

# Tools For Computational Biology – Lectures 18 & 19

## Introduction to Single-cell genomics

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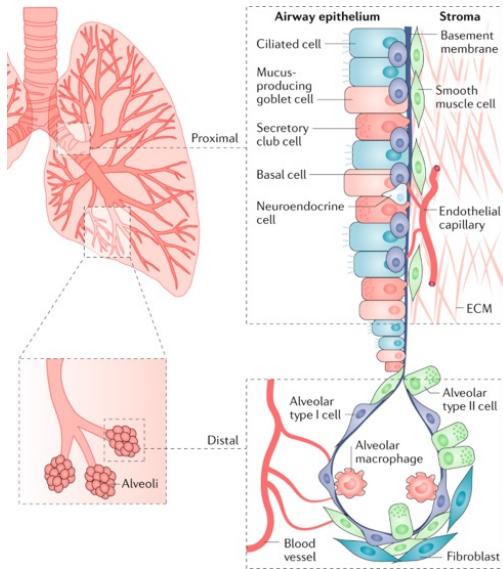
# Today's agenda

- Single-cell genomics: Why?
- Single-cell genomics: How?
- Single-cell RNA-seq preprocessing and analysis

# Why single-cell genomics

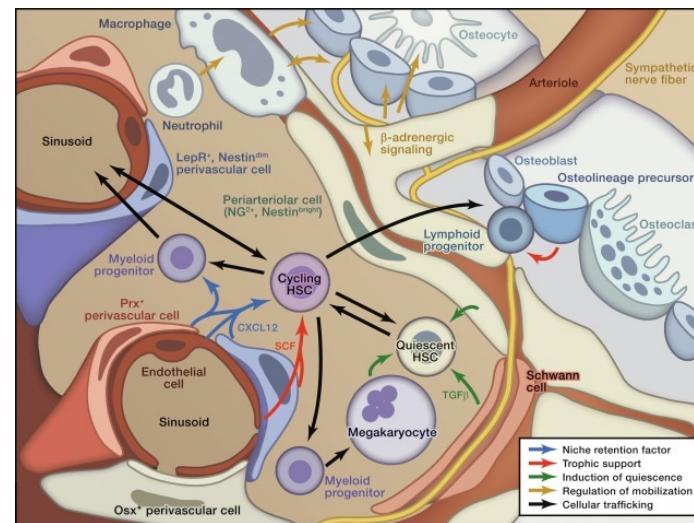
# Heterogeneity in tissue homeostasis

## Lung



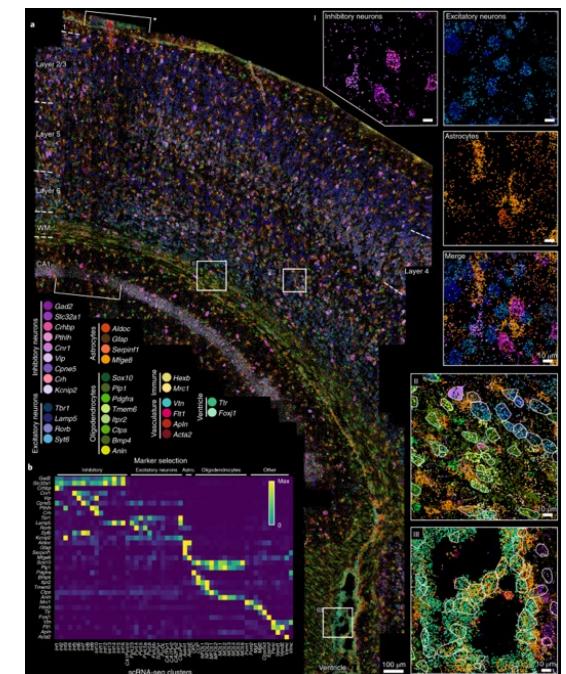
Altorki et. al., 2019

## Bone Marrow



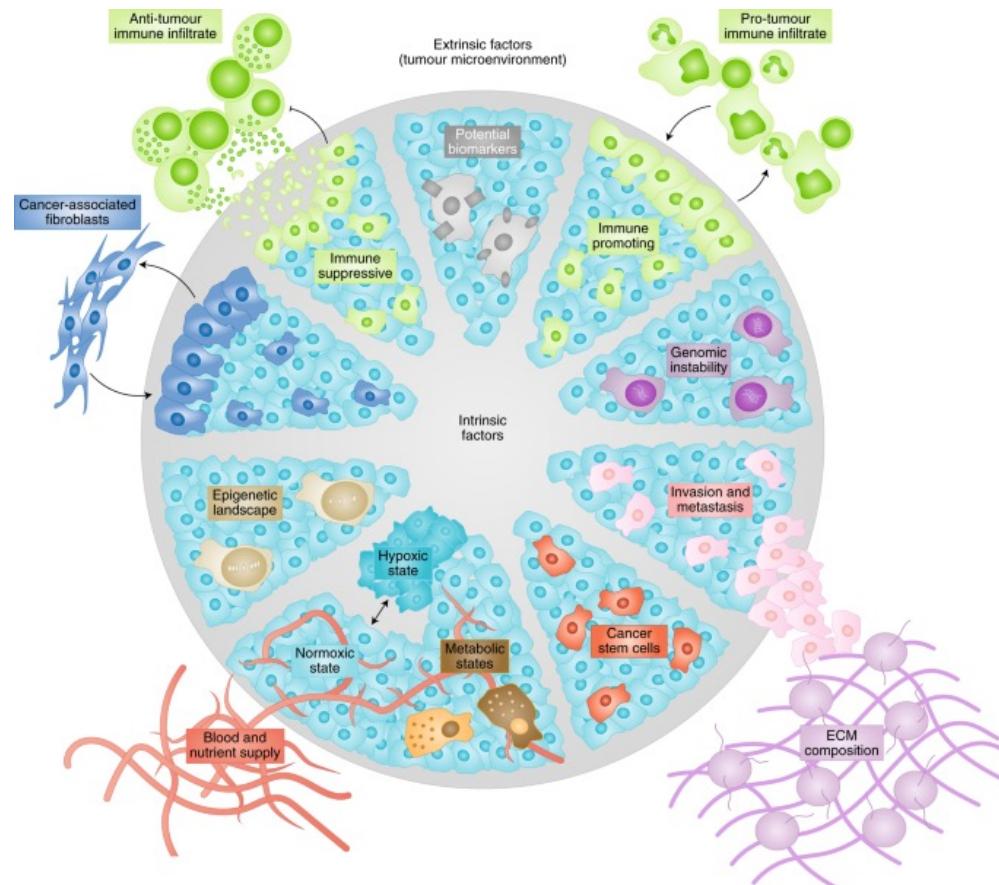
Hoffman et. al., 2020

# Somatosensory Cortex



Codeluppi et. al., 2018

# Tumor heterogeneity



Lawson et. al., 2018

Single-cell technologies profile heterogeneity  
instead of average



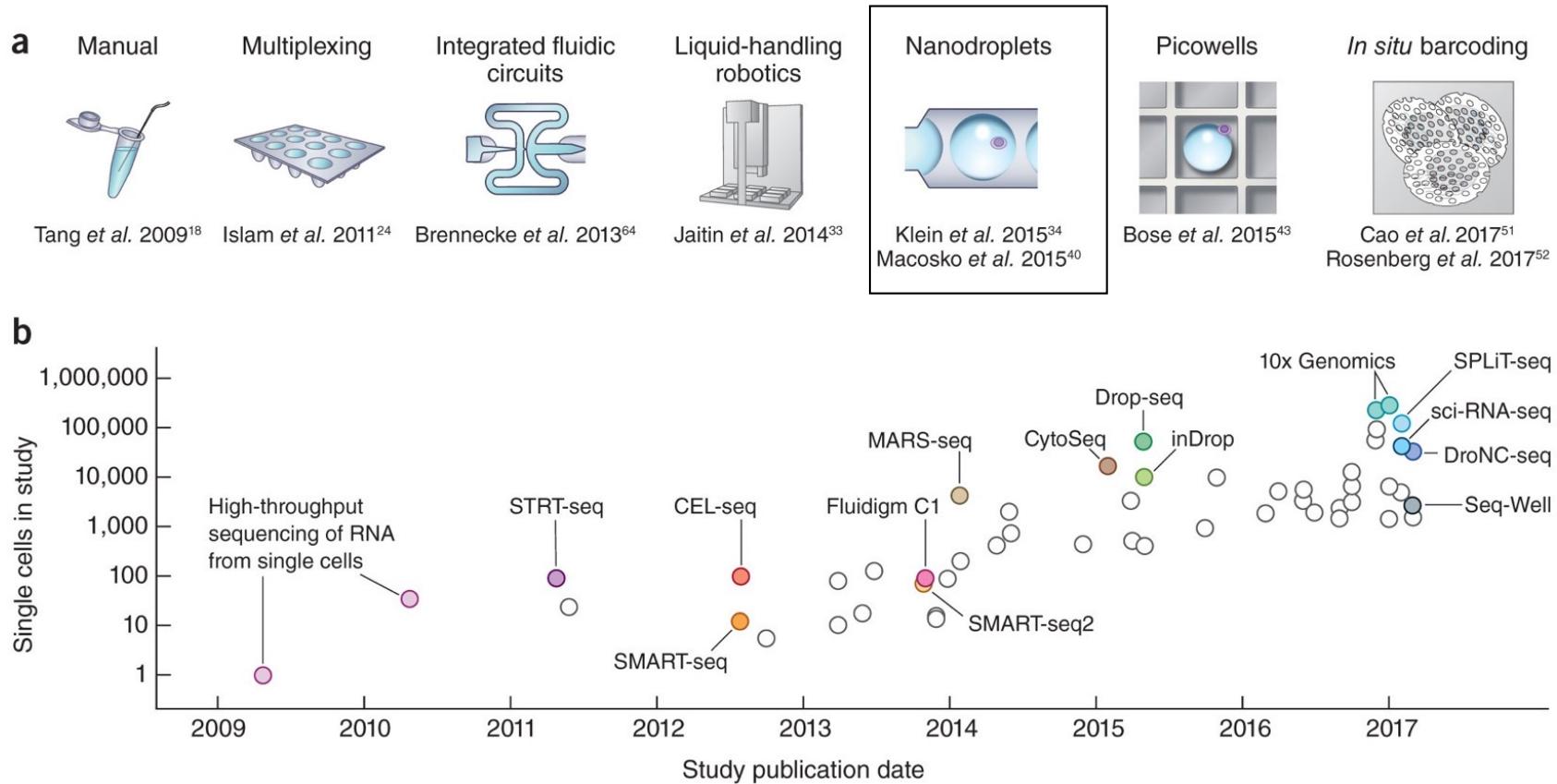
Bulk Genomics



Single-cell Genomics

# Single-cell genomics: How?

# Evolution of single-cell RNA-seq



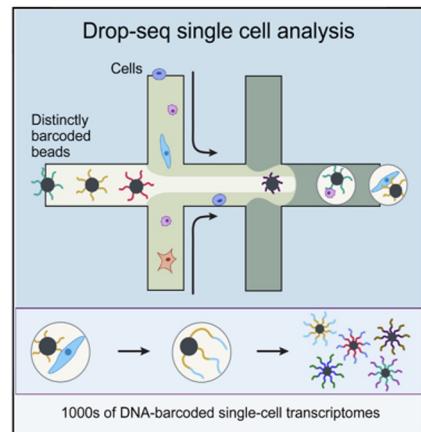
# Microfluidic single-cell technologies

Moved throughput from hundreds to thousands of cells

## Cell

### Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

#### Graphical Abstract



#### Resource

#### Authors

Evan Z. Macosko, Anindita Basu, ..., Aviv Regev, Steven A. McCarroll

#### Correspondence

emacosko@genetics.med.harvard.edu (E.Z.M.), mccarroll@genetics.med.harvard.edu (S.A.M.)

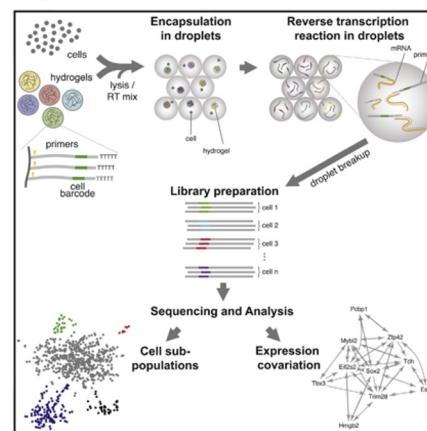
#### In Brief

Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.

## Cell

### Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells

#### Graphical Abstract



#### Resource

#### Authors

Allon M. Klein, Linas Mazutis, ..., David A. Weitz, Marc W. Kirschner

#### Correspondence

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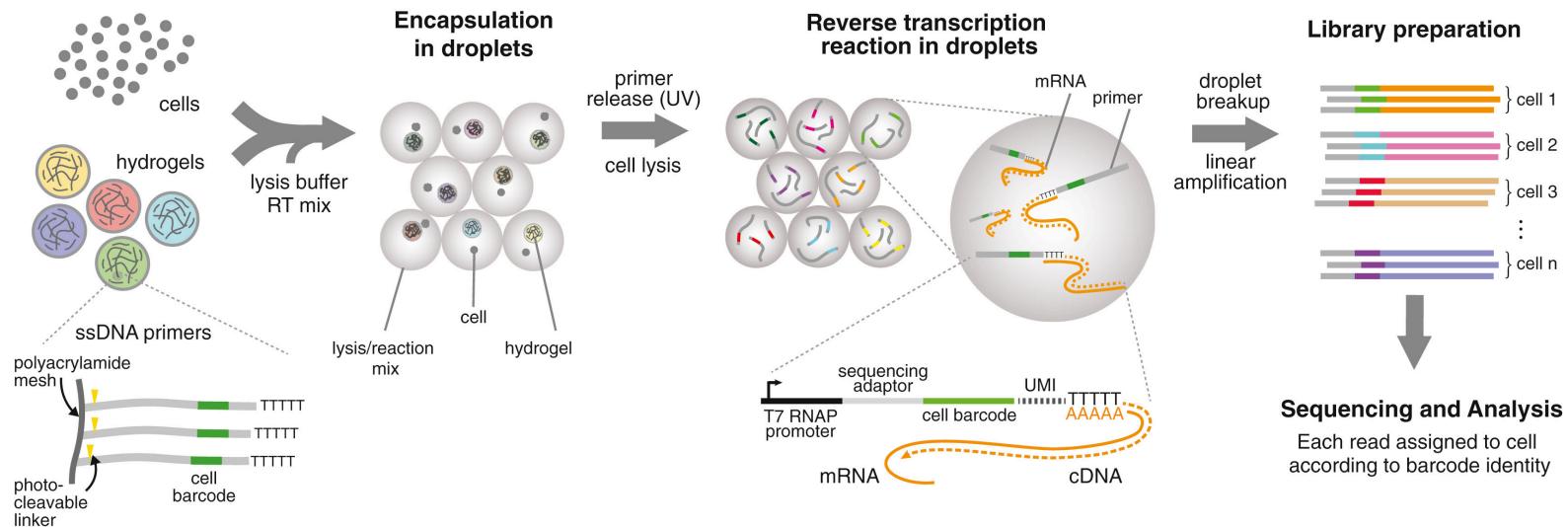
#### In Brief

Capturing single cells along with a set of uniquely barcoded primers in tiny droplets enables single-cell transcriptomics of a large number of cells in a heterogeneous population. Applying this analysis to mouse embryonic stem cells reveals their population structure, gene expression relationships, and the heterogeneous onset of differentiation.

Klein et al *Cell* 2015  
Macosko et al *Cell* 2015

# Microfluidic single-cell technologies

Moved throughput from hundreds to thousands of cells

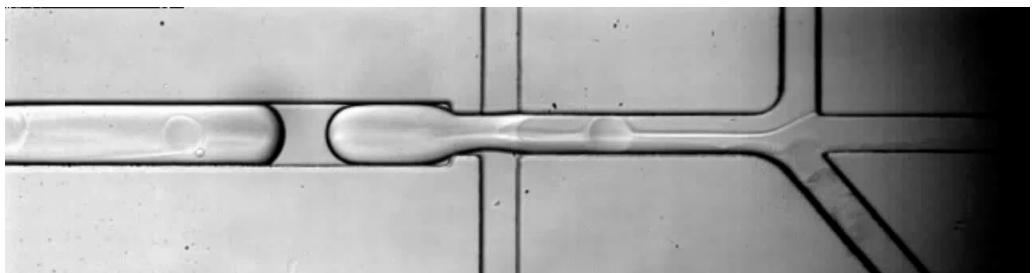
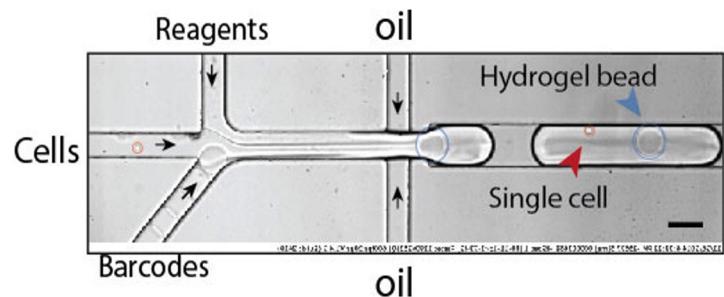


- Droplet-based processing using microfluidics
- Nanoliter scale aqueous drops in oil
- 3' End
- Bead based for cell barcoding
- Uses UMI (Unique Molecular Identifier).

Klein et al *Cell* 2015  
Macosko et al *Cell* 2015

# Microfluidic single-cell technologies

Moved throughput from hundreds to thousands of cells



Klein et al *Cell* 2015  
Macosko et al *Cell* 2015

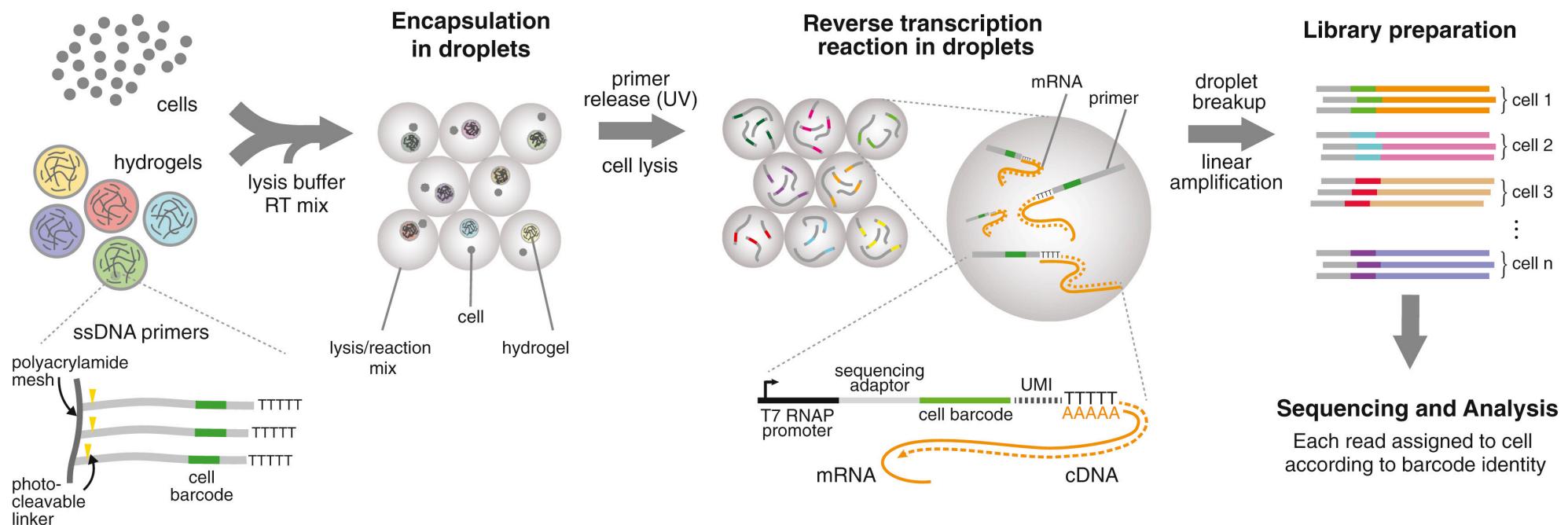
# CellXGene interactive tool

```
uvx cellxgene launch 10x_pbmc.h5ad
```

```
http://localhost:5005
```

