

# 第四课 空间转录物组计算方法与数据分析

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20250403

# 罗伯特 胡克 ( 1635-1703 , 英国 )

1665 Robert Hooke first described cells

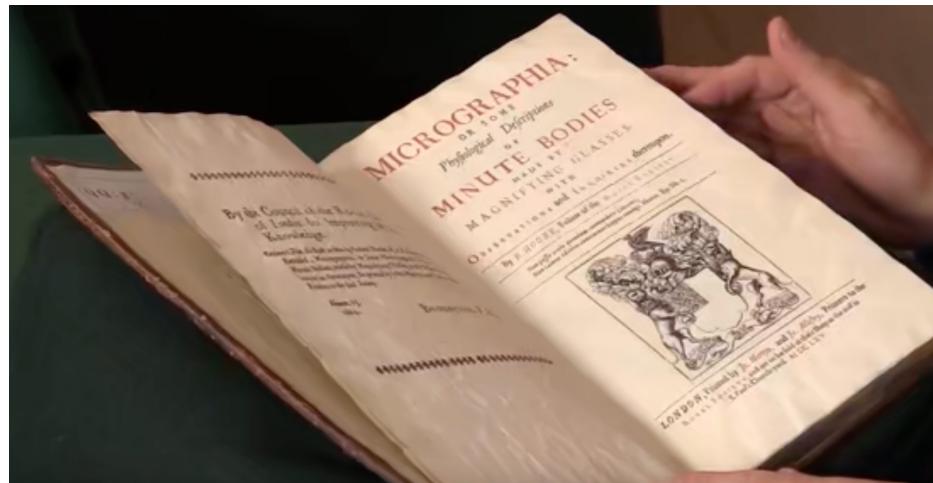
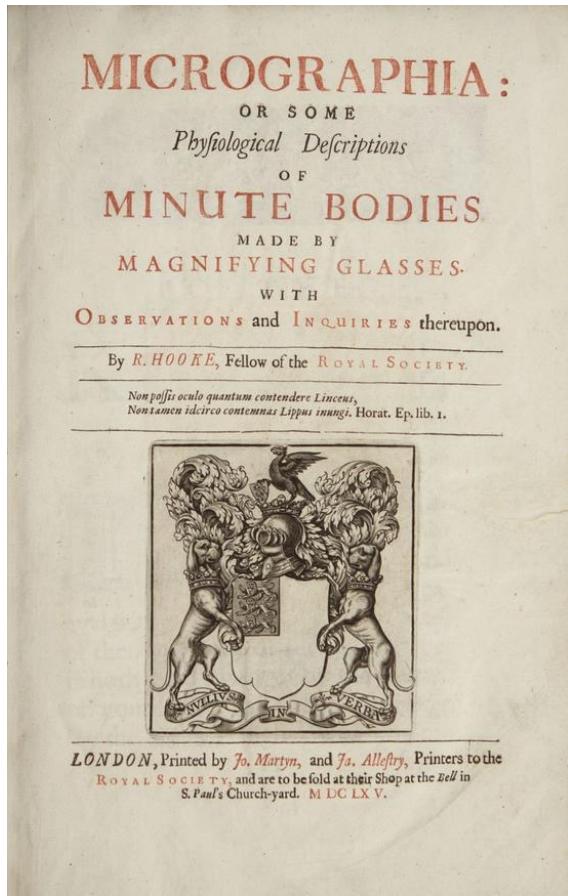


