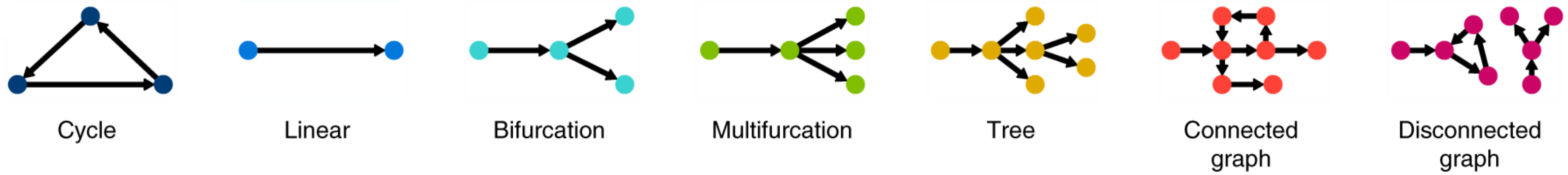
Lecture 7
Trajectory analysis

Trajectory inference (TI) for scRNA-seq

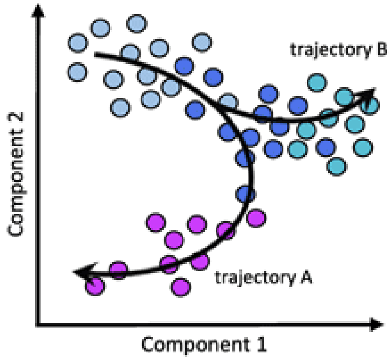
- Understand the cell fate decisions in biological processes, such as differentiation, immune response, or cancer expansion with scRNA-seq data
- Infer or assume a type of underlying trajectory structure



Saelens W. et. al., *Nat. Biotech.* **37**, 547–554(2019)

- Computationally project and order the cells along the trajectory

- The orders of the cells are also called the pseudotimes

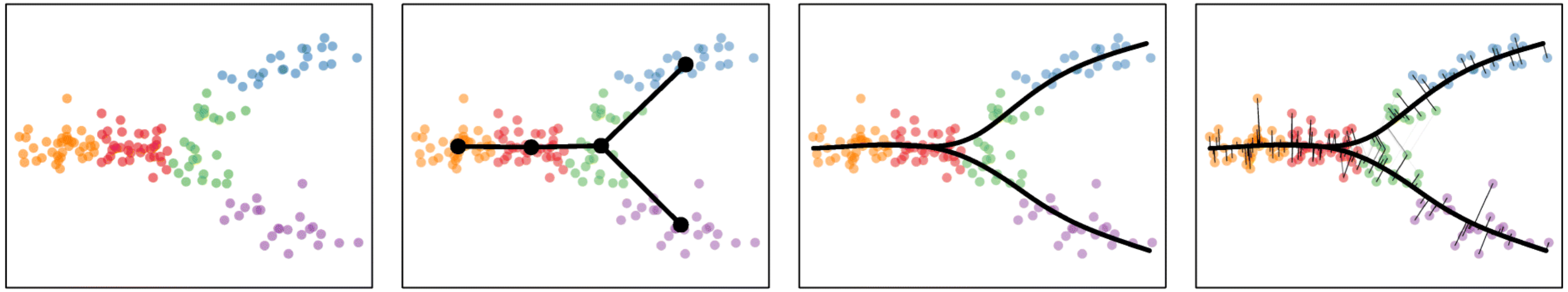


Liu S. *F1000Research* 5 (2016)

- There already exists more than 70 TI methods
(For a comprehensive benchmarking, see Saelens W. et. al., *Nat. Biotech.* **37**, 547–554(2019))

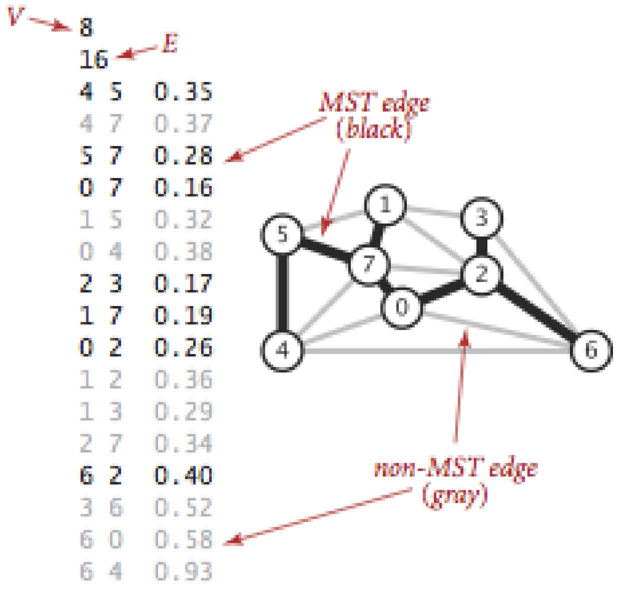
Slingshot (Street et. al., BMC Genomics, 2018)

