

ISB in collaboration with the NCI Cancer Systems Biology Consortium and MC2 Centers presents a free virtual workshop

# Spatial Multi-Omics for Cancer Systems Biology

Monday, February 12 from 8 - 3:15 PST



Learn more and register  
[isbscience.org/spatial-multiomics-workshop](https://isbscience.org/spatial-multiomics-workshop)  
or scan the QR code

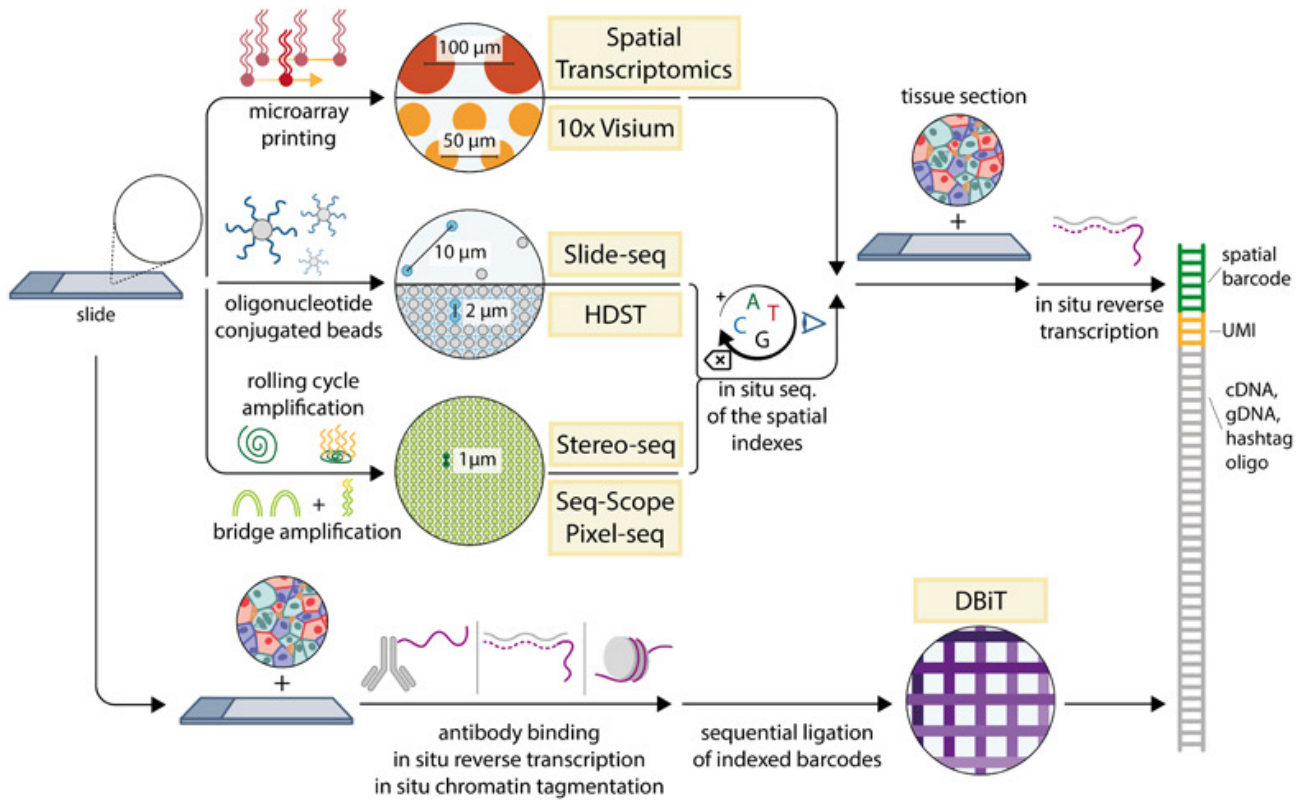


# Practical Methods in Spatial-Omics

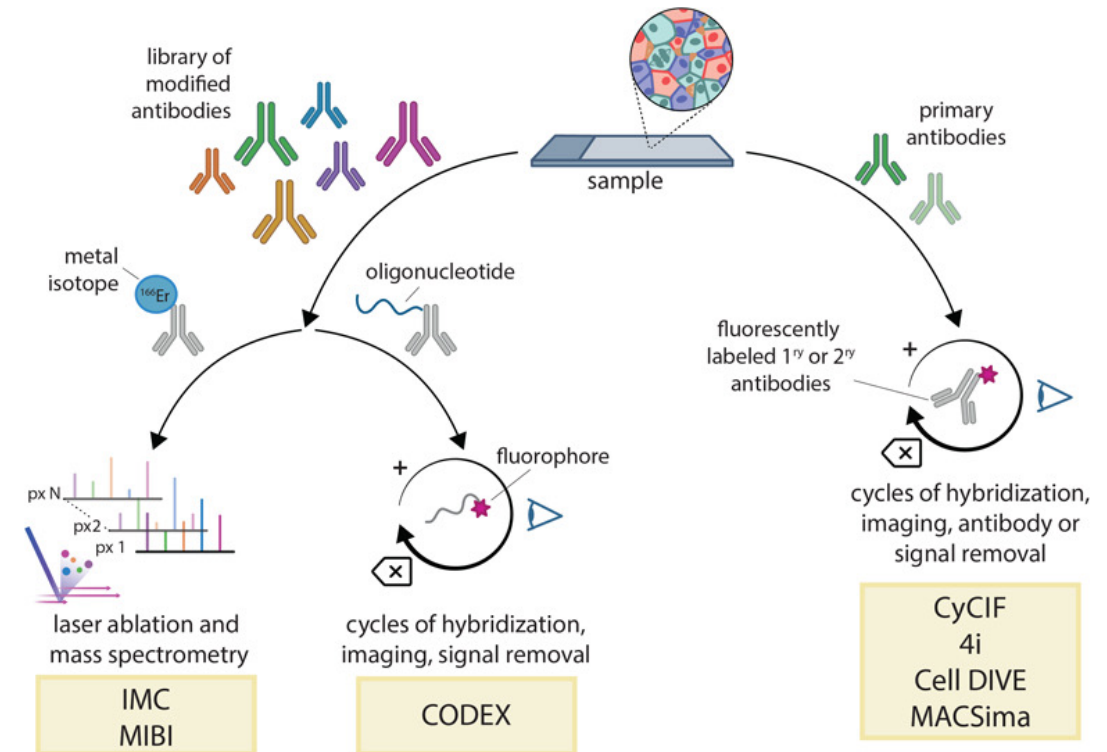
Shuozhen Bao  
Alev Baysoy  
Dr. Rong Fan Lab  
Yale University