# Single-cell RNA-seq: Preprocessing and Analysis

# Microfluidic single-cell technologies



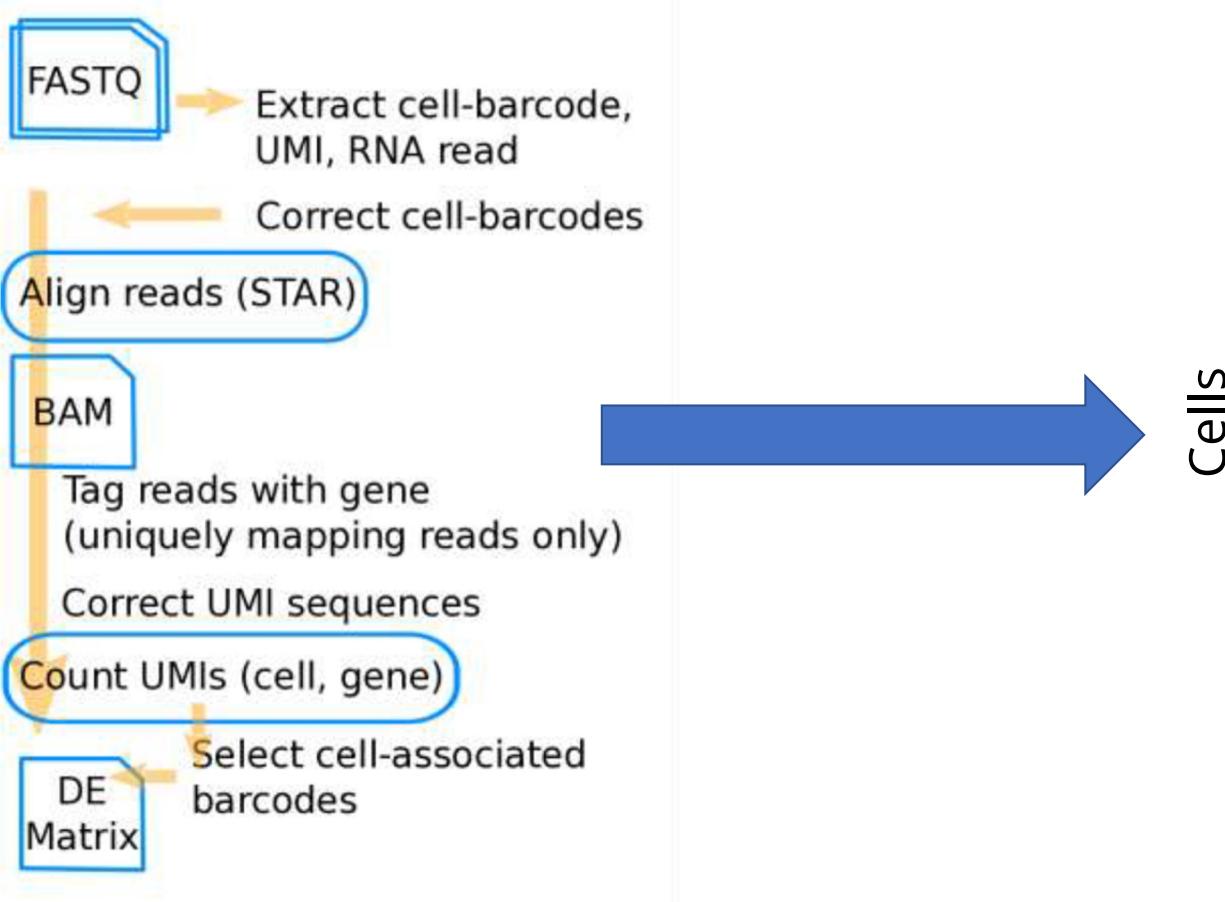
Klein et al *Cell* 2015

# Cell Barcodes and UMIs

- Cell Barcode: Unique cell identifier – Whitelist
- UMI: Unique molecular identifier – Random 8mer

	Cellular barcode	UMI	
Cell 1	TTGCCGTGGTGT	GGCGGGGA	CGGTGTTA ] <i>DDX51</i>
	TTGCCGTGGTGT	TATGGAGG	CCAGCACC ] <i>NOP2</i>
	TTGCCGTGGTGT	TCTCAAGT	AAAATGGC ] <i>ACTB</i>
Cell 2	CGTTAGATGGCA	GGGCCGGG	CTCATAGT ] <i>LBR</i>
	CGTTAGATGGCA	ACGTTATA	ACCGGTAC ] <i>ODF2</i>
	CGTTAGATGGCA	TCGAGATT	AGCCCTTT ] <i>HIF1A</i>
Cell 3	AAATTATGACCGA	AGTTTGTA	GGGAATTAA ] <i>ACTB</i> ← 2 reads, 1 molecule
	AAATTATGACCGA	AGTTTGTA	AGATGGGG ]
	AAATTATGACCGA	TGTGCTTG	GACTGCAC ] <i>RPS15</i>
Cell 4	GTAAACGTACC	CTAGCTGT	GATTTCT ] <i>GTPBP4</i>
	GTAAACGTACC	GCAGAACT	GTGGCGT ] <i>GAPDH</i>
	GTAAACGTACC	AAGGCTTG	CAAAGTTC ]
	GTAAACGTACC	TTCCGGTC	TCCAGTCG ] <i>ARL1</i> ← 2 reads, 2 molecules
.....			
(Thousands of cells)			

# 10X Preprocessing Pipeline: CellRanger



# Genes

Cells

0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	76	0	0	0	62	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87	0	0	0	
0	38	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	9	0	0	0	
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	
0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	40	0	0	0	0	0	5	0	84	0	0	0	0	0	0	0	0	0	
10	36	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	48	0	95	0	3	0	73	0	0	0	0	8	0	0	0	0	0	0	0	0	
0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	52	0	0	0	0	
0	0	3	0	0	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	52	0	0	0	0	94	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	90	0	0	0	85	45	0	0	0	0	0	0	0	0	0	0	0	0	
0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	
0	0	22	0	0	0	0	0	0	0	65	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0	0	0	0	0	0	0	
0	0	0	0	0	0	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	26	0	0	0	0	
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	69	0	0	77	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	67	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	62	0	0	0	5	0	0	0	0	0	0	0	0	0	
0	0	0	0	30	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	30	74	0	0	22	0	0	52	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	73	0	35	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	90	0	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0	0	
0	90	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	
0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	12	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	8	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	11	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	75	0	0	63	0	0	16	0	0	19	0	36	0	0	0	0	0	
0	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	96	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	57	0	0	92	0	0	0	0	46	0	0	0	99	0	0	0	0	0	

Genes

Scale & Resolution X \*Noise & Sparsity\*



Dendritic  
CD14 Monocytes  
FCGR3A Monocytes

Megakaryocytes

CD4 T  
CD8 T  
NK

B

# VSCode

→ Lecture18-scRNA-seq-analysis.ipynb

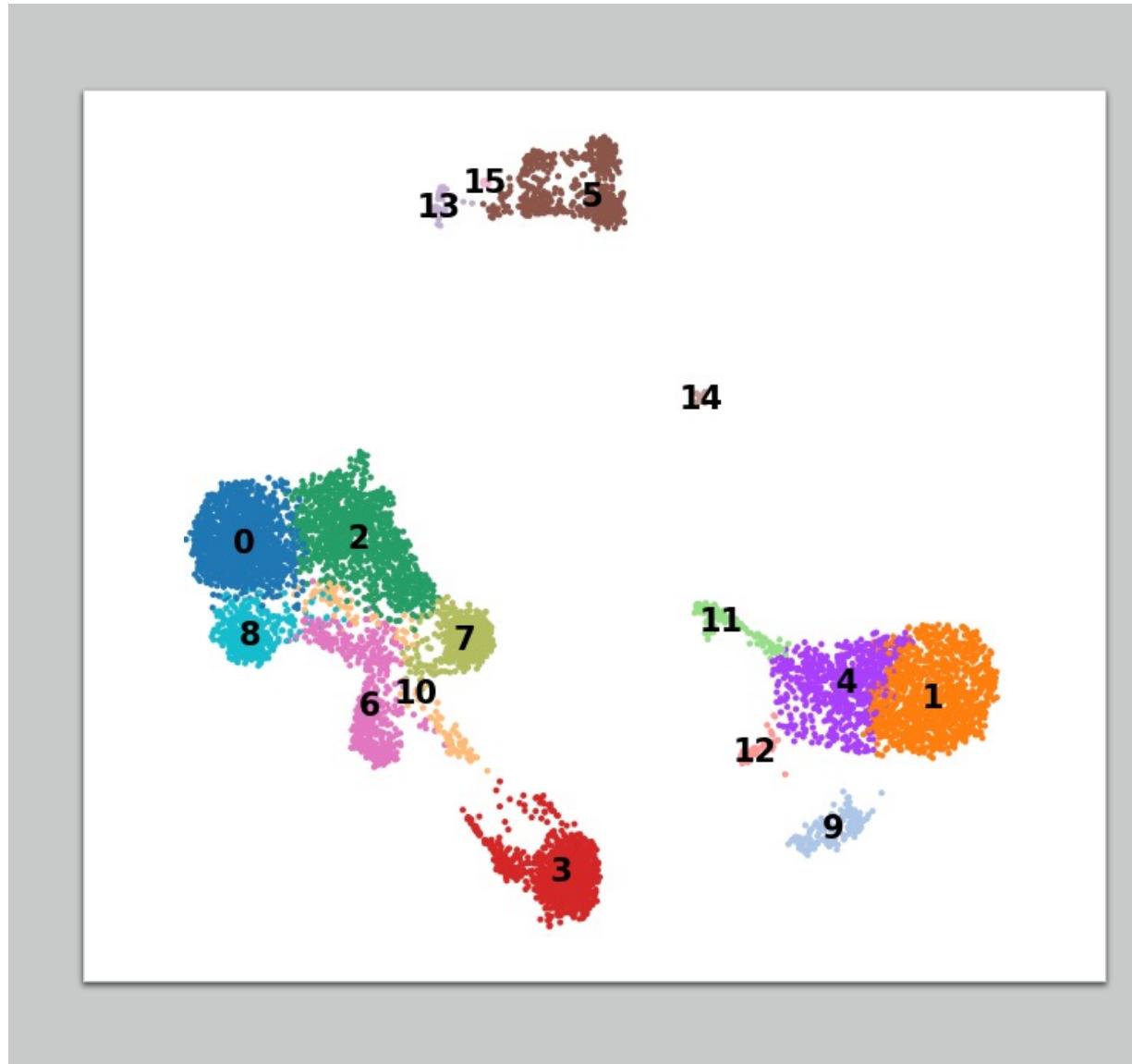
# A black-box view

```
import scanpy as sc

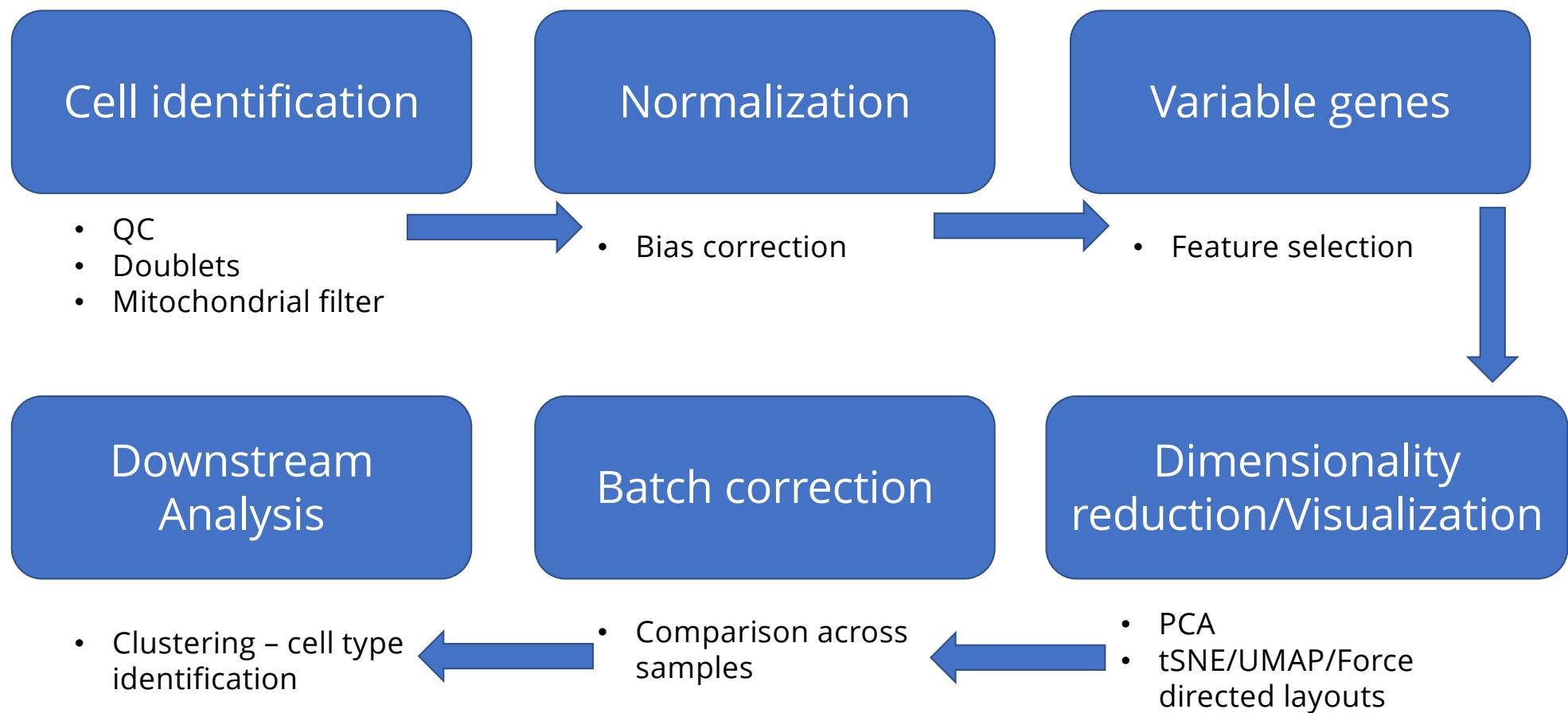
ad = sc.read( <counts file> )
sc.pp.normalize_total(ad)
sc.pp.log1p(ad)

sc.pp.highly_variable_genes(ad)
sc.pp.pca(ad)

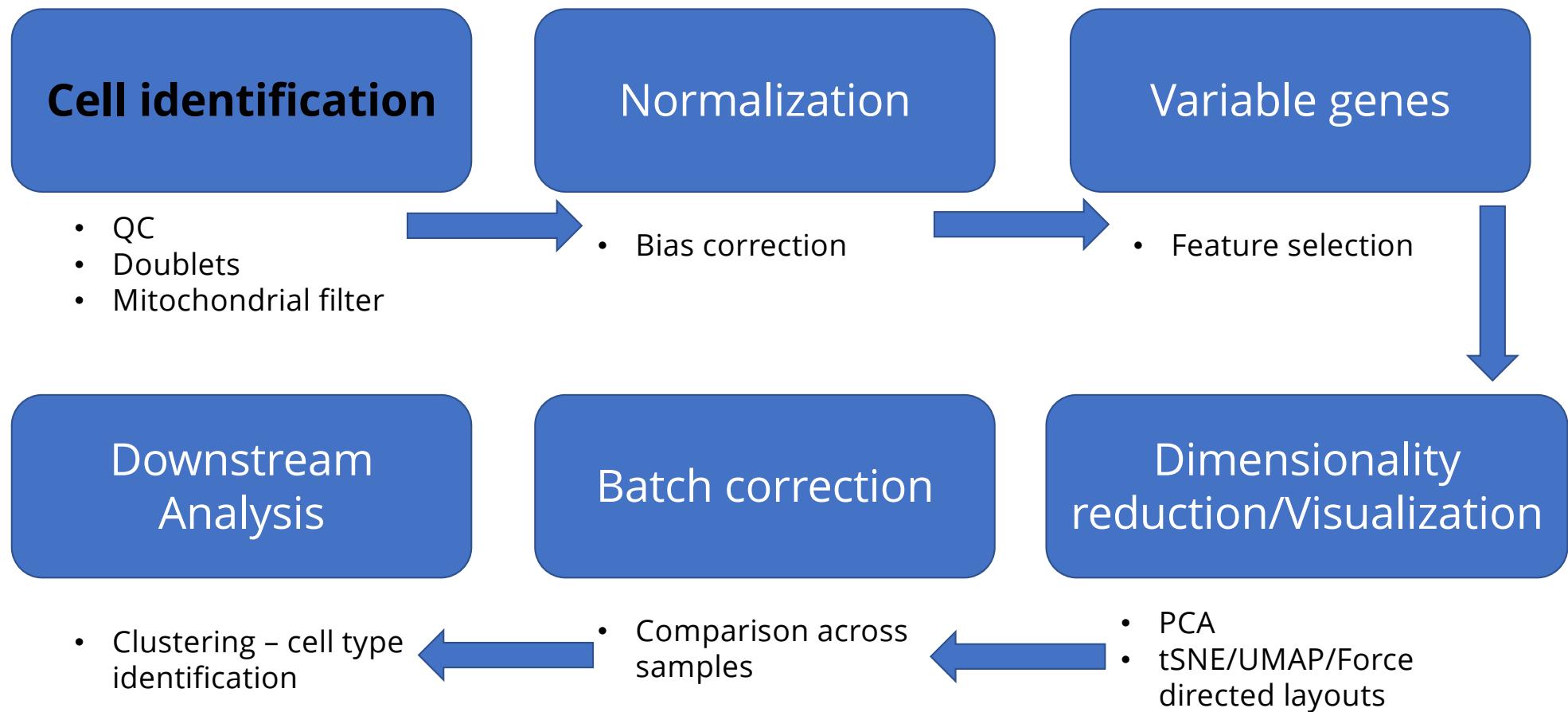
sc.pp.neighbors(ad)
sc.tl.leiden(ad)
sc.tl.umap(ad)
```



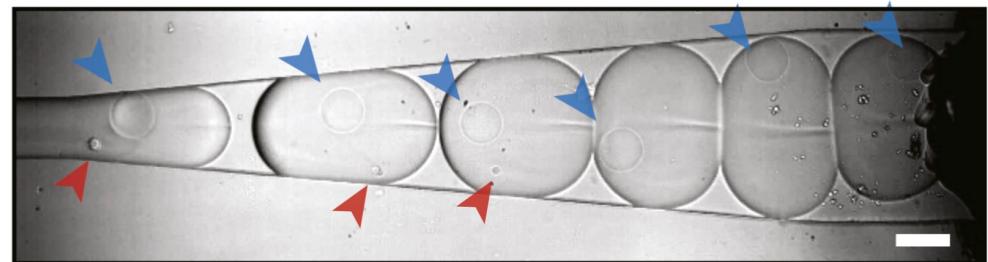
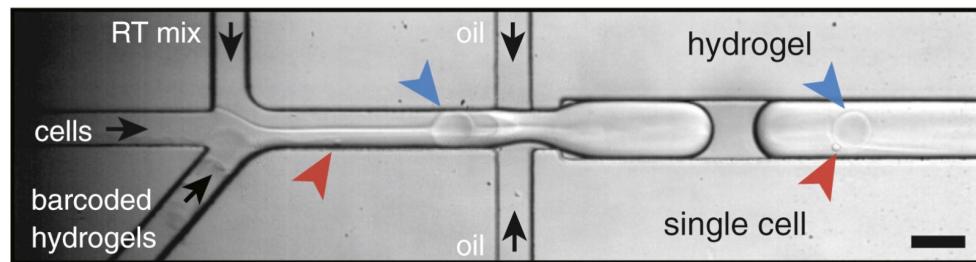
# scRNA-seq analysis steps



