Estimation of single-cell and tissue perturbation effect in spatial transcriptomics via Spatial Causal Disentanglement

Stathis Megas*, Daniel Chen*, … & Sarah Teichmann

Published in ICLR 2025

14.10.2025

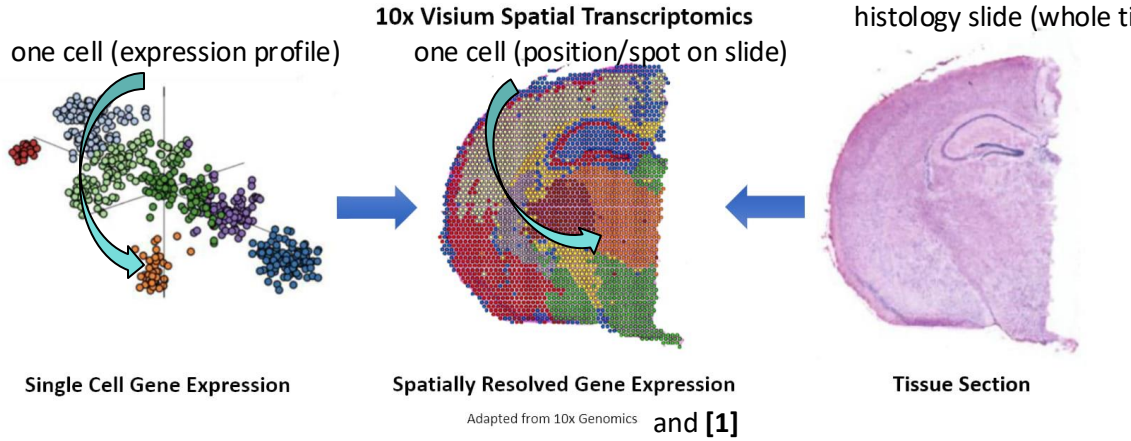
Clemence Reda

Background

1. Spatial transcriptomics
2. Causal disentanglement
3. K-hop Graph Neural Networks

Spatial transcriptomics a high-throughput technology which captures gene expression and position of cell

Get transcriptomic and morphological information at the single-cell level

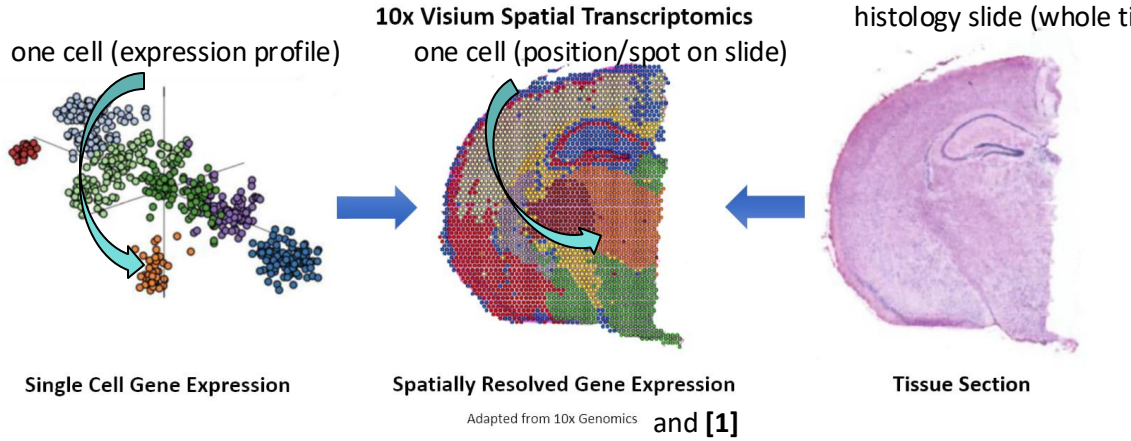


Adapted from 10x Genomics and **[1]**

[1] https://sites.dartmouth.edu/cqb/2020/11/24/new-services-sample-multiplexing-spatial-transcriptomics-and-multiomics/

Spatial transcriptomics a high-throughput technology which captures gene expression and position of cell