# 一台双镜片的复合光学显微镜

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# 《显微图谱：放大镜下微小实体的展示》（1665）



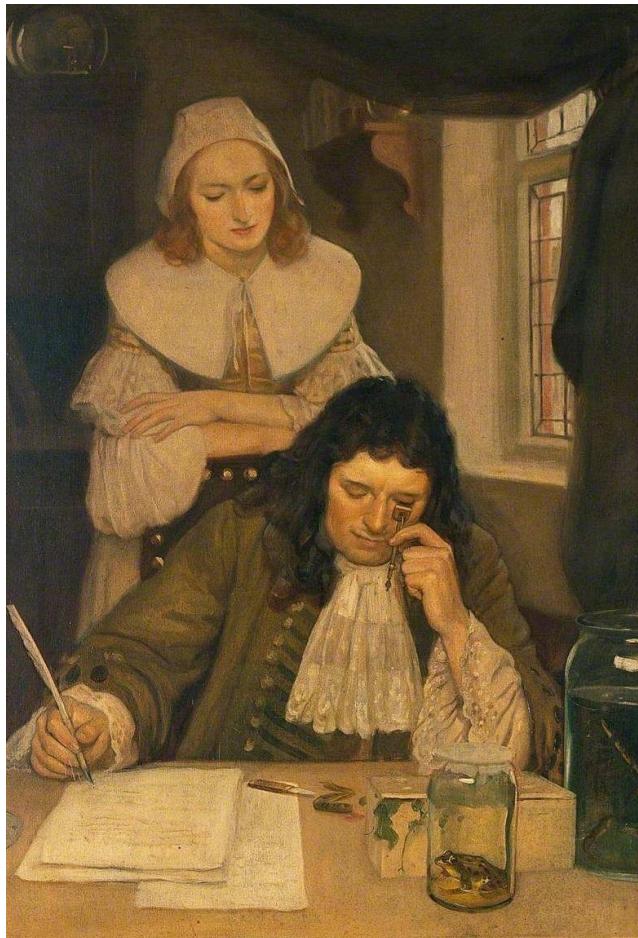
<https://www.bilibili.com/video/BV1rX4y1c7ce/>

<https://finance.sina.com.cn/tech/2021-07-03/doc-ikqcfnca4527637.shtml>

# 列文虎克（1632-1723，荷兰）

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1677 列文虎克第一个观测到活细胞



# 显微镜与微生物学的开拓者

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# 微生物学的开拓者（1675）

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# 《戴珍珠耳环的少女》, 扬-维米尔, 1665

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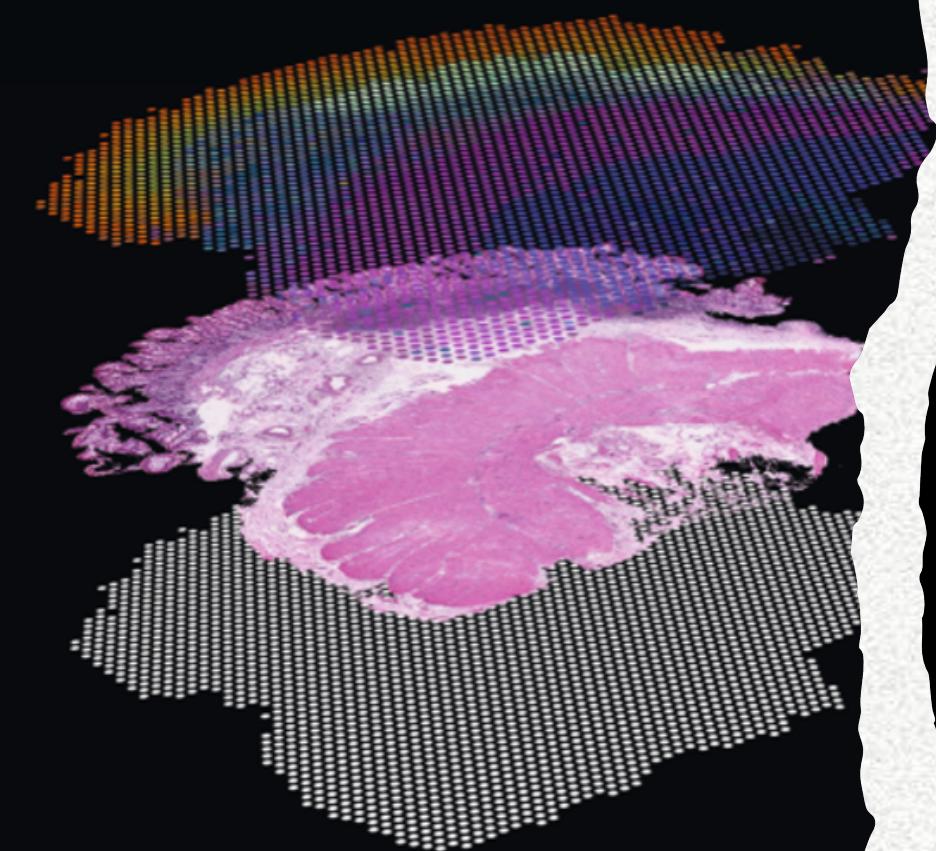
# 涂晕画法



