Single-cell RNA-seq analysis

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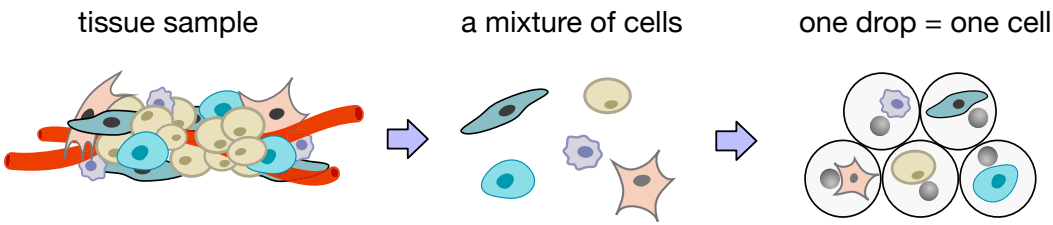
University of British Columbia

27 March, 2022

Today's lecture

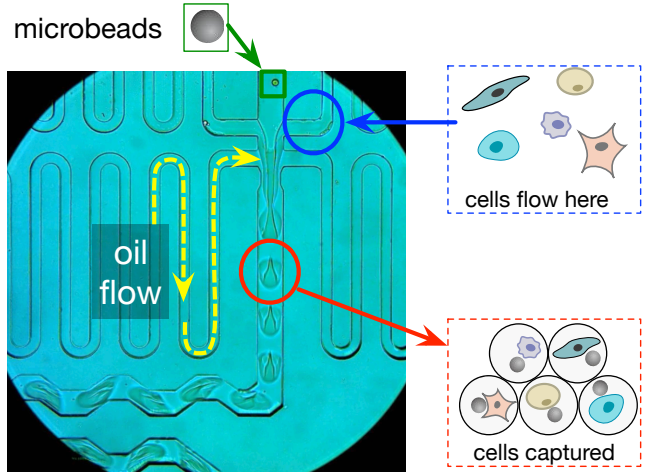
- Single-cell sequencing technology
- Basic Data Q/C
- Doublet detection in single-cell data
- Data normalization across many batches
- Latent topic modelling
- Other interesting topics in scRNA-seq analysis

Droplet-based single-cell sequencing technology



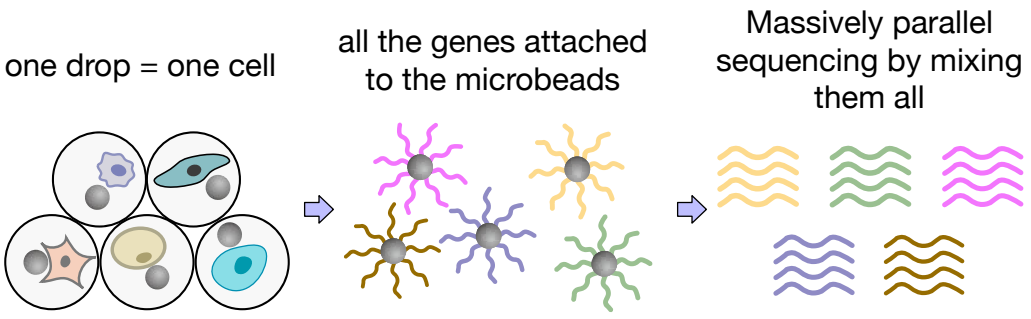
Macosko *et al.*, *Cell* (2015)

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



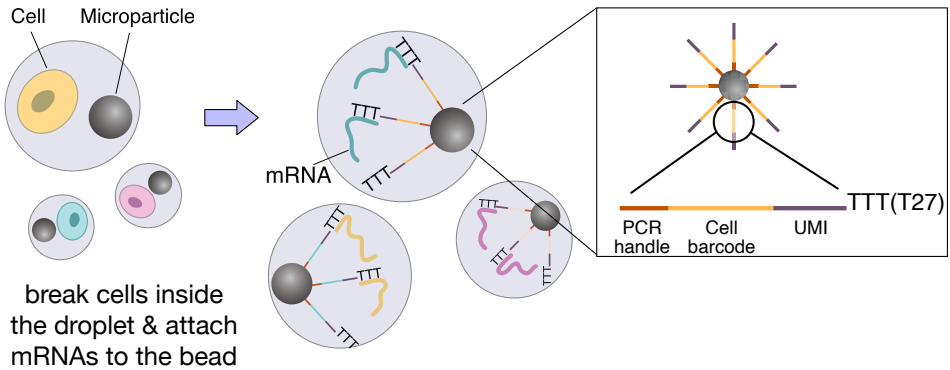
Macosko *et al.*, *Cell* (2015)

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



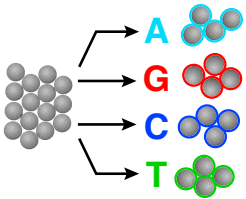
Macosko *et al.*, *Cell* (2015)

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



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)

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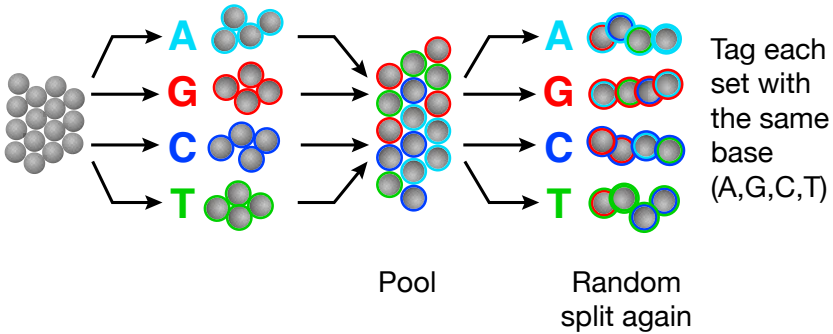

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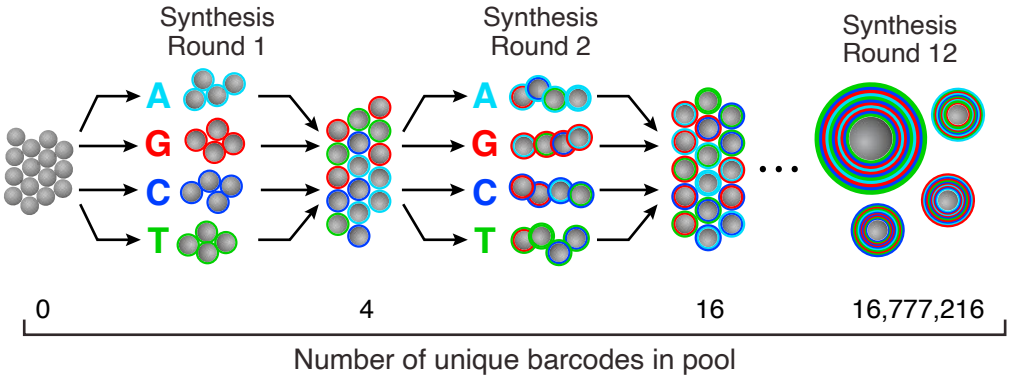
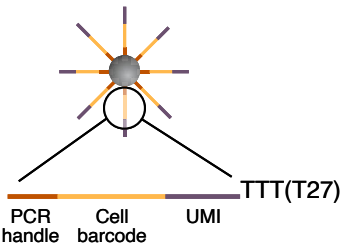
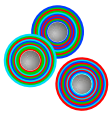


Macosko *et al.*, *Cell* (2015)

The lengths of barcode sequences determine data dimensionality



4^{12} 4^{8}



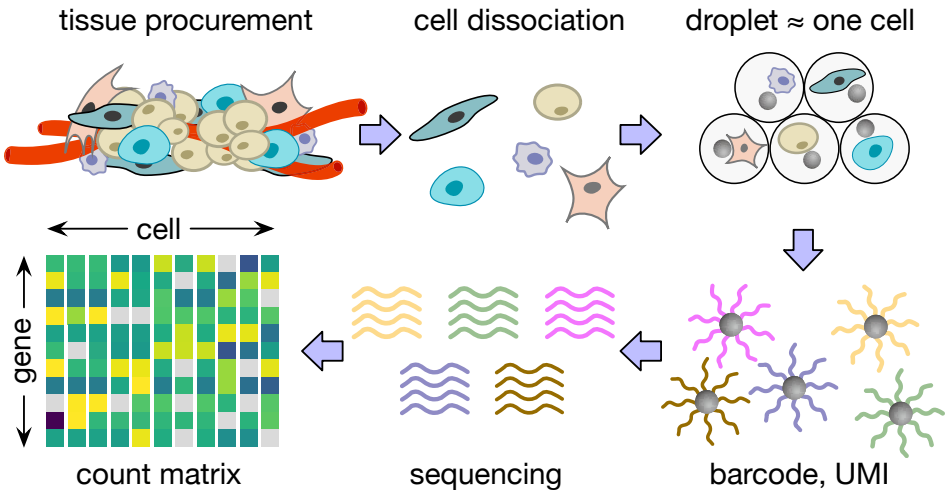
+ × 8 rounds of synthesis

- 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)

Droplet-based single-cell sequencing pipeline



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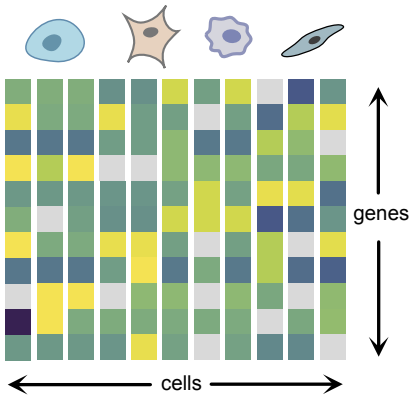
People love to see this high-dimensional data matrix in 2D/3D space



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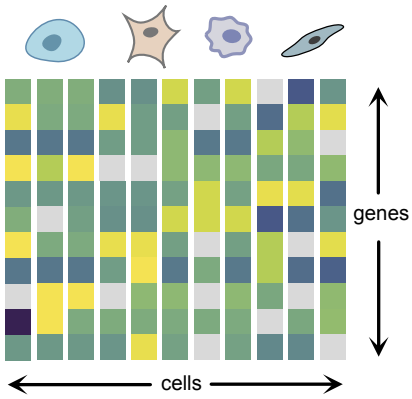
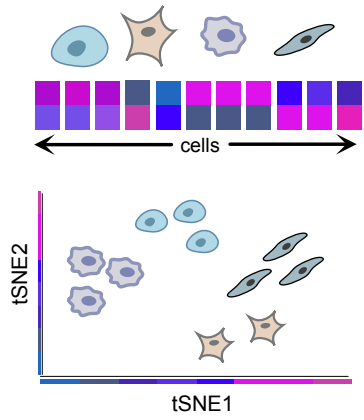
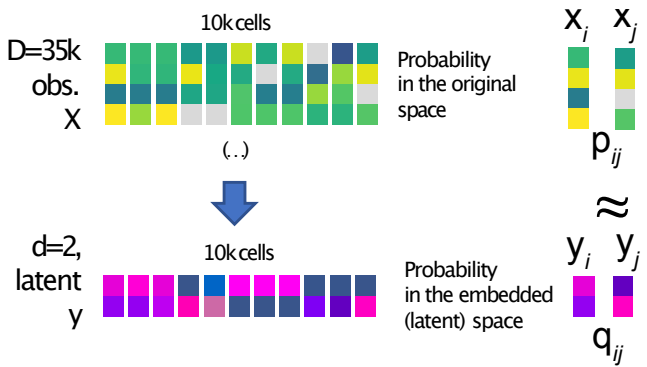




`tSNE`: t-distributed Stochastic Neighbourhood Embedding (Van der Maaten & Hinton, 2008).

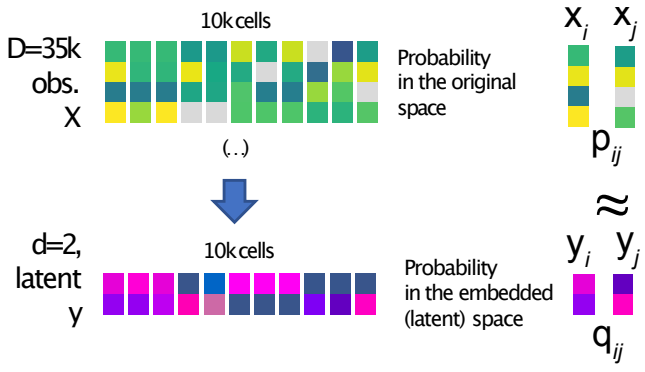
SNE: What is "stochastic neighbourhood embedding?"



