# Single-cell analysis workshop

Sydney Precision Bioinformatics Group

Workshop presenters:
Hani Kim
Yingxin Lin
Shila Ghazanfar

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# Sydney Precision Bioinformatics Research Group

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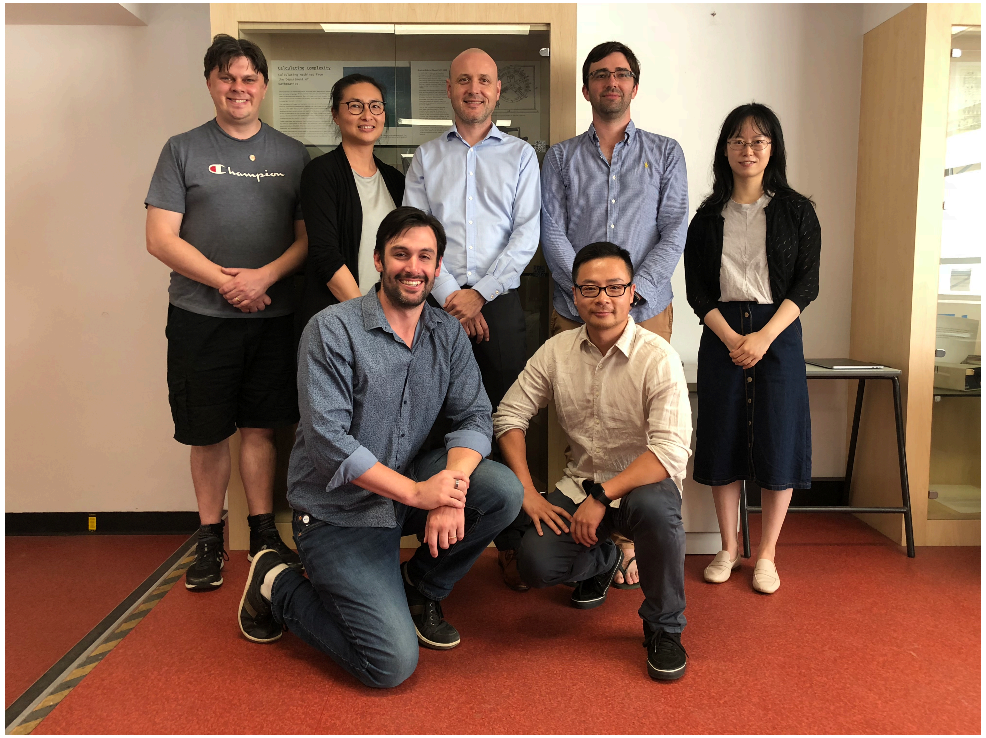
We share an interest in developing statistical and computational methodologies to tackle the foremost significant challenges posed by modern biology and medicine.

Meet our senior and junior research leaders:

A/Prof. John Ormerod; Prof. Jean Yang; Prof. Samuel Mueller; Dr. Garth Tarr; Dr. Rachel Wang



Dr. Ellis Patrick; Dr. Pengyi Yang

and senior research associates, PhD candidates, Honours and TSP students.

Find out more:

http://www.maths.usyd.edu.au/bioinformatics/

Shiny apps: http://shiny.maths.usyd.edu.au/

Github: https://github.com/SydneyBioX

# Roadmap for the workshop

- Setting up: 13:30 – 13:45 Google cloud set up
- Session 1: 13:45 – 14:15 Single cell analysis overview (scdney)
- Session 2: 14:15 – 15:00 Quality control and data integration
- AFTERNOON TEA: 1500-1530
- Session 3: 15:30 – 16:00 Overview of single-cell downstream analysis
- Session 4: 16:00 – 16:45 Downstream analysis: cell type identification, identify marker genes & cell type composition
- Extension: cell type identification via supervised classification and single cell trajectory analysis

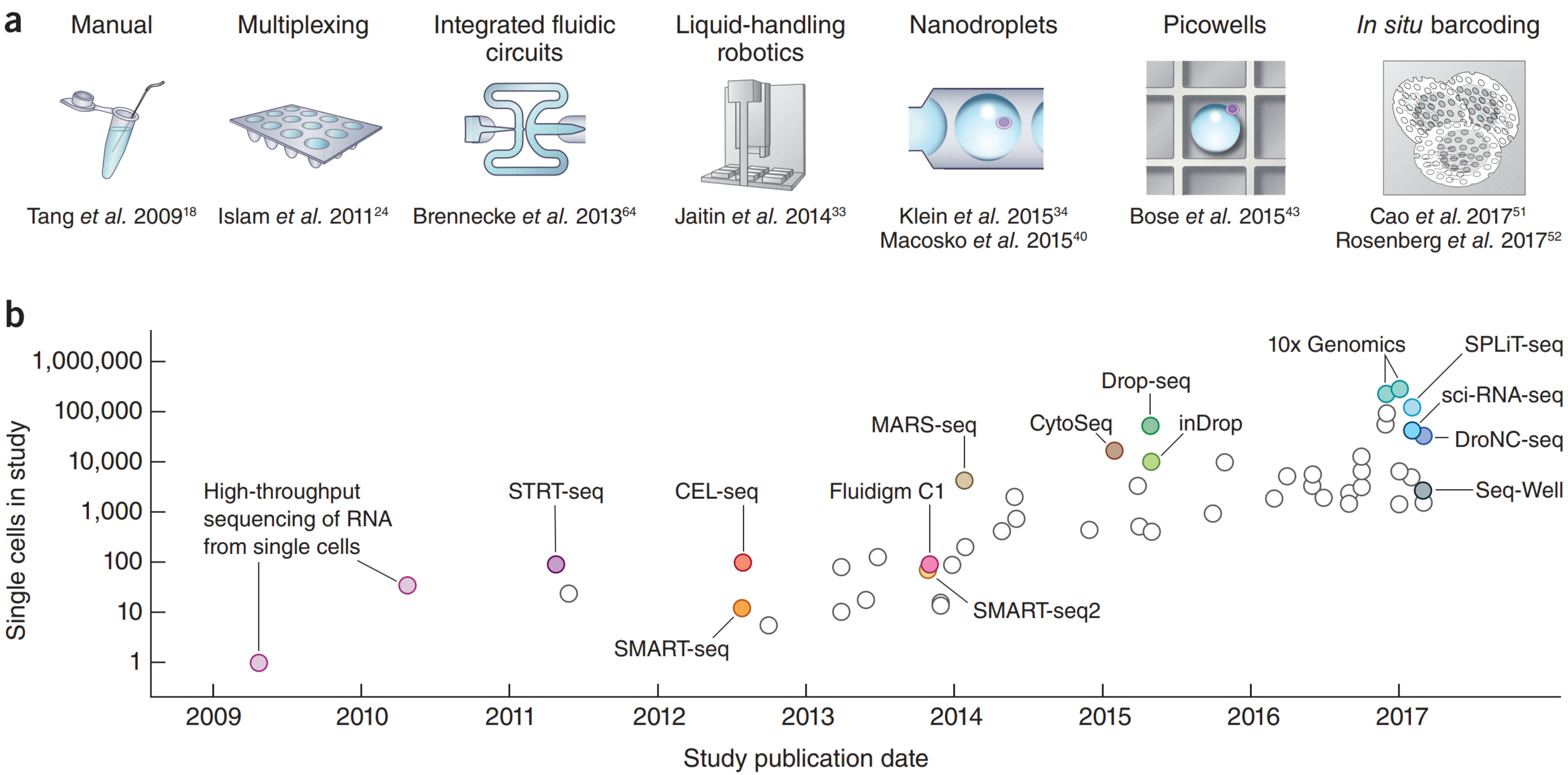
## Configuring Google Cloud and workshop materials

- Workshop materials: https://sydneybiox.github.io/BIS2019_SC/index.html

- Machine 1: 34.68.240.36
- Machine 2: 34.94.37.174

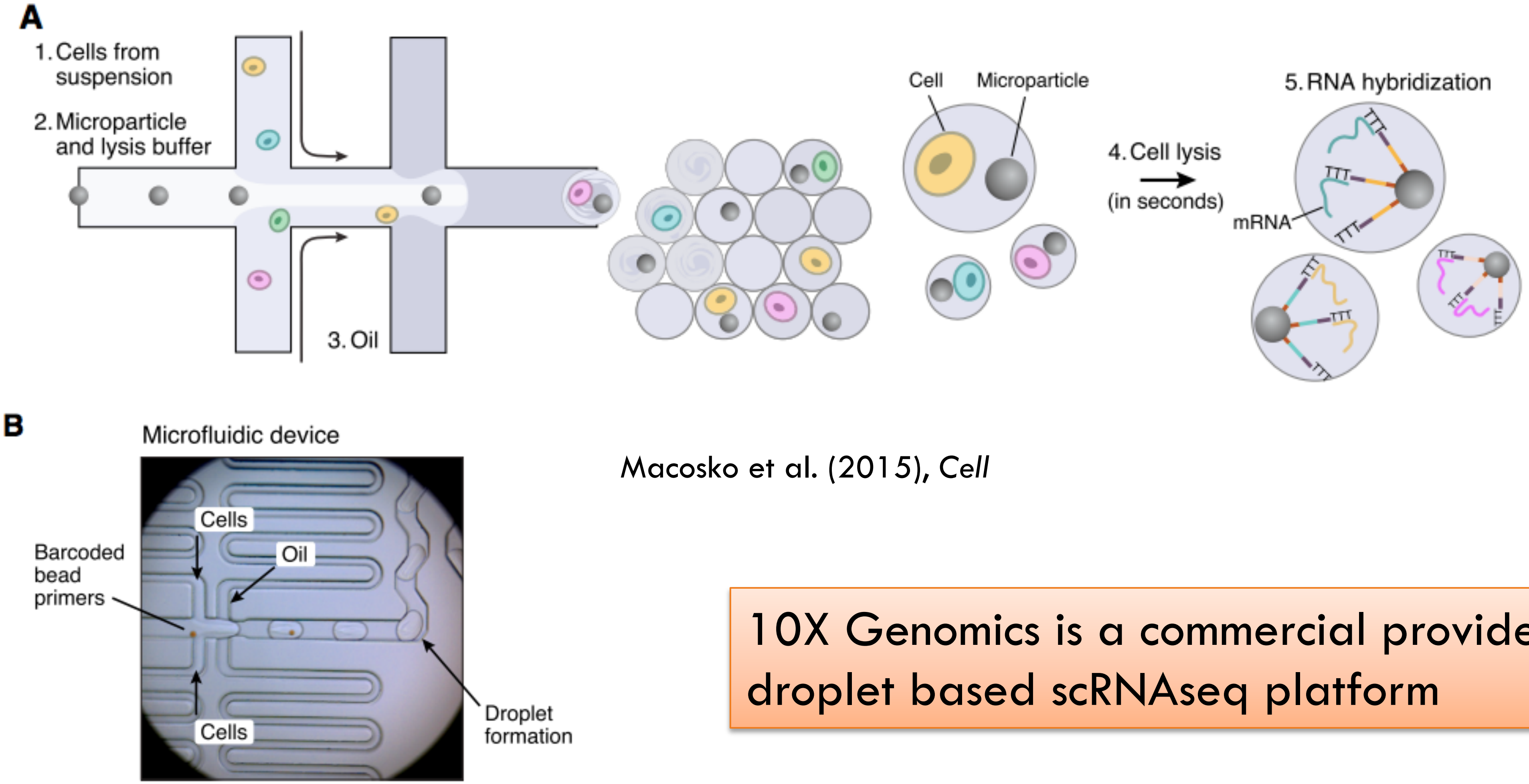
```
source("/home/user_setup.R")
```

# Exponential growth in single cell RNA seq technologies



Svensson et al. *Nature Protocols* (2018)

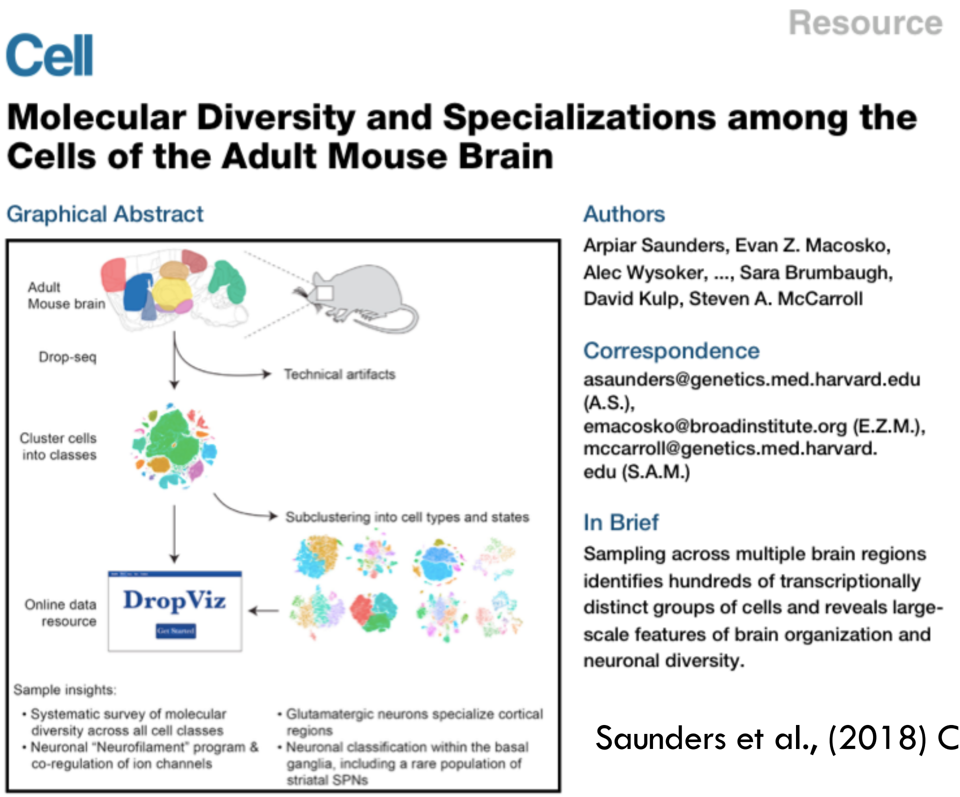
# Droplet based technologies are now dominating



Macosko et al. (2015), *Cell*

10X Genomics is a commercial provider of droplet based scRNAseq platform

# scRNAseq experiments approaching 1 million cells



Resource

Cell

## Molecular Diversity and Specializations among the Cells of the Adult Mouse Brain

### Graphical Abstract

Sample insights:

- Systematic survey of molecular diversity across all cell classes
- Neuronal "Neurofilament" program & co-regulation of ion channels
- Glutamatergic neurons specialize cortical regions
- Neuronal classification within the basal ganglia, including a rare population of striatal SPNs

### Authors

Arpiar Saunders, Evan Z. Macosko, Alec Wysoker, ..., Sara Brumbaugh, David Kulp, Steven A. McCarroll

### Correspondence

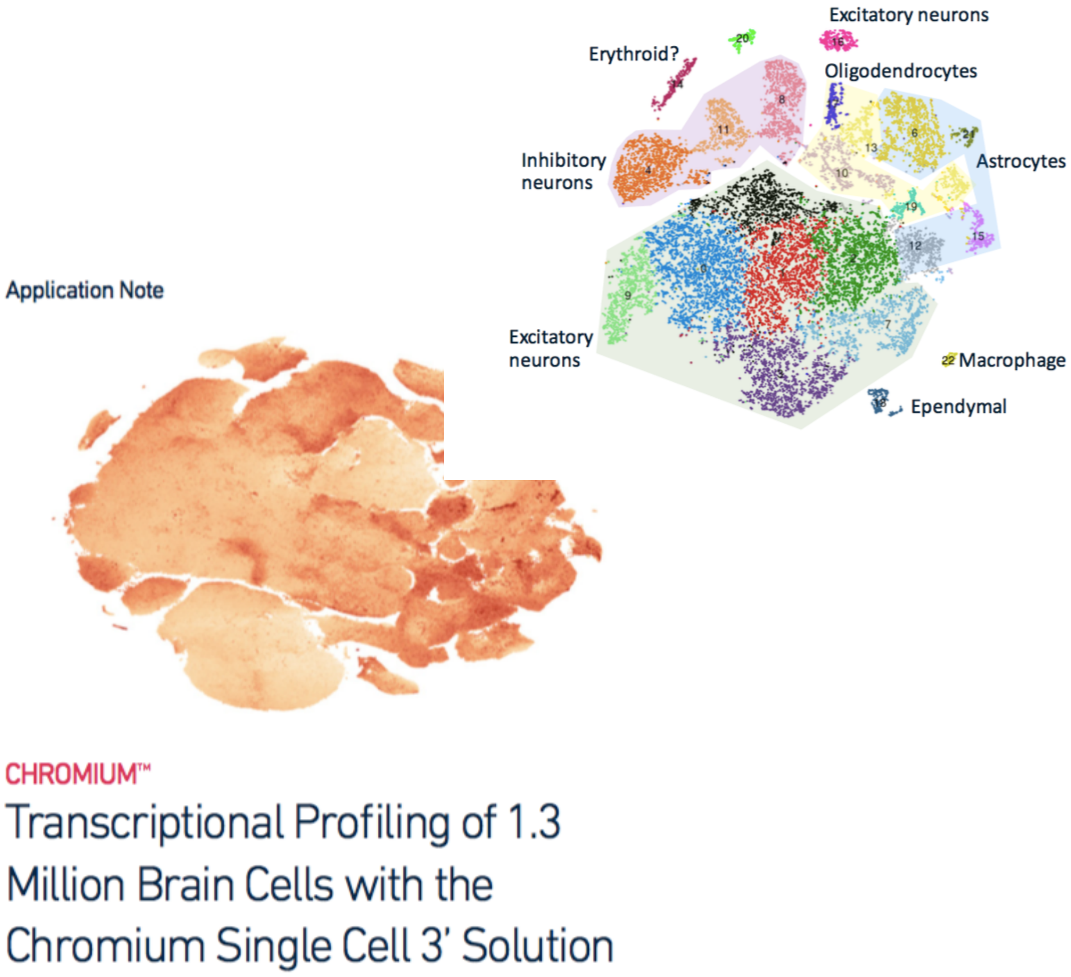
asaunders@genetics.med.harvard.edu (A.S.),
emacosko@broadinstitute.org (E.Z.M.),
mccarroll@genetics.med.harvard.edu (S.A.M.)

### In Brief

Sampling across multiple brain regions identifies hundreds of transcriptionally distinct groups of cells and reveals large-scale features of brain organization and neuronal diversity.

Saunders et al., (2018) Cell

**690,000 individual cells** from 9 regions of adult mouse brain

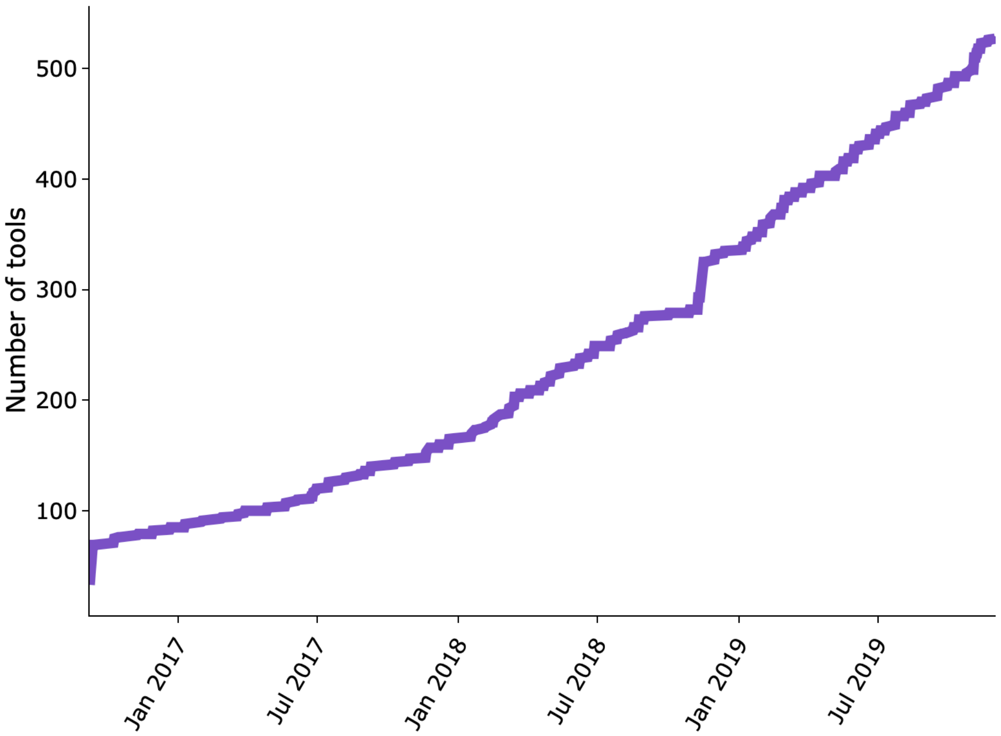


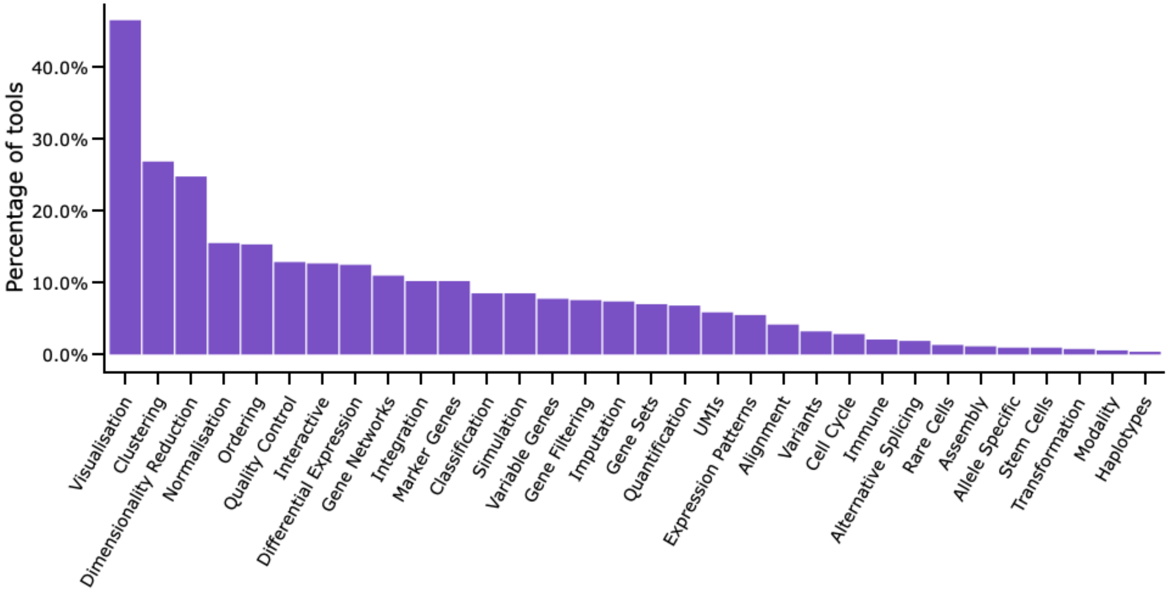
Application Note

CHROMIUM™

Transcriptional Profiling of 1.3 Million Brain Cells with the Chromium Single Cell 3' Solution

