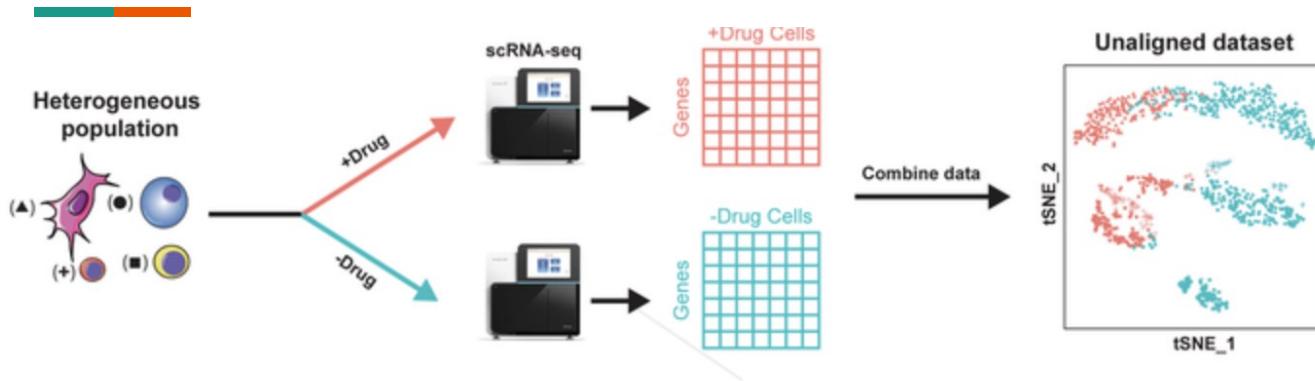
$$\log \mu_{gj}^* = \log \hat{\mu}_{gij} - \hat{\gamma}_{gi}$$
$$\phi_g^* = \frac{1}{N_{batch}} \sum_i \hat{\phi}_{gi}$$

Mutual nearest neighbor (MNN)



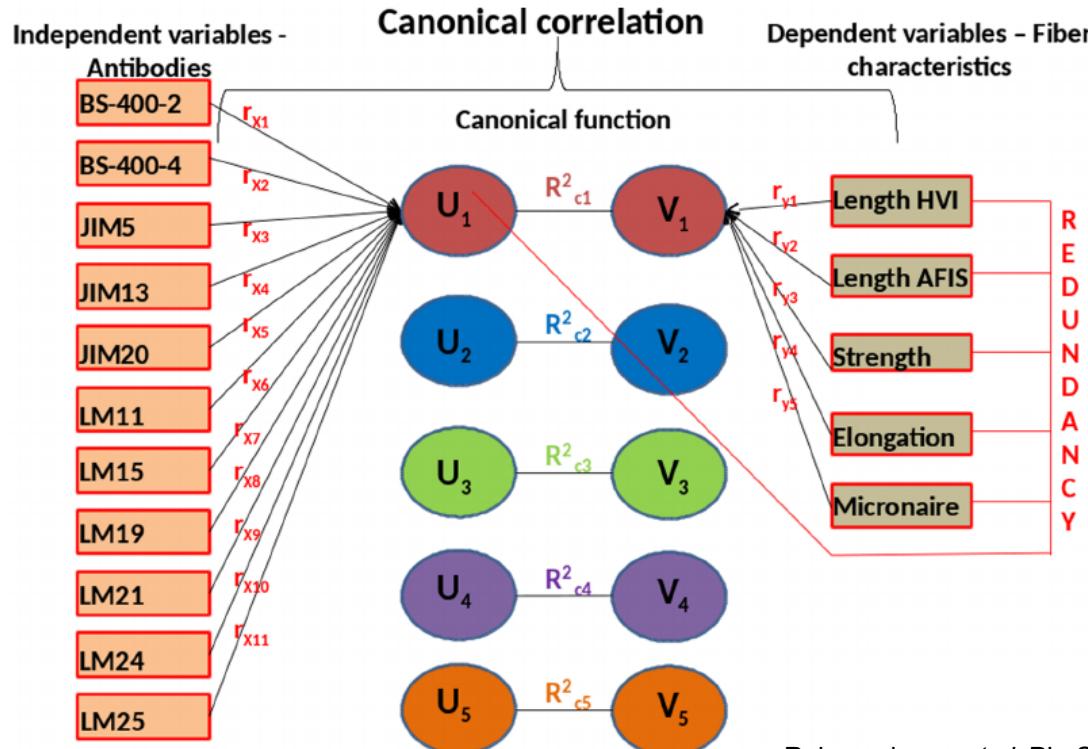
- Map clusters of cells together rather than individual cells
- Similar to reciprocal best hits from BLAST for identifying orthologs
- Apply the average mapping vector to unique cell types

Integration via canonical correlation



Butler et al. Nat Biotech 36:411-420 (2018)

Canonical correlation analysis (CCA)



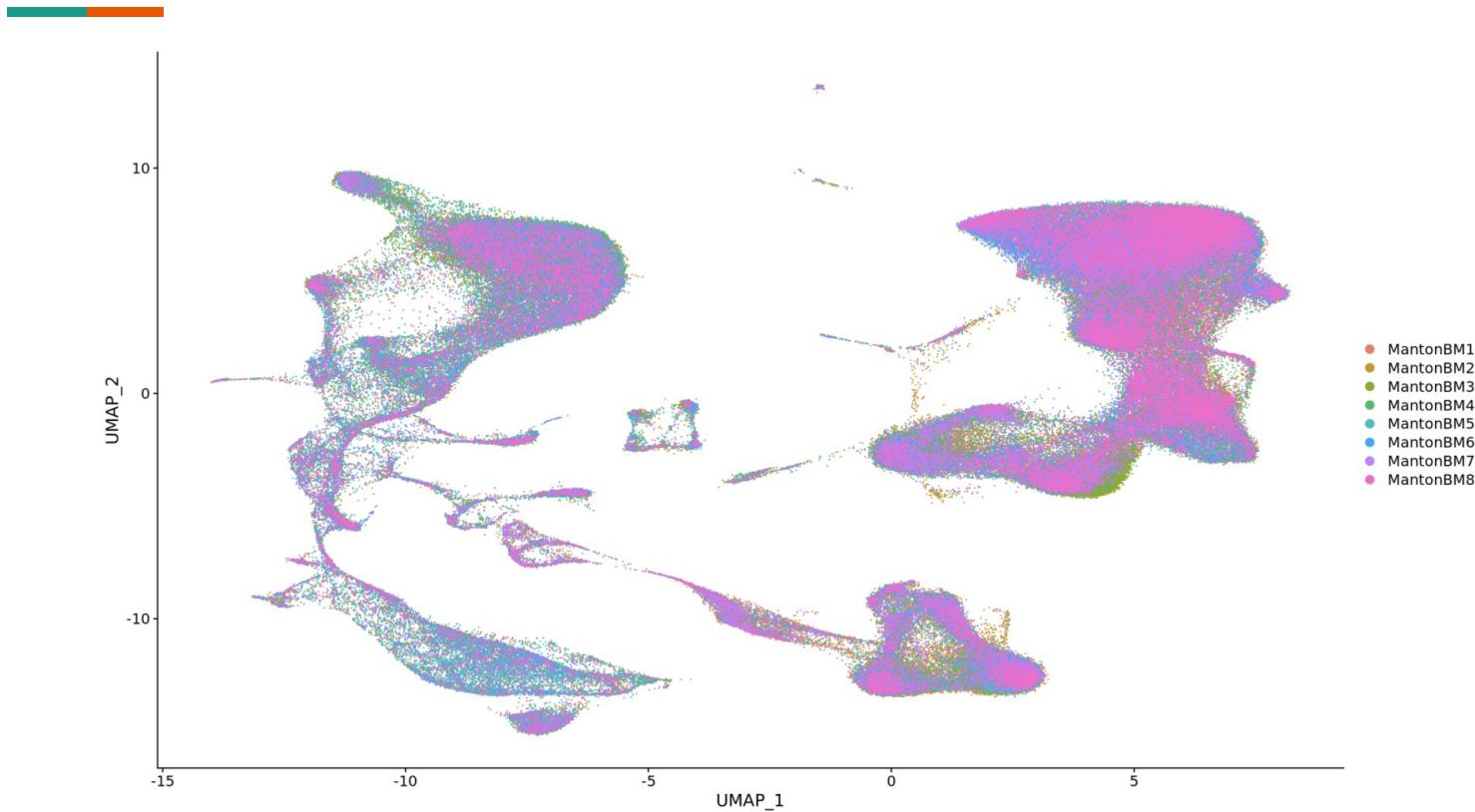
- Same samples with two different systems of observations