# Common Spatial Technologies

## Sequence-based



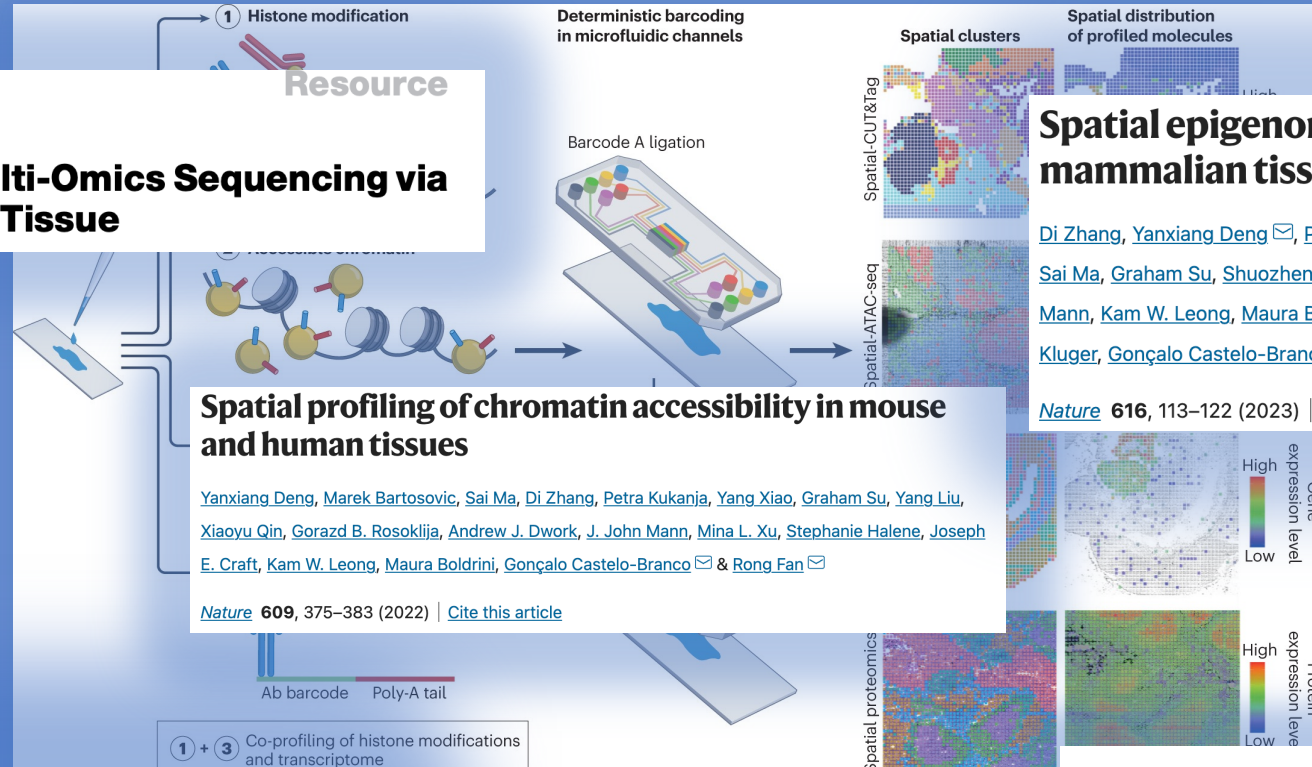
## Image-based



Bressan D. et al. The dawn of spatial omics. *Science*. 2023 Aug 4;381(6657):eabq4964. doi: 10.1126/science.abq4964.  
 For more info on spatial transcriptomics: <https://spatialomics.net/talks/>

# Deterministic Barcoding in-tissue (DBiT-seq)

Broad applications of different modalities



Cell

## High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue

### Spatial profiling of chromatin accessibility in mouse and human tissues

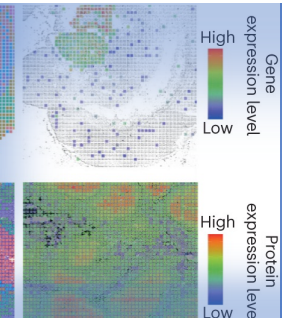
[Yanxiang Deng](#), [Marek Bartosovic](#), [Sai Ma](#), [Di Zhang](#), [Petra Kukanja](#), [Yang Xiao](#), [Graham Su](#), [Yang Liu](#), [Xiaoyu Qin](#), [Gorazd B. Rosoklija](#), [Andrew J. Dwork](#), [J. John Mann](#), [Mina L. Xu](#), [Stephanie Halene](#), [Joseph E. Craft](#), [Kam W. Leong](#), [Maura Boldrini](#), [Gonçalo Castelo-Branco](#) & [Rong Fan](#)

*Nature* **609**, 375–383 (2022) | [Cite this article](#)

### Spatial epigenome–transcriptome co-profiling of mammalian tissues

[Di Zhang](#), [Yanxiang Deng](#), [Petra Kukanja](#), [Eneritz Agirre](#), [Marek Bartosovic](#), [Mingze Dong](#), [Cong Ma](#), [Sai Ma](#), [Graham Su](#), [Shuozen Bao](#), [Yang Liu](#), [Yang Xiao](#), [Gorazd B. Rosoklija](#), [Andrew J. Dwork](#), [J. John Mann](#), [Kam W. Leong](#), [Maura Boldrini](#), [Liya Wang](#), [Maximilian Haeussler](#), [Benjamin J. Raphael](#), [Yuval Kluger](#), [Gonçalo Castelo-Branco](#) & [Rong Fan](#)

*Nature* **616**, 113–122 (2023) | [Cite this article](#)



### High-plex protein and whole transcriptome co-mapping at cellular resolution with spatial CITE-seq

[Yang Liu](#), [Marcello DiStasio](#), [Graham Su](#), [Hiromitsu Asashima](#), [Archibald Enniful](#), [Xiaoyu Qin](#), [Yanxiang Deng](#), [Jungmin Nam](#), [Fu Gao](#), [Pino Bordignon](#), [Marco Cassano](#), [Mary Tomayko](#), [Mina Xu](#), [Stephanie Halene](#), [Joseph E. Craft](#), [David Hafler](#) & [Rong Fan](#)

*Nature Biotechnology* (2023) | [Cite this article](#)

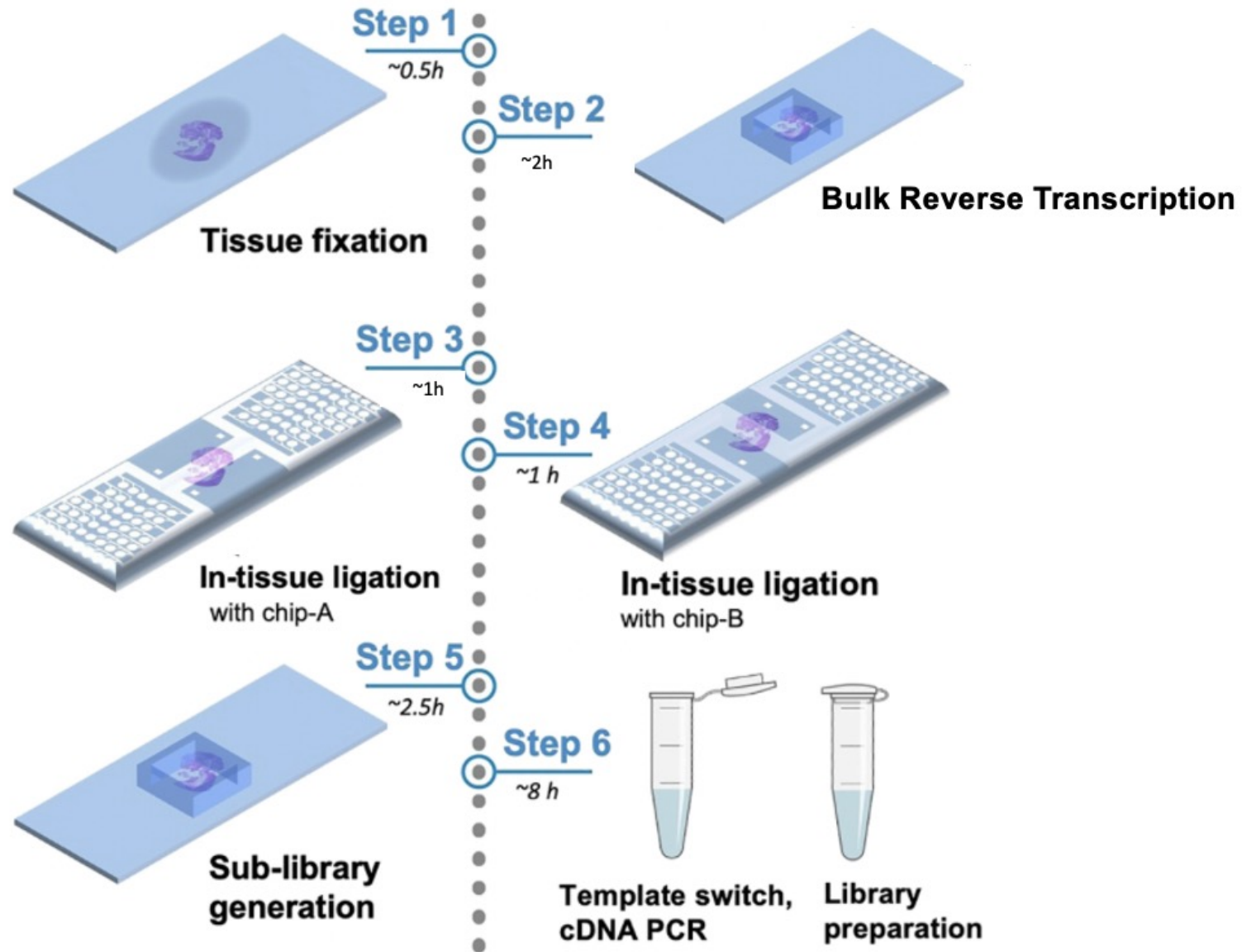
Deng, Y., Bai, Z. & Fan, R. *Nat Rev Bio*

> *Science*. 2022 Feb 11;375(6581):681–686. doi: 10.1126/science.abg7216. Epub 2022 Feb 10.

