Pseudotime and lineage tree reconstruction from single cell transcriptomics data

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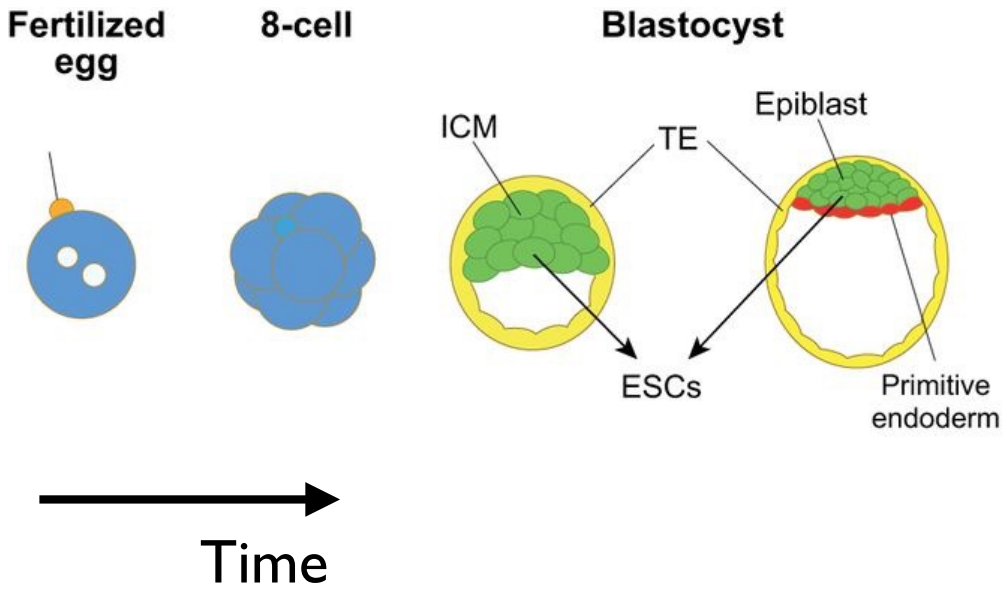
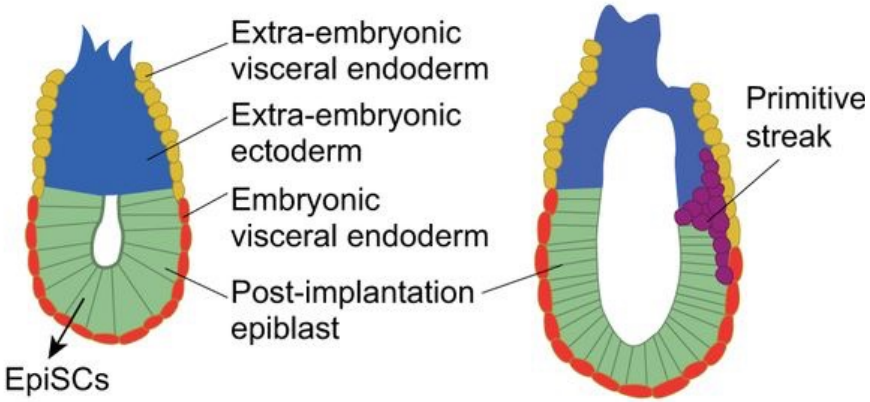
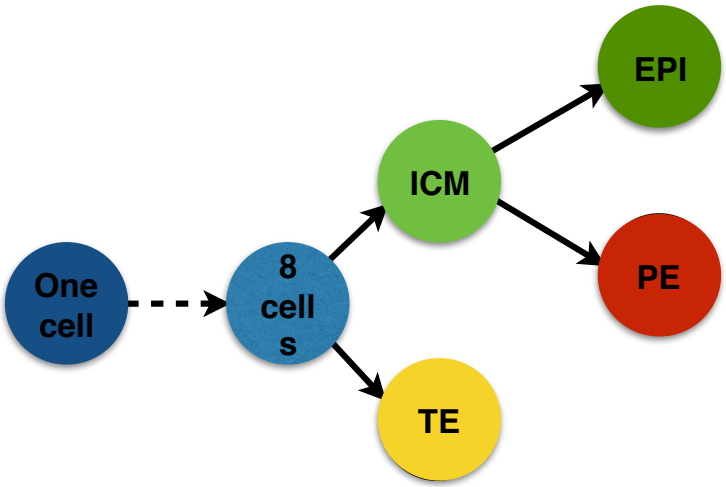
July 2018

EMBL

Cell differentiation, lineage trees

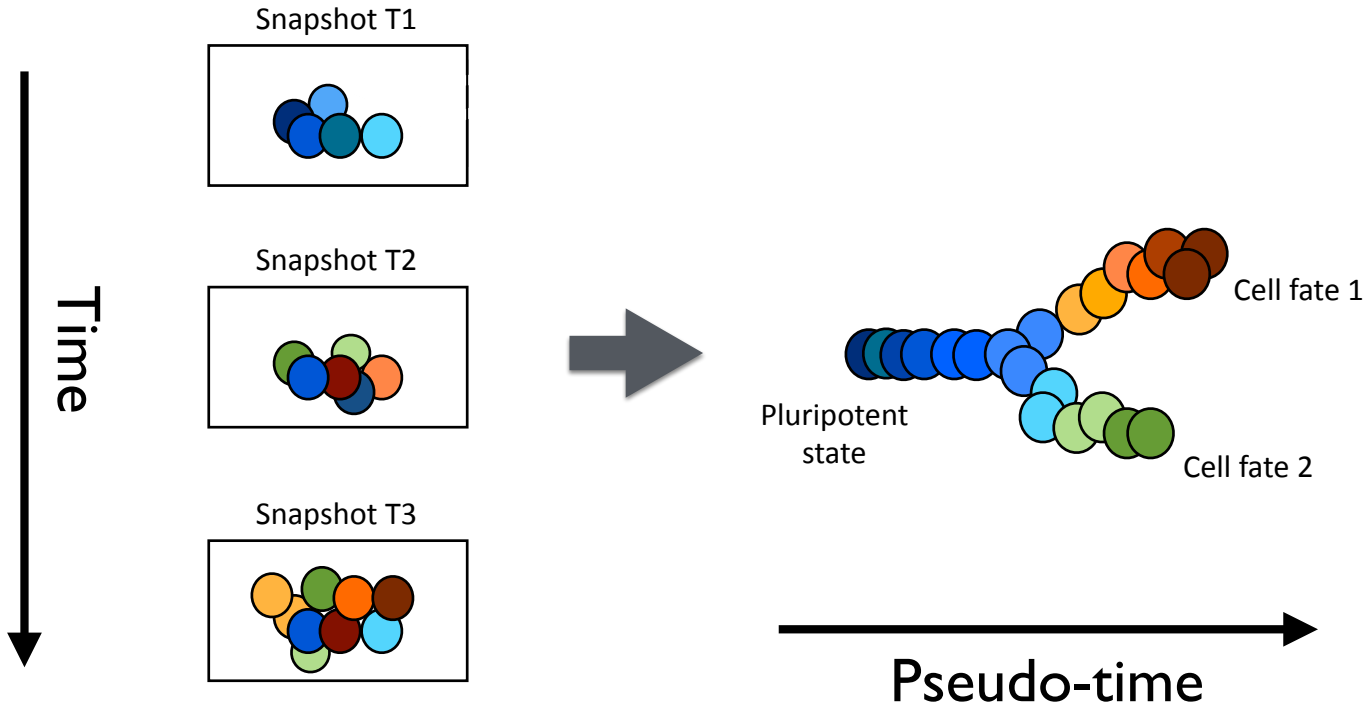






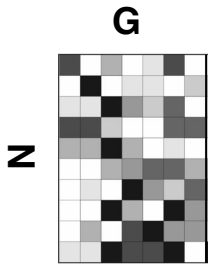
- Change of transcriptional profile

Lineage tree reconstruction



- Asynchronous development of cells —> bulk measurements fail

Single cell transciptomics data and challenges



- scRNA-seq, sc-qPCR, flow cytometry, mass cytometry.
- Data matrix of size n*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 $X \quad [N, G]$

