



Pseudo-temporal ordering and RNA velocity

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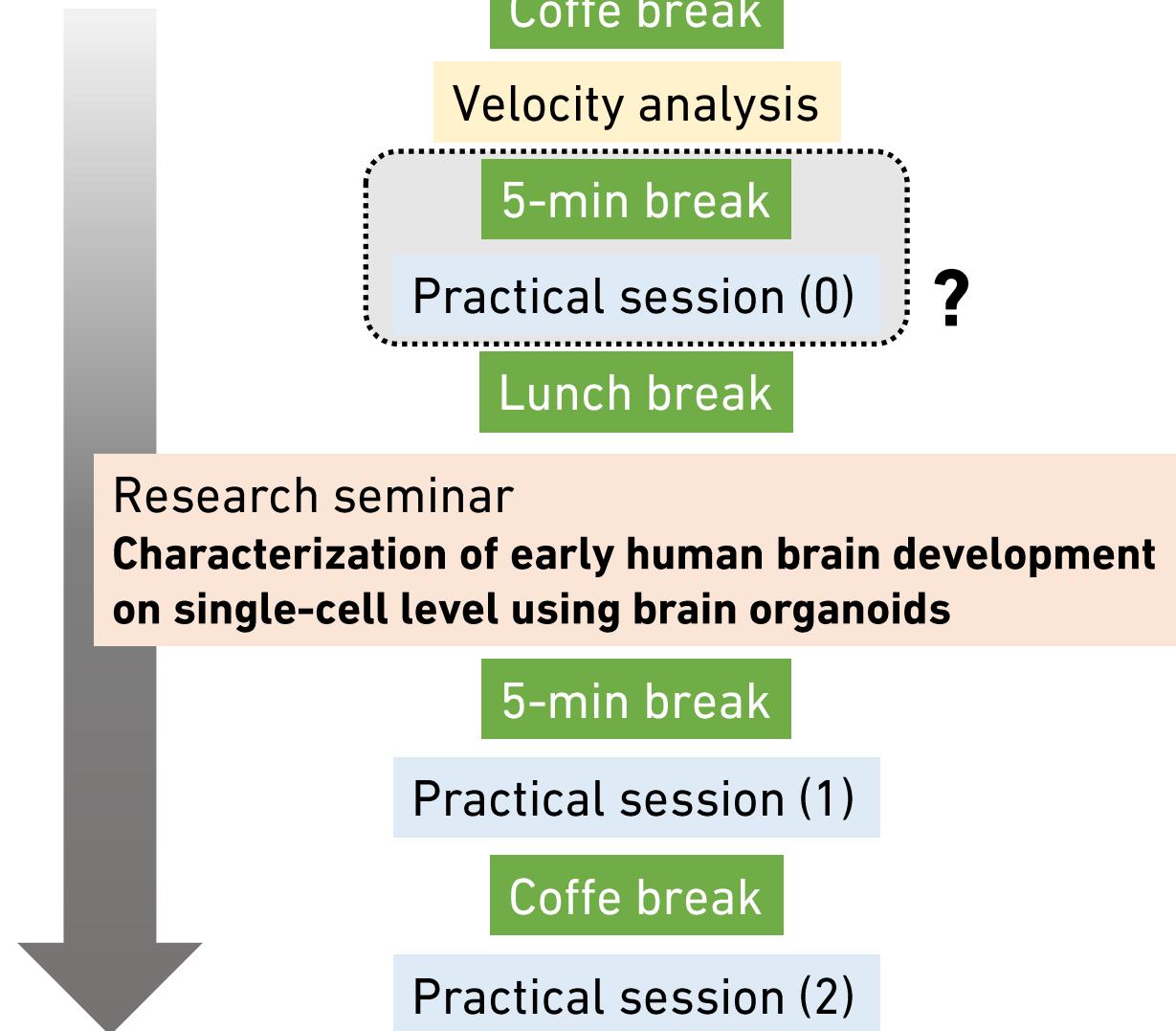
11.07.2024



Workshop
**The
Hitchhiker's
Guide
to scRNA-seq**

| The Hitchhiker's Guide to scRNA-seq | | | | | |
|--|--|---|---|--------------------------------------|--|
| Workshop program | | | | | |
| | Monday | Tuesday | Wednesday | Thursday | |
| 9h00 | Welcome | Good morning | Good morning | Good morning | Good morning |
| 9h30 | Get to know each other | Integration António Sousa | Adaptive immune receptor Lisa Dratva | Velocity Zhisheng He | Spatial Transcriptomics Anna Maguza |
| 10h40 | Coffee break | Coffee break | Coffee break | Coffee break | Coffee break |
| 11h00 | Fundamentals of scRNA-seq Tomás Gomes | Integration António Sousa | Adaptive immune receptor Lisa Dratva | Velocity Zhisheng He | Spatial Transcriptomics Anna Maguza |
| 12h30 | Lunch break | Lunch break | Lunch break | Lunch break | Lunch break |
| 13h30 | Fundamentals of scRNA-seq Tomás Gomes | Seminar Yang Li HZI Braunschweig | Seminar Nuno Morais IMM | Seminar Zhisheng He ETH Zurich | Spatial Transcriptomics Anna Maguza |
| 14h00 | Integration António Sousa | Adaptive immune receptor Lisa Dratva | Velocity Zhisheng He | | |
| 15h30 | Coffee break | Coffee break | Coffee break | Coffee break | Coffee break |
| 16h30 | Fundamentals of scRNA-seq Tomás Gomes | Integration António Sousa | Adaptive immune receptor Lisa Dratva | Velocity Zhisheng He | Closing session |
| 17h00 | | | | | |
| 19h00 | Art Gallery | Dinner with speakers | Dinner with speakers | | |
| 20h00 | All group dinner | | | | |
| 21h00 | | | | | |
| More info: scworkshop2024@medicina.ulisboa.pt | | | | | |

Pseudotime, trajectory analysis and fate probability



Interior Design Style



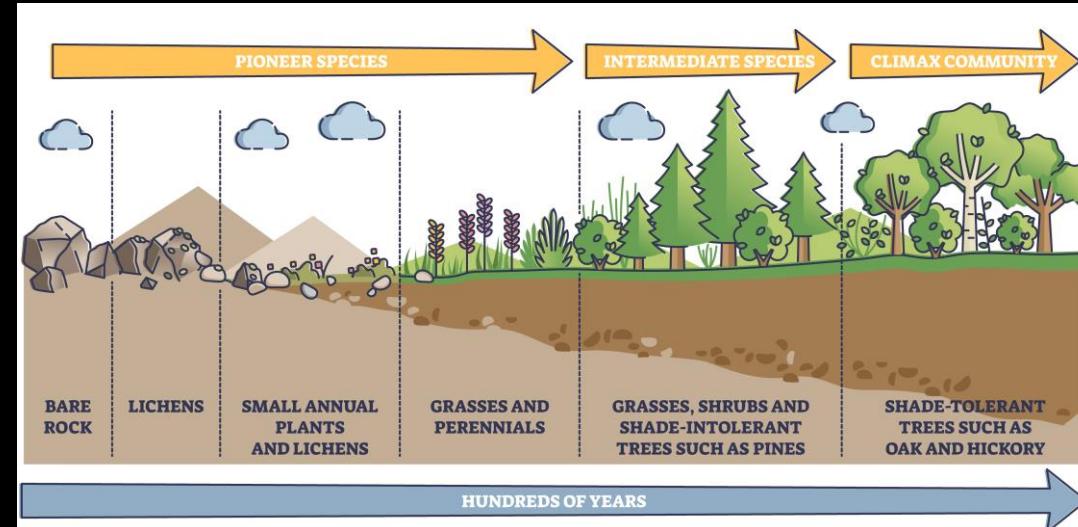
Urban Expansion



"The Only Constant in Life Is Change"

- Heraclitus

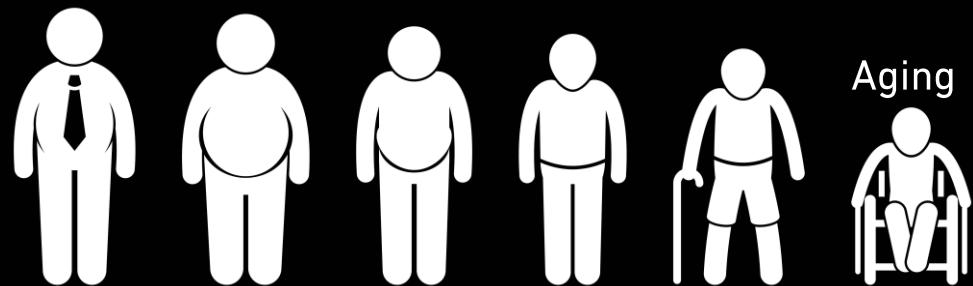
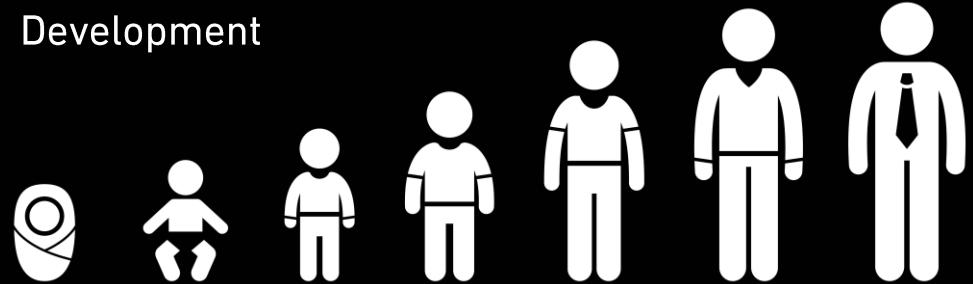
Ecological Primary Succession