- Identify correlation structure observation systems (features)

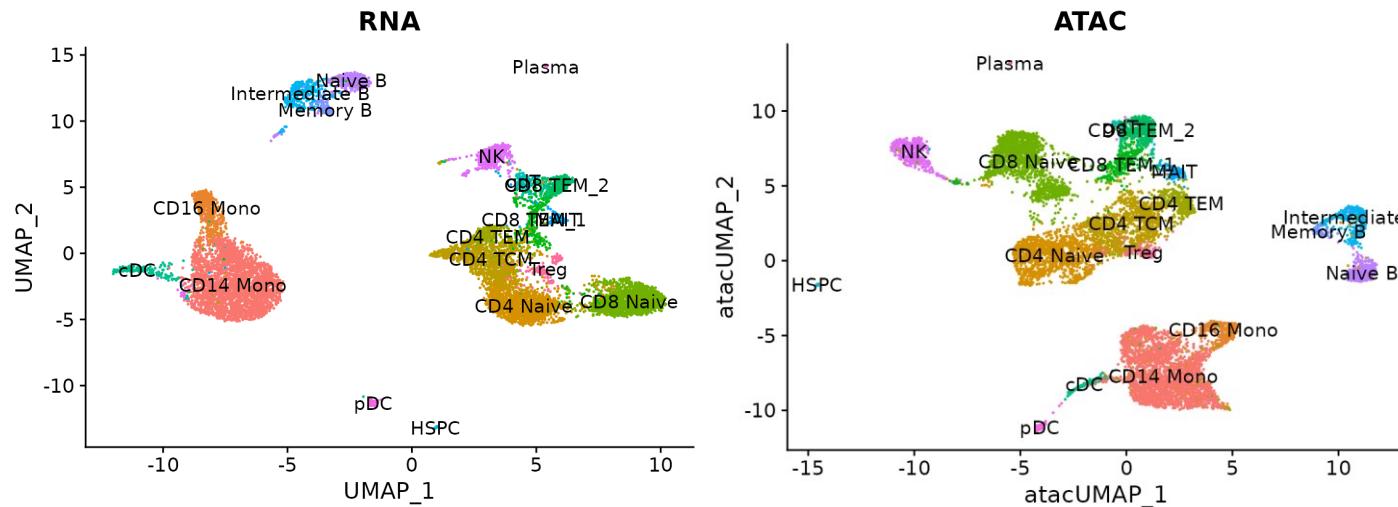
CCA for single-cell data

- Two single-cell experiments contain **different cells** (samples) but the **same genes** (features)
- CCA for single-cell data treats cells as feature describing the genes
 - Identify correlation structure between cells
 - **Expectation:** gene expressions of the same cell type will be most correlated
- Follow up by MNN-styled technique

Good data integration



Integrating RNA-seq with ATAC-seq

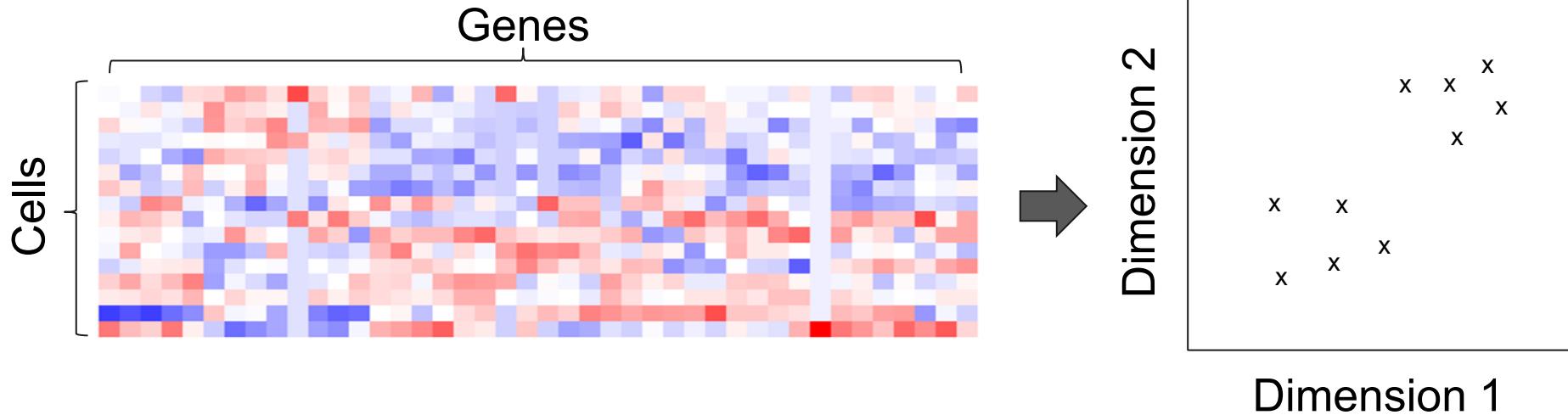


- ATAC-seq = open chromatin ~ gene expression level
- Transfer cell type label



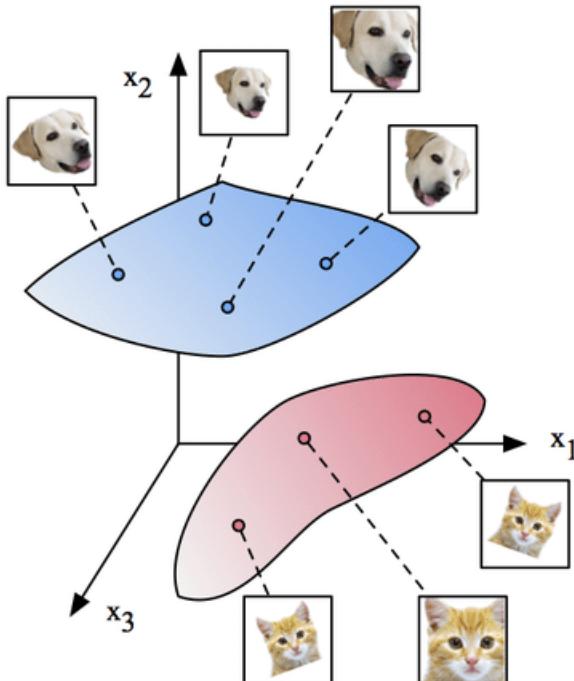
Visualizing single-cell data with dimensionality reduction techniques

Dimensionality reduction

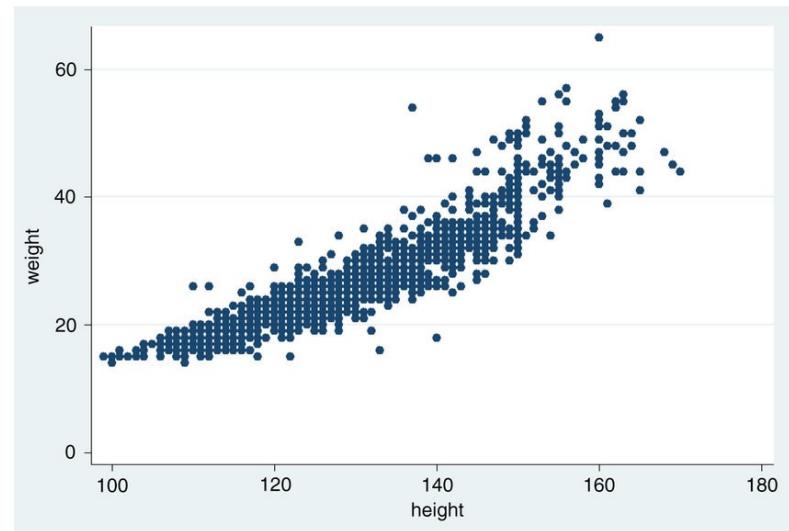


- Collapse high-dimensional data on to 2D or 3D scatter plot that **preserve some information in original dimension**

