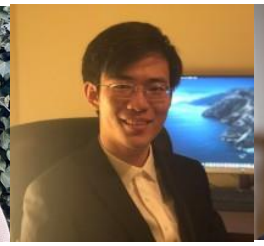
RECOMB
Seoul | 서울 | 2025

Learning Latent Trajectories in Developmental Time Series with Hidden-Markov OT

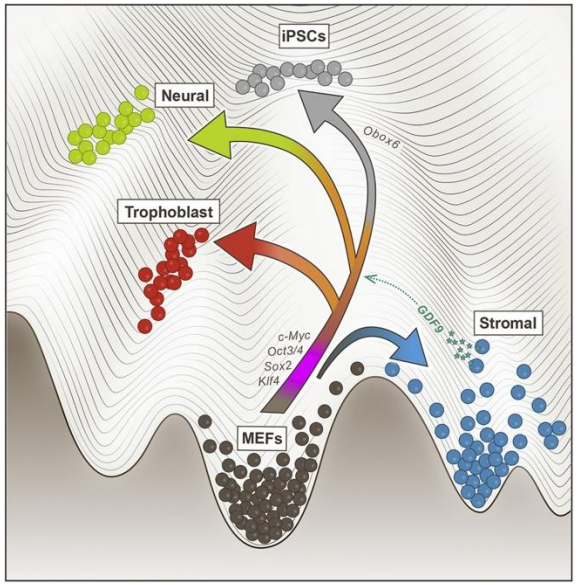
Peter Halmos*, **Julian Gold***, Xinhao Liu, and Ben Raphael

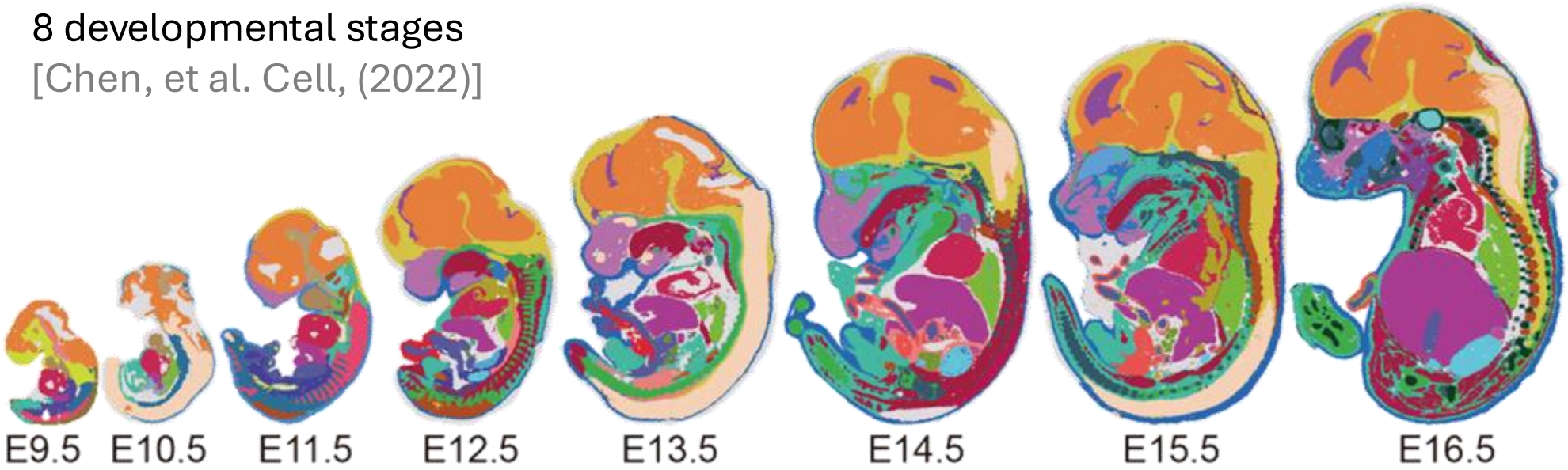

PRINCETON UNIVERSITY

Temporal and Spatiotemporal transcriptomics: Sequencing across multiple time points during developmental and reprogramming processes

Reprogramming of fibroblasts to induced pluripotent stem cells [Schiebinger, et al. Cell, (2019)]



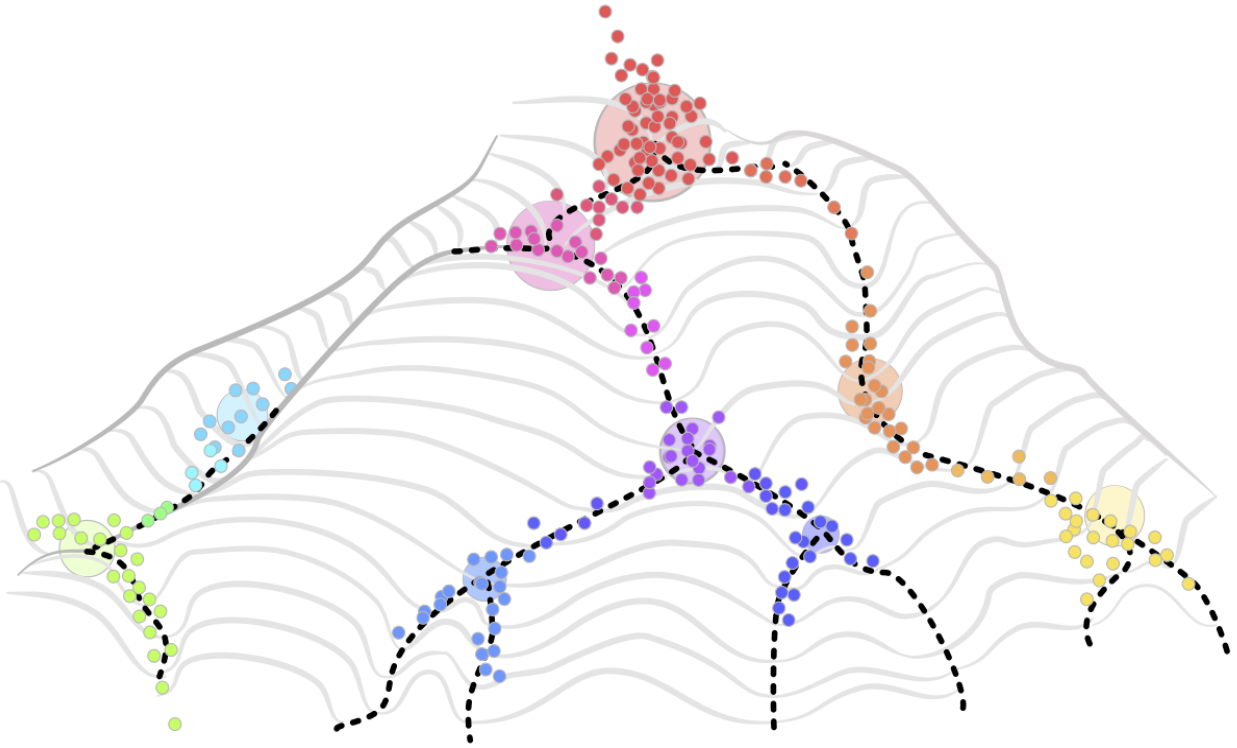
Spatial Transcriptomics of mouse embryos across 8 developmental stages
[Chen, et al. Cell, (2022)]



**And others!*
(Pijuan-Sala et al., Nature, 2019)
(Liu et al. *Developmental Cell*, 2022)

...

Temporal and Spatiotemporal transcriptomics: Opens up the Analysis of Fundamental Biological Questions!



The "Waddington Landscape"
Photo cred: (Waddington, 1957)

Questions:

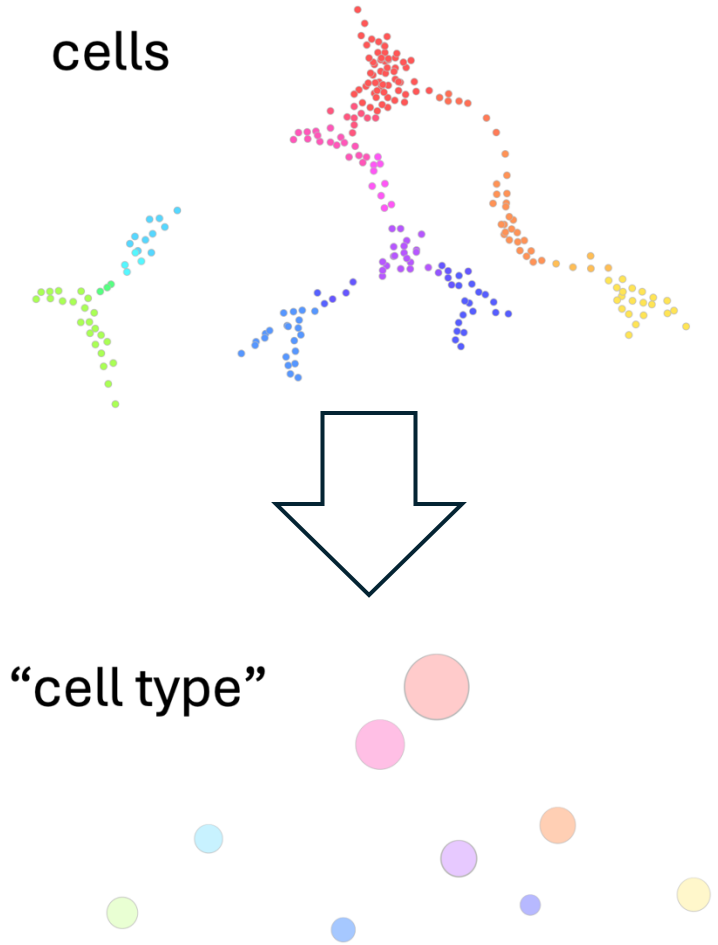
1. Ancestor-descendant relationships between cells across two timepoints?
2. Cell-states or types which index the temporal process of development?
3. Trajectories between these cell types?

Limitations:

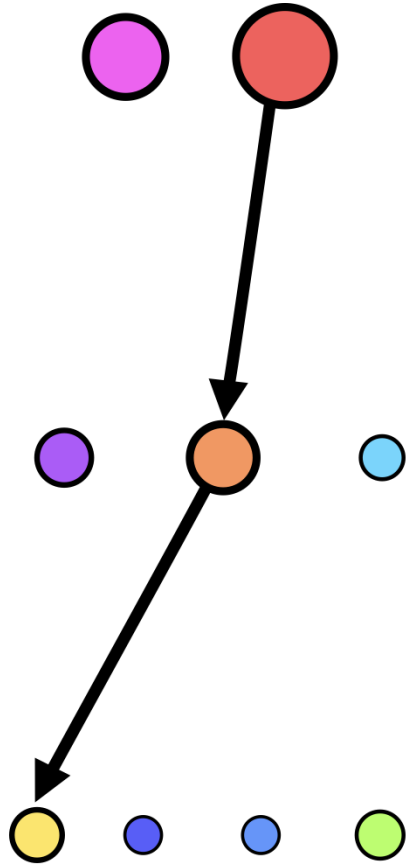
- Technology is destructive – each sample from a different individual
- Do not have ground-truth trajectories!

Differentiation maps and Cell Types (States)

A *cell-type* is a coarse-graining of cells into clusters.



