Statistical Methods for High-dimensional Biology

Single-cell RNA-seq Analysis

Yongjin Park, UBC Path&Lab, STAT, BC Cancer

Goal of today's lecture

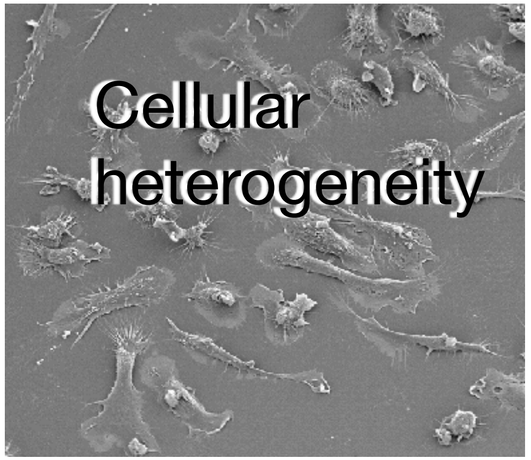
- Grasp the underlying basic ideas of scRNA-seq methods (not math).

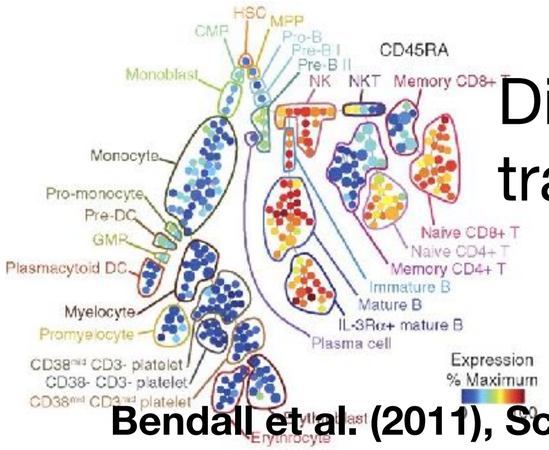
- Next week: multiomics, more advanced techniques and problems

Disclaimer

- We won't discuss a specific toolbox frequently used in a typical single-cell analysis (Seurat, Scanpy, etc.)
- Don't need to know every detail to implement and run your single-cell analysis.
- Large sample size, high-dimensional, so I used torch library (any other ML libraries will do).
- https://github.com/STAT540-UBC/lectures/tree/main/lect14-single_cell

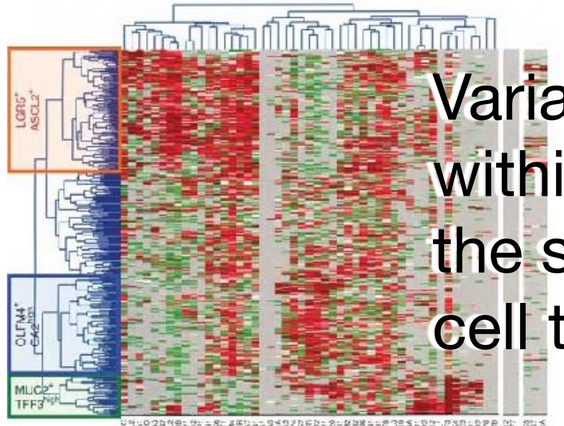
Why do we do single-cell assays?





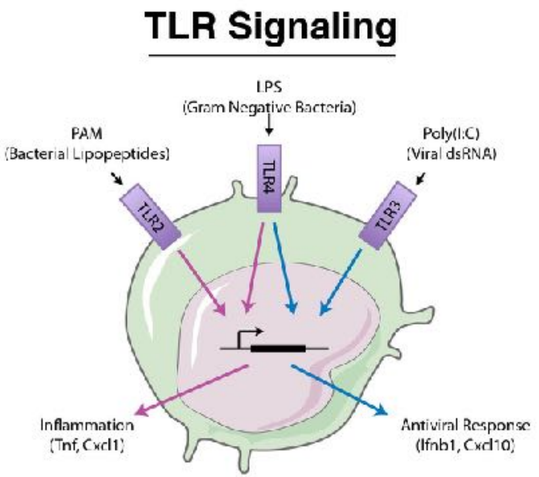
Differentiation trajectory

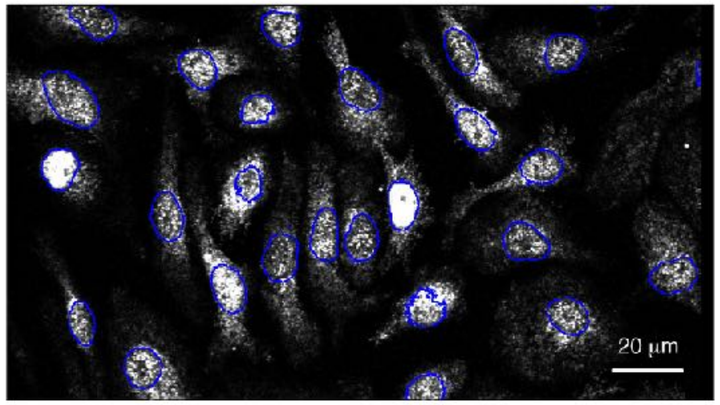
Bendall et al. (2011), Science

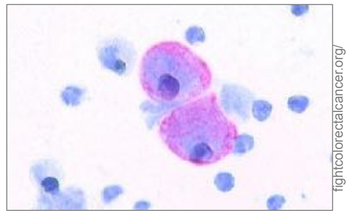


Variation within the same cell types

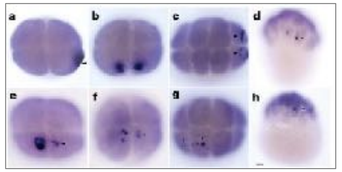
Dalerba et al. (2011), Nature Biotech







Circulating Tumor Cells



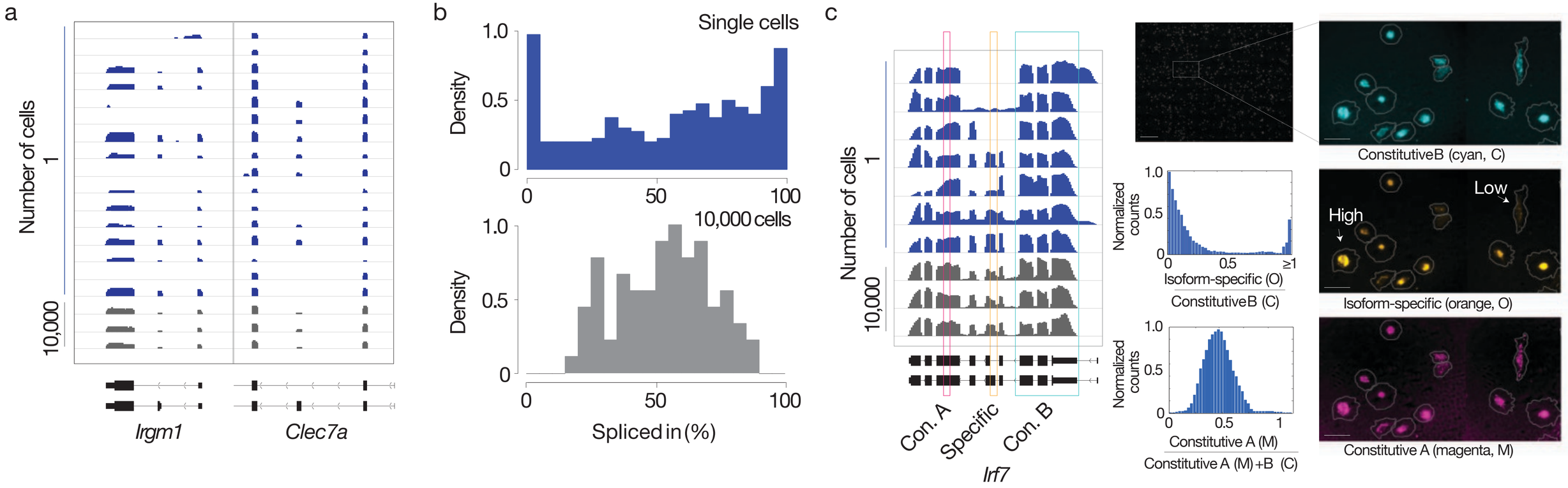
Zebrafish early embryo

Cellular responses can vary substantially between “identical” cells.

Overcome low input

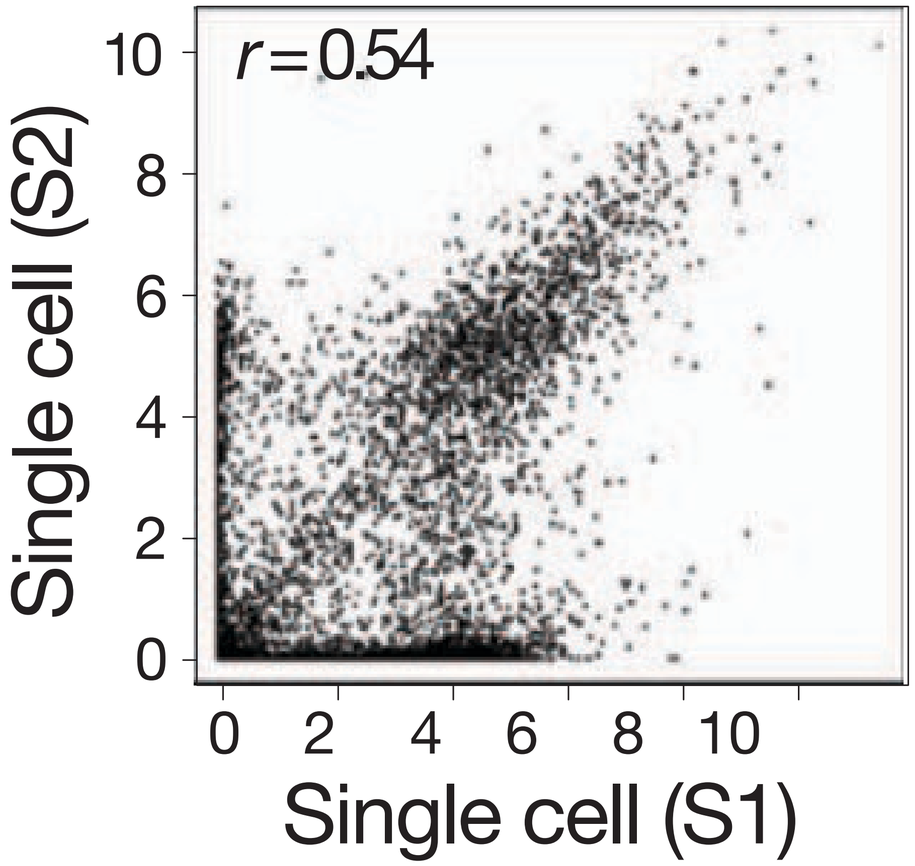
Slides from Manolis Kellis

Single-cell RNA-seq captures isoform variation across cells



Shalek *et al.* (2013)

Single-cell ≠ cell sorted assays



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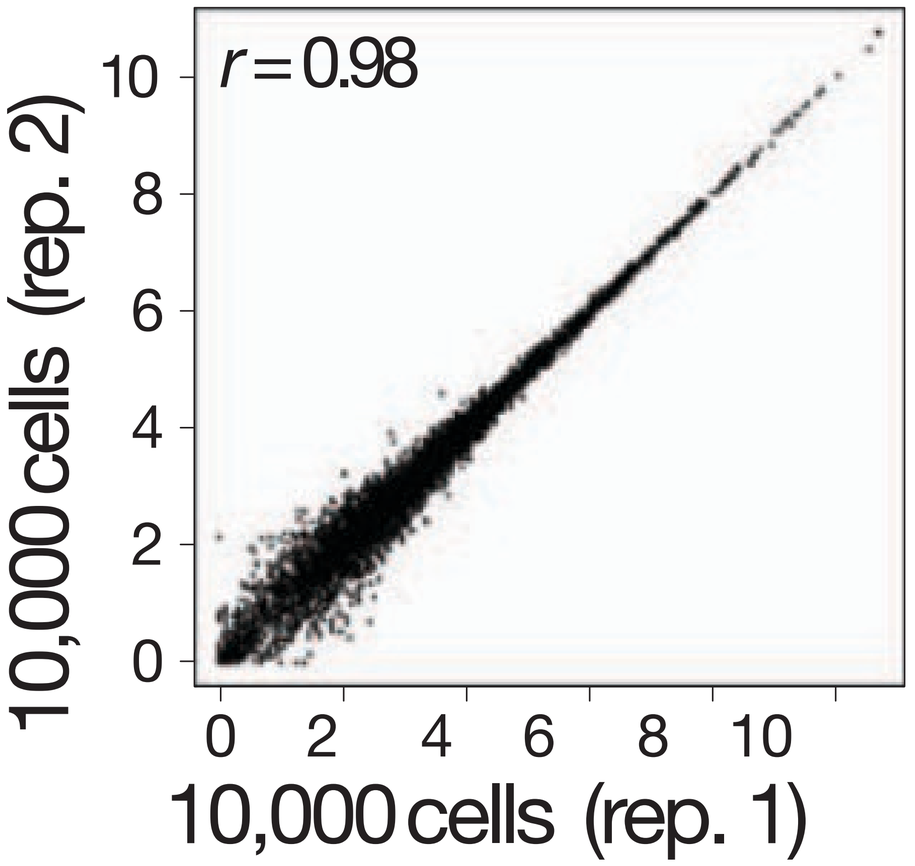
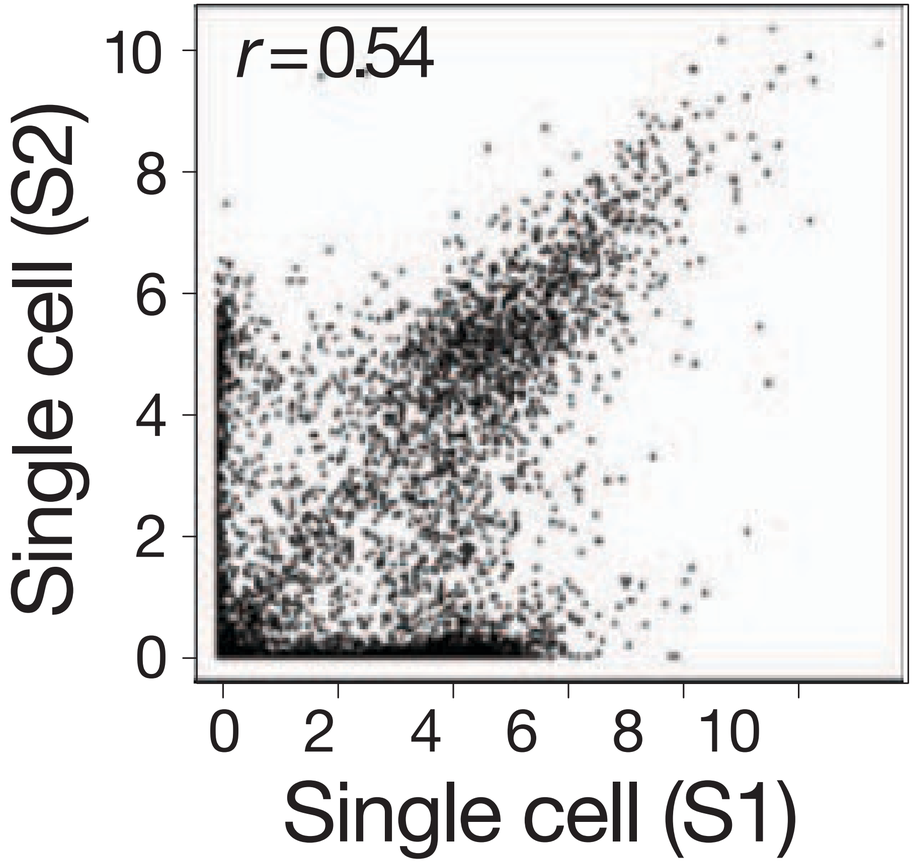


Shalek *et al.* (2013)

Single-cell ≠ cell sorted assays



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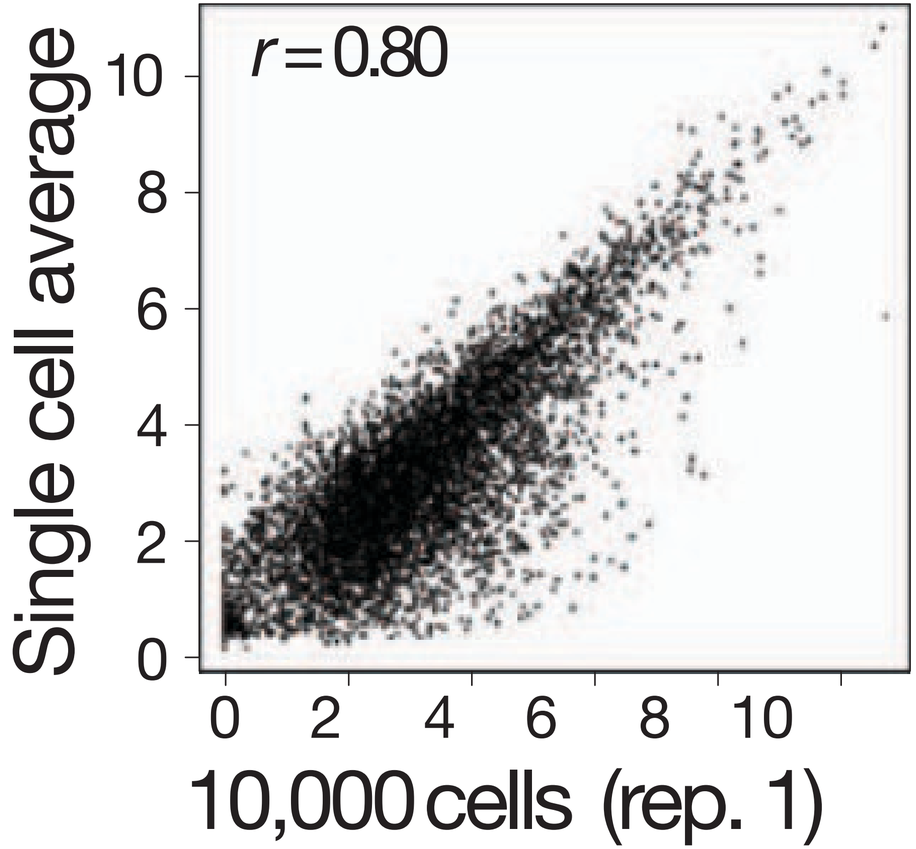
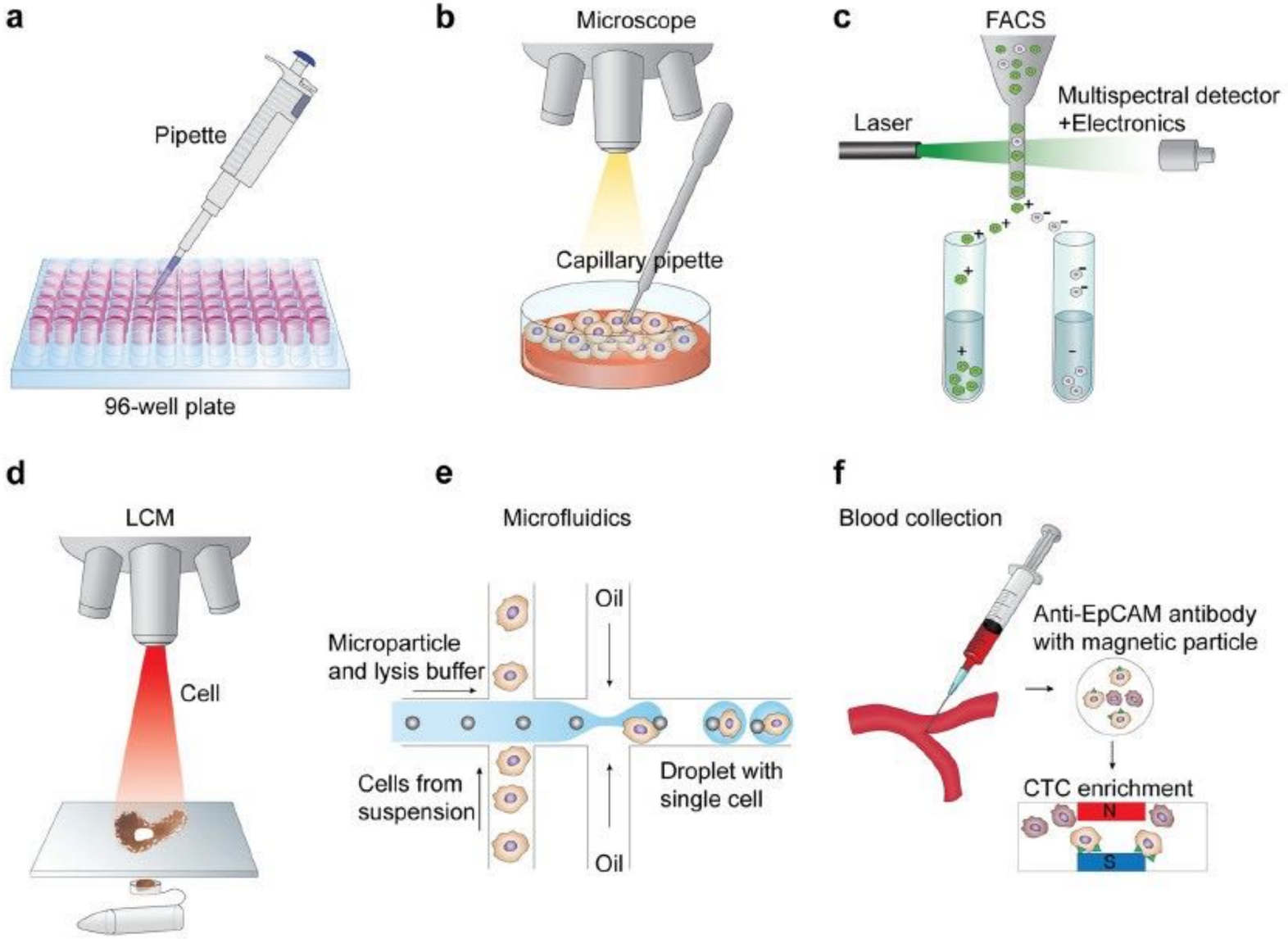


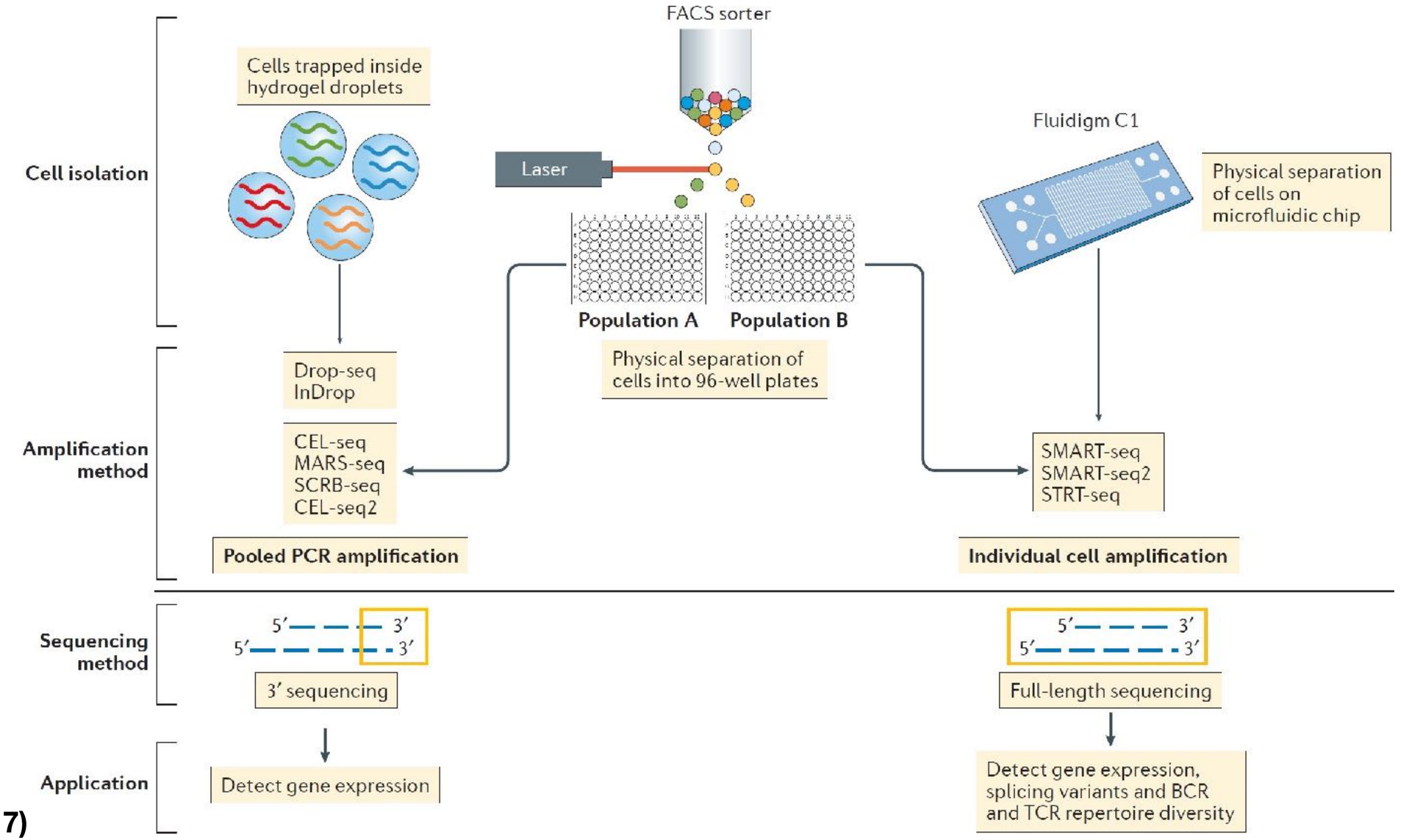
Shalek *et al.* (2013)

Evolution of methods for isolating single cells



Paplexi *et al.* (2017)

Theme: separate cells, amplify RNA, sequence



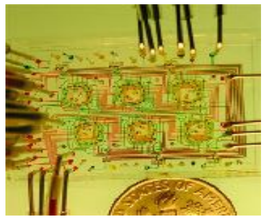
Paplexi *et al.* (2017)

Single-cell Profiling technologies

1. Cells in wells, traps, and valves (nanowell, Flow sorting, Fluidigm C1)

- Screen for and retrieve single cells of interest
- Enrich for rare cells with desired properties
- Control the cellular microenvironment
- Monitor or control cell-cell interactions
- Precise/extensive manipulation of single cells

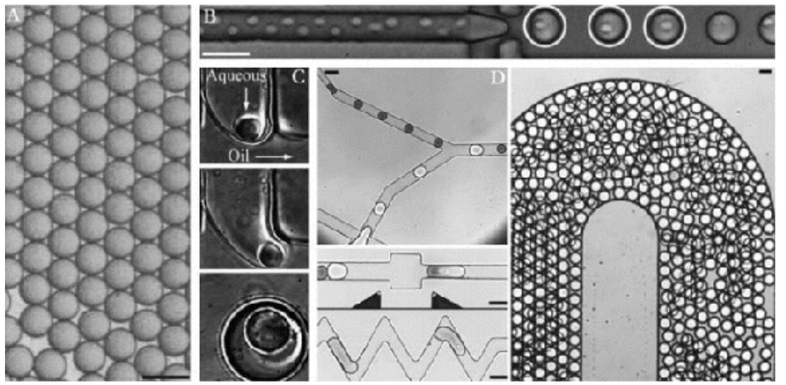
Passive wells



Active pumps and valves

2. Droplets (Drop-seq, ddPCR)

- Introduce distinct “packets” of reagents to single cells
- Perform amplification on individual cells
- Sort large populations of single cells



Slides from Manolis Kellis

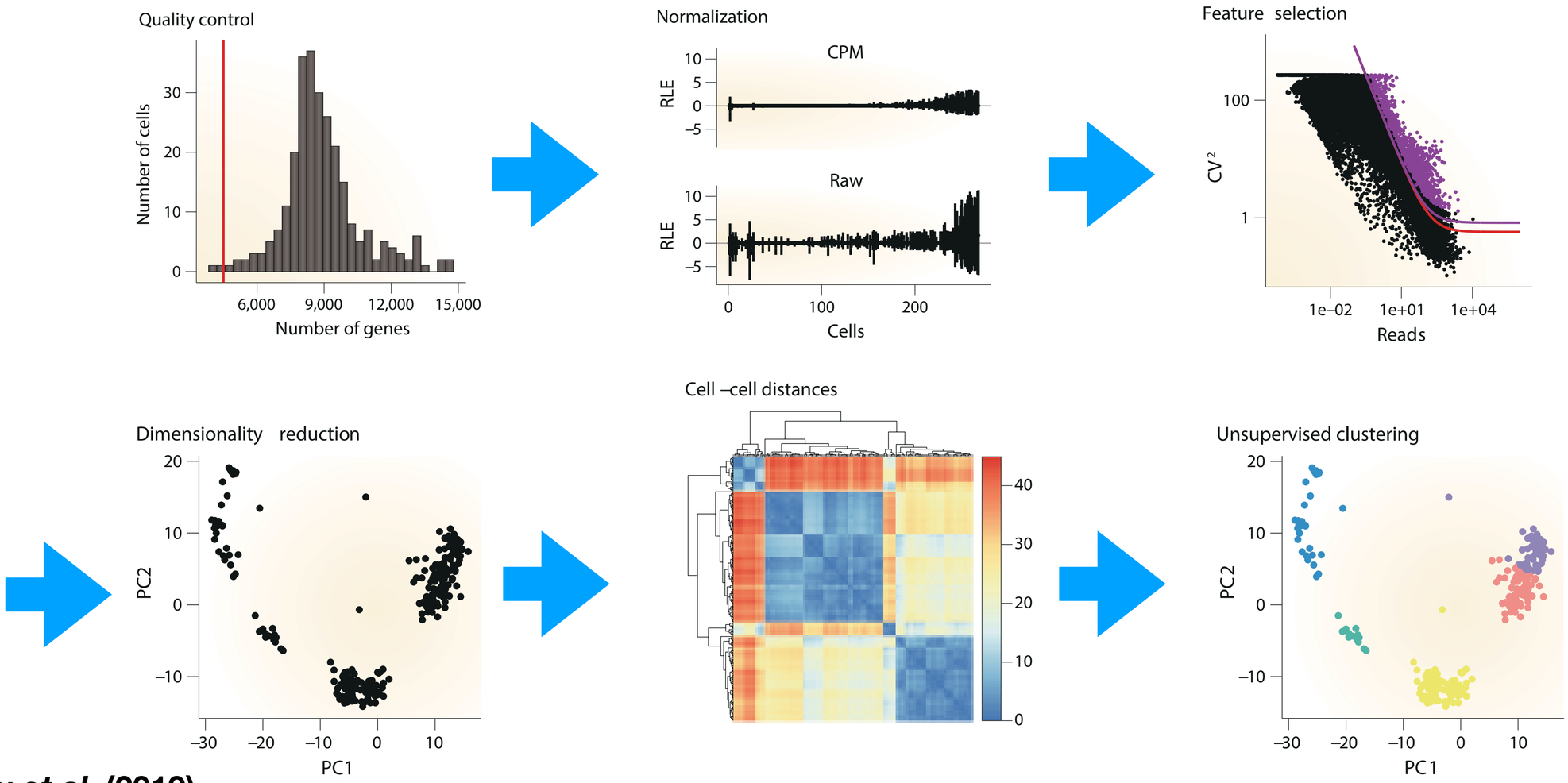
3. Combinatorial indexing (SCI-seq, SPLiT-seq)

- Economic use of reagents for cell separation
- Efficiency of handling larger populations than Drop-seq
- Maintain complexities of population without bias from droplet or well.

“The ability to define cell types through unsupervised clustering on the basis of transcriptome similarity has emerged as one of the most powerful applications of scRNAseq.”

Kiselev *et al*. (2019)

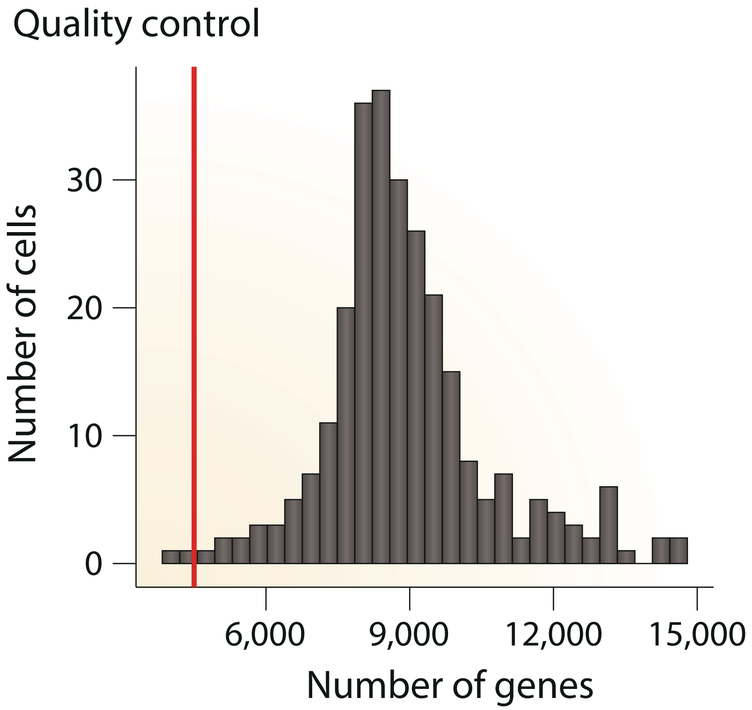
Unsupervised ML is a crucial step in a typical scRNA-seq clustering analysis

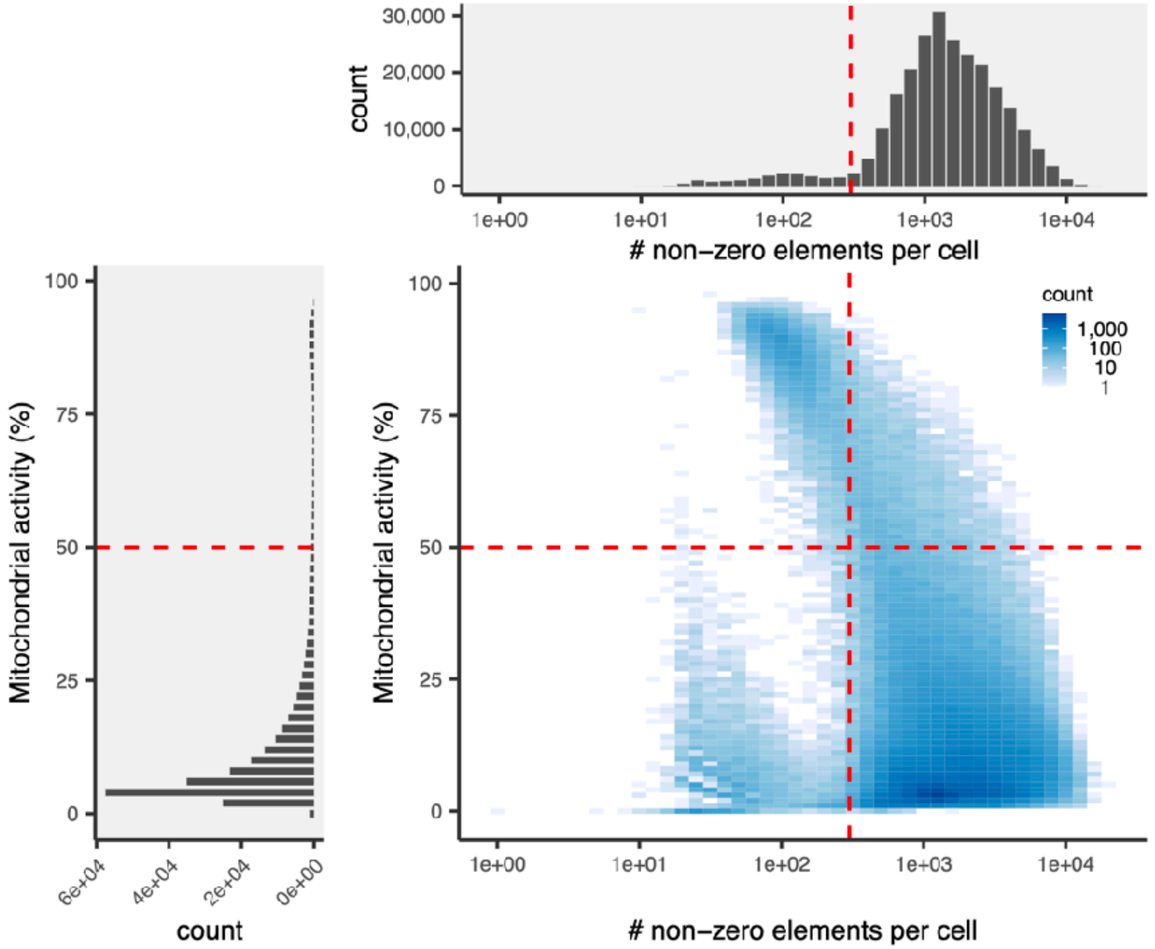


Kiselev *et al*. (2019)

1. Basic Q/C to keep statistically viable cells

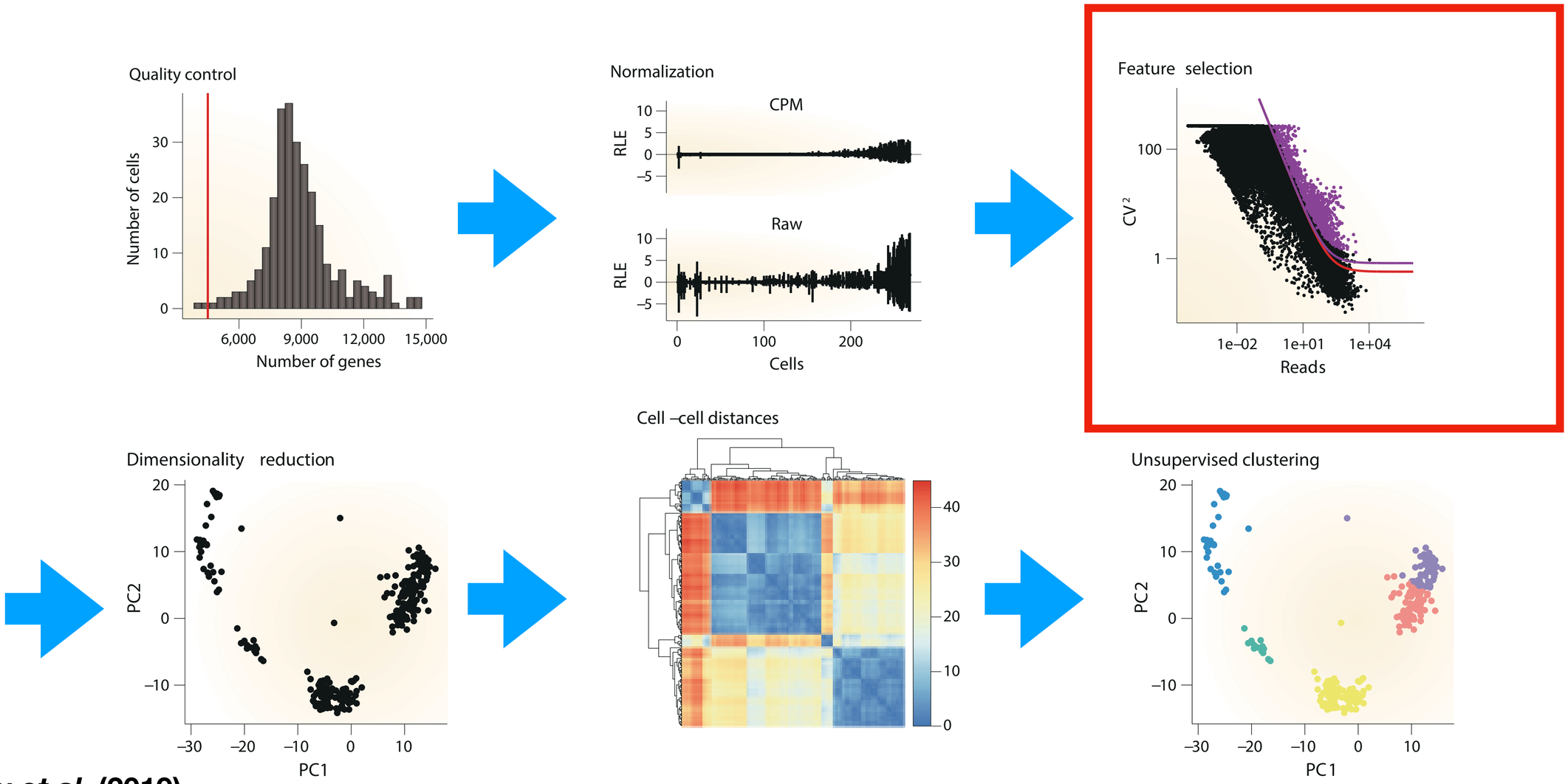
Too few expression ≈ dying/ambient cells
too high mitochondrial% ≈ bursting cells





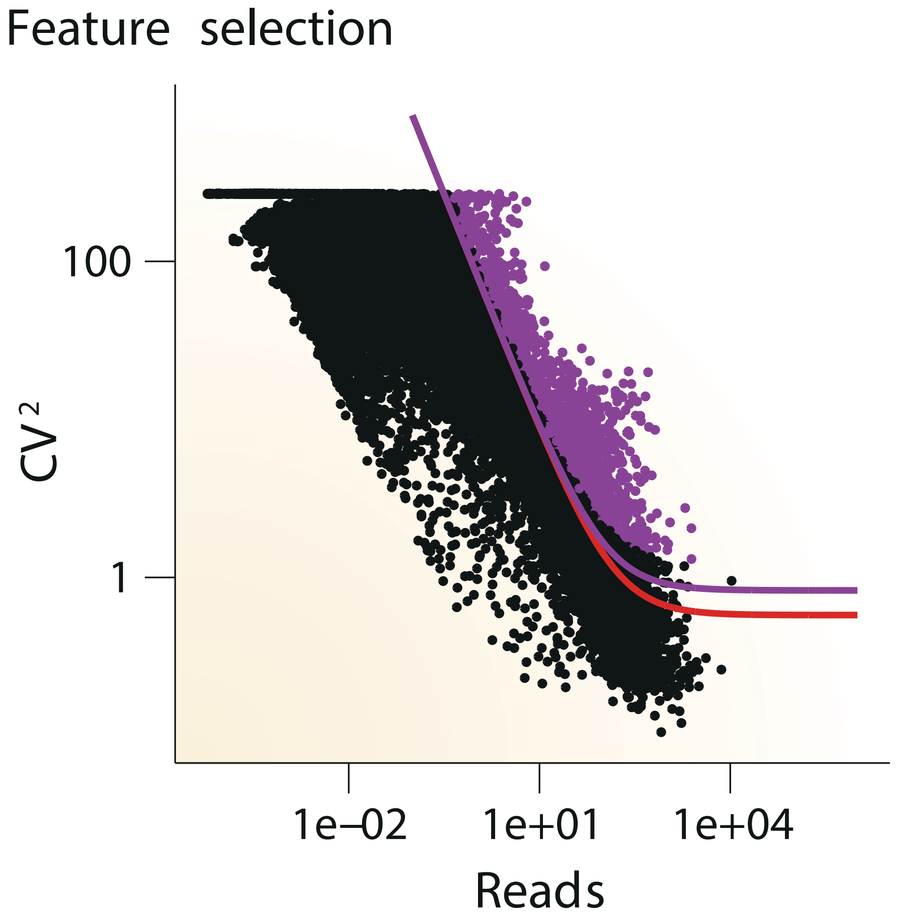
Kiselev *et al*. (2019)

Unsupervised ML is a crucial step in a typical scRNA-seq data analysis



Kiselev *et al*. (2019)

2. Selecting (perhaps) the most informative features/genes to avoid the curse of dimensionality



What is a good feature?

★ High variance across cells

★ High expression to serve as a landmark

★ Coefficient of variation = Standard Dev / Mean

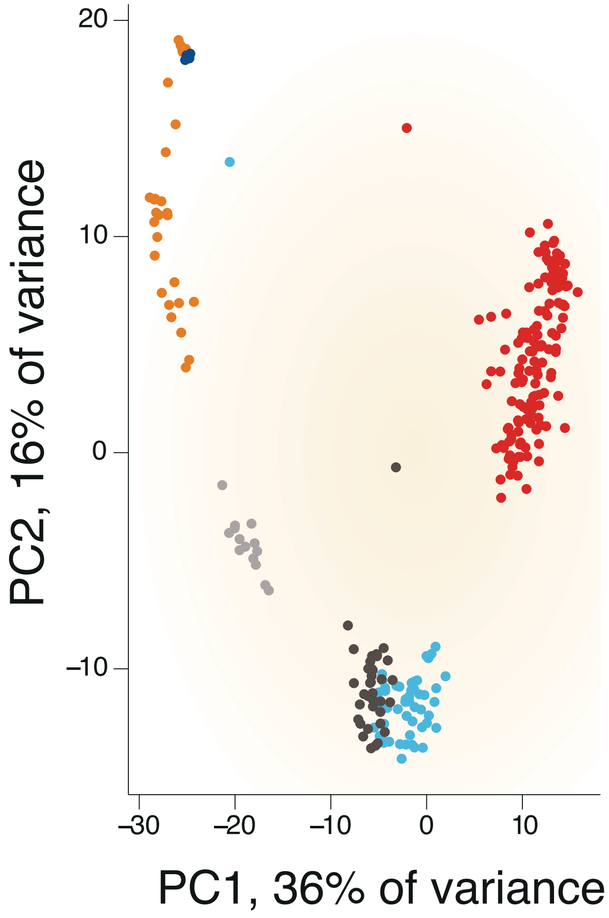
How many should we keep them?

Some grey area: Which one do you think is better?

Maybe it's obvious why we explain higher % of variance with fewer genes

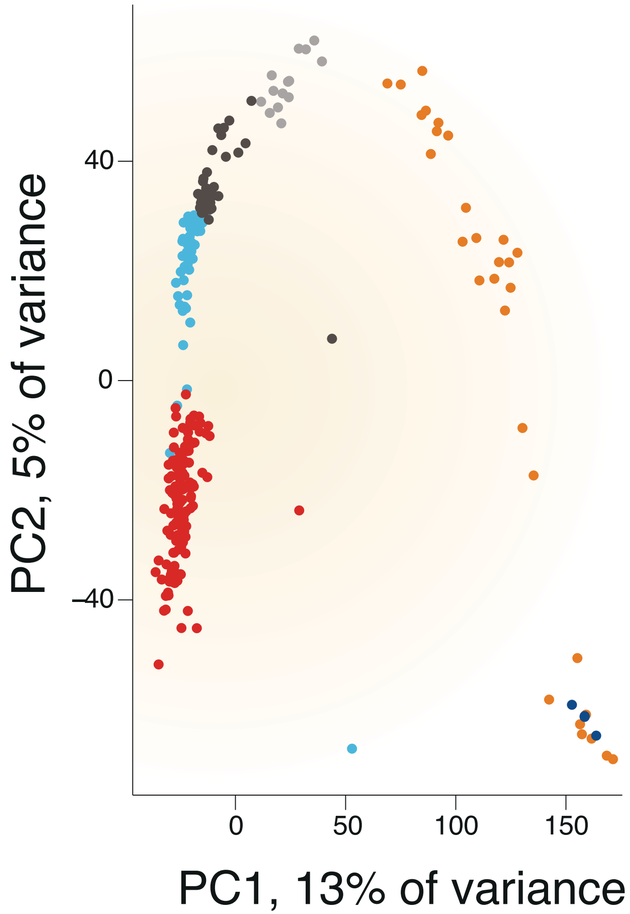
Because we retained high CV, high average expression genes

500 genes



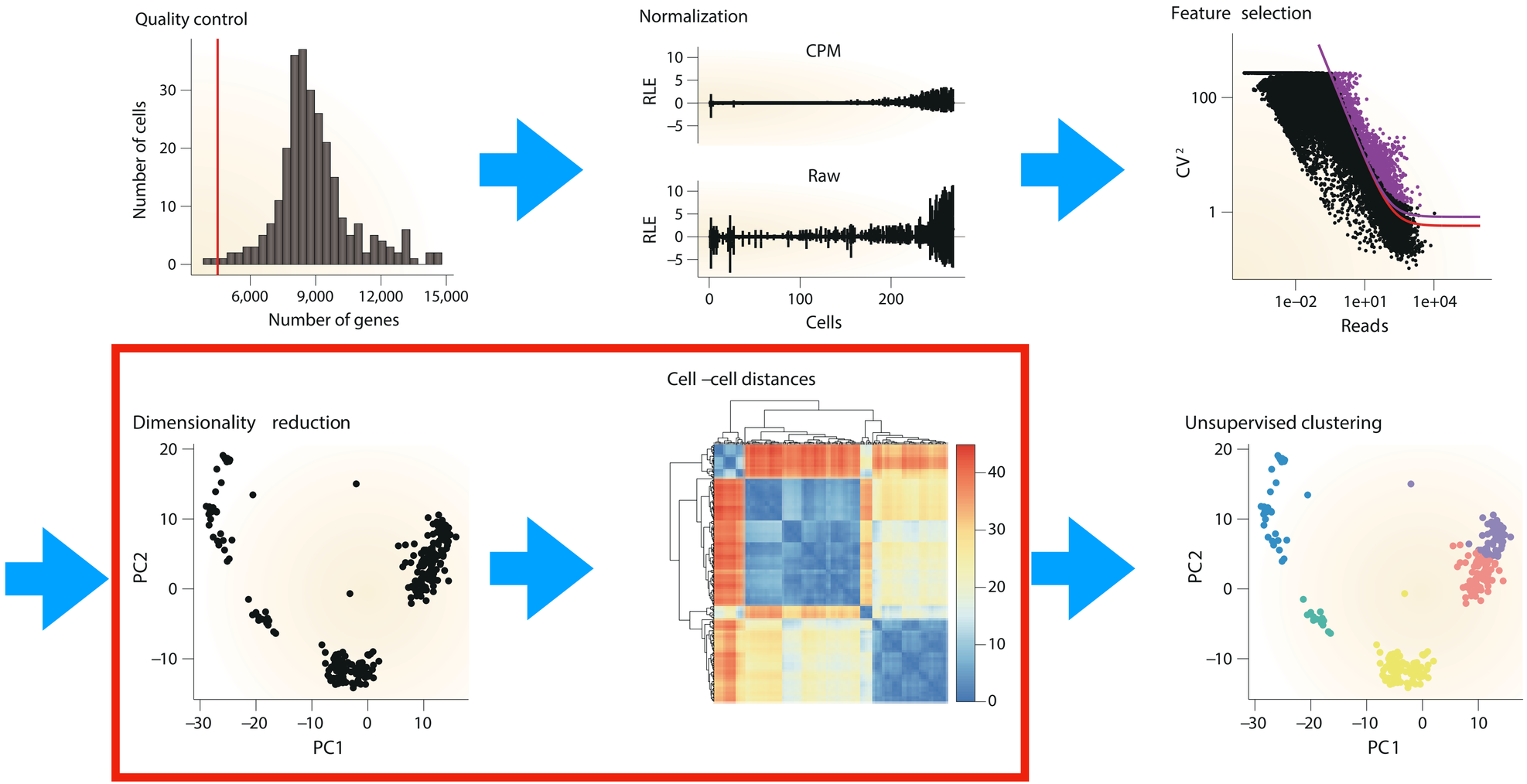
vs.