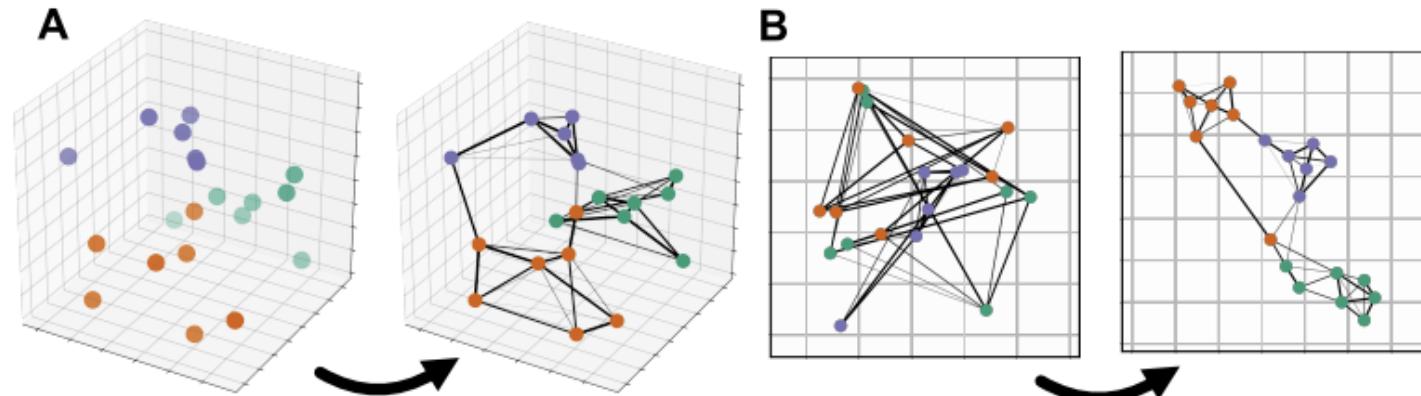
Manifold hypothesis



“Real-world, high-dimensional data lie on some low-dimensional manifolds”



Dimensionality reduction algorithm sketch



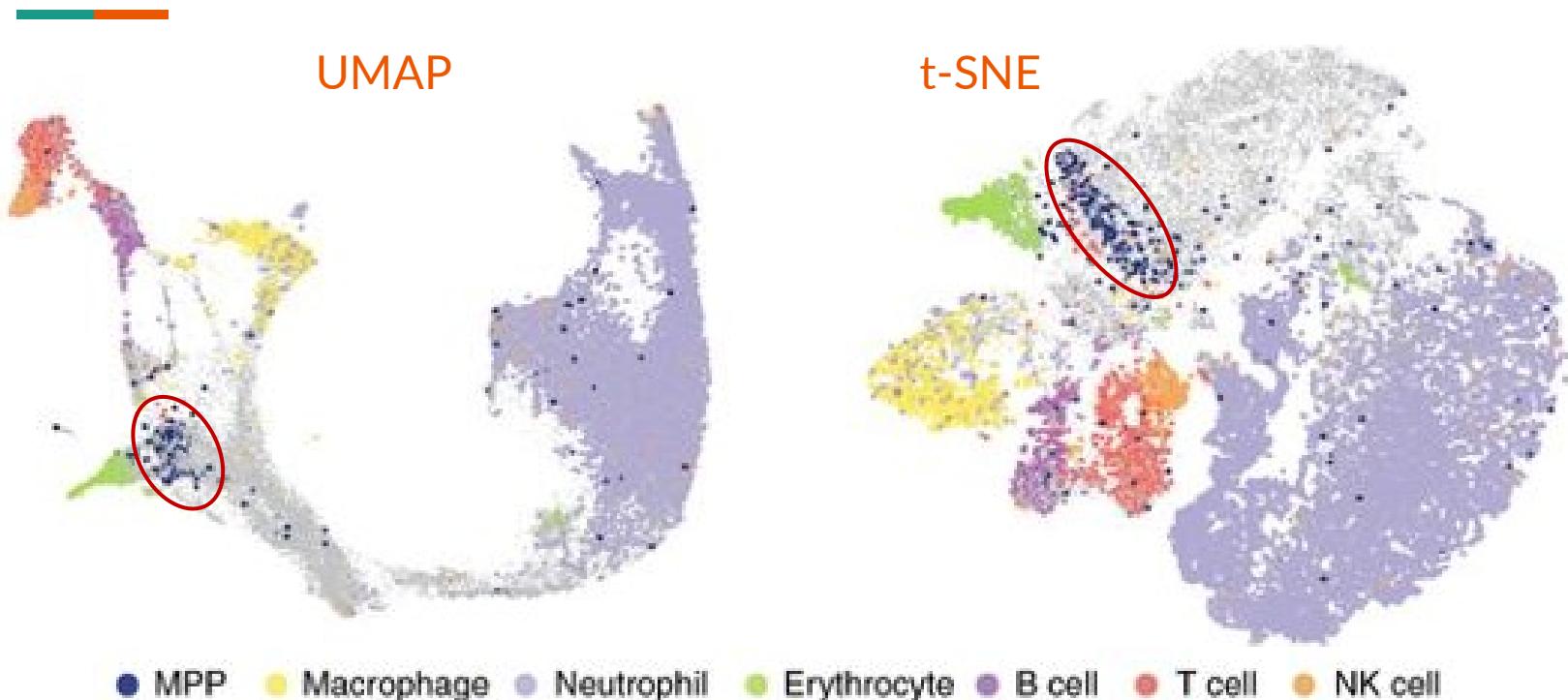
Step 1: Compute a graphical representation of the dataset

Step 2 (non-parametric): Learn an embedding that preserves the structure of the graph

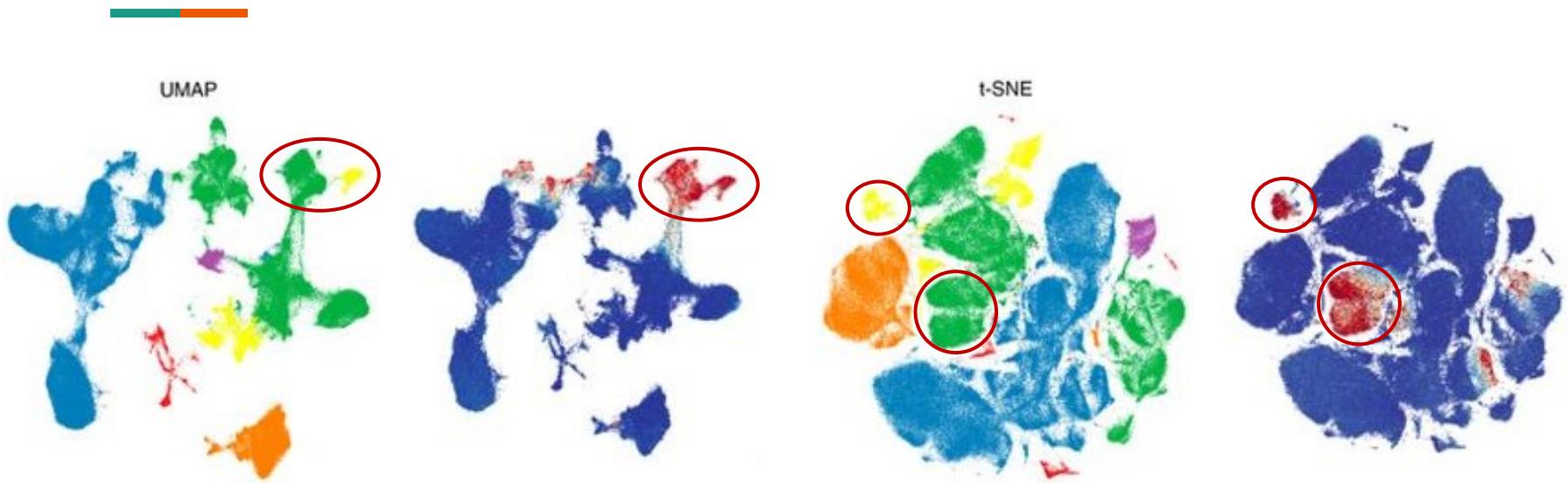
Sainburg, T. et al., Neural Comput 33(11):2881-2907 (2021)