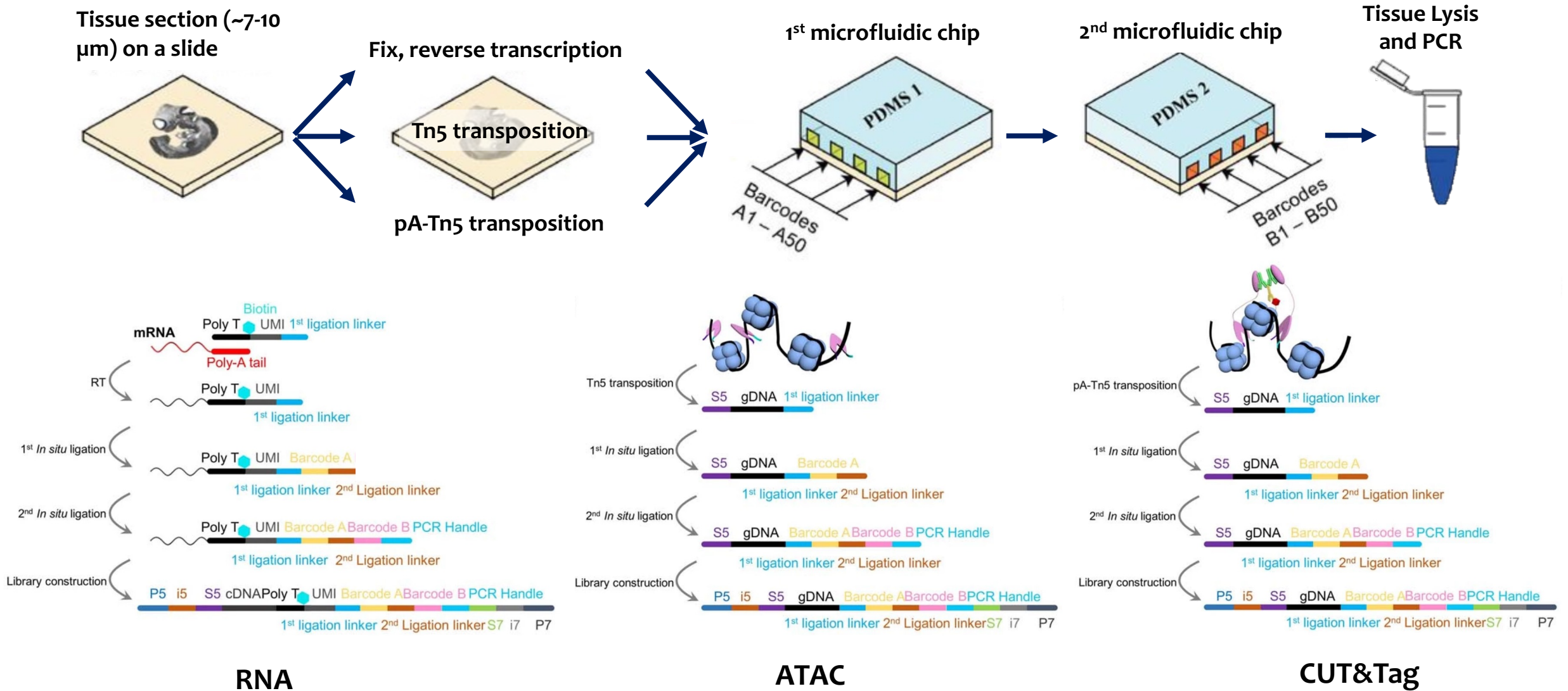
### Spatial-CUT&Tag: Spatially resolved chromatin modification profiling at the cellular level

[Yanxiang Deng](#)<sup>1,2</sup>, [Marek Bartosovic](#)<sup>3</sup>, [Petra Kukanja](#)<sup>3</sup>, [Di Zhang](#)<sup>1</sup>, [Yang Liu](#)<sup>1,2</sup>, [Graham Su](#)<sup>1,2</sup>, [Archibald Enniful](#)<sup>1,2</sup>, [Zhiliang Bai](#)<sup>1</sup>, [Gonçalo Castelo-Branco](#)<sup>3,4</sup>, [Rong Fan](#)<sup>1,2,5</sup>

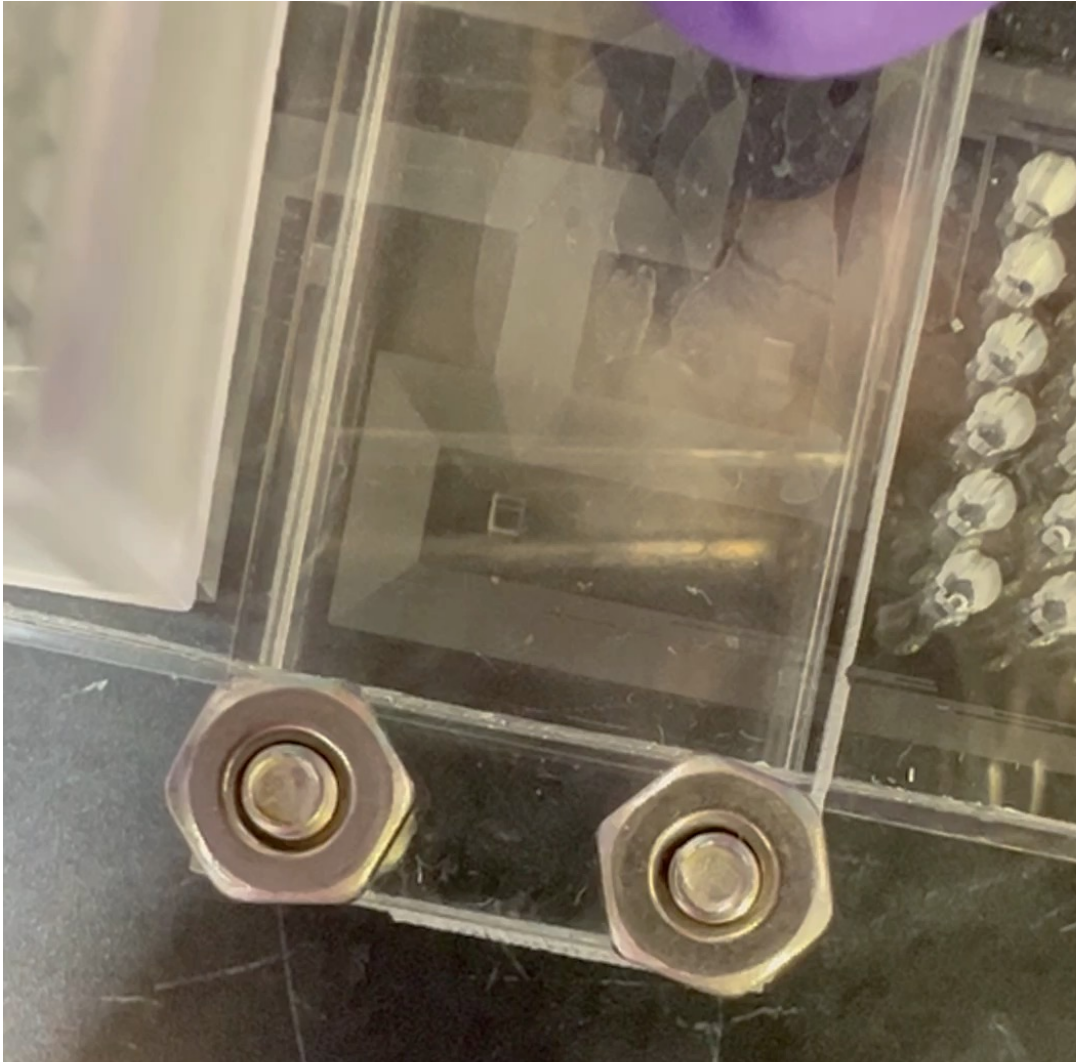
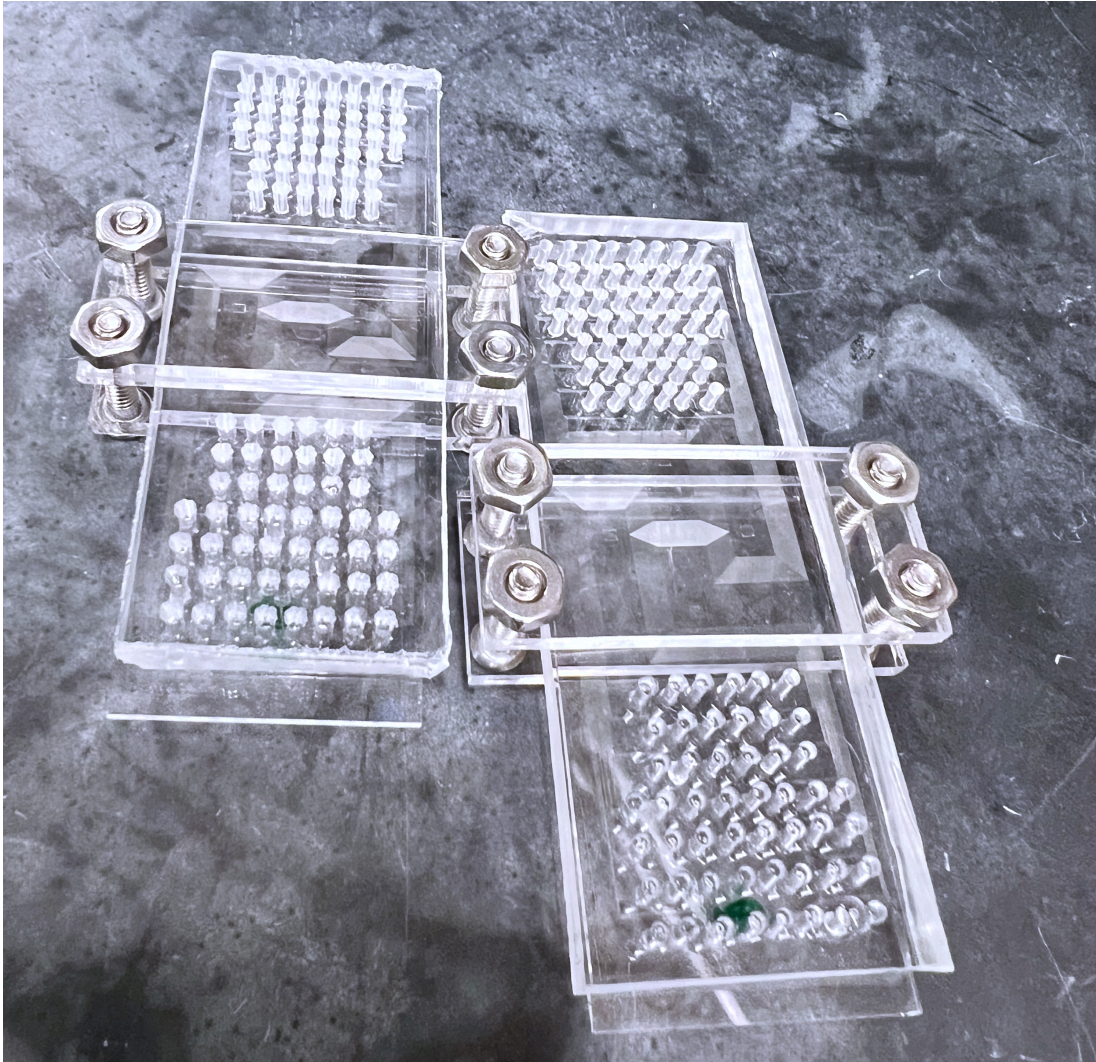
# Experiment Workflow for DBiT