# scRNA-seq analysis steps



# scRNA-seq: Empty droplets & Ambient RNA



- Most droplets do not have cells!
  - Ambient RNA

# Single-cell RNA-seq

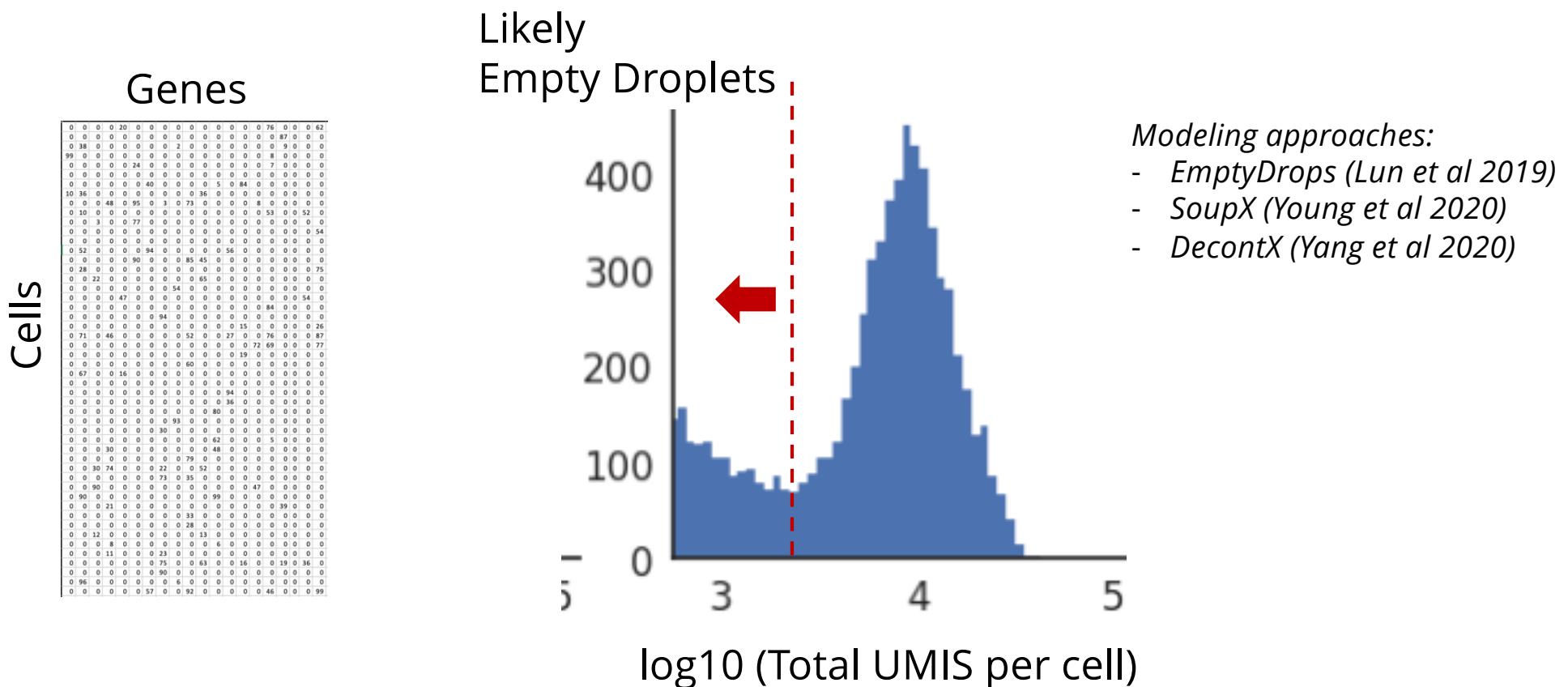
*All rows are not real cells*

Genes

Cells

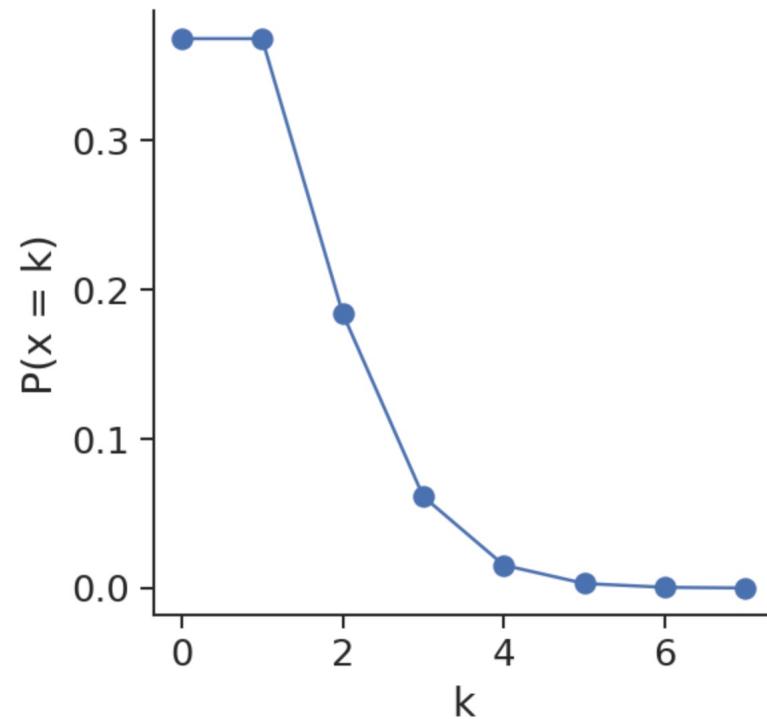
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87	0	0	0		
0	38	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	9	0	0	0		
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0		
0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0		
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0	0	0	0	0	40	0	0	0	0	5	0	84	0	0	0	0	0	0	0	0	0	0	
10	36	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	48	0	95	0	3	0	73	0	0	0	0	8	0	0	0	0	0	0	0	0	
0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	52	0	0	0	0	
0	0	3	0	0	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
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0	52	0	0	0	94	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	90	0	0	0	85	45	0	0	0	0	0	0	0	0	0	0	0	0	0
0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	
0	0	22	0	0	0	0	0	0	65	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	26	
0	71	0	46	0	0	0	0	0	52	0	0	27	0	0	76	0	0	0	87	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	69	0	0	0	77	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	67	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	62	0	0	0	5	0	0	0	0	0	0	0
0	0	0	30	0	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	30	74	0	0	0	22	0	0	52	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	73	0	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	90	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0	0	0	0
0	90	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	12	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	8	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	11	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	75	0	0	63	0	0	16	0	0	19	0	36	0	0	0	0	0
0	0	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	96	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	57	0	0	92	0	0	0	0	46	0	0	0	99	0	0	0	0	0

# scRNA-seq: Removal of empty droplets

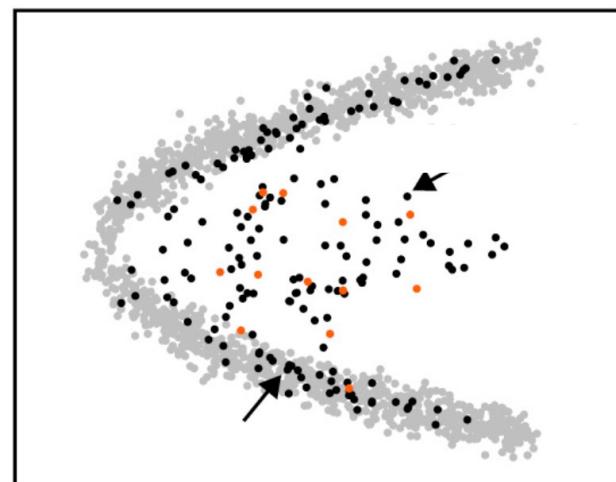
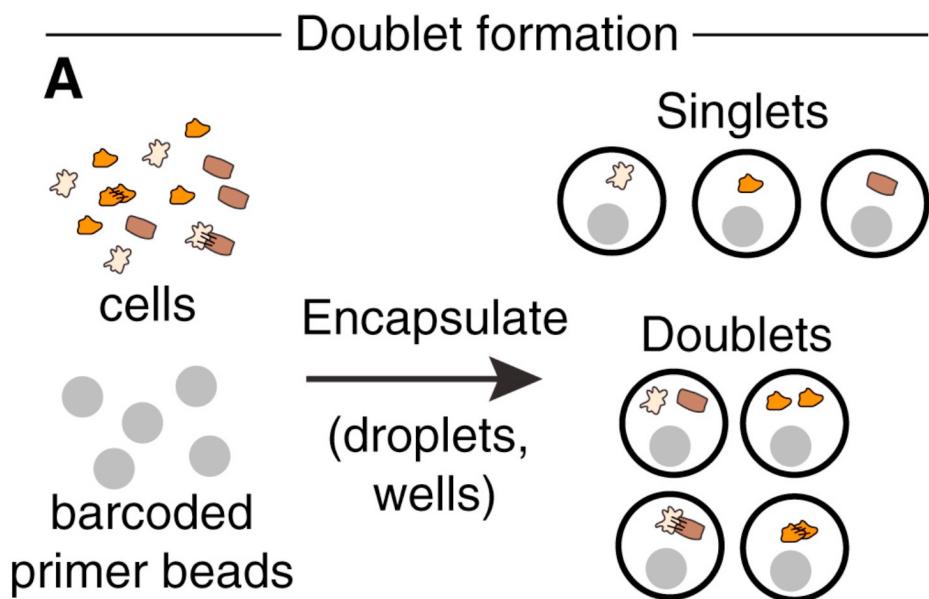


## Cell containing droplets

- Cell encapsulation follows a Poisson distribution – are there reasons beyond ambient RNA, that can lead to misleading biology



# Doublets

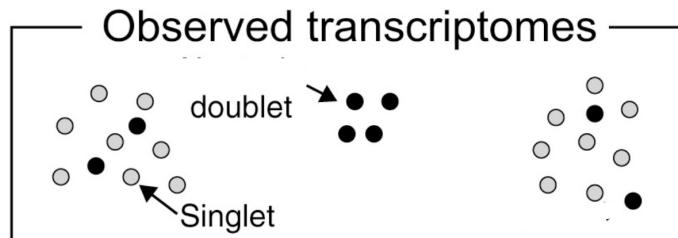


Wolock et al 2019

# How to detect doublets?

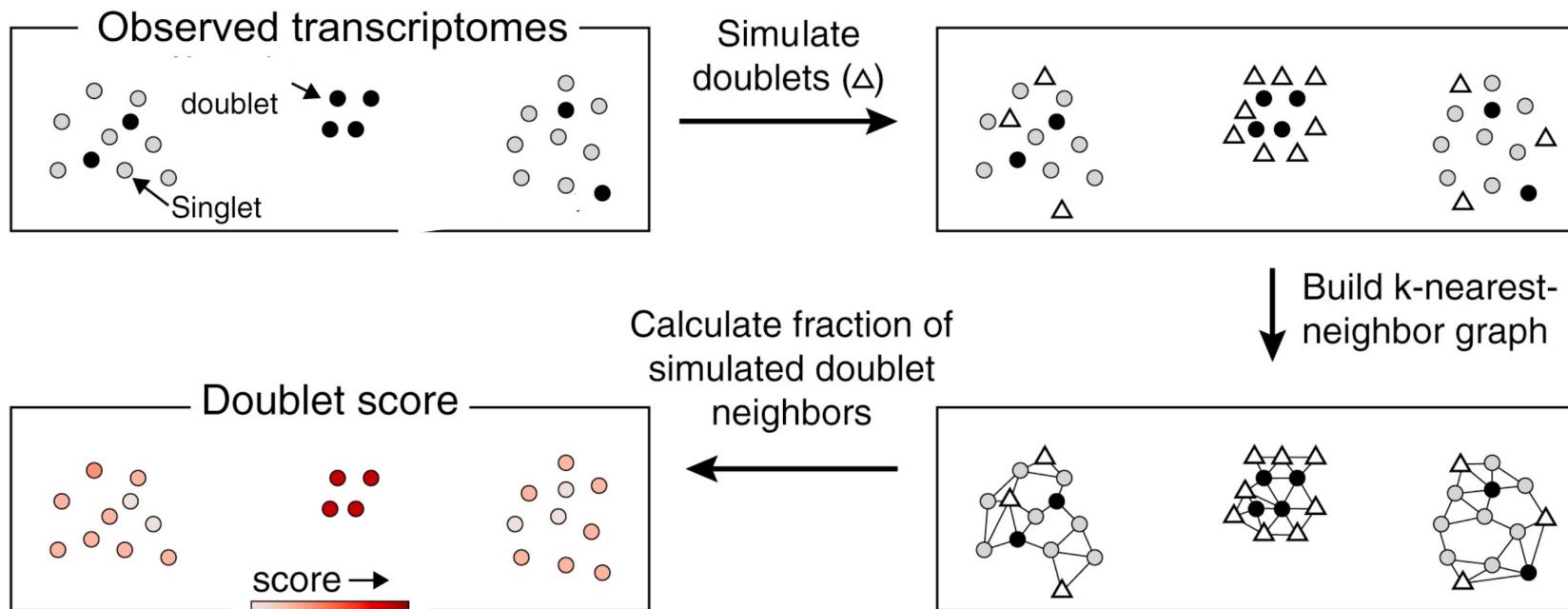
- Assume:

- Multiplets / Doublets are rare
- Constituent singlets are present



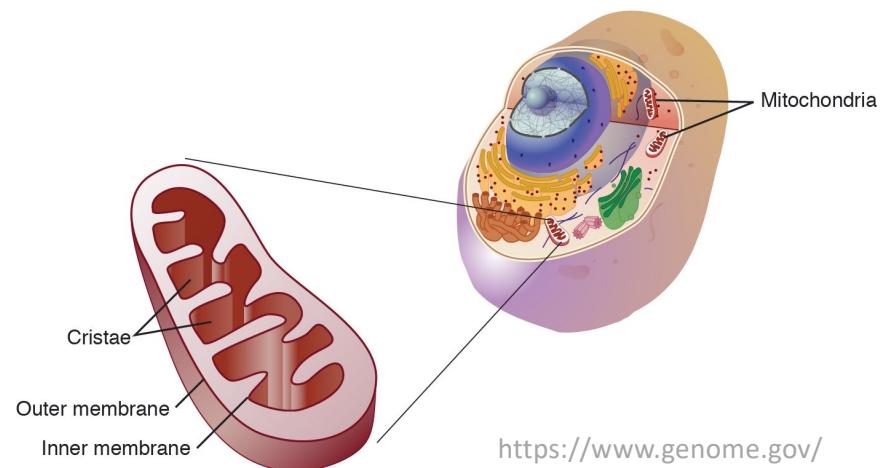
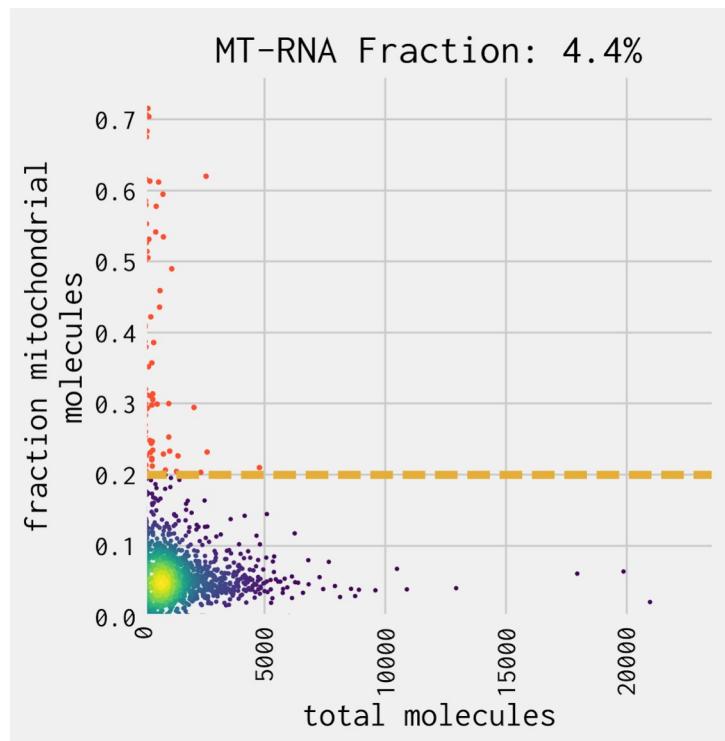
# How to detect doublets?

- Assume:
  - Multiplets / Doublets are rare
  - Constituent singlets are present



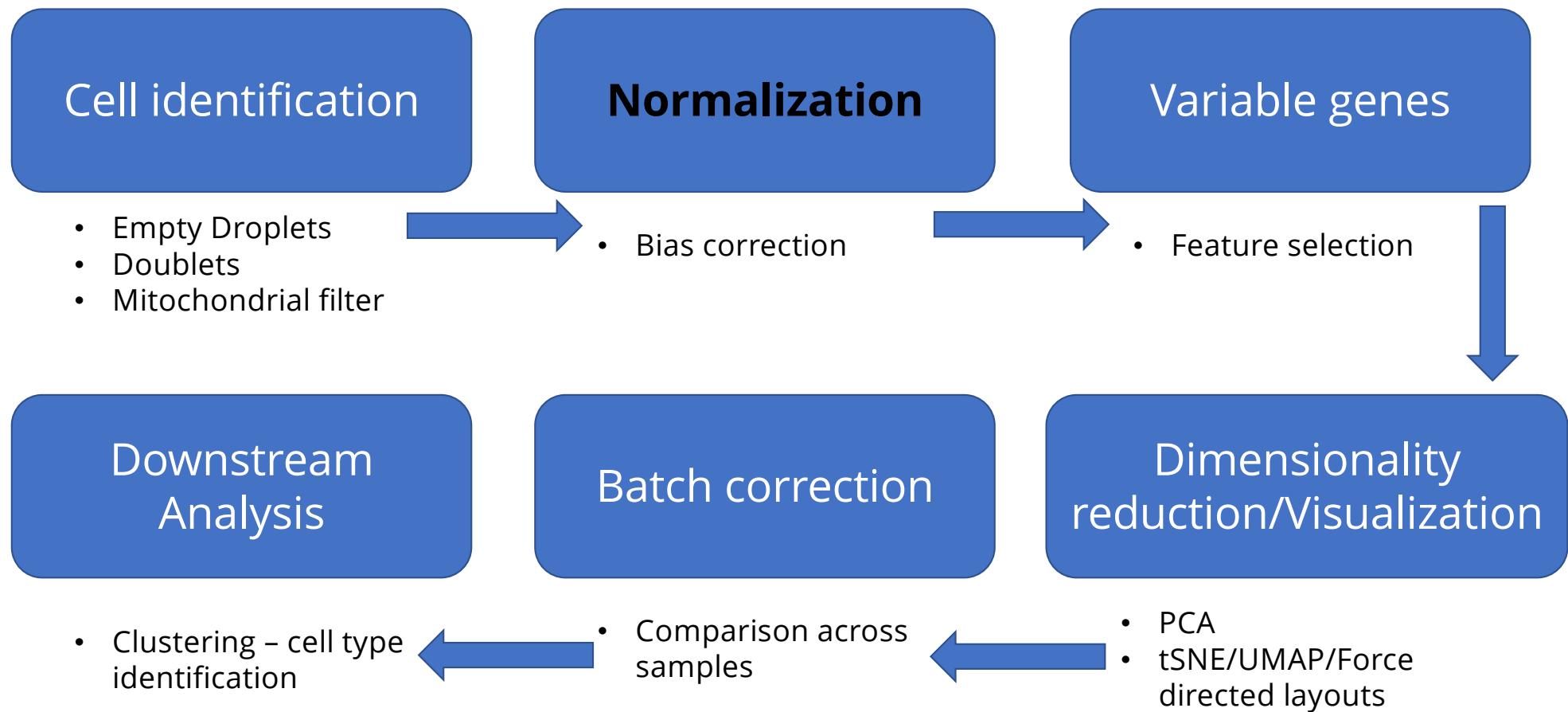
- Can this detect all possible multiplets?

