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Investigating the Tumor Microenvironment in Cancer using Nonnegative Matrix Factorization and Spatial Analysis

Atul Deshpande,
Postdoctoral Fellow,
Fertig Laboratory,
Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University
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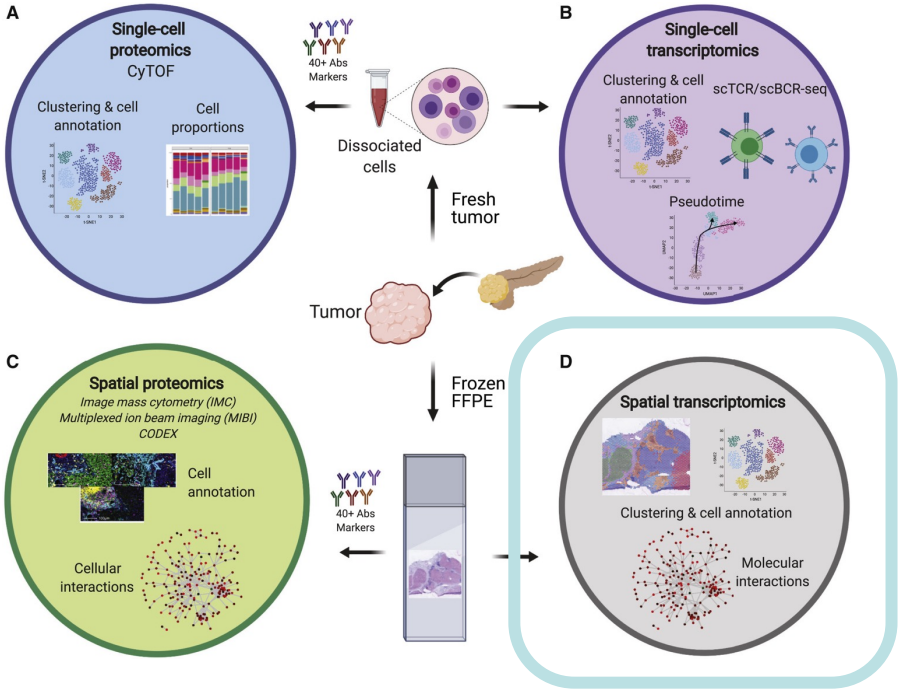
Introduction

- The composition of the tumor microenvironment is a key factor in tumor growth and immunotherapy response
  - immunosuppressors enable immune evasion
- Single-cell and spatial omics technologies capture intra-tumor heterogeneity
- Spatial transcriptomics technologies measure the expression of thousands of genes at near single-cell resolution



Davis-Marcisak et al, 2021, *Cancer Cell*

10X Visium Spatial Transcriptomics

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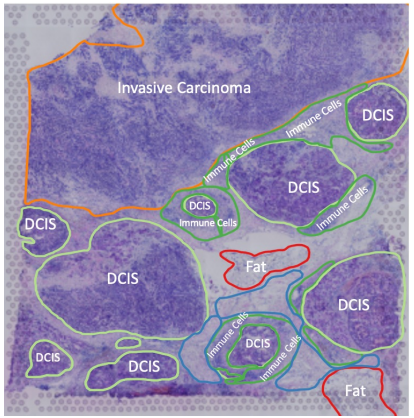
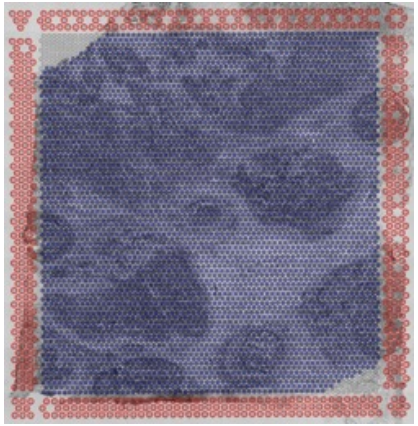
- Example: 10x Visium sample of invasive breast ductal carcinoma
- Retains the spatial context of molecular profiles of cellular neighborhoods
  - Whole transcriptome
    - High dropouts like scRNAseq
  - Spot-based technology (50um dia.)
    - One to ten cell per spot
    - Cell deconvolution

Annotated H & E slide



H & E Slide with overlaid spots



@10x Genomics

10X Visium Spatial Transcriptomics

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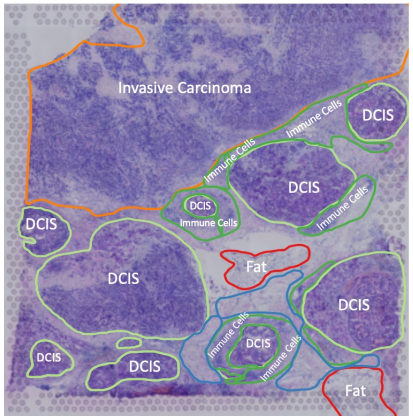
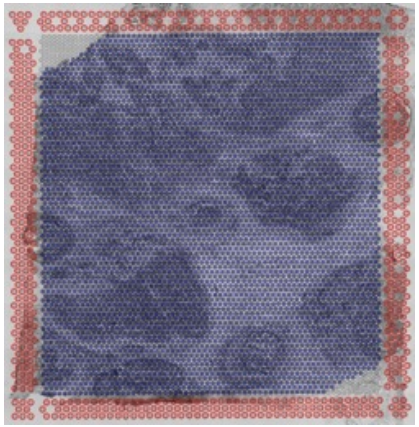
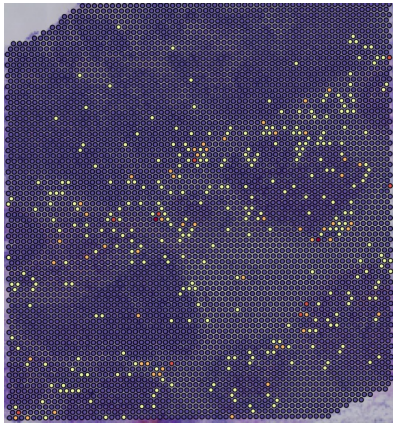
Annotated H & E slide

