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USC Office of Research and Innovation  
USC Kidney Research Center  
USC Center for Translational Exposomics Research

AIMS@USC presents:

# The Spatial Summit

**Bringing together** investigators to explore cutting-edge spatial technologies and analysis strategies.

**AGENDA**

- 2:30 p.m. Introduction to AIMS@USC
- 3:00 p.m. Spatial Technologies at USC
- 3:30 p.m. Bioinformatic Analysis

March 18<sup>th</sup>  
2:30 p.m. – 4:00 p.m.

University of Southern California  
Health Science Campus

**Mudd Memorial Building: Room 608**



Register Here



Contact: Jonathan Nelson at [j.nelson@med.usc.edu](mailto:j.nelson@med.usc.edu)

# Welcome Everyone!

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# Agenda

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- **Introduction and Vision for AIMS@USC** – Overview of the initiative, its goals, and how it aims to support the USC spatial research community.
- **Answering Submitted Questions** – Addressing questions and topics that participants shared ahead of the summit.
- **Advanced Cell Segmentation Approaches** – Discussion of emerging computational strategies for cell segmentation in spatial data, including **Baysor** (PMID: 34650268) and **Ficture** (PMID: 39266749)
- **Survey and Open Q&A** – An opportunity for feedback, discussion, and shaping future AIMS activities.

# Today...Spatial Transcriptomics! Tomorrow...

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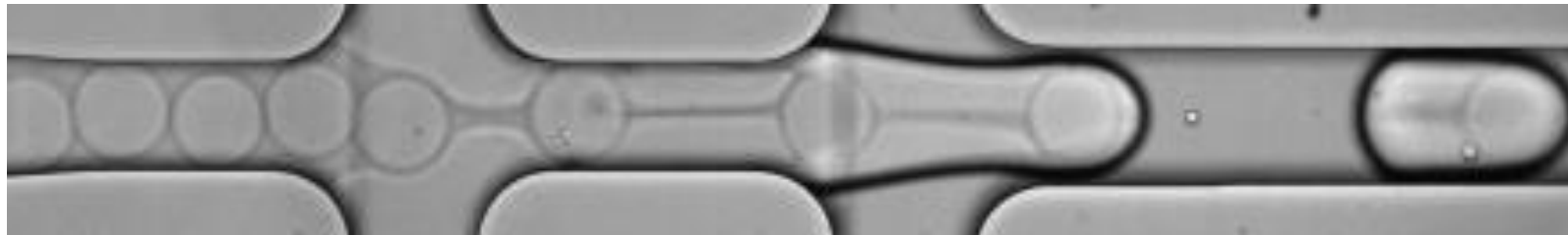
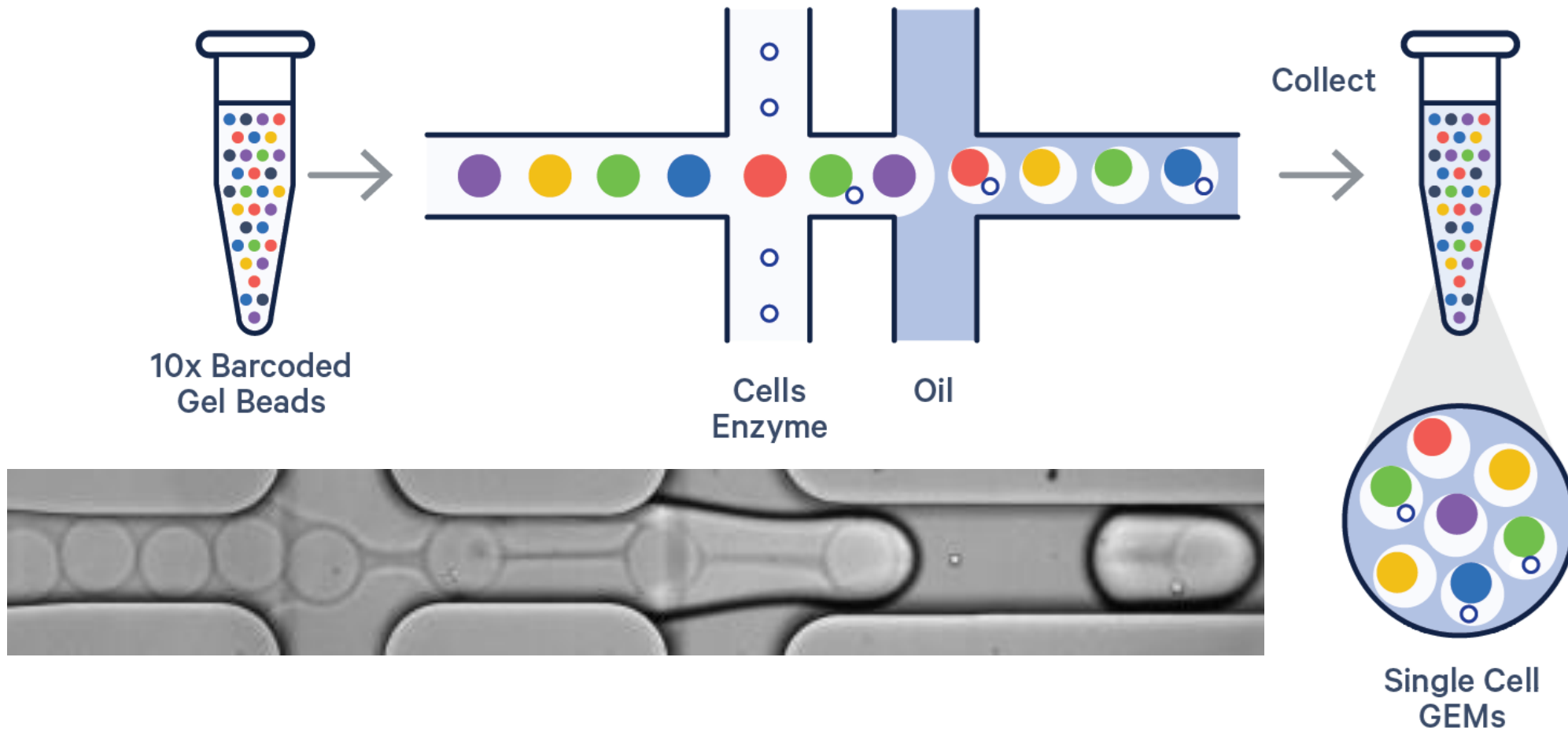
- ...Proteomics
- ...Metabolomics
- ...Genomics
  
- We hope to incorporate other data modalities in the future.
  
- Many of the same challenges and computational approaches will apply to all these kinds of dataset.

# Perspective

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# Dissociated Single Nucleus RNAsequencing



# Studying the Kidney

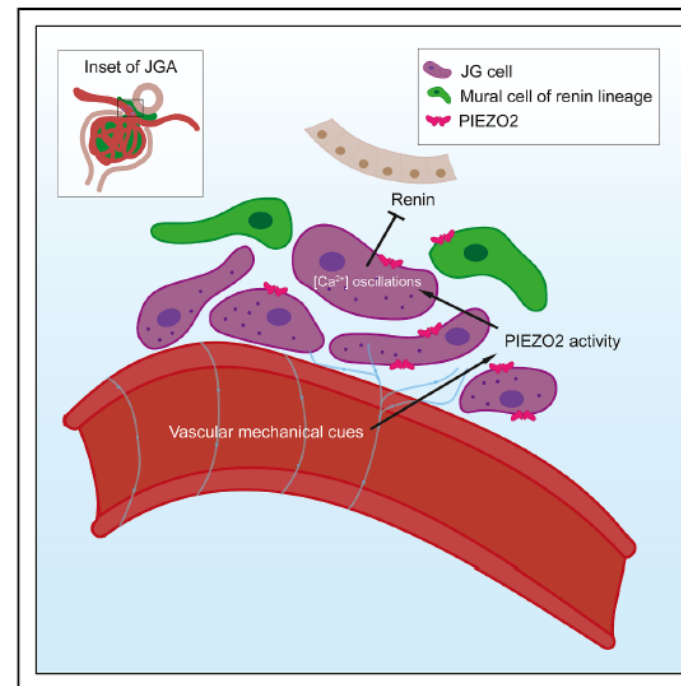


Cell

Article

## Renal PIEZO2 is an essential regulator of renin

Graphical abstract



Authors

Rose Z. Hill, Jonathan W. Nelson, Georgina Gyarmati, ..., Jeffrey H. Miner, Janos Peti-Peterdi, Ardem Patapoutian

Correspondence

hillros@ohsu.edu (R.Z.H.),  
ardem@scripps.edu (A.P.)

In brief

The force-sensing ion channel PIEZO2 is an essential regulator of the renin-angiotensin-aldosterone system (RAAS), a pathway that controls blood volume. Loss of PIEZO2 in the kidney cells that produce renin disrupts cellular calcium dynamics and levels of the enzyme in mice, linking mechanotransduction in the kidney to the RAAS.

# \*Monthly\* Bioinformatics Club

## USC Single-Cell Omics Research and Education About

**University of Southern California Single-Cell Omics Research and Education (USC-SCORE)** is dedicated to driving excellence in the field of single-cell omics. We focus on applying and developing advanced methodologies to explore gene expression, epigenetics, proteomics, and spatial biology at the single-cell resolution. Our community is committed to training the next generation of scientists through workshops, seminars, and collaborative projects. We strive to uncover new insights into cellular diversity and its implications for personalized medicine and therapeutic innovation.

We meet on the **last Monday of the month from 4pm-5pm PST**.

Organizers: Jonathan Nelson ([jnelson4@usc.edu](mailto:jnelson4@usc.edu))

## Email Signup

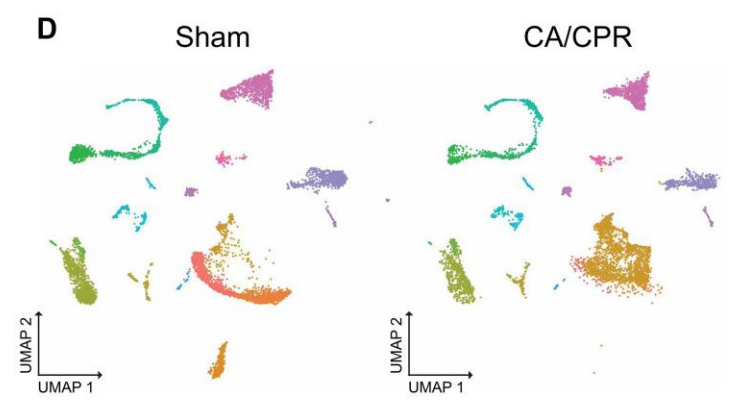
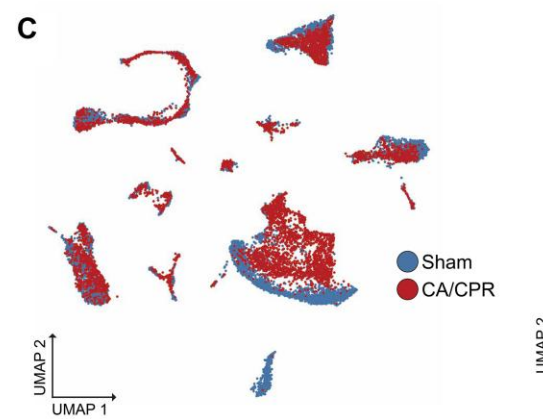
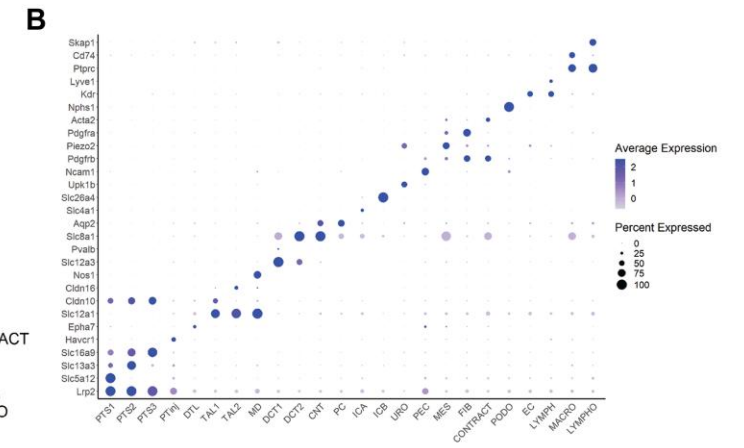
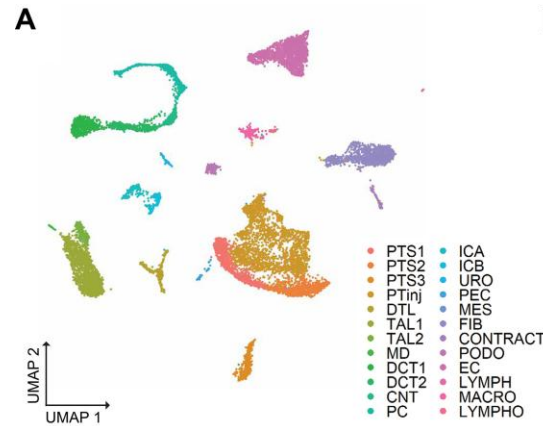
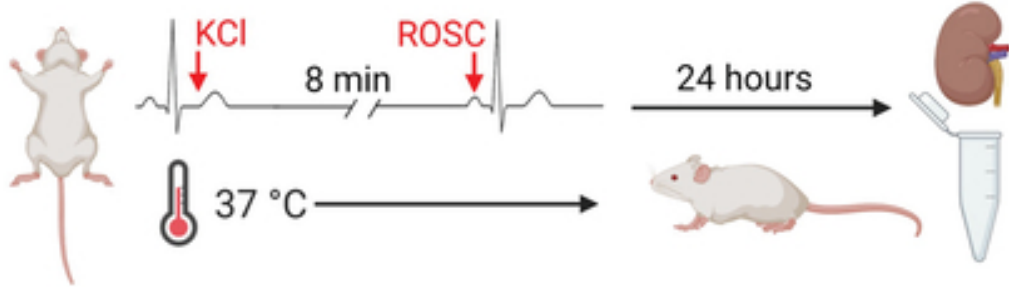
Click [here](#) to join USC-SCORE and receive emails with the zoom link to attend.

## Upcoming Presentation Topics:

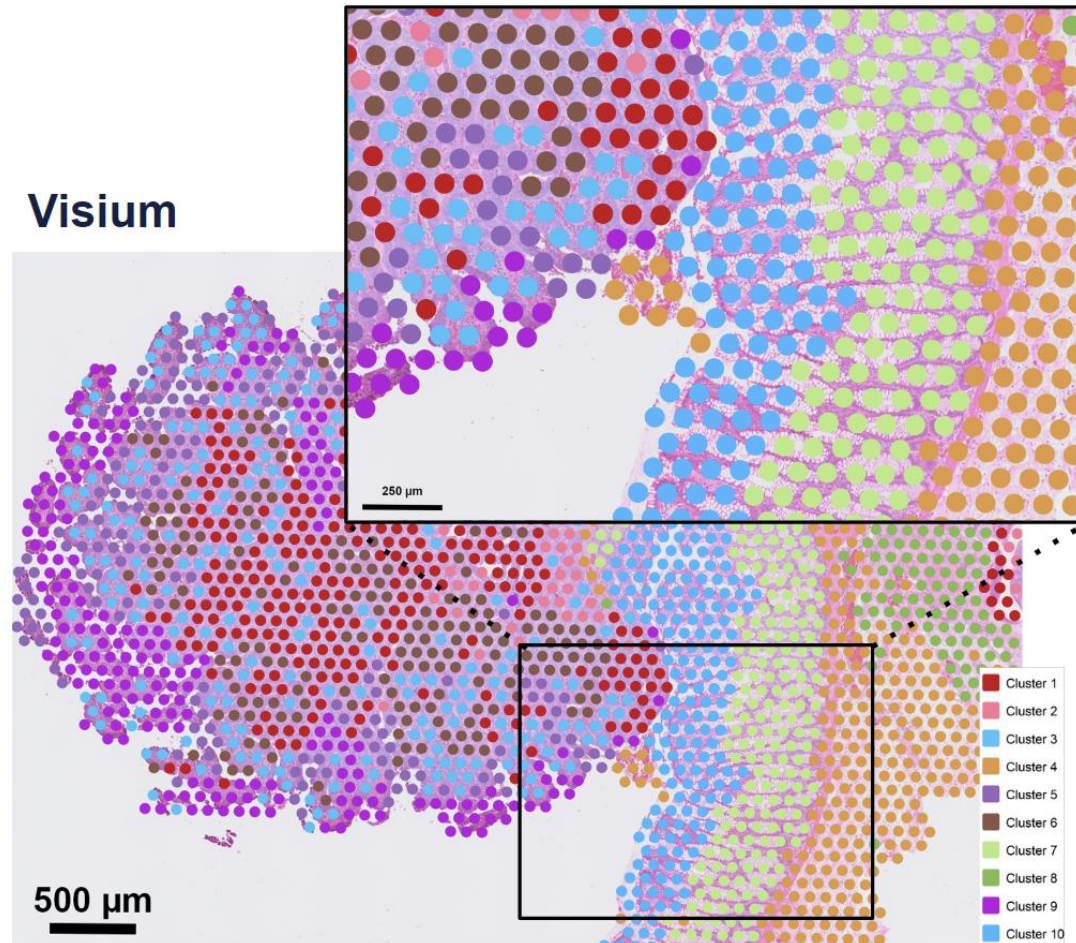
Date	Topic	Presenter	Materials
January 27th	Updates to Seurat V5	Jonathan Nelson	<a href="#">SCORE #1</a>
February 24th	Advances in scRNASeq Technologies	Jeremiah Reyes	<a href="#">SCORE #2</a>
March 31st	Best Practices for Reporting Data (GitHub/GEO)	Jessica Bahena-Lopez	<a href="#">SCORE #3</a>
April 28th	Cell-Cell Interactions with CellChat	Kevin Burfeind	<a href="#">SCORE #4</a>
June 30th	Access to KPMP Datasets	Xiao-Tong Su	<a href="#">SCORE #5</a>
August 25th	Spatial Transcriptomic Analysis	Lu Zhang	<a href="#">SCORE #6</a>
September 29th	Getting Started with Xenium	Jonathan Nelson	<a href="#">SCORE #7</a>
November 24th	Annotating a Xenium Dataset	Jonathan Nelson	<a href="#">SCORE #8</a>
February 23rd	Putting the "Spatial" in Spatial Analysis	Jonathan Nelson	



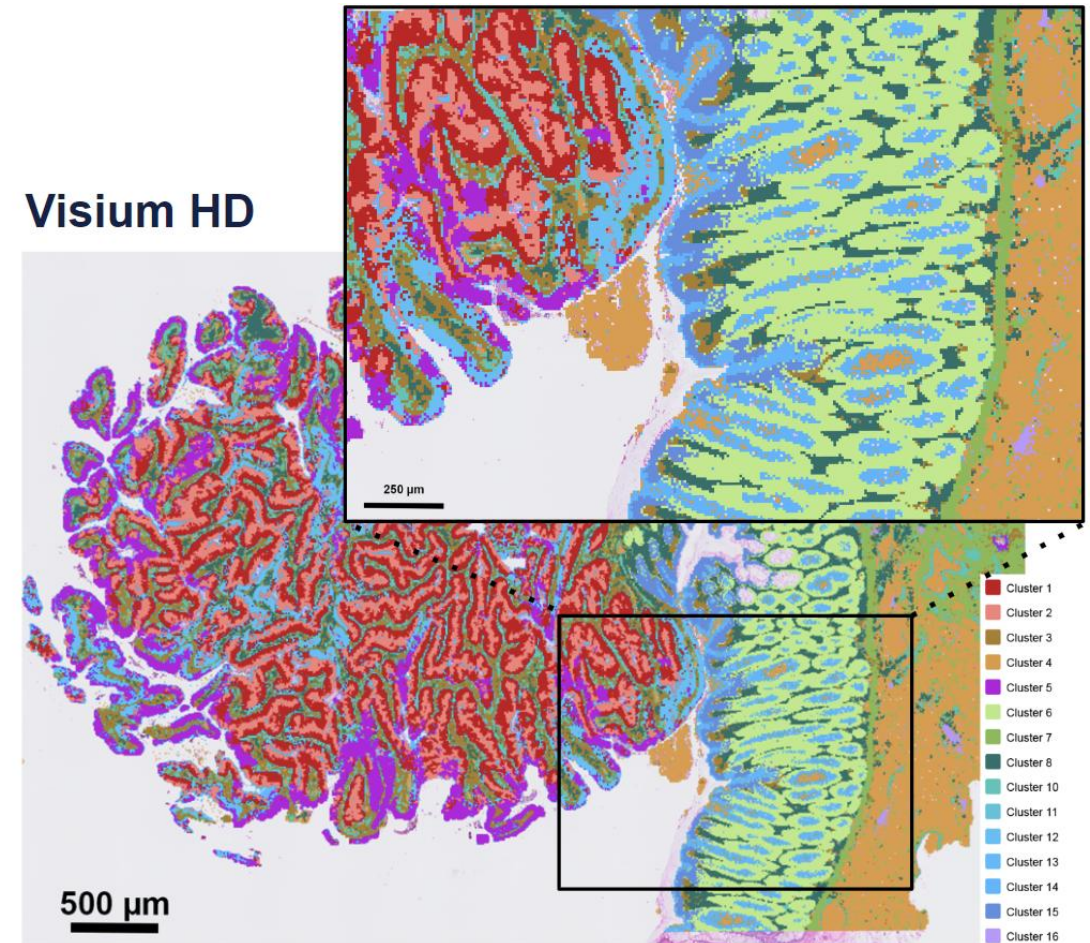
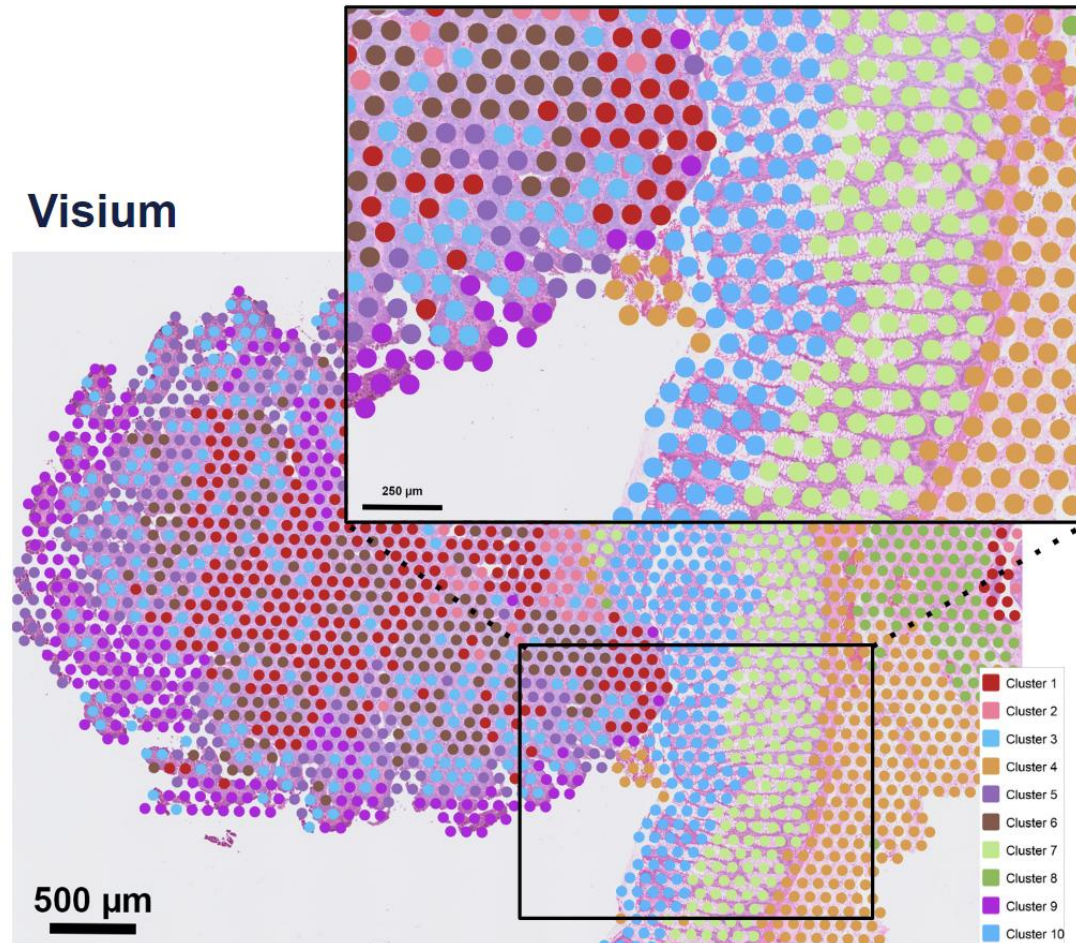
# Missing Context From Dissociation