- Similarity = correlation in gene expression across cells

t-SNE vs UMAP on single-cell data



t-SNE vs UMAP on single-cell data



Becht, E. et al. Nature Biotechnology 37:38-44 (2019)

- Both are equally good at detecting individual cell types
- But UMAP is better at capturing transitions across cell types



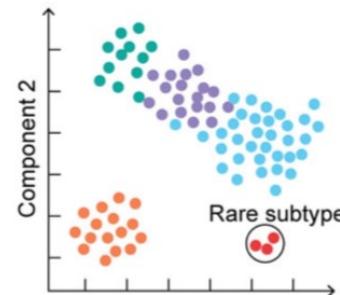
Single-cell analysis overview

Cell clustering and trajectory reconstruction

Heterogeneous tissue or tumor



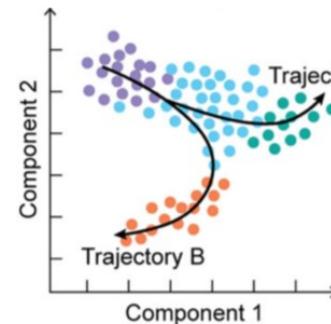
Dimensionality reduction
(e.g. PCA)



Hwang et al. Exp & Mol Med 2018

Clustering of cells with similar omics signatures reveal groups of different cell types and developmental stages

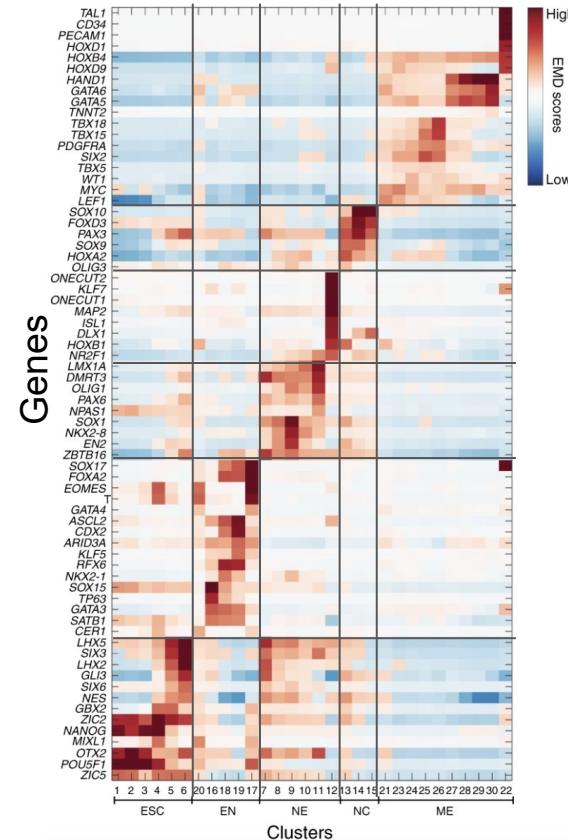
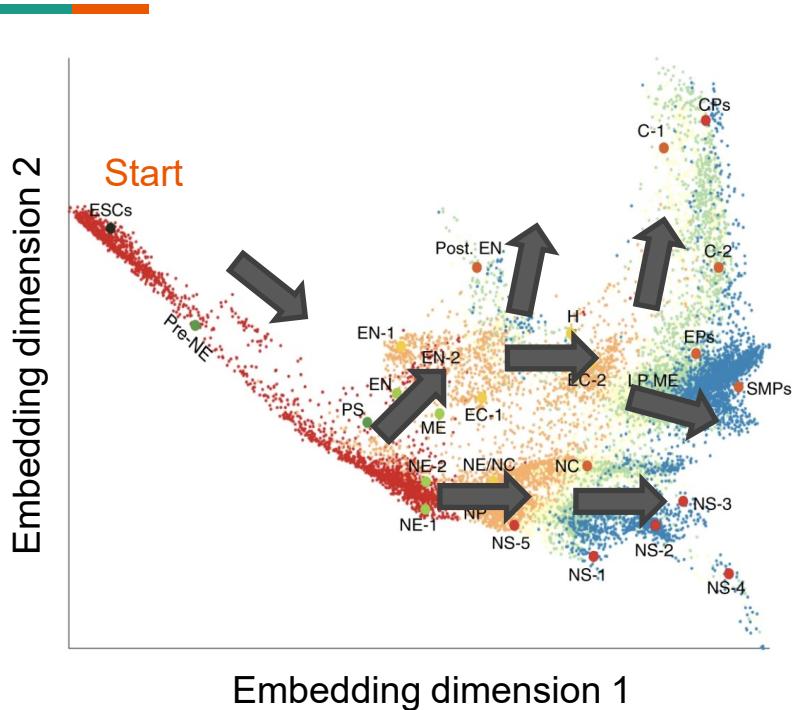
Trajectory modeling with random walk, diffusion, or Markov chain reconstruct the paths of cell development