# Mitochondrial filter



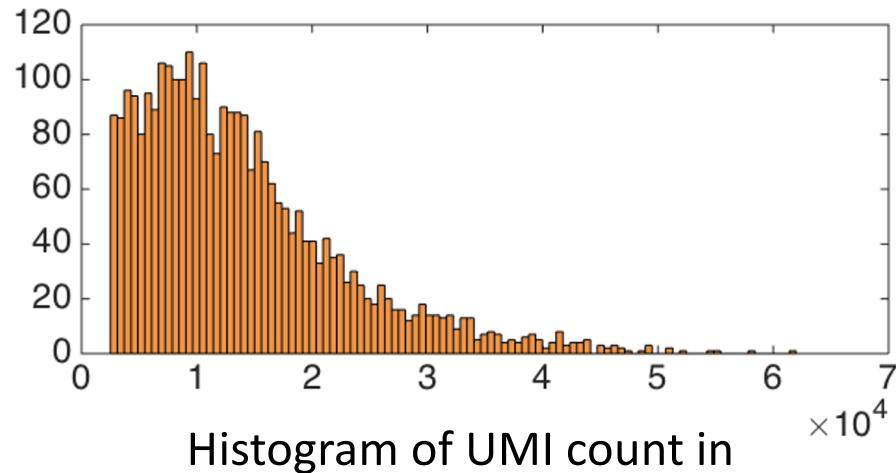
- Calculate fraction of molecules from MT genes
- Exclude cells with > 20% (optional)

# scRNA-seq analysis steps



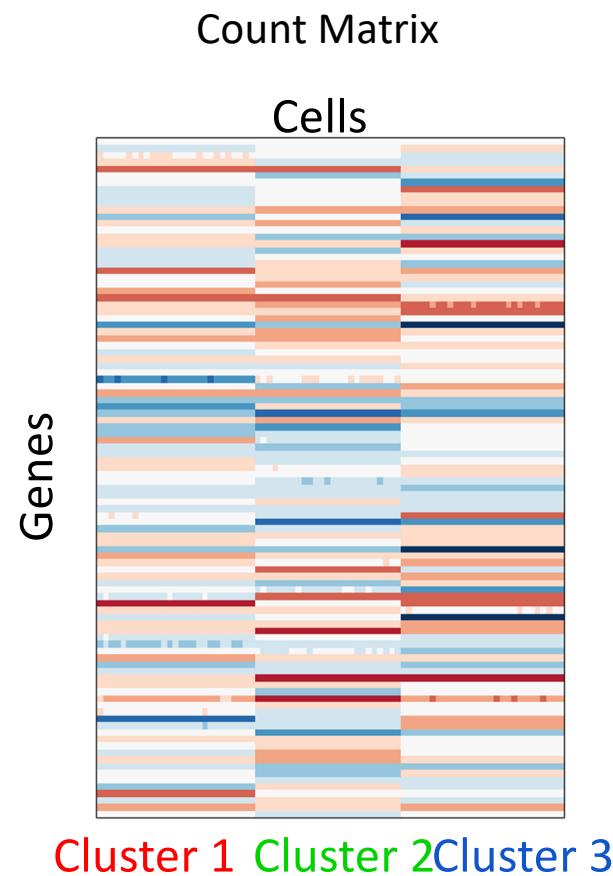
# Why Normalize?

- Larger skew in distribution of total molecules (UMIs) per cell, i.e. library size
- Expression values not comparable across cells
  - *Measuring distance between cells*



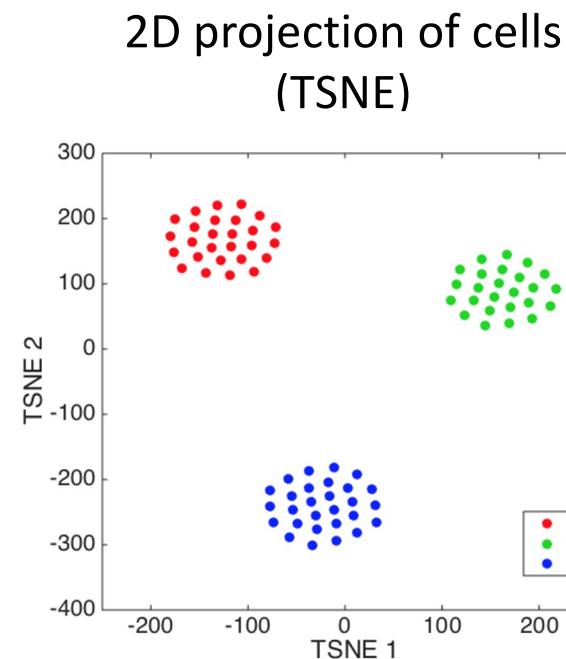
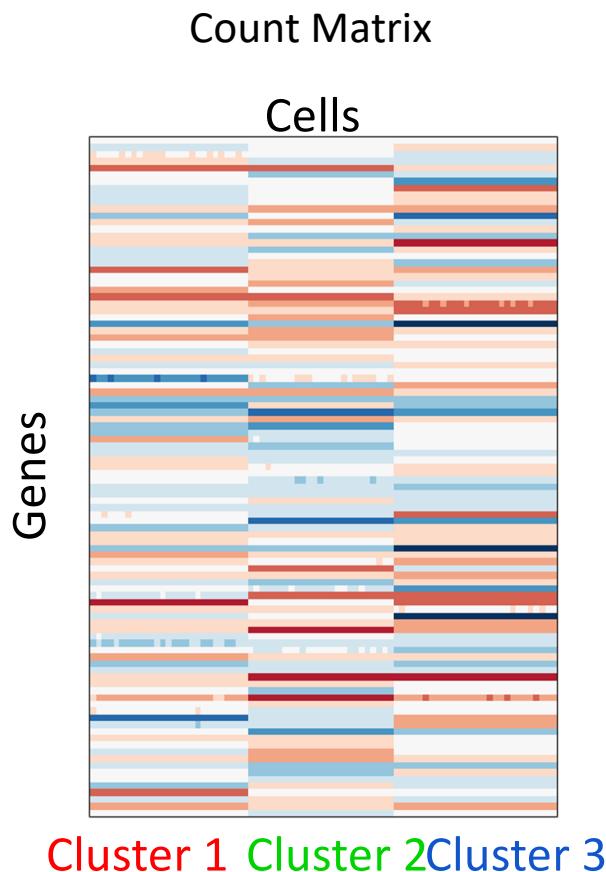
Histogram of UMI count in  
example SC dataset  
From Zeisel, Science 2014

# Why is this problematic?

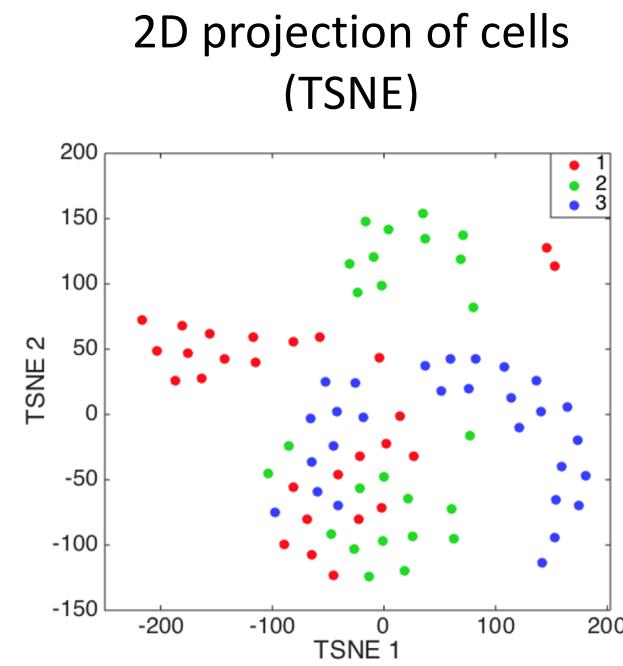
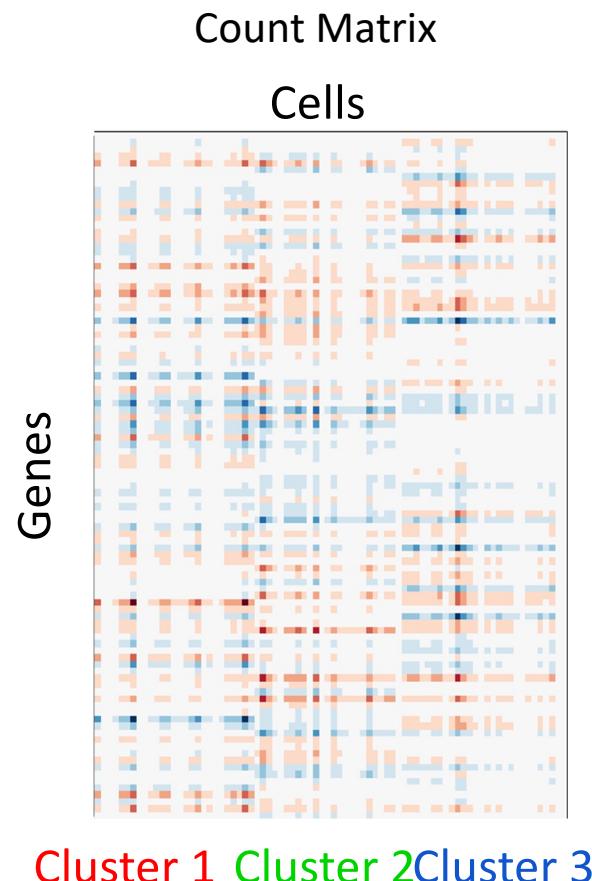


Slides courtesy of Elham Azizi

# Why is this problematic?

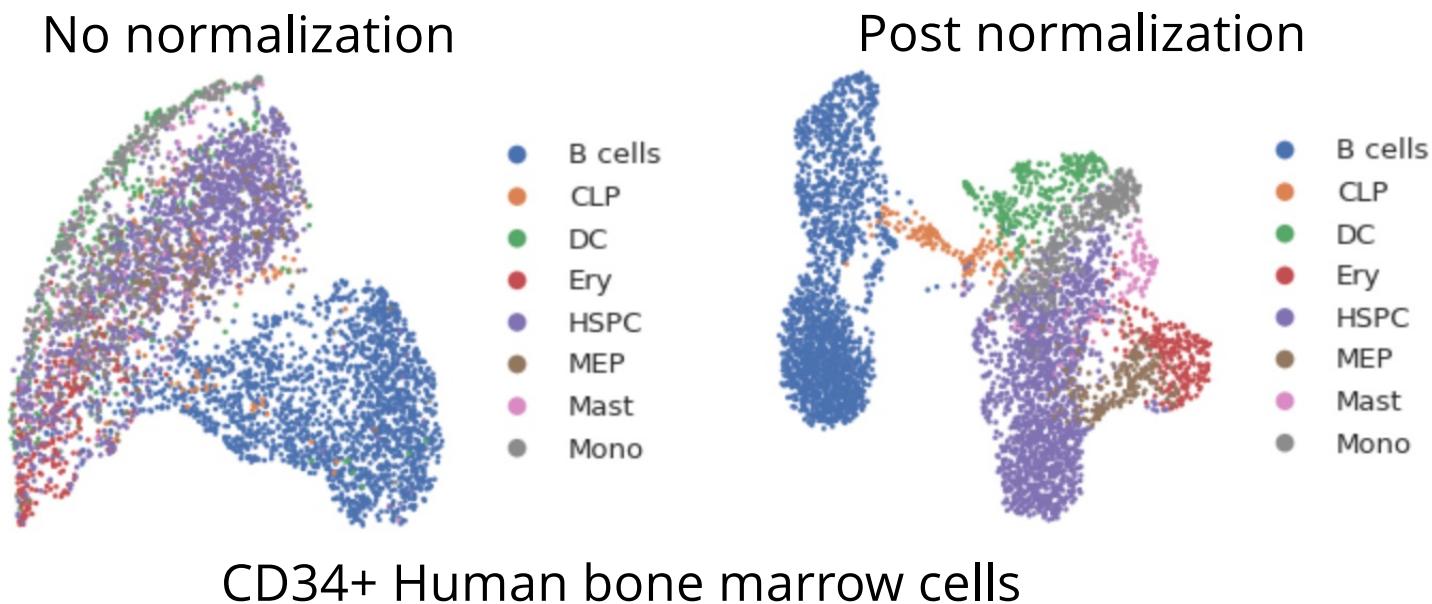


# Why is this problematic?

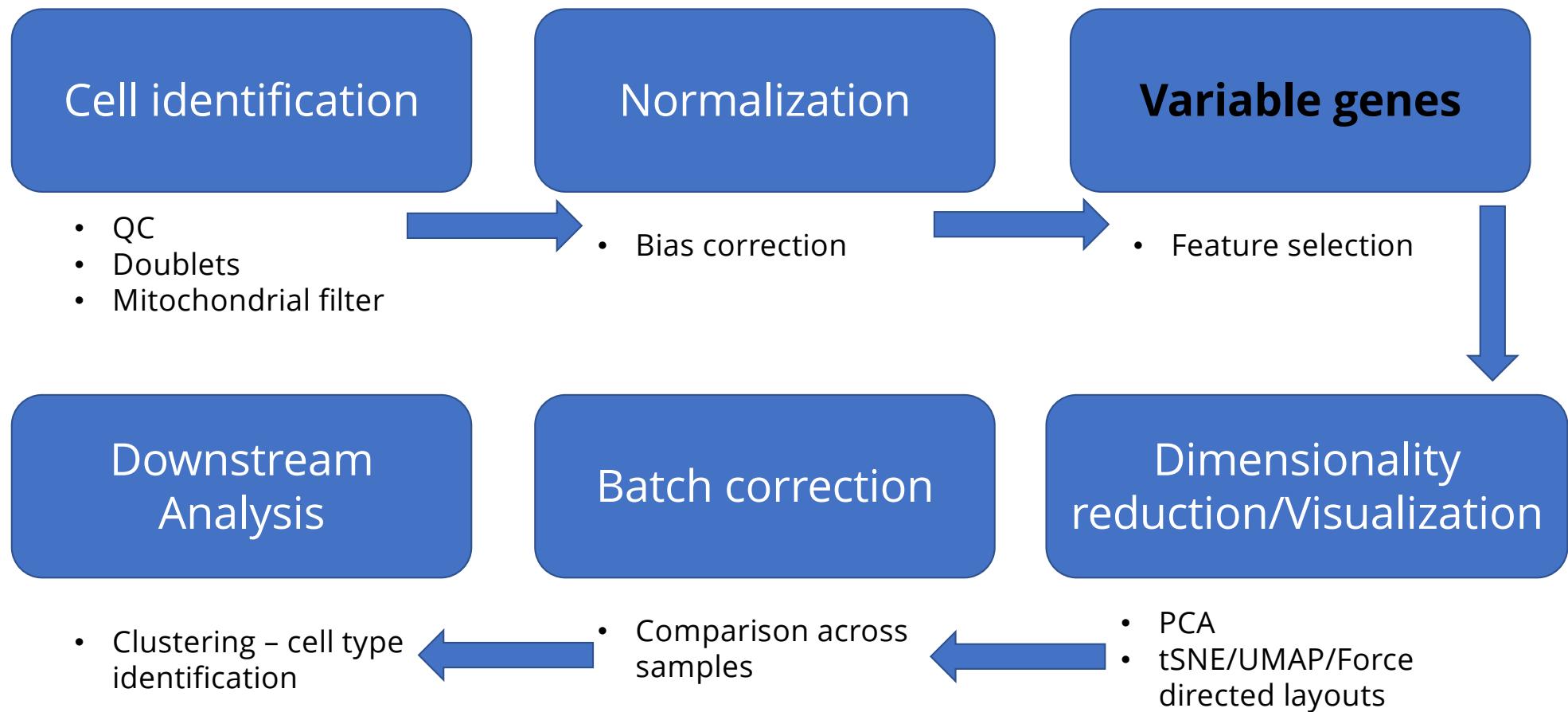


# Normalization

- Global normalization:
  - Divide counts by total molecules in each cell
  - Multiply by median [To avoid numerical issues]
- Log transform of the data

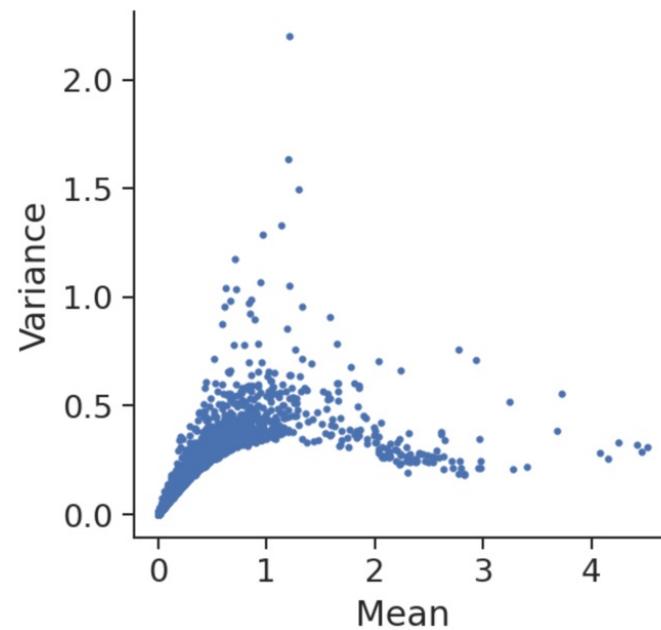


# scRNA-seq analysis steps



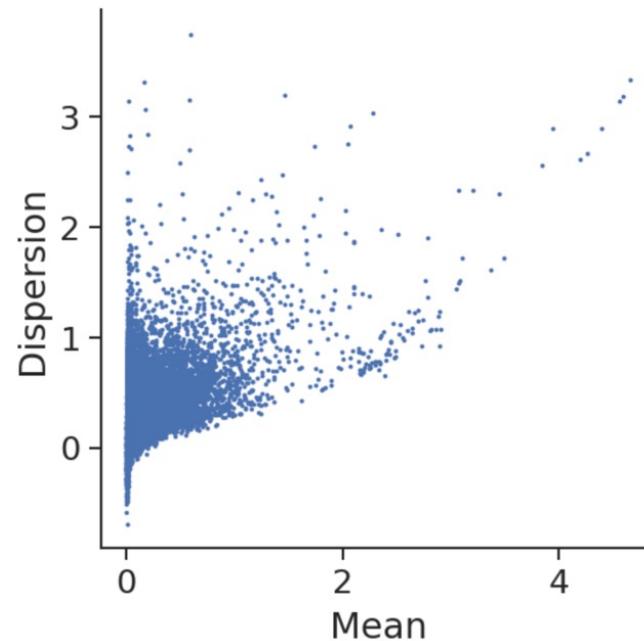
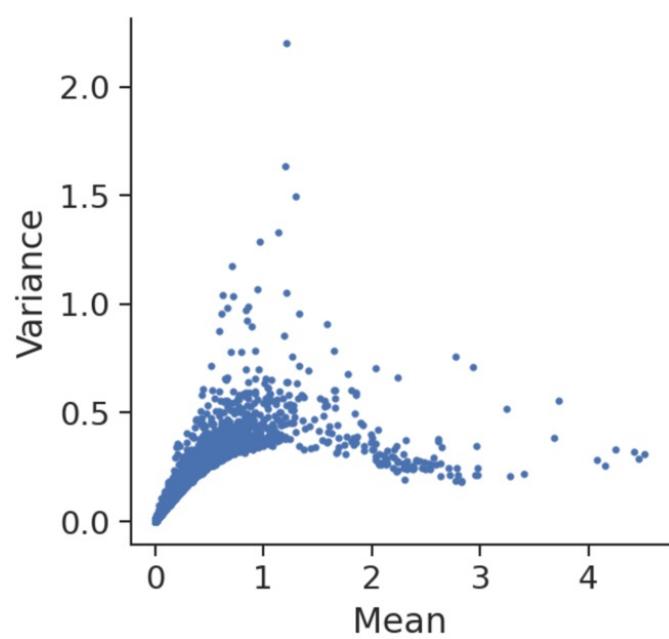
# Feature selection

- Cell states are defined by expression of subsets of genes
- Goal of feature selection: Select genes that inform the biology rather than genes that represent random noise
- Possible Solution: How variable is the gene across cells?