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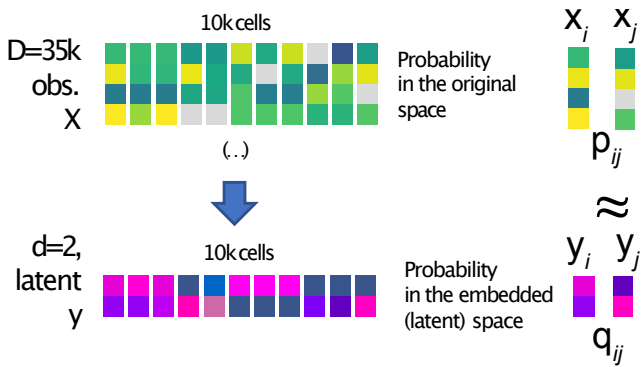
$$q_{ij} \propto \exp(\|\mathbf{y}_i - \mathbf{y}_j\|^2 / 2\sigma^2)$$

SNE: What is "stochastic neighbourhood embedding?"



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

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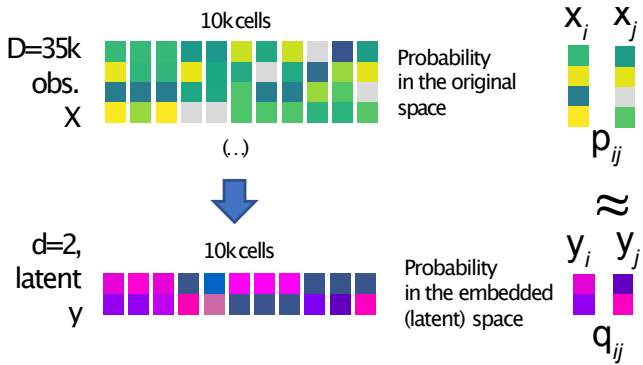

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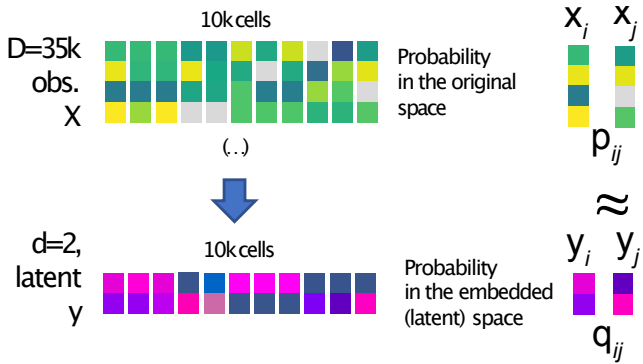
$$\min D_{\mathrm{KL}}\left(p_{ij} \| q_{ij}\right) = \sum_{ij} p_{ij} \frac{p_{ij}}{q_{ij}}$$

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



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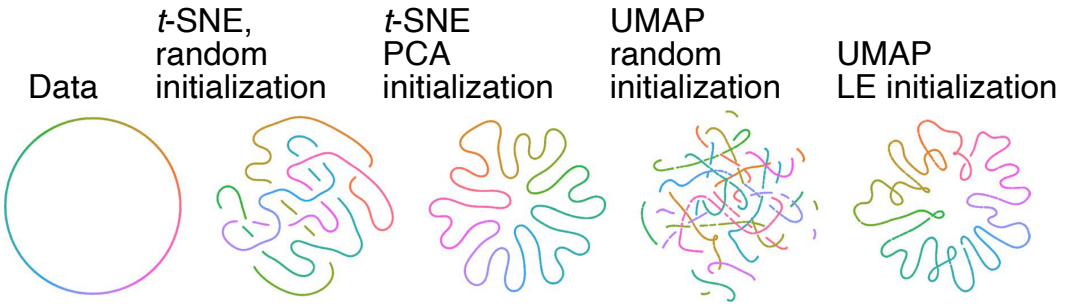
- q_{ij}: probability between cells i and j in the embedded low-dimensional space

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

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

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

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

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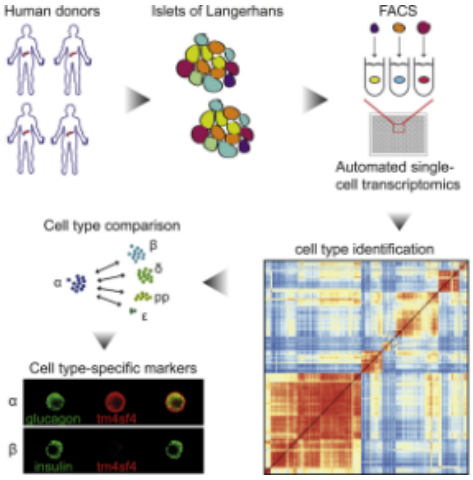
- Other interesting topics in scRNA-seq analysis

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

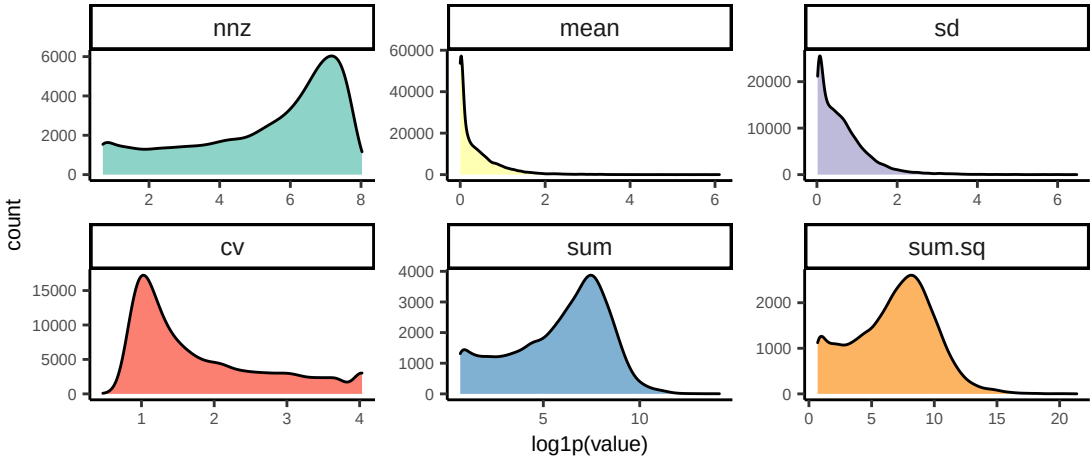


Muraro *et al. Cell Systems* (2016)

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

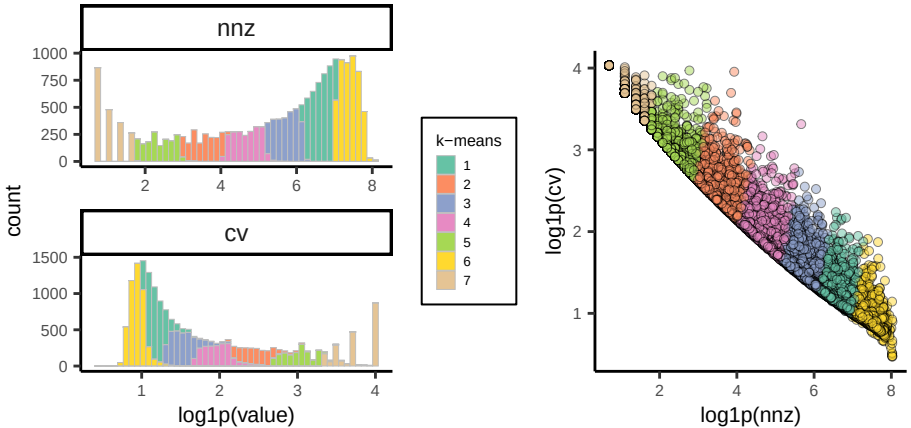
- genes/features/rows: 19,140
- cells/columns: 3,072
- non-zero elements: 12,442,034
- $\sim$ 21 % non-zero

Gene-level statistics across cells



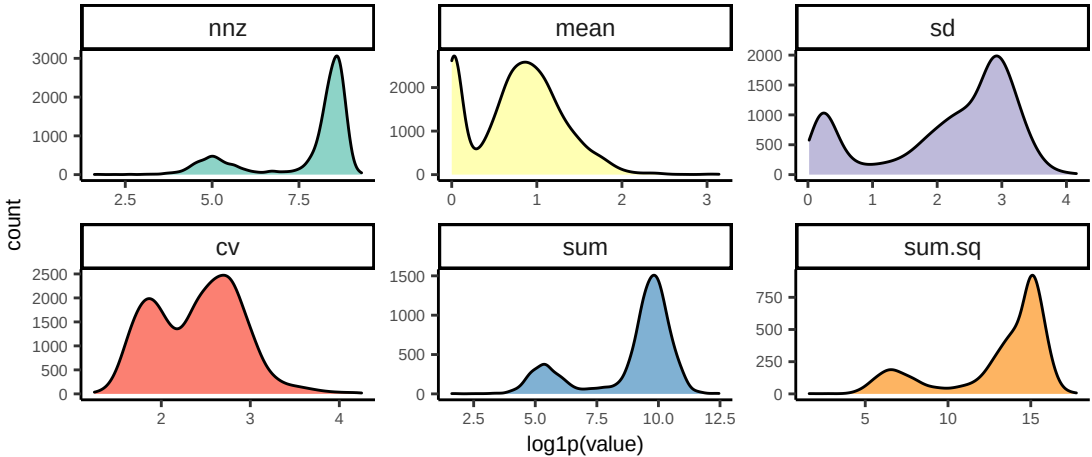
`nnz`: number of non-zero elements, `sd`: standard deviation, `cv`: coefficient of variation (`sd`/`mean`), `sum.sq`: sum of squares.

Can we drop any genes?



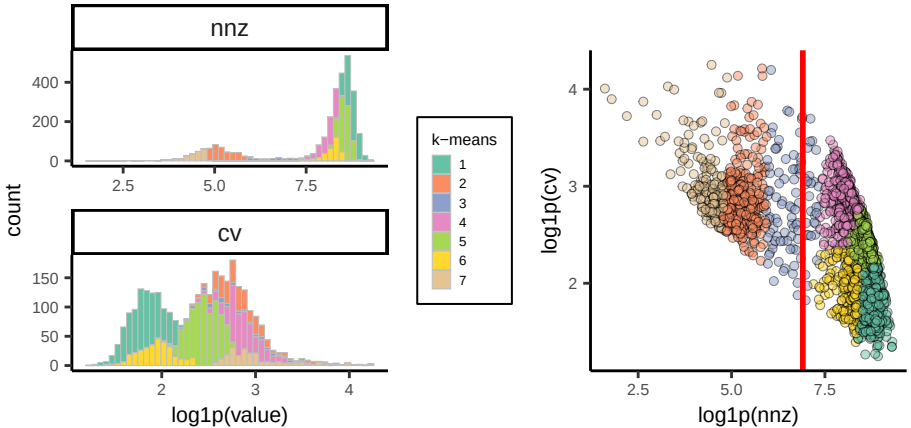
We don't like unstable genes with low average expressions and high CV.

Cell-level statistics across genes within each cell



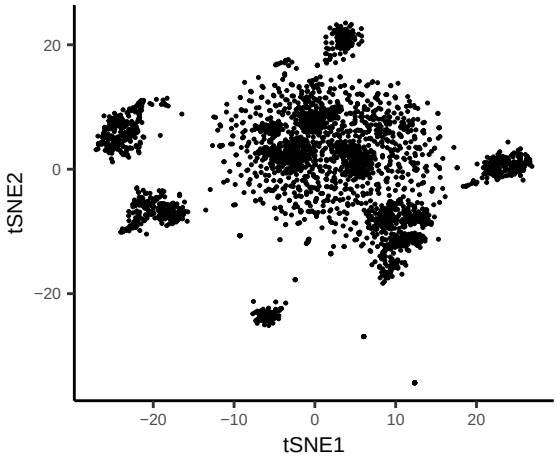
`nnz`: number of non-zero elements, `sd`: standard deviation, `cv`: coefficient of variation (`sd`/`mean`), `sum.sq`: sum of squares.

Filter out cells that are not informative...?