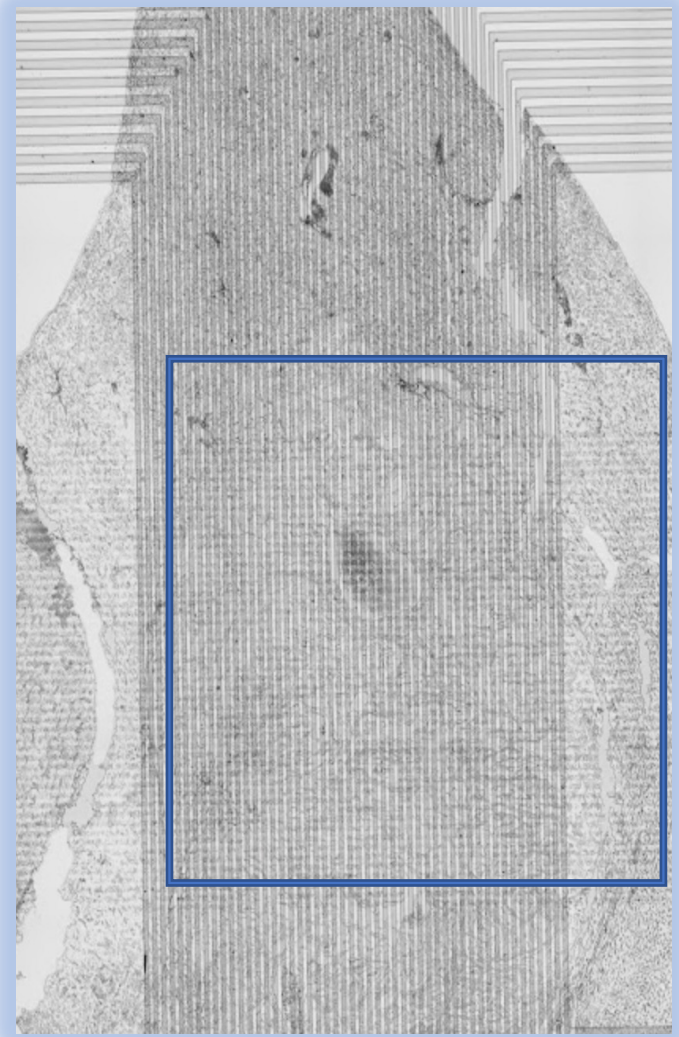
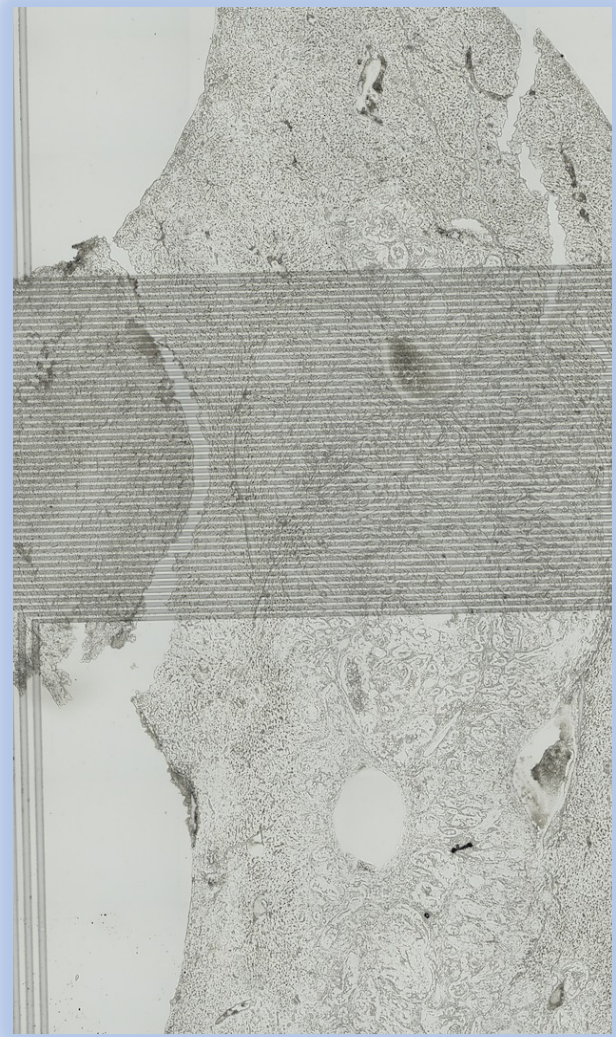
# Experiment Workflow for DBiT