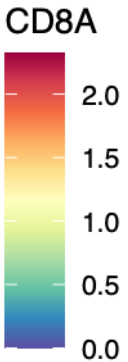


H & E Slide with overlaid spots



CD8A expression

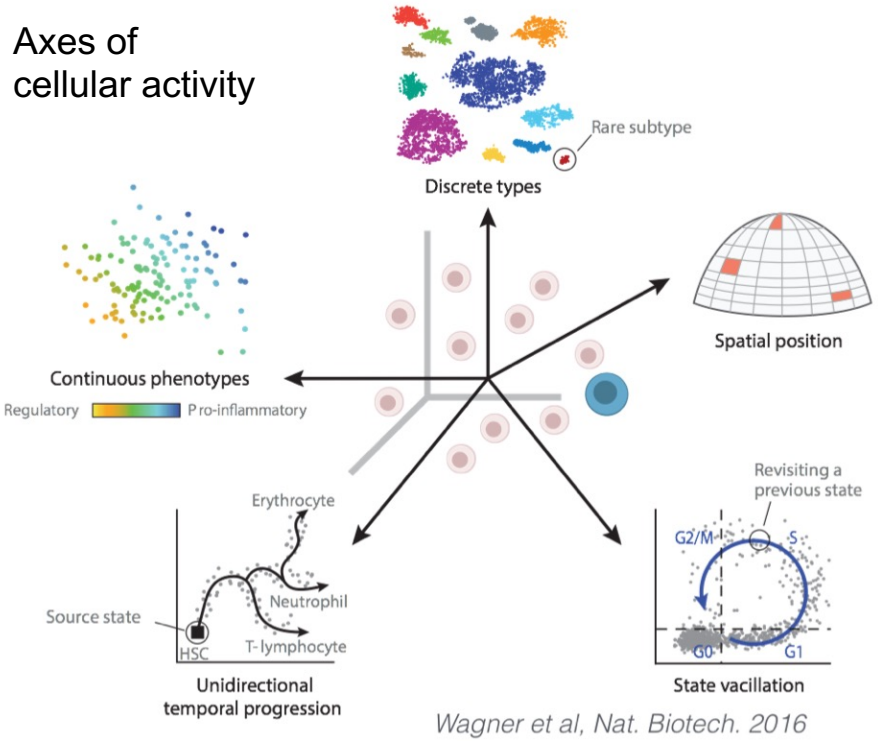




Can we quantify the intra-tumor heterogeneity in terms of underlying biological factors?

Quantifying the underlying biological factors using matrix factorization

Axes of cellular activity



Wagner et al, Nat. Biotech. 2016

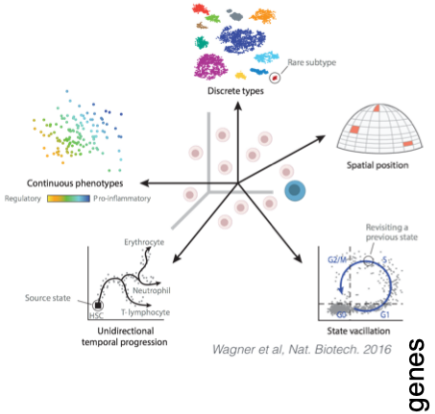
Cell's molecular profile affected by multiple biological factors

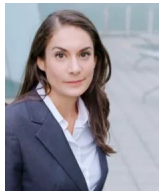
Multiple cells contribute to a spot's molecular profile

