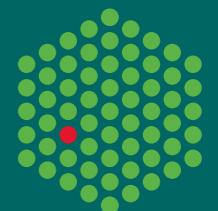


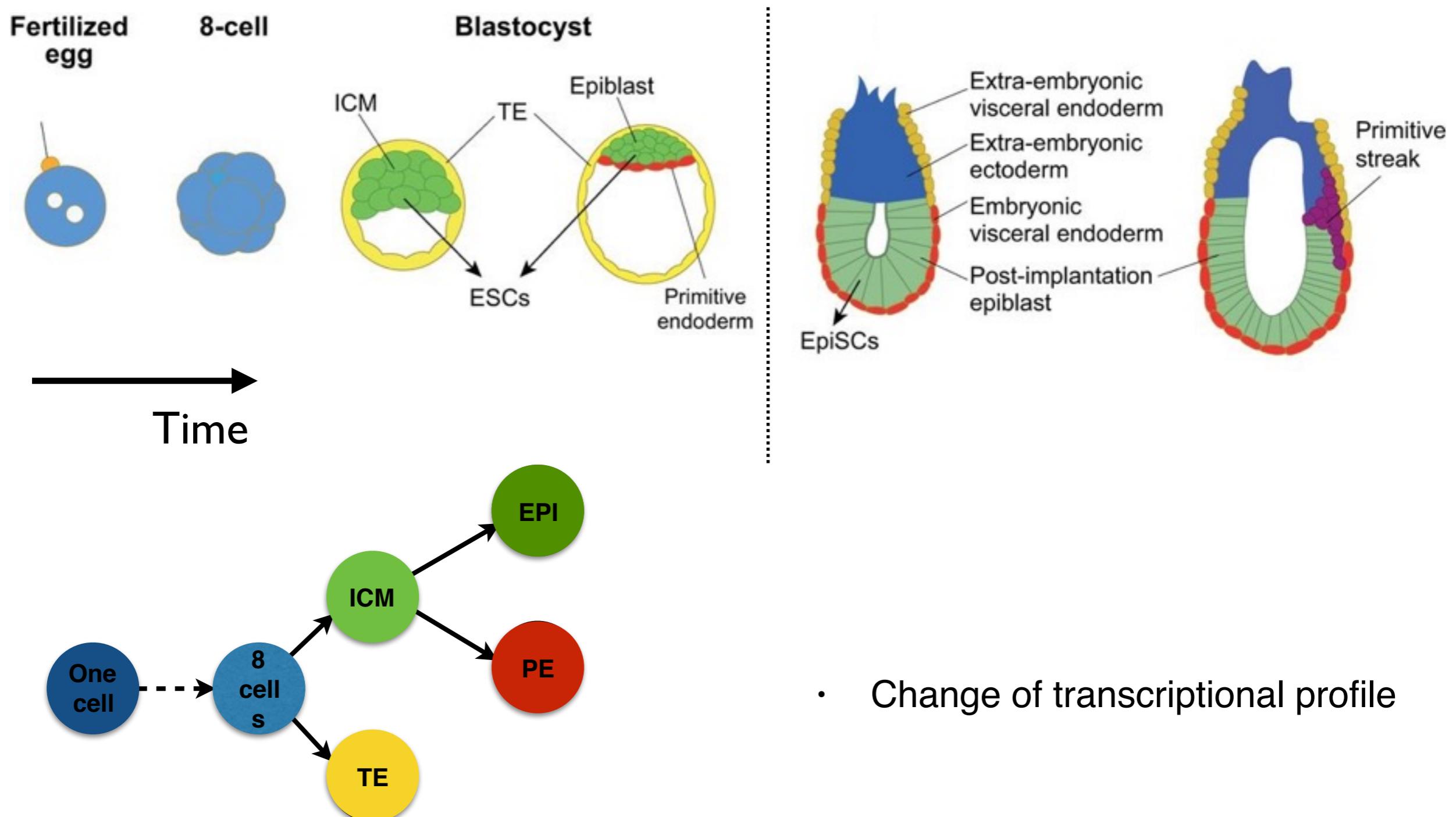
# Pseudotime and lineage tree reconstruction from single cell transcriptomics data

Laleh Haghverdi  
July 2018

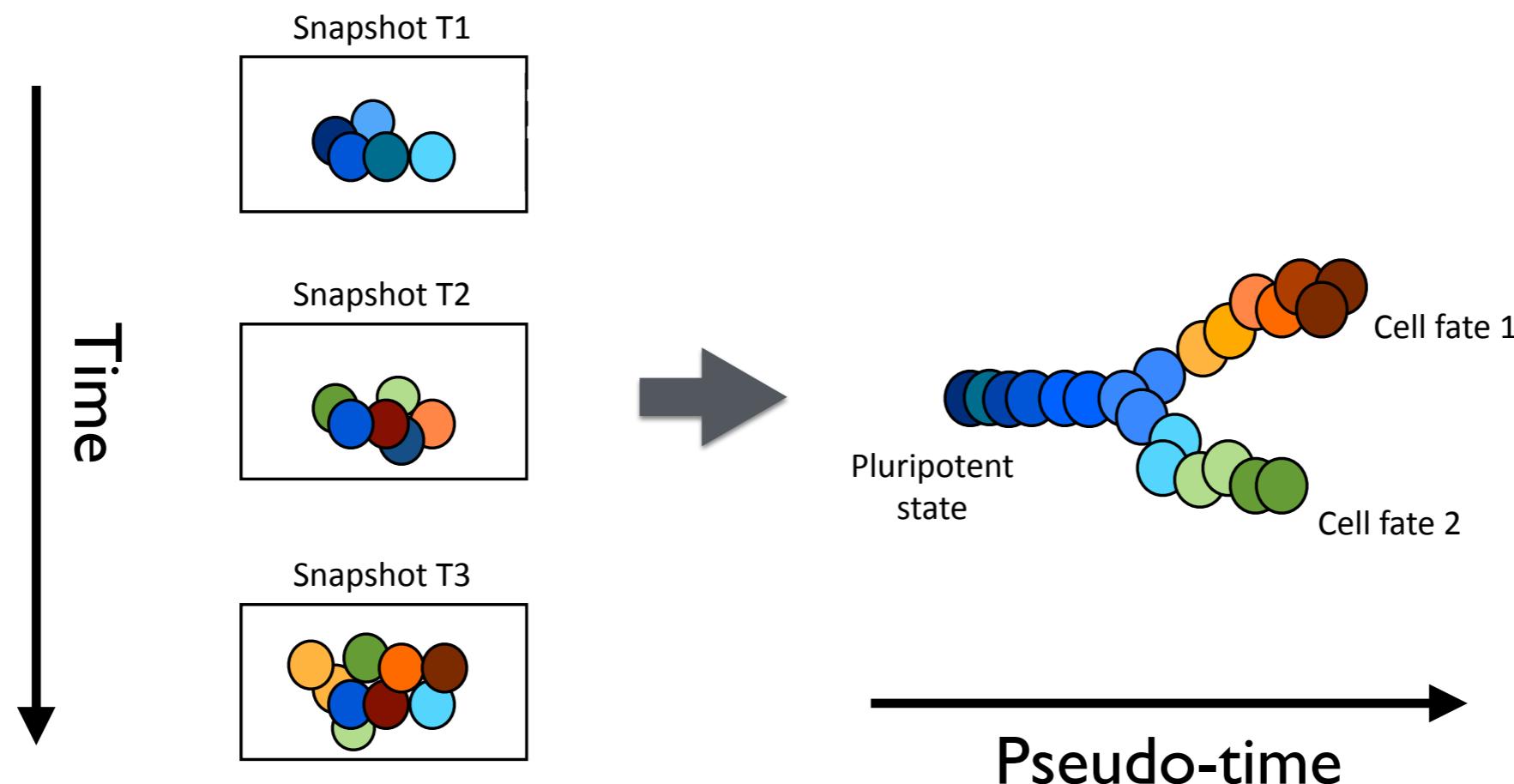
EMBL



# Cell differentiation, lineage trees



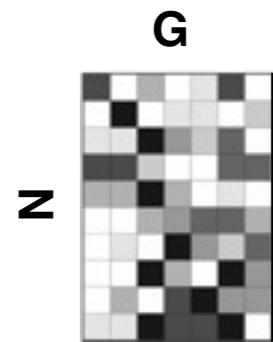
# Lineage tree reconstruction



- Asynchronous development of cells → bulk measurements fail

# Single cell transcriptomics data and challenges

- scRNA-seq, sc-qPCR, flow cytometry, mass cytometry.
- Data matrix of size  $n \times G$
- High-dimensional data, with too many irrelevant features (i.e. genes) noise dominates the signal —> selection of highly variable genes
- Big noise and dropouts



# Single-cell data embedding and lineage tree analysis

- PCA (Wold et al. *Chemometrics and intelligent laboratory systems* 1987)
- t-SNE (Maaten, *Journal of machine learning research* 2008)
- Diffusion maps (Coifman et al. *PNAS* 2005)  
*(non-linear methods try to accommodate more information in lower dimensions)*
- SPADE (Qiu, Peng, et al. *Nature biotechnology* 2011)
- *monocle2* (Qiu, Xiaojie, et al. *Nature methods* 2017)
- *Wishbone* (Setty et al. *Nature biotechnology* 2016)
- *DPT* (Haghverdi et al. *Nature methods* 2016)

# PCA

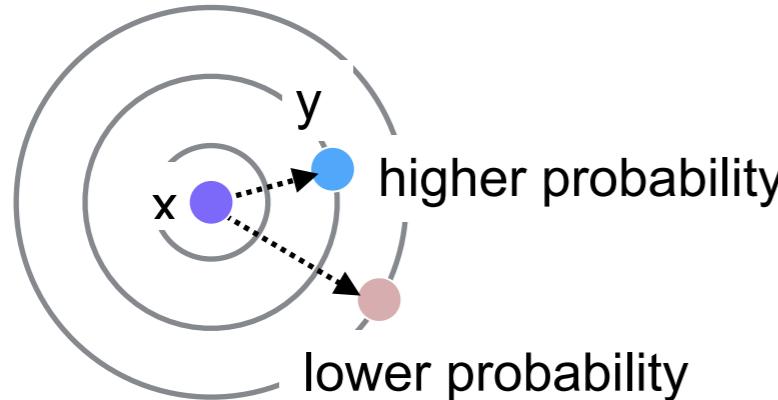
centred data matrix  $X [N, G]$

$$\operatorname{argmax}_W \{ W^T X^T X W \}$$
$$\underbrace{\phantom{W^T}}_{Y^T} \quad \underbrace{\phantom{X^T X}}_{*} \quad Y$$

$Y$  is orthonormal, so what we are maximizing is just the variance of the embedded data  $Y$

# Diffusion maps

Gaussian kernel



$$T_{n \times n}$$

$$\psi_i \mathbf{T} = \lambda_i \psi_i$$

$$1 = \lambda_0 \geq \lambda_1 \geq \dots \geq \lambda_{n-1}$$

$$D_t^2(x, y) = \|\mathbf{T}^t(x, \cdot) - \mathbf{T}^t(y, \cdot)\|^2 \quad (\text{for symmetric } T)$$

$$= \sum_{i=1}^{n-1} \lambda_i^{2t} (\psi_i(x) - \psi_i(y))^2$$

# t-SNE

Original (high-dim) space X

$$p_{ij} = \frac{1}{z_X(i)} \cdot \exp\left(-\frac{\|\mathbf{X}_i - \mathbf{X}_j\|^2}{2\sigma^2}\right)$$

$$z_X(i) = \sum_j \exp\left(-\frac{\|\mathbf{X}_i - \mathbf{X}_j\|^2}{2\sigma^2}\right)$$

$$\mathbf{P} \leftarrow \frac{\mathbf{P} + \mathbf{P}'}{2n}$$

embedding (e.g. 2D) space Y

$$q_{ij} = \frac{1}{z_Y} \cdot \frac{1}{1 + \|\mathbf{Y}_i - \mathbf{Y}_j\|^2}$$

$$z_Y = \sum_{k \neq l} \frac{1}{1 + \|\mathbf{Y}_k - \mathbf{Y}_l\|^2}$$

- Minimize the cost function

$$C = KL(\mathbf{P} || \mathbf{Q}) = \sum_{i \neq j} p_{ij} \frac{\log(p_{ij})}{\log(q_{ij})}$$

