

Cell lineage and pseudotime inference for single-cell transcriptomics (Monocle & Slingshot)

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Resources

Trapnell et al. [The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells](#). *Nat Biotechnol*, 32(4):381-6, 2014.

Street et al. [Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics](#). *BMC Genomics*, 19:477, 2018.

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- 1 Monocle
- 2 Monocle2
- 3 Slingshot
- 4 Monocle3 (beta)

Monocle

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Monocle: main idea

Order cells by progress through a biological process
(development, dose-response, disease progression)

Cells undergo gradual transcriptional changes

Clustering vs. trajectory inference

Clustering:

- assumes biologically distinct groups (discrete cell types / states)
- the basis for most scRNA-seq analyses

Trajectory inference (pseudotemporal ordering):

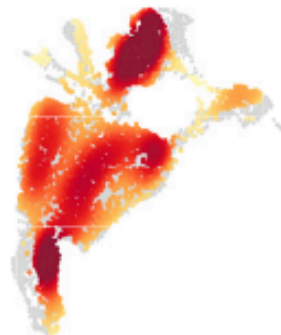
- assumes continuous transitions between cells (lineage / trajectory)
- allows better interpretation of biological processes

CLUSTER ANALYSIS

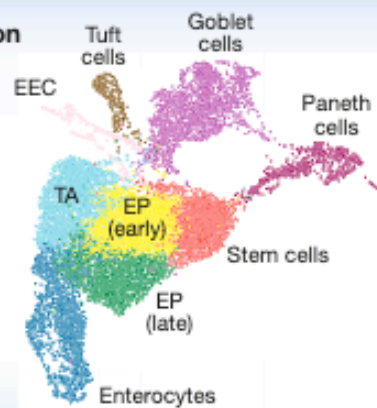
Clustering



Compositional analysis



Cluster annotation

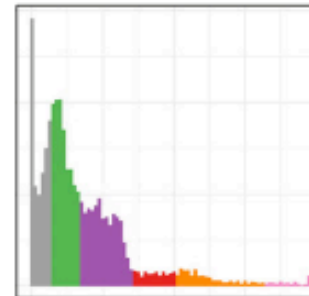


TRAJECTORY ANALYSIS

Trajectory inference

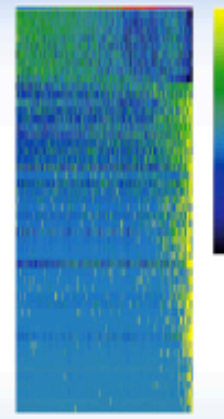


Metastable states



Pseudotime

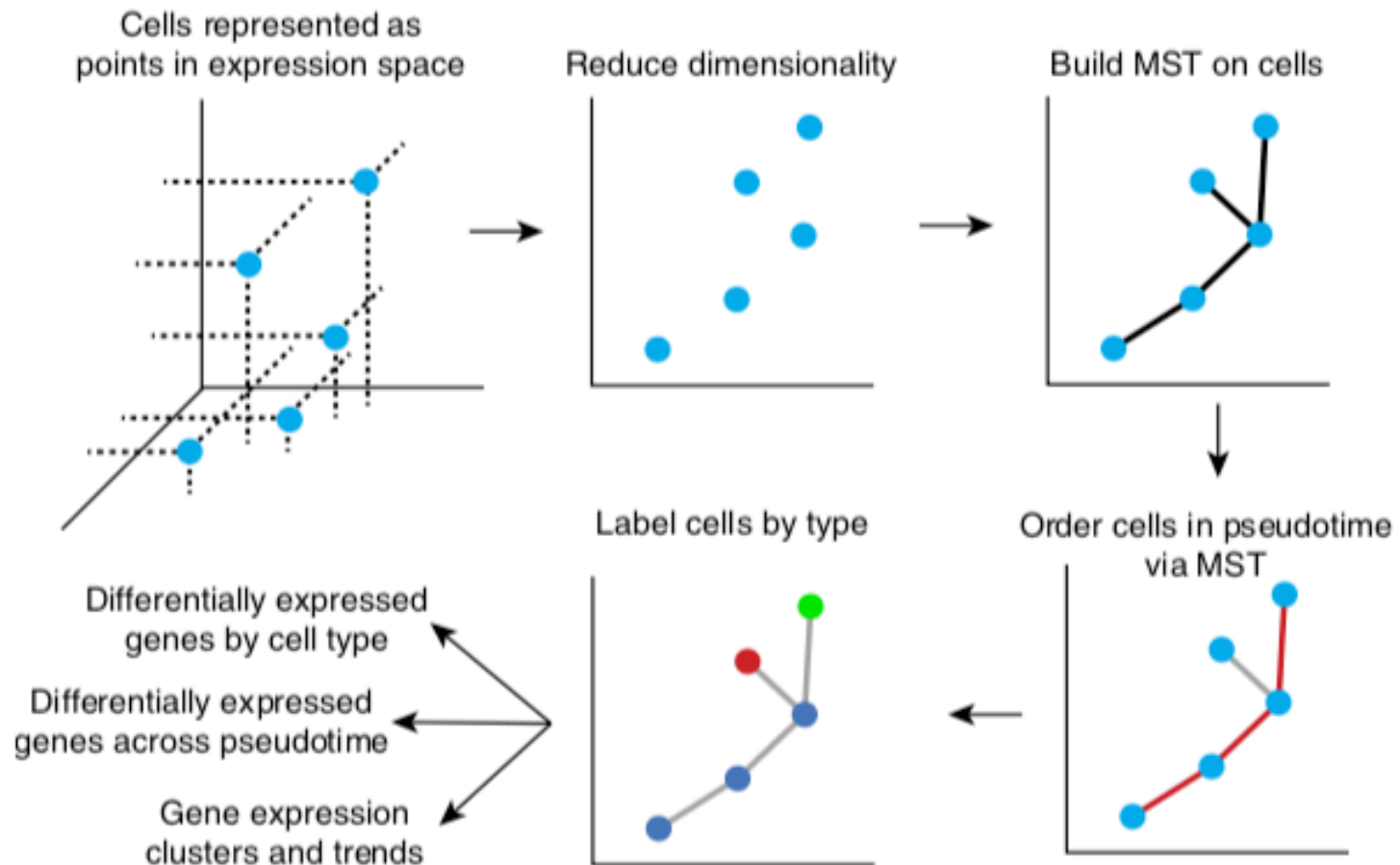
Gene expression dynamics



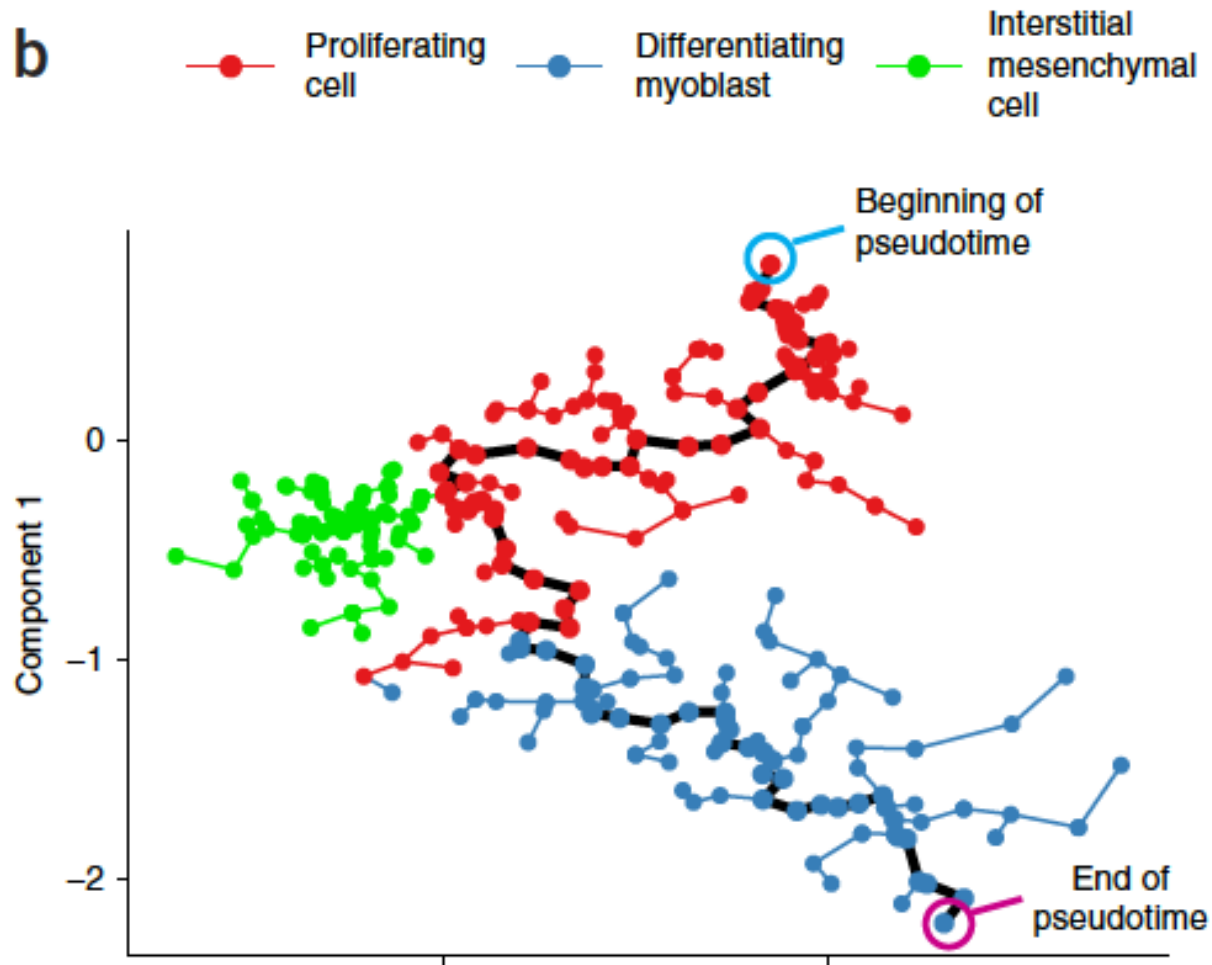
Pseudotime

Monocle: key steps

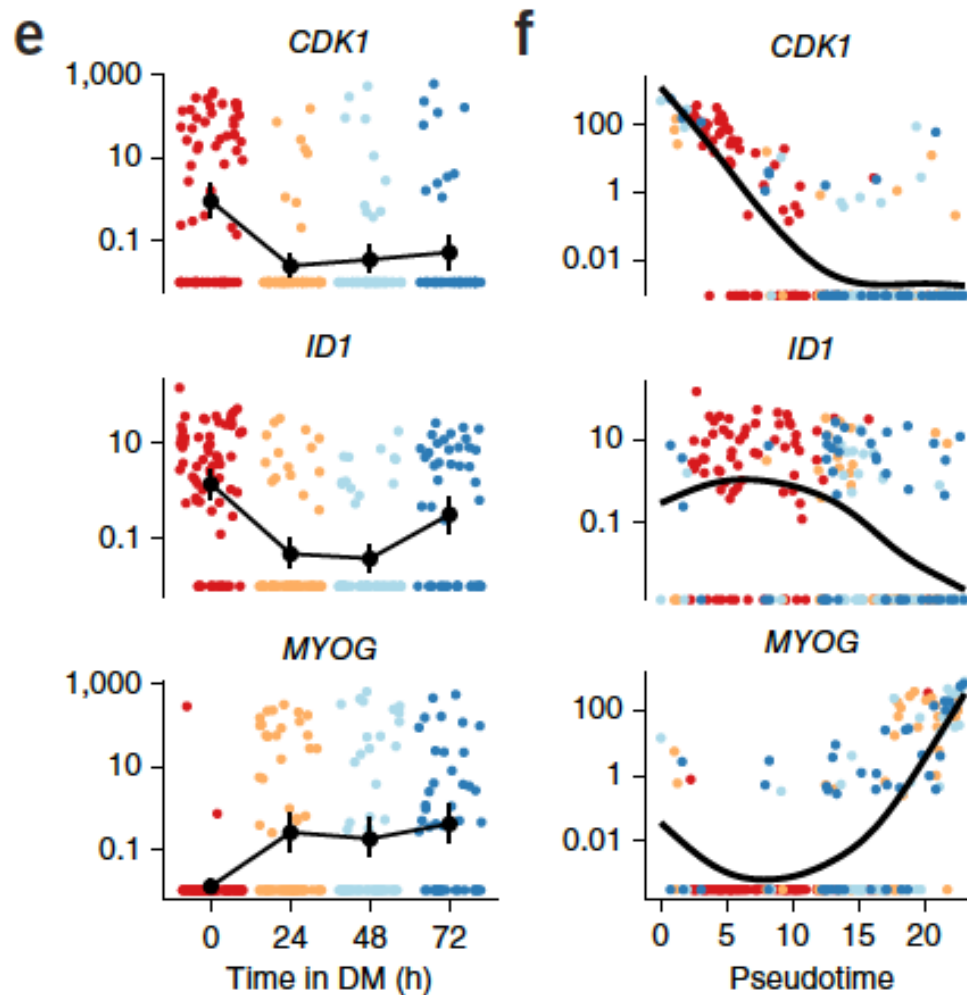
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Monocle: pseudotemporal ordering of cells