# Unsatisfied With Early Iterations of Spatial Transcriptomics

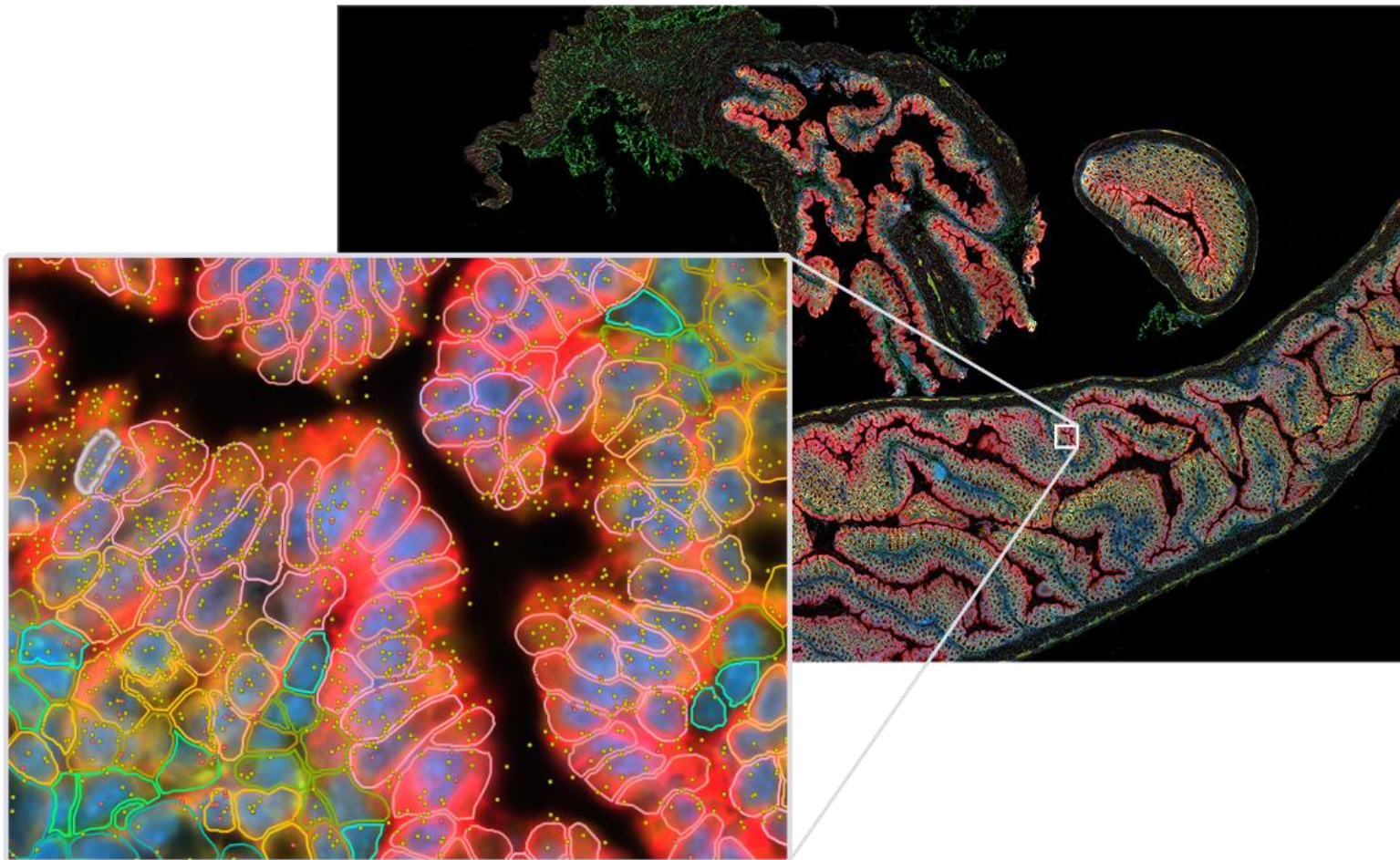


# Unsatisfied With Early Iterations of Spatial Transcriptomics



# Impressed with Xenium Resolution

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INTERNAL

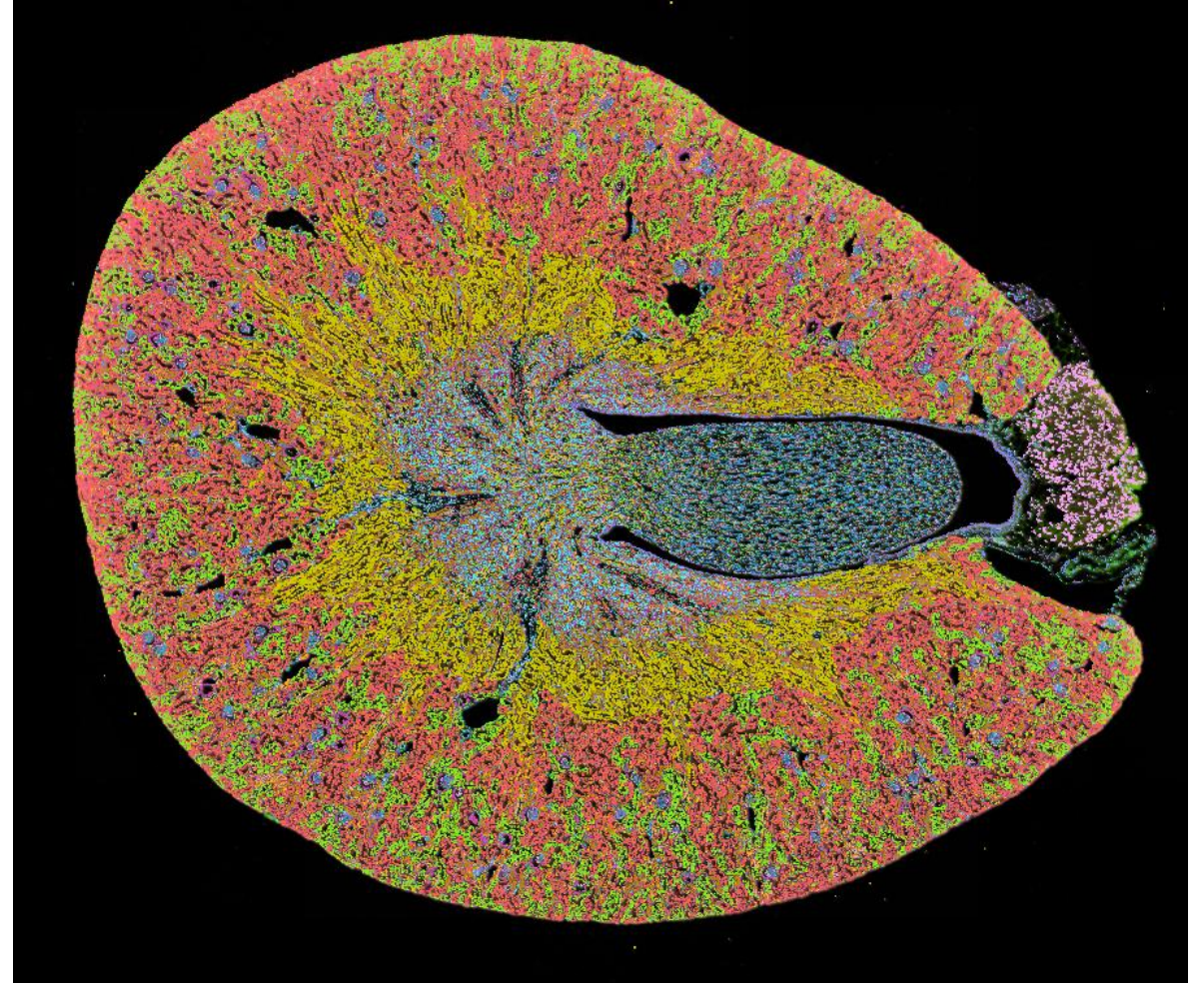
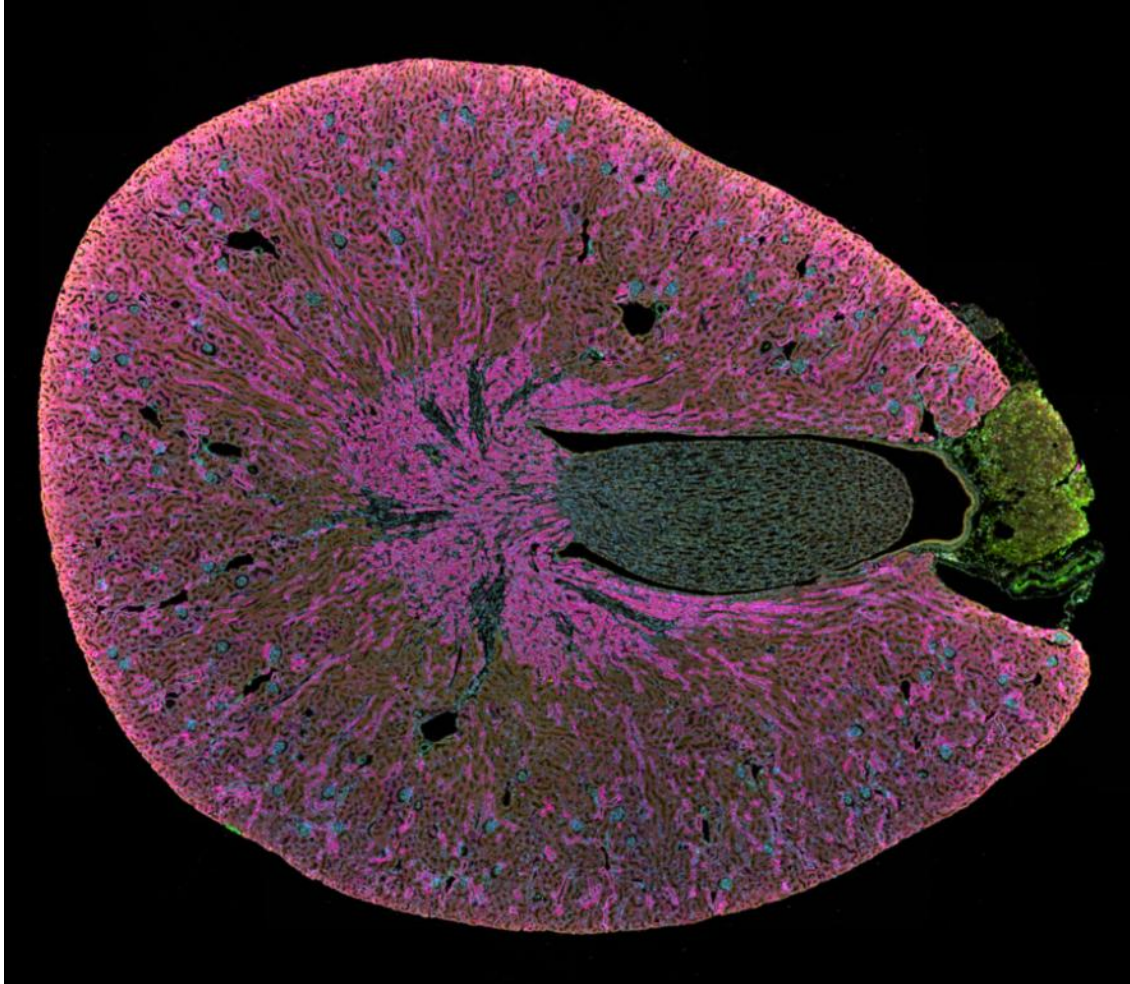
# We Jumped Off the High Dive With Xenium

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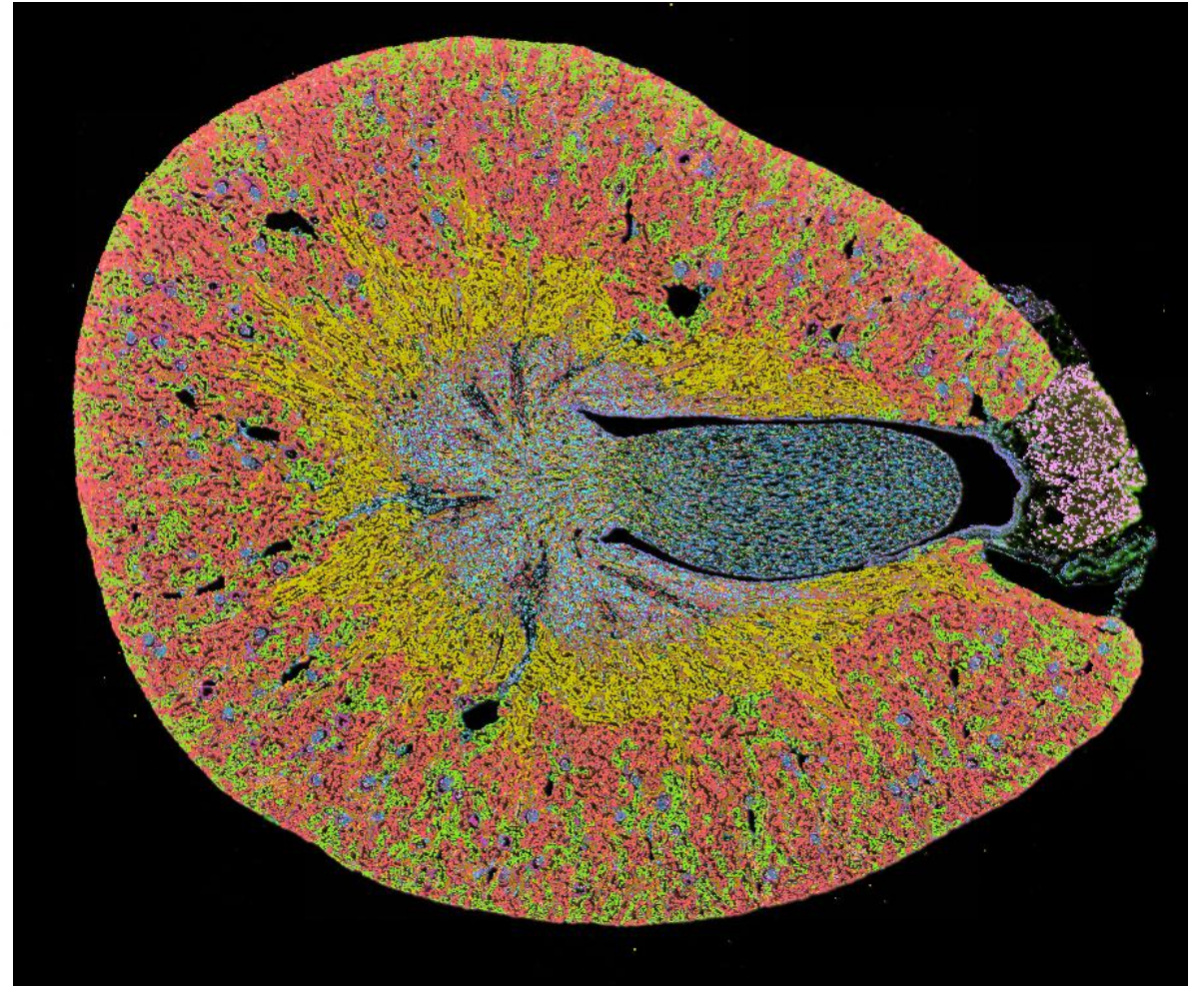
INTERNAL

# Our First Xenium Dataset!

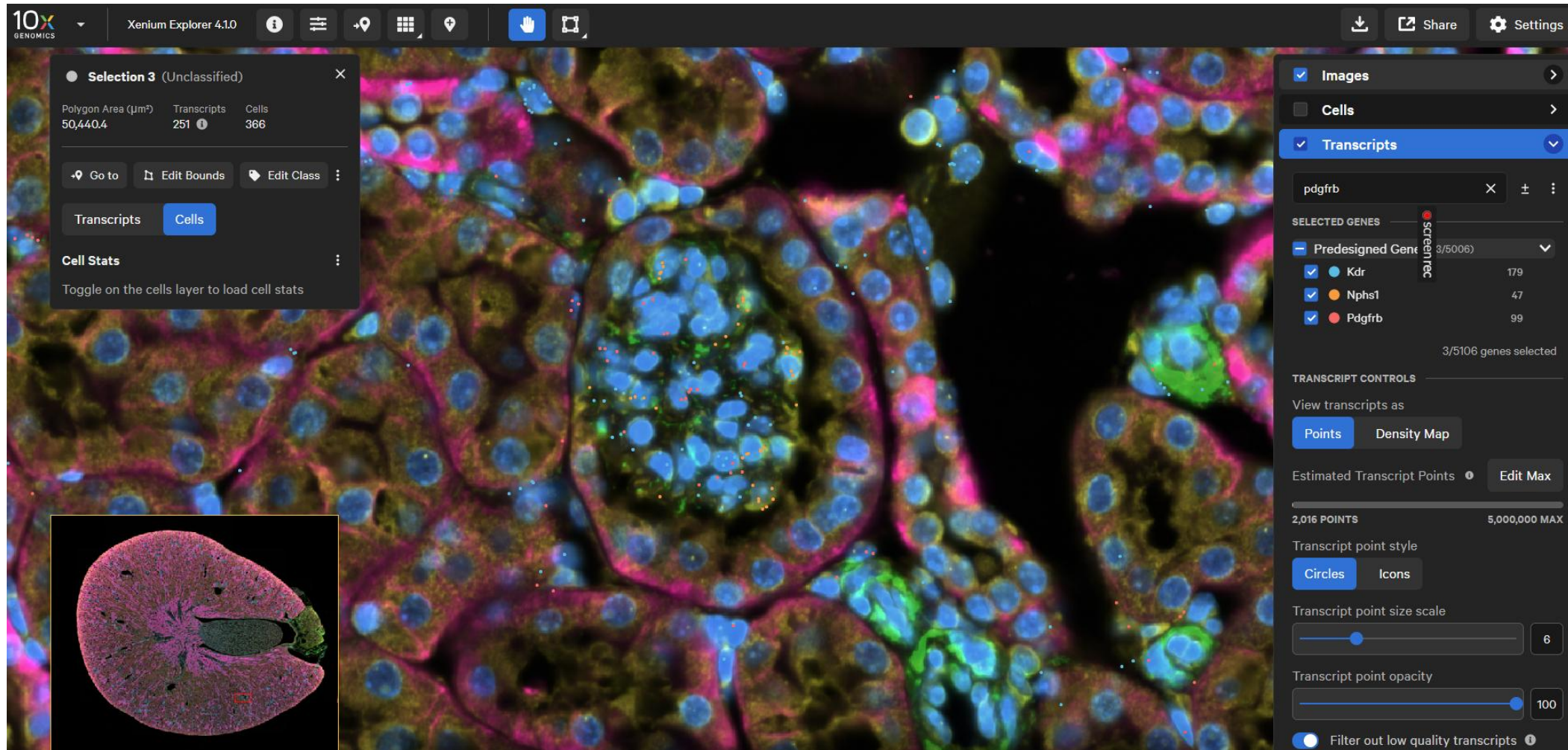


# Our First Xenium Dataset!

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# The Resolution Breathtaking!