- Initialise with  $\mathbf{Y}^{(0)}$

$$\frac{\partial C}{\partial \mathbf{Y}_i} = 4 \sum_j (p_{ij} - q_{ij})(\mathbf{Y}_i - \mathbf{Y}_j)(1 + ||\mathbf{Y}_i - \mathbf{Y}_j||^2)^{-1}$$

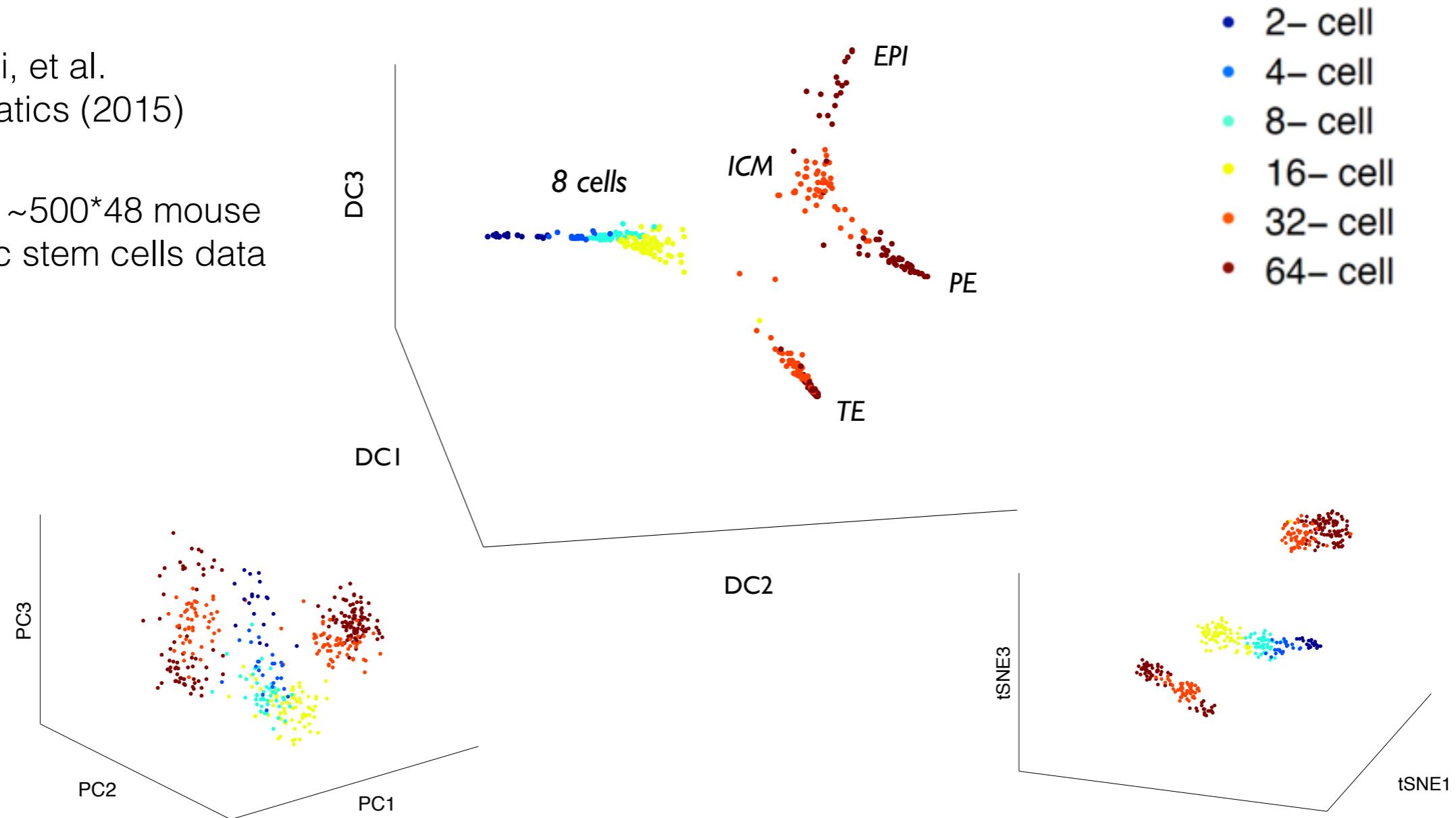
$$\mathbf{Y}^{(t)} = \mathbf{Y}^{(t-1)} + \eta \frac{\partial C}{\partial \mathbf{Y}_i} + \alpha(t)(\mathbf{Y}^{(t-1)} - \mathbf{Y}^{(t-2)})$$

- The final solution for Y depends on the initialisation  $\mathbf{Y}^{(0)}$
- t-SNE does not respect the global continuity of data manifold

# PCA, t-SNE, Diffusion maps

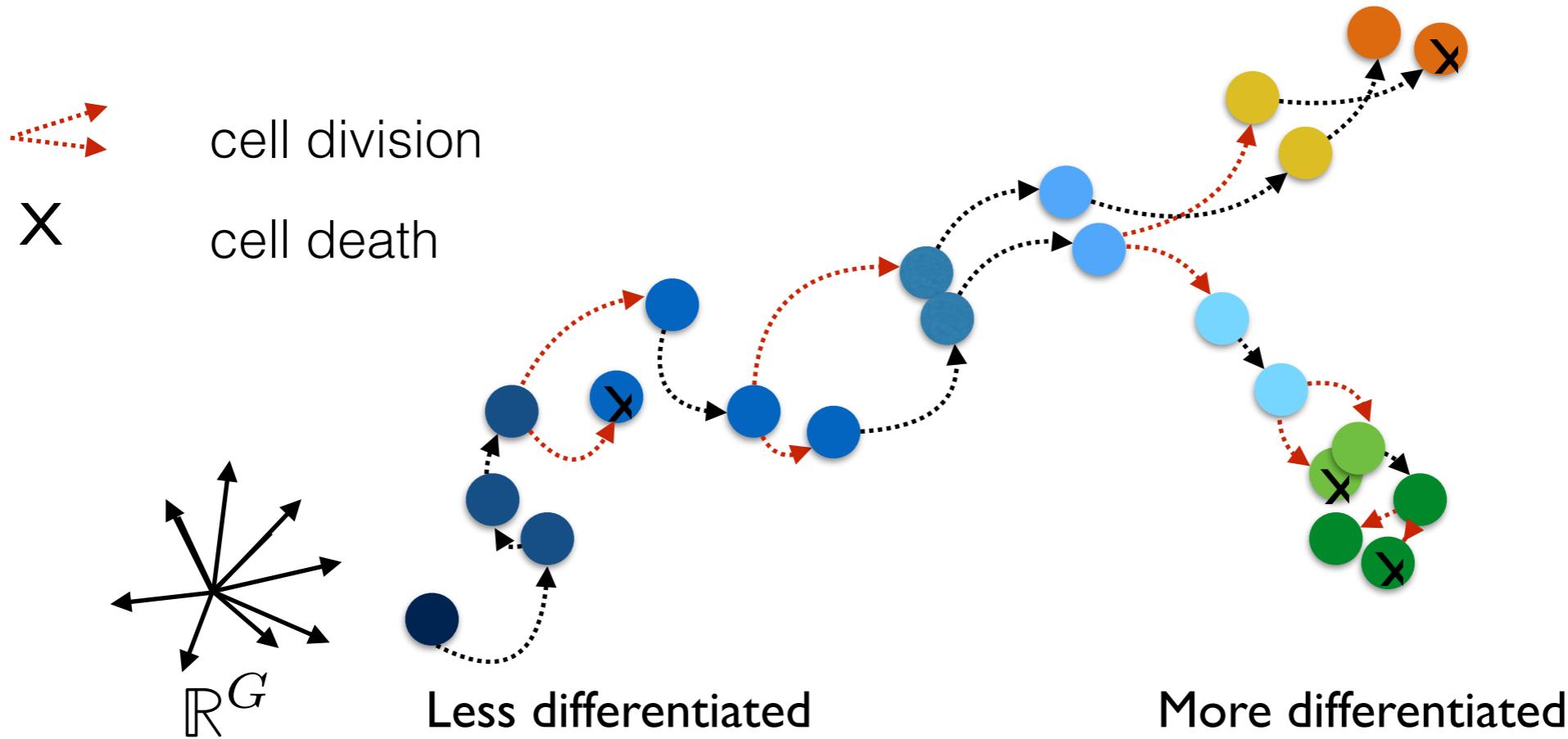
Haghverdi, et al.  
Bioinformatics (2015)

sc-qPCR ~500\*48 mouse  
embryonic stem cells data  
set



- Keeps the global continuity structure
- Robust to noise
- Intrinsically related to the biological process

# Cell differentiation; a diffusion-like process



$$\frac{\partial p}{\partial t} = \frac{\partial^2}{\partial x^2} (Dp(t)) - \frac{\partial}{\partial x} (\mu p(t)) + S$$

$$p(t) = p(t-1) + \Delta p$$

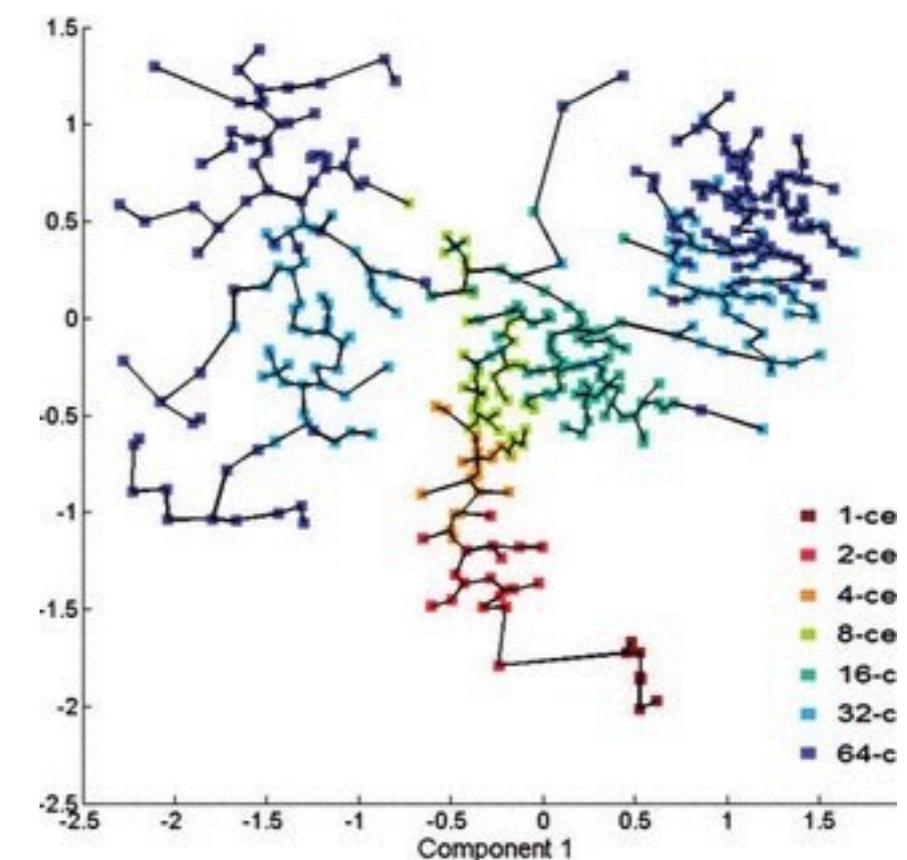
$$p(t) = p(t-1)T$$

- Different types of time evolution dynamics can take place on the same manifold (Butler et al. Electronic journal of linear algebra 2007)