Insect Metamorphosis



Development

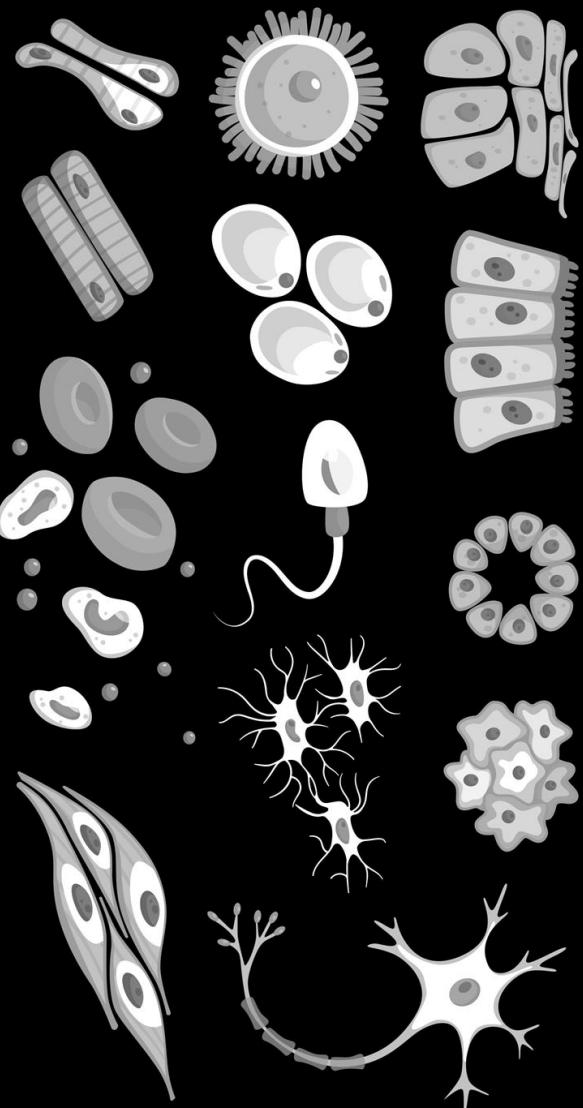


Aging

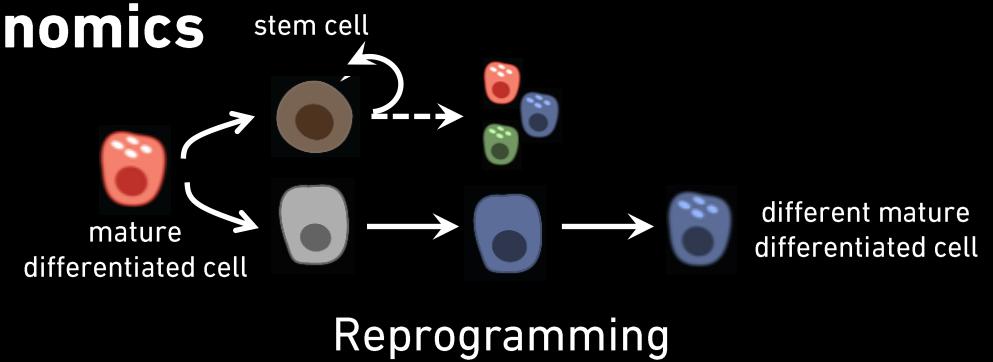
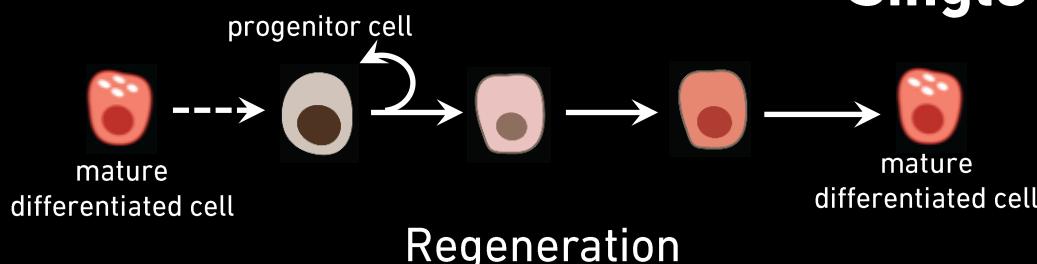
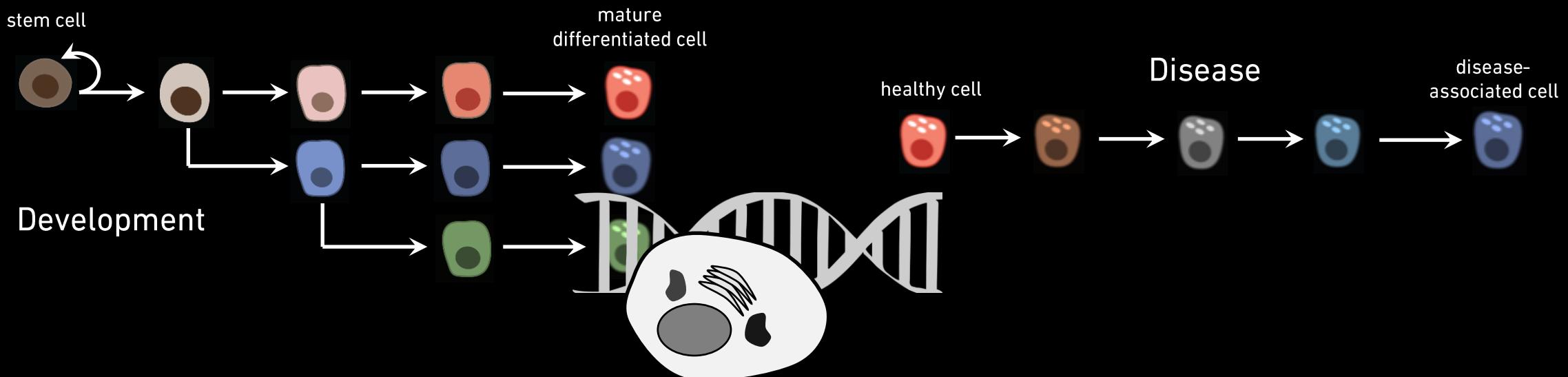
Diseases



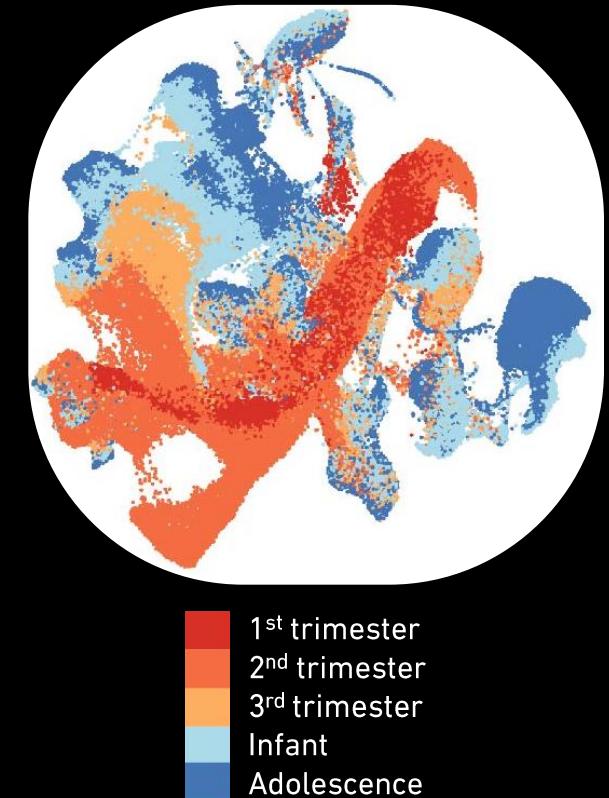
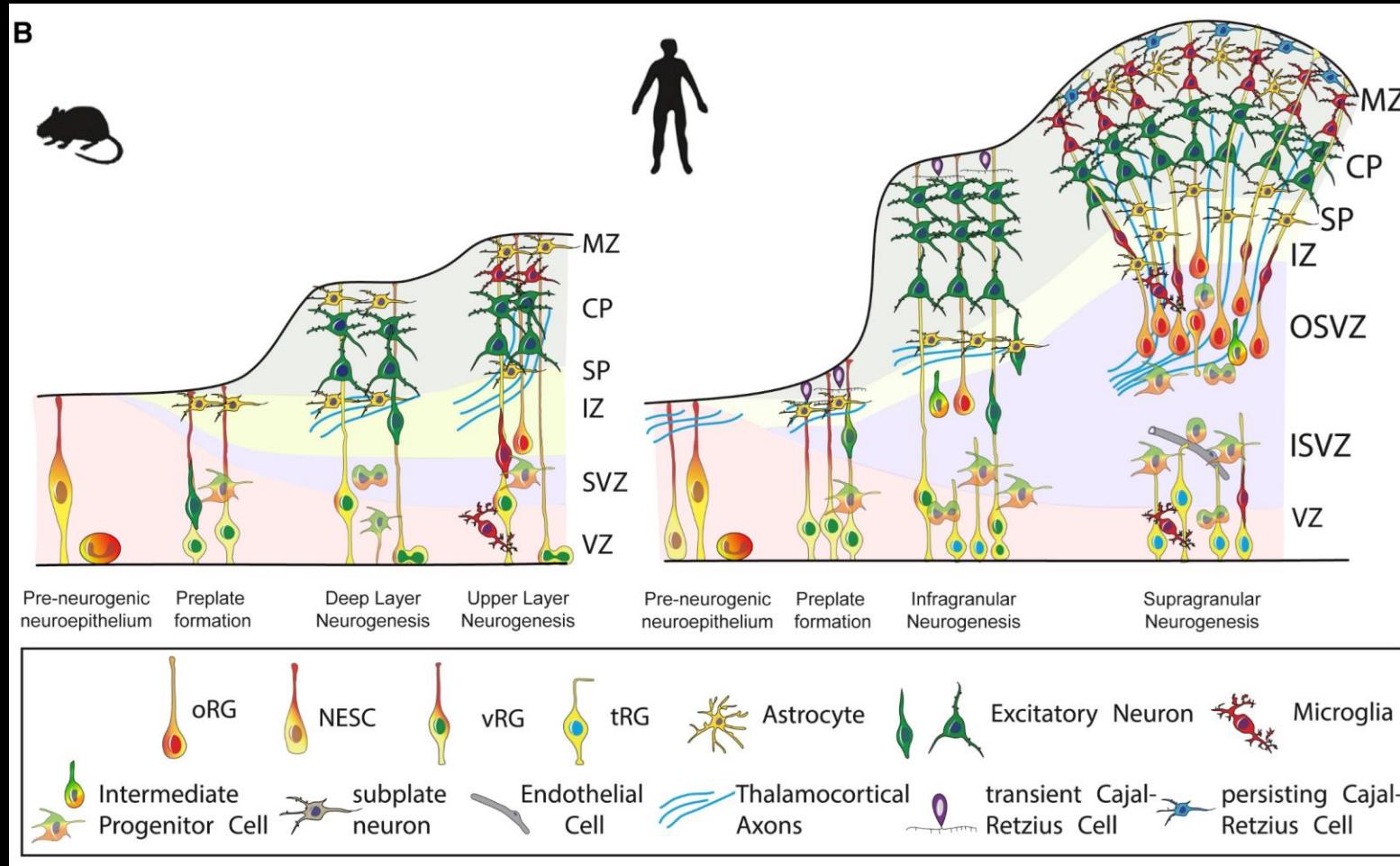
Changes in cells



Cell state transitions



Cell state transitions: Neurodevelopment



Re-processed data from Wang, et al. (2024)
Molecular and cellular dynamics of the developing
human neocortex at single-cell resolution. bioRxiv.
<https://doi.org/10.1101/2024.01.16.575956>.

scRNA-seq analysis to study cell state transitions



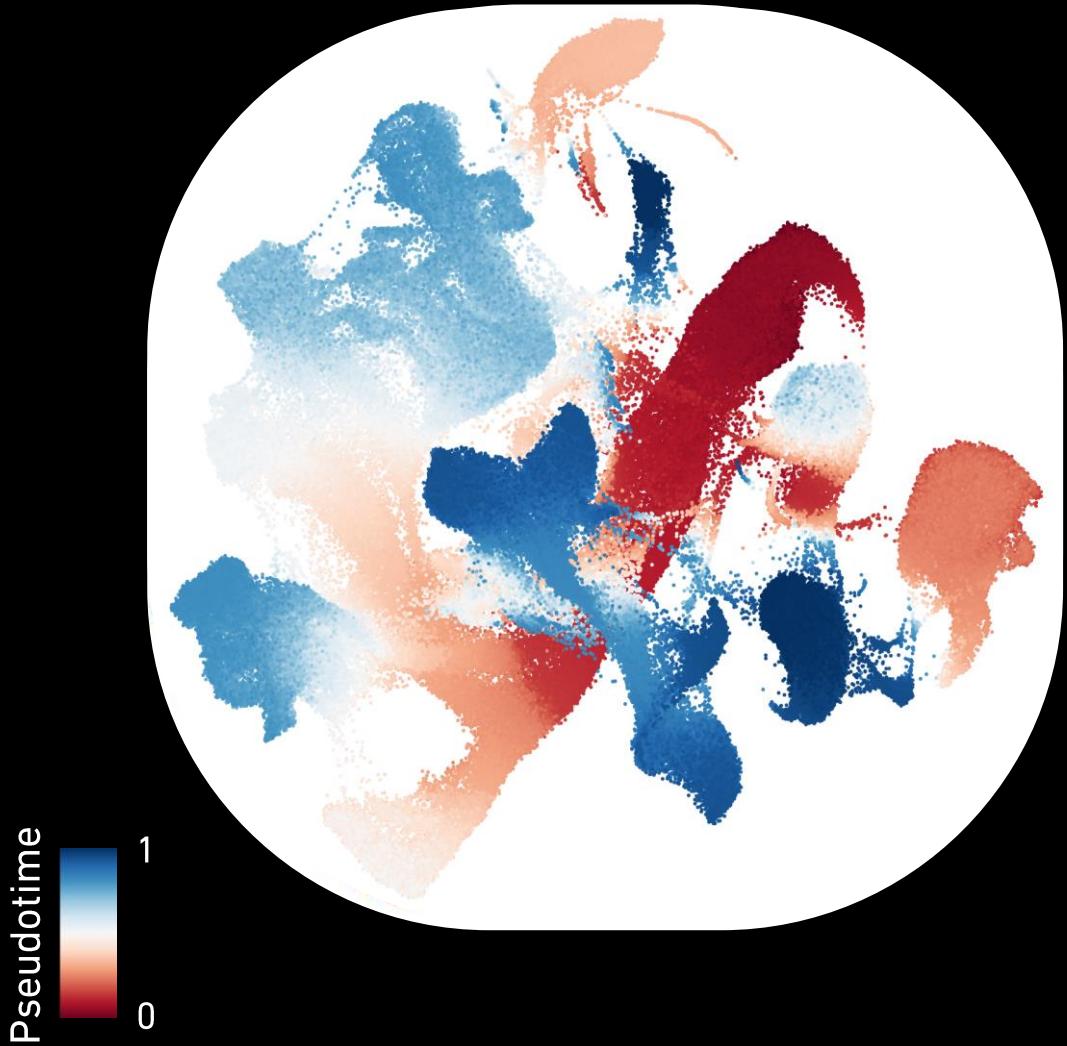
1st trimester
2nd trimester
3rd trimester
Infant
Adolescence