# Number of scRNAseq tools also increasing rapidly

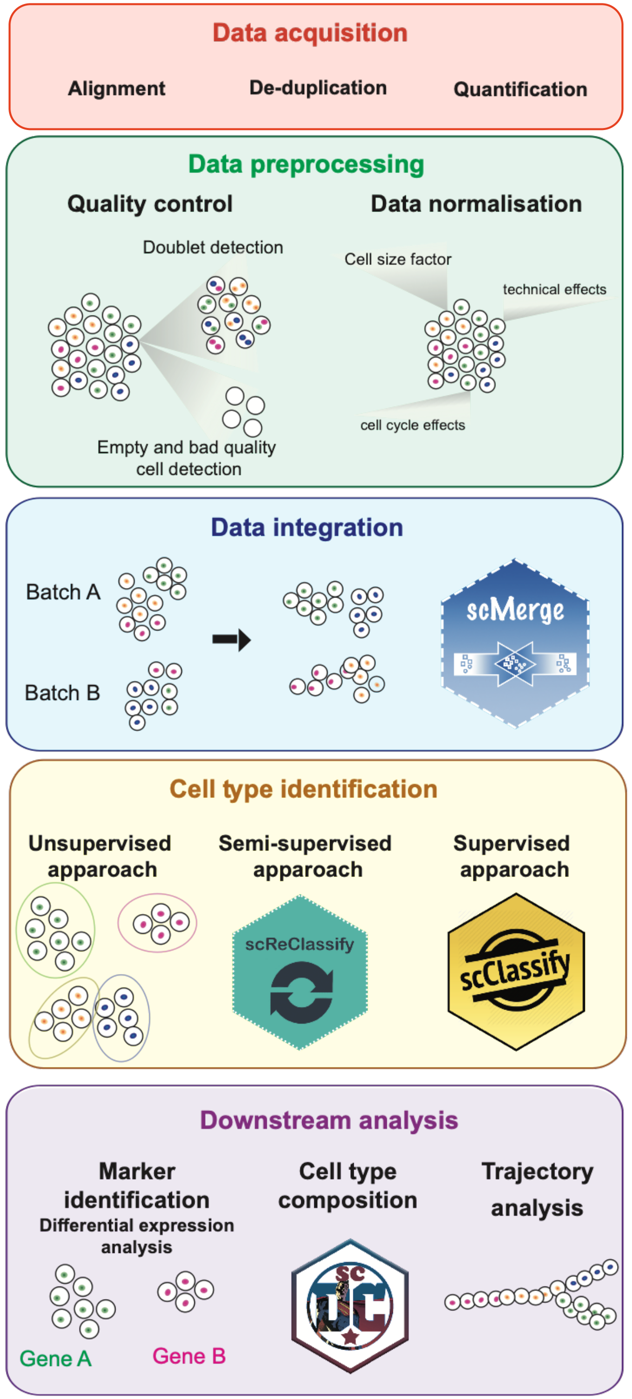




Downloaded from www.scrna-tools.org

# Single-cell RNA-seq analysis

# Components of a typical scRNA-seq analysis process



Data acquisition

Alignment De-duplication Quantification

Data preprocessing

Quality control

Doublet detection

Empty and bad quality cell detection

Data normalisation

Cell size factor

technical effects

cell cycle effects

Data integration

Batch A

Batch B

Cell type identification

Unsupervised apparoach

Semi-supervised apparoach

Supervised apparoach

Downstream analysis

Marker identification

Differential expression analysis

Cell type composition

Trajectory analysis

# Component 1: Data acquisition

Data acquisition

Alignment

De-duplication

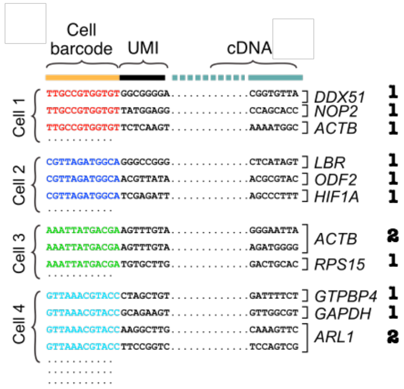
Quantification

## Input

- BCL or fastq file from the sequencer

## Output

- Gene/cell counts matrix


Cell barcode
UMI
cDNA
Cell 1
TTGCCGTGGTGTGGCGGGGA............CGGTGTTA DDX51 1
TTGCCGTGGTGTTATGGAGG............CCAGCACC NOP2 1
TTGCCGTGGTGTTCTCAAGT............AAAATGGC ACTB 1
Cell 2
CGTTAGATGGCAGGGCCGGG............CTCATAGT LBR 1
CGTTAGATGGCAACGTTATA............ACGCGTAC ODF2 1
CGTTAGATGGCATCGAGATT............AGCCCTTT HIF1A 1
Cell 3
AAATTATGACGAAGTTTGTA............GGGAATTA
AAATTATGACGAAGTTTGTA............AGATGGGG ACTB 2
AAATTATGACGATGTGCTTG............GACTGCAC RPS15 1
Cell 4
GTTAAACGTACCCTAGCTGT............GATTTTCT GTPBP4 1
GTTAAACGTACCGCAGAAGT............GTTGGCGT GAPDH 1
GTTAAACGTACCAAGGCTTG............CAAAGTTC
GTTAAACGTACCTTCCGGTC............TCCAGTCG ARL1 2

(Thousands of cells)

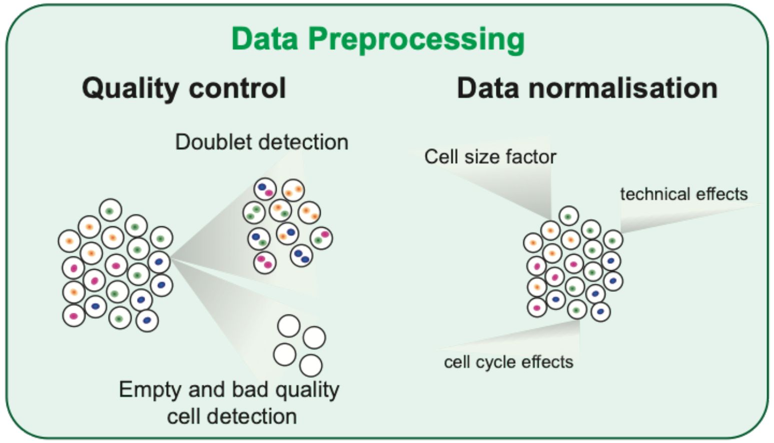
## Software

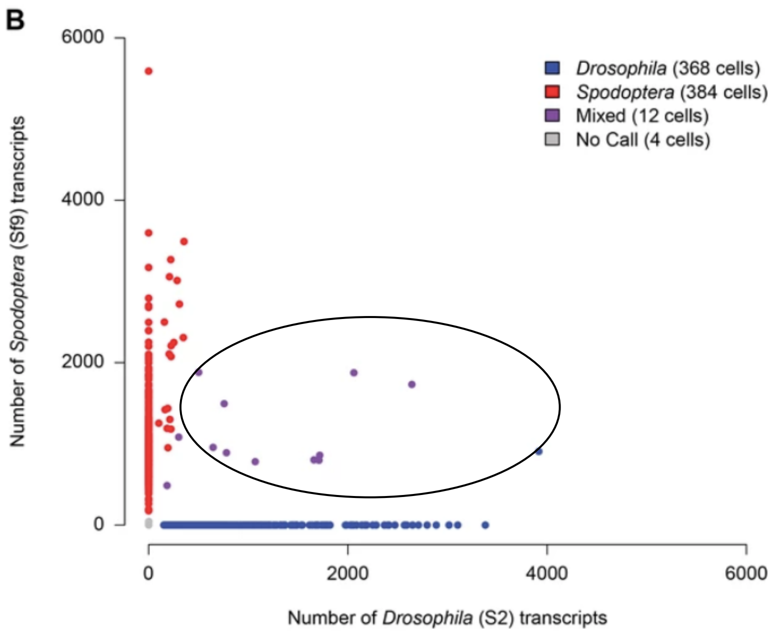
- CellRanger for 10X Genomics data
- Macosko’s custom scripts for DropSeq data
- STAR for alignment plus custom scripts (or there is STAR-solo)

## Considerations

- Single or mix of species? Does it include ERCC spike-ins? May need to build a custom reference
- Barcode and/or UMI sequencing errors – CellRanger takes care of this automatically
- Align to exon or exon and intron?

# Component 2: Data preprocessing – Quality control





Croset (2018), eLife

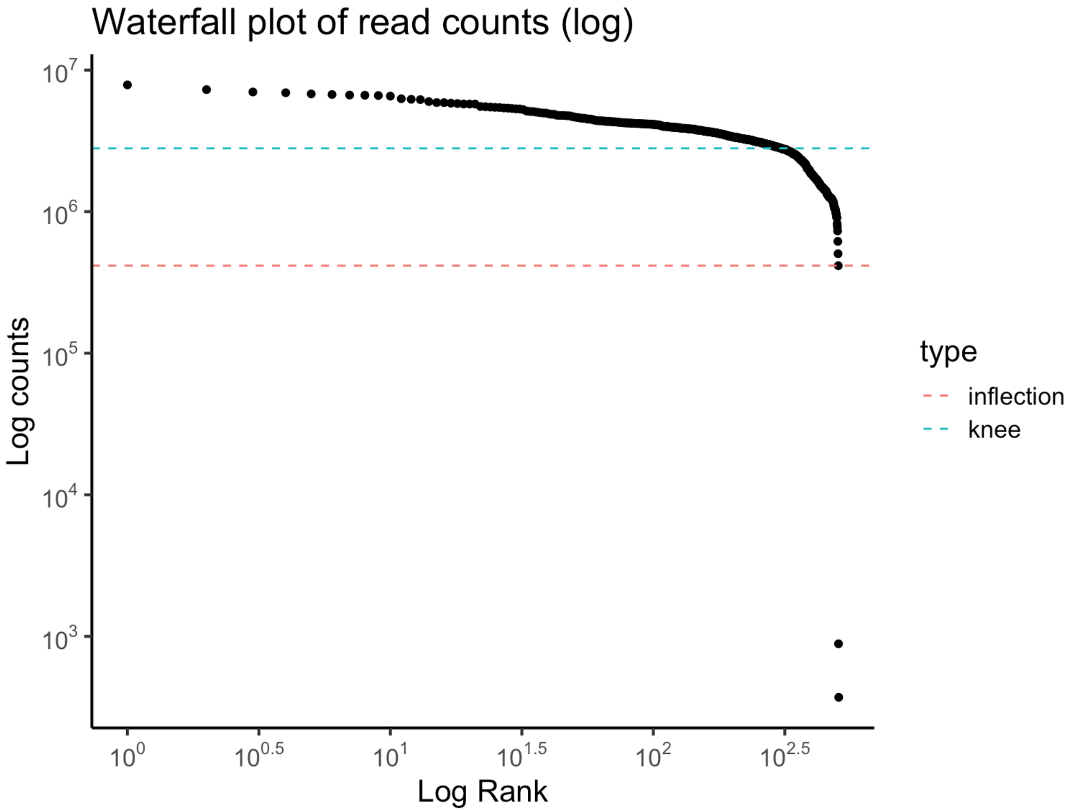
## Software

- Seurat (all-purpose single cell R package)
- Scater
- DropletUtils (R package with a number of handy utility functions)
- Your own custom scripts

## Considerations

- Filter out droplets with doublets – may be difficult to find. Can estimate expected rate by doing species mixture experiment

# Component 2: Data preprocessing – Quality control



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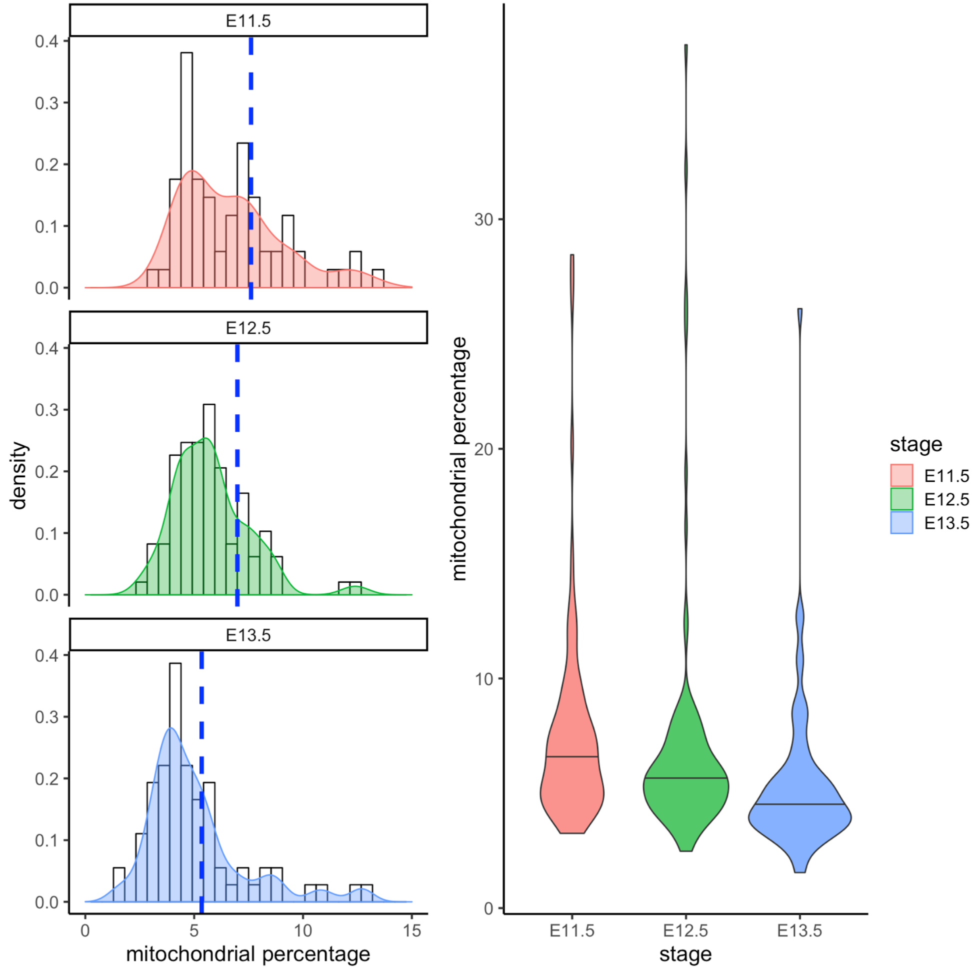
- Filter out droplets with no cells

# Component 2: Data preprocessing – Quality control



## Software

- Seurat (all-purpose single cell R package)
- Scater
- DropletUtils (R package with a number of handy utility functions)
- Your own custom scripts

## Considerations

- Filter out droplets with doublets – may be difficult to find. Can estimate expected rate by doing species mixture experiment
- Filter out droplets with no cells
- Filter out droplets with damaged cells – look for high mitochondrial gene content or high spike-in

# Component 2: Data normalisation

## Software

- scran for non-full-length datasets (Lun et al. Genome Biology 2016)
- bulk methods for full-length datasets (TPM normalisation)

## Normalisation aims to address

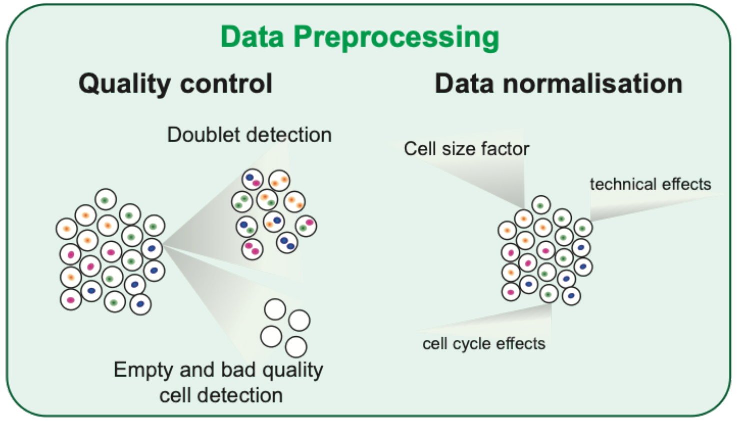
- Removing sampling effects
- Scaling count data to obtain correct relative gene expression abundances between cells

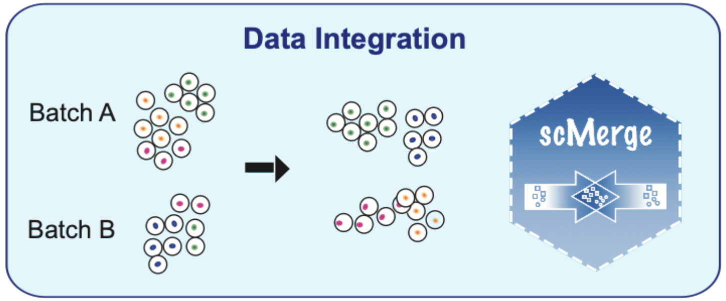
## After normalisation, data matrices are typically log(x+1)-transformed

- Distances represent log-fold changes
- log transformation mitigates (but does not remove) the mean–variance relationship in single-cell data
- reduces the skewness of the data

Luecken et al. Molecular Systems Biology 2019

# Component 3: Data integration

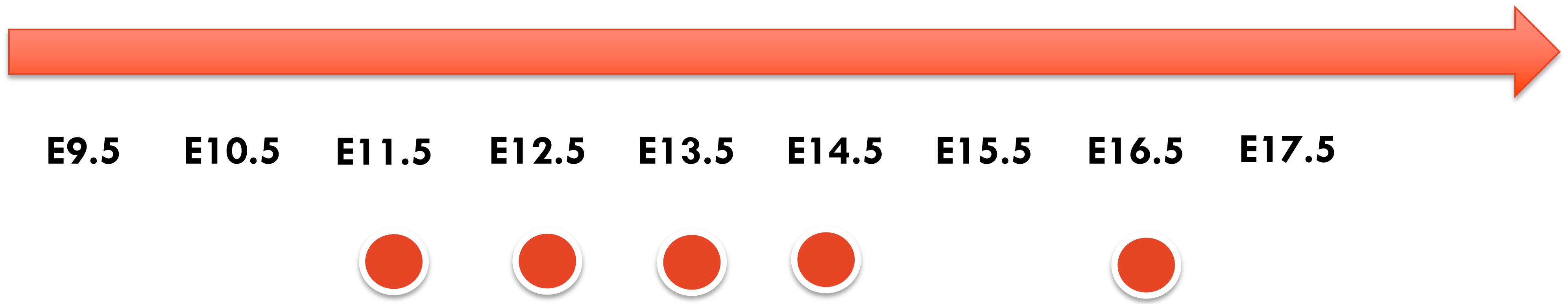




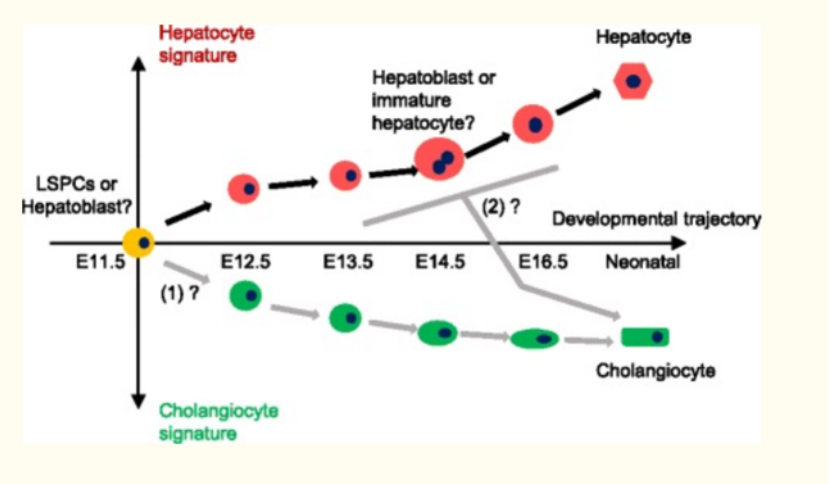
## Software

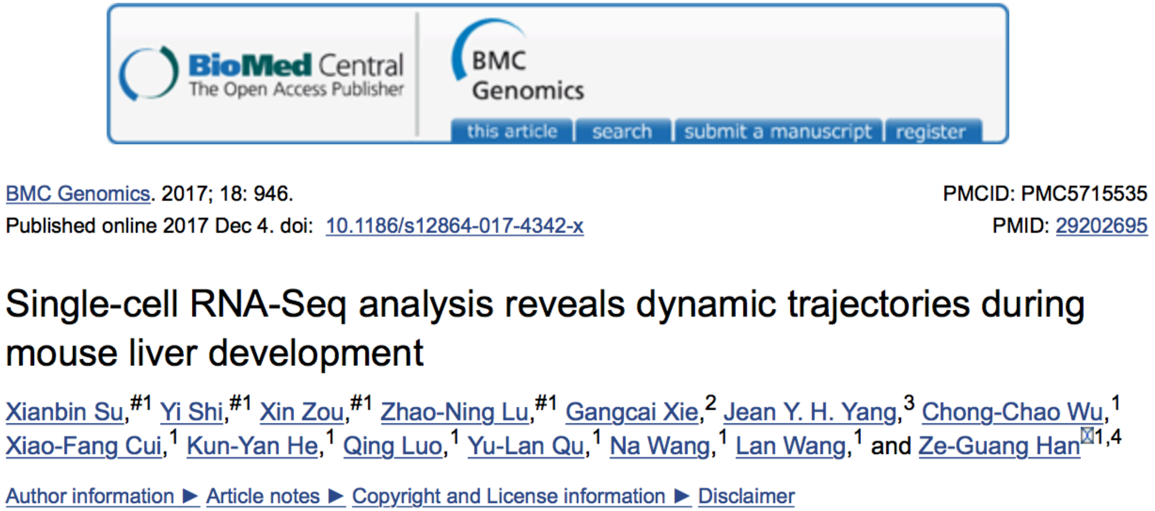
- Seurat (all-purpose single cell R package) for very basic normalization
- Batch effect correction
  - mnnCorrect
  - Harmony
  - Liger
  - **scMerge**

# scMerge motivation - Liver fetal development time course dataset



GSE87795
Su et al.





BMC Genomics. 2017; 18: 946.
Published online 2017 Dec 4. doi: 10.1186/s12864-017-4342-x

PMCID: PMC5715535
PMID: 29202695

## Single-cell RNA-Seq analysis reveals dynamic trajectories during mouse liver development

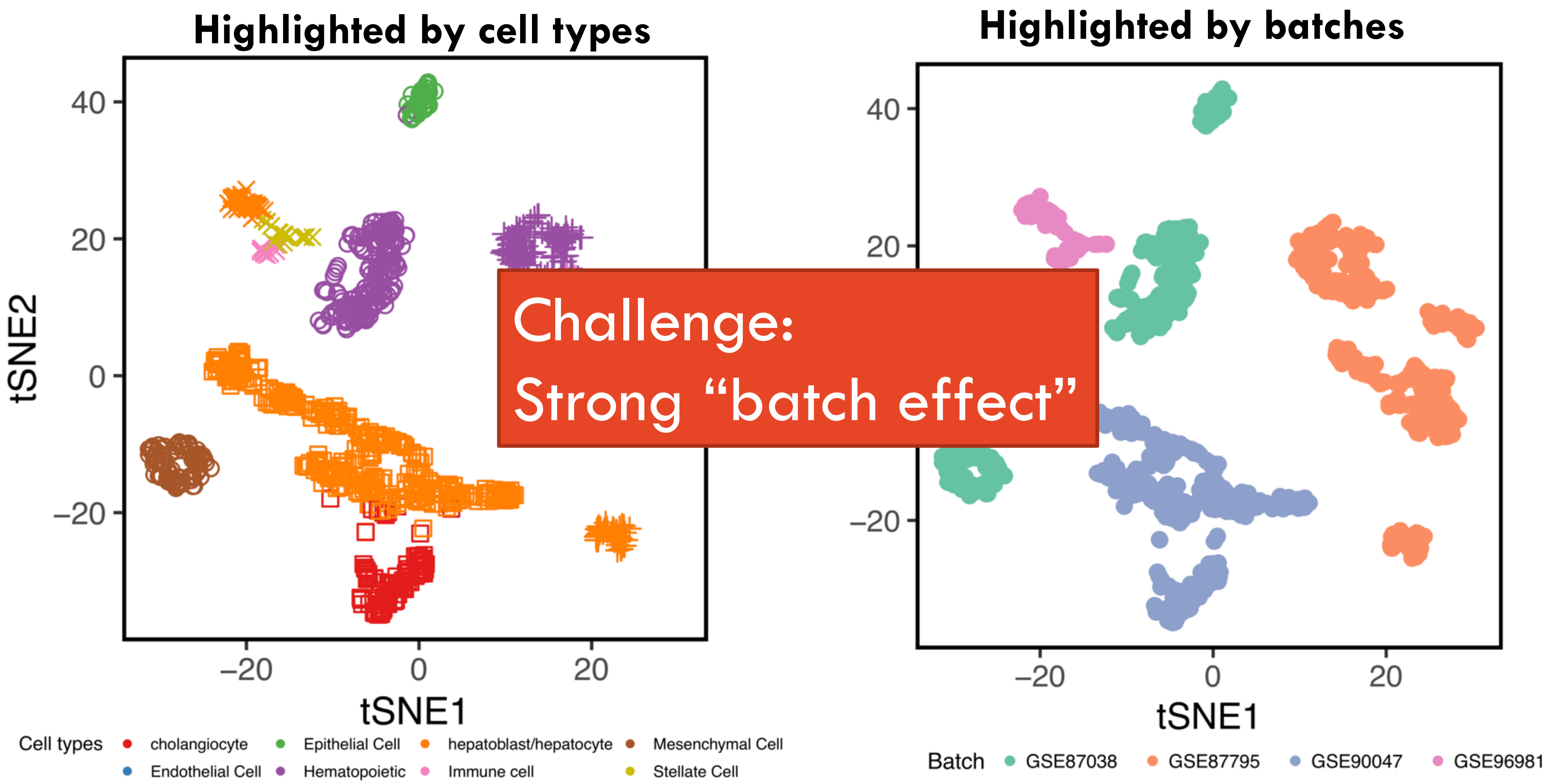
Xianbin Su,[#1] Yi Shi,[#1] Xin Zou,[#1] Zhao-Ning Lu,[#1] Gangcai Xie,[2] Jean Y. H. Yang,[3] Chong-Chao Wu,[1] Xiao-Fang Cui,[1] Kun-Yan He,[1] Qing Luo,[1] Yu-Lan Qu,[1] Na Wang,[1] Lan Wang,[1] and Ze-Guang Han[1,4]

Author information ▶ Article notes ▶ Copyright and License information ▶ Disclaimer

# Liver fetal development time course datasets

|  | E9.5 | E10.5 | E11.5 | E12.5 | E13.5 | E14.5 | E15.5 | E16.5 | E17.5 |  |
|---|---|---|---|---|---|---|---|---|---|---|
| GSE87795<br>Su et al. |  |  | ● | ● | ● | ● |  | ● |  | N = 389 cells |
| GSE90047 Yang et al. |  | ● | ● | ● | ● | ● | ● |  | ● | N = 448 cells |
| GSE87038 Dong et al. | ● | ● | ● |  |  |  |  |  |  | N = 320 cells |
| GSE96981 Camp et al. |  |  |  |  |  | ● | ● | ● |  | N = 79 cells |

# tSNE of liver fetal development time course datasets



Challenge:
Strong “batch effect”

# Breaking observed data into components

For *n* cells with data collected for *m* genes

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{W\alpha} + \boldsymbol{\epsilon}$$