# Going beyond mere embedding and visualisation; pseudo-time and branch identification

## Minimum Spanning tree

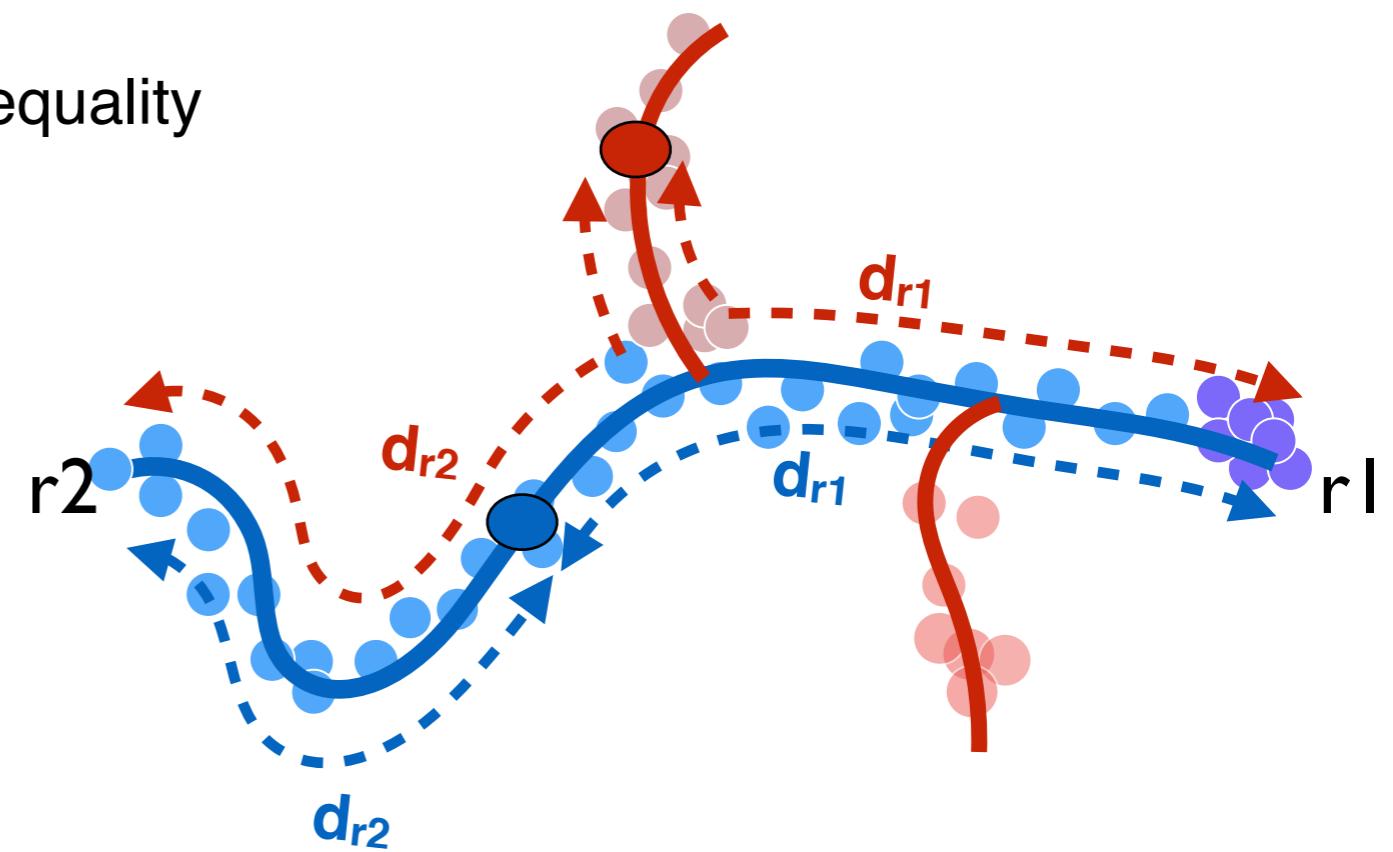
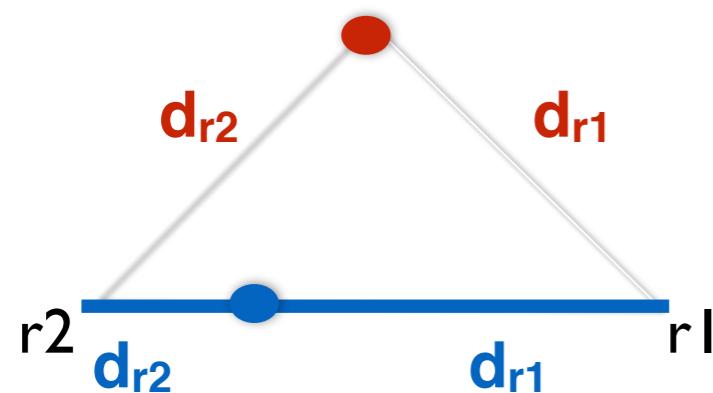
- Minimum total edges distance that goes through all nodes
- In general not robust; requires a neat low-dimensional embedding



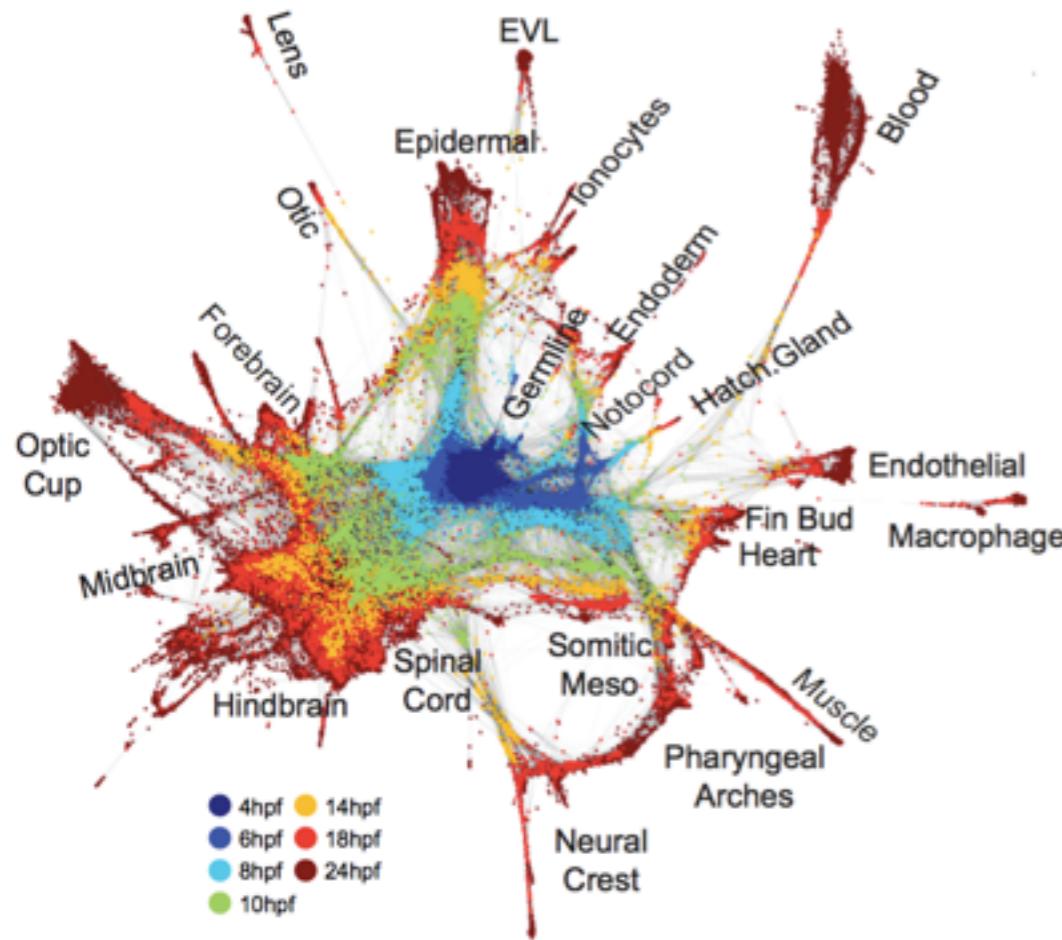
# Going beyond mere embedding and visualisation; pseudo-time and branch identification

## Using an on-manifold metric

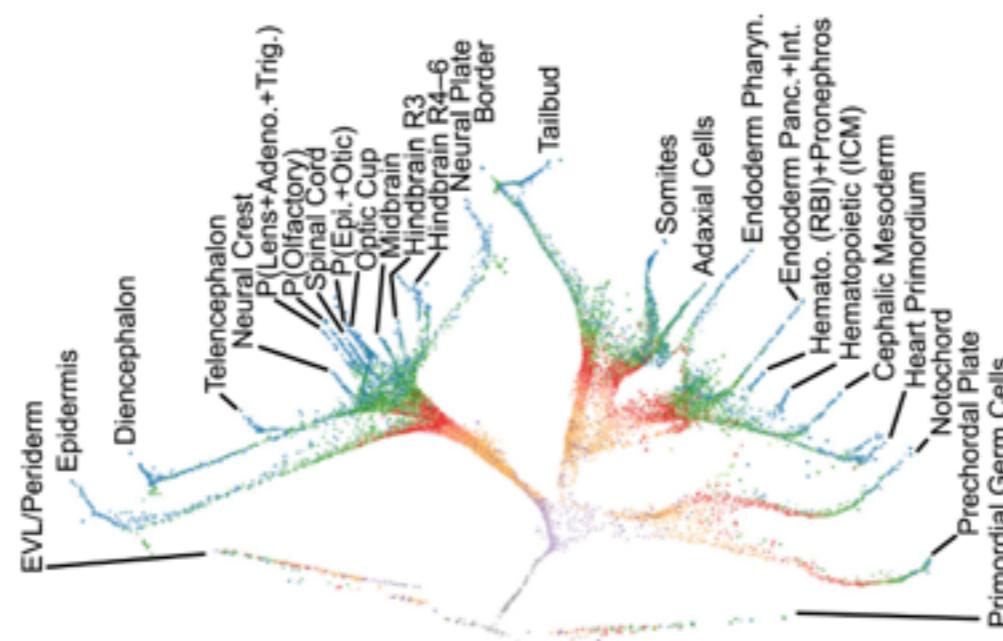
- Pseudotime defined by on-manifold distance from the root cell
- Branch identification using triangle inequality



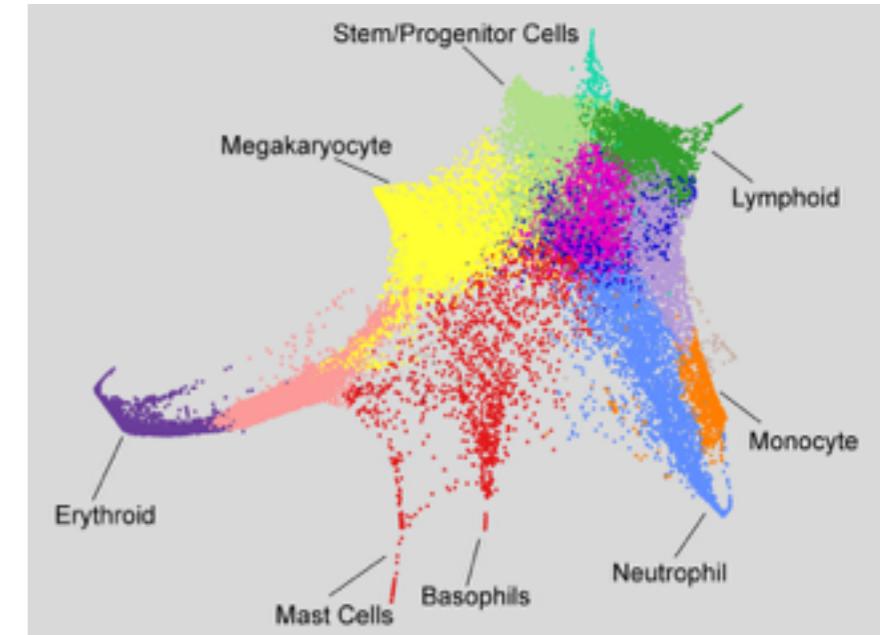
# Complex manifolds with several branching events



Zebrafish (Wagner, Daniel E., et al. *Science* 2018)



Zebrafish (Farrell, Jeffrey A., et al. 2018)