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

Exploratory Data Analysis with tSNE



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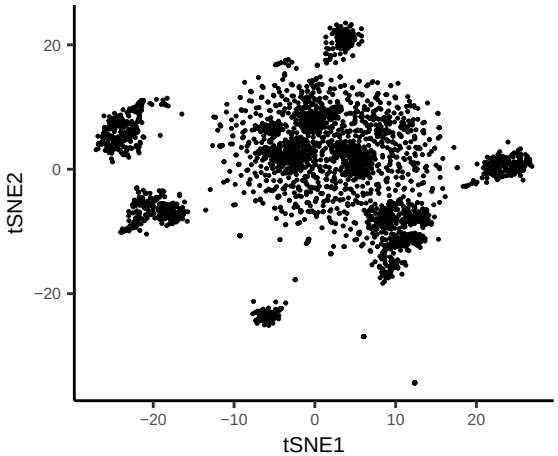

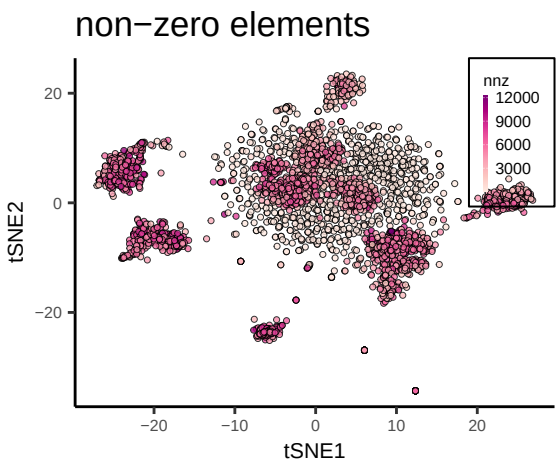

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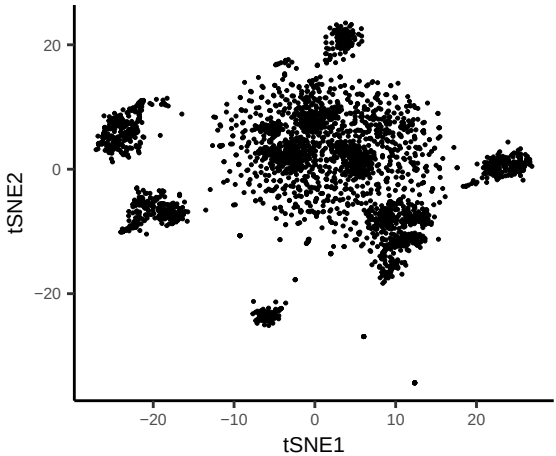

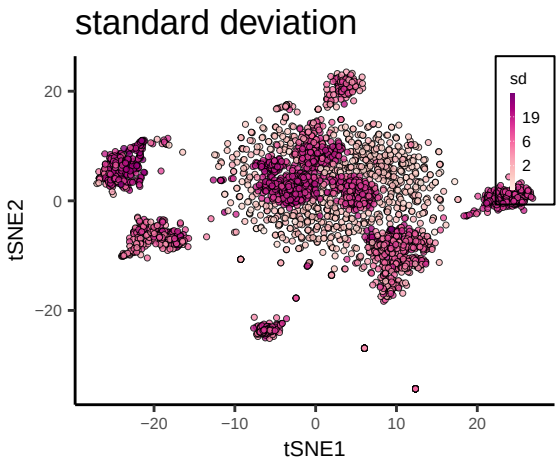

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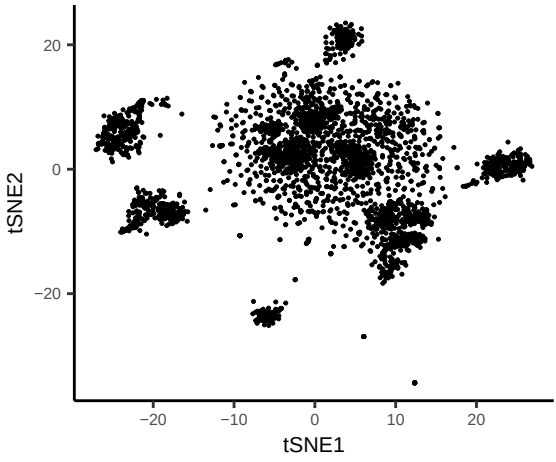

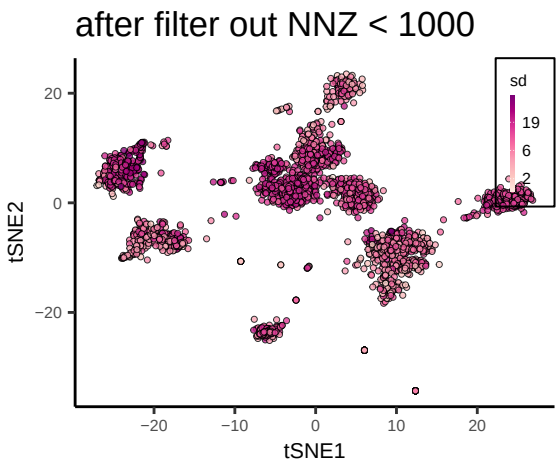

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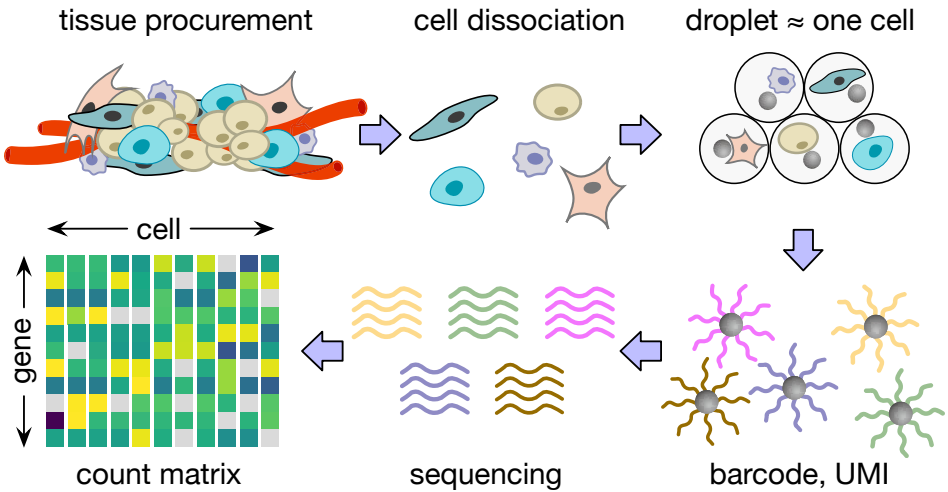
scRNA-seq pipeline: What if we capture more than one cell in a droplet?



Macosko *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:

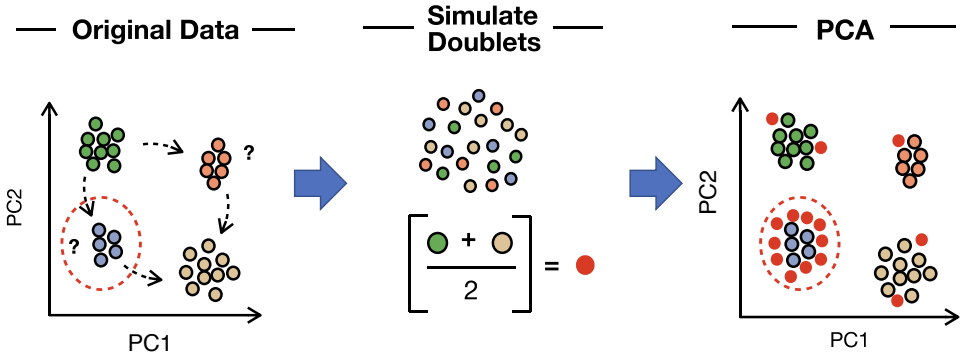
- Take some other j by random selection
- Create an artificial doublet

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

Some thought questions:

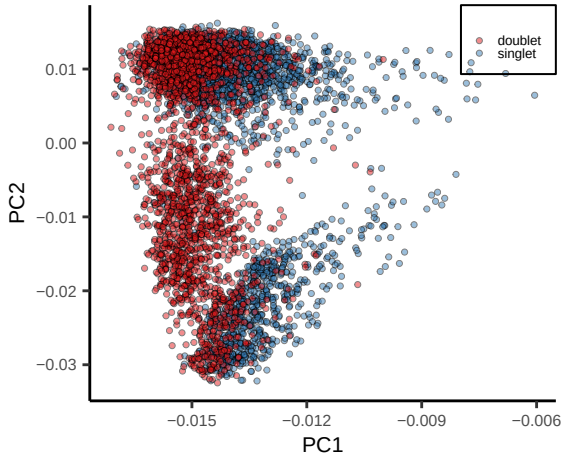
- Doublets within the same cell type?
- Doublets between the different cell types?

k-Nearest Neighbour classification for doublet detection



McGinnis et al. Cell Systems (2019)

Can you tell the difference by a quick visual inspection?



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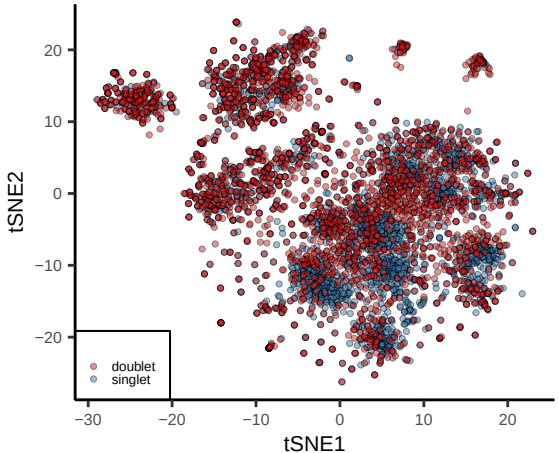

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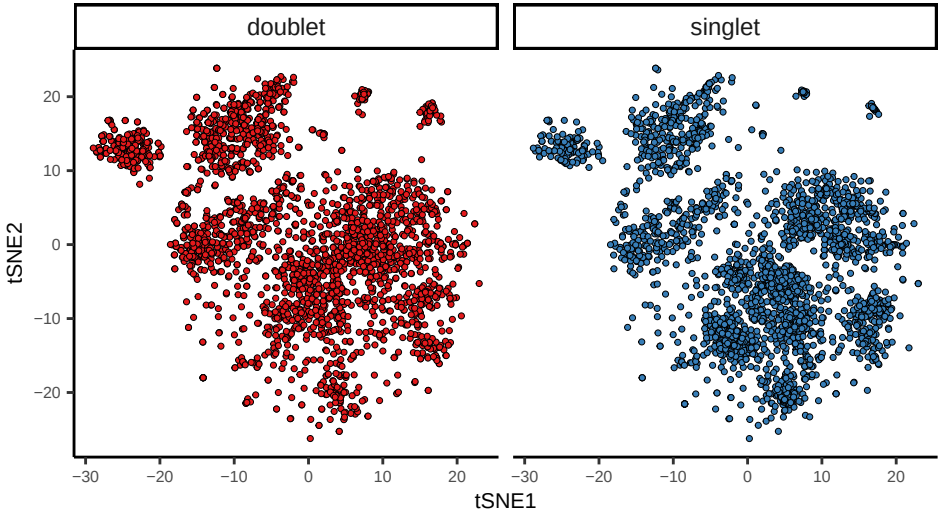


Can we design a classifier to distinguish singlets vs. doublets?

k-Nearest Neighbour classification for doublet detection

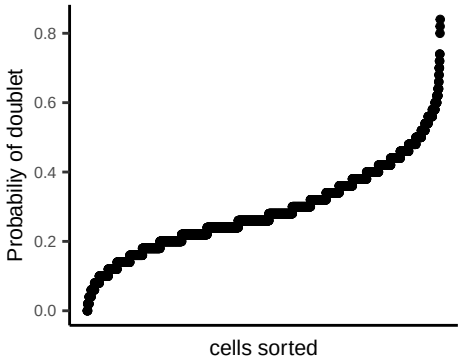
- Step 1. Create artificial doublets, $\tilde{\mathbf{x}}$
- Step 2. Mix them with the original cells and perform PCA
- Step 3. Find nearest neighbours of the original cells (using #PC=50)
- Step 4. Count the number of doublets in the neighbourhood