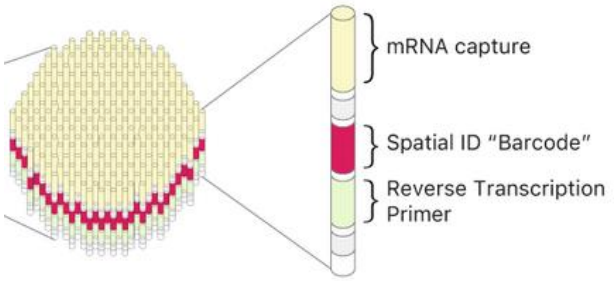
Get transcriptomic and morphological information at the single-cell level



and **[1]**

<u>For sequencing-based ST</u>

(a) Glass slide is arrayed with barcoded spots
(b) Frozen tissue is laid onto the slide
(c) Spatially-marked cDNA is synthesized
(d) Deconvolution (assignation of an expression value to a spot and a gene)



adapted from **[2]**

Oligo(dT) initiates synthesis of cDNA from mRNA

Uniquely identifies the x,y coordinates of the spot

increases coverage of transcriptome in combination with Oligo(dT)

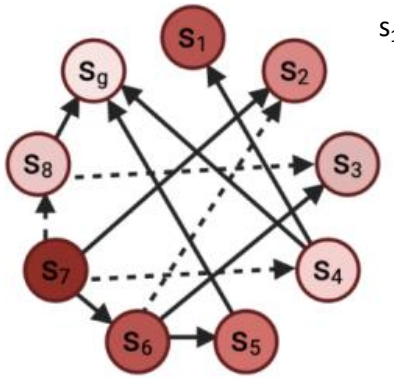
+ amplification handle
+ UMI (specific to transcript)

[1] https://sites.dartmouth.edu/cqb/2020/11/24/new-services-sample-multiplexing-spatial-transcriptomics-and-multiomics/
[2] https://en.wikipedia.org/wiki/Spatial_transcriptomics

Causal disentanglement inference of the intra- and cross-cell interactions explaining spatial transcriptomics

Causal inference reconstruct from data the causal diagram of *independent and identifiable* actions between variables which accounts for the observations

disentanglement



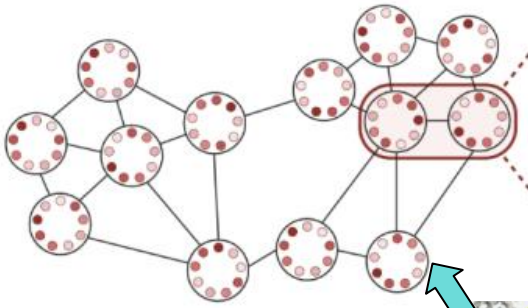
s_1, s_2, …, are variables

within-node interaction

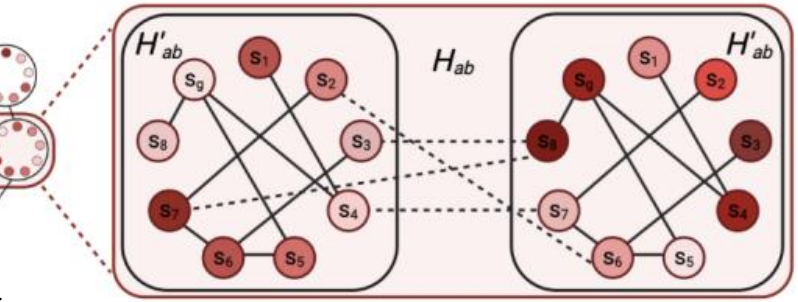
interaction across NN

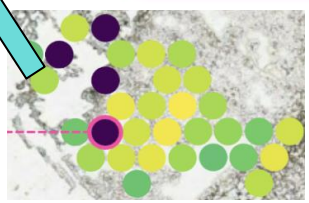
disentanglement

Node neighborhood graph
1-hop neighbors < 15 µm away
2-hop neighbors < 30 µm away