# DBiT Barcode Flow

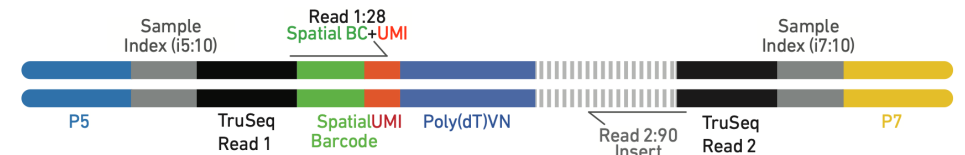
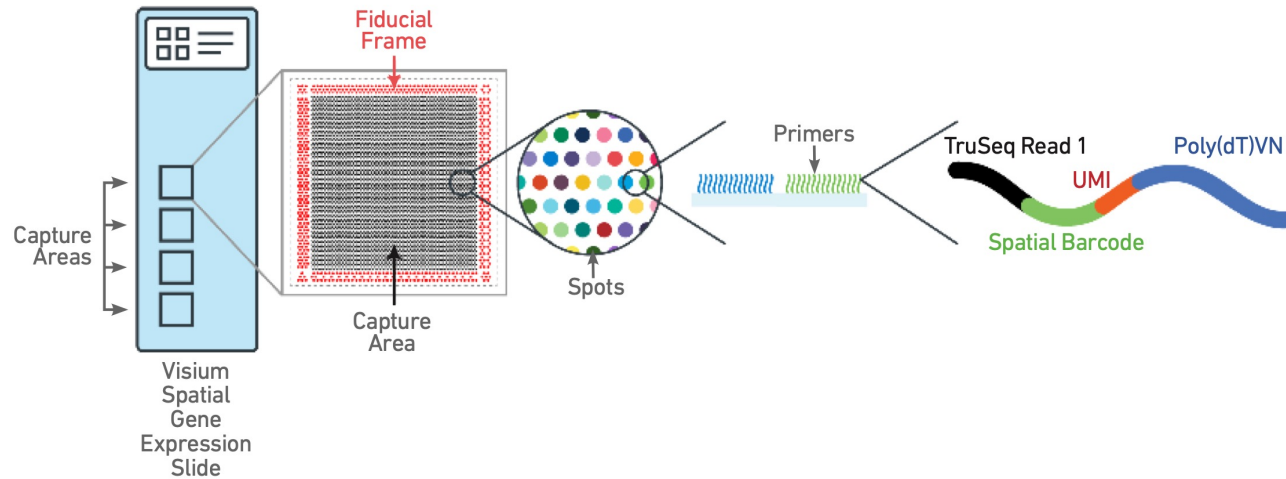
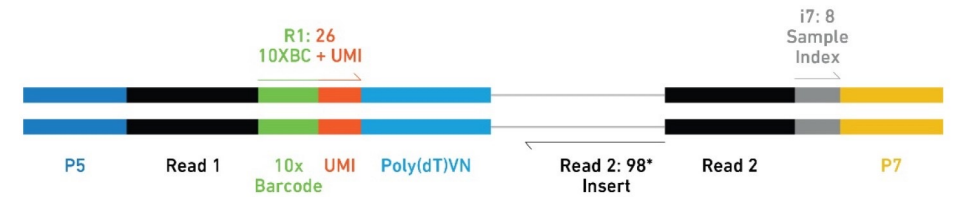
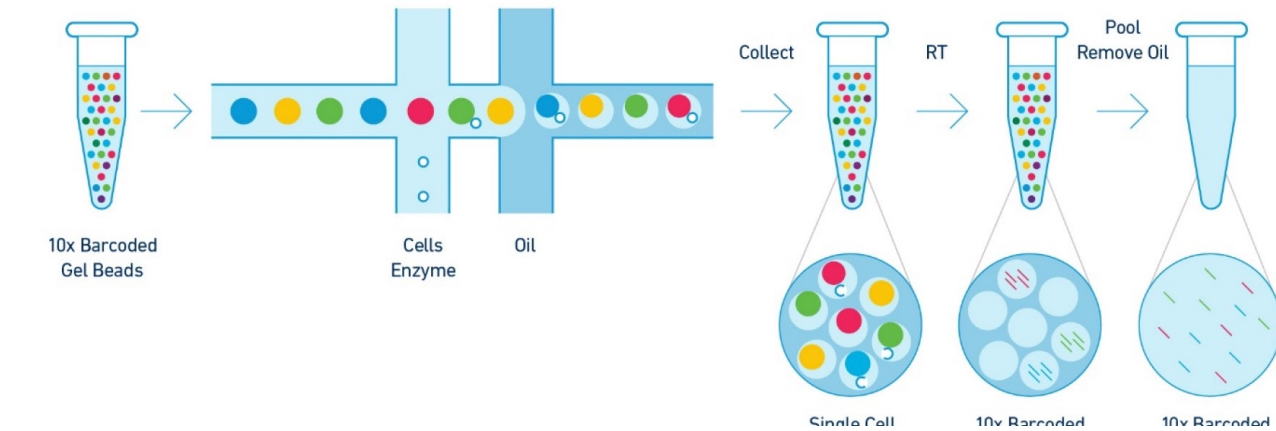


# DBiT Chip A and Chip B Scan





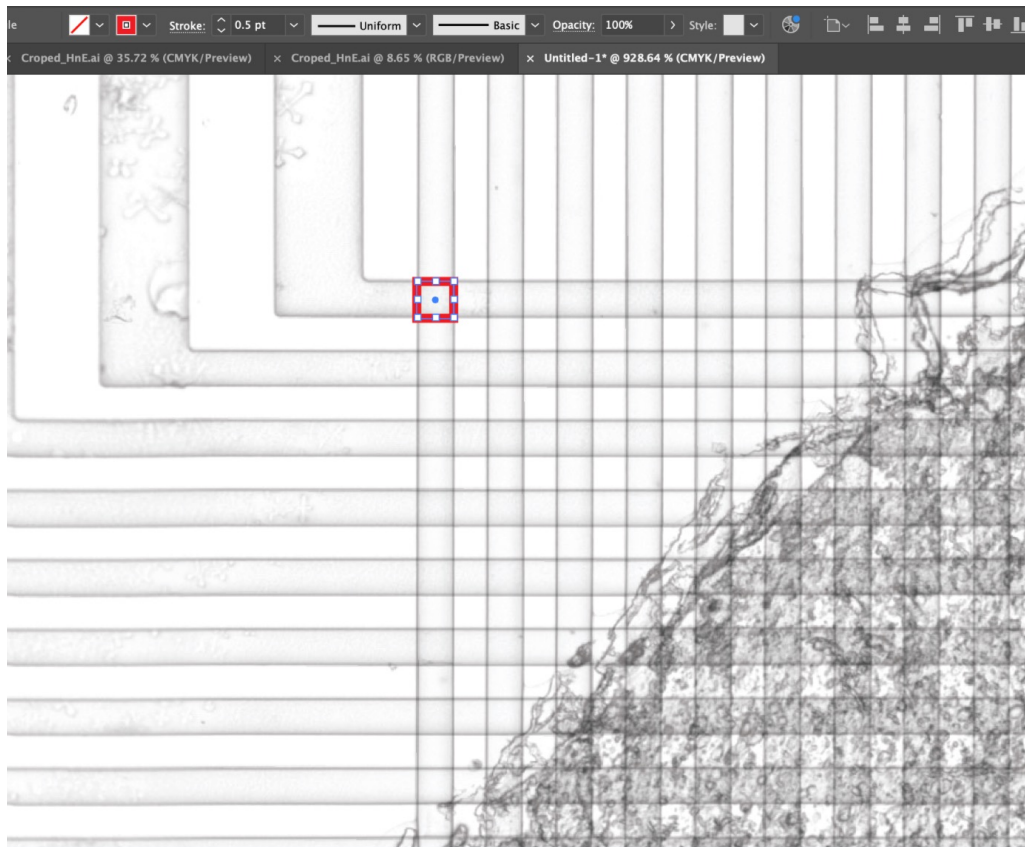
# Sequencing-based: From Single-cell to Spatial