- Idea: build a connection graph for the clusters



- Main steps:
  - Dimension reduction and clustering
  - Treat clusters as nodes in a graph and draw a minimum spanning tree (MST)
    - MST: spanning tree whose weights (sum of its edge weights) is the smallest among spanning trees
    - Greedy MST algorithm to find the solution
      - Tutorial: https://algs4.cs.princeton.edu/43mst/
    - Edge weight: distance between two clusters

$$d^2(\mathcal{C}_i,\mathcal{C}_j) \equiv (\bar{X}_i - \bar{X}_j)^T (S_i + S_j)^{-1} (\bar{X}_i - \bar{X}_j)$$

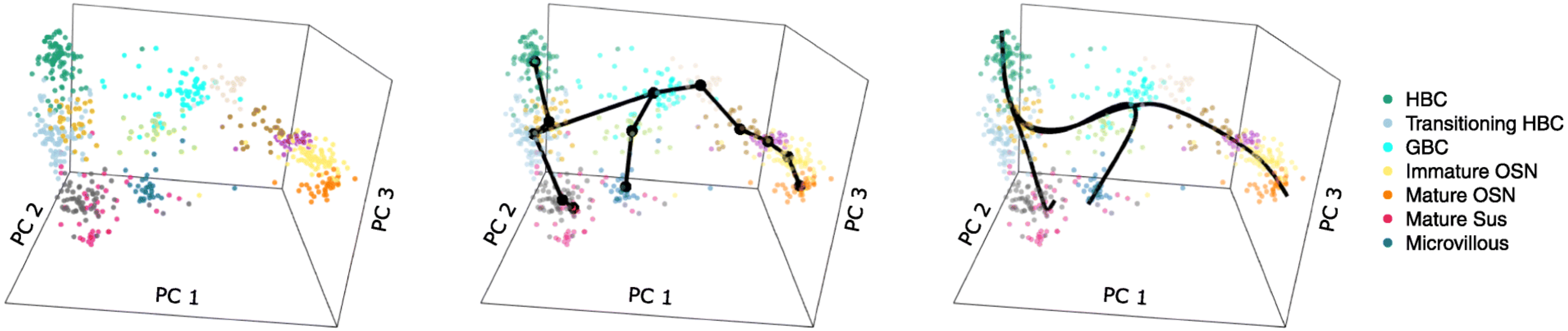


```
8
16
4 5  0.35
4 7  0.37
5 7  0.28
0 7  0.16
1 5  0.32
0 4  0.38
2 3  0.17
1 7  0.19
0 2  0.26
1 2  0.36
1 3  0.29
2 7  0.34
6 2  0.40
3 6  0.52
6 0  0.58
6 4  0.93
```

An edge-weighted graph and its MST

Slingshot (Street et. al., BMC Genomics, 2018)

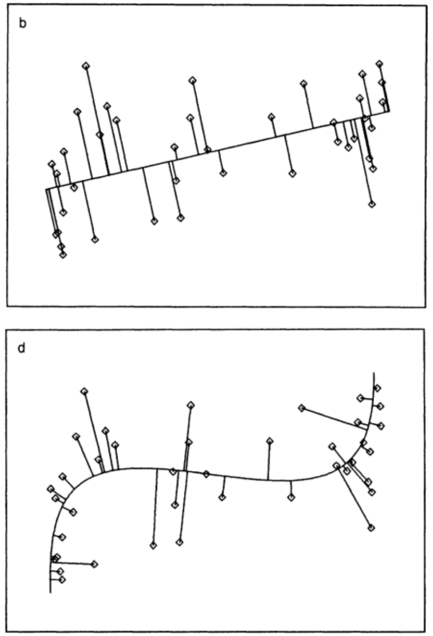
- Main steps:
  - Estimate the lineage (trajectory) structure
    - Dimension reduction and clustering
    - Treat clusters as nodes in a graph and draw a minimum spanning tree (MST)
    - Undirected tree -> directed tree: user provided initial cluster
      - Perform constrained MST if users provide the leaf node
  - Drawback: what if the lineage structure is not a tree?
  - Estimate a cell pseudotime
    - For each lineage (path from initial node to a leaf node), fit a principal curve and project the cells onto the principal curve to determine the pseudotime



    - Challenge: shared lineages should have overlapping principal curves and cells belonging to multiple lineages should have similar pseudotime estimates

Slingshot (Street et. al., BMC Genomics, 2018)