# Then..the DREADD

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# Struggling with Basic Questions

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- **How Do You Load Spatial Data Into R studio?**
- **How Do You QC The Data?**
- **How Do You Annotate Cells?**
- **How Do You Process Spatial Coordinates?**
- **How Do You Compare to snRNAseq Results?**

# Computational Requirements

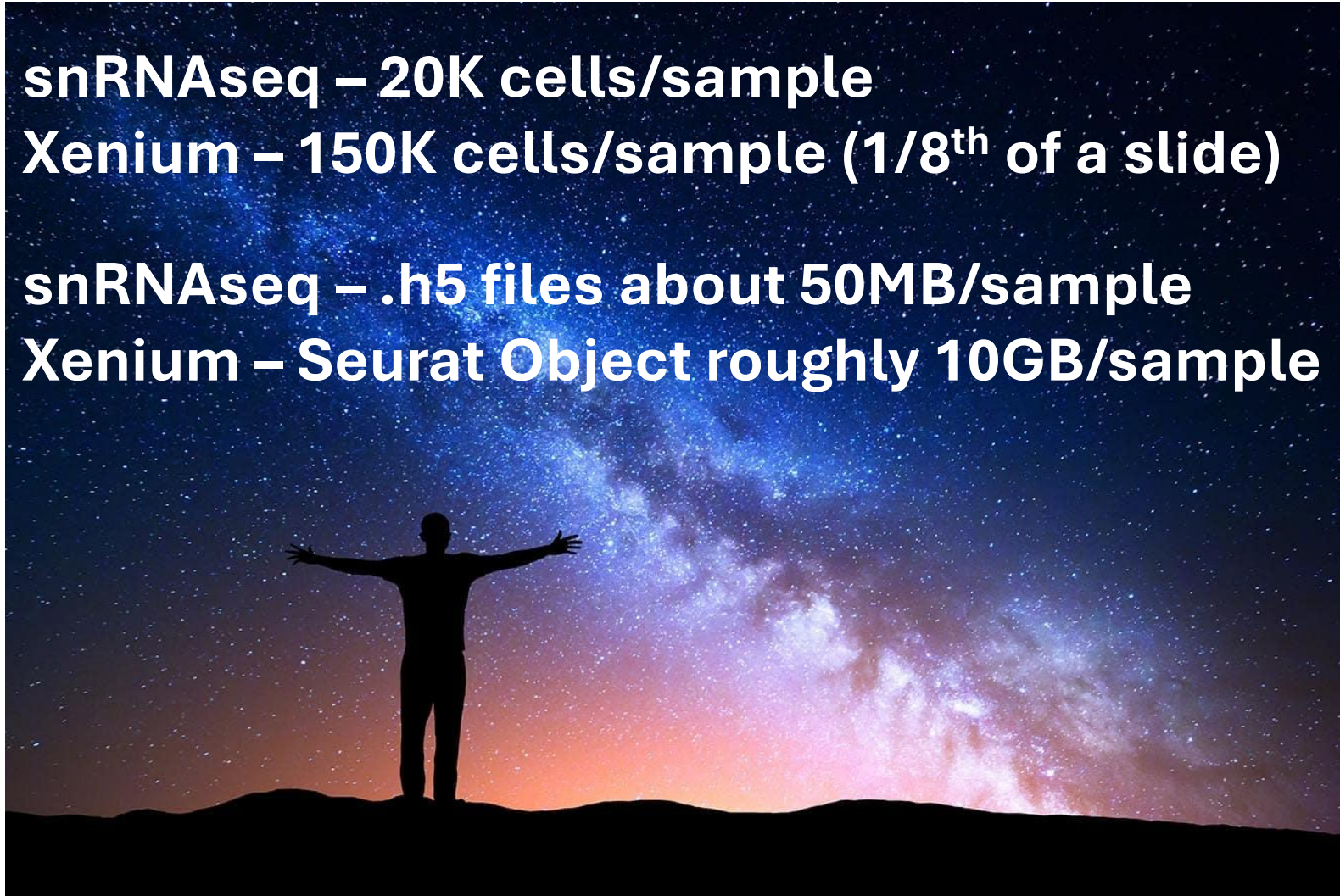
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**snRNAseq – 20K cells/sample**

**Xenium – 150K cells/sample (1/8<sup>th</sup> of a slide)**

**snRNAseq – .h5 files about 50MB/sample**

**Xenium – Seurat Object roughly 10GB/sample**



# Struggling with Basic Questions

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- I like to do analysis **locally** on my own computer.
- My setup:
  - Dell XPS laptop
  - Intel Core i9 (parallelization oesn't help much in R)
  - 64GB of RAM
  - 2TB SSD
- Storage of data is an issue...especially as you iterate segmentation approaches

# Analysis Tools Growing at “Ludicrous Speed”

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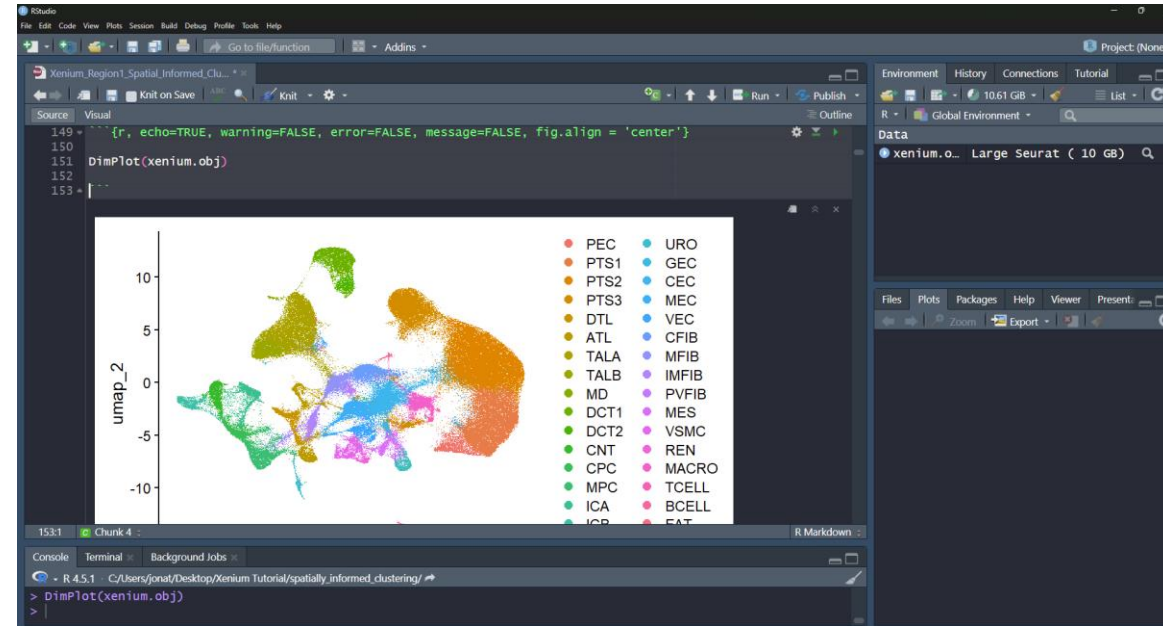
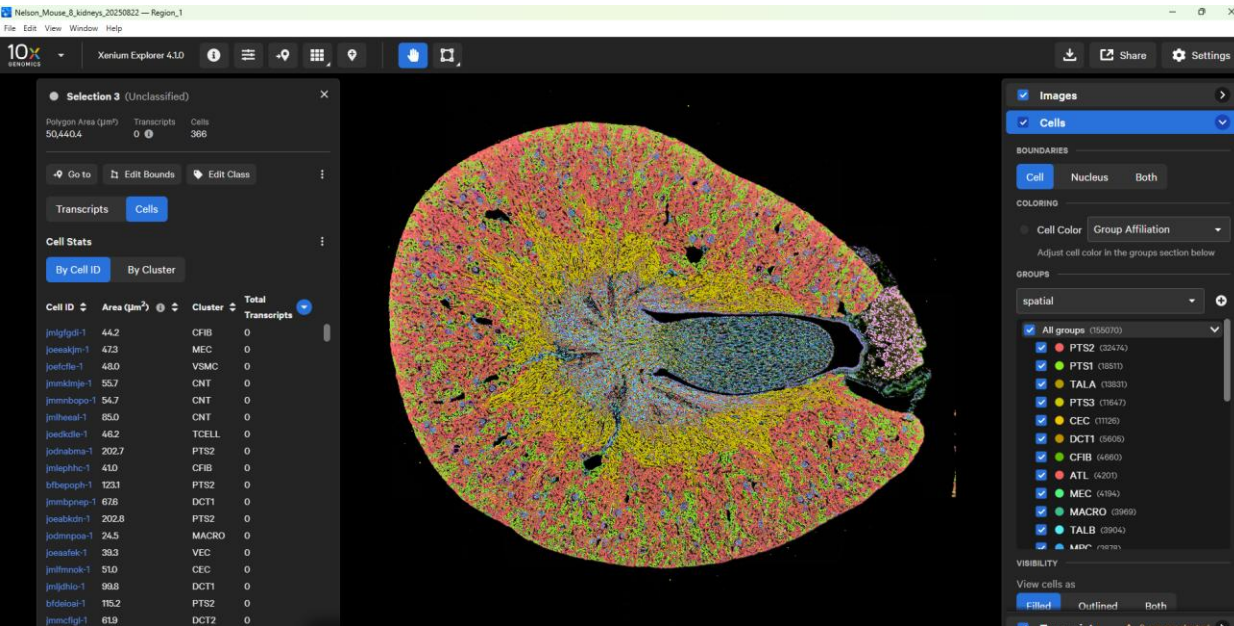
# There is a Gap Between Paper and Implementation

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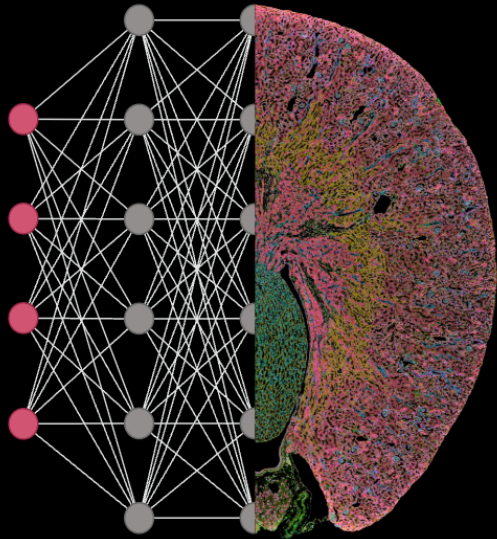


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# Communication Between Analysis Tools



INTERNAL



# AIMS@USC

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AI Methods for Spatial Omics Analysis at USC

# AIMS@USC: A Brief History

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2024

Baxter Foundation Award & Southern California P30ES007048 Pilot Grant: First Spatial Omics Datasets  
*(Spatial transcriptomics and metabolomics)*

2026

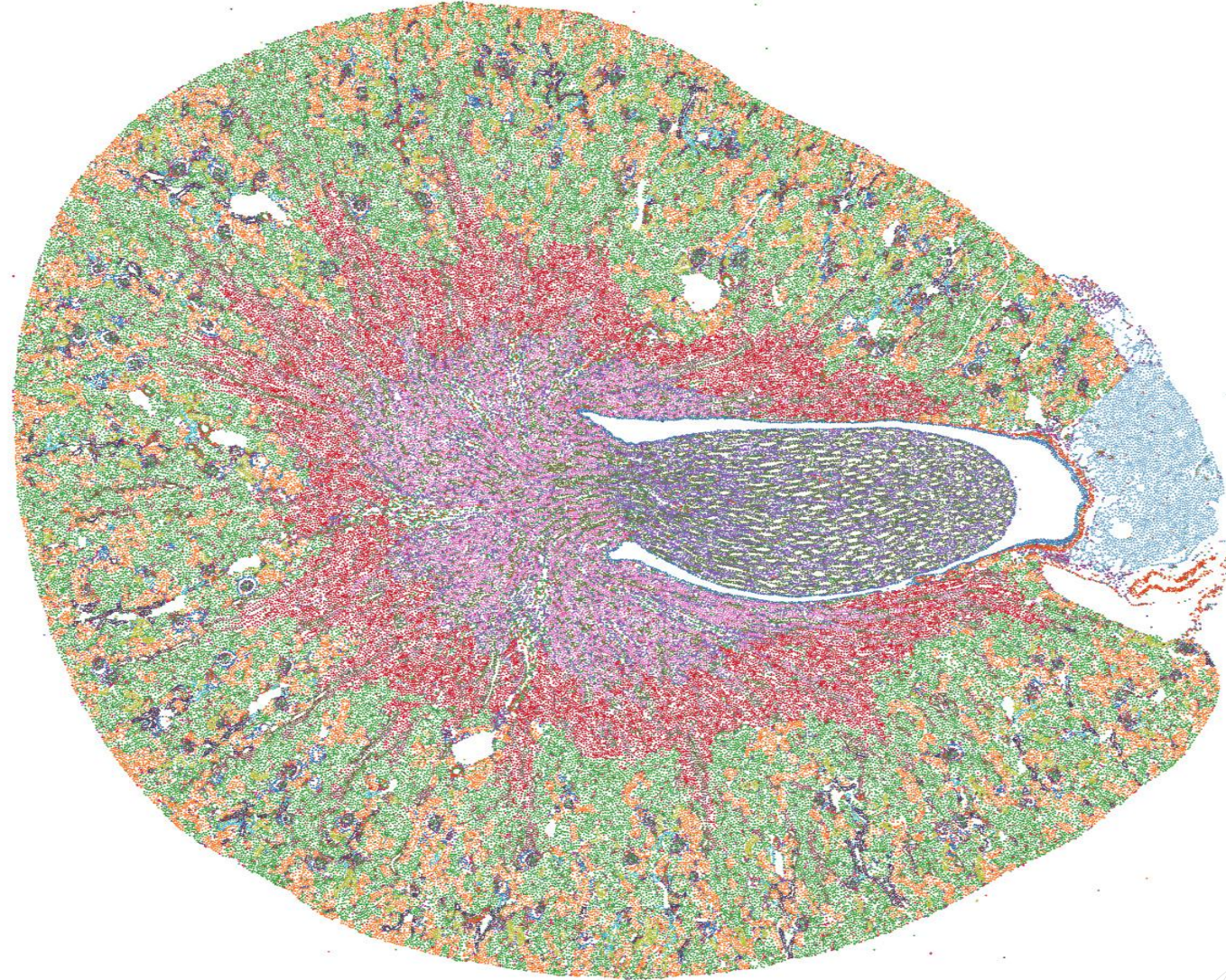
USC Office of Research and Innovation Research Catalyst Grant on Multidisciplinary Artificial Intelligence: AIMS@USC!

2025

SC-CTSI Team Building Voucher: Initialization of Team

# AIMS@USC: A Brief History

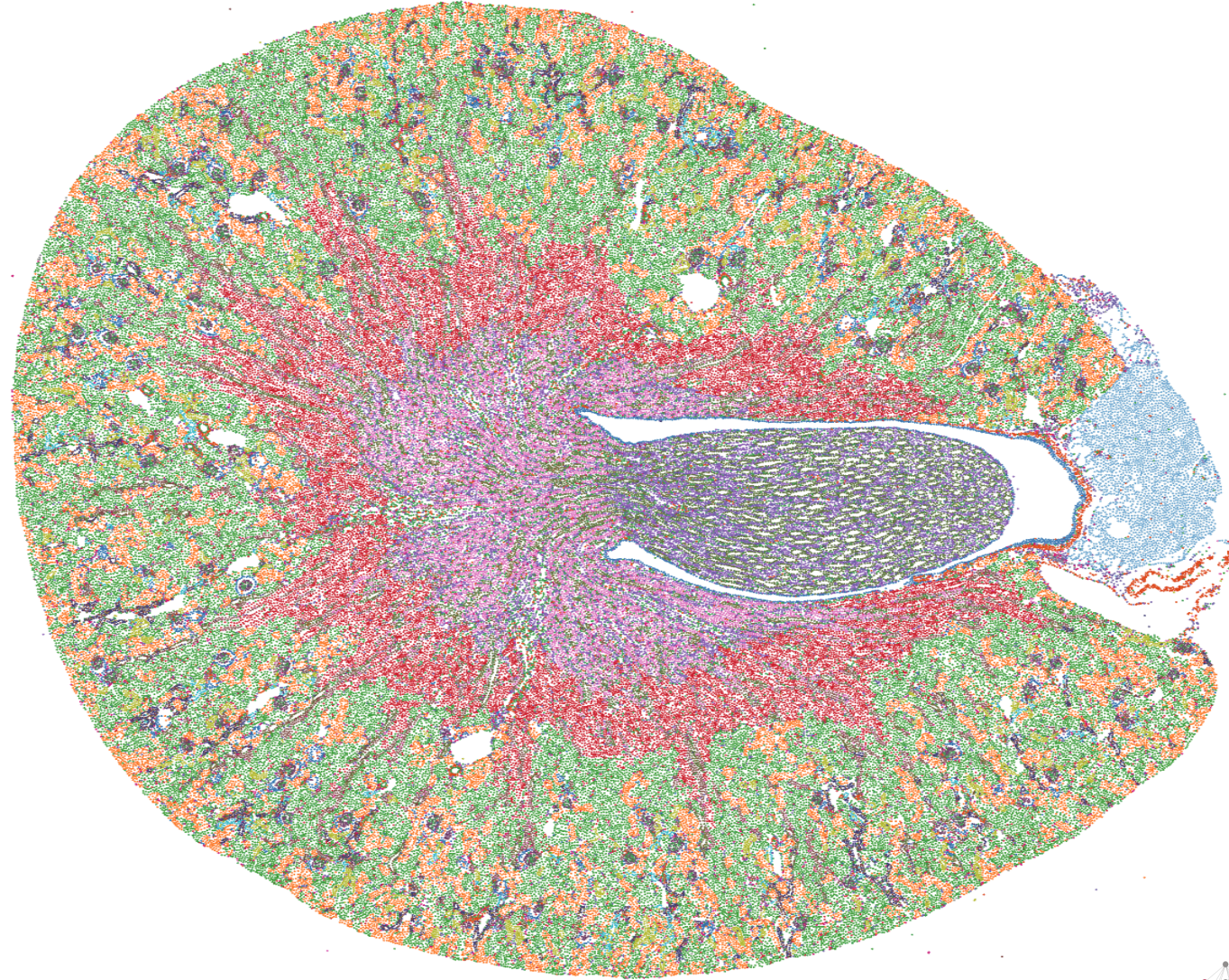
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*Data: Xenium 5000 gene panel plus 100 custom genes, segmented using Baysor*

# Spatial data can give unprecedented insights

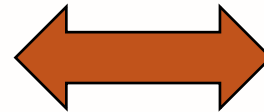
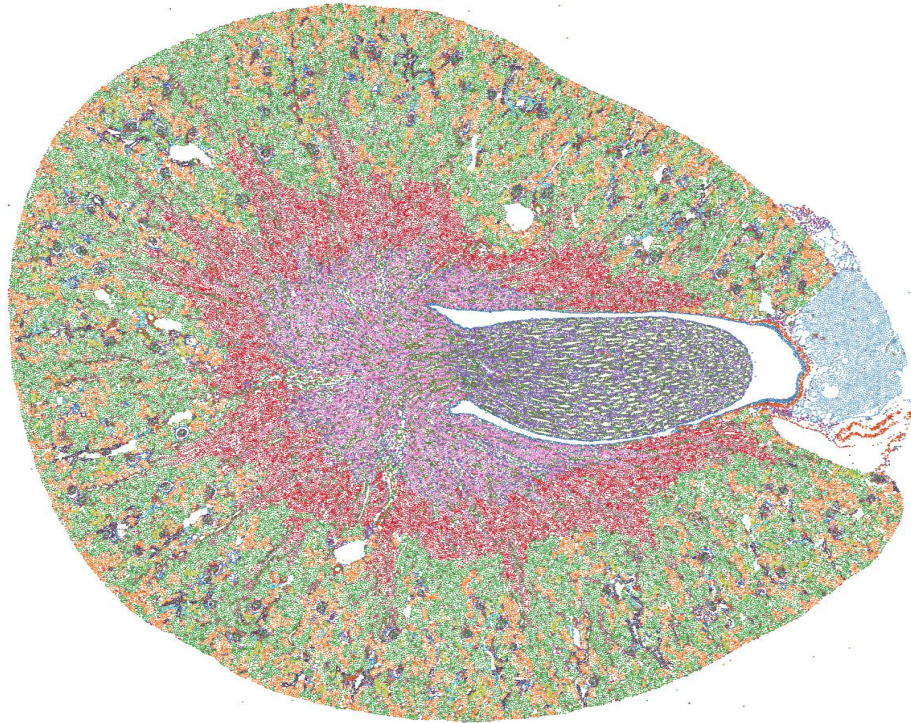
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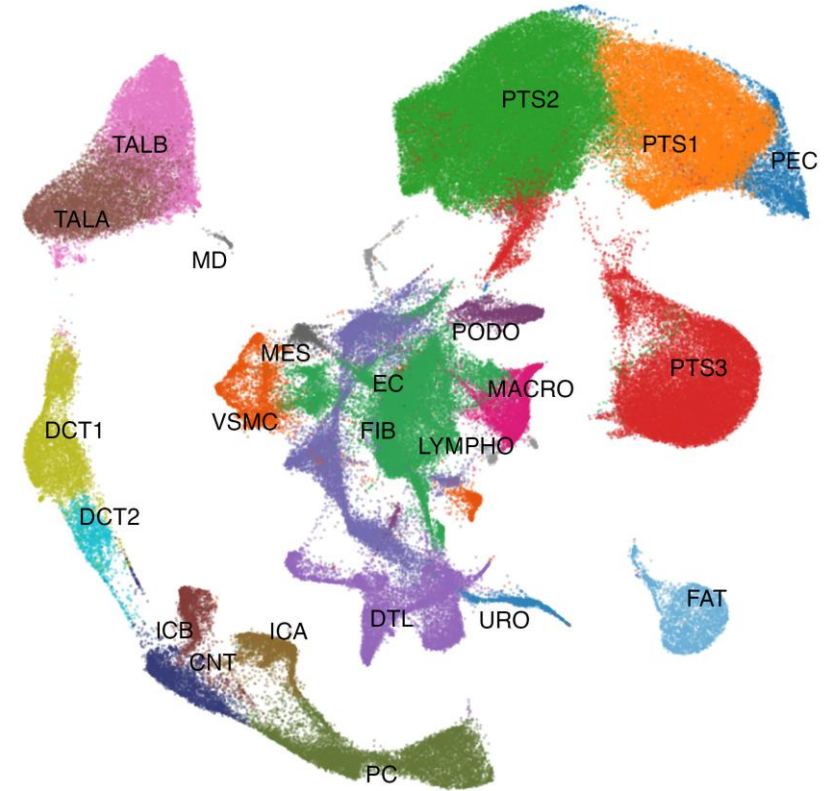
Data: Xenium 5000 gene panel plus 100 custom genes, segmented using Baysor