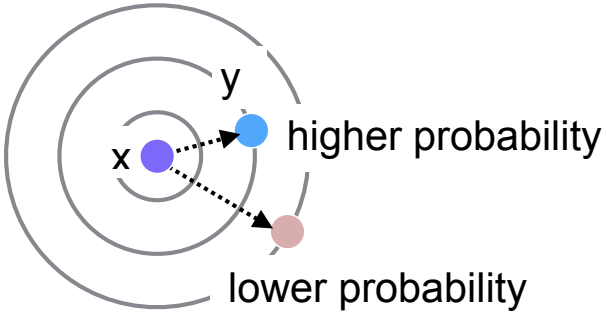
$$\text{argmax}_W \{ \underbrace{W^T X^T}_{Y^T} \underbrace{XW}_{Y} \}$$

$$Y^T * Y$$

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

Diffusion maps

Gaussian kernel



$$T_{n\times n}$$

$$\psi_i \mathbf{T} = \lambda_i \psi_i \qquad 1 = \lambda_0 \geq \lambda_1 \geq ... \geq \lambda_{n-1}$$

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

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

t-SNE

Original (high-dim) space X

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

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

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

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 $\mathbf{Y}^{(0)}$

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

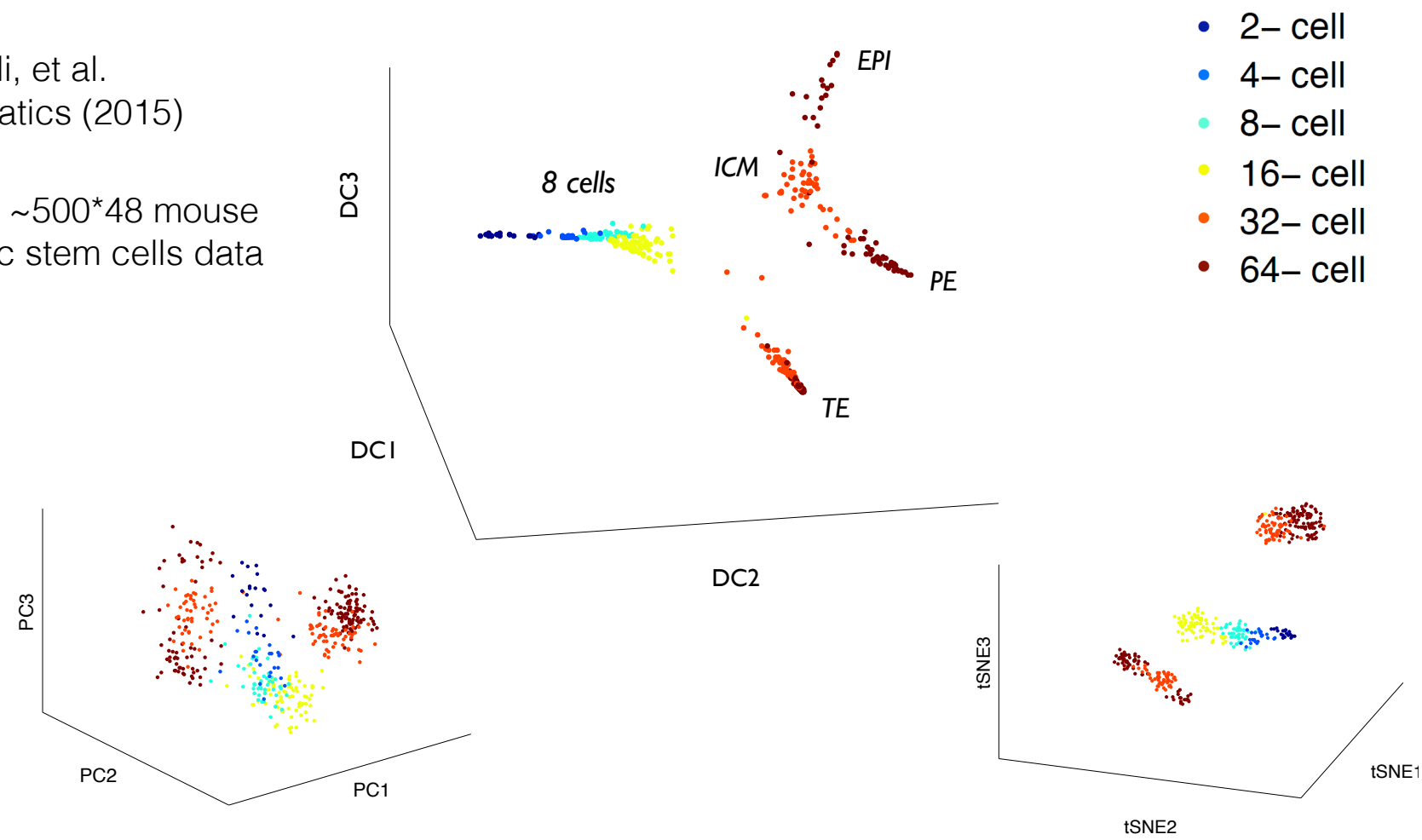
$$\mathbf{Y}^{(t)} = \mathbf{Y}^{(t-1)} + \eta \frac{\partial C}{\partial \mathbf{Y}_i} + \alpha(t)(\mathbf{Y}^{(t-1)} - \mathbf{Y}^{(t-2)})$$

- The final solution for Y depends on the initialisation Y^0
- t-SNE does not respect the global continuity of data manifold

PCA, t-SNE, Diffusion maps

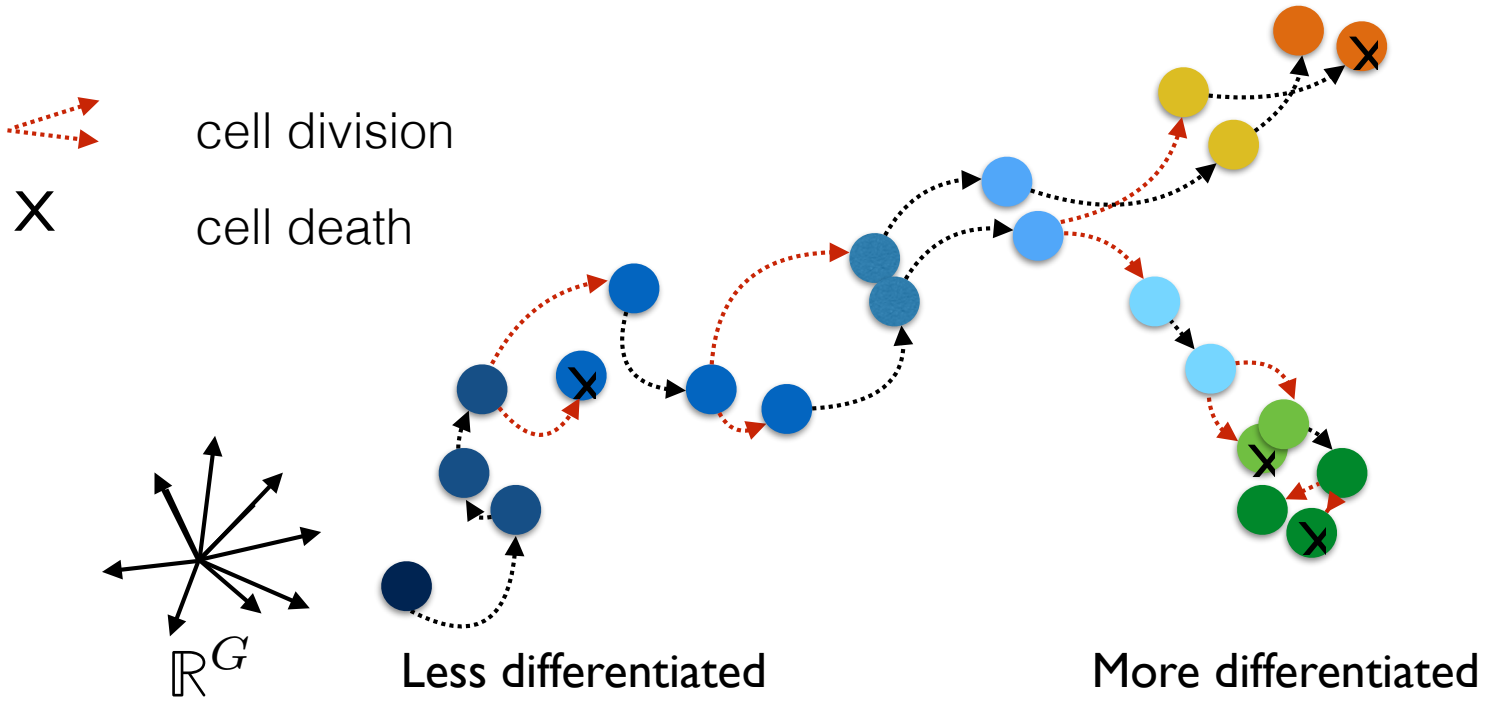
Haghverdi, et al.
Bioinformatics (2015)