Monocle: trajectories of individual genes



Monocle: definition of pseudotime

What is pseudotime?

Pseudotime is a measure of how much progress an individual cell has made through a process such as cell differentiation.

- Biological process (BP): cells don't progress in synchrony
- scRNA-seq of BPs: cells display different progress states
- Pseudotime = distance to the start of the trajectory

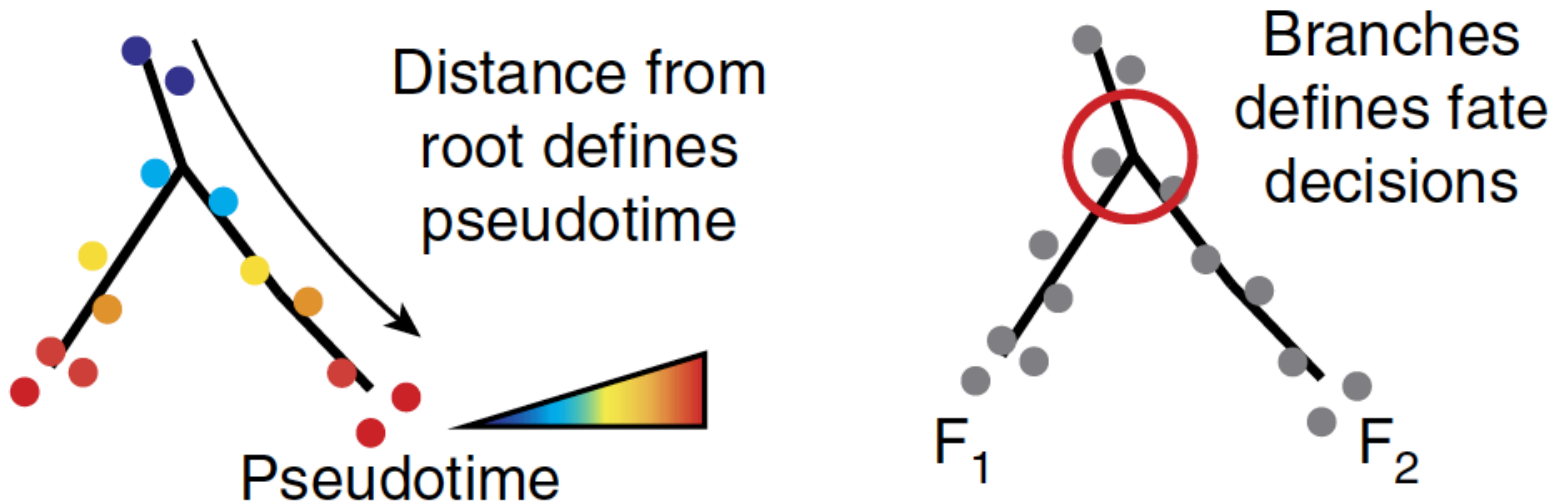
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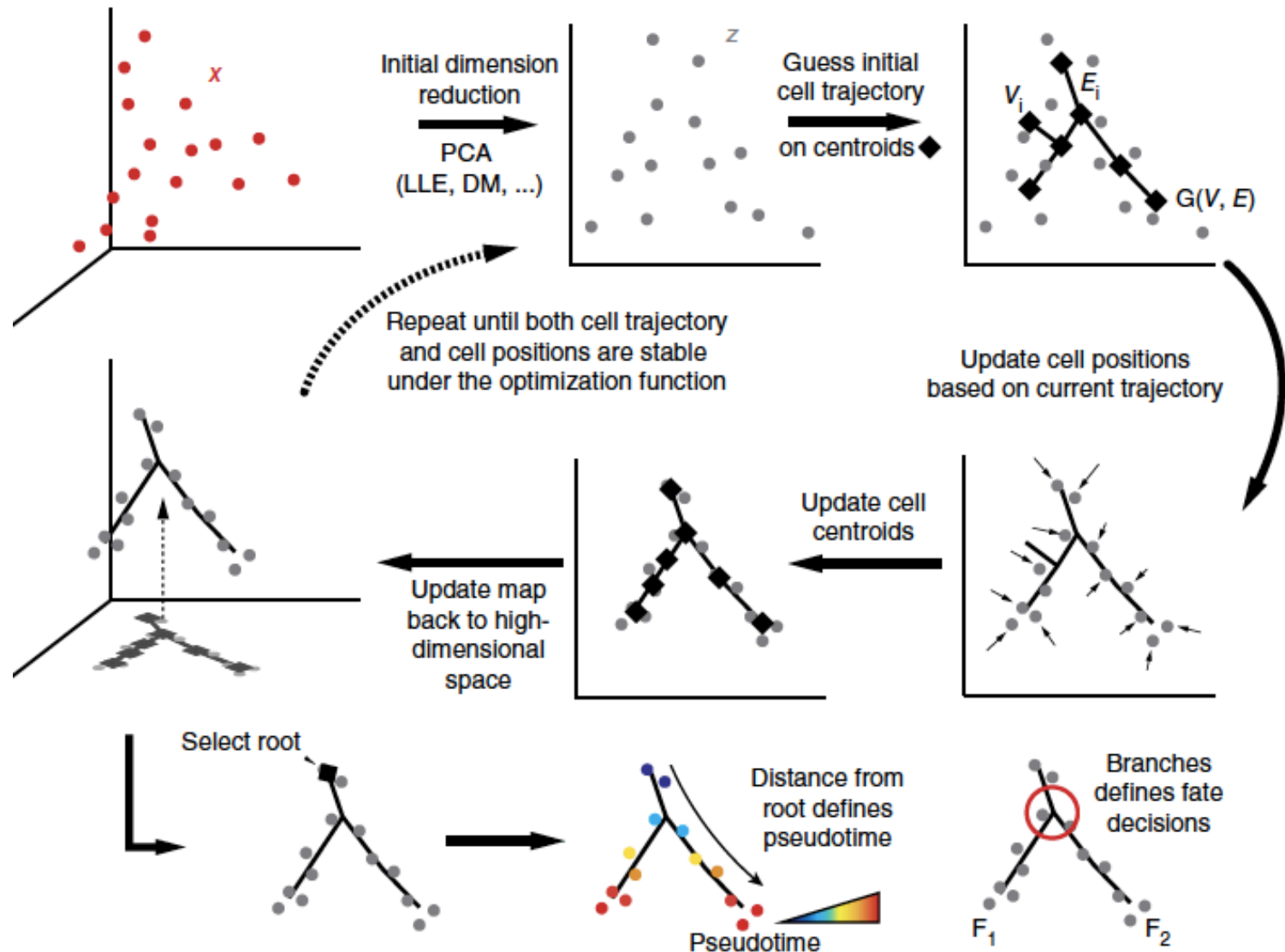
Qiu et al. [Reversed graph embedding resolves complex single-cell trajectories](#). *Nat Methods*, 14(10):979-82, 2017.