# Spatial data can give unprecedented insights

Spatial Context

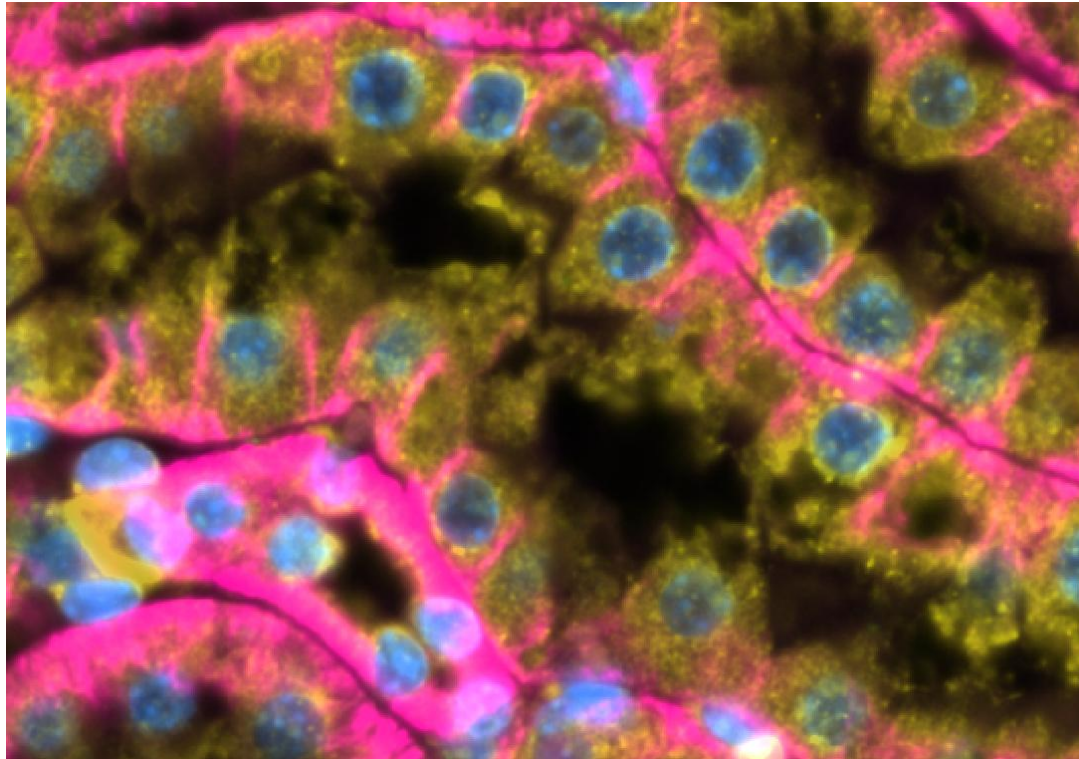


Single cell or subcellular resolution



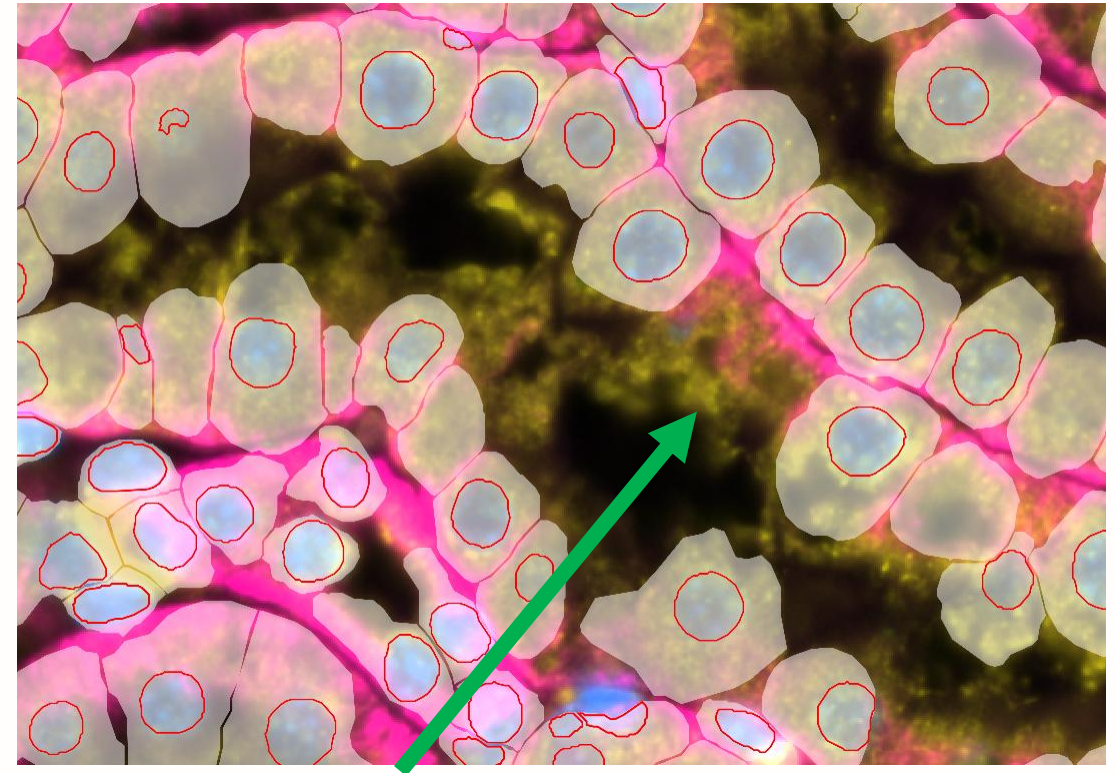
# ... But unlocking those insights isn't that simple

Cell Staining



- Nuclear
- Boundary
- Interior - Prot
- Interior - RNA

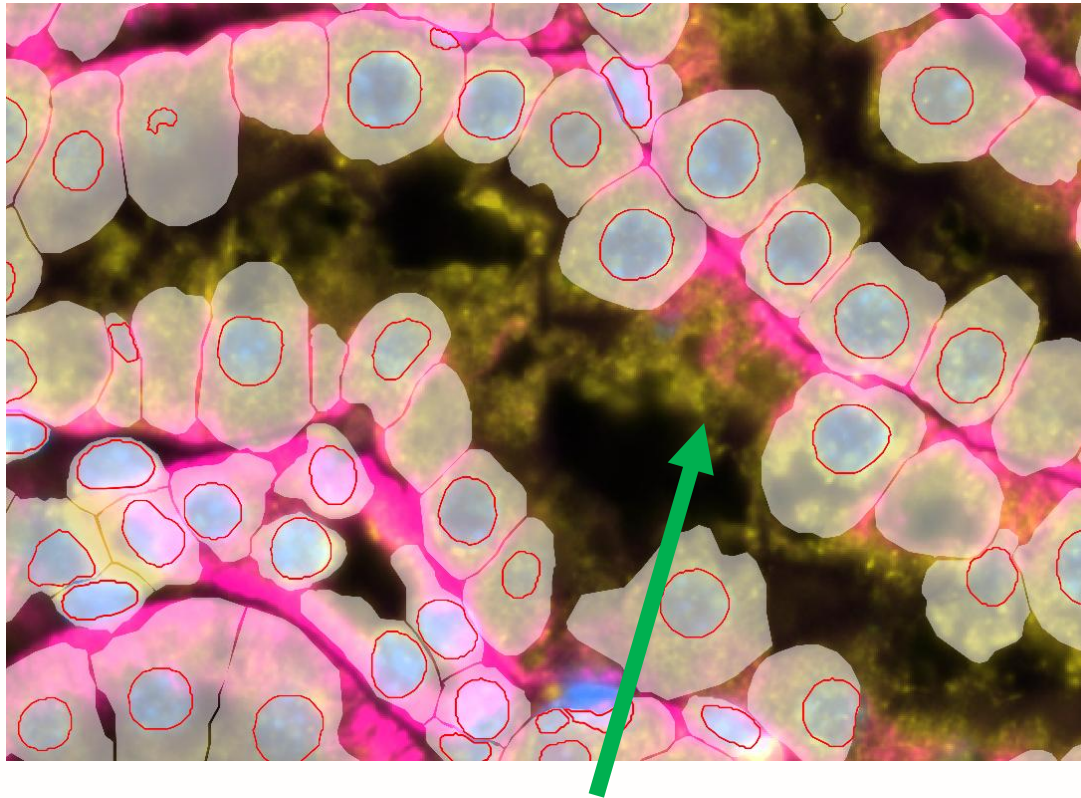
“Standard” Segmentation Algorithm Misses Some Cells



Missing Proximal Tubule Cell

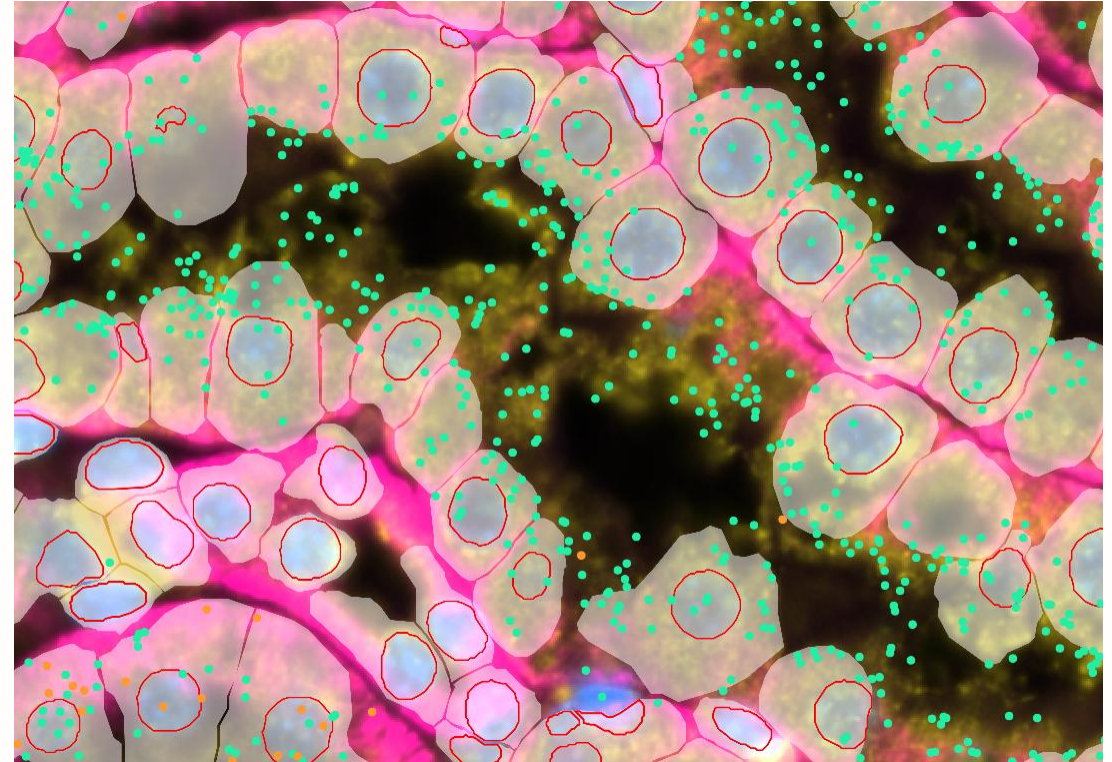
# ... But unlocking those insights isn't that simple

Standard Segmentation Algorithm



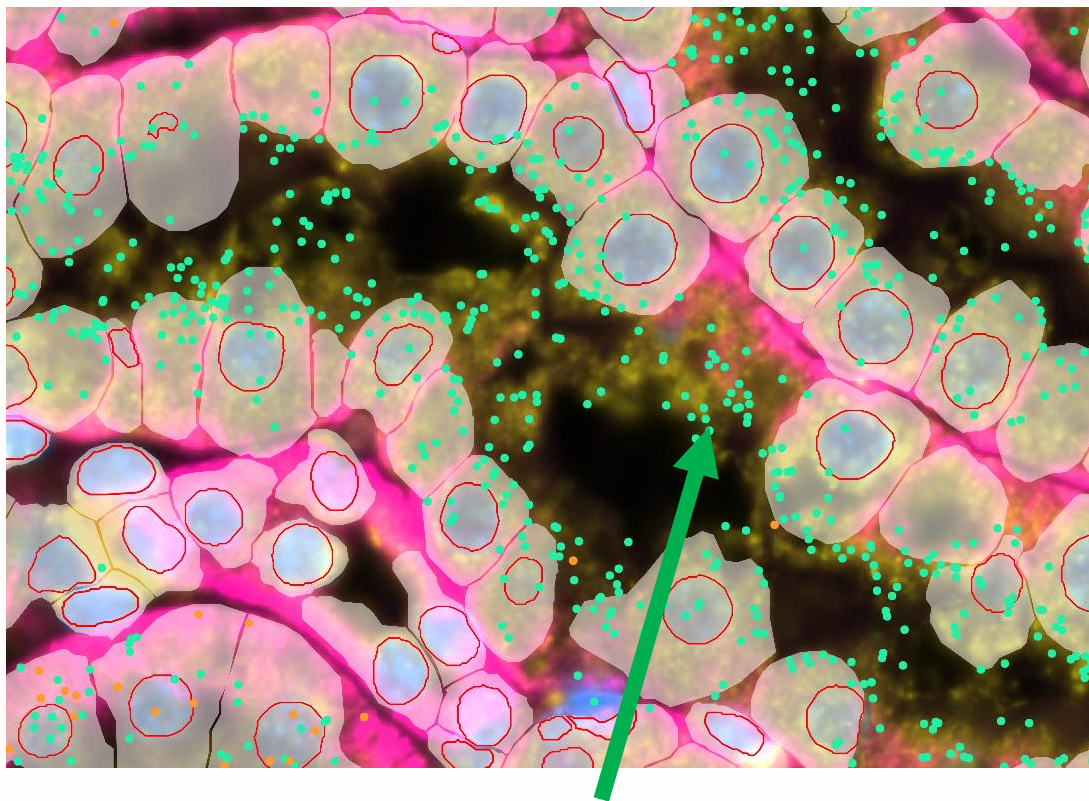
"Missing" Proximal Tubule Cell

25% of *Lrp2* (key renal gene; blue dots) isn't registered to actual cells!



# Early Insights

25% of *Lrp2* (key renal gene; blue dots) isn't registered to actual cells!

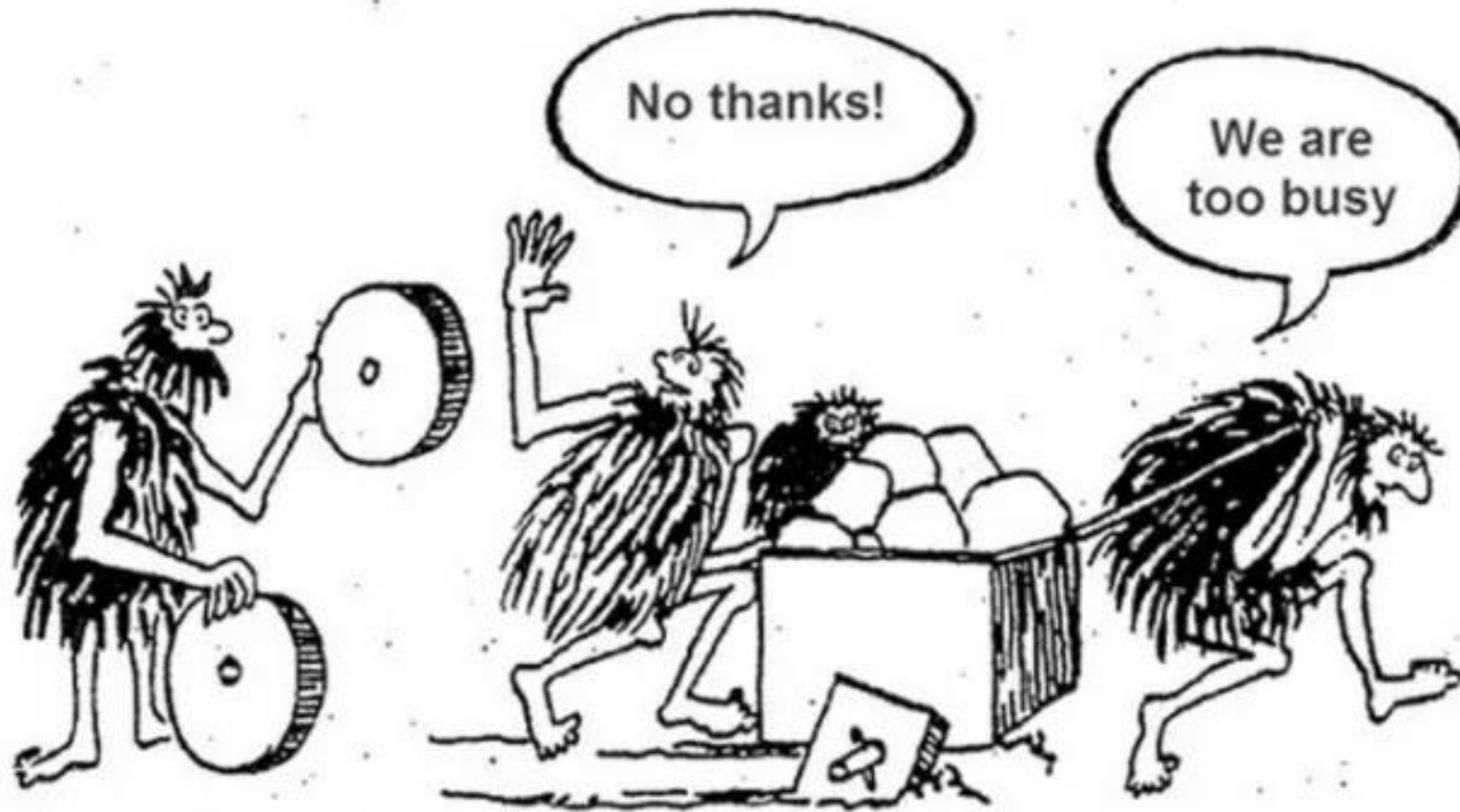


“Missing” Proximal Tubule Cell

1. Stain-based features alone can meaningfully improve segmentation (*inspired by advances in computer vision*)
2. Integrating molecular features with imaging yields better segmentation (*a machine learning perspective*)
3. Cell-free, pattern-based approaches offer a complementary approach (*drawing on spatial statistics*)

## Most importantly: we needed a team of experts

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# AIMS@USC Team



**J. Goodrich, PhD, Co-Director**  
Data science, toxicology



**J. Nelson, PhD, Co-Director**  
Molecular biology, informatics



**M. Khayatkhoei, PhD**  
Computer Science, AI



**K. Street, PhD**  
Single Cell/Spatial Omics



**L. Zhang, PhD**  
Spatial Methods



**Thomas Kowal, PhD**  
Postdoctoral scholar

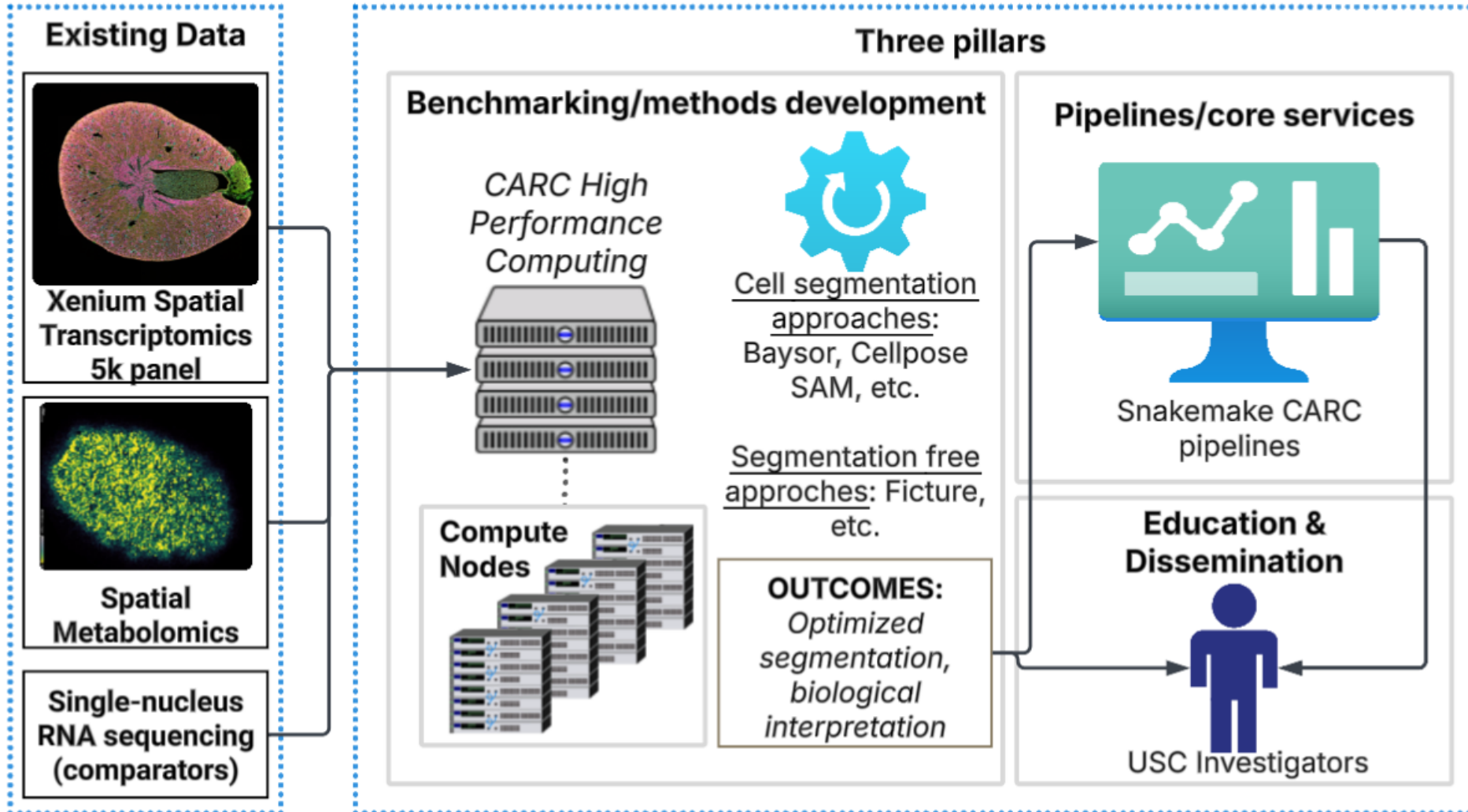


**Hongxu Wang, MS**  
Bioinformatician

**Xinran Wang**  
Biostatistics PhD Student

**Daniella Carrillo**  
Biostatistics MS Student

# AIMS@USC Goals



# You Have Questions...We Have Answers

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- What are the experiential costs?
- What are the tissue prep requirements?
- How do I QC and annotate a spatial datasets?