Response of naive immune cells to infection

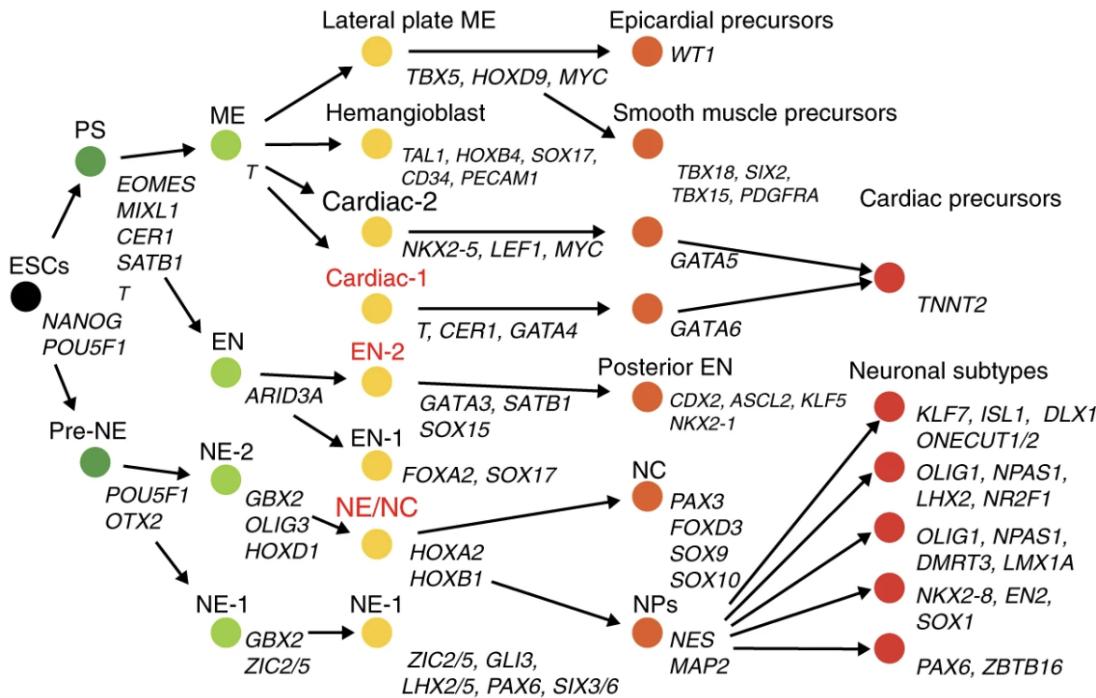


An end-to-end example



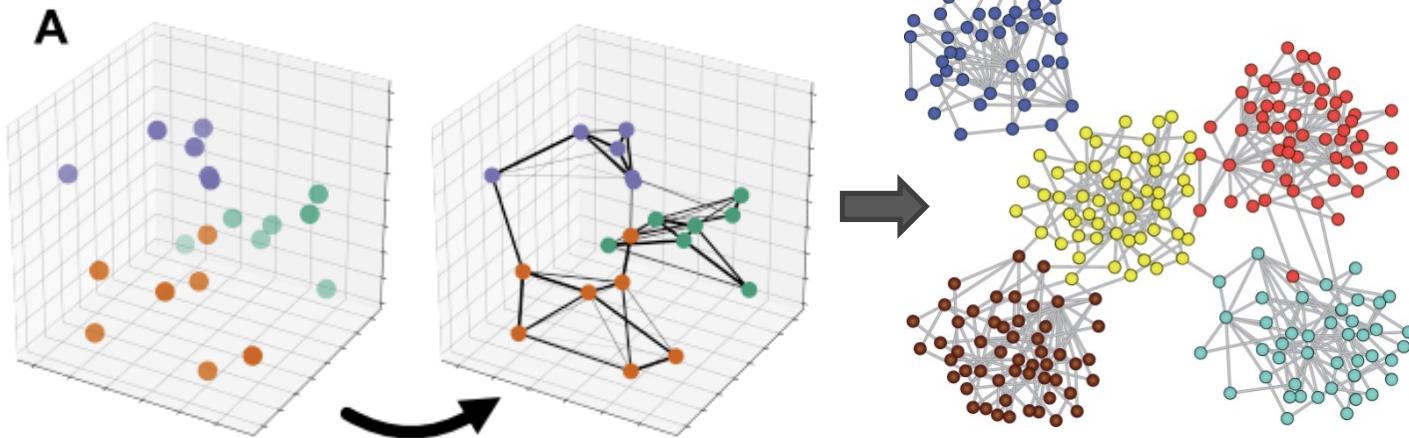
Source: Moon et al. Nature Biotechnology 37:1482-92 (2019)

An end-to-end example



Source: Moon et al. Nature Biotechnology 37:1482-92 (2019)

Algorithm sketch for cell type clustering



Sainburg, T. et al., Neural Comput 33(11):2881-2907 (2021)

<https://github.com/topics/graph-clustering>

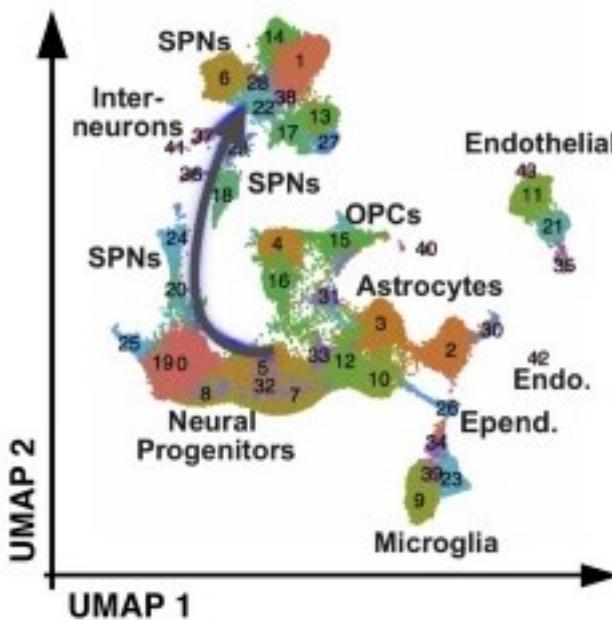
- Connect cells with similar gene expression profile
- Split network into **modules with dense edges**



Cell developmental trajectory

Cells can be arranged along developmental path

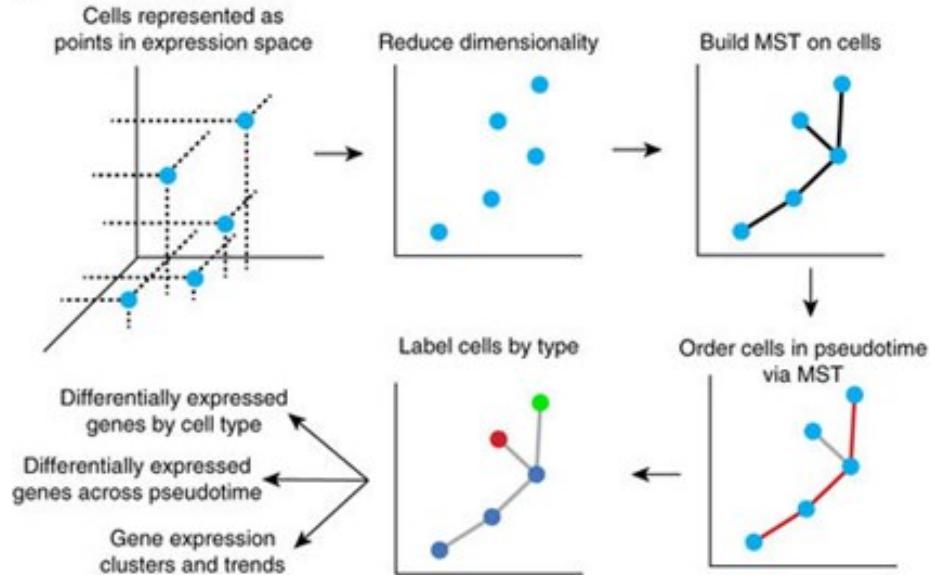
(b) UMAP Embeddings



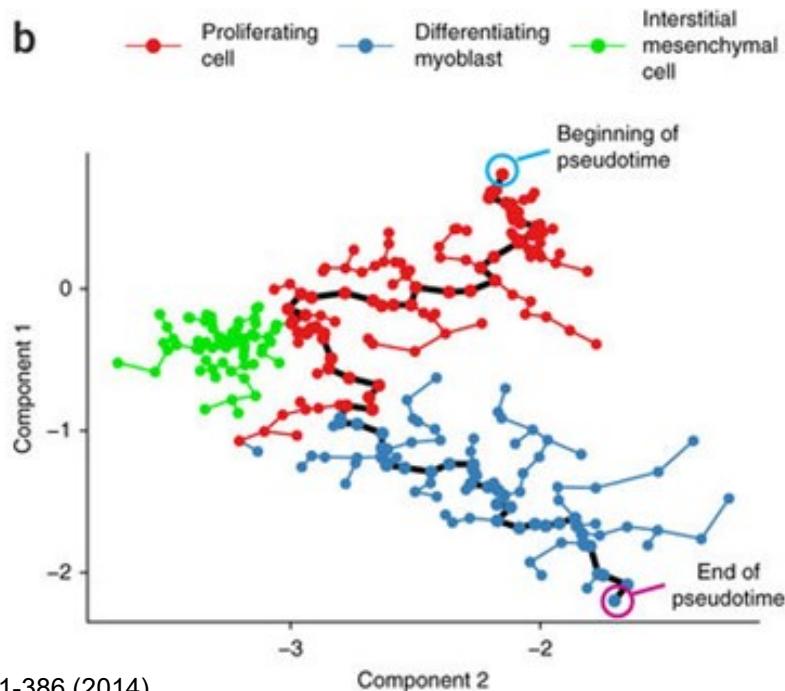
- Cells are in **continuous developmental states**
- Similar gene expression implies similar state
- Reconstruct **pseudotime**
- Identify important genes for development
 - Expression change along the trajectory
 - Expression switch at a particular time point