The data we observe

Biologically relevant variation
cell types
p *wanted* variables

Unwanted variation
batch and technical effects
k *unwanted* variables

Random noise

# scMerge algorithm

Estimated by **stably expressed genes** by factor analysis

$$Y = X\beta + W\alpha + \epsilon$$

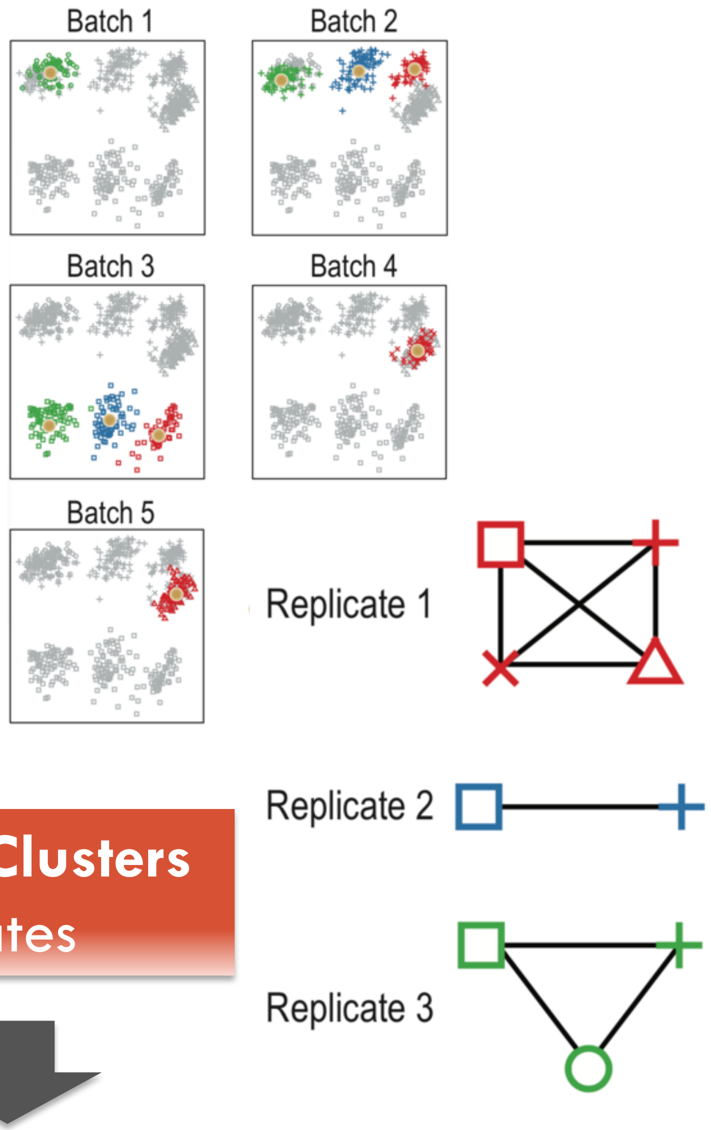
Estimated with **replicates** by factor analysis

RUVIII algorithm Molania et al. (2019), Nuclei Acids Res

# scMerge algorithm

**Clustering** for each batch

(k-means by default)



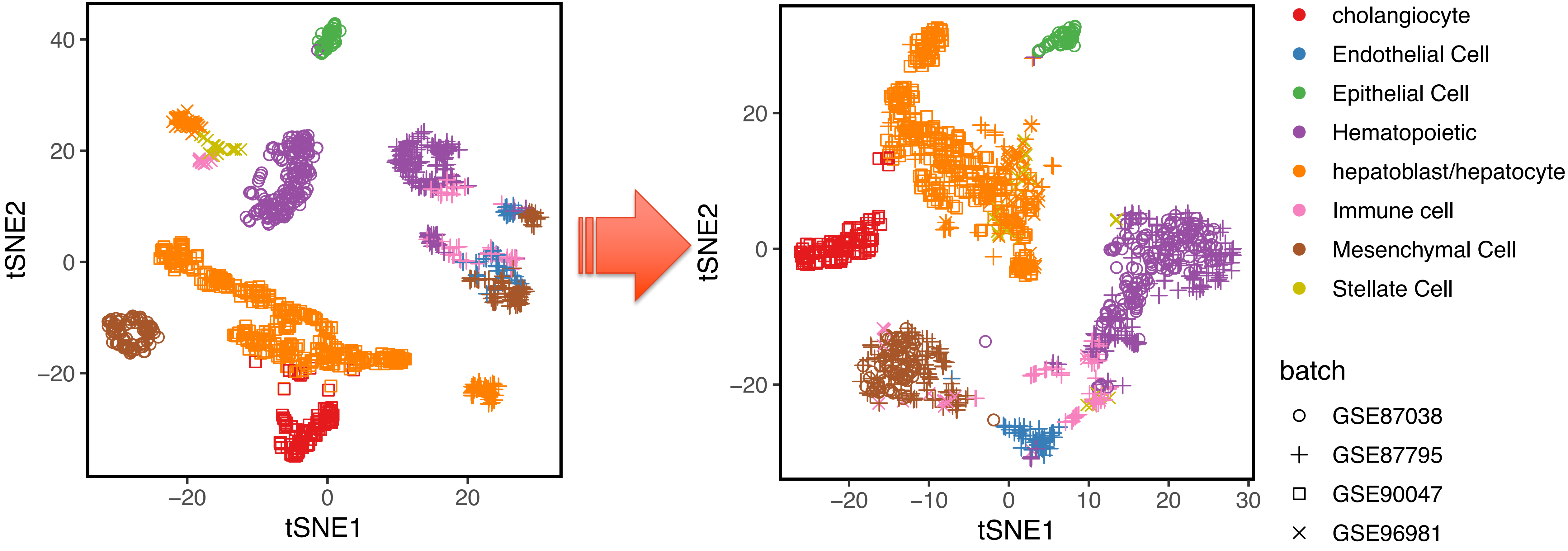
Pseudo-replicates

Find **Mutual Nearest Clusters** as pseudo-replicates

**Frame as pseudo-replicate information**

$$\begin{array}{r|ccc|}
\text{Cell 1} & 1 & 0 & 0 \\
\text{Cell 2} & 1 & 0 & 0 \\
\text{Cell 3} & 0 & 1 & 0 \\
\vdots & \vdots & \vdots & \vdots \\
\text{Cell C} & 0 & 0 & 1 \\
\hline
 & \text{Replicate 1} & \text{Replicate 2} & \text{Replicate 3}
\end{array}$$

# Coming back to our motivational data – Liver fetal development time course datasets



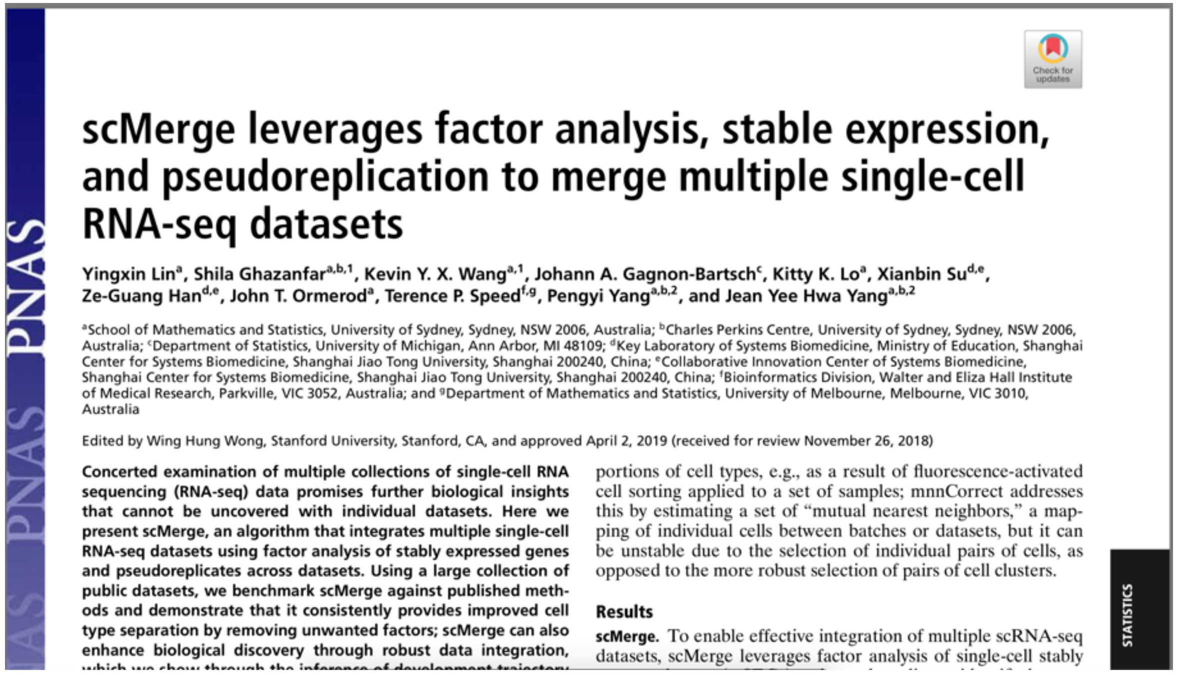
Before scMerge

After scMerge

# More information

## PNAS:

https://doi.org/10.1073/pnas.1820006116



# scMerge leverages factor analysis, stable expression, and pseudoreplication to merge multiple single-cell RNA-seq datasets

Yingxin Lin[a], Shila Ghazanfar[a,b,1], Kevin Y. X. Wang[a,1], Johann A. Gagnon-Bartsch[c], Kitty K. Lo[a], Xianbin Su[d,e], Ze-Guang Han[d,e], John T. Ormerod[a], Terence P. Speed[f,g], Pengyi Yang[a,b,2], and Jean Yee Hwa Yang[a,b,2]

[a]School of Mathematics and Statistics, University of Sydney, Sydney, NSW 2006, Australia; [b]Charles Perkins Centre, University of Sydney, Sydney, NSW 2006, Australia; [c]Department of Statistics, University of Michigan, Ann Arbor, MI 48109; [d]Key Laboratory of Systems Biomedicine, Ministry of Education, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China; [e]Collaborative Innovation Center of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China; [f]Bioinformatics Division, Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia; and [g]Department of Mathematics and Statistics, University of Melbourne, Melbourne, VIC 3010, Australia

Edited by Wing Hung Wong, Stanford University, Stanford, CA, and approved April 2, 2019 (received for review November 26, 2018)

**Concerted examination of multiple collections of single-cell RNA sequencing (RNA-seq) data promises further biological insights that cannot be uncovered with individual datasets. Here we present scMerge, an algorithm that integrates multiple single-cell RNA-seq datasets using factor analysis of stably expressed genes and pseudoreplicates across datasets. Using a large collection of public datasets, we benchmark scMerge against published methods and demonstrate that it consistently provides improved cell type separation by removing unwanted factors; scMerge can also enhance biological discovery through robust data integration, which we show through the inference of development trajectory**

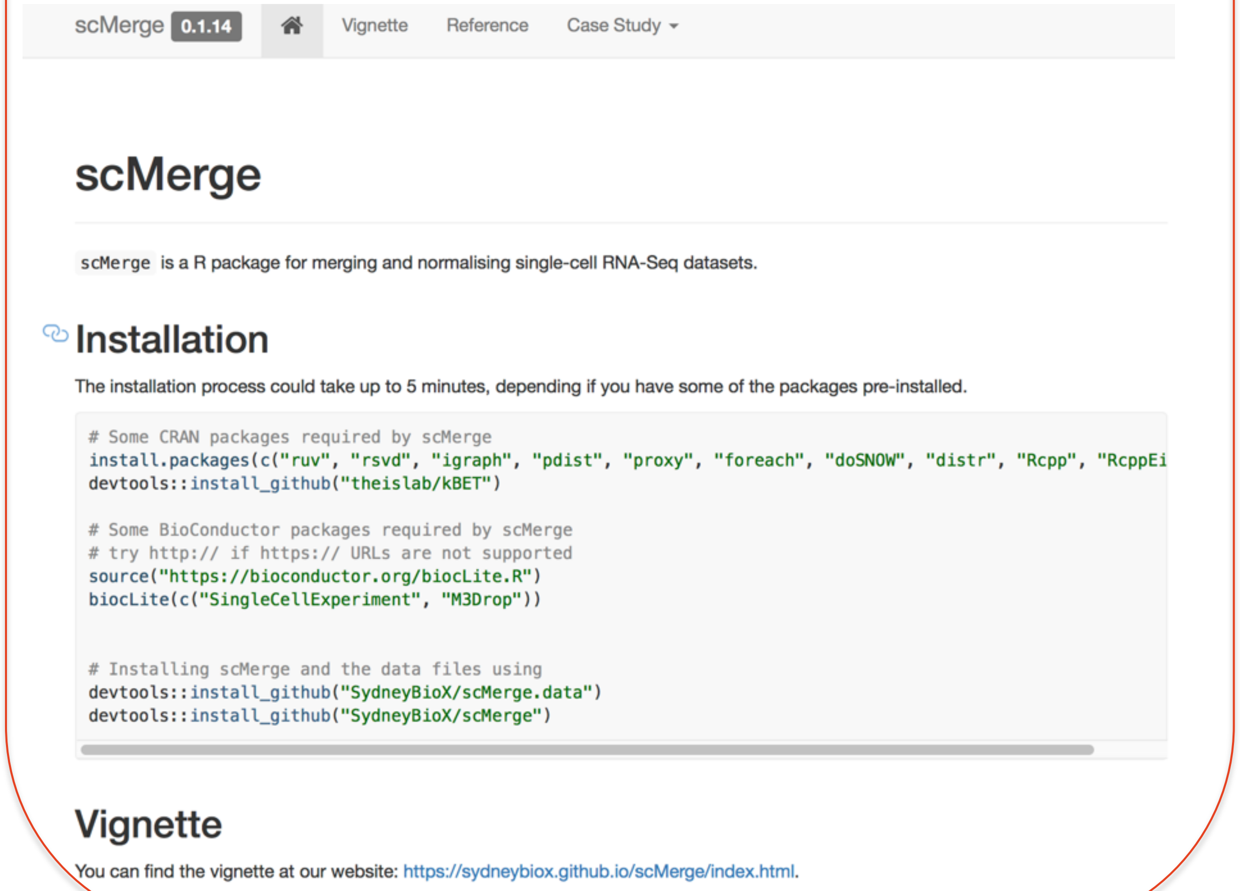
portions of cell types, e.g., as a result of fluorescence-activated cell sorting applied to a set of samples; mnnCorrect addresses this by estimating a set of "mutual nearest neighbors," a mapping of individual cells between batches or datasets, but it can be unstable due to the selection of individual pairs of cells, as opposed to the more robust selection of pairs of cell clusters.

### Results

**scMerge.** To enable effective integration of multiple scRNA-seq datasets, scMerge leverages factor analysis of single-cell stably

## scMerge R package and website:

https://sydneybiox.github.io/scMerge/



# scMerge

`scMerge` is a R package for merging and normalising single-cell RNA-Seq datasets.

## Installation

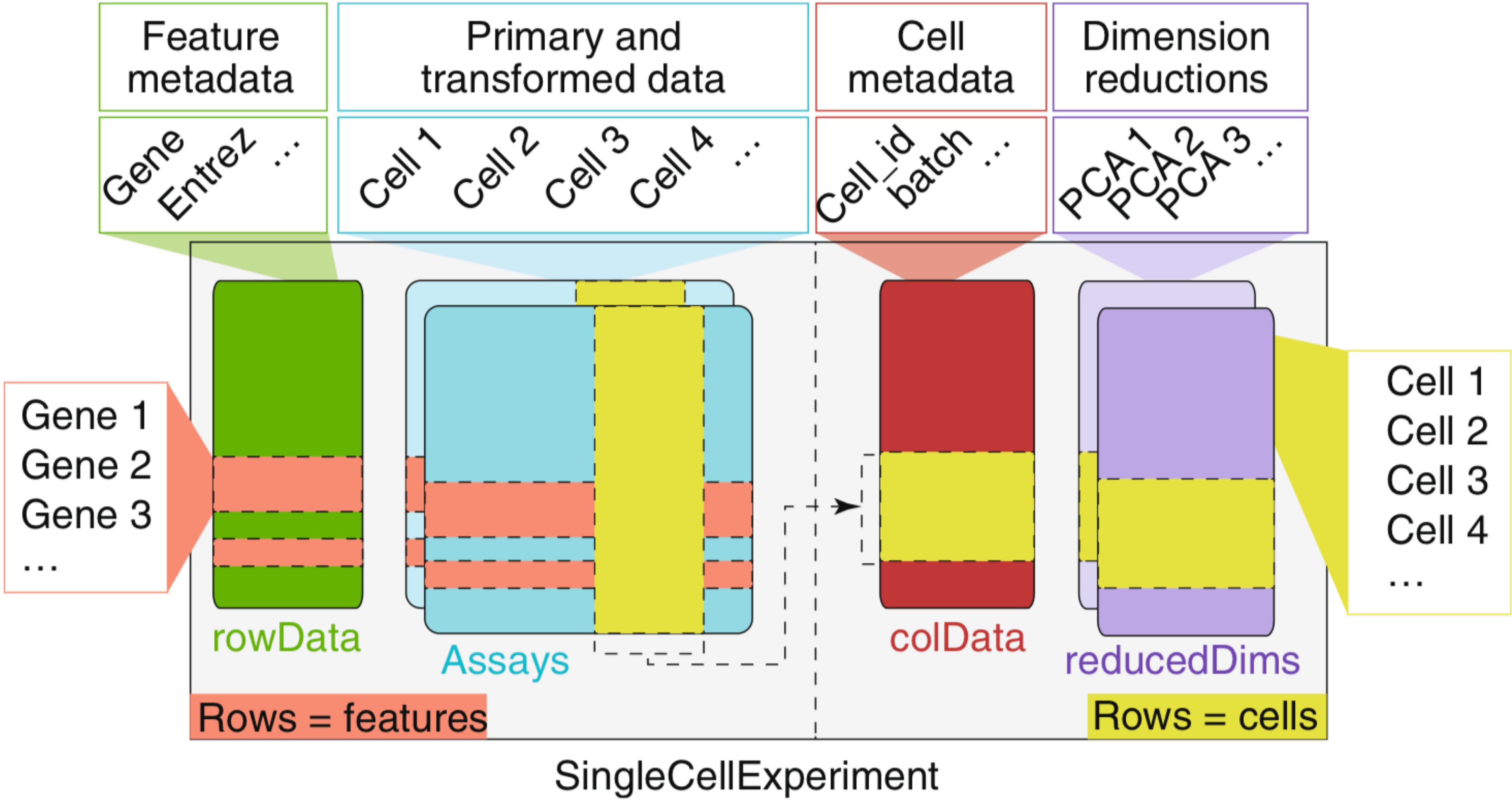
The installation process could take up to 5 minutes, depending if you have some of the packages pre-installed.

```
# Some CRAN packages required by scMerge
install.packages(c("ruv", "rsvd", "igraph", "pdist", "proxy", "foreach", "doSNOW", "distr", "Rcpp", "RcppEi
devtools::install_github("theislab/kBET")

# Some BioConductor packages required by scMerge
# try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite(c("SingleCellExperiment", "M3Drop"))


# Installing scMerge and the data files using
devtools::install_github("SydneyBioX/scMerge.data")
devtools::install_github("SydneyBioX/scMerge")
```

## Vignette

You can find the vignette at our website: https://sydneybiox.github.io/scMerge/index.html.

# SingleCellExperiment Object



Amezquita et al. Nature Methods 2019

We will try this soon …

14:15 – 15:00 Quality control and data integration

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# Roadmap for the workshop

Setting up: 13:30 – 13:45 Google cloud set up

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AFTERNOON TEA: 1500-1530

Session 3: 15:30 – 16:00 Overview of single-cell downstream analysis

Session 4: 16:00 – 16:45 Downstream analysis: cell type identification, identify marker genes & cell type composition

Extension: cell type identification via supervised classification and single cell trajectory analysis

# Summary and Q&A

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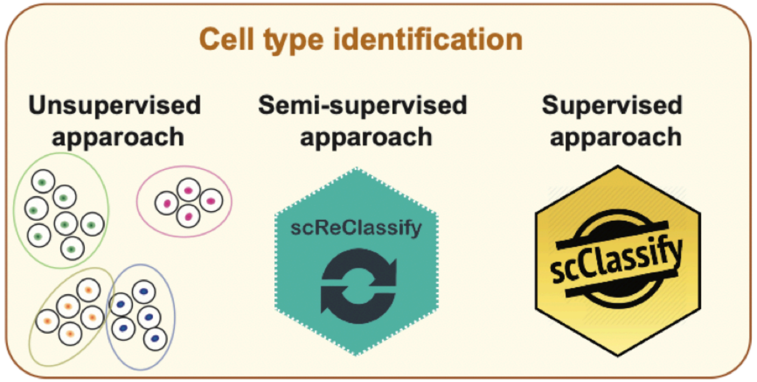
# Afternoon Tea

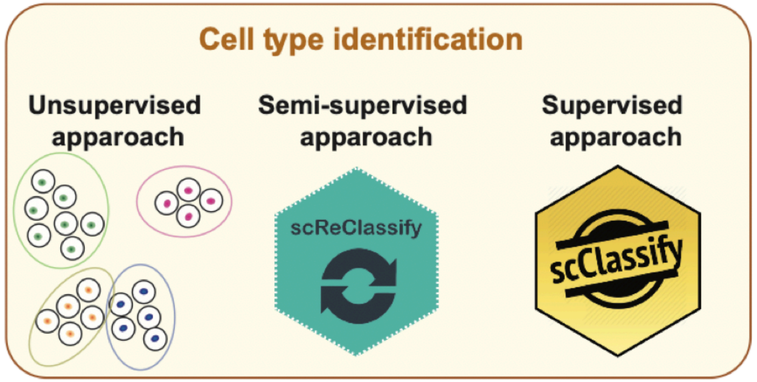
# Component 4: Cell type identification



## Science questions

- What cell types are present in the dataset?
- Can we identify the cell types?

# Component 4: Cell type identification



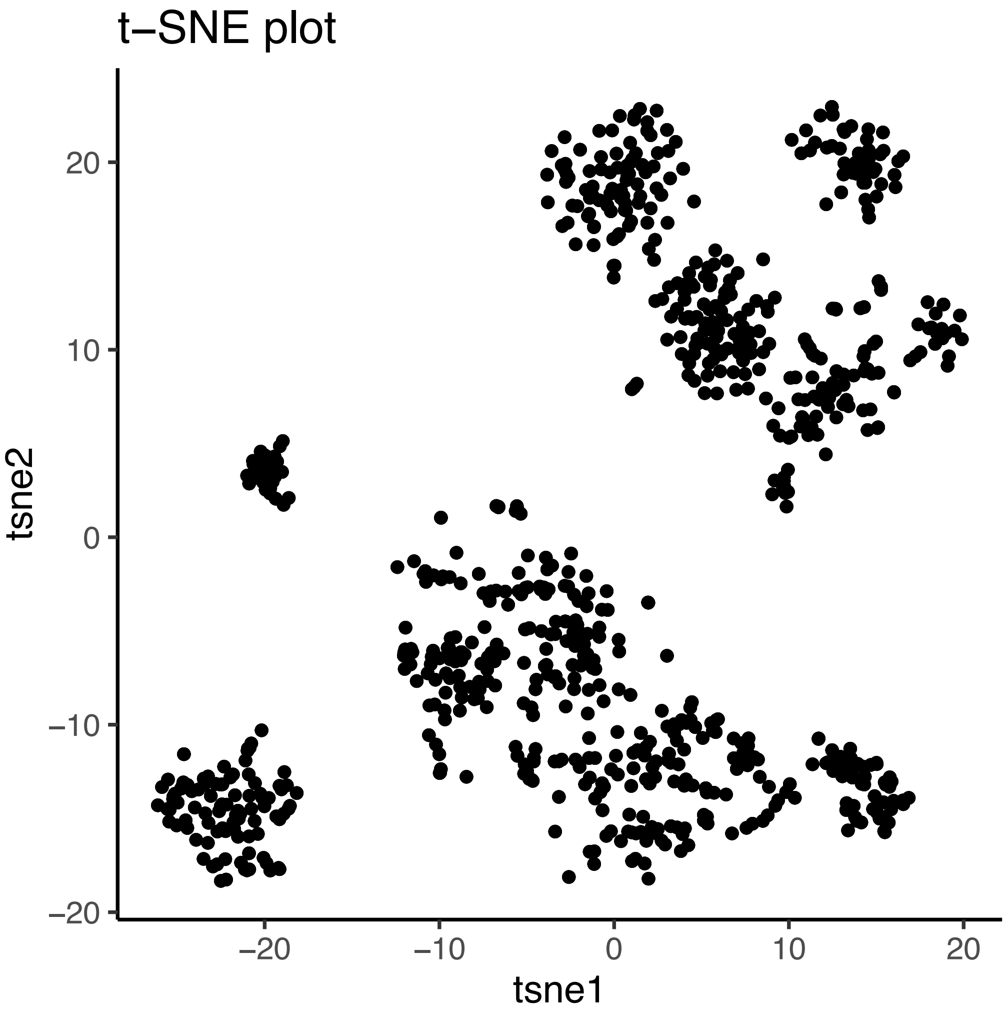
## Science questions

- What cell types are present in the dataset?
- Can we identify the cell types?