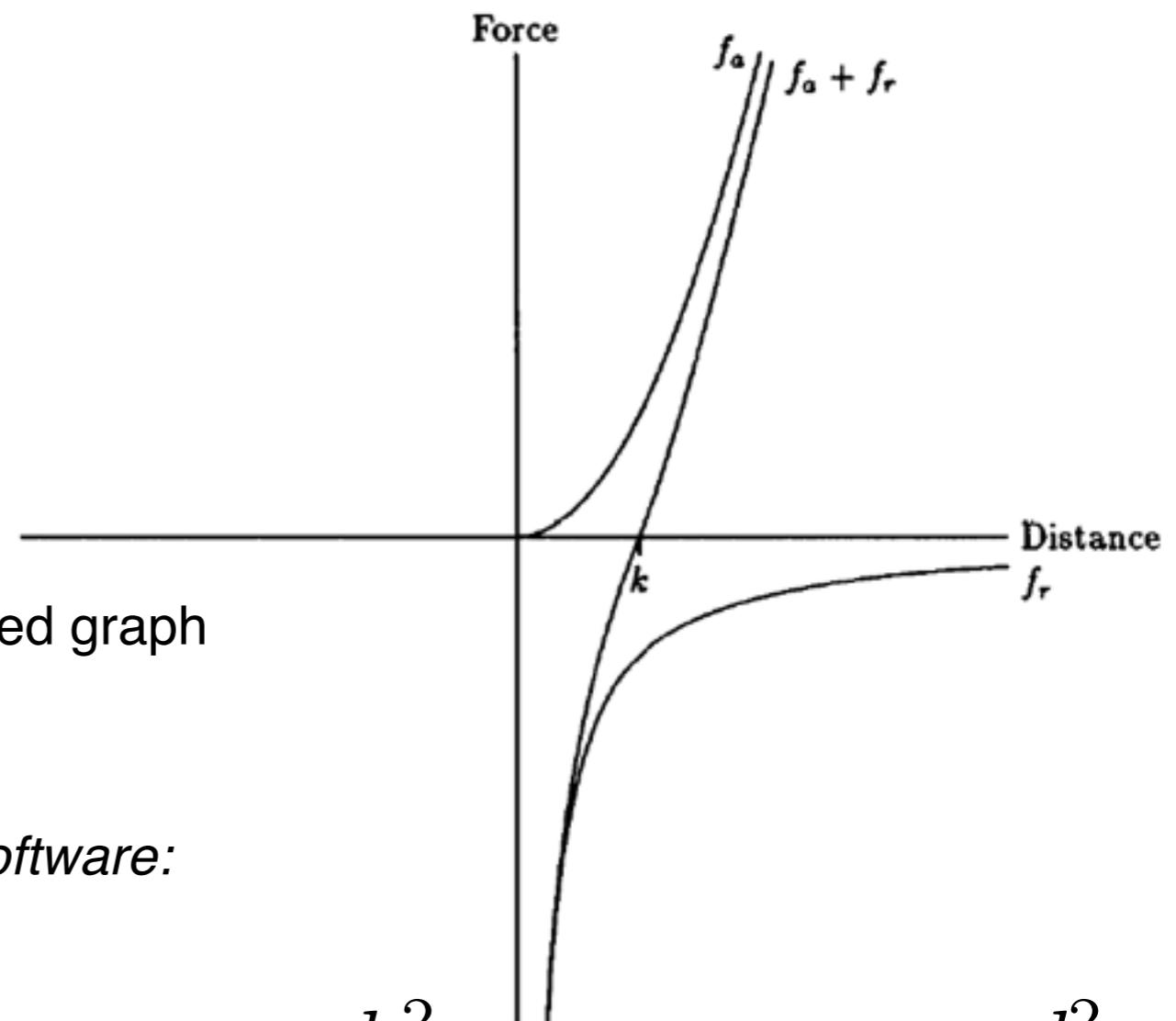
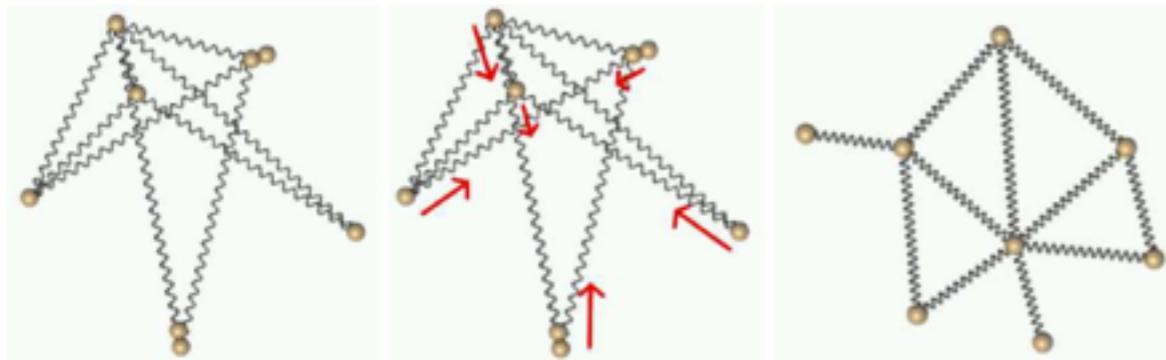
Blood (Berthold Goetgens lab 2018)

# Complex manifolds with several branching events

- Force-directed graphs
- Taking directed dynamics into account
- SPRING (Weinreb et al.  
*Bioinformatics* 2017) → User-defined root cell and endpoints  
to infer directed diffusions
- URD (Farrell et al. *Science* 2018)
- Velocyto (La Manno, *bioRxiv* 2017) → Estimate the velocity vector for each cell

# Force-directed embedding

- Minimize the energy of a springs system



- Just a visualisation; requires a prior neatly weighted graph (e.g. careful knn graph construction)
- Fruchterman and Reingold (Fruchterman et al. *Software: Practice and experience*, 1991)

skips local minima

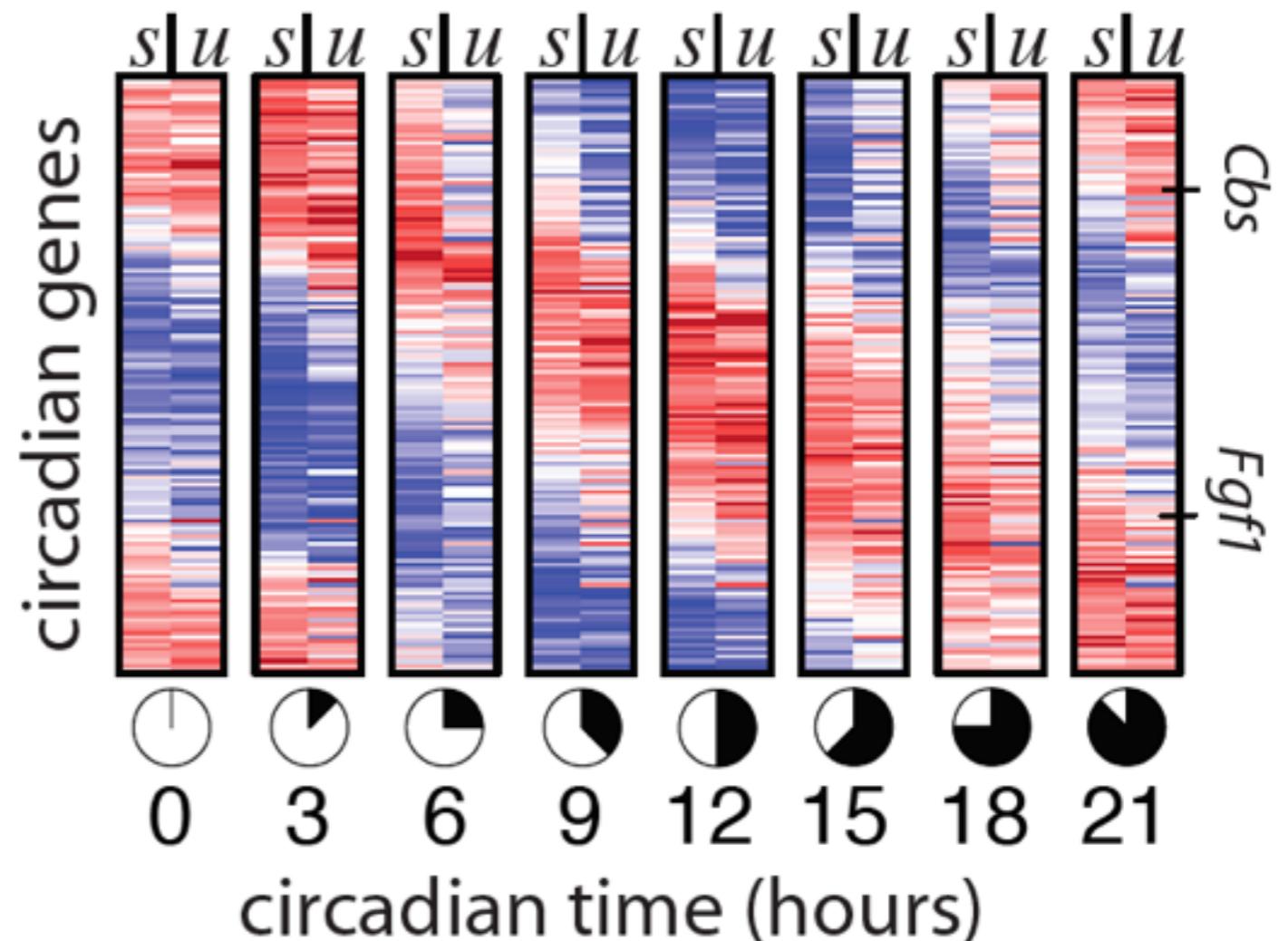
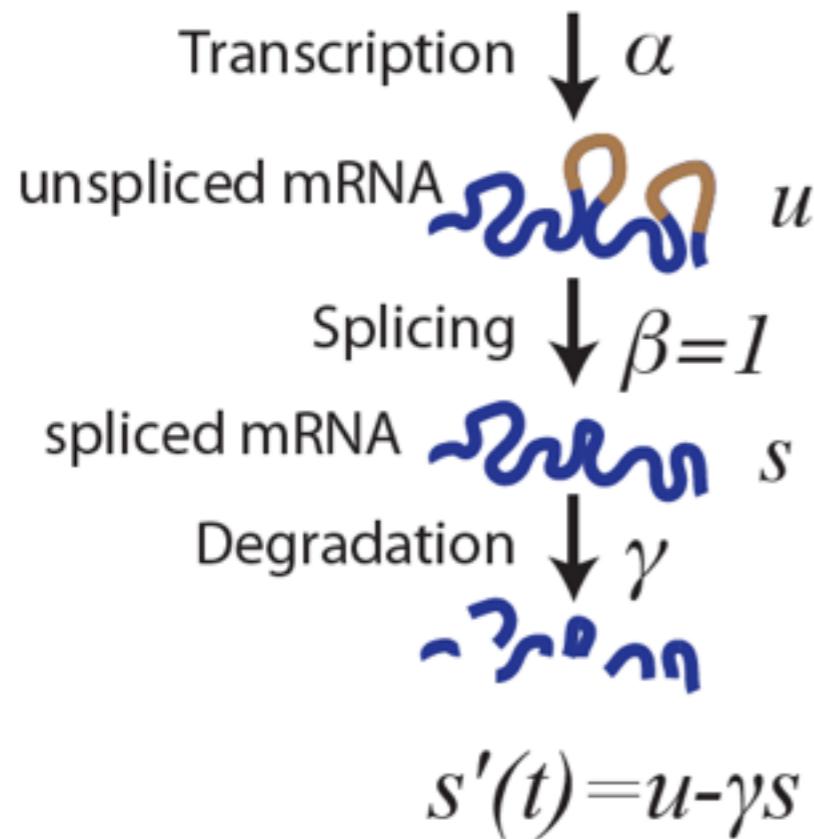
$$f_r(d) = \frac{k^2}{d^2} \quad f_a(d) = -\frac{d^2}{k}$$

# t-SNE (as a force-directed method)

$$\begin{aligned} \frac{\partial C}{\partial \mathbf{Y}_i} &= 4 \left( \sum_j p_{ij} q_{ij} z_Y (\mathbf{Y}_i - \mathbf{Y}_j) - \sum_j q_{ij}^2 z_Y (\mathbf{Y}_i - \mathbf{Y}_j) \right) \\ &= 4 \left( \underbrace{\sum_j p_{ij} \frac{\mathbf{Y}_i - \mathbf{Y}_j}{1 + \|\mathbf{Y}_i - \mathbf{Y}_j\|^2}}_{\text{Repulsive}} - \underbrace{\sum_j \frac{1}{z_Y} \cdot \frac{\mathbf{Y}_i - \mathbf{Y}_j}{(1 + \|\mathbf{Y}_i - \mathbf{Y}_j\|^2)^2}}_{\text{Attractive}} \right) \end{aligned}$$