20,000 genes



Kiselev *et al*. (2019)

Unsupervised ML is a crucial step in a typical scRNA-seq data analysis



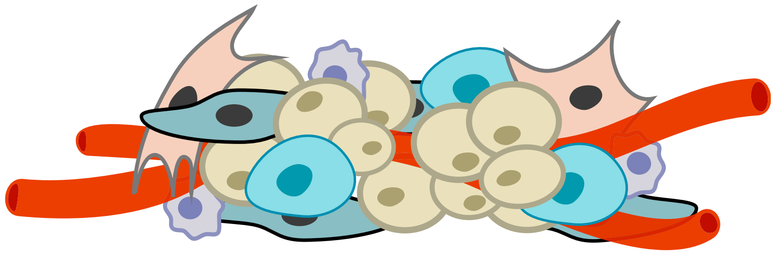
Kiselev *et al*. (2019)

Today's lecture

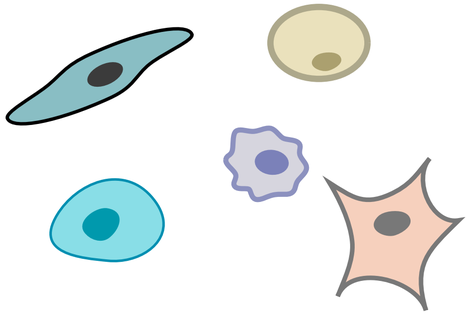
- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

Droplet-based single-cell sequencing technology

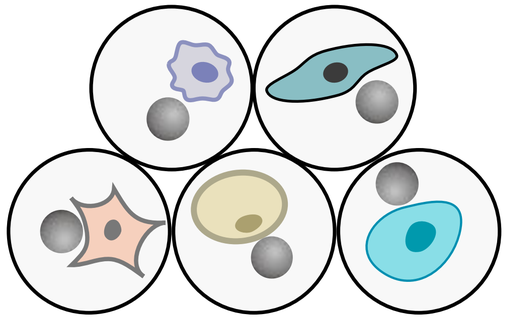
tissue sample



a mixture of cells

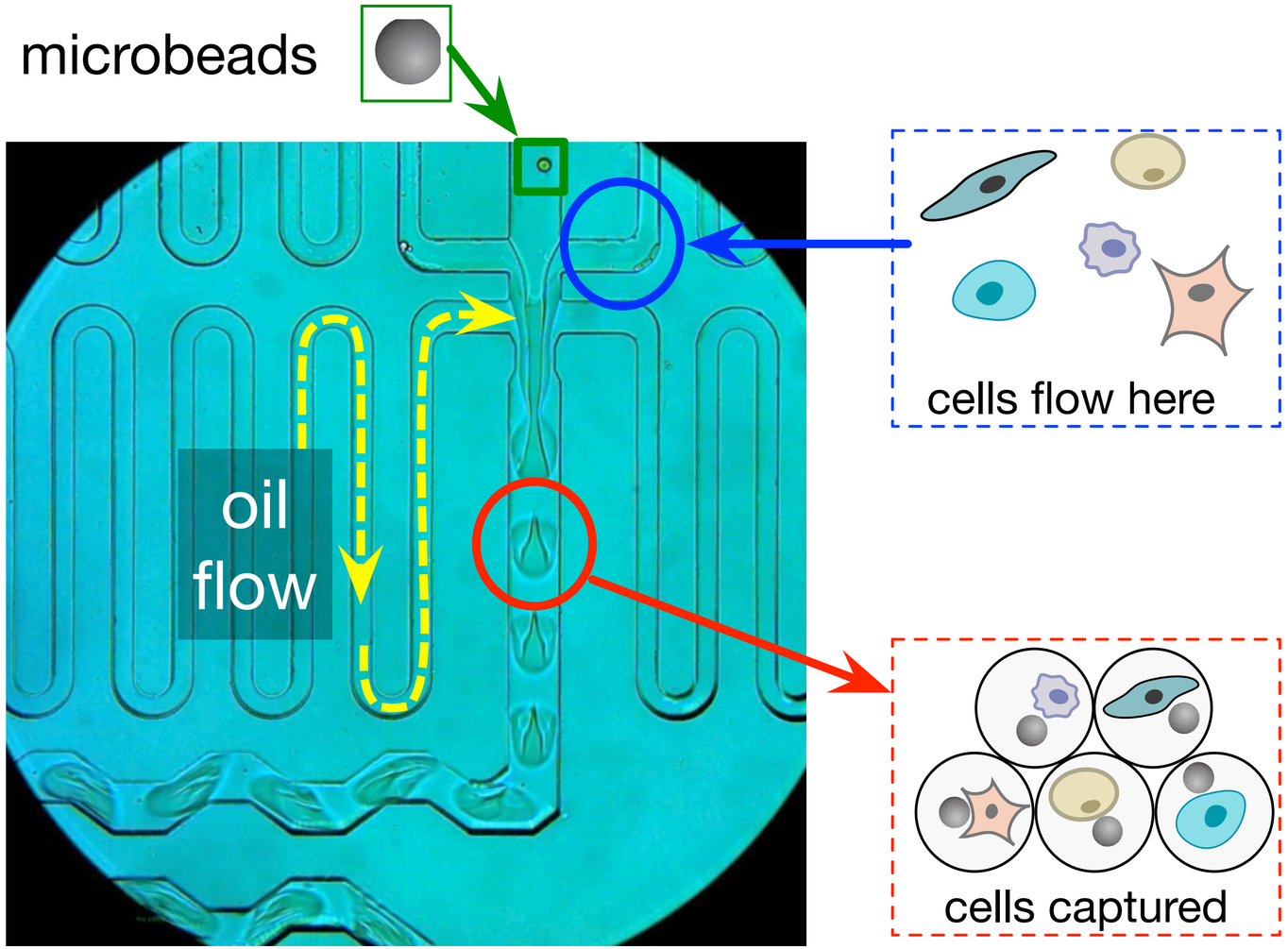


one drop = one cell

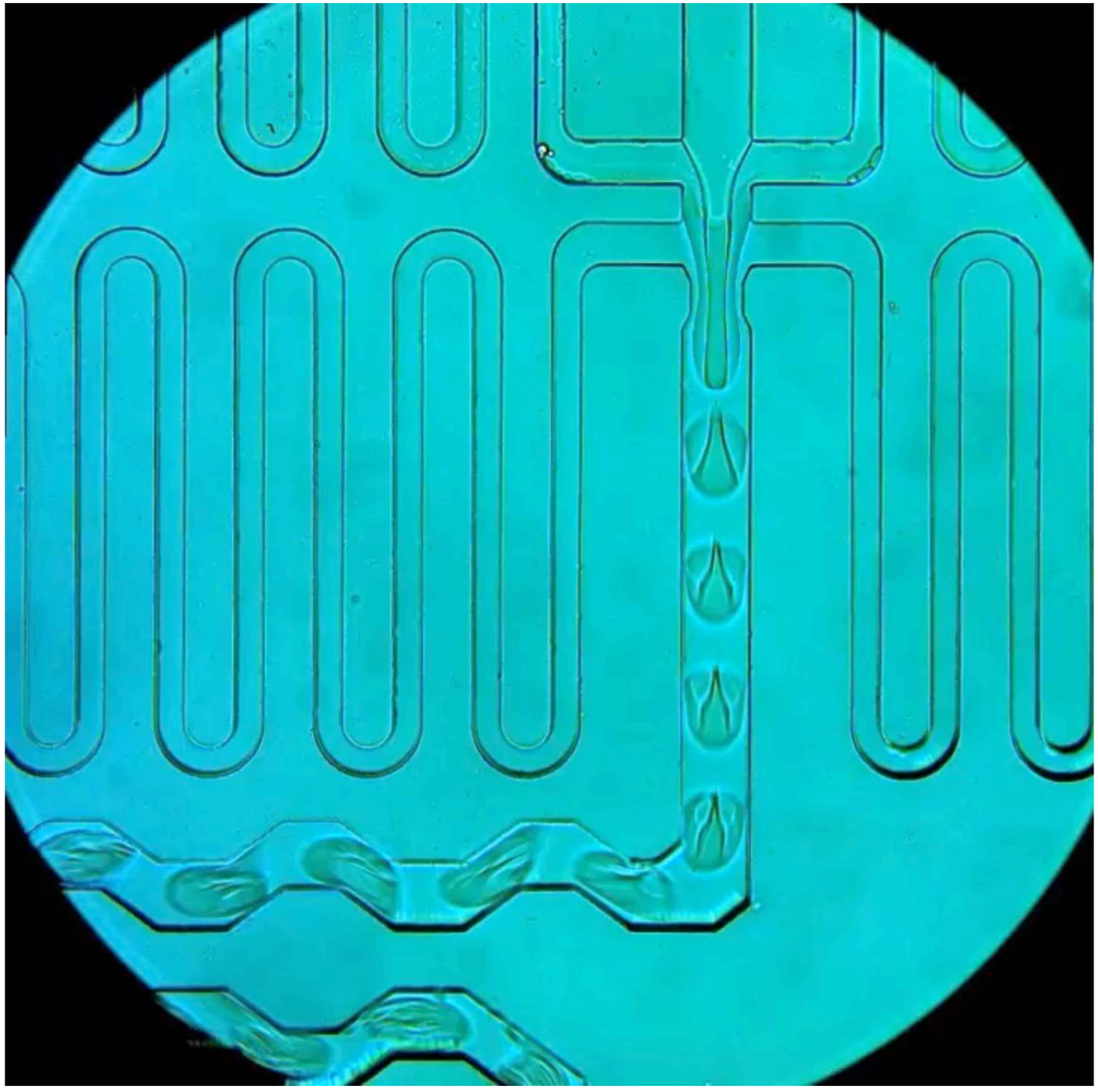


Macosko *et al.*, *Cell* (2015)

Drop-seq idea 1: Capture one cell with a microbead in a droplet

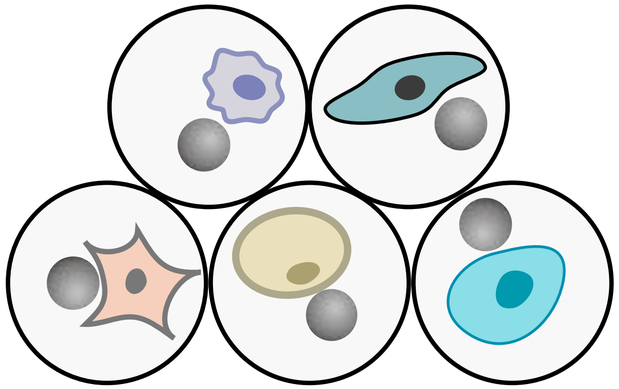


Macosko *et al.*, *Cell* (2015)

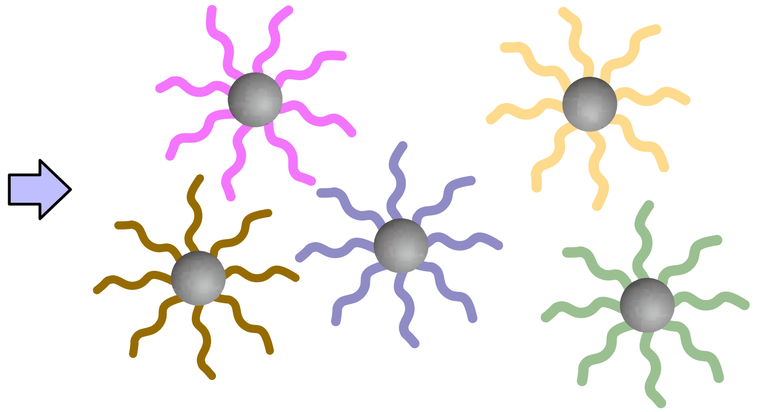


Drop-seq idea 2: Massively-parallel sequencing followed by cell-specific barcoding

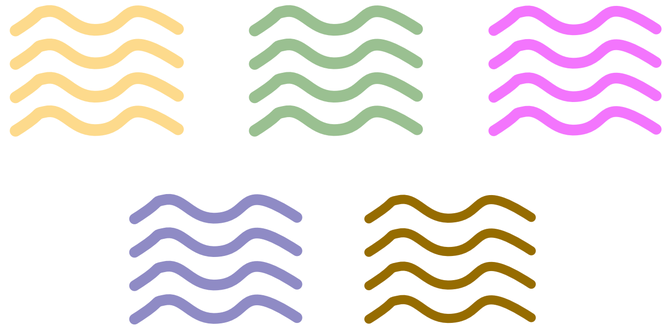
one drop = one cell



all the genes attached to the microbeads

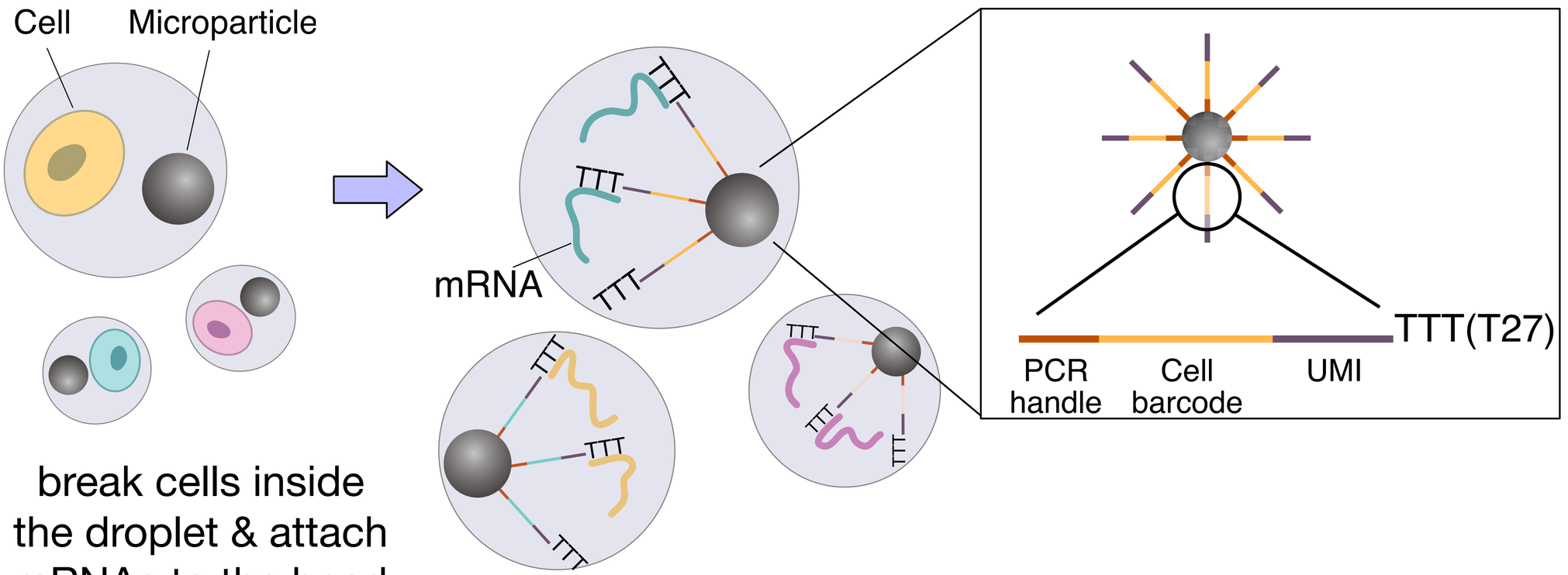


Massively parallel sequencing by mixing them all



Macosko *et al.*, *Cell* (2015)

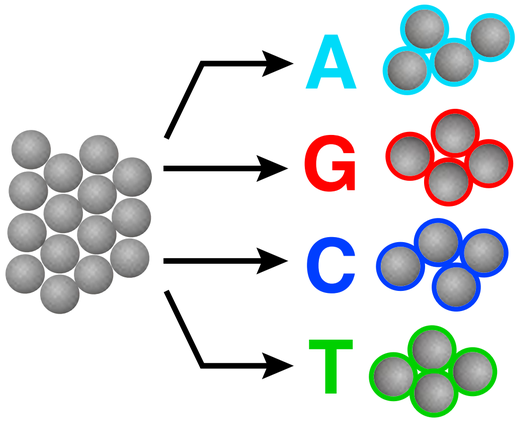
Drop-seq idea 3: How do we keep track of mRNA short reads' membership to a certain droplet?



break cells inside the droplet & attach mRNAs to the bead

Macosko *et al.*, *Cell* (2015)

How do we construct millions of unique barcodes? Use DNA as a hashing function!



Randomly split the beads into 4 sets

Tag each set with the same base (A,G,C,T)

Macosko *et al.*, *Cell* (2015)

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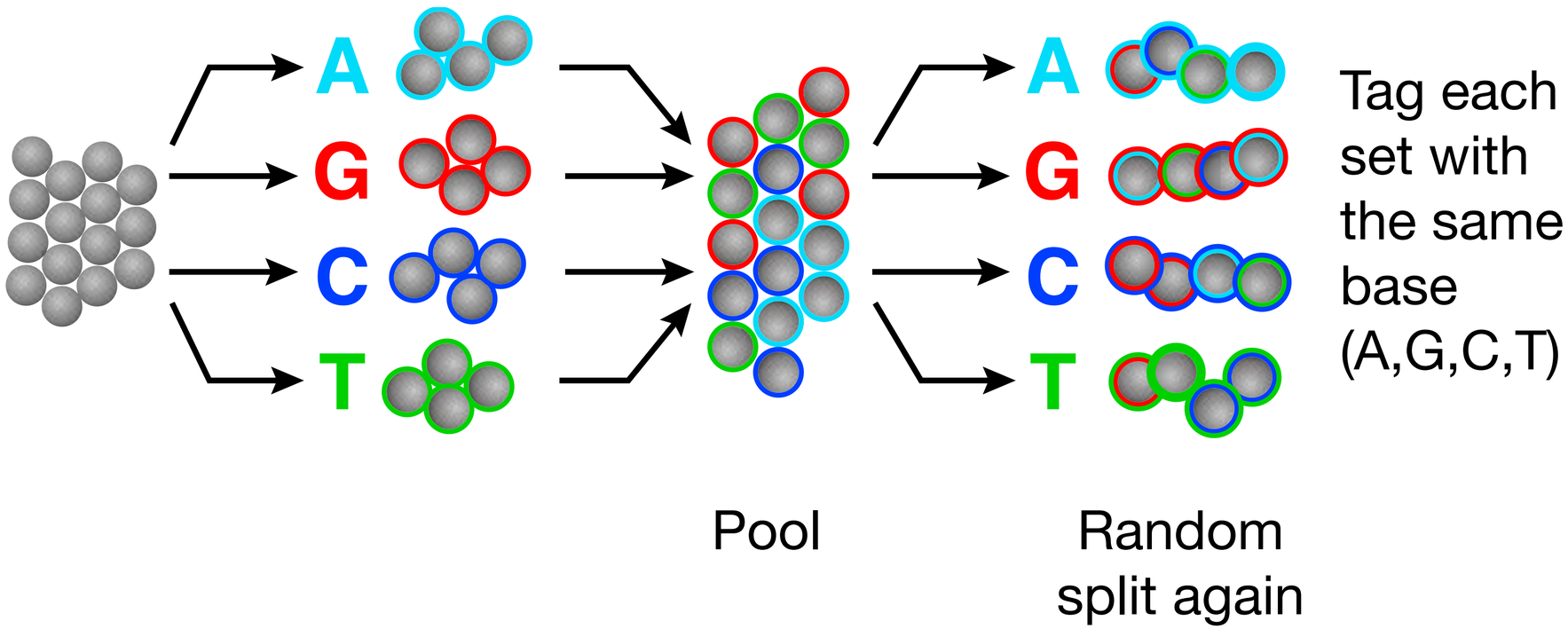

Macosko *et al.*, *Cell* (2015)

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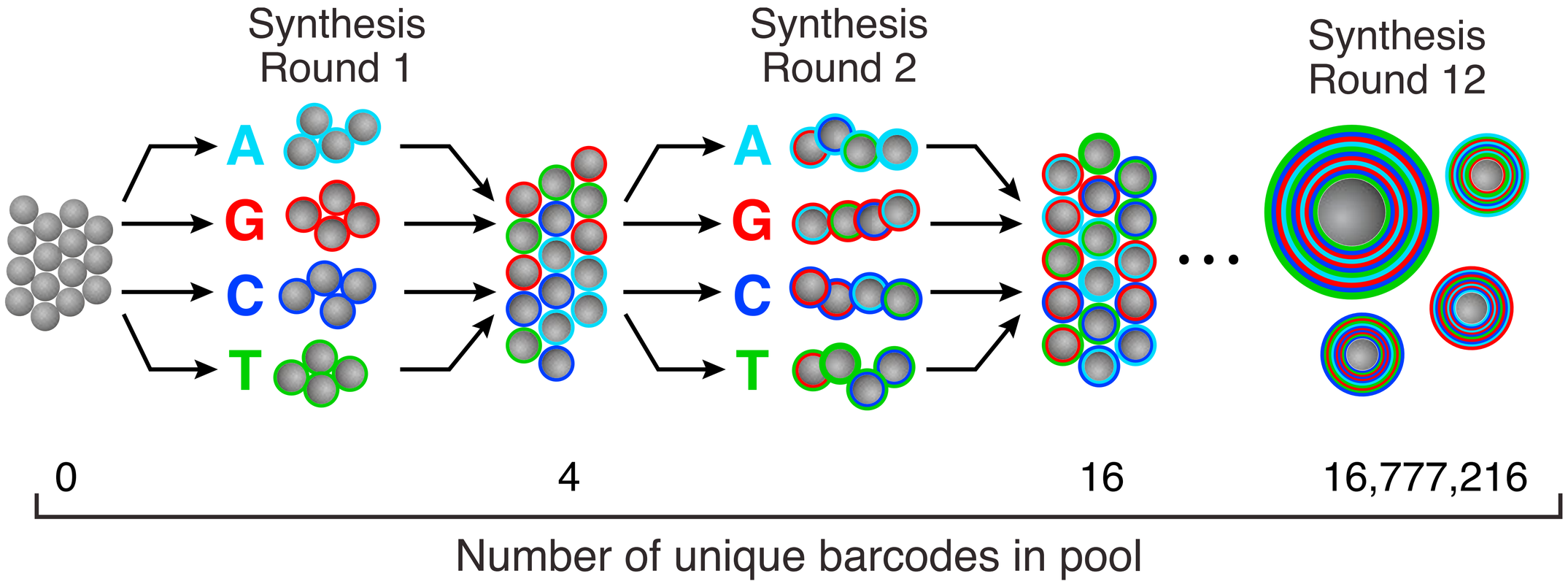
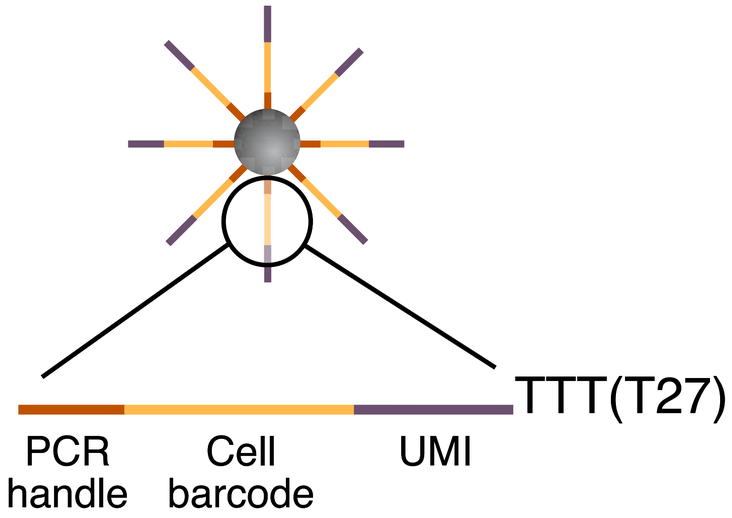
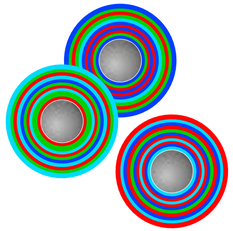


Macosko *et al.*, *Cell* (2015)

The lengths of barcode sequences determine data dimensionality



4^{12} 4^{8}



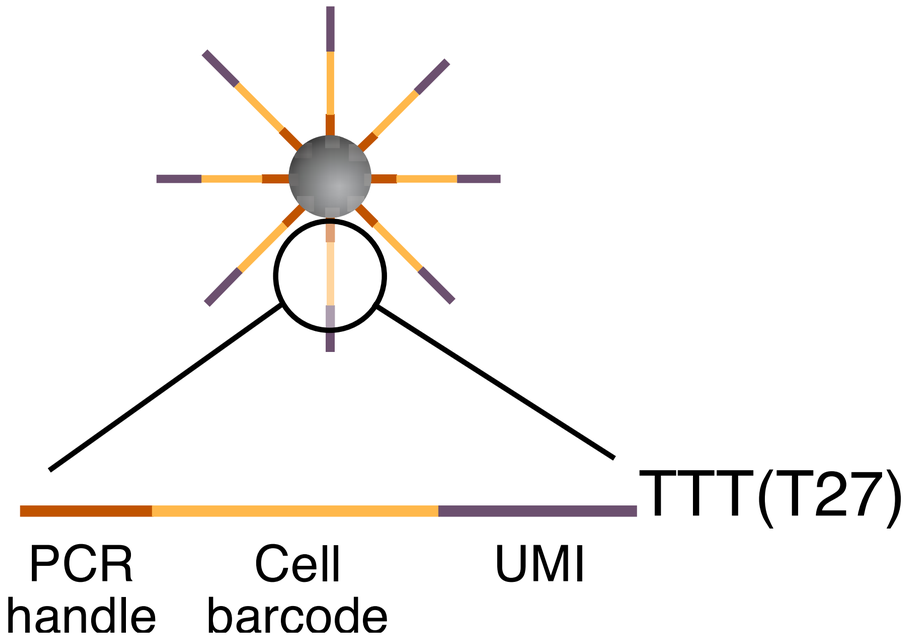
+ × 8 rounds of synthesis

- Millions of the same **cell barcode** per bead
- 4^8 different **molecular barcodes** (UMIs) per bead

Technically, we can build up to a 65,536 × 16,777,216, gene × cell expression matrix in one single-cell RNA-seq experiment.

Macosko *et al.*, *Cell* (2015)

Single-cell technology defines the shape of a resulting data matrix



30K genes

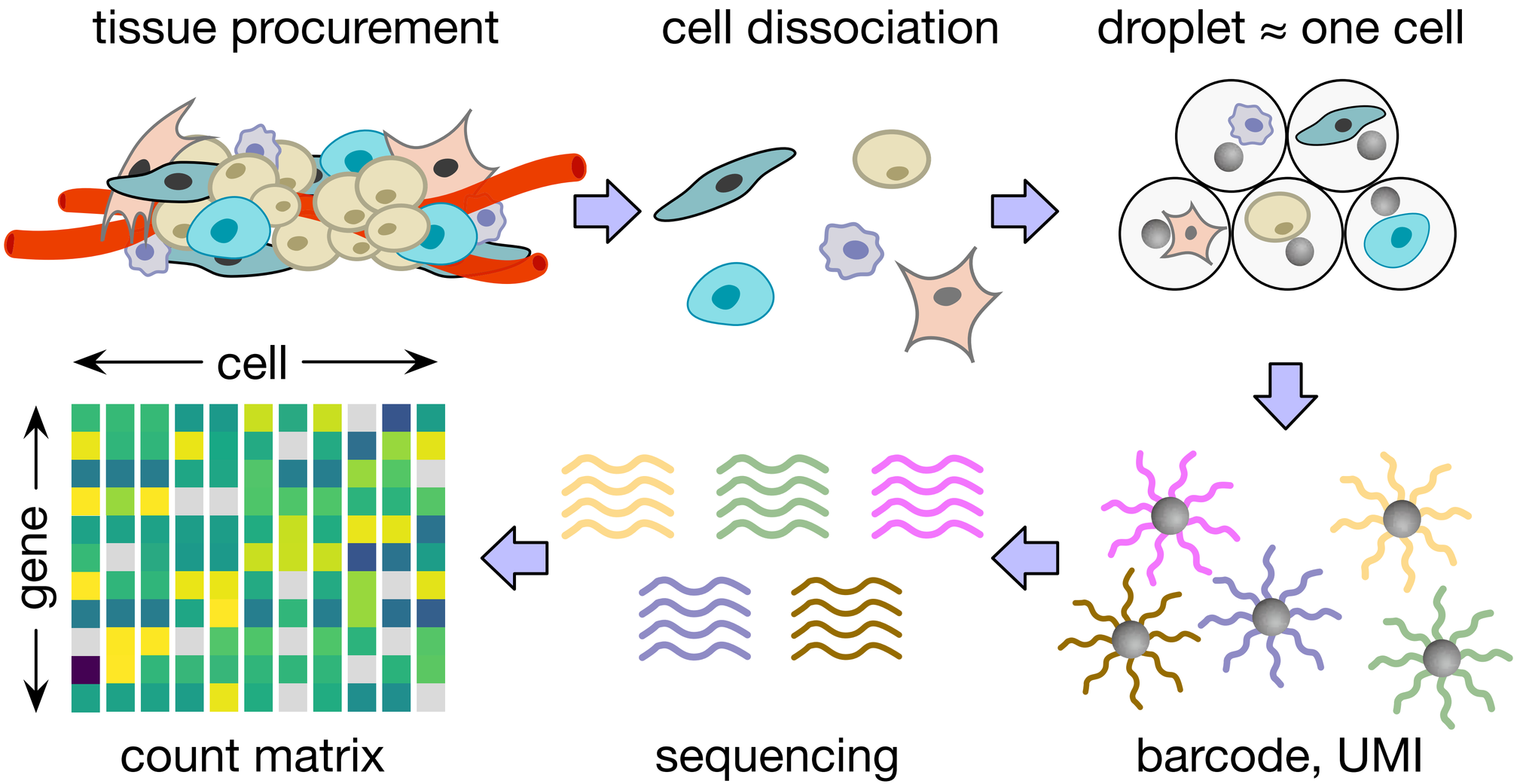
$X_{ij} \in [0, 4^8]$ 65K

$0 \leq \log(1 + X) < 12$

$< 4^{12} \approx 16M$ cells

Makosco *et al.* Cell (2015)

Droplet-based single-cell sequencing pipeline



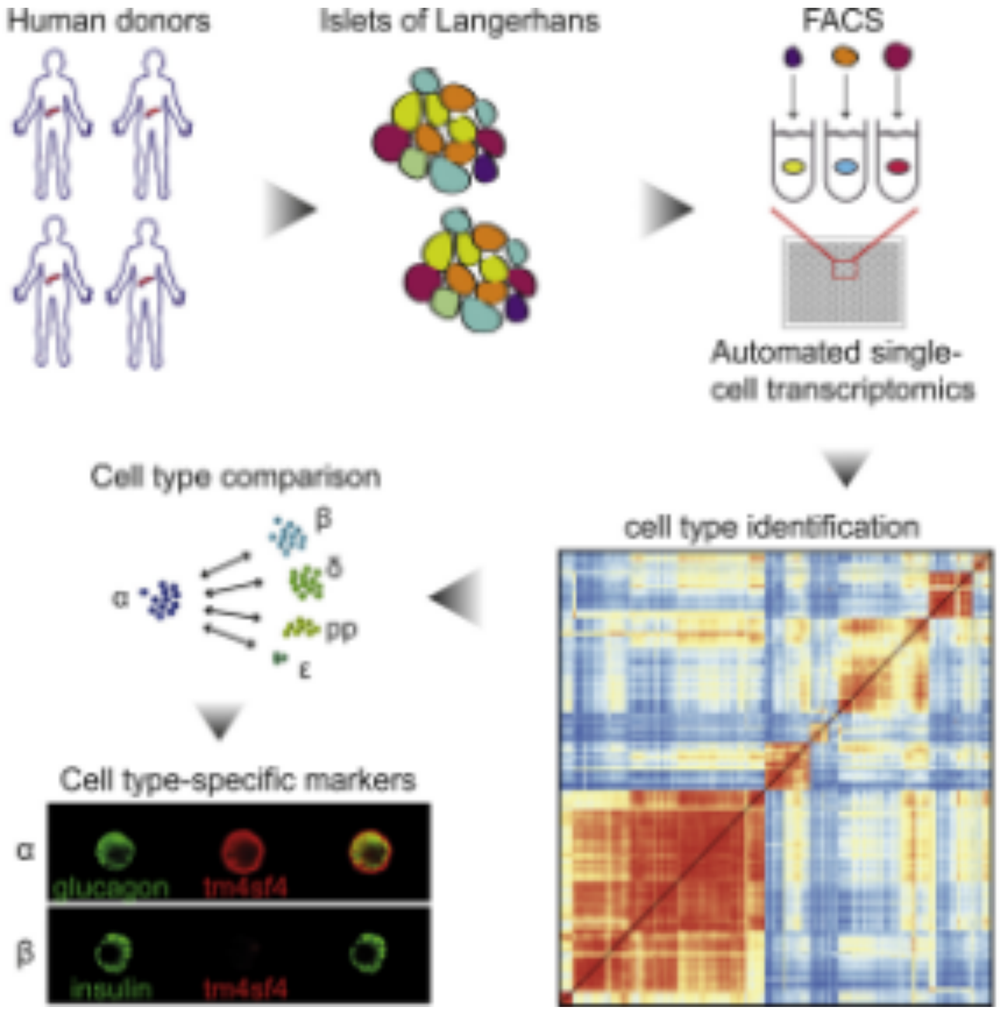
Macosko *et al.*, *Cell* (2015)

Today's lecture

- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

Example: single-cell RNA-seq data of human pancreatic cells

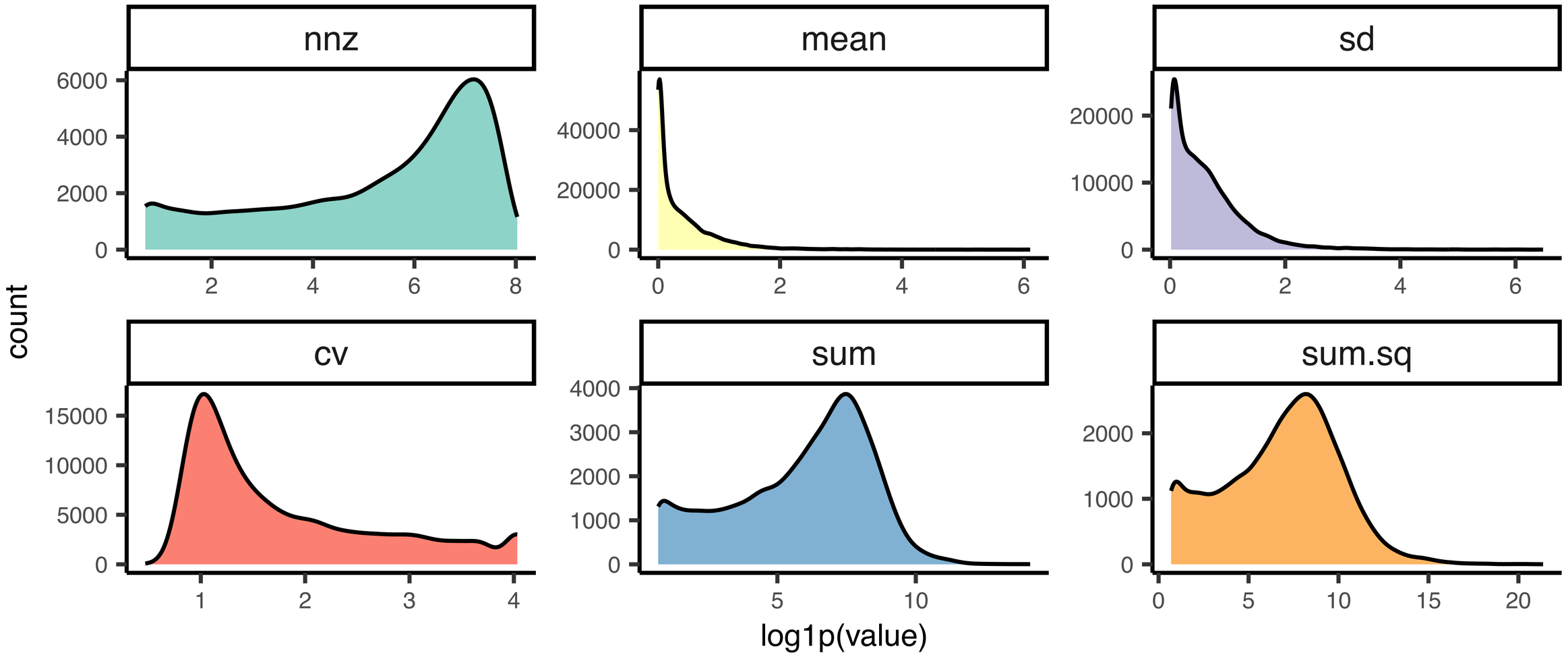
We will use scRNA-seq data (GEO accession: GSE85241) as a working example.



- genes/features/rows: 19,140
- cells/columns: 3,072
- non-zero elements: 12,442,034
- $\sim$ 21 % non-zero

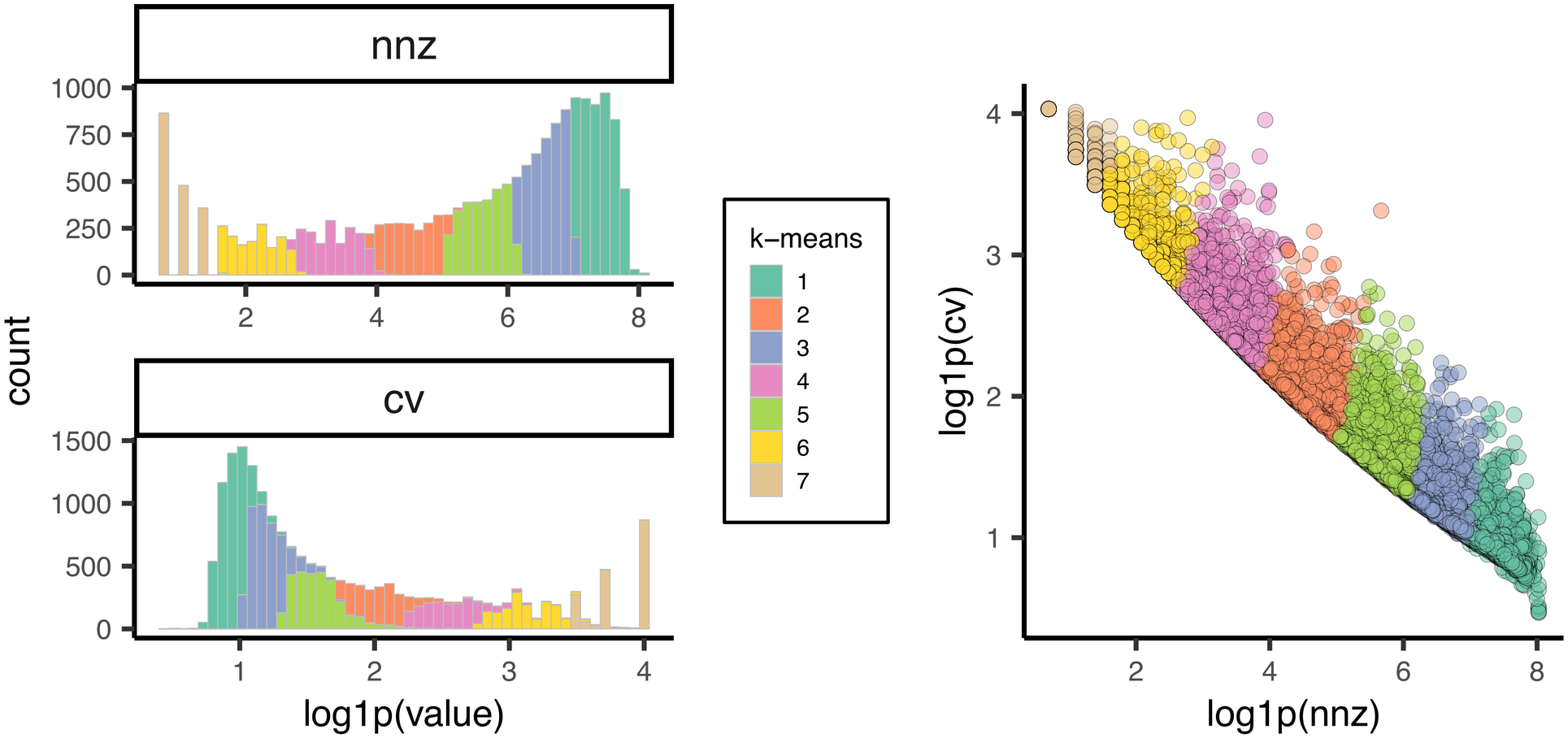
Muraro *et al. Cell Systems* (2016)

Gene-level statistics across cells



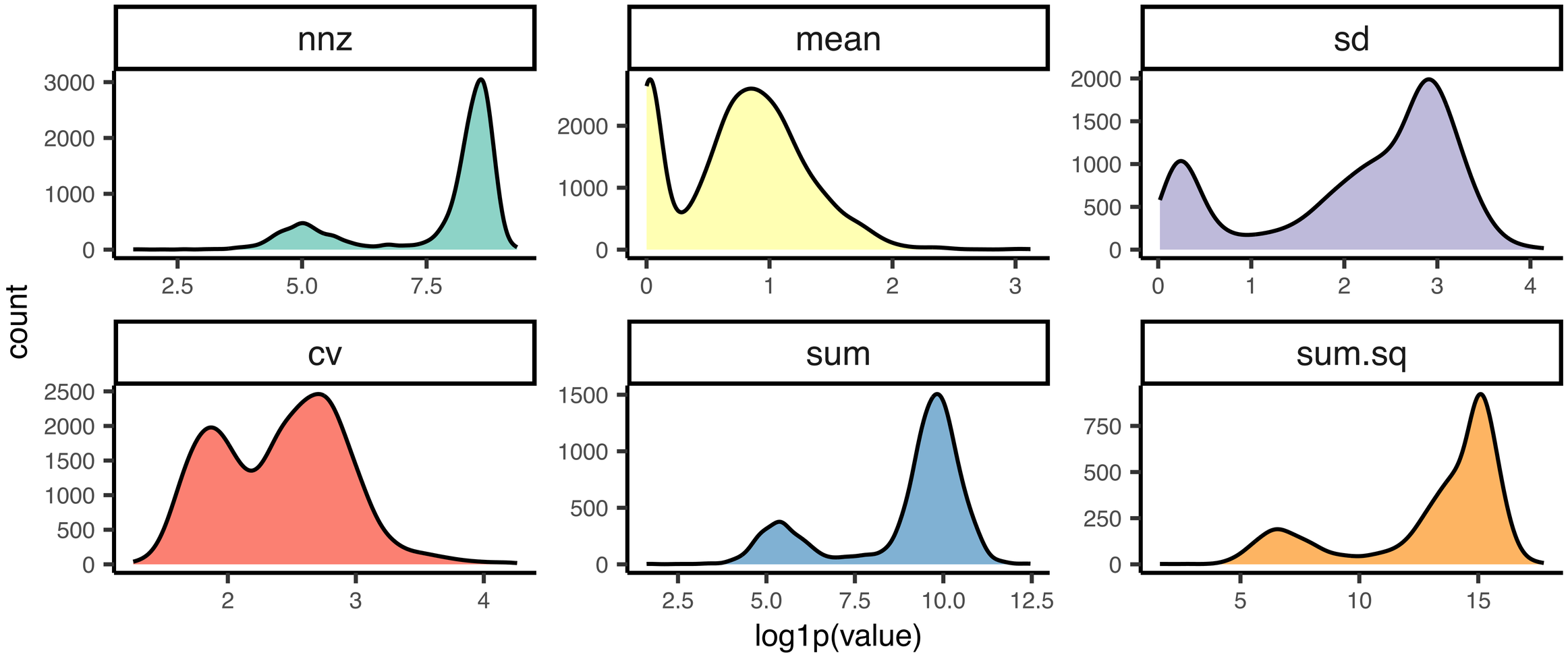
`nnz`: number of non-zero elements, `sd`: standard deviation, `cv`: coefficient of variation (`sd/mean`), `sum.sq`: sum of squares.

Can we drop any genes?



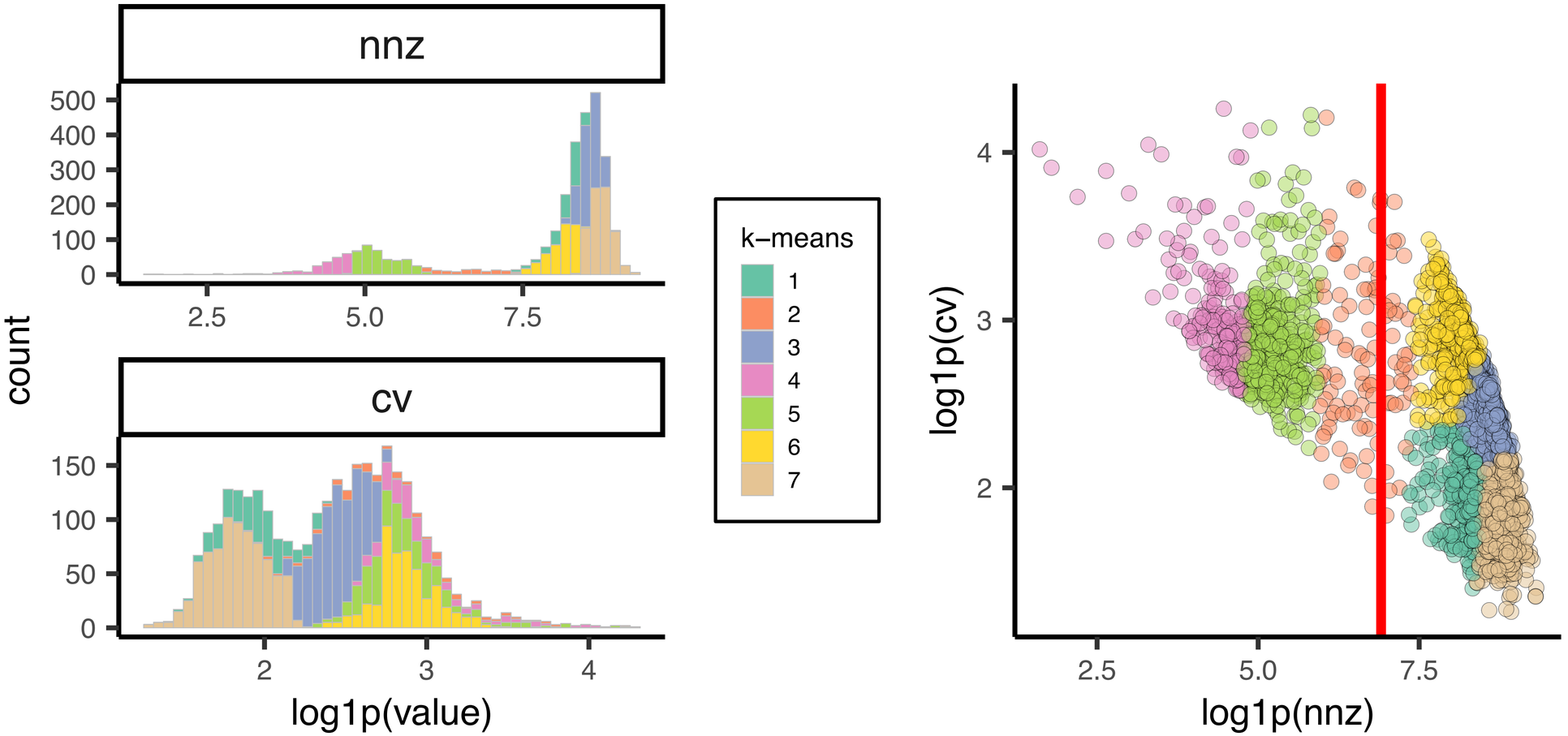
We don't like unstable genes with low average expressions and high CV.

Cell-level statistics across genes within each cell



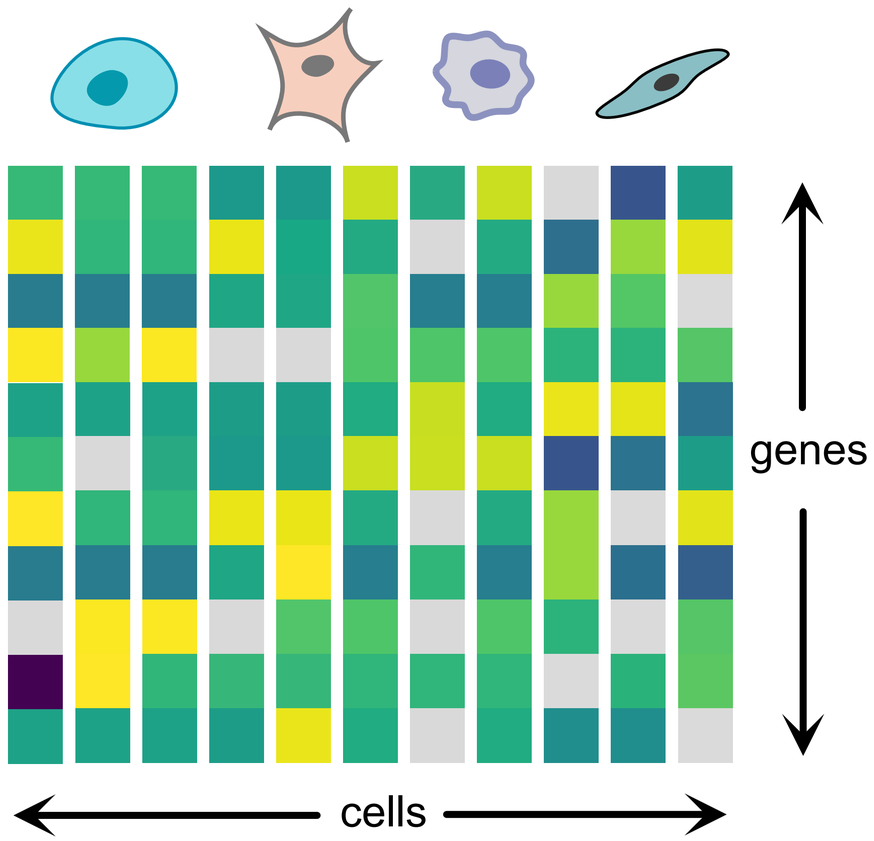
`nnz`: number of non-zero elements, `sd`: standard deviation, `cv`: coefficient of variation (`sd/mean`), `sum.sq`: sum of squares.

What about cells? Can we filter out some cells not informative?



We may remove cells with too few non-zero elements (e.g., NNZ $<$ 1000).

Was it a good technical decision? How do we know? Let's visualize it!