可能借助了暗箱  
进行辅助观察

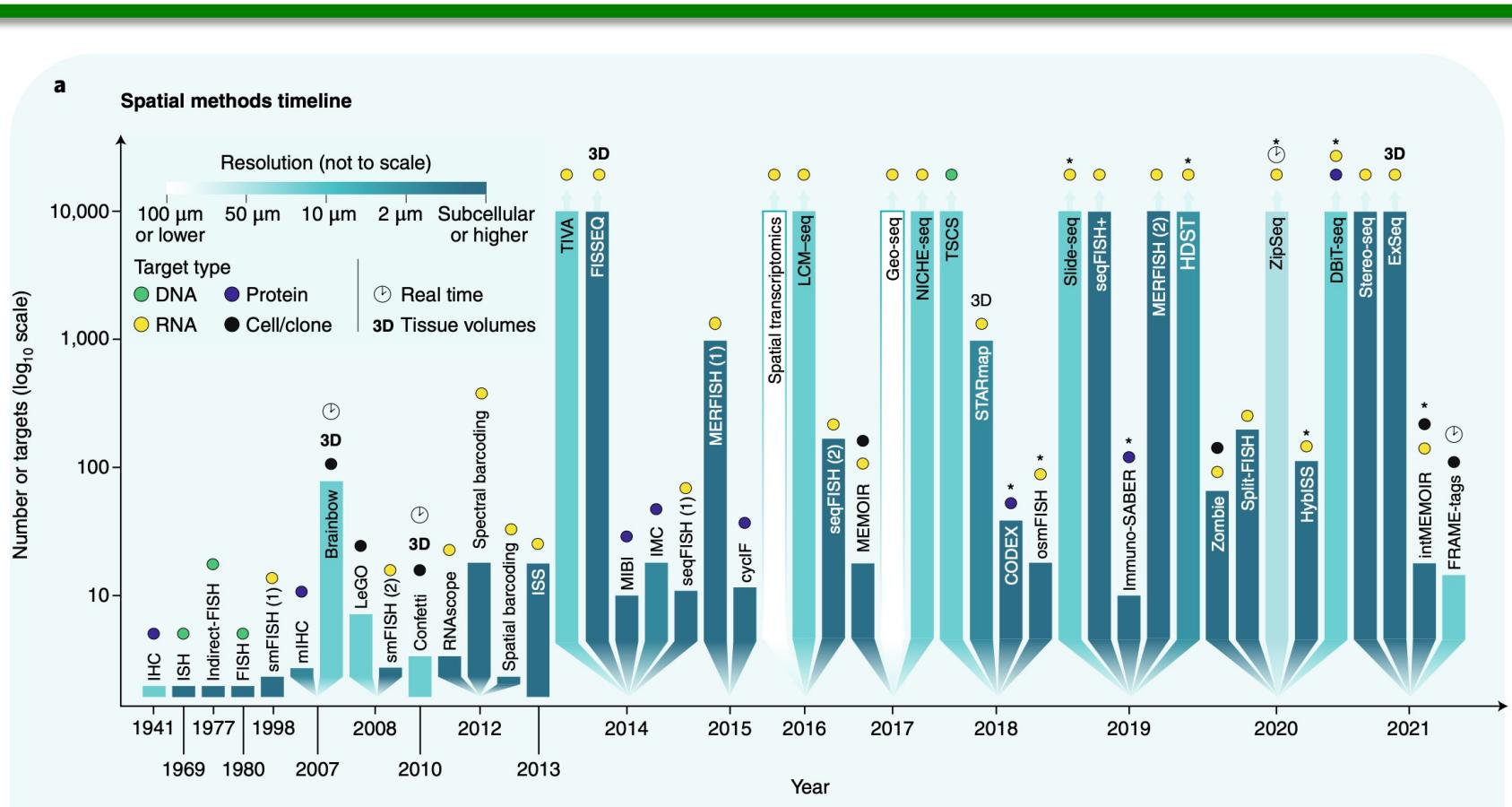
# nature method

Method of the Year 2020:  
Spatially resolved transcriptomics



空间转录组学

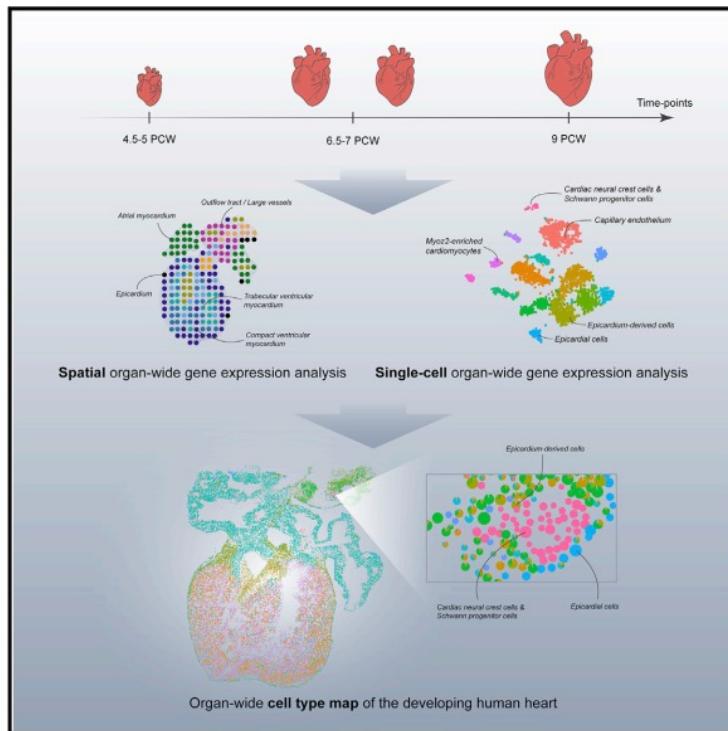
# 空间组学技术的发展历史



空间转录组学是一项开创性的技术，让科学家能够测定组织样本中的所有基因活动，并定位该活动发生的位置。

# A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the Developing Human Heart

## Graphical Abstract



## Authors

Michaela Asp, Stefania Giacomello,  