Factorization methods reveal latent features associated with biological factors

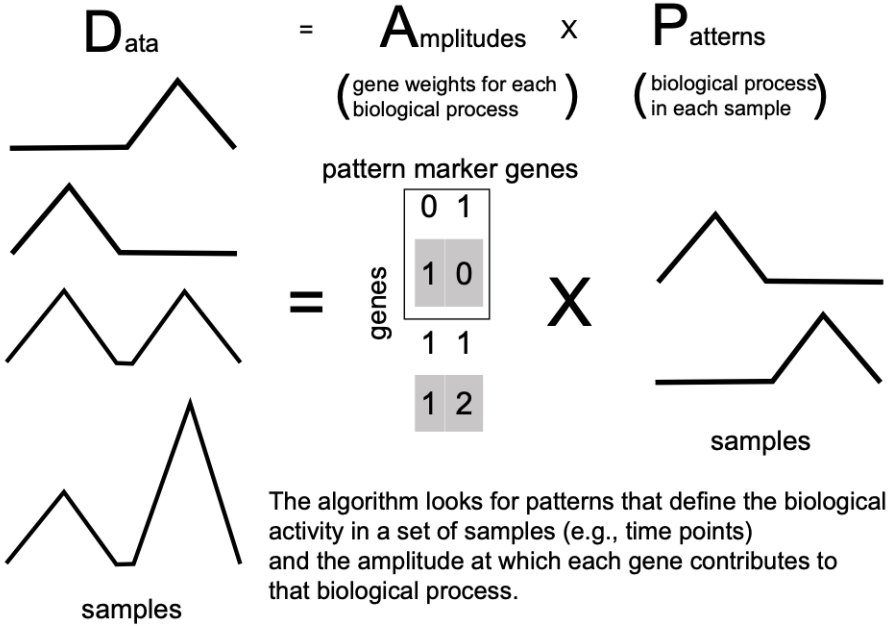
They are also used to deconvolve a spot into its constituent cell types

CoGAPS (Coordinated Gene Activity in Pattern Sets) Nonnegative Matrix Factorization



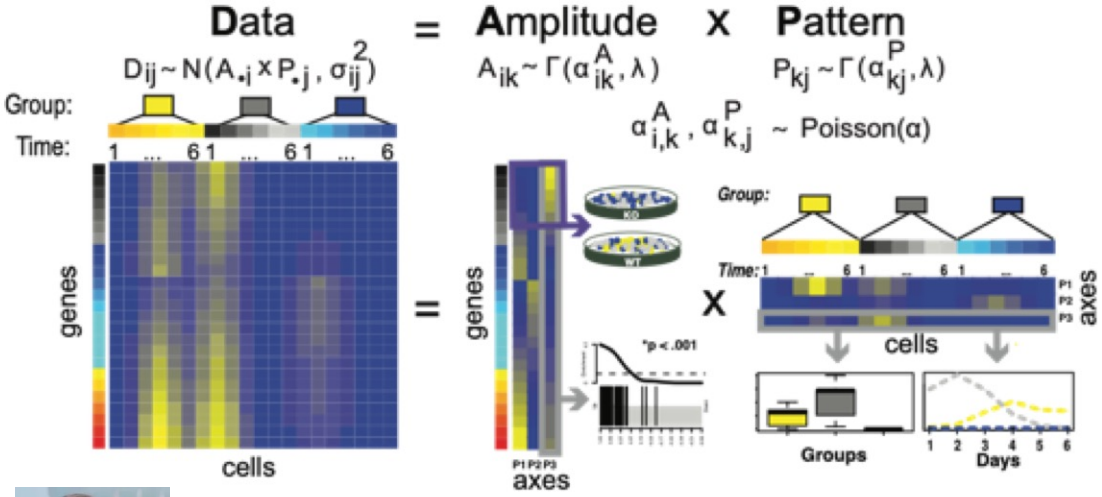


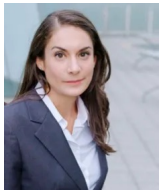
Genevieve Stein-O'Brien



The algorithm looks for patterns that define the biological activity in a set of samples (e.g., time points) and the amplitude at which each gene contributes to that biological process.

CoGAPS as a Bayesian sparse nonnegative matrix factorization for genomics data



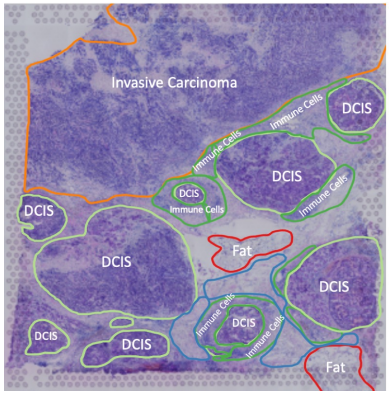


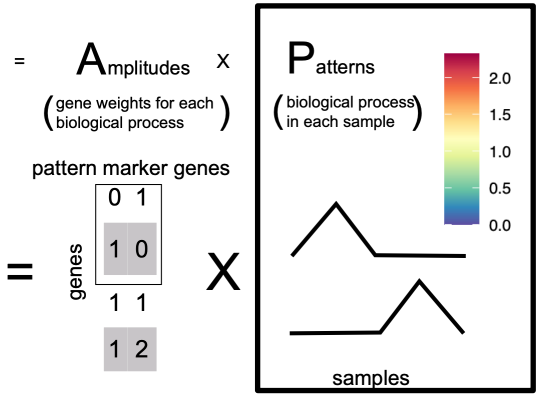
Genevieve Stein-O'Brien

Bioconductor Package CoGAPS
Seurat and GenePattern Workflows

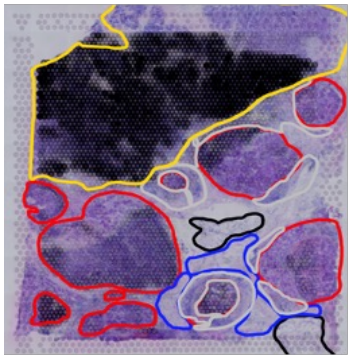
Fertig et al *Bioinformatics* 2010;
Stein-O'Brien et al *Bioinformatics* 2017;
Stein-O'Brien et al *Cell Systems* 2019;
Sherman et al *BMC Bioinformatics* 2020