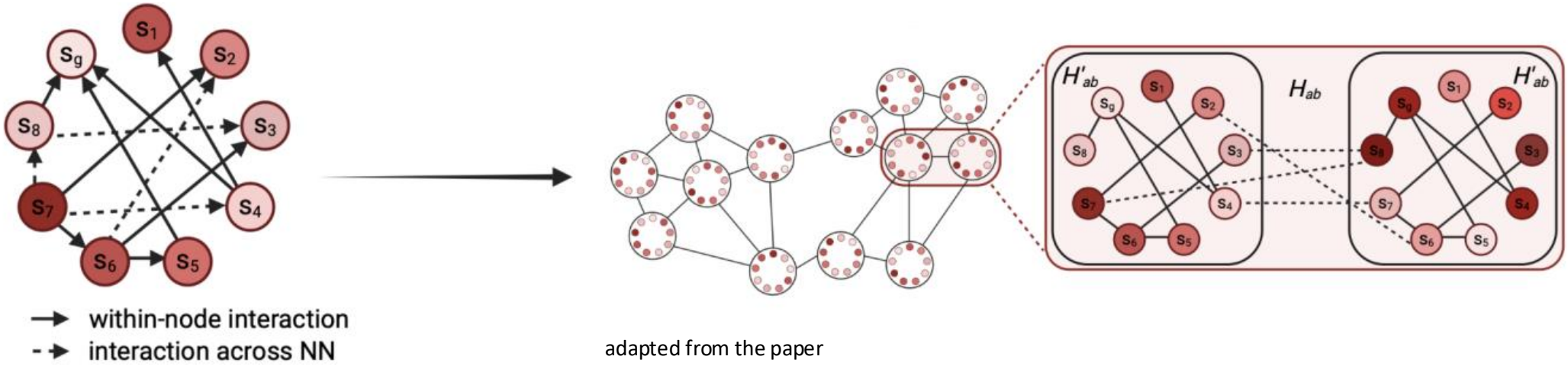


adapted from the paper





Causal disentanglement inference of the intra- and cross-cell interactions explaining spatial transcriptomics



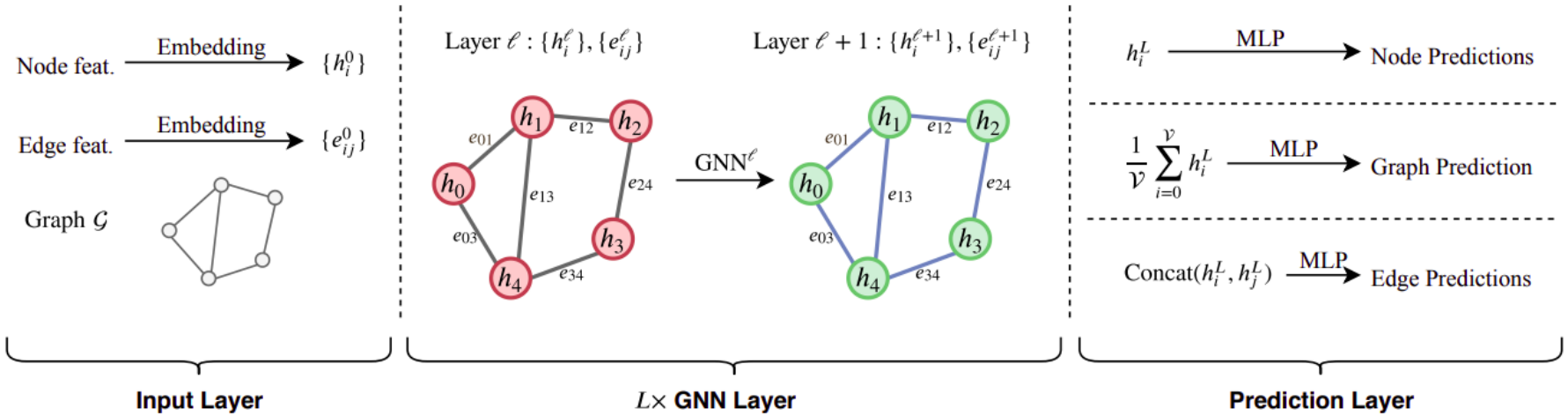
adapted from the paper

Objective learn the distribution of spatial omics by recovering variable-variable interaction matrices H' (intra-node) and H (cross-node)

+ identifiability: there are unique matrices that correspond to a given data set

- Nodes are (single-cell) spots
- Variables are HVGs

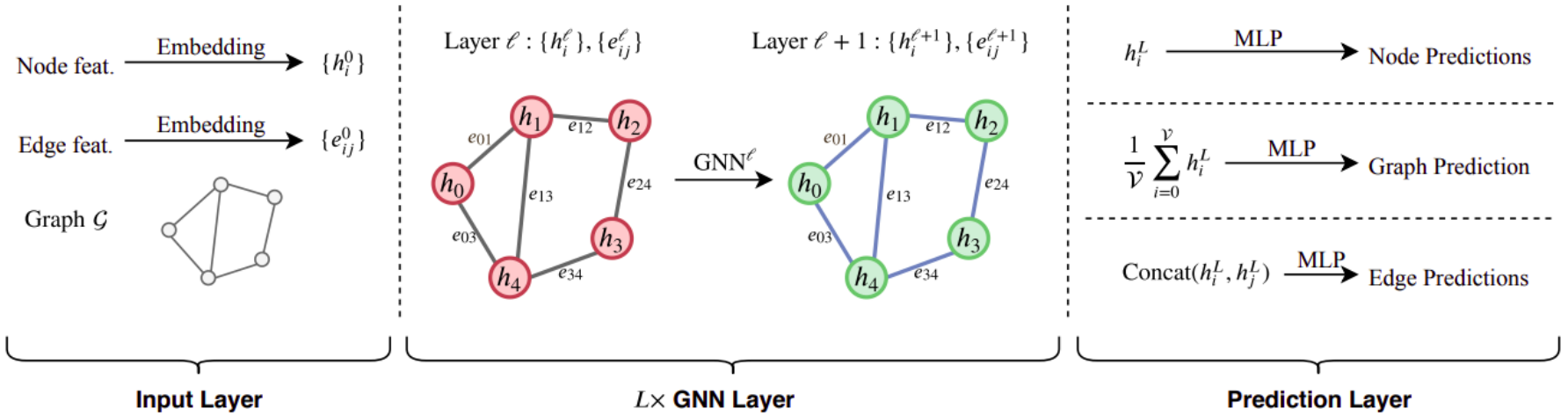
K-hop Graph Neural Networks aggregate information from neighbors at distance = K for node representations