Ludvig Larsson, ..., Mats Nilsson,  
Christer Sylvén, Joakim Lundeberg

## Correspondence

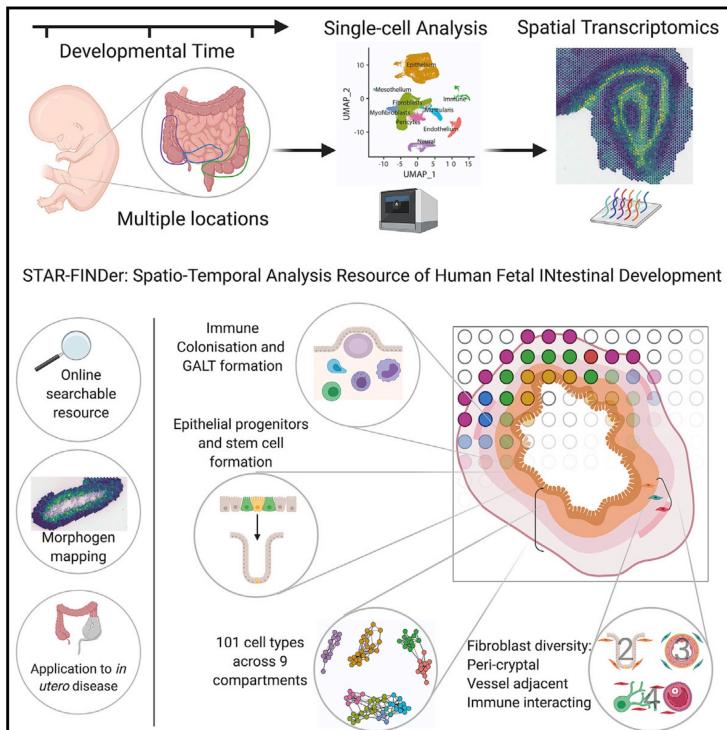
michaela.asp@scilifelab.se (M.A.),  
joakim.lundeberg@scilifelab.se (J.L.)

## In Brief

The gene expression landscape of human heart development is explored at single-cell resolution with spatial transcriptomic approaches to construct a 3D organ-wide atlas.