Minimum spanning tree

a



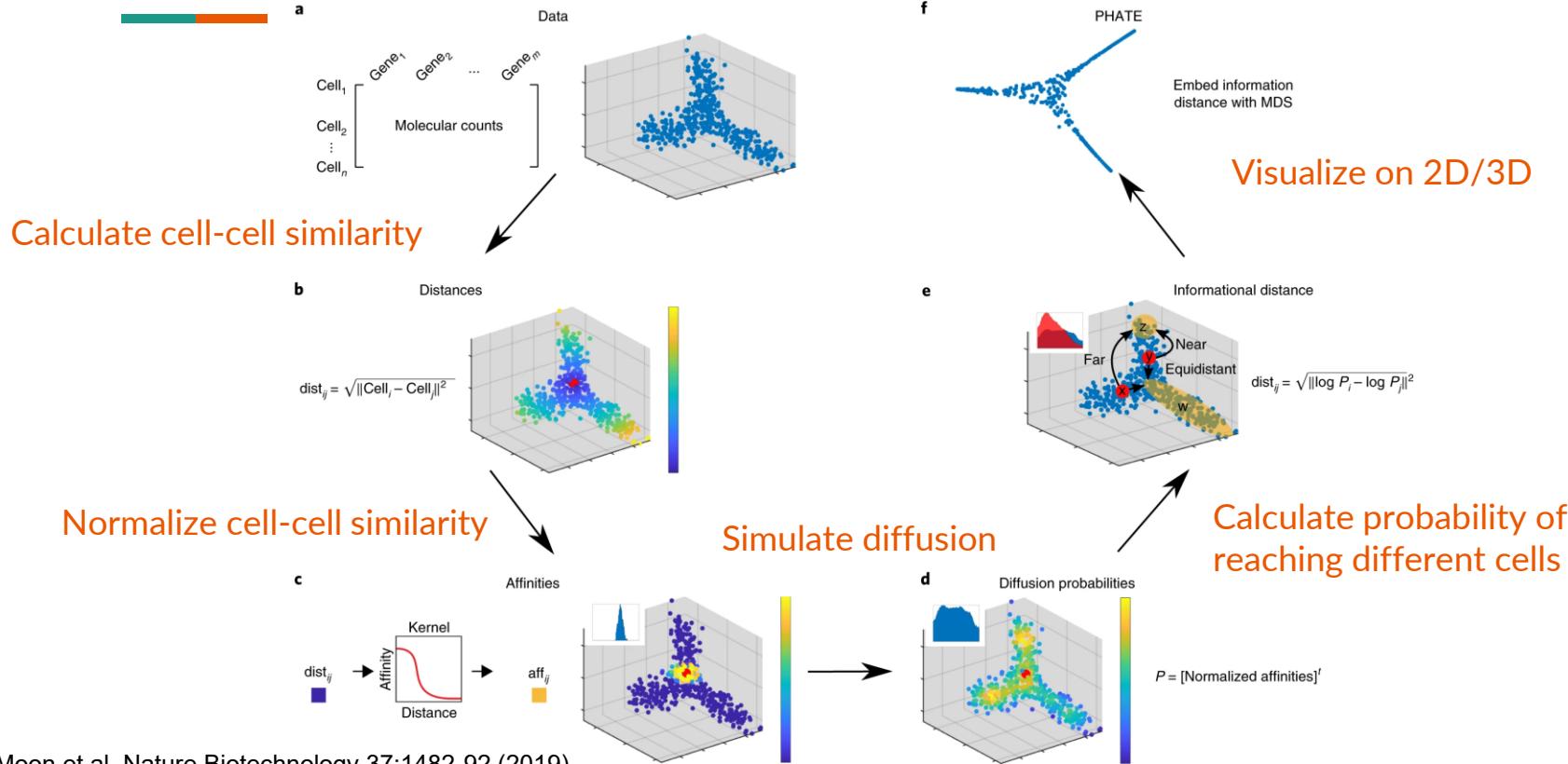
b



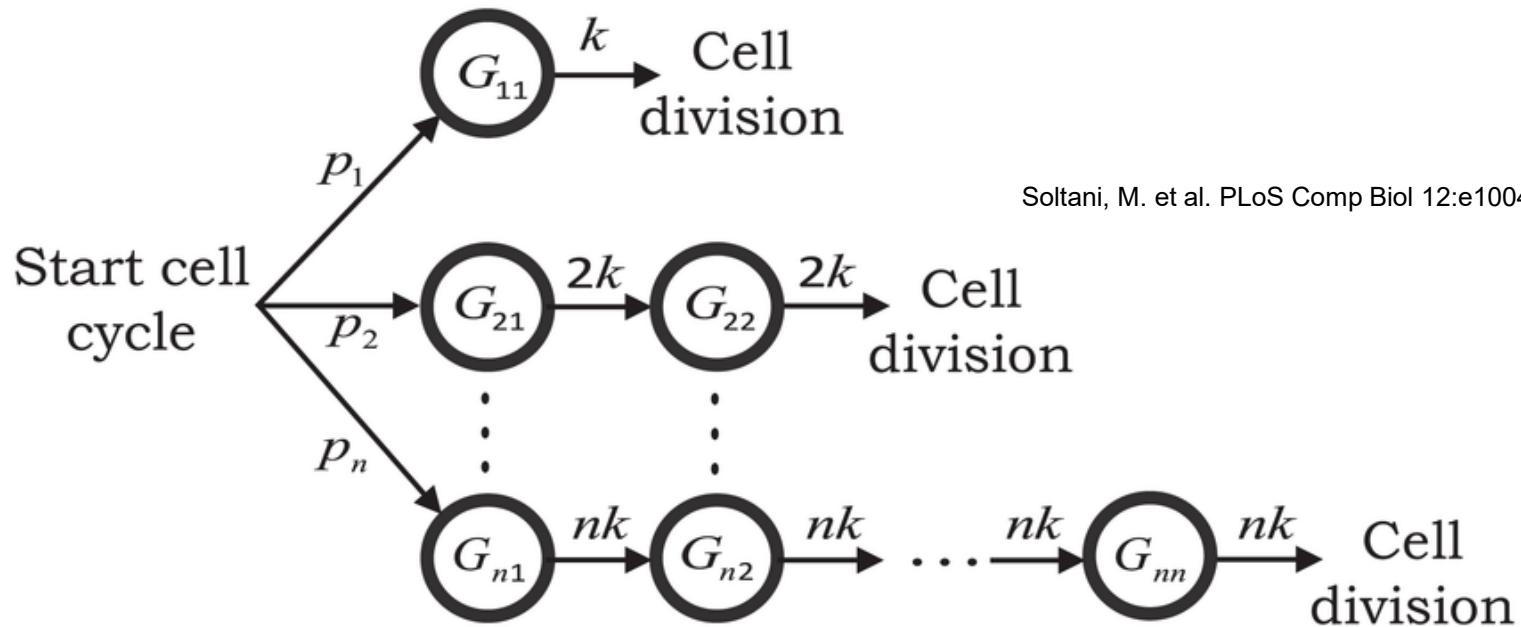
Trapnell et al. Nat Biotech 32:381-386 (2014)