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



$$\frac{\partial p}{\partial t} = \frac{\partial^2}{\partial x^2}\left(Dp(t)\right) - \frac{\partial}{\partial x}\left(\mu p(t)\right) + \nabla \cdot p$$

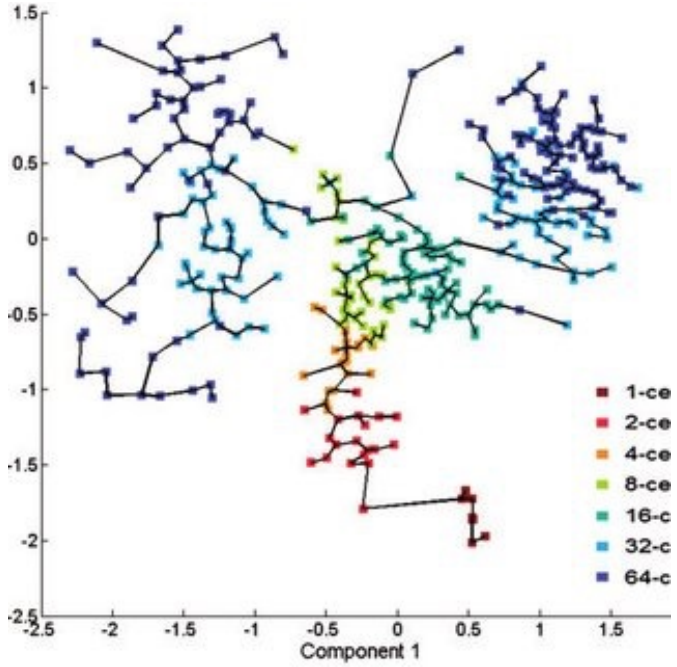
$$p(t) = p(t-1) + \Delta p \qquad p(t) = p(t-1)T$$

- 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

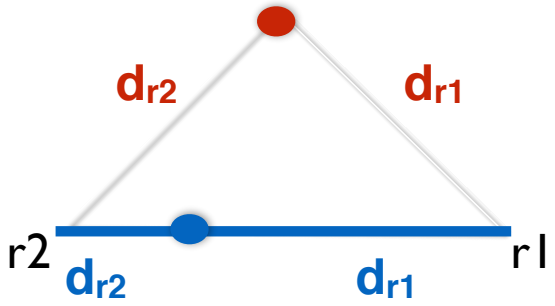
- Minimum total edges distance that goes through all nodes
- In general not robust; requires a neat low-dimensional embedding

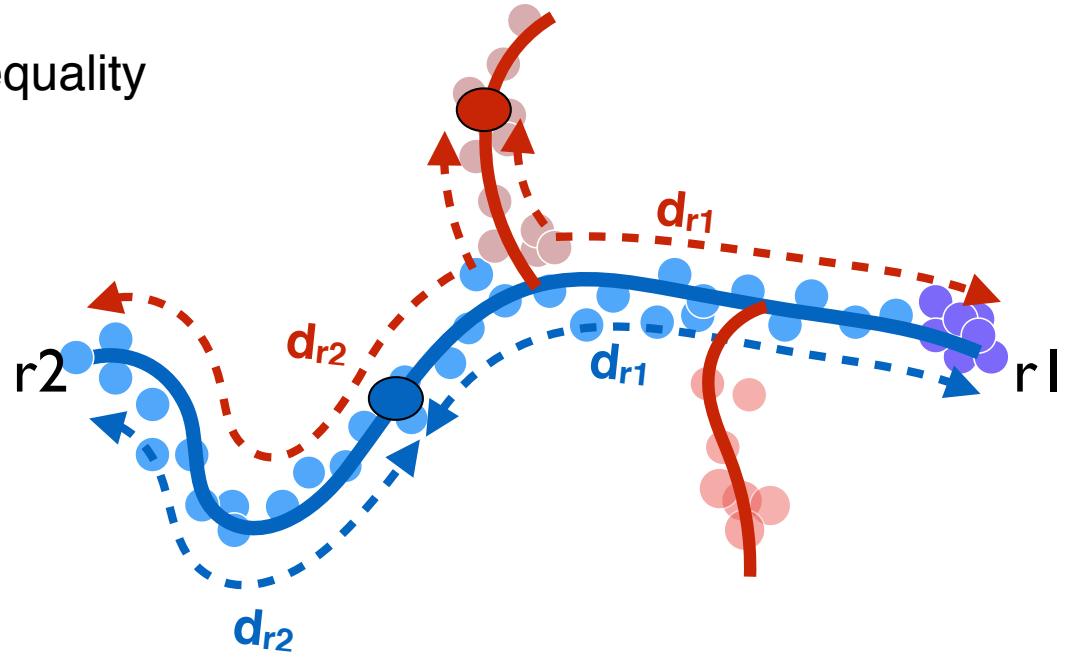


Going beyond mere embedding and visualisation; pseudo-time and branch identification

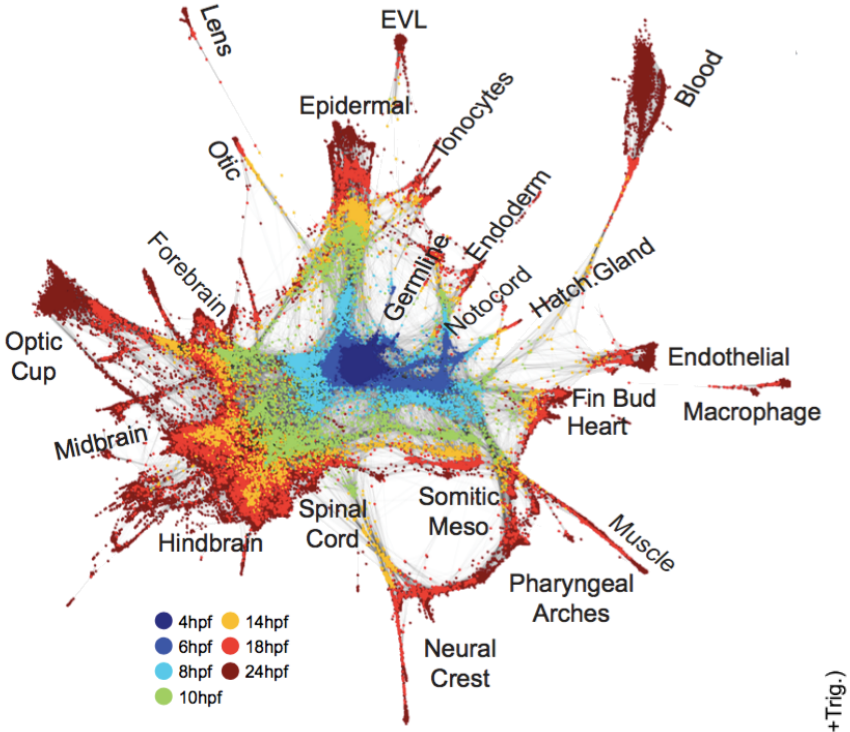
Using an on-manifold metric

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

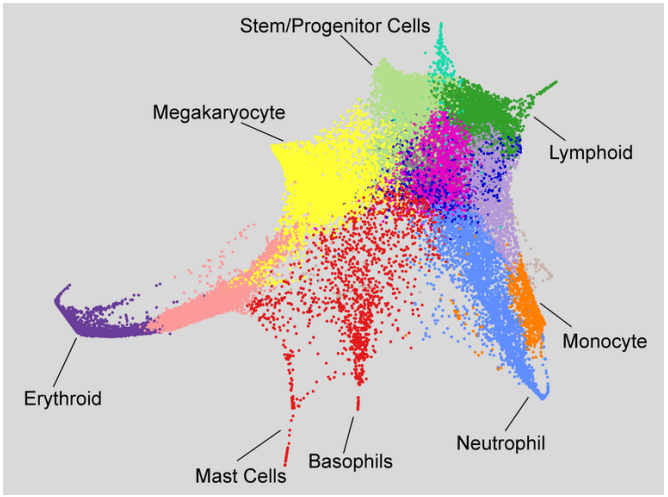




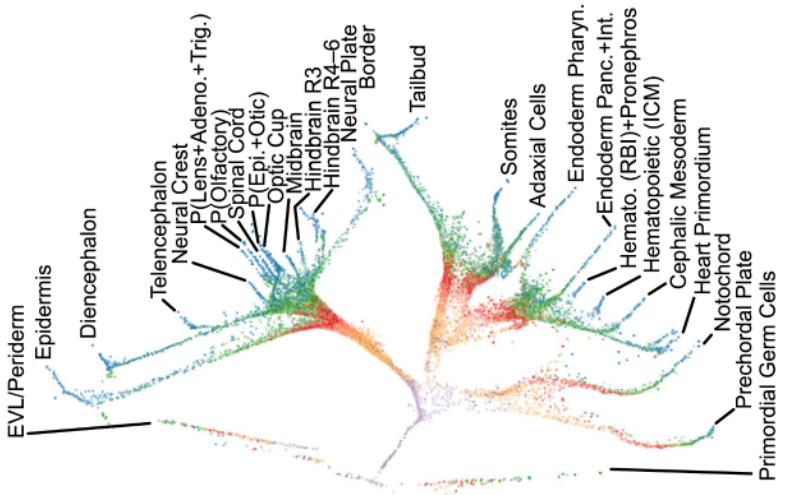
Complex manifolds with several branching events