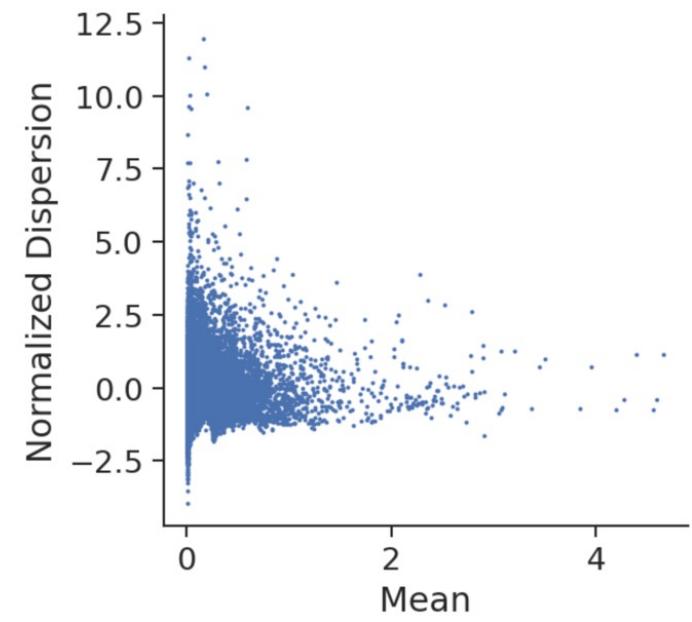
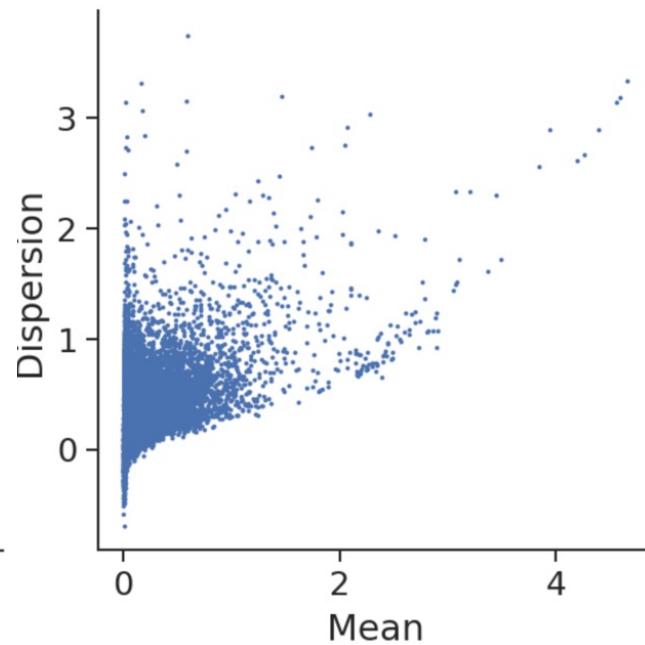
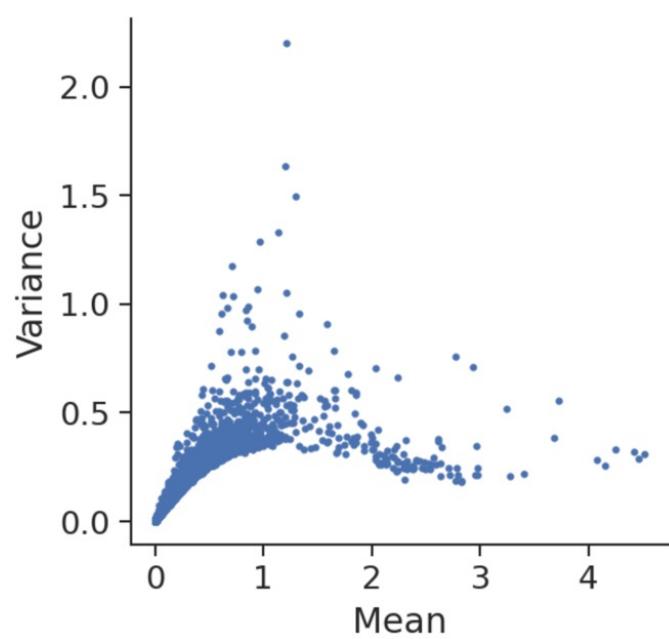
# Feature selection

$$Dispersion = \frac{Variance}{Mean}$$



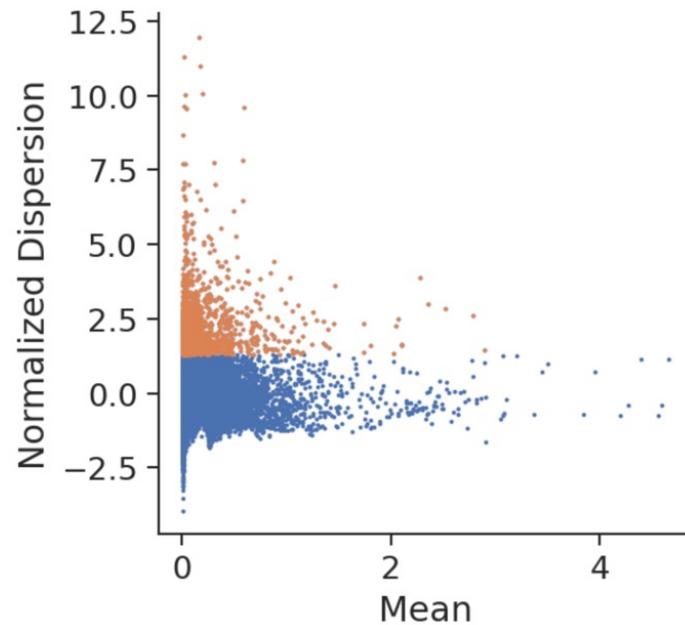
# Feature selection: Share information across genes

$$\begin{aligned} \text{Normalized Dispersion} \\ = \frac{(\text{Dispersion} - \text{Mean}(\text{Dispersion in expression mean bin}))}{\text{Std}(\text{Dispersion in expression mean bin})} \end{aligned}$$



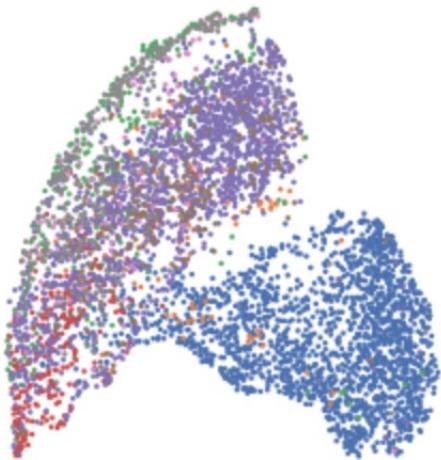
# Feature selection: Share information across genes

$$\begin{aligned} \text{Normalized Dispersion} \\ = \frac{(\text{Dispersion} - \text{Mean}(\text{Dispersion in expression mean bin}))}{\text{Std}(\text{Dispersion in expression mean bin})} \end{aligned}$$

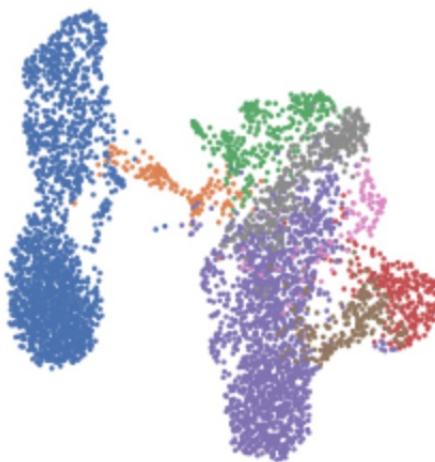


# Feature selection: highly variable genes

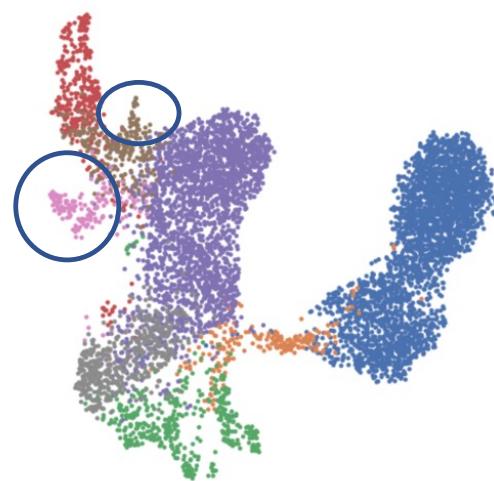
No normalization



Post normalization

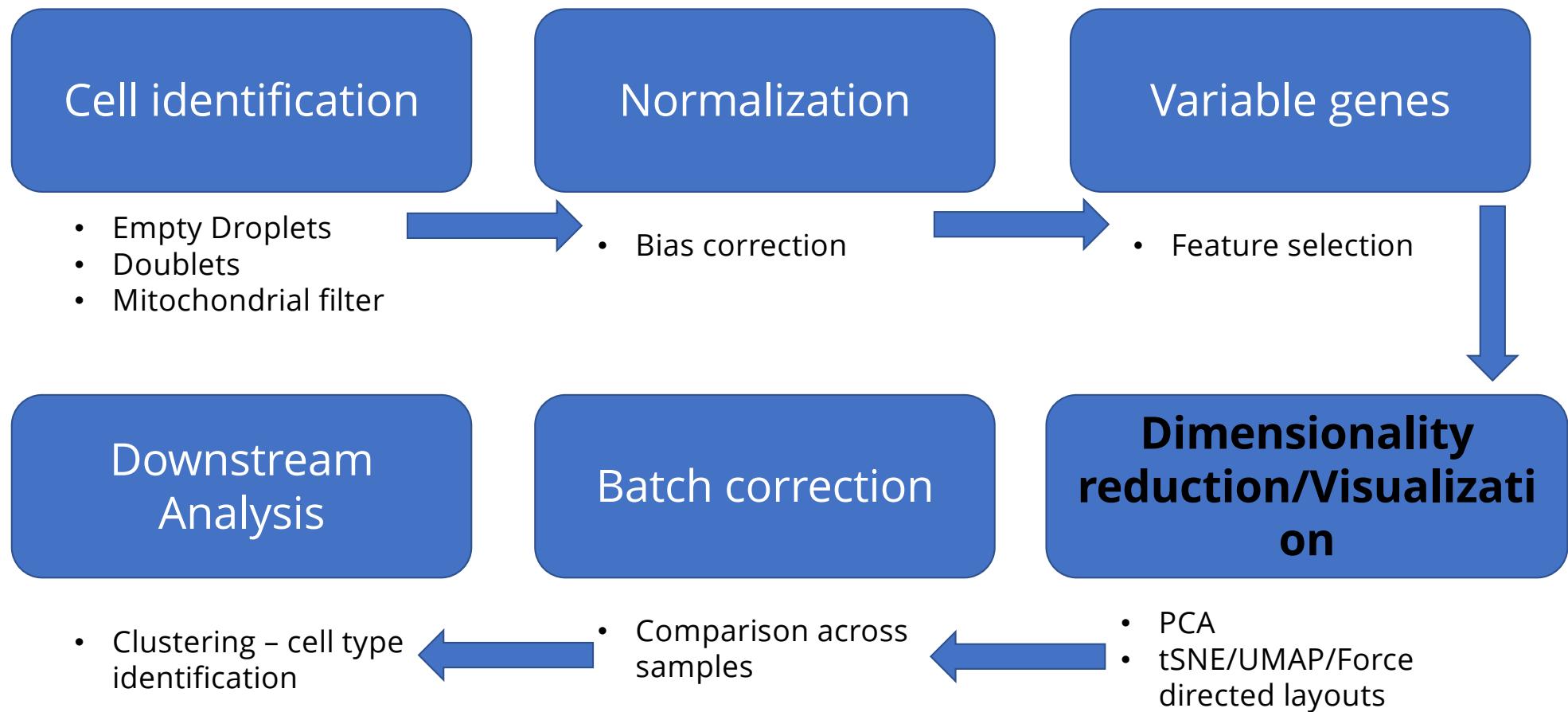


Feature selection



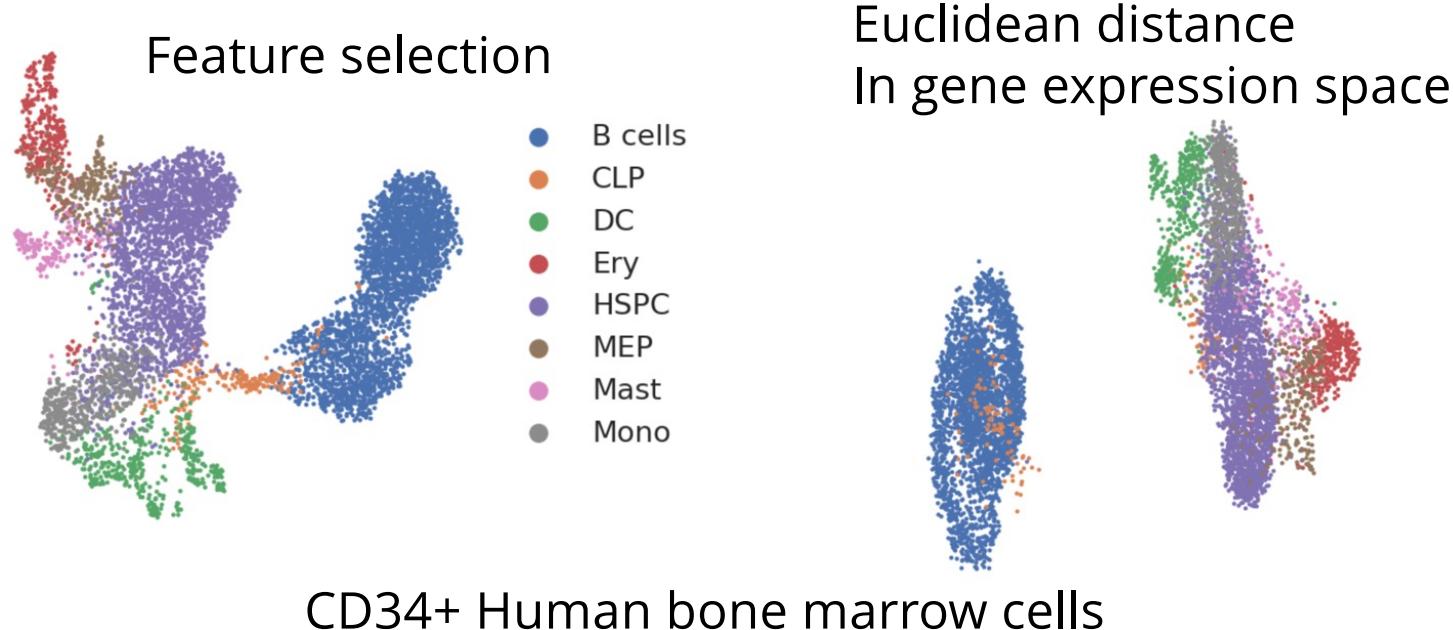
CD34+ Human bone marrow cells

# scRNA-seq analysis steps



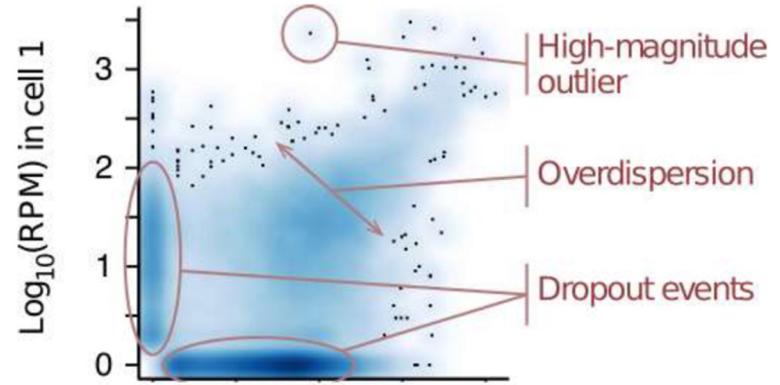
# Measuring distance between cells

- Possible Solution: Euclidean distance between normalized, selected genes



# Single-cell RNA Noise: Dropouts

- ~5-10% of transcripts in a cell are captured
- Further loss during reverse transcription
- Genes with higher expression have fewer zeros
- Non-zero values are also under-estimates of true counts



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## BRIEF COMMUNICATIONS

Bayesian approach to  
single-cell differential  
expression analysis



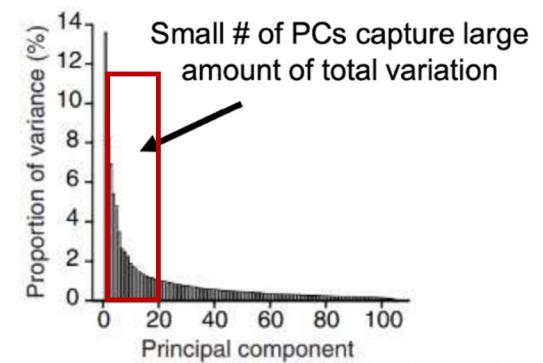
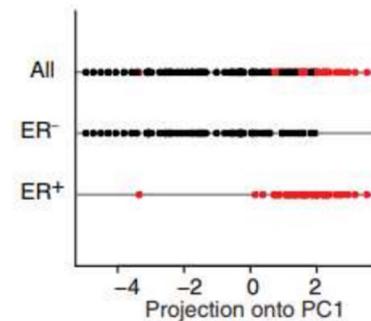
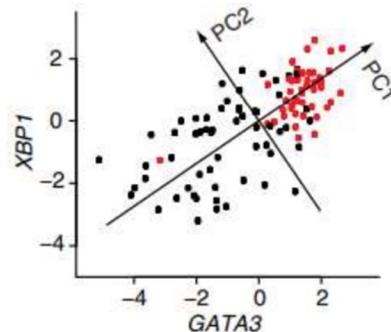
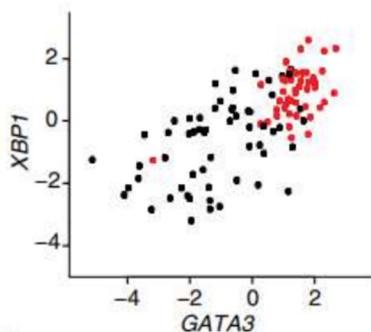
Peter V Kharchenko<sup>1-3</sup>, Lev Silberstein<sup>3-5</sup> &  
David T Scadden<sup>3-5</sup>

© 2014 Nature America, Inc.

# Alternative solution: PCA

- Cell states are defined by co-regulated gene modules
- PCA as a proxy to identify these genes modules

PCA effectively defines new axes through the data that capture the highest amount of variation possible



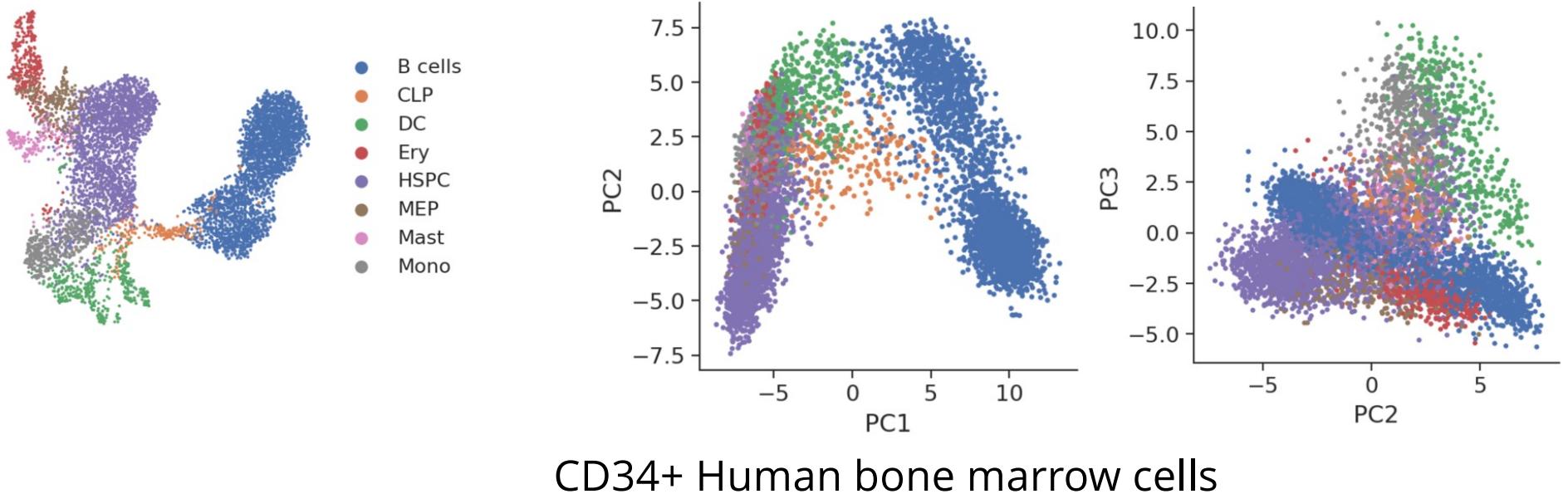
Ringér, *Nature Biotech*, 2008

- Selection of subset of PCs: Dimensionality reduction

# Dimensionality Reduction

- Reduce the number of dimensions of data while preserving high dimensional information
- Overcome noise in high dimensions
- Computational efficiency
- Visualization