- Trajectory along the most similar cells

Diffusion approach for cell-cell transitions



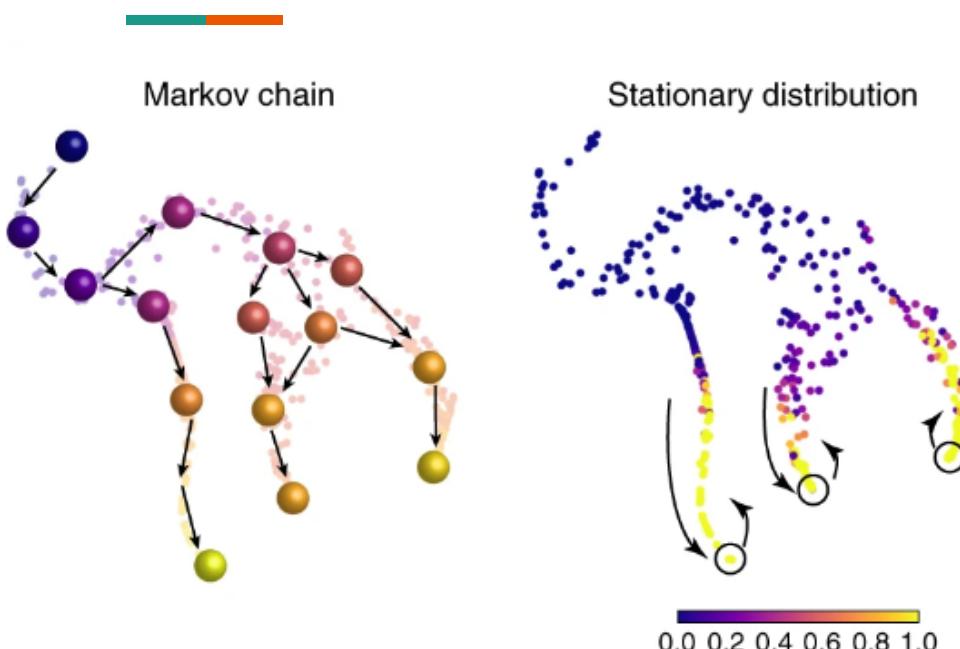
Markov chain model for cell development



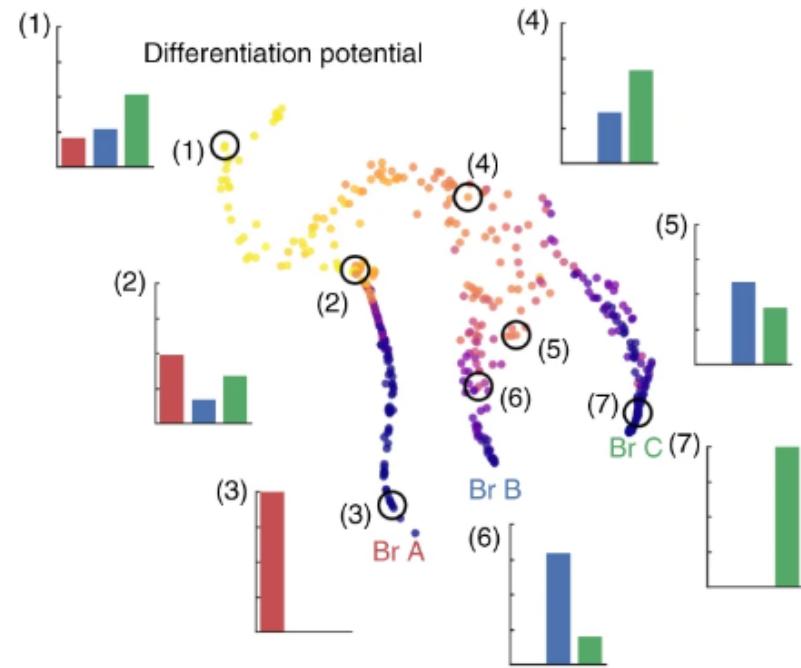
Soltani, M. et al. PLoS Comp Biol 12:e1004972

- Cell state + state transition probability + dependency on previous state

Estimating differentiation potential

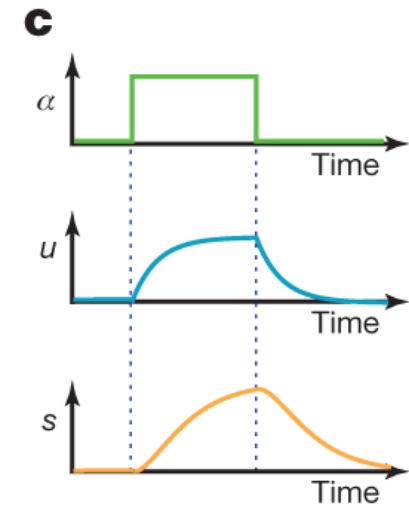
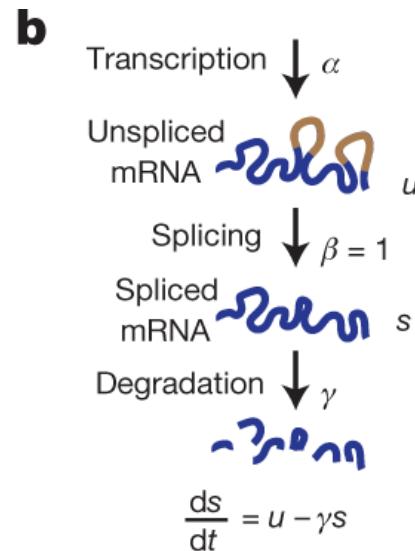
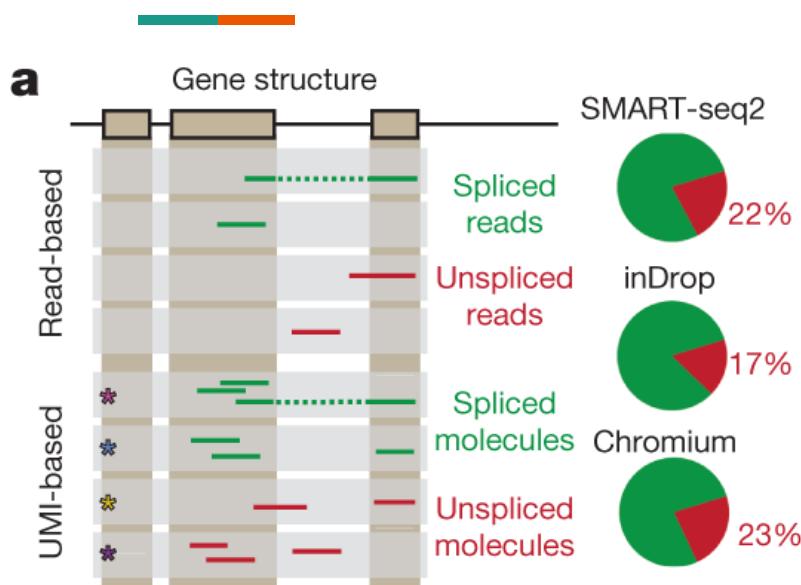


Setty et al. Nat Biotech 37:451-460 (2019)



- Differentiation potential = probability of reaching multiple final cell types

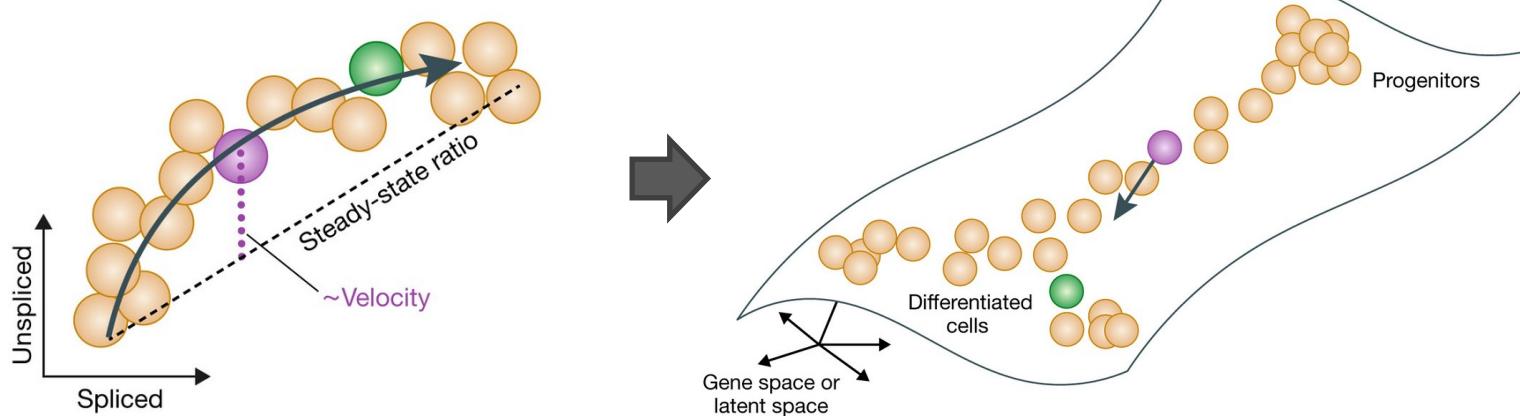
Dynamics of unspliced transcript



La Manno et al. Nature 2018

- When a gene is activated, level of unspliced transcripts rises first
- When a gene is repressed, level of unspliced transcripts drops first