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



Blood (Berthold Goetgens lab 2018)



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

Complex manifolds with several branching events

- Force-directed graphs
- Taking directed dynamics into account

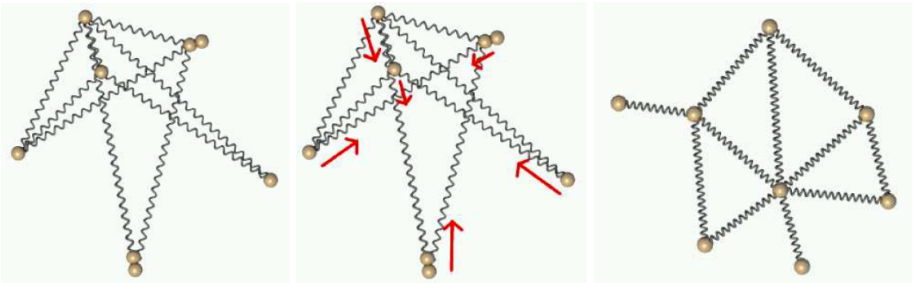
- SPRING (Weinreb et al. *Bioinformatics* 2017)
- URD (Farrell et al. *Science* 2018)
- Velocyto (La Manno, *bioRxiv* 2017)

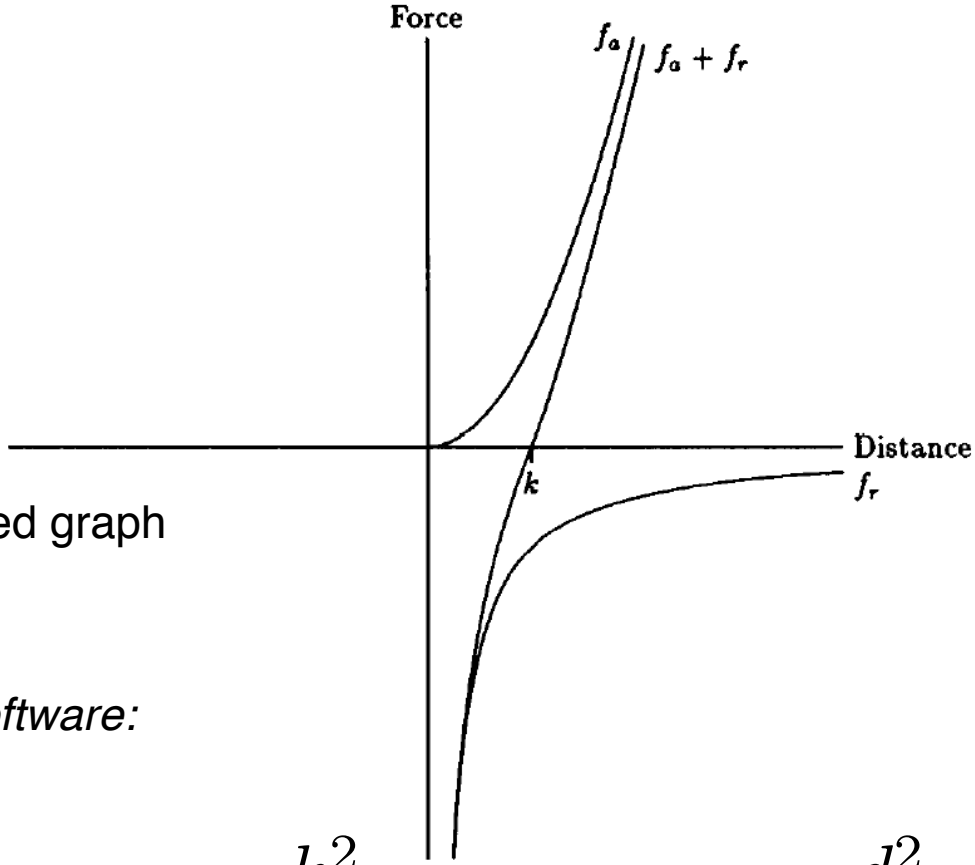
—> User-defined root cell and endpoints to infer directed diffusions

—> 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 Reinhold (Fruchterman et al. *Software: Practice and experience,* 1991)

skips local minima

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

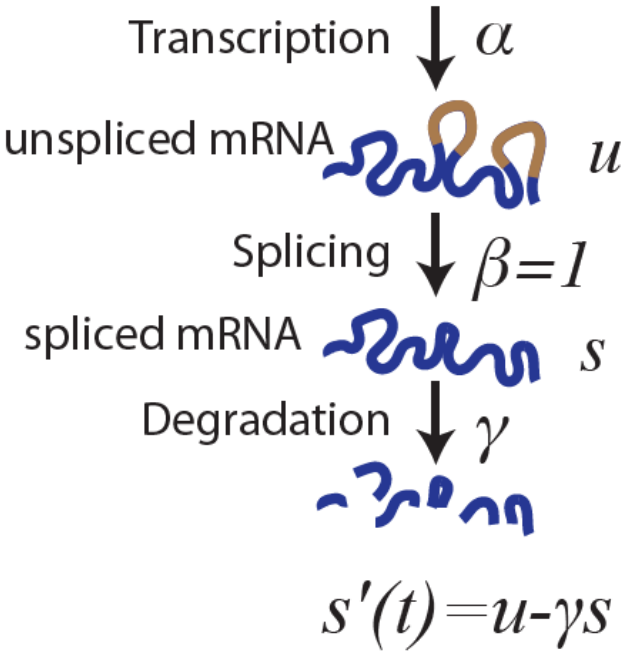
t-SNE (as a force-directed method)

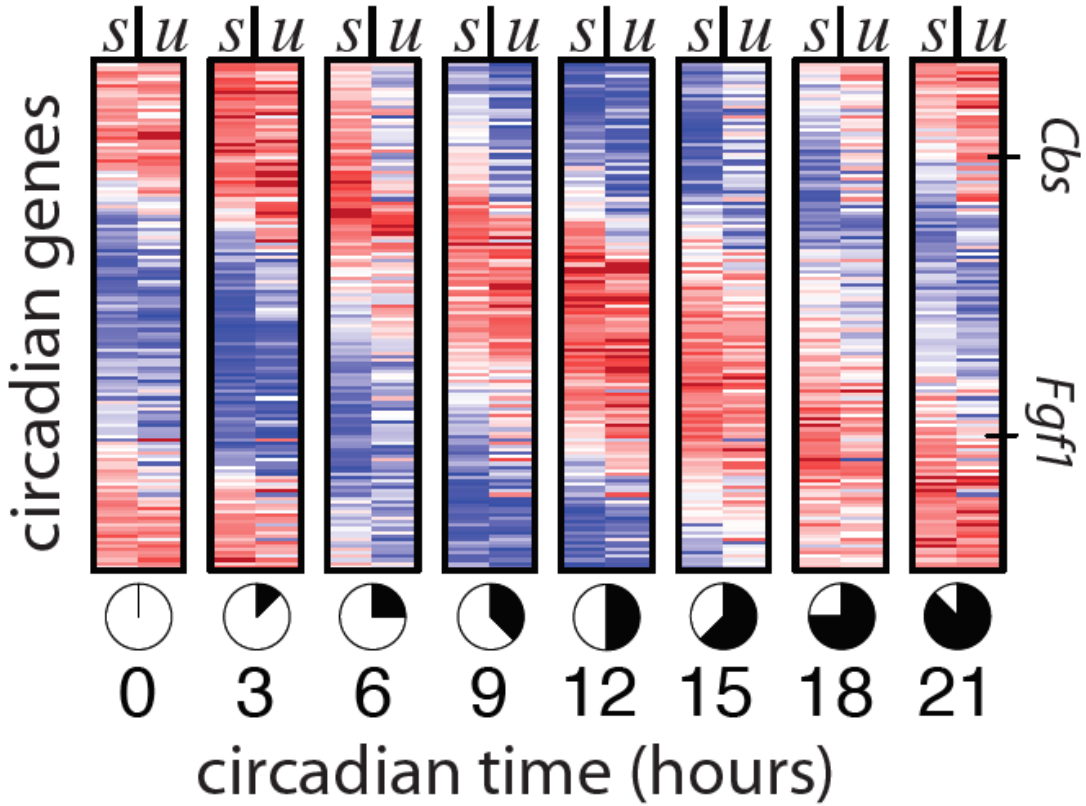
$$\frac{\partial C}{\partial \mathbf{Y}_i} = 4 \left(\sum_j p_{ij} q_{ij} z_Y (\mathbf{Y}_i - \mathbf{Y}_j) - \sum_j q_{ij}^2 z_Y (\mathbf{Y}_i - \mathbf{Y}_j) \right)$$