## Spatiotemporal analysis of human intestinal development at single-cell resolution

### Graphical Abstract



### Authors

David Fawkner-Corbett,  
Agne Antanaviciute, Kaushal Parikh, ...,  
Paul R.V. Johnson, Hashem Koohy,  
Alison Simmons

### Correspondence

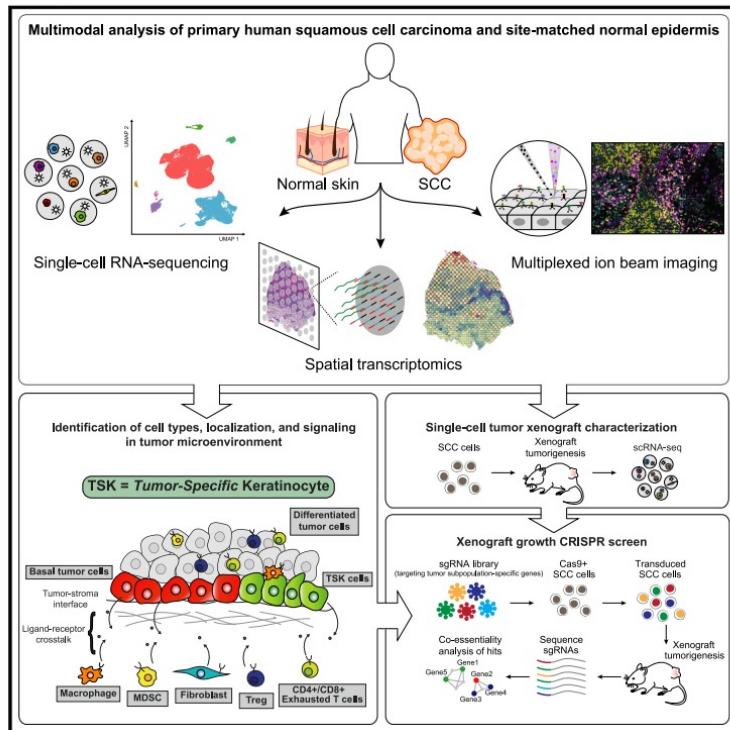
hashem.koohy@imm.ox.ac.uk (H.K.),  
alison.simmons@imm.ox.ac.uk (A.S.)

### In Brief

Fawkner-Corbett et al. chart human intestinal morphogenesis across time, location, and cellular compartments using a combination of single-cell RNA sequencing and spatial transcriptomics. The resulting online searchable atlas describes the principles of crypt-villus axis formation as well as neural, vascular, mesenchymal morphogenesis, and immune populations of the developing gut.

# Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma

## Graphical Abstract



## Authors

Andrew L. Ji, Adam J. Rubin,  
Kim Thrane, ..., Garry P. Nolan,  
Joakim Lundeberg, Paul A. Khavari