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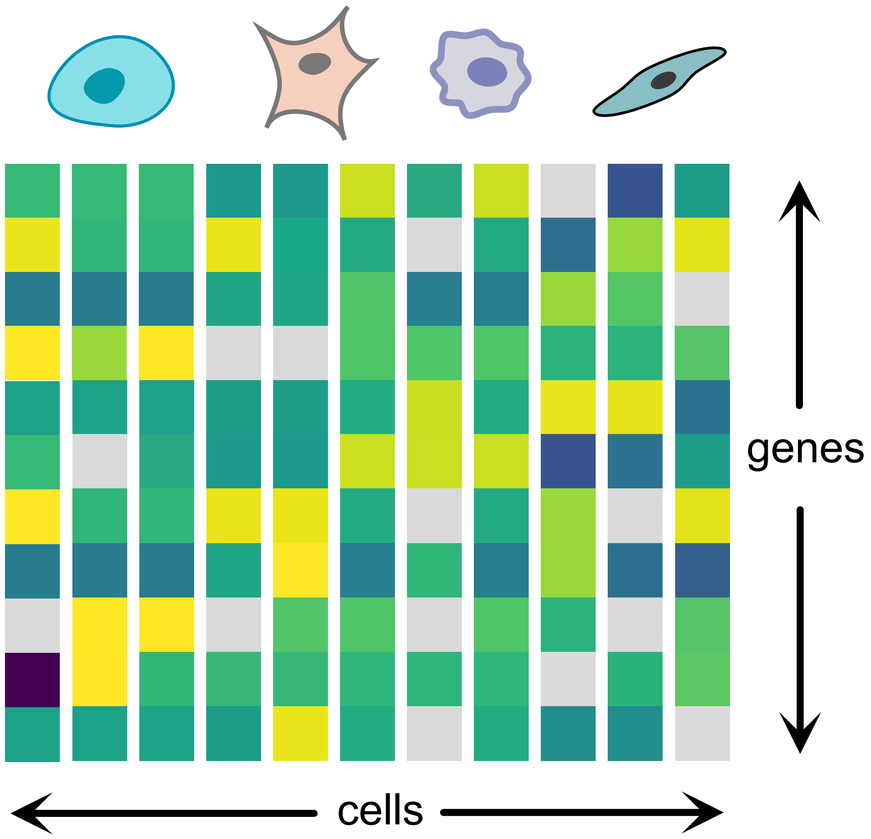
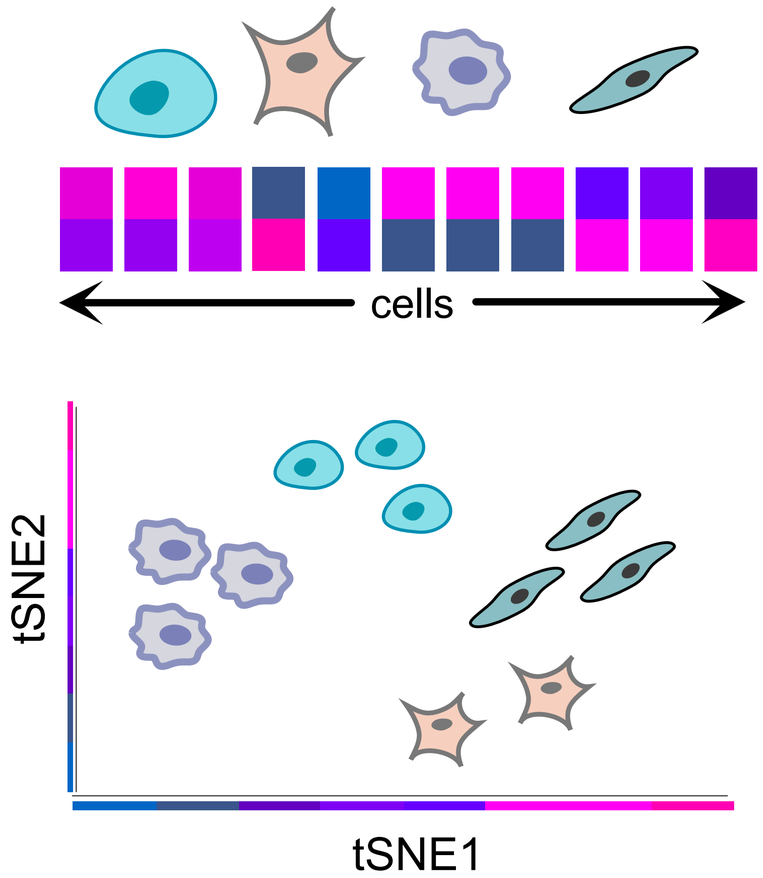
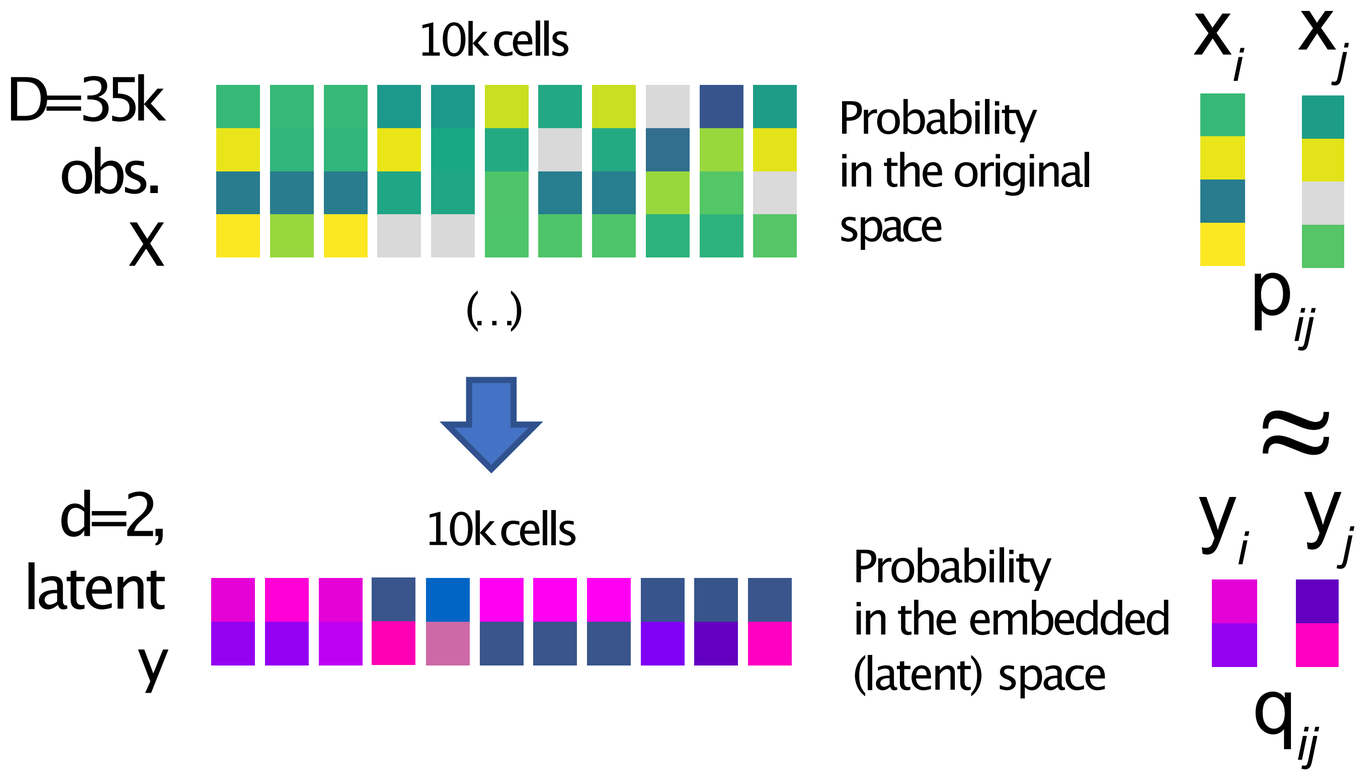




`tSNE`: t-distributed Stochastic Neighbourhood Embedding (Van der Maaten & Hinton, 2008).

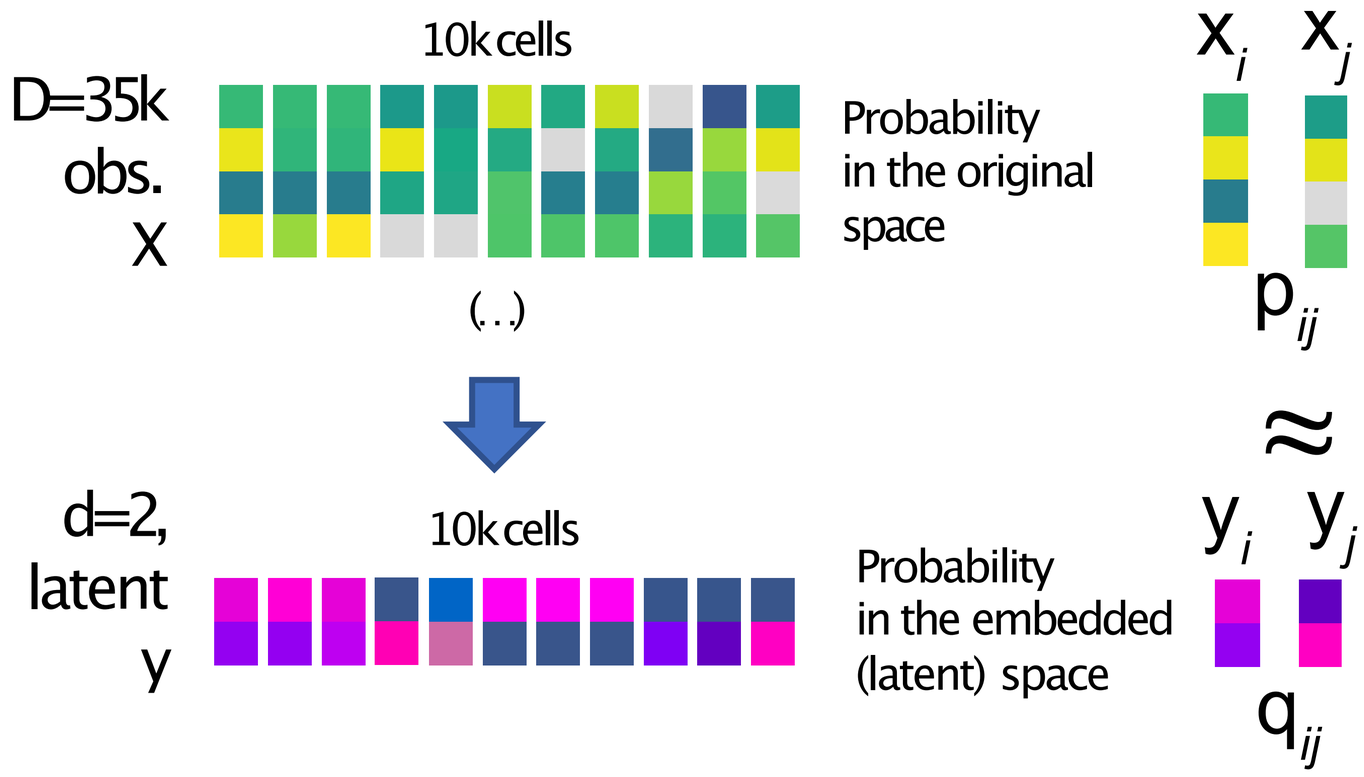
SNE: What is “stochastic neighbourhood embedding?”



- p_{ij}: probability between cells i and j in the original high-dimensional space

$$p_{ij} \propto \exp(-\|\mathbf{x}_i - \mathbf{x}_j\|^2 / 2\sigma^2)$$

SNE: What is “stochastic neighbourhood embedding?”



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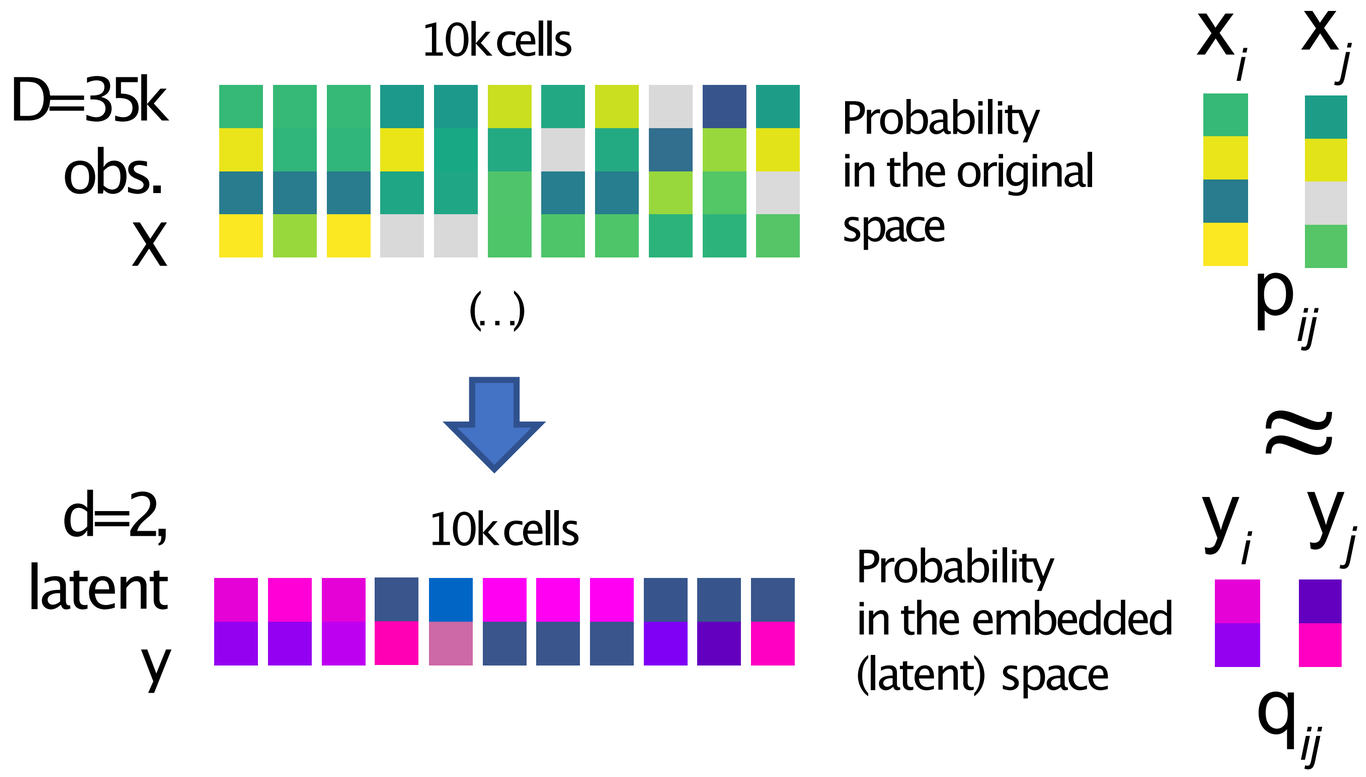
- q_{ij}: probability between cells i and j in the embedded low-dimensional space

$$q_{ij} \propto \exp(-\|\mathbf{y}_i - \mathbf{y}_j\|^2/2\sigma^2)$$

SNE: What is “stochastic neighbourhood embedding?”



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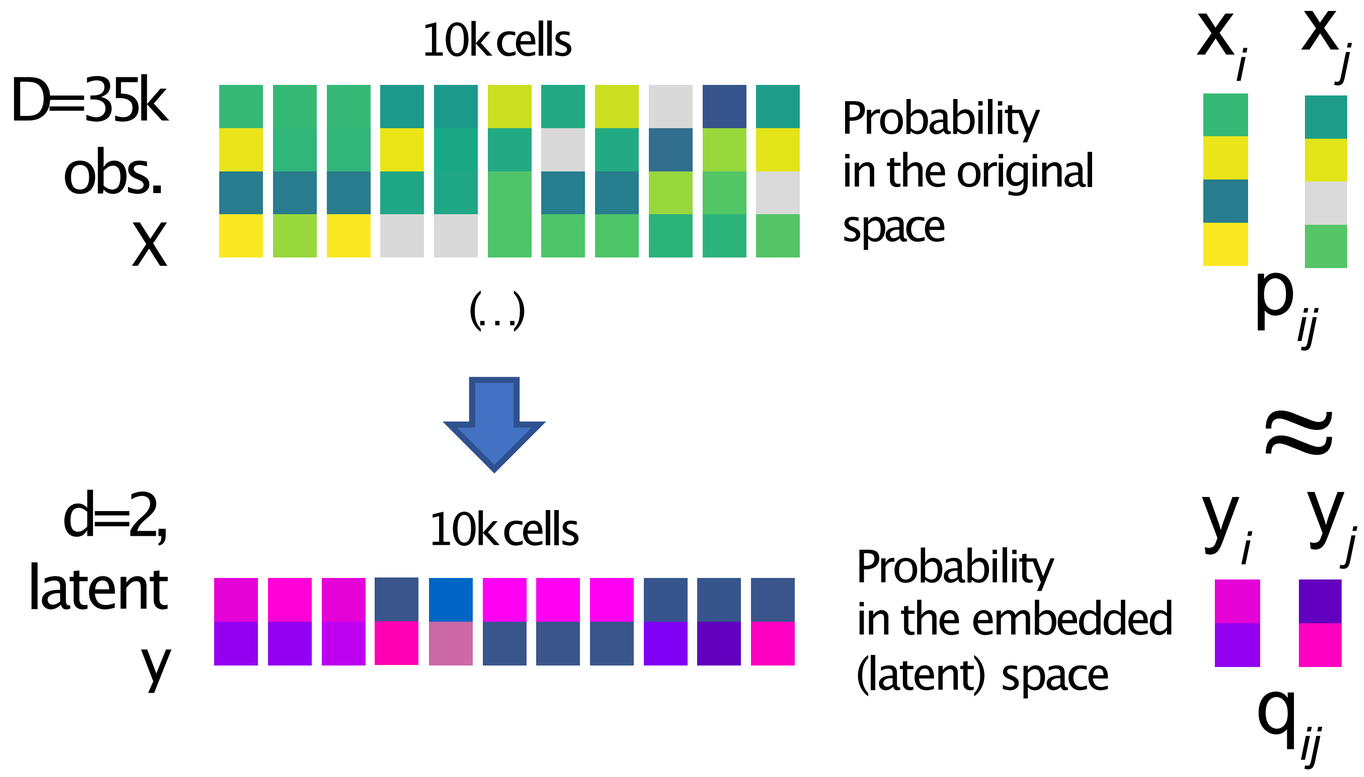

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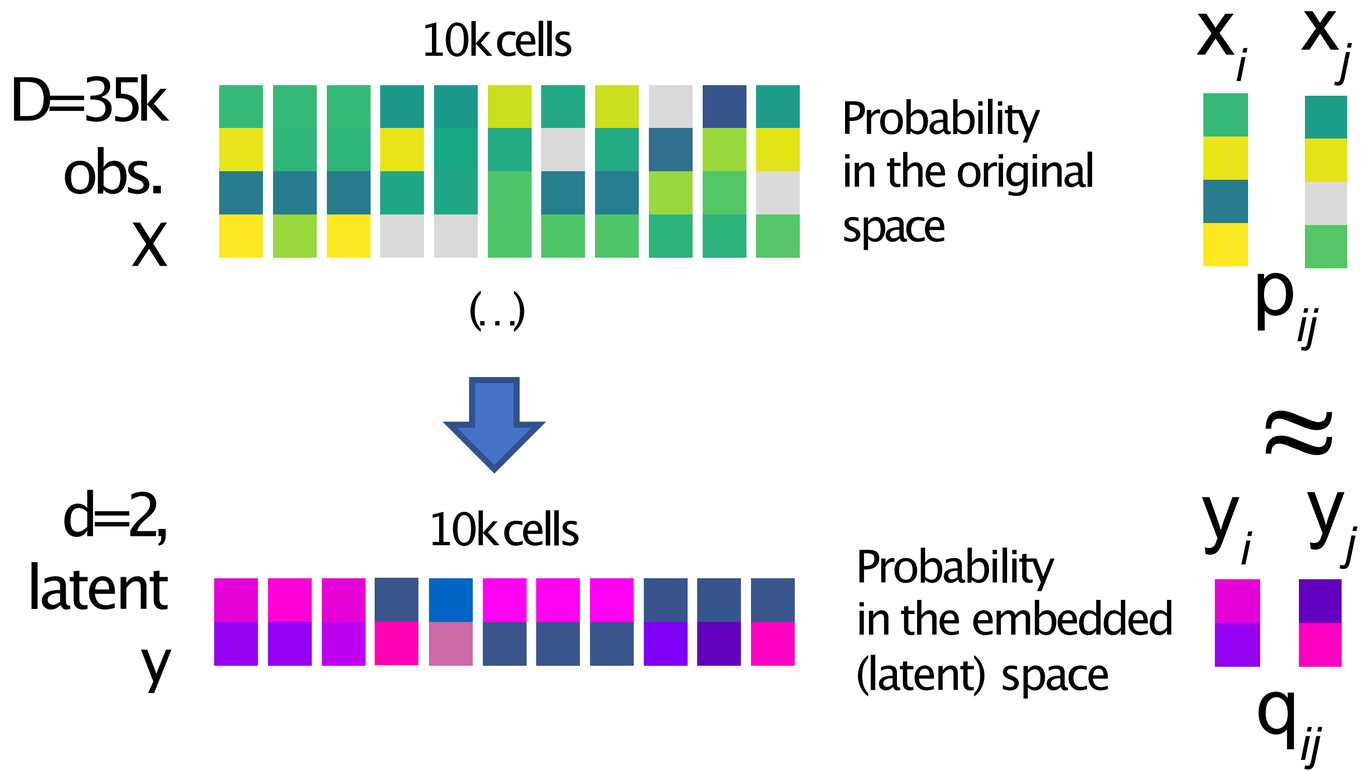
$$\min D_{\mathsf{KL}}\left(p_{ij}\|q_{ij}\right) = \sum_{ij} p_{ij} \log \frac{p_{ij}}{q_{ij}}$$

tSNE: What is t-distributed "stochastic neighbourhood embedding?"



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$$p_{ij} \propto \exp(-\|\mathbf{x}_i - \mathbf{x}_j\|^2/2\sigma^2)$$

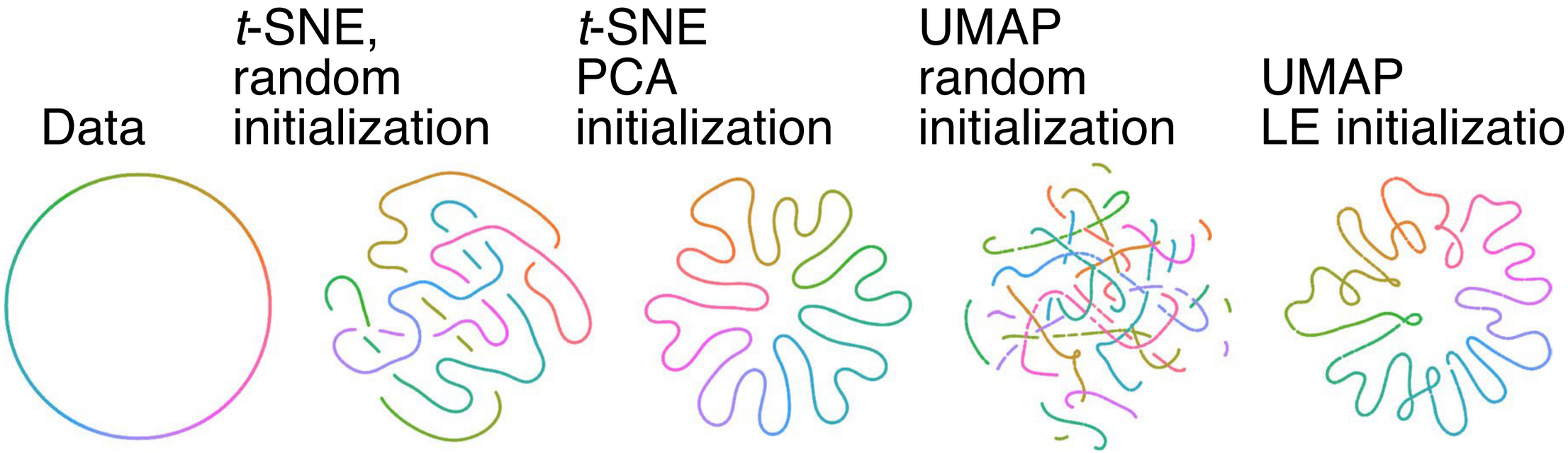
- q_{ij}: probability between cells i and j in the embedded low-dimensional space

$$q_{ij} \propto \left(1 + \|\mathbf{y}_i - \mathbf{y}_j\|^2\right)^{-1}$$

Goal: make pairwise probabilities between cells in the observed and latent space as close as possible.

$$\min D_{\mathsf{KL}}\left(p_{ij} \| q_{ij}\right) = \sum_{ij} p_{ij} \log \frac{p_{ij}}{q_{ij}}$$

Warning: Don't make over-interpretation on embedding results



Kobak and Berens, *Nature Biotech* (2021)

Check out these papers:

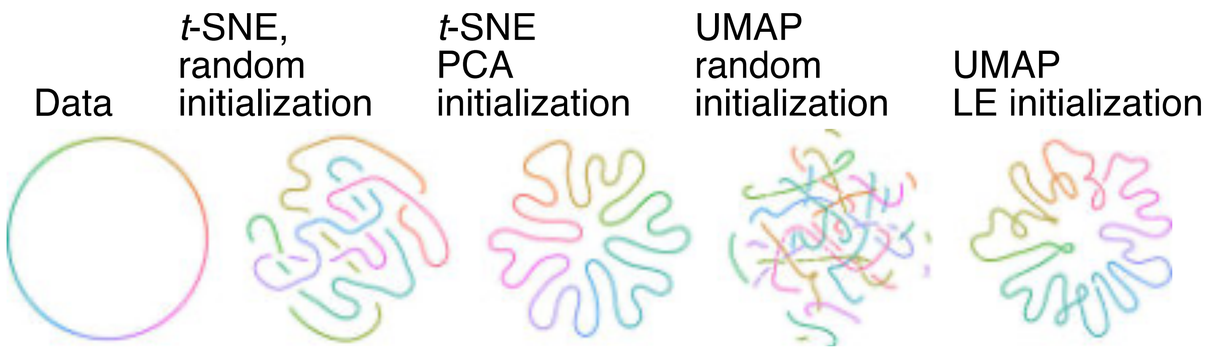
Initialization is critical for preserving global data structure in both t-SNE and UMAP

The art of using t-SNE for single-cell transcriptomics

Dimensionality reduction for visualizing single-cell data using UMAP

Don’t over-interpret embedding results

Initialization matters;
the coordinates are locally-optimized <u>without</u> biological relevance… and PCA is descent initialization

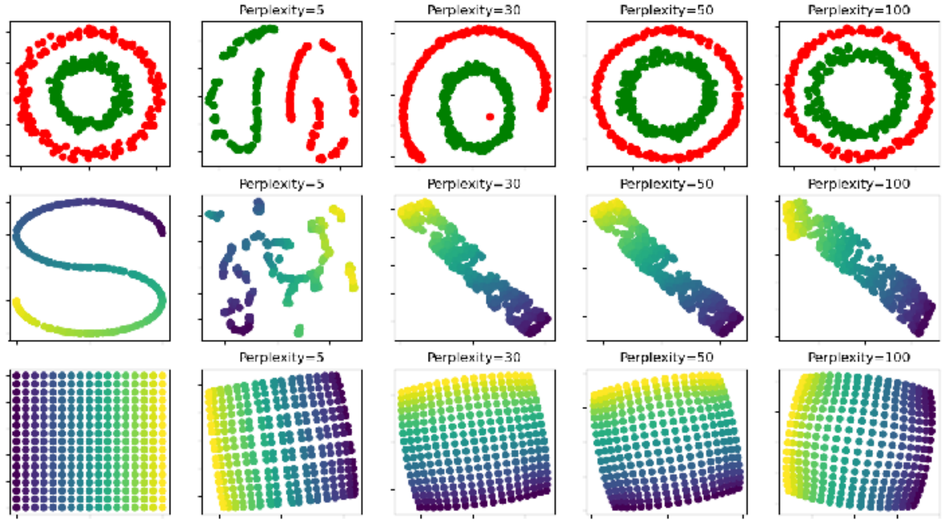


Kobak & Linderma, Nat. Biotech. (2021)

Hyperparameters change the embedding results greatly…

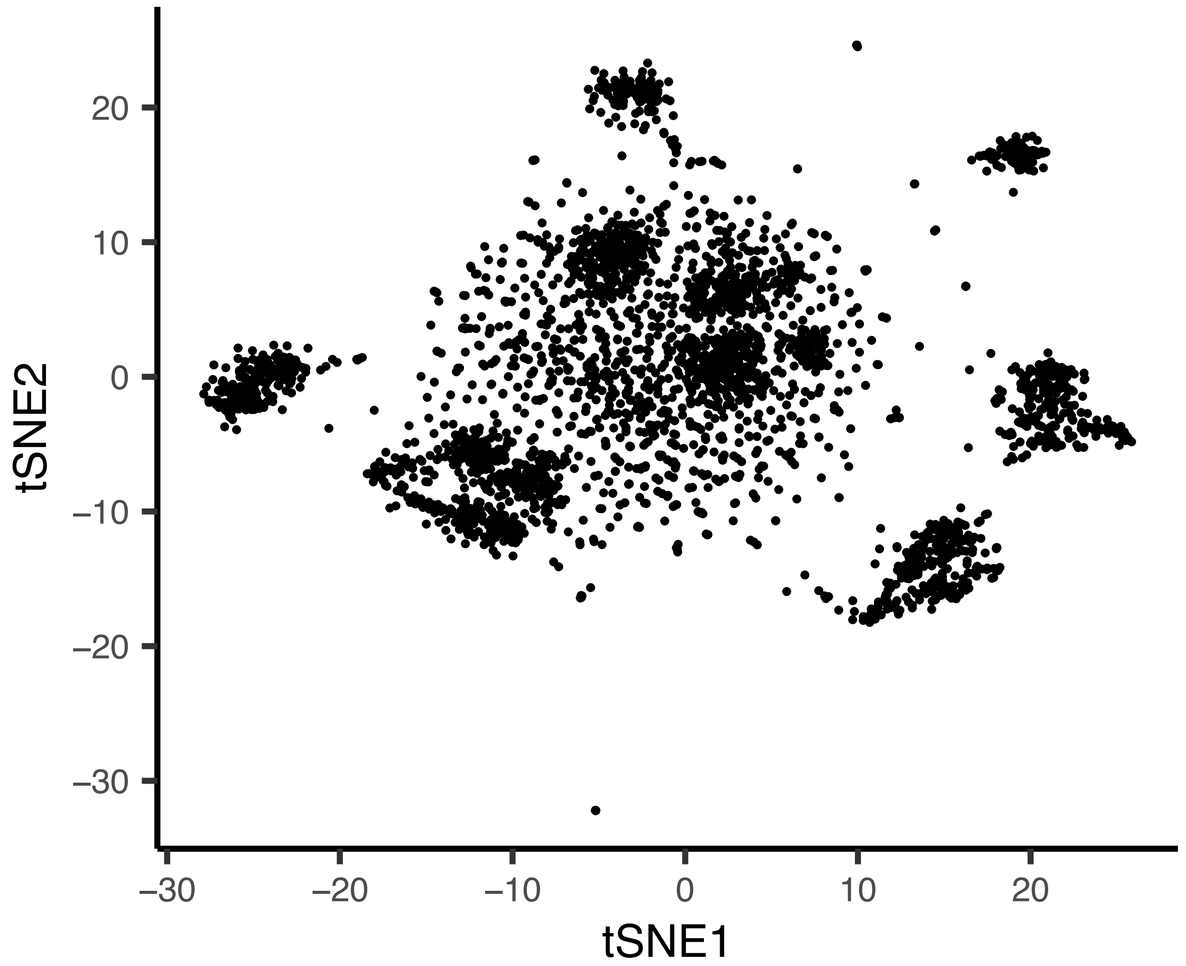
e.g., prefixed perplexity → σ

$$p_{ij} \propto \exp\left(-\left\|x_i - x_j\right\|^2 / 2\sigma^2\right)$$

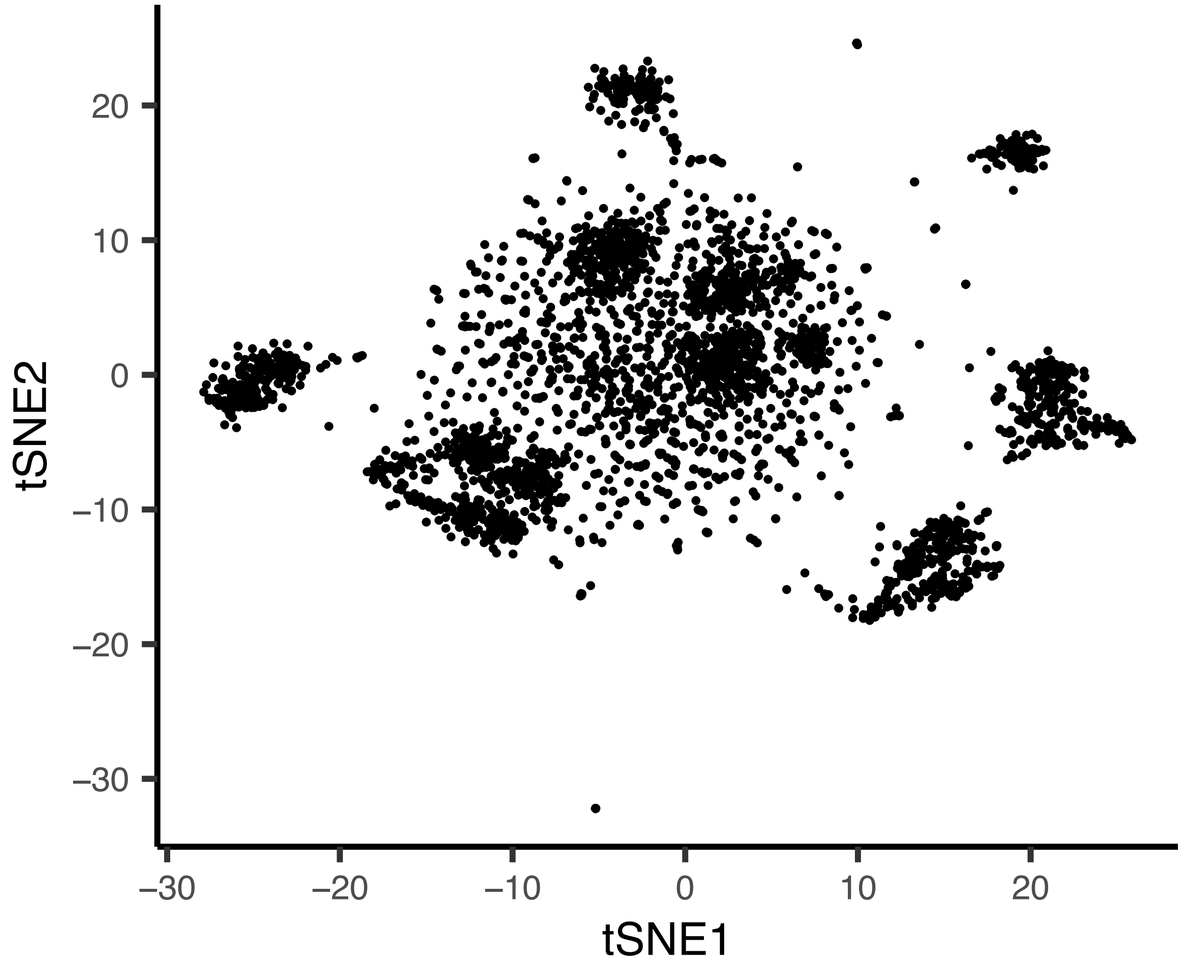


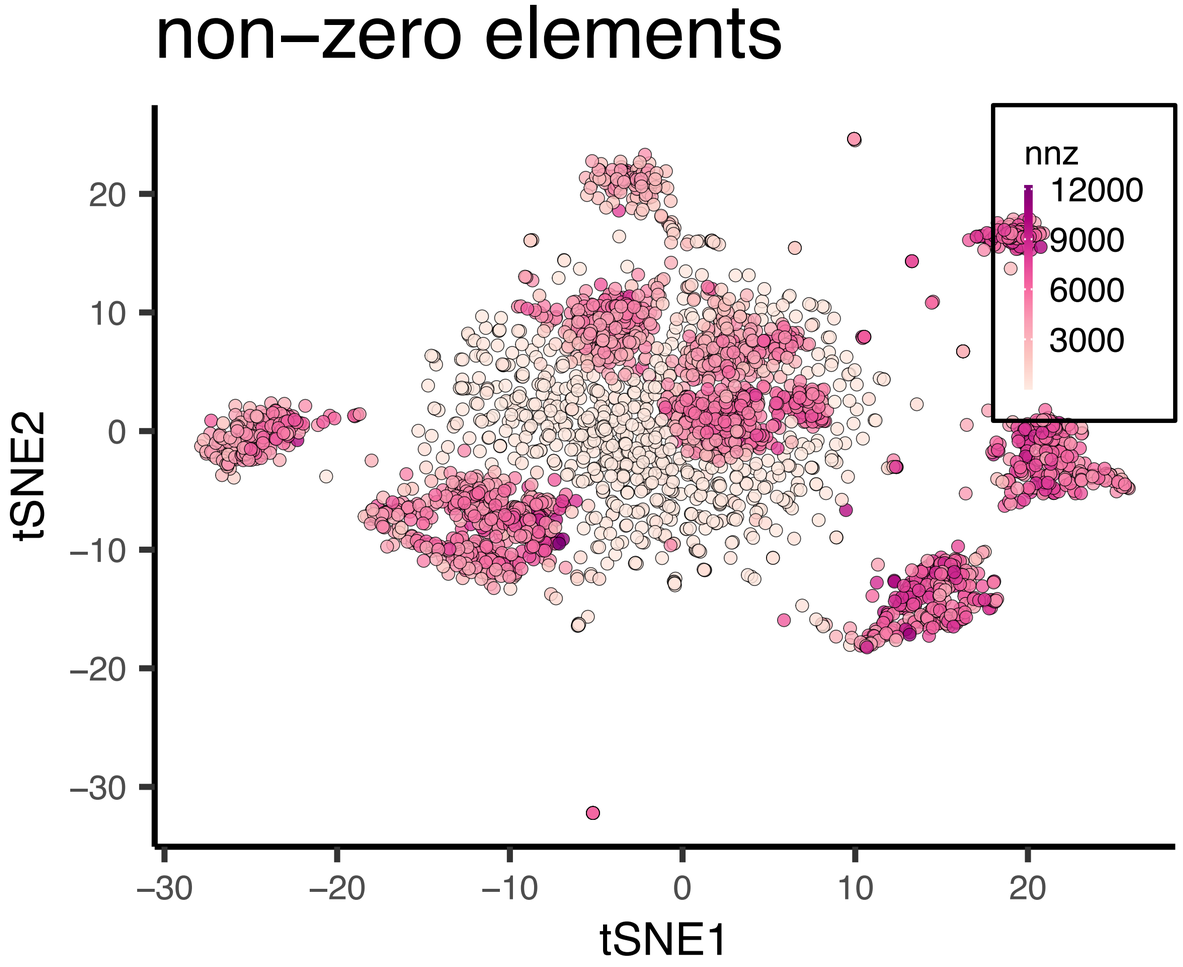
https://scikit-learn.org/stable/auto_examples/manifold/plot_t_sne_perplexity.html

Exploratory Data Analysis with tSNE

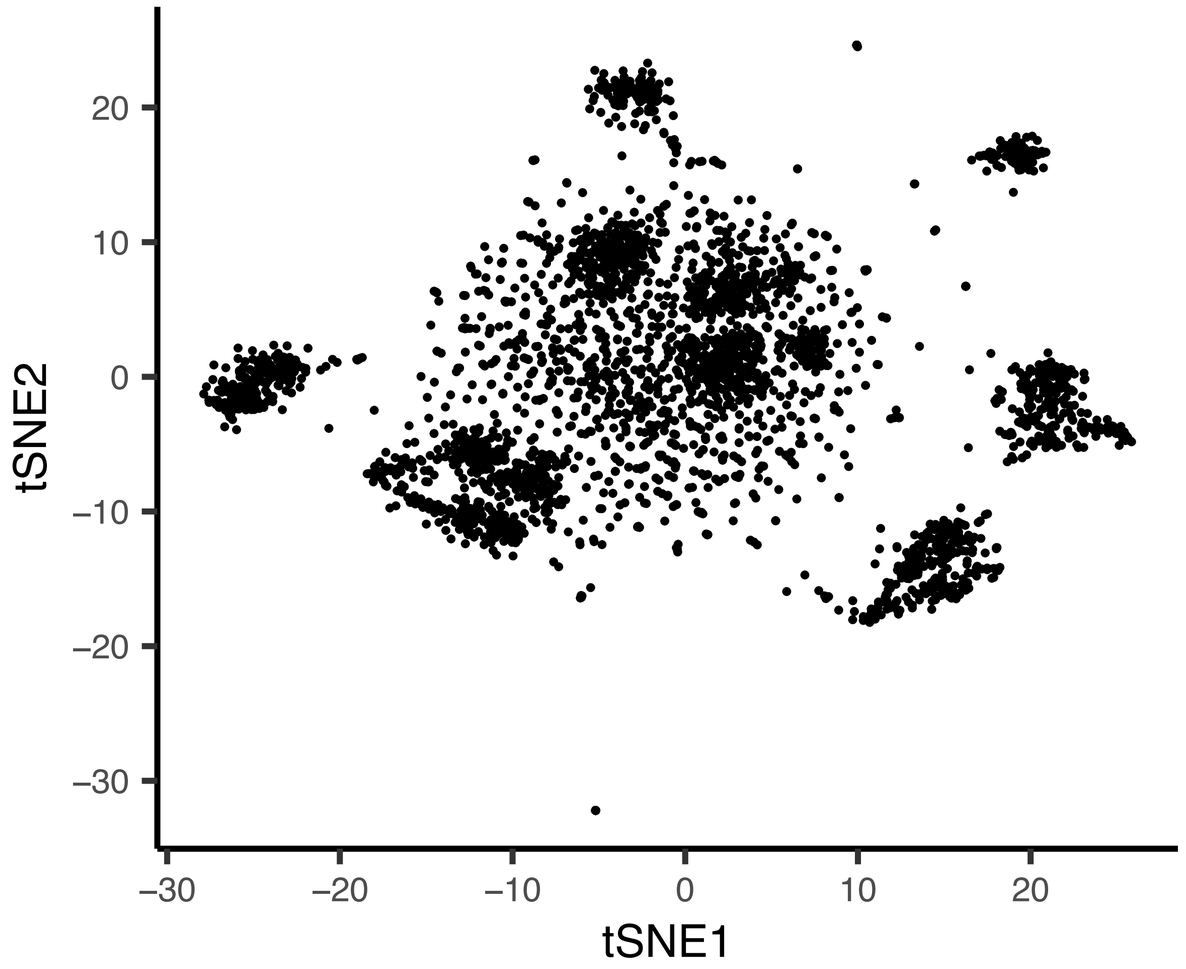


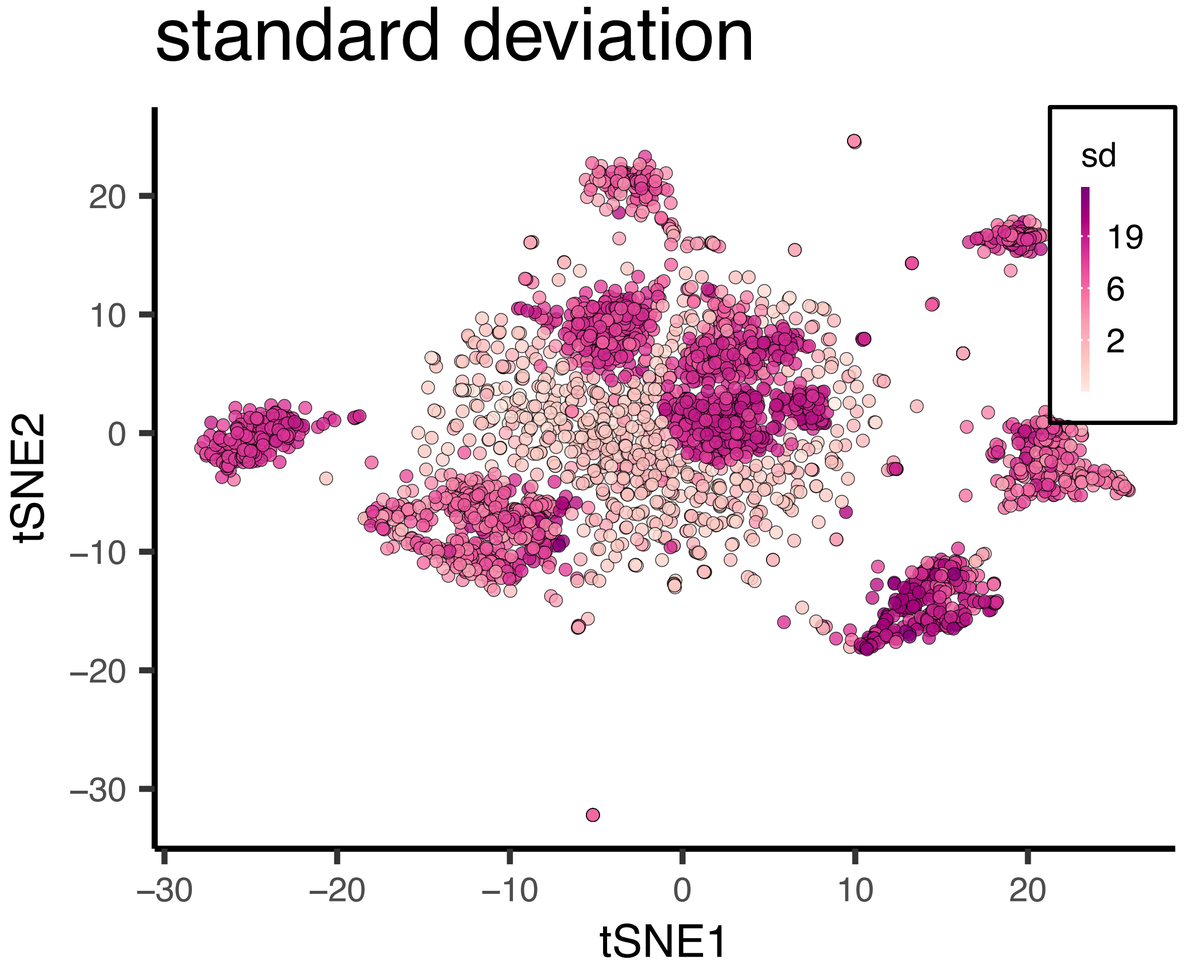
Exploratory Data Analysis with tSNE



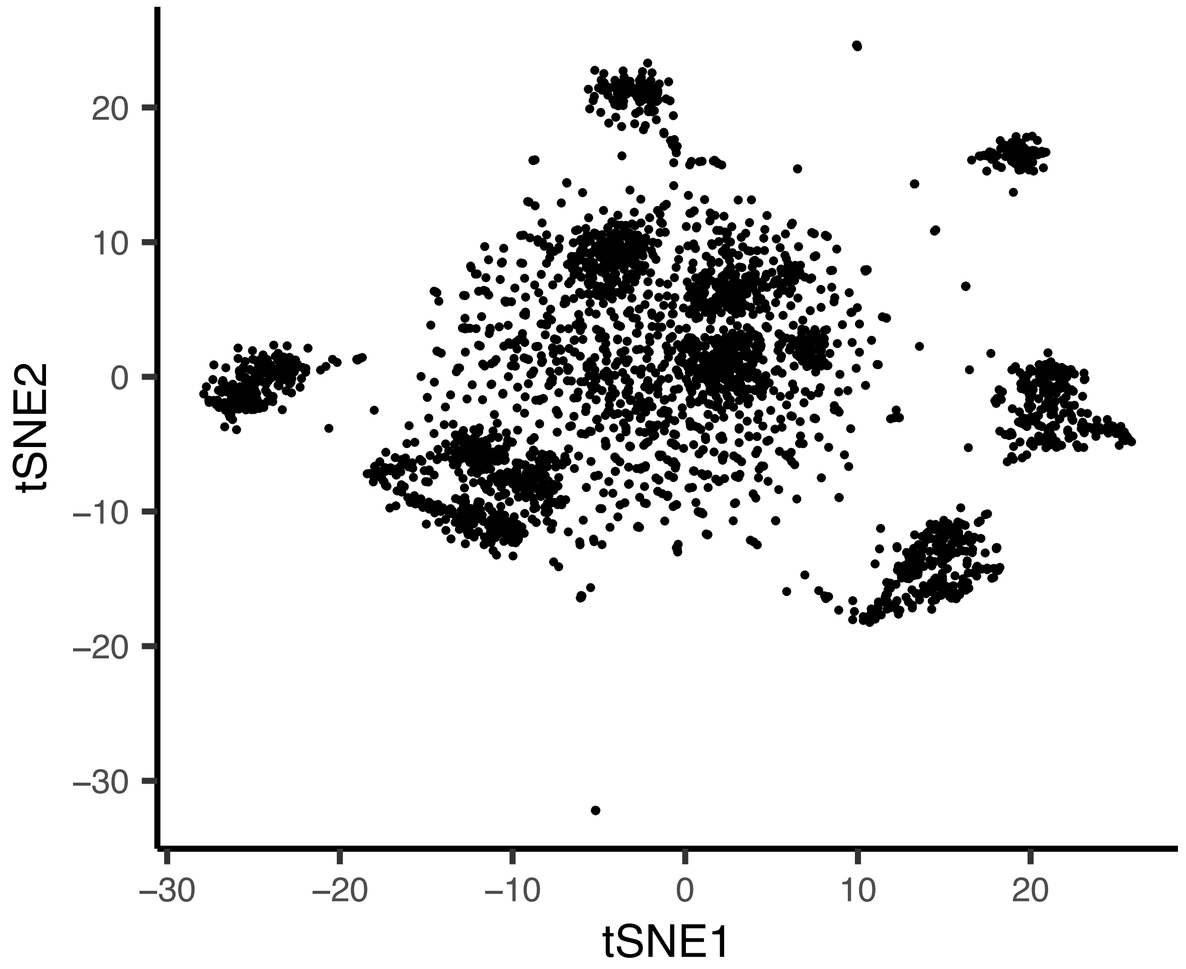


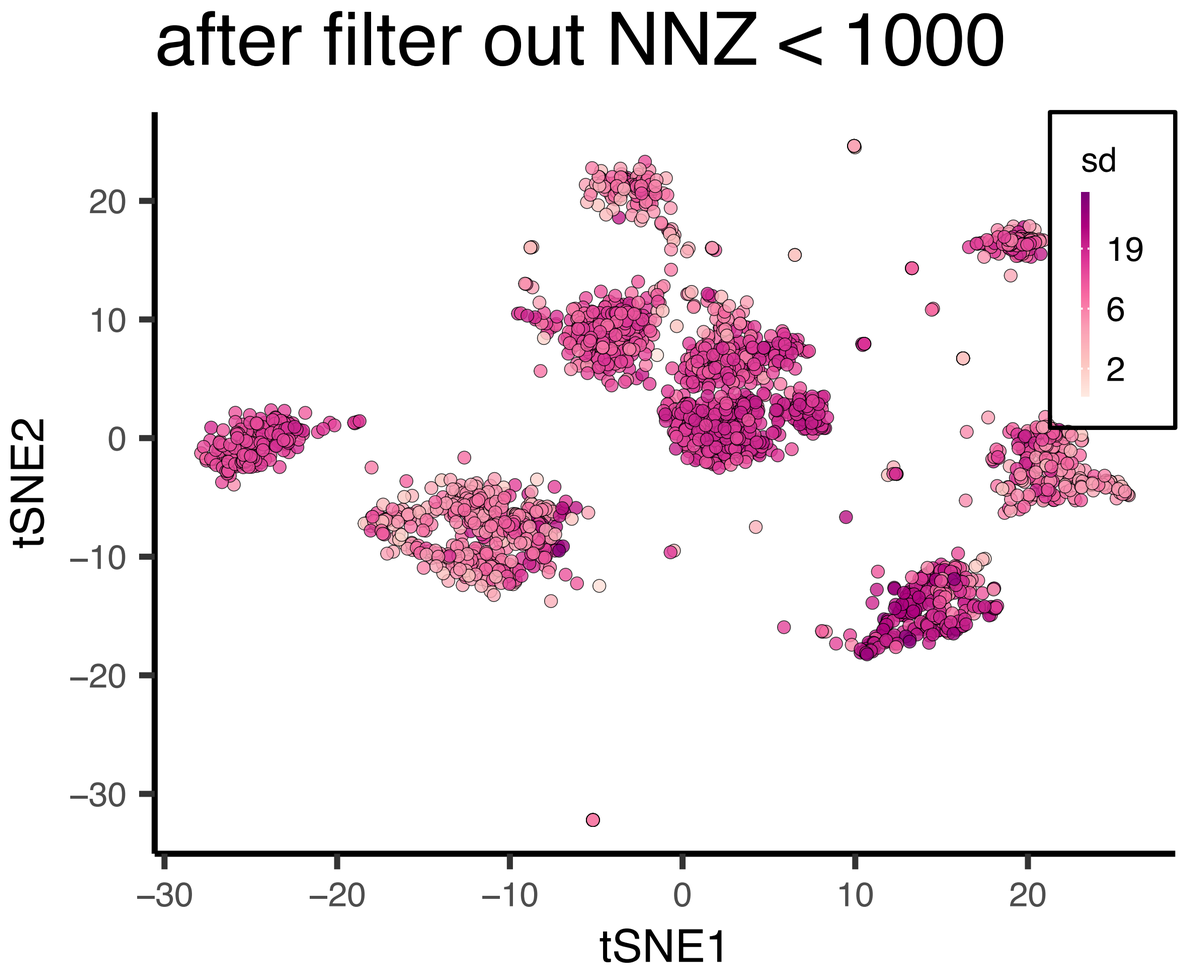
Exploratory Data Analysis with tSNE





Exploratory Data Analysis with tSNE



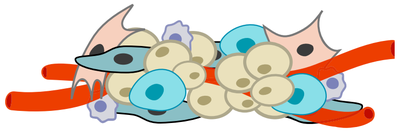


Today's lecture

- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

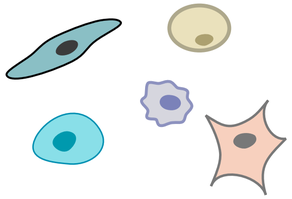
Each step is vulnerable to experimental/technical noise and human errors

Tissue procurement



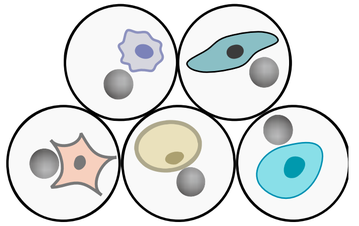
- Postmortem intervals?
- When and how did we get this sample?

A mixture of cells

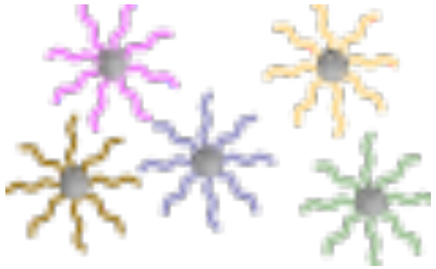


Sampling bias
(where to cut from bulk tissue)

One drop ≈ one cell

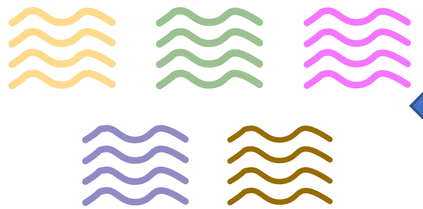


Each droplet could have more than one cell



STAMPs
(sc-Transcriptome Attached to Micro-Particles)

PCR artifacts, sequencing error



Sequencing

Gene x Cell counting matrix

Makosco *et al.* Cell (2015)

What is a doublet in single-cell data?

Biological/technical definition:

- One or more cells captured (usually at most two cells by chance)
- Thus, multiple cells accidental share the same cell barcode sequence
- Not so clear in general... since we missed the chance to assign different tags to different cells encapsulated in the same droplet.

Statistical definition:

- If we could find marker genes of multiple cell types are simultaneously expressed...
- An unvetted approach: Find ambiguous/intermediate coordinates in PCA/tSNE/UMAP (after removing ambient cells).

Can we create artificial doublets?