# Experimental Costs

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## 6.3 Cost of a Xenium Experiment

### 6.3.1 Does **NOT** Informatics Time

### 6.3.2 Some “Back of the Envelope” Math

6.3.2.0.1 **Vanilla** Xenium Experiment

6.3.2.1 2x Xenium Slides: **\$15,000**

6.3.2.2 8 samples/slide x2 slides + \$1,000 Sample Prep/Labor/QC

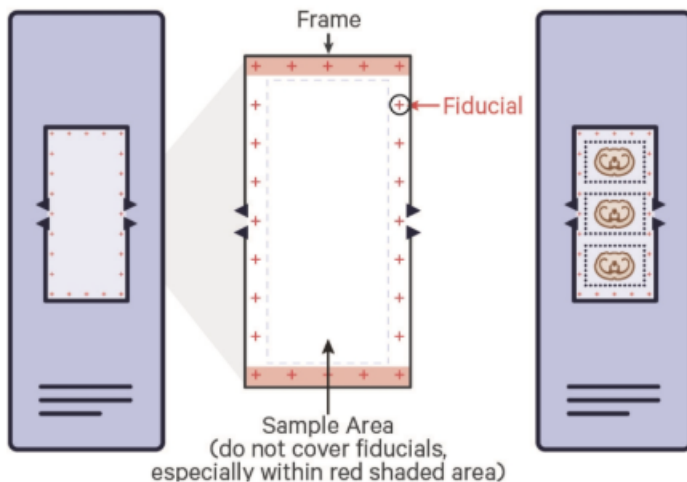
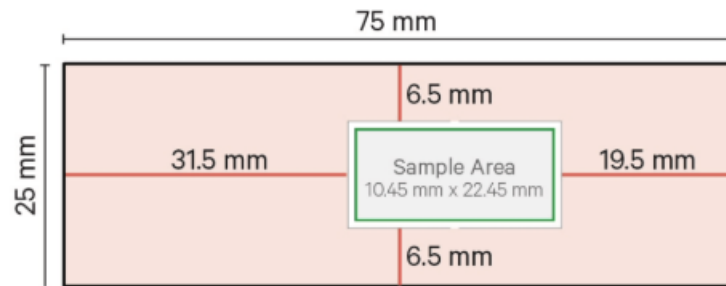
6.3.2.3 Roughly **\$1,000** per sample

6.3.2.4 Could be less with Discounts from 10X Rep

# Tissue Requirements

## 6.4.5 Practice and Preparation

<https://kb.10xgenomics.com/hc/en-us/articles/12811210821133-How-can-I-more-easily-visualize-the-fiducial-frame-on-Xenium-slides>



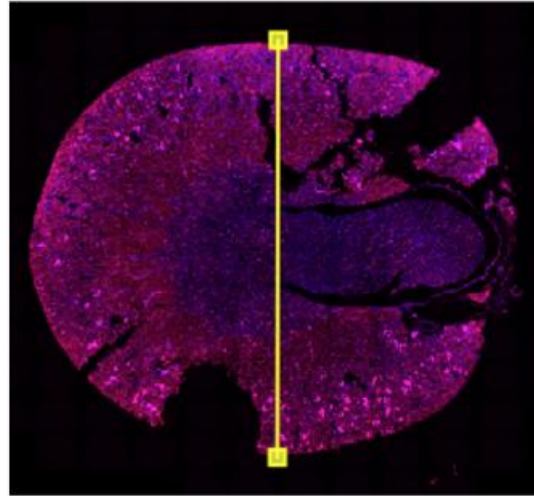
I've had great experience with freshly prepared FFPE blocks.

RNAase-free reagents at tissue collection.

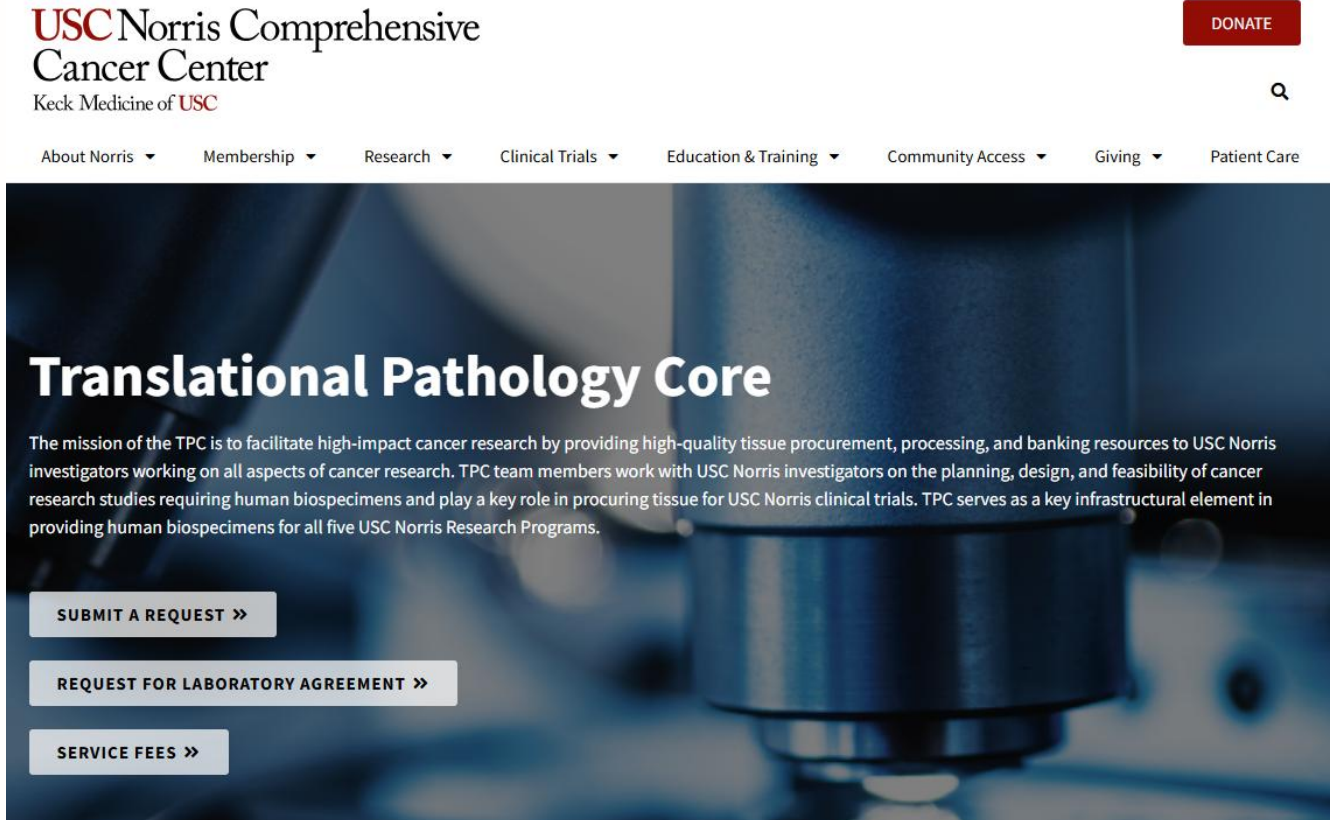
Used within weeks of tissue collection.

# Tissue Requirements

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# Tissue Requirements



**USC Norris Comprehensive Cancer Center**  
Keck Medicine of USC

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## Translational Pathology Core

The mission of the TPC is to facilitate high-impact cancer research by providing high-quality tissue procurement, processing, and banking resources to USC Norris investigators working on all aspects of cancer research. TPC team members work with USC Norris investigators on the planning, design, and feasibility of cancer research studies requiring human biospecimens and play a key role in procuring tissue for USC Norris clinical trials. TPC serves as a key infrastructural element in providing human biospecimens for all five USC Norris Research Programs.

[SUBMIT A REQUEST >>](#)

[REQUEST FOR LABORATORY AGREEMENT >>](#)

[SERVICE FEES >>](#)



Ronald Vaca is THE MAN!

# Quality Control and Annotation

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- Minimum number of transcripts/cell?
  - Depends on analysis and segmentation.
  - Currently not filtering based in low transcript number
- Challenges with Label Transfer for Annotation
  - snRNAseq markers **does not** equate to a good in situ marker
  - Many panels **don't include** key informative genes
  - We're working on this...but so far...the **best label transfer** has been between two spatial datasets with the same panel.

# Comparing and Optimizing Cell Segmentation Approaches

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Thomas Kowal, PhD

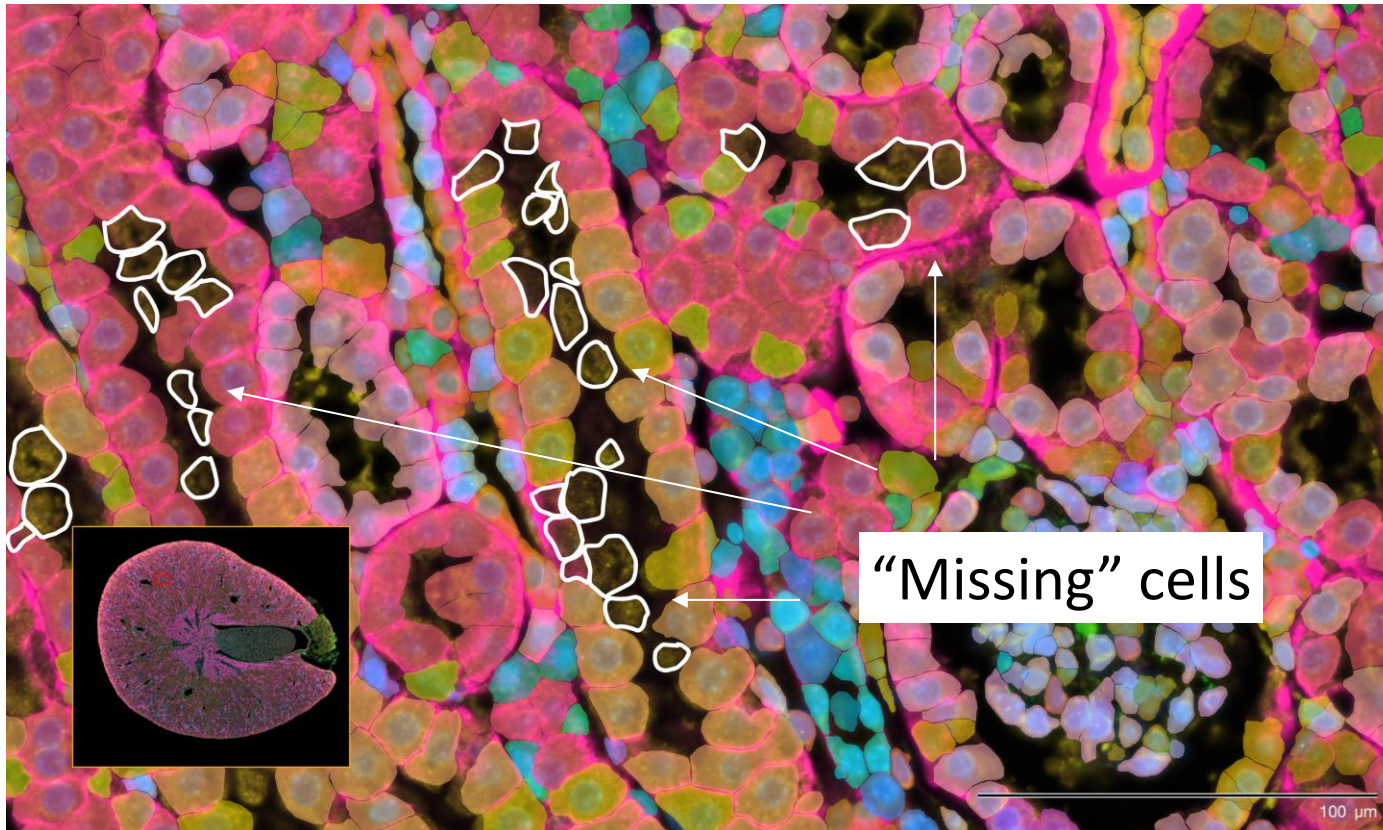
# Importance of cell segmentation

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- Robust image-based analysis and interpretation requires accurately defined cell boundaries
- Manual segmentation is impractical for large-scale, high-throughput studies
- Many automated segmentation approaches differ in their philosophies (e.g. transcript clustering-based, image learning-based) and are applied without systemic evaluation of differing strengths and weaknesses

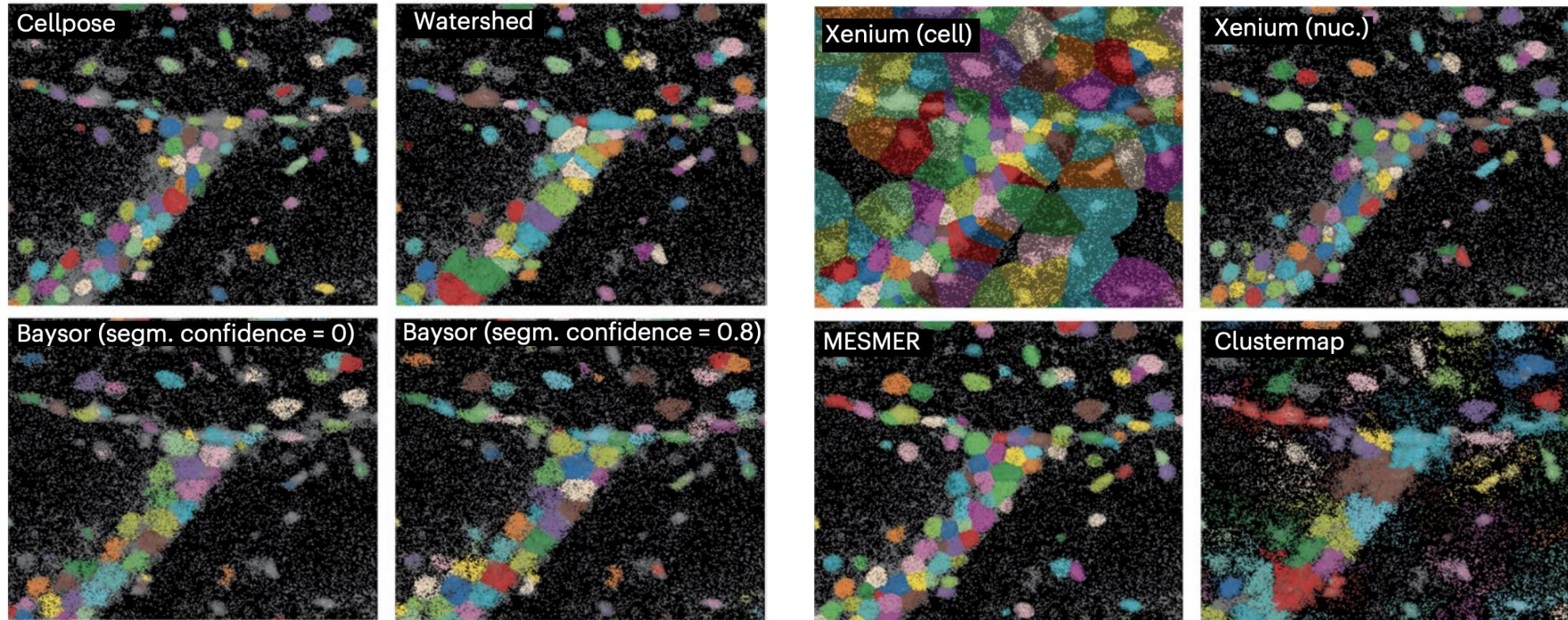
1. Salas et al. Optimizing Xenium In Situ data utility by quality assessment and best-practice analytical workflows. *Nat. Methods*. 22:813-823 (2025).
2. Yu et al. Segmentation Matters: Recognizing the Cell Segmentation Challenge in Spatial Transcriptomics. bioRxiv preprint. DOI: 10.1101/2025.08.25.672145.

# Xenium's native segmentation algorithm is sub-optimal



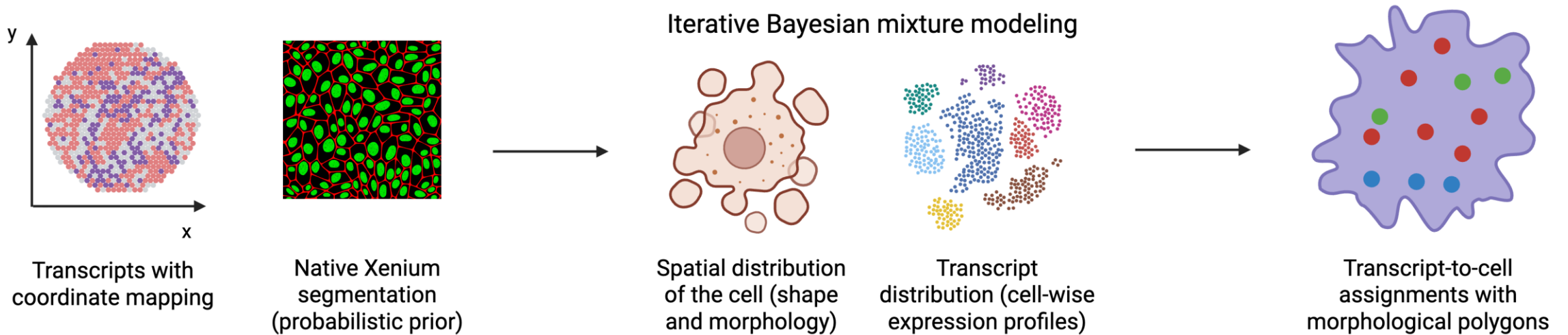
- Relying on Xenium's native segmentation algorithm could negatively impact downstream analysis
- Entire cells were missed in our murine kidney data
- AIMS@USC objective: identify and optimize best options for automated segmentation in high-dimensional (large gene panels, e.g. Xenium's 5K panel) and large-area datasets

# Identifying viable segmentation approaches



- Salas et al. (2022) compared various segmentation approaches on Xenium data
- This study only used a 248-gene panel; further investigation of higher dimensional data is needed