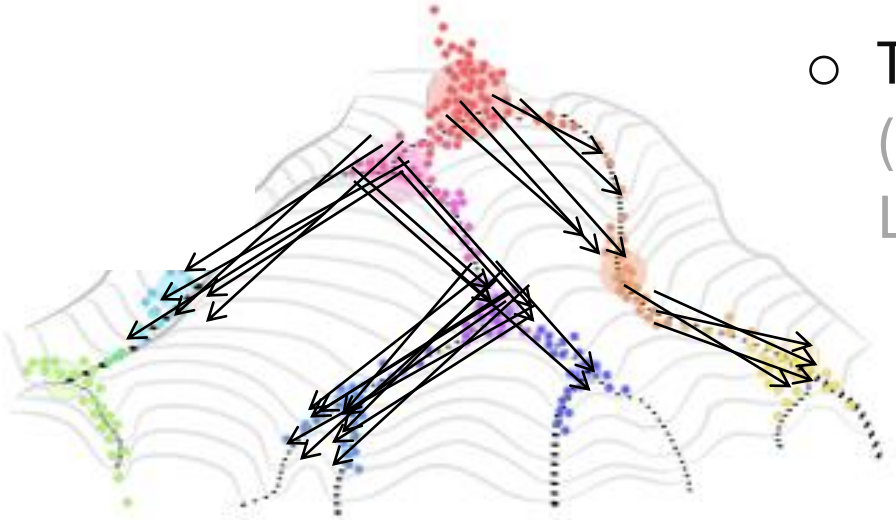
A differentiation map is a directed acyclic graph giving the ancestral relationship between cell-types



Existing Methods Infer Cell-Cell Coupling Independent of Cell Type

1. What are the ancestor-descendant relationship between cells across two timepoints?

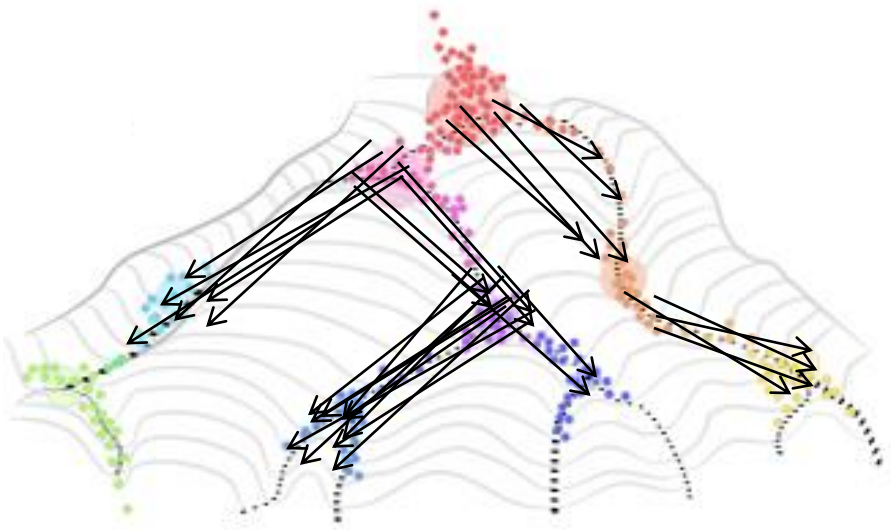
Cell-to-cell Coupling (Waddington OT, DeST-OT moscot)



- Many existing methods model the dynamics of cells using the technique of *optimal transport* (OT).
- These infer the least-cost mapping between individual cells (Schiebinger et al 2019, Zeira et al 2022, Klein et al 2025, Liu & Halmos et al 2025), building cell-to-cell trajectories

Existing Methods Infer Cell-Cell Coupling Independent of Cell Type

Cell-to-cell Coupling (Waddington OT, DeST-OT moscot)



However, these methods **do not**:

- Learn cell-types (assume cell-type inference is *distinct*)
- Find a **differentiation map** *jointly* with cell-type

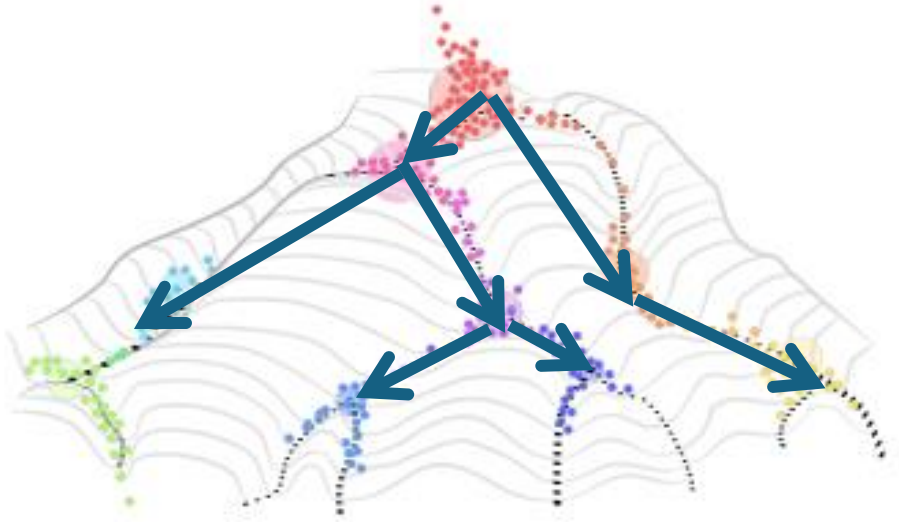
Finding Latent Trajectories over Latent Cell-State

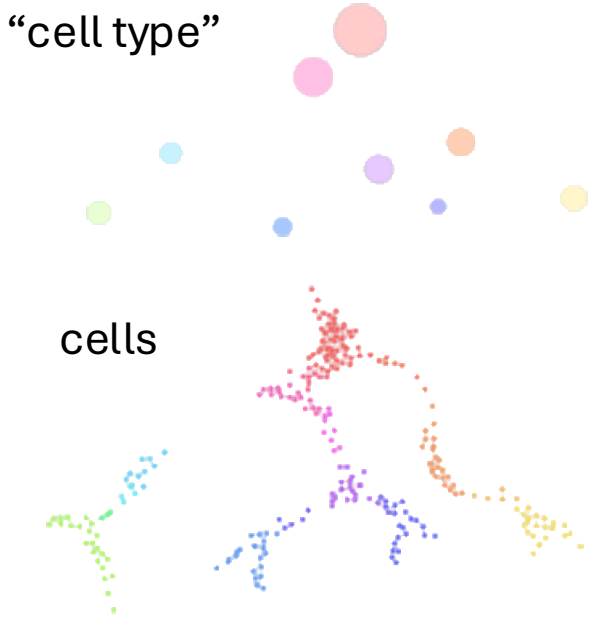
Our work addresses:

2. What are the cell-states or types which "index" the temporal process of development?

3. What is the *differentiation map* between these cell types?

Cell-type and Trajectory Inference



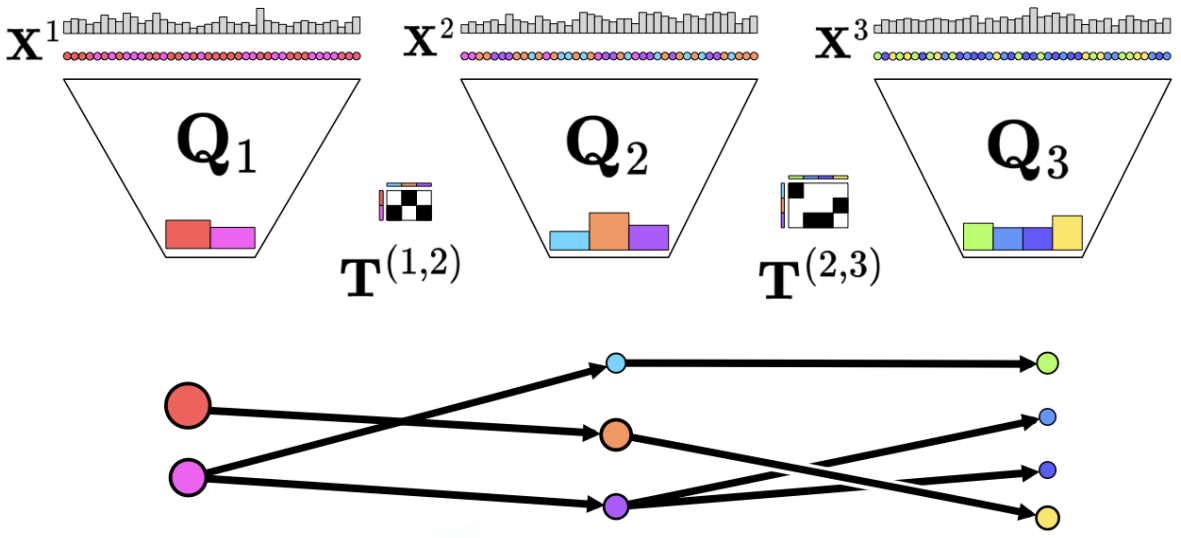


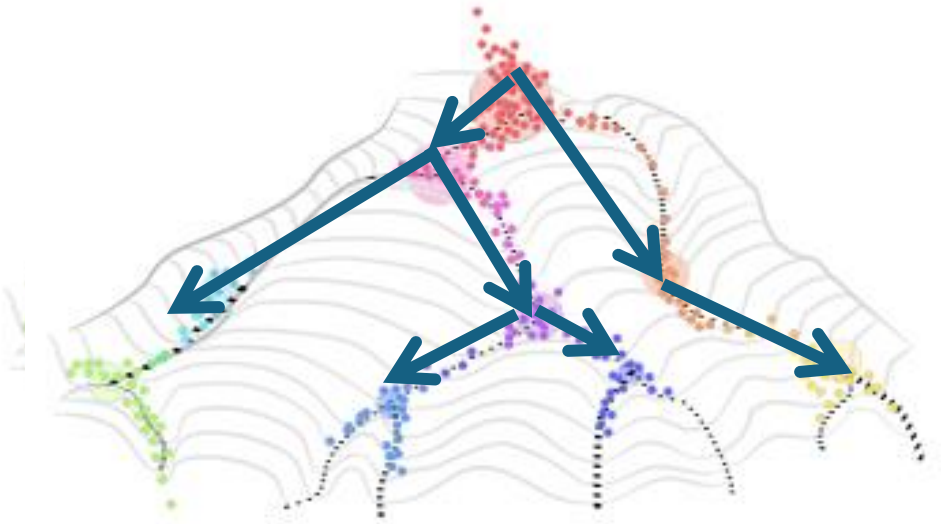
Hidden Markov Optimal Transport (HM-OT)

(1) Discovers latent cell types and aligns individual cells to them.

(2) Maps between the cell types while minimizing an optimal transport cost.

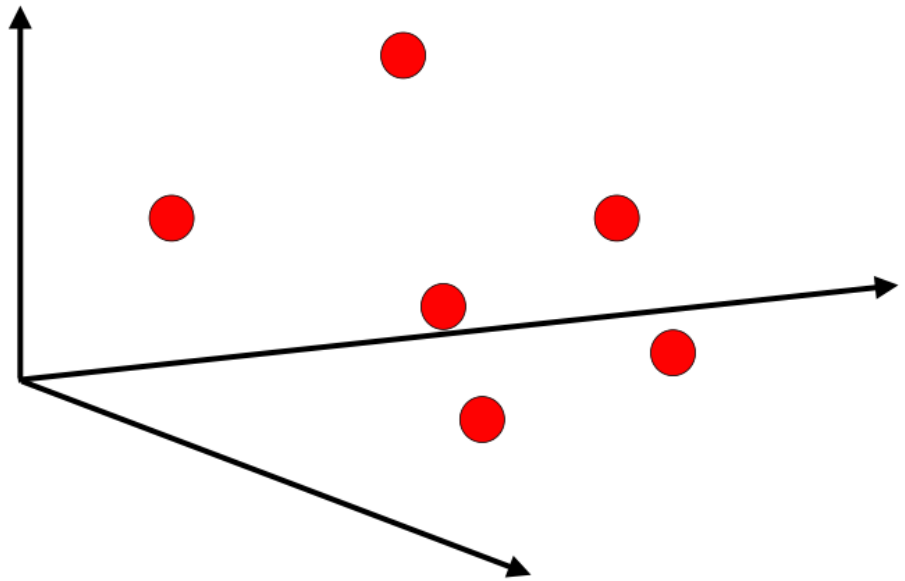
(3) Uses *low-rank optimal transport* (Forrow et al '19, Scetbon et al '20, Lin et al '21, Halmos et al '24) to do (1) and (2) simultaneously across multiple timepoints.





Optimal Transport (source: shamelessly lifted from Marco Cuturi!)