A straightforward definition (used in `DoubletFinder`):

For each cell i:

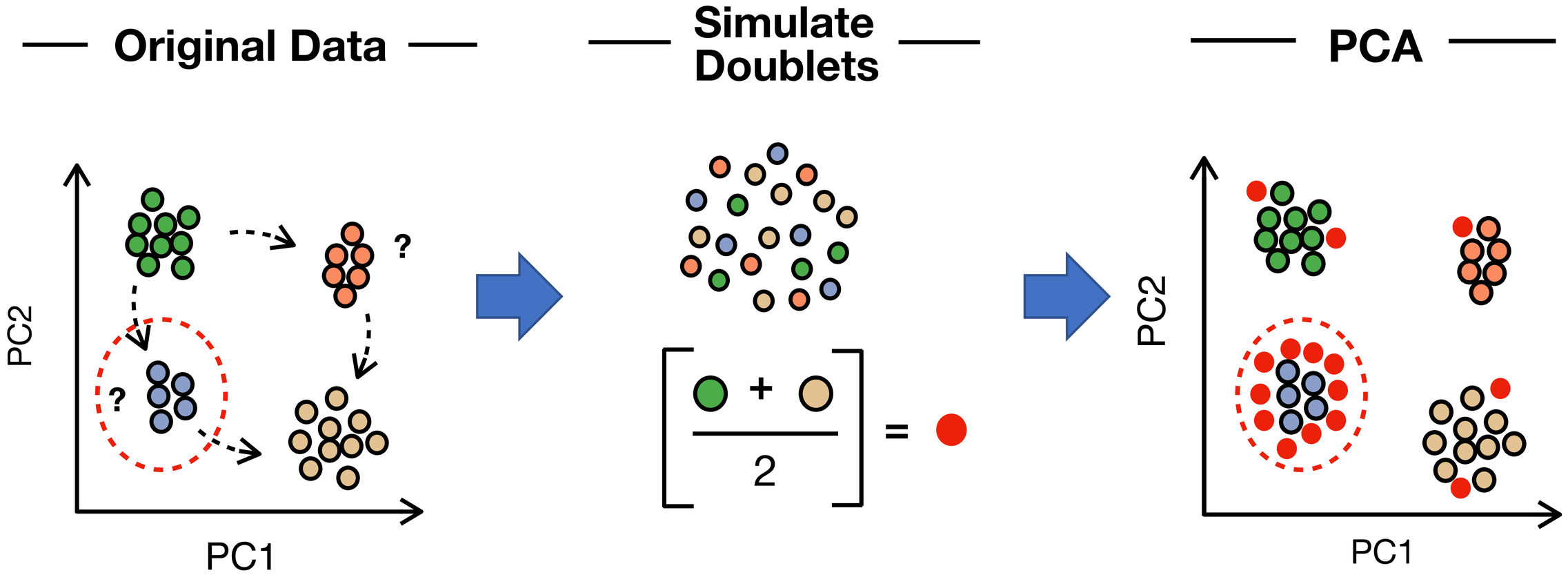
- Take some other j by random selection
- Create an artificial doublet

$$\tilde{\mathbf{x}} \leftarrow \frac{1}{2}(\mathbf{x}_i + \mathbf{x}_j)$$

Some thought questions:

- Doublets within the same cell type?
- Doublets between the different cell types?

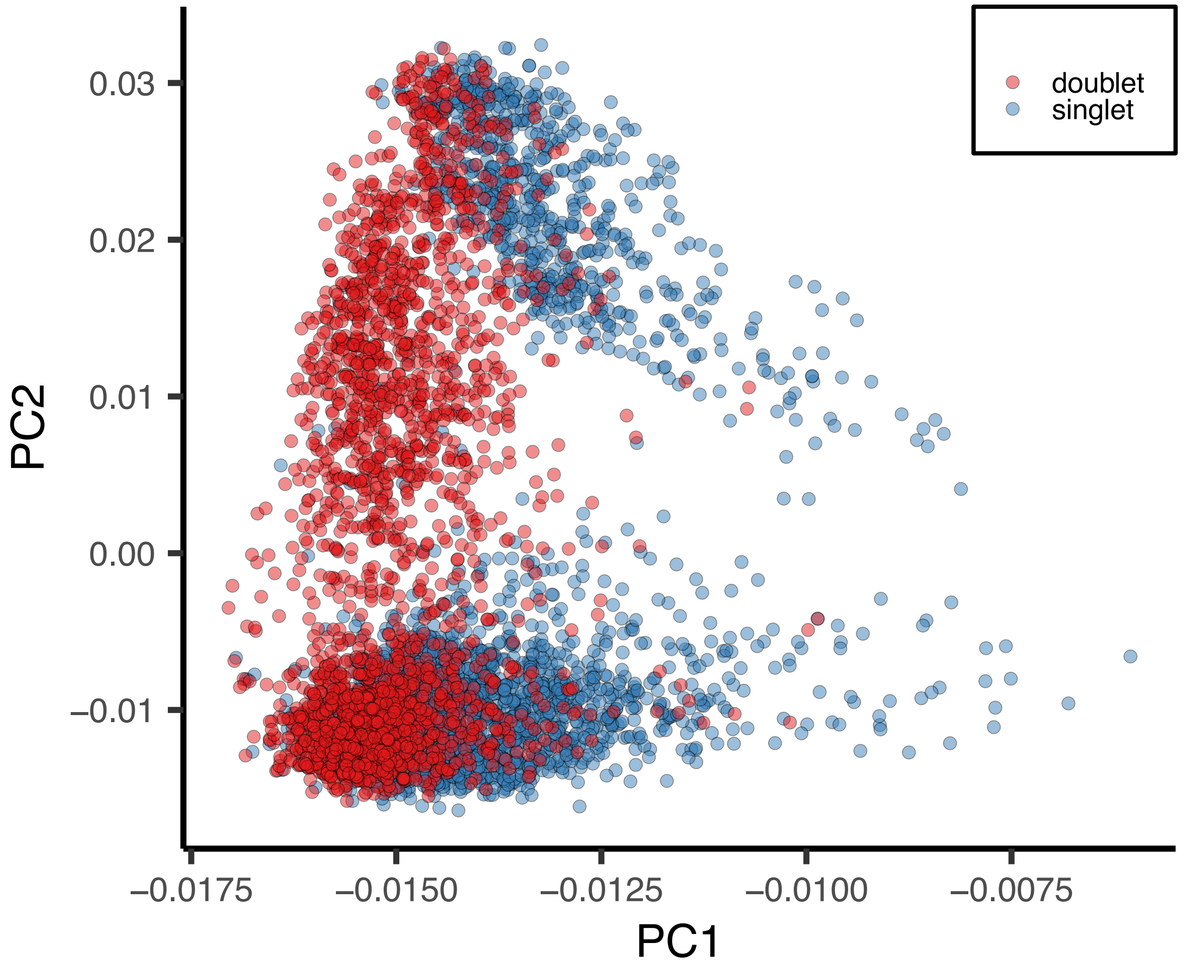
k-Nearest Neighbour classification for doublet detection



McGinnis et al. Cell Systems (2019)

McGinnis *et al.*, *Cell Systems* (2019)

Can you tell the difference by a quick visual inspection?



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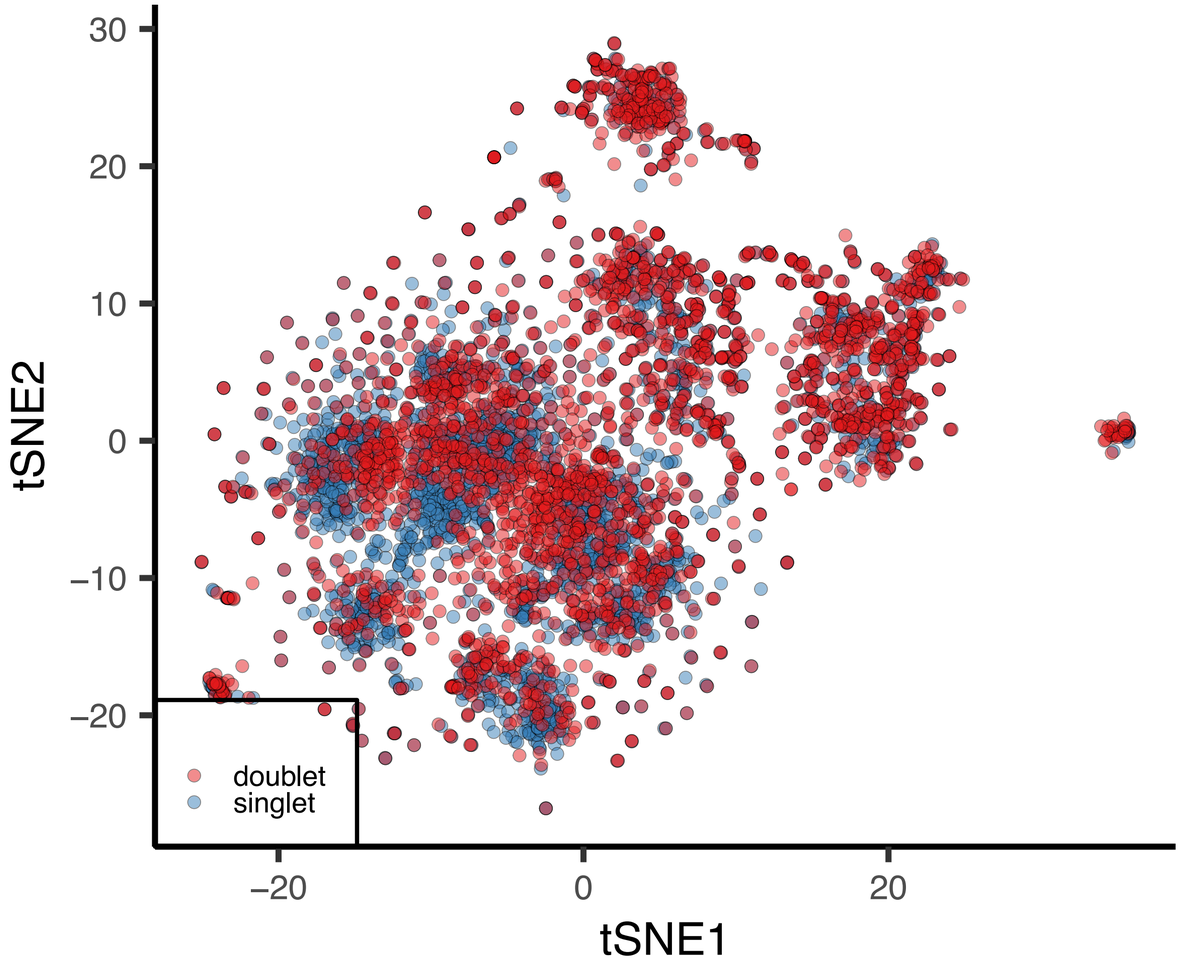

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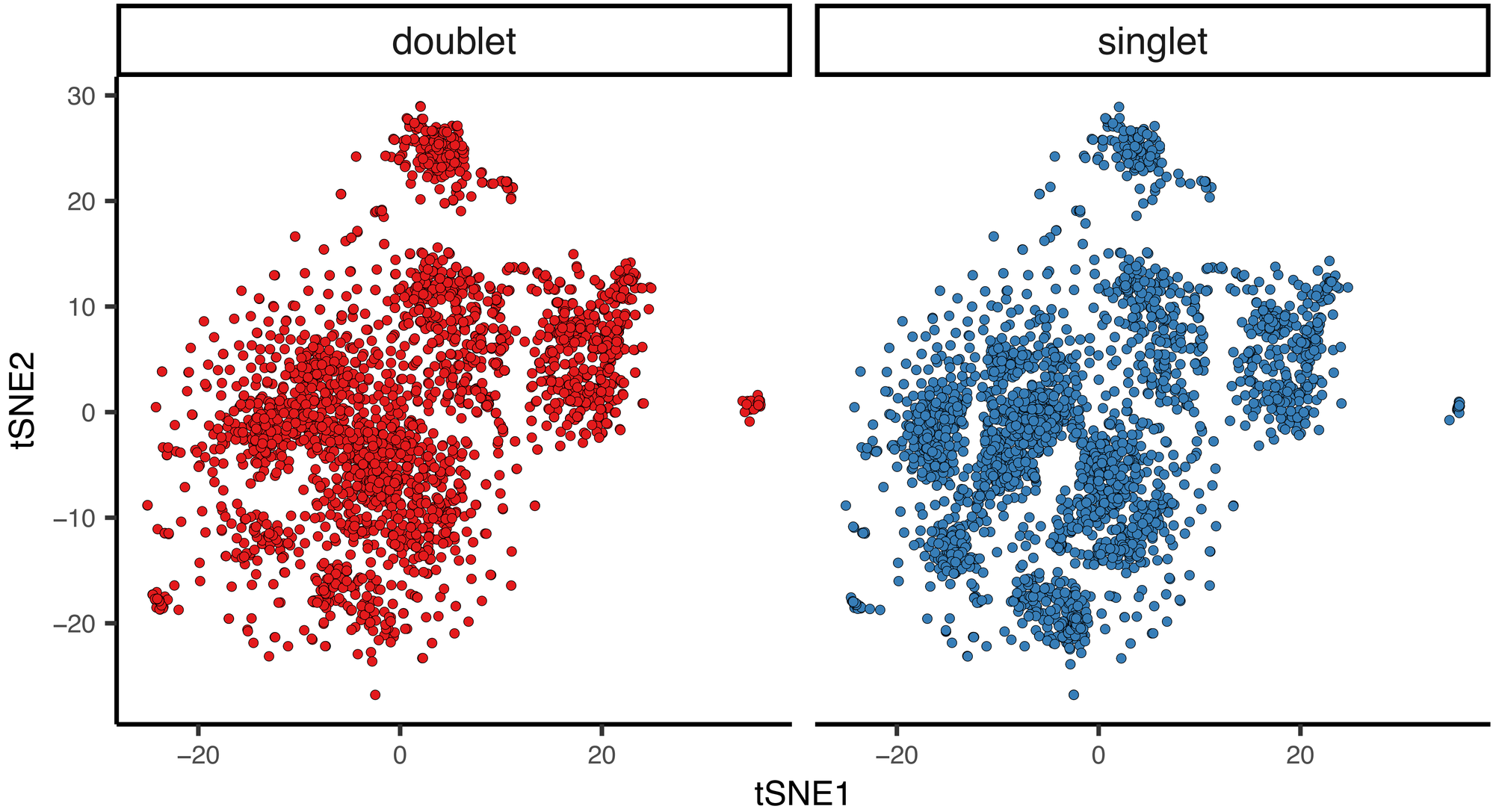
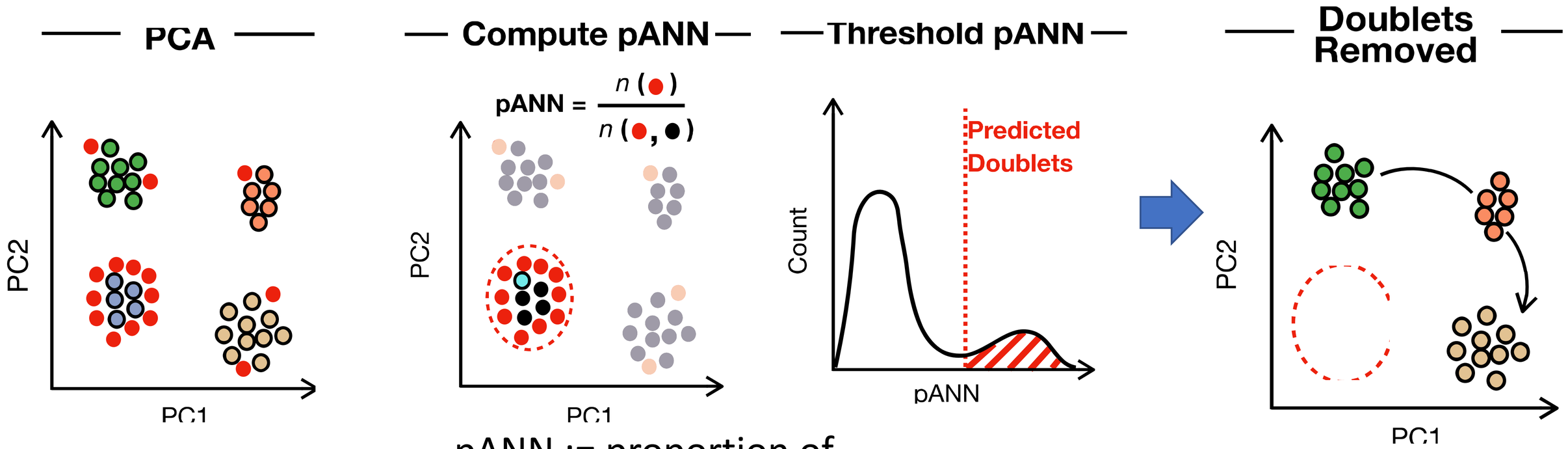


Doublet detection ≈ Identifying cell clusters mostly consisting of droplets



pANN := proportion of artificial nearest neighbors

McGinnis *et al.* Cell Systems (2019)

k-Nearest Neighbour classification for doublet detection

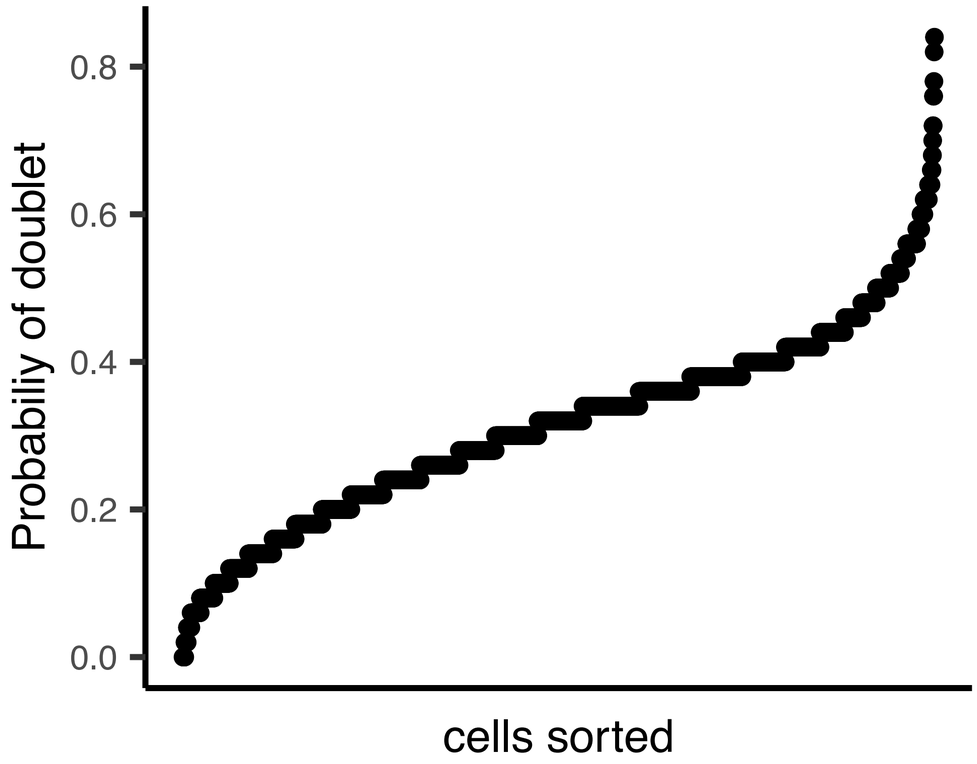
- Step 1. Create artificial doublets, $\tilde{\mathbf{x}}$
- Step 2. Mix them with the original cells and perform PCA
- Step 3. Find nearest neighbours of the original cells (using #PC=50)
- Step 4. Count the number of doublets in the neighbourhood

k-Nearest Neighbour classification for doublet detection

- **Q: How many of my neighbours are indeed a doublet?**

$$\hat{P}_i = \frac{1}{|\mathcal{N}(i)|} \sum_{j \in \mathcal{N}(i)} I\{j \text{ is a doublet}\}$$

Key assumption: There is a principal component that can set apart hidden doublets from the most of singlets.



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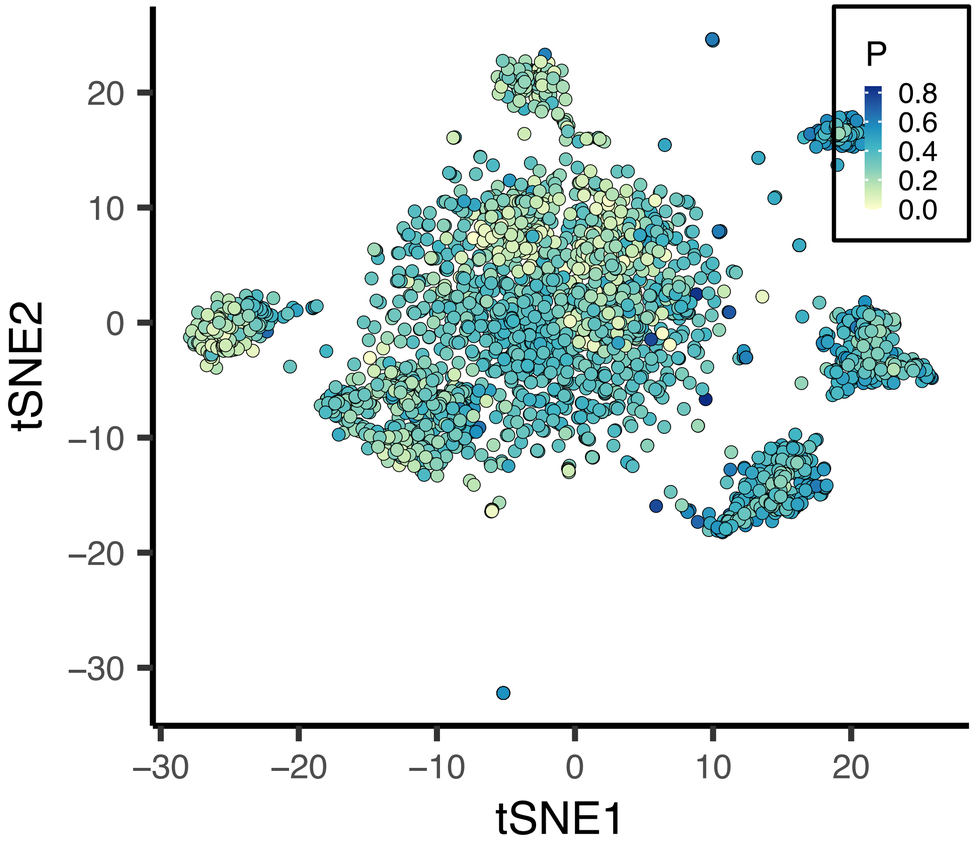

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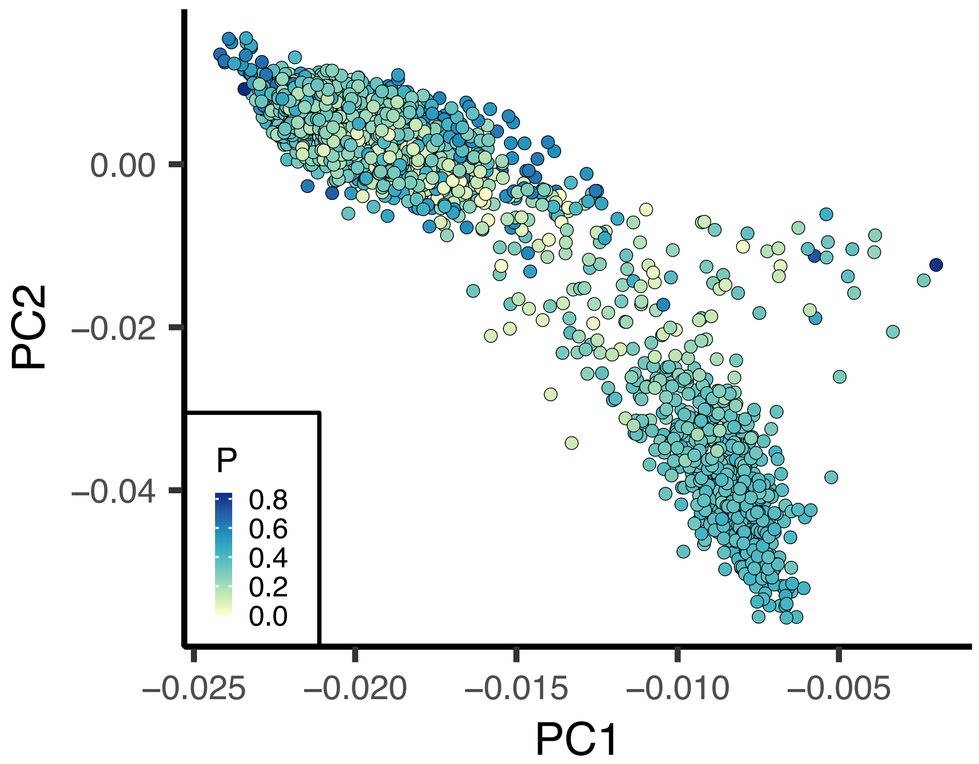

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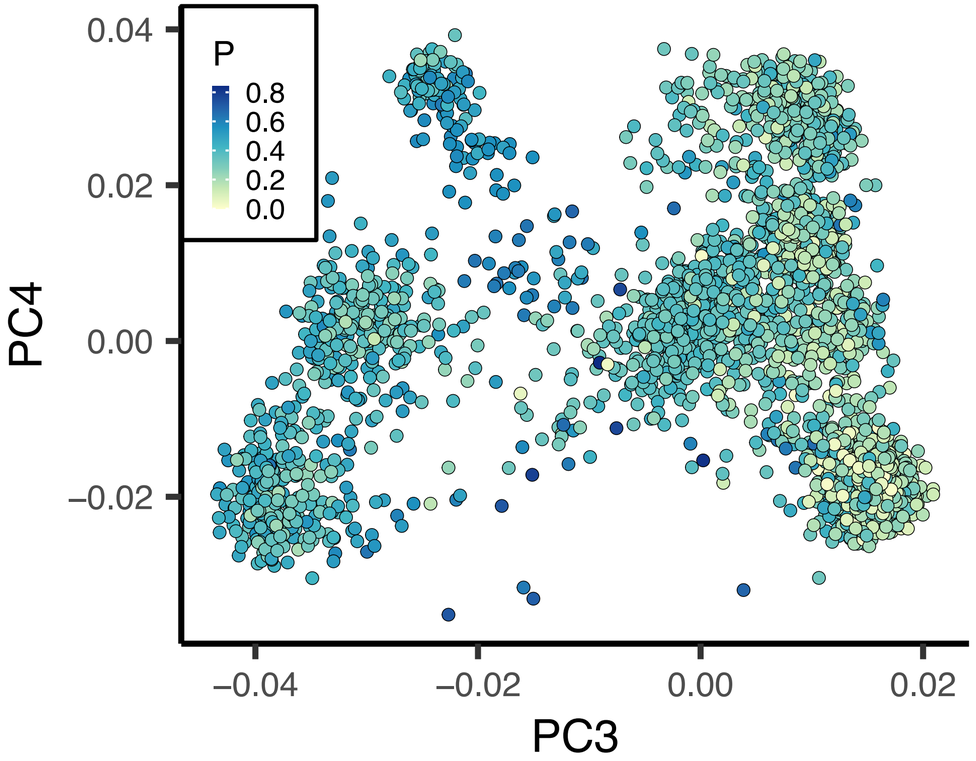

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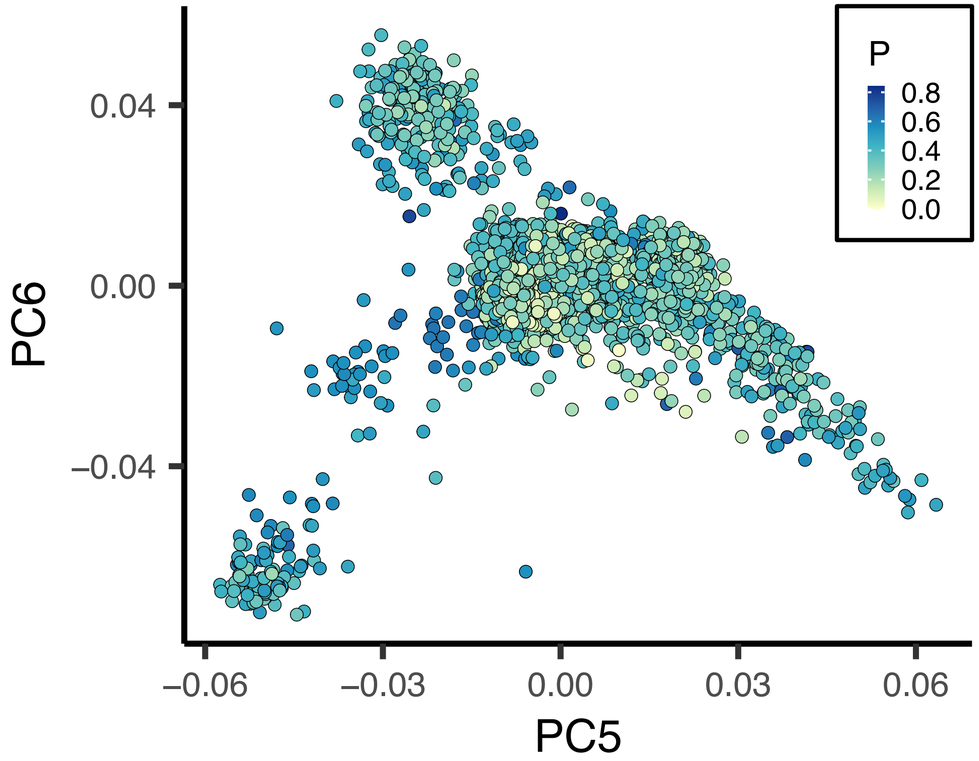
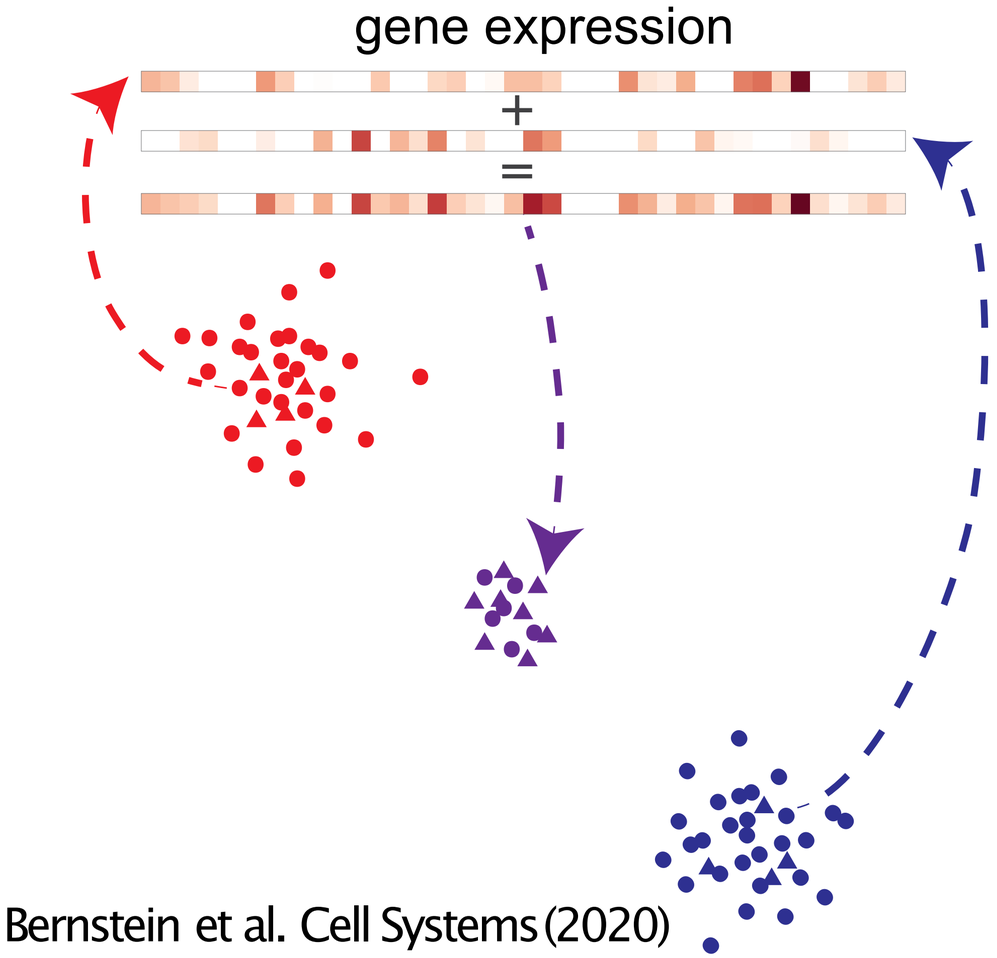
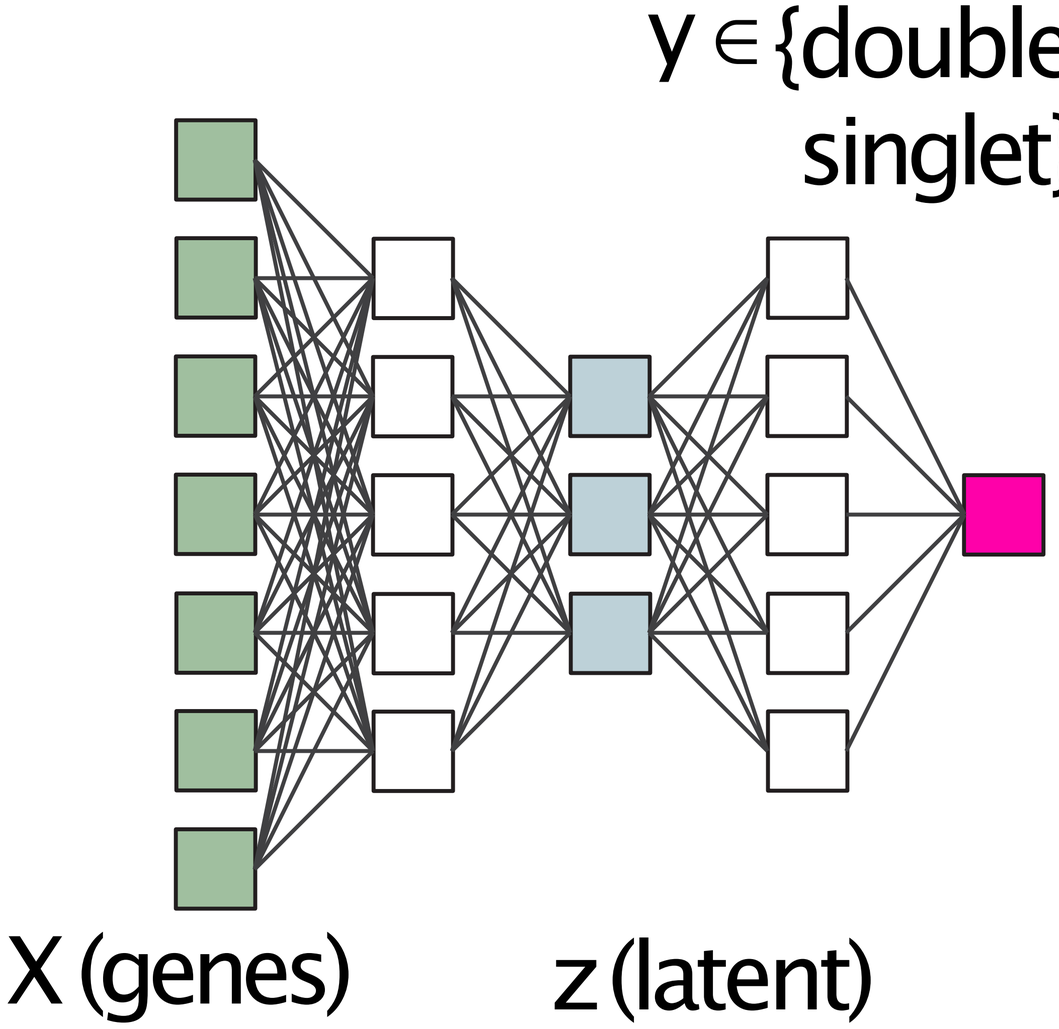


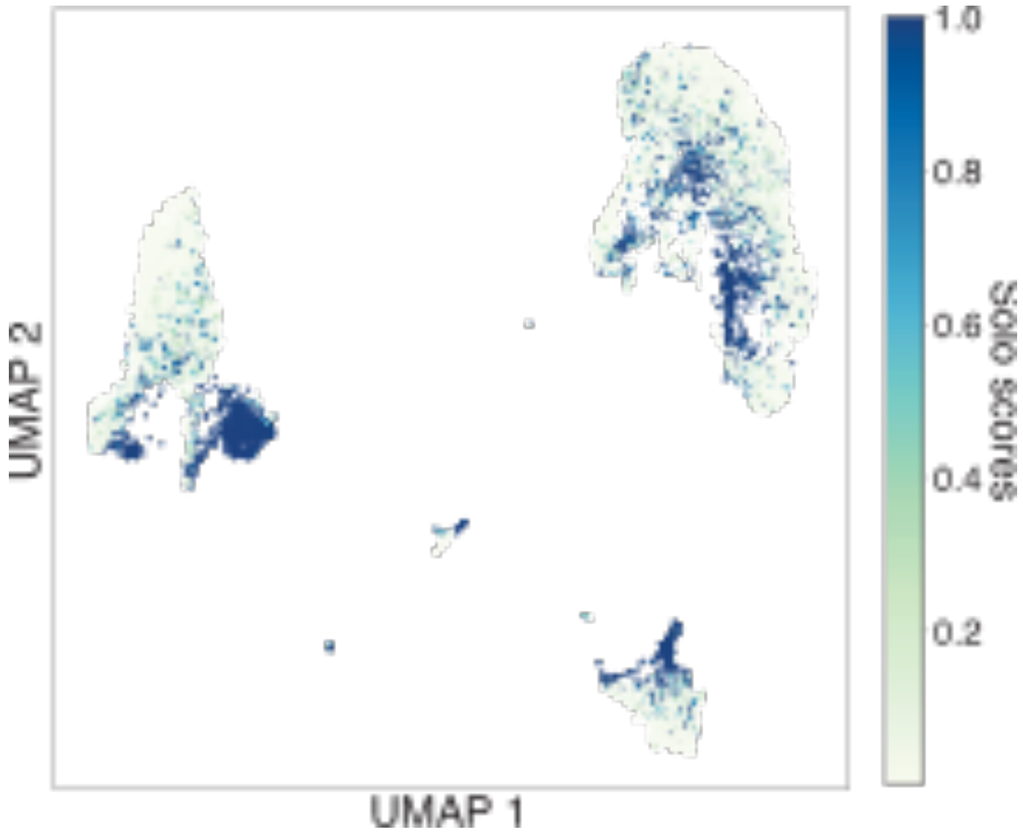
Artificial Neural Network-based classification for doublet detection

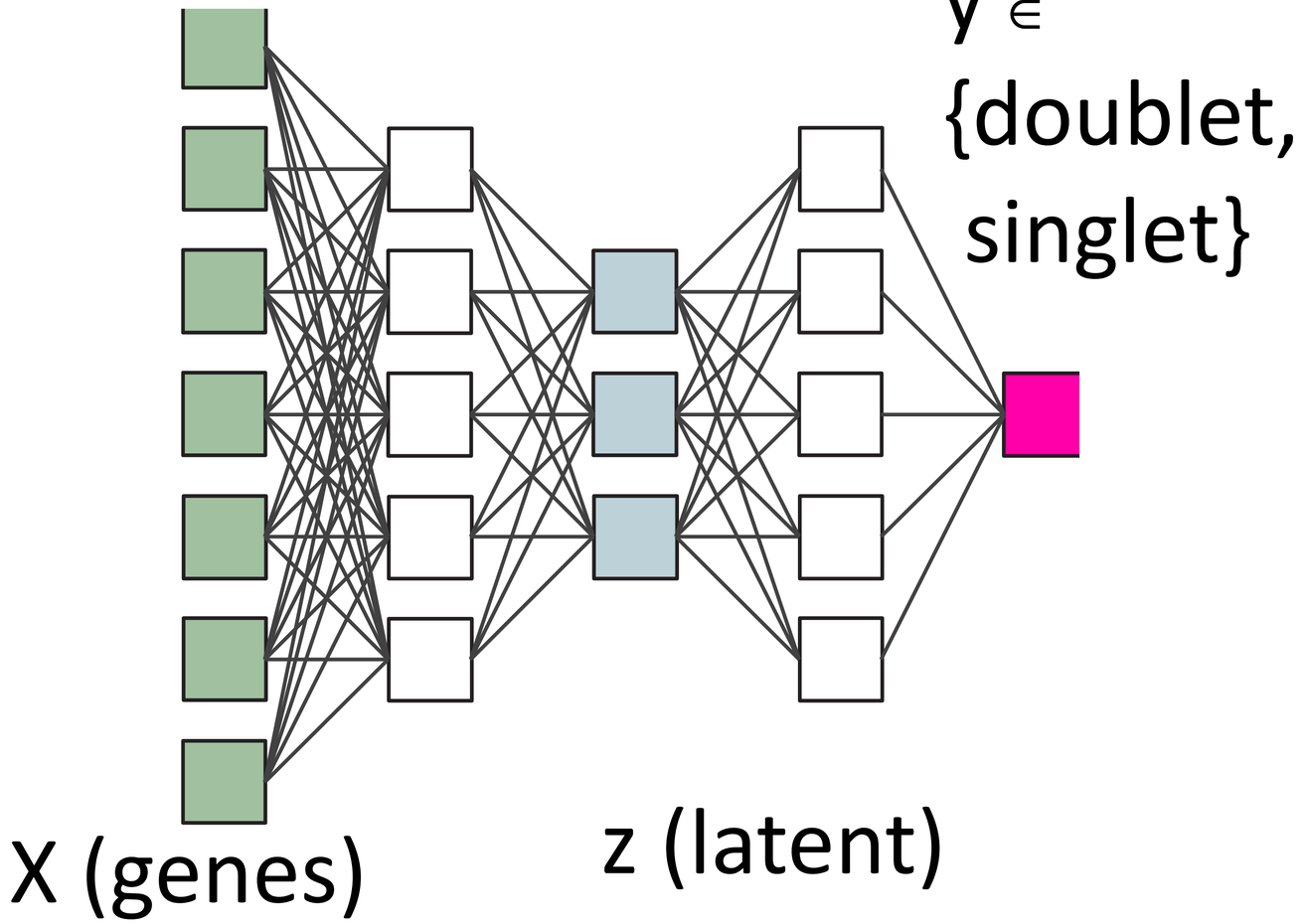


Bernstein et al. Cell Systems (2020)



Doublet classification by supervised ML (a deep Neural Network model)





Bernstein *et al.* Cell Systems (2020)