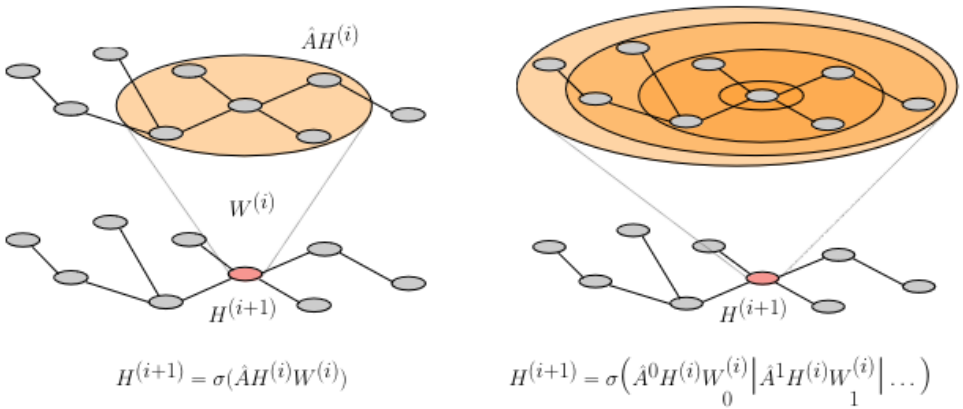
example for K=1: adapted from **[3]**

[3]
https://wandb.ai/yashkotadia/benchmarking-gnns/reports/Part-2-Comparing-Message-Passing-Based-GNN-Architectures--VmlldzoyMTk4OTA

K-hop Graph Neural Networks aggregate information from neighbors at distance ≤ K for node representations



example for K=1: adapted from **[3]**



K=1 versus K>1: adapted from **[4]**

[3] https://wandb.ai/yashkotadia/benchmarking-gnns/reports/Part-2-Comparing-Message-Passing-Based-GNN-Architectures--VmlldzoyMTk4OTA

[4] Abu-El-Haija et al. *ICML*, 2019.

Content of the paper

"Celcomen [...] a novel framework of causal structure learning for feature interaction in graph data, such as [...] gene regulation inference"

Specific issues:

- Extend seamlessly learning intracell regulations to cell communication
- Identifiable model (leading to interpretation)
- Produce perturbation counterfactuals

Prior approaches:

- Manual modeling of PPI/GRN
- Deep learning without strong guarantees

Content of the paper

"Celcomen [...] a novel framework of causal structure learning for feature interaction in graph data, such as [...] gene regulation inference"

1. Assumptions for the distribution on spatial omics
2. Simplified loss function for the k-hop GNN
3. Identifiability guarantees
4. Synthesis of spatial omics with Simcomen
5. Experimental results

Assumptions for the distribution on spatial omics to derive properties of Celcomen (inference module)

P : distribution on spatial omics data sets with N(=500) genes and M(=2,500) spots
O^{α}_{c} : observed normalized gene expression value for gene α and spot c
S^{α}_{c} : normalized gene expression value for gene α and spot c under distribution P

maximize the enthropy h(P) under
(A1) for all genes α and β, for all spot c, $O^{\alpha}_{c} \times O^{\beta}_{d} = E_{S\sim P}[S^{\alpha}_{c} \times S^{\beta}_{d}]$ (intra-spot gene-gene correlations)
(A2) for all genes α and β, for all 1-hop neighboring spots c and d, $O^{\alpha}_{c} \times O^{\beta}_{d} = E_{S\sim P}[S^{\alpha}_{c} \times S^{\beta}_{d}]$ (cross-spot)
(A3) no batch effect, same cell type, steady state, monotonic interactions (e.g., no synergy)