A puzzle:

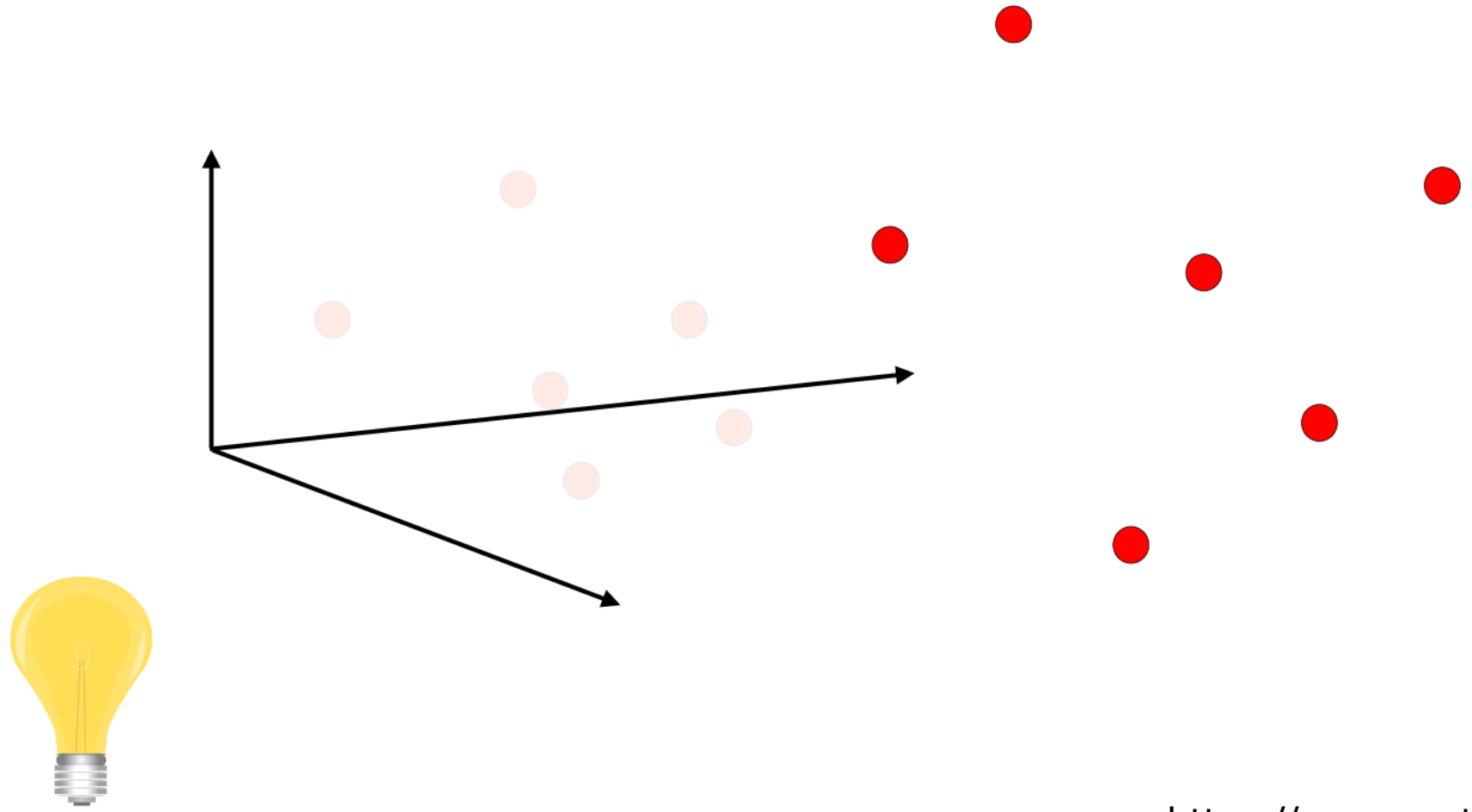


https://marcocuturi.net/ot.html

Optimal Transport

https://marcocuturi.net/ot.html

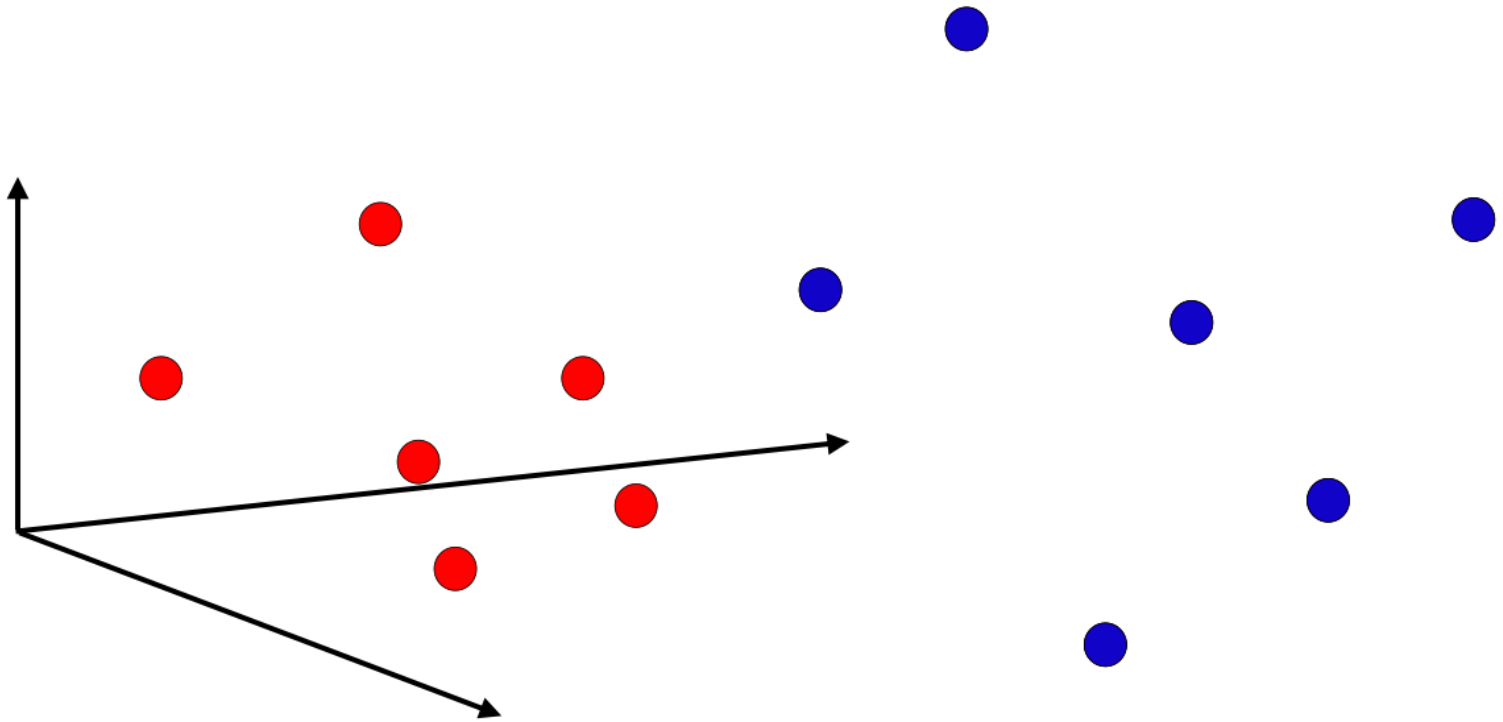
Optimal Transport



https://marcocuturi.net/ot.html

Optimal Transport

The puzzle is: who went where?



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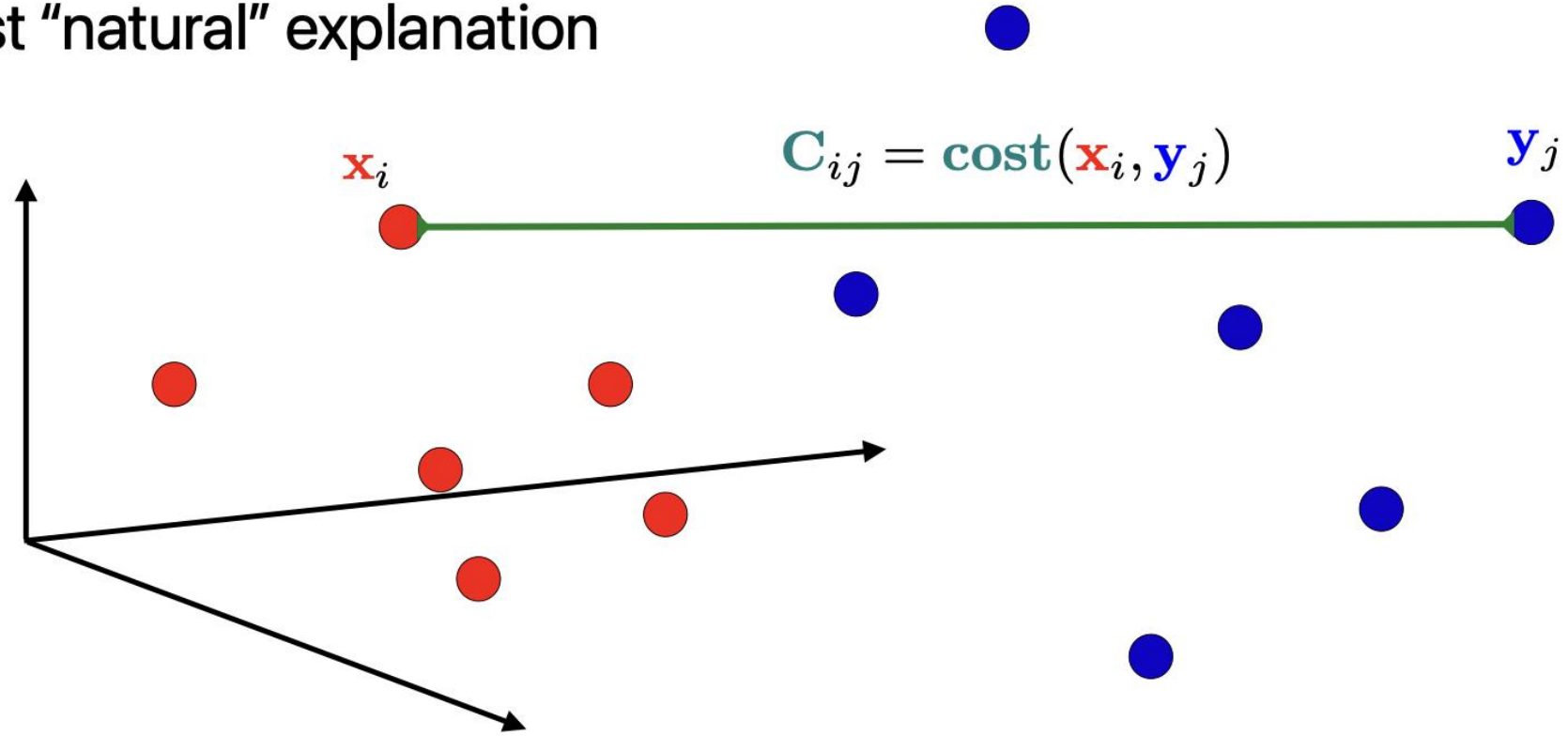
Optimal Transport