Training a parametric classifier to discern doublets vs. singlets

$$f : \mathbf{x}_i \rightarrow y_i,\ y \in \{0, 1\}$$

$$\prod_{i=1}^{n} f(\mathbf{x}_i)^{y_i} (1 - f(\mathbf{x}_i))^{1-y_i}$$

```
build.classifier <- nn_module(
 classname = "classfier",
 initialize = function(d, k = 5) {
  self$fc <- nn_sequential(
   nn_batch_norm1d(d), nn_linear(d, k),
   nn_batch_norm1d(k), nn_relu(),
   nn_linear(k, 2*k),
   nn_batch_norm1d(2*k),
   nn_relu(), nn_linear(2*k, 1),
   nn_sigmoid())
 },
 forward=function(x, min_=.01, max_=.99) {
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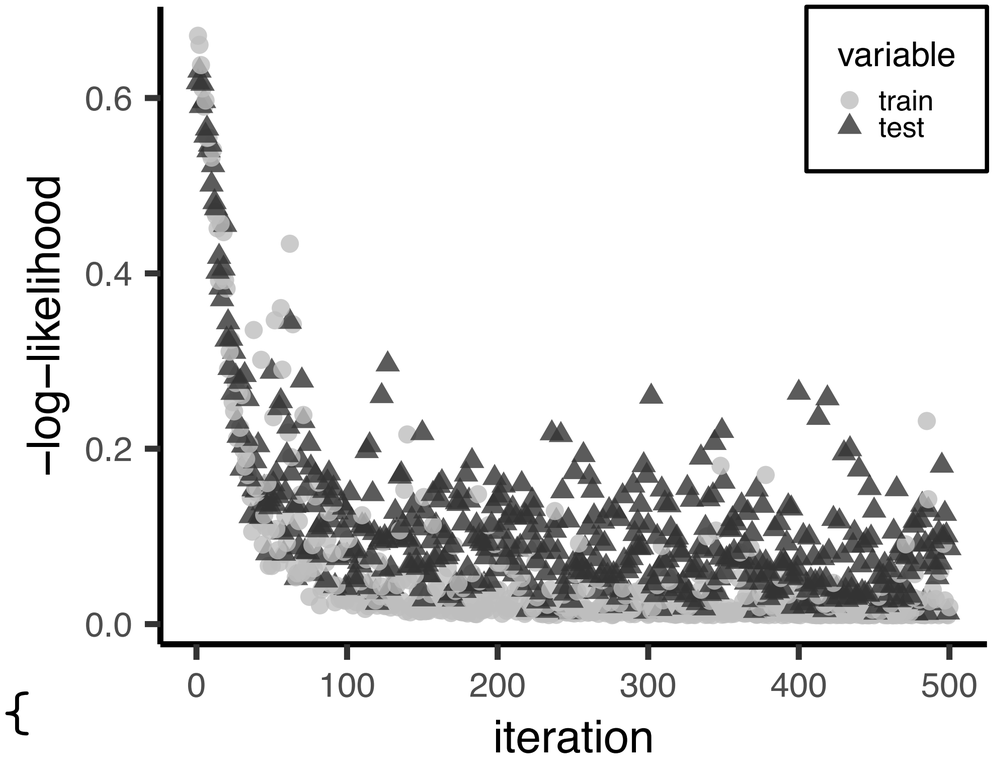

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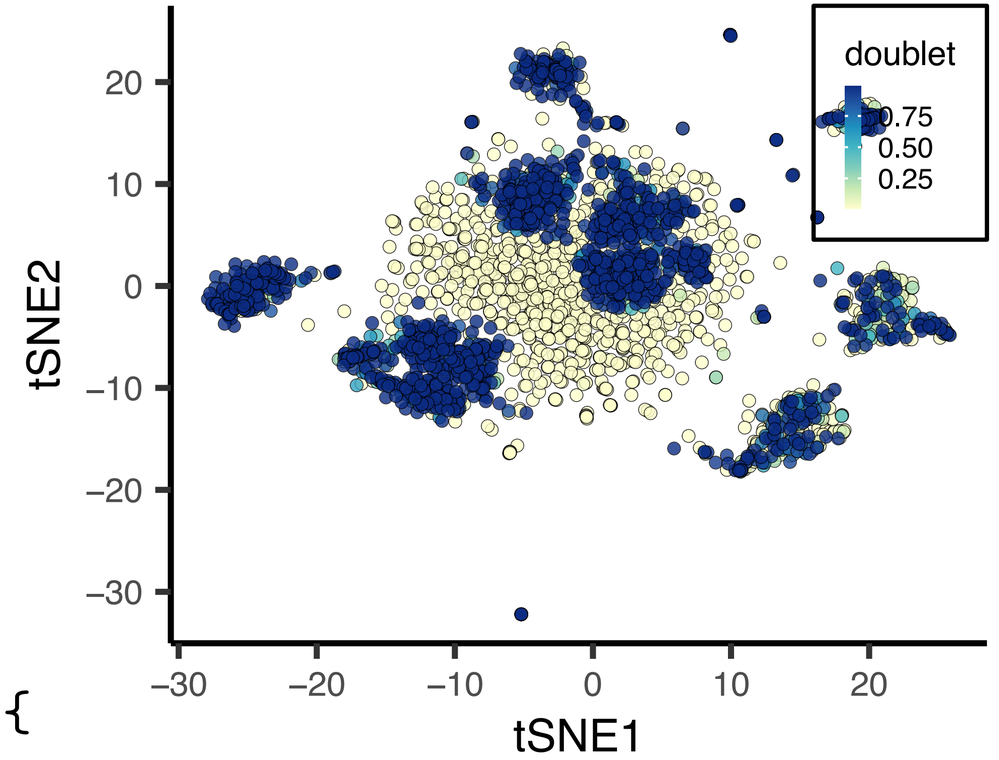
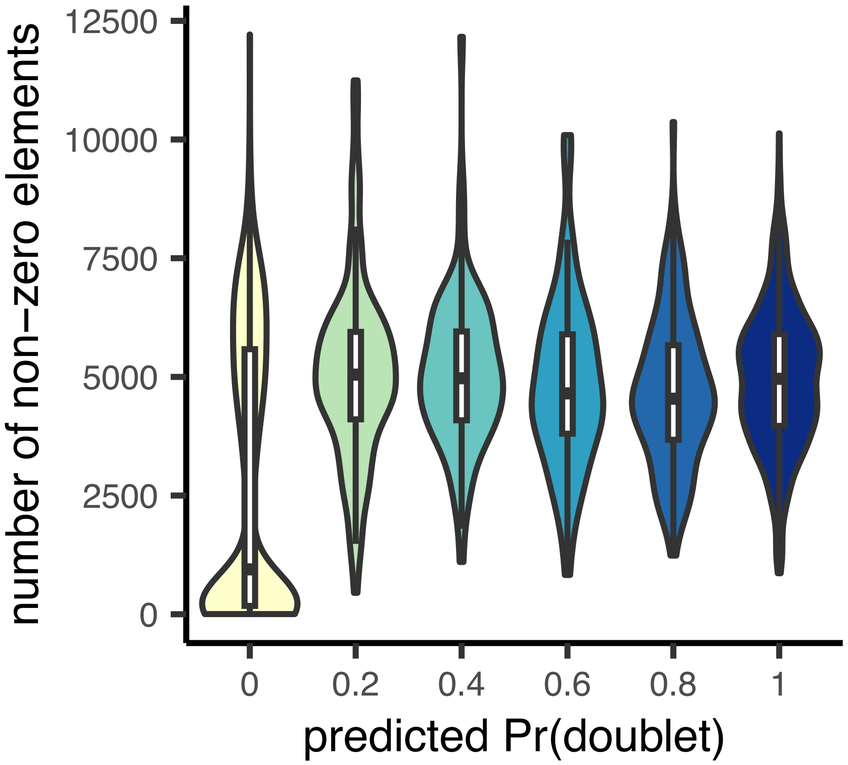
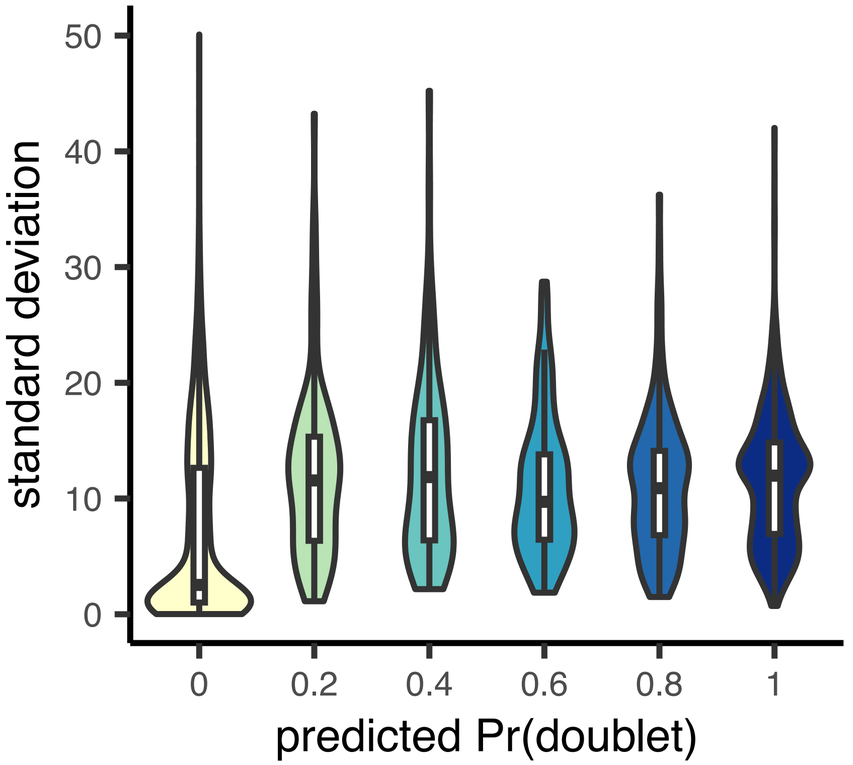


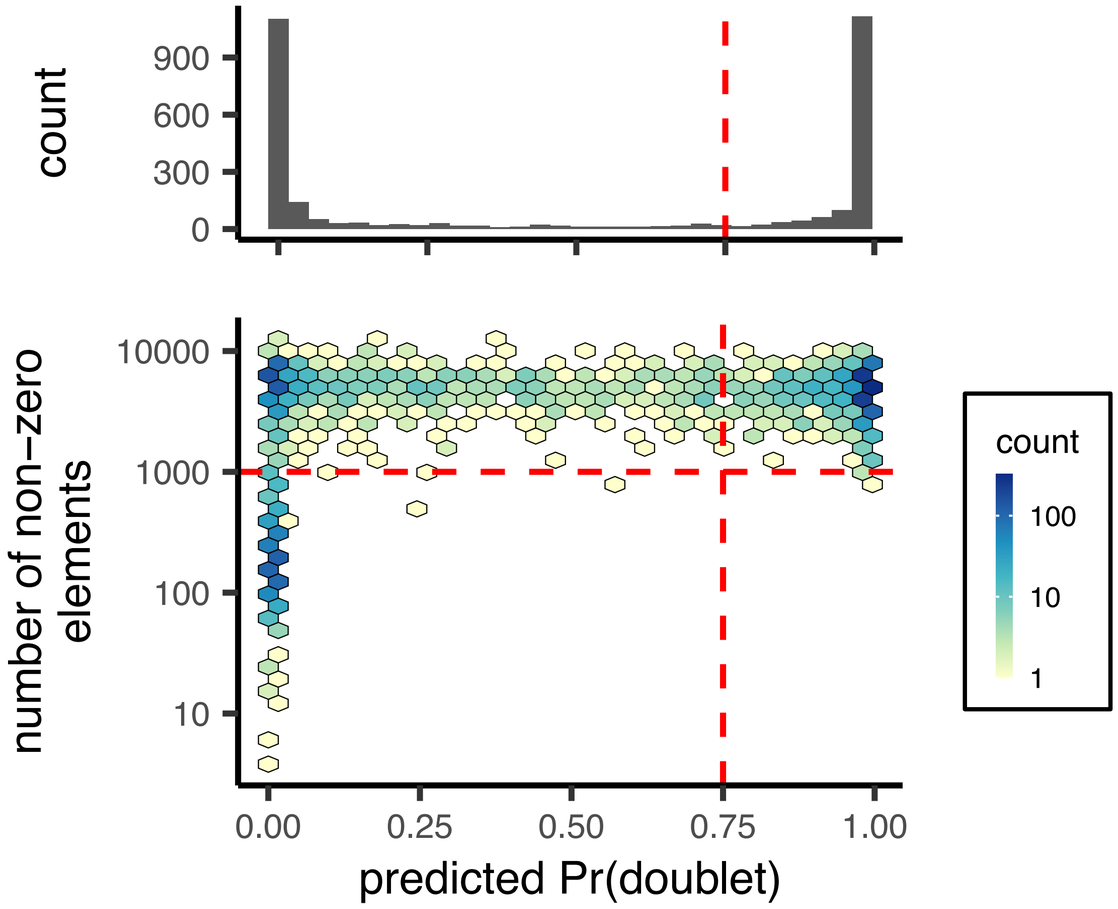
The predicted doublets generally correspond to cells with few non-zero elements





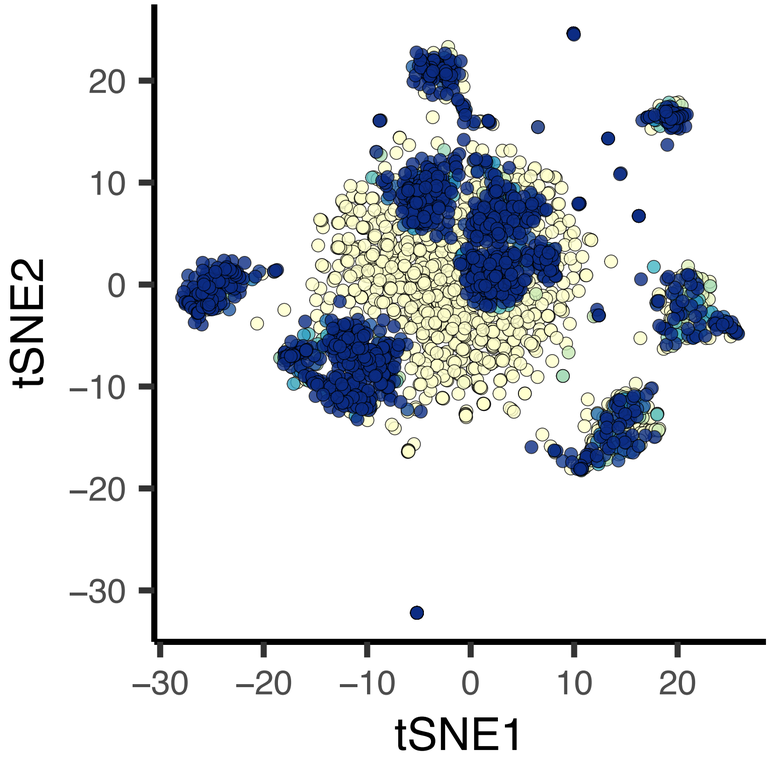
Low expression within a cell may stem from unwanted burst-out cells or ambient RNA molecules.

The predicted doublets generally correspond to cells with few non-zero elements

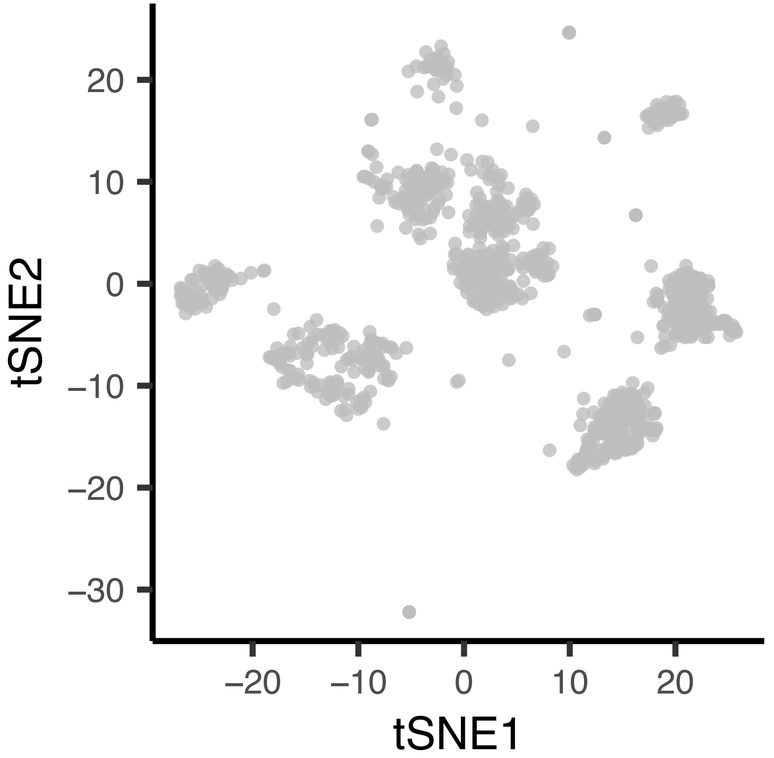


After removing potential doublets

- All the cells

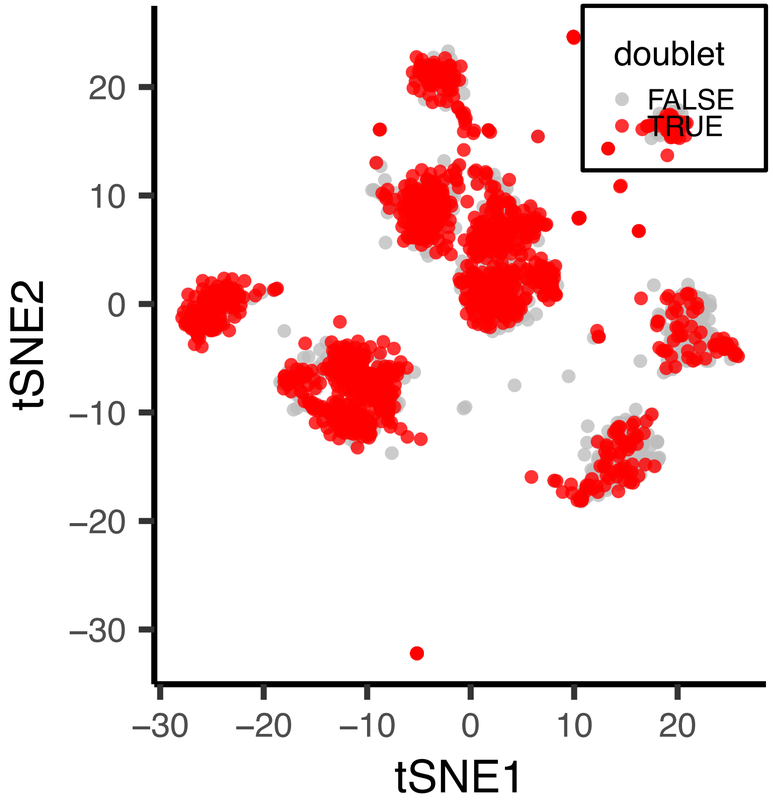


- P(doublet) $<$ .75, #non-zeros $>$ 1k



Maybe it is more than just low expression cells

- #non-zeros $>$ 1k



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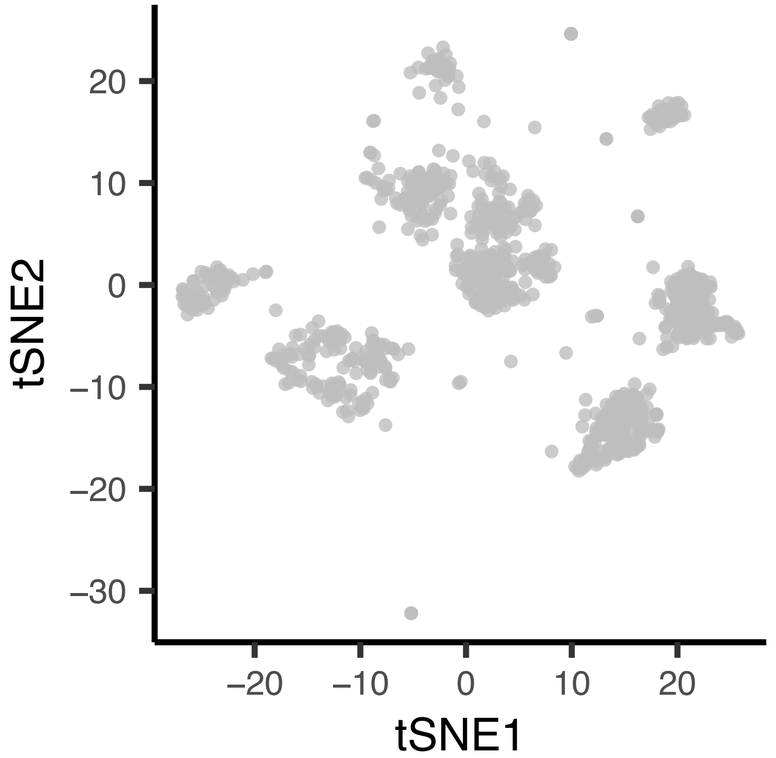


Discussion on doublet Q/C

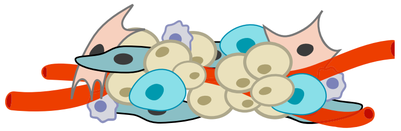
- It's a routine unique in single-cell sequencing data analysis
- Do we need it in practice? How frequently doublets emerge?
- Perhaps a majority of them simply stem from "dying" cells or broken cells... If so, we can just filter out low-expressed cells?
- **Big assumption**: There are doublet cells. Is it true?

Today's lecture

- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

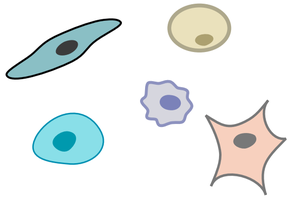
Each step is vulnerable to experimental/technical noise and human errors

Tissue procurement



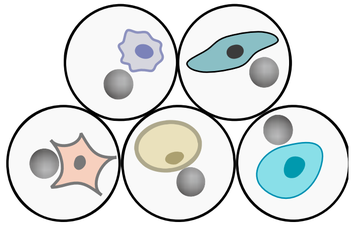
- Postmortem intervals?
- When and how did we get this sample?

A mixture of cells

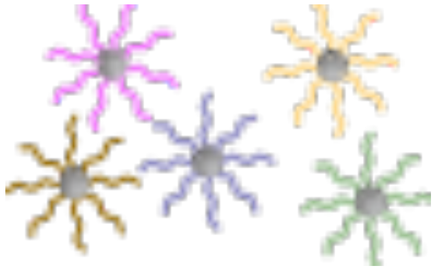


Sampling bias
(where to cut from bulk tissue)

One drop ≈ one cell

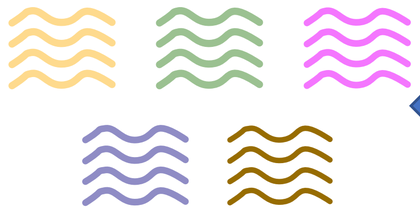


Each droplet could have more than one cell



STAMPs
(sc-Transcriptome Attached to Micro-Particles)

PCR artifacts, sequencing error



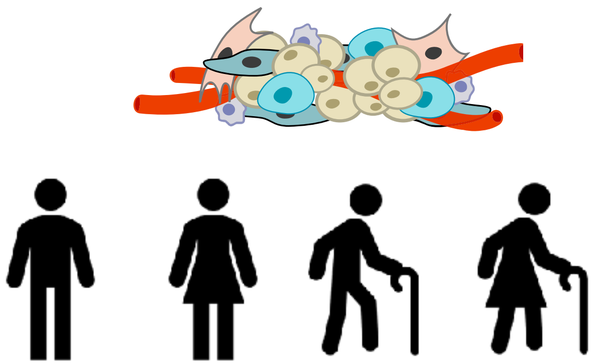
Sequencing

Gene x Cell counting matrix

Makosco *et al.* Cell (2015)

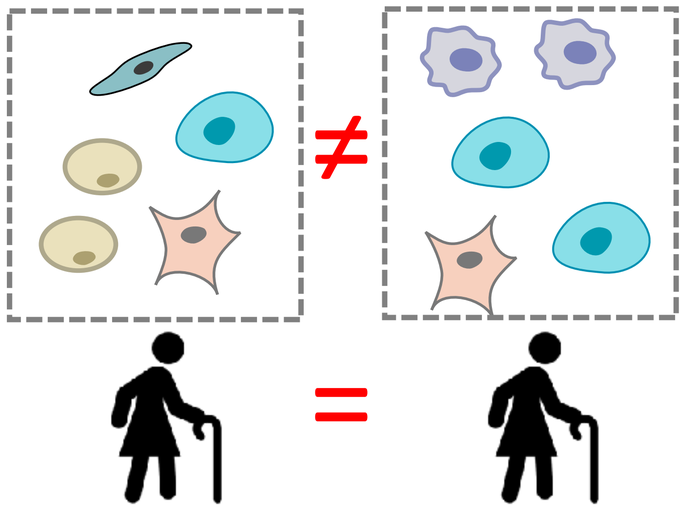
Batch effect & skewed distrib. of cell types

Tissue procurement on **multiple** samples (batches)



Batch effect can distort inherent biological expression levels

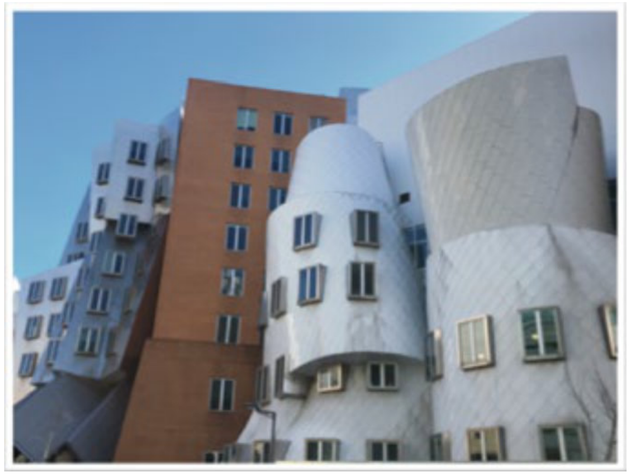
A bag of **multiple** cell types



Different batches could contain different cell types (due to sampling bias, technical noise)

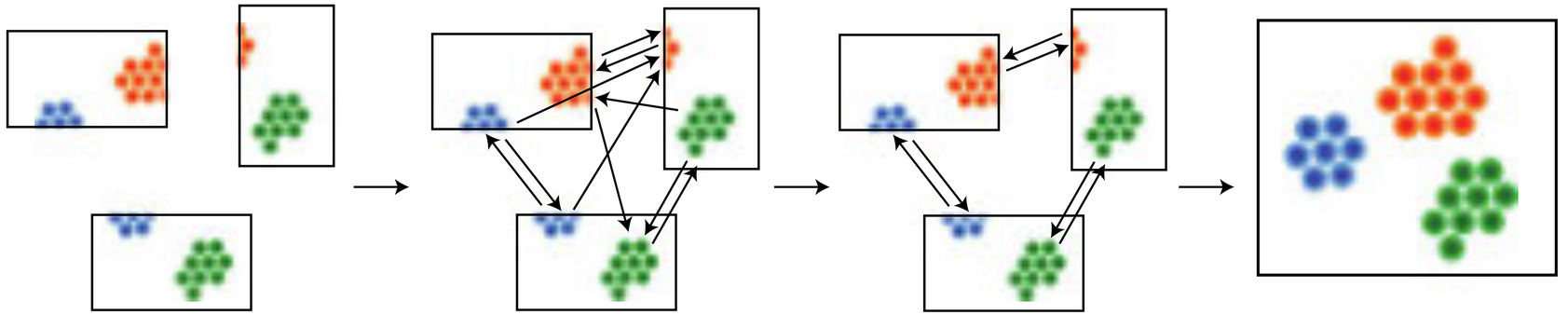
Batch normalization for joint analysis across multiple scRNA-seq data

snapshots
of many data



panorama stitched
together

How do we integrate multiple samples/batches?



Collect many single-cell RNA-seq experiments

Find nearest neighbours across data sets

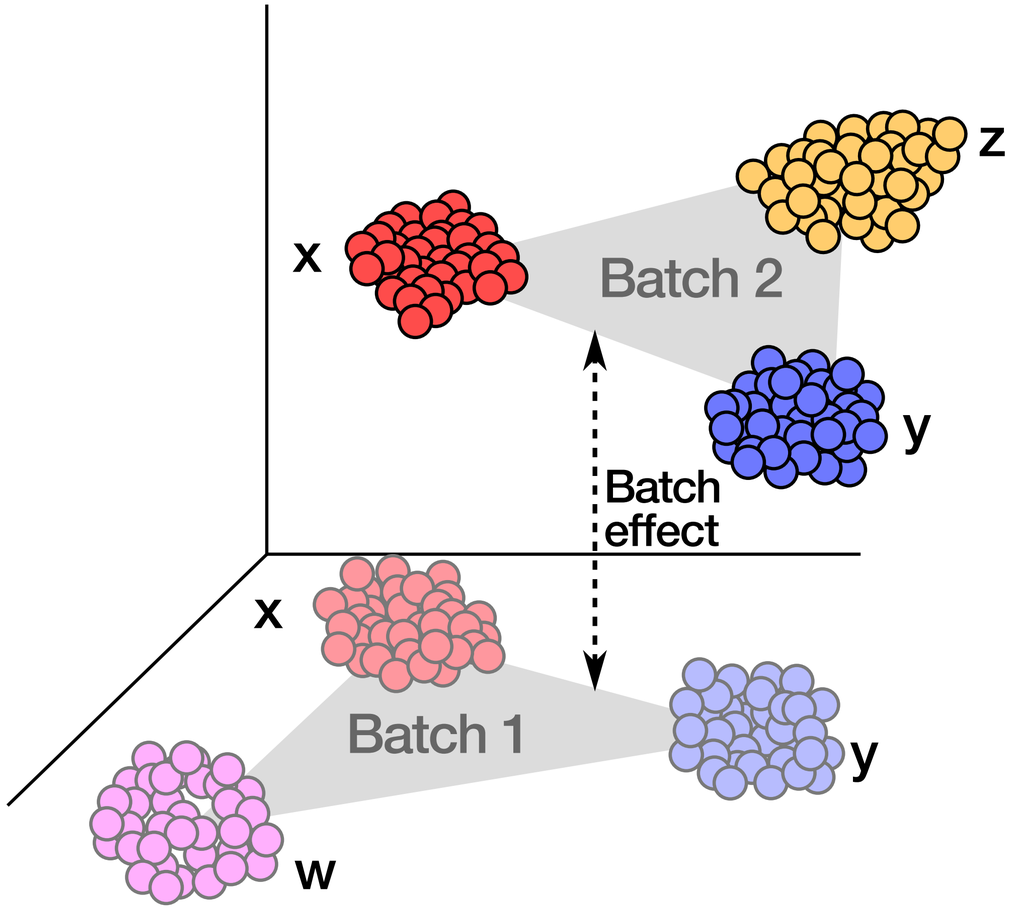
Keep mutually neighbouring cell pairs

Create single-cell panorama

Scanorama: mutual nearest neighbourhood-based data integration

Hie, Bryson, Berger *Nature Biotechnology* (2019)

Batch normalization aims to minimize the difference between nearest cells across different batches



Haghverdi, .., and Marioni, *Nature Biotechnology* (2018)

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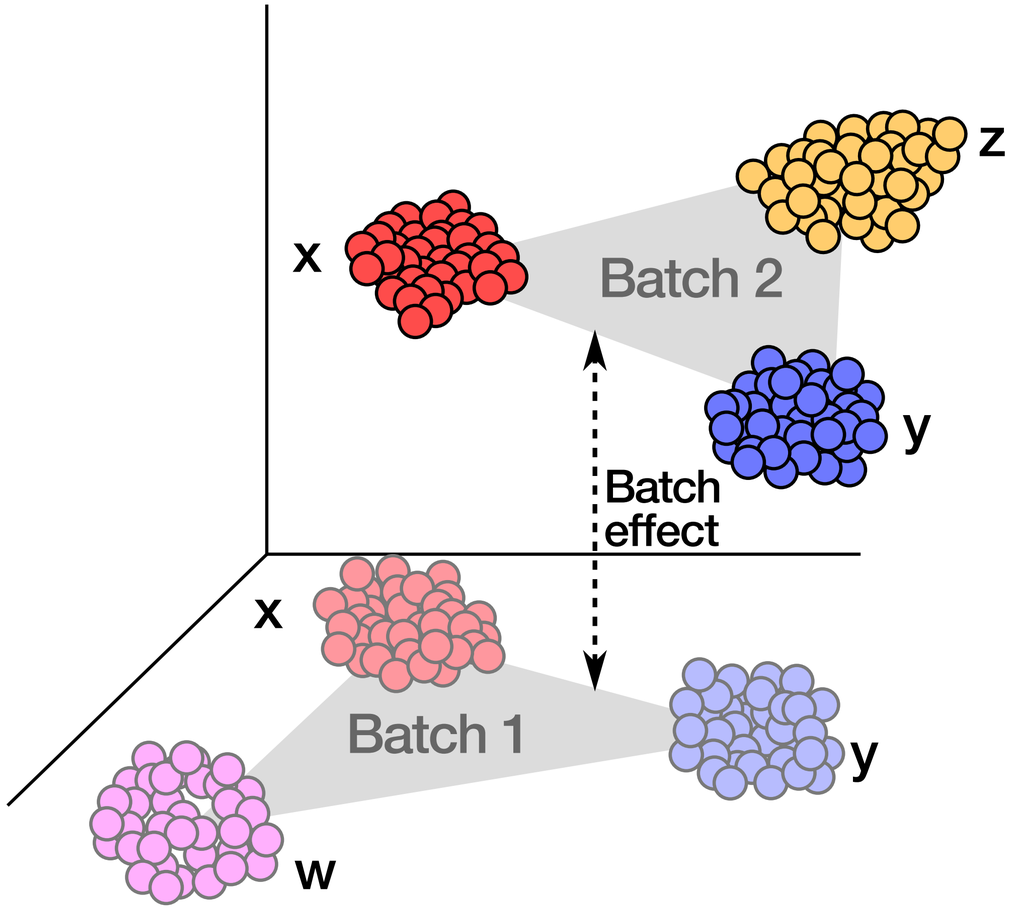

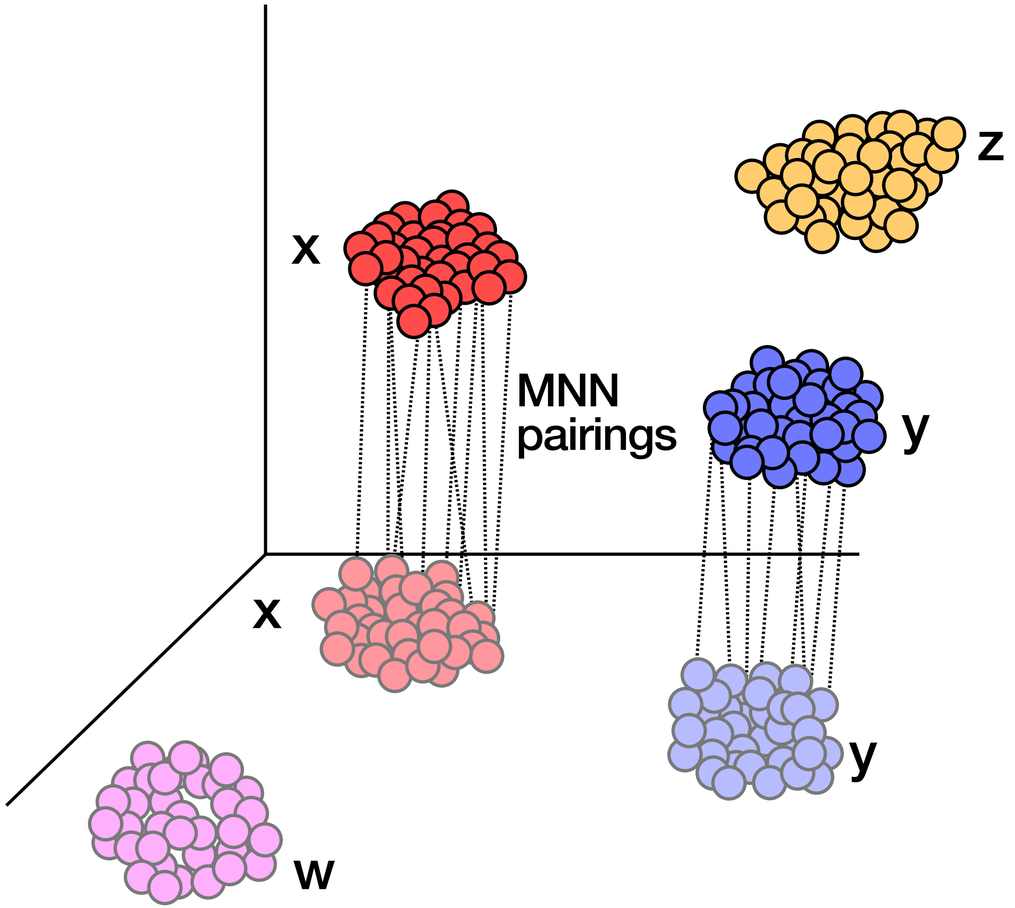

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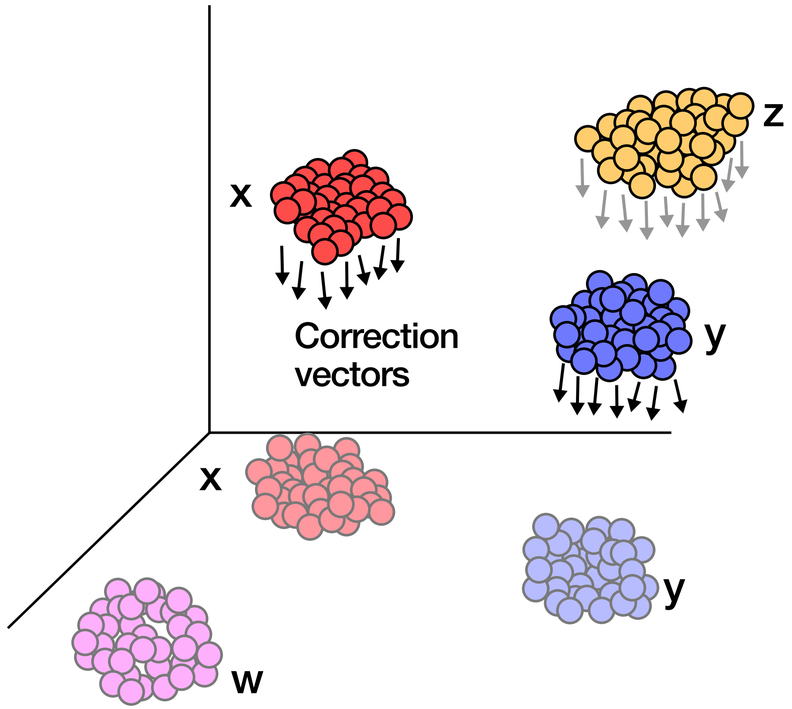

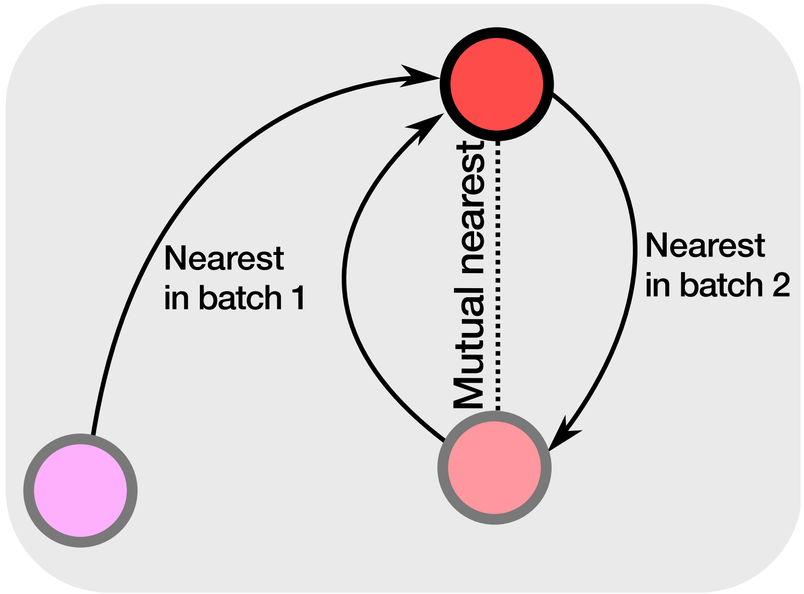

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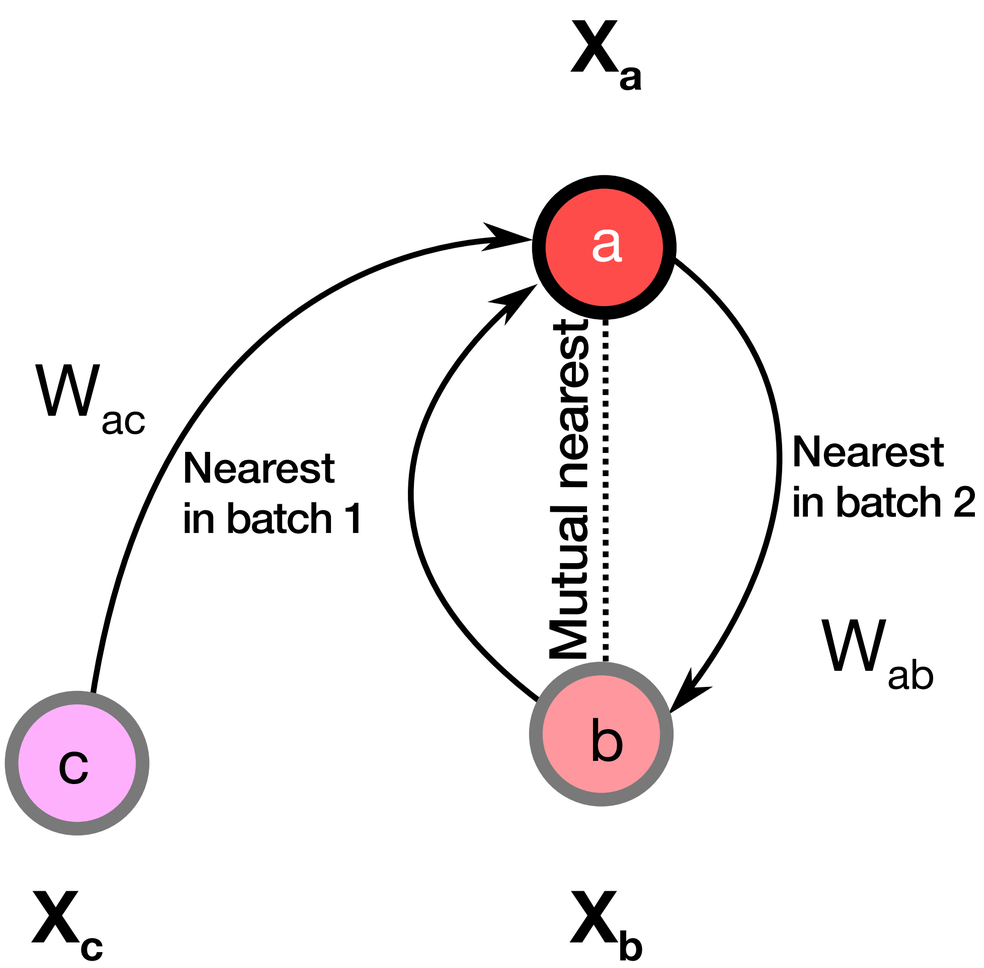


What is the gap Δ between the batches?

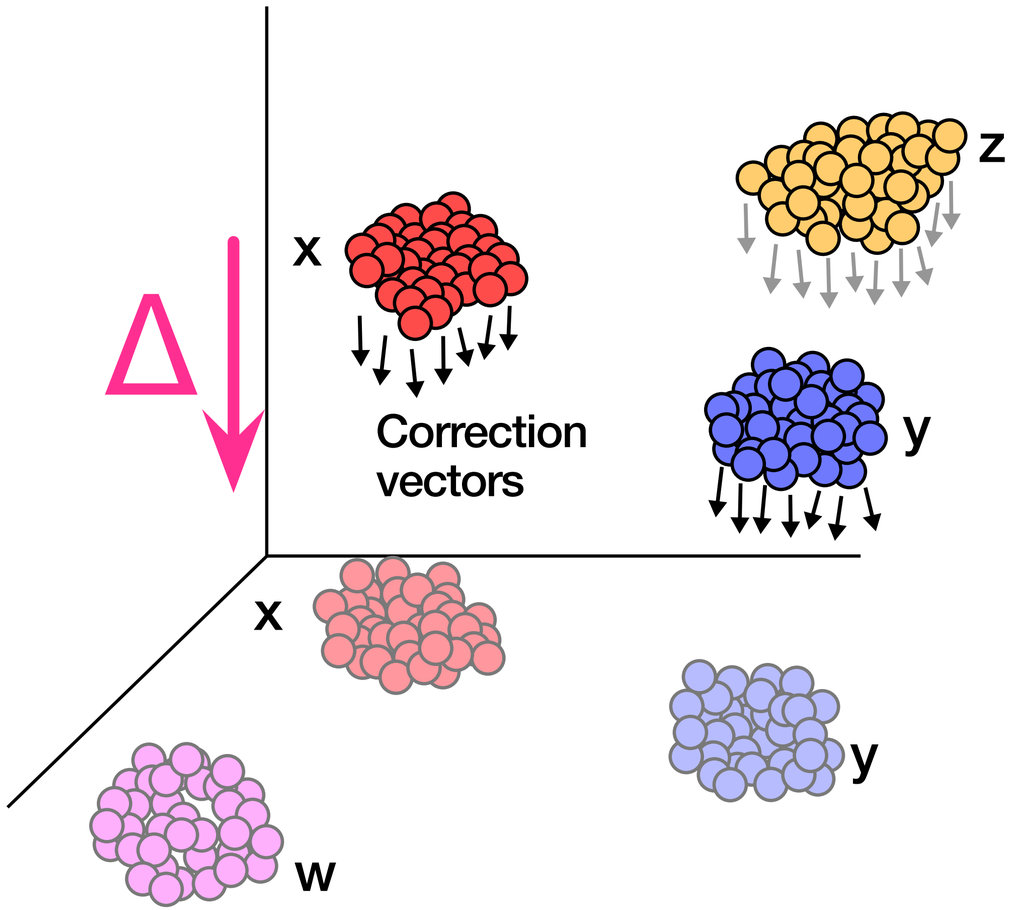
$$\min_{\Delta} \sum_{a,b} W_{ab} \|\mathbf{x}_a - \mathbf{x}_b - \Delta\|_2$$

Assume that the similarity between cells

$$0 \approx W_{ac} < W_{ab}$$

Haghverdi, .., and Marioni, *Nature Biotechnology* (2018)

Batch normalization aims to minimize the difference between nearest cells across different batches



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$$\min_{\Delta} \sum_{a,b} W_{ab} \|\mathbf{x}_a - \mathbf{x}_b - \Delta\|_2$$

Fixed point (local) optimal solution:

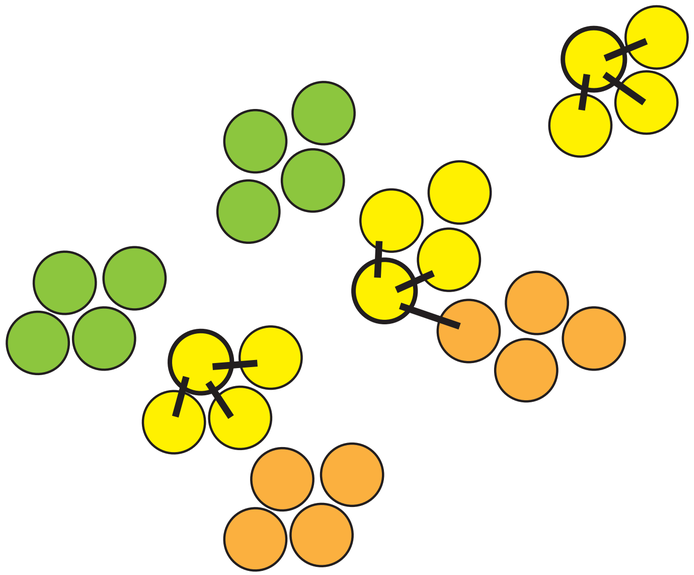
$$\Delta \leftarrow \frac{\sum_{a,b} W_{ab}(\mathbf{x}_a - \mathbf{x}_a)}{\sum_b W_{ab}}$$

Haghverdi, .., and Marioni, *Nature Biotechnology* (2018)

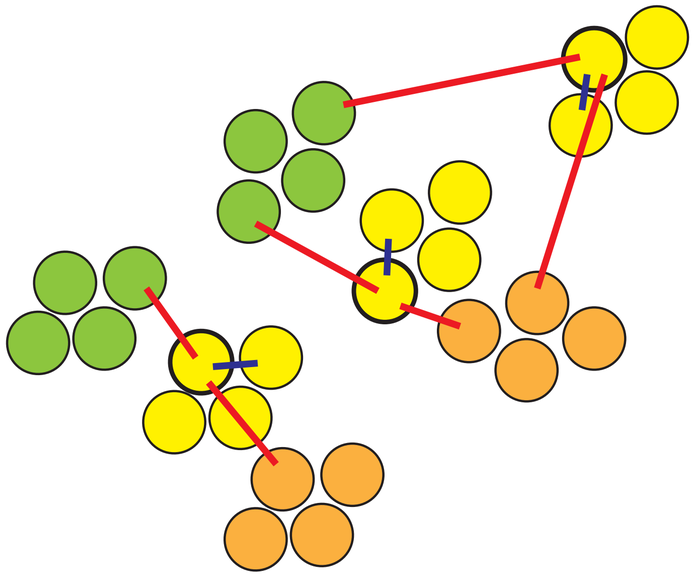
A batch-balancing k-nearest neighbour graph

BBKNN method strikes balance between over- and under-normalization

K-Nearest Neighbour



Batch Balanced K-Nearest Neighbour

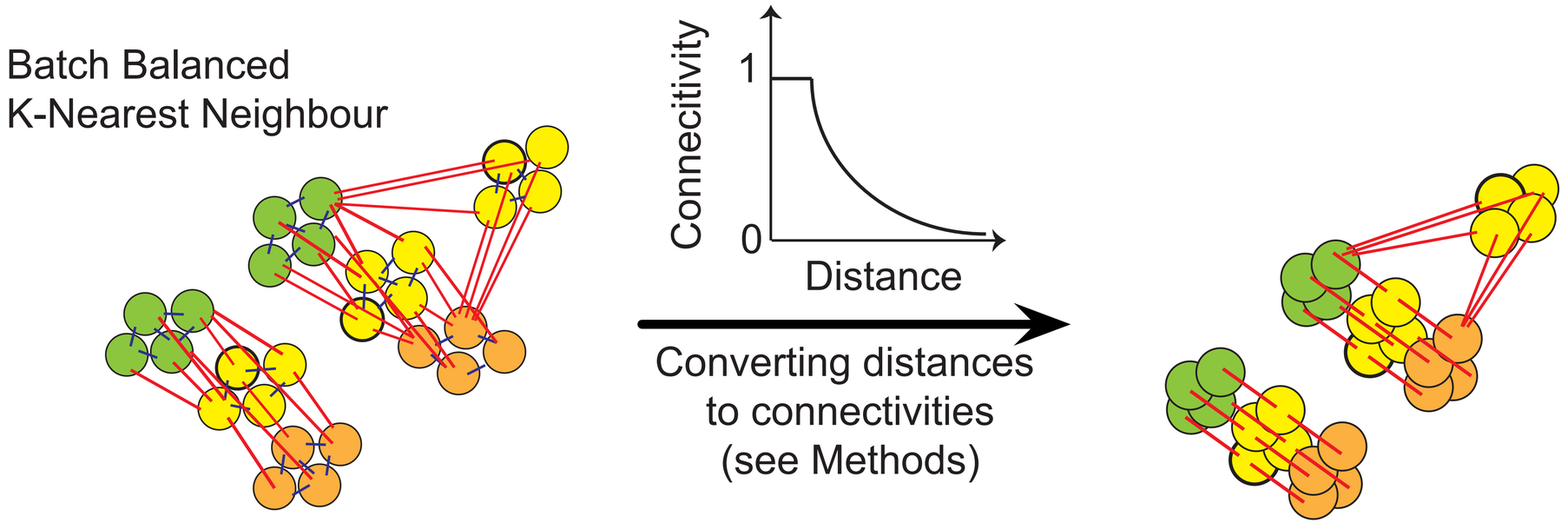


What kind of differences in due to inter-batch, technical discrepancy, not inter-cell-type divergence?