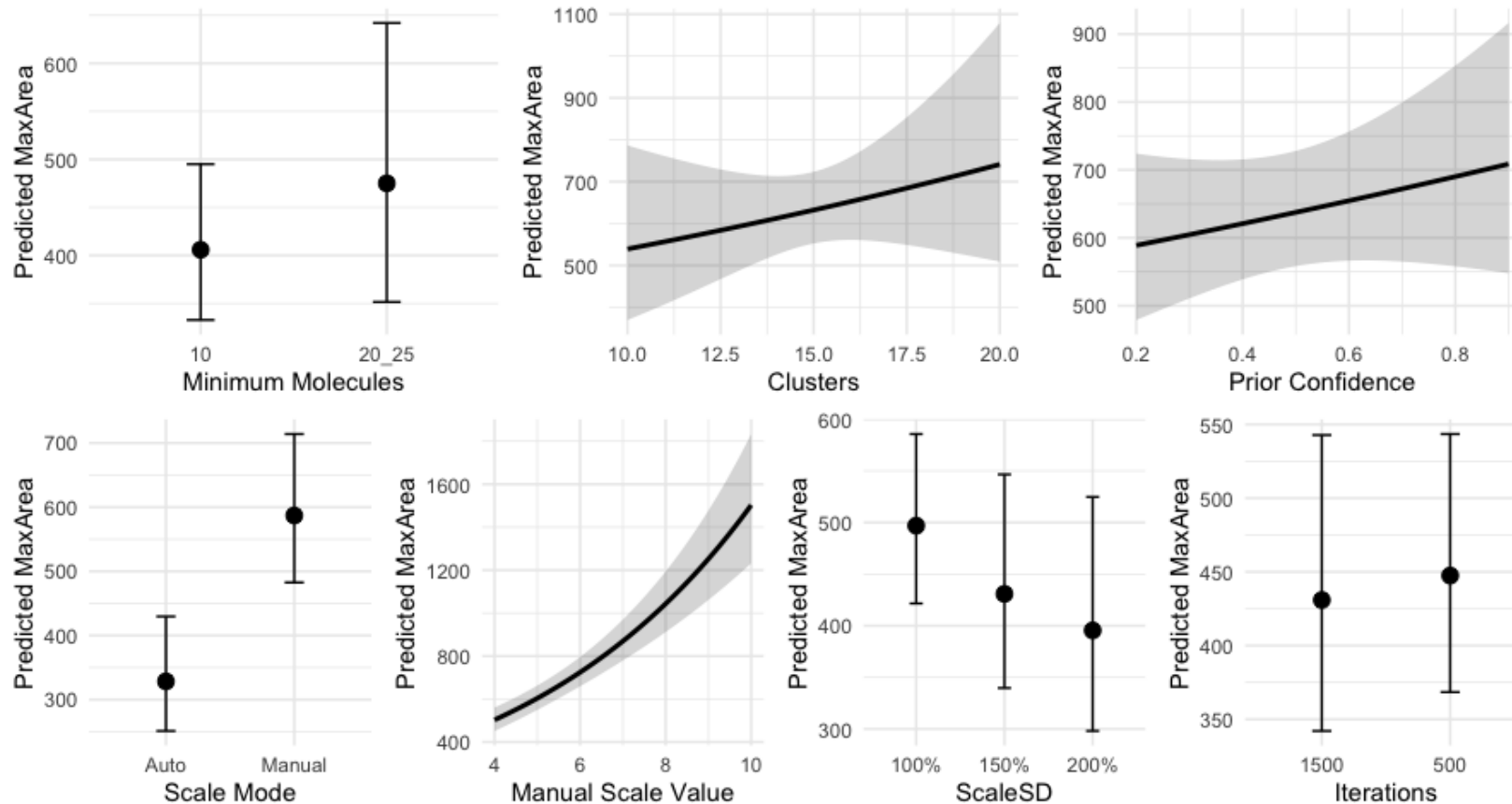
# Baysor is a very promising method



**BUT: Baysor has 6 different tunable parameters that need to be optimized for the best segmentation results**

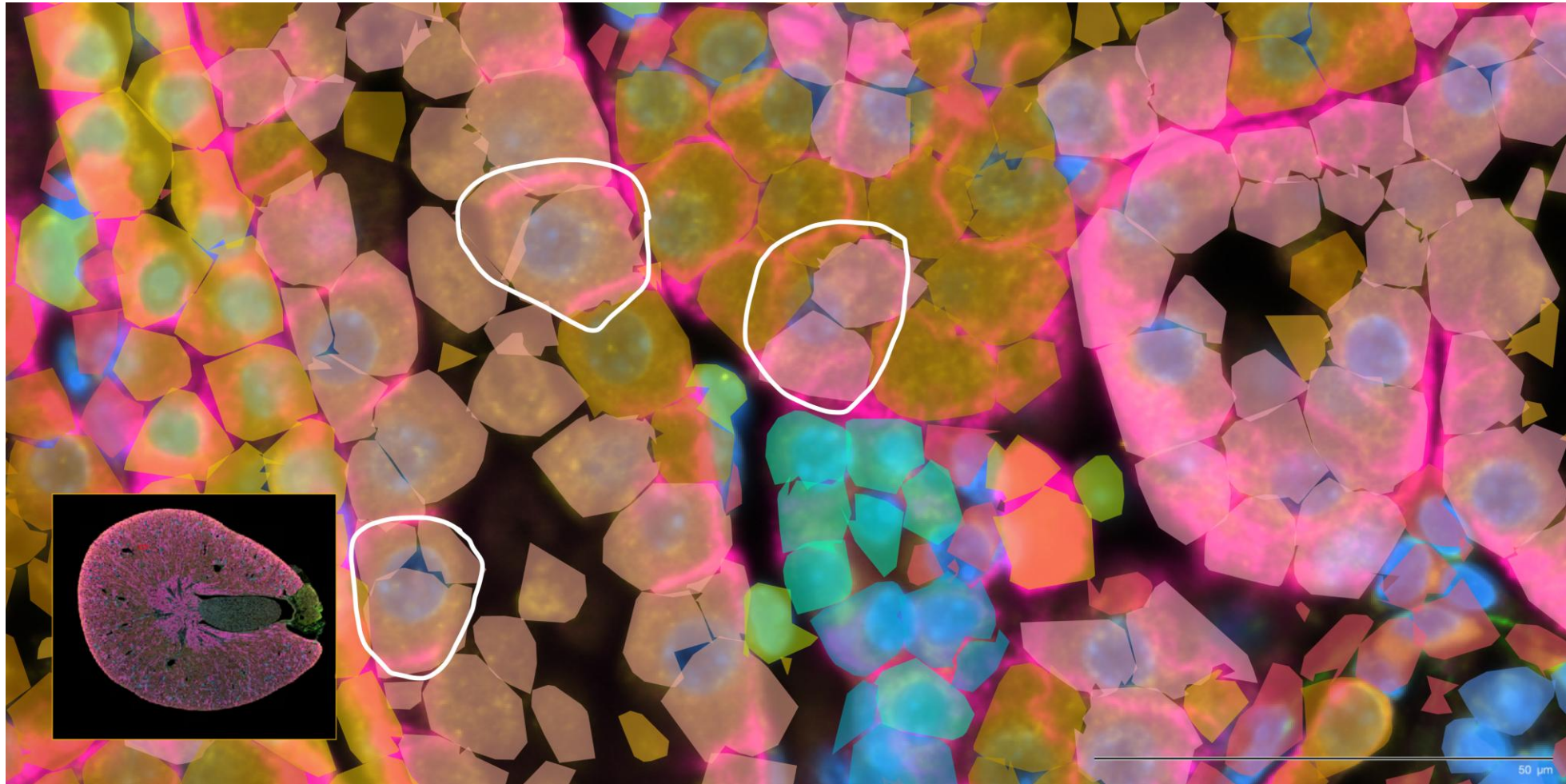
Petukhov et al. Cell segmentation in imaging-based spatial transcriptomics. *Nat. Biotechnol.* 40:345-354 (2022).

# Baysor parameters dramatically impact segmentation results and need to be optimized



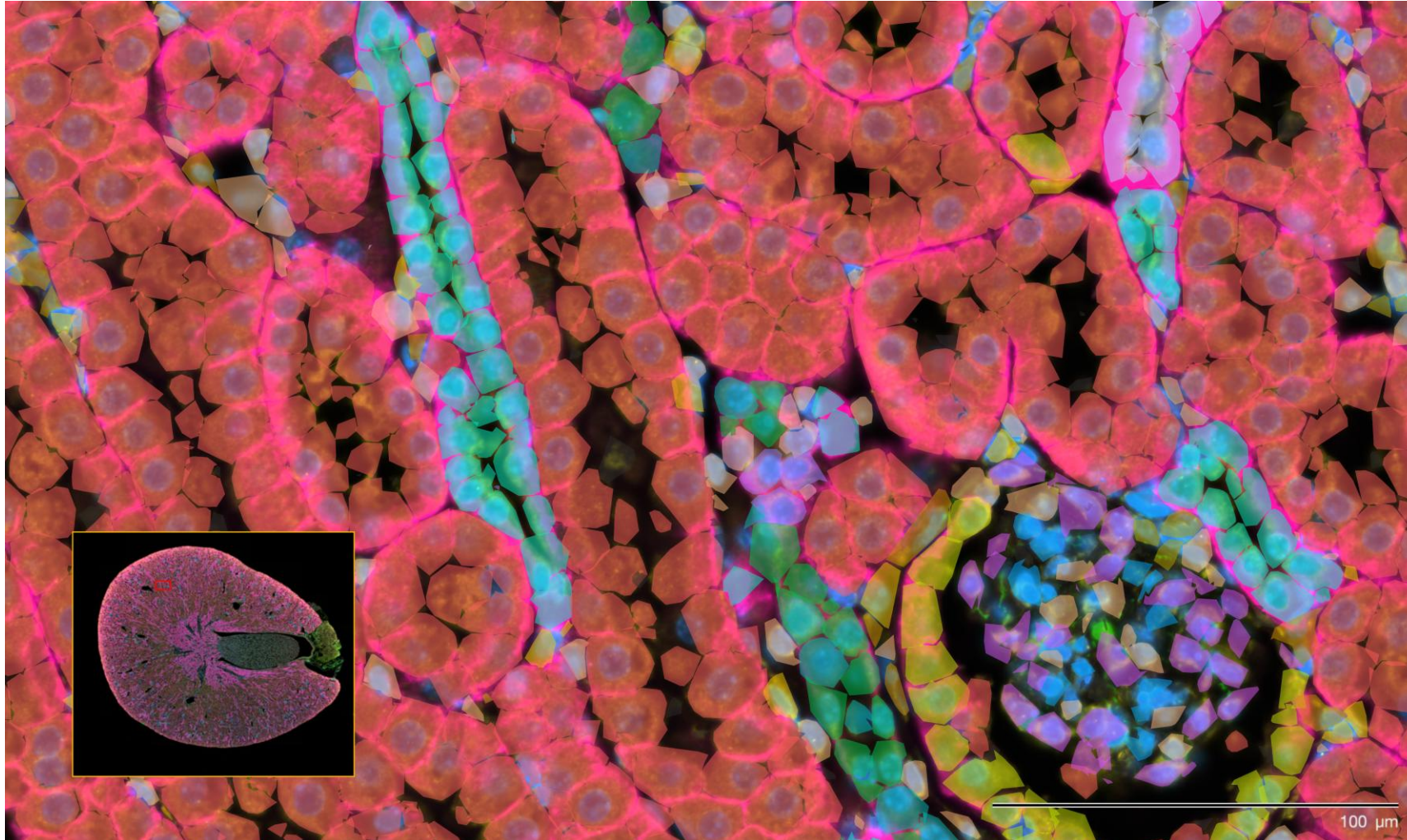
Baysor parameter values affect the **maximum cell area**, number of cells, degree of oversegmentation

# Baysor before optimization



INTERNAL

# Baysor after optimization

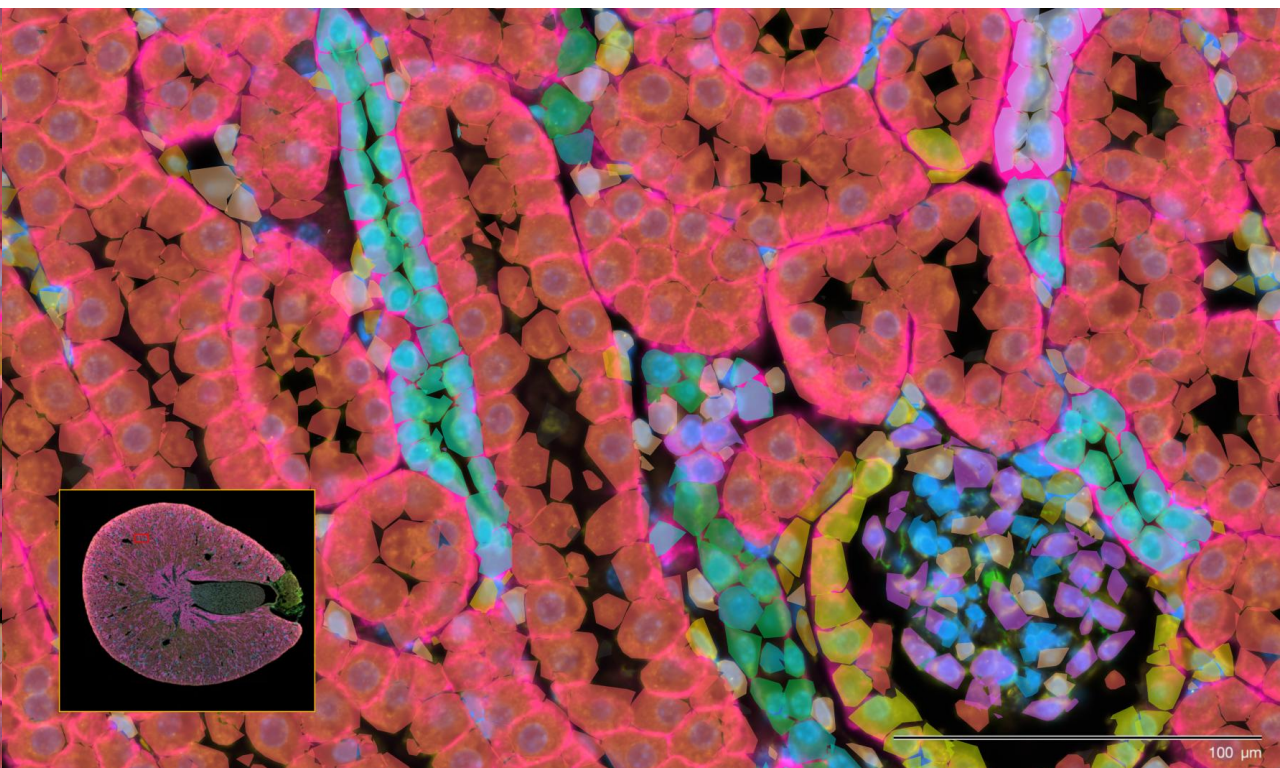
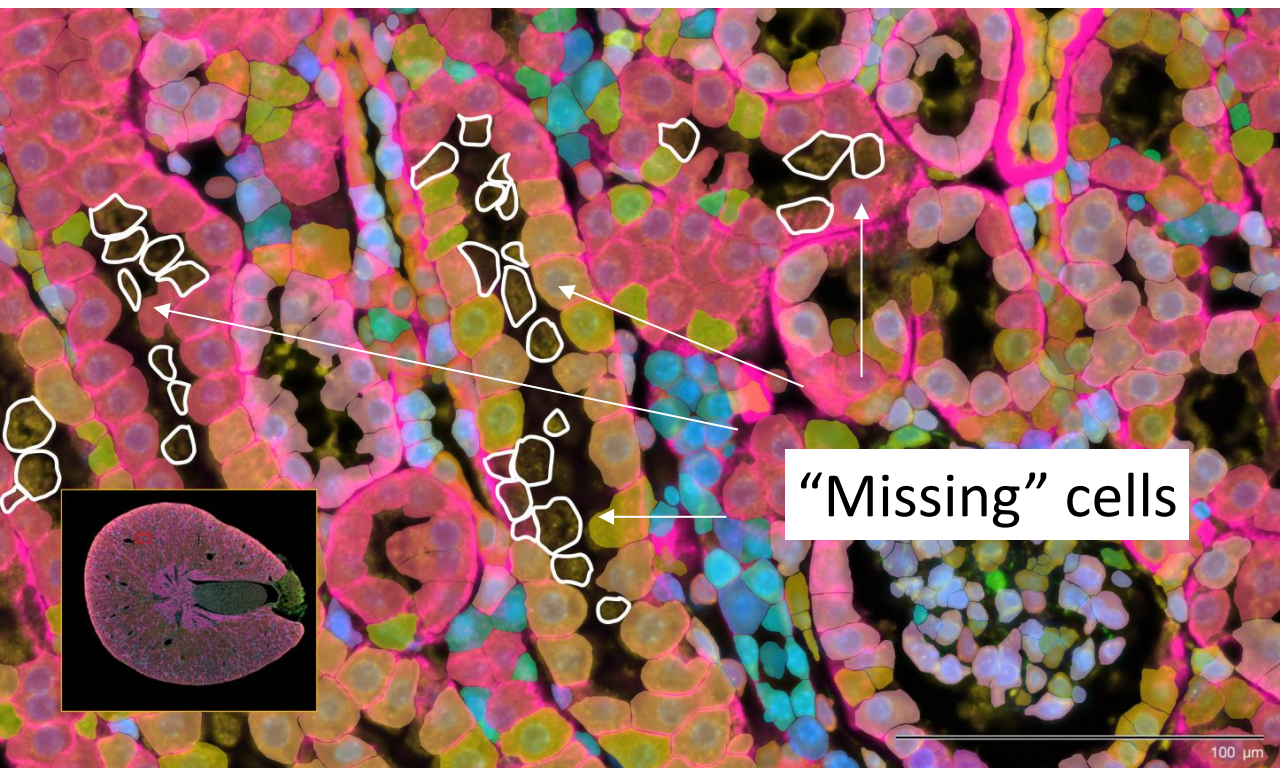


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# Optimized Baysor outperforms Xenium's native segmentation

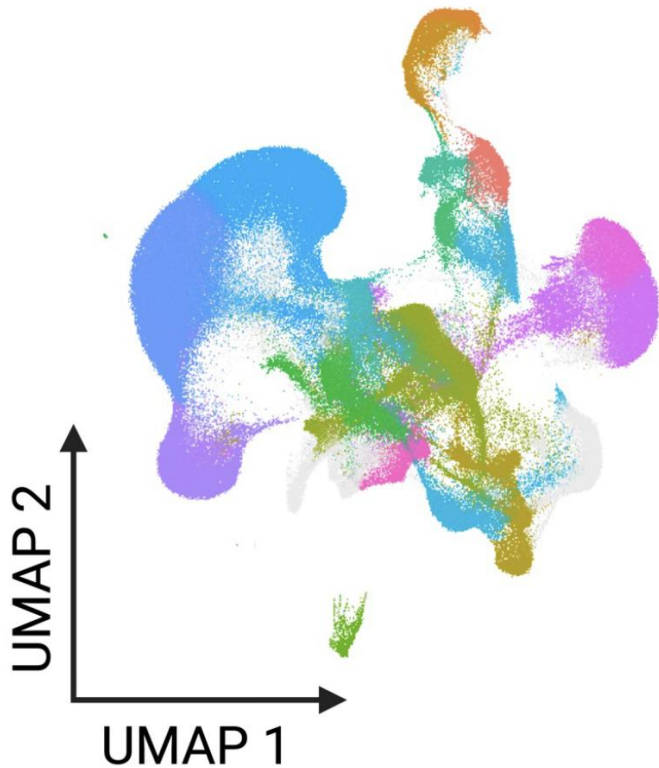
Original Xenium Segmentation

Optimized Baysor Segmentation



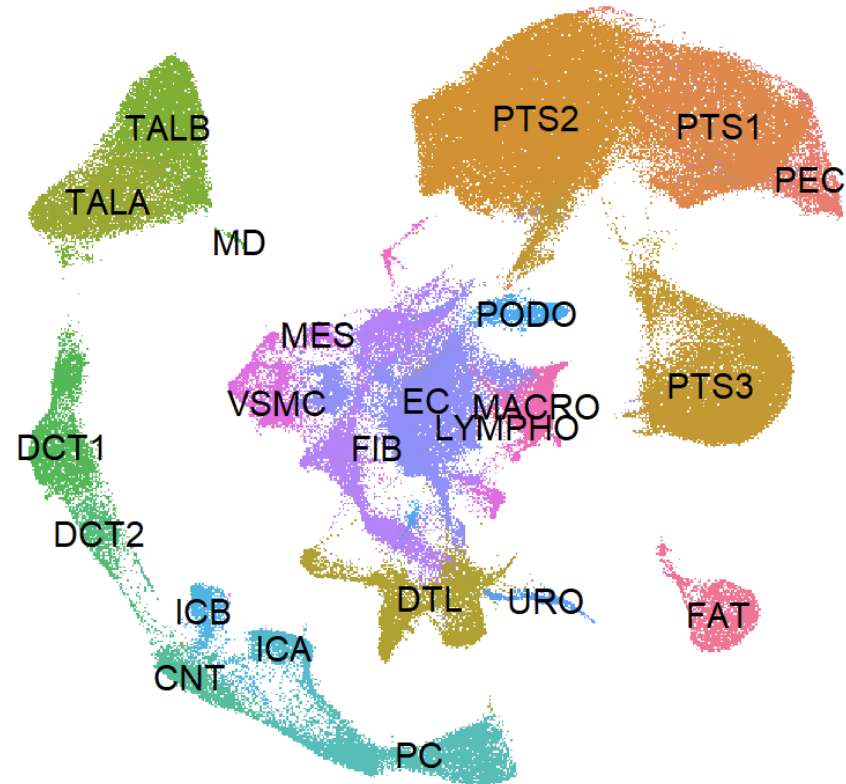
# Optimized Baysor improves cell type clustering compared to Xenium's native segmentation

Xenium segmentation



- CNT
- DCT1
- DCT2
- DTL
- EC
- FAT
- FIB
- ICA
- ICB
- IMM
- PC
- PTS1
- PTS2
- PTS3
- TALA
- TALB
- VSMC
- Other

Optimized Baysor segmentation



- PEC
- PTS1
- PTS2
- PTS3
- DTL
- TALA
- TALB
- MD
- DCT1
- DCT2
- CNT
- PC
- ICA
- ICB
- PODO
- URO
- EC
- FIB
- MES
- VSMC
- LYMPHO
- MACRO
- FAT

# General Baysor workflow

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1. Gene subset selection (CARC; 15 minutes) – need to select top genes to overcome Baysor memory limitations in large datasets
2. Running Baysor (CARC; 4-12 hours)
3. Rejoining all transcripts using Xenium Ranger (CARC; ~24 hours), working on optimizing via parallelization (expected < 2 hours)
4. Visualization in Xenium Explorer (local) – must first download Xenium Ranger output as Xenium Explorer can't run on CARC

CARC usage for these steps ranges from 32-128 GB memory, and 2-16 CPU

# Future directions

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- Devise comprehensive segmentation pipelines optimized for large, high dimensional (e.g. Xenium 5K panel) spatial transcriptomics datasets (~1 year timeline)
- Further optimization of Baysor to overcome computational limitations (very resource-intensive to run)
- Optimizing “computer vision” type models (i.e. Cellpose SAM which uses image learning) to determine “gold standard” segmentation methodology for application to spatial data with staining across multiple omics layers