## Analysis techniques

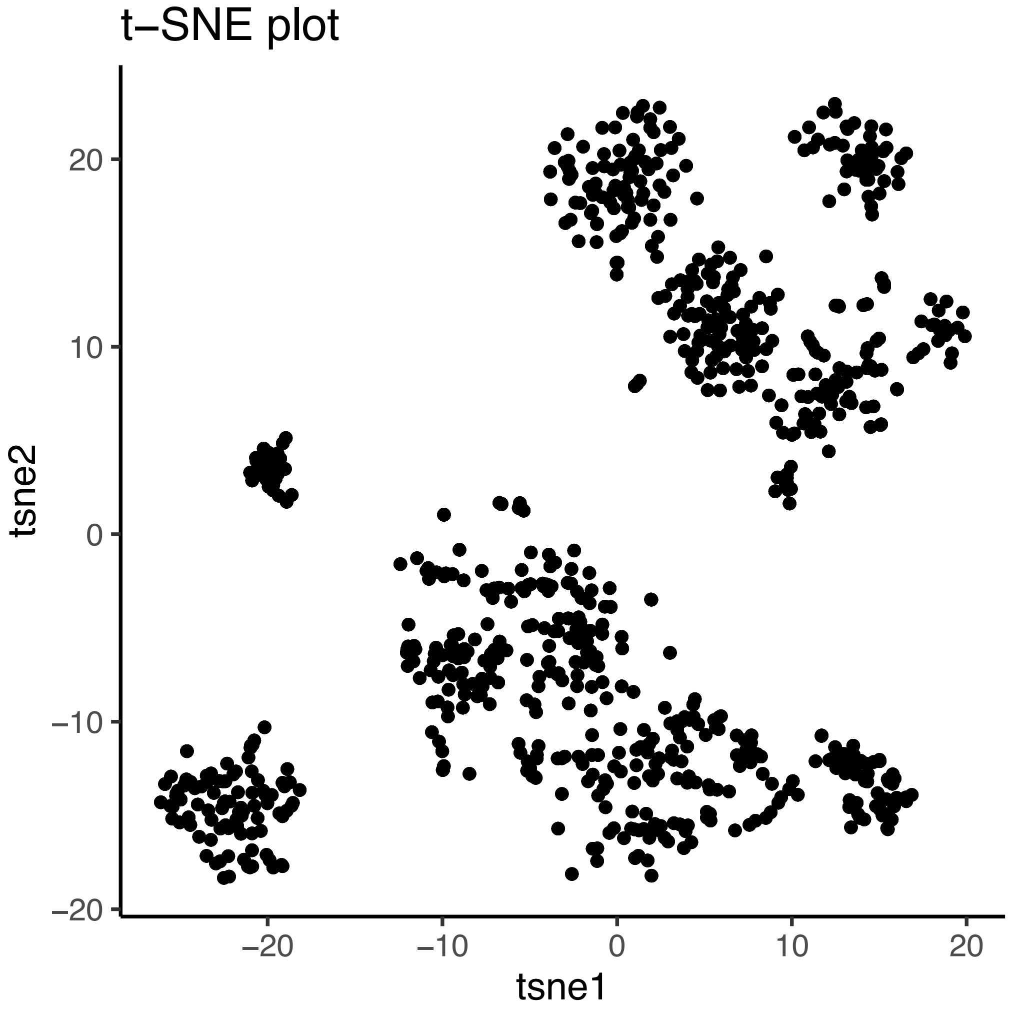
- Visualization (dimension reduction)
- Clustering (unsupervised learning)
- Classification (supervised learning)

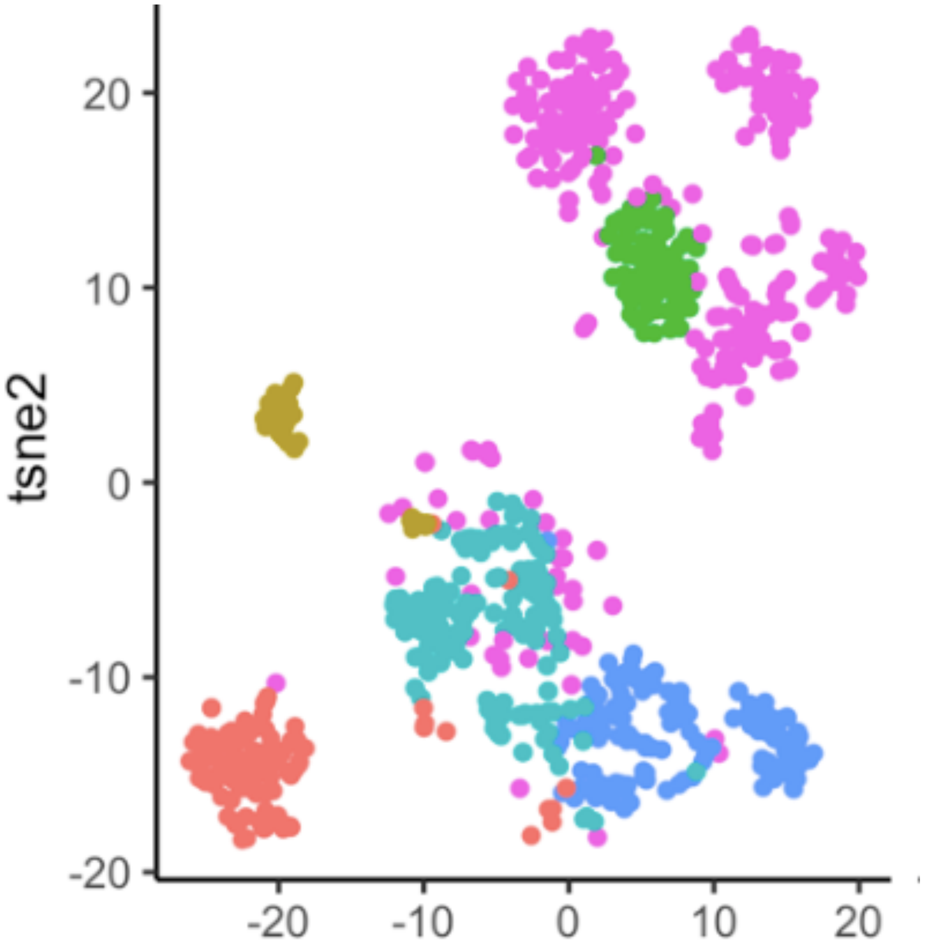
# Dimension reduced plot of our data (tSNE plot)



How many cell types are there?
What are the cell types?

# k-means clustering





# Clustering algorithms for scRNA-seq

*k*-means

Hierarchical

RaceID

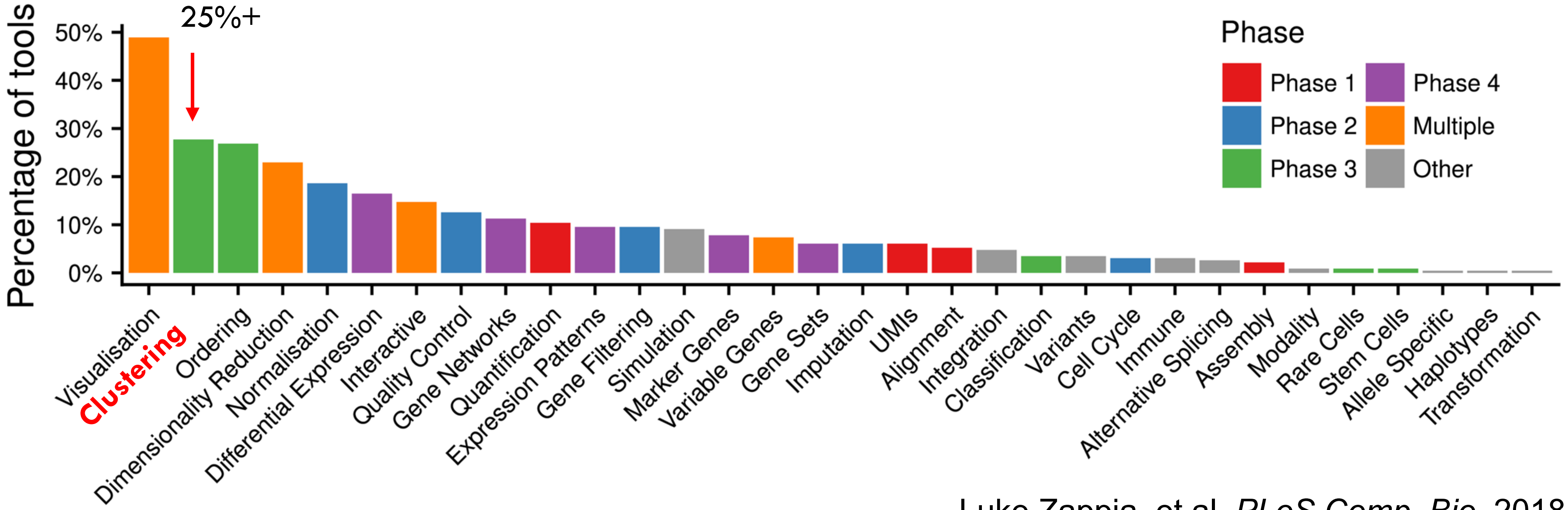
SC3

CIDR

countClust

RCA

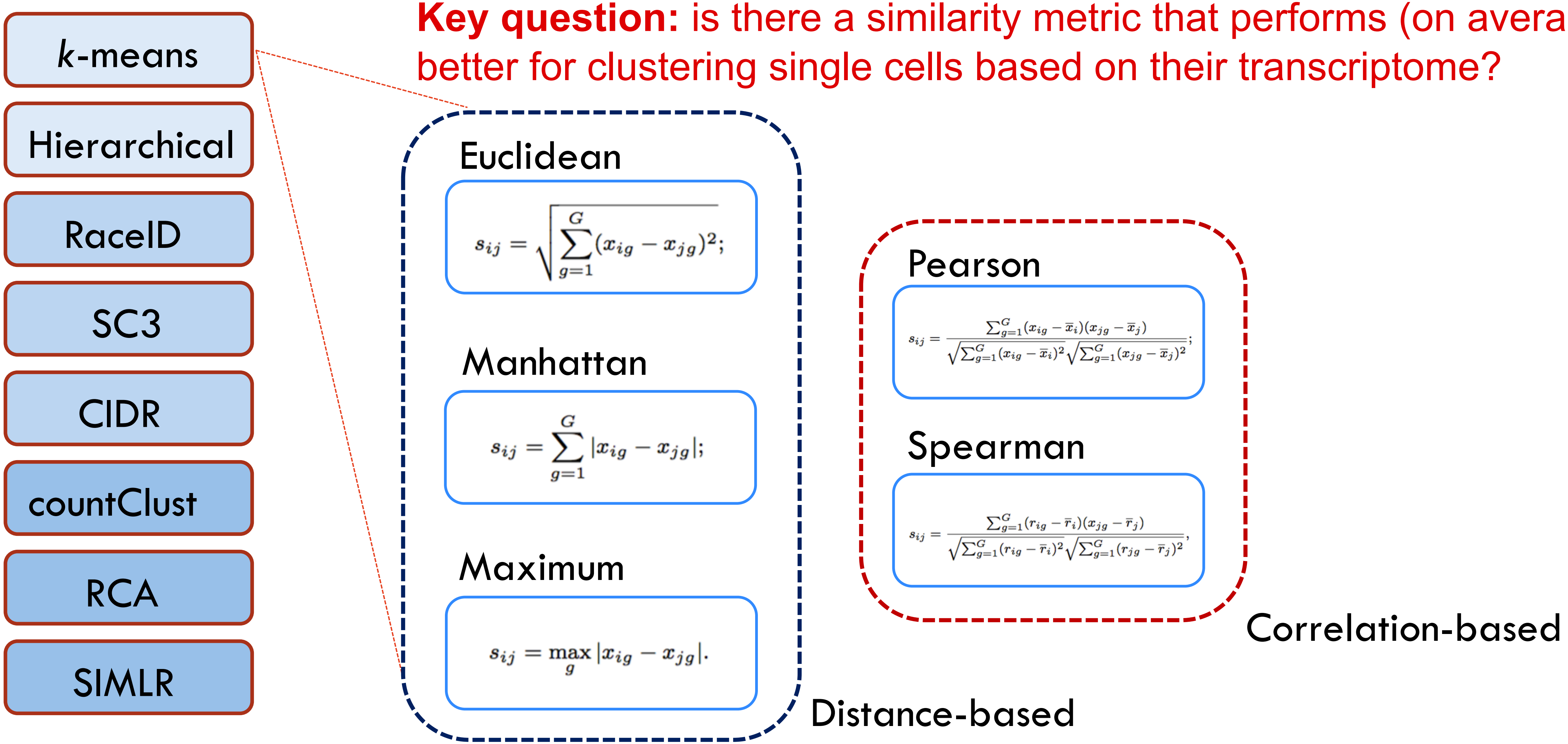
SIMLR



Luke Zappia, et al. *PLoS Comp. Bio.* 2018

# Similarity metric is the core of clustering algorithm

**Key question:** is there a similarity metric that performs (on average) better for clustering single cells based on their transcriptome?



*k*-means

Hierarchical

RaceID

SC3

CIDR

countClust

RCA

SIMLR

Euclidean

$$s_{ij} = \sqrt{\sum_{g=1}^{G} (x_{ig} - x_{jg})^2};$$

Manhattan

$$s_{ij} = \sum_{g=1}^{G} |x_{ig} - x_{jg}|;$$

Maximum

$$s_{ij} = \max_{g} |x_{ig} - x_{jg}|.$$

Distance-based

Pearson

$$s_{ij} = \frac{\sum_{g=1}^{G} (x_{ig} - \overline{x}_i)(x_{jg} - \overline{x}_j)}{\sqrt{\sum_{g=1}^{G} (x_{ig} - \overline{x}_i)^2} \sqrt{\sum_{g=1}^{G} (x_{jg} - \overline{x}_j)^2}};$$

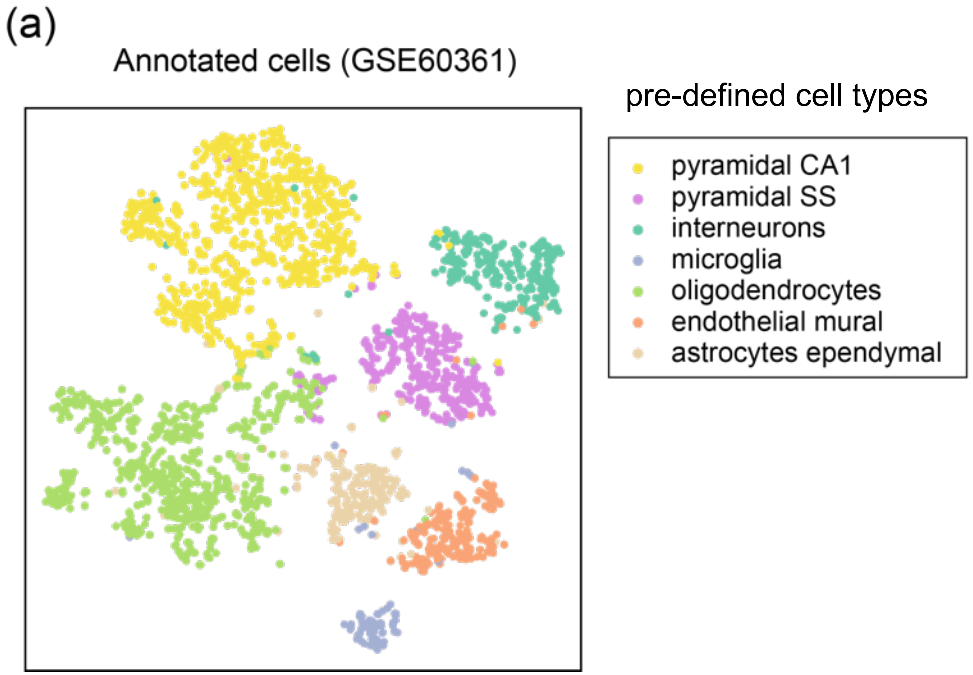
Spearman

$$s_{ij} = \frac{\sum_{g=1}^{G} (r_{ig} - \overline{r}_i)(x_{jg} - \overline{r}_j)}{\sqrt{\sum_{g=1}^{G} (r_{ig} - \overline{r}_i)^2} \sqrt{\sum_{g=1}^{G} (r_{jg} - \overline{r}_j)^2}},$$

Correlation-based

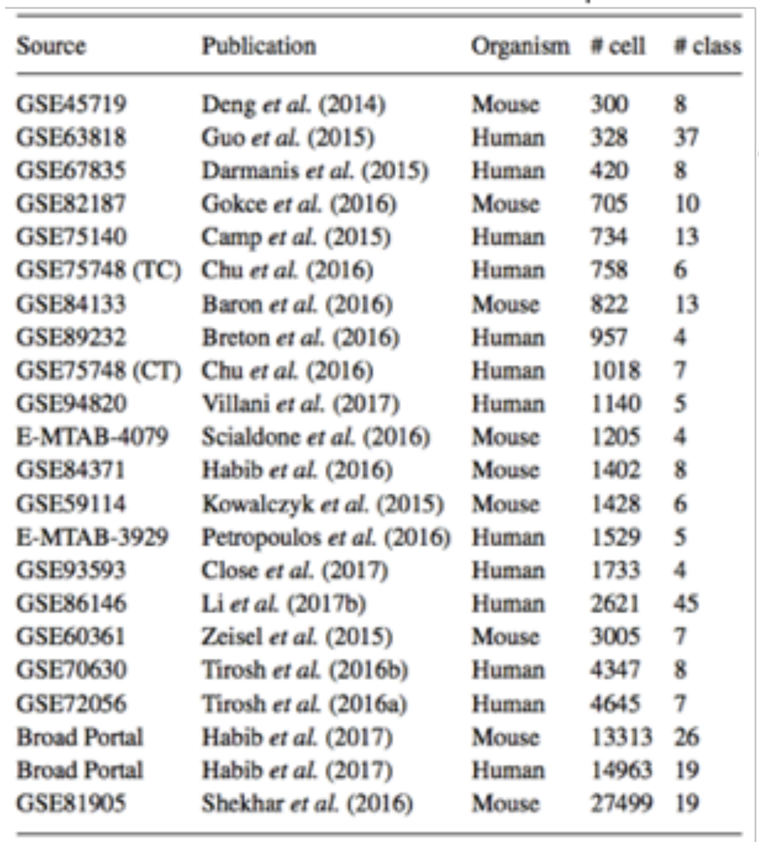
# *k*-means Clustering on GSE60361

*k*-means

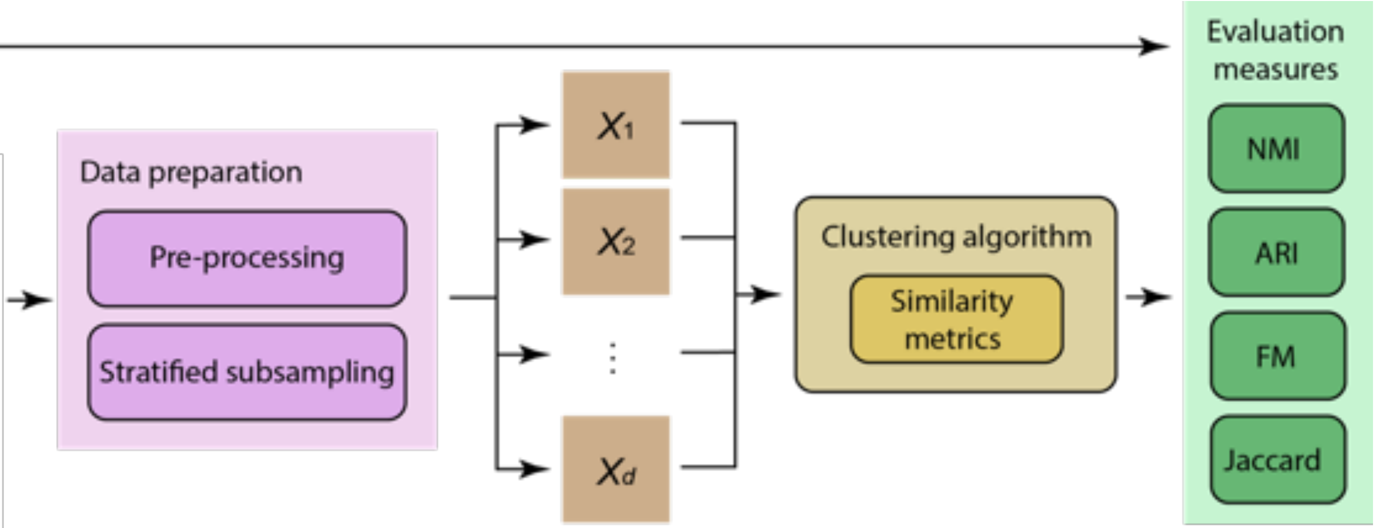


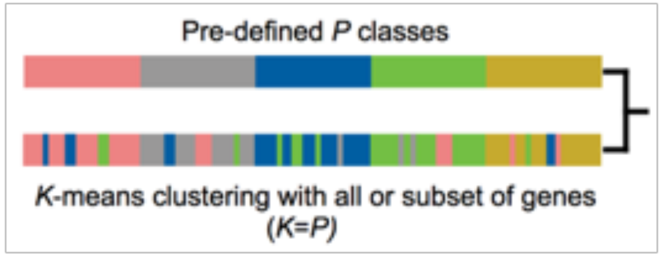
Zeisel A, et al. *Science* 2015

# Evaluation framework



| Source | Publication | Organism | # cell | # class |
|---|---|---|---|---|
| GSE45719 | Deng *et al.* (2014) | Mouse | 300 | 8 |
| GSE63818 | Guo *et al.* (2015) | Human | 328 | 37 |
| GSE67835 | Darmanis *et al.* (2015) | Human | 420 | 8 |
| GSE82187 | Gokce *et al.* (2016) | Mouse | 705 | 10 |
| GSE75140 | Camp *et al.* (2015) | Human | 734 | 13 |
| GSE75748 (TC) | Chu *et al.* (2016) | Human | 758 | 6 |
| GSE84133 | Baron *et al.* (2016) | Mouse | 822 | 13 |
| GSE89232 | Breton *et al.* (2016) | Human | 957 | 4 |
| GSE75748 (CT) | Chu *et al.* (2016) | Human | 1018 | 7 |
| GSE94820 | Villani *et al.* (2017) | Human | 1140 | 5 |
| E-MTAB-4079 | Scialdone *et al.* (2016) | Mouse | 1205 | 4 |
| GSE84371 | Habib *et al.* (2016) | Mouse | 1402 | 8 |
| GSE59114 | Kowalczyk *et al.* (2015) | Mouse | 1428 | 6 |
| E-MTAB-3929 | Petropoulos *et al.* (2016) | Human | 1529 | 5 |
| GSE93593 | Close *et al.* (2017) | Human | 1733 | 4 |
| GSE86146 | Li *et al.* (2017b) | Human | 2621 | 45 |
| GSE60361 | Zeisel *et al.* (2015) | Mouse | 3005 | 7 |
| GSE70630 | Tirosh *et al.* (2016b) | Human | 4347 | 8 |
| GSE72056 | Tirosh *et al.* (2016a) | Human | 4645 | 7 |
| Broad Portal | Habib *et al.* (2017) | Mouse | 13313 | 26 |
| Broad Portal | Habib *et al.* (2017) | Human | 14963 | 19 |
| GSE81905 | Shekhar *et al.* (2016) | Mouse | 27499 | 19 |

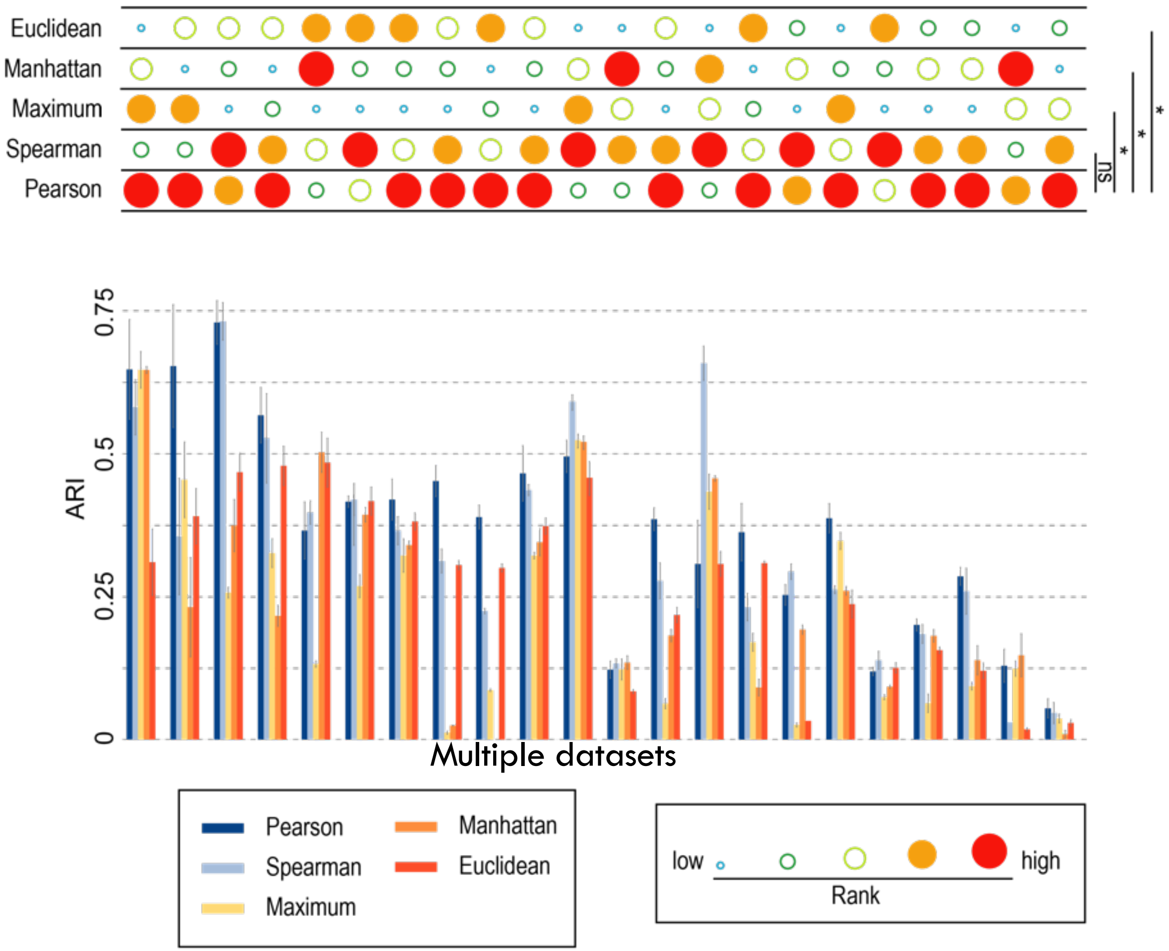




**Agreement to pre-defined classes:**
Normalized Mutual Information (NMI)
Adjusted Rand Index (ARI)
Fowlkes-Mallows Index (FM)
Jaccard Index (Jaccard)