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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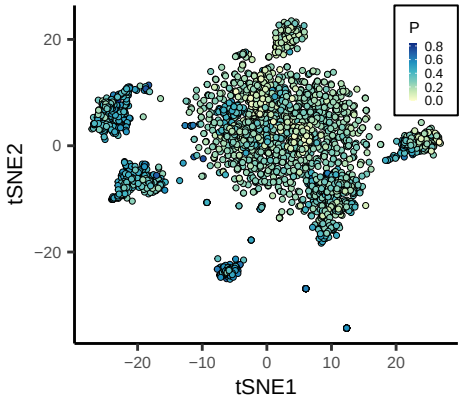

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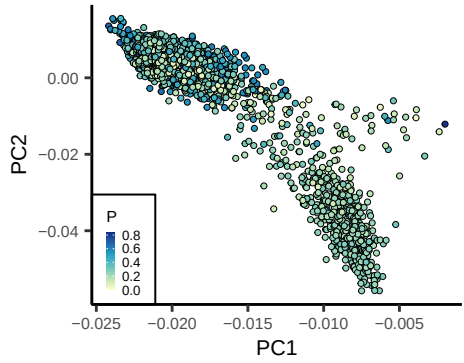

k-Nearest Neighbour classification for doublet detection

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

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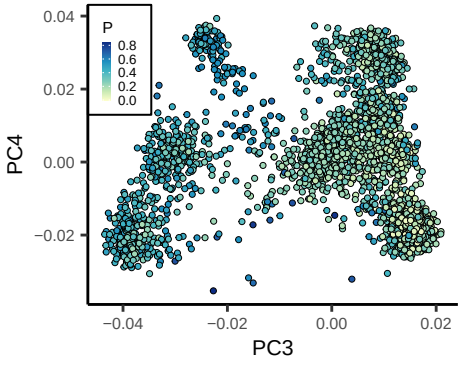

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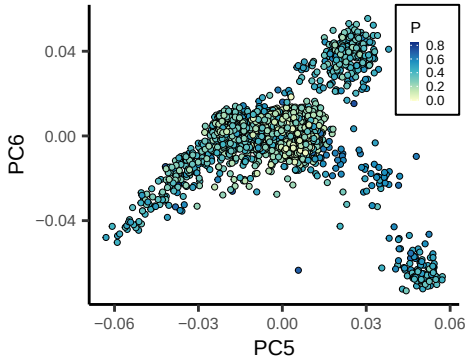
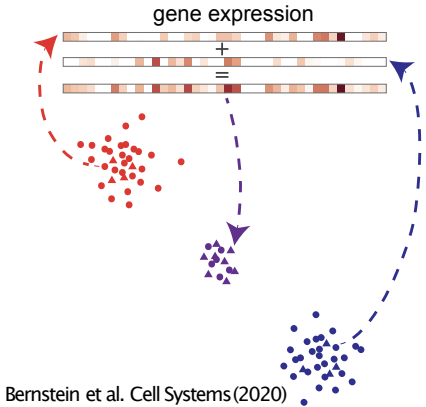
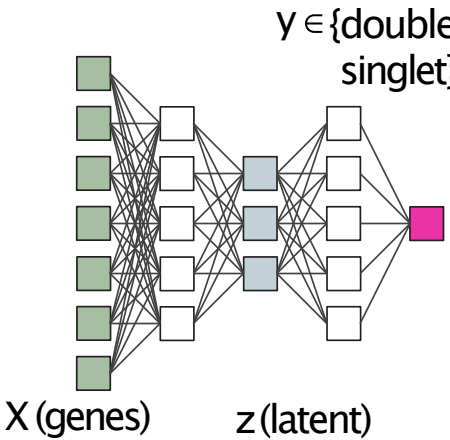


Artificial Neural Network-based classification for doublet detection



Bernstein et al. Cell Systems (2020)