# Spatial Information Extraction

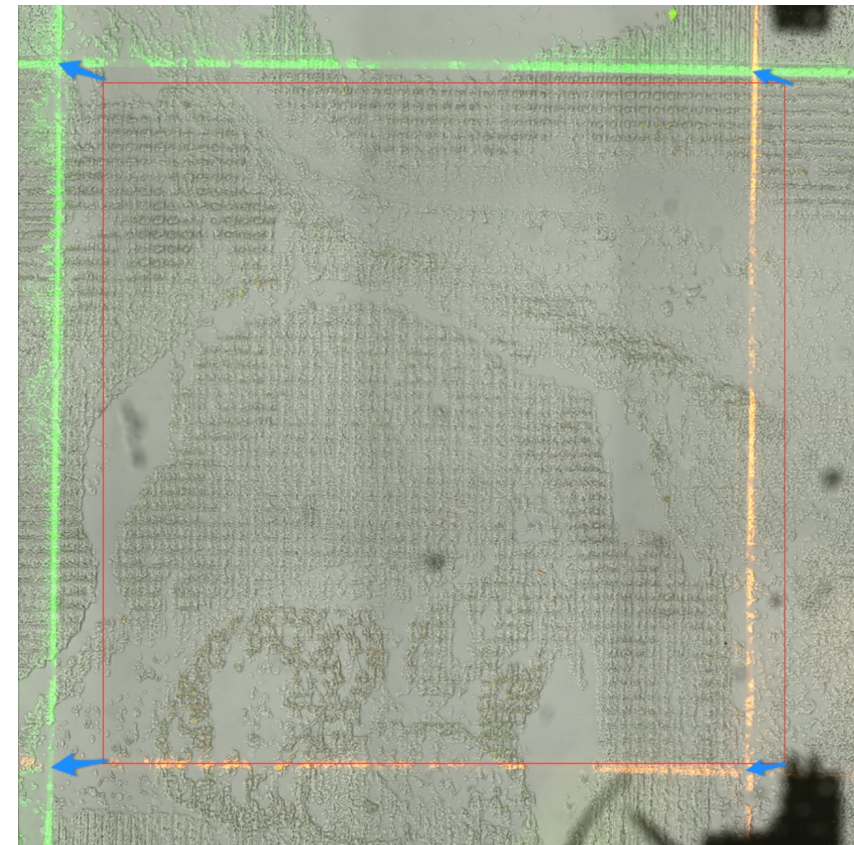
Manually

Adobe Illustrator + Python



Software

AtlasXomics Browser



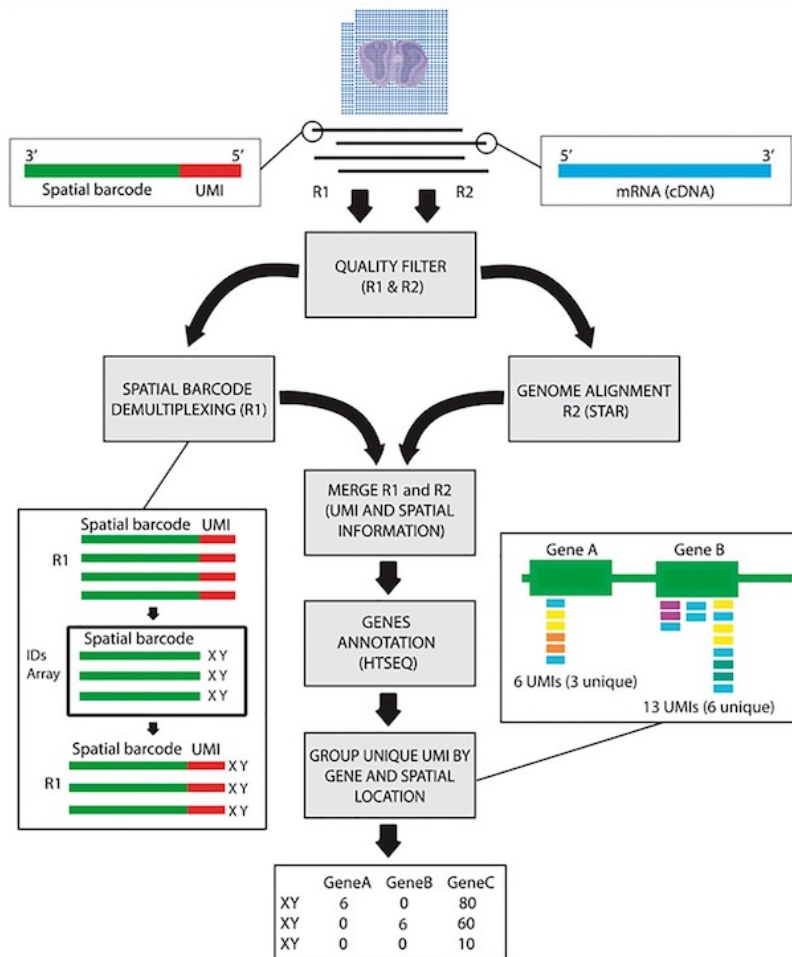
[https://github.com/ShuoZhen/2024\\_ISB\\_workshop\\_Practical\\_Methods\\_in\\_Spatial-Omics/tree/main/Spatial\\_folder\\_generation](https://github.com/ShuoZhen/2024_ISB_workshop_Practical_Methods_in_Spatial-Omics/tree/main/Spatial_folder_generation)

<https://docs.atlasxomics.com/projects/AtlasXbrowser/en/latest/Overview.html>

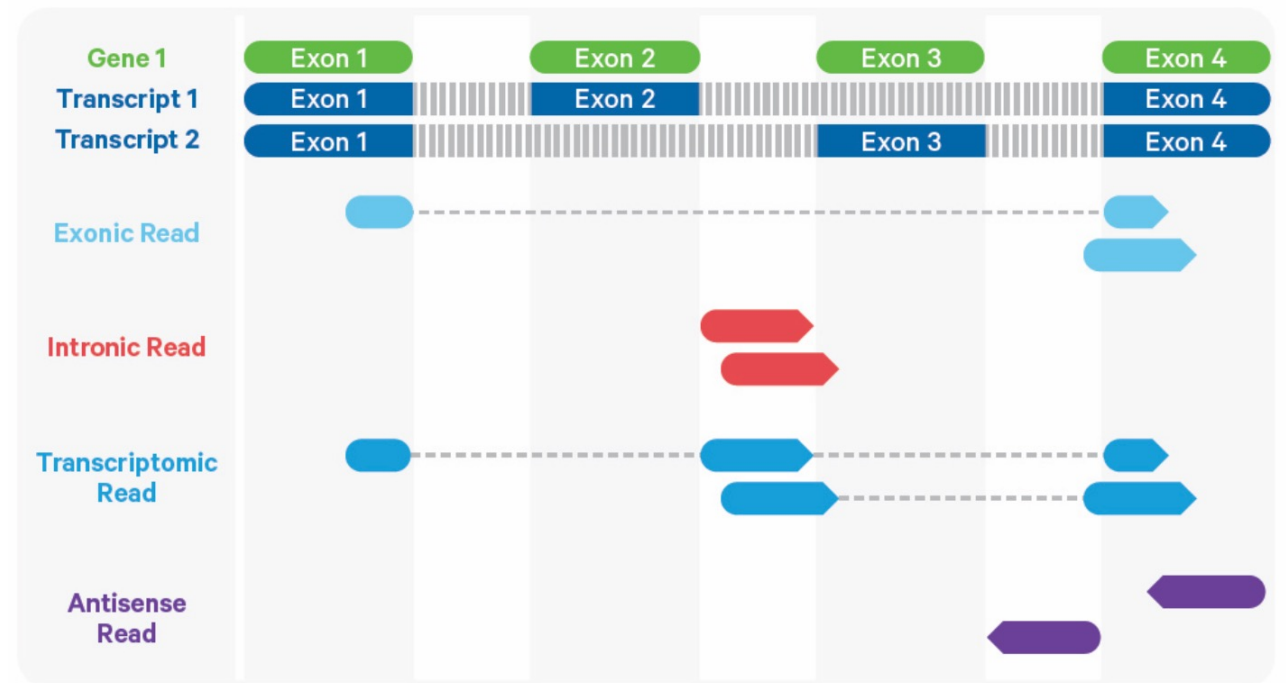


# Data Pre-processing -> Gene Matrix or Fragments.tsv

ST Pipeline



Cell Ranger



Both use STAR for genome alignment

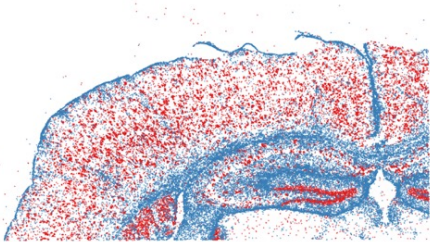
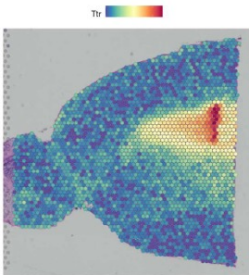
[https://github.com/jfnavarro/st\\_pipeline](https://github.com/jfnavarro/st_pipeline)

<https://www.10xgenomics.com/cn/support/software/cell-ranger/latest/algorithms-overview/cr-gex-algorithm>