Taiyun Kim

# Evaluation results (against the pre-defined cell types)



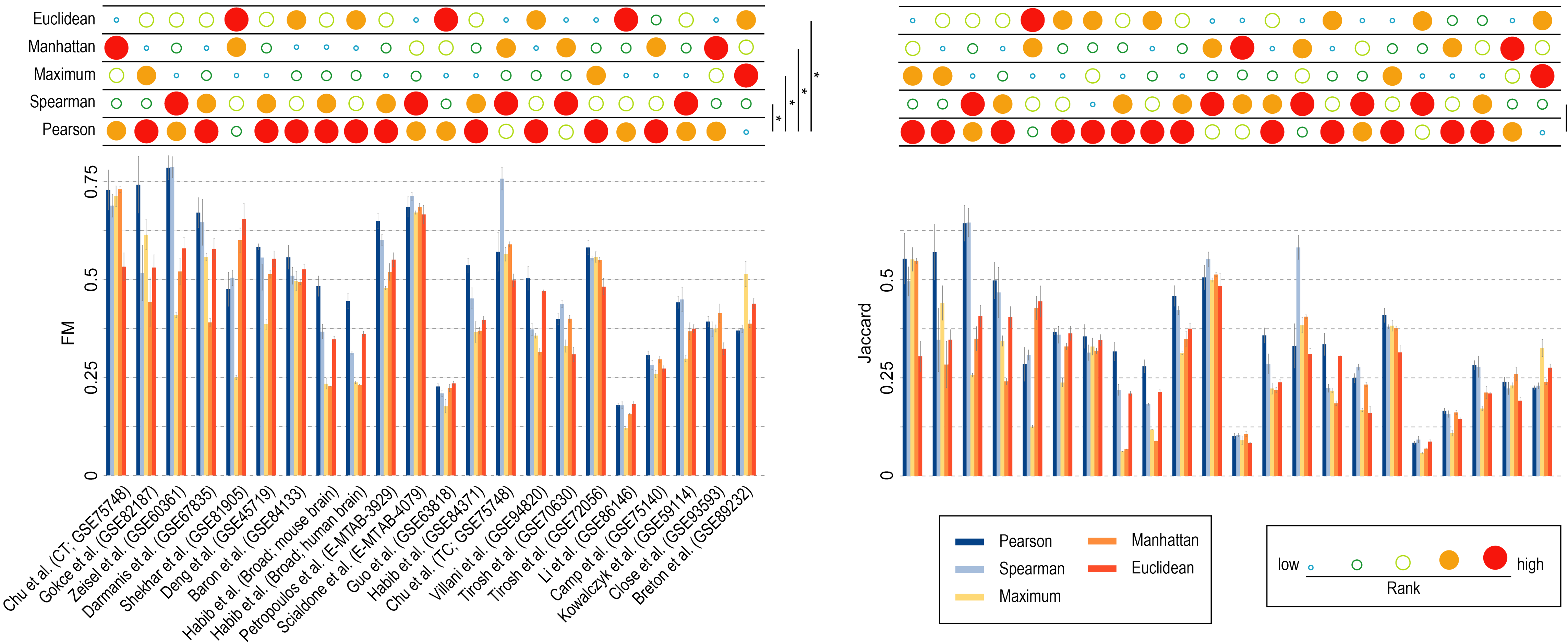
**Impact of similarity metrics on single-cell RNA-seq data clustering**

Taiyun Kim, Irene Rui Chen, Yingxin Lin, Andy Yi-Yang Wang, Jean Yee Hwa Yang, Pengyi Yang

*Briefings in Bioinformatics*, bby076,

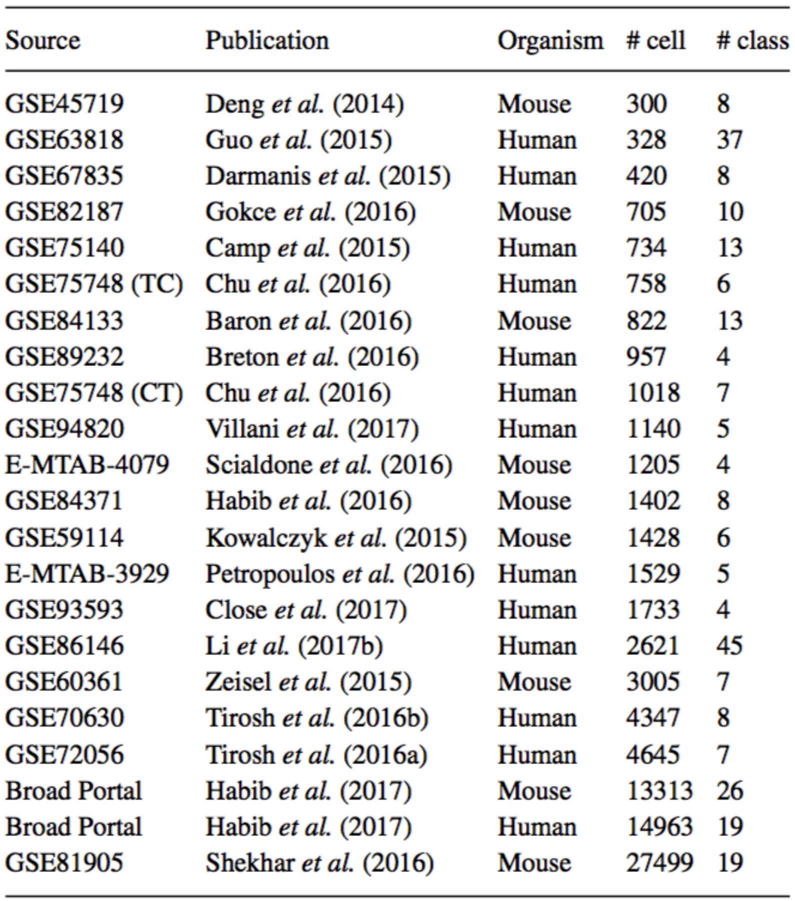
**PhD student: Taiyun Kim**

# Evaluation results (against the pre-defined cell types) using other measures



On average, correlation-based metrics improved on distance-based metrics by 31.5% (NMI), 39.6% (ARI), 16% (FM), 23% (Jaccard)

# Account for data scaling and zero-counts



| Source | Publication | Organism | # cell | # class |
|---|---|---|---|---|
| GSE45719 | Deng *et al.* (2014) | Mouse | 300 | 8 |
| GSE63818 | Guo *et al.* (2015) | Human | 328 | 37 |
| GSE67835 | Darmanis *et al.* (2015) | Human | 420 | 8 |
| GSE82187 | Gokce *et al.* (2016) | Mouse | 705 | 10 |
| GSE75140 | Camp *et al.* (2015) | Human | 734 | 13 |
| GSE75748 (TC) | Chu *et al.* (2016) | Human | 758 | 6 |
| GSE84133 | Baron *et al.* (2016) | Mouse | 822 | 13 |
| GSE89232 | Breton *et al.* (2016) | Human | 957 | 4 |
| GSE75748 (CT) | Chu *et al.* (2016) | Human | 1018 | 7 |
| GSE94820 | Villani *et al.* (2017) | Human | 1140 | 5 |
| E-MTAB-4079 | Scialdone *et al.* (2016) | Mouse | 1205 | 4 |
| GSE84371 | Habib *et al.* (2016) | Mouse | 1402 | 8 |
| GSE59114 | Kowalczyk *et al.* (2015) | Mouse | 1428 | 6 |
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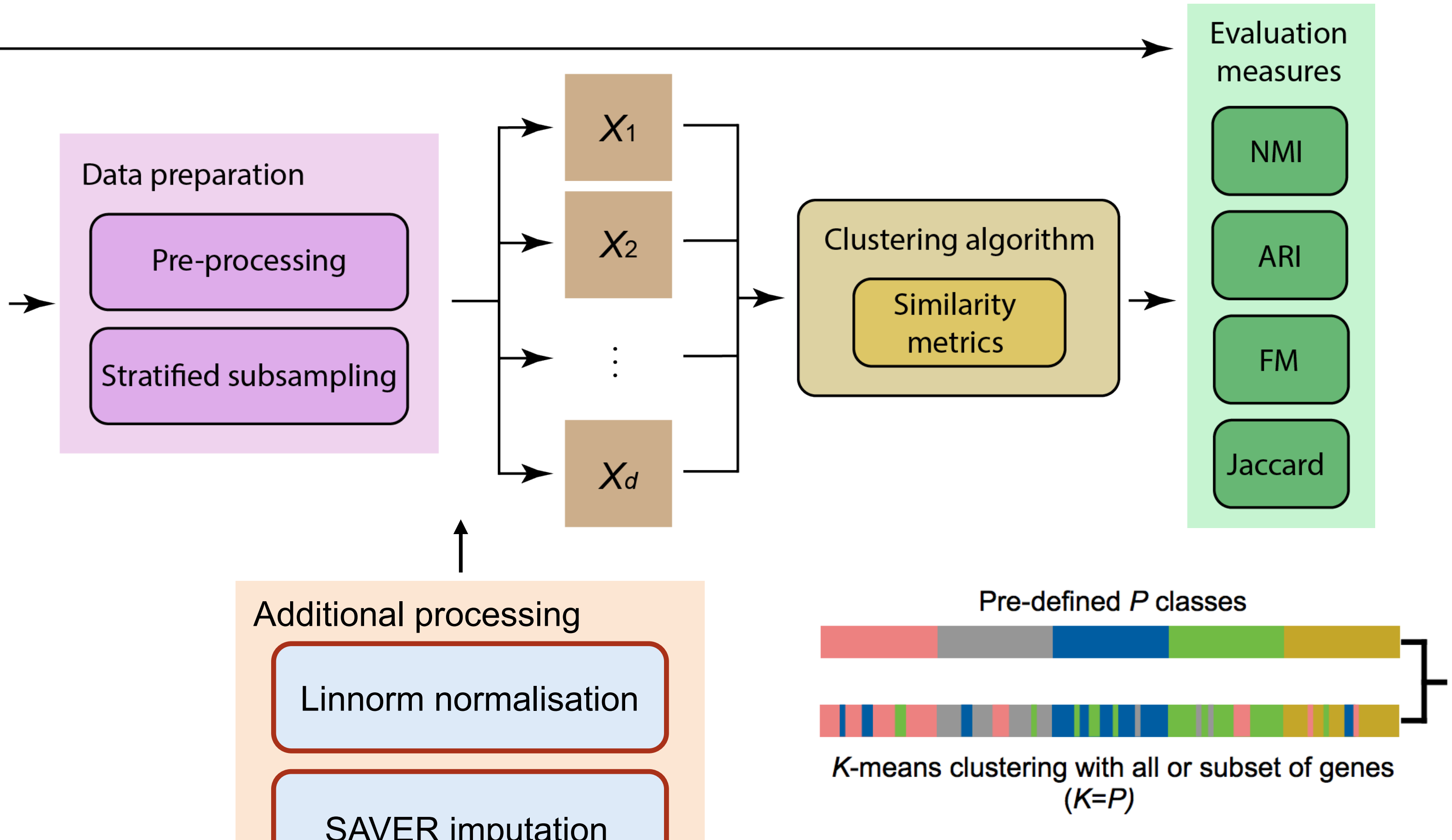
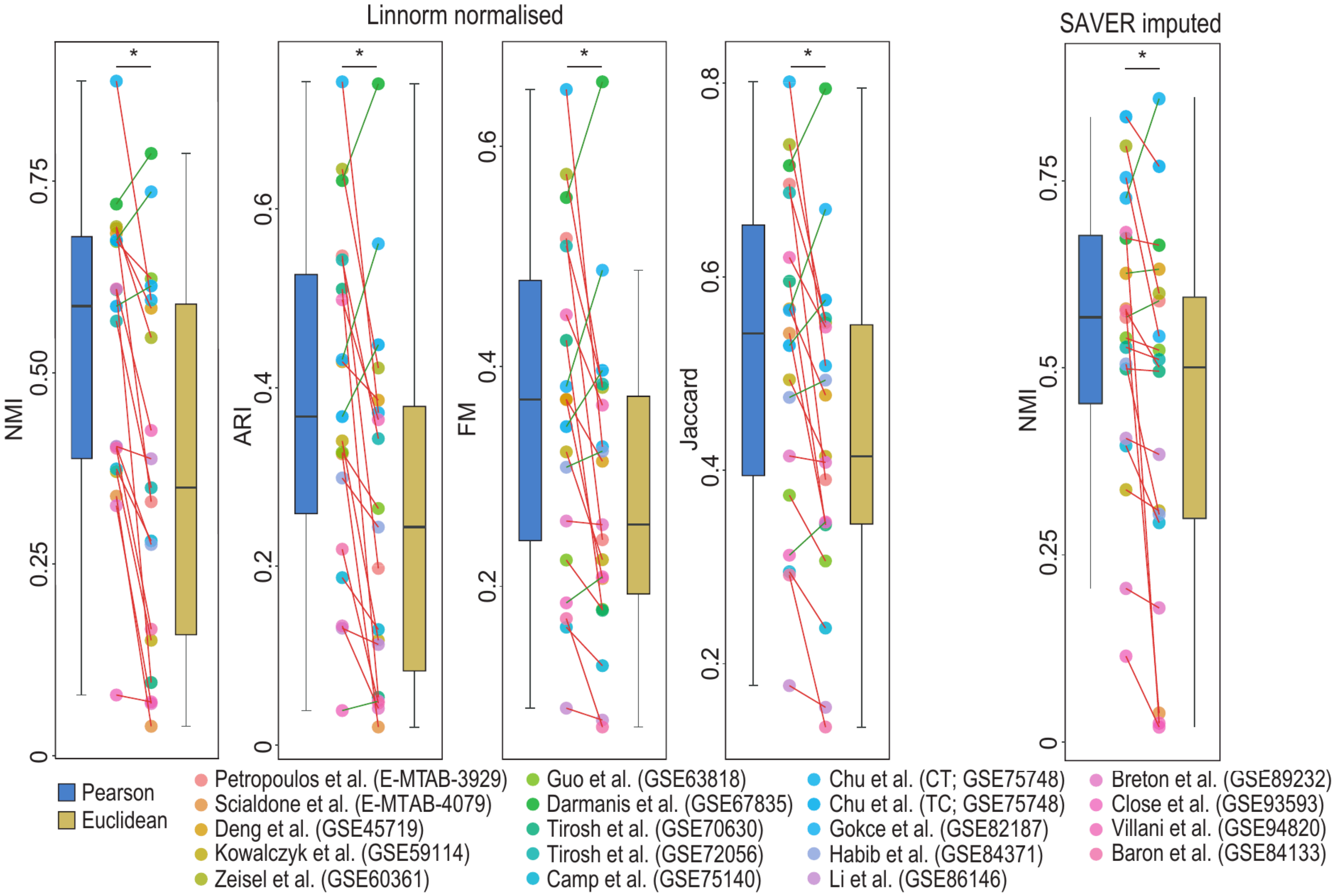
| GSE81905 | Shekhar *et al.* (2016) | Mouse | 27499 | 19 |



**Agreement to pre-defined classes:**
Normalized Mutual Information (NMI)
Adjusted Rand Index (ARI)
Fowlkes-Mallows Index (FM)
Jaccard Index (Jaccard)

# Account for normalisation and imputation

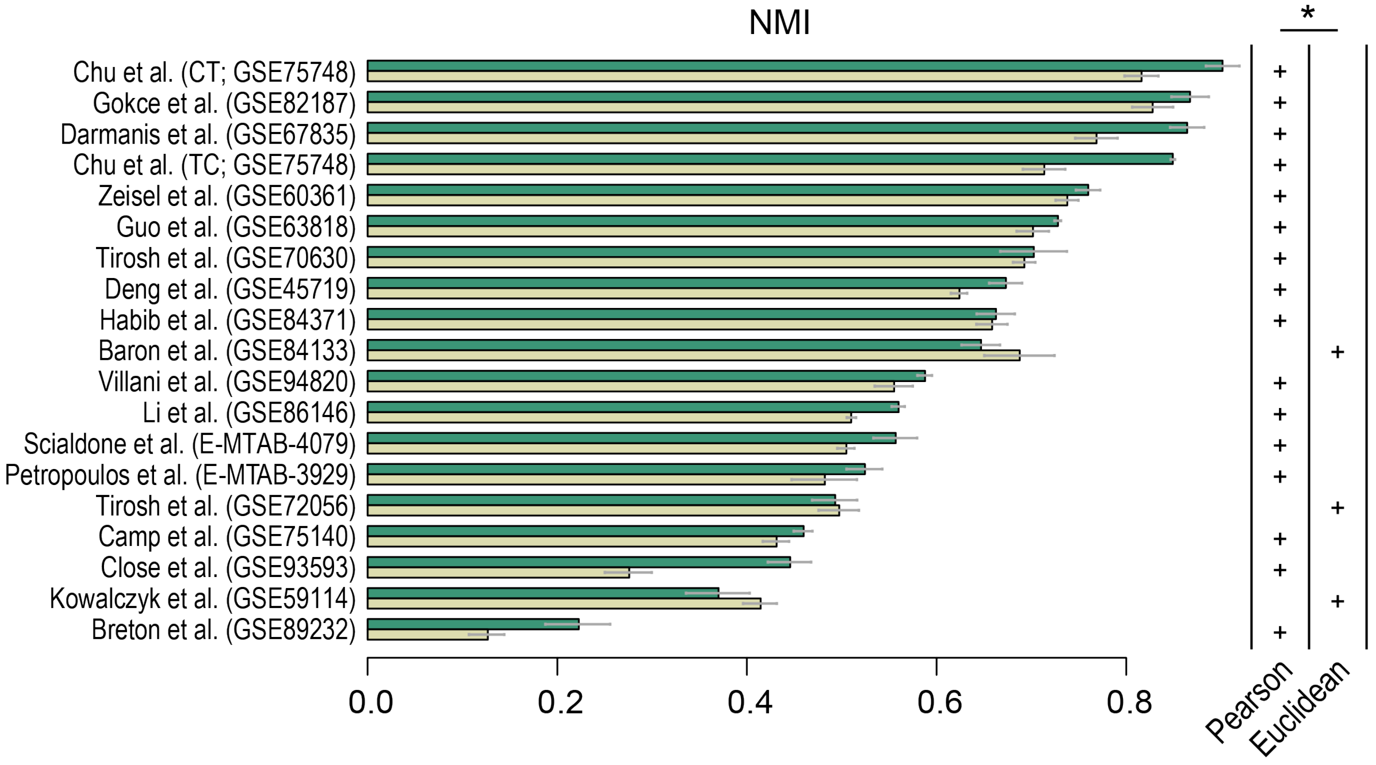


# Improving the state-of-the-art clustering method using correlation metric

SIMLR

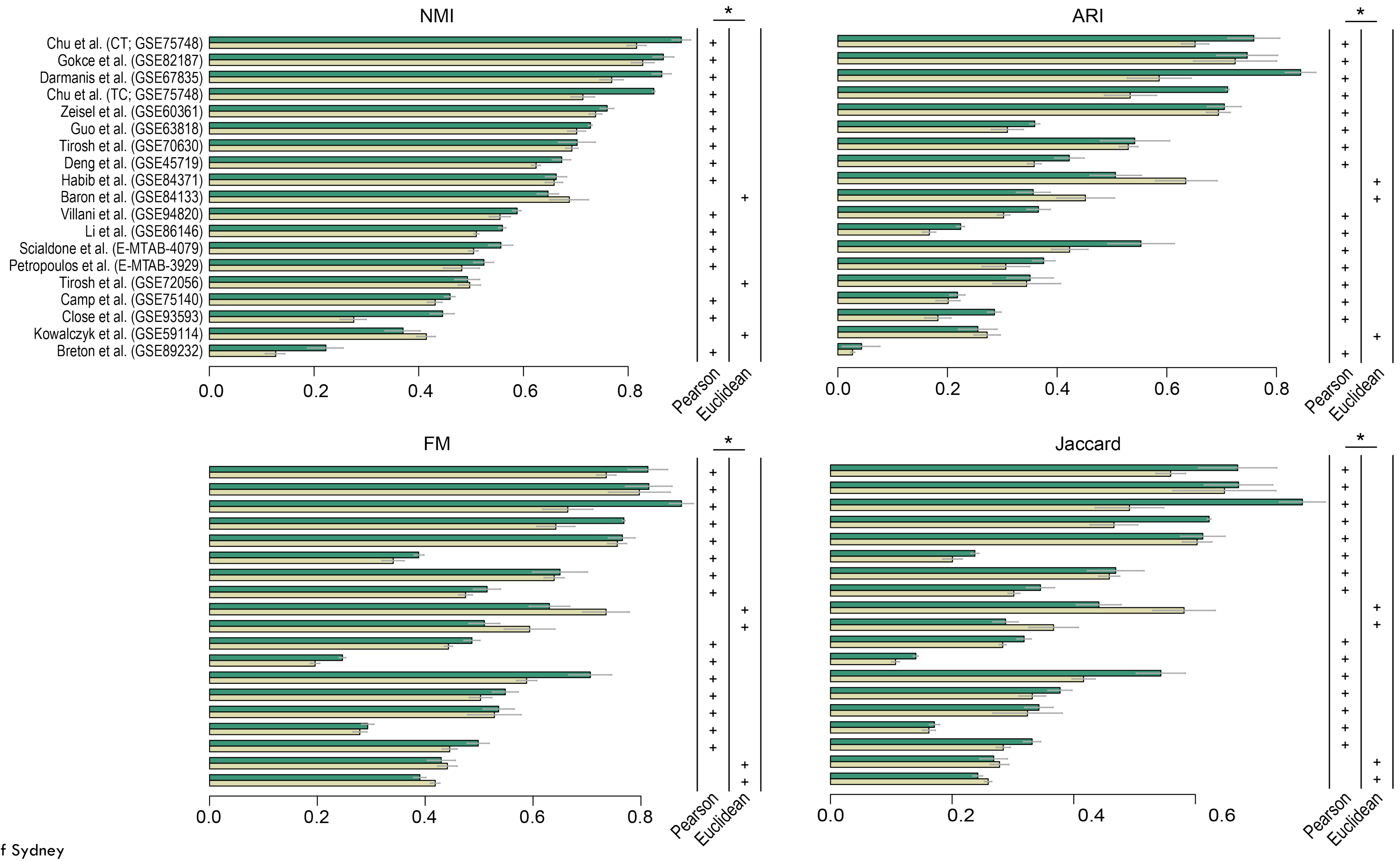
$$K(x_i, x_j) = \frac{1}{\epsilon_{ij}\sqrt{2\pi}} \exp\left(-\frac{\qquad}{2\epsilon_{ij}^2}\right)$$

$$s_{ij} = \frac{\sum_{g=1}^{G}(x_{ig} - \overline{x}_i)(x_{jg} - \overline{x}_j)}{\sqrt{\sum_{g=1}^{G}(x_{ig} - \overline{x}_i)^2}\sqrt{\sum_{g=1}^{G}(x_{jg} - \overline{x}_j)^2}};$$

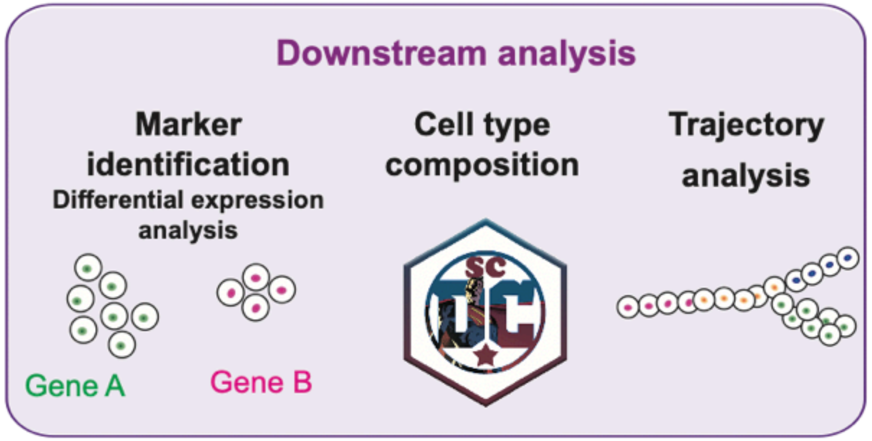


Wang, B., Zhu, J., Pierson, E., Ramazzotti, D., and Batzoglou, S. (2017). Visualization and analysis of single-cell rna-seq data by kernel-based similarity learning. *Nature Methods*, **14**(4), 414.

# Evaluation results of SIMLR with Pearson or Euclidean metrics



# Component 5: Downstream analysis



## Science questions

- Which genes are differentially expressed between cell types?
- What are the marker genes for each cell type?
- What is the cell type composition?
- Are the cells transitioning from one state to another?