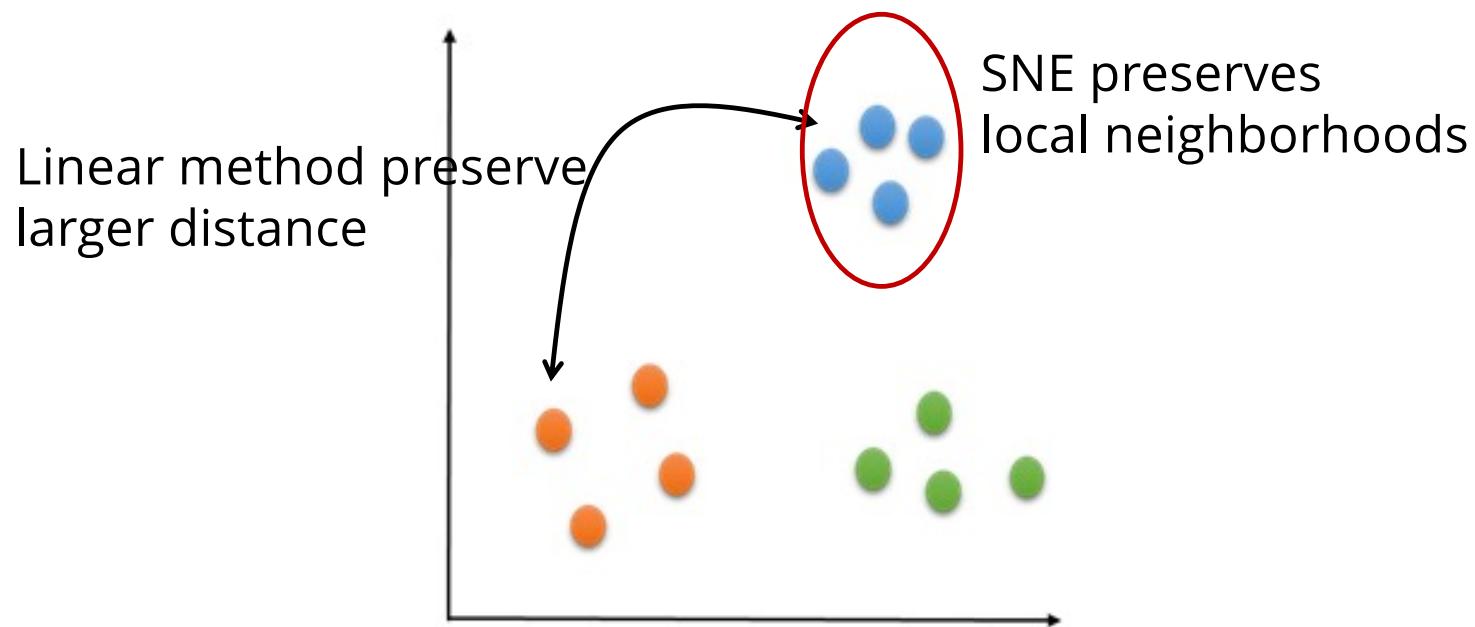
# PCA for visualization



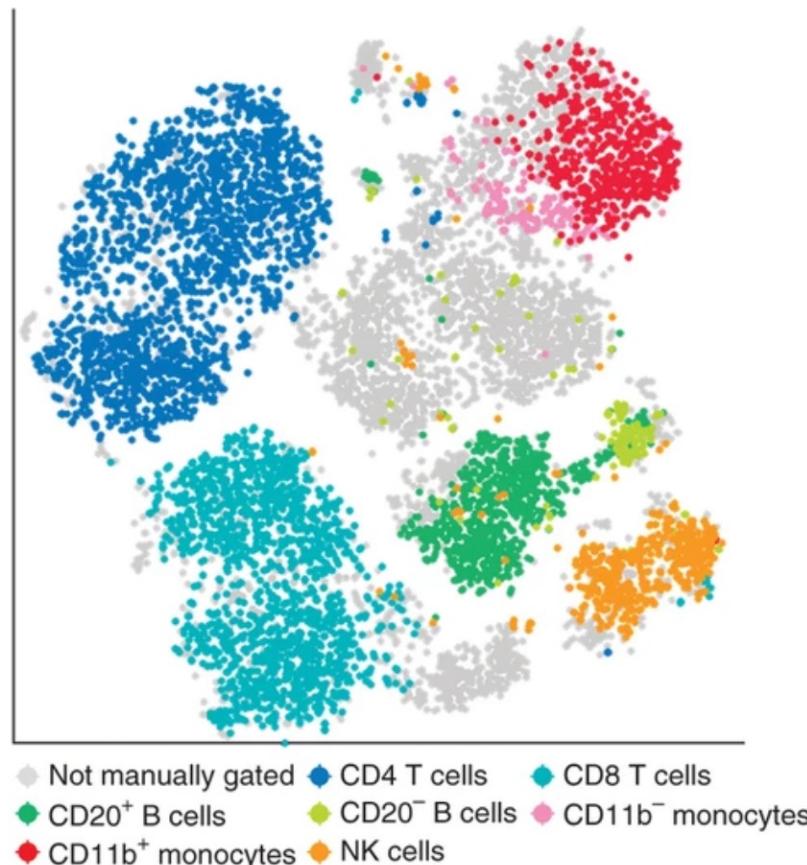
- Two axes can only capture so much information
  - Not explicitly modeled to capture as much of higher order information in 2D

## Dimensionality Reduction for Visualization: tSNE

- SNE: Stochastic Neighborhood Embedding
- Goal: Compute a low dimensional representation that best preserves the local neighborhoods of cells



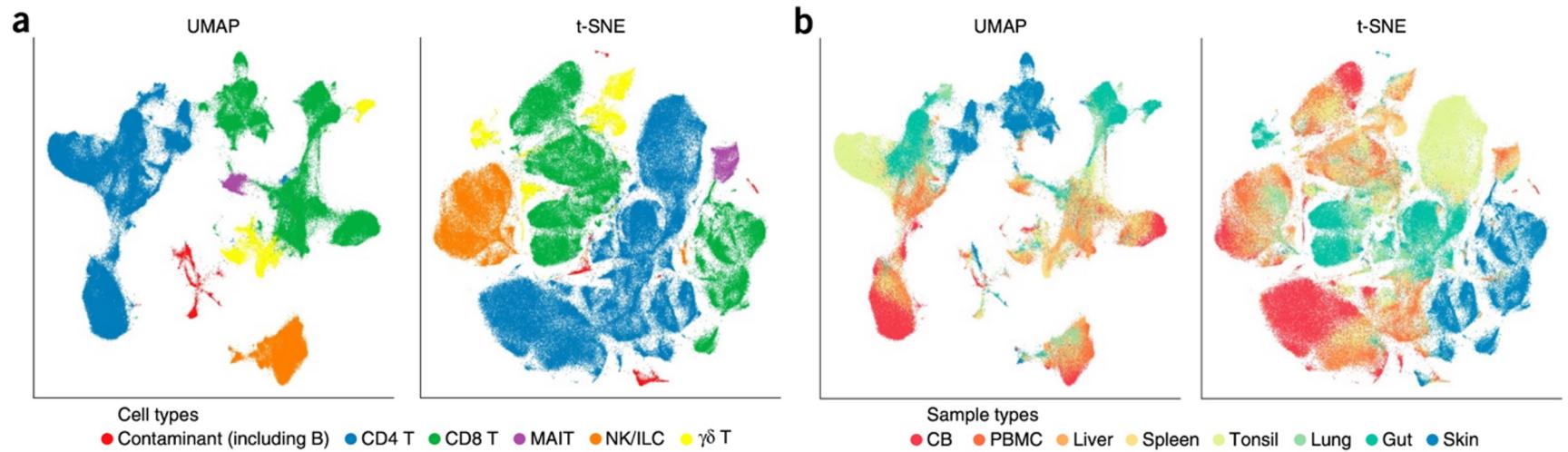
# tSNE for single-cells



David Amir et. al, 2013

# Visualization: UMAP

- Claim: Better preservation of global structure compared to tSNE while also preserving local structure



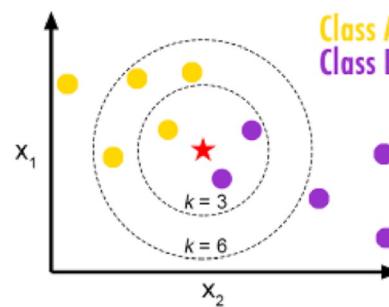
Becht et. al., 2019

# Visualization: Force directed layouts

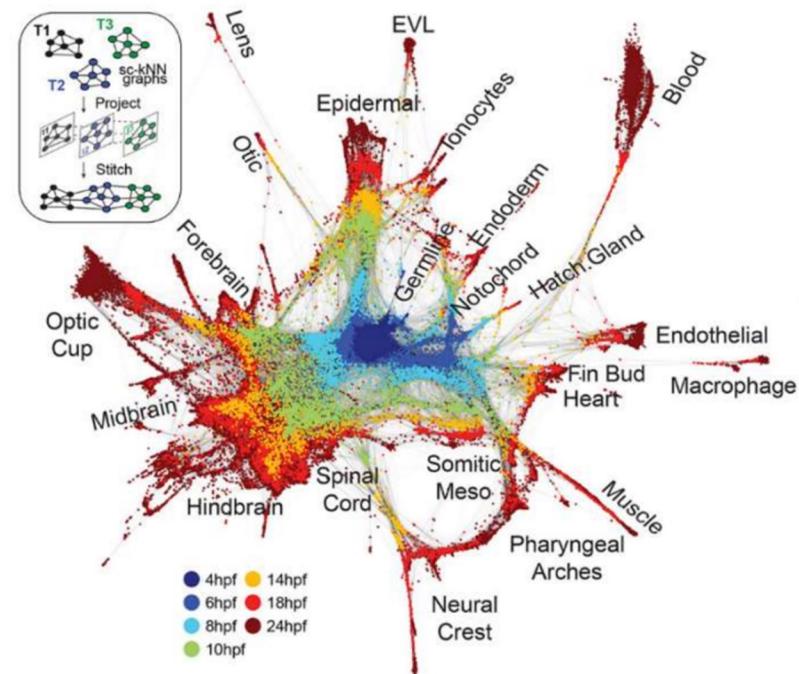
**Goal:** To visualize the structure of our data

## Force-directed graphs

Visualize cells based on nearest neighbor structures

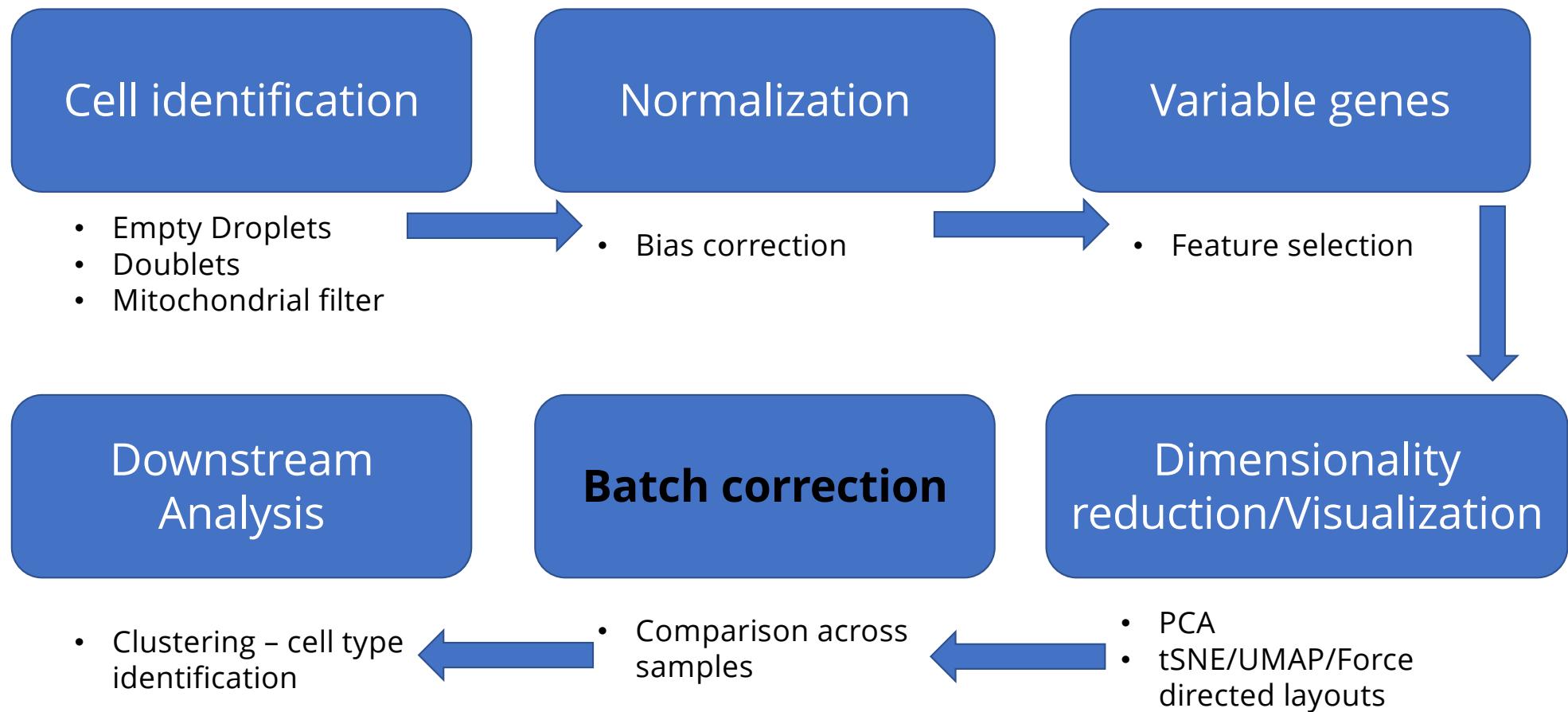


Repulsion between nodes  
Attractive forces added  
to edges connecting  
nodes (spring functions)