Assumptions for the distribution on spatial omics to derive properties of Celcomen (inference module)

P : distribution on spatial omics data sets with N(=500) genes and S(=2,500) spots
o^{α}_{c} : observed normalized gene expression value for gene α and spot c
s^{α}_{c} : normalized gene expression value for gene α and spot c under distribution P

Loss function with symmetric Lagrangian multipliers H and H' in $R^{N \times N}$ **(A3)**
where ⊙ Hadamard product, A adjacency matrix of node neighborhood graph A_{cd}=1 iff (c,d) exists, otherwise =0

cross-spot **(A2)** *intra-spot* **(A1)** *interactions*

$$\text{Loss}(P, H, H') = - h(P) - \sum_{\alpha,\beta} (H \odot E_{S\sim P}[sAs^{T} - oAo^{T}] + H' \odot E_{S\sim P}[ss^{T} - oo^{T}])_{\alpha,\beta}$$

$$(H \odot sAs^{T})_{\alpha,\beta} = H_{\alpha,\beta} \sum_{c,d<S} s^{\alpha}_{c} \times A_{c,d} \times s^{\beta}_{d}$$

⚠ Optimization over all possible P is untractable!

Simplified loss function for the k-hop GNN

(1) extremization over P

Problem (★) with symmetric Lagrangian multipliers H and H' in $R^{N\times N}$

$$\min_P \text{Loss}(P, H, H') = - h(P) - \sum_{\alpha,\beta} (H \odot E_{S\sim P}[sAs^T - oAo^T] + H' \odot E_{S\sim P}[ss^T - oo^T])_{\alpha,\beta}$$

$$= G(o;H,H')$$

Problem (1) $\max_{H,H'} \text{Extr}(H, H') = \log(Z) - \sum_{\alpha,\beta} (H \odot (oAo^T) + H' \odot (oo^T))_{\alpha,\beta}$

where $Z = \int_s \exp(G(s;H,H'))ds$

No more optimization on P!

Theorem 1 (1) is equivalent to (★)

Simplified loss function for the k-hop GNN

(1) extremization over P

Problem (★) with symmetric Lagrangian multipliers H and H' in $R^{N\times N}$

$$\min_P \text{Loss}(P, H, H') = - h(P) - \sum_{\alpha,\beta} (H \odot E_{S\sim P}[sAs^T - oAo^T] + H' \odot E_{S\sim P}[ss^T - oo^T])_{\alpha,\beta}$$

$$= G(o;H,H')$$

Problem (1) $\max_{H,H'} \text{Extr}(H, H') = \log(Z) - \sum_{\alpha,\beta} (H \odot (oAo^T) + H' \odot (oo^T))_{\alpha,\beta}$

where $Z = \int_s \exp(G(s;H,H'))ds$

No more optimization on P!

Theorem 1 (1) is equivalent to (★)

Proof Under the Karush–Kuhn–Tucker (KKT) conditions,
(P*, H*, H'*) is a saddle point of Loss with respect to P ⇔ P* is an optimal point for (★)

Then P* satisfies $\nabla_P \text{Loss}(P, H, H') = 0 \Rightarrow P^*(s) = \exp(G(s;H,H'))/Z$ (stationarity condition)
Since H*, H'* ≠ 0, $E_{S\sim P}[sAs^T] = oAo^T$ and $E_{S\sim P}[ss^T] = oo^T$ (primal feasibility)

⇒ Loss(P*, H*, H'*) = Extr(H*, H'*)

Then $\min_{H,H'} \text{Extr}(H, H')$ is the dual problem of (★) and (H*,H'*) are dual-optimal

Simplified loss function for the k-hop GNN

(1) extremization over P + **(2)** mean-field theory approx.

Problem (★) with symmetric Lagrangian multipliers H and H' in $\mathbb{R}^{N \times N}$

$$\min_P \text{Loss}(P, H, H') = - h(P) - \sum_{\alpha,\beta} (H \odot E_{S\sim P}[sAs^T - oAo^T] + H' \odot E_{S\sim P}[ss^T - oo^T])_{\alpha,\beta}$$

$$= G(o;H,H')$$

Problem (1) $\max_{H,H'} \text{Extr}(H, H') = \log(Z) - \sum_{\alpha,\beta} (H \odot (oAo^T) + H' \odot (oo^T))_{\alpha,\beta}$

where $Z = \int_s \exp(G(s;H,H'))ds$

⍰ Computation of Z unfeasible

Problem (2) $\max_{H,H'} \text{ExtrMFT}(H, H') = \log(Z') - G(o;H,H')$

where $Z \approx Z' = \int_s \exp(G'(s;H,H'))ds$ and $G'(s;H,H') = O(s^\alpha_c)$