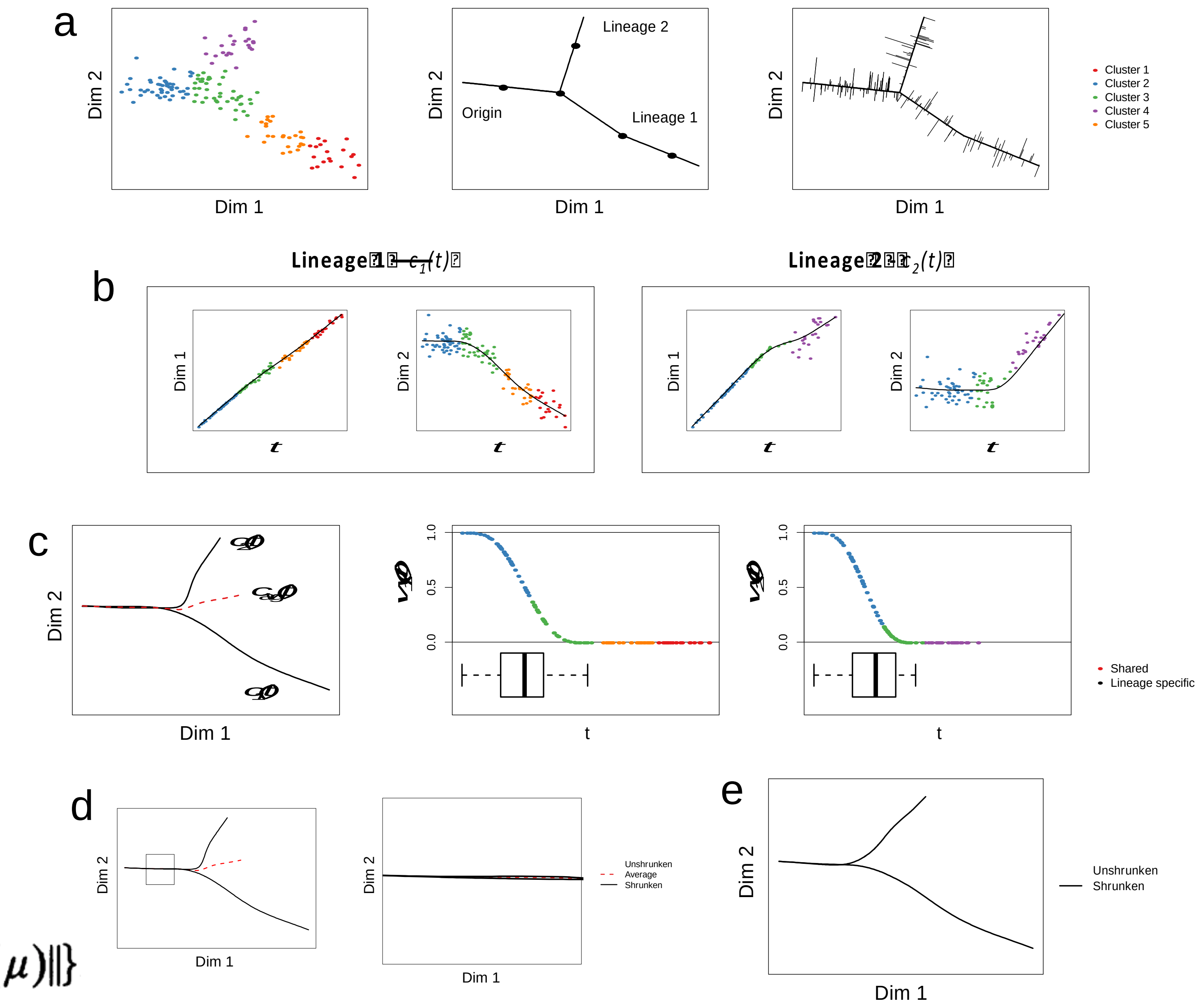
- Principal curve (Hastie and Stuetzle, JASA 1989)



- Generalization of getting first (linear) PC

$$\mathbf{x}_i = \mathbf{f}(\lambda_i) + \mathbf{e}_i$$

$$\lambda_{\mathbf{f}}(\mathbf{x}) = \sup_{\lambda}\{\lambda : \|\mathbf{x} - \mathbf{f}(\lambda)\| = \inf_{\mu}\|\mathbf{x} - \mathbf{f}(\mu)\|\}$$



PAGA (Wolf et. al., Genome Biology, 2019)

- Construct KNN graph of the cells (use any reasonable method, can apply denoising first)