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

RNA velocity

(La Manno, *bioRxiv* 2017)

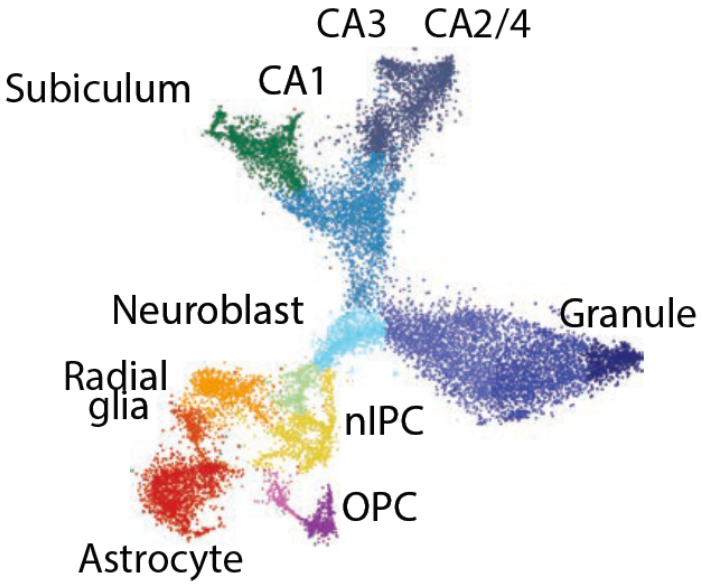


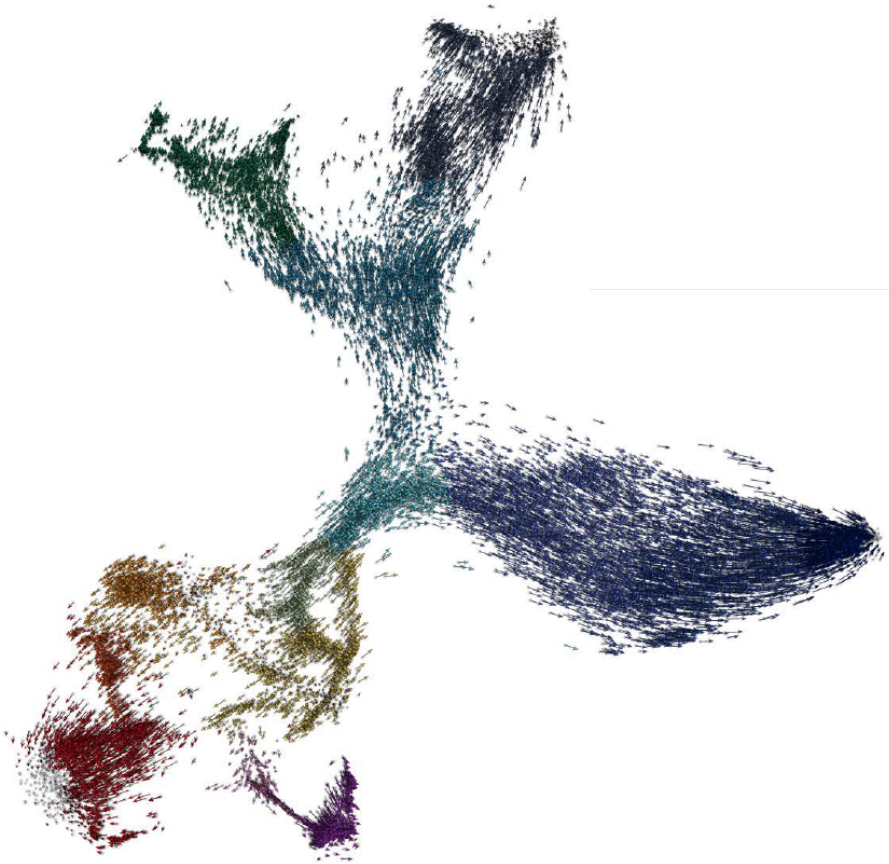


RNA velocity

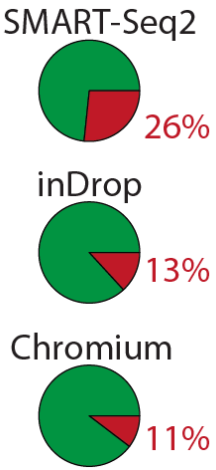
(La Manno, *bioRxiv* 2017)