RNA velocity model

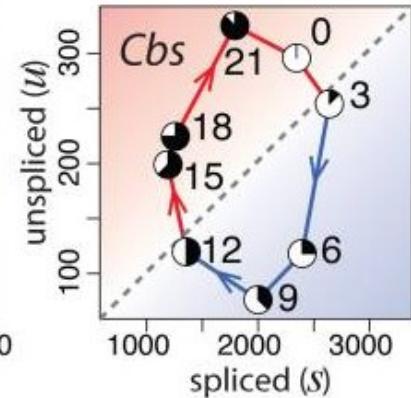
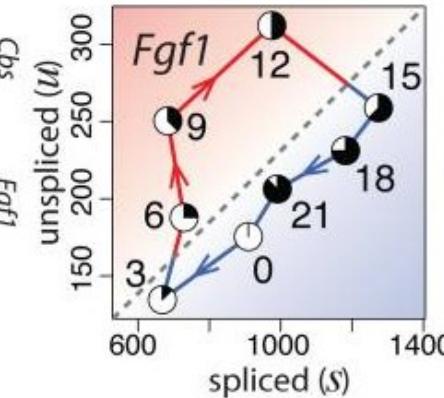
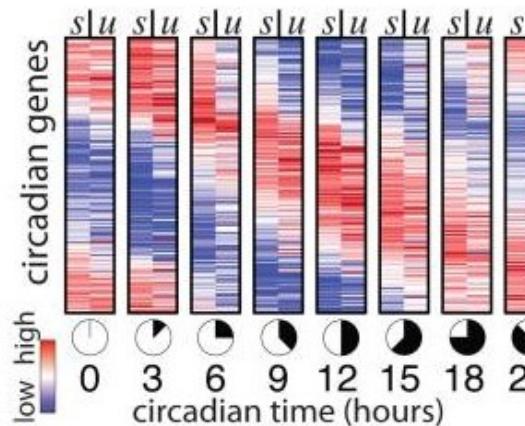
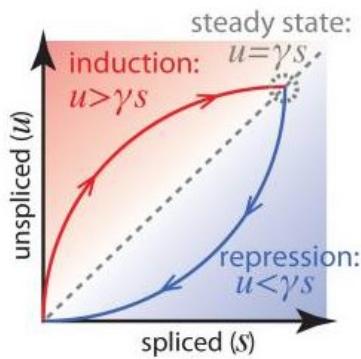


Bergen, V. et al. Mol Sys Biol 17:e10282 (2021)

- Ratio of spliced and unspliced isoforms tells gene activation state
- Compare to nearby cells to identify direction of activation or repression

Proof of RNA velocity

Expected pattern



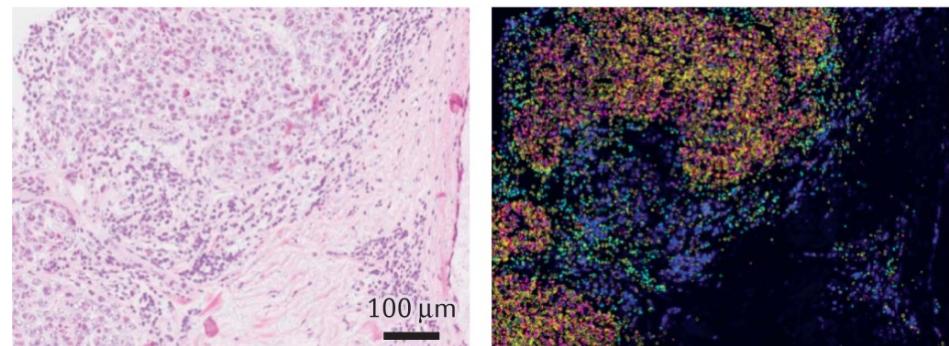
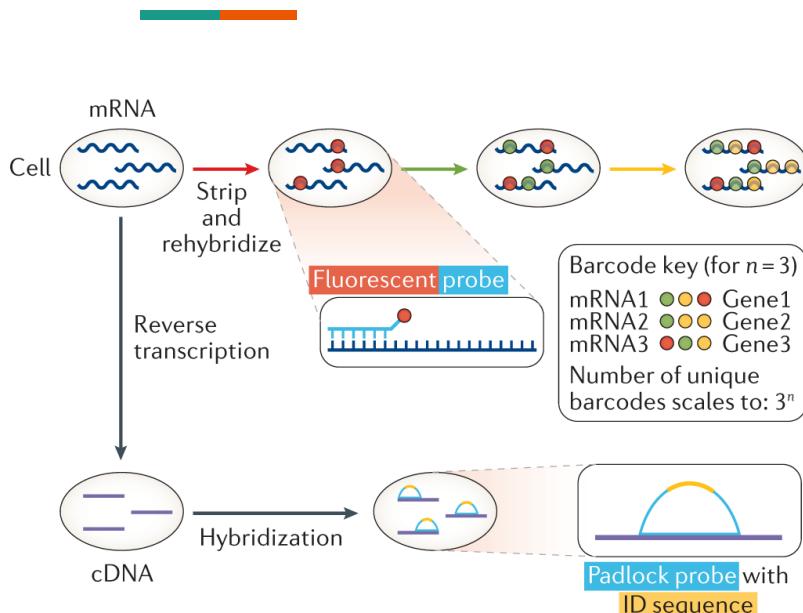
La Manno et al. Nature 2018

- Circadian genes are genes whose expression cycle with the time of day



Spatial transcriptomics

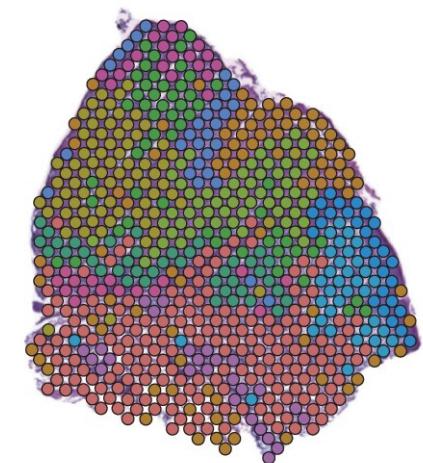
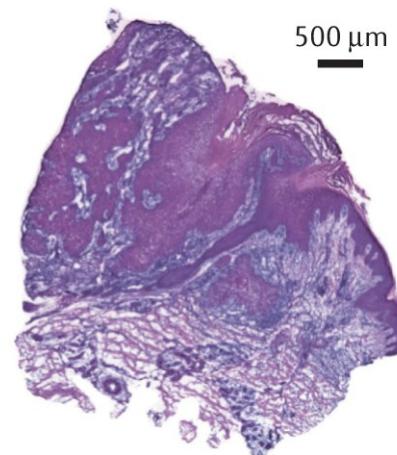
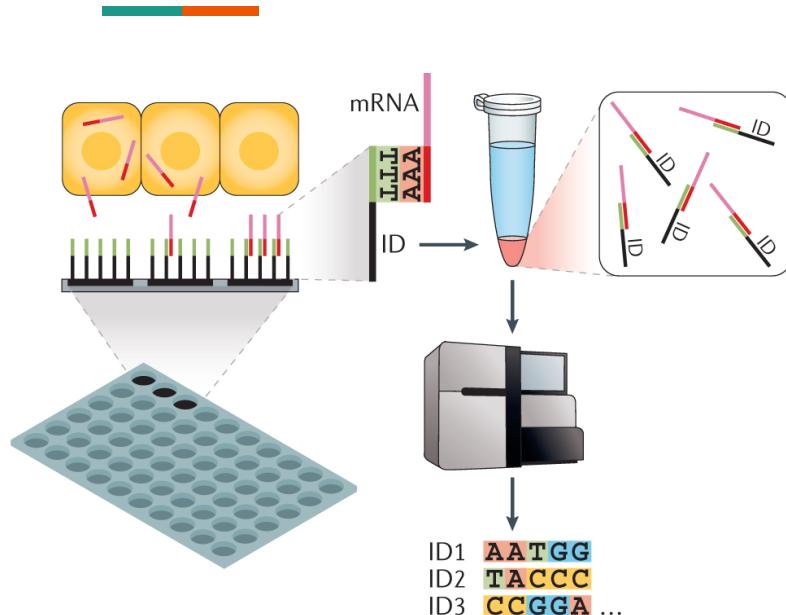
Extended NanoString



Longo et al. Nature Reviews Genetics 22:627-644 (2021)

- In-situ fluorescence labeling of selected RNA transcripts

Spatial barcoding



Longo et al. Nature Reviews Genetics 22:627-644 (2021)

- Spatial cell isolation + barcoding

Single-nucleus sequencing

- Isolate nuclei instead of whole-cells
 - Only capture RNA expression in nucleus
- Good for cells that are difficult to isolate: adipocyte, neuron, etc.
 - Also works well with preserved tissues

Summary

- Benefits of single-cell technology
- Difference between single-cell and bulk transcriptomics
- New analysis ideas
 - Visualization of high-dimensional data (preview)
 - Cell type clustering
 - Developmental trajectory reconstruction
- Spatial transcriptomics

Any question?

- See you on October 9