- Clustering and determine connectivity between clusters based on the KNN graph
    - ε_{ij}^{sym}: number of edges (outgoing and ingoing) between cluster i and j
    - Under the “null” where there is no connection between the two clusters

$$p_{\text{arbit}}(\varepsilon|e_i, e_j, n_i, n_j, n) \simeq \mathcal{N}(\varepsilon|\hat{\varepsilon}^{\text{sym}}(e_i, e_j, n_i, n_j, n), \hat{\sigma}^{\text{sym}}(e_i, e_j, n_i, n_j, n))$$

$$\text{with} \quad \hat{\varepsilon}^{\text{sym}}(e_i, e_j, n_i, n_j, n) = \tfrac{e_i n_j + e_j n_i}{n-1},$$

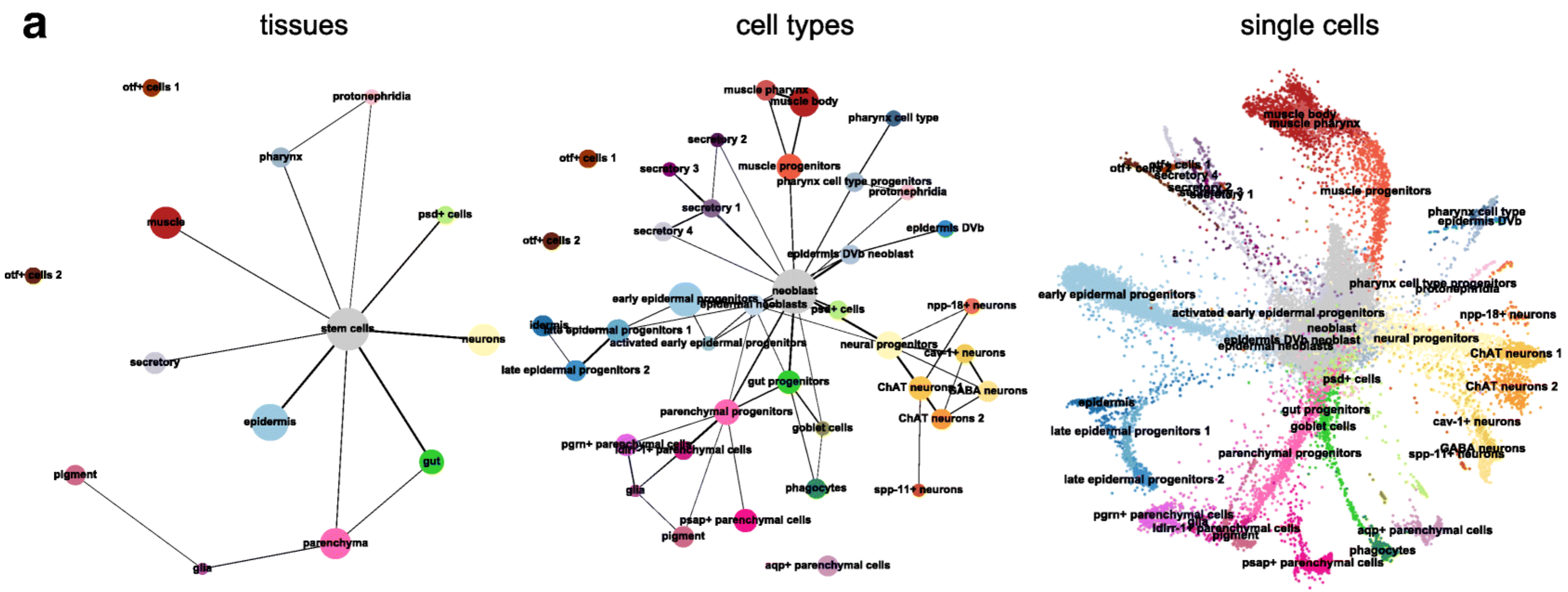
$$\hat{\sigma}^{\text{sym}}(e_i, e_j, n_i, n_j, n) = \tfrac{e_i n_j (n-n_j-1) + e_j n_i (n-n_i-1)}{(n-1)^2}.$$

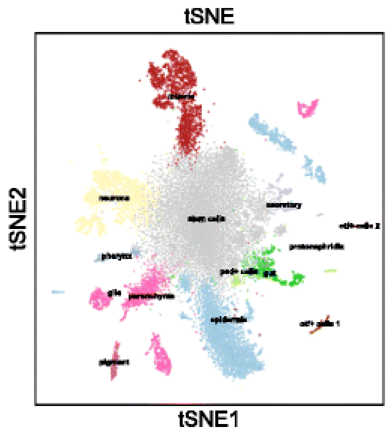
        - n_i: number of nodes in cluster i, e_i: number of outgoing edges of cluster i
    - Cluster connectivity score:

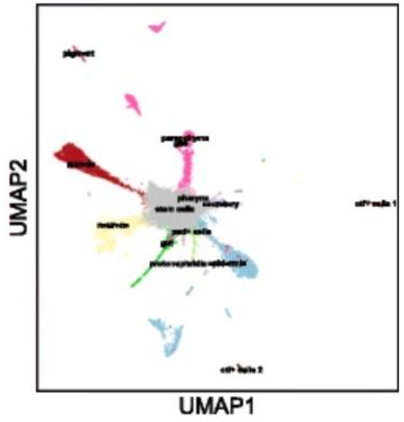
$$c_{ij} = \begin{cases} \frac{\varepsilon_{ij}^{\text{sym}}}{\hat{\varepsilon}^{\text{sym}}(e_i, e_j, n_i, n_j, n)} & \text{if } \varepsilon_{ij}^{\text{sym}} < \hat{\varepsilon}^{\text{sym}}(e_i, e_j, n_i, n_j, n) \\ 1 & \text{else.} \end{cases}$$

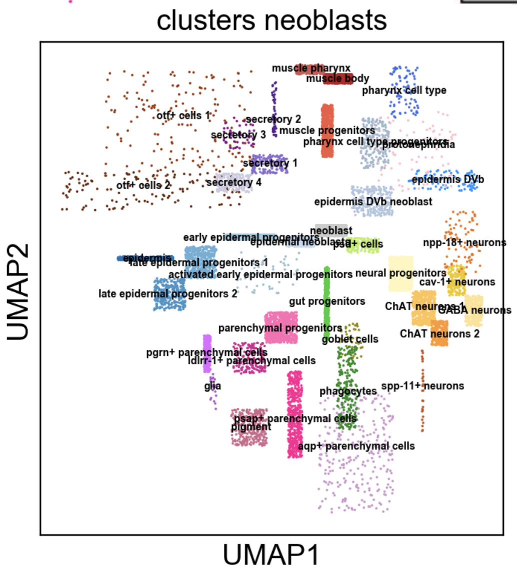
- Thresholding cluster connectivity score to get the final trajectory structure

PAGA (Wolf et. al., Genome Biology, 2019)









- Initialize UMAP with the coarse cluster graph leads to better visualization of the data

PAGA (Wolf et. al., Genome Biology, 2019)

- Pseudotime estimation for each cell (DPT)
  - Pseudotime defined as the distance of a continuous progression along a manifold
  - Based on a diffusion maps model on the cell-cell graph (like MAGIC, cell-cell transition matrix T)
  - Some highlights of the algorithm
    - Laplace transformation

$$\widetilde{L} = I - \widetilde{T}, \quad \widetilde{T} = D^{\frac{1}{2}} T D^{-\frac{1}{2}}$$

    - Calculate diffusion pseudotime based on the eigenvectors and eigenvalues of L (or equivalently, T)

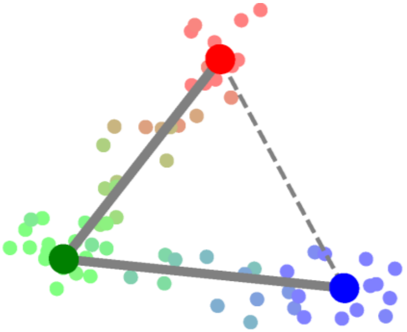
$$\widetilde{\text{dpt}}^2(\iota_1, \iota_2) = \sum_{r=2}^{n_{\text{nodes}}} \left(\frac{\lambda_r}{1-\lambda_r}\right)^2 (\widetilde{v}_{r\iota_1} - \widetilde{v}_{r\iota_2})^2$$

    - Making using of trajectory structure: assign ∞ to cell-cell distance for cells in disconnected clusters

$$\widetilde{\text{dpt}}(\iota_1, \iota_2) = \sum_{r=n_{\text{comps}}+1}^{n_{\text{nodes}}} \left(\frac{\lambda_r}{1-\lambda_r}\right)^2 (\widetilde{v}_{r\iota_1} - \widetilde{v}_{r\iota_2})^2 + \sum_{r=1}^{n_{\text{comps}}} (\widetilde{v}_{r\iota_1} - \widetilde{v}_{r\iota_2})^2.$$

VITAE (Du et. al., BioRXiv, 2023)

- Combine a graph-based method and direct modeling of the data using variational autoencoder
- Assume a complete graph $\mathcal{G} = (\mathcal{N}, \mathcal{E})$
  - $\mathcal{N}(\mathcal{G})$: a vertex denotes a distinct cell state / type
  - $\mathcal{E}(\mathcal{G})$: an edge denotes a possible transition between two cell states/types