Trajectory identification and characterization

Trajectories represent paths of cell state transitions

- Look for trajectory backbone
- Look for branching points
- Assign cells to the trajectory

scRNA-seq analysis to study cell state transitions



Trajectory identification and characterization

Trajectories represent paths of cell state transitions

- Look for trajectory backbone
- Look for branching points
- Assign cells to the trajectory

Pseudotime / pseudo-temporal cell ordering

Pseudotime quantifies progression of cell state transitions

scRNA-seq analysis to study cell state transitions



Trajectory identification and characterization

Trajectories represent paths of cell state transitions

- Look for trajectory backbone
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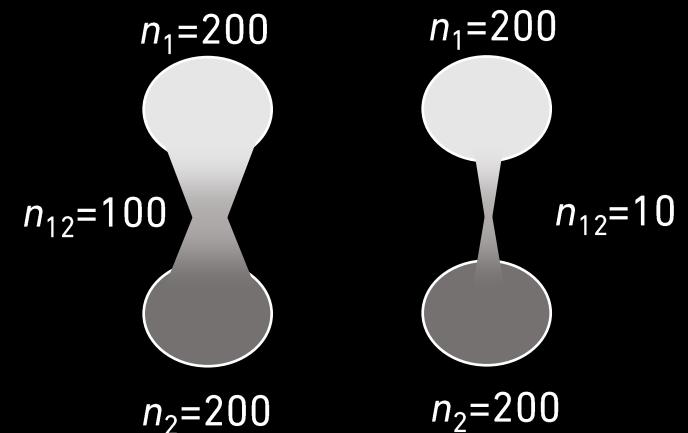
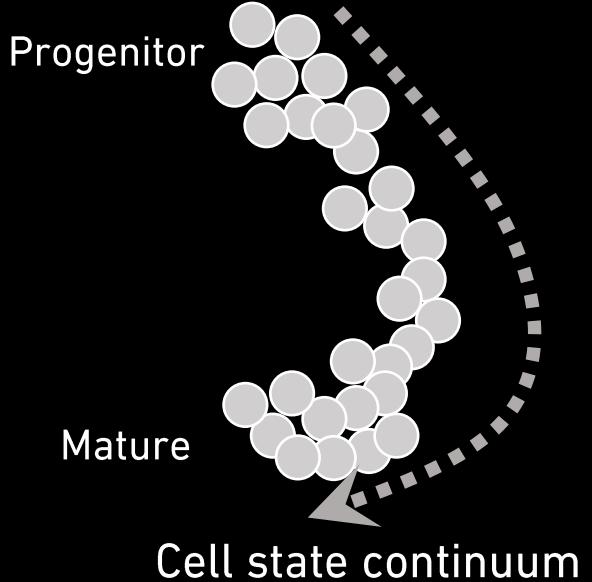
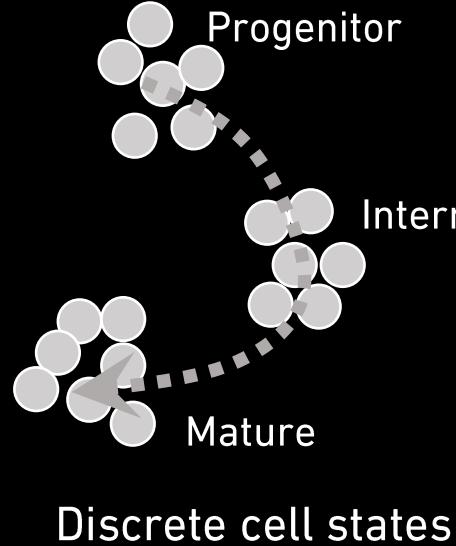
Pseudotime / pseudo-temporal cell ordering

Pseudotime quantifies progression of cell state transitions

Cell fate probability estimation

Estimate the fate commitment of cells

Trajectory & Pseudotime: WHEN

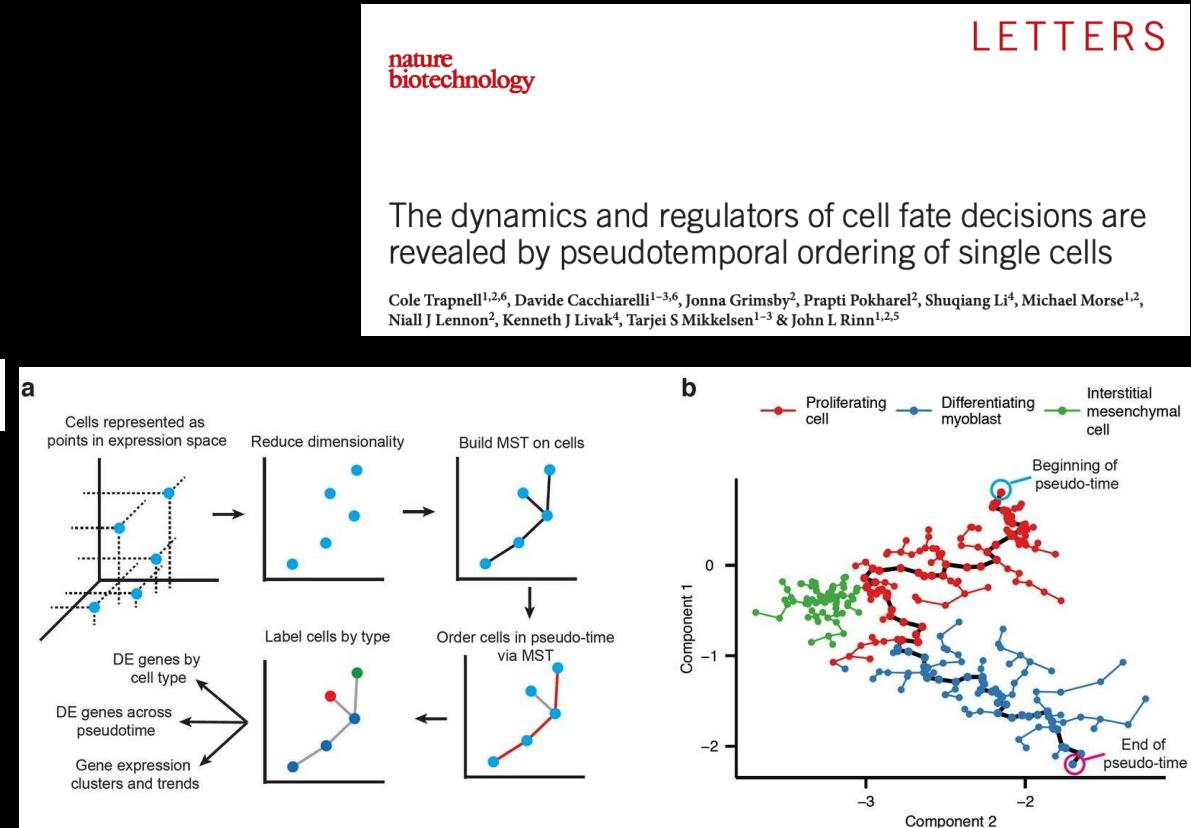
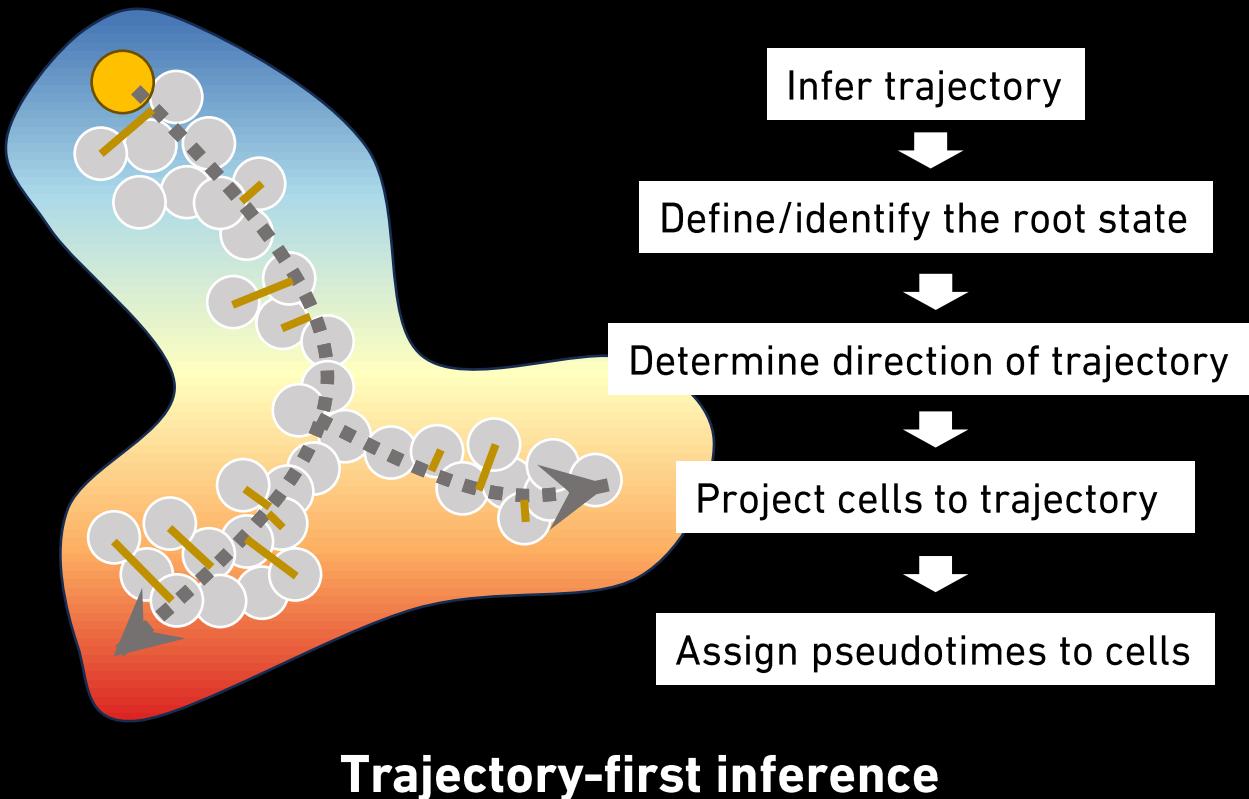


Artificial
continuum by
doublets?