# RNA velocity

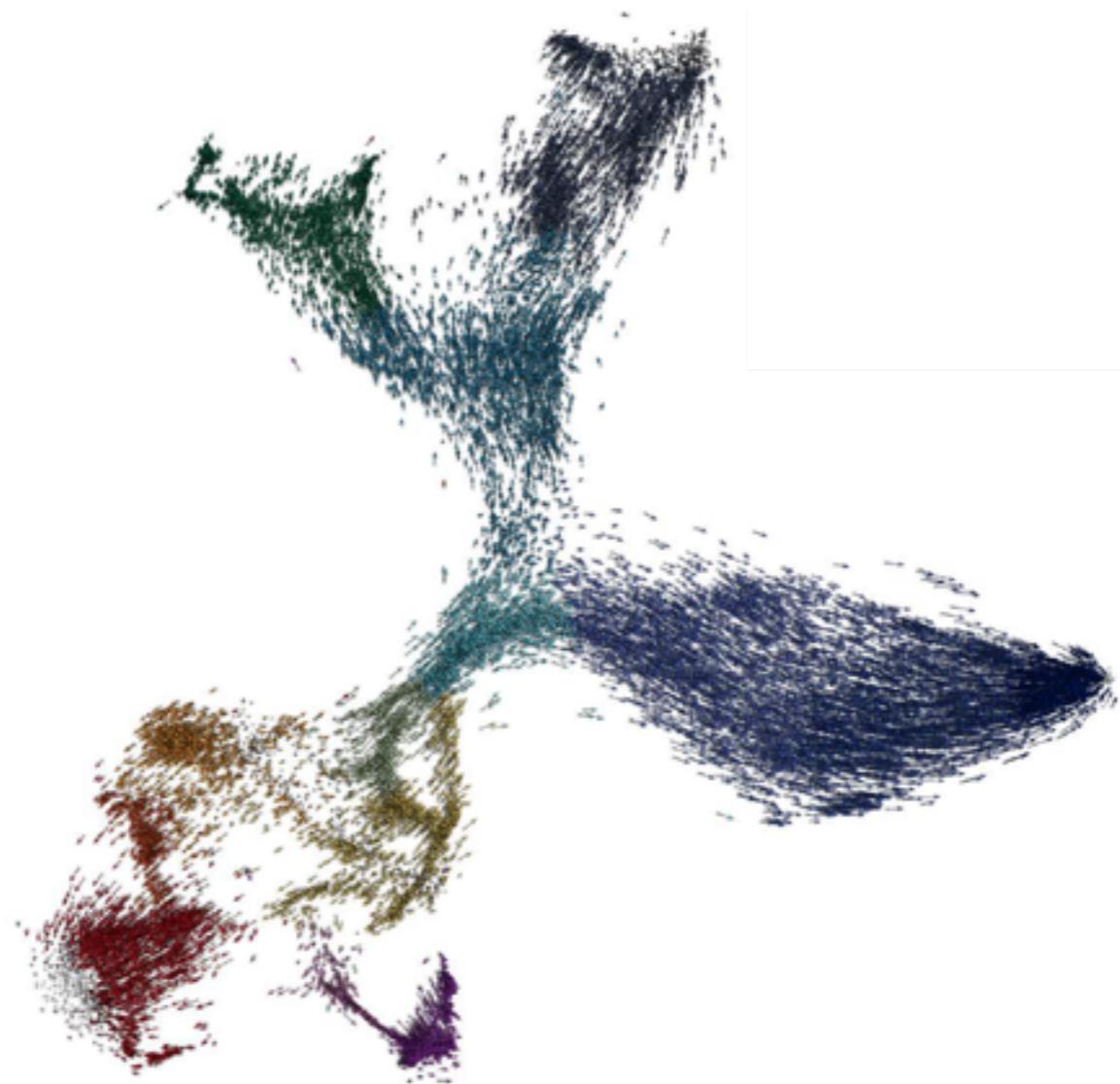
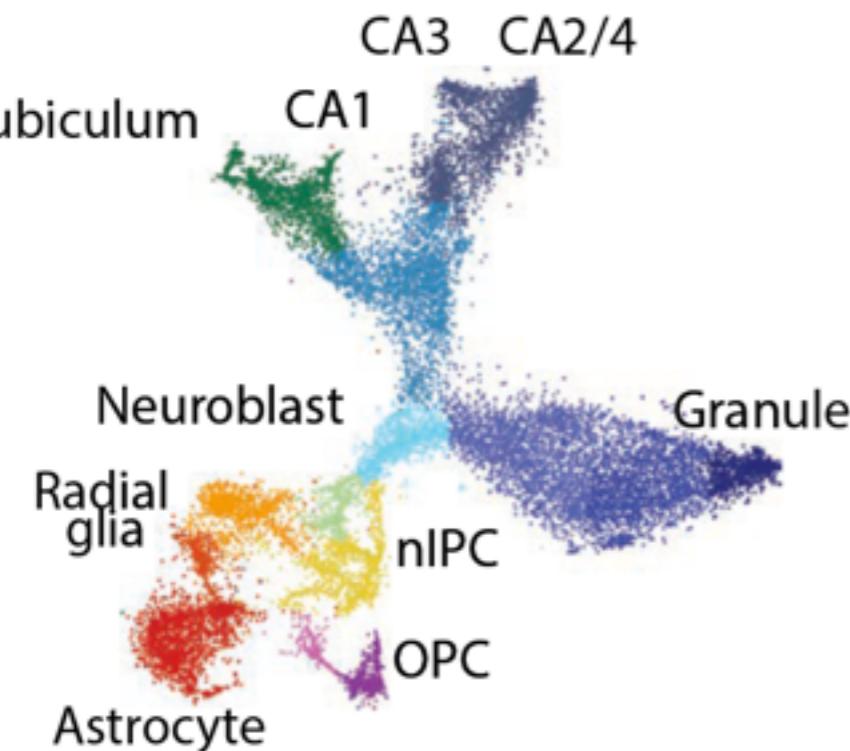
(La Manno, *bioRxiv* 2017)



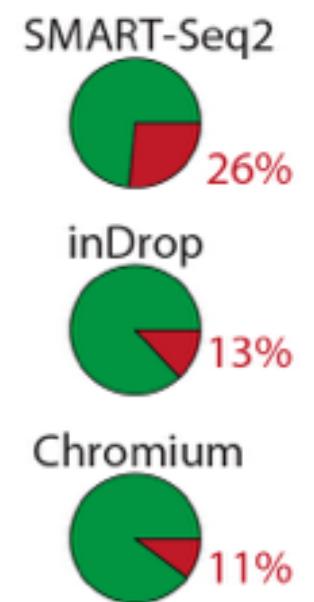
# RNA velocity

(La Manno, *bioRxiv* 2017)