MFT approximation for all gene α and spot c, $s^\alpha_c = m^\alpha + \delta s^\alpha_c$, where $\delta s^\alpha_c \ll m^\alpha$

average expression of gene α across spots

Z' can be turned into a simple closed-form expression

Identifiability of the Lagrange multipliers

i.e., the intra/cross-spot gene-gene interaction matrices

Problem (★) with symmetric Lagrangian multipliers H and H' in $R^{N \times N}$

$$\min_P \text{Loss}(P, H, H') = - h(P) - \sum_{\alpha,\beta} (H \odot E_{S\sim P}[sAs^T - oAo^T] + H' \odot E_{S\sim P}[ss^T - oo^T])_{\alpha,\beta}$$

$$= G(o;H,H')$$

Problem (1) $\max_{H,H'} \text{Extr}(H, H') = \log(Z) - \sum_{\alpha,\beta} (H \odot (oAo^T) + H' \odot (oo^T))_{\alpha,\beta}$

where $Z = \int_s \exp(G(s;H,H'))ds$

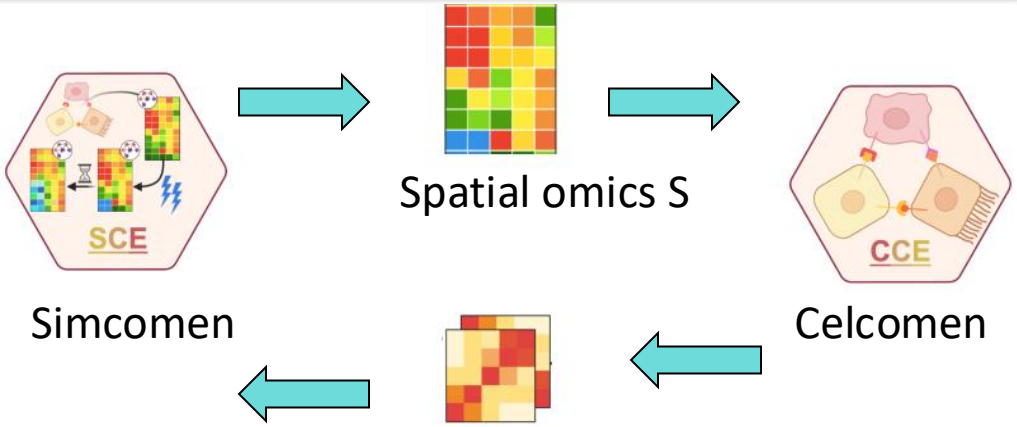
Theorem 2 If Extr(H, H') = Extr(K, K'), then H=K and H'=K'

Proof Extr is quadratic in o (the observed spatial omics matrix)
For each pair of genes (α,β),

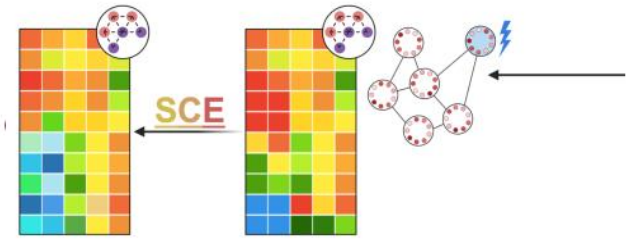
- taking the second derivative with respect to spot o_i leads to $(H')_{\alpha,\beta}=(K')_{\alpha,\beta}$
- taking the second derivative with respect to spot o_i and neighboring o_j leads to $H_{\alpha,\beta}=K_{\alpha,\beta}$

Synthesis of spatial omics with Simcomen Generate spatial omics matrices according to learned P(S)