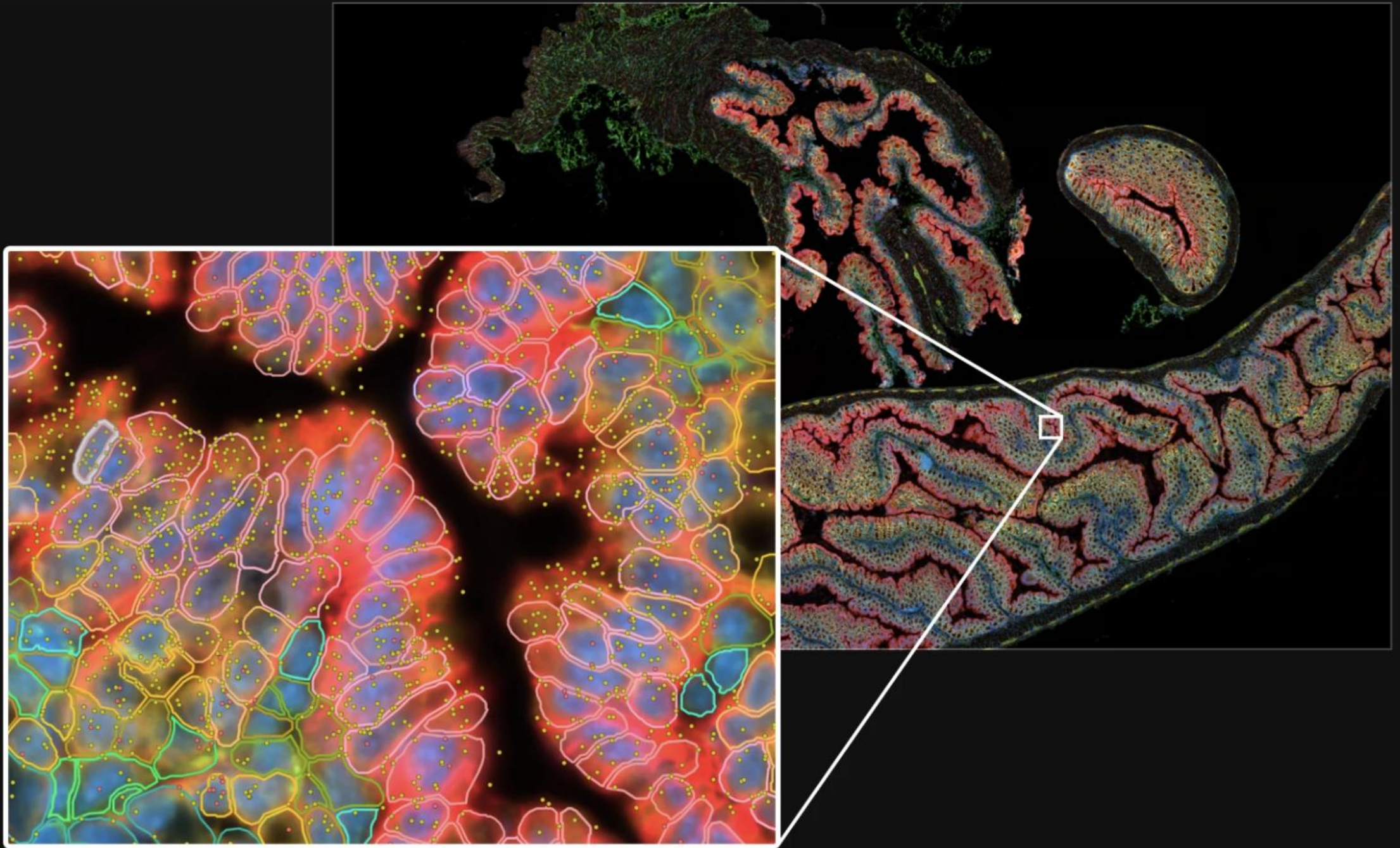
# Segmentation-free algorithm for Kidney ST data

Presenter: Lu Zhang

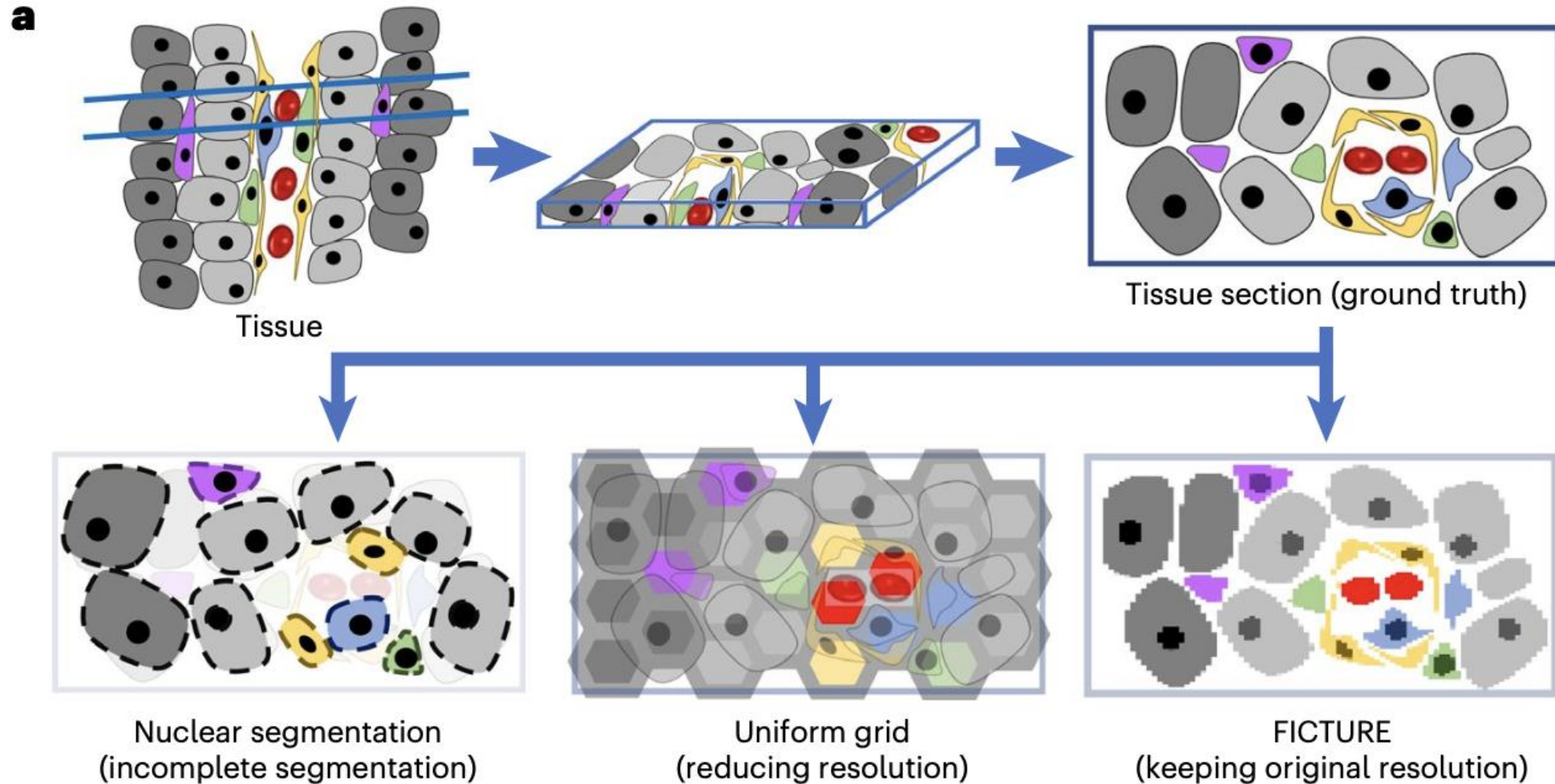
University of Southern California, Biostatistics Division

[lzhang63@usc.edu](mailto:lzhang63@usc.edu)





# The Bottleneck of Cell Segmentation

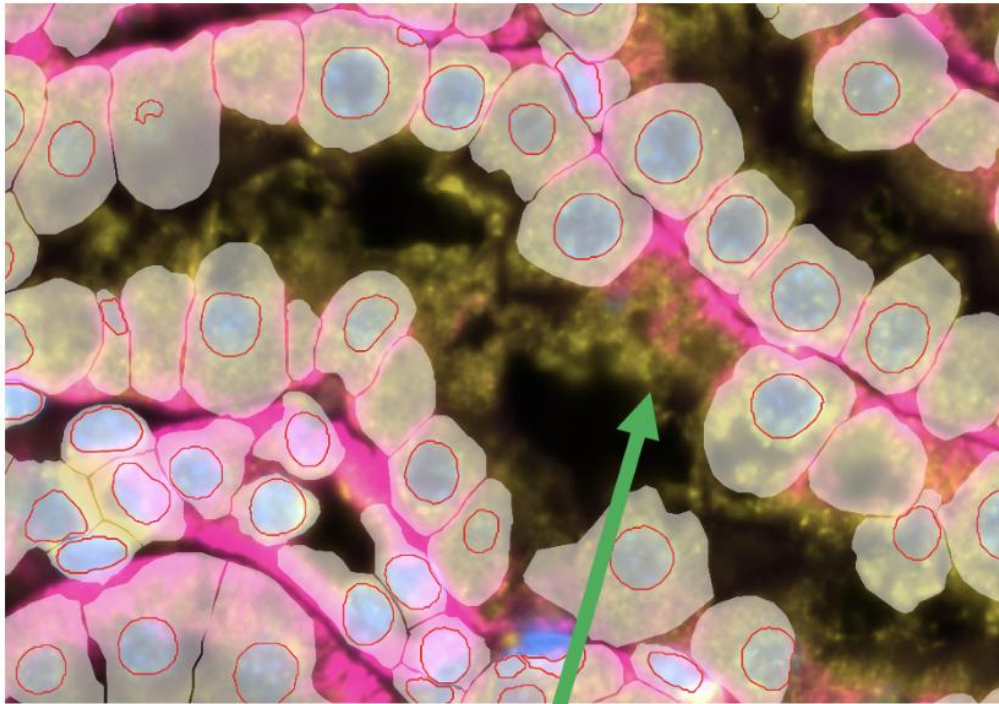


Source: Si et al., Nature Methods 2024

INTERNAL

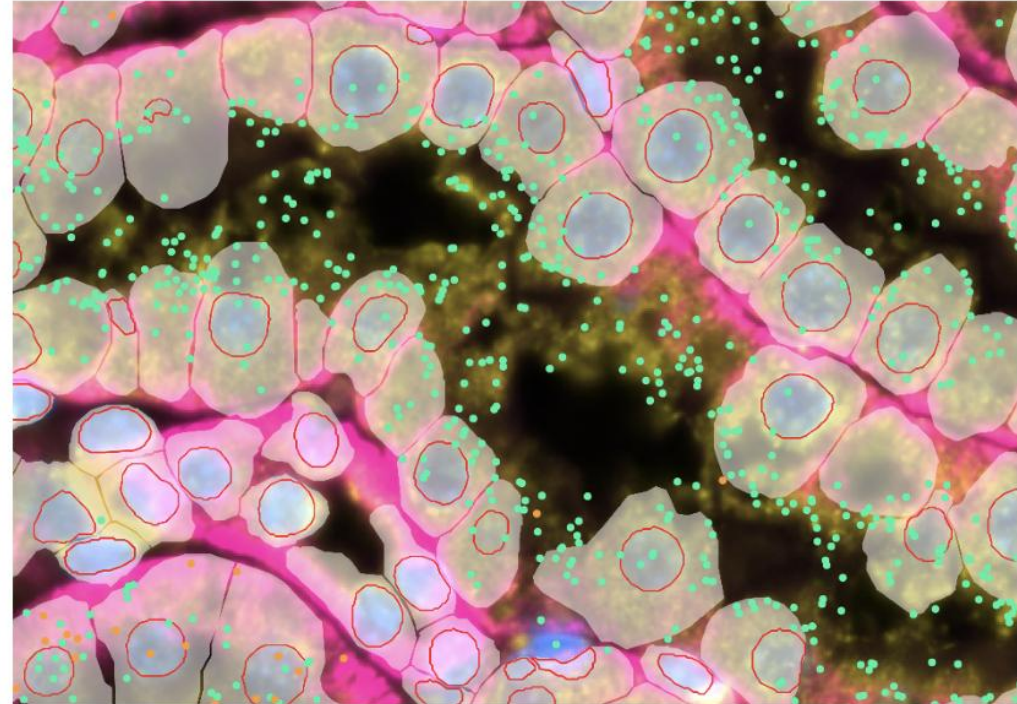
# Standard Segmentation is Sub-Optimal

Segmentation Algorithm



“Missing” Proximal Tubule Cell

Majority of *Lrp2* (key renal gene; blue dots) isn't registered to actual cells



**Summary**   Decoding   Cell Segmentation   Analysis   Image QC

## Key Metrics ?

155,086

Number of cells detected

540

Median transcripts per cell

785.9

Nuclear transcripts per 100  
 $\mu\text{m}^2$

121,304,221

Total high quality decoded  
transcripts

## Sample Region Summary

Region name

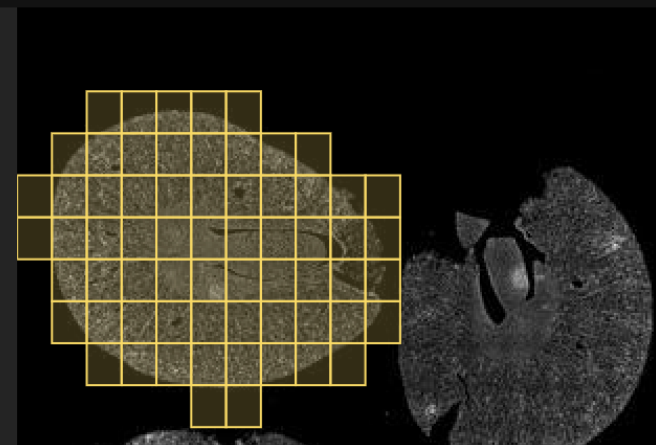
Region\_1

Slide ID

0054800

Cassette name

Mouse\_8\_kidneys



## Segmentation Metrics ?

Number of cells detected	<b>155,086</b>
Percent of transcripts within cells	<b>82.3%</b>
Cells per 100 $\mu\text{m}^2$	<b>0.546</b>
Median genes per cell	<b>362</b>
Median predesigned genes per cell	<b>352</b>
Median custom genes per cell	<b>11</b>
Median transcripts per cell	<b>540</b>

## Segmentation Methods ?

Cells segmented by boundary stain (ATP1A1/CD45/E-Cadherin)	<b>33,927 (21.9%)</b>
Cells segmented by interior RNA stain (18S)	<b>119,672 (77.2%)</b>
Cells segmented by nucleus expansion (5.0 $\mu\text{m}$ )	<b>1,487 (1.0%)</b>
Number of cells detected	<b>155,086 (100%)</b>

# The Segmentation-Free Paradigm

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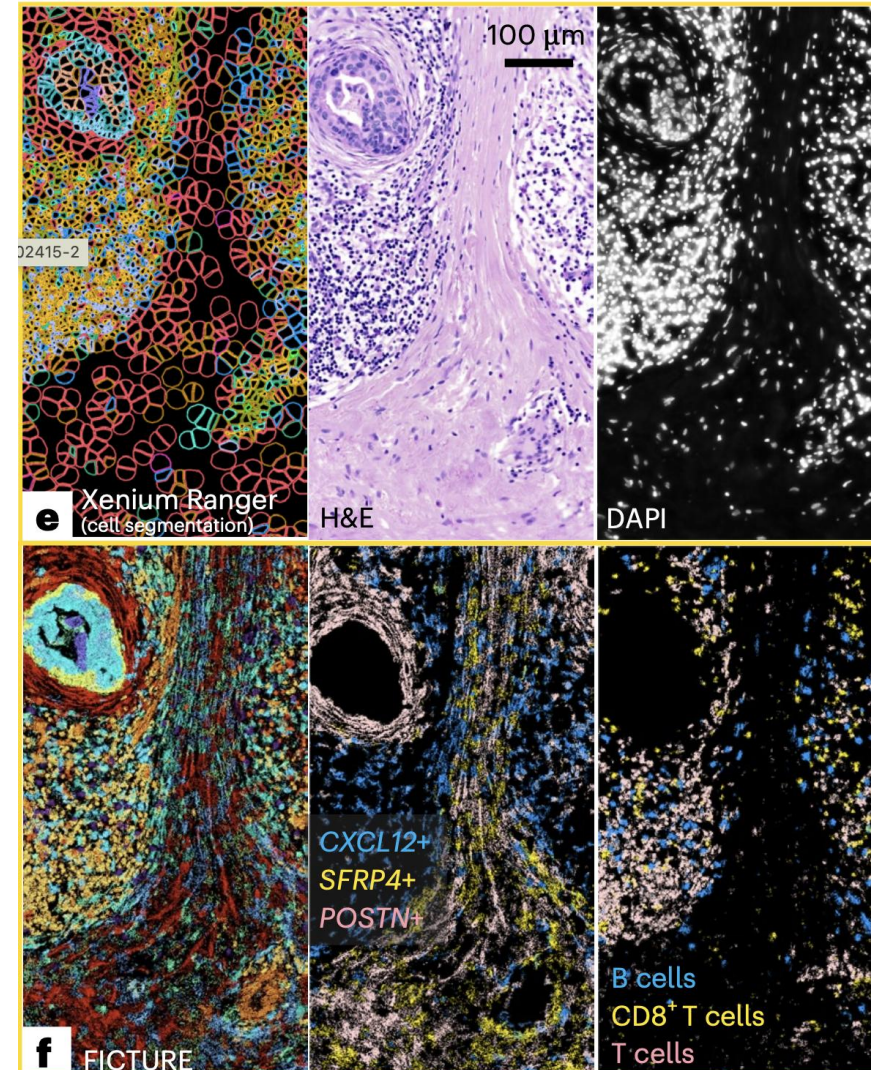
## Core Idea:

- Treat the tissue as a continuous field of gene expression or a set of spatial points rather than discrete cell objects.
- **General Workflow:**
  1. **Spatial Encoding:** Convert discrete spot coordinates into a continuous field (e.g., KDE) or grid features.
  2. **Feature Extraction:** Identify recurring transcriptional signatures (cell types) from local neighborhoods or pixel vectors.
  3. **Pixel/Spot Classification:** Assign specific cell types to spatial coordinates (pixels) rather than pre-defined segments.

# Advantages of Segmentation-Free Approaches

## Key Benefits:

- **Data Utilization:** Uses nearly 100% of detected transcripts.
- **Resolution:** Subcellular precision; captures cell shapes based on molecular diffusion.
- **Discovery:** Identifies cell types with low total counts or irregular shapes (e.g., astrocytes, fibroblasts).



# FIGTURE (Si et al., Nature Methods 2024)

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**Title:** FIGTURE: scalable segmentation-free analysis of submicron-resolution spatial transcriptomics

## Key Idea

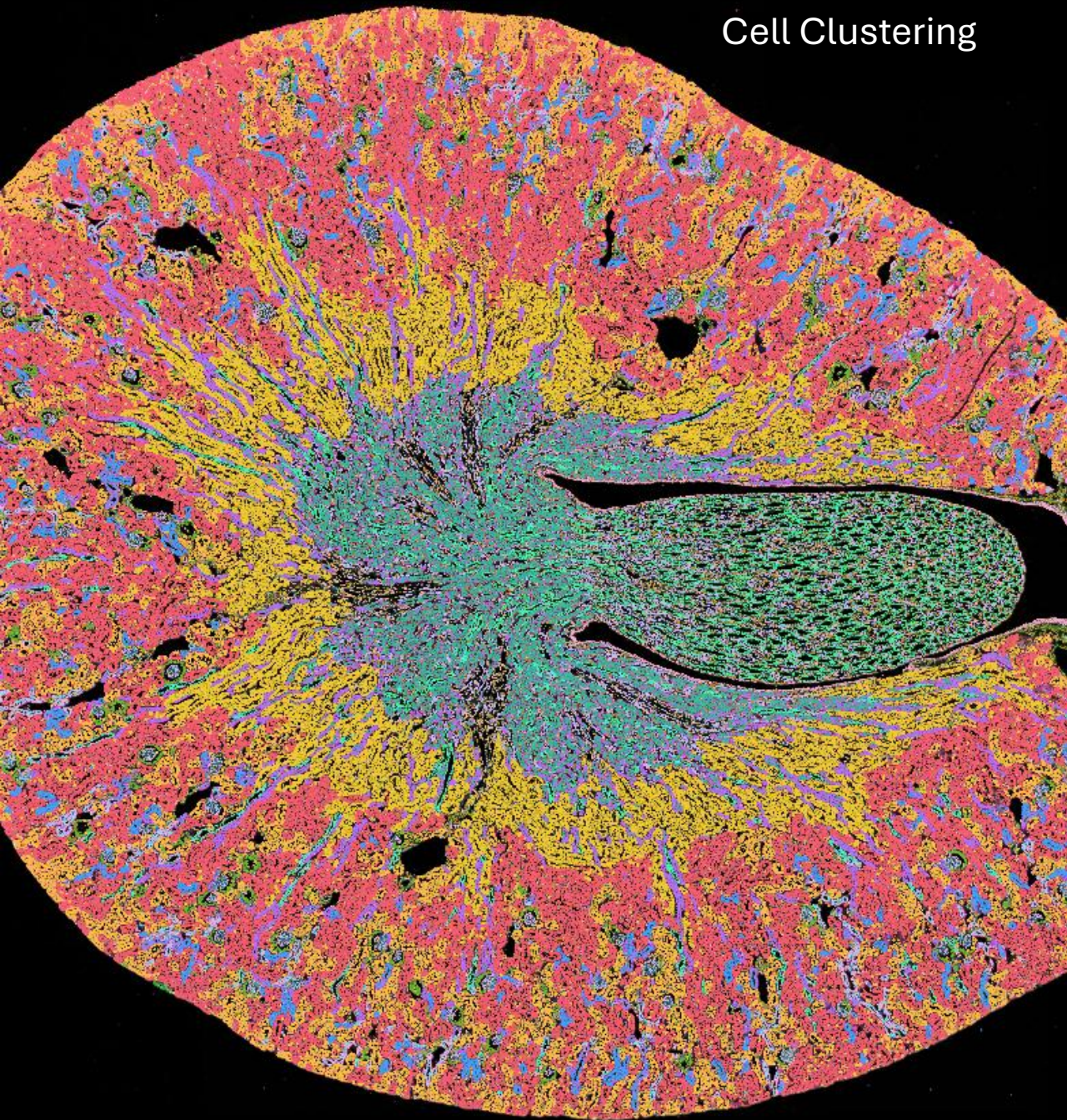
- Treat each pixel as a mixture of latent biological factors.