Unsupervised CoGAPS matrix factorization mirrors pathology annotations

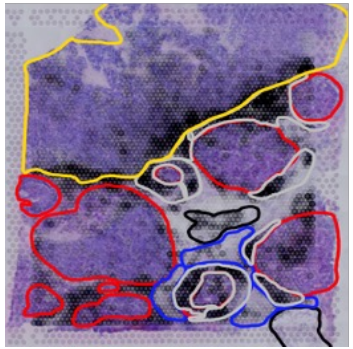




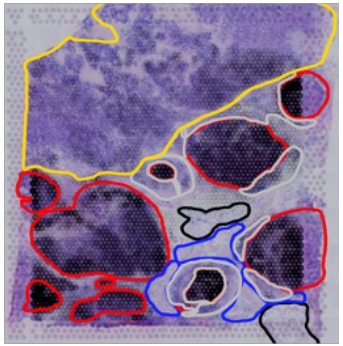
Tumor pattern

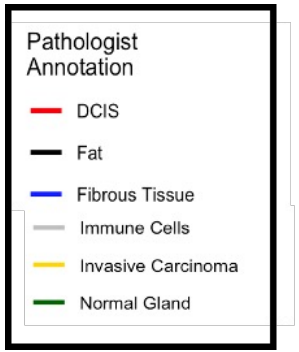


Immune pattern



DCIS pattern





Melanie Loth

(Deshpande, Loth, et. al. 2022, biorxiv)

Higher dimensional CoGAPS analysis leads to further resolution of the heterogeneity

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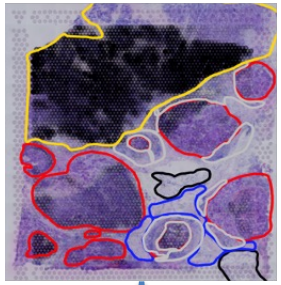
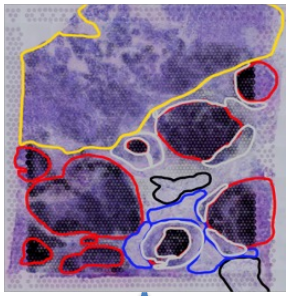
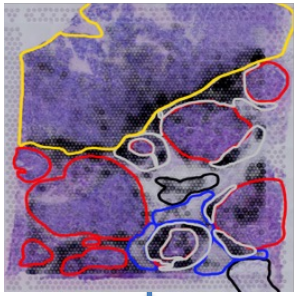
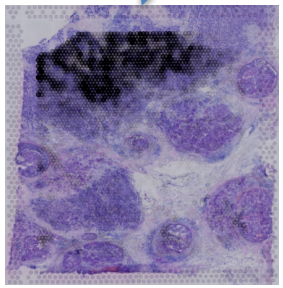
Low (5)

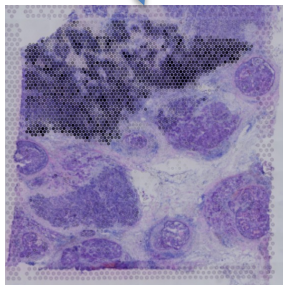
High (16)