Training Adversarial process between Celcomen and Simcomen

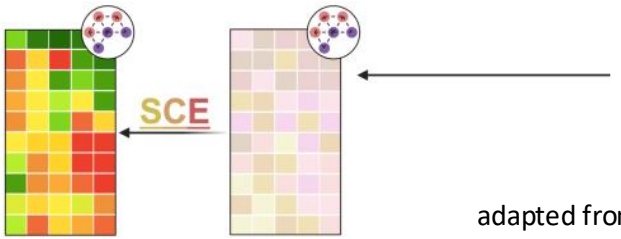


Counterfactual perturbations
Generate the most likely S



Apply KO on a gene in a spot

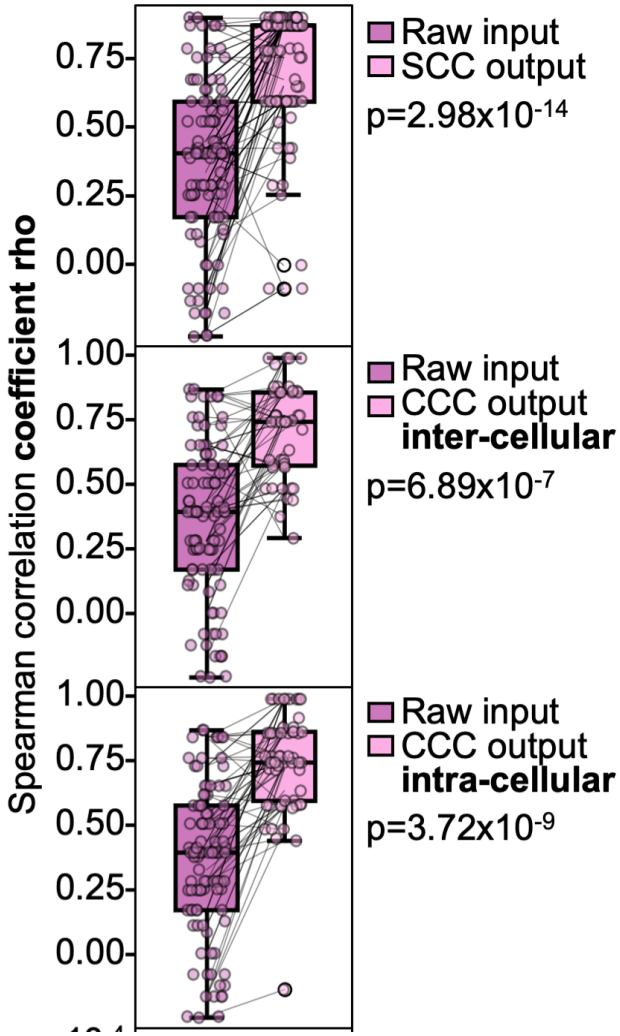
Synthetic data generation



Start from random init

adapted from the paper

Experimental results Identifiability on synthetic data, disentanglement on real-life data, counterfactual KO



[vec(H), vec(H')]
VS [vec(H*), vec(H'*)]

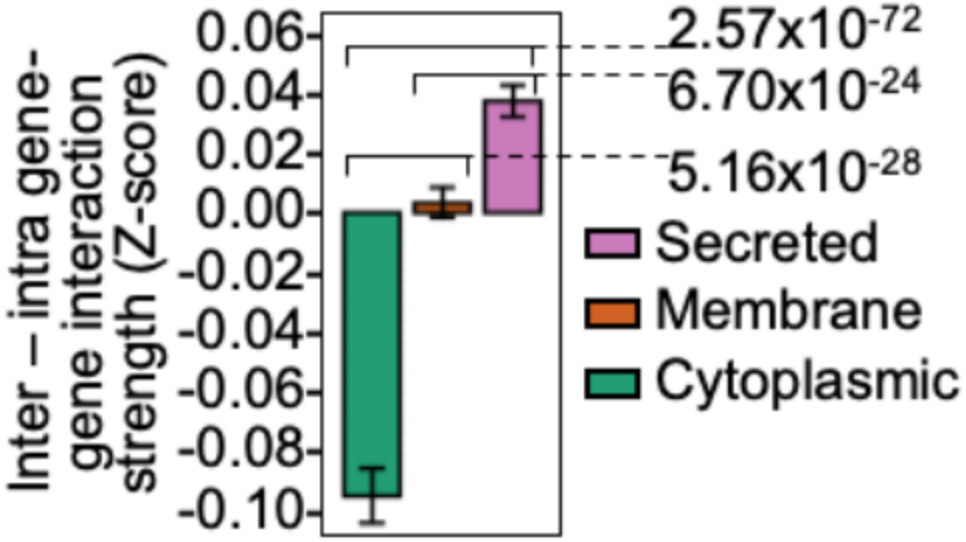
vec(H) VS vec(H*)

vec(H') VS vec(H'*)

Identifiability on synthetic data

Generate H*, H'* at random (N=4)
S ← Simcomen(H*, H'*)
H, H' ← Celcomen(S)

Experimental results Identifiability on synthetic data, disentanglement on real-life data, counterfactual KO