Developing mouse  
hippocampus dataset

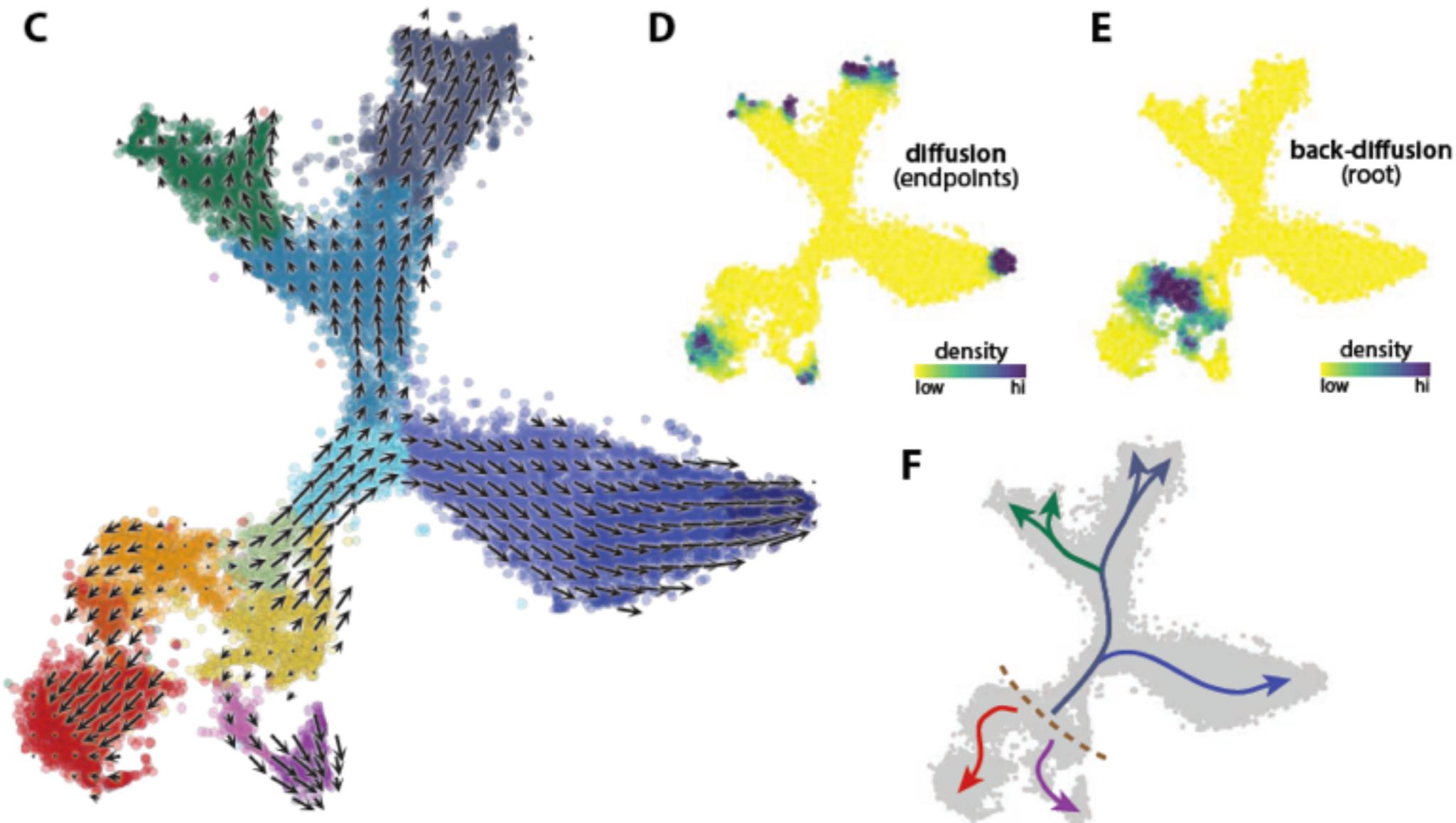


Unspliced mRNA  
percentage



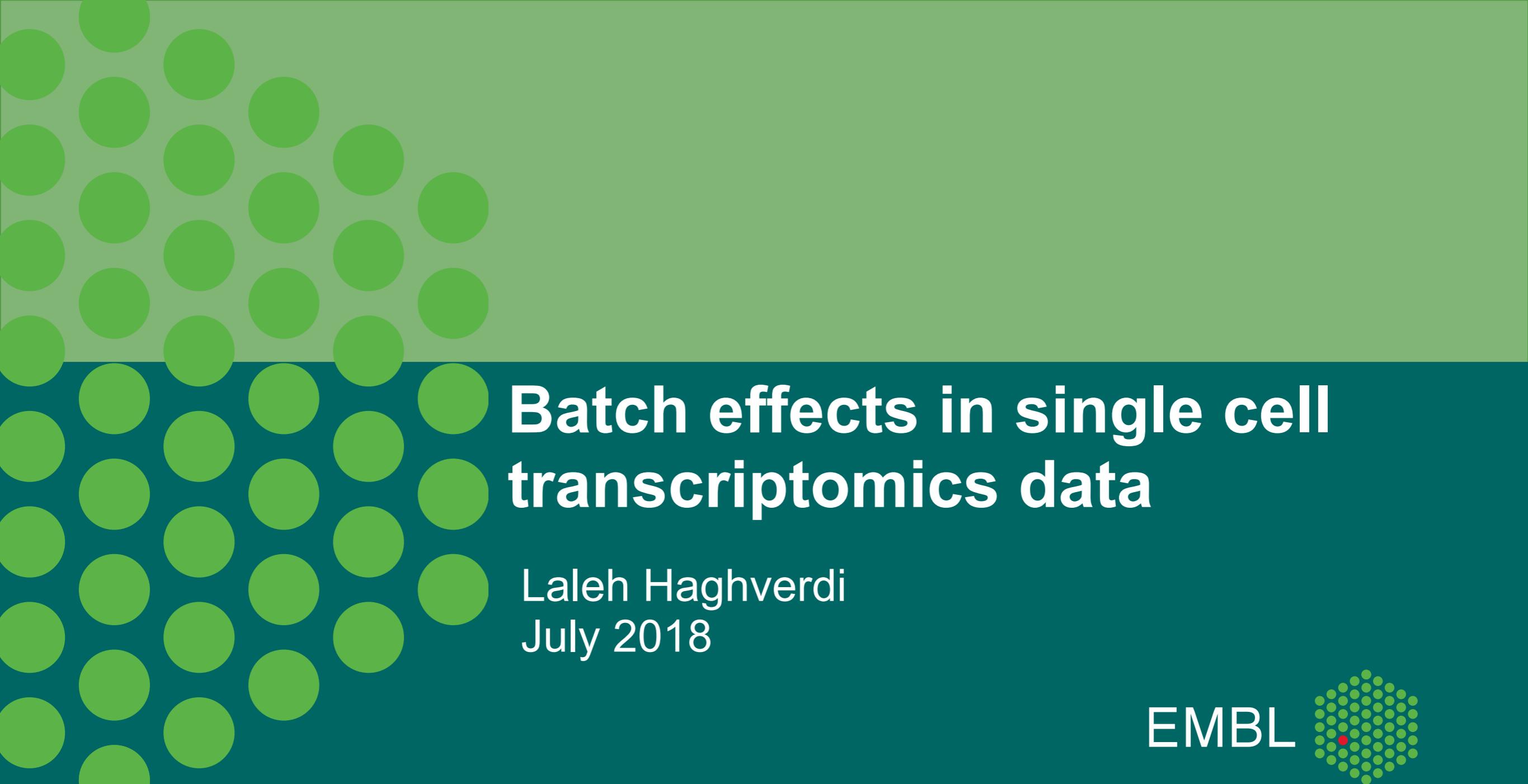
$$T_{ij} = \underbrace{pW_{ij}}_{\text{Geometrical diffusion}} + \underbrace{(1 - p)D_{ij}}_{\text{Velocity-driven (drift)}}$$

Geometrical diffusion      Velocity-driven (drift)



# Summary

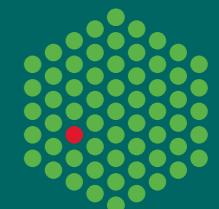
- Development and lineage trees
- Single- cell transcriptomics
- Dimension reduction methods
- Lineage reconstruction methods with few branchings
- Lineage reconstruction methods for complex topologies
- Integrating actual time information (RNA velocity)



# Batch effects in single cell transcriptomics data

Laleh Haghverdi  
July 2018

EMBL



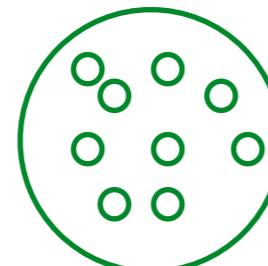
# Batch correction in single-cell data

- Single-cell data growing in scales
- Human Cell Atlas project
- Split data collection between different labs possibly using different facilities and different protocols

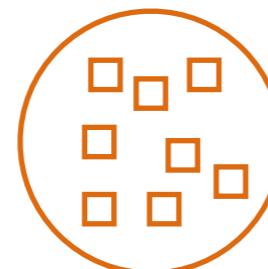
# Confounded vs. balanced experimental design

- Totally confounded

Batch1

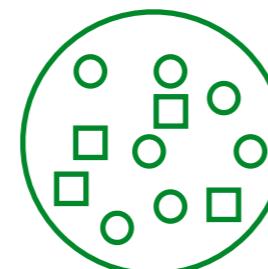


Batch2

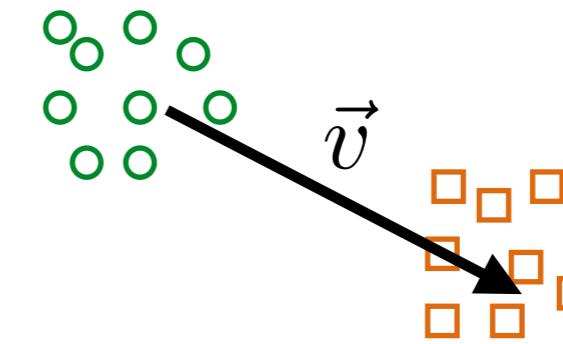


- Balanced

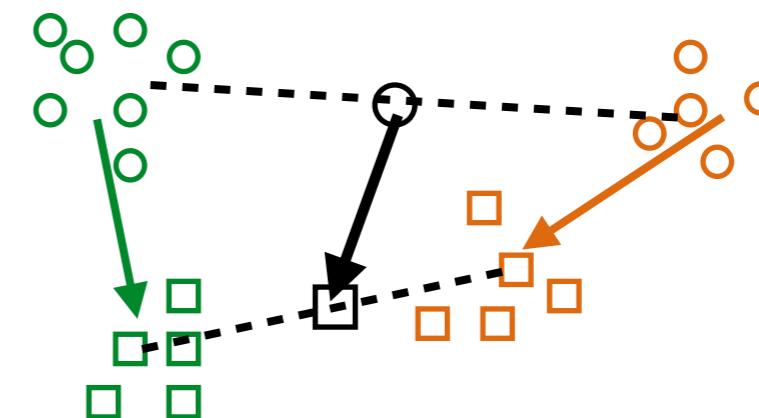
Batch1



Batch2



$$\vec{v} = \overrightarrow{\text{biological}} + \overrightarrow{\text{batch}}$$



average biological vector  
e.g. limma and Combat

# Batch correction for single-cell RNA-seq data

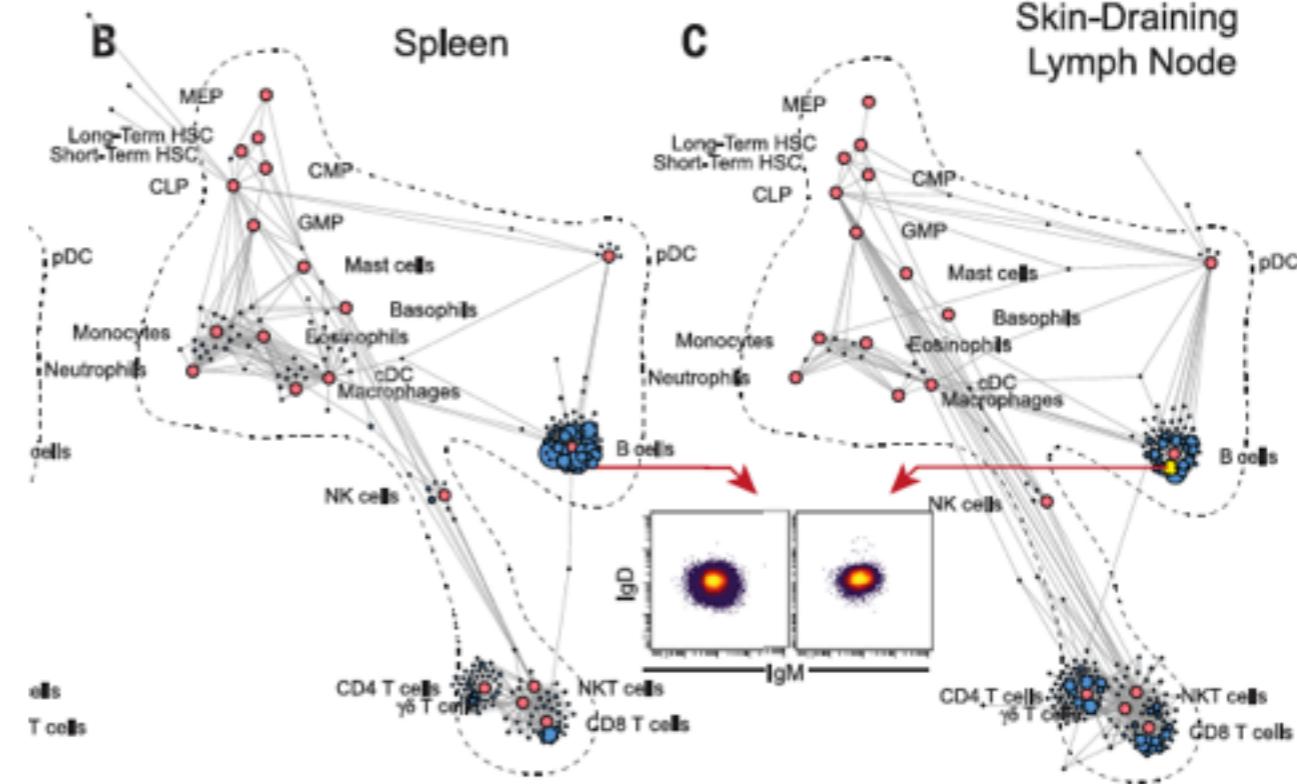
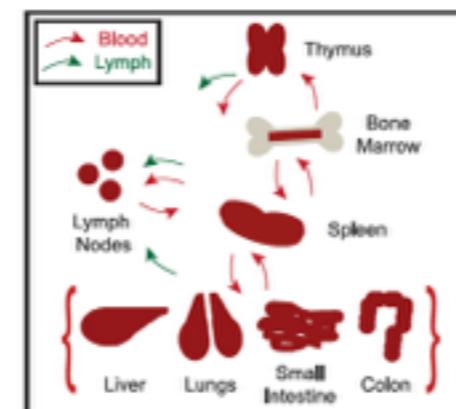
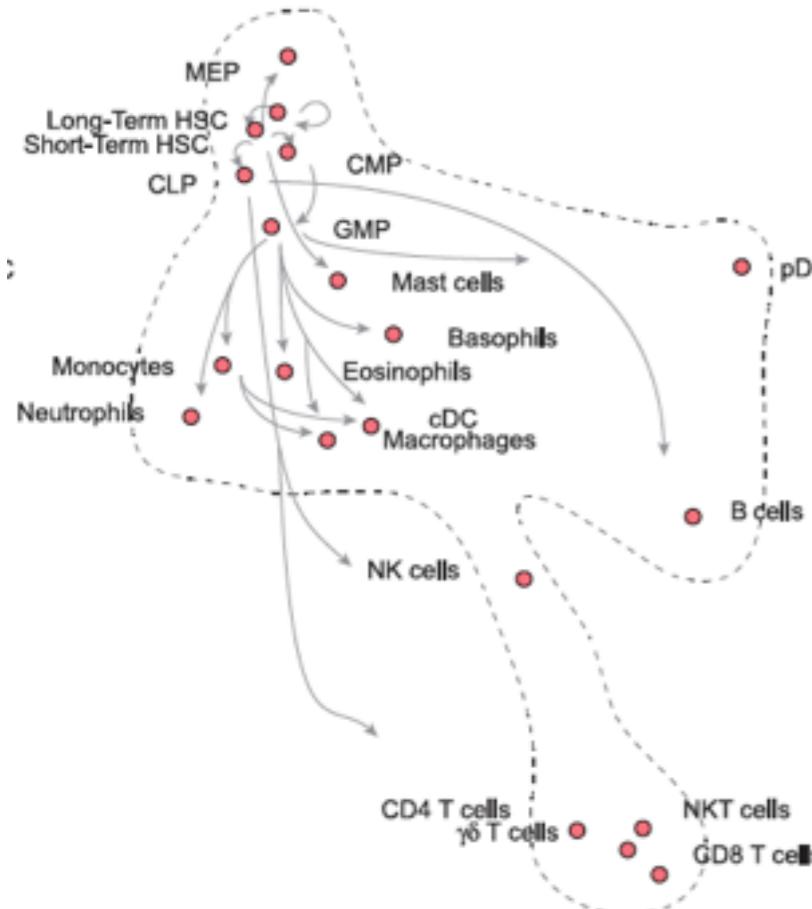
- Different laboratories, different protocols and different cell type compositions
- Scalable
- limma (Ritchie et al. Nucleic Acids Res. 2015)
- Combat (Johnson et al. Bioinformatics 2007)
- RUVseq (Risso et al. Nature biotechnology 2014)
- Projection on a reference embedding
- Scaffold (Spitzer, et al. *Science* 2015)
- Mutual Nearest Neighbours matching (Haghverdi, et al. *Nature biotechnology* 2018)
- CCA approach + alignment (Butler, et al. *Nature biotechnology* 2018)
- Autoencoders approach (Lopez et al. arxiv 2017, Eraslan et al. biorxiv 2018)