- A cell position $\tilde{\boldsymbol{w}}_i \in [0, 1]^k$ on the graph

$$\tilde{\boldsymbol{w}}_i = \begin{cases} \boldsymbol{e}_j & \text{if cell } i \text{ is on vertex } j \in \{1, \cdots, k\} \\ w_i \boldsymbol{e}_{j_1} + (1 - w_i)\boldsymbol{e}_{j_2} & \text{if cell } i \text{ is on the edge between vertices } j_1 \text{ and } j_2 \ (j_1 \neq j_2) \end{cases}$$

- The trajectory backbone, $\mathcal{B}$, as a subgraph of $\mathcal{G}$

$$\mathcal{N}(\mathcal{B}) = \mathcal{N}(\mathcal{G})$$

$$\mathcal{E}(\mathcal{B}) = \left\{ (j_1, j_2) \in \mathcal{E}(\mathcal{G}) : \sum_i \mathbb{1}_{\{\tilde{w}_{ij_1} > 0, \tilde{w}_{ij_2} > 0\}} > 0 \right\}$$

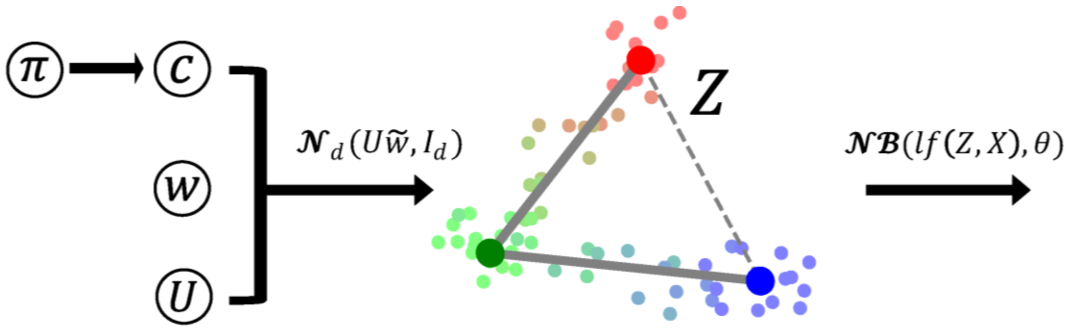
VITAE (Du et. al., BioRXiv, 2023)

$$w_i \overset{\text{i.i.d.}}{\sim} \text{Uniform}(0,1)$$

$$c_i \overset{\text{i.i.d.}}{\sim} \text{Multinomial}(1, \boldsymbol{\pi}),$$

$$w_i \perp\!\!\!\perp c_i$$

$$\tilde{\boldsymbol{w}}_i = w_i \boldsymbol{a}_{c_i} + (1 - w_i) \boldsymbol{b}_{c_i}$$



π
c
w
U
$\mathcal{N}_d(U\tilde{w}, I_d)$
Z
$\mathcal{NB}(lf(Z,X),\theta)$

Observed count data Y_{ig}

	Gene$_1$	Gene$_2$	Gene$_3$...
Cell$_1$	1	2	4
Cell$_2$	0	1	0
Cell$_3$	1	1	3
Cell$_4$	0	2	1
Cell$_5$	1	1	5
Cell$_6$	3	0	1
Cell$_7$	2	0	2
...			...

- Assume latent variables $\boldsymbol{Z}_i \in \mathbb{R}^d$ satisfy

$$\boldsymbol{Z}_i | \tilde{\boldsymbol{w}}_i \sim \mathcal{N}_d(\boldsymbol{U}\tilde{\boldsymbol{w}}_i, \boldsymbol{I}_d)$$

A non-linear mapping from the latent space to the high-dimensional observed data

Model f_g by a neural network

  - $\boldsymbol{U}$: unknown positions of the vertices in $\mathbb{R}^d$
  - $\boldsymbol{X}_i$: cell-specific confounding covariates (data source, cell cycle, et. al.)

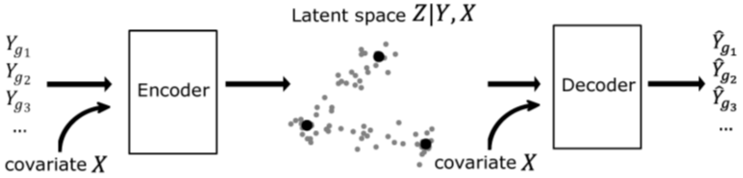
- We also assume a mixture prior on $\tilde{\boldsymbol{w}}_i$

VITAE (Du et. al., BioRXiv, 2023)

- Key contribution: Simultaneous batch effect removal and trajectory analysis
- Loss function:

Reconstruction loss

$$
\begin{aligned}
L = & -(1-\alpha)\sum_{i=1}^{N}\mathbb{E}_{q(\boldsymbol{Z}_i|\mathbf{Y}_i,\boldsymbol{X}_i)}\log p(\mathbf{Y}_i|\boldsymbol{Z}_i,\boldsymbol{X}_i) \\
& +\beta\sum_{i=1}^{N}D_{\mathrm{KL}}(q(\boldsymbol{Z}_i|\mathbf{Y}_i,\boldsymbol{X}_i)\|p(\boldsymbol{Z}_i)) \\
& -\alpha\sum_{i=1}^{N}\log p(\mathbf{Y}_i|\boldsymbol{Z}_i=\mathbf{0}_d,\boldsymbol{X}_i) \\
& +\kappa\,\Omega_{\mathrm{MMD}}(\mathcal{D}_N) \\
& +\gamma\,\Omega_{\mathrm{Jacobian}}(\mathcal{D}_N).
\end{aligned}
$$



- Four penalty terms:
  - β-VAE:
    - Set $\beta > 1$ to encourage posteriors of $\boldsymbol{Z}_i$ to lie along trajectory backbone
  - Adjust for confounding $\boldsymbol{X}_i$ and batch effects
    - Soft penalty: help decorrelate $\boldsymbol{Z}_i$ from $\boldsymbol{X}_i$
    - MMD loss: used across replicates where the cell populations are known to be the same
  - Jacobian regularizer
    - enhance stability in optimization

$$
\Omega_{\mathrm{Jacobian}}(\mathcal{D}_N)=\sum_{i=1}^{N}\sum_{j=1}^{d}\sum_{g=1}^{G}\mathbb{E}_{q(\boldsymbol{Z}_i|\mathbf{Y}_i,\boldsymbol{X}_i)}\left[\left(\frac{\partial \boldsymbol{Z}_{ij}}{\partial \mathbf{Y}_{ig}}\right)^2\right]
$$

GPfates (Lonnberg et. al., Science Immunology, 2017)

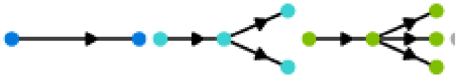
- Model (normalized and dimension-reduced) scRNA-seq data as generated from a mixture of Gaussian processes

$$X = f_c(t) + \varepsilon \quad p(F|T) = \prod_{c=1}^{C} \mathcal{N}(f_c|0, \boldsymbol{K}_t^c)$$

$$k(t_{n_1}, t_{n_2}) = \sigma_{\mathrm{SE}}^2 \exp\left(-\frac{|t_{n_1} - t_{n_2}|^2}{2l_{\mathrm{SE}}^2}\right)$$

  - Infer posterior $t|X$ to estimate each cell's pseudotime
  - Prior distribution $p(t_n) = \mathcal{N}(\mathrm{day}_n, \sigma_{\mathrm{prior}}^2)$
    - Make use of the calendar time

  - Use variational Bayes and EM to infer parameters
  - For interpretation of each GP component, only allow one branching point