Developing mouse
hippocampus dataset

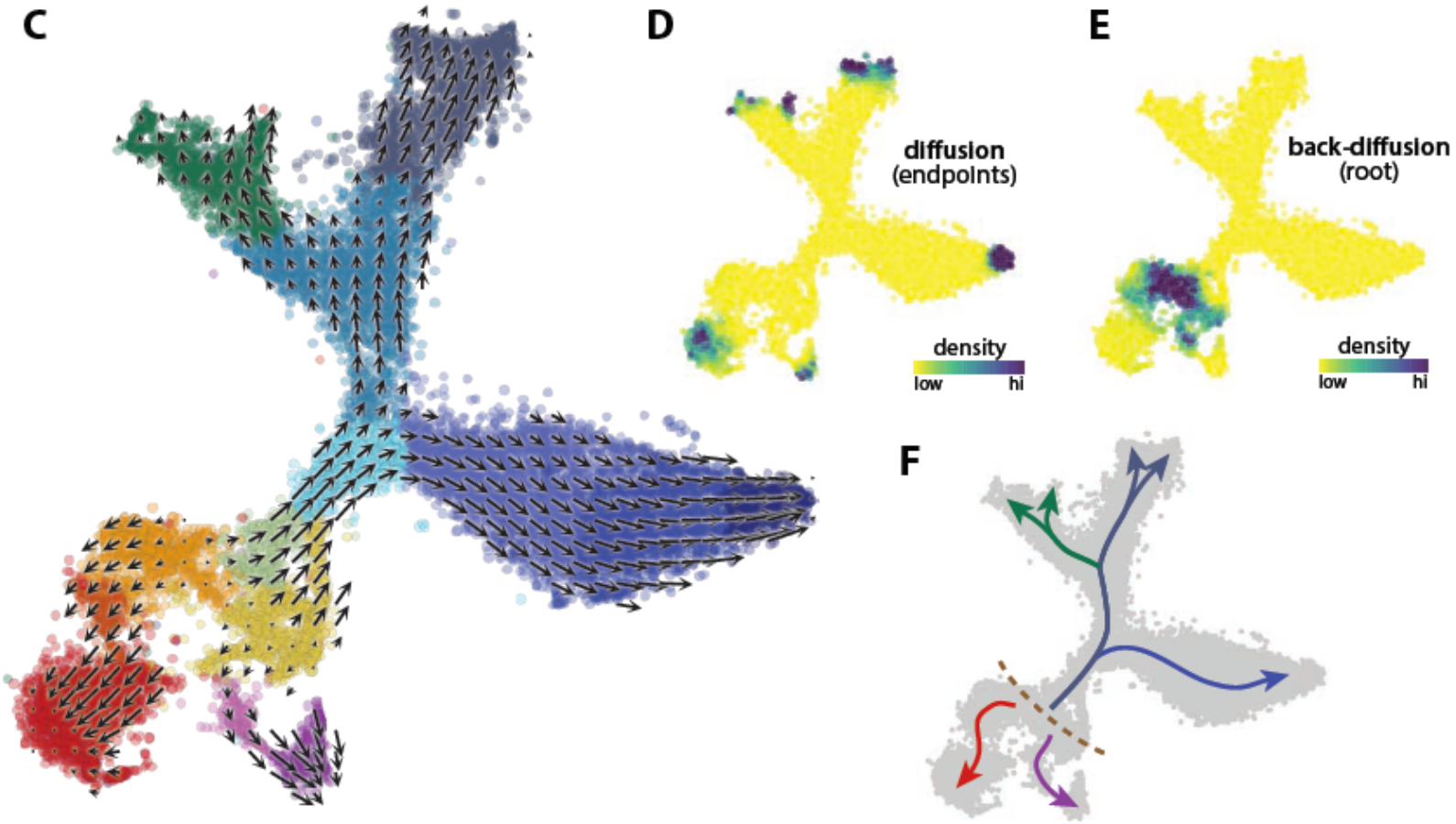




Unspliced mRNA
percentage



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



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

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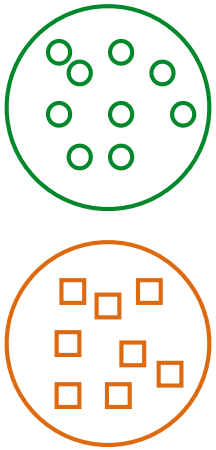
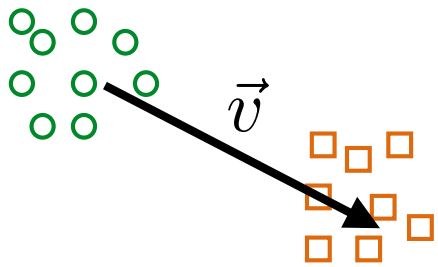
Batch correction in single-cell data

- 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

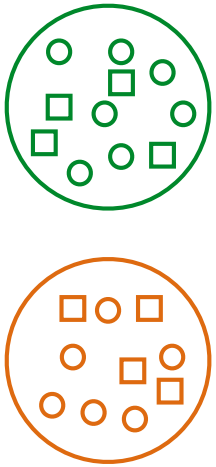
- Totally confounded

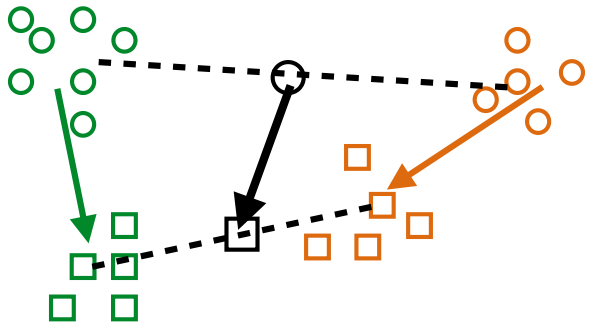




$$\overrightarrow{v} = \overrightarrow{biological} + \overrightarrow{batch}$$

- Balanced





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)

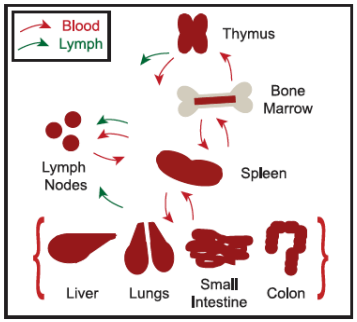
- Projection on a reference embedding
- Scaffold (Spitzer, et al. *Science* 2015)
- Mutual Nearest Neighbours matching (Haghverdi, et al. *Nature biotechnology* 2018)
- CCA approach + alignment (Butler, et al. *Nature biotechnology* 2018)
- Autoencoders approach (Lopez et al. arxiv 2017, Eraslan et al. biorxiv 2018)

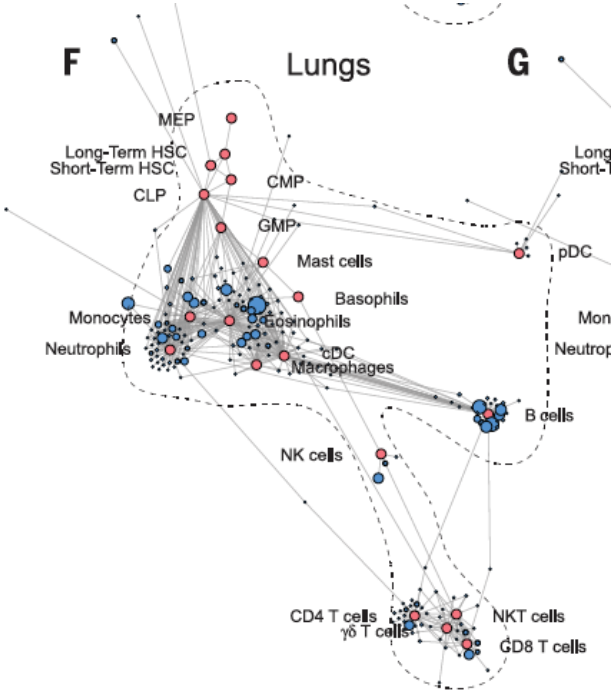
Projection methods

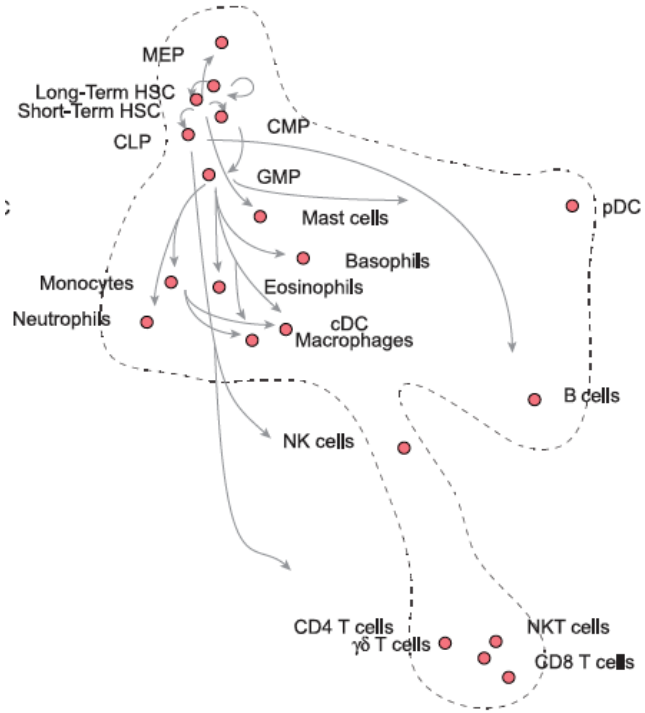
- Inter-batch and intra-batch distances are not comparable and can not be mixed
- Embedding of the reference batch (inter-batch only) + projection of the other batch on that embedding (intra-batch)
- projection with PCA, t-SNE, diffusion maps, etc.
- Requires that the reference batch is a super set of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

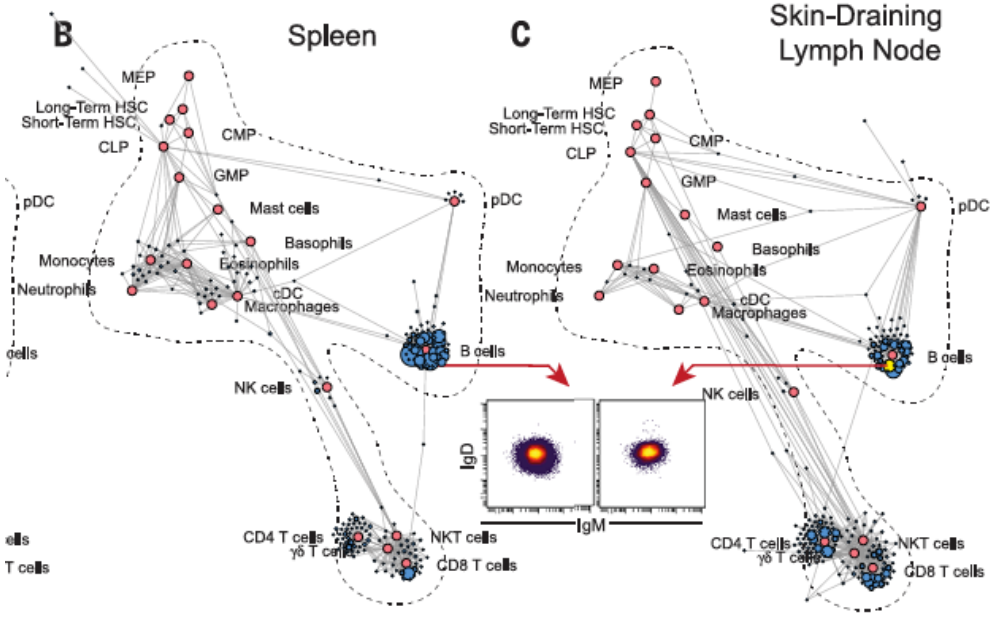
Projection example

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

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

batch **i**
sample (cell) **j**
gene **g**

biological effect

- Centre and scale within each condition

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

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

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

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

RUV, RUVseq

(Risso et al. Nature biotechnology 2014)