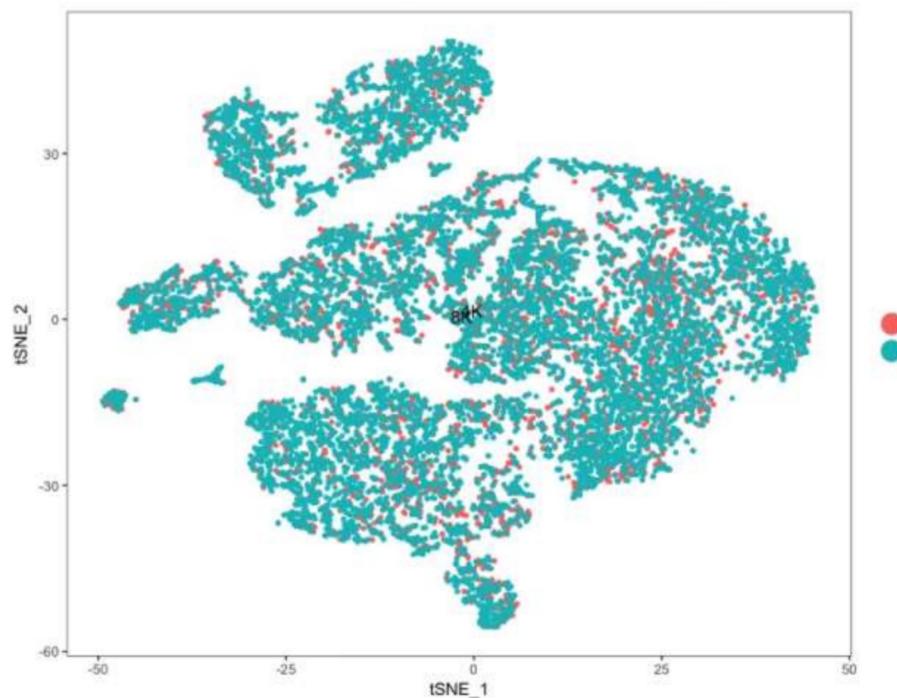
Wagner et al., *Science*, 2018

# scRNA-seq analysis steps

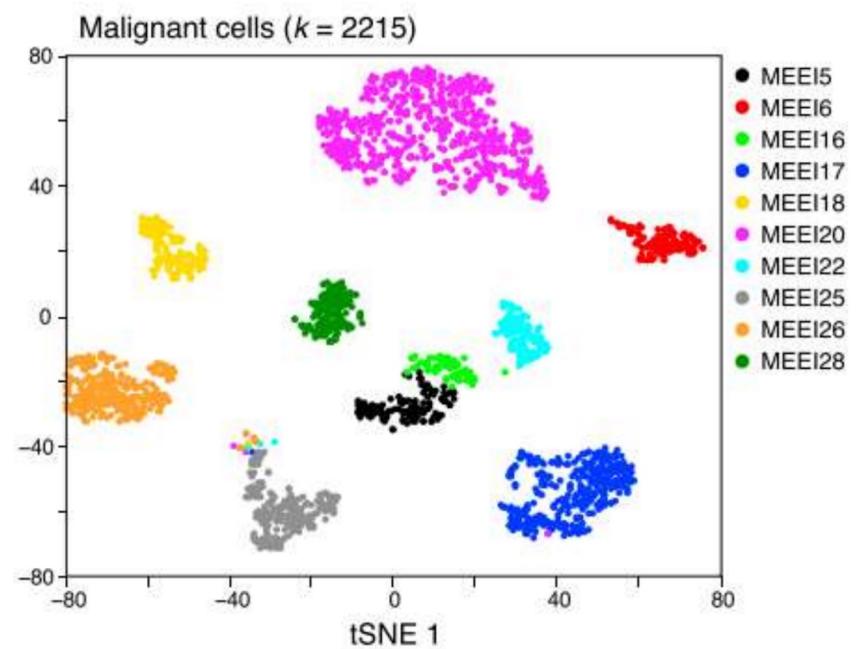


# Real heterogeneity –vs- technical noise

**Technical replicate of PBMCs  
has near-perfect overlap**

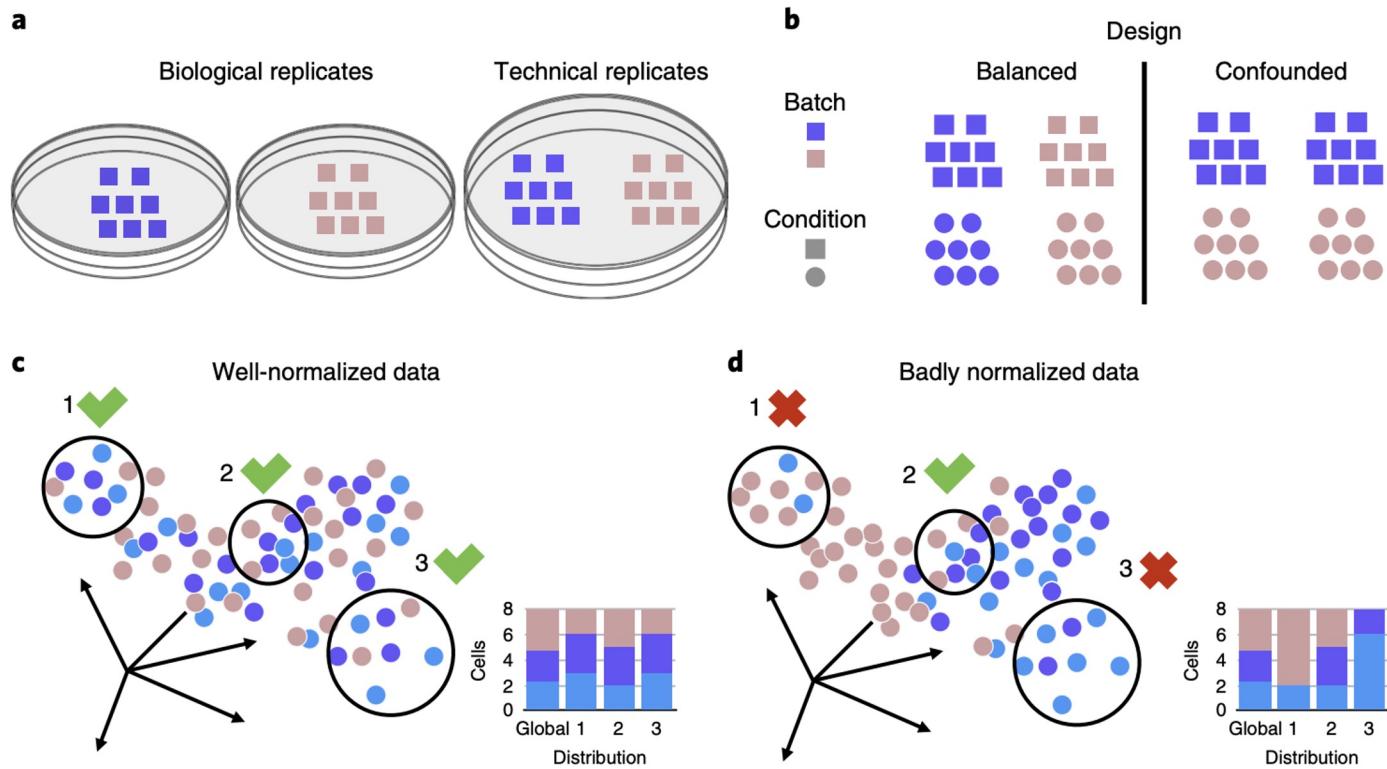


**Cancer cells dramatically  
different between patients**



# Assessing normalization and batch effects

Evaluate mixing of samples



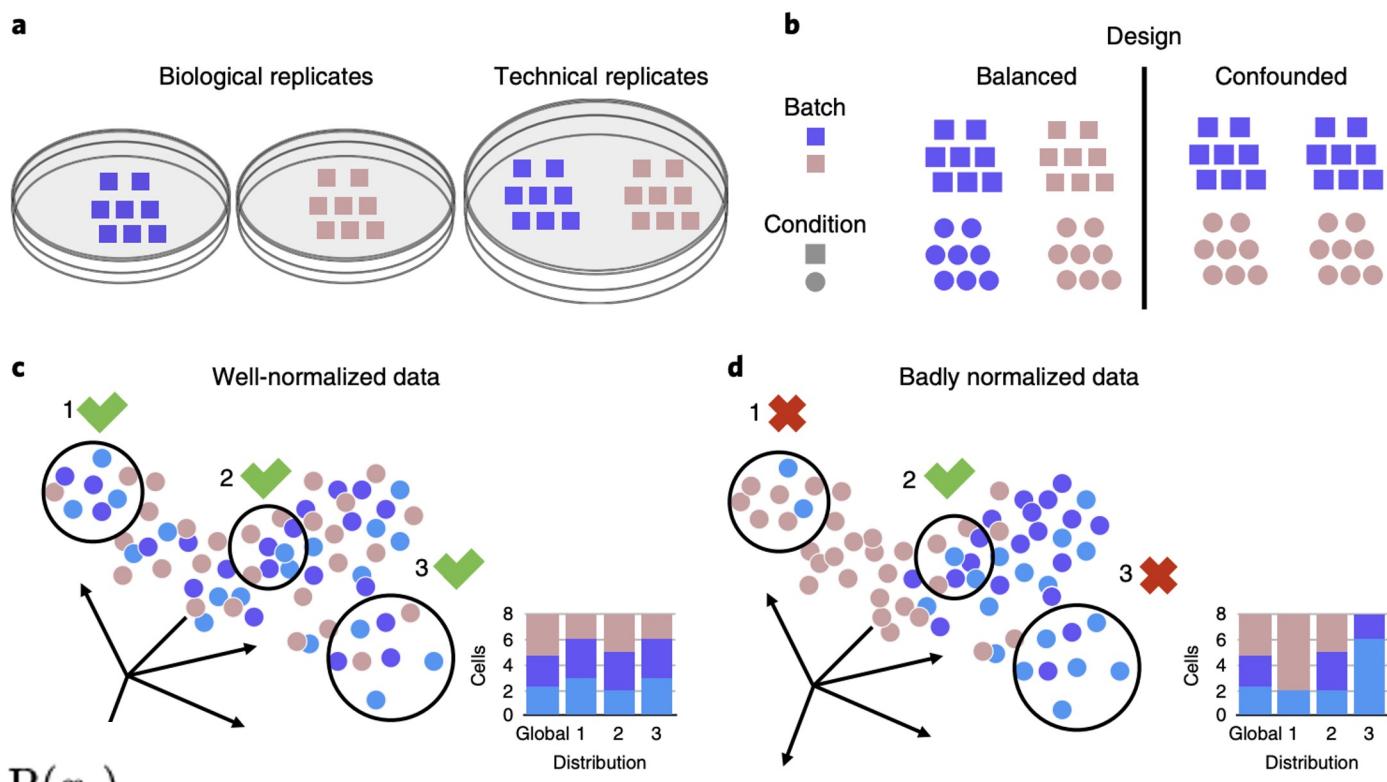
Buttner et. al, 2018

# Assessing normalization and batch effects

Select random neighborhoods of fixed size

Compute Shannon Entropy of distribution across samples

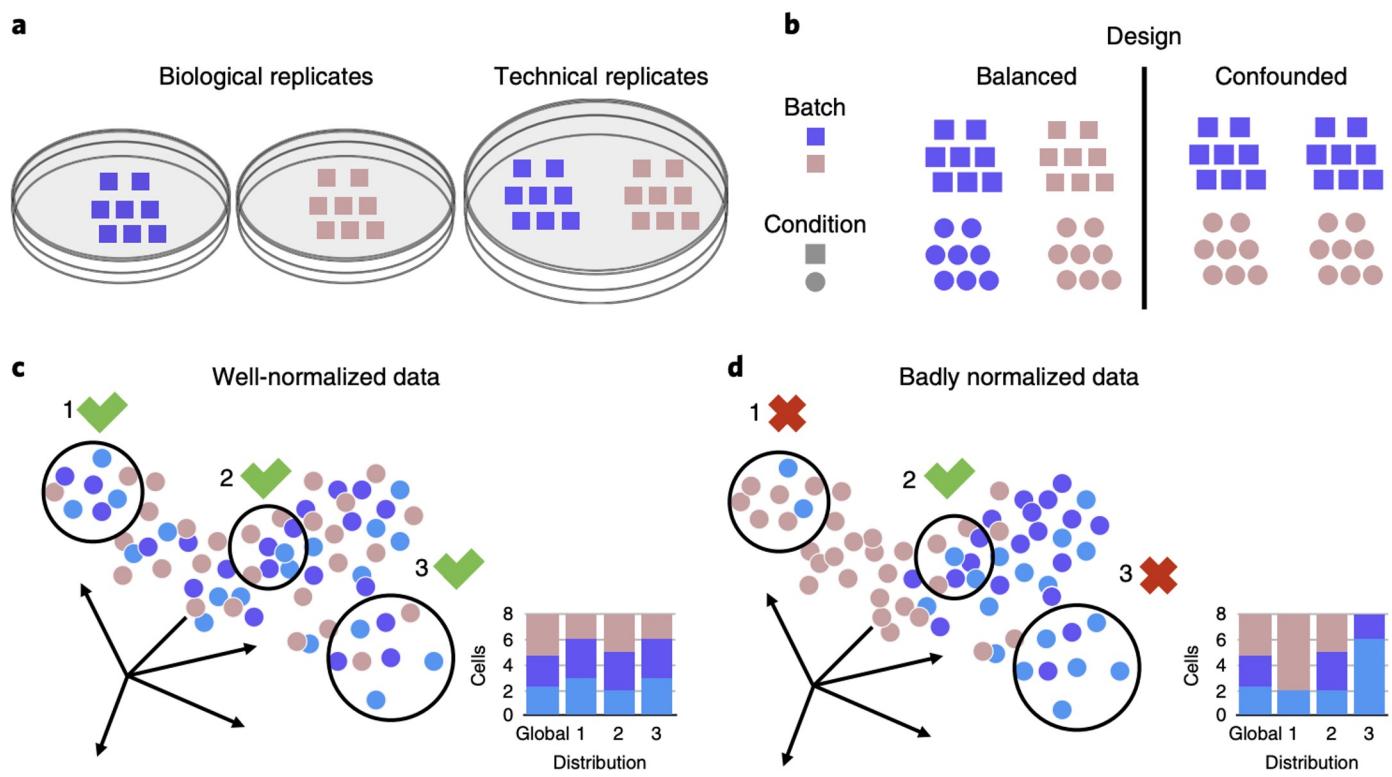
$$H(X) = - \sum_{i=1}^n P(x_i) \log_b P(x_i)$$



Buttner et. al, 2018

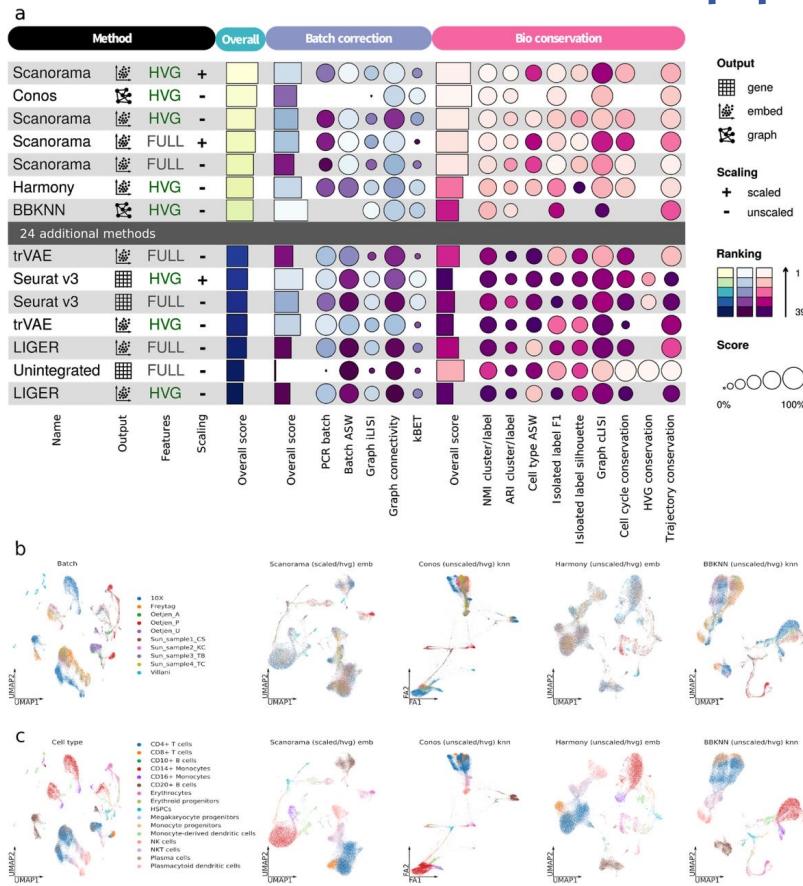
# Assessing normalization and batch effects

kBET:  
Chi-squared test in  
random  
neighborhoods,  
followed by  
averaging of binary  
test results



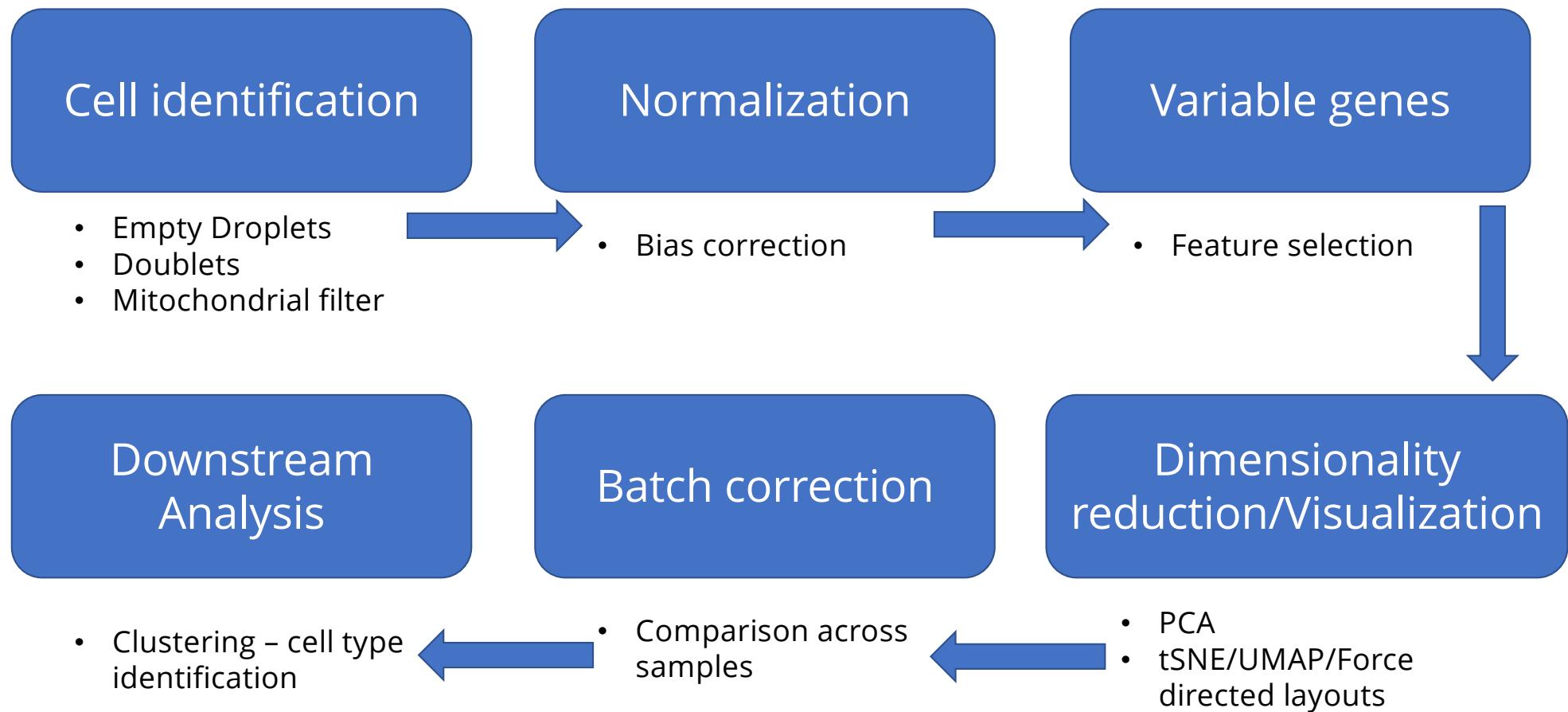
Buttner et. al, 2018

# Batch effect correction approaches



Luecken et. al, 2020

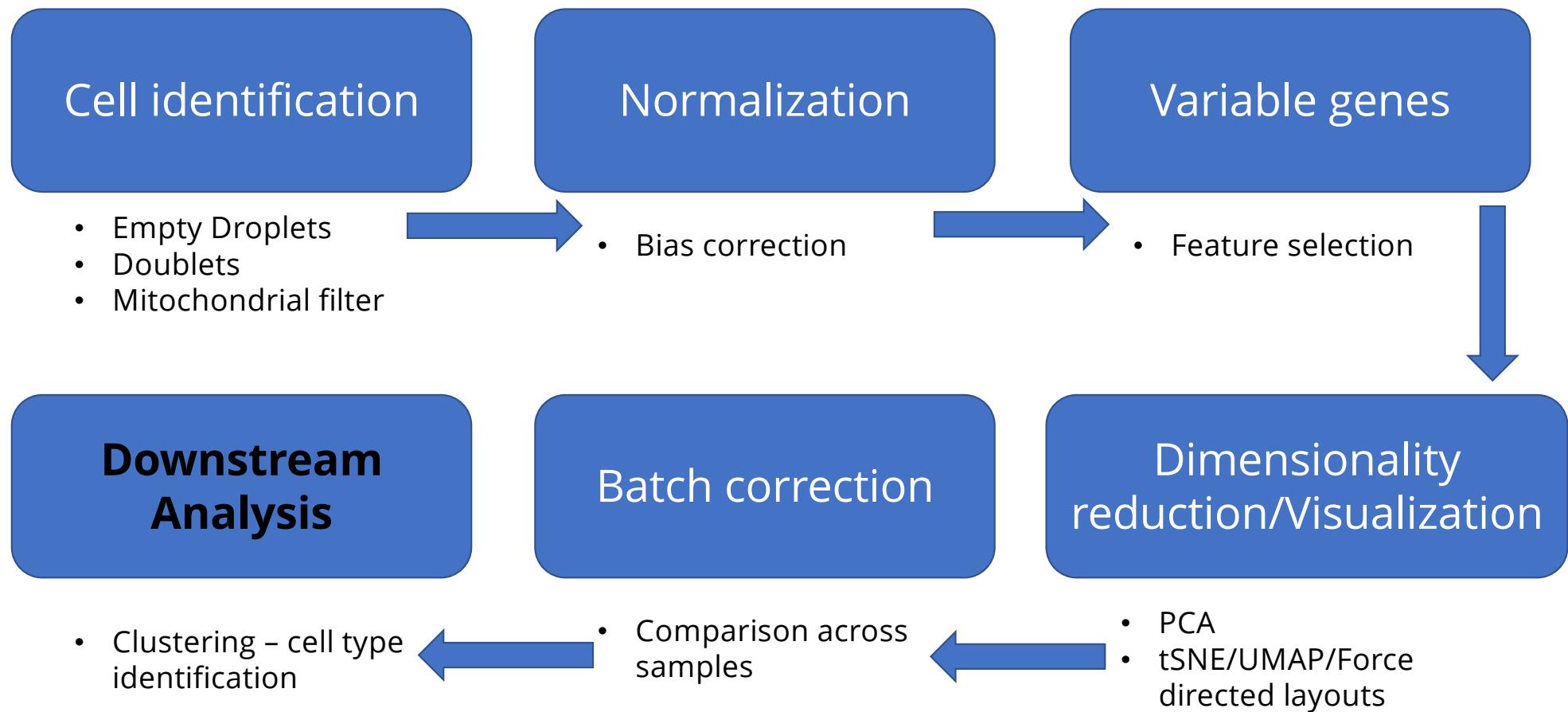
# scRNA-seq analysis steps



## Other corrections

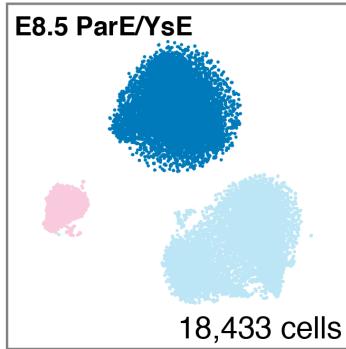
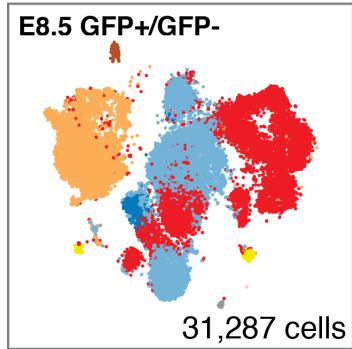
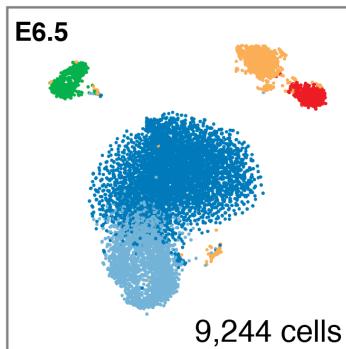
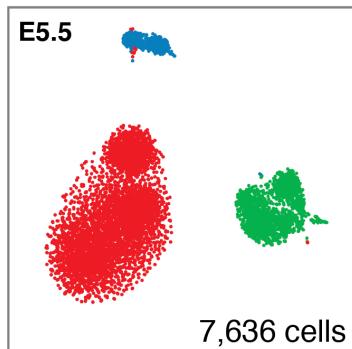
- Cell cycle correction
- Gene expression imputation