# Downstream Data Analysis - RNA

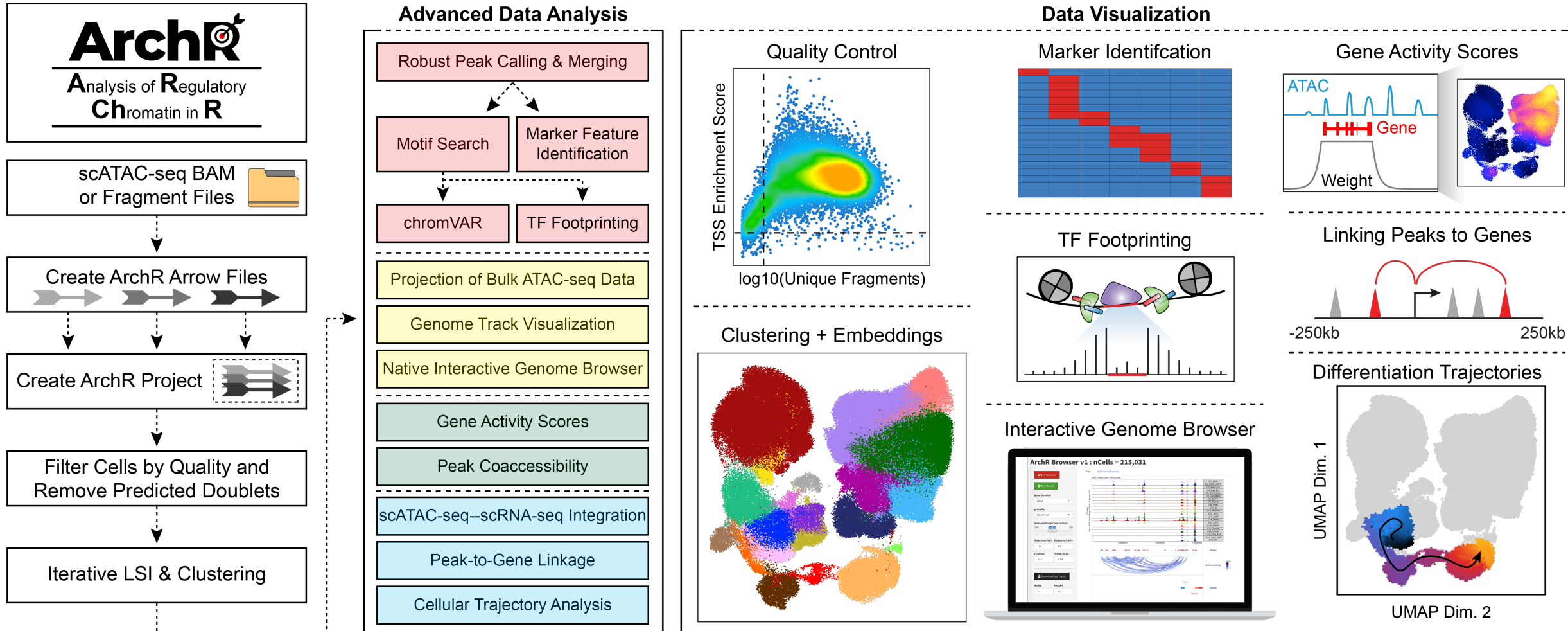
## Spatial analysis

These vignettes will help introduce users to the analysis of spatial datasets in Seurat v5, including technologies that leverage sequencing-based readouts, as well as technologies that leverage in-situ imaging-based readouts. The vignettes introduce data from multiple platforms including 10x Visium, SLIDE-seq, Vizgen MERSCOPE, 10x Xenium, Nanostring CosMx, and Akoya CODEX.

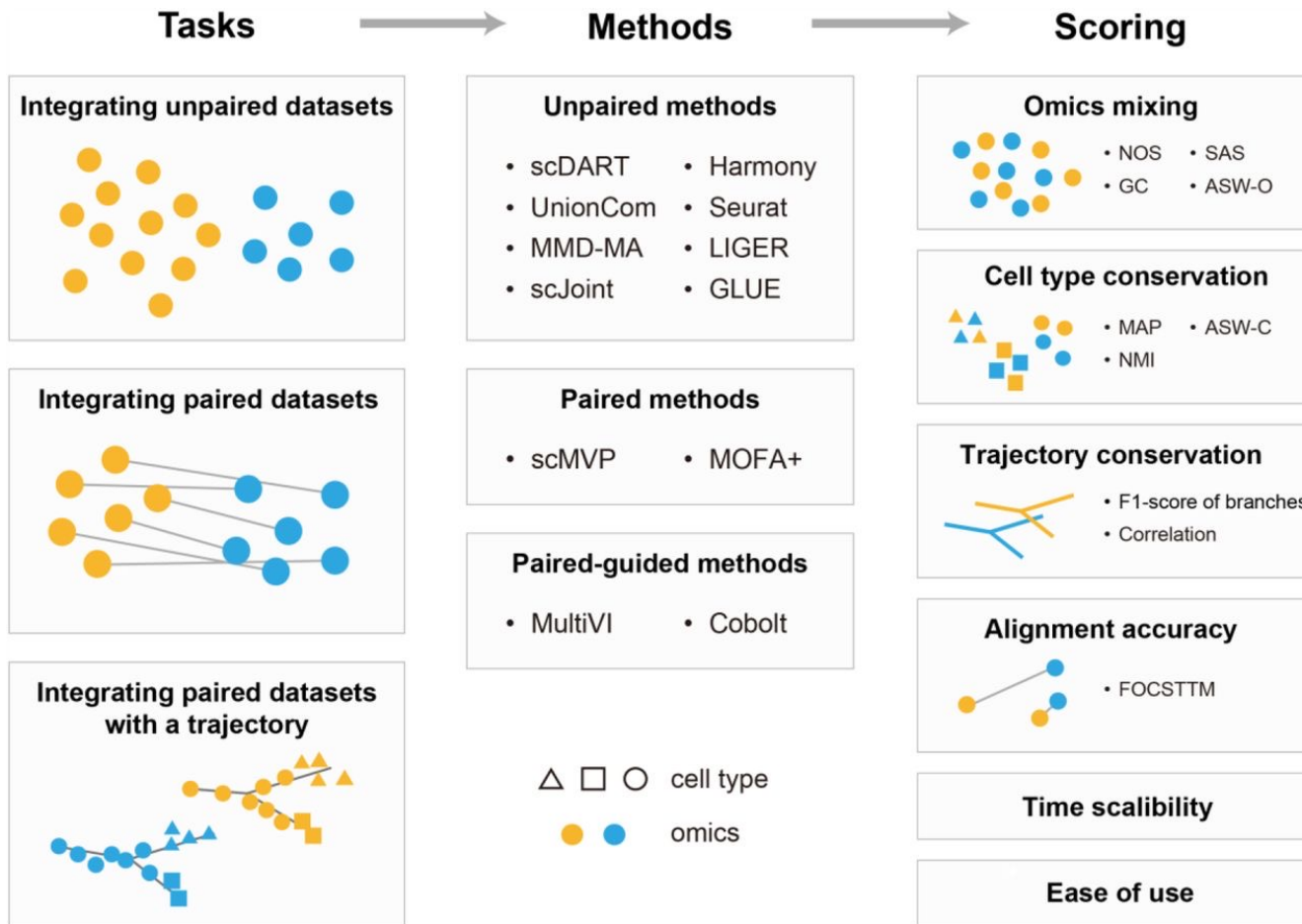
<p><u><a href="#">Analysis of spatial datasets (Imaging-based)</a></u></p>  <p>Learn to explore spatially-resolved data from multiplexed imaging technologies, including MERSCOPE, Xenium, CosMx SMI, and CODEX.</p> <p>GO</p>	<p><u><a href="#">Analysis of spatial datasets (Sequencing-based)</a></u></p>  <p>Learn to explore spatially-resolved transcriptomic data with examples from 10x Visium and Slide-seq v2.</p> <p>GO</p>
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- Spatial physical context
  - Localized analysis
  - Pseudo-time analysis
- Like Single-cell analysis
  - **Not exact single-cell**
  - Demultiplexing

# Downstream Data Analysis – ATAC/CUT&Tag

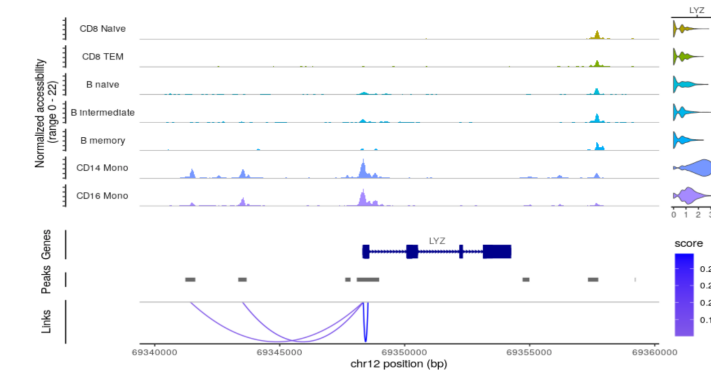


# Downstream Data Analysis – Multi-omics

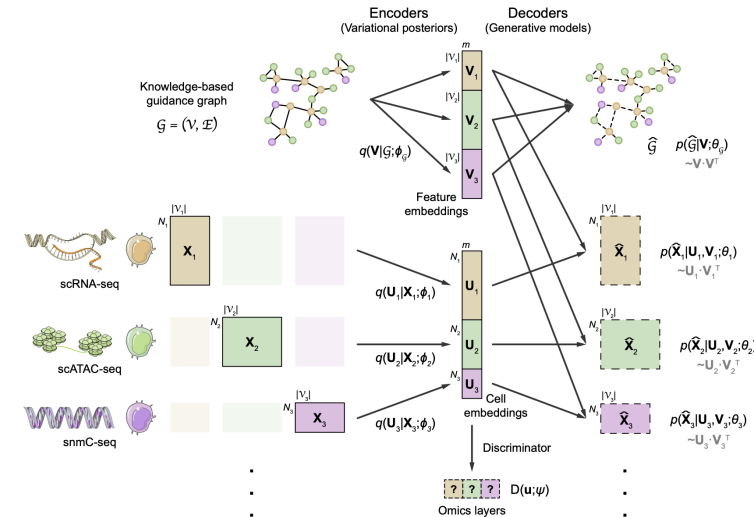


## Joint scRNA-seq and scATAC-seq analysis: 10x Multiomic

In this tutorial we demonstrate a joint analysis of combined gene expression and DNA accessibility data, measured in the same human PBMCs using the 10x Genomics multiomic kit.