- Doublet detection algorithms (e.g. *DoubleFinder*, *Scrublet*)
- TF markers for intermediates

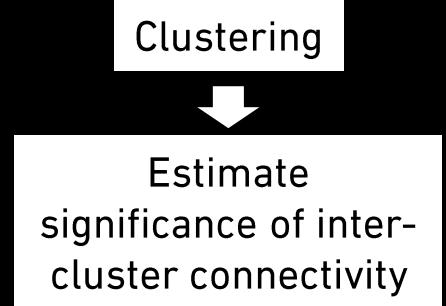
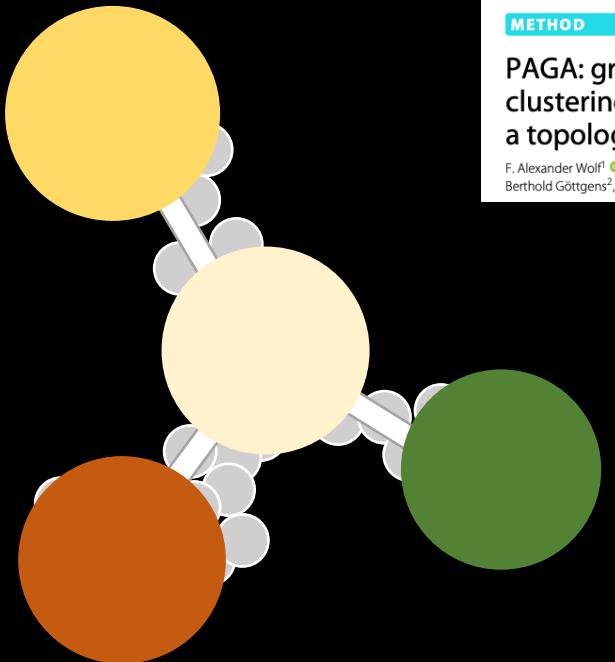
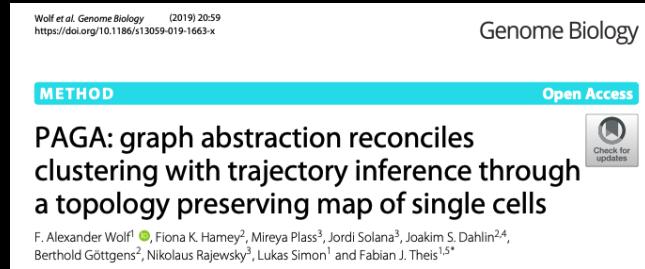
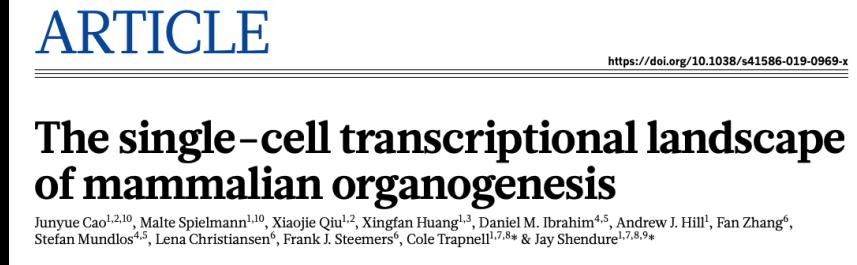
Trajectory & Pseudotime: HOW

Monocle (2014)
(*monocle* in R)



Trajectory & Pseudotime: HOW (2)

Monocle 3 (2019)
(*monocle3* in R, *py-monocle* in Python)



Trajectory & Pseudotime: HOW (2)

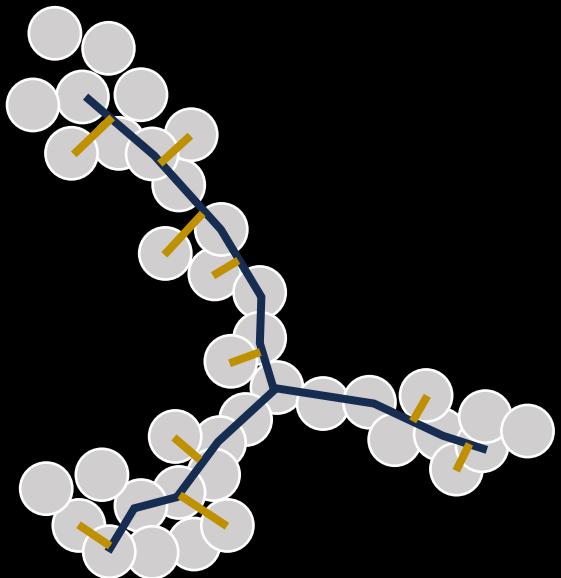
Monocle 3 (2019)
(*monocle3* in R , *py-monocle* in Python)

ARTICLE

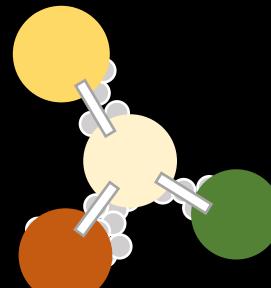
<https://doi.org/10.1038/s41586-019-0969-x>

The single-cell transcriptional landscape of mammalian organogenesis

Junyue Cao^{1,2,10}, Malte Spielmann^{1,10}, Xiaojie Qiu^{1,2}, Xingfan Huang^{1,3}, Daniel M. Ibrahim^{3,5}, Andrew J. Hill¹, Fan Zhang⁶, Stefan Mundlos^{4,5}, Lena Christiansen⁶, Frank J. Steemers⁶, Cole Trapnell^{1,7,8,*} & Jay Shendure^{1,7,8,9,*}



Connectivity constraint



Principal graph

Maximize similarities between a cell and the average of its connected cells in the graph

SimplePPT: A Simple Principal Tree Algorithm

Qi Mao*

Le Yang†

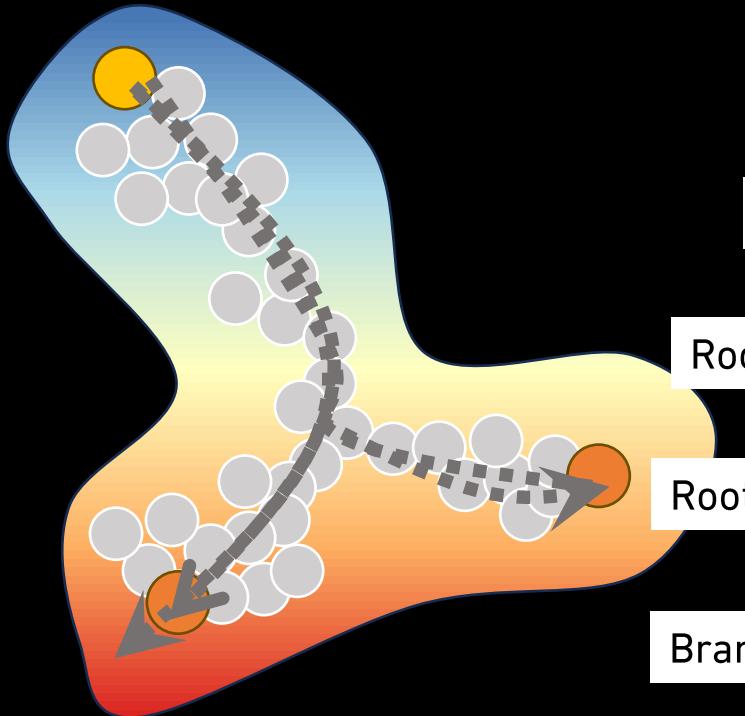
Li Wang‡

Steve Goodison§

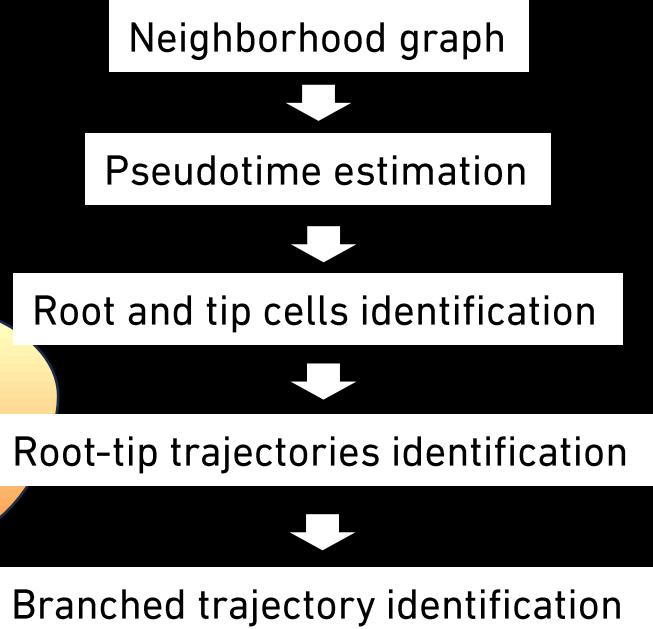
Yijun Sun¶

$$\min_{f_{\mathcal{G}} \in \mathcal{F}_{\mathcal{G}, \ell}} \frac{1}{N} \sum_{i=1}^N \min_{\mathbf{z} \in \mathcal{Z}} c(\mathbf{x}_i, f_{\mathcal{G}}(\mathbf{z})), \quad \mathcal{F}_{\mathcal{G}, \ell} = \{f_{\mathcal{G}} \in \mathcal{F}, \ell(\mathcal{G}) \leq \ell\},$$
$$\ell(\mathcal{G}) = \sum_{(V_i, V_j) \in \mathcal{E}} w_{i,j} \|f_{\mathcal{G}}(\mathbf{z}_i) - f_{\mathcal{G}}(\mathbf{z}_j)\|^2.$$

Trajectory & Pseudotime: HOW (3)



Pseudotime-first inference

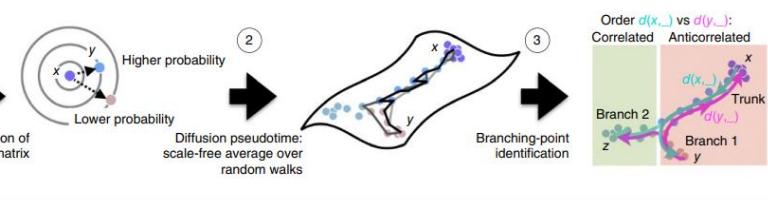


Branch point

Cell with largest
pseudotime (tip cell)

Tip cell

Anti-correlated



Diffusion Pseudotime (2016)
(*destiny* in R, *scanpy* in Python)

Diffusion pseudotime robustly reconstructs lineage branching

Laleh Haghverdi^{1,2}, Maren Büttner¹, F Alexander Wolf⁴,
Florian Büttner^{1,3} & Fabian J Theis^{1,2}

Neighborhood graph

Transition matrix

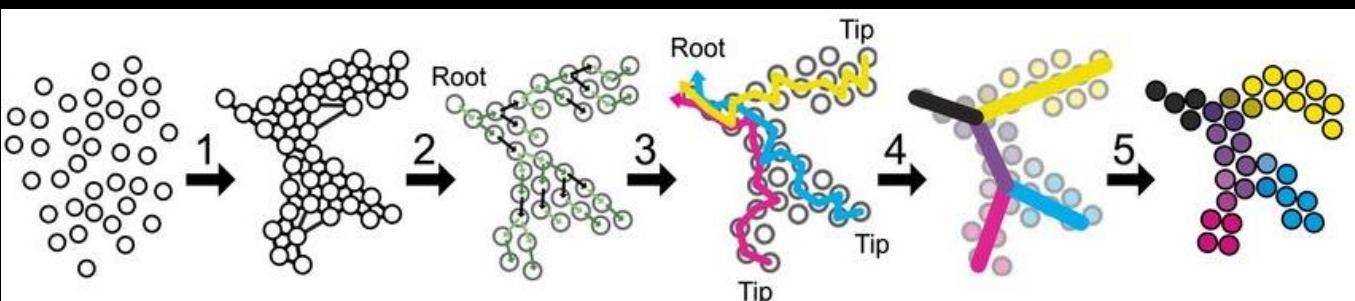
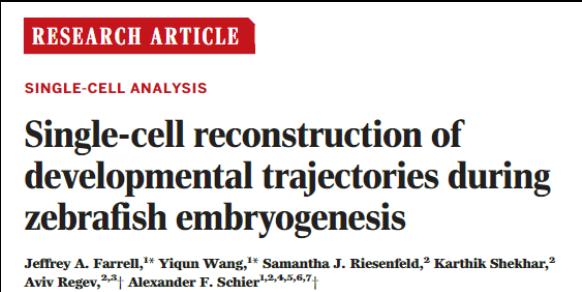
Diffusion map