## Correspondence

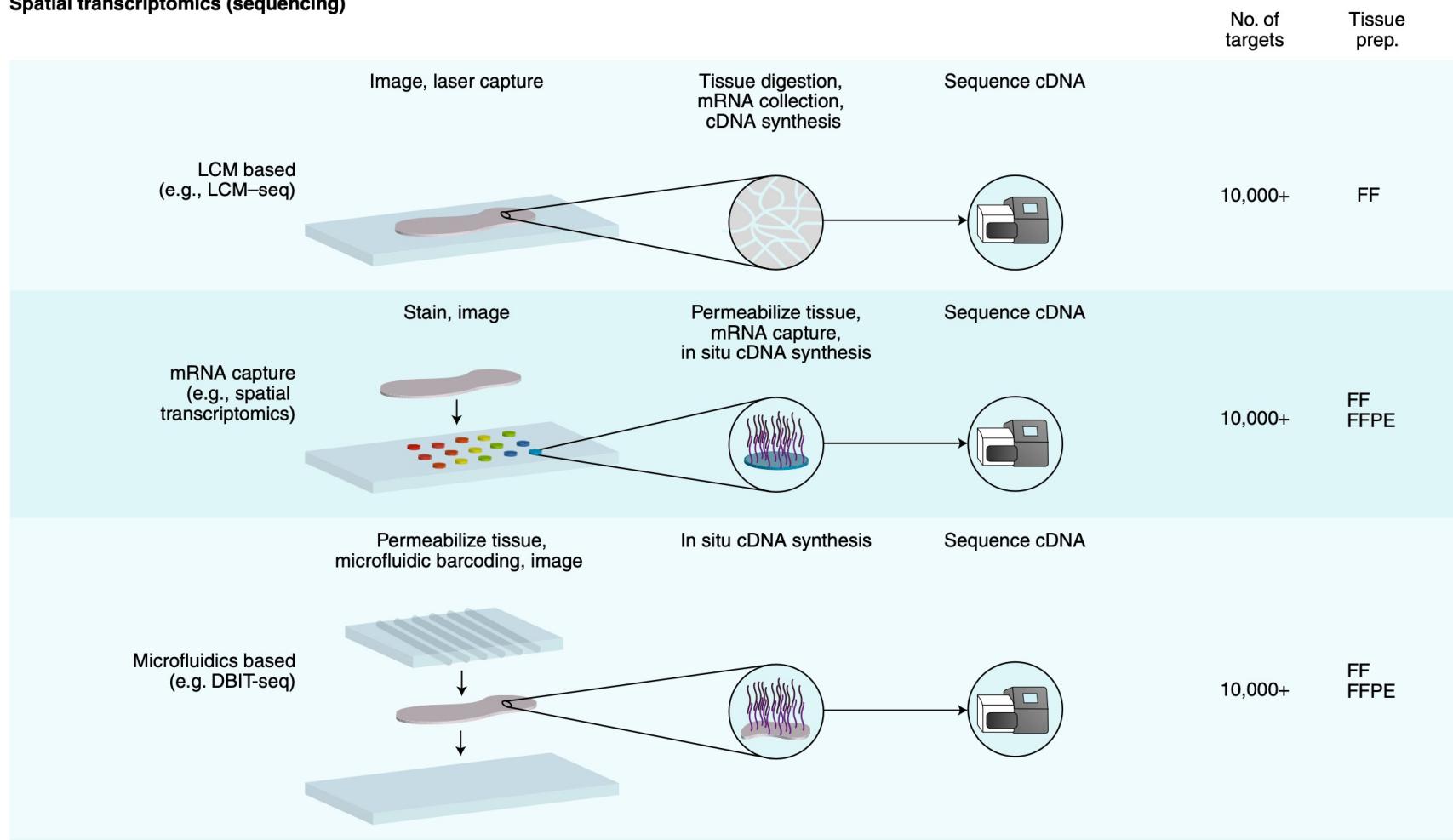
khavari@stanford.edu

## In Brief

Integration of high-dimensional multi-omics approaches to characterize human cutaneous squamous cell carcinoma identifies a tumor-specific keratinocyte population as well as the immune infiltrates and heterogeneity at tumor leading edges.