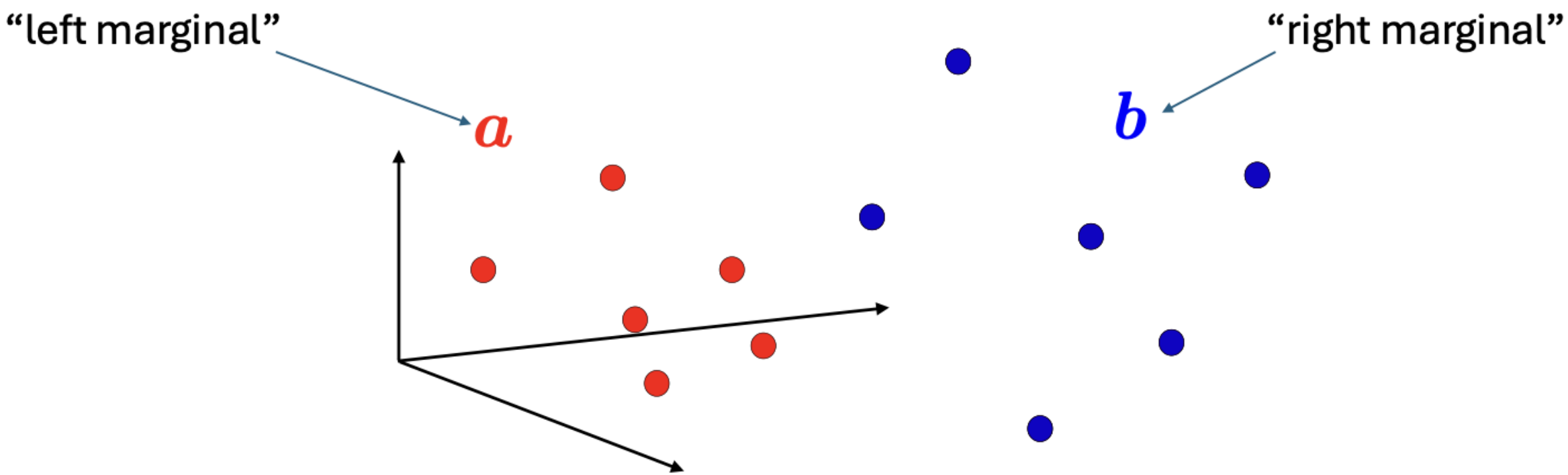
Goal: Find most “natural” explanation



https://marcocuturi.net/ot.html

Optimal Transport Feasible Set

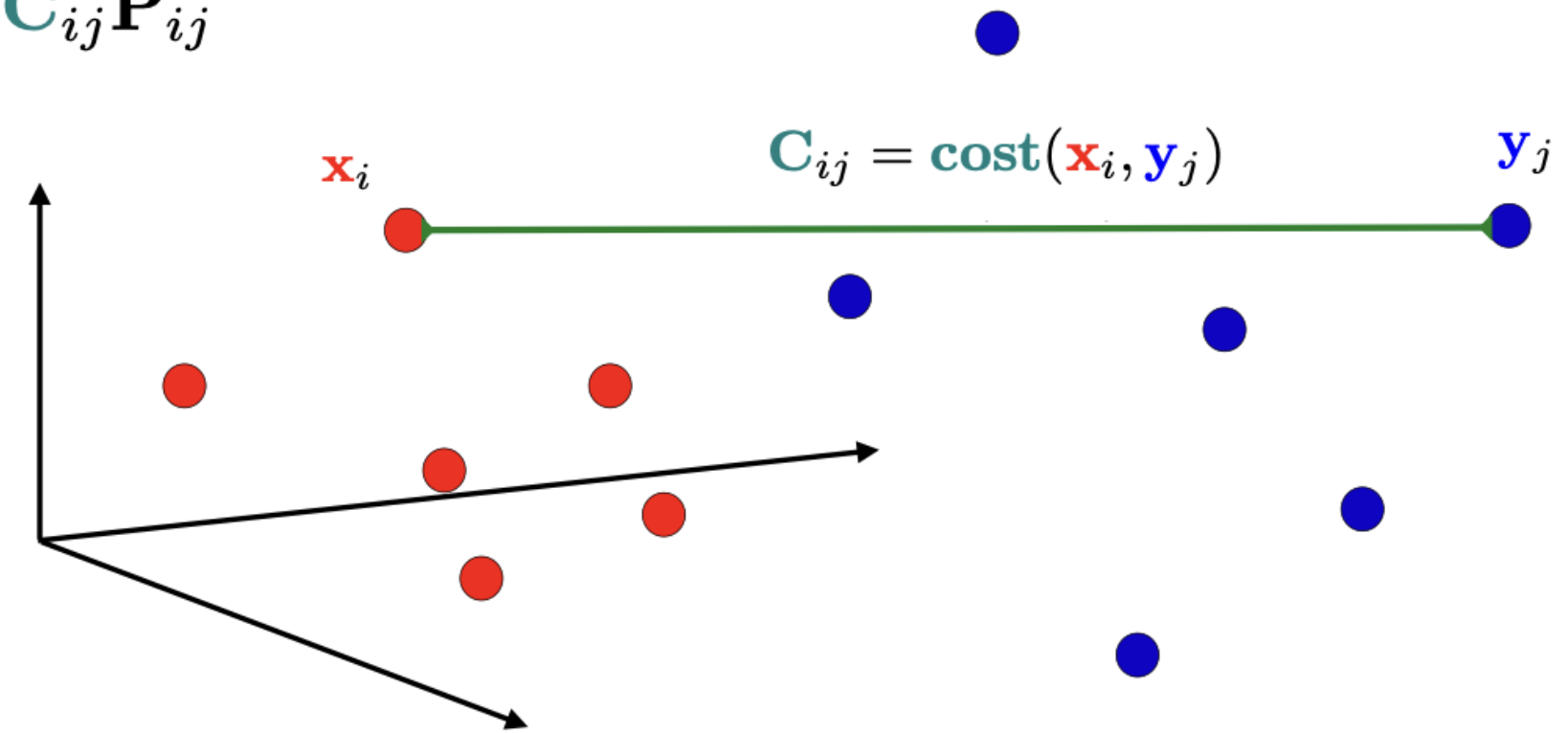
$$\Pi(\boldsymbol{a}, \boldsymbol{b}) = \{\mathbf{P} \in \mathbb{R}_{+}^{n \times m} : \mathbf{P}\mathbf{1}_m = \boldsymbol{a},\ \mathbf{P}^T\mathbf{1}_n = \boldsymbol{b}\}$$



Wasserstein Problem

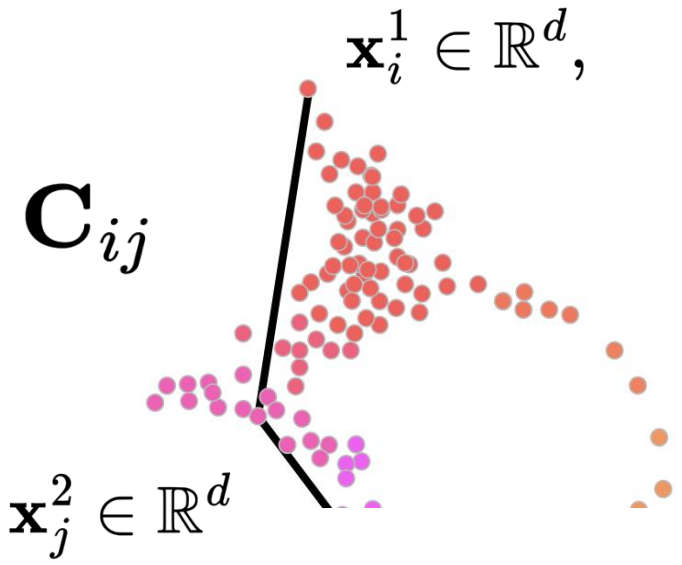
$$\mathbf{P}^\star = \underset{\mathbf{P}\in\Pi(\boldsymbol{a},\boldsymbol{b})}{\operatorname{argmin}} \langle \mathbf{C}, \mathbf{P} \rangle$$

$$= \underset{\mathbf{P}\in\Pi(\boldsymbol{a},\boldsymbol{b})}{\operatorname{argmin}} \sum_{i,j} \mathbf{C}_{ij}\mathbf{P}_{ij}$$



Why use OT for temporal alignment?

The most "natural" alignment minimizes the transcriptional distance between these cells, represented with cost C_{ij}



$$\mathbf{C}_{ij} := \mathbf{C}_{ij}^{(1,2)} = \|\mathbf{x}_i^1 - \mathbf{x}_j^2\|_2$$

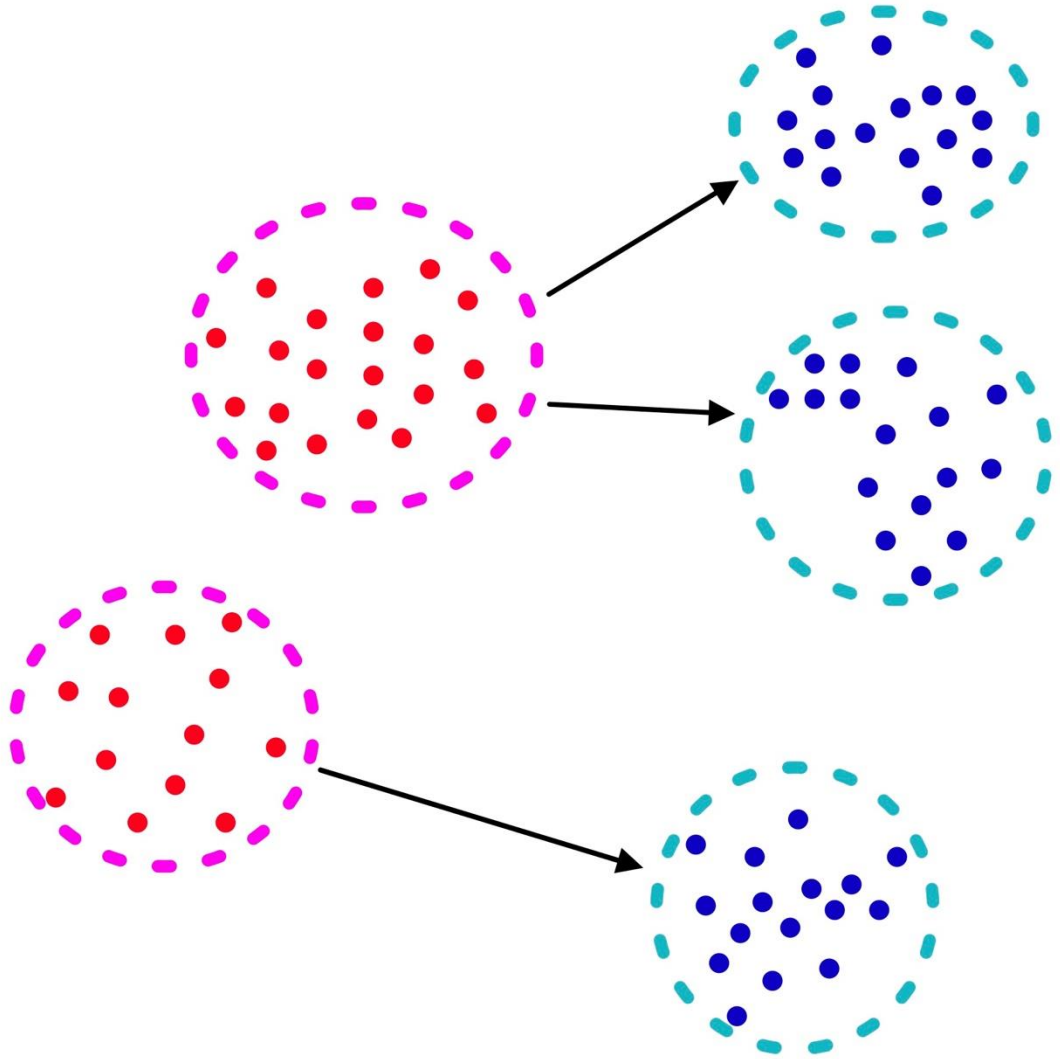
*Low-Rank** Wasserstein Problem

Often the data has some cluster structure, e.g. cell types, and the most interesting biological question is to understand the mapping at that resolution.

Consider a modification of the puzzle:

(1) What are the "best" cell types

(2) Which cell types transitioned to which?



* Latent-coupling (LC) formulation
(Lin et al 2021, Halmos et al 2024)

Low-Rank Wasserstein Problem

$$\mathbf{P}^\star = \arg\min_{\mathbf{P}\in\Pi_{\mathbf{g}_1,\mathbf{g}_2}(\mathbf{a},\mathbf{b})} \langle \mathbf{C}, \mathbf{P} \rangle$$

LC (latent coupling) factorization of P imposes *rank constraint* and decomposes P into 3 factors while keeping it a feasible coupling