Monocle2: main idea

Unsupervised reconstruction of more complex trajectories that include one or more *branching points* (cell fates)



Monocle2: key steps



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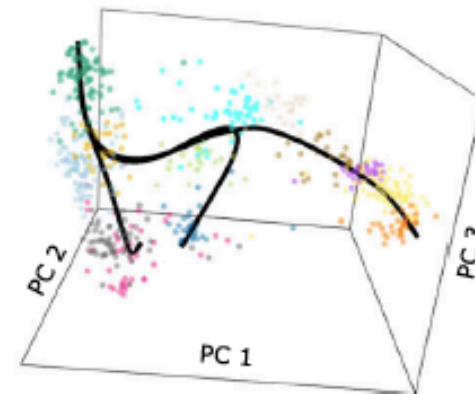
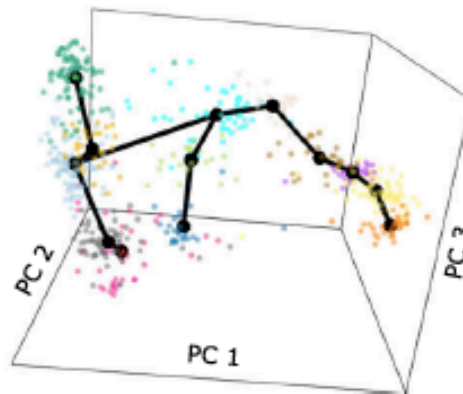
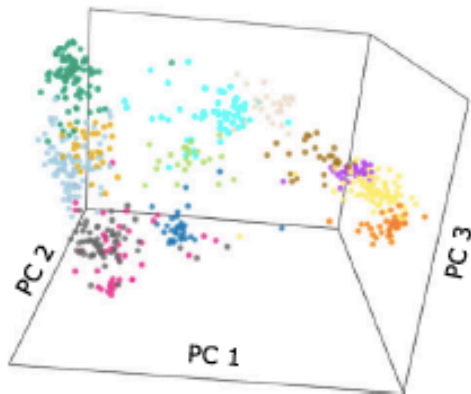
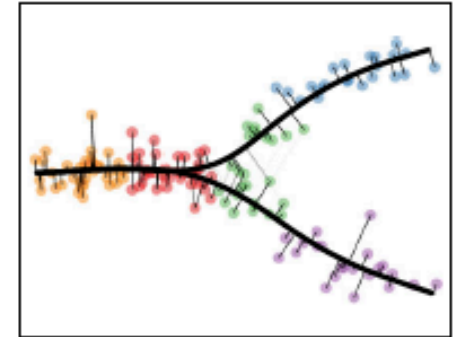
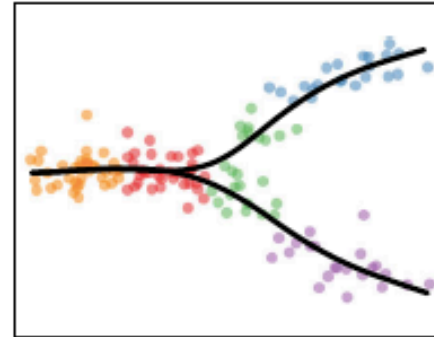
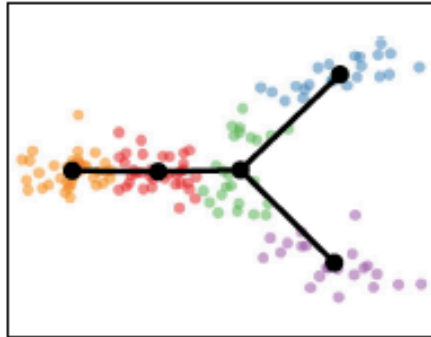
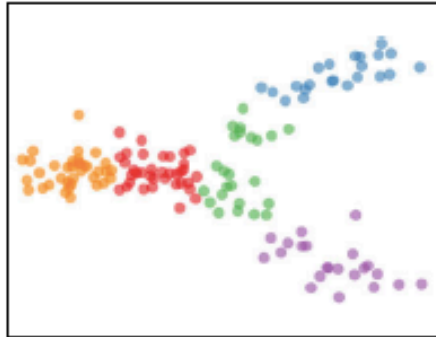
<http://bioconductor.org/packages/slingshot>