Polanski, .., Teichmann *Bioinformatics* (2019)

A batch-balancing k-nearest neighbour graph

BBKNN method strikes balance between over- and under-normalization



Polanski, .., Teichmann *Bioinformatics* (2019)

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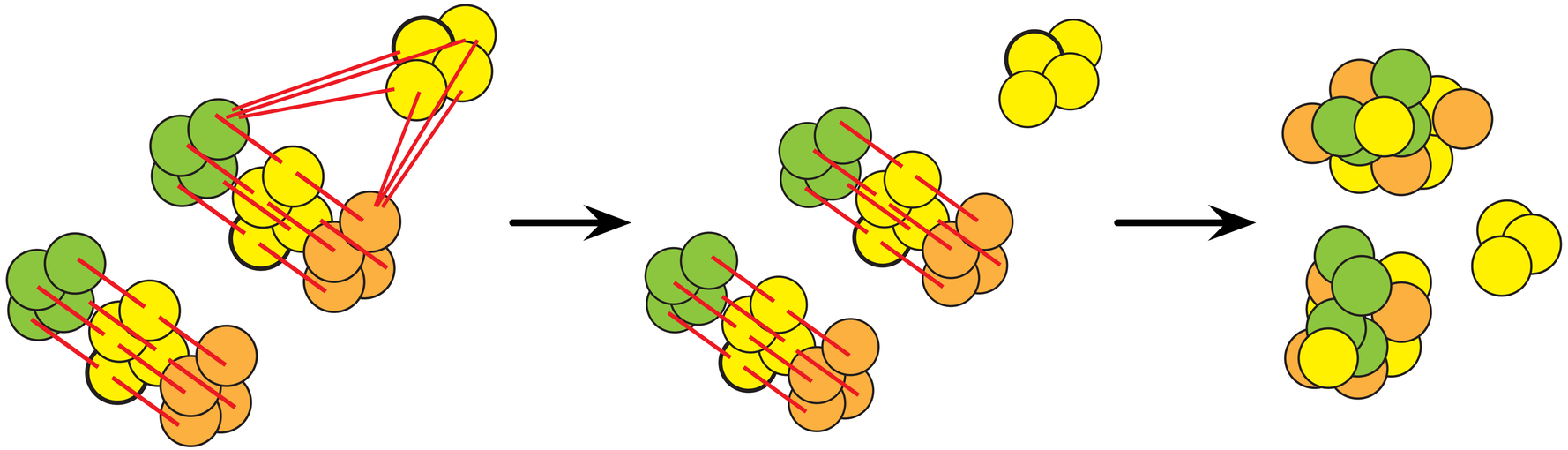


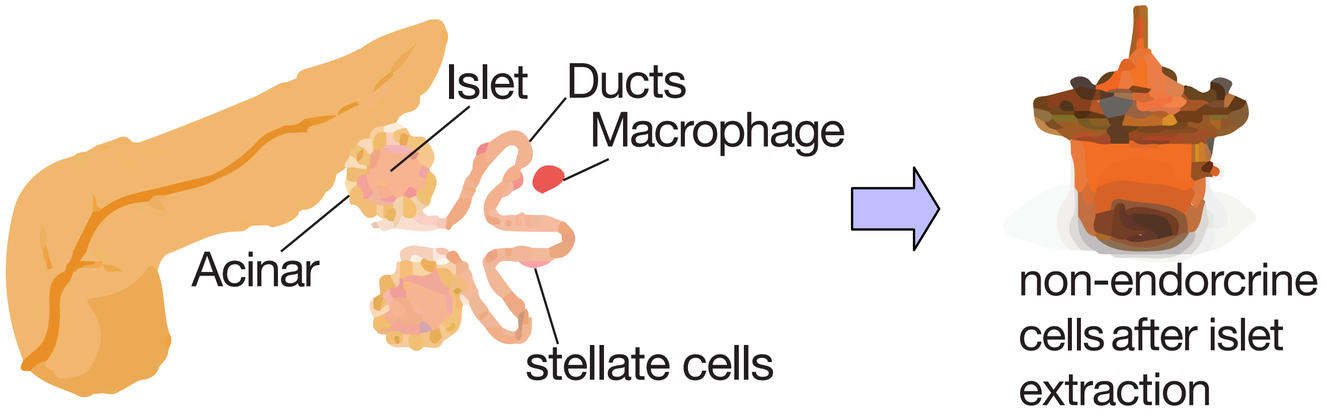
Polanski, .., Teichmann *Bioinformatics* (2019)

Example: another scRNA-seq data on human pancreatic islet cells

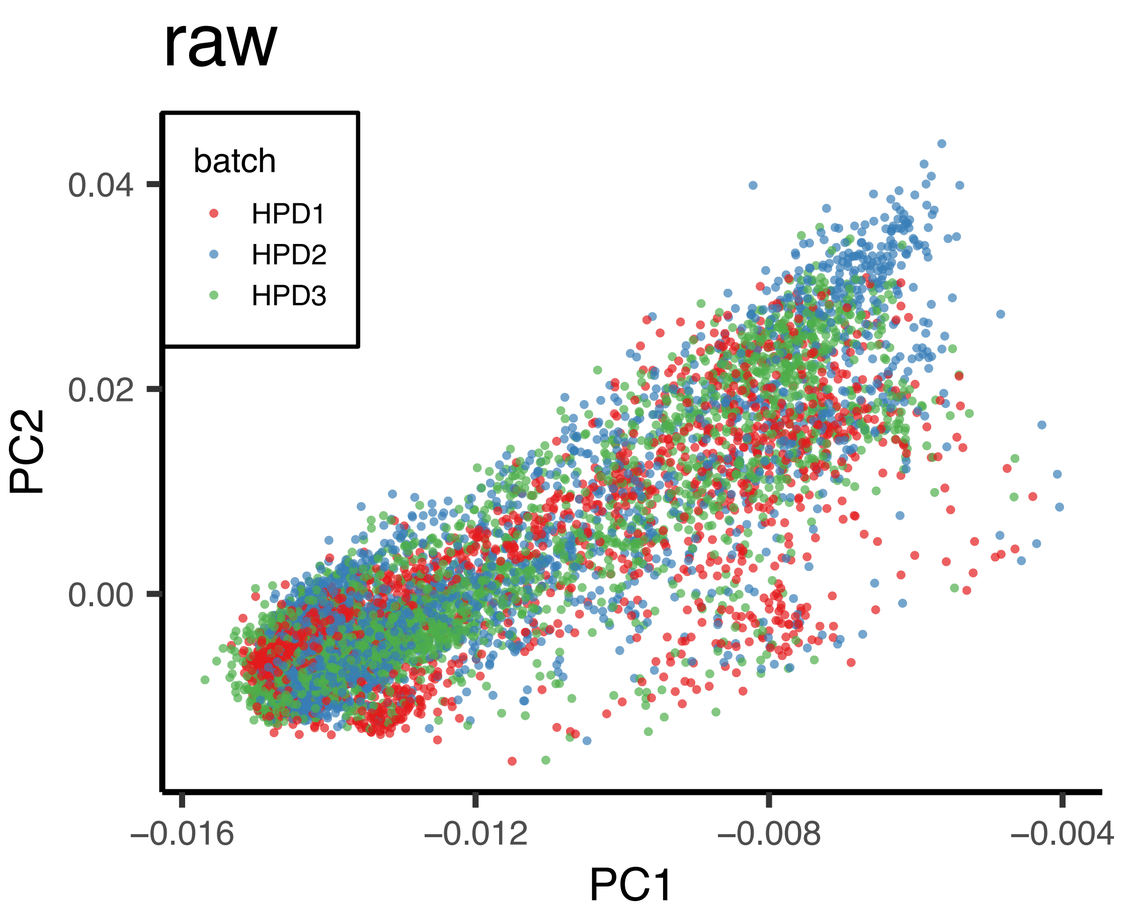
Single-cell RNA-seq data from three donors (three batches)

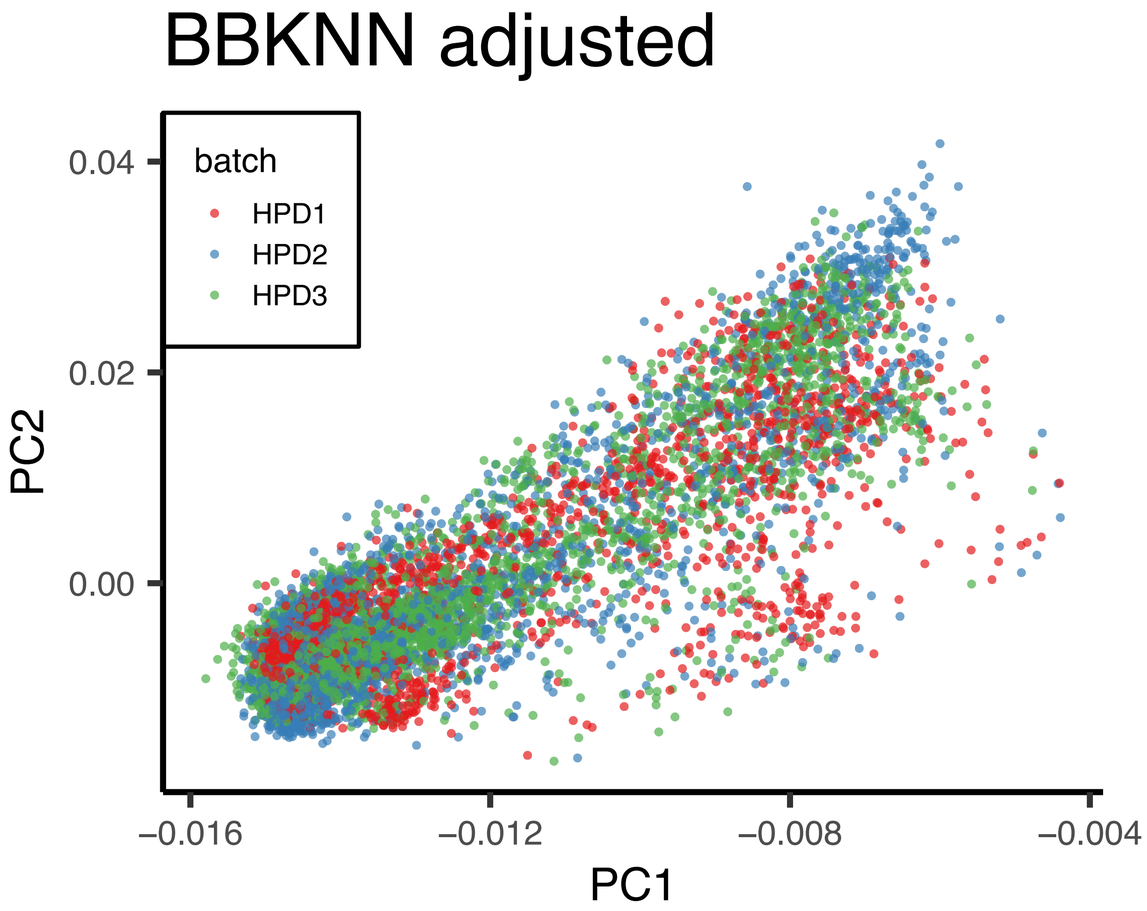
Goal: Remove potential batch effects across different donors. (1) Construct BBKNN graphs between cells; (2) compute average discrepancy Δ between batches in the PC space; (3) adjust them.

Qadir, *et al.*, *PNAS* (2020)



BBKNN-guided normalization (Δ) adjusts the inter-batch discrepancy





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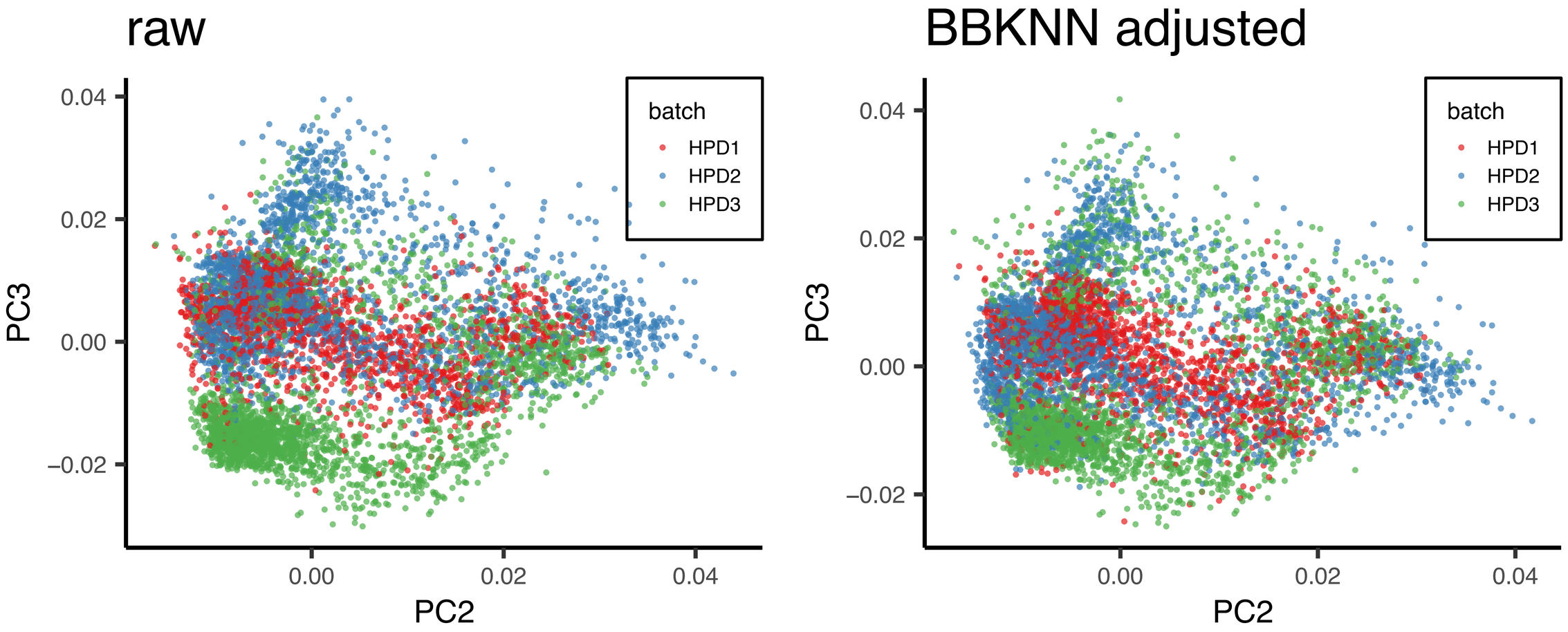

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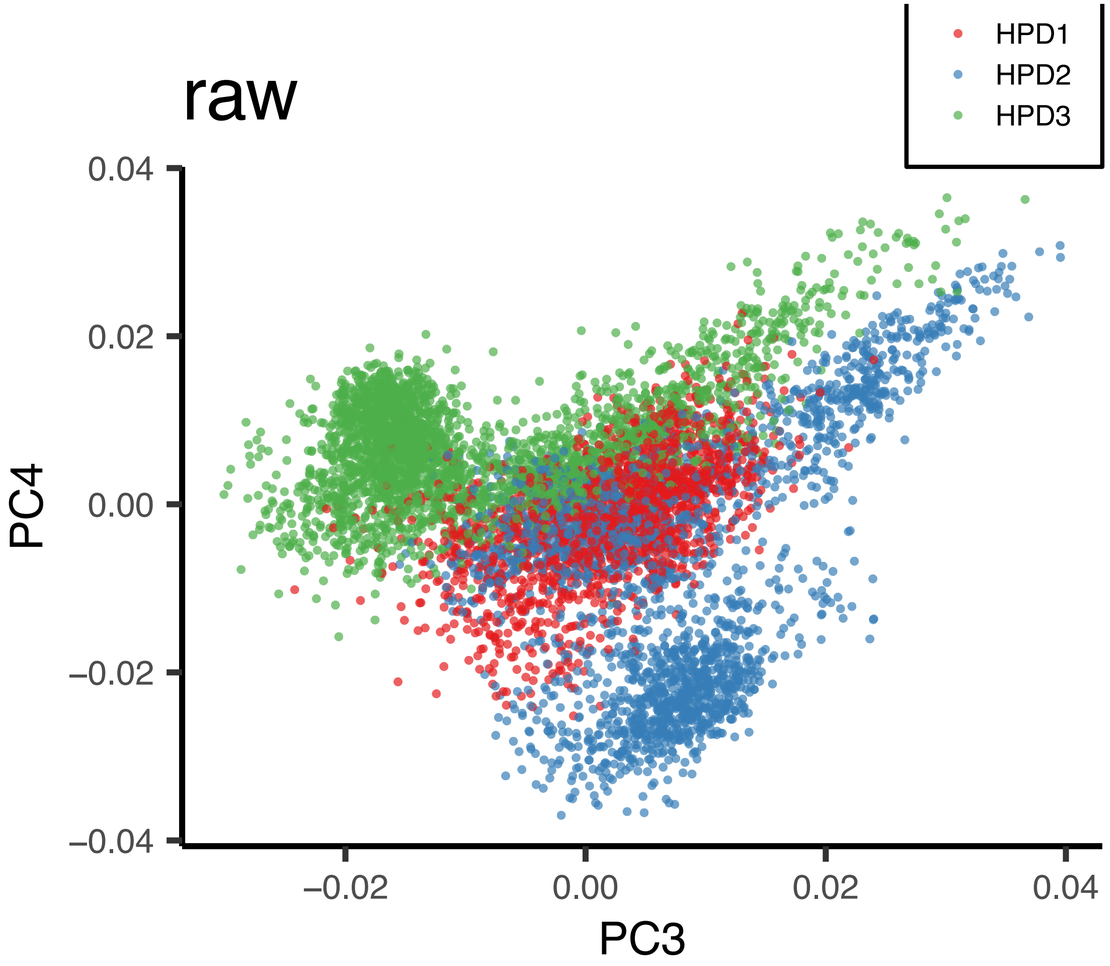

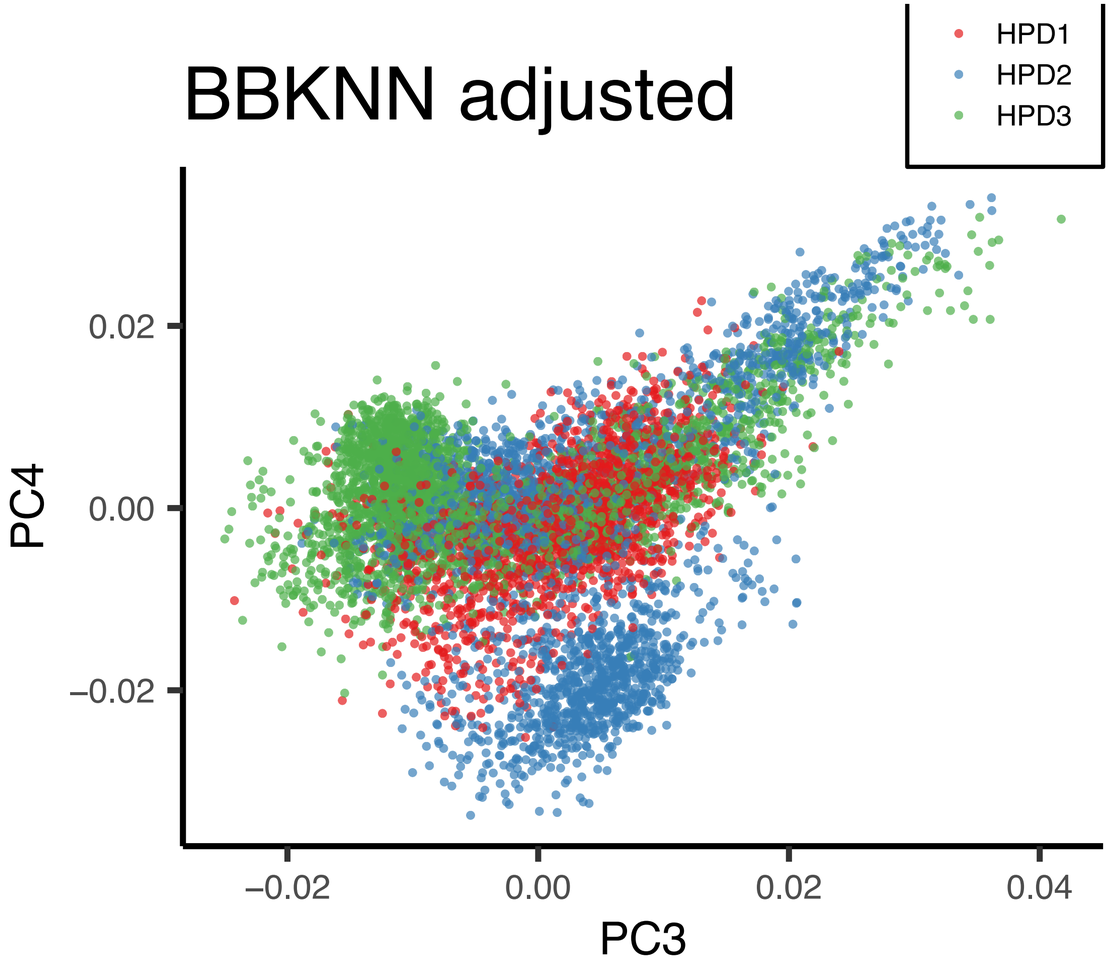

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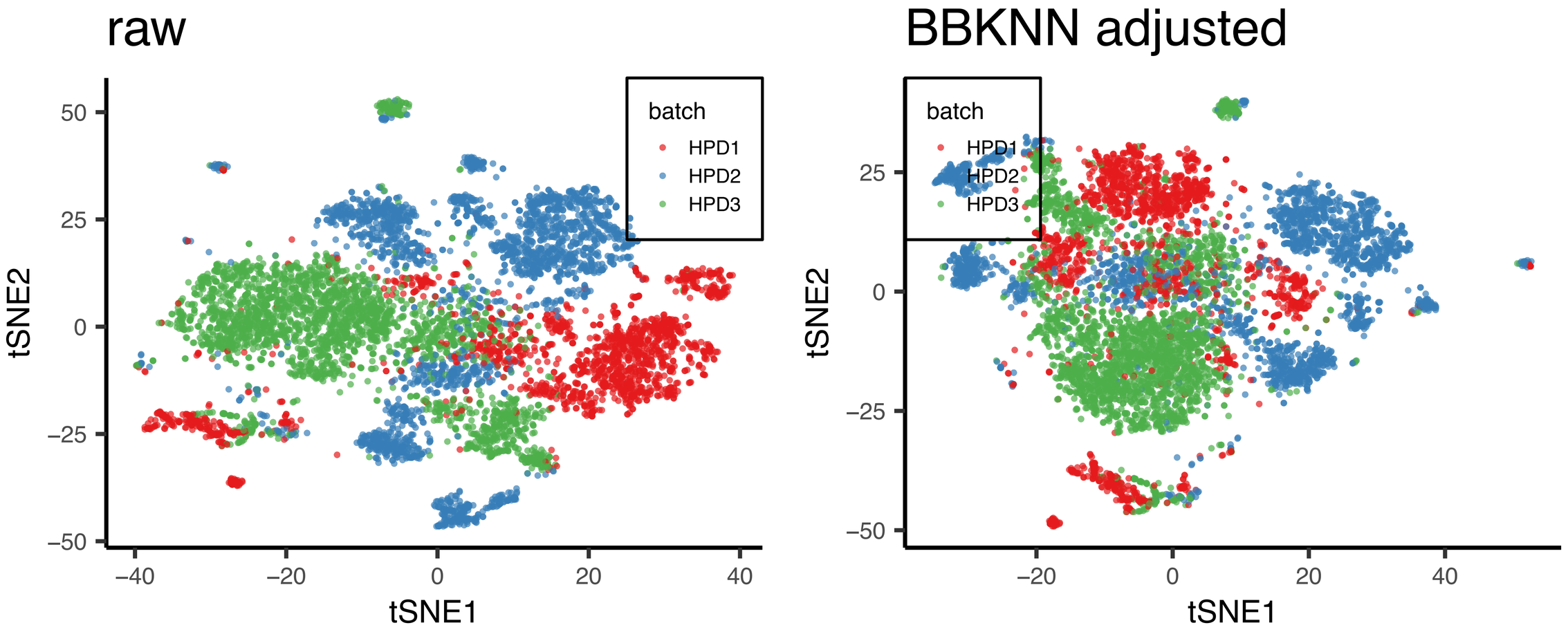
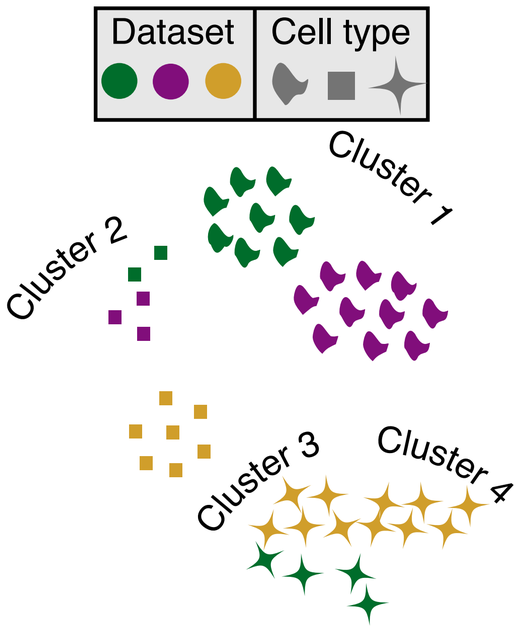


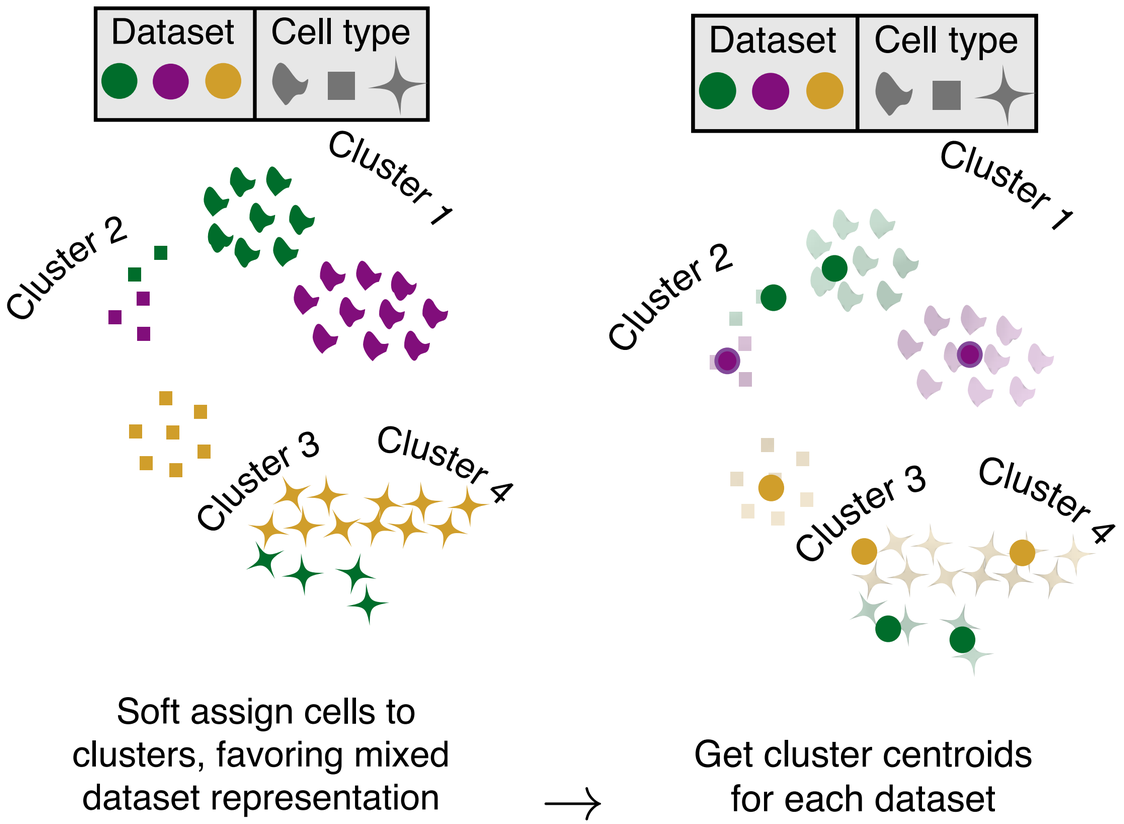
Harmony: clustering-based data normalization



Soft assign cells to clusters, favoring mixed dataset representation

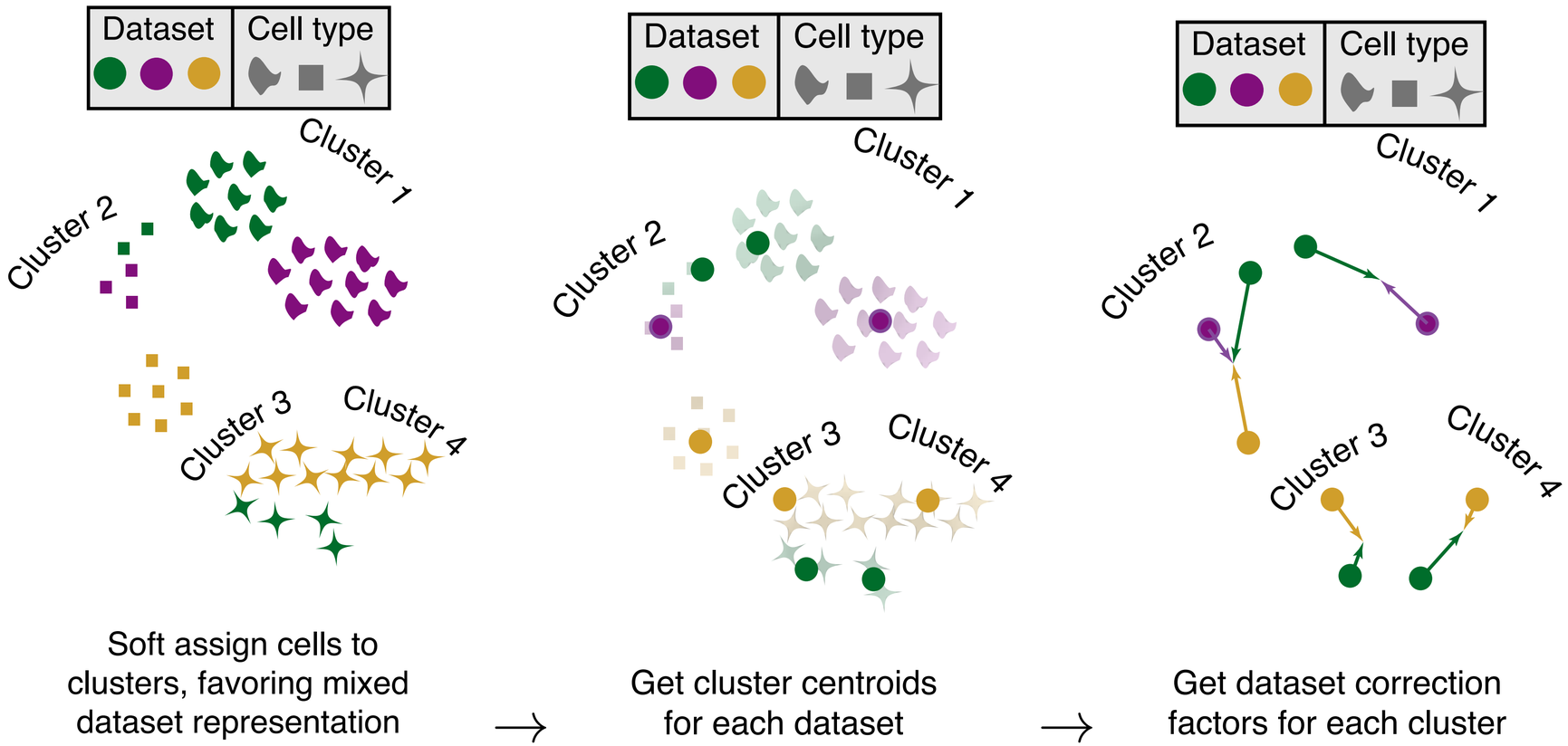
Korsunsky, .., Loh, Raychaudhuri *Nature Methods* (2019)

Harmony: clustering-based data normalization



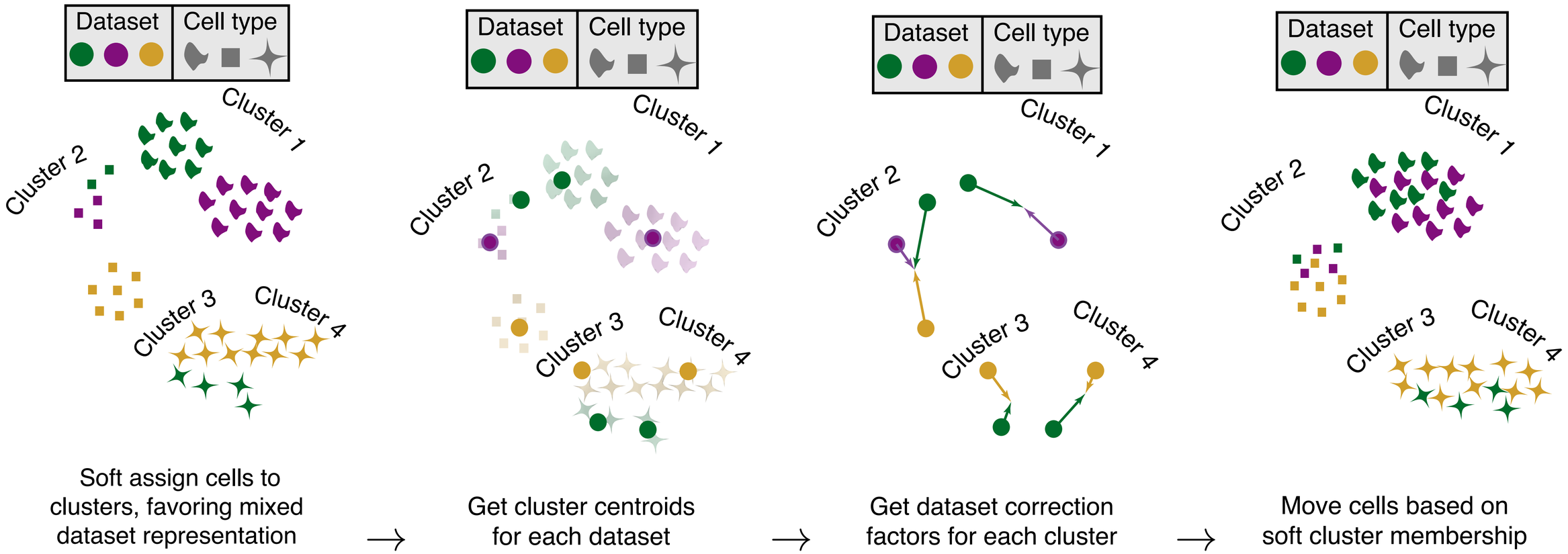
Korsunsky, .., Loh, Raychaudhuri *Nature Methods* (2019)

Harmony: clustering-based data normalization



Korsunsky, .., Loh, Raychaudhuri *Nature Methods* (2019)

Harmony: clustering-based data normalization



Korsunsky, .., Loh, Raychaudhuri *Nature Methods* (2019)

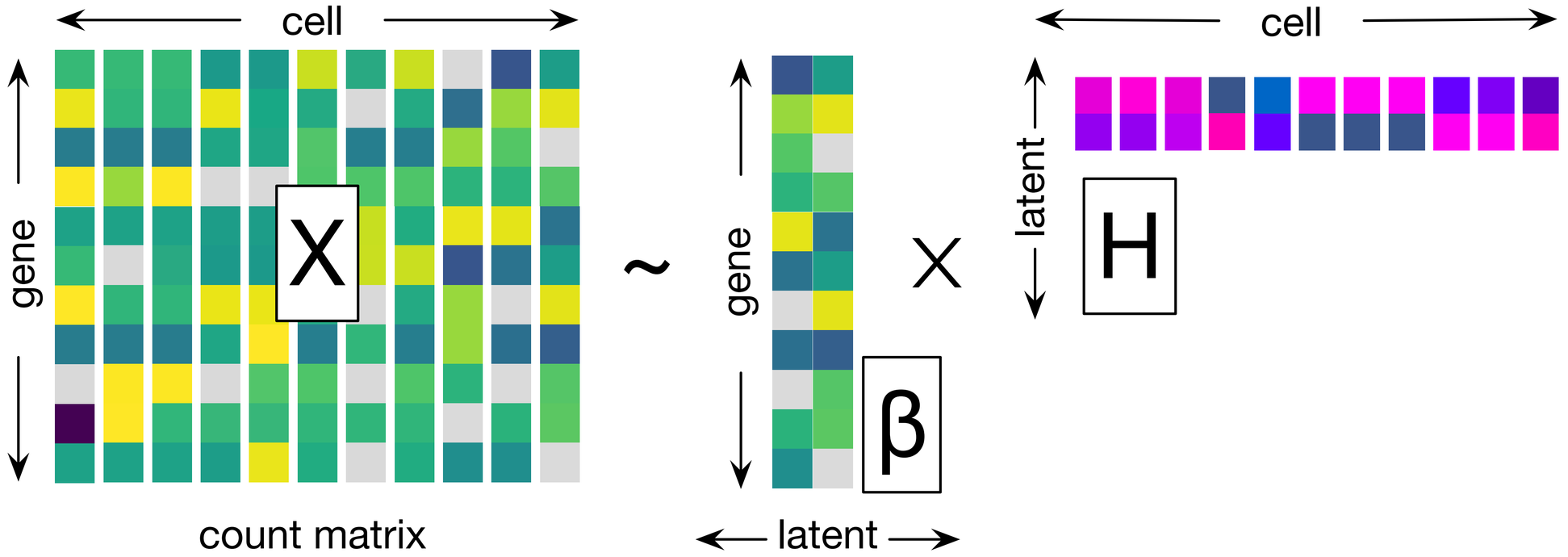
Discussions

- What can we do with BBKNN graphs?
- Why do we need batch normalization?
- Is it possible to over-correct the differences?
- Is it also possible to under-correct the differences?

Today's lecture

- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

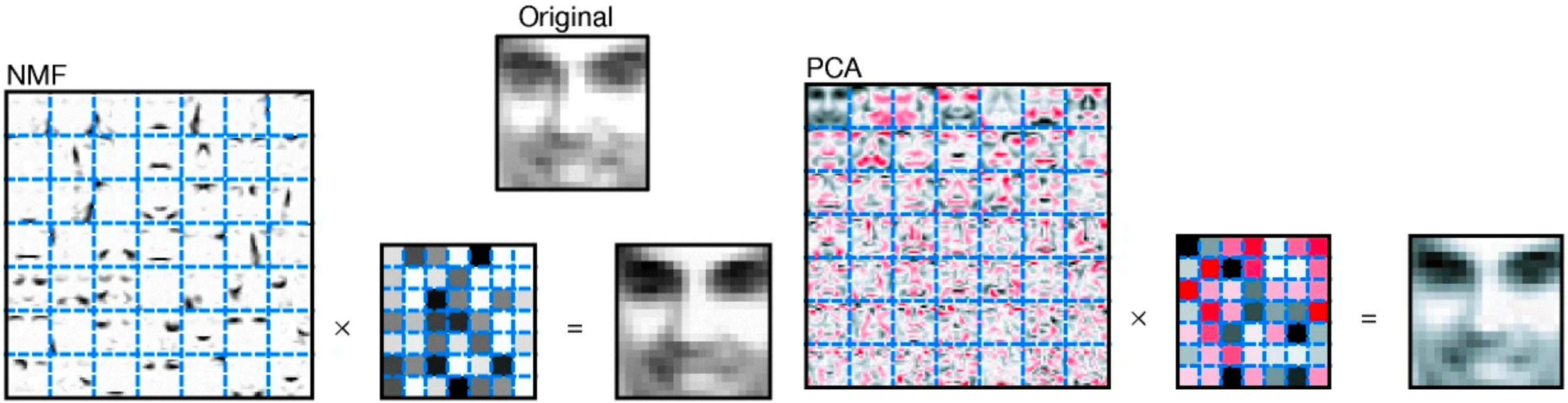
A generative model of single-cell data?



$$\mathbb{E}[X] \approx f\left(H\beta\right)$$

Can we assign tens of thousands of cells to some hidden probability space (H)?

Recall: Lect #12 about NMF

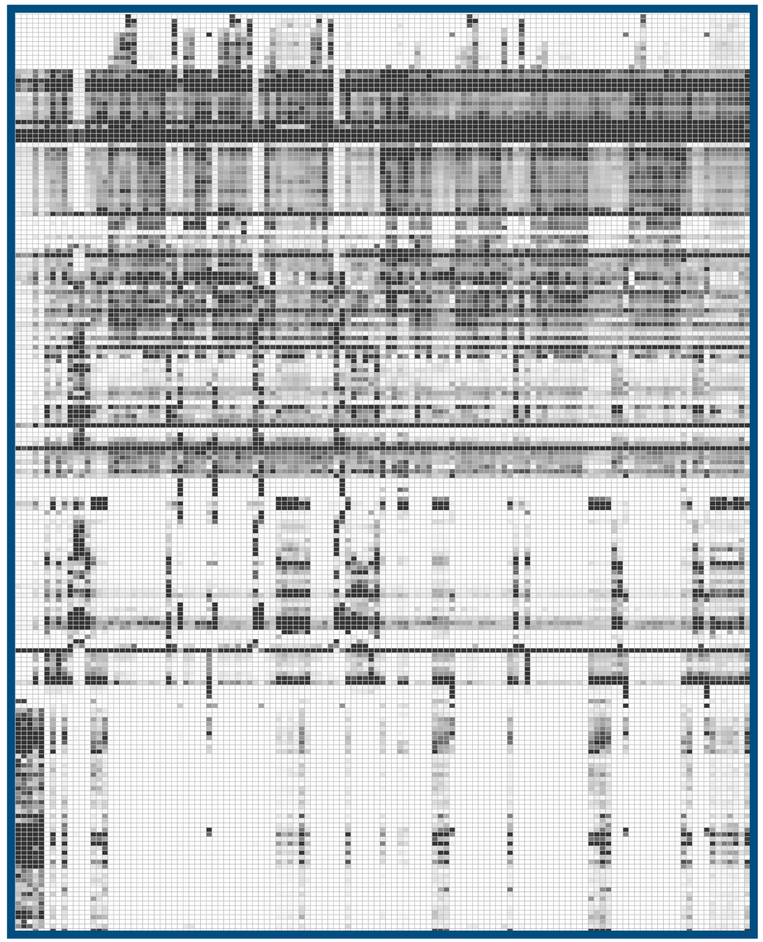


Decomposition by "parts"

Positive + negative can cancel each other

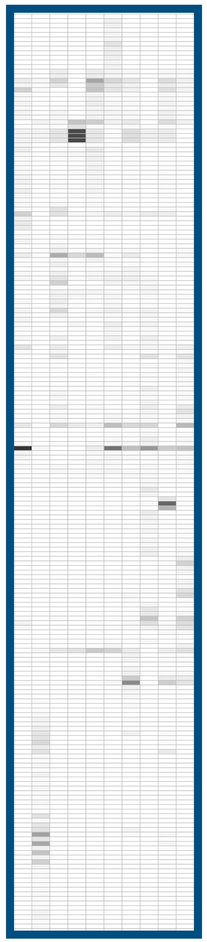
Lee and Seung, *Nature* (1999)

NMF to factorize expression matrix



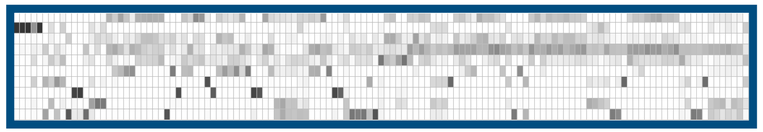
X

$\sim$



β

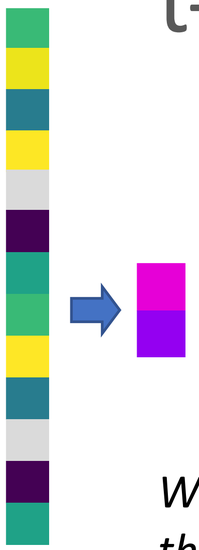
$\times$



H

A model-based approach goes beyond visualization of the high-dim. scRNA-seq

t-SNE



D=35k obs. X

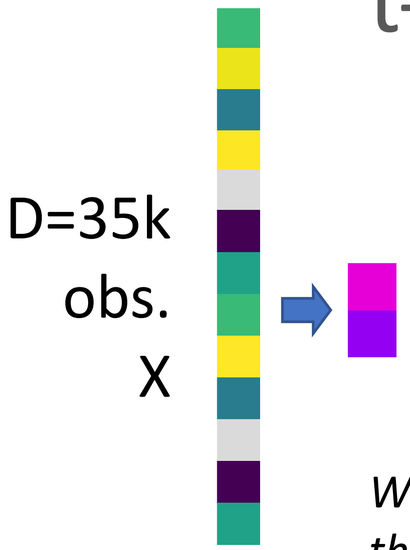
d=2, latent z

Where are the model parameters?

$$\min_{z} \quad D_{KL}\left(p_{ij}||q_{ij}\right) = \sum_{ij} p_{ij}\log\frac{p_{ij}}{q_{ij}}$$

A model-based approach goes beyond visualization of the high-dim. scRNA-seq

t-SNE

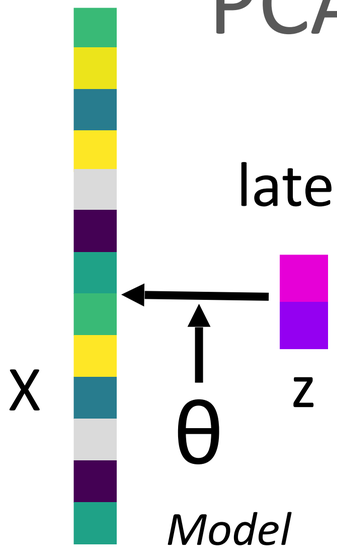


d=2,
latent
z

Where are the model parameters?

$$\min_{z} \quad D_{KL}\left(p_{ij} \,||\, q_{ij}\right) = \sum_{ij} p_{ij} \log \frac{p_{ij}}{q_{ij}}$$

PCA, MF



latent

z

θ

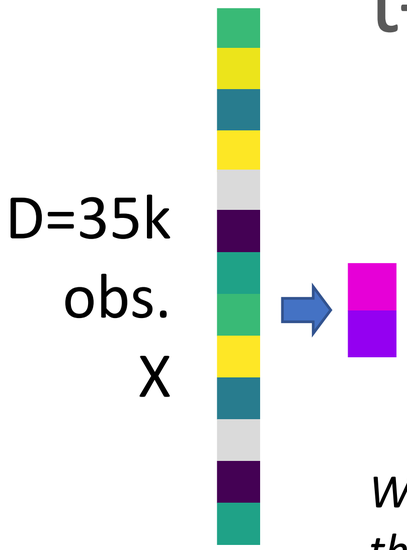
Model parameters

Can we generate/
estimate/impute
latent states
on unobserved data?

$$z \leftarrow f(x^*; \theta)$$

A model-based approach goes beyond visualization of the high-dim. scRNA-seq

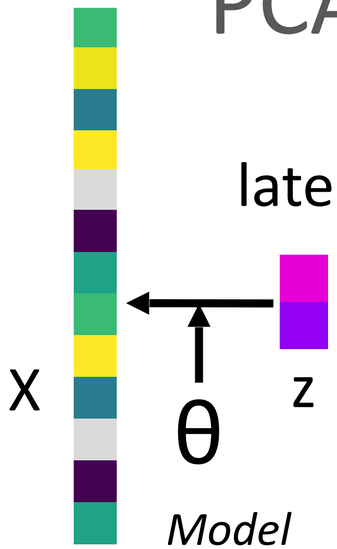
t-SNE



Where are the model parameters?

$$\min_{z} \quad D_{KL}\left(p_{ij}\,||\,q_{ij}\right) = \sum_{ij} p_{ij} \log \frac{p_{ij}}{q_{ij}}$$

PCA, MF



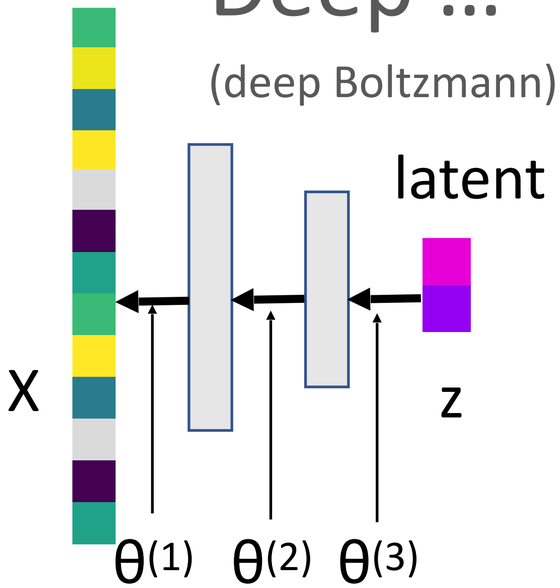
Model parameters

Can we generate/ estimate/impute latent states on unobserved data?

$$z \leftarrow f(x^*; \theta)$$

Deep …

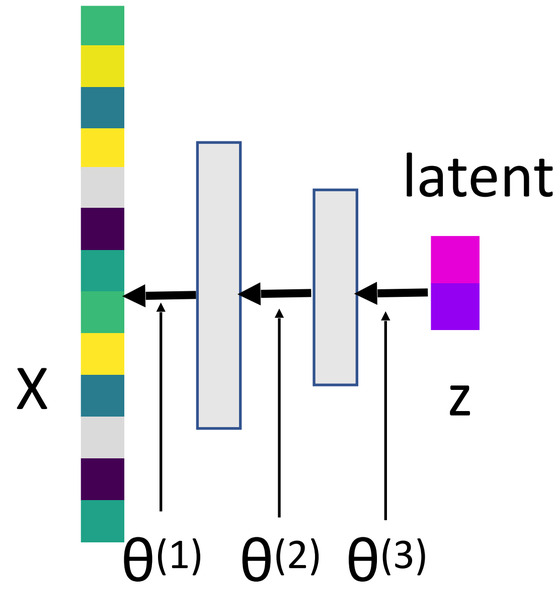
(deep Boltzmann)



Can we represent high-dimensional data using multiple functions?

$$z \leftarrow f(f(f(x^*; \theta^{(1)}); \theta^{(2)}); \theta^{(3)})$$

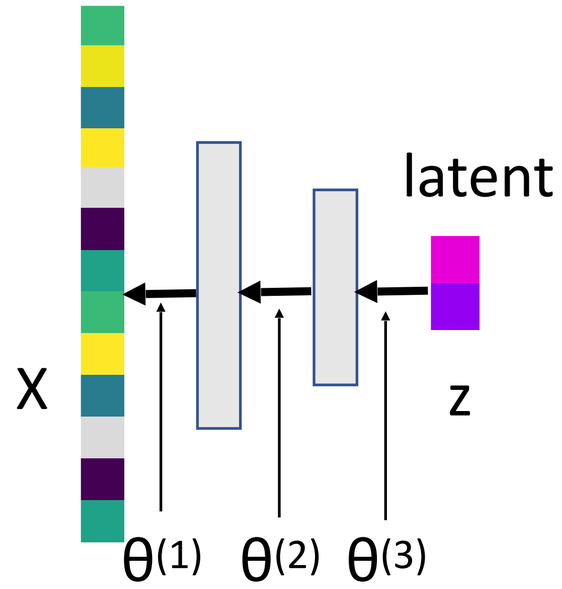
How do we estimate the parameters of a deep latent variable model?



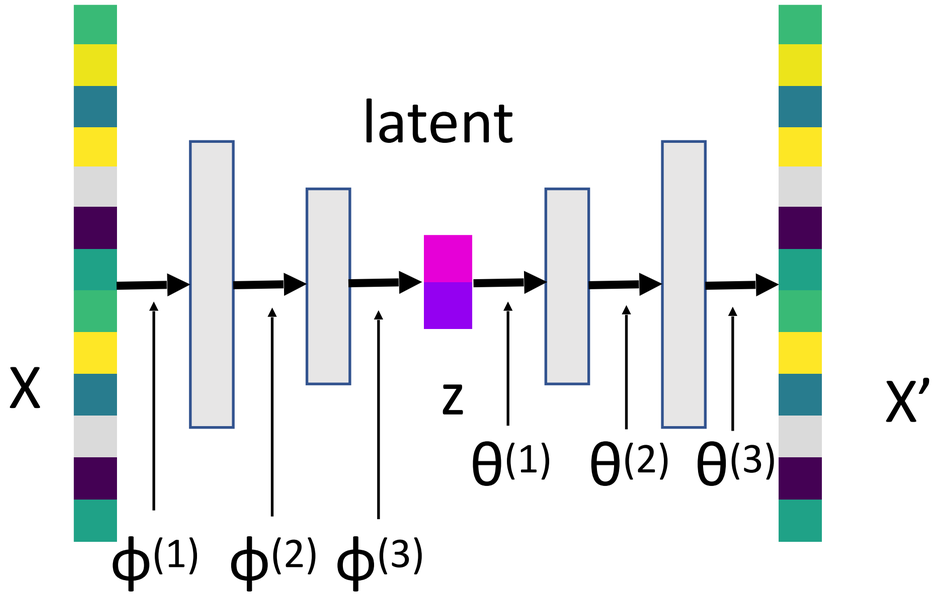
$$\int dZ\ p(Z)p(X \mid \theta, Z)$$

- E-step: Estimate/sample the latent Z
- M-step: Maximize the parameter θ

How do we estimate the parameters of a deep latent variable model?



vs.



$$\int dZ\ p(Z)p(X \mid \theta, Z)$$

- E-step: Estimate/sample the latent Z
- M-step: Maximize the parameter θ

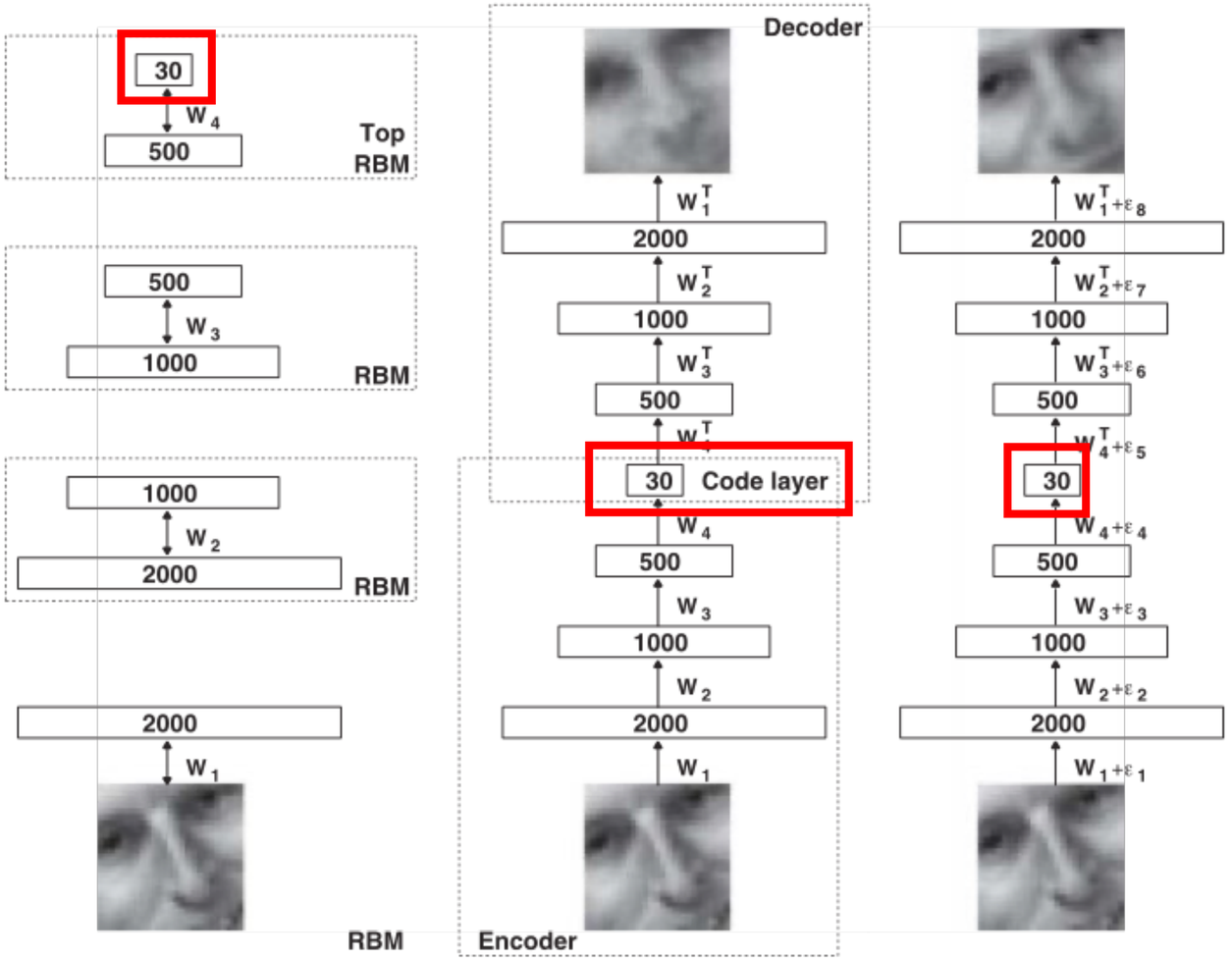
Goal: Make X and X' similar with respect to a data-generating model

No need to carry out an EM-type of algorithm
Just straight optimization of the parameters

Digression

Geoff Hinton's Deep Autoencoder model

Greedy layer-by-layer pretraining



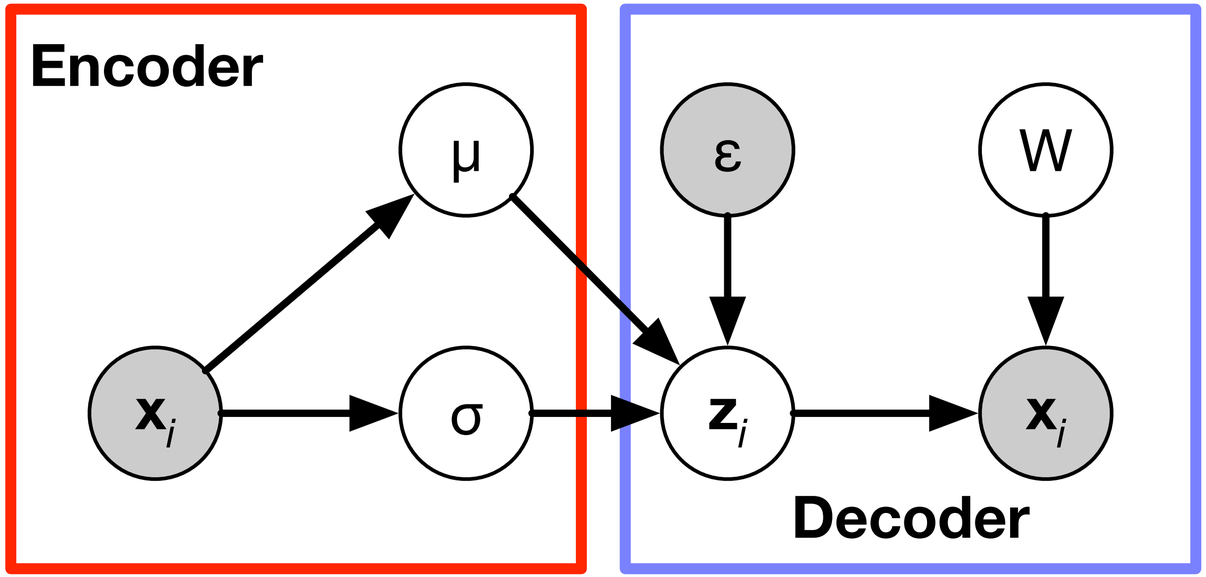
Fine-tuning by taking gradient steps

Hinton and Salakhutdinov, Science (2006)

Why do we need unsupervised learning for single-cell RNA-seq data?

- Probabilistic interpretation of latent states
- Incomplete single-cell data, lots of drop-out measurements