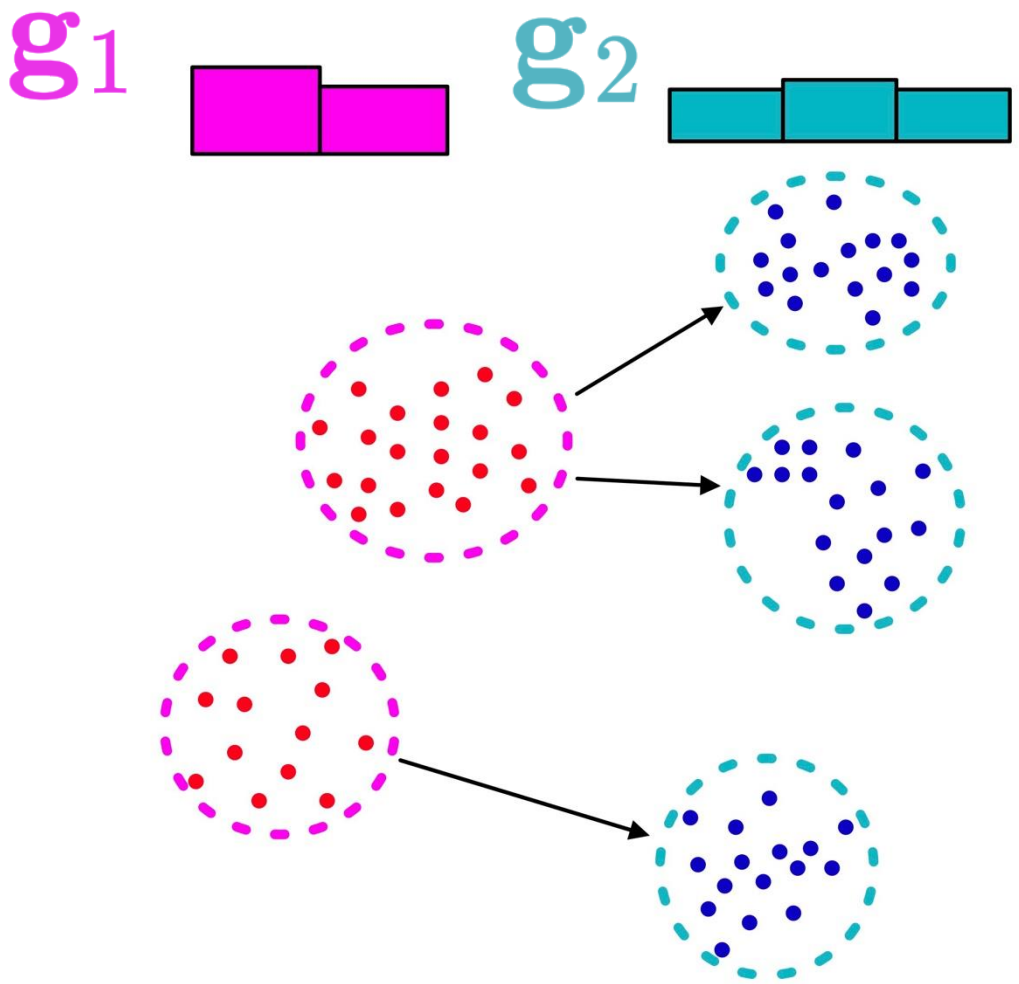
$$\mathbf{P} \in \Pi_{\mathbf{g}_1,\mathbf{g}_2}(\mathbf{a}, \mathbf{b})$$

$$\implies \mathbf{P} = \mathbf{Q}_1 \mathrm{diag}(1/\mathbf{g}_1) \mathbf{T} \mathrm{diag}(1/\mathbf{g}_2) \mathbf{Q}_2^{\mathrm{T}}$$

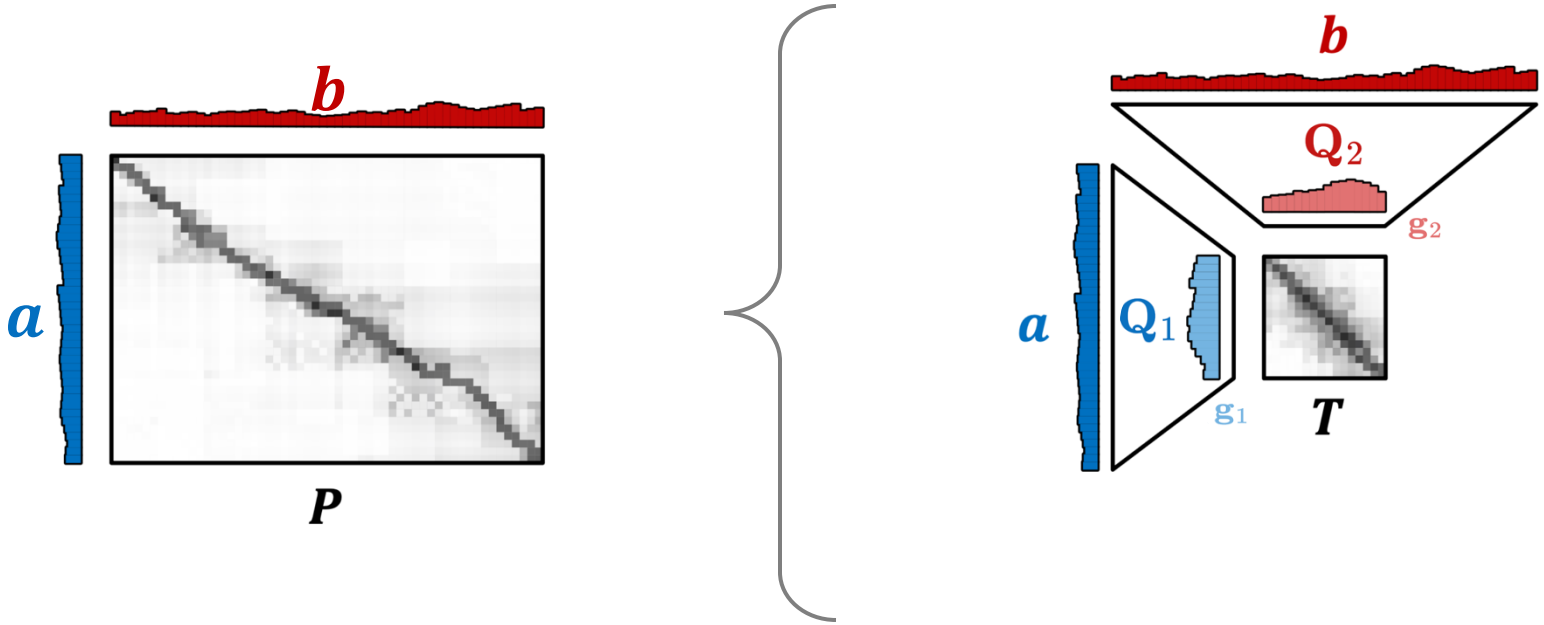
$\mathbf{Q}_1 \in \Pi(\mathbf{a}, \mathbf{g}_1)$ — Couples point distribution at time 1 to cell-type distribution at time 1

$\mathbf{T} \in \Pi(\mathbf{g}_1, \mathbf{g}_2)$ — Couples cell-type distribution at time 1 to cell-type distribution at time 2

$\mathbf{Q}_2^{\mathrm{T}} \in \Pi(\mathbf{g}_2, \mathbf{b})$ — Couples cell-type distribution at time 2 to point distribution at time 2



Low-Rank Optimal Transport: A Special Parametrization for a Coupling



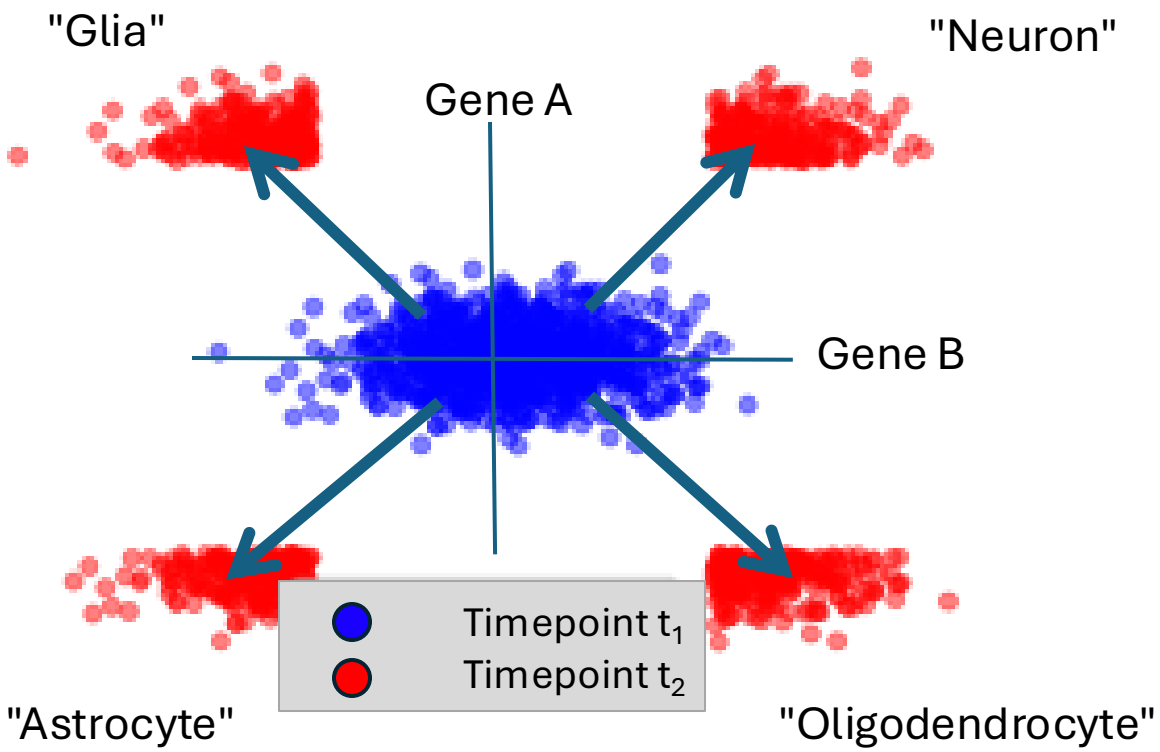
Factor relaxation with latent coupling (FRLC)

Low rank approximation of optimal transport

Halmos*, Liu*, Gold*, R. NeurIPS (2024)

Why use Low-Rank OT for temporal alignment?

Sometimes the clustering / cell types at one timepoint are *not enough* to build a map of cell differentiation!

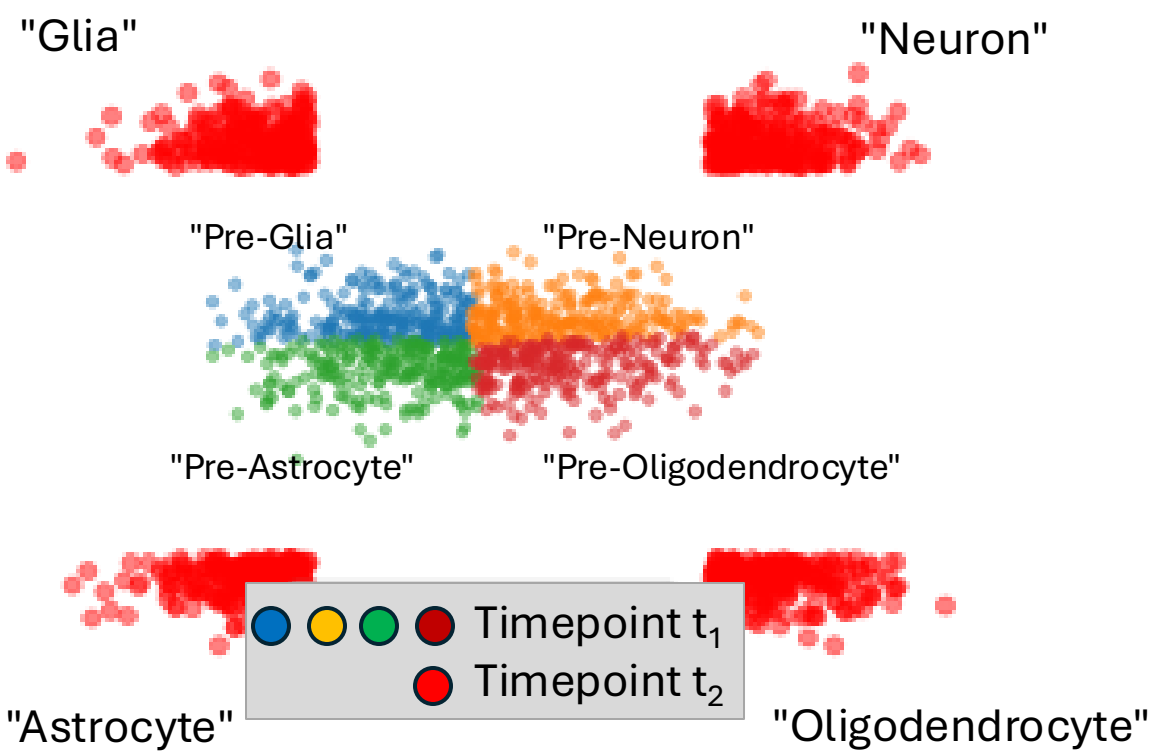


Why use Low-Rank OT for temporal alignment?

Need to leverage temporal information to get both differentiation map and cell-state correct!