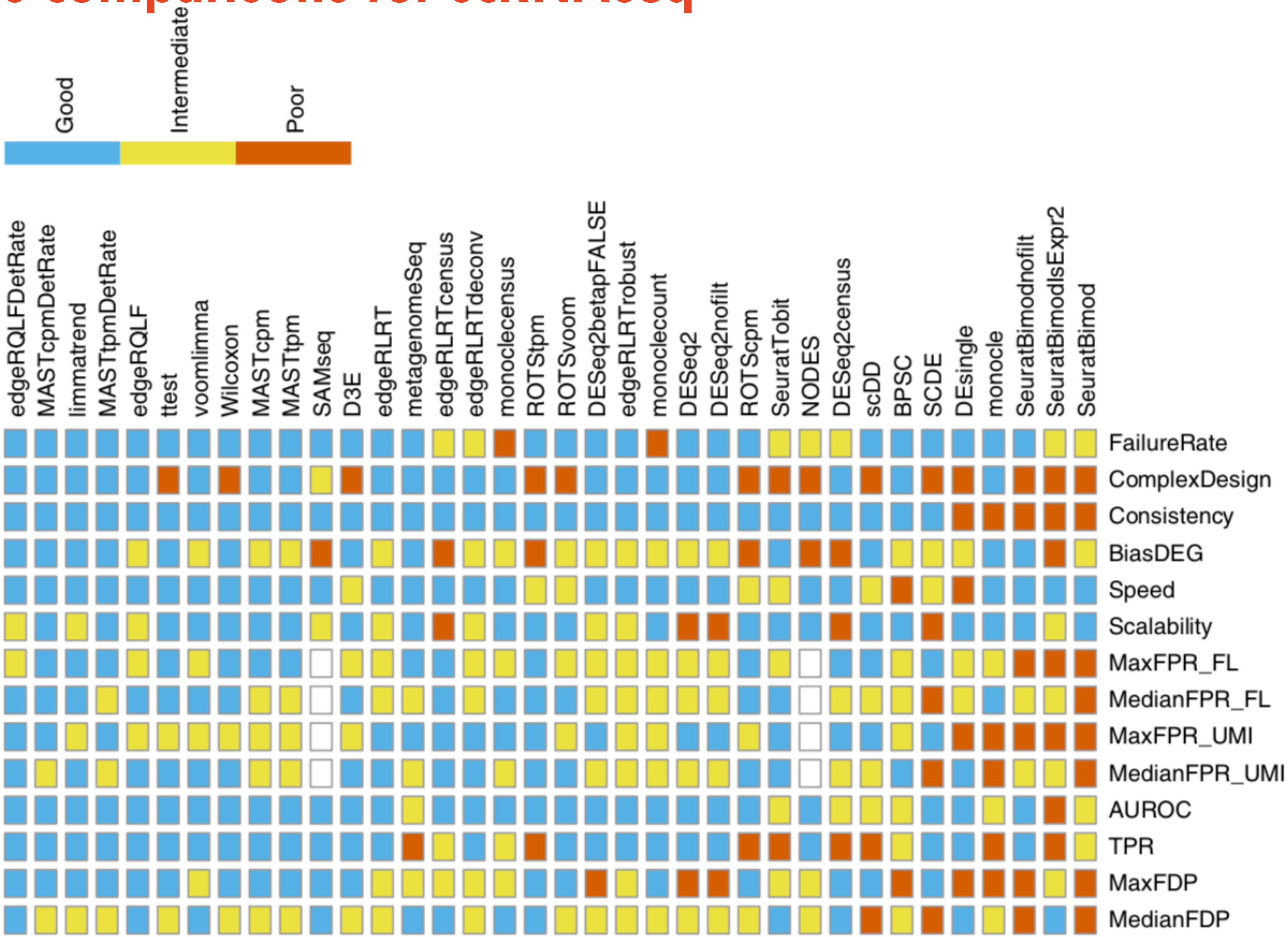
# Differential expression testing: Differences between single cell and bulk RNAseq

- Advantage of single-cell:

  Account for cellular heterogeneity: DE tests can be now performed within cell-identity clusters across experimental conditions.

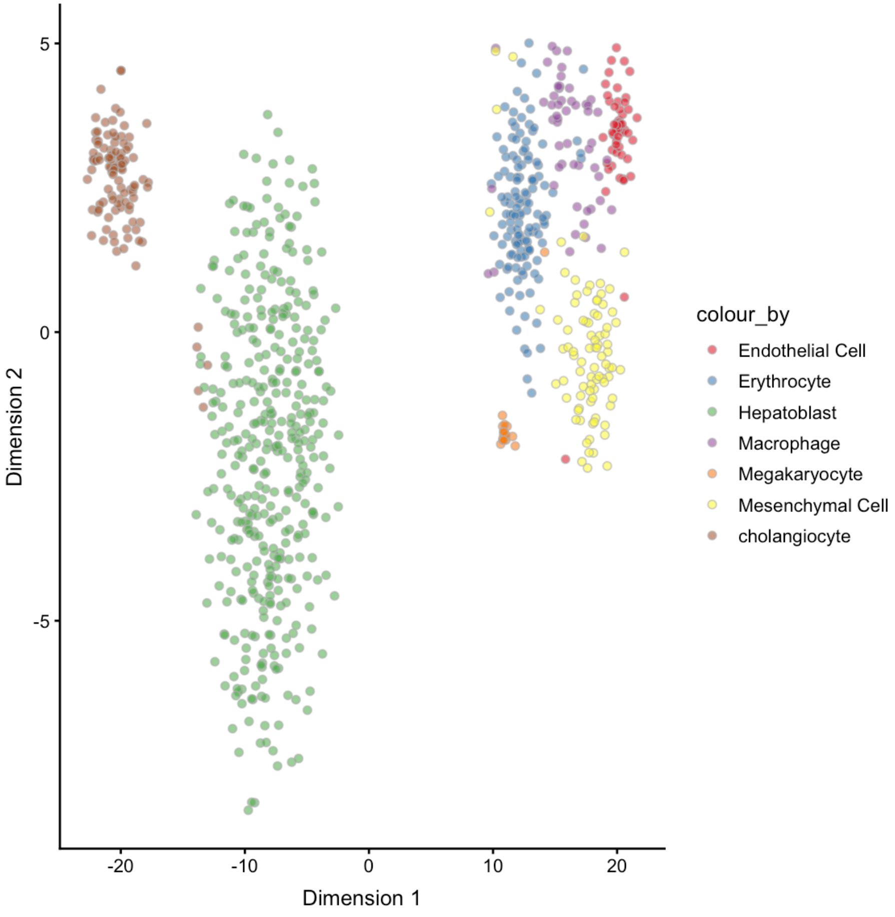
- Unique challenges for single-cell:
  - Dropout
  - High cell-to-cell variability
- Bulk DE methods
  - edgeR
  - limma
  - DESeq2
- Single-cell DE methods
  - MAST
  - ZINB-WaVE
  - DECENT
  - …

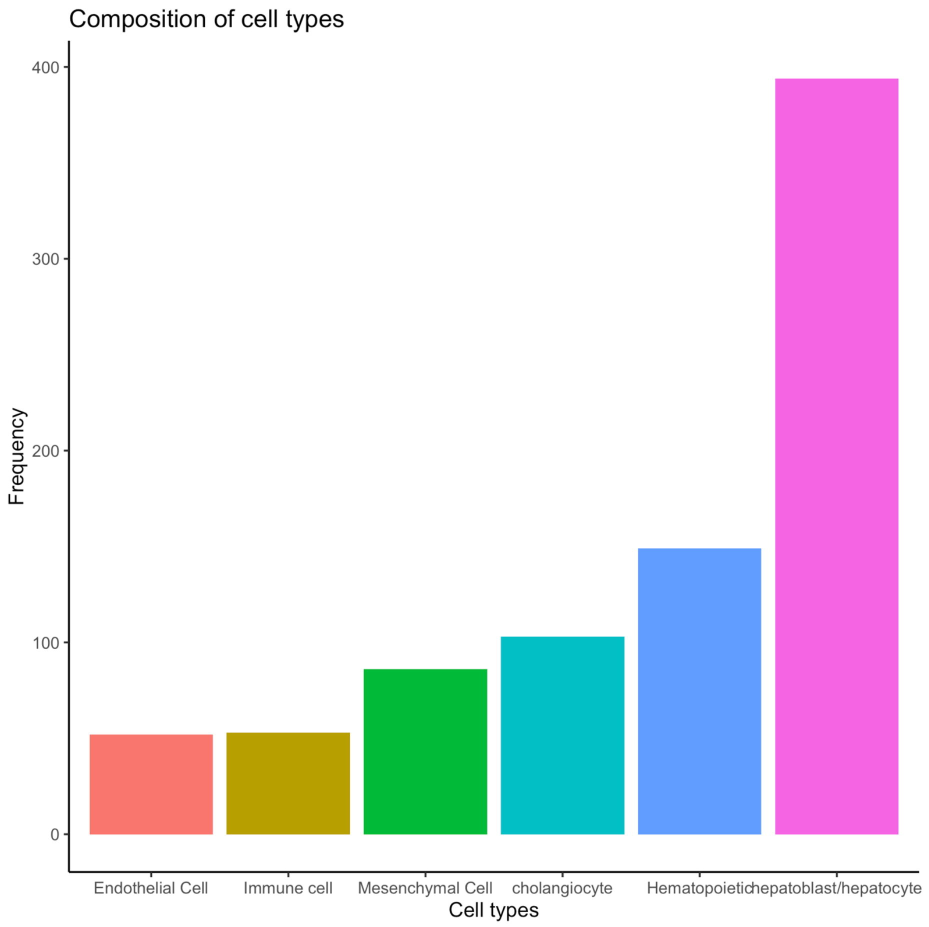
# DE methods comparisons for scRNAseq



Soneson and Robinson (2018) Nature methods

# Cell type composition





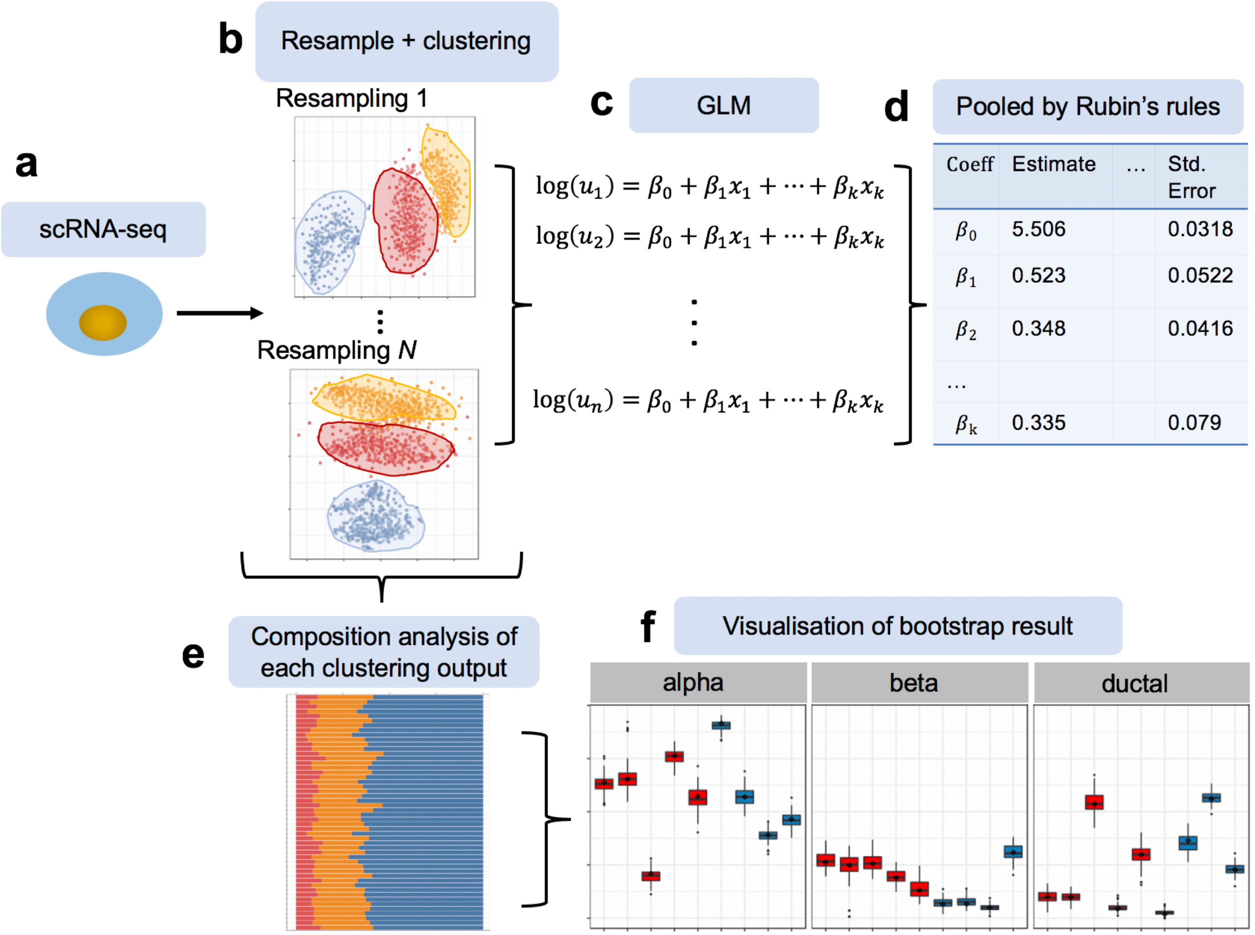
Can we conclude that there are more cholangiocytes than mesenchymal cells?

# Single cell Differential Composition (scDC)

scDC simulates ***uncertainty*** in cell-type proportions via bootstrapping

Main components:

- Sample with replacement from count matrix, stratified by patient
- Cell type identification via clustering (PCA -> Kmeans (Pearson correlation)
- Calculations of cell – type proportions standard error from bootstrap samples
- Calculation of pooled log-linear model using Rubin's pooled estimate



$$\log(u_1) = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k$$

$$\log(u_2) = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k$$

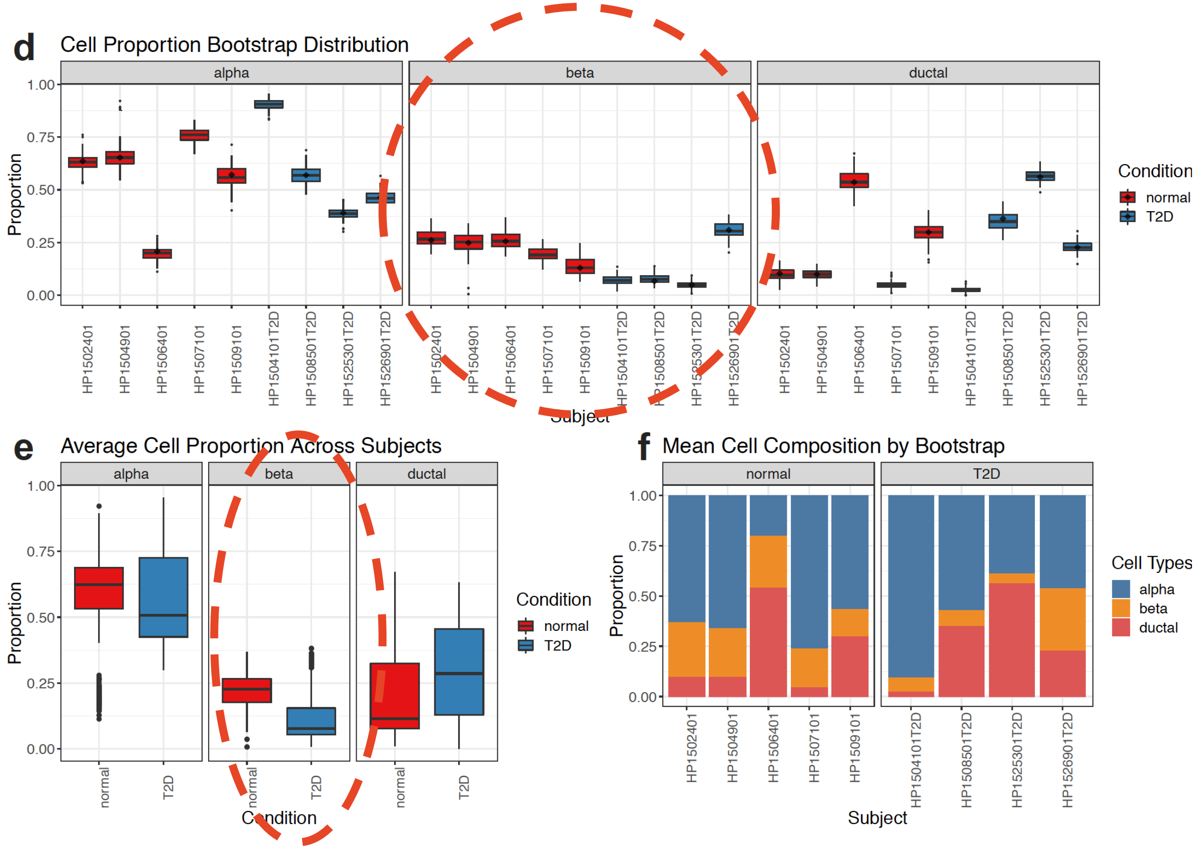
$$\vdots$$

$$\log(u_n) = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k$$

| Coeff | Estimate | … | Std. Error |
|---|---|---|---|
| $\beta_0$ | 5.506 | | 0.0318 |
| $\beta_1$ | 0.523 | | 0.0522 |
| $\beta_2$ | 0.348 | | 0.0416 |
| … | | | |
| $\beta_k$ | 0.335 | | 0.079 |

# Single cell Differential Composition (scDC)

- Examined two synthetic datasets constructed from two sets of real experimental data — Pancreas (T2D vs healthy) and Neuronal (developing mouse)
- In pancreas dataset
  - confirmed the original finding that 1 of the 4 subjects has a higher beta cell value, as IQR non overlap
- In neuronal dataset
  - Revealed new finding that progenitor cells percentage increase over time



We will try this soon…

16:00 – 16:45 Downstream analysis: identify marker genes & cell type composition

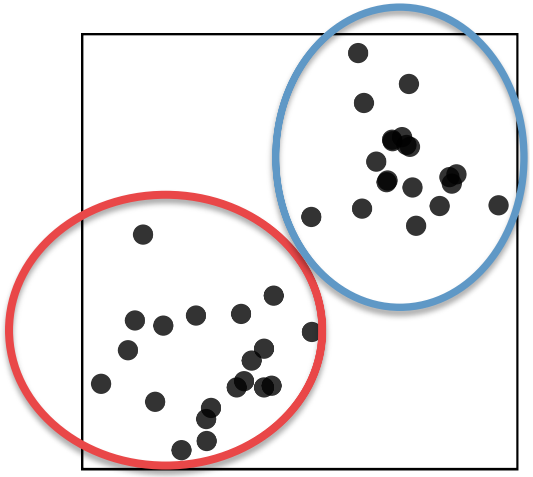
Extension:

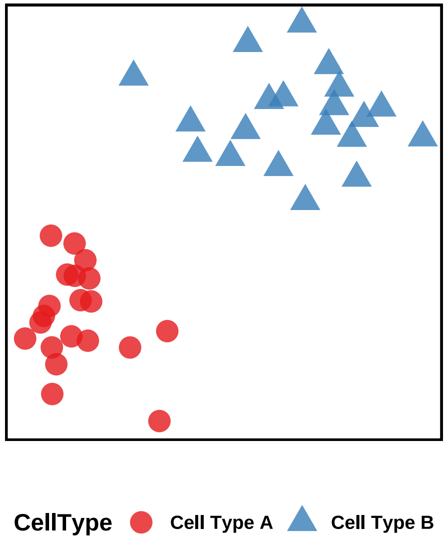
1. cell type identification via supervised classification
2. single cell trajectory analysis

# An alternative approach of cell type identification: supervised learning

## Clustering (unsupervised learning)

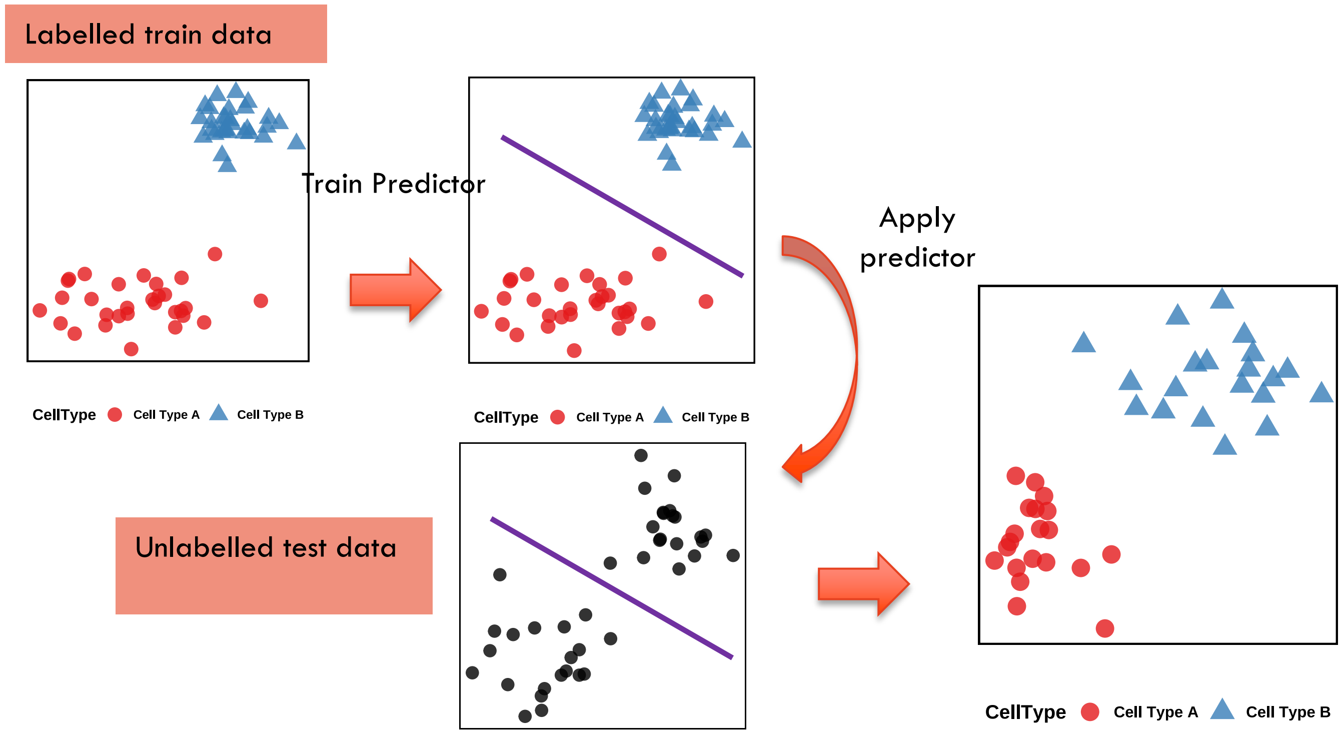
- Group the cells that are "close" to each other
- Annotated each cluster by DE genes or other characteristics
- Identify the novel cell type





## Classification (supervised learning)

- Required reference labelled datasets
- Predict cell types label directly
- What if there are cell types that are not in the reference data?