Signac



scGLUE

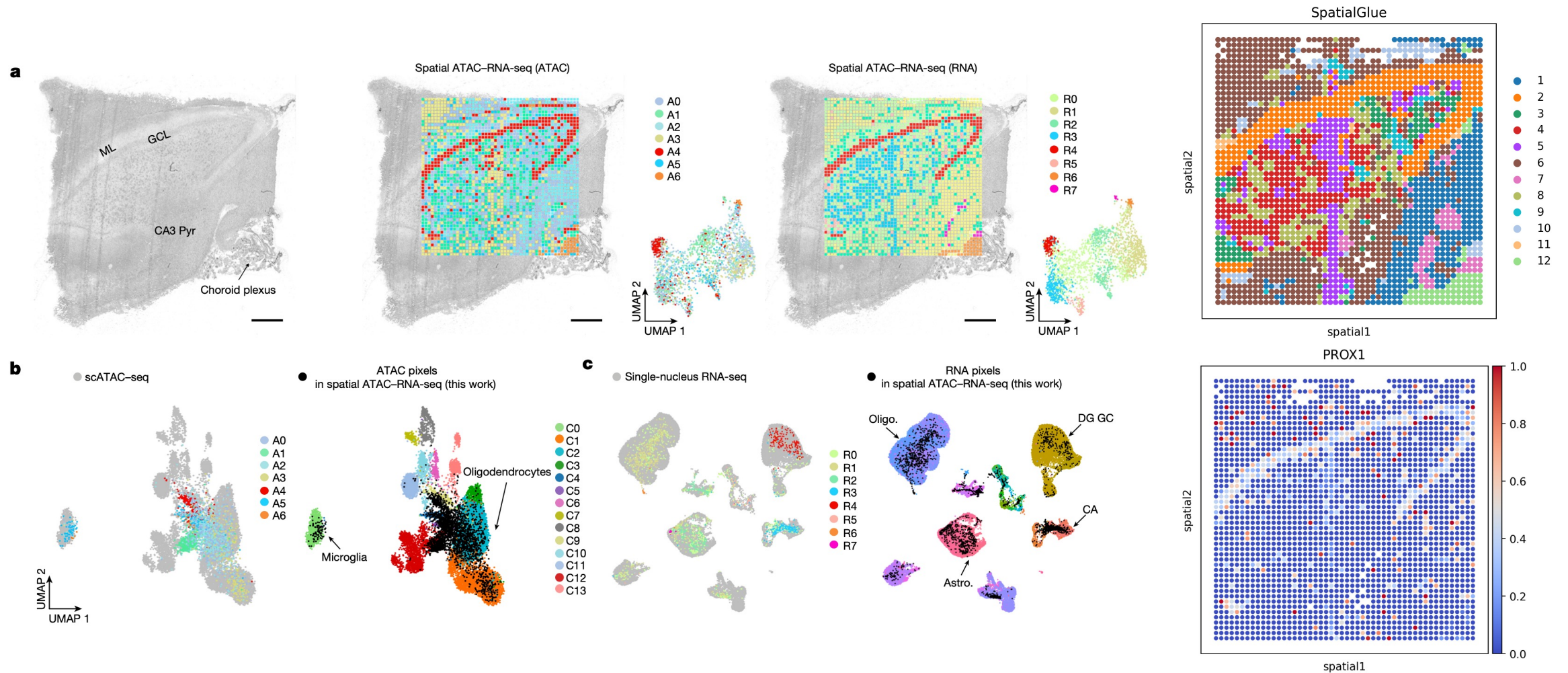
<https://stuartlab.org/signac/articles/overview>

<https://scglue.readthedocs.io/en/latest/>

<https://www.biorxiv.org/content/10.1101/2023.11.15.564963v1>

scJoint/squidpy/seurat/harmony

# Downstream Data Analysis – Multi-omics

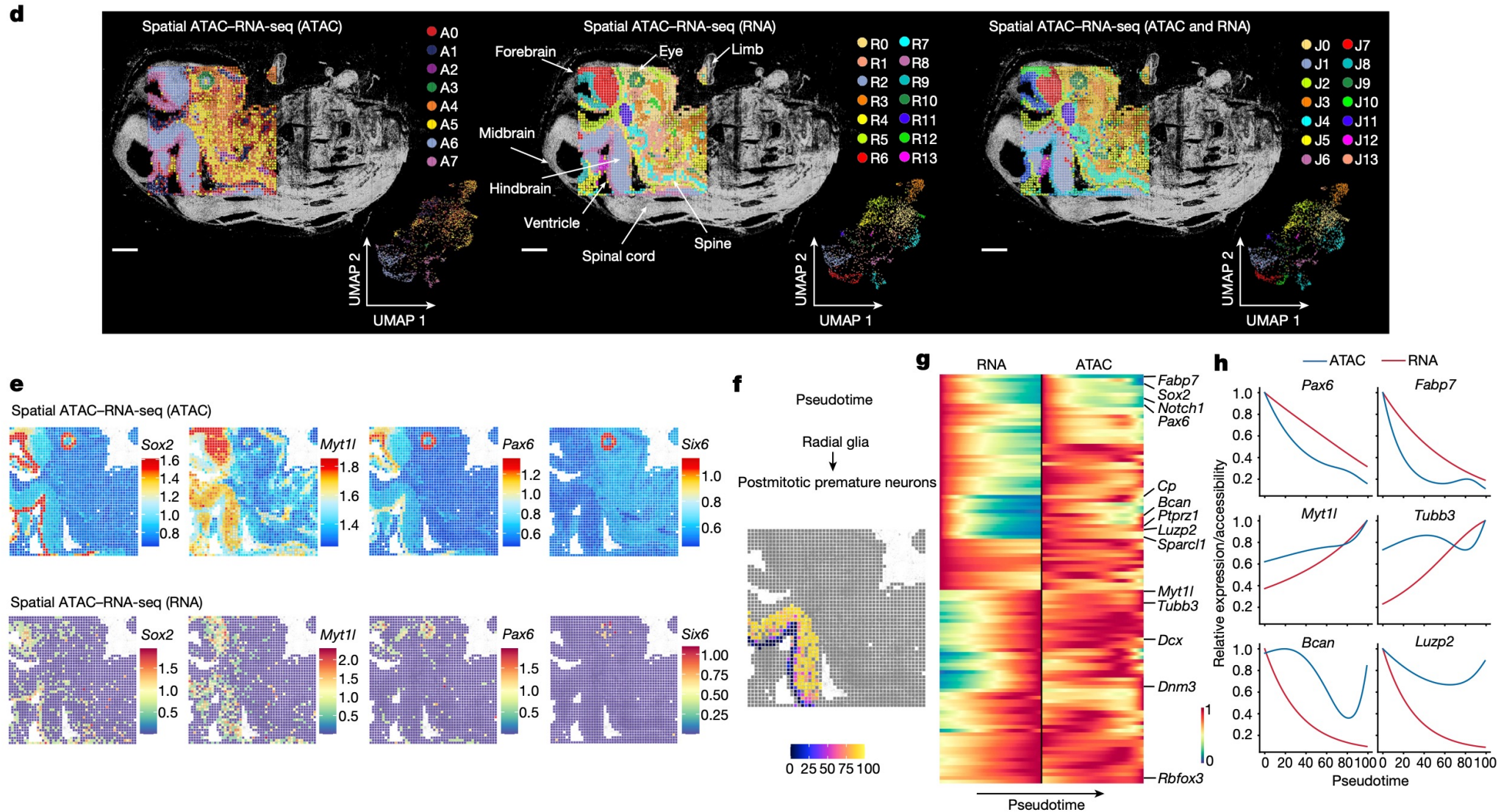


SpatialGlue data generated by Yao Lu, unpublished

Zhang, D. et al. Spatial epigenome–transcriptome co-profiling of mammalian tissues. *Nature* 616, 113–122 (2023). Long, Y. et al. Deciphering spatial domains from spatial multi-omics with SpatialGlue. *Biorxiv* (2023).



# Downstream Data Analysis – Pseudo-time Analysis





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Thank you!