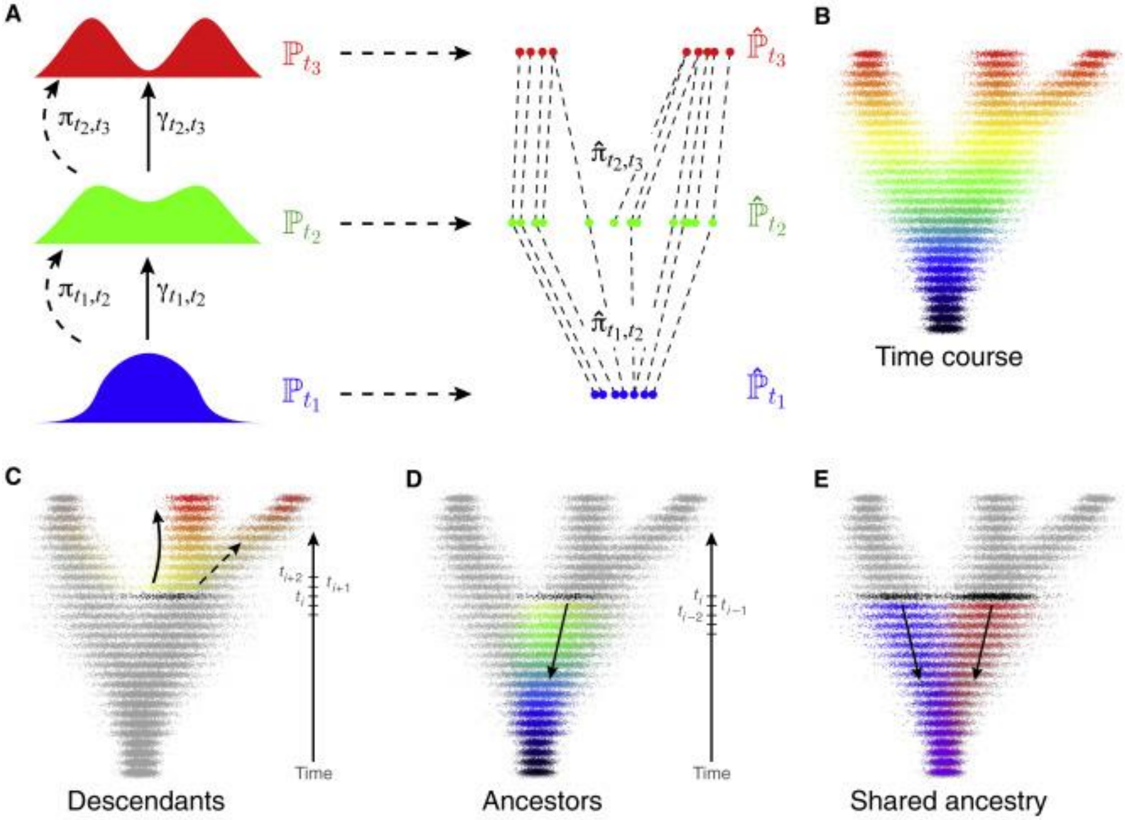


Waddington-OT (Schiebinger et. al., Cell, 2019)

- Make use the cell collection time and assume that cells having a later collection time are descendants of the earlier collected cells
- Estimate transition between cells
  - Optimal transport coupling

$$\begin{aligned} \pi_{s,t}(\epsilon) = \underset{\pi}{\text{minimize}} \quad & \iint c(x,y)\pi(x,y)dxdy - \epsilon \iint \pi(x,y)\log\pi(x,y)dxdy \\ \text{subject to} \quad & \int \pi(x,\cdot)dx = \mathbb{Q}_s \\ & \int \pi(\cdot,y)dy = \mathbb{P}_t. \end{aligned}$$

    - $\pi(x,y)$: joint distribution at two time points
    - $c(x,y)$: pre-defined cost function
  - Corresponding optimization problem



$$\begin{aligned} \hat{\pi}_{t_i,t_{i+1}} \quad = \quad \underset{\pi}{\arg\min} \quad & \sum_{x\in S_i}\sum_{y\in S_{i+1}} c(x,y)\pi(x,y) - \epsilon \iint \pi(x,y)\log\pi(x,y)dxdy \\ & + \lambda_1 \mathrm{KL}\left[\sum_{x\in S_i}\pi(x,y)\middle\| d\hat{\mathbb{P}}_{t_{i+1}}(y)\right] + \lambda_2 \mathrm{KL}\left[\sum_{y\in S_{i+1}}\pi(x,y)\middle\| d\hat{\mathbb{Q}}_{t_i}(x)\right] \end{aligned}$$

Related papers

- Street, K., Risso, D., Fletcher, R. B., Das, D., Ngai, J., Yosef, N., ... & Dudoit, S. (2018). Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics. *BMC genomics*, *19*, 1-16.
- Wolf, F. A., Hamey, F. K., Plass, M., Solana, J., Dahlin, J. S., Göttgens, B., ... & Theis, F. J. (2019). PAGA: graph abstraction reconciles clustering with trajectory inference through a topology preserving map of single cells. *Genome biology*, *20*, 1-9.
- Haghverdi, L., Büttner, M., Wolf, F.A., Buettner, F. and Theis, F.J., 2016. Diffusion pseudotime robustly reconstructs lineage branching. Nature methods, 13(10), pp.845-848.
- Du, J. H., Chen, T., Gao, M., & Wang, J. (2023). Model-based trajectory inference for single-cell rna sequencing using deep learning with a mixture prior. *bioRxiv*, 2020-12.
- Lönnberg, T., Svensson, V., James, K. R., Fernandez-Ruiz, D., Sebina, I., Montandon, R., ... & Teichmann, S. A. (2017). Single-cell RNA-seq and computational analysis using temporal mixture modeling resolves TH1/TFH fate bifurcation in malaria. *Science immunology*, *2*(9), eaal2192.
- Schiebinger, G., Shu, J., Tabaka, M., Cleary, B., Subramanian, V., Solomon, A., ... & Lander, E. S. (2019). Optimal-transport analysis of single-cell gene expression identifies developmental trajectories in reprogramming. *Cell*, *176*(4), 928-943.

- Coifman, R. R., & Lafon, S. (2006). Diffusion maps. *Applied and computational harmonic analysis*, *21*(1), 5-30.
- Hastie, T., & Stuetzle, W. (1989). Principal curves. *Journal of the American statistical association*, *84*(406), 502-516.