Labelled train data

Train Predictor

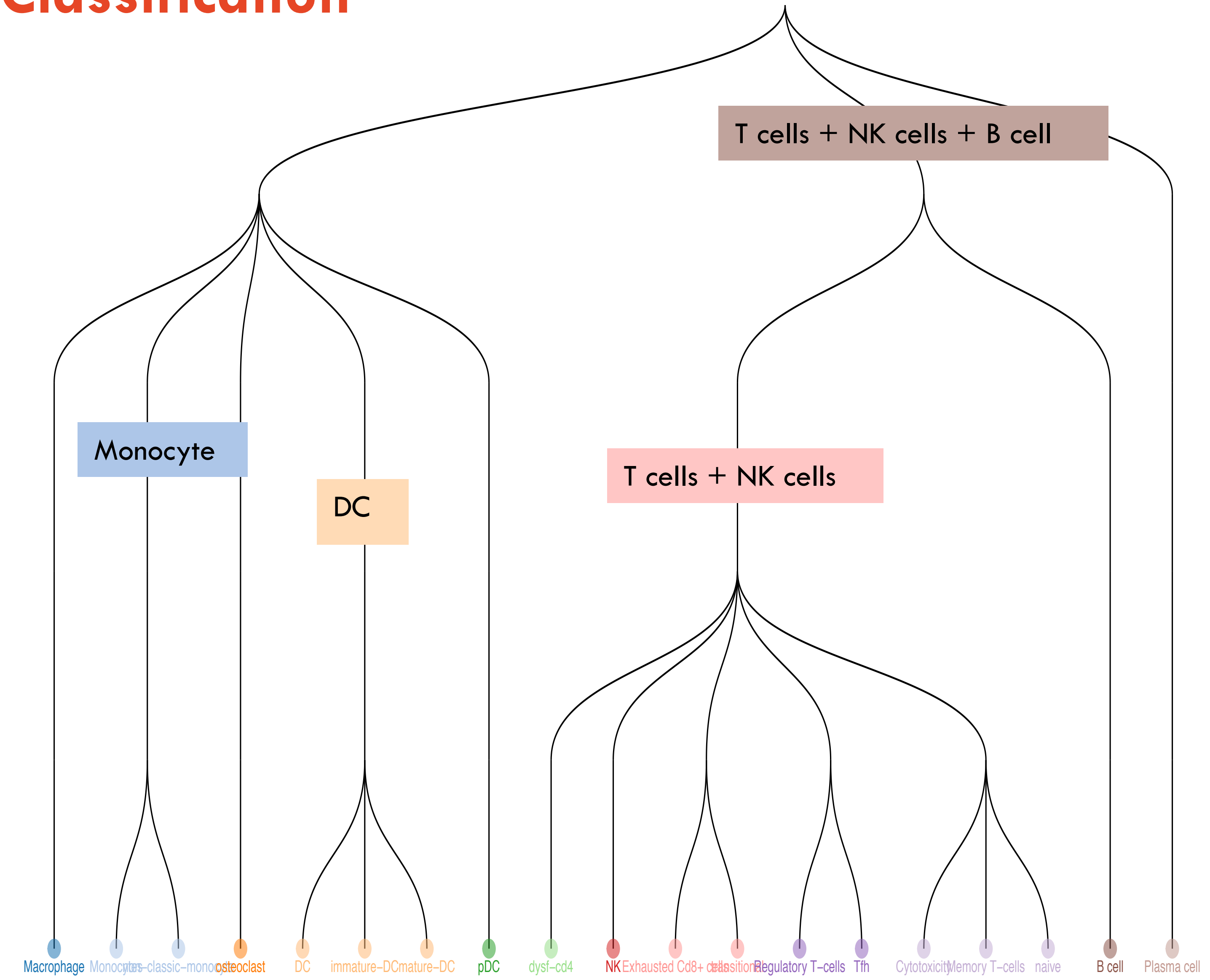
Apply predictor

Unlabelled test data

# scClassify: Hierarchical Classification

## *Step 1: Constructing cell type hierarchical tree:*

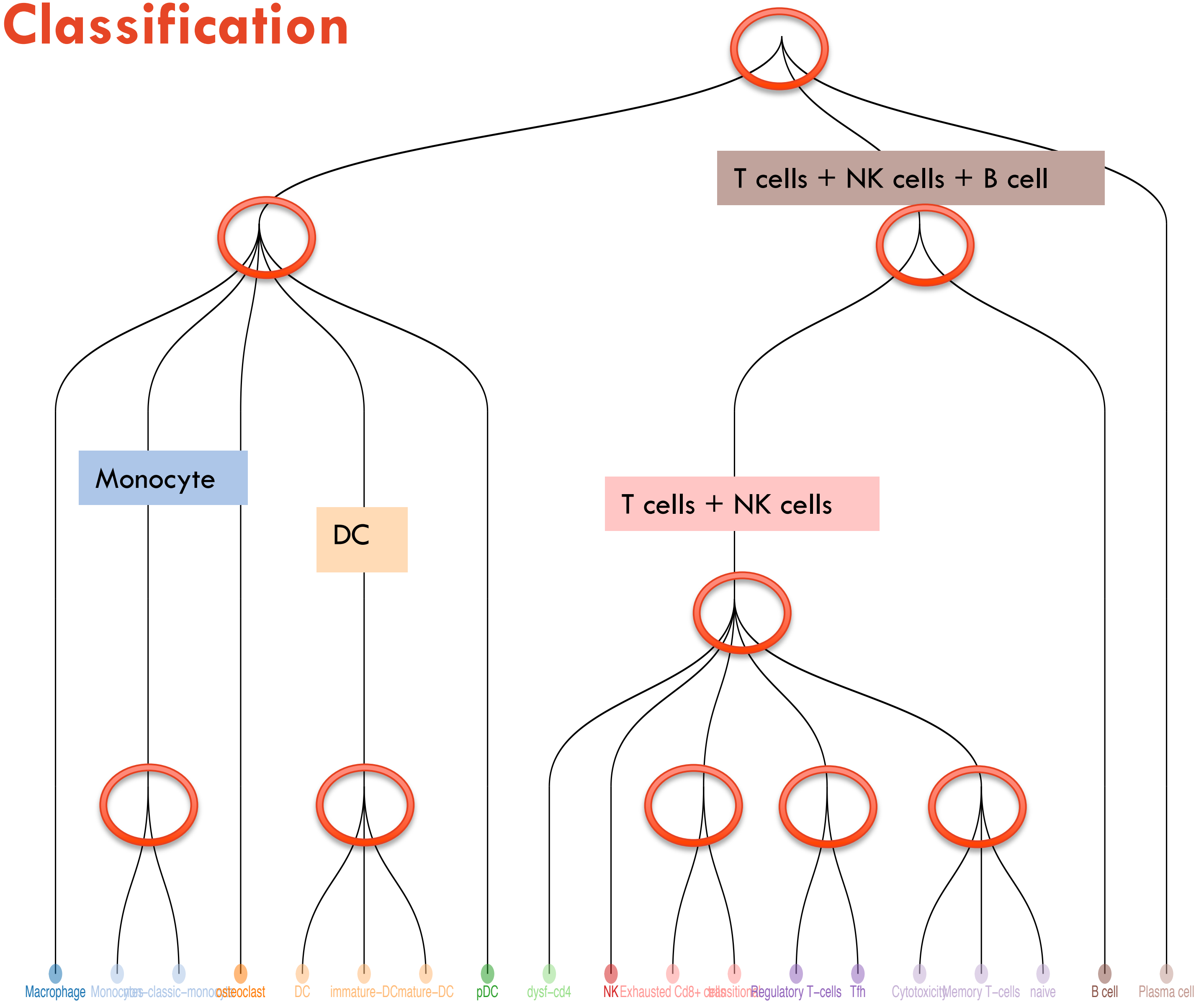
***We use hierarchical ordered partitioning and collapsing hybrid (HOPACH) to generate the cell type hierarchical tree based on the reference dataset.***



van der Laan, M. J. and Pollard, K. S. (2003), *Journal of Statistical Planning and Inference.*

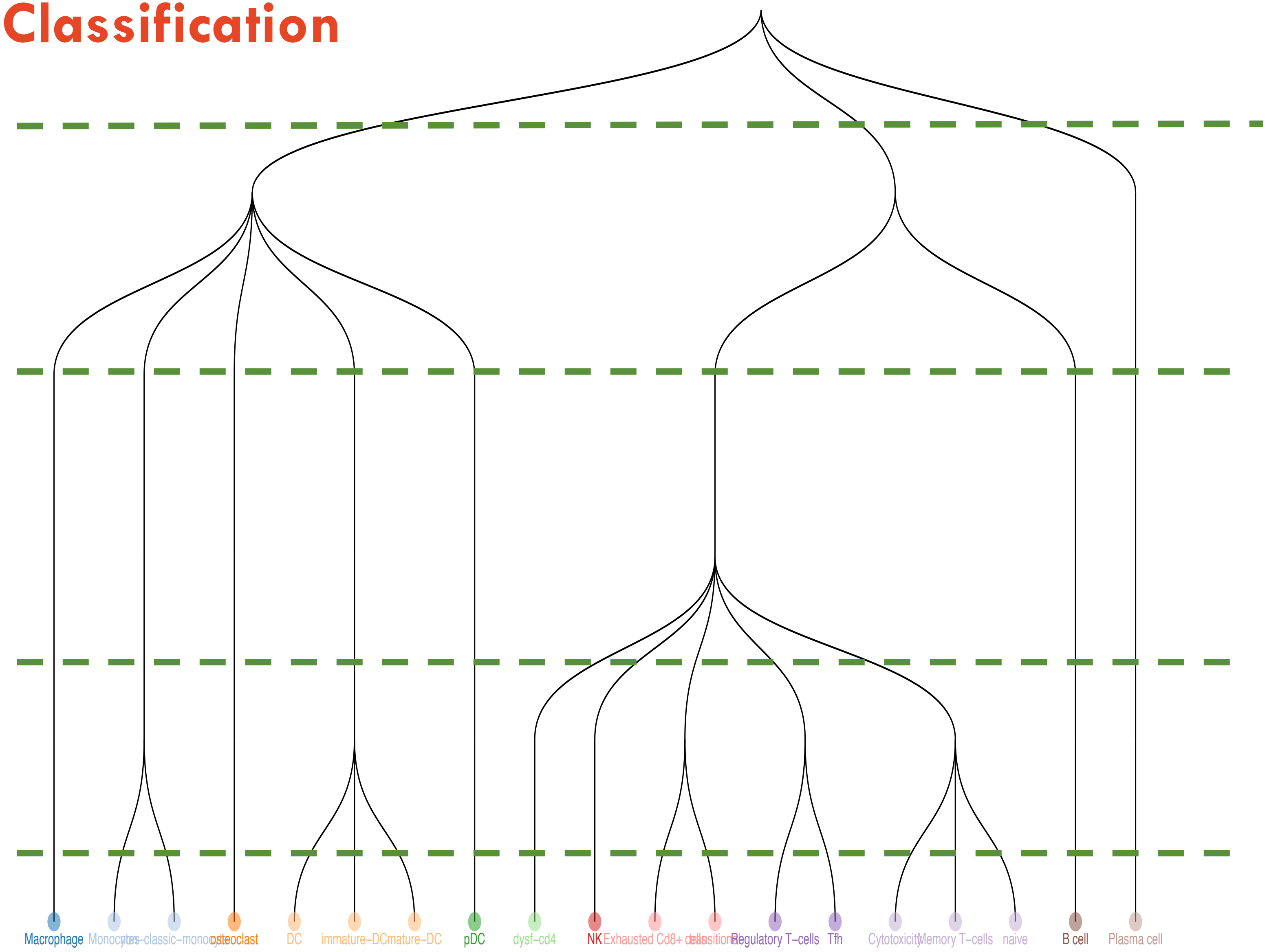
# scClassify: Hierarchical Classification

***Step 2: Feature selection at each branch point.***



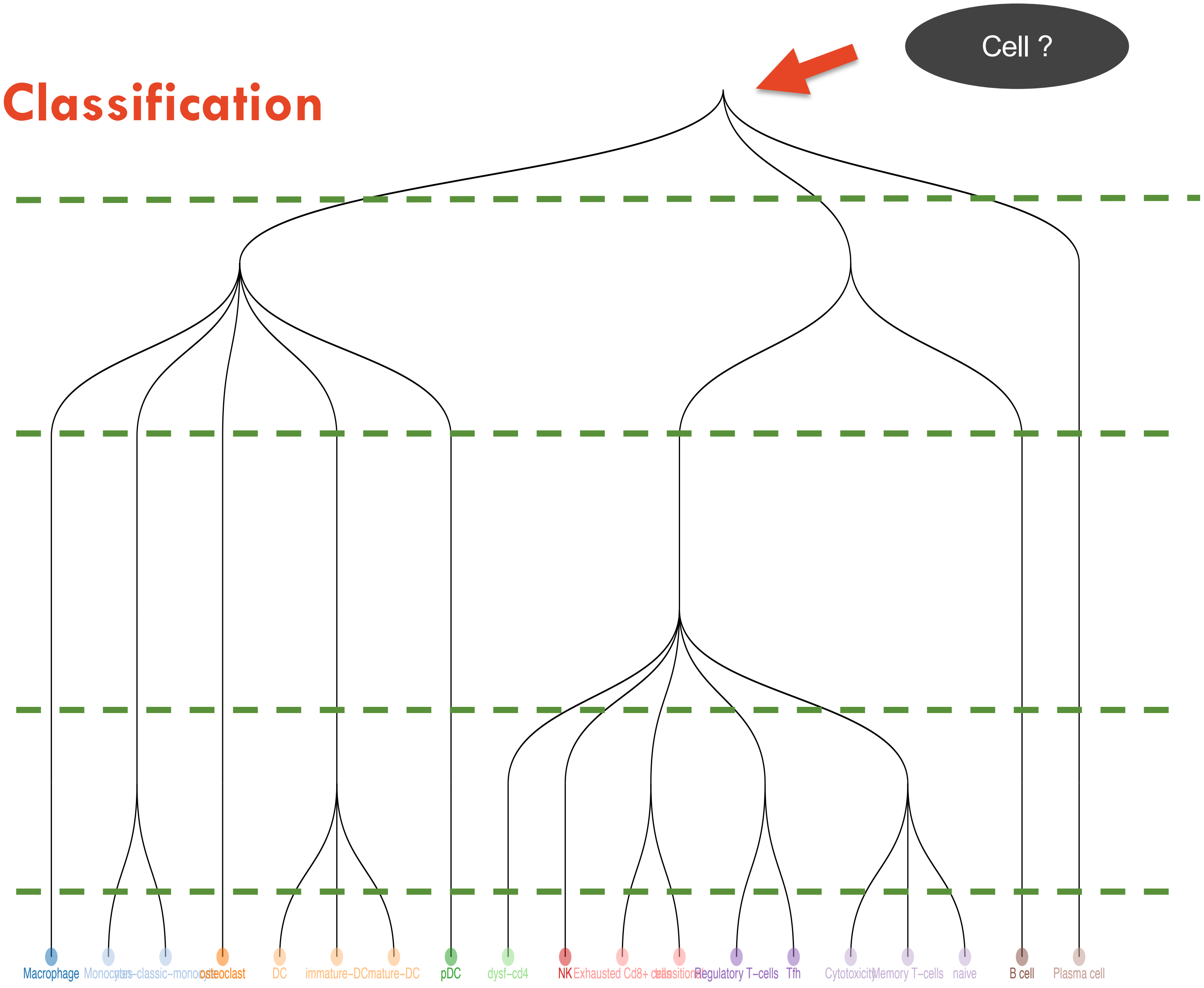
# scClassify: Hierarchical Classification

*Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:*

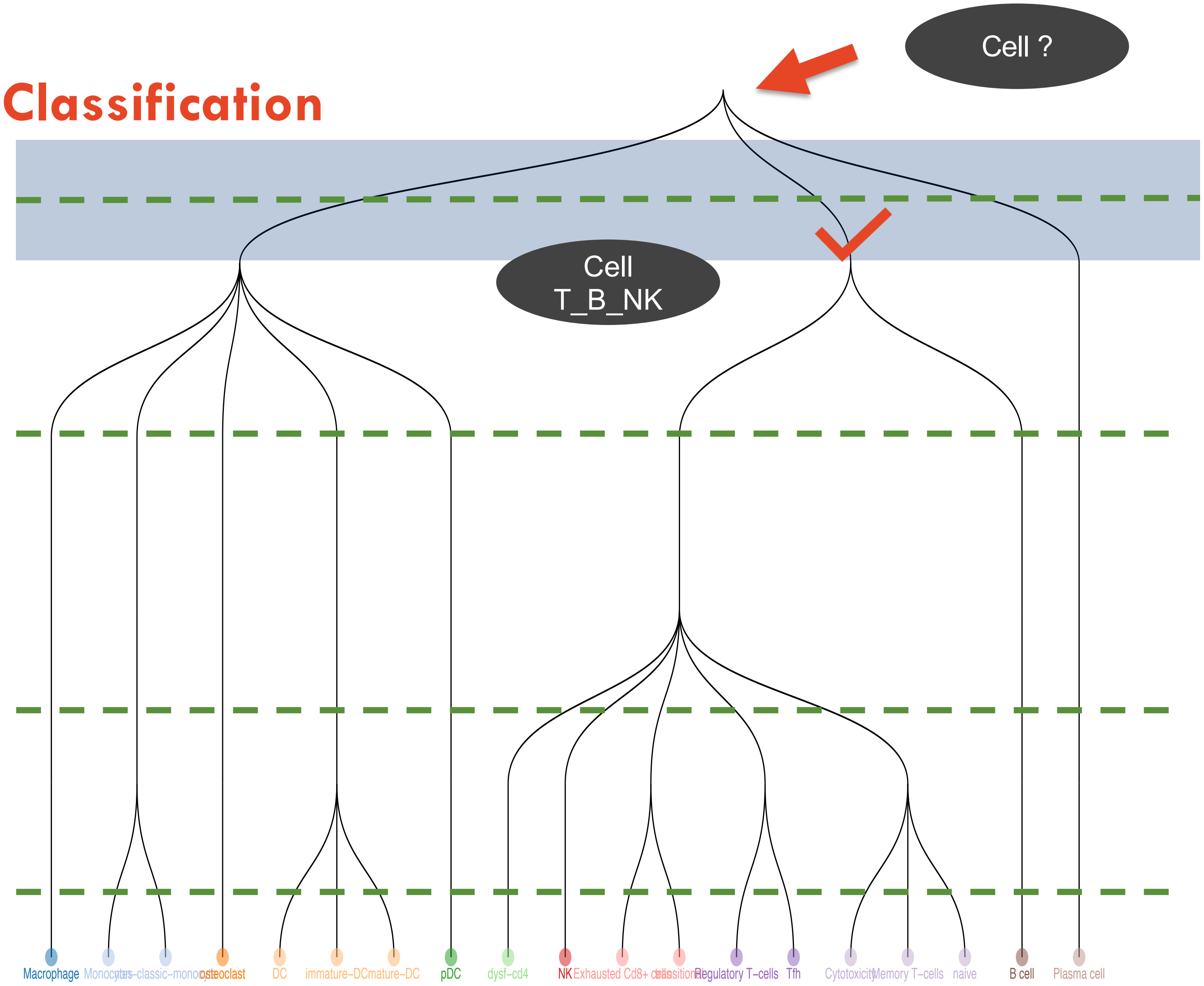


# scClassify: Hierarchical Classification

***Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:***



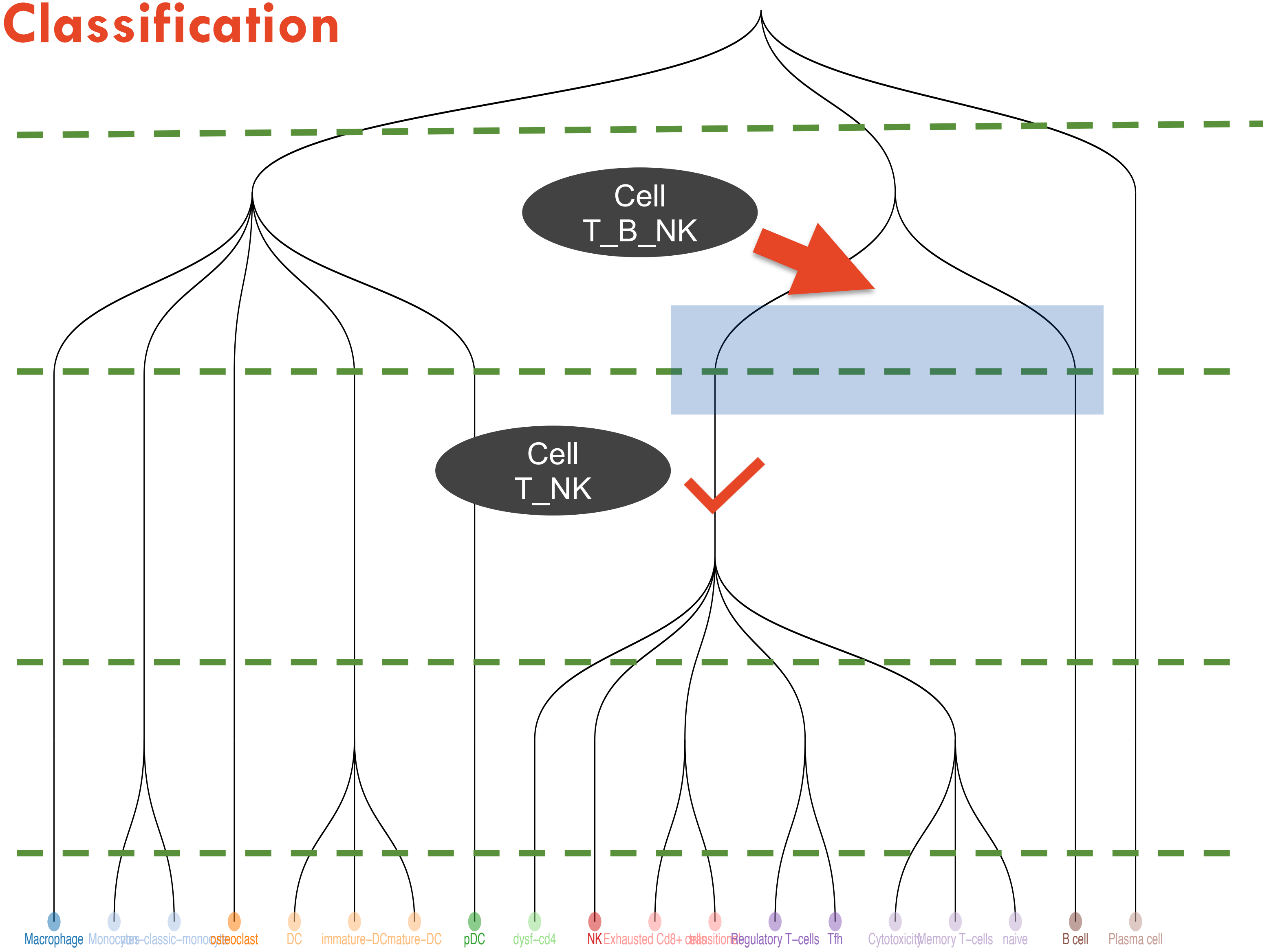
# scClassify: Hierarchical Classification



***Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:***

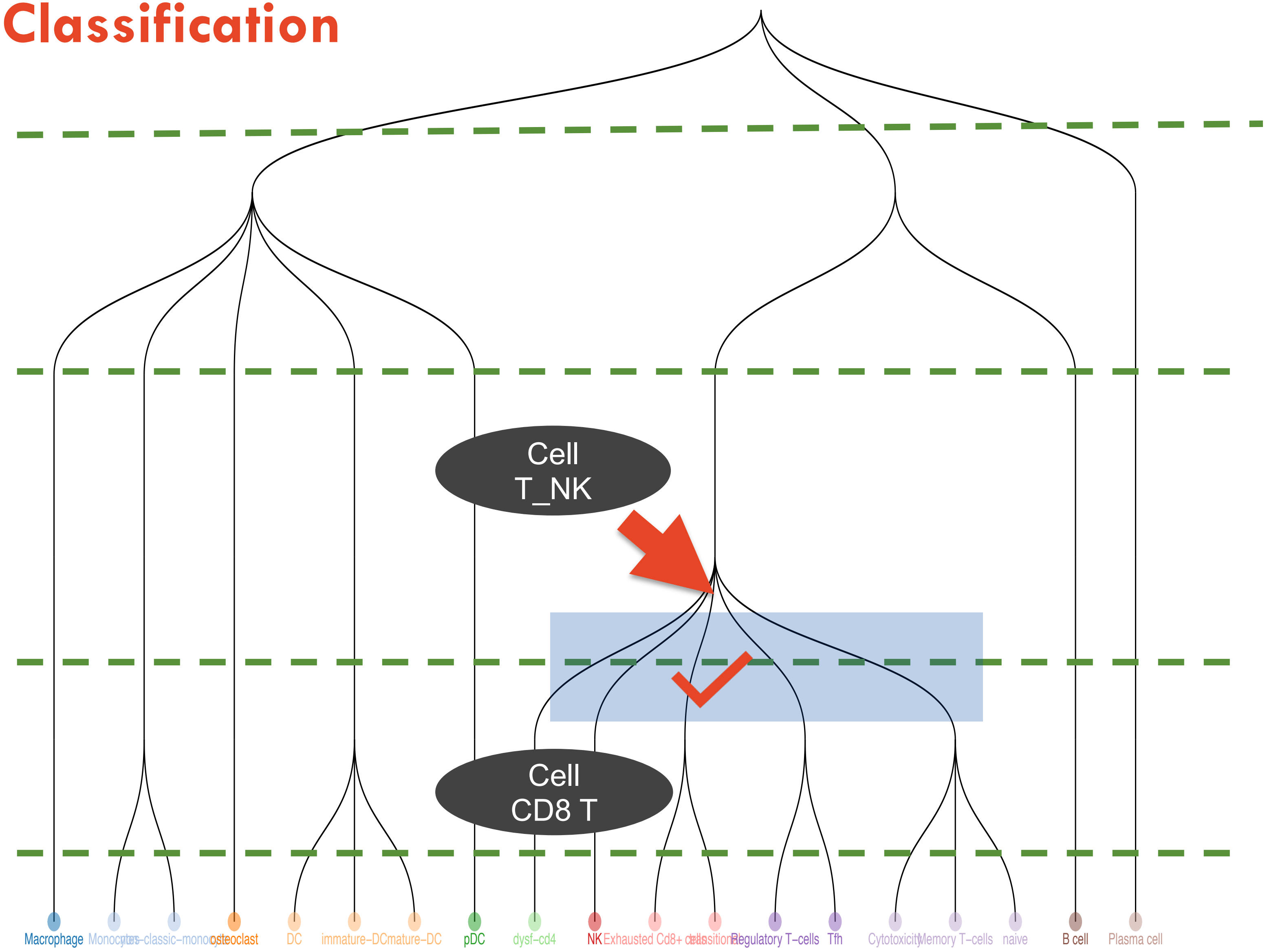
# scClassify: Hierarchical Classification

***Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:***



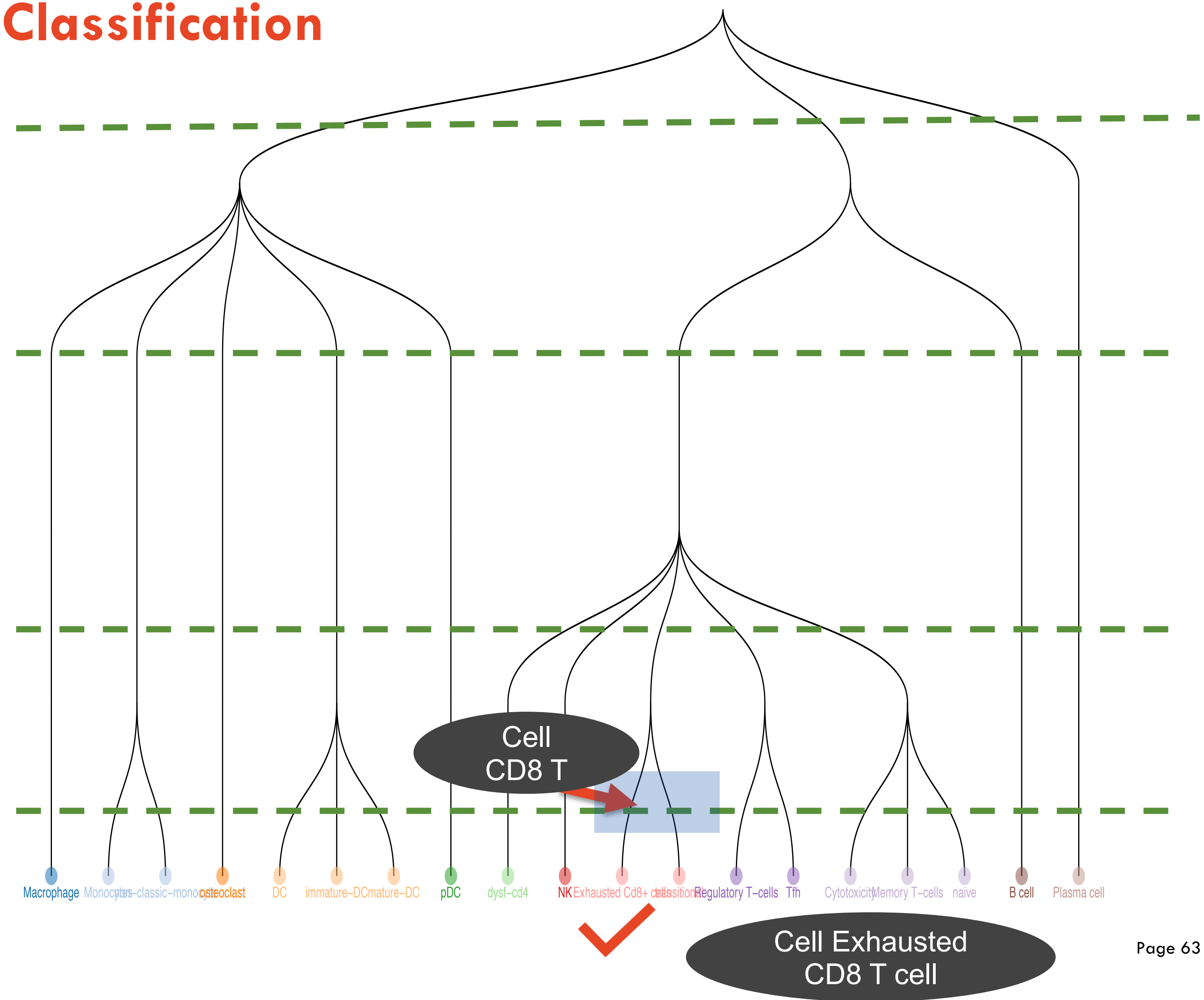
# scClassify: Hierarchical Classification

*Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:*



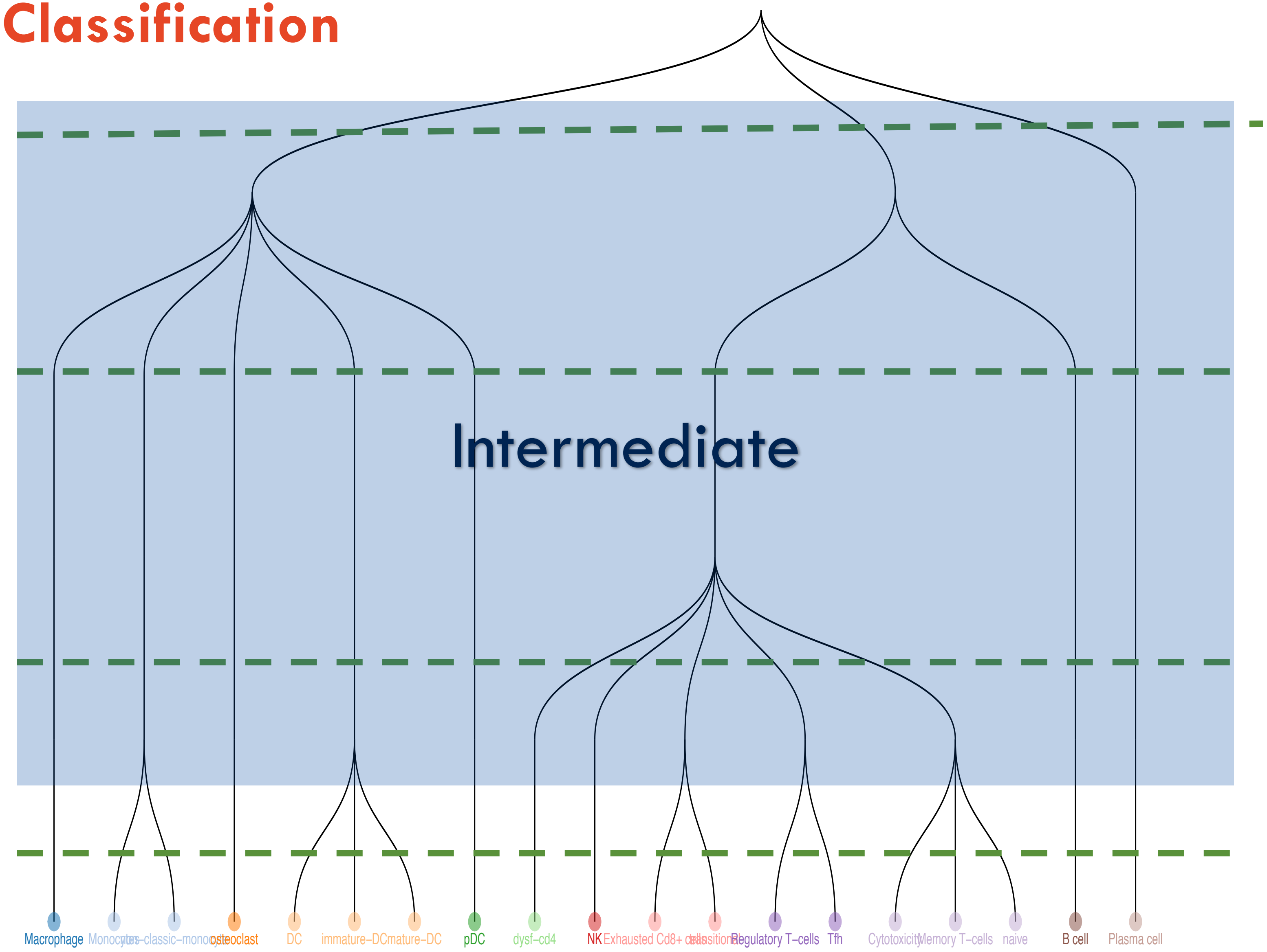
# scClassify: Hierarchical Classification

***Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:***



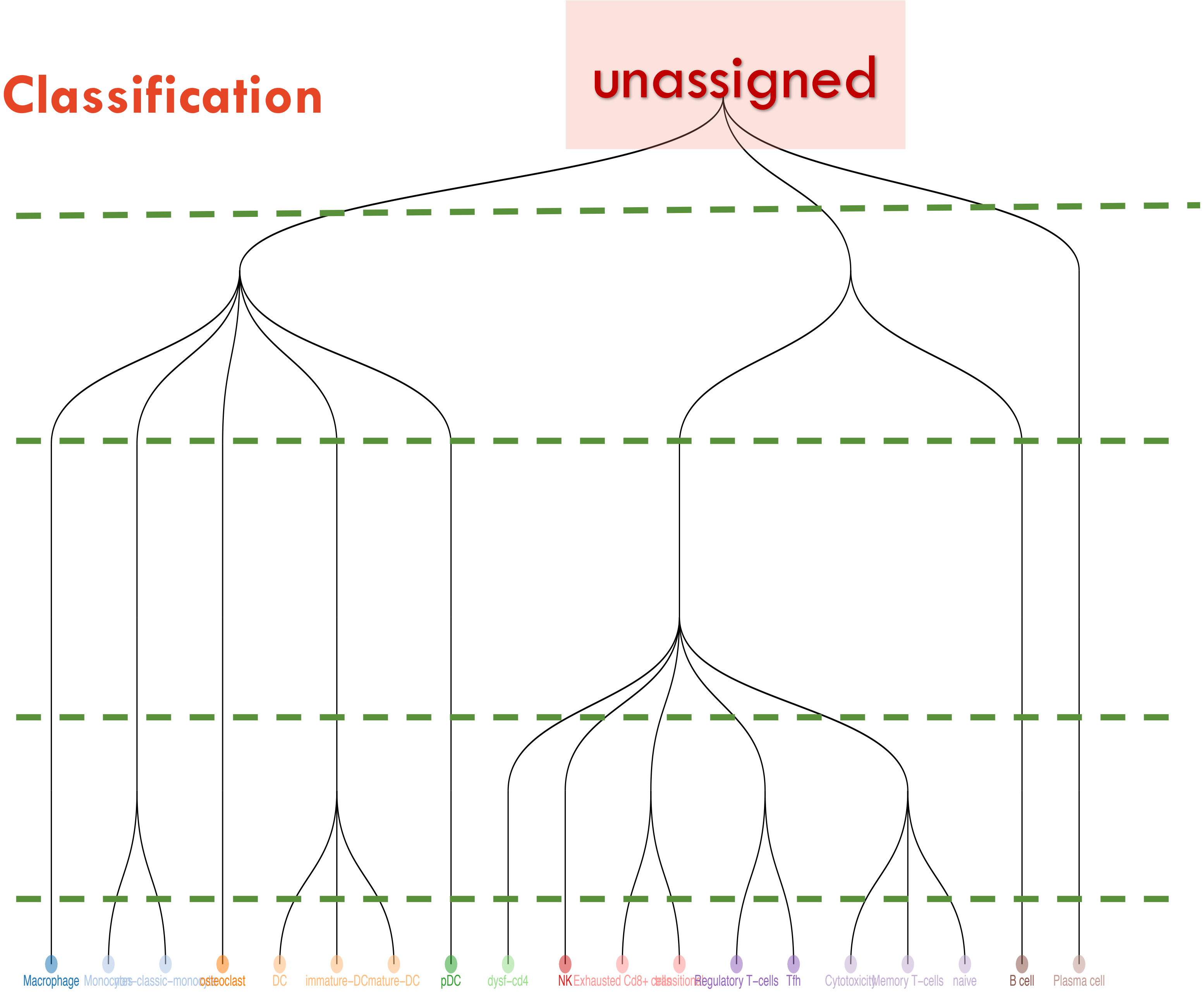
# scClassify: Hierarchical Classification

*Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:*



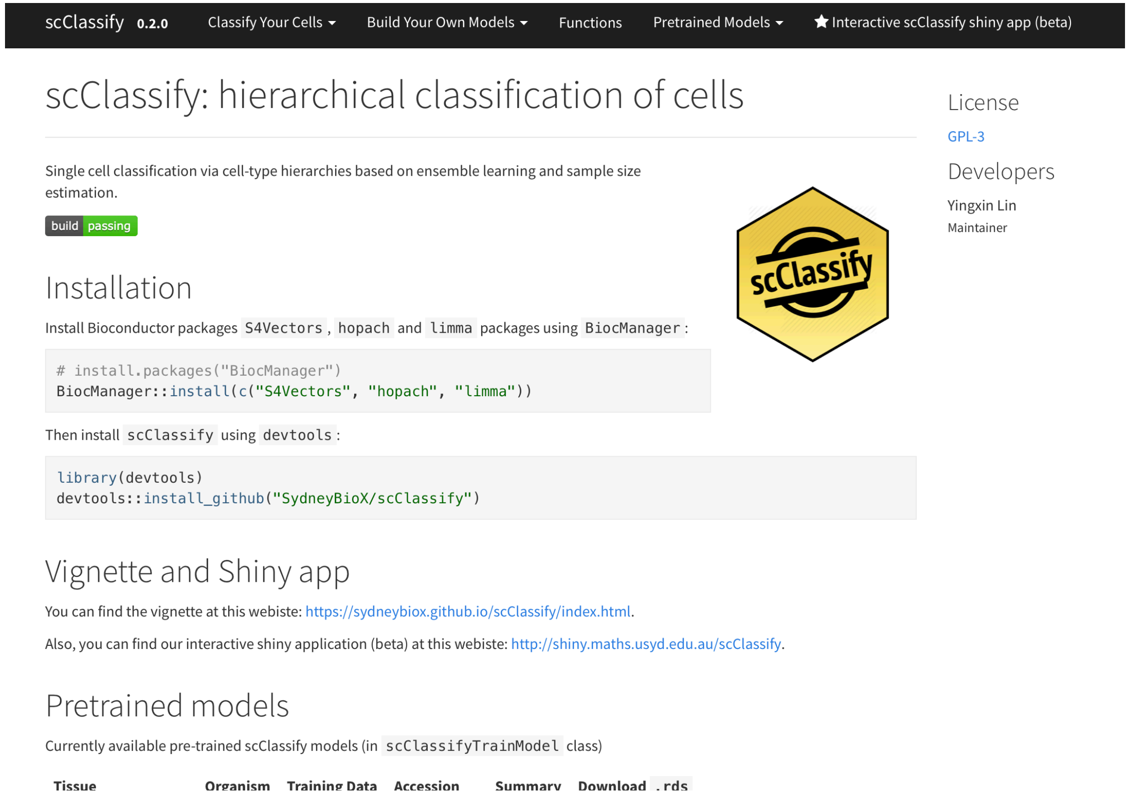
# scClassify: Hierarchical Classification

*Step 3: Performing correlation-based weighted kNN for each level of the cell type hierarchical tree:*



# scClassify

Try scClassify: https://sydneybiox.github.io/scClassify/



## scClassify: hierarchical classification of cells

Single cell classification via cell-type hierarchies based on ensemble learning and sample size estimation.

### Installation

Install Bioconductor packages `S4Vectors`, `hopach` and `limma` packages using `BiocManager`:

```
# install.packages("BiocManager")
BiocManager::install(c("S4Vectors", "hopach", "limma"))
```

Then install `scClassify` using `devtools`:

```
library(devtools)
devtools::install_github("SydneyBioX/scClassify")
```

### Vignette and Shiny app

You can find the vignette at this webiste: https://sydneybiox.github.io/scClassify/index.html.

Also, you can find our interactive shiny application (beta) at this webiste: http://shiny.maths.usyd.edu.au/scClassify.

### Pretrained models

Currently available pre-trained scClassify models (in `scClassifyTrainModel` class)

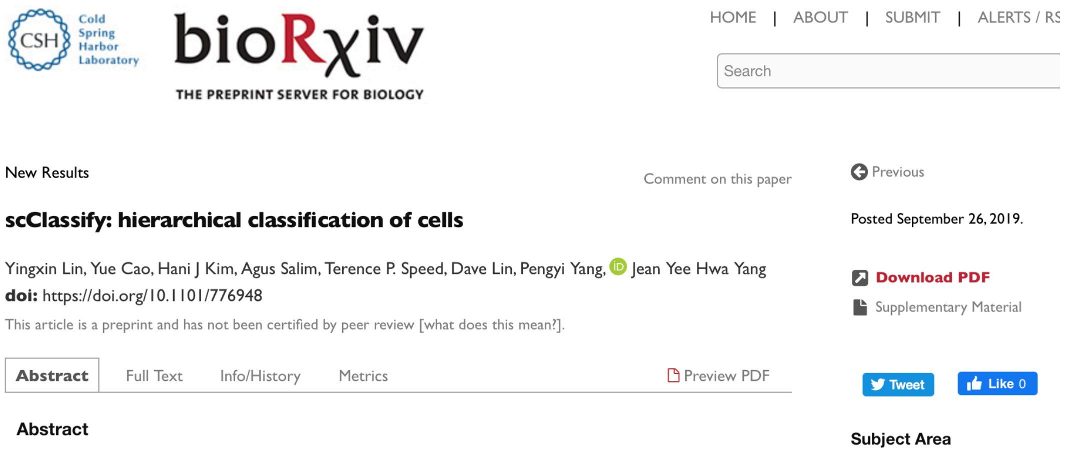
| Tissue | Organism | Training Data | Accession | Summary | Download .rds |
|---|---|---|---|---|---|

License

GPL-3

Developers

Yingxin Lin
Maintainer



New Results

**scClassify: hierarchical classification of cells**

Yingxin Lin, Yue Cao, Hani J Kim, Agus Salim, Terence P. Speed, Dave Lin, Pengyi Yang, Jean Yee Hwa Yang

**doi:** https://doi.org/10.1101/776948

This article is a preprint and has not been certified by peer review [what does this mean?].

Posted September 26, 2019.

**Abstract**

**Subject Area**

# Trajectory inference

## Why trajectory analysis?

- Cells may not be sufficiently be described by a discrete classification system such as clustering
- Biological processes drive the development are usually continuous process
- Trajectory inference therefore can be used to model
  - the transitions between cell identities
  - Branching differentiation process
  - Dynamic gene regularization model

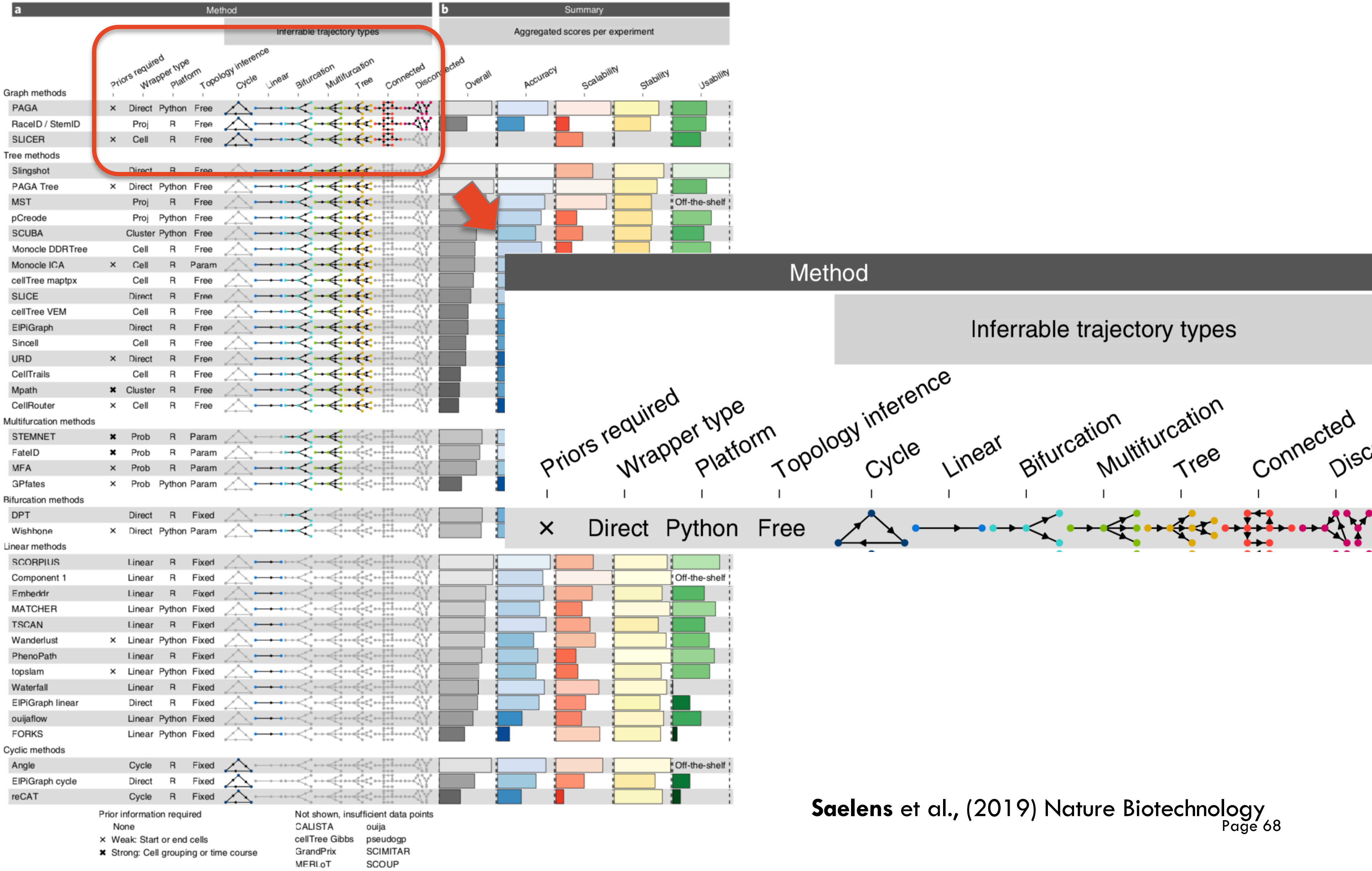
## What is trajectory inference?

- Interpret single-cell data as a snapshot of a continuous process.

## Typical steps involved in trajectory inference:

- Reduce the dimensionality of the single cell data
- Finding paths through the reduced dimension space, by minimizing the changes between neighboring cells
- Order the cells by pseudotime

# Comparisons of pseudotime inference methods



| | Priors required | Wrapper type | Platform | Topology inference |
|---|---|---|---|---|
| **Graph methods** | | | | |
| PAGA | × | Direct | Python | Free |
| RaceID / StemID | | Proj | R | Free |
| SLICER | × | Cell | R | Free |
| **Tree methods** | | | | |
| Slingshot | | Direct | R | Free |
| PAGA Tree | × | Direct | Python | Free |
| MST | | Proj | R | Free |
| pCreode | | Proj | Python | Free |
| SCUBA | | Cluster | Python | Free |
| Monocle DDRTree | | Cell | R | Free |
| Monocle ICA | × | Cell | R | Param |
| cellTree maptpx | | Cell | R | Free |
| SLICE | | Direct | R | Free |
| cellTree VEM | | Cell | R | Free |
| ElPiGraph | | Direct | R | Free |
| Sincell | | Cell | R | Free |
| URD | × | Direct | R | Free |
| CellTrails | | Cell | R | Free |
| Mpath | ✖ | Cluster | R | Free |
| CellRouter | × | Cell | R | Free |
| **Multifurcation methods** | | | | |
| STEMNET | ✖ | Prob | R | Param |
| FateID | ✖ | Prob | R | Param |
| MFA | × | Prob | R | Param |
| GPfates | × | Prob | Python | Param |
| **Bifurcation methods** | | | | |
| DPT | | Direct | R | Fixed |
| Wishbone | × | Direct | Python | Param |
| **Linear methods** | | | | |
| SCORPIUS | | Linear | R | Fixed |
| Component 1 | | Linear | R | Fixed |
| Embeddr | | Linear | R | Fixed |
| MATCHER | | Linear | Python | Fixed |
| TSCAN | | Linear | R | Fixed |
| Wanderlust | × | Linear | Python | Fixed |
| PhenoPath | | Linear | R | Fixed |
| topslam | × | Linear | Python | Fixed |
| Waterfall | | Linear | R | Fixed |
| ElPiGraph linear | | Direct | R | Fixed |
| ouijaflow | | Linear | Python | Fixed |
| FORKS | | Linear | Python | Fixed |
| **Cyclic methods** | | | | |
| Angle | | Cycle | R | Fixed |
| ElPiGraph cycle | | Direct | R | Fixed |
| reCAT | | Cycle | R | Fixed |

Prior information required
None
× Weak: Start or end cells
✖ Strong: Cell grouping or time course

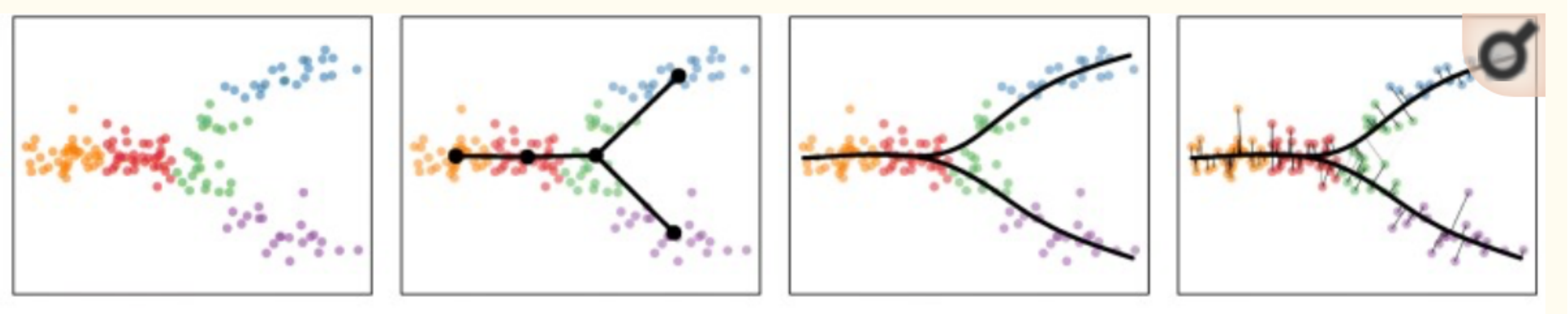
| Not shown, insufficient data points | |
|---|---|
| CALISTA | ouija |
| cellTree Gibbs | pseudogp |
| GrandPrix | SCIMITAR |
| MERLoT | SCOUP |

**Saelens** et al., (2019) Nature Biotechnology

# Slingshot example (Street et al., 2018)

Three stages:

1. Reduced dimension of the data
2. Inference of the global lineage structure. Uses cluster-based minimum spanning tree
3. Inference of pseudotime variables for cells along each lineage. Fits simultaneous principal curves



# Summary

# Acknowledgment

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**Taiyun Kim**
Mengbo Li
**Yingxin Lin**
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**Andy Tran**
Andy Wang
**Kevin Wang**
Xiangnan Xu
Weichang Yu
Yunwei Zhang

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**Shila Ghazanfar**