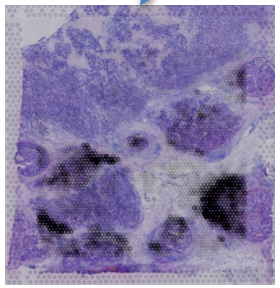

Invasive Carcinoma pattern

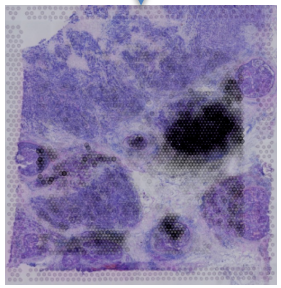

DCIS pattern

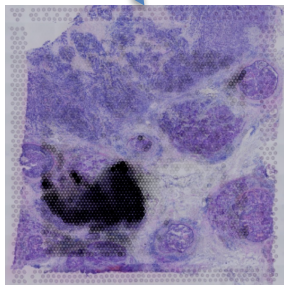

Immune pattern

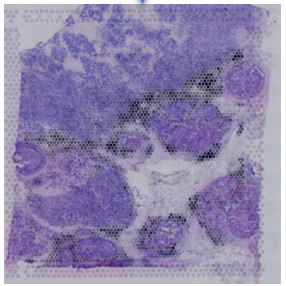

Invasive.1


Invasive.2

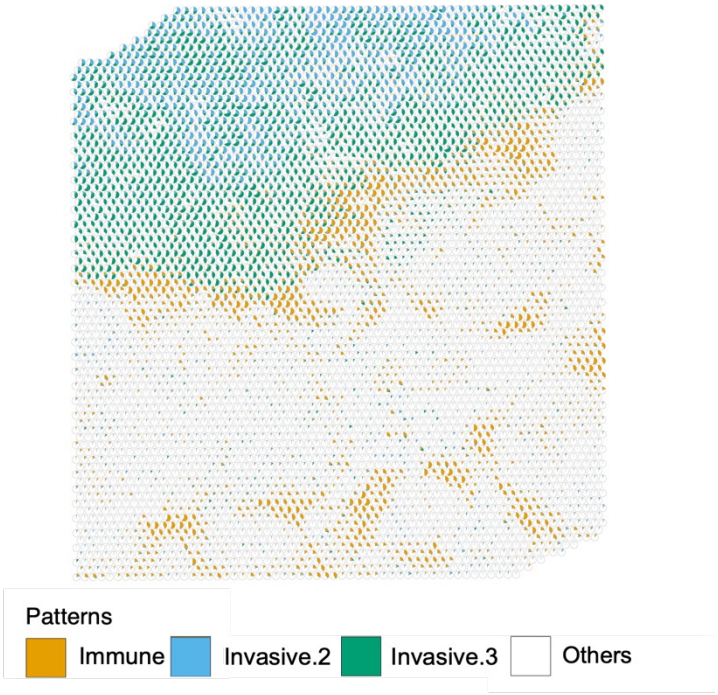

DCIS.1

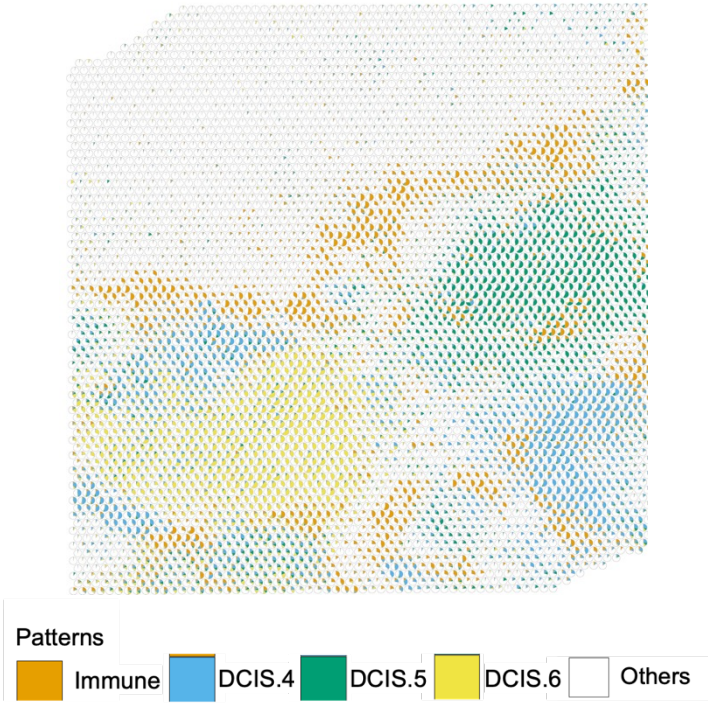

DCIS.2


DCIS.3

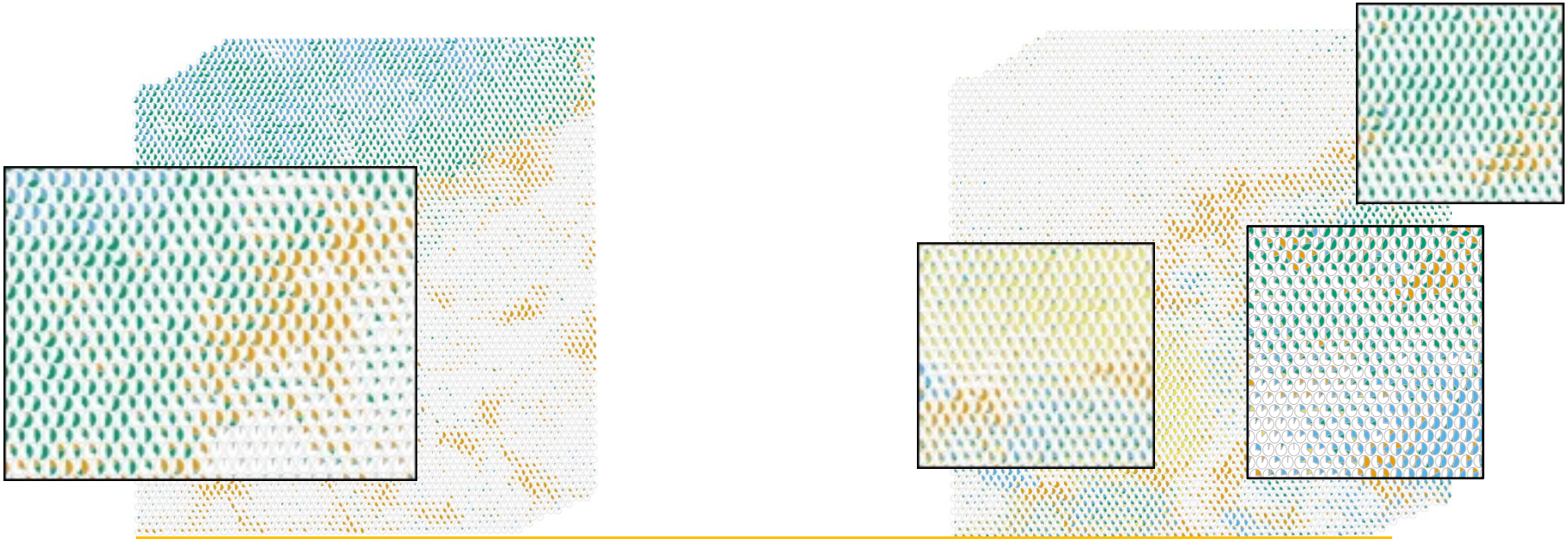

Immune

Unsupervised CoGAPS analysis also reveals spatially overlapping features