# 基于测序的空间转录组学技术

## Spatial transcriptomics (sequencing)



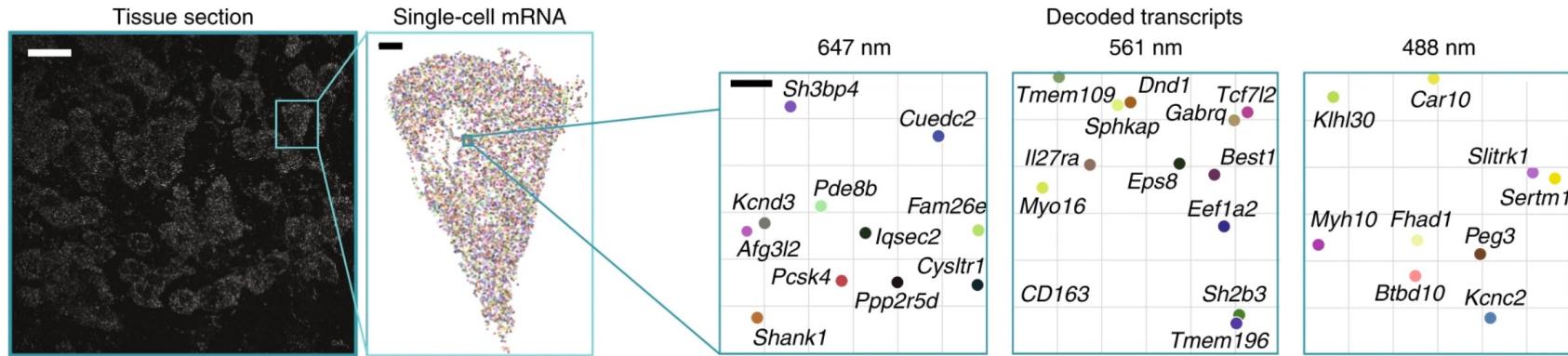
# 基于成像的空间转录组学技术

## Spatial transcriptomics (FISH)

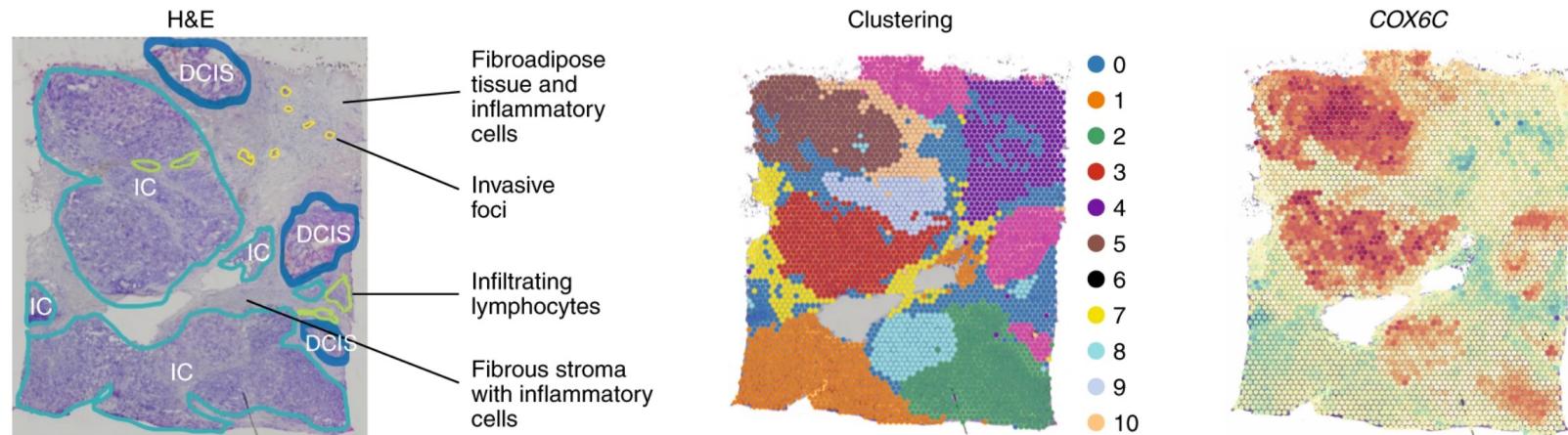
	Barcode	No. of targets	Tissue prep.
smFISH	NA	<10	FF FFPE
Spectral barcoding	● + ● + ● = ●	32 (792)	NA
Spatial barcoding	● ● ●	<10	NA
Round 1	Round 2	Round <i>n</i>	
osmFISH	Formamide	Formamide	NA 33 FF
MERFISH	Photobleach	Photobleach	1 0 ... 1 10,000 FF
seqFISH	DNase	DNase	● ● ... ● 249 FF

## 空间转录组技术特点比较

## Spatial transcriptomics (FISH)



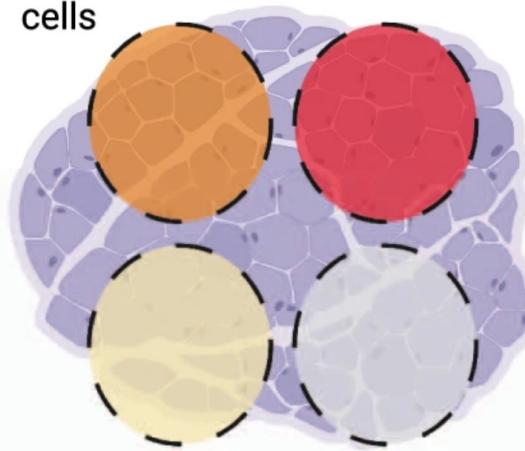
## Spatial transcriptomics (sequencing)