# Projection methods

- Inter-batch and intra-batch distances are not comparable and can not be mixed
- Embedding of the reference batch (inter-batch only) + projection of the other batch on that embedding (intra-batch)
- projection with PCA, t-SNE, diffusion maps, etc.
- Requires that the reference batch is a super set of all appearing populations

# Projection example

Scaffold map of cell types within the immune system for various organs  
(Spitzer, et al. *Science* 2015)



Embedding of the reference batch  
(bone marrow)

# Combat

(Johnson et al. Bioinformatics 2007)

- Shrunken (empirical Bayesian) inference on a linear model

$$Y_{ijg} = \alpha_g + X\beta_g + \gamma_{ig} + \delta_{ig}\varepsilon_{ijg},$$

## batch i

sample (cell) j

gene **g**

1

## biological effect

- Centre and scale within each condition

$$Z_{ijg} = \frac{Y_{ijg} - \hat{\alpha}_g - X\hat{\beta}_g}{\hat{\sigma}_g}.$$

- Instead of adjusting each gene independently, borrow information from all other genes

$$Z_{ig} \sim N(\gamma_{ig}, \delta_{ig}^2)$$

$$\gamma_{ig} \sim N(Y_i, \tau_i^2) \quad \text{and} \quad \delta_{ig}^2 \sim \text{Inverse Gamma } (\lambda_i, \theta_i).$$

# RUV, RUVseq

(Risso et al. Nature biotechnology 2014)

$[N, G]$

$$Y = \underbrace{X\beta}_{\text{Biological effects}} + \underbrace{W\alpha}_{\text{Unwanted effects}} + \varepsilon,$$
$$[N, K] * [K, G] \quad [N, L] * [L, G]$$

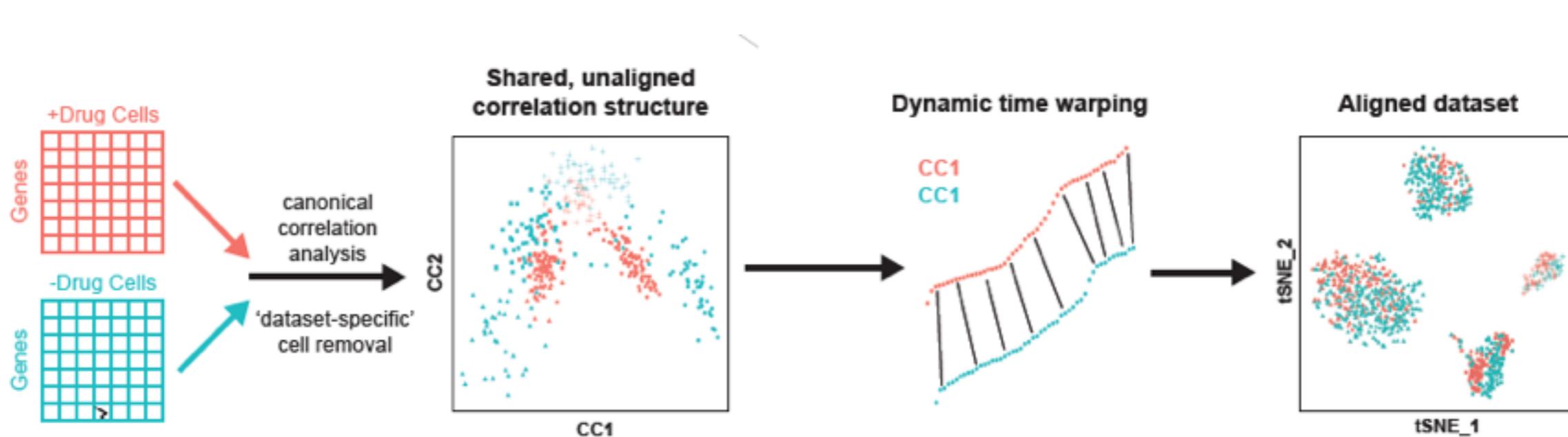
- Supervised approach (e.g. with negative control genes or known biological effects)

$$Y_c = W\alpha_c + \varepsilon_c,$$

# CCA based approach

Butler, et al. *Nature biotechnology* 2018

- Find directions of maximal correlation between batches;  
Canonical Correlation analysis (Bhatia, 1998)
- Align cells on the canonical correlation components

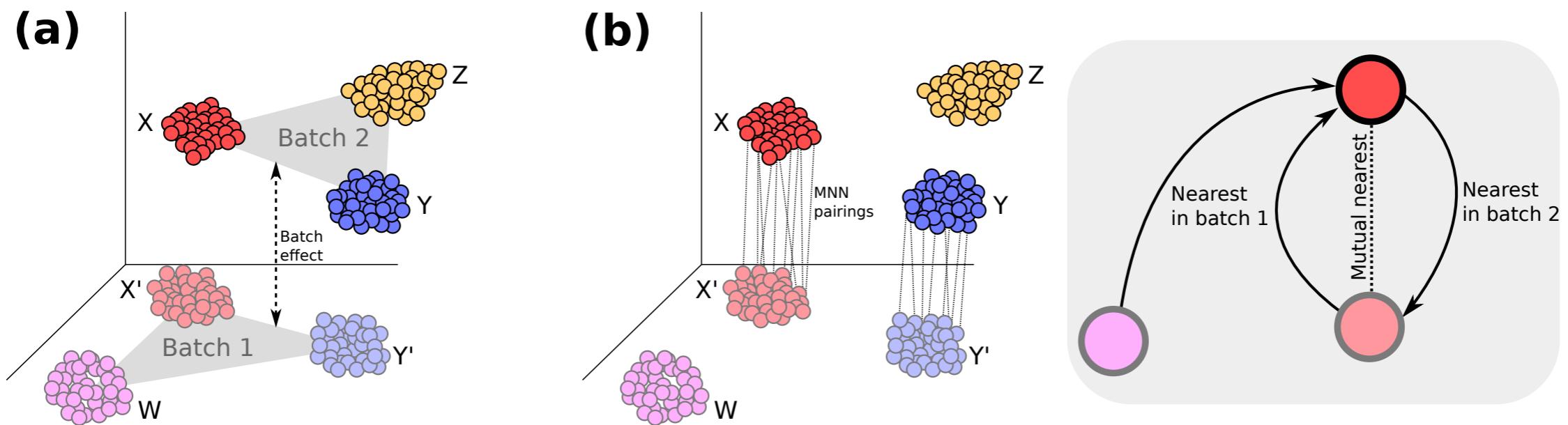


# Mutual Nearest Neighbours Analysis

Haghverdi et al. *Nature biotechnology* 2018

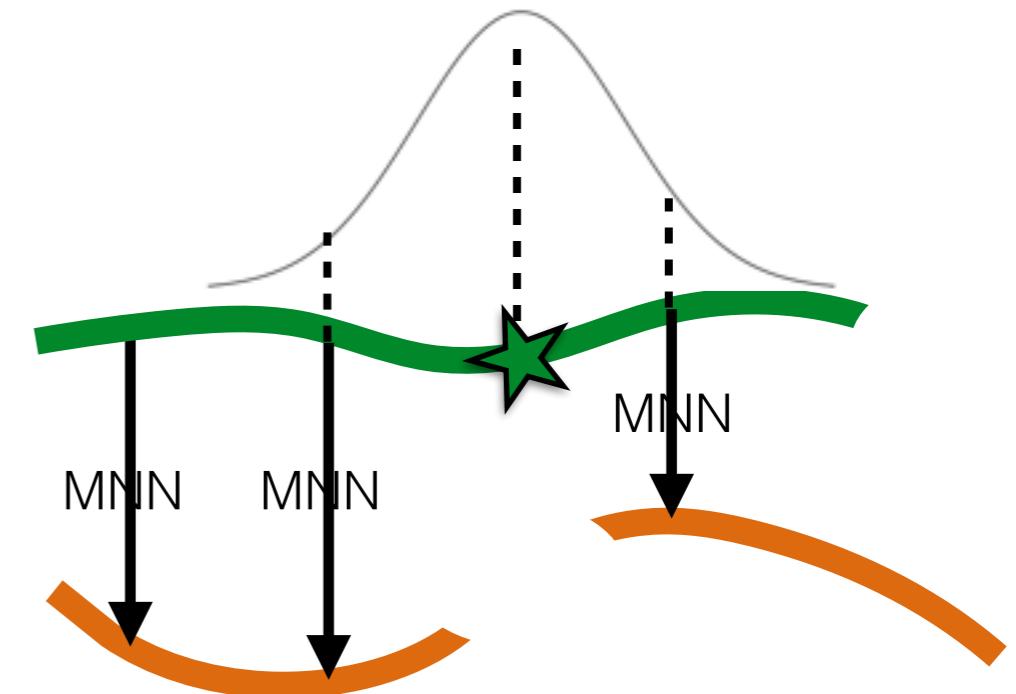
- Batch effect is almost orthogonal to the biological hyper plane (Central limit theorem)