Training a parametric classifier to discern doublets vs. singlets

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

```
build.classifier <-
    nn_module(
        classname = "classfier",
        initialize = function(d, k = 5) {
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                nn_linear(d, k),
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                nn_relu(),
                nn_linear(k, 2 * k),
                nn_batch_norm1d(2 * k),
                nn_relu(),
                nn_linear(2 * k, 1),
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        },
        forward = function(x, min_=.01, max_=.99) {
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$$\prod_{i=1}^{n} f(\mathbf{x}_i)^{y_i}(1 - f(\mathbf{x}_i))^{1-y_i}$$

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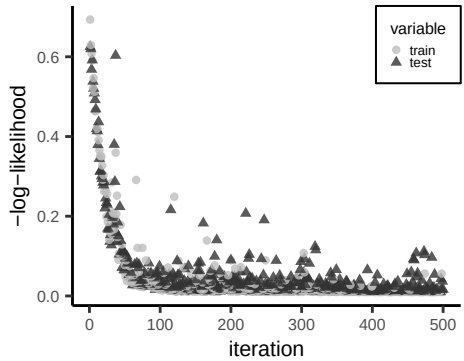

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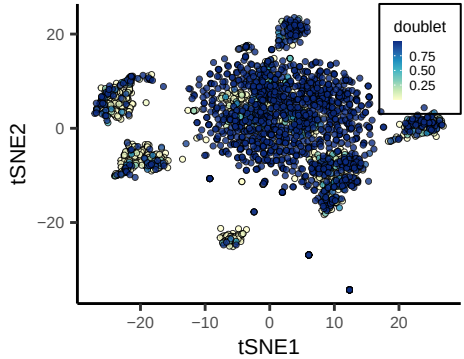
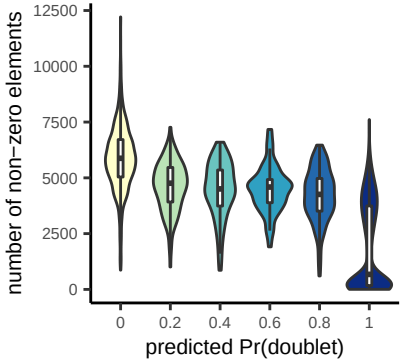
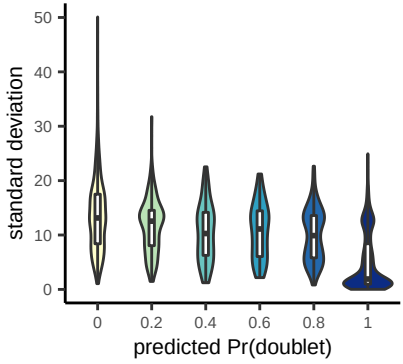


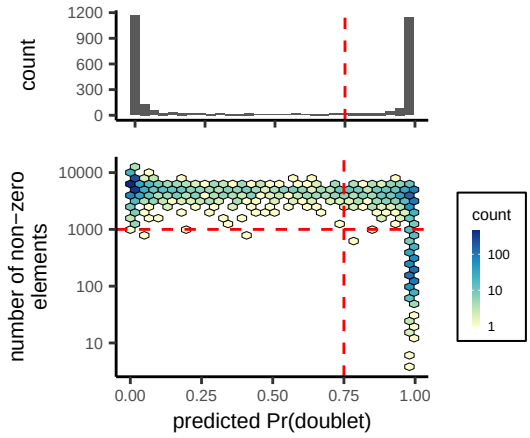
The predicted doublets generally correspond to cells with few non-zero elements





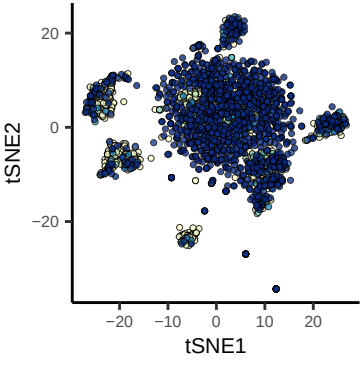
Low expression within a cell may stem from unwanted burst-out cells or ambient RNA molecules.

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

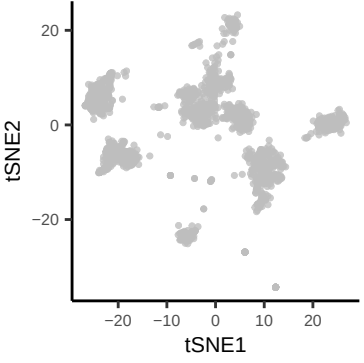


After removing potential doublets

- All the cells

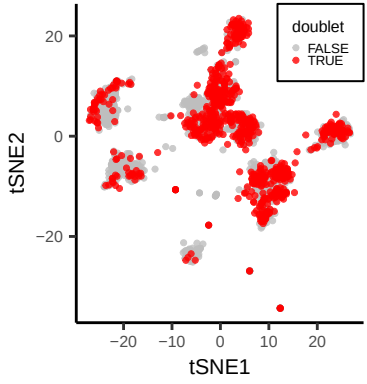


- P(doublet) $< .75$, #non-zeros > 1k



Maybe it is more than just low expression cells

- #non-zeros > 1k



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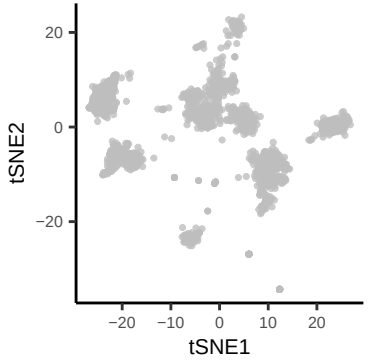


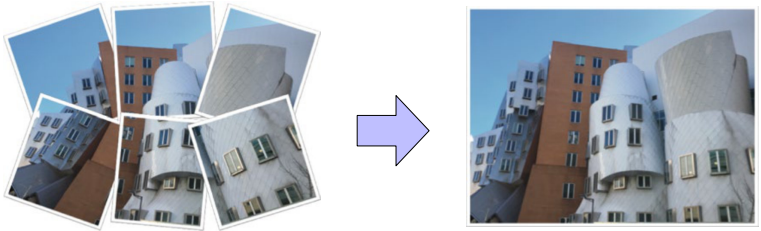
Discussion on doublet Q/C

- It's a unique routine 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.

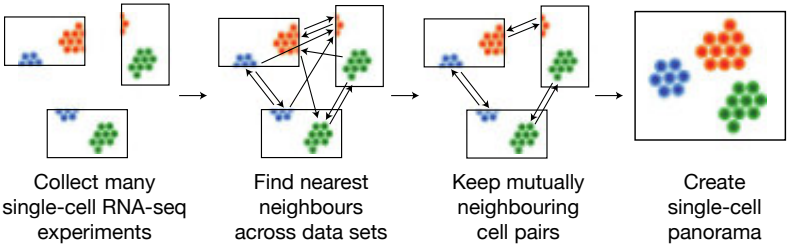
Today's lecture

- 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

Batch normalization for joint analysis across multiple single-cell RNA-seq data

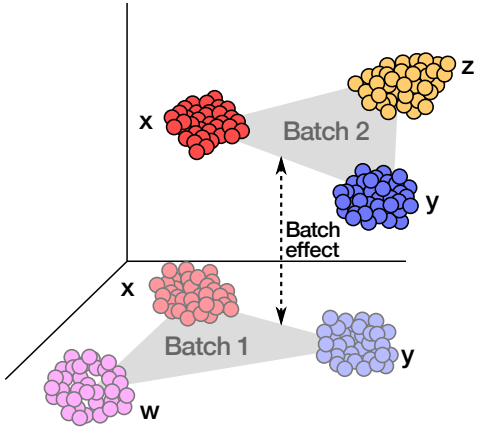


How do we integrate multiple samples/batches?



Scanorama: mutual nearest neighbourhood-based data integration

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



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

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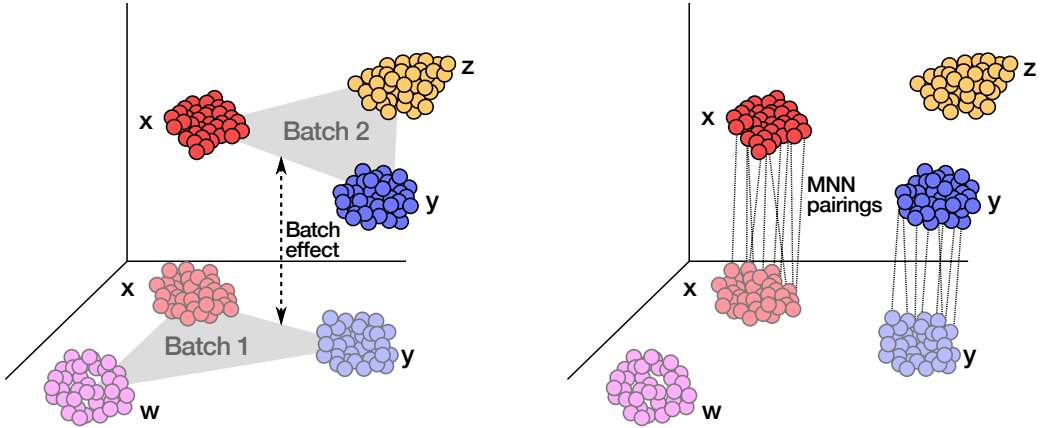

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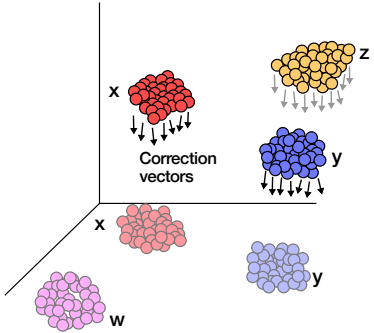

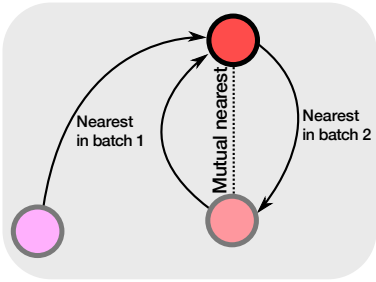