$[N, G]$

$$Y = \underbrace{X\beta}_{\text{Biological effects}} + \underbrace{W\alpha}_{\text{Unwanted effects}} + \varepsilon,$$

$[N, K] * [K, G]$ $\qquad$ $[N, L] * [L, G]$

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

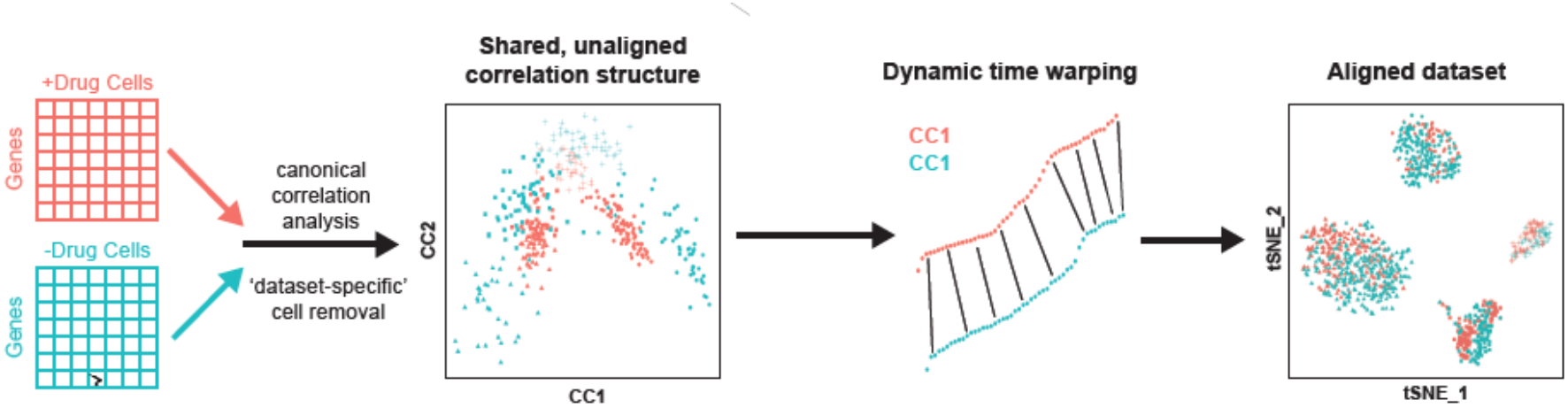
$$Y_c = W\alpha_c + \varepsilon_c,$$

- How to know which genes will not be affected?

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

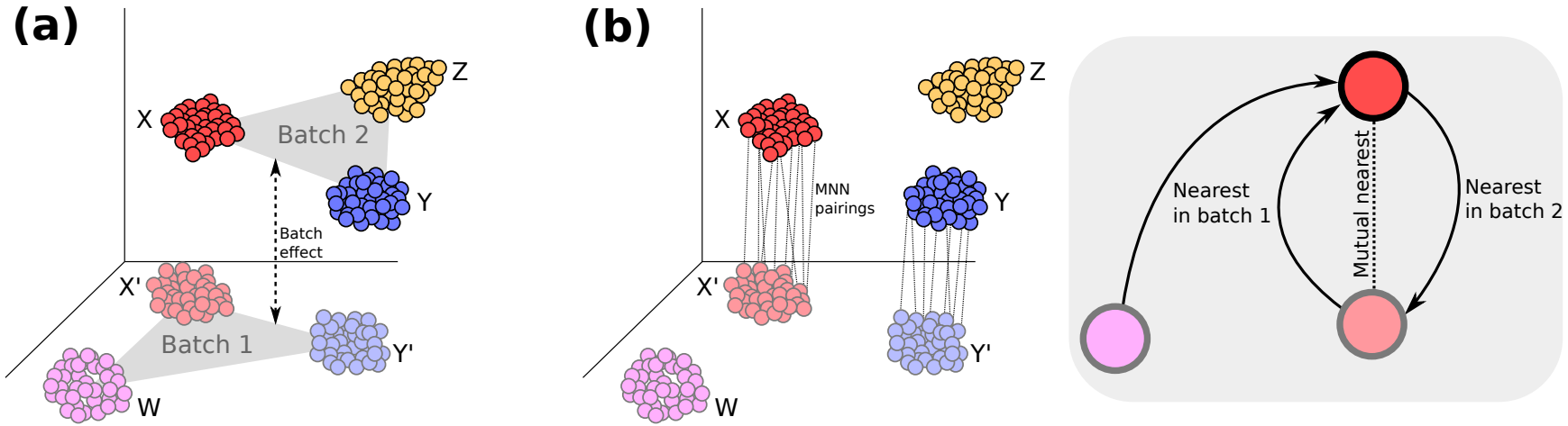


Mutual Nearest Neighbours Analysis

Haghverdi et al. *Nature biotechnology* 2018

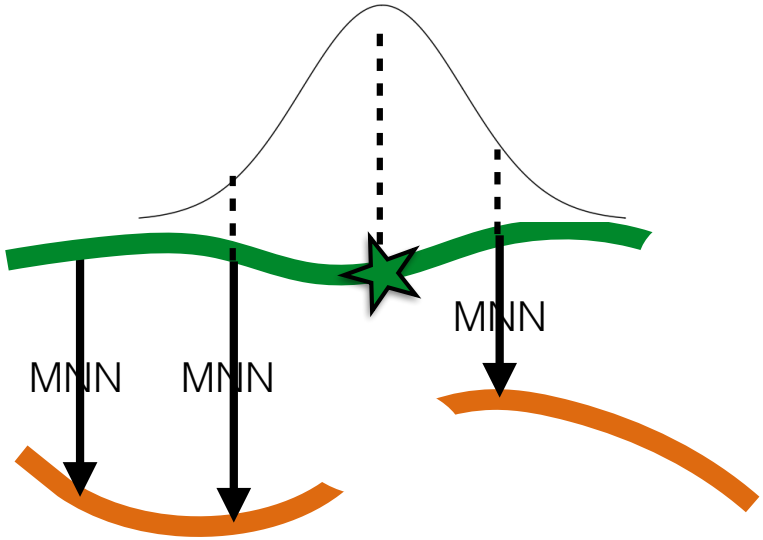
- Batch effect is almost orthogonal to the biological hyper plane (Central limit theorem)