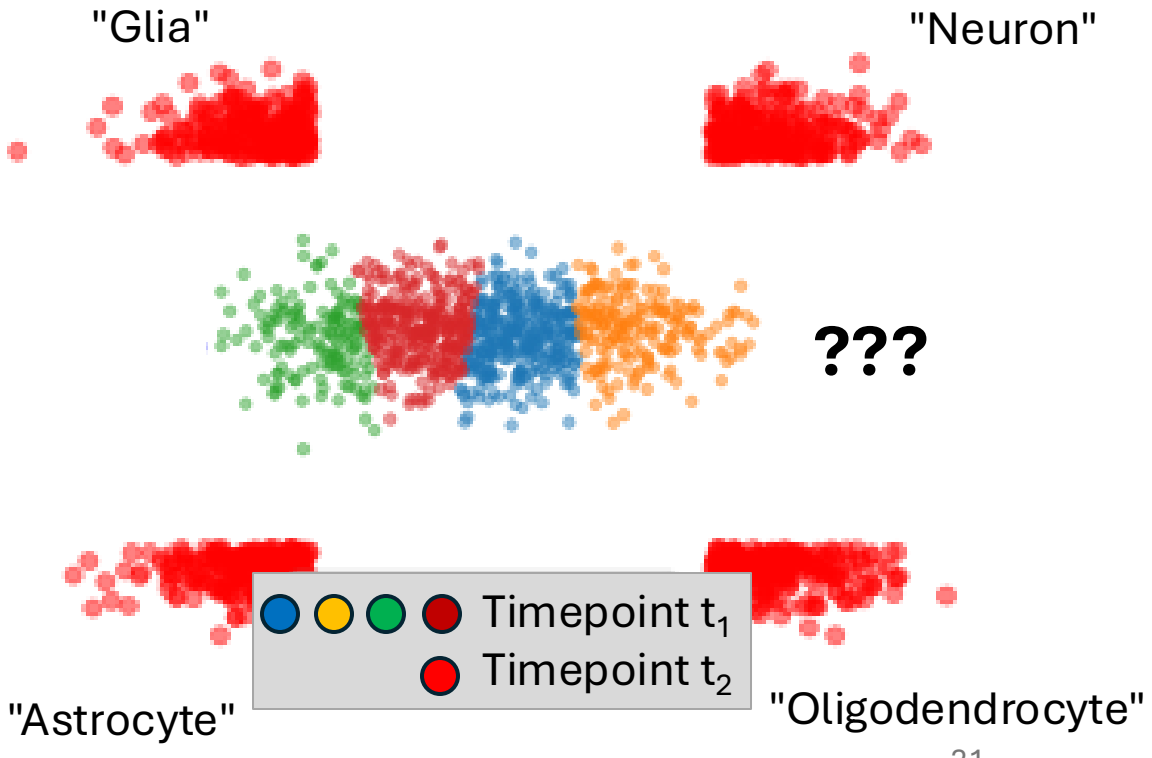
HM-OT / Low-Rank OT:

Clusters by ancestry using multiple timepoints.



Single-timepoint clustering (e.g. k-means)

Only uses information from one timepoint.

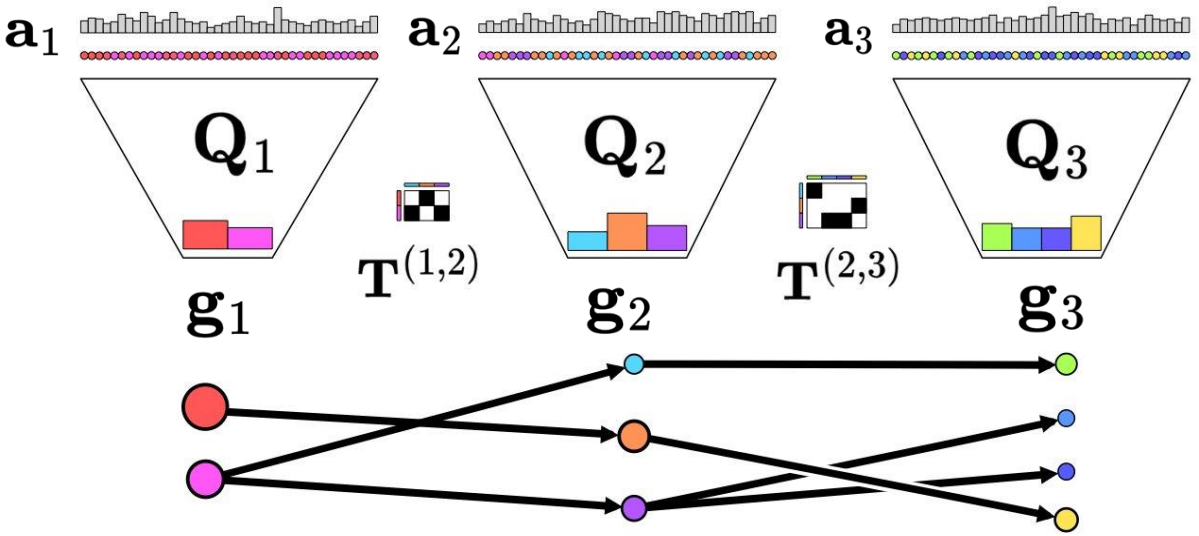


Hidden-Markov Optimal Transport

Problem: Given empirical distributions $(a_t)_{t=1,..,N}$ find the latent factors $(Q_t)_{t=1,..,N}$ and differentiation maps $(T^{(t,t+1)})_{t=1,..,N-1}$ that minimize the Wasserstein distance traveled by the clusters through time.

$$\min_{\mathbf{Q},\mathbf{T}\,:\,(\mathbf{Q}_t,\mathbf{Q}_{t+1},\mathbf{T}^{(t,t+1)})\in \mathsf{LC}_{\mathbf{a}_t,\mathbf{a}_{t+1}}(r_t,r_{t+1})} \sum_{t=1}^{N-1} \langle \mathbf{C}^{(t,t+1)}, \mathbf{P}^{(t,t+1)} \rangle_F$$

$$\mathbf{P}^{(t,t+1)} := \mathbf{Q}_t \mathrm{diag}(1/\mathbf{g}_t) \mathbf{T}^{(t,t+1)} \mathrm{diag}(1/\mathbf{g}_{t+1}) \mathbf{Q}_{t+1}^T$$



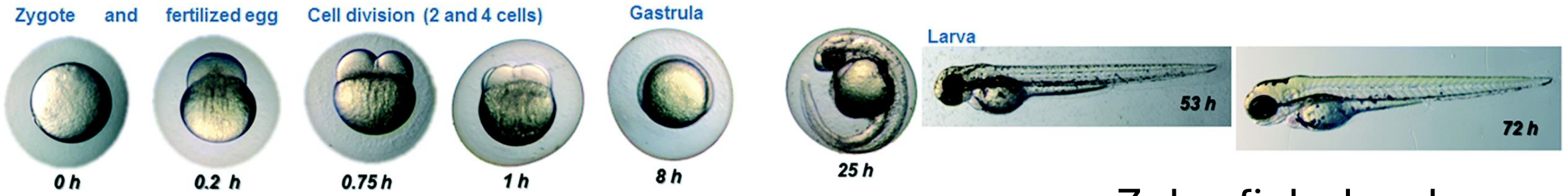
HM-OT: Algorithm

- Computes MAP estimates of Q_t , $T^{(t,t+1)}$, g_t using an algorithm analogous to forward-backward for Hidden Markov Models (HMM)

- Highly flexible in terms of input information! One can either run it unsupervised and learn all variables or fix/initialize any subset of the following and learn the rest:
  - Cell-type proportions ($\mathbf{g}_t$) [i.e. if you know there are "rare" cell-types]
  - Cell to cell-type mappings ($\mathbf{Q}_t$) [i.e. if you know cell-types]

- More algorithmic details are in the paper!

Zebrafish development is a well-studied model of organismal development and cell differentiation



Zebrafish development

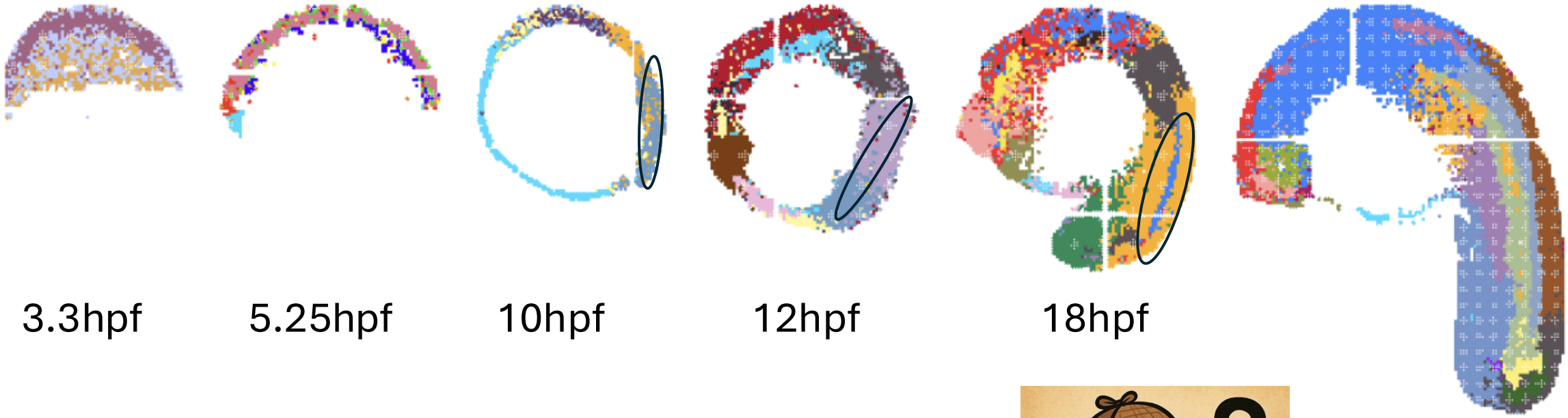
[García-Cambero,et al. *Env.Sci.* 2019]

Spatiotemporal transcriptomics

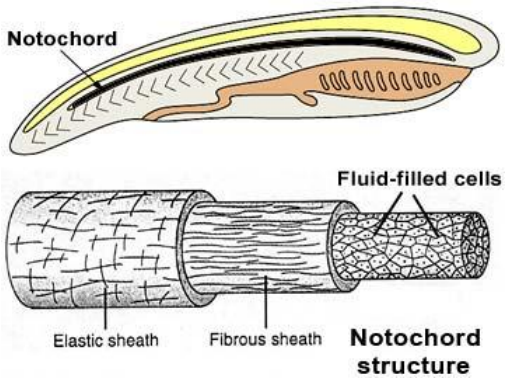
Stereo-Seq [Liu et al. *Developmental Cell* (2022)]

Spatiotemporal transcriptomics of zebrafish embryogenesis

Liu et al. *Developmental Cell* (2022)

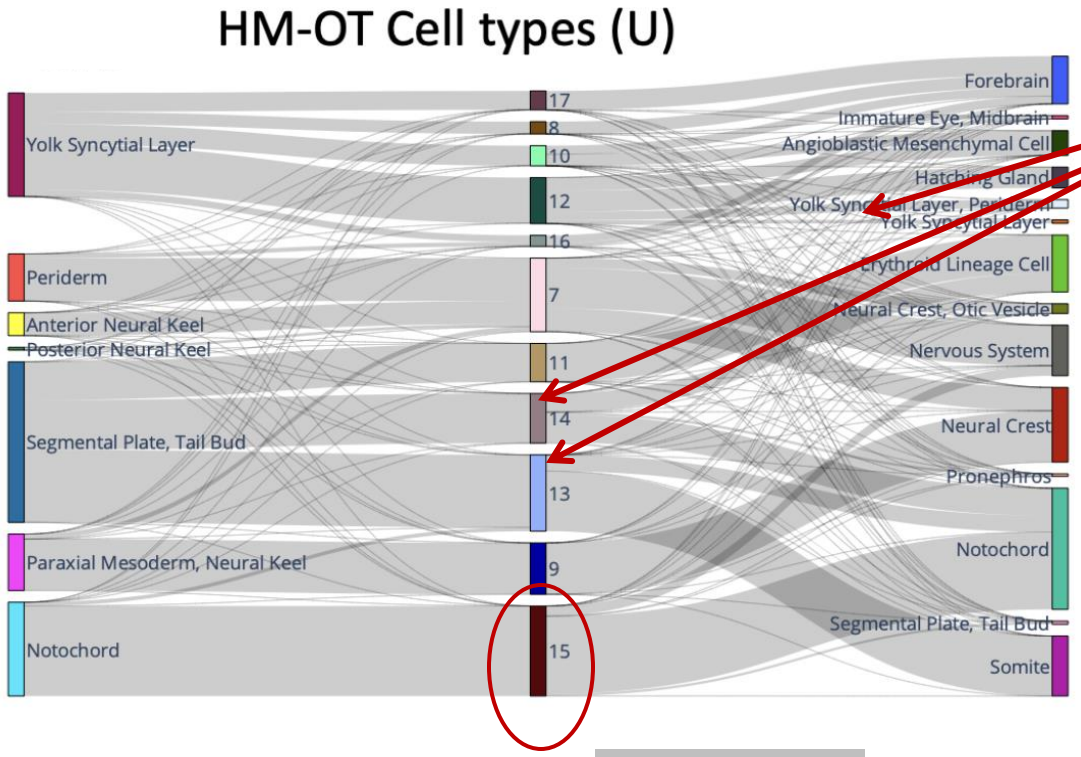


3.3hpf 5.25hpf 10hpf 12hpf 18hpf 24hpf



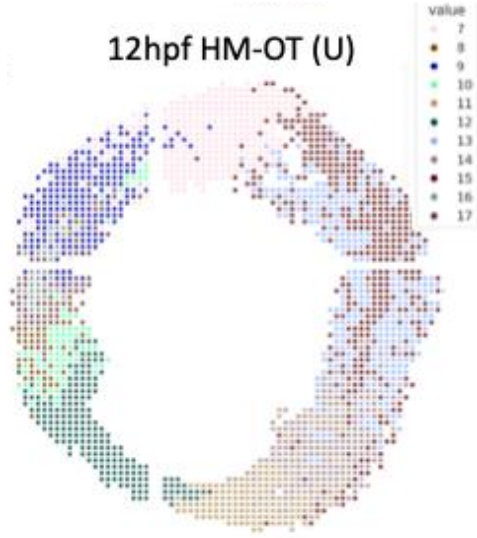
Notochord cell-type "disappears" in the published annotation!