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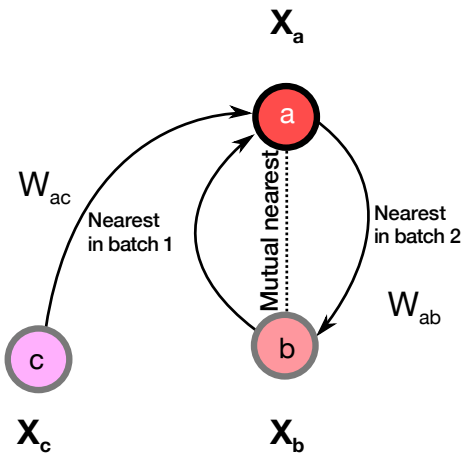


What is the gap Δ between the batches?

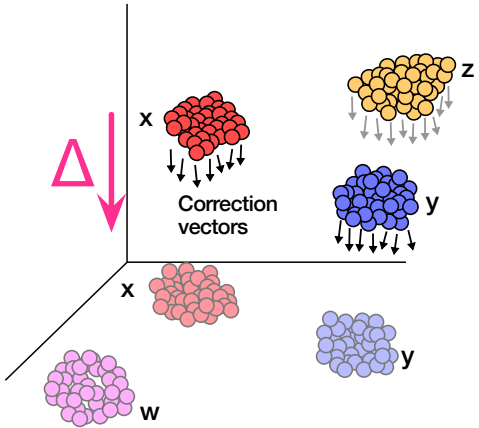
$$\min_{\Delta} \sum_{a,b} W_{ab} \|\mathbf{x}_a - \mathbf{x}_b - \Delta\|_2$$

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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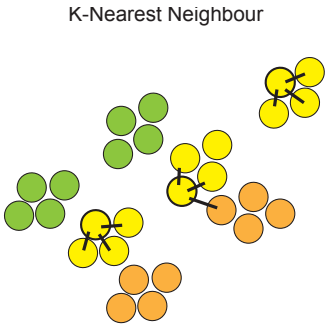
Fixed point (local) optimal solution:

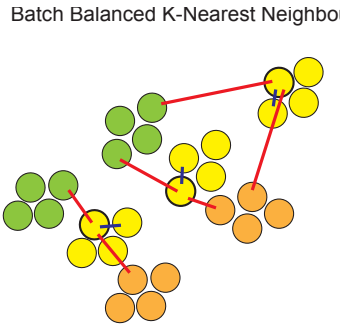
$$\Delta \leftarrow \frac{\sum_{a,b} W_{ab}(\mathbf{x}_a - \mathbf{x}_a)}{\sum_b W_{ab}}$$

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

A batch-balancing k-nearest neighbour graph

BBKNN method strikes balance between over- and under-normalization



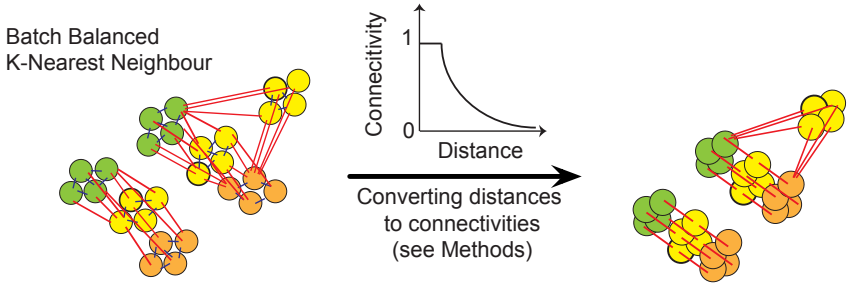


What kind of differences in due to inter-batch, technical discrepancy, not inter-cell-type divergence?

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

A batch-balancing k-nearest neighbour graph

BBKNN method strikes balance between over- and under-normalization



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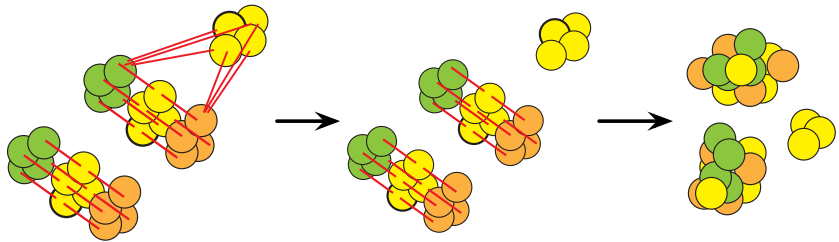
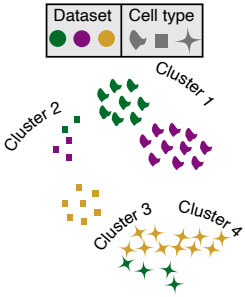


Polanski, .., Teichmann *Bioinformatics* (2019)

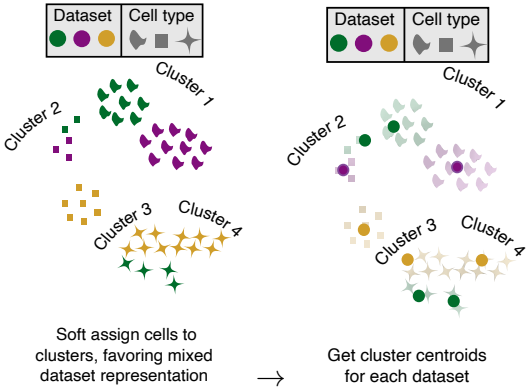
Harmony: clustering-based data normalization



Soft assign cells to clusters, favoring mixed dataset representation

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

Harmony: clustering-based data normalization



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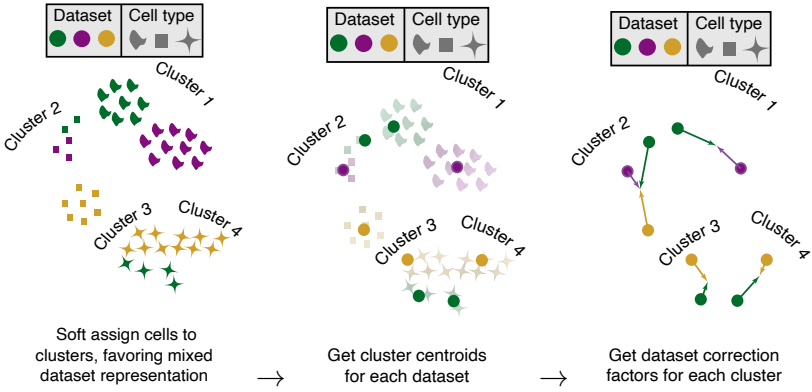

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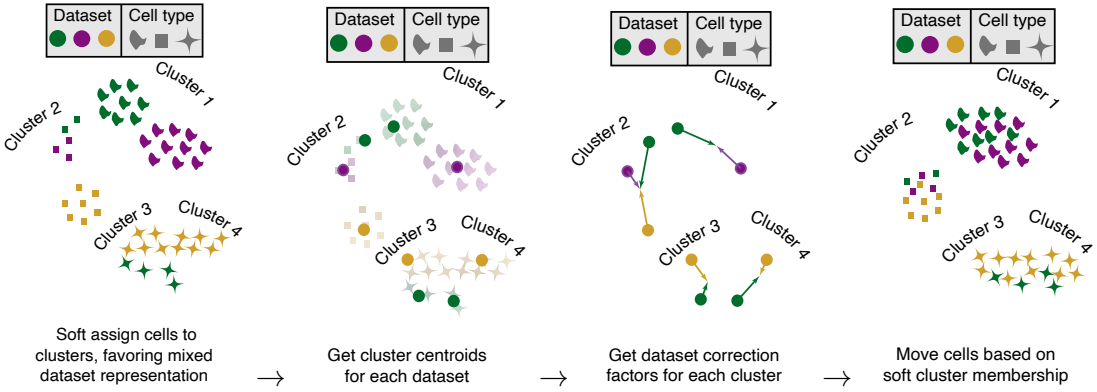
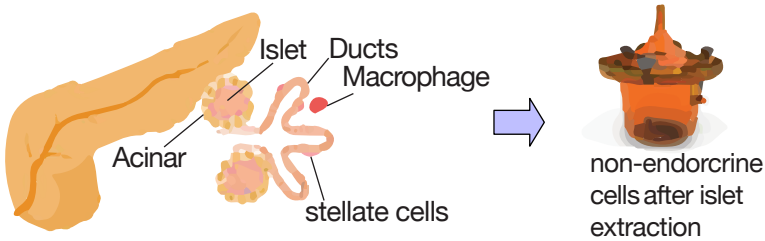


Korsunsky, .., Loh, Raychaudhuri *Nature Methods* (2019)

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

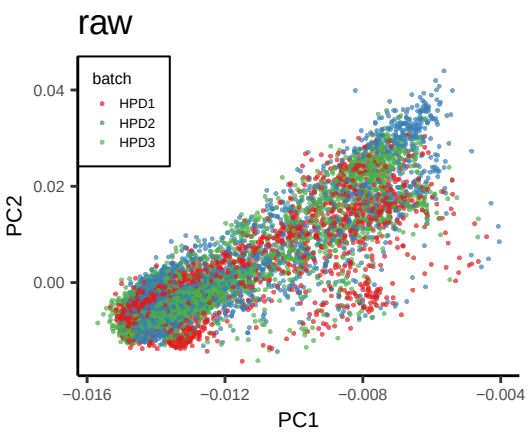
Single-cell RNA-seq data from three donors (three batches)

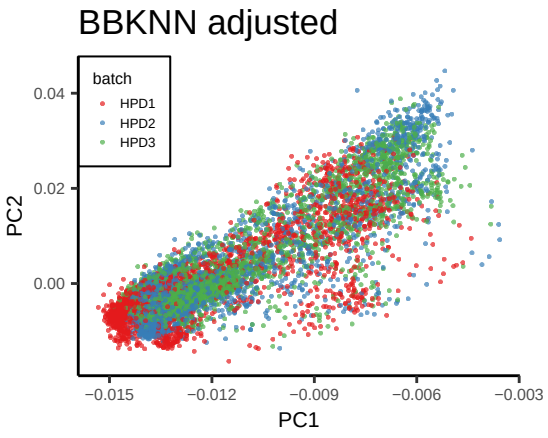


Goal: Remove potential batch effects across different donors. (1) Construct BBKNN graphs between cells; (2) compute average discrepancy Δ between batches in the PC space; (3) adjust them.

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

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





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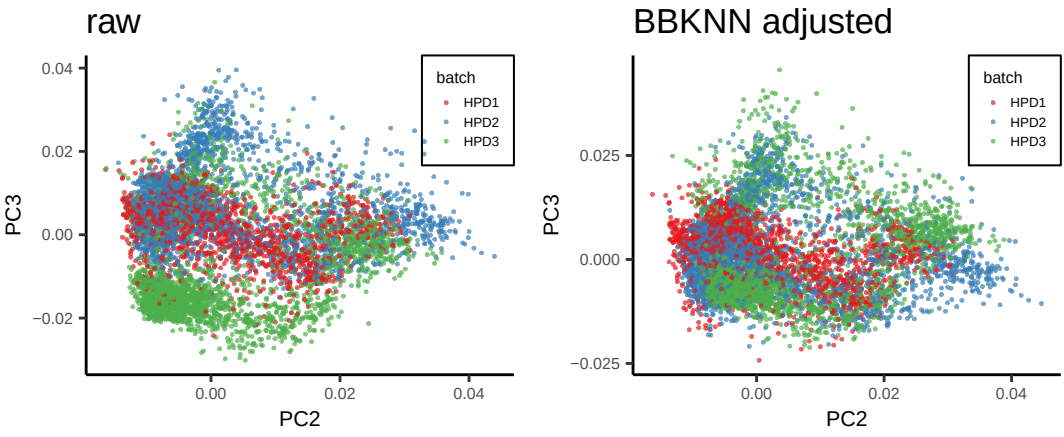

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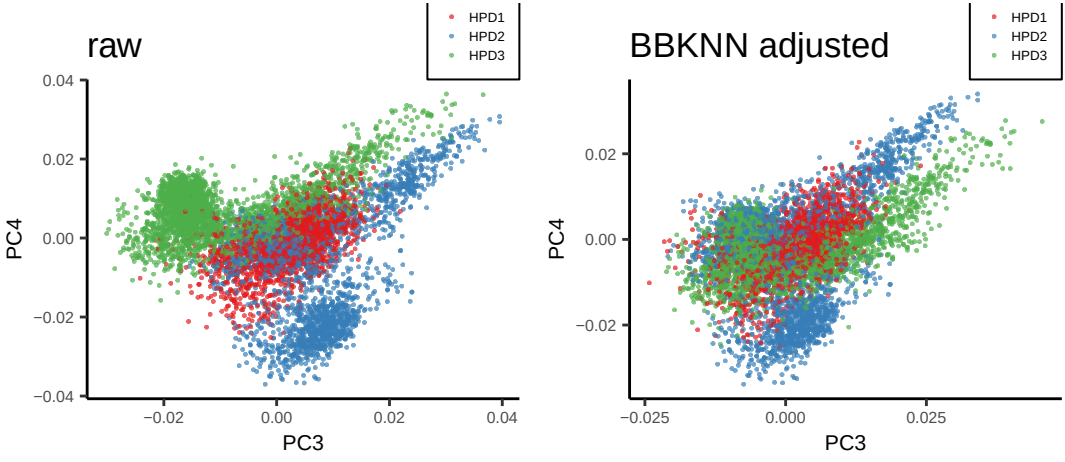

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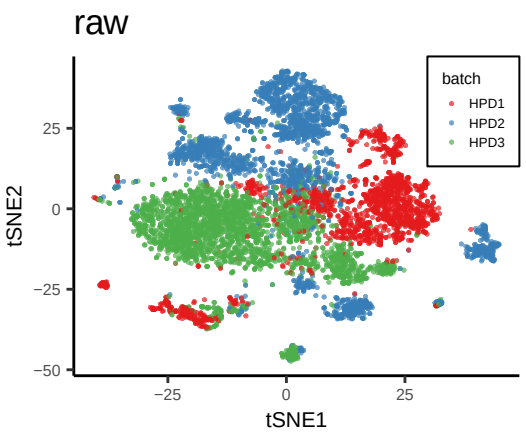