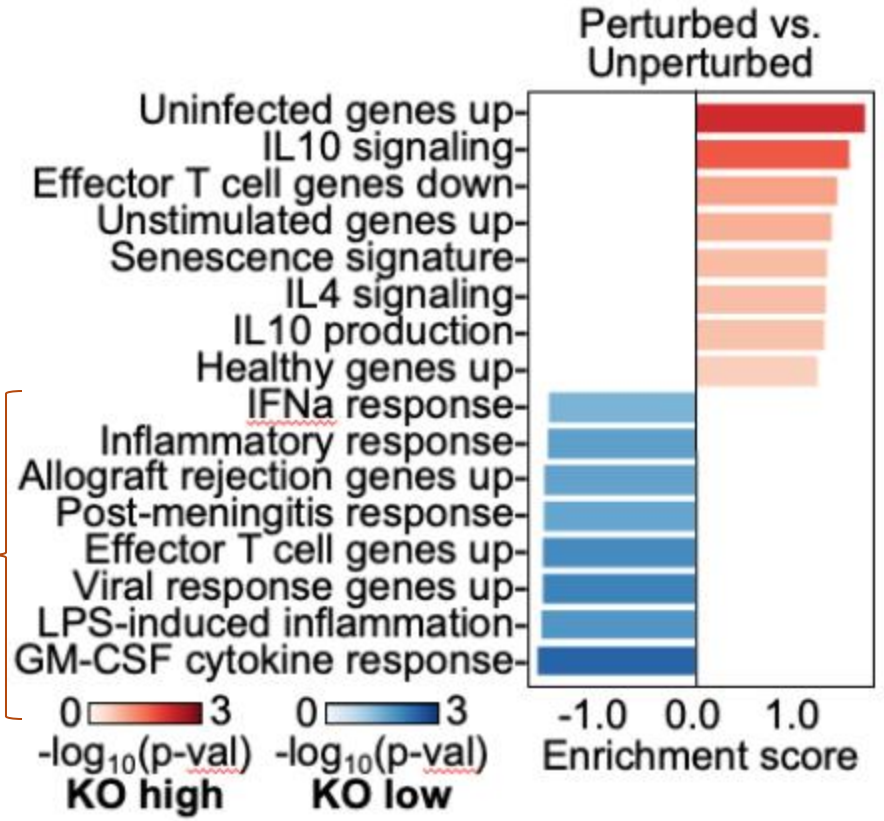


Disentanglement on real-life data

H, H' ← Celcomen(O) observed data
Compare for each pair of genes the relative intra/cross-spot score

< 0 : mostly active inside a spot
> 0 : mostly active across spots

Experimental results Identifiability on synthetic data, disentanglement on real-life data, counterfactual KO



Immune response related genes are downregulated in KO spot (+ neighbors)

Counterfactual KO

H, H' ← Celcomen(O) observed data
S_{KO} ← Simcomen(H, H' + interferon KO)
scores ← DiffAnalysis(S_{KO}, O)
Enrichments ← GSEA(scores)

Enrichment = group of genes
Enrichment score = strength
> 0 : very upregulated in KO
< 0 : very downregulated in KO

Intro to GSEA: https://recess-eu-project.github.io/flash%20lecture/pathway-enrichment-2/

Perspectives

1. Comments on the paper
2. Why is it interesting for BioComp?

My comments on the paper

Strengths:
- The framework is elegant (once you've understood it)
- Lots of meaningful experiments on real-life data (including on Perturb-Map)
- Open-source: https://github.com/Teichlab/celcomen/

Weaknesses:
- The math is awful (sorry)
- **(A3)** no batch effect, same cell type, steady state, monotonic undirected interactions
- Results of the experiment on identifiability are not so great (I think)
- No morphological features (but not so easy to integrate I guess)

Your comments?

Why is it interesting for BioComp?

Counterfactual perturbations on spatial omics: Virtual Cells VS Virtual Tissues

Cell
Leading Edge

Perspective

How to build the virtual cell with artificial intelligence: Priorities and opportunities

Charlotte Bunne,[1,2,3,4,50] Yusuf Roohani,[1,3,5,50] Yanay Rosen,[1,3,50] Ankit Gupta,[3,6] Xikun Zha
Theo Alexandrov,[8,9] Mohammed AlQuraishi,[9] Patricia Brennan,[3] Daniel B. Burkhardt,[11] And

[1]Department of Computer Science, Stanford University, Stanford, CA, USA
[2]Genentech, South San Francisco, CA, USA

CellPress
OPEN ACCESS

wellcome connecting science

Our events Abc

Home / Our events / Conferences / AI x BIO

AI x BIO

23–25 June 2025

Wellcome Genome Campus, UK and online

Enter the era of AI in Biology