Unsupervised CoGAPS analysis also reveals spatially overlapping features



Latent features interact in regions of spatial overlap

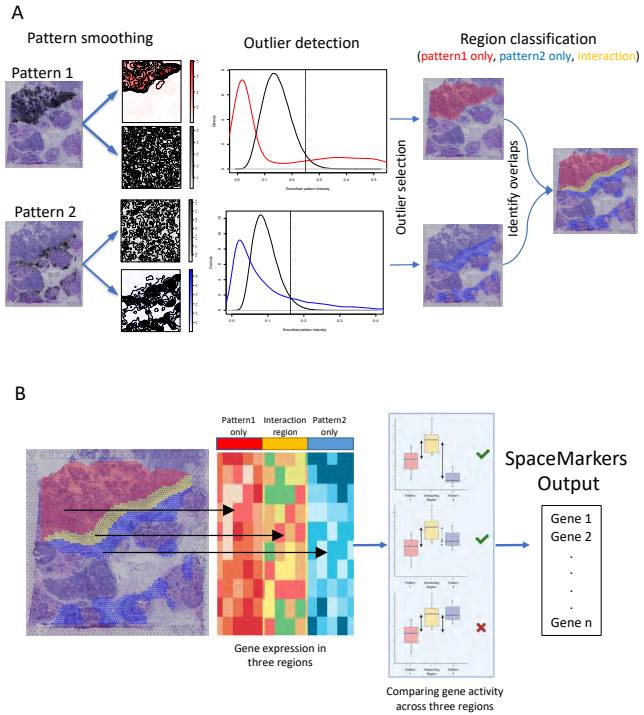
Can we identify markers associated with spatially interacting features?

SpaceMarkers identifies pathways altered from tumor and immune interactions

CoGAPS reveals spatially overlapping patterns

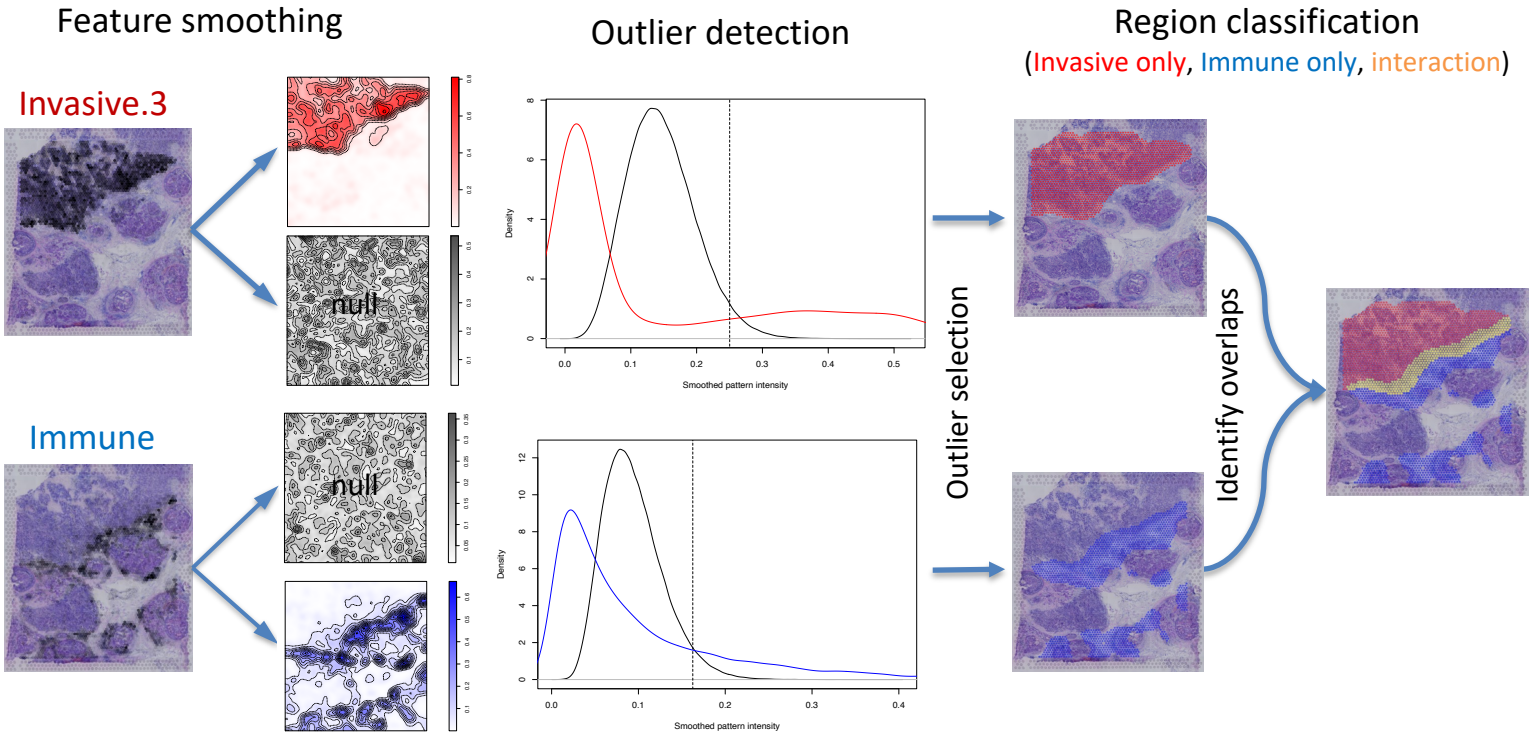
1. The interaction region of two patterns defined as spots with overlapping influence from both