$$\vec{X} \cdot \vec{Y} = \sum_{i=1}^D x_i y_i$$

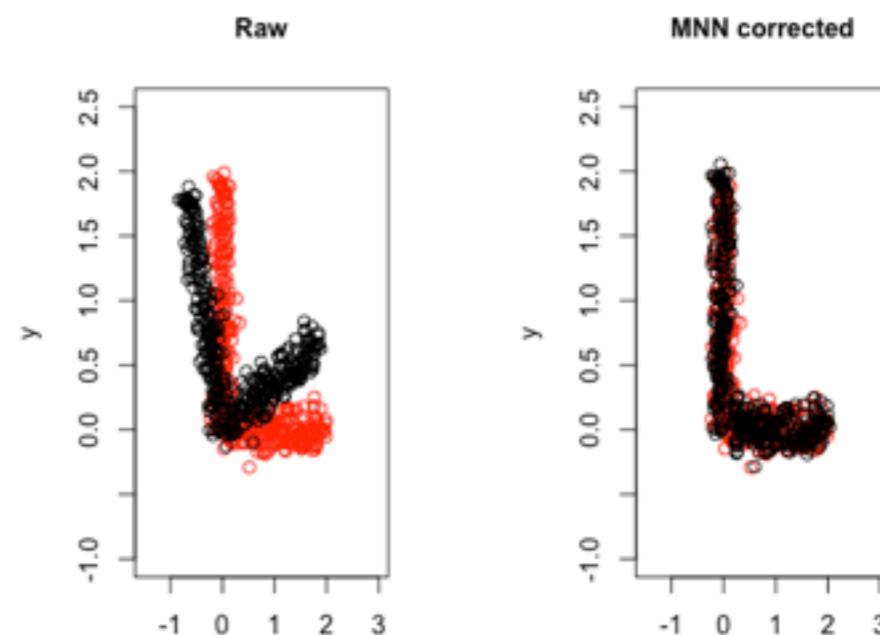


# Mutual Nearest Neighbours Analysis

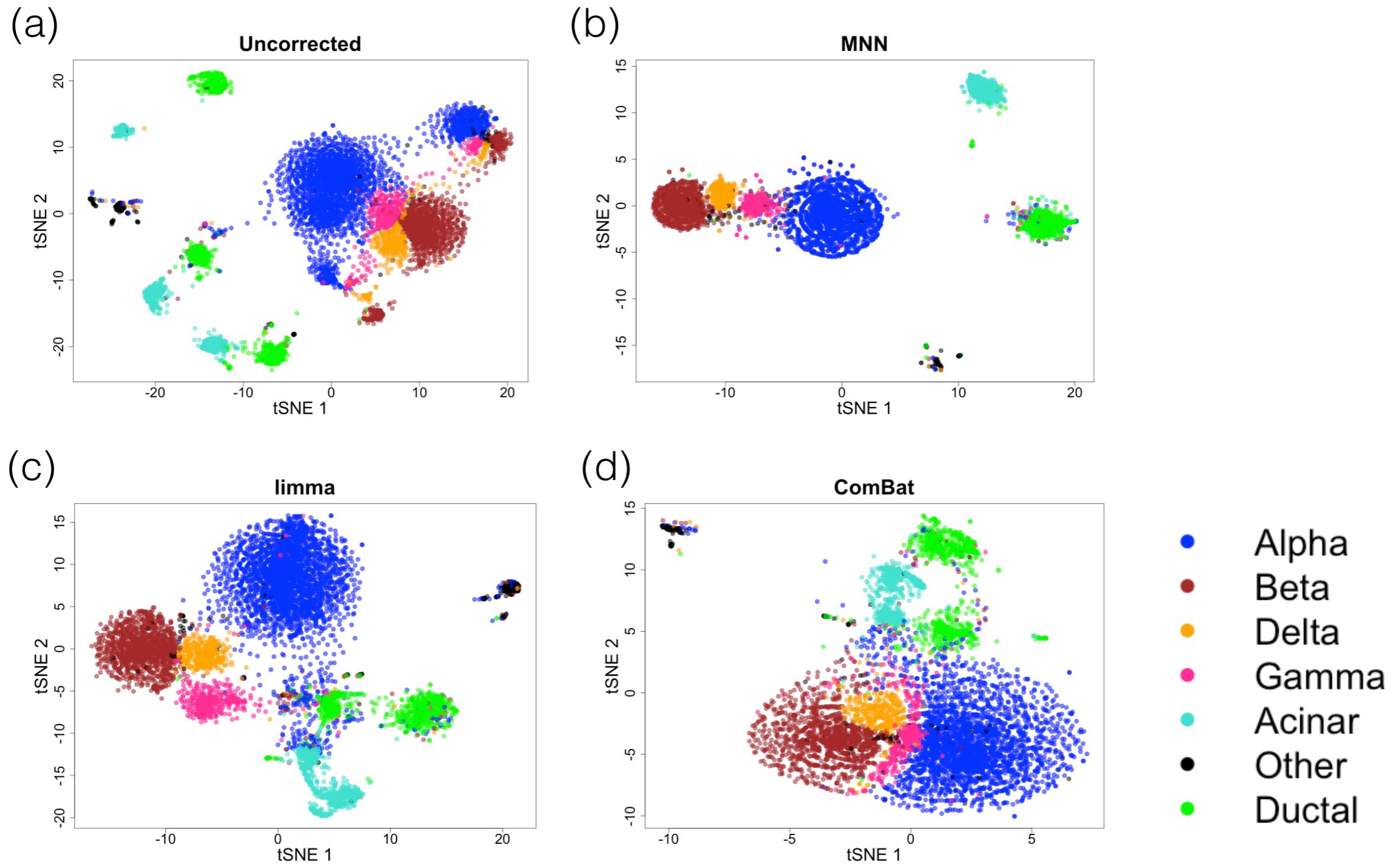
- Once a few MNN pairs have been identified at a few locations, batch effects for everywhere else is estimated using a smoothing Gaussian kernel



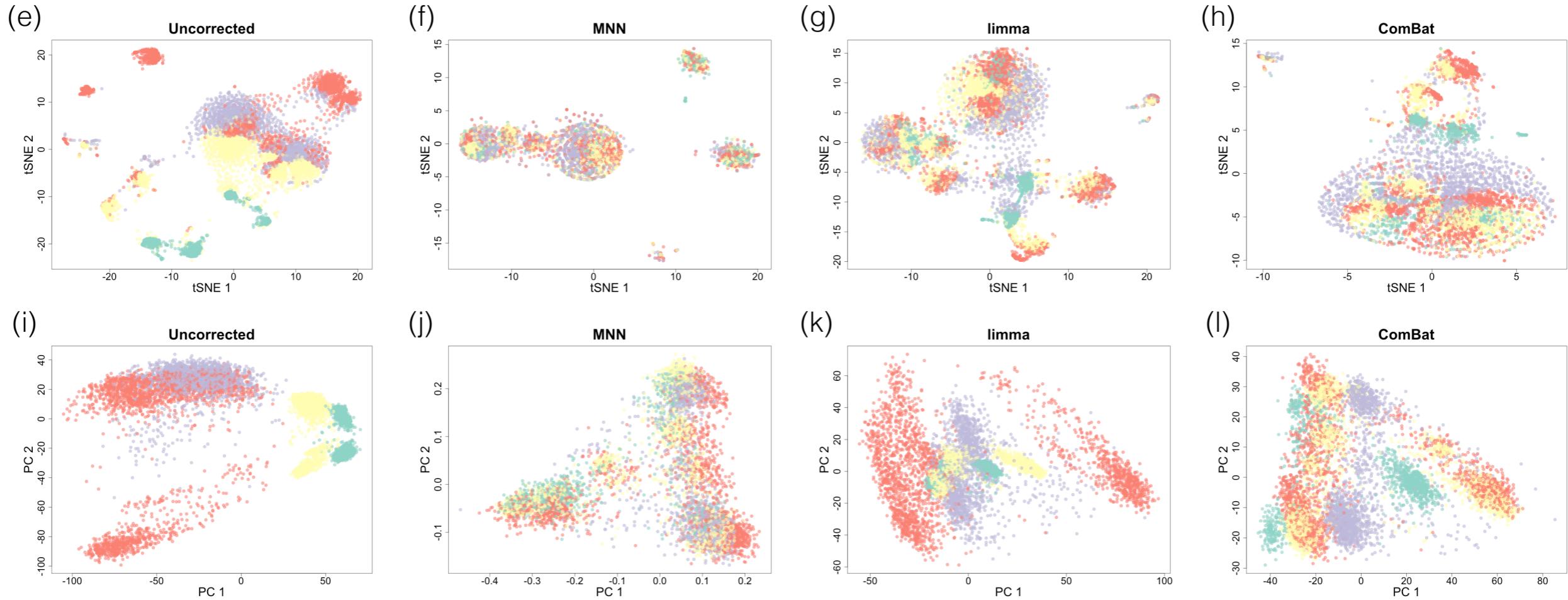
- Locally linear (overall nonlinear)
- Tolerates small non-orthogonalities



# MNN on four Pancreas data sets

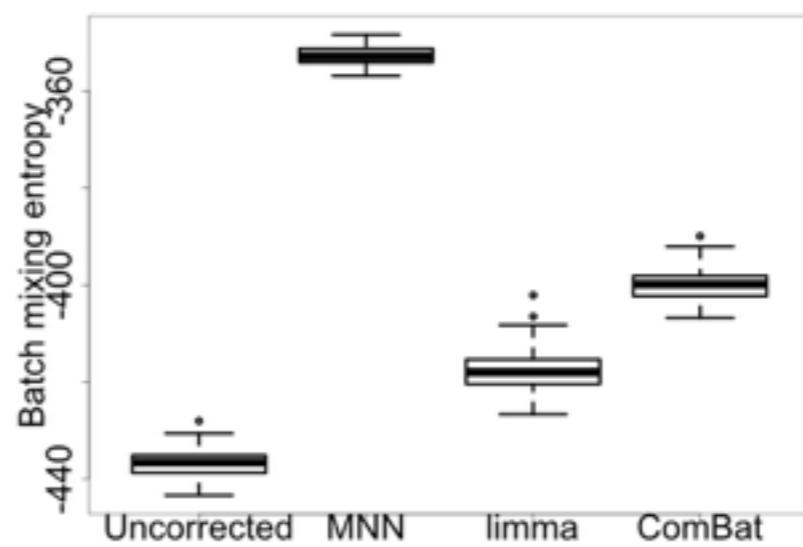


# MNN on four Pancreas data sets

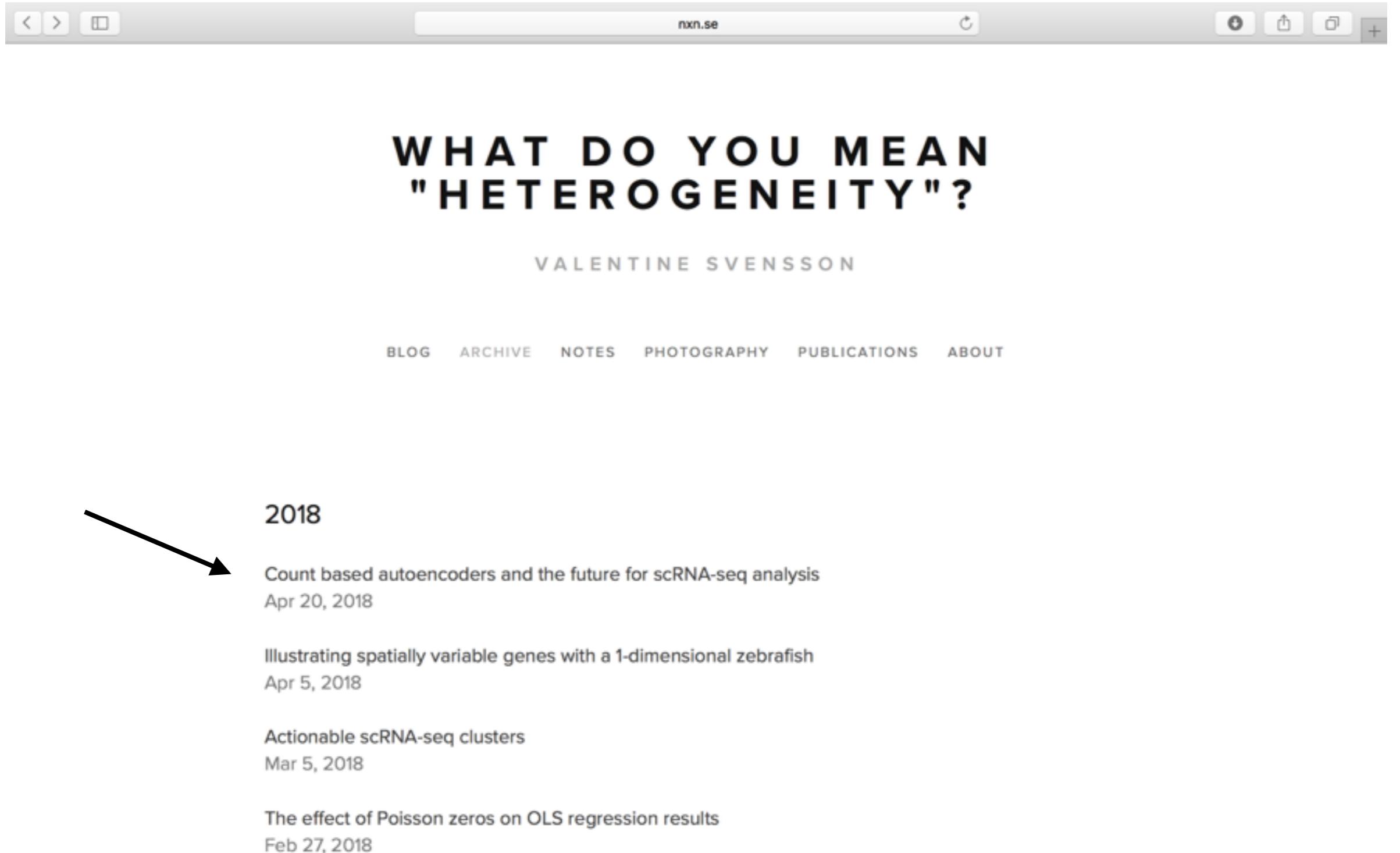


**Batch**

- GSE81076
- GSE86473
- GSE85241
- E-MTAB-5061



# With autoencoders



The screenshot shows a web browser window with the URL `nxn.se` in the address bar. The page title is "WHAT DO YOU MEAN \"HETEROGENEITY\"?". Below the title is the author's name, VALENTINE SVENSSON. A navigation menu at the top includes links for BLOG, ARCHIVE, NOTES, PHOTOGRAPHY, PUBLICATIONS, and ABOUT. The main content area displays four blog posts from 2018, each with a title and a date. A black arrow points from the year "2018" to the first post.

2018

Count based autoencoders and the future for scRNA-seq analysis  
Apr 20, 2018

Illustrating spatially variable genes with a 1-dimensional zebrafish  
Apr 5, 2018

Actionable scRNA-seq clusters  
Mar 5, 2018

The effect of Poisson zeros on OLS regression results  
Feb 27, 2018