- We can design generative model parameters as interpretable as possible!

Variation autoencoder (VAE): a Bayesian inference framework for easy/scalable inference of latent variable model

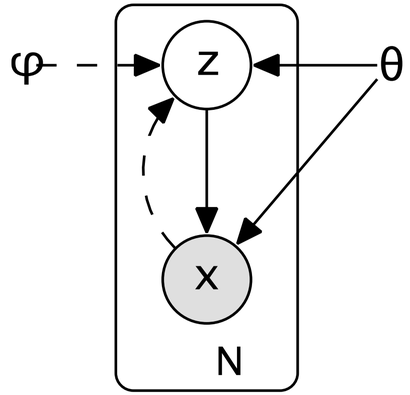


- Define relationships between variables (auto generative process)
- Usually, the decoder side captures our scientific hypothesis
- We can use an "auto-diff" algorithm (e.g., Facebook `torch` or Google `tensorflow`) to calculate gradients for the model parameters to optimize.

Variational Inference, VI by Neural Net & SGD

True log-probability
(difficult due to integration
over all the latent variables)

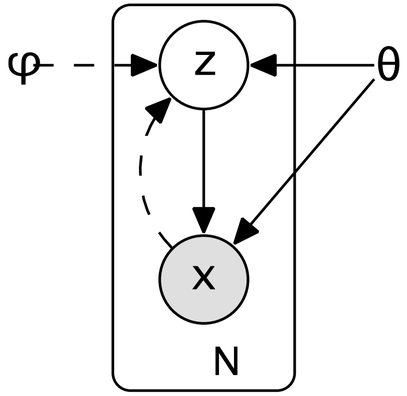
$$\ln \int dZ\, p(Z) p(X \mid \theta, Z)$$



Variational Inference, VI by Neural Net & SGD

True log-probability
(difficult due to integration
over all the latent variables)

$$\ln \int dZ\, p(Z)p(X\,|\,\theta, Z)$$
$$= \ln \int dZ\, p(Z)p(X\,|\,\theta, Z)\frac{q(Z)}{q(Z)}$$



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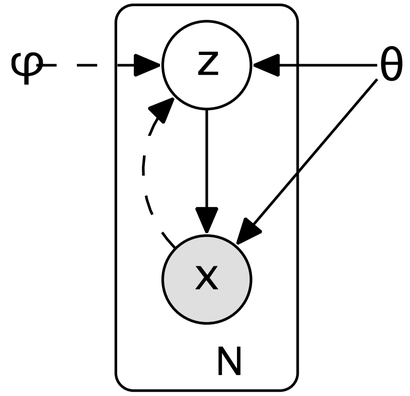
$$\geq \int dZ \ln\left(\frac{p(Z)p(X\,|\,\theta, Z)}{q(Z)}\right) q(Z)$$

Jensen's inequality



What is your choice of q(Z)?

$q(Z) = \prod_d q(z_d)$

Variational Inference, VI by Neural Net & SGD

True log-probability
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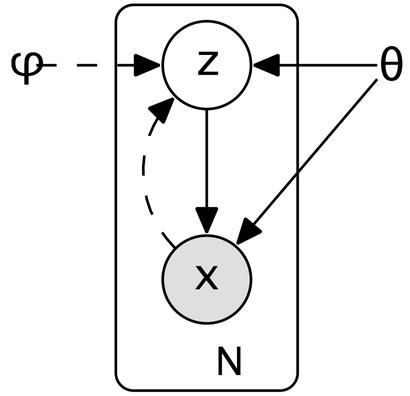
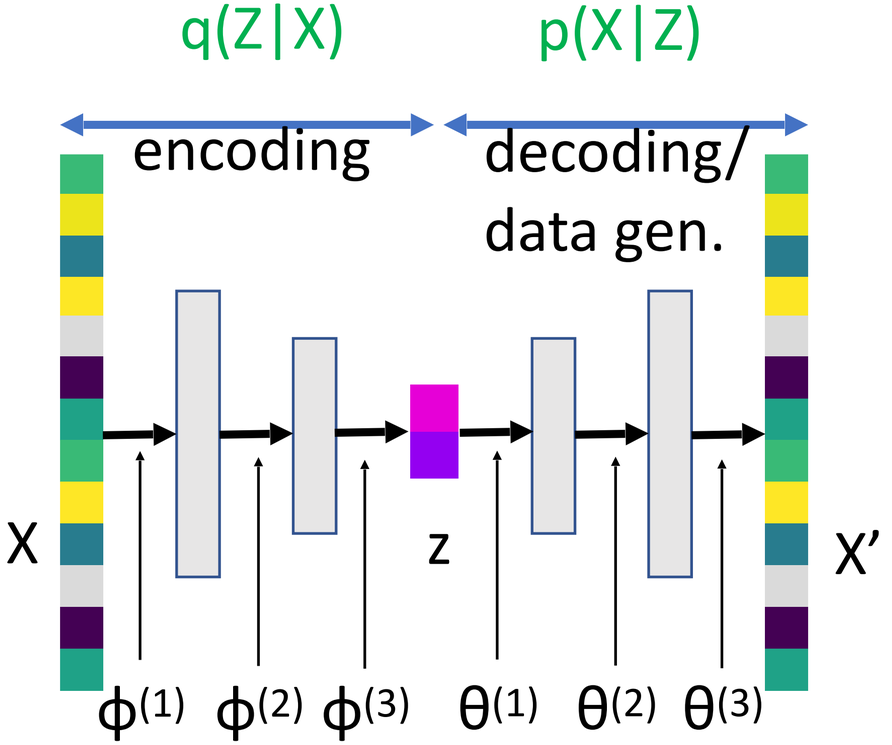
$$\ln \int dZ\, p(Z)p(X\,|\,\theta, Z)$$
$$= \ln \int dZ\, p(Z)p(X\,|\,\theta, Z)\frac{q(Z)}{q(Z)}$$
$$\geq \int dZ \ln\left(\frac{p(Z)p(X\,|\,\theta, Z)}{q(Z)}\right) q(Z)$$
$$= \mathbb{E}_q\left(\ln p(X\,|\,\theta, Z)\right)$$
$$+ \mathbb{E}_q\left(\ln p(Z)/q(Z)\right)$$

Lower-bound tractable
if variational q(Z) makes the function
easier to take average (E).



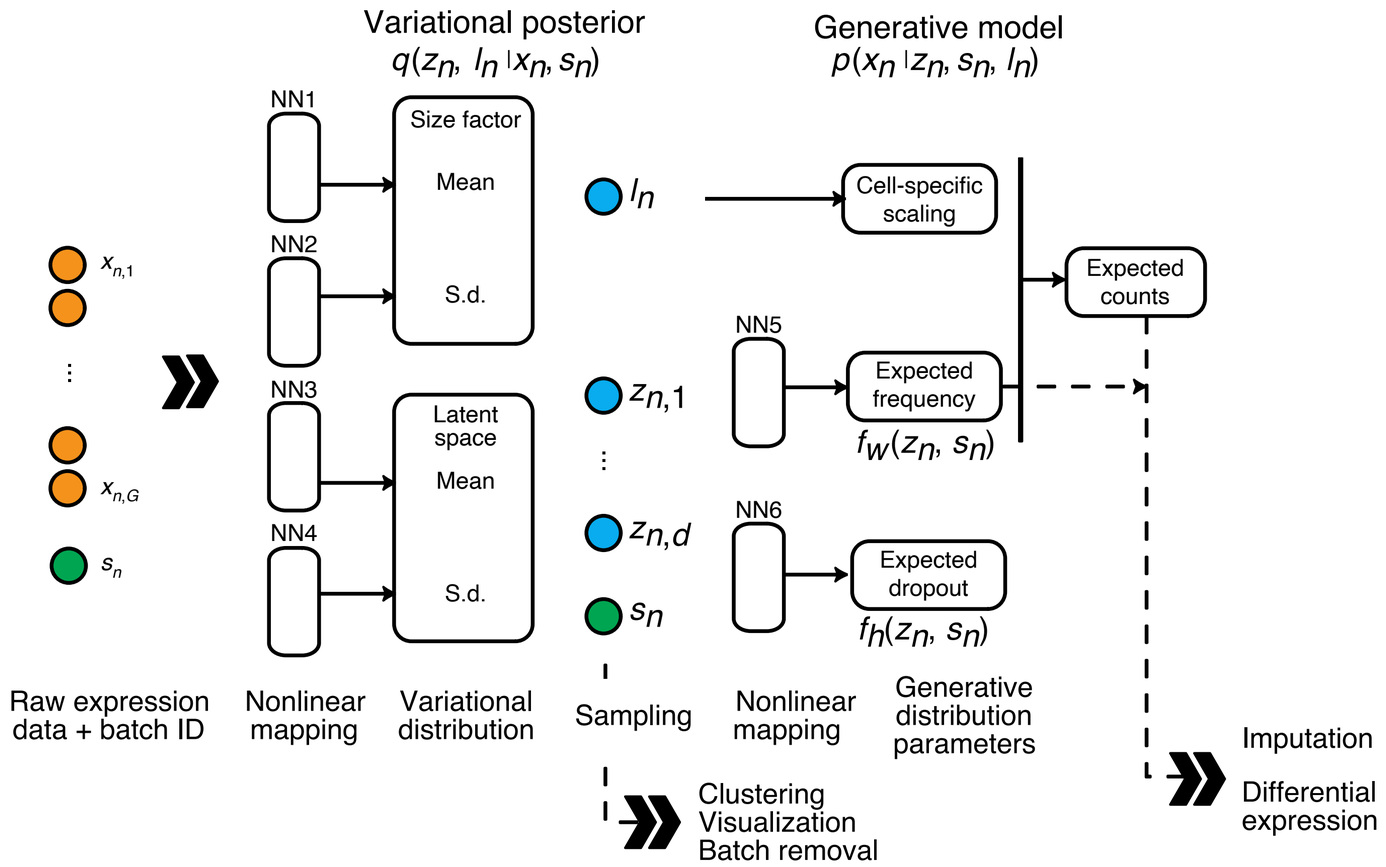
What is your choice of q(Z)?

What if taking expectation w.r.t. q(Z) is hard? **Sample z**



Kingma & Welling (2013)

Deep generative modeling for single-cell transcriptomics

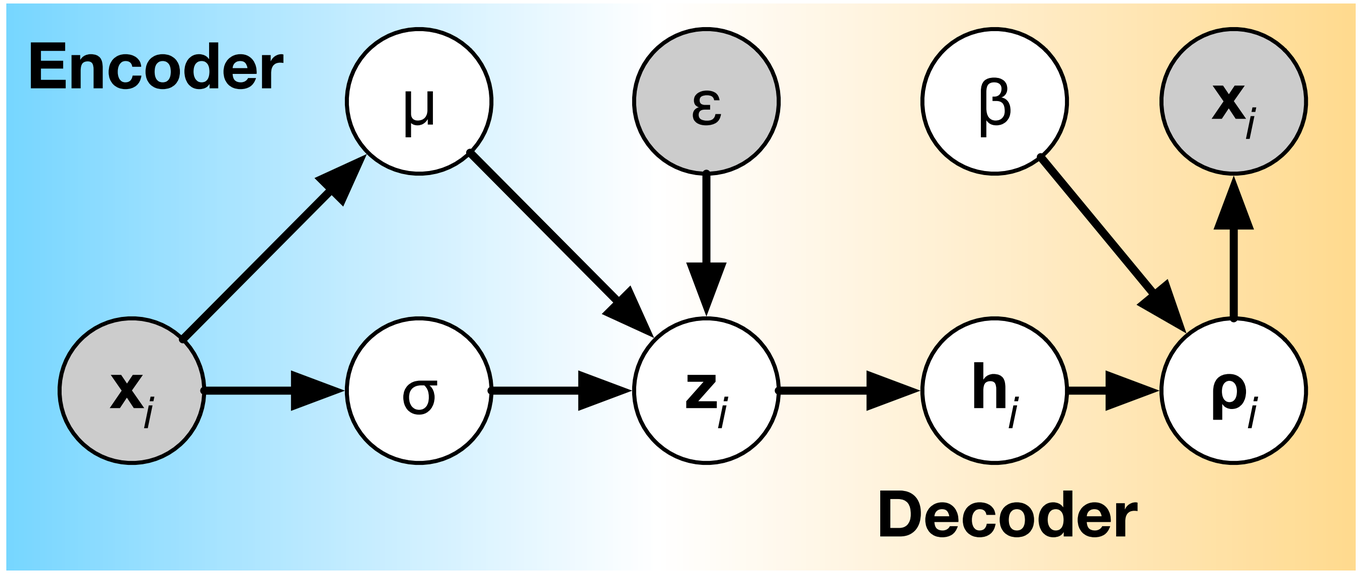


Generative model: zero-inflated negative binomial distribution

Lopez, .., Jordan, Yosef, *Nature Methods* (2018)

Multinomial topic modelling for (incomplete) single-cell expression data

Can we simply model scRNA-seq counts by multinomial distribution?



- X_{ig}: gene expression of a gene g in a single cell i
- H_{ik}: latent topic proportion of a cell i to a topic k
- β_{kg}: topic k-specific gene probability

Multinomial topic modelling for (incomplete) single-cell expression data

Can we simply model scRNA-seq counts by multinomial distribution?

Likelihood model:

$$\mathcal{L} = \prod_{i=1}^{n} \prod_{g=1}^{\text{genes}} \left(\sum_{k} H_{ik} \beta_{kg} \right)^{X_{ij}}$$

- X_{ig}: gene expression of a gene g in a single cell i
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Likelihood model:

$$\mathcal{L} = \prod_{i=1}^{n} \prod_{g=1}^{\text{genes}} \left(\underbrace{\sum_{k} H_{ik}\beta_{kg}}_{\text{a gene } g\text{'s probability in a cell } \iota \equiv \rho_{ig}} \right)^{X_{ij}}$$

- X_{ig}: gene expression of a gene g in a single cell i
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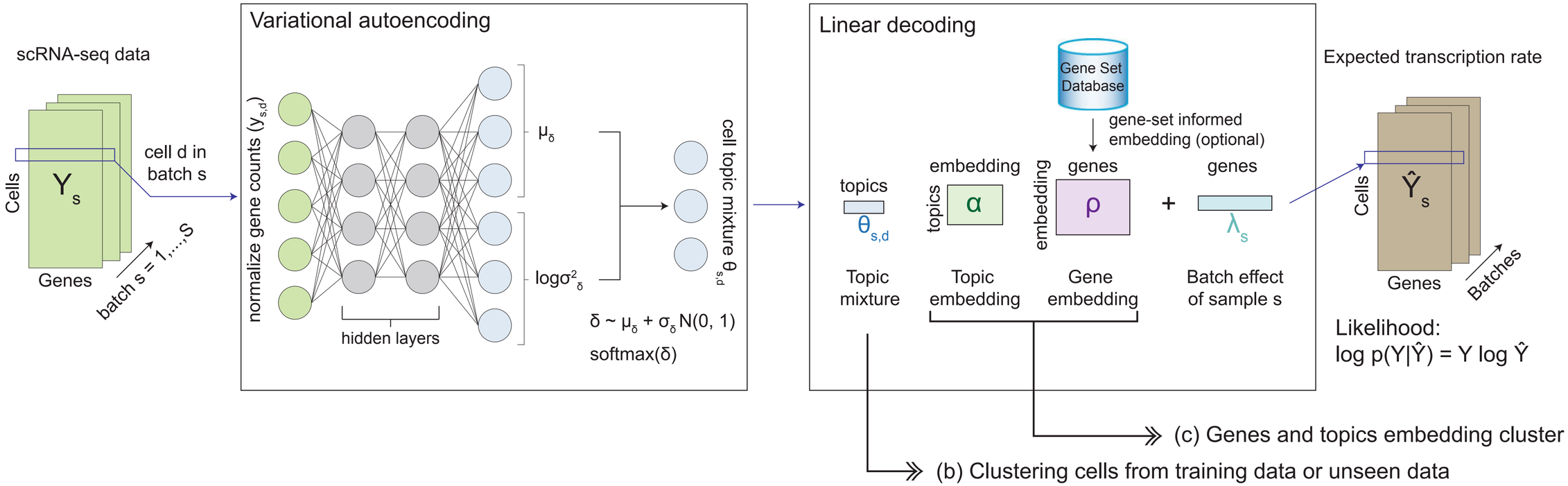
- β_{kg}: topic k-specific gene probability

Single-cell Embedded Topic Model



We can factorize $\beta = \alpha\rho$.

Zhao, Cai, .., Li, *Nature Comm.* (2021)

Topic Modelling: Comparison between document vs. single-cell

We think of a cell as a document, which is $\approx$ a bag of words, or $\approx$ a bag short mRNA reads.

variables	in document topic model	in single cell ETM
D	Total number of documents (corpus)	Total number of cells
d	Document index	Cell index
N_d	Number of words in a document d	Number of read counts in a cell d
j	Word index, $j \in [N_d]$	Read index
K	Total number of topics	Total number of cell type topics
k	Topic index, $k \in [K]$	Cell topic index
V	Size of vocabulary	Total number of genes
v	Vocabulary index $v \in [V]$	Gene index
W_{dj}^v	Indicator for a word to vocabulary $\in \{0, 1\}$	Indicator for a read to a gene $\in \{0, 1\}$
X_{dv}	Vocabulary v occurrence in a document d	Gene expression of a gene v in a cell $d \in [0, N_d]$

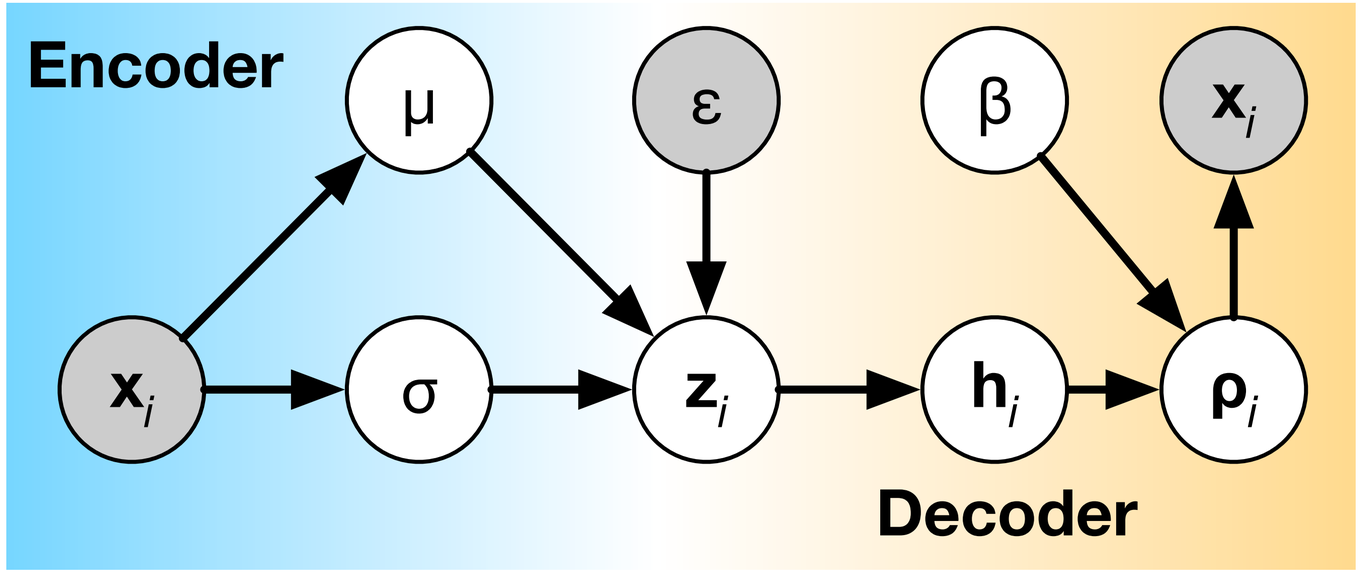
$W_{dj}^v = 1$ if and only if a word j in a document d takes v-th word in the vocabulary; otherwise, $W_{dj}^v = 0$.

Single-cell Embedded topic model's latent states and model parameters

variables	in document topic model	in single cell ETM
Z_{dj}^k	Indicator for assigning a word to a topic k	Indicator for assigning a read to a topic k
H_{dk}	Hidden state k of a document d	Hidden state k of a cell d
β_{kv}	topic k-specific vocabulary v frequency	topic k-specific, a gene v's exression

- In Latent Dirichlet Allocation: $\sum_{k=1}^{K} H_{dk} = 1$ and $H_{dk} > 0$, and $\mathbf{h}_d \sim \text{Dirichlet}(\alpha/K, \ldots, \alpha/K)$ *a priori*. Approximately, we have $\hat{H}_{dk} = \sum_j^{N_d} Z_{dj}^k / N_d$.
- In Embedded Topic model, H_{dk} with the simplex constraints; $H_{dk} = \exp(\delta_{dk}) / \sum_{k'} \exp(\delta_{dk'})$ where $\delta_{dk} \sim \mathcal{N}(0, 1)$ *a priori*.