Slingshot vs Monocle1/2

Table 1 Summary of existing lineage and pseudotime inference methods

	Dimensionality reduction	Cluster based	Graph	Pseudotime calculation	Branching	Supervision
Diffusion Pseudotime	Diffusion maps	No	Weighted k-NN graph on cells	Transition probabilities over arbitrary length random walks	Yes	Starting cell
Embeddr	Laplacian eigenmaps	No	N/A	Principal curve, orthogonal projection	No	Path direction ¹ , subsetting ²
Monocle	ICA	No	MST on cells	Diameter path, PQ trees	Yes ³	Path direction ¹ , number of lineages
Monocle 2	Reversed graph embedding	No	Principal graph on cells	Distance to root	Yes	Starting cluster
TSCAN	PCA	Yes	MST on clusters	Cluster centers, orthogonal projection	Yes	Starting cluster
Waterfall	PCA	Yes	MST on clusters	Cluster centers, orthogonal projection	Yes ⁴	Path direction ¹
Wishbone	Diffusion maps	No	Ensemble of k-NN graphs on cells	Distance refinement by waypoints	Yes ⁵	Starting cell
Slingshot	Any	Yes	MST on clusters	Simultaneous principal curves, orthogonal projection	Yes	Starting cluster, end clusters (optional)

Slingshot: key steps



- HBC
- Transitioning HBC
- GBC
- Immature OSN
- Mature OSN
- Mature Sus
- Microvillous

1) Dim. reduction
& Clustering

2) Identification of
lineage structure

3) Smoothing of branching
& pseudotime estimation

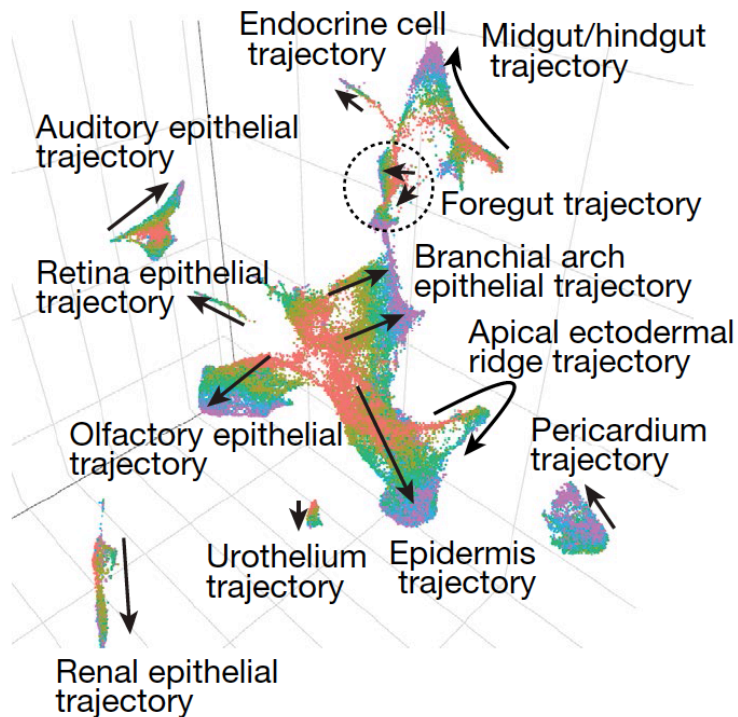
Monocle

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Wolf et al. [PAGA: graph abstraction reconciles clustering with trajectory inference through a topology preserving map of single cells](#). *Genome Biol*, 20:59, 2019.

Monocle3: major updates

- UMAP algorithm to initialize trajectory inference
- Support for trajectories with multiple roots
- Unsupervised partition of cells to learn *disjoint / parallel trajectories*



<https://cole-trapnell-lab.github.io/monocle3/>

Many more tools + related approaches!

- Benchmarking study of 45 trajectory inference methods

Saelens et al. [A comparison of single-cell trajectory inference methods](#).
Nat Biotechnol, 37:547-554, 2019.

- RNA velocity: infers direction + progression from spliced vs. unspliced mRNA

La Manno et al. [RNA velocity of single cells](#). *Nature*, 560(7719):494-8, 2018.