Denoised transition matrix

Random walk for pseudotime

Trajectory & Pseudotime: HOW (4)

URD (2018)
(URD in R)



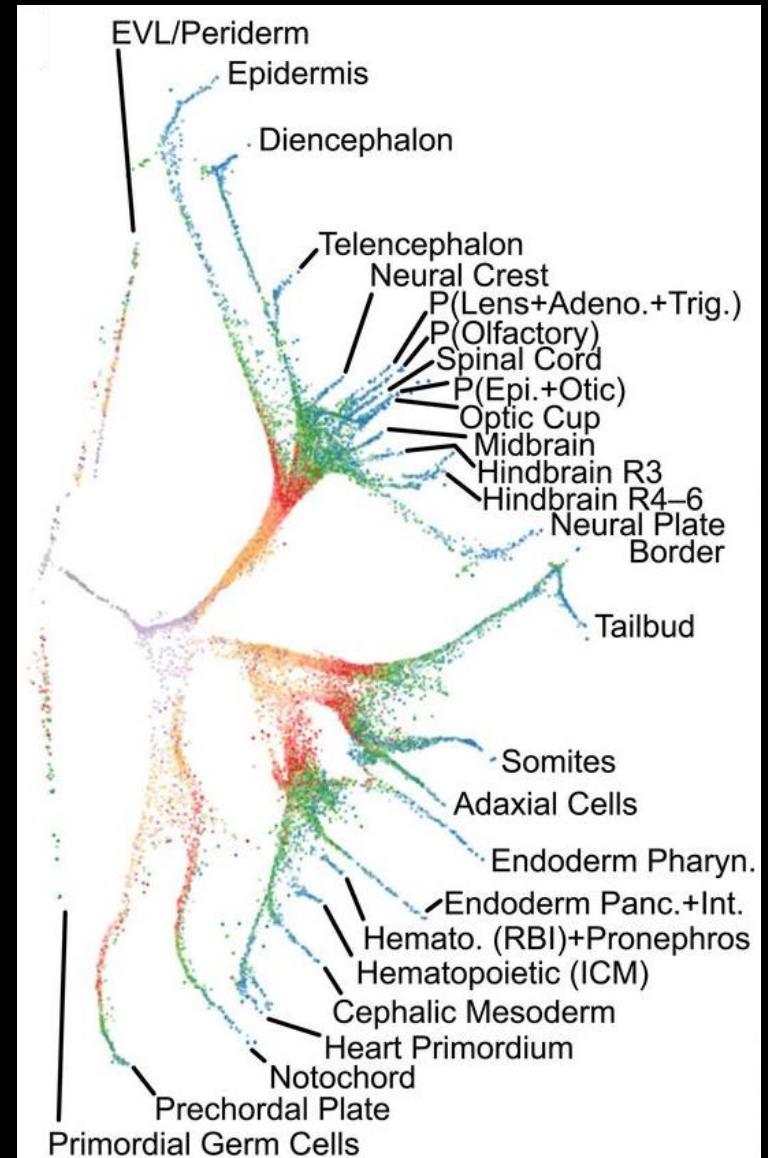
Diffusion map
(*destiny*)

Probabilistic
breadth-first
search (flood
simulation)

Random walk
simulation on
biased transition
matrix

Segment-
and-join

Force-directed
layout based on
visitation
frequency from
tips

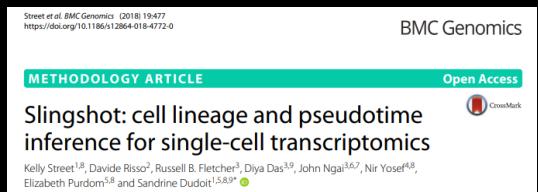


Trajectory & Pseudotime: MORE

Slingshot (2018)

(*slingshot* in R, *pyslingshot* in Python)

- Trajectory-first inference of trajectory and pseudotime



Palantir (2019)

(*palantir* in Python)

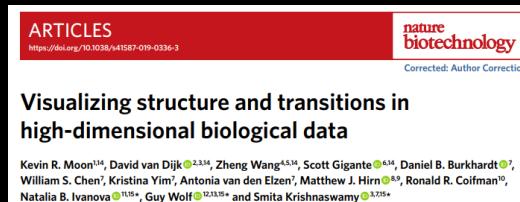
- Pseudotime-first inference of pseudotime and fate probability



PHATE (2019)

(*phate* in Python, *phateR* in R as a wrapper to the Python package)

- Diffusion-based pseudotime-first inference of pseudotime and trajectories
- Data visualization approach which can highlight cell state transitions



moscot (2023)

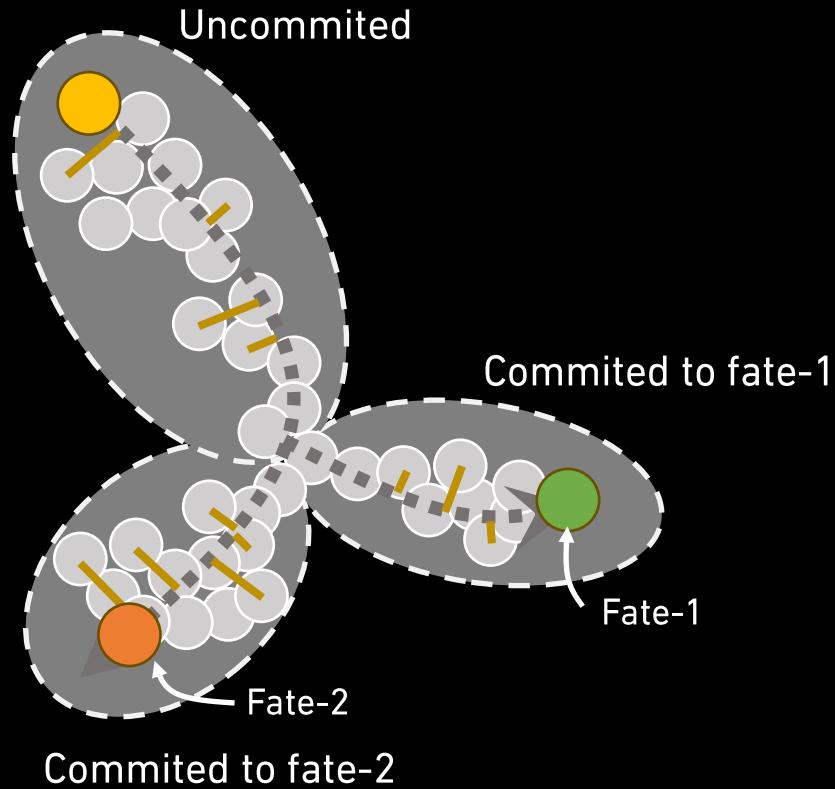
(*moscot* in Python)

- A general framework based on optimal transport to map cells across time and space
- Incorporate real time information for pseudotime estimation

Mapping cells through time and space with moscot

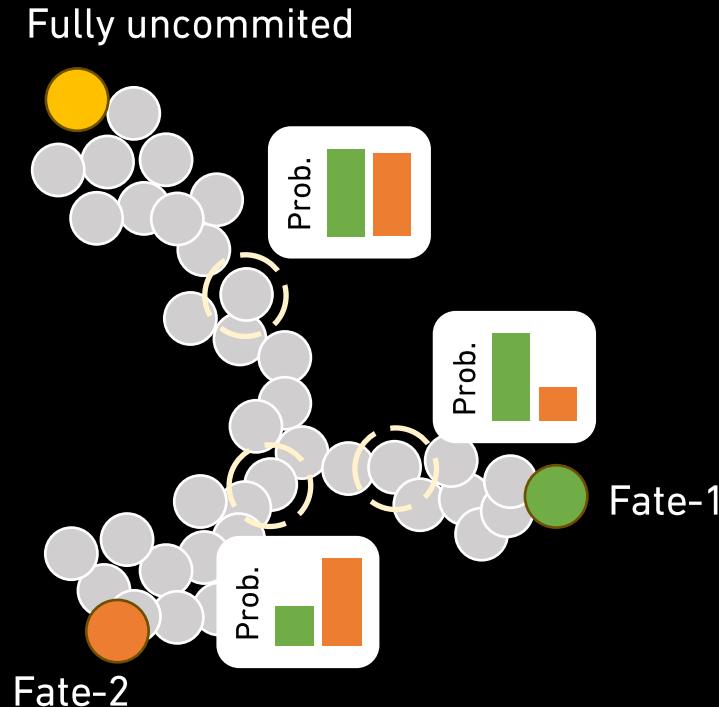
Dominik Klein^{1,2}, Giovanni Palla^{1,3}, Marius Lange^{*1,4}, Michal Klein^{*7}, Zoe Piran⁵, Manuel Gander¹, Laetitia Meng-Papaxanthos⁶, Michael Stern^{8,9}, Aimée Bastidas-Ponce^{8,9}, Marta Tarquis-Medina^{8,9}, Heiko Lickert^{8,9,10}, Mostafa Bakhti^{8,9}, Mor Nitzan^{5,11,12}, Marco Cuturi⁷, Fabian J. Theis^{1,2,3*}

Trajectory vs. fate probability



Trajectory analysis

Assume one decision per branch point
Intuitive and simple



Fate probability estimation

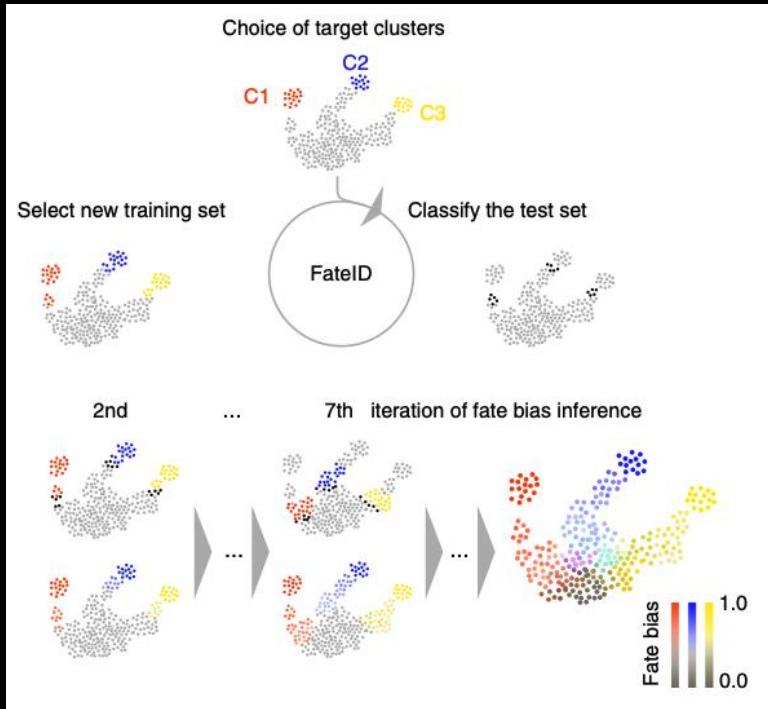
Assume continuous decision making
Flexible and likely better reflect the real biology