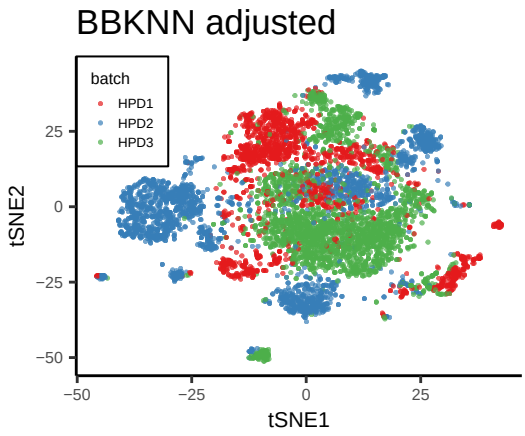




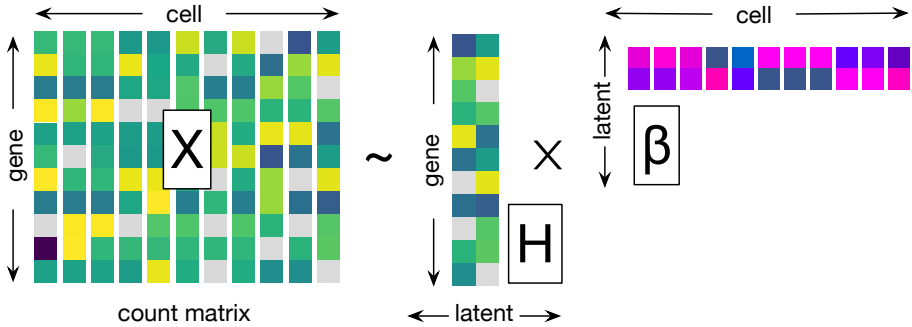
Discussions

- 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

What is a generative model of single-cell data?



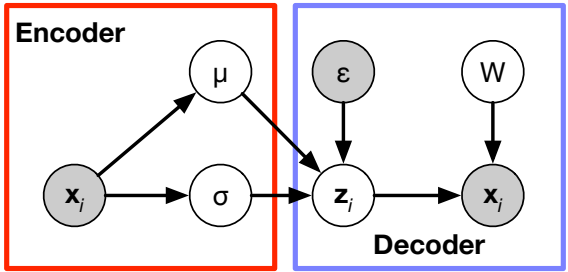
$$\mathbb{E}[X] \approx f\left(H\beta\right)$$

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

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

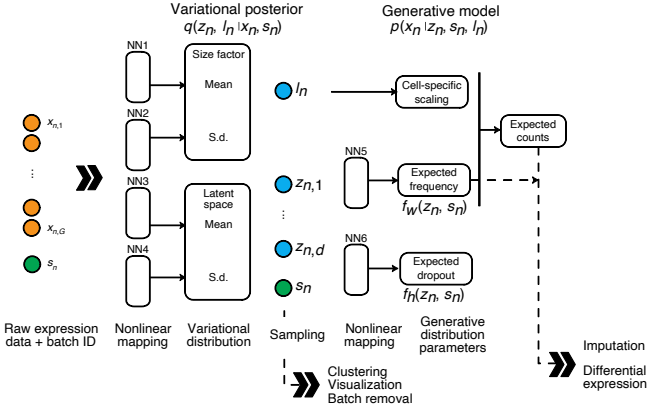
- Probabilistic interpretation of latent states
- Incomplete single-cell data, lots of drop-out measurements
- We can design generative model parameters as interpretable as possible!

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.

Deep generative modeling for single-cell transcriptomics

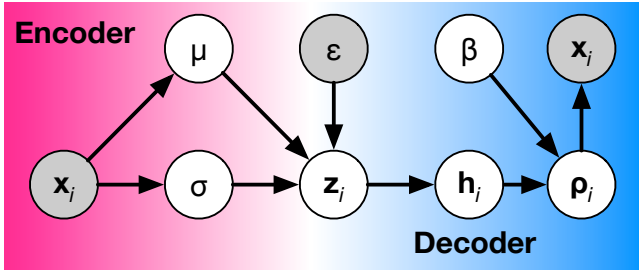


Generative model: zero-inflated negative binomial distribution

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

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Likelihood model:

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

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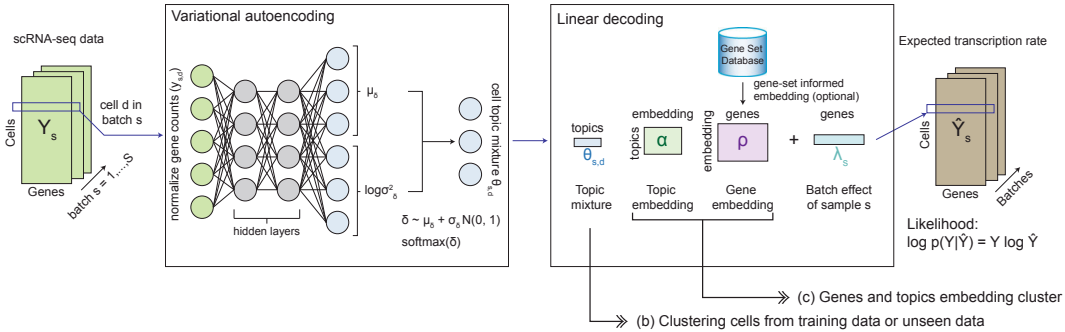
Single-cell Embedded Topic Model



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

Topic Modelling: Compare 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, $W_{dj}^v = 0$.

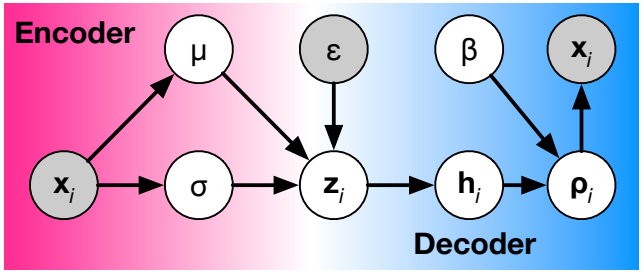
Single-cell Embedded topic model's latent states and model parameters

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

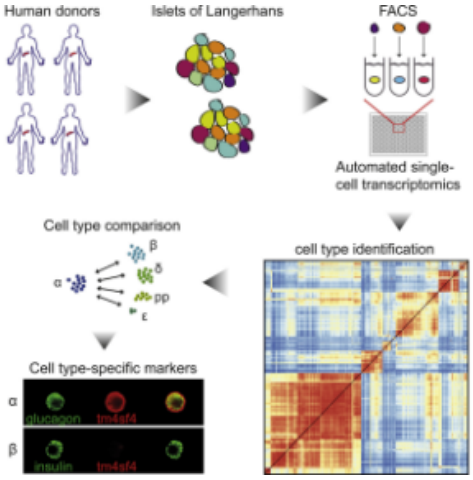
- In Latent Dirichlet Allocation: $\sum_{k=1}^{K} H_{dk} = 1$ and $H_{dk} > 0$, and $\mathbf{h}_d \sim \text{Dirichlet}(\alpha/K, \ldots, \alpha/K)$ *a priori*. Approximately, we have $\hat{H}_{dk} = \sum_j^{N_d} Z_{dj}^k / N_d$.
- In Embedded Topic model, H_{dk} with the simplex constraints; $H_{dk} = \exp(\delta_{dk}) / \sum_{k'} \exp(\delta_{dk'})$ 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

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



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



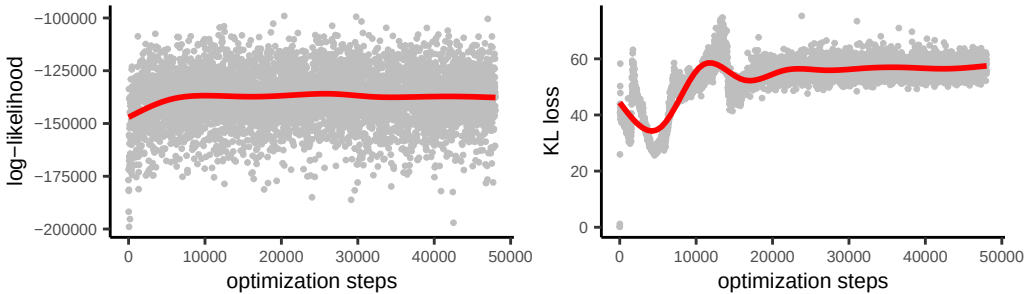
Muraro *et al. Cell Systems* (2016)

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

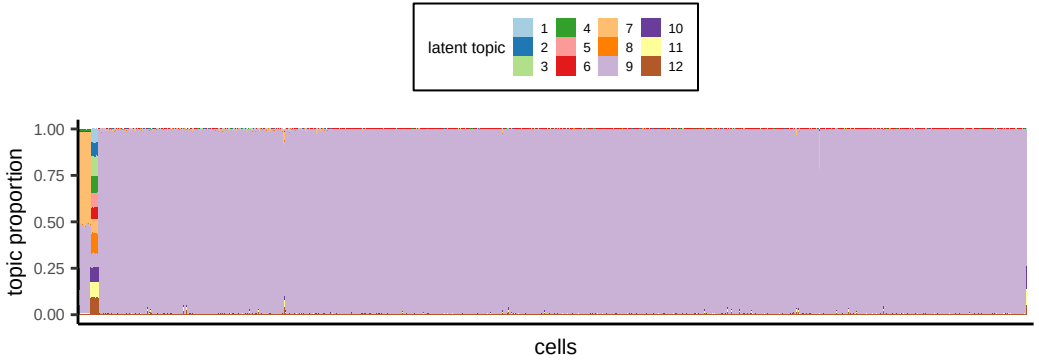
Variational inference $\approx$ maximum likelihood regularized by a KL-divergence term

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



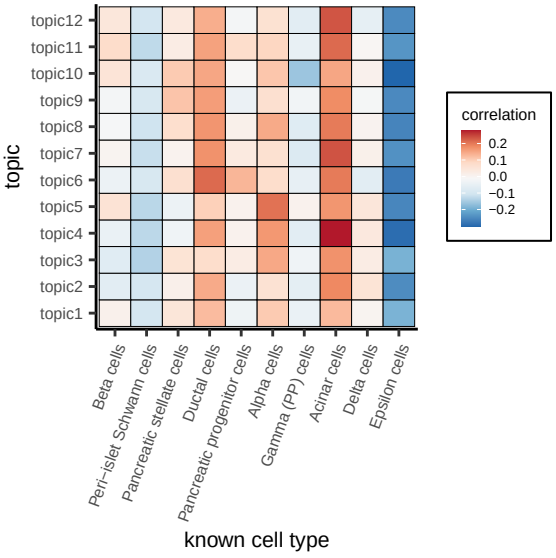
We may need to train longer than usual... (don't be fooled by log-likelihood)

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