# scRNA-seq analysis steps

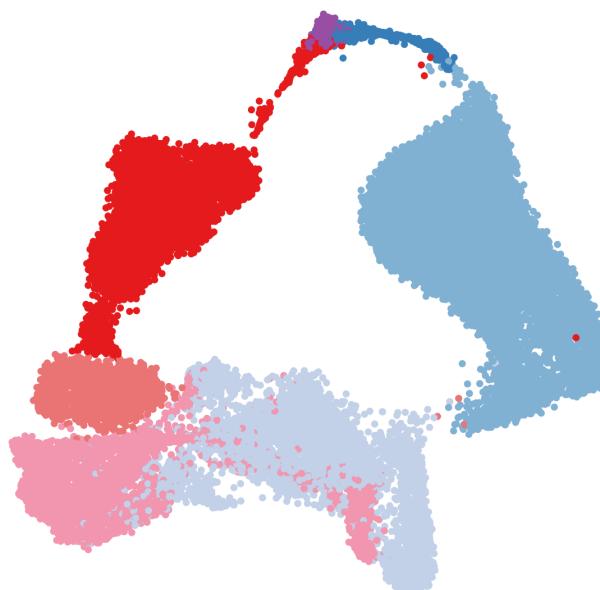


# Single-cell data

## Discrete clusters



## Continuous trajectories



ICM  
PrE  
VE  
Gut tube: VE  
EPI  
DE  
Gut tube: DE

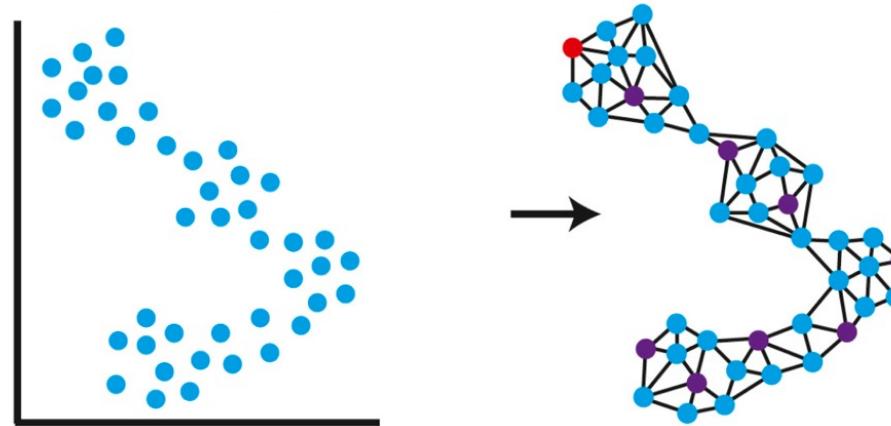
# Nearest neighbor graphs

- For each point, find  $k$  nearest points using Euclidean distance

$Graph G = (V, E)$

$V$ : Set of vertices

$E$ : Set of edges



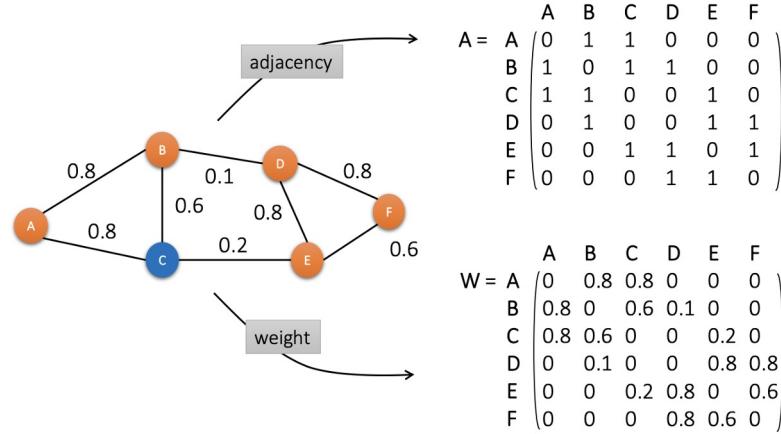
# Graph adjacency matrices

- Graph can also be represented as an adjacency matrix o

*Graph*  $G = (V, E)$

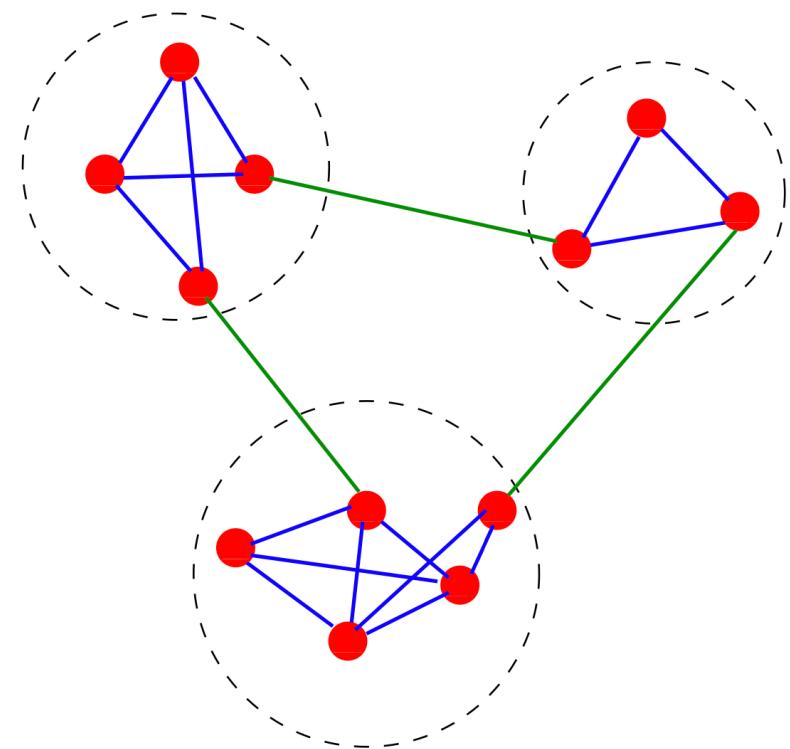
*Adjacency matrix*  $A \in \mathbb{R}^{|V| \times |V|}$

$A_{ij} = E(i, j)$



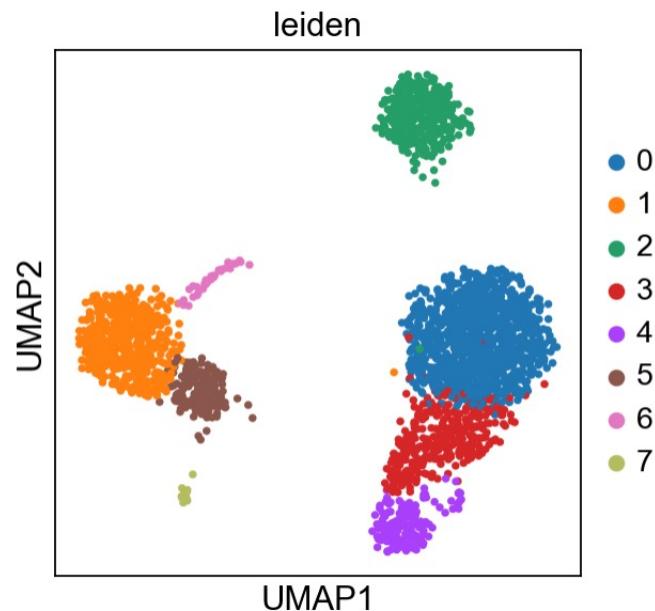
# Graph based clustering

- Community detection
- Identify clusters of nodes or “communities” with high density of edges within and low density of edges across communities



Fortunato 2009

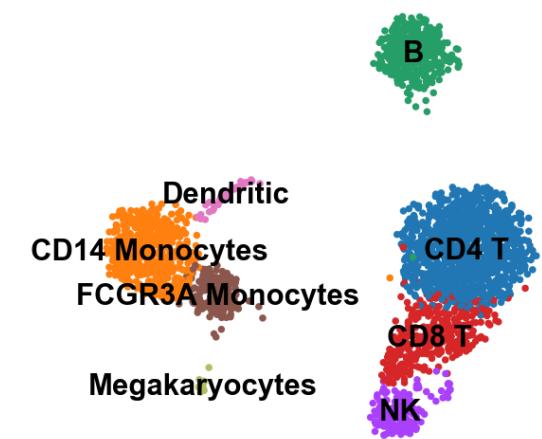
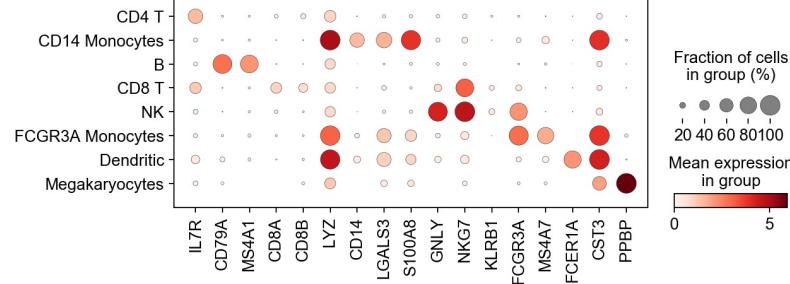
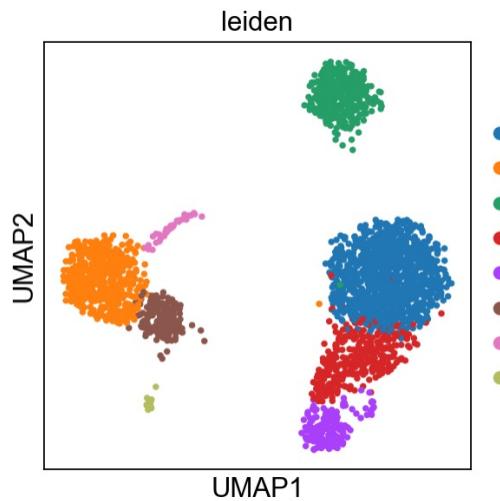
# Leiden clustering in single-cell data



Butler et. al. 2018

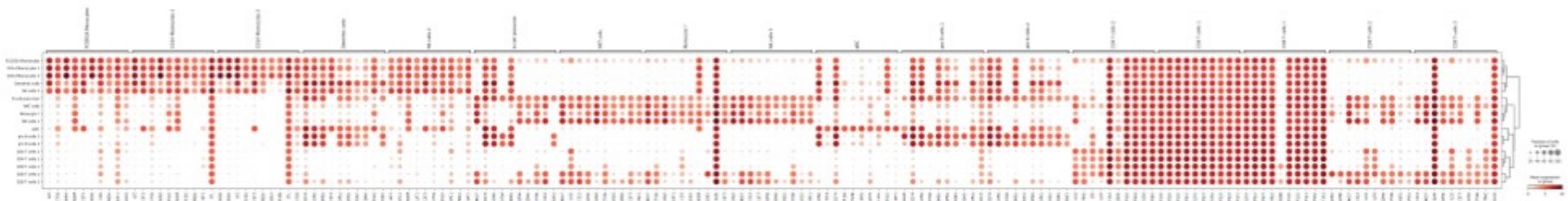
# Cell type identification

- Marker based identification



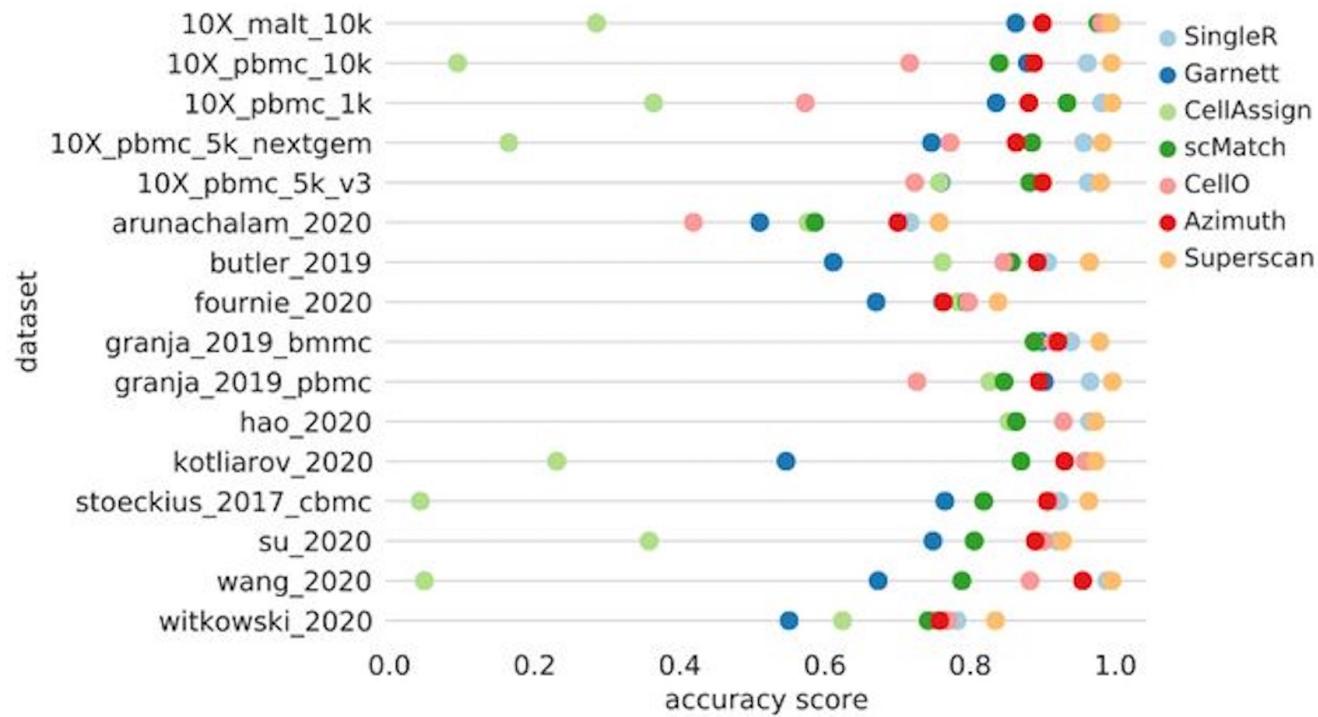
# Cell type identification

- Differential expression



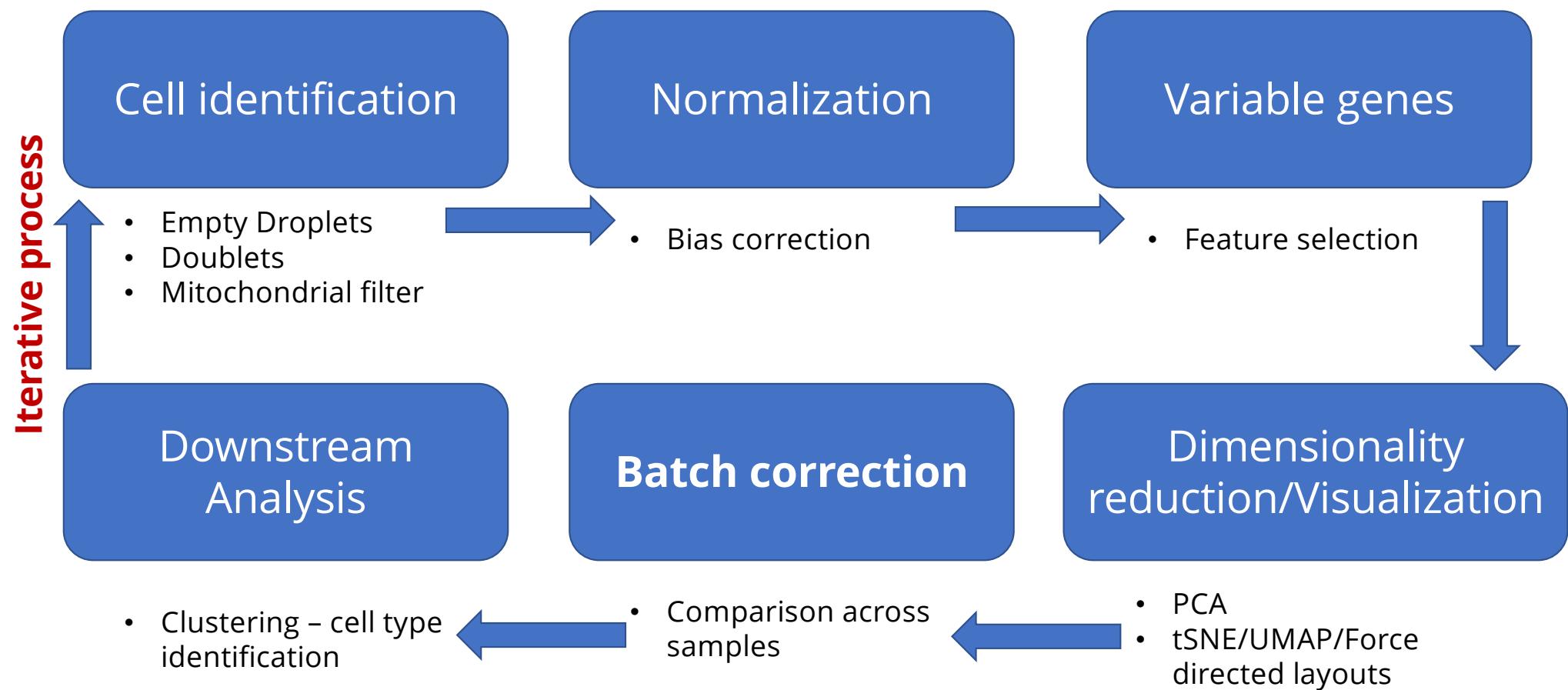
# Cell type identification

- Supervised approaches: Train on manually labeled cells
- Superscan



Shasha et. al. 2021

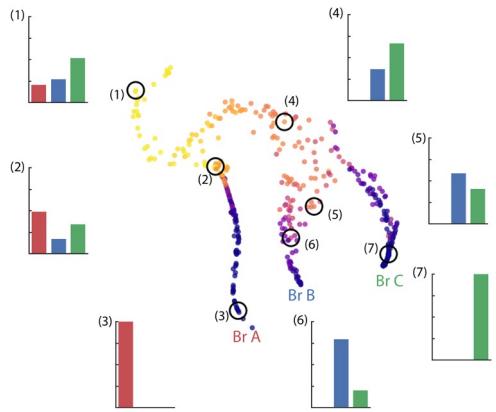
# scRNA-seq analysis steps



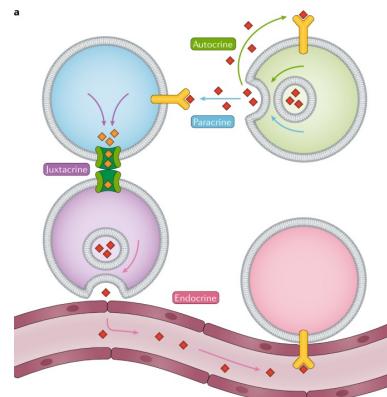
# Interpreting single-cell data

# Interpreting single-cell data

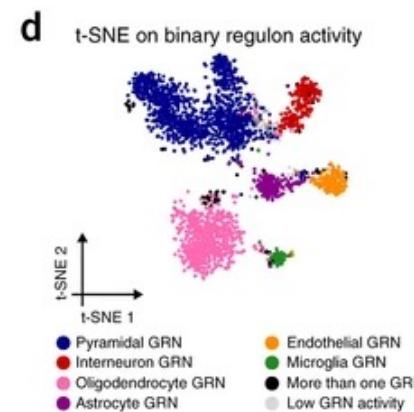
## Trajectory analysis



## Cell communication



## Regulatory Networks

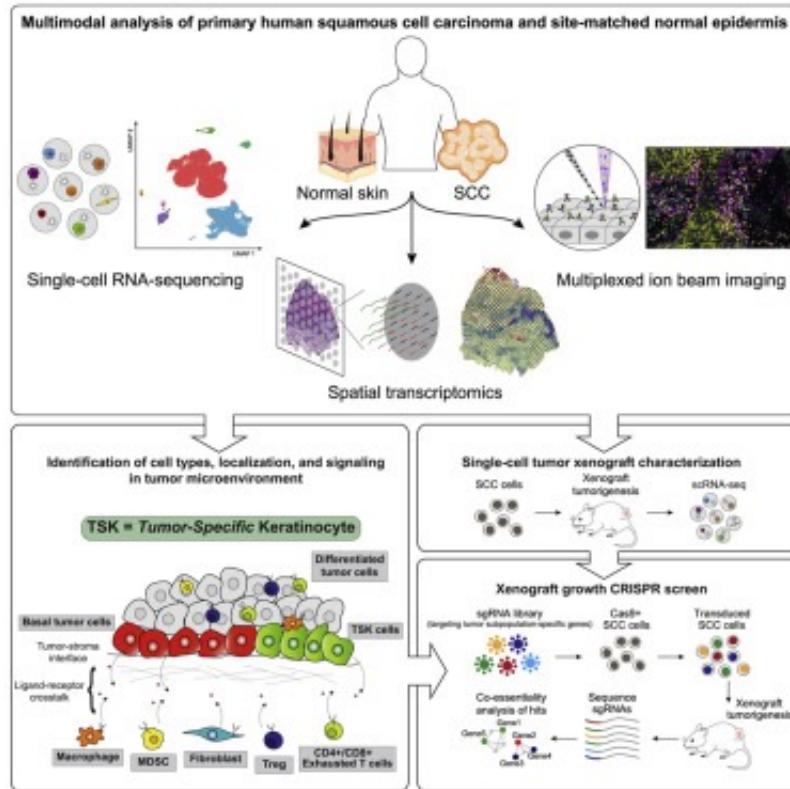


# Where to find data?

## Major data resources – Healthy/Normal

- Human Cell Atlas
  - Fetal Cell Atlas
- Tabular Muris

# Major Data resources - GEO



Supplementary file	Size	Download	File type/resource
GSE144236_CAL27_counts.txt.gz	20.9 Mb	(ftp)(http)	TXT
GSE144236_CAL27_vitro_counts.txt.gz	20.0 Mb	(ftp)(http)	TXT
GSE144236_SCC13_counts.txt.gz	11.2 Mb	(ftp)(http)	TXT
GSE144236_XG_TME_counts.txt.gz	3.4 Mb	(ftp)(http)	TXT
GSE144236_cSCC_counts.txt.gz	127.2 Mb	(ftp)(http)	TXT
GSE144236_patient_metadata_new.txt.gz	648.8 Kb	(ftp)(http)	TXT

SRA Run Selector [?](#)

Processed data are available on Series record

Raw data are available in SRA

Count matrices (post QC) and metadata are typically made available

# Interactive browsers

## Mouse endoderm atlas



[endoderm-explorer.com](http://endoderm-explorer.com)

A large number of studies set up webapps for interacting with the data