HM-OT: Clustering and Differentiation Map of Zebrafish



Corrects transitions which were incorrect with annotated cell-types

12hpf



Notochord recovered!

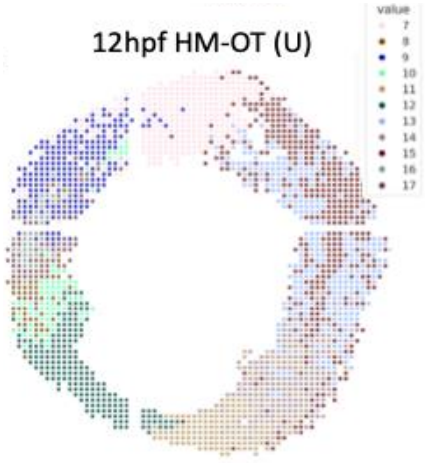
Stereo-Seq data and cell type annotations from Liu et al. *Developmental Cell* (2022)

HM-OT: Differentiation Map of Zebrafish

HM-OT inferred cell-types substantially improve the NPMI (pointwise mutual information) to ground truth trajectories relative to annotated types:

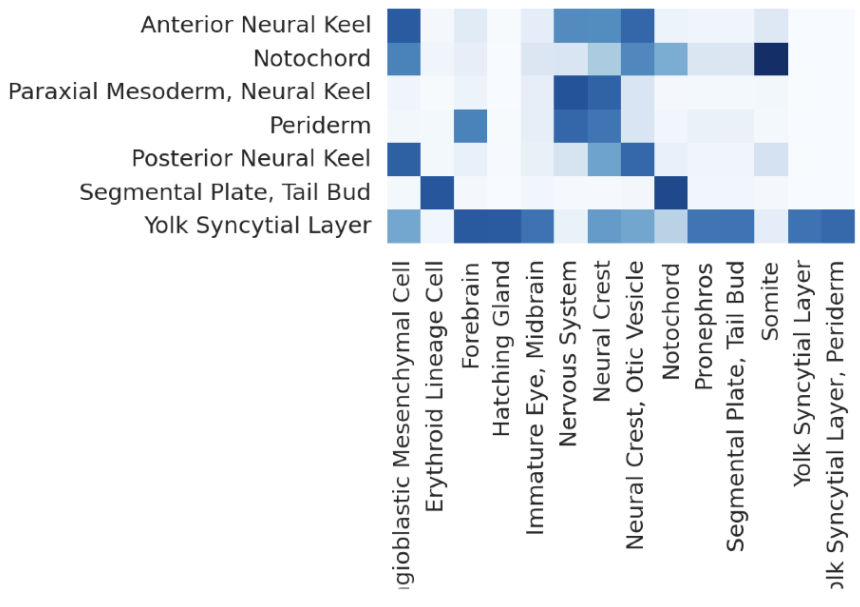
12hpf

12hpf HM-OT (U)

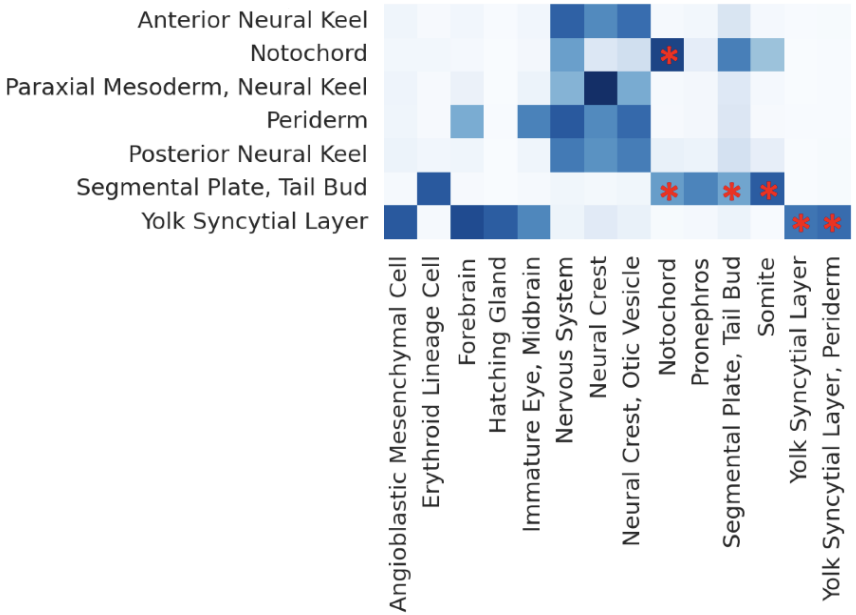


NPMI of Cell-types between 10hpf and 18hpf

HM-OT (S)

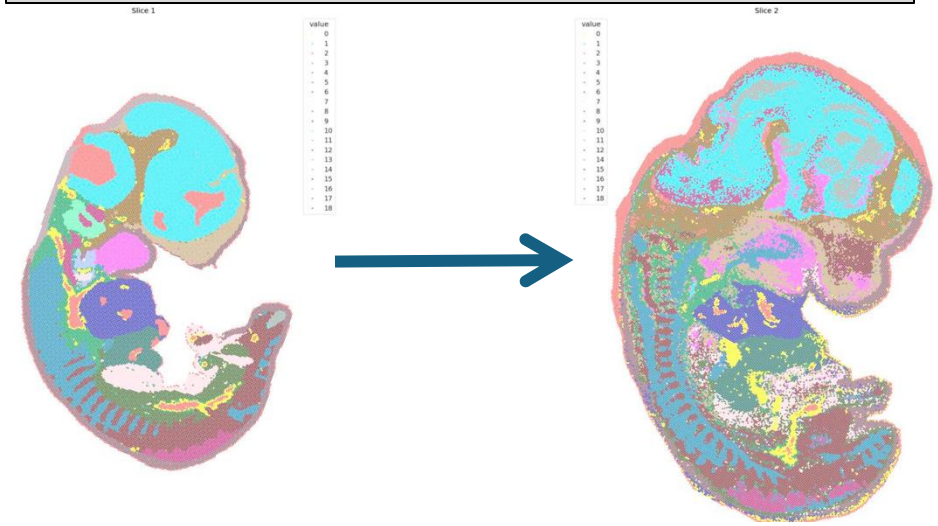


HM-OT (U)

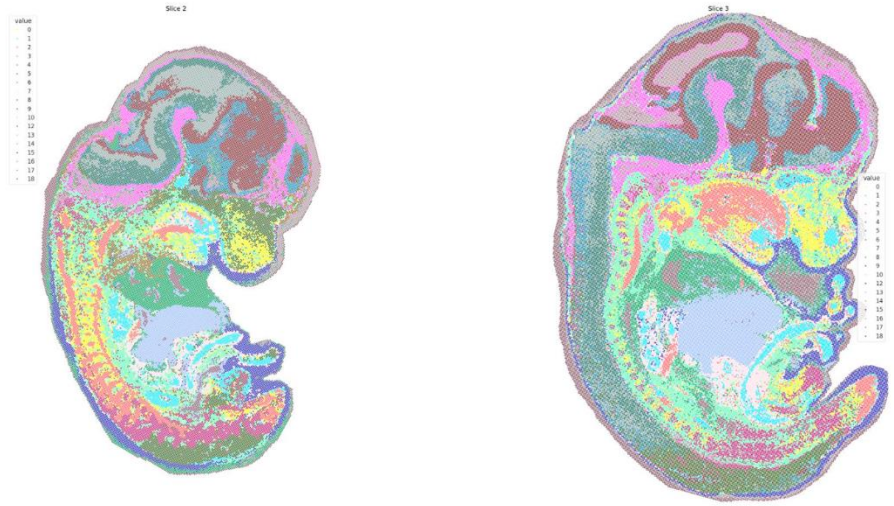


HM-OT is a Flexible Toolbox for (Co) Clustering

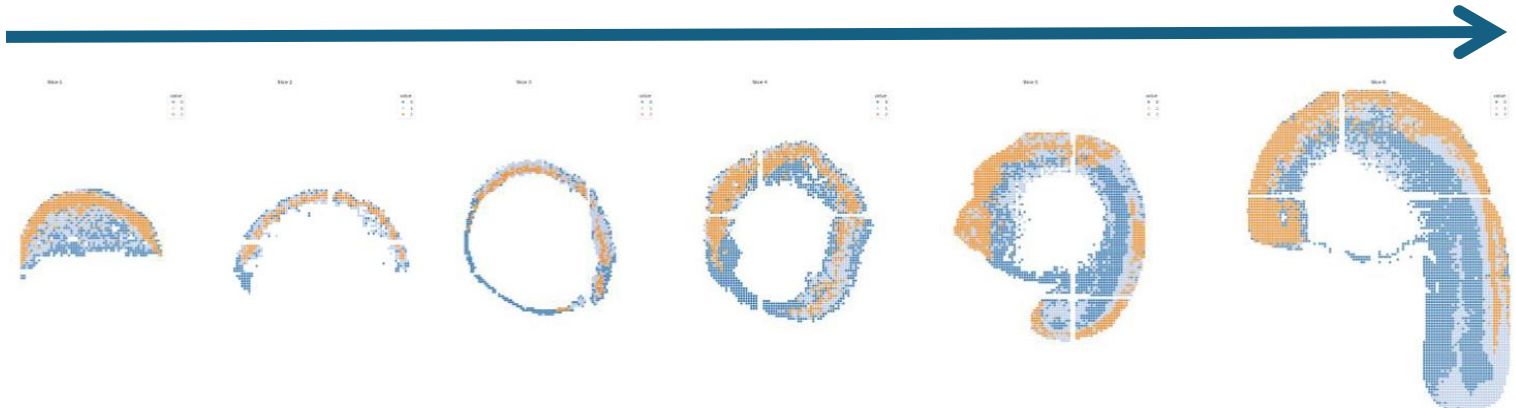
Transfer known clusters forwards or backwards in time to other data

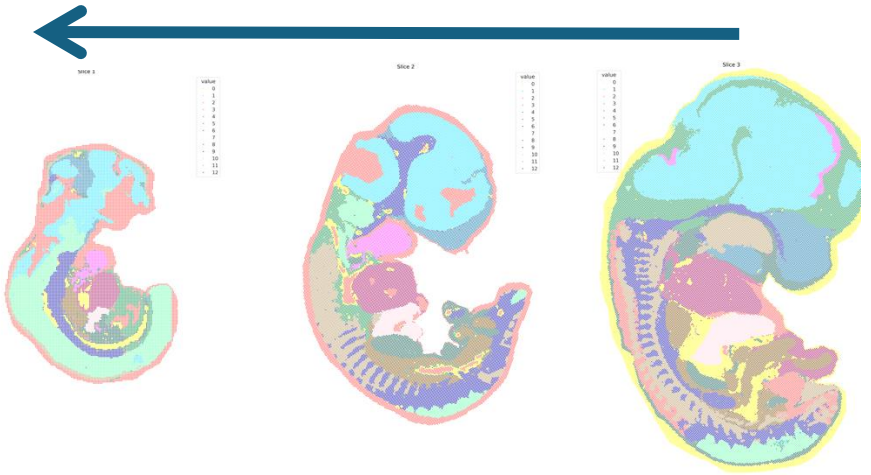


Learn cell state/type from scratch to minimize HM-OT objective



Project or co-cluster cell-types forward and backward in time through differentiation map