Fate probability estimation: HOW

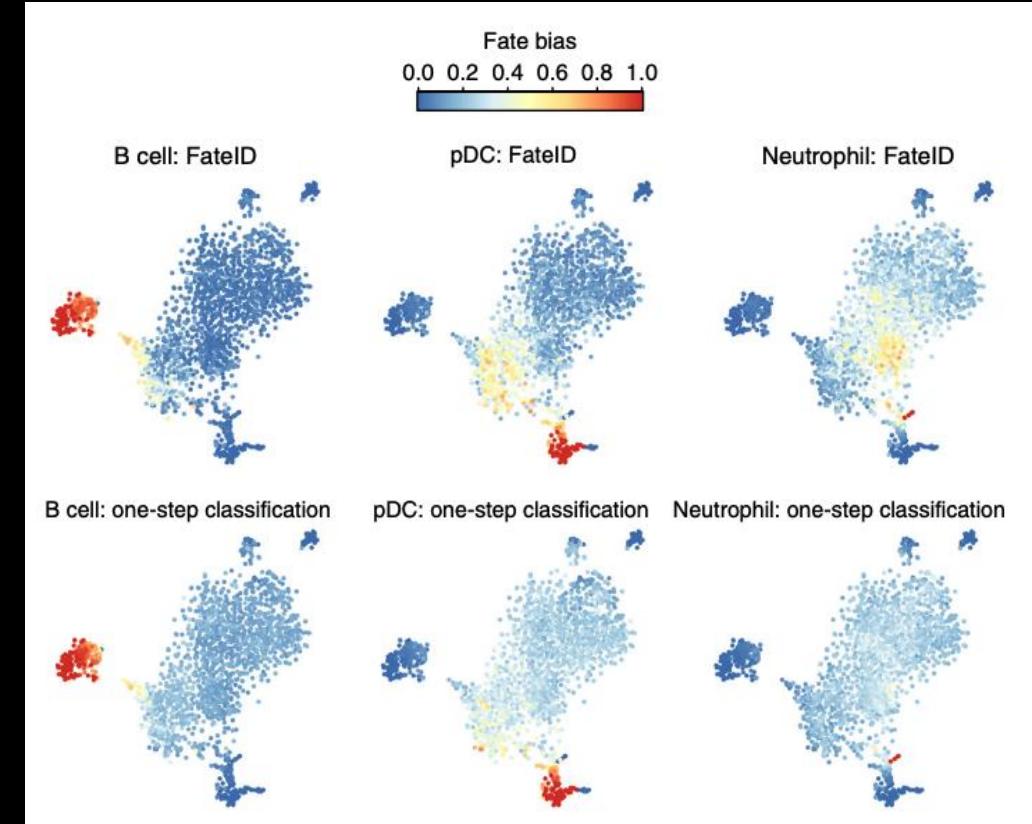
FateID (2018)
(*FateID* in R)

FateID infers cell fate bias in multipotent progenitors from single-cell RNA-seq data

Josip S Herman¹⁻³, Sagar¹ & Dominic Grün¹

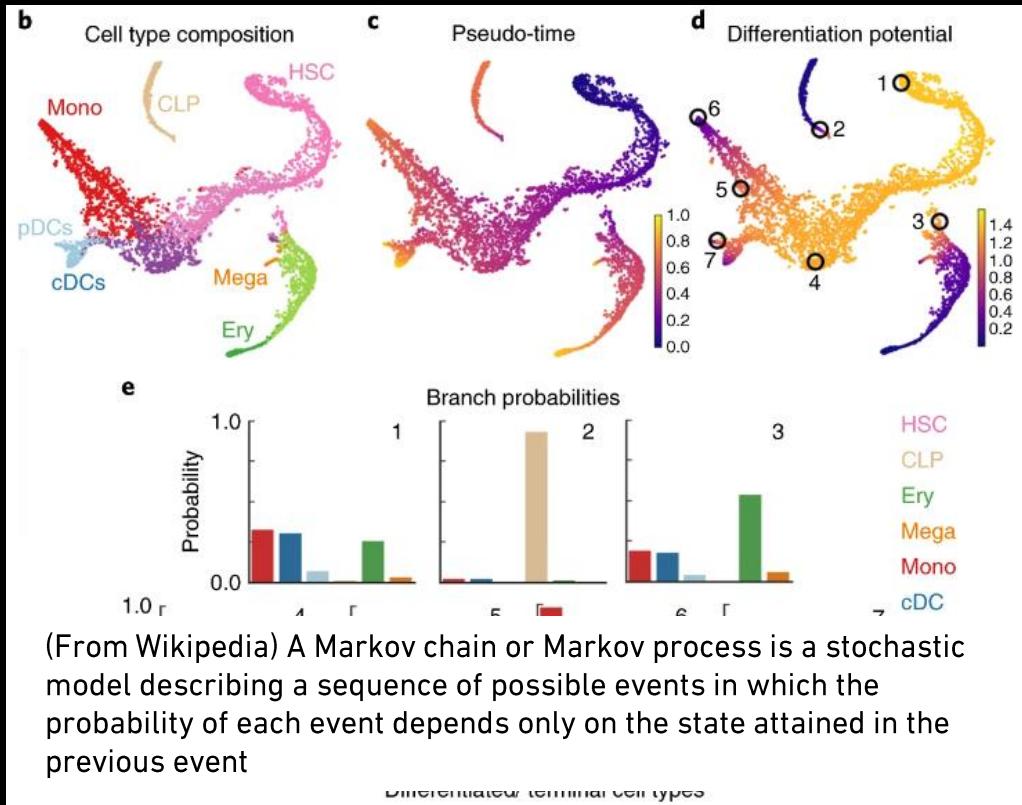
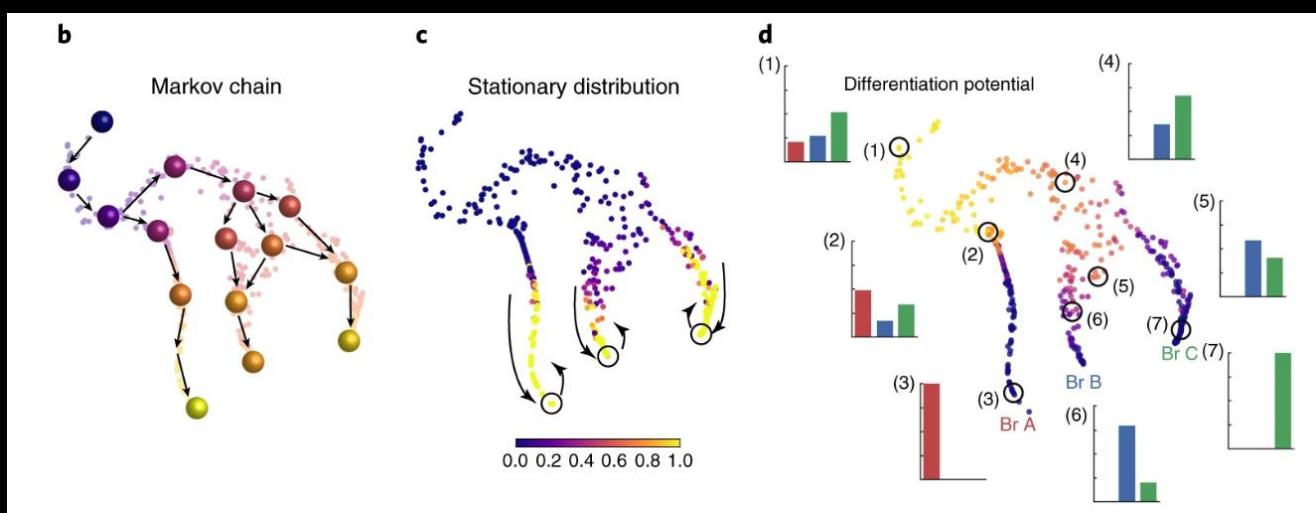


Pseudotime-free iterative cell type classification with random forest



Fate probability estimation: HOW (2)

Palantir (2019)
(*palantir* in Python)



Pseudotime
(by shortest paths on diffusion neighborhood graph)

Pseudotime-biased transition matrix

Terminal states specification

Random walk through the absorbing Markov chain on "waypoint cells"

Propagate the information from "waypoint cells" to all cells

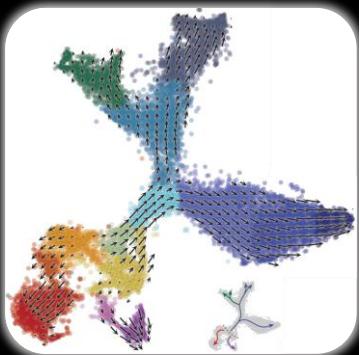
Limitations of trajectory and pseudotime analysis

scRNA-seq: a snapshot measure on every cell

- No real measurement on cell and cell state dynamics
- Cell state dynamics are inferred based on transcriptomic similarity
- Domain-specific knowledge is needed to define root/initial (and terminal) states
- Difficult to characterize unknown initial/terminal states

Can we recover cell dynamics from scRNA-seq data?