2. Identify genes associated with spatial pattern interaction



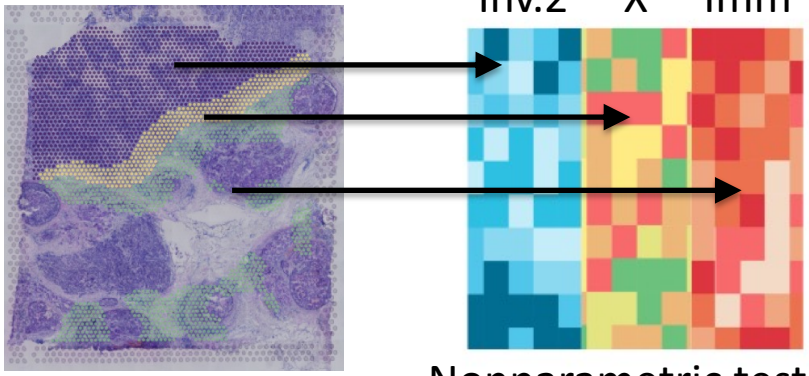
(Deshpande, Loth, et. al. 2022, biorxiv)

SpaceMarkers identifies interaction region between two spatially overlapping latent features



2. SpaceMarkers detects molecular changes associated with pattern interactions

Invasive.2 X Immune



Nonparametric test

Original CoGAPS model

$$D \sim AP + N(0, \Sigma)$$

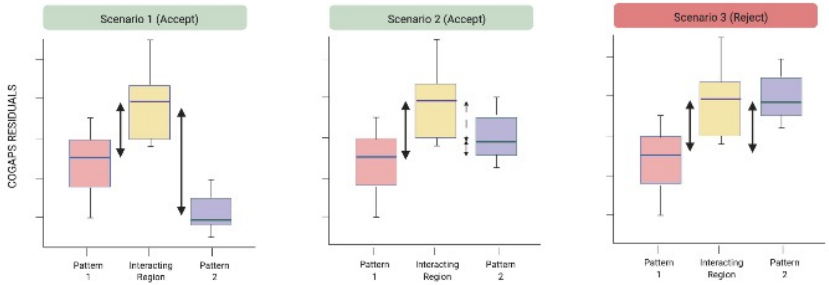
Updated model

$$D \sim AP + f(A, P) + N(0, \Sigma)$$

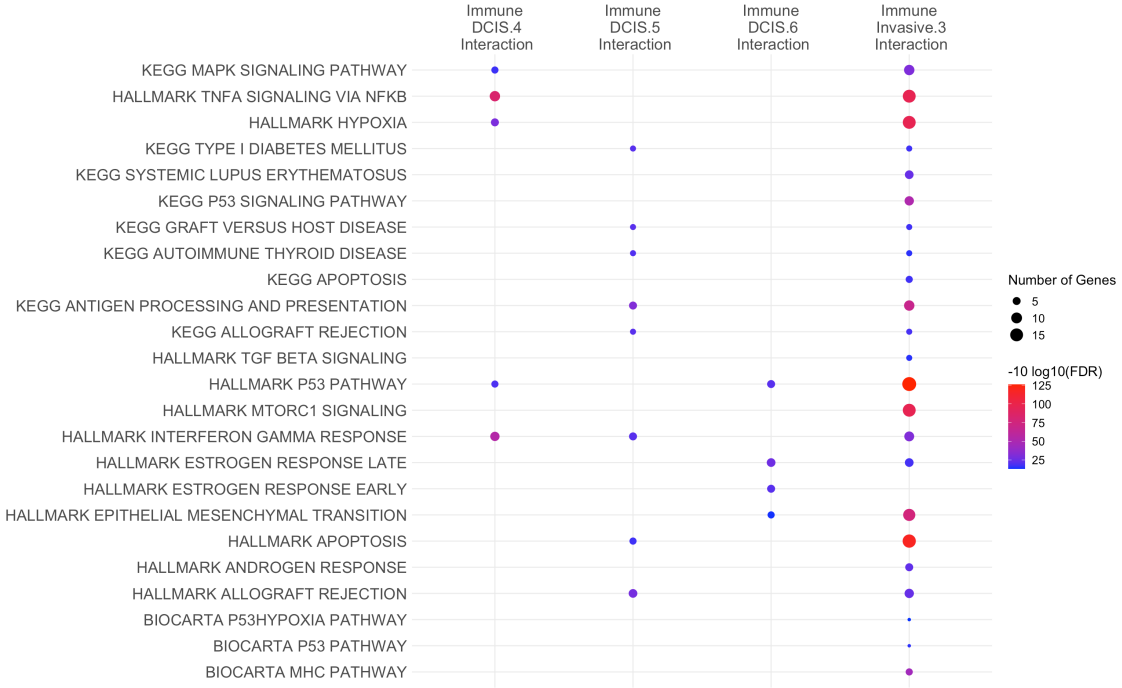
residuals

a. Differential expression

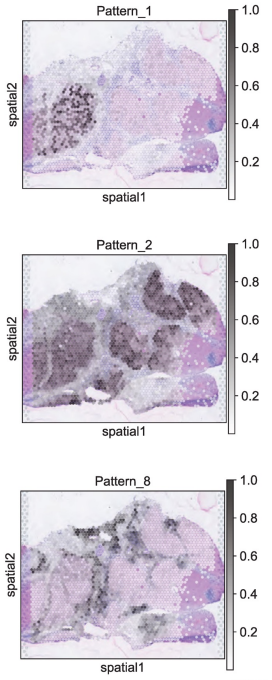
- confounding factors



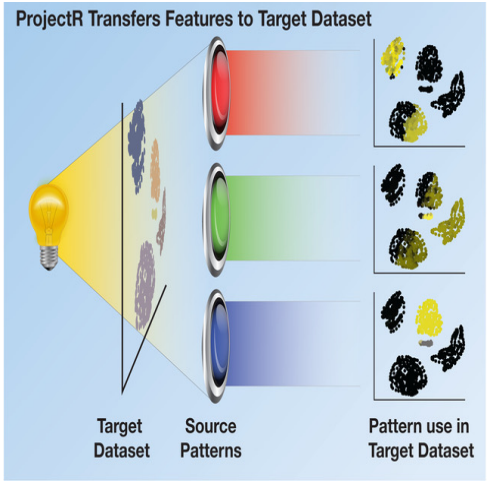
SpaceMarkers output helps identify pathways altered from tumor and immune interactions



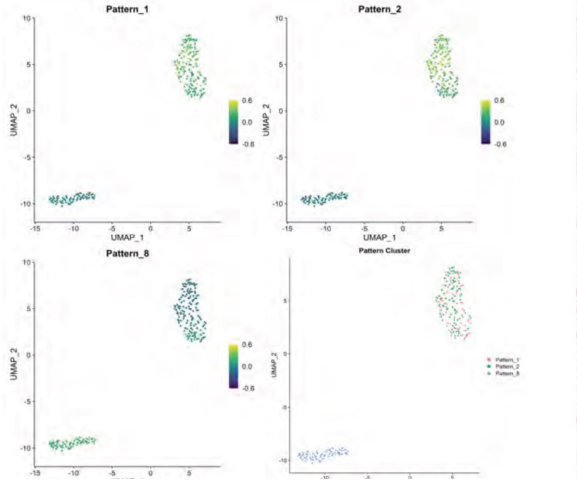
Associating cells in matched scRNAseq data using transfer learning and SpaceMarkers

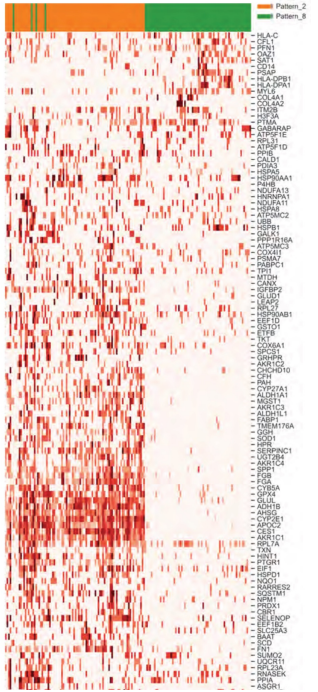


ProjectR (Stein-O'Brien 2019)



Matched scRNAseq





SpaceMarkers annotations

Conclusion

- CoGAPS nonnegative matrix factorization can reveal underlying biological features or cell populations in spatial data.