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

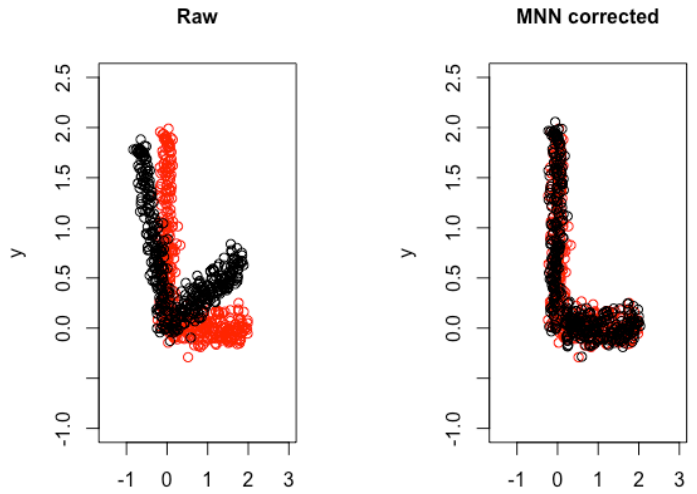


Mutual Nearest Neighbours Analysis

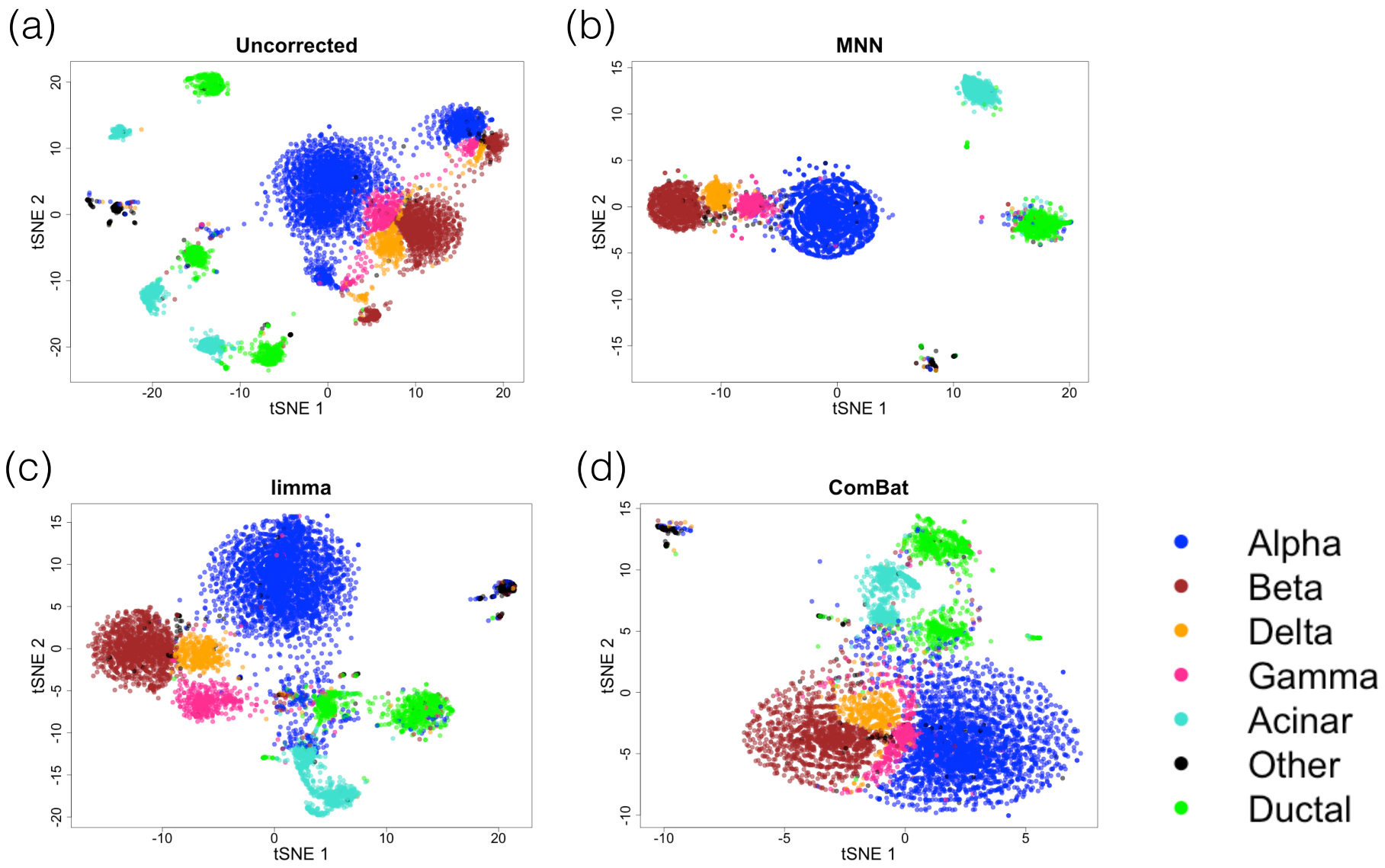
- Once a few MNN pairs have been identified at a few locations, batch effects for everywhere else is estimated using a smoothing Gaussian kernel



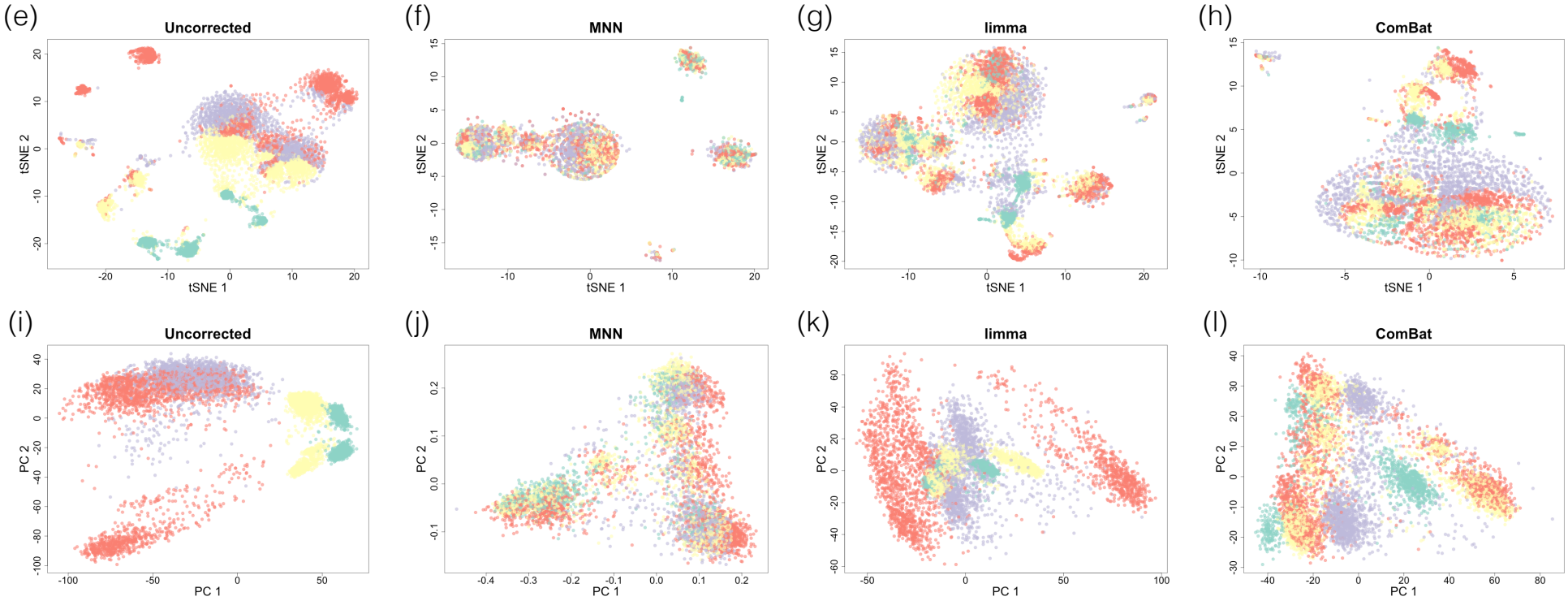
- Locally linear (overall nonlinear)
- Tolerates small non-orthogonalities

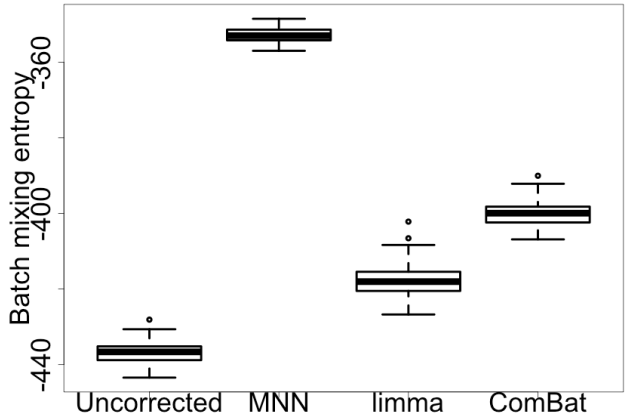


MNN on four Pancreas data sets

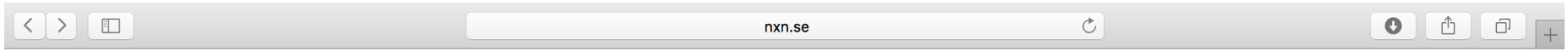


MNN on four Pancreas data sets





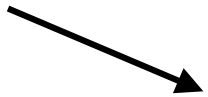
With autoencoders



WHAT DO YOU MEAN "HETEROGENEITY"?

VALENTINE SVENSSON

BLOG ARCHIVE NOTES PHOTOGRAPHY PUBLICATIONS ABOUT



2018

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