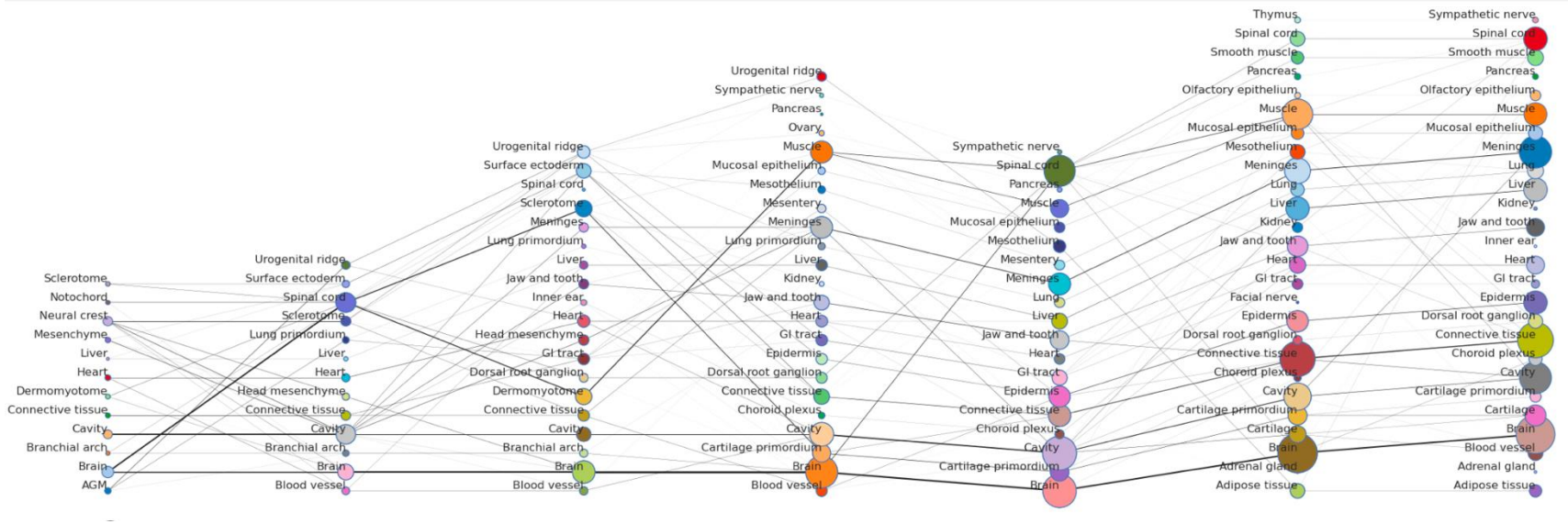




Large-Scale Inference of Differentiation Maps

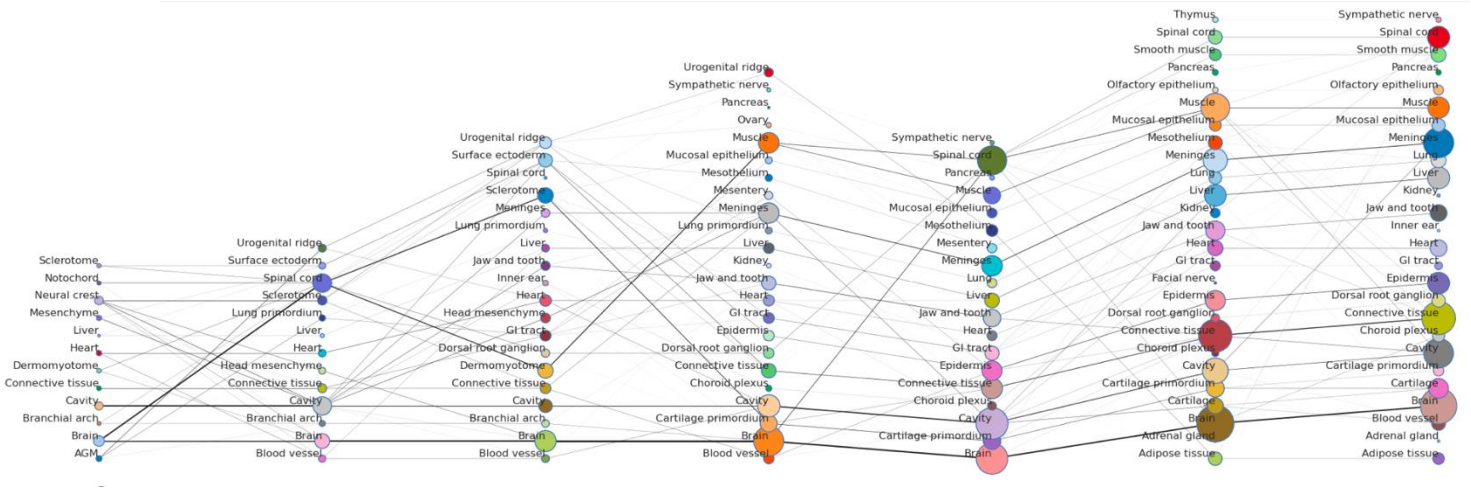
Lightning fast and space-efficient; can scale maps to millions of points!

Spatial (Stereo-Seq) Mouse Development (Chen et al '22)

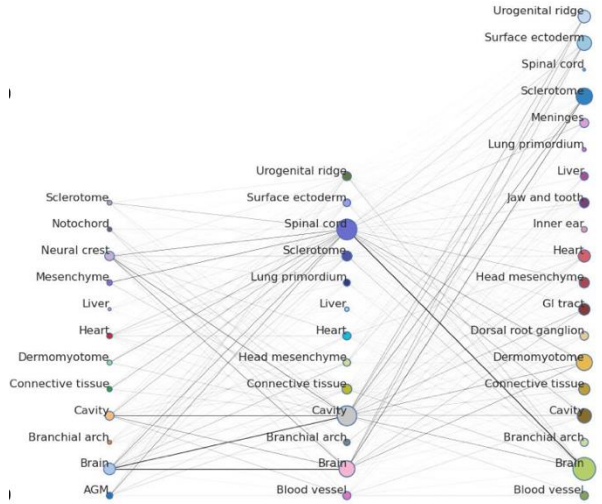


Temporal (Single-Cell) Mouse Embryogenesis (Qiu et al '24)

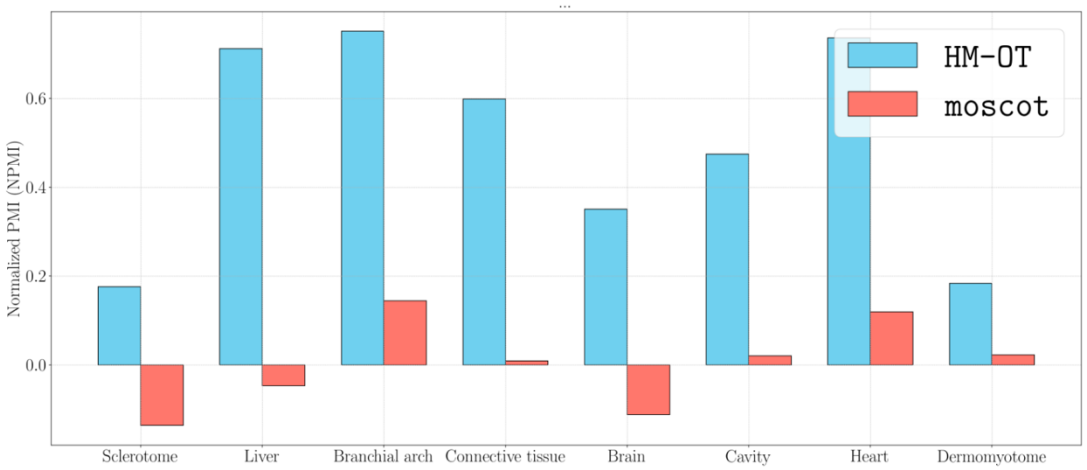
Large-Scale Inference of Differentiation Maps



HM-OT



moscot spatiotemporal



Other true transitions identified:

- urogenital ridge transitioning to ovary (NPMI = **0.560**, E11.5-12.5)
- lung primordium transitioning to lung (NPMI=**0.927**, E12.5-13.5)
- dermomyotome transitioning to muscle (NPMI=**0.968**, E11.5-12.5)
- sclerotome transitioning to cartilage primordium (NPMI=**0.866**, E11.5-12.5)
- surface ectoderm transitioning to the epidermis (NPMI=**0.705**, E11.5-12.5)

Spatial (Stereo-Seq) Mouse Development (Chen et al '22)

Summary

HM-OT: a scalable algorithm to infer differentiation maps, discover temporal co-clusters, and track cell-types through time and space.

- HM-OT introduces a novel factorization of optimal transport to model cell-type differentiation
- Optimizes this factorization across the full time-series of temporal transcriptomics data

Thank you!

https://github.com/raphael-group/HM-OT/

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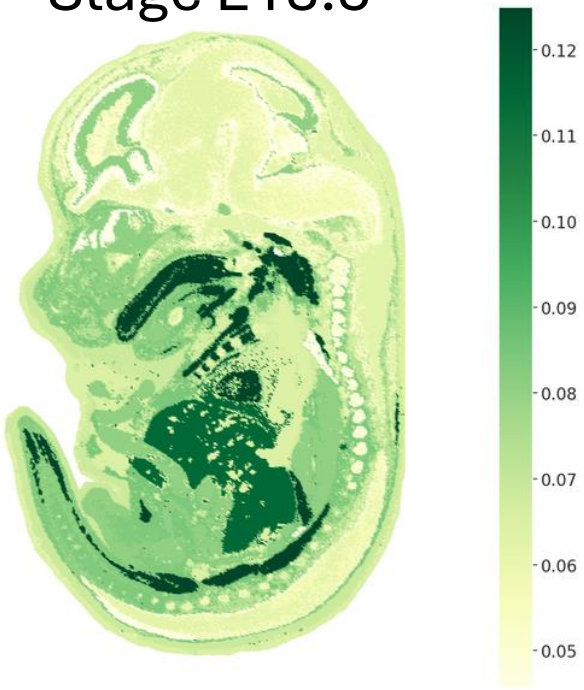
NIH NATIONAL CANCER INSTITUTE Informatics Technology for Cancer Research

SCHMIDT FUTURES

Scaling DeST-OT with low rank optimal transport

Stage E13.5

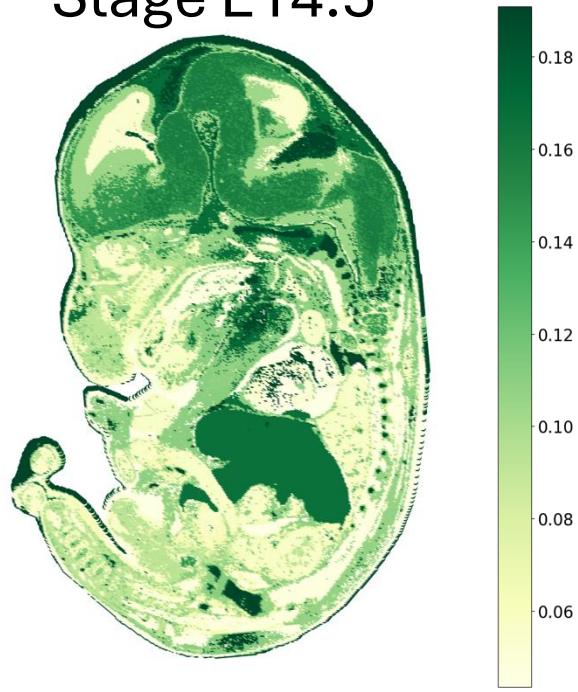


77K spots

Stereo-seq of mouse embryo

[Chen et al. *Cell*, 2022]

Stage E14.5



102K spots