- SpaceMarkers identifies markers of intercellular interaction associated with spatially overlapping latent features.
- SpaceMarkers output is dependent on the latent space method.
- Applied SpaceMarkers with features learned from CoGAPS and STdeconvolve.

Availability

- Preprint of manuscript available on biorxiv (https://www.biorxiv.org/content/10.1101/2022.06.02.490672v1)
- SpaceMarkers R package available at https://github.com/FertigLab/SpaceMarkers

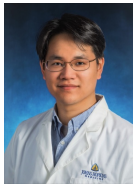
Future Work

- Apply SpaceMarkers with more latent space methods
- Extend the algorithm to more than two overlapping patterns

Acknowledgements

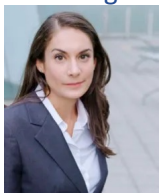
Elana Fertig

Melanie Loth



Won Jin Ho

Luciane Kagohara



Genevieve Stein-O'Brien

Johns Hopkins

Robert Anders
Ana Cordova
Ludmila Danilova
Alexander Favorov
Melanie Loth
Elizabeth Jaffee
Cesar Santa-Maria
Daniele Gilkes
Qingfeng Zhu
Aleksander Popel
Mark Yarchoan
Lei Zheng

Michael Ochs (TCNJ)

10X Genomics

Courtney Anderson
Nikhil Rao
Jennifer Chew
Cedric Uytingco
Stephen Williams
Andrej Hartnett
Zachary W. Bent