RNA velocity



RNA velocity

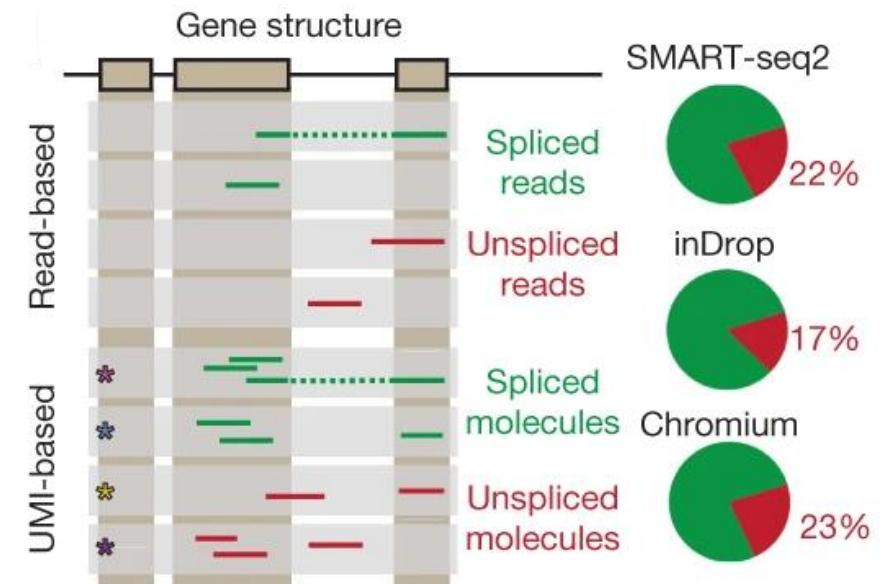
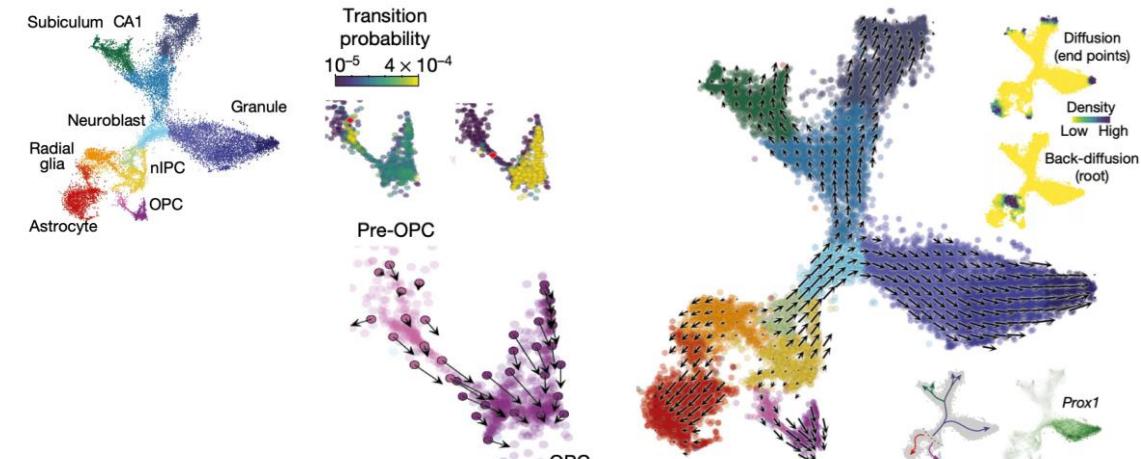
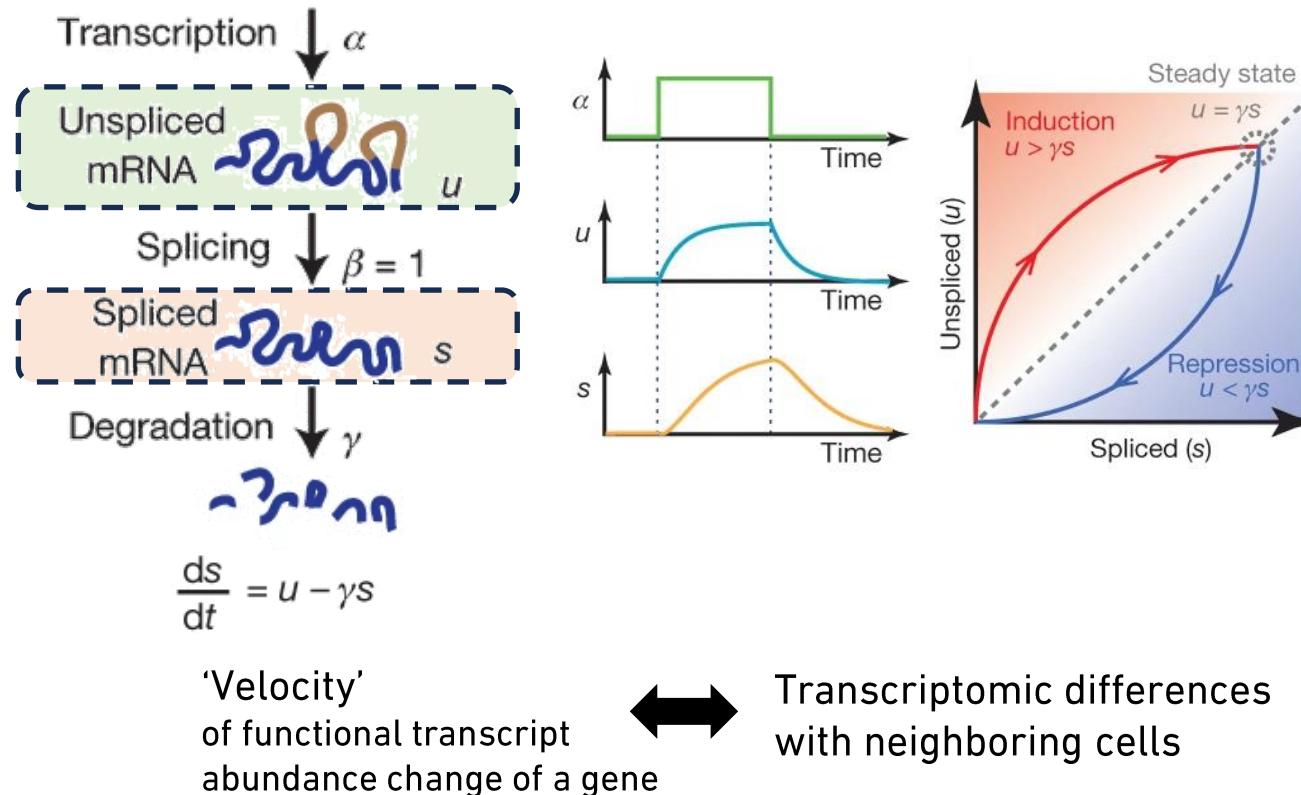
LETTER

La Manno et al. 2018

<https://doi.org/10.1038/s41586-018-0414-6>

RNA velocity of single cells

Gioele La Manno^{1,2}, Ruslan Soldatov³, Amit Zeisel^{1,2}, Emelie Braun^{1,2}, Hannah Hochgerner^{1,2}, Viktor Petukhov^{3,4}, Katja Lidschreiber⁵, Maria E. Kastriti⁶, Peter Lönnerberg^{1,2}, Alessandro Furlan¹, Jean Fan³, Lars E. Borm^{1,2}, Zehua Liu³, David van Bruggen¹, Jimin Guo³, Xiaoling He⁷, Roger Barker⁷, Erik Sundström⁸, Gonçalo Castelo-Branco¹, Patrick Cramer^{5,9}, Igor Adameyko⁶, Sten Linnarsson^{1,2*} & Peter V. Kharchenko^{3,10*}



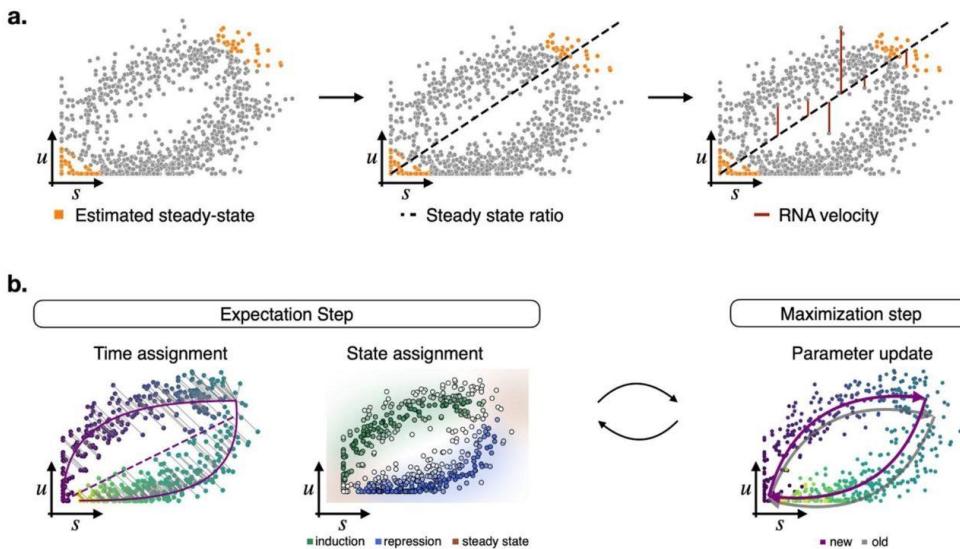
RNA velocity (2)

Limitations of *velocyto*

Assumptions must be made to infer the parameters

- Constant splicing rate for all genes
- Steady-state assumption for degradation rates
- Constant change rate of spliced molecule; or constant unspliced molecules

The R implementation is slow



Weiler et al. (2021) A guide to trajectory inference and RNA velocity. bioRxiv.
<https://doi.org/10.1101/2021.12.22.473434>

Bergen et al. 2020
(*scvelo* in Python)

ARTICLES

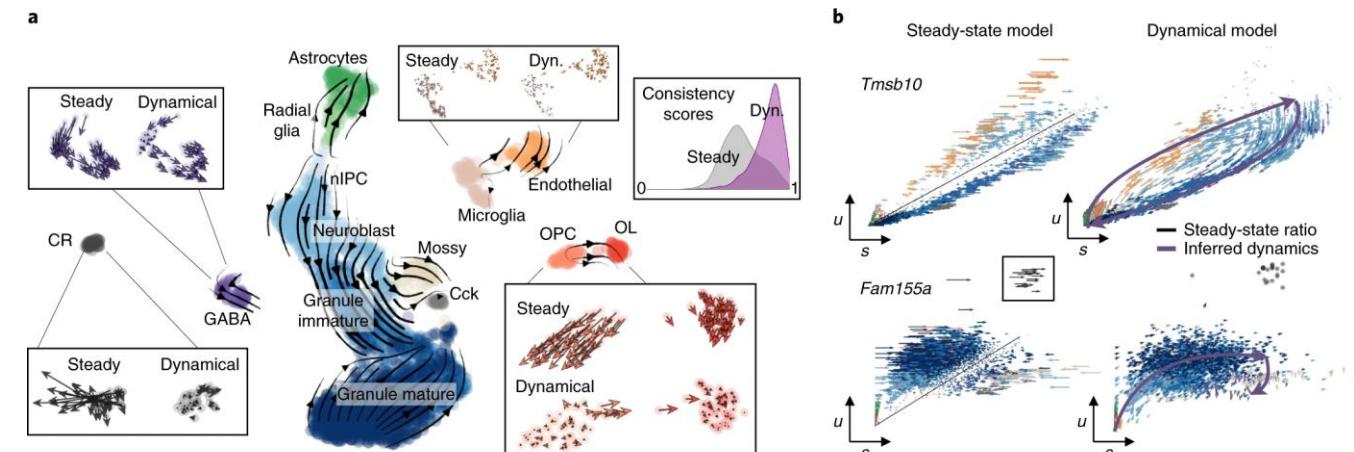
<https://doi.org/10.1038/s41587-020-0591-3>

nature
biotechnology

Check for updates

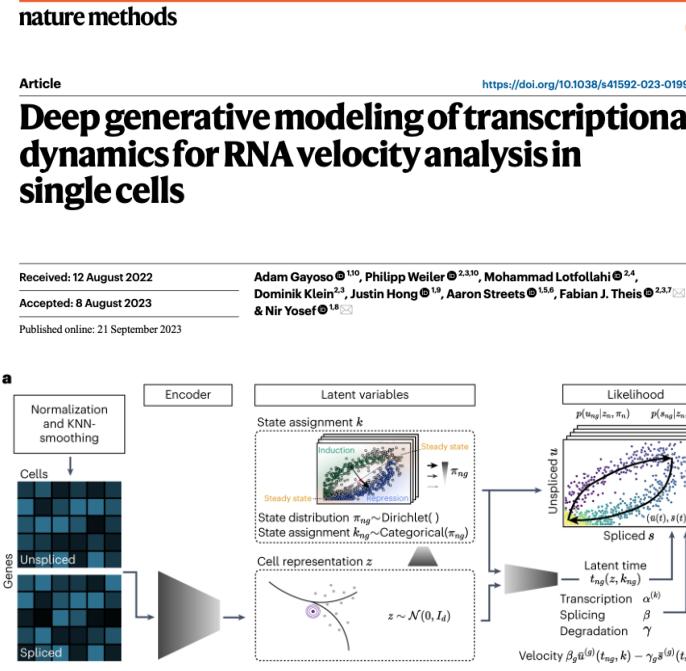
Generalizing RNA velocity to transient cell states through dynamical modeling

Volker Bergen^{1,2}, Marius Lange^{1,2}, Stefan Peidli¹, F. Alexander Wolf¹ and Fabian J. Theis^{1,2}



RNA velocity (3)

veloVI (2023)
(*velovi* in Python)

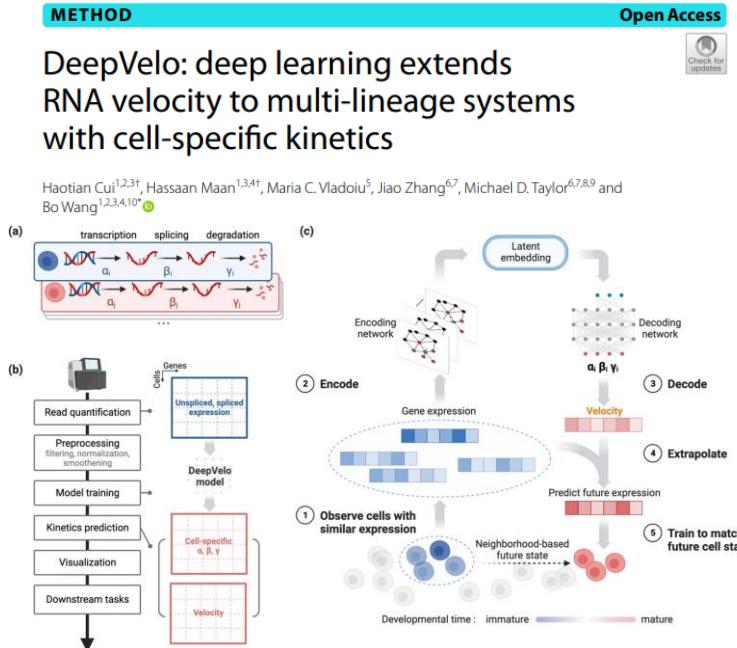


cellDancer (2023)
(*celldancer* in Python)