There is no obvious pattern... yet

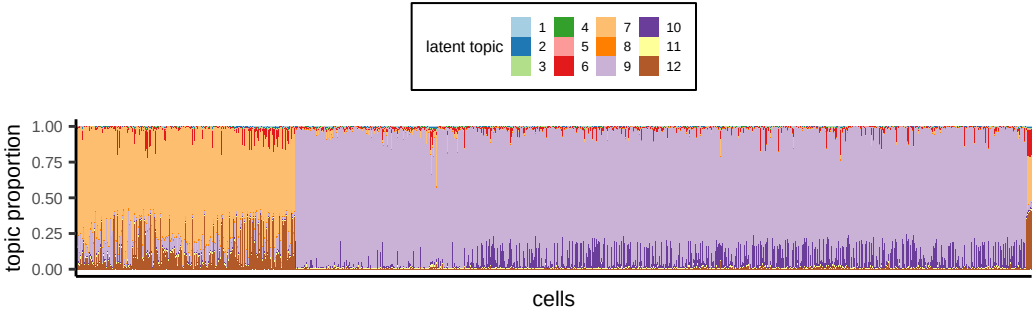
ETM learning just started ... (weight parameters β)



- 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...

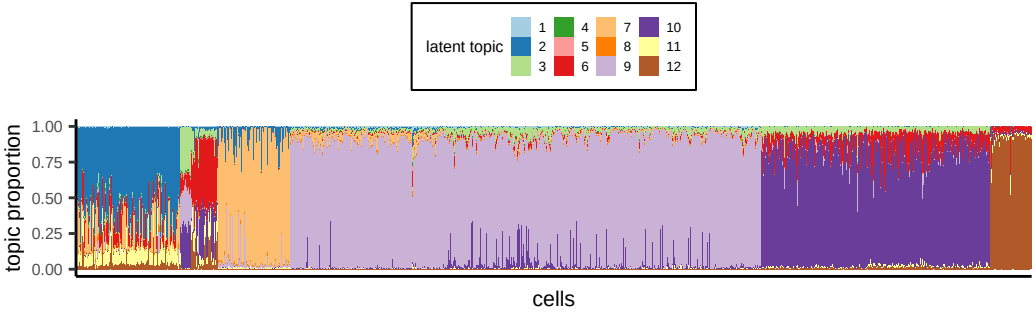
If we keep on training ETM (hidden states) ..

epoch = 300



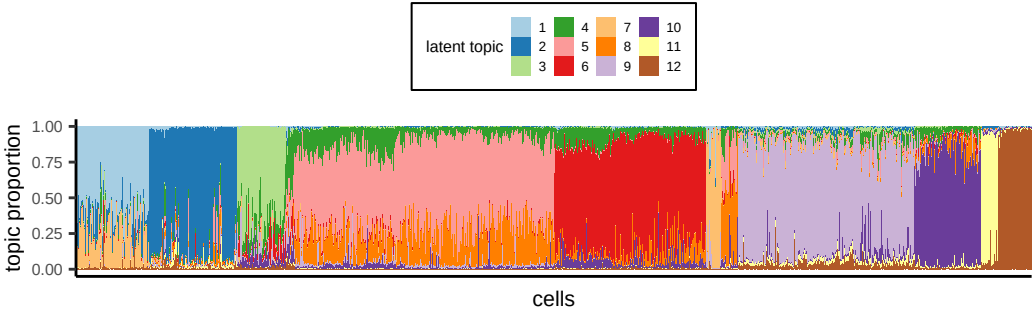
If we keep on training ETM (hidden states) ..

epoch = 600



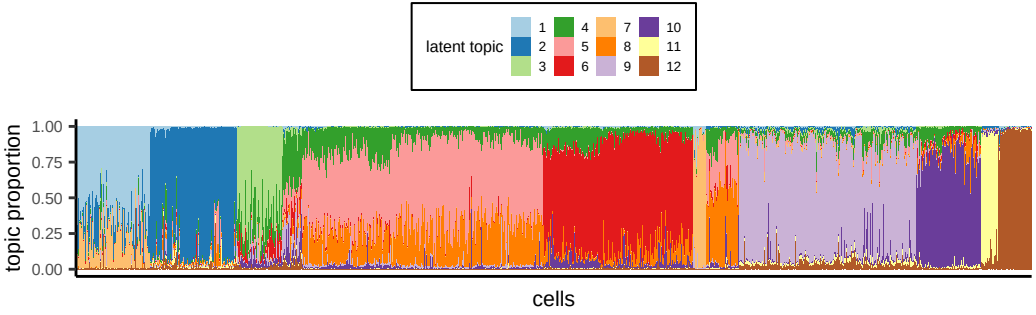
If we keep on training ETM (hidden states) ..

epoch = 900

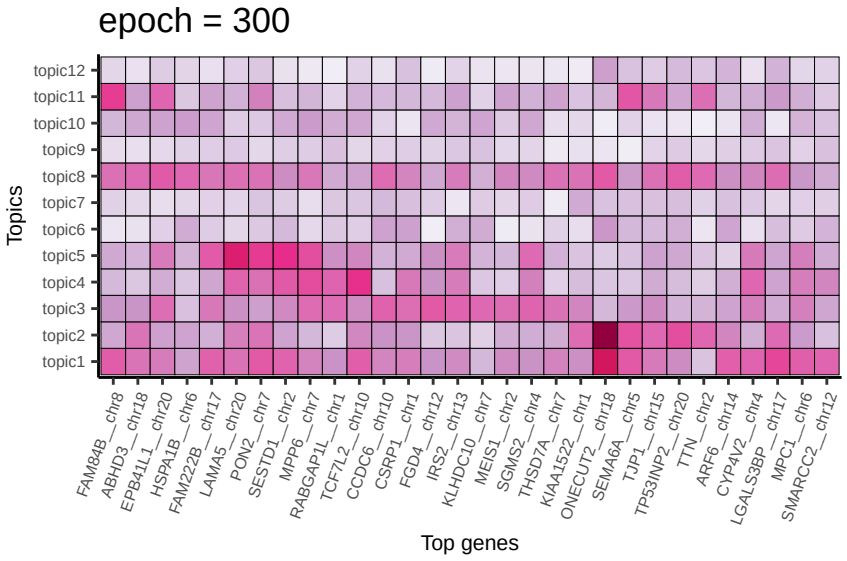


If we keep on training ETM (hidden states) ..

epoch = 1200



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



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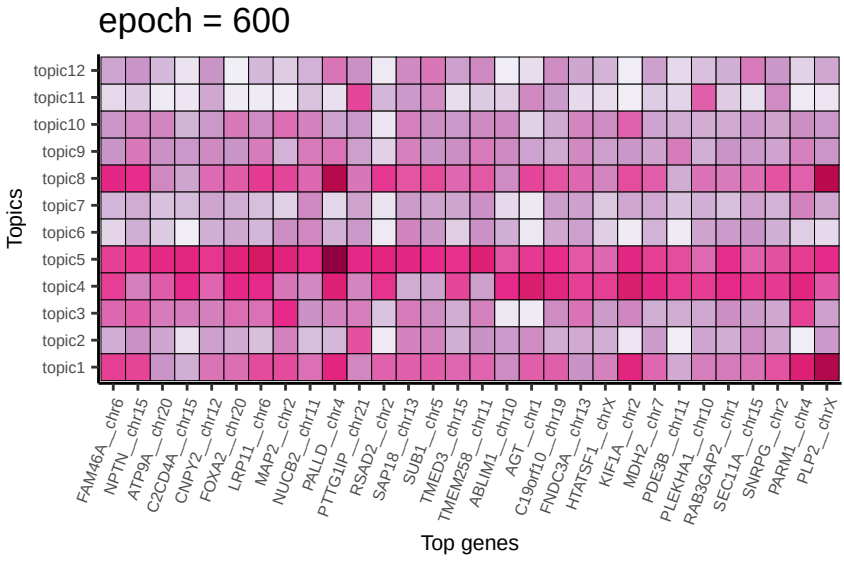

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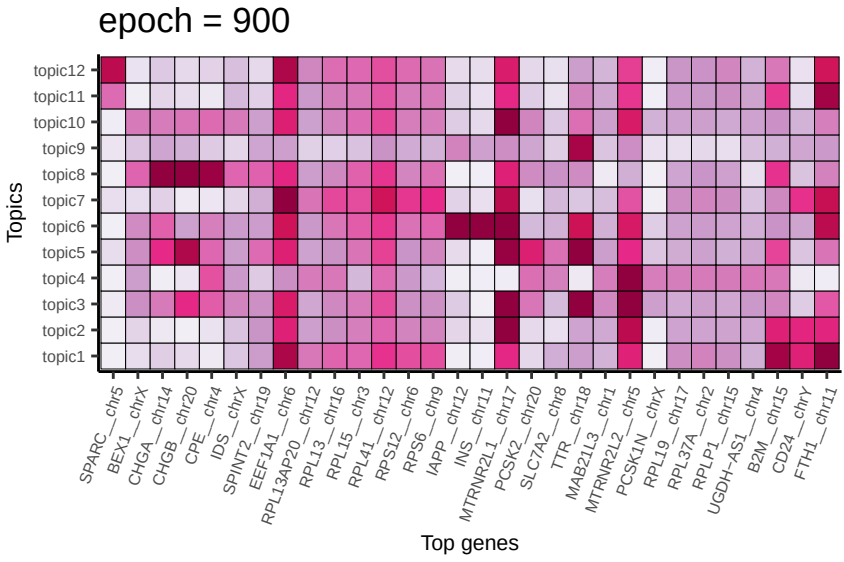

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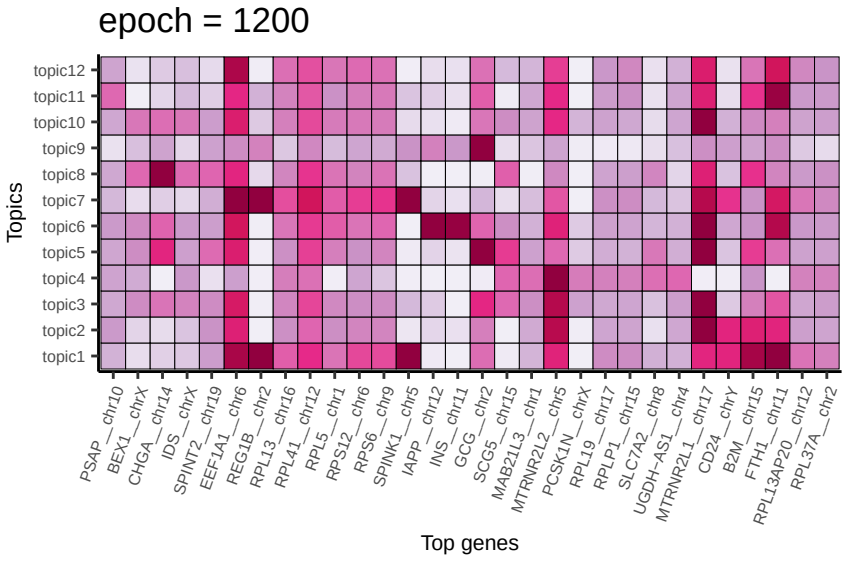

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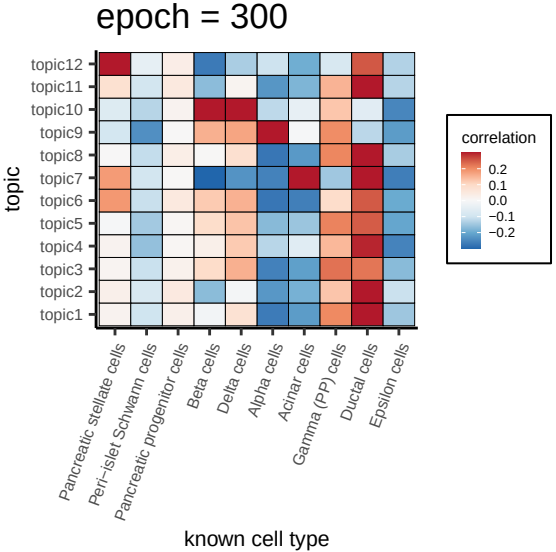
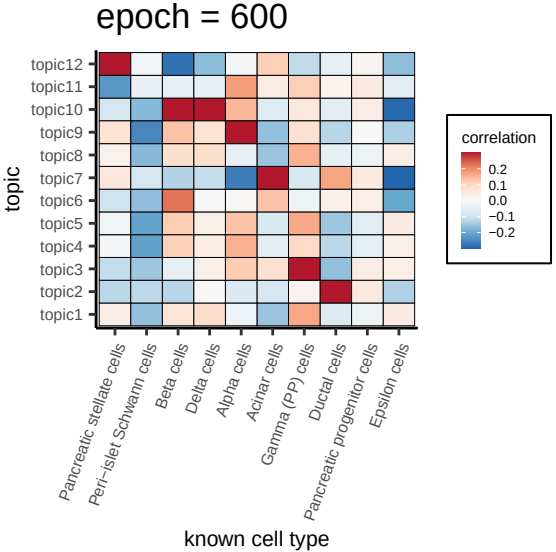


- We can correlate each topic-specific gene $\times 1$ weight vector, β_k, with known cell type-specific marker genes
- We retrieved marker gene information of known cell types from PangaloDB

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



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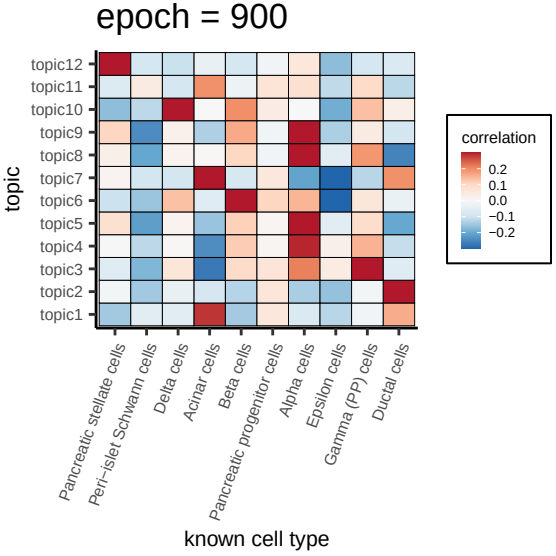

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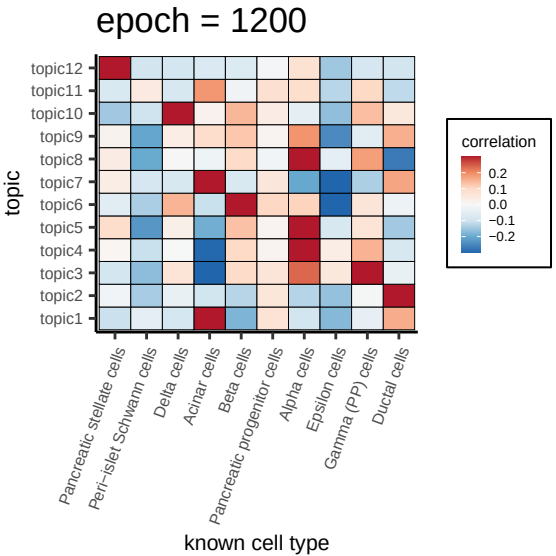

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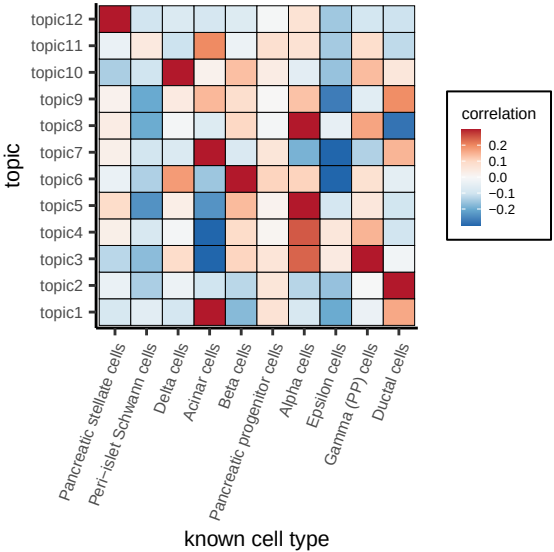
- We retrieved marker gene information of known cell types from PangaloDB

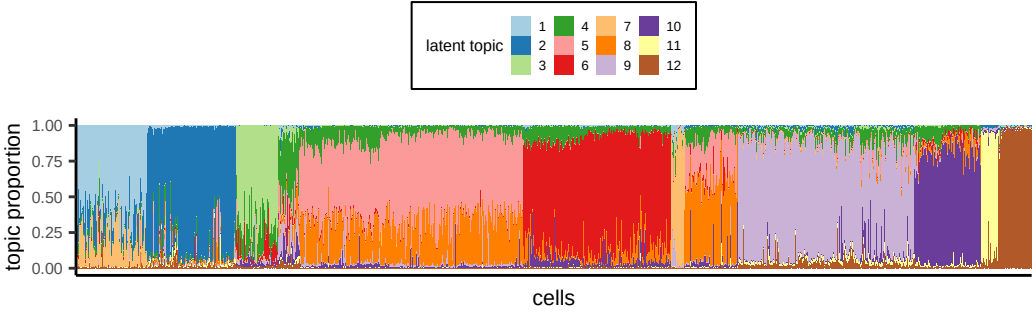
After enough training steps...



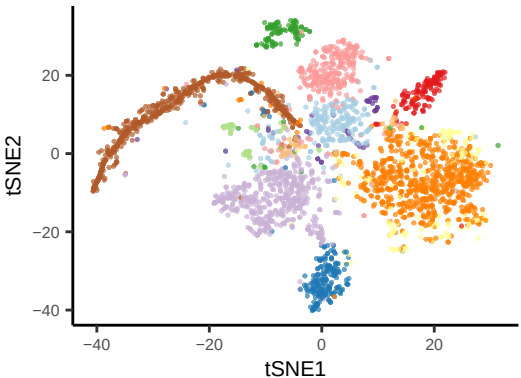
- We have recapitulated most known Pancreatic cell types in our single-cell analysis

Single-cell ETM effectively learns cellular admixture model

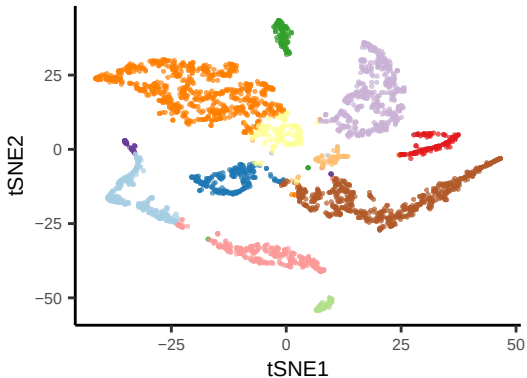
epoch = 2010



using top 50 PCs

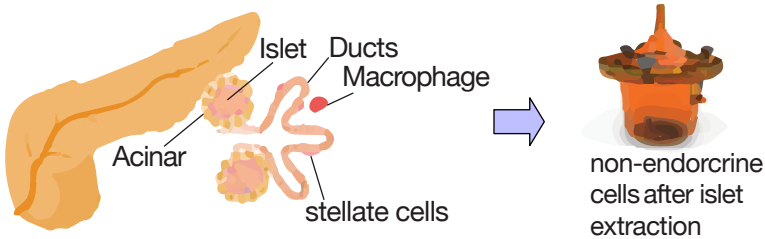


tSNE on the latent topic space



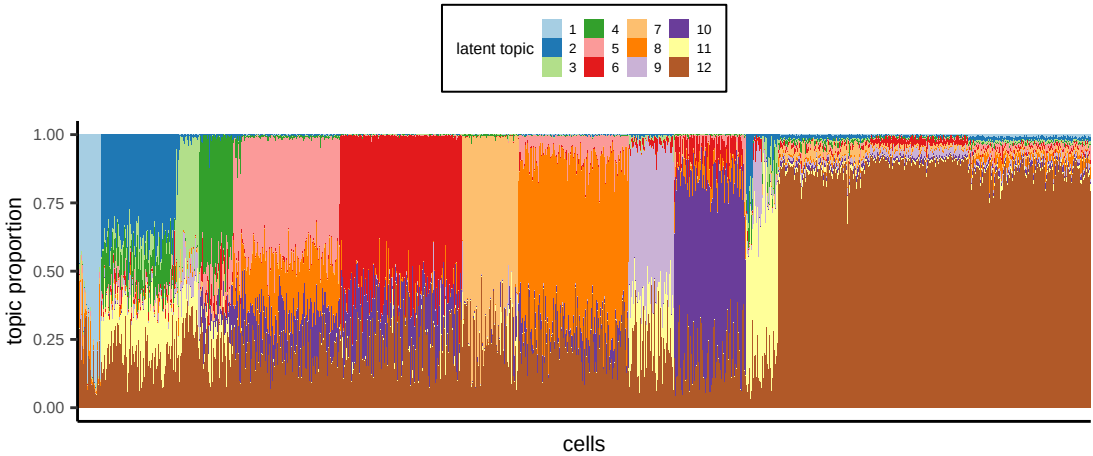
A latent topic model robustly capture cell states, avoiding batch effects

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

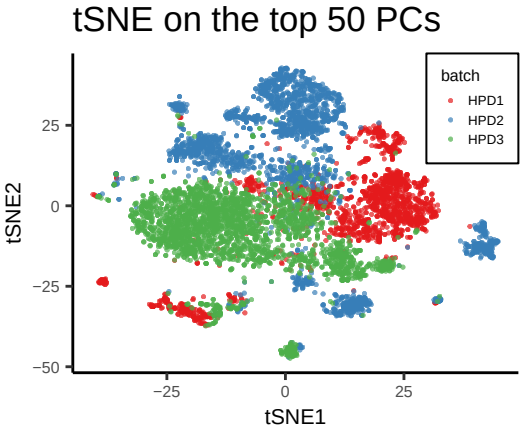


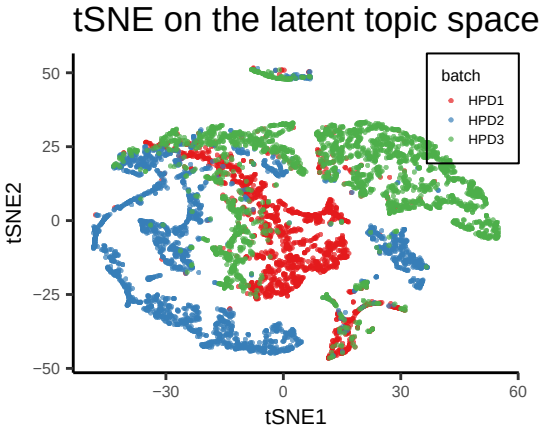
Goal: Topic space for 6,873 cells shared across multiple donors (batches).

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 are well mixed!





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