- Additional constraints: $\beta_{kv} > 0$ and $\sum_v \beta_{kv} = 1$, meaning that only a handful of vocabulary v contribute to a topic k.

Multinomial topic modelling for (incomplete) single-cell expression data

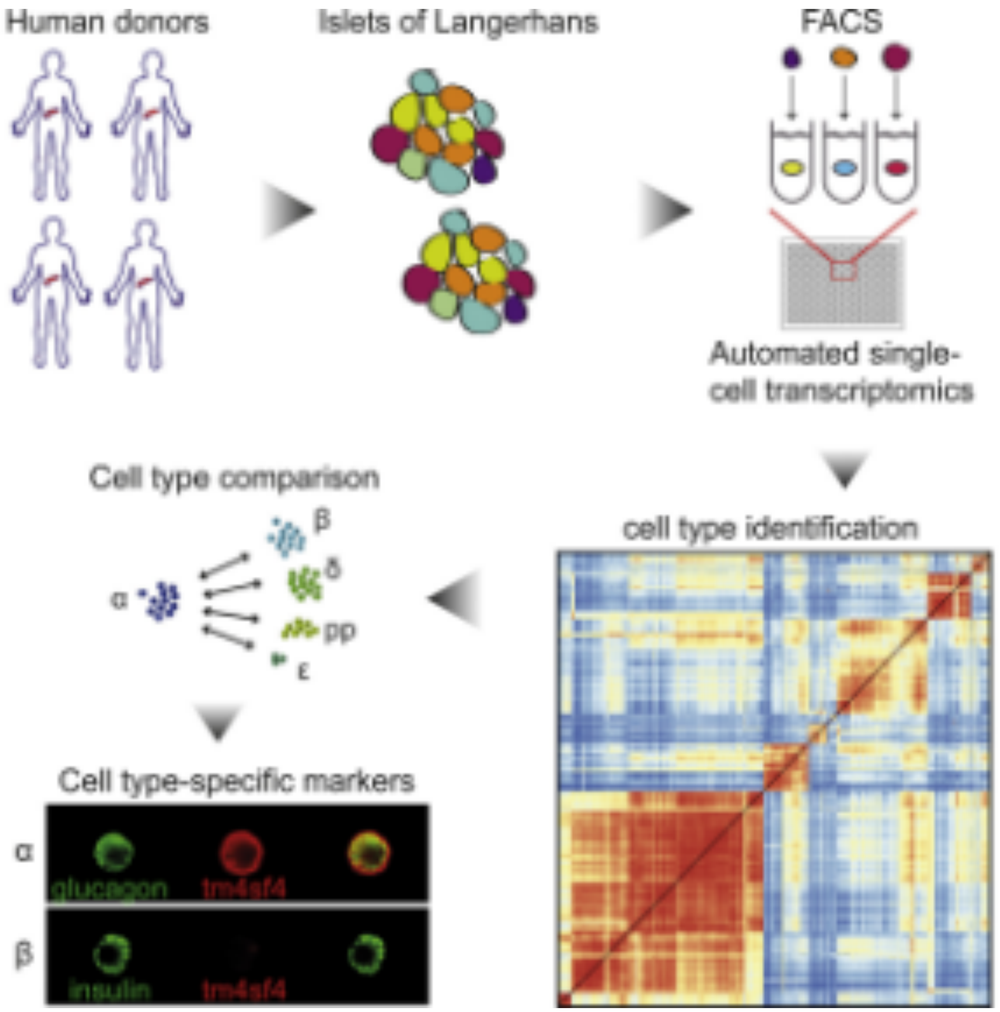


Probability of gene g in a cell i:

$$\rho_{ig} = \sum_{k \in \text{topics}} H_{ik} \beta_{kg}$$

By **not** normalizing the probability of each cell, we do not worry about modelling sequencing depths.

Example: single-cell RNA-seq data of human pancreatic cells



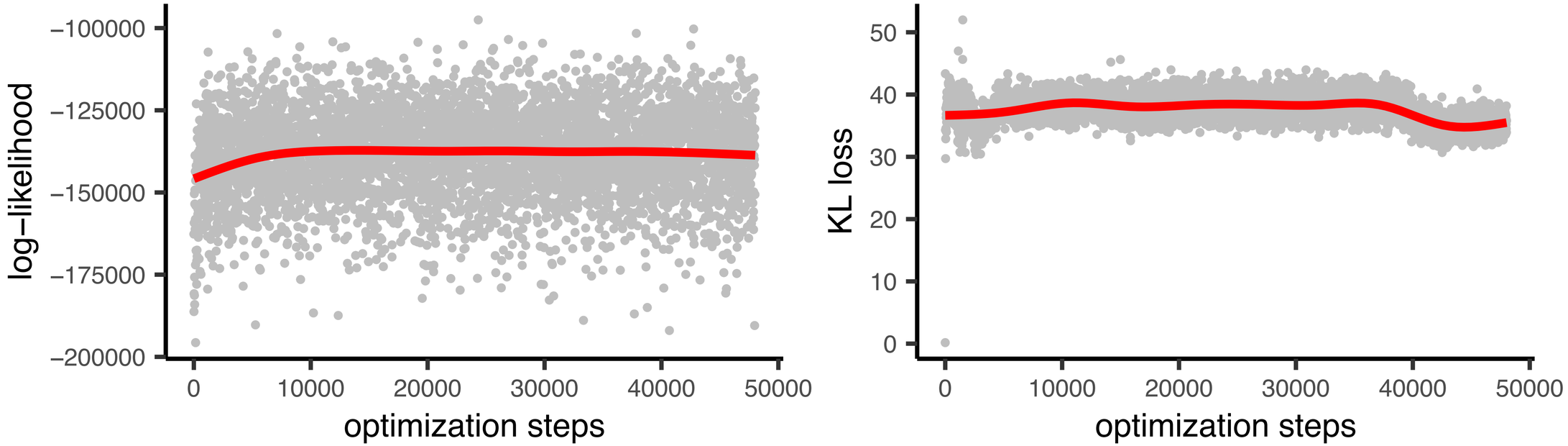
Muraro *et al. Cell Systems* (2016)

We will use scRNA-seq data (GEO accession: GSE85241) as a working example.

- genes/features/rows: 19,140
- cells/columns: 3,072
- non-zero elements: 12,442,034
- $\sim$ 21 % non-zero

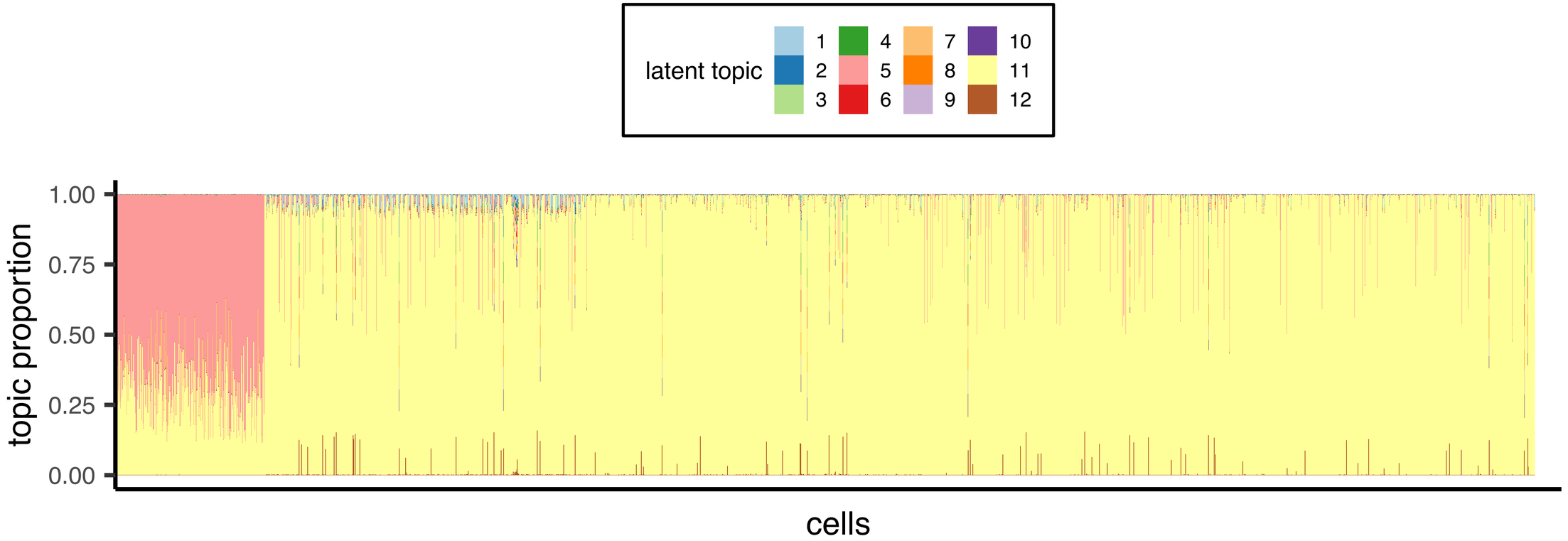
Variational inference $\approx$ maximum likelihood regularized by a KL-divergence term

$$\underbrace{\mathbb{E}[\log p(\mathbf{x}|\mathbf{h}(\mathbf{z}))]}_{\text{expected data likelihood}} - \underbrace{\mathbb{E}[\log q(\mathbf{z})/p(\mathbf{z})]}_{\text{KL loss}}$$



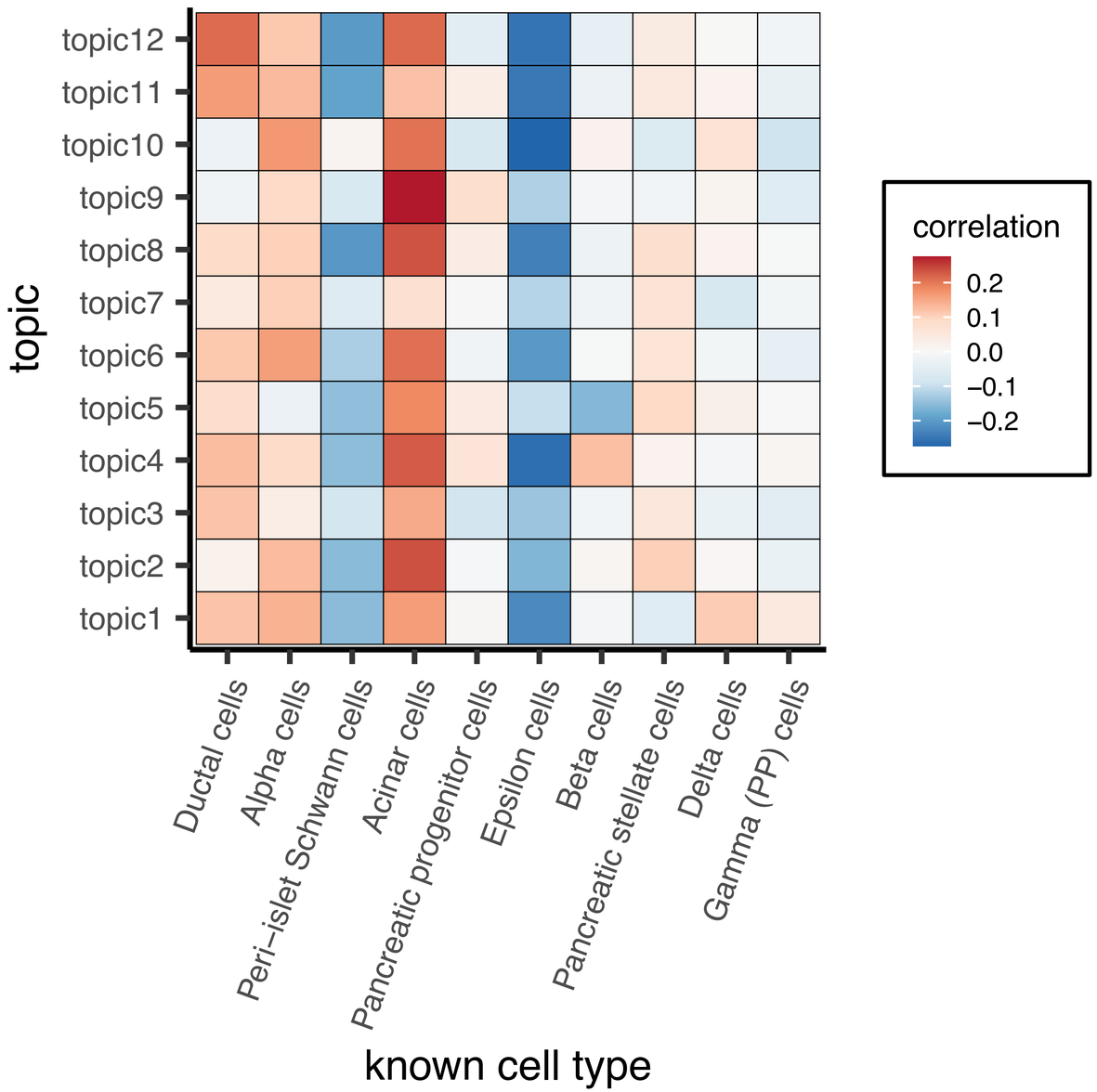
We may need to train longer than usual... (don't be fooled by log-likelihood)

ETM learning just started ... (hidden states $\mathbf{h}$)



There is no obvious pattern... yet

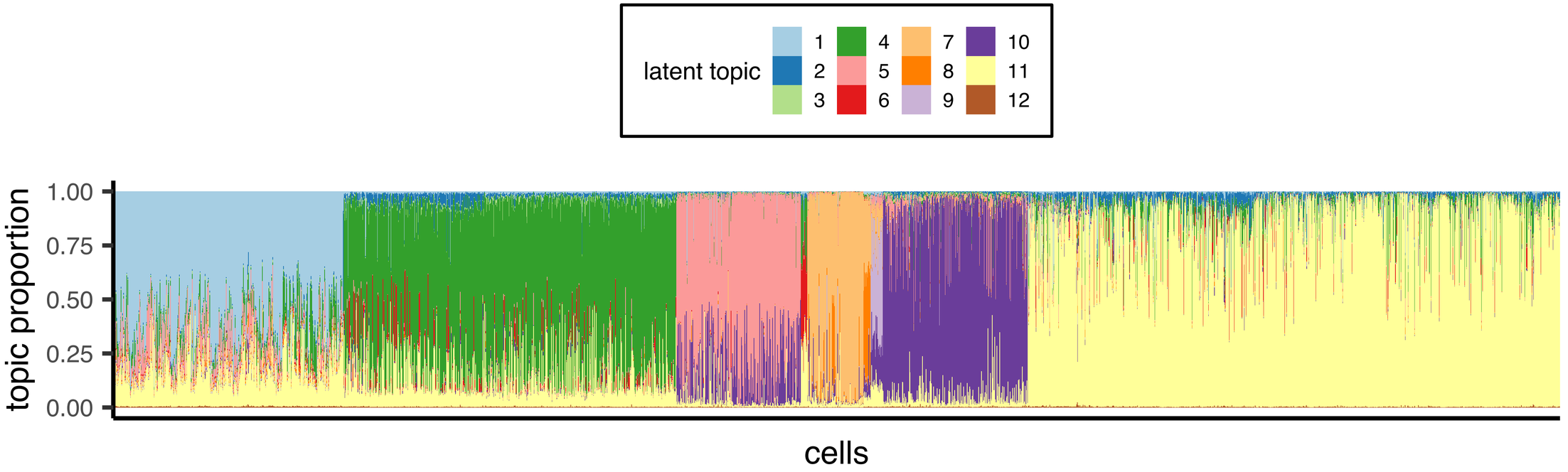
ETM learning just started ... (weight parameters β)



- We can correlate each topic-specific gene $\times 1$ weight vector, β_k, with known cell type-specific marker genes
- No obvious concepts emerged yet, not so specific correlation patterns, yet...

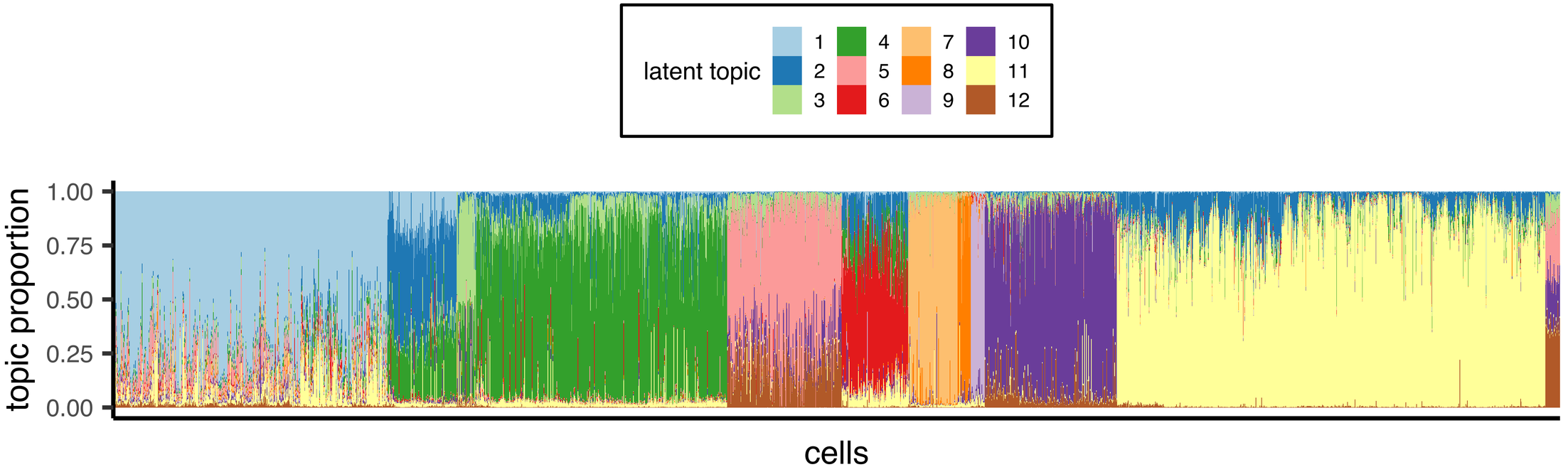
If we keep on training ETM (hidden states) ..

epoch = 270



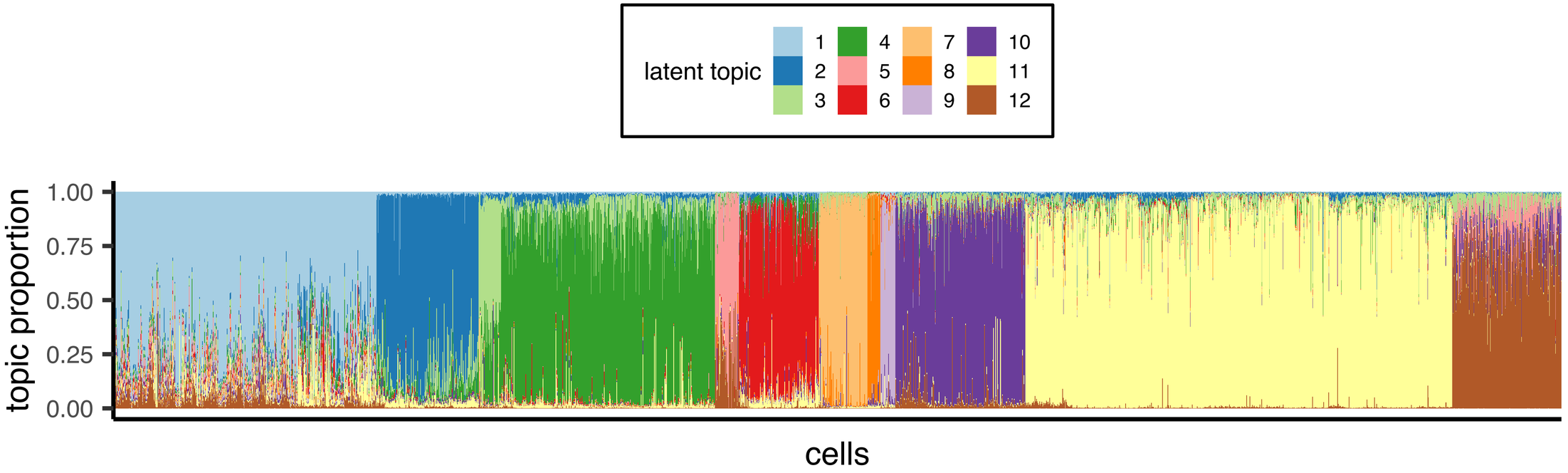
If we keep on training ETM (hidden states) ..

epoch = 570



If we keep on training ETM (hidden states) ..

epoch = 870



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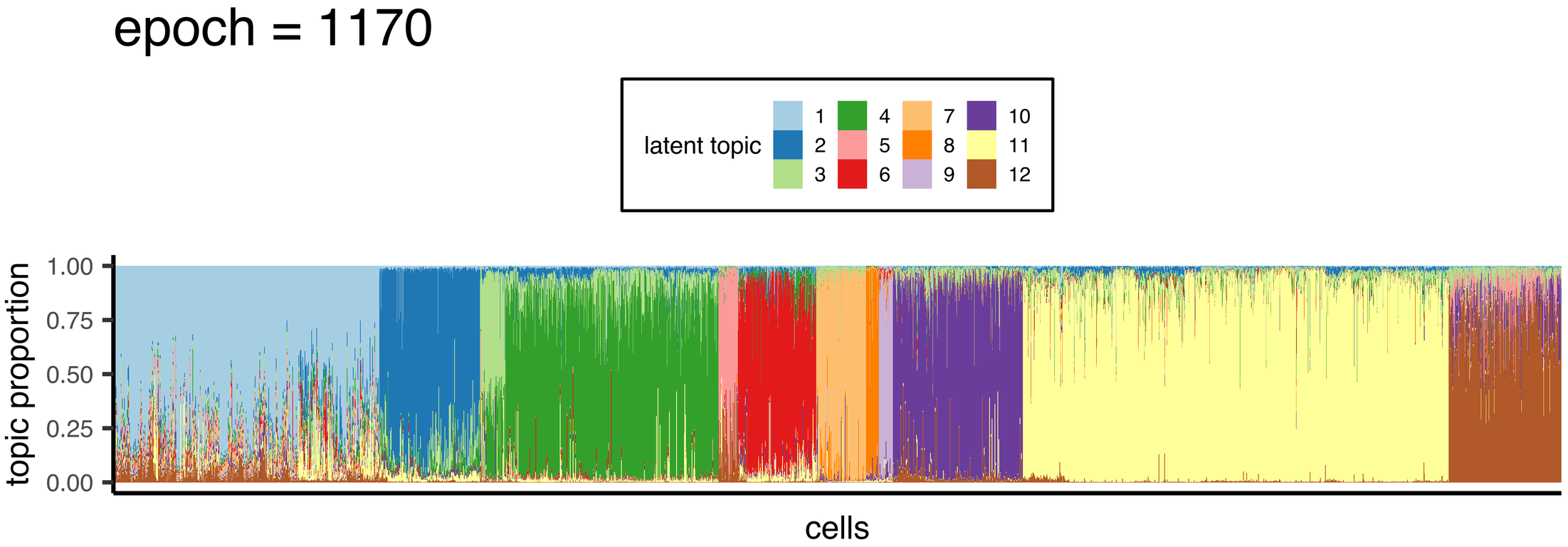
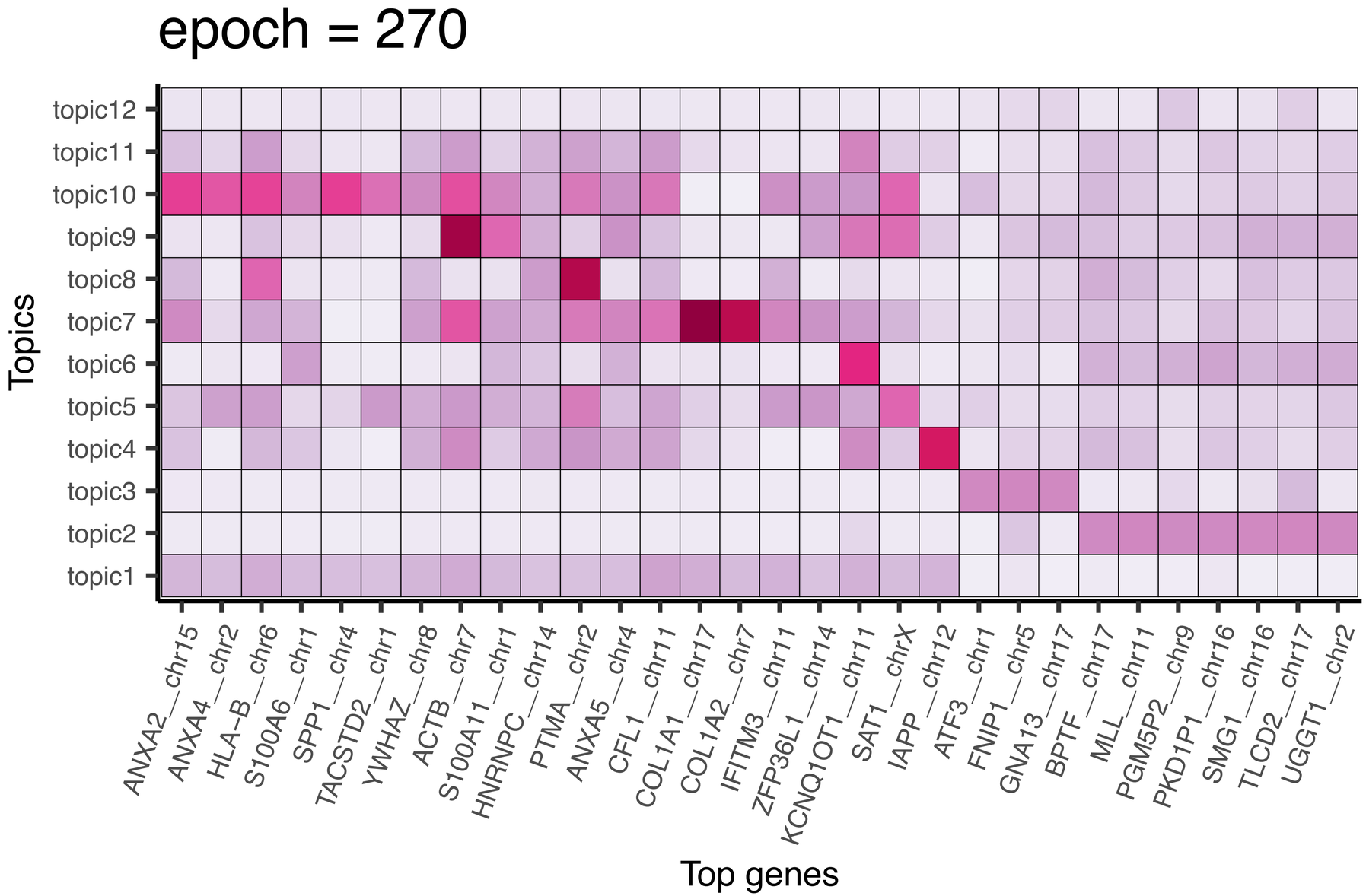


If we keep on training ETM (weight parameters) ...



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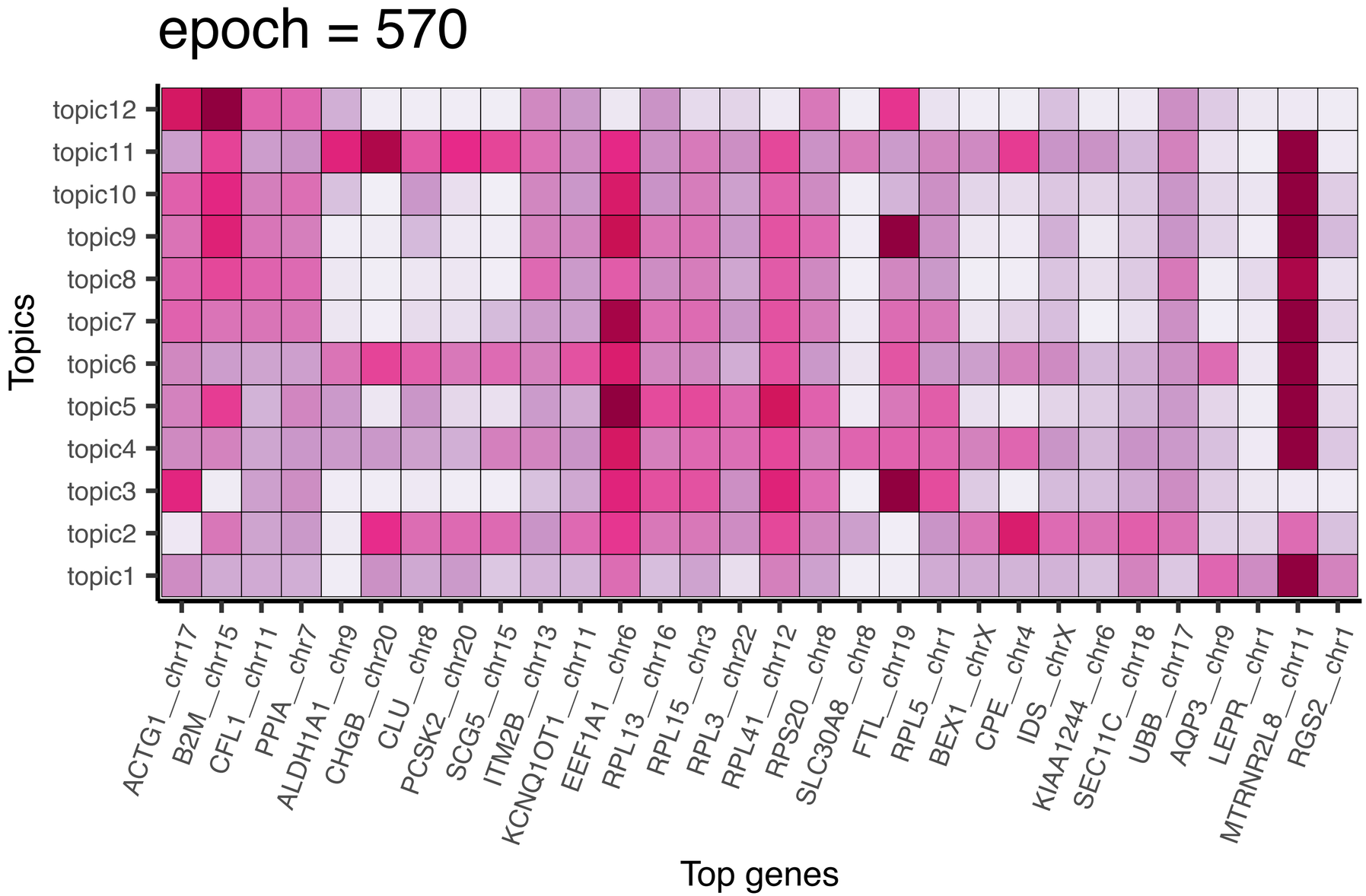

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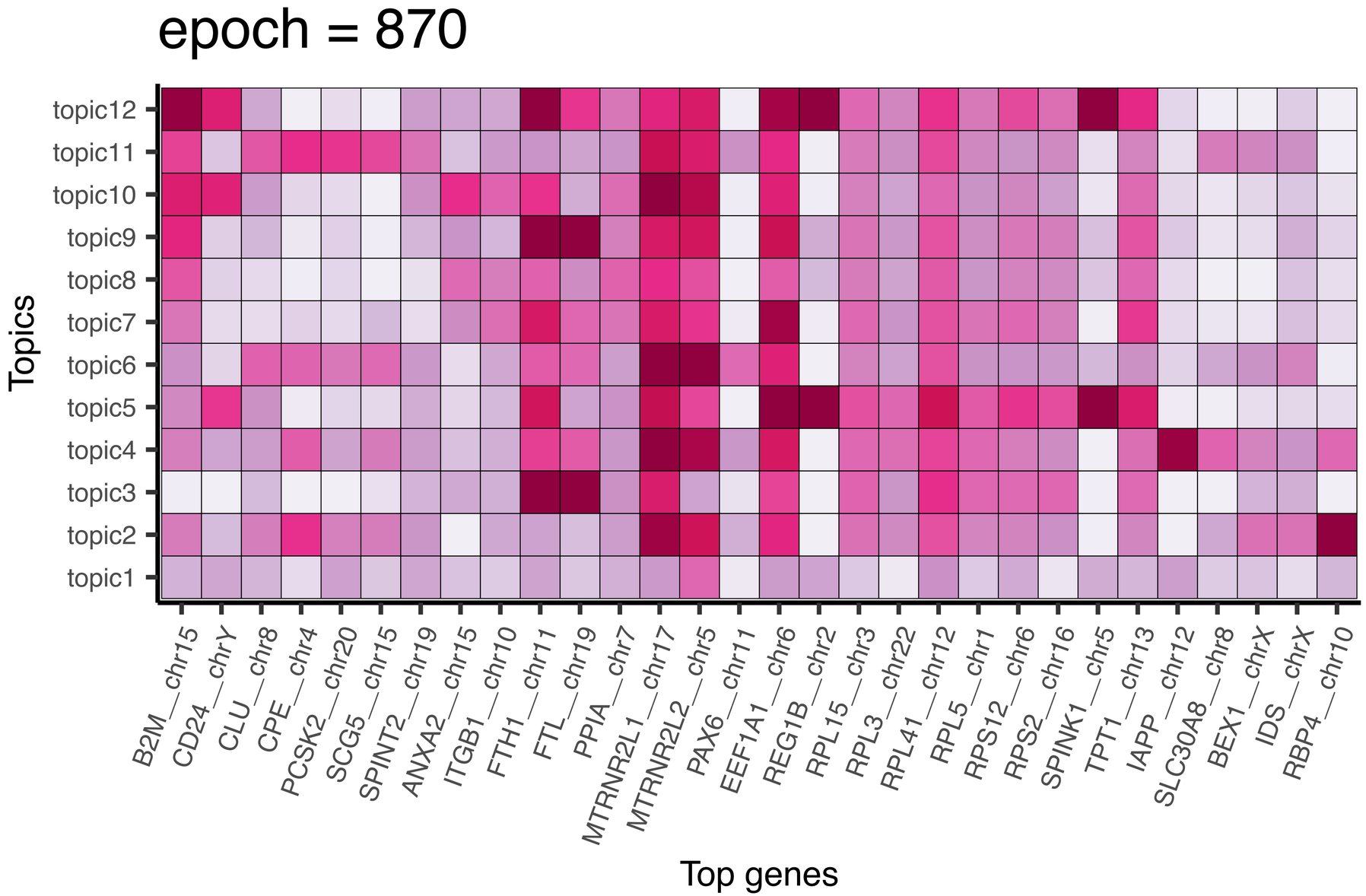

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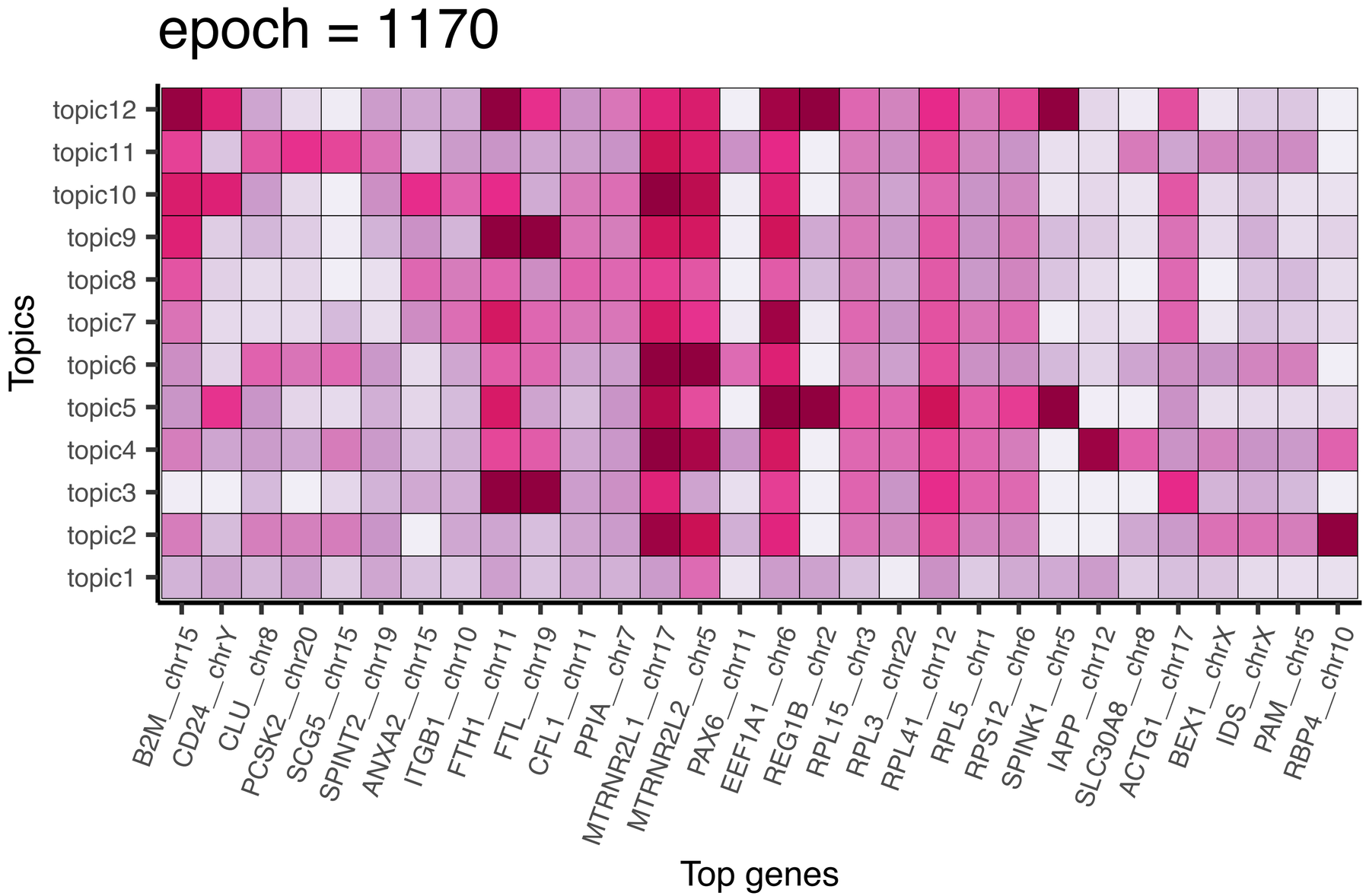

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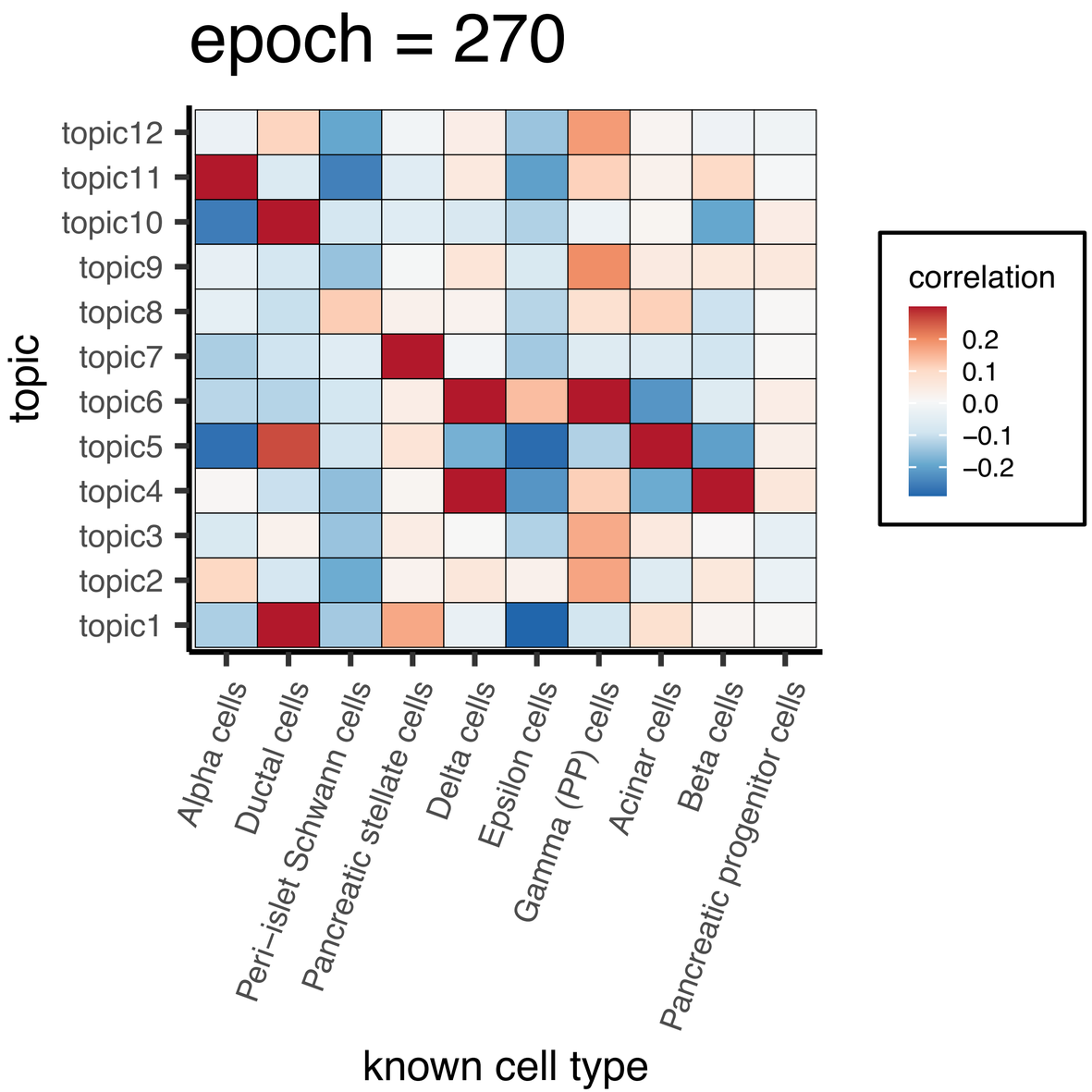

- We can correlate each topic-specific gene $\times 1$ weight vector, β_k, with known cell type-specific marker genes
- We retrieved marker gene information of known cell types from PangaloDB

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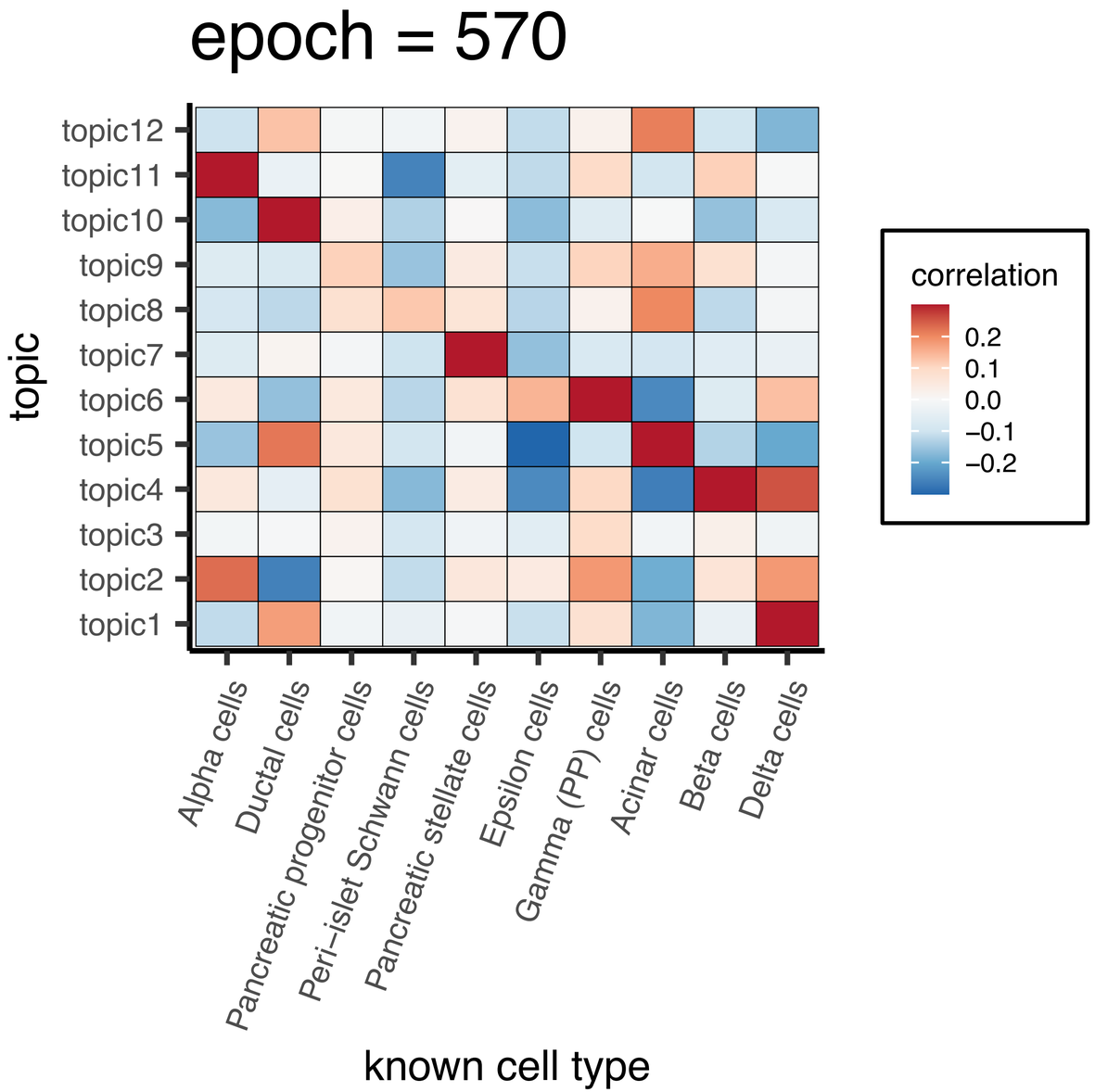

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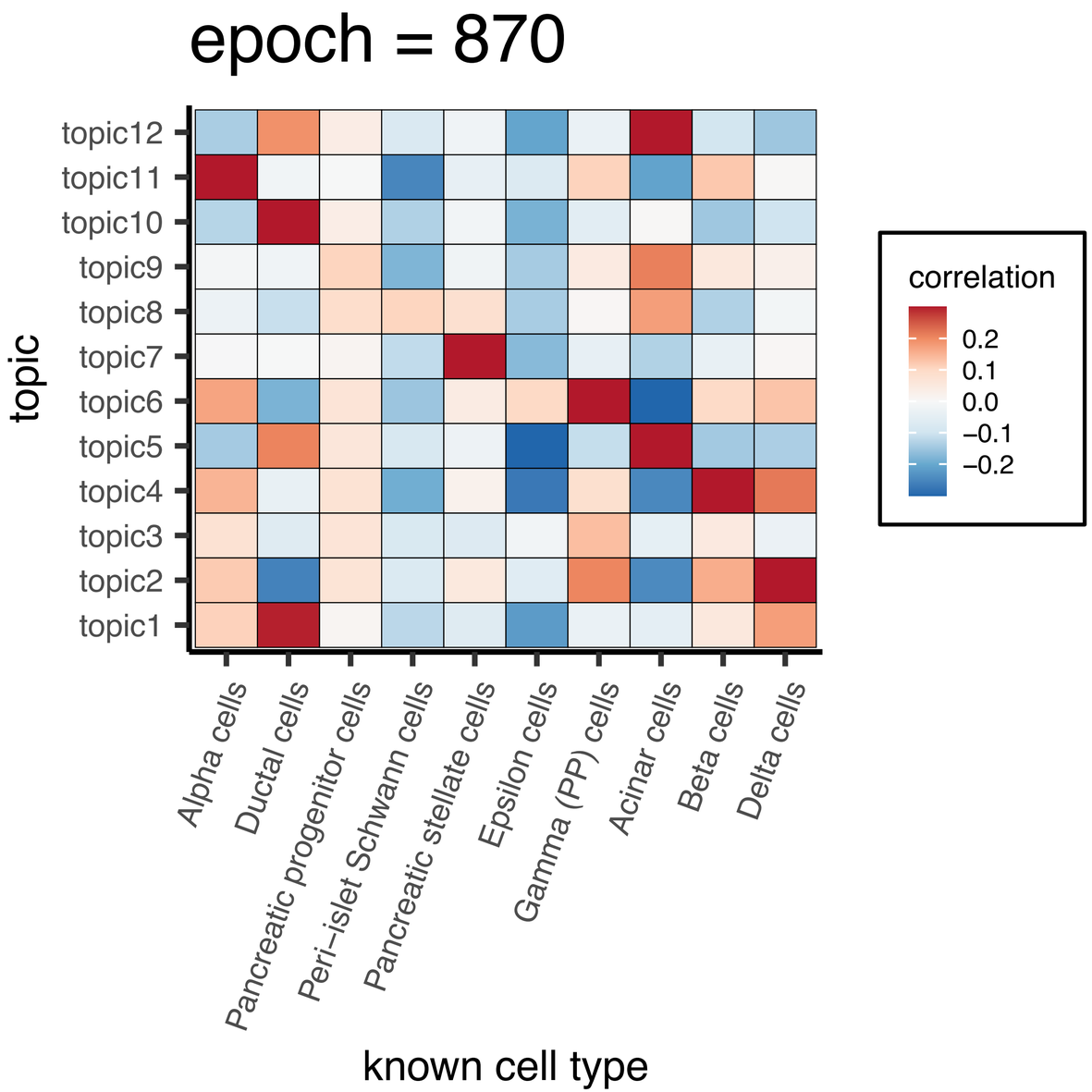

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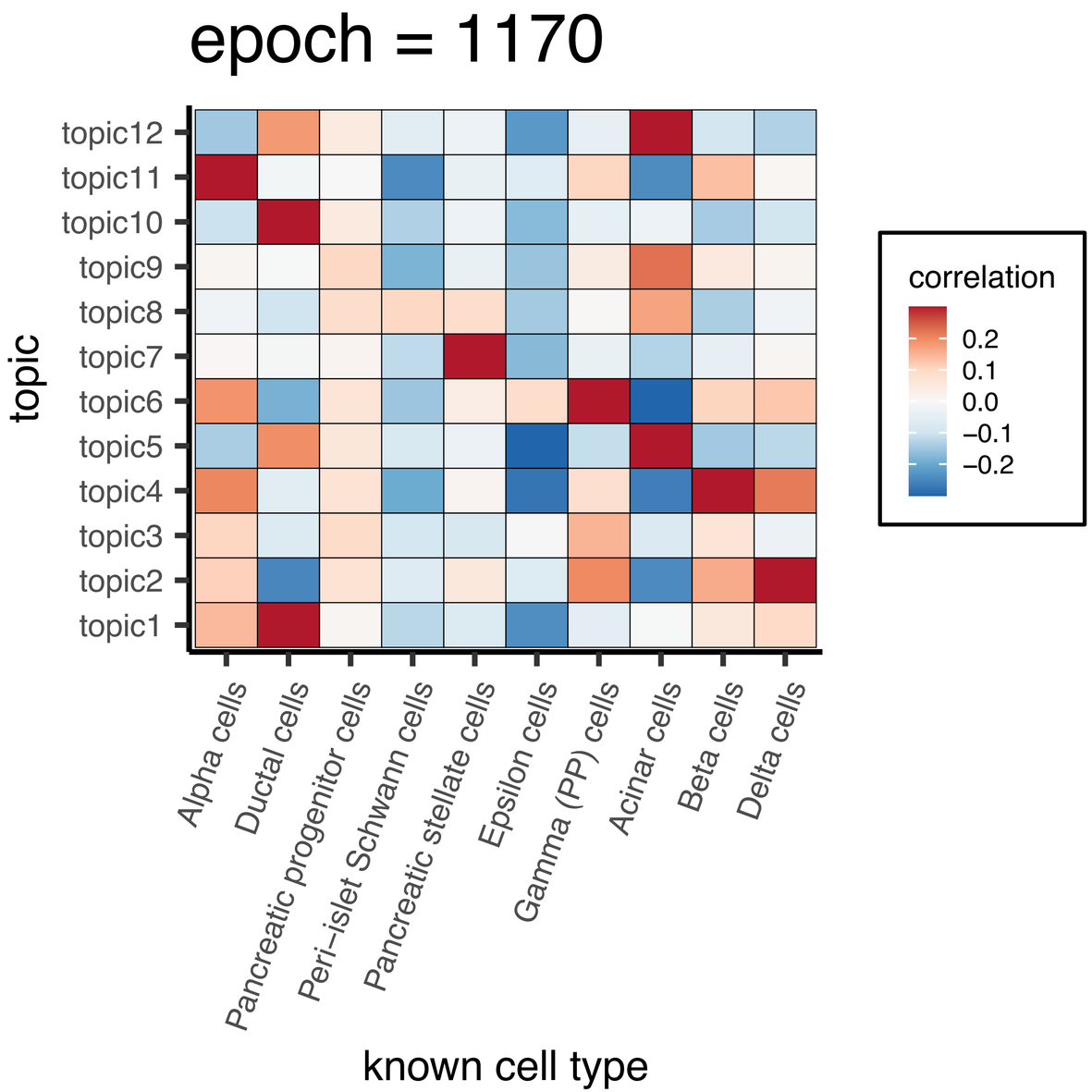

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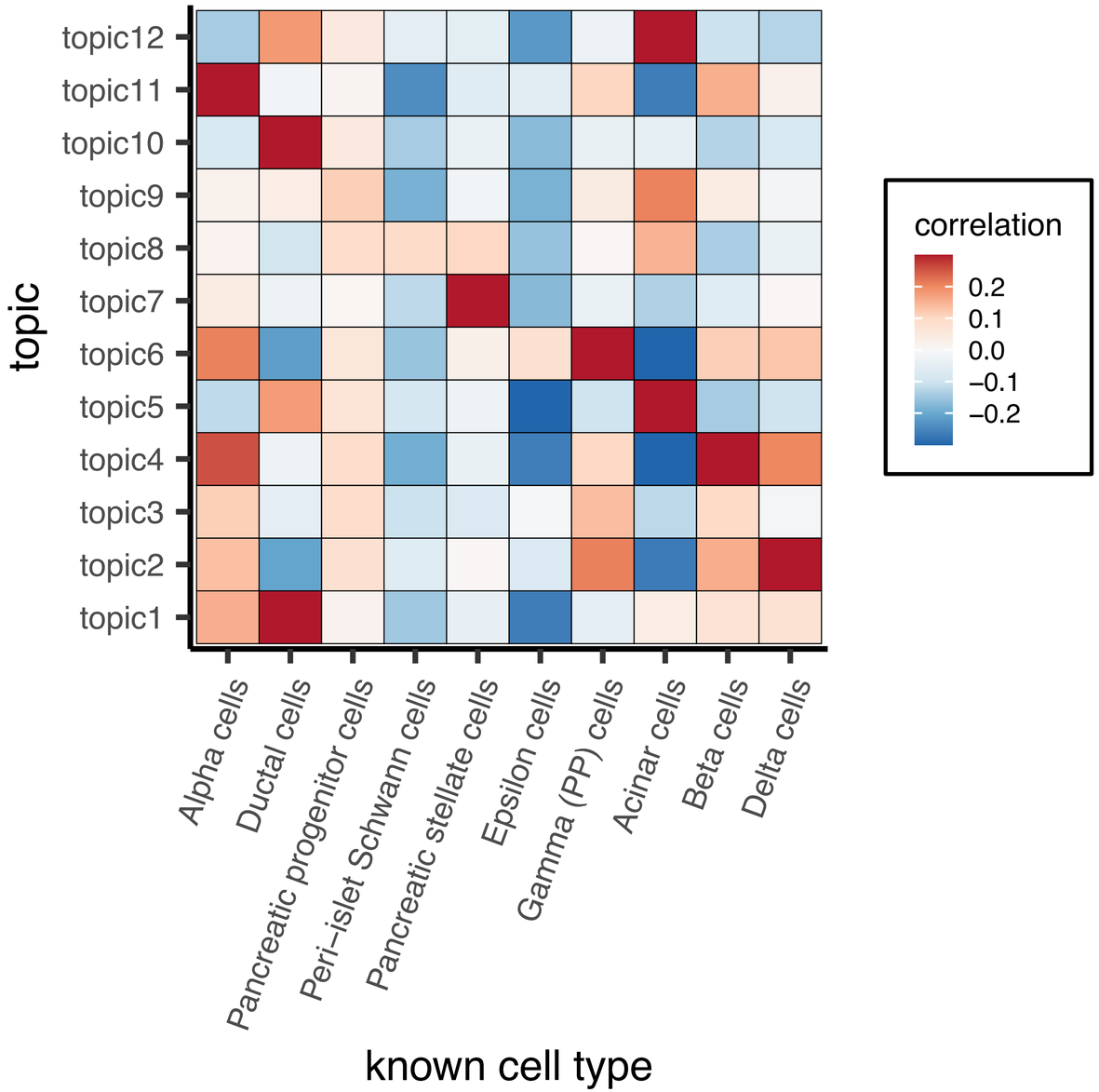
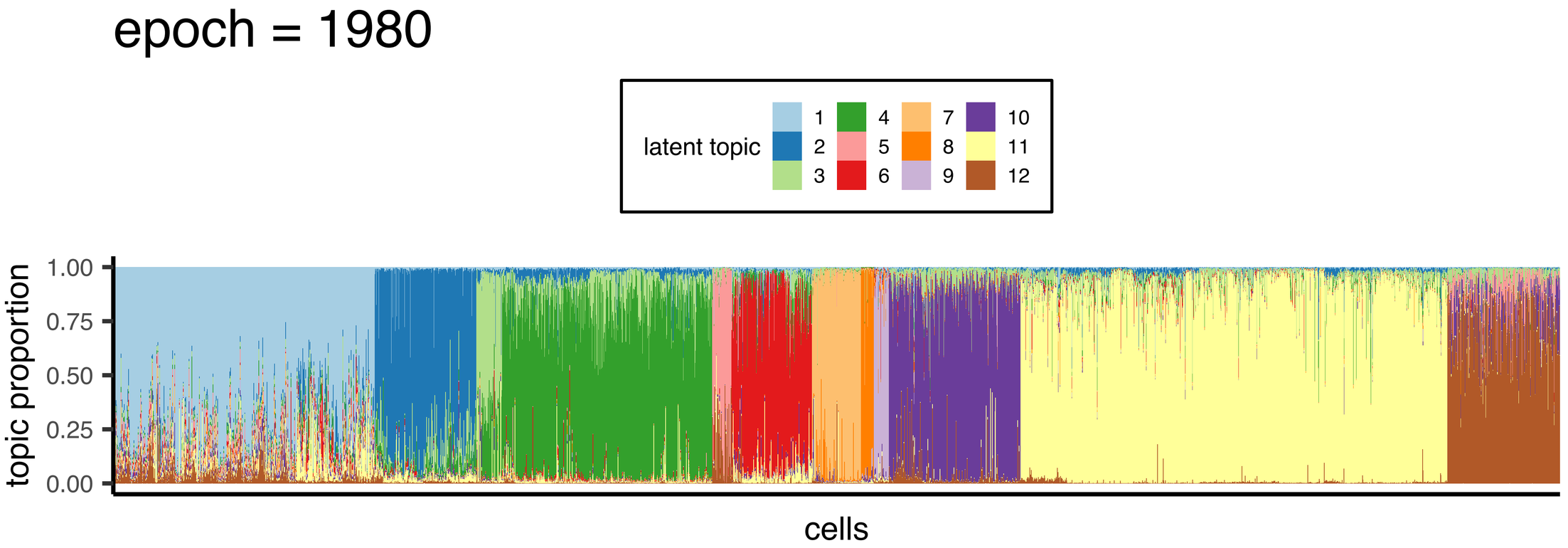
After enough training steps...

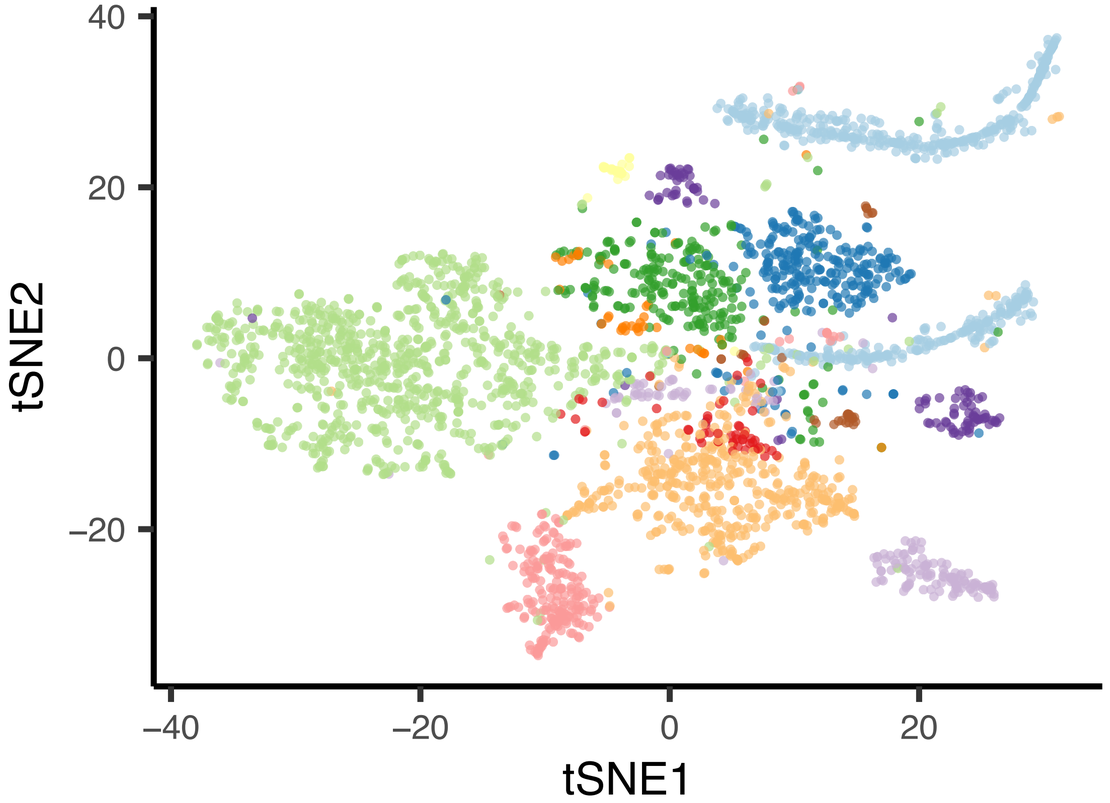


- We have recapitulated most known Pancreatic cell types in our single-cell analysis

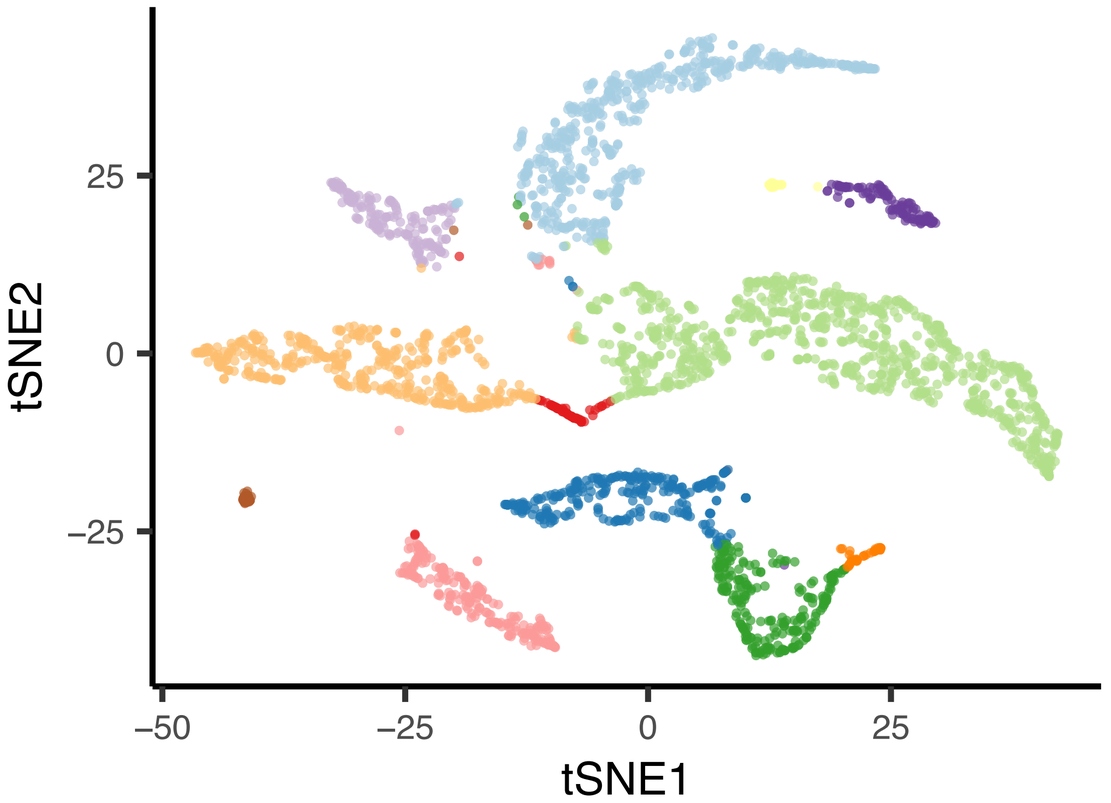
Single-cell ETM effectively learns cellular admixture model



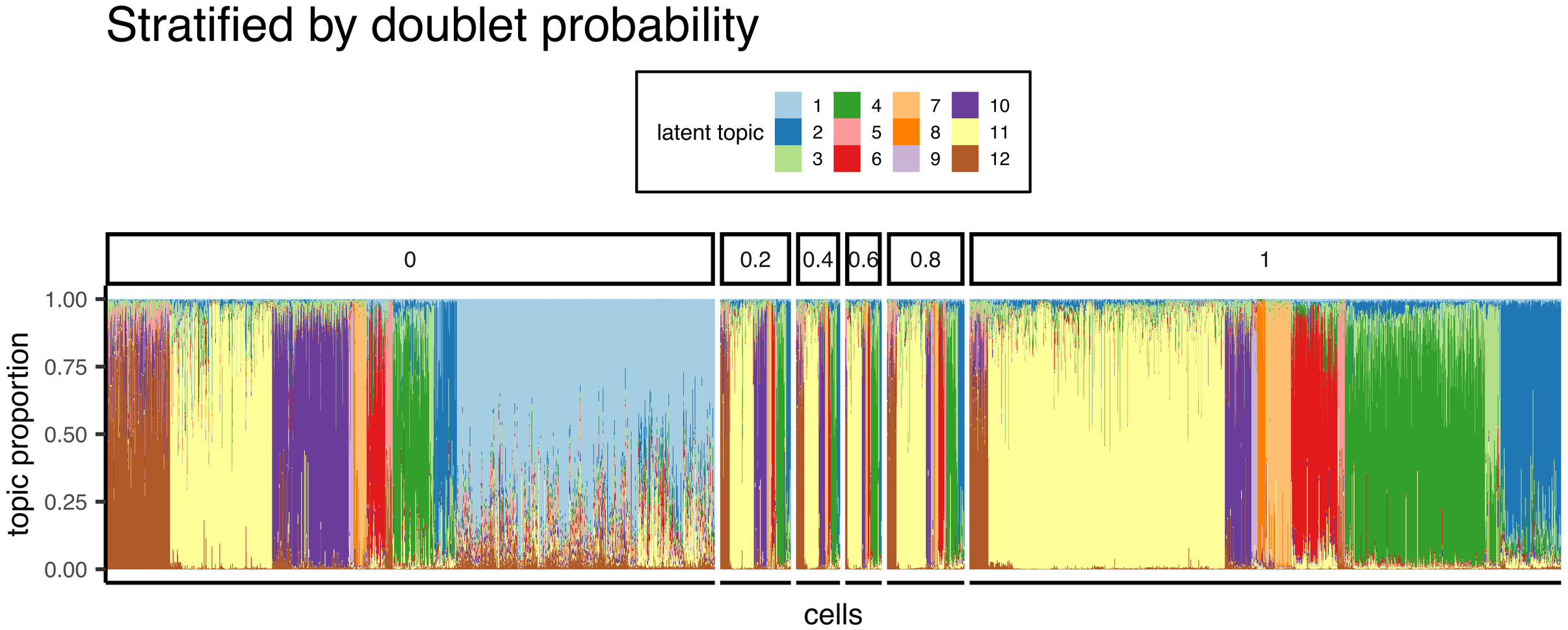
using top 50 PCs



tSNE on the latent topic space



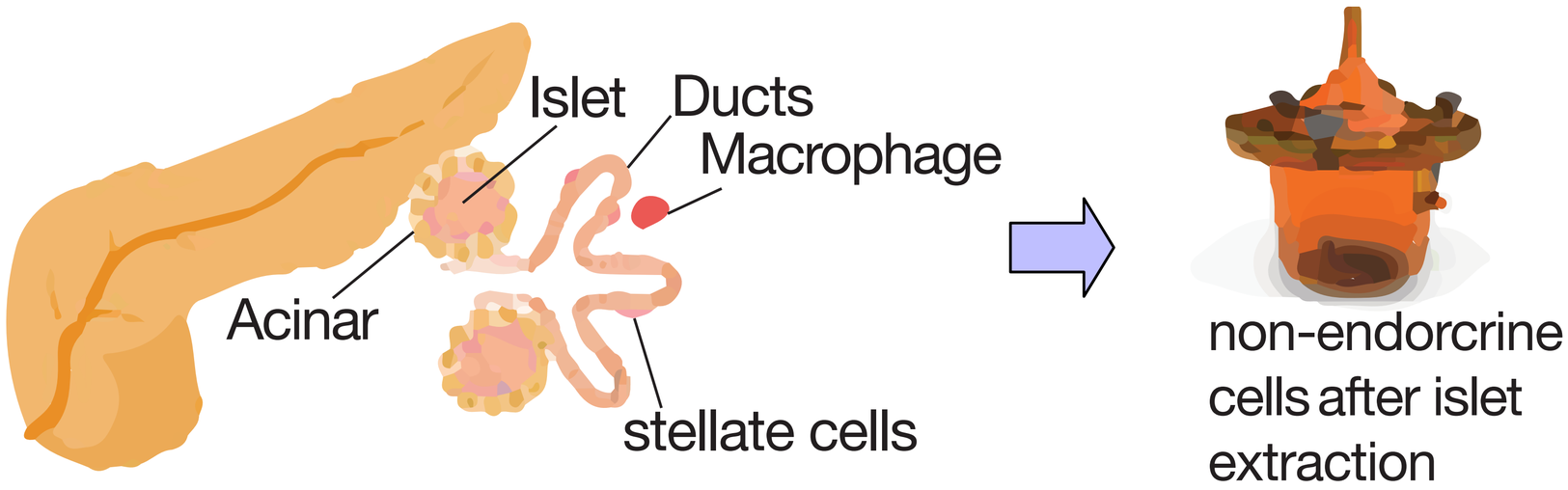
Wait, what about the doublets?



What do you think?

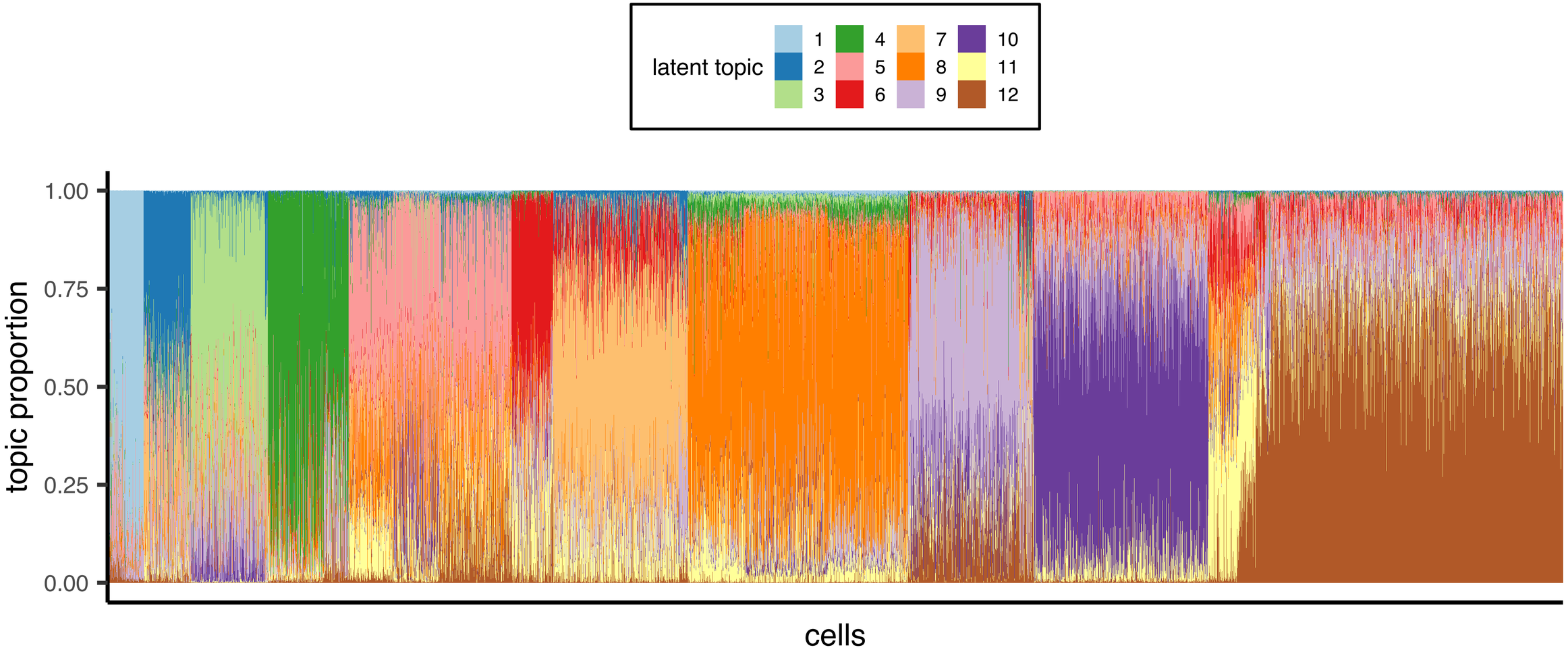
A latent topic model robustly capture cell states, avoiding batch effects

Single-cell RNA-seq data from three donors (three batches)

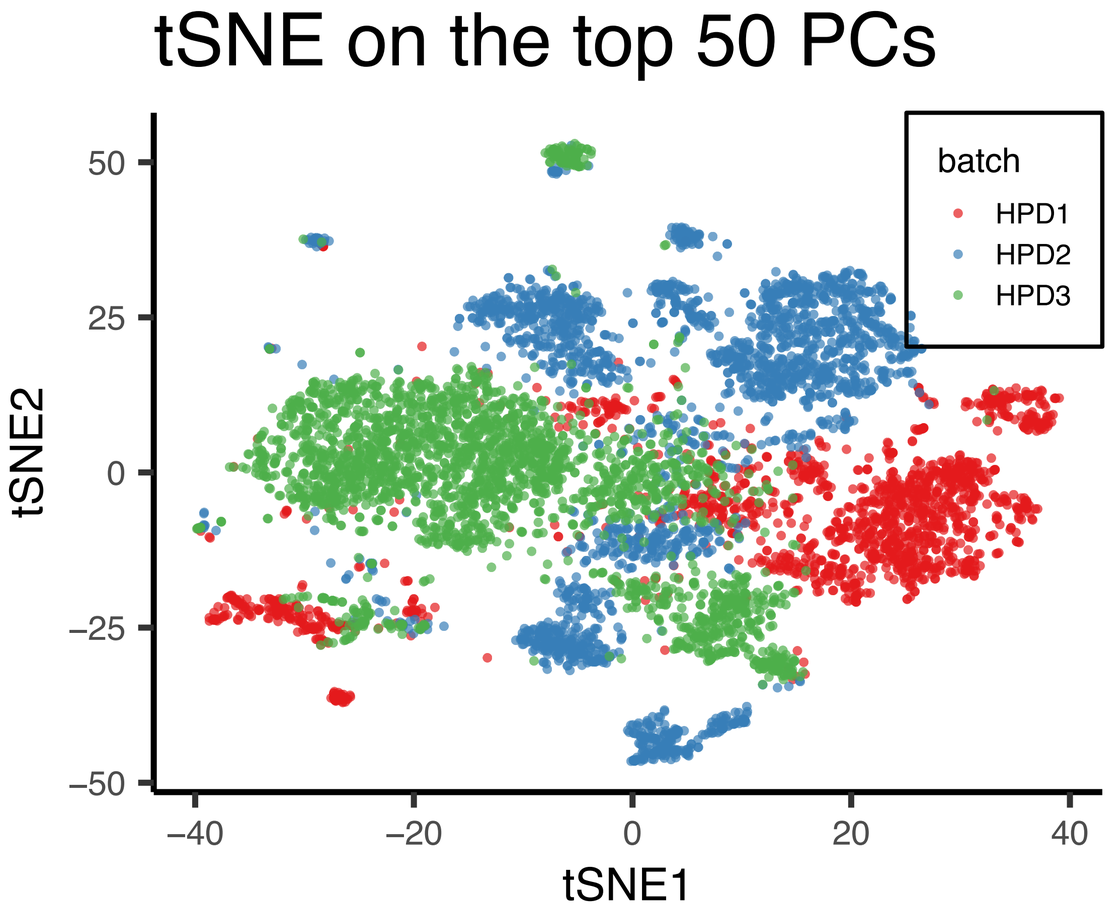


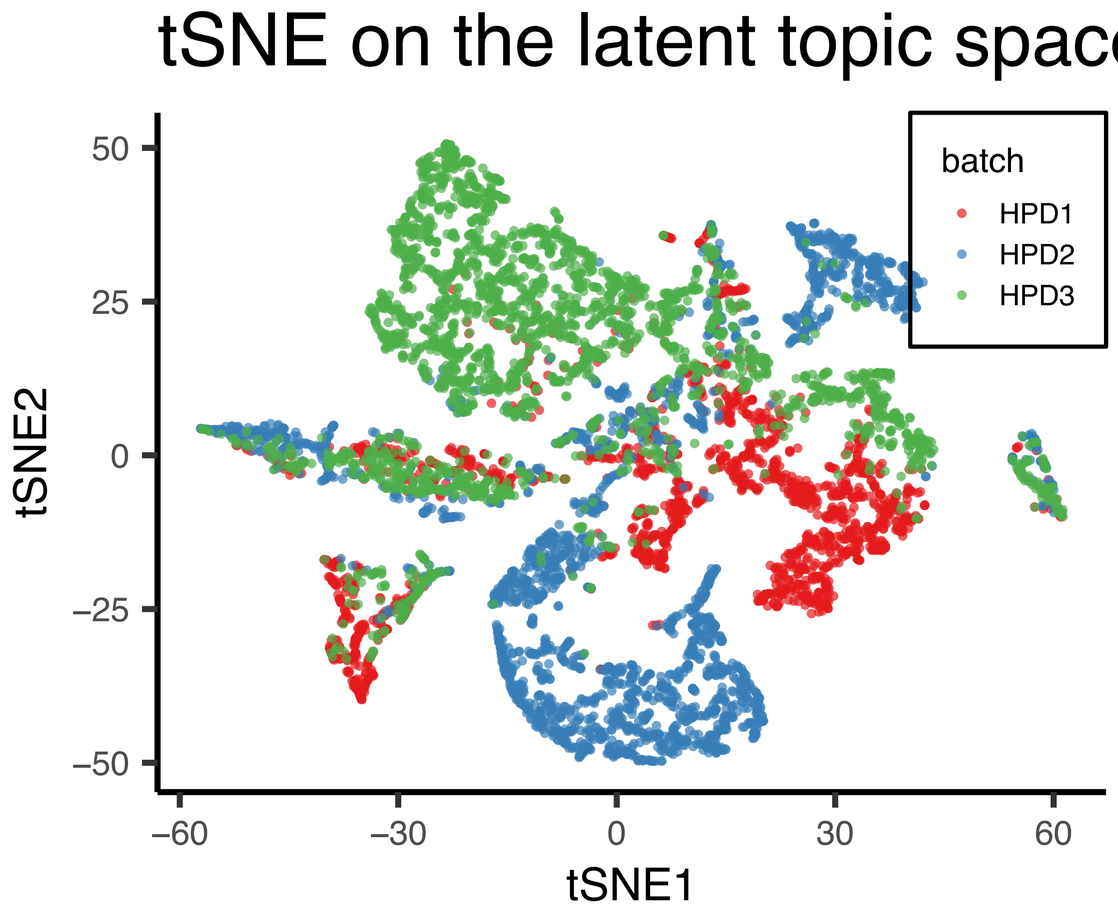
Goal: Topic space for 6,873 cells shared across multiple donors (batches).

Qadir, *et al.*, *PNAS* (2020)

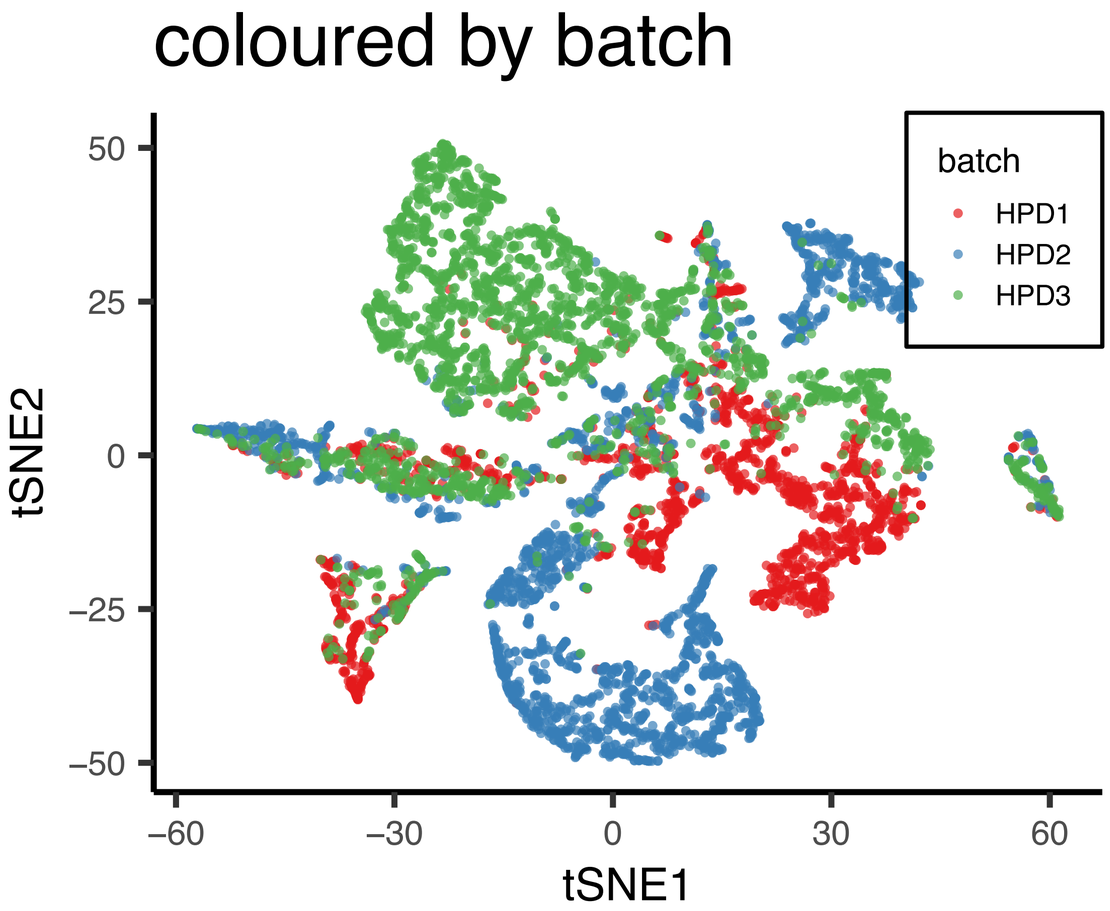

latent topic
1
2
3
4
5
6
7
8
9
10
11
12
1.00
0.75
0.50
0.25
0.00
topic proportion
cells

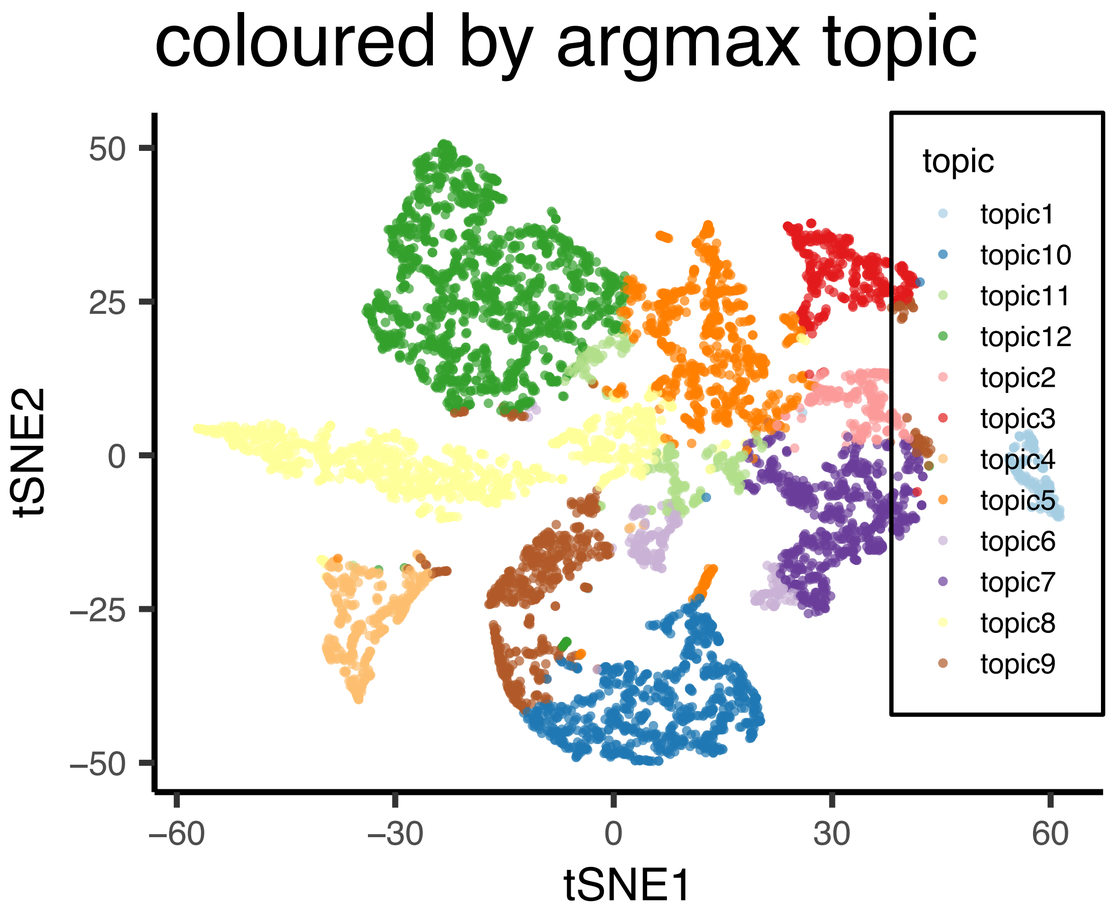
Multiple batches mingle well in latent topic space!





Multiple batches mingle well in latent topic space!





Discussions on latent topic modelling

- Most cells predominantly belong to one topic (one colour). Why?
- If we model cells as a mixture of cell topics, we can capture doublets or triplets
- The underlying generative model assumes no sequencing depth! This can help avoid batch-specific differences in practice.
- VAE offers a flexible framework with which our scientific hypothesis can be formulated in a probabilistic language
- Potentially, this pure unsupervised learning framework can be combined with supervised, semi-supervised learning models.

Today's lecture

- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

Other topics that we don't have time to discuss now

1. Differential expression analysis
2. RNA velocity and pseudo-time analysis
3. Multiomics data integration
4. Spatial transcriptomics
5. Joint analysis with bulk sequencing data

Summary

- Single-cell RNA-seq technology
- Doublet finding and other Q/C in scRNA-seq anlaysis
- Data normalization across multiple batches
- Model-based latent representation identification