DeepVelo (2024)
(*deepvelo* in Python)

Cui et al. *Genome Biology* (2024) 25:27
<https://doi.org/10.1186/s13059-023-03148-9> **Genome Biology**

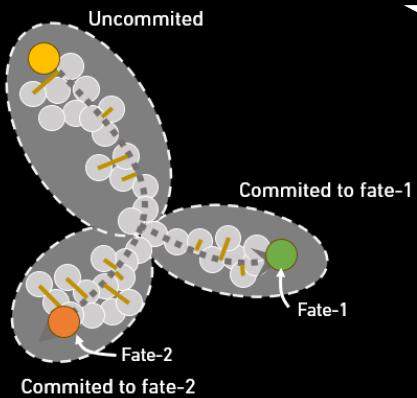
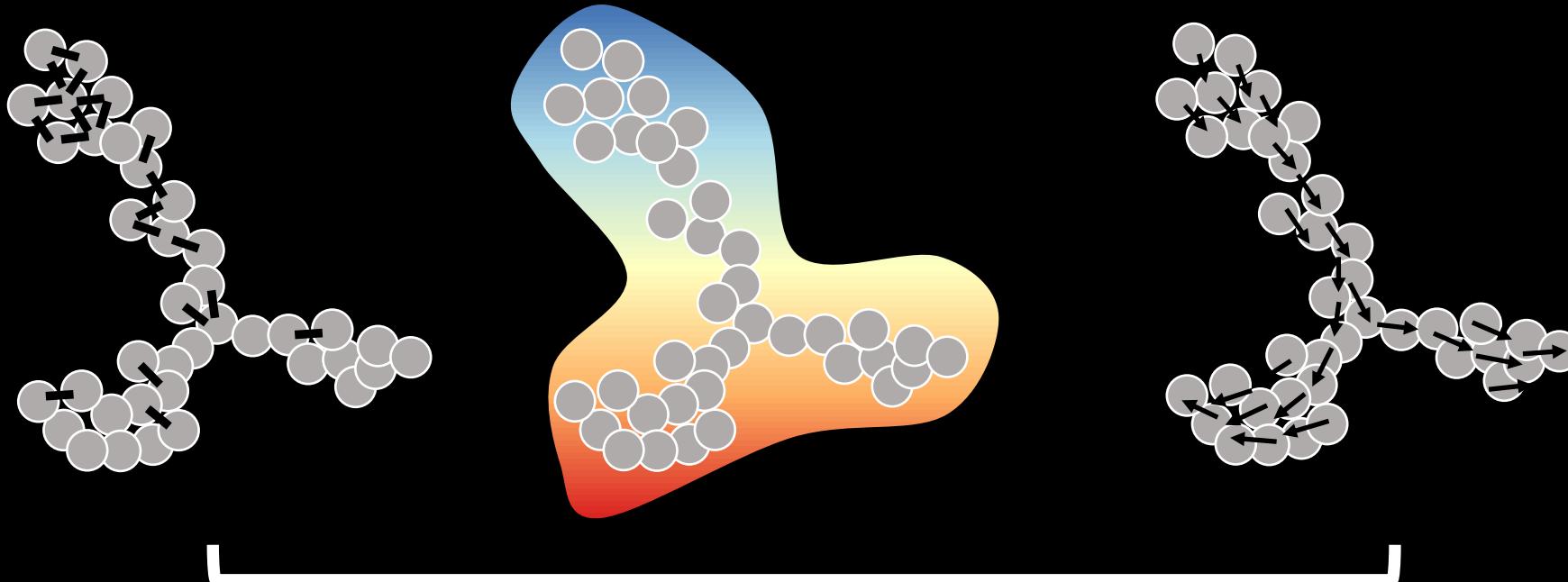


Use deep learning algorithms to infer gene-specific reaction rates for each cell

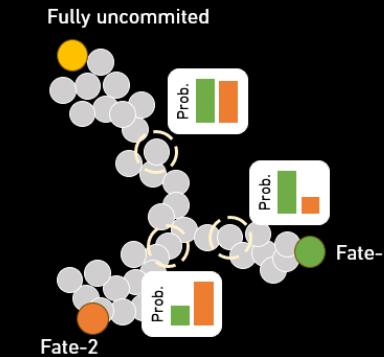
Transcriptomic
similarities

Pseudotimes

RNA velocities



Trajectory analysis



Fate probability estimation

Fate mapping combining different information

CellRank 2 (2024)
(*cellrank* in Python)

nature methods

Article

<https://doi.org/10.1038/s41592-024-02303-9>

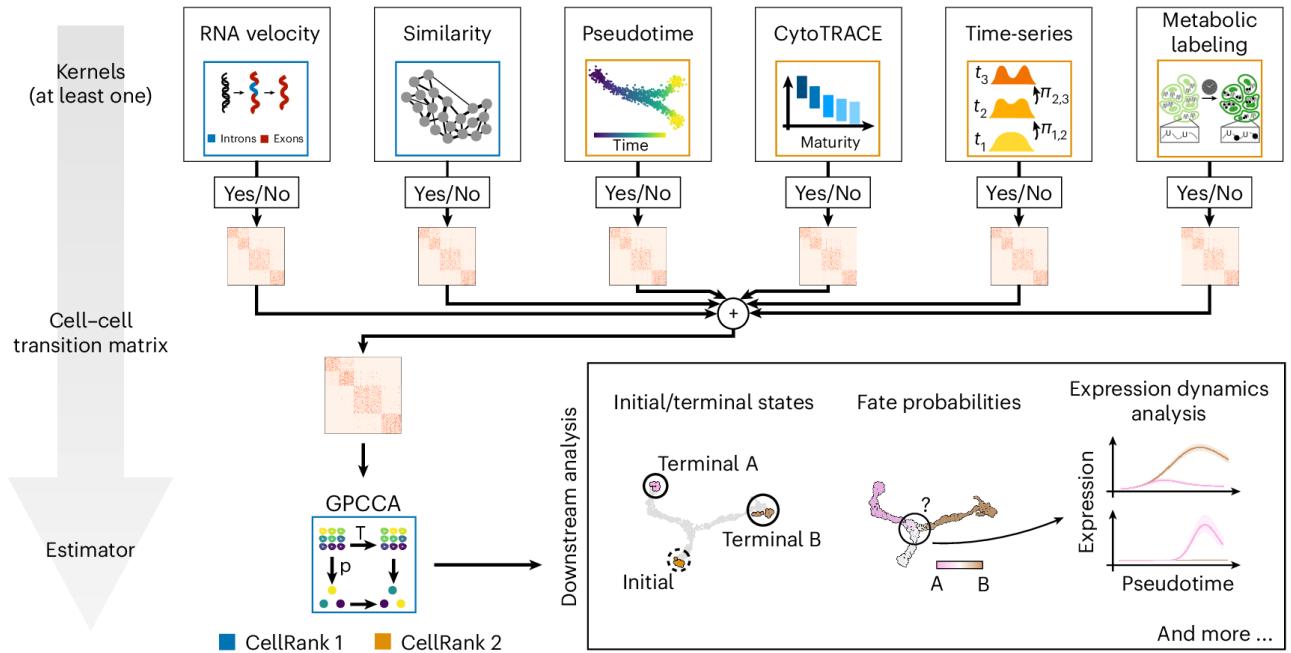
CellRank 2: unified fate mapping in multiview single-cell data

Received: 18 July 2023

Philipp Weiler^{1,2,8}, Marius Lange^{1,2,3,8}, Michal Klein^{1,4}, Dana Pe'er^{5,6} & Fabian Theis^{1,2,7}

Accepted: 9 May 2024

- Integrate different transition matrices based on different information
- Random walk on an absorbing Markov chain (very efficient implementation)



nature biotechnology

Article

<https://doi.org/10.1038/s41587-023-01747-2>

Multimodal spatiotemporal phenotyping of human retinal organoid development

Received: 3 March 2022

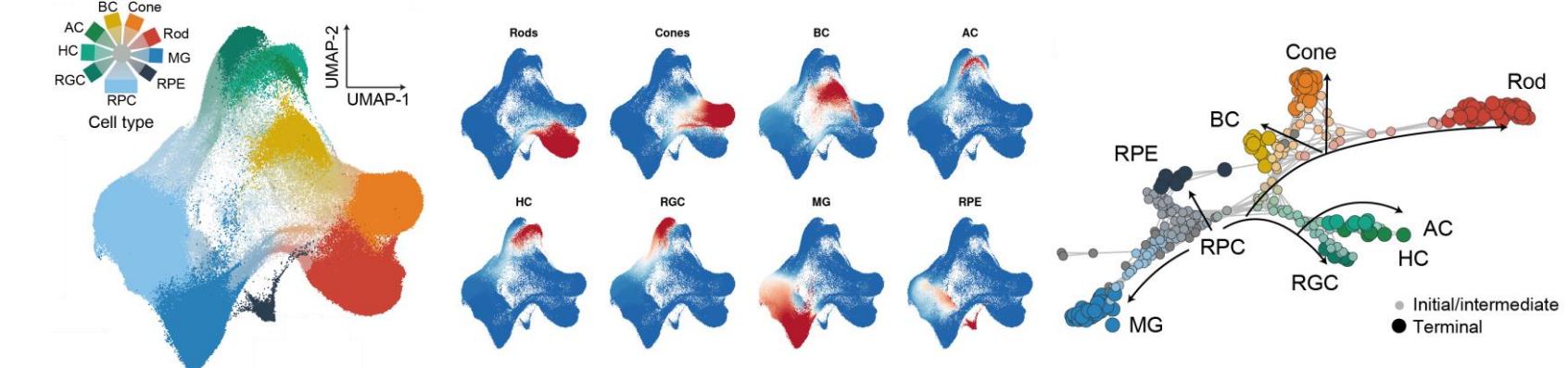
Philipp Wahle^{1,2,3,6}, Giovanna Brancati^{1,2,3,6}, Christoph Harmel^{1,2,3,5,6}, Zhisong He^{1,6}, Gabriele Gut¹, Jacobo Saravia del Castillo^{1,4}, Aline Xavier da Silveira dos Santos^{1,2,3,5}, Qianhui Yu^{2,3,5}, Pascal Noser¹, Jonas Simon Fleck¹, Bruno Cjetz^{2,3,5}, Dinko Pavlinić^{2,3}, Simone Picelli^{1,2,3}, Max Hess^{1,4}, Gregor W. Schmidt¹, Tom T. A. Lummen¹, Yanyan Hou^{2,3}, Patricia Gallikker^{2,3}, David Goldblum^{2,3}, Marton Balogh^{2,3}, Cameron S. Cowan², Hendrik P. N. Scholl^{2,3}, Botond Roska^{2,3}, Magdalena Renner^{2,3}, Lucas Pelkmans^{1,4}, Barbara Treutlein^{1,2,3} & J. Gray Camp^{1,2,3,5}

Accepted:

13 March 2023

Published online: 8 May 2023

Check for updates



Last but not the least...

All analysis can output wrong and misleading results,
even if you don't make any technical mistake

Limitations on the computational methods

Data quality

Questions?