## Method

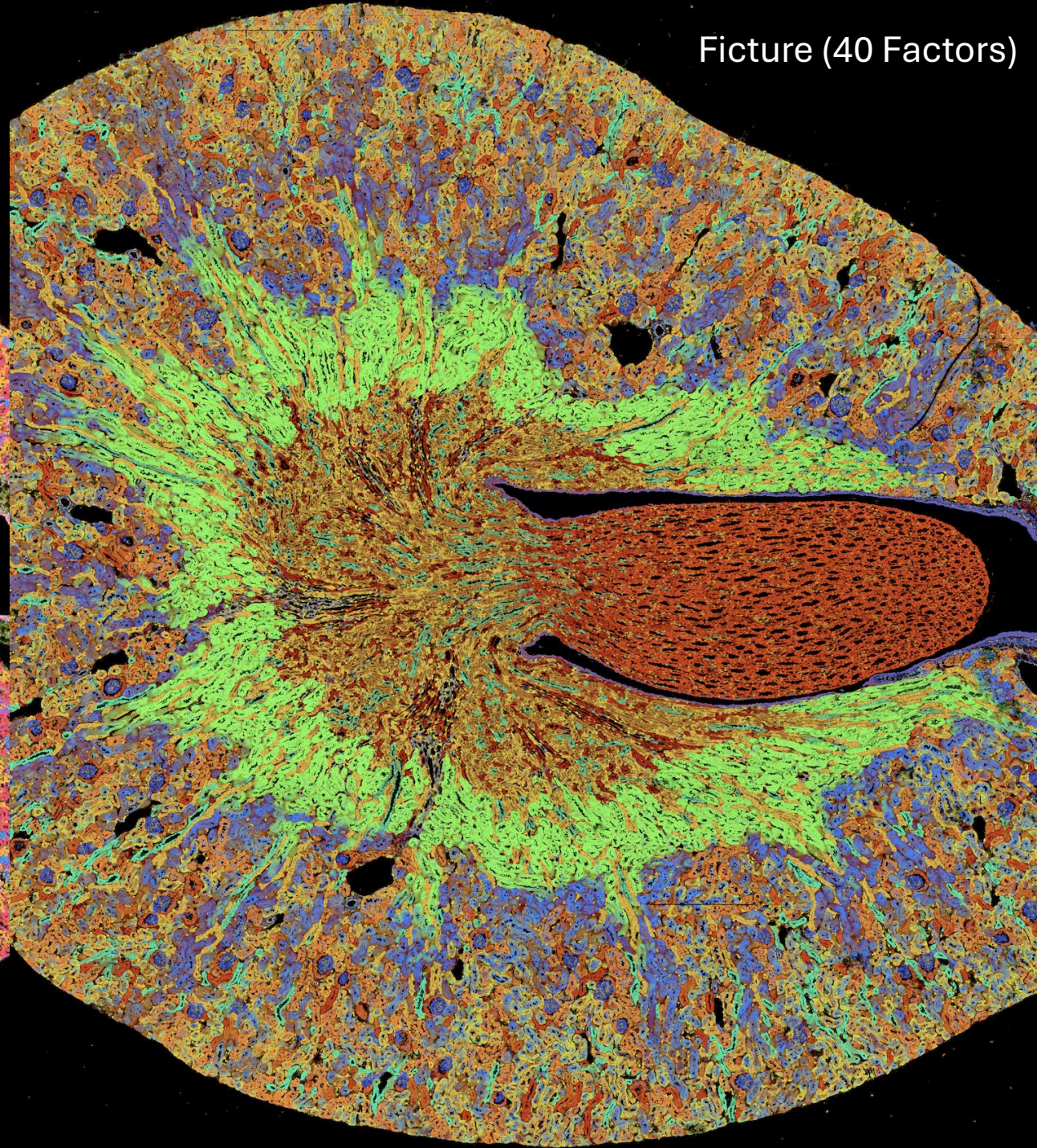
- Latent Dirichlet Allocation (LDA) models pixel-level gene expression.
- Spatial “anchors” aggregate local transcript information.

**Tutorial:** <https://seqscope.github.io/figure/>

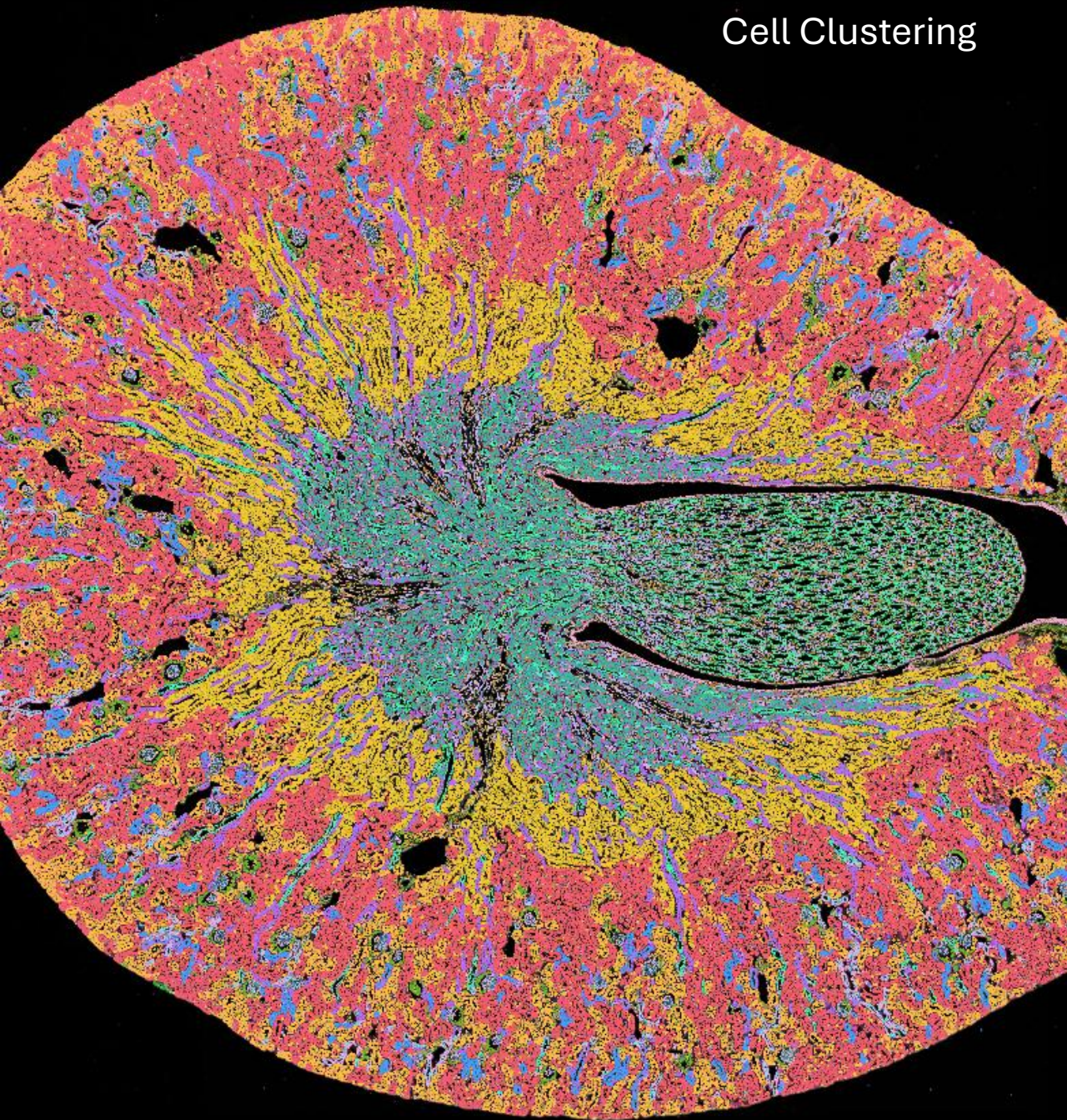
Cell Clustering



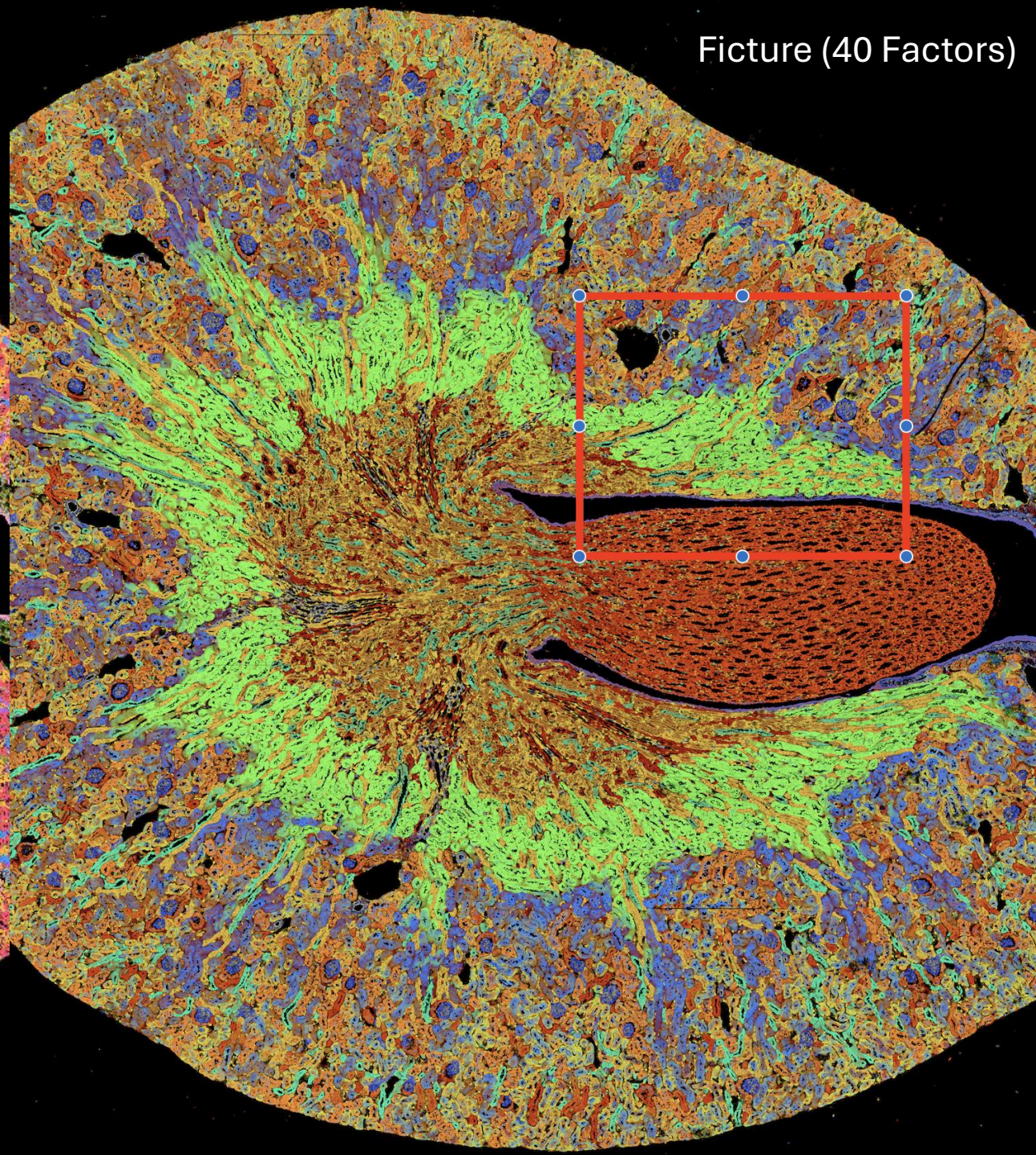
Picture (40 Factors)



Cell Clustering



Picture (40 Factors)



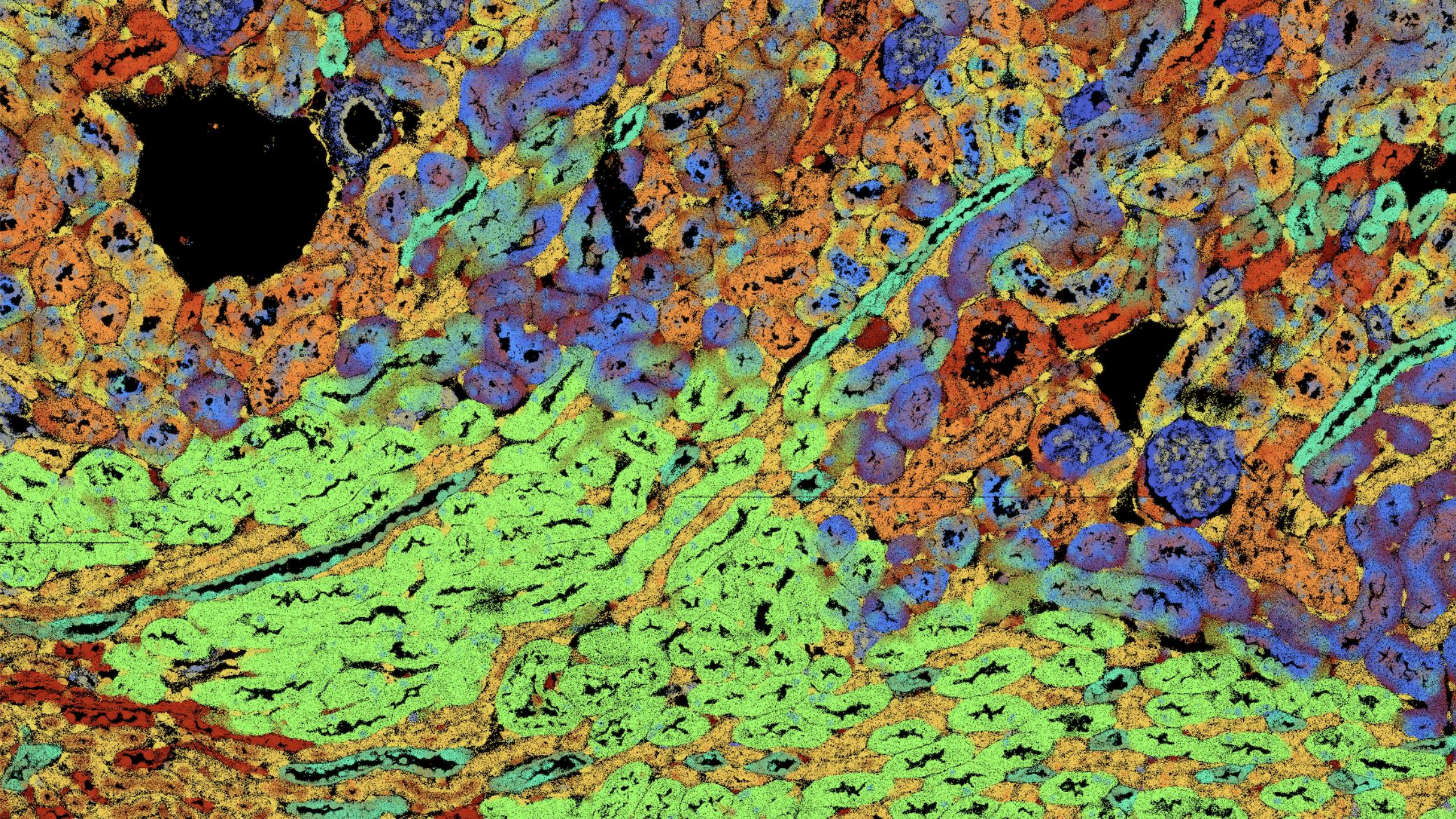


Figure Factor 0

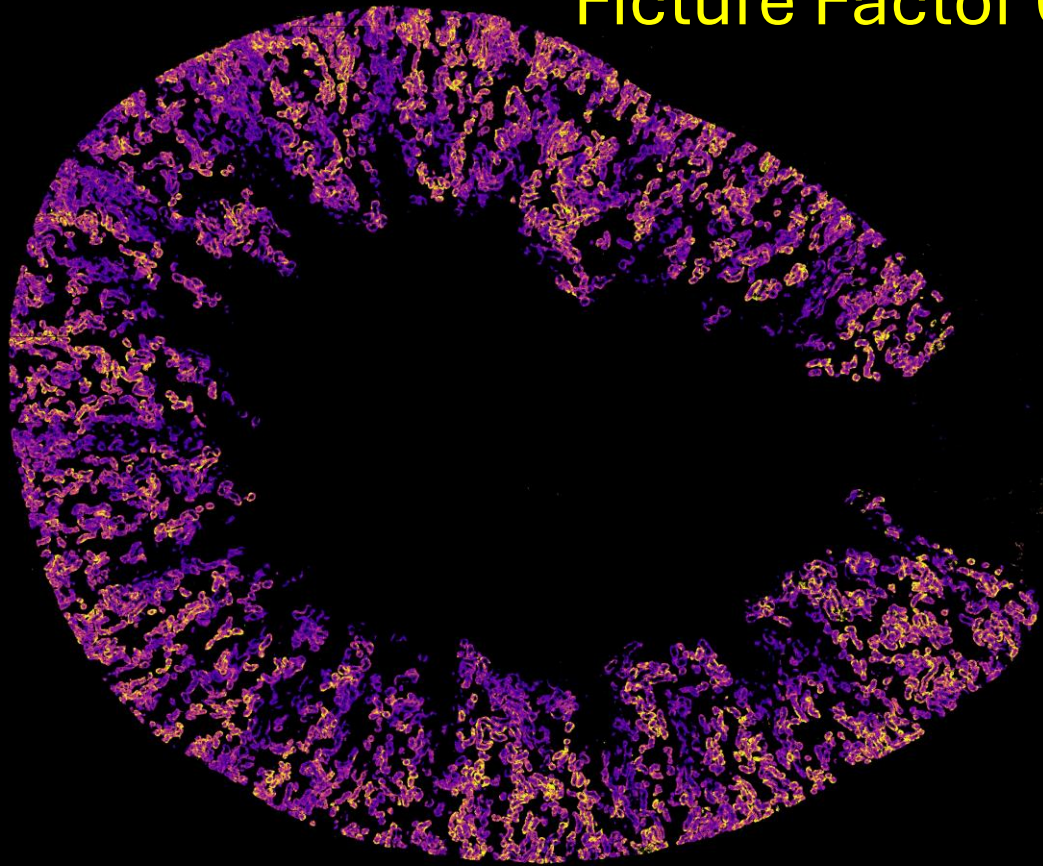
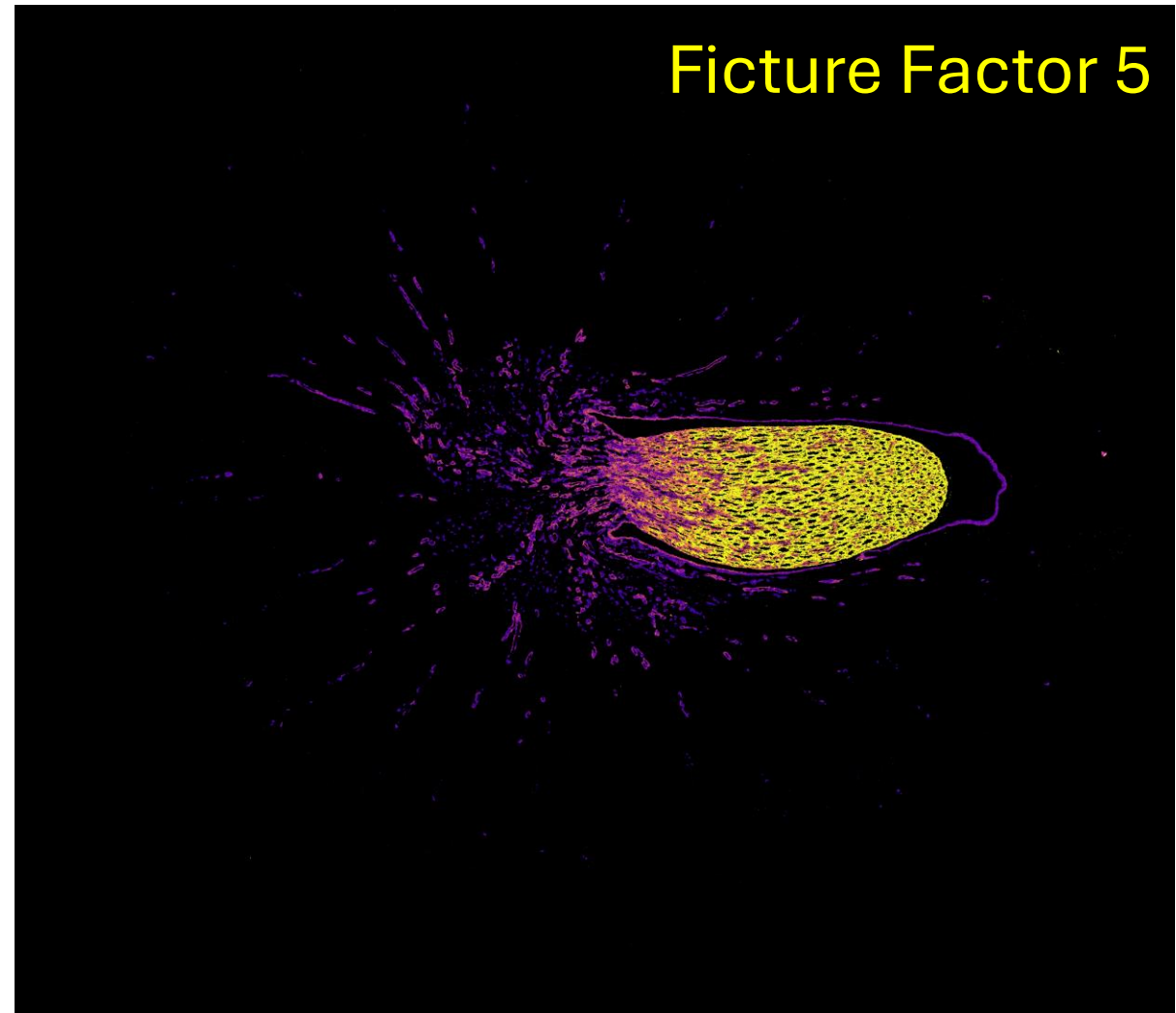


Figure Factor 5



# FIGTURE (Si et al., Nature Methods 2024)

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**Title:** FIGTURE: scalable segmentation-free analysis of submicron-resolution spatial transcriptomics

## Key Idea

- Treat each pixel as a mixture of latent biological factors.

## Method

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- Spatial “anchors” aggregate local transcript information.

**Tutorial:** <https://seqscope.github.io/figure/>

# Open Floor For Questions and Discussion

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# Looking Towards the Future..

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# Looking Towards the Future..

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WE WANT TO  
HEAR FROM YOU



# Shaping AIMS@USC's Future

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- **What Should We Discuss in Future “Spatial Summit” Meetings?**
- **Should we host coding “hackathons” to solve pressing questions?**
- **Should we host a bootcamp to teach spatial analysis?**
- **Should we form a “spatial analysis core” to support researchers?**
- **Should we publish practical spatial analysis guides online?**

# Looking Towards the Future...

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## AIMS@USC presents: **The Spatial Summit**

### **The Spatial Summit Survey**

Thank you for attending the AIMS@USC Spatial Summit. We truly appreciate you taking the time to join us and contribute to the discussion. Your thoughtful and honest feedback is invaluable as we plan future events, resources, and initiatives for the AIMS@USC community. The short survey below will help us understand what was most useful, what could be improved, and how we can best support spatial research across USC moving forward.