# 空间转录组的分辨率

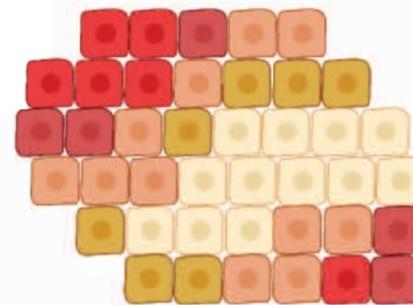
## c Multi-cell resolution

Each spot collects expression among several cells



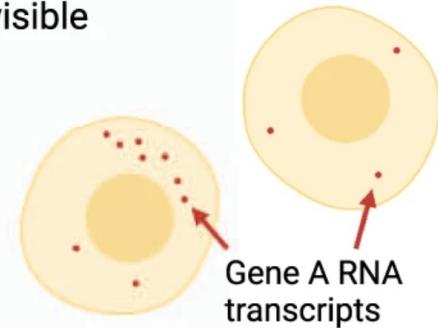
## d Single-cell resolution

Data reveals expression patterns of individual cells



## e Sub-cellular resolution

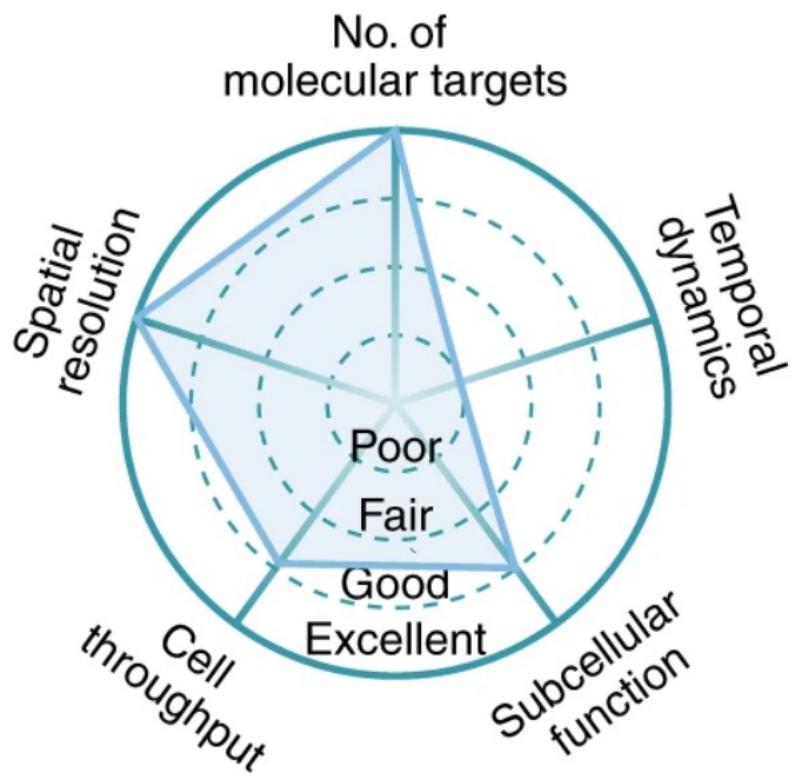
In addition to variation between cells, variation in RNA location within cells is visible



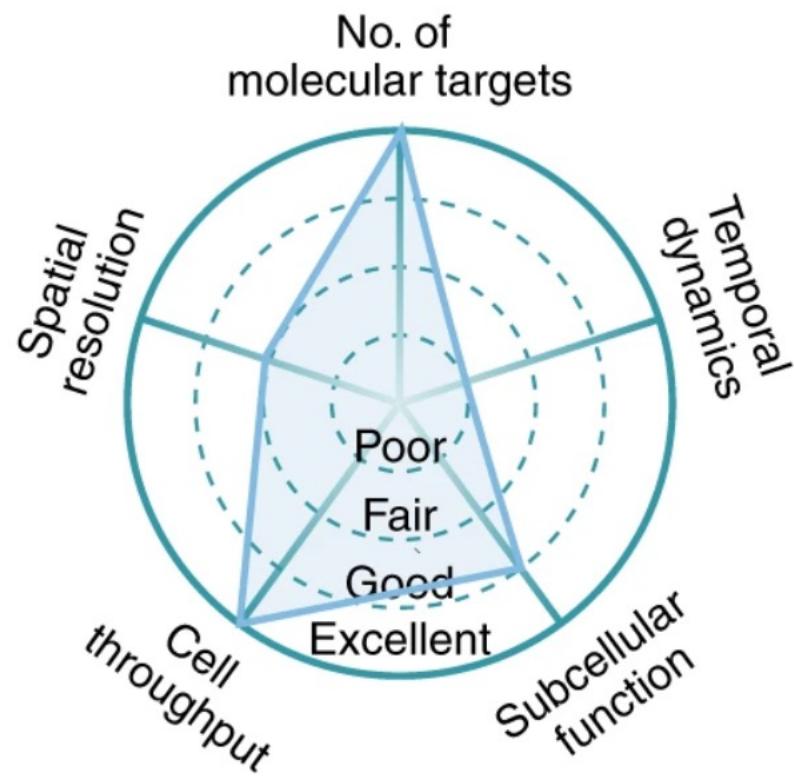
(Depicted: single molecule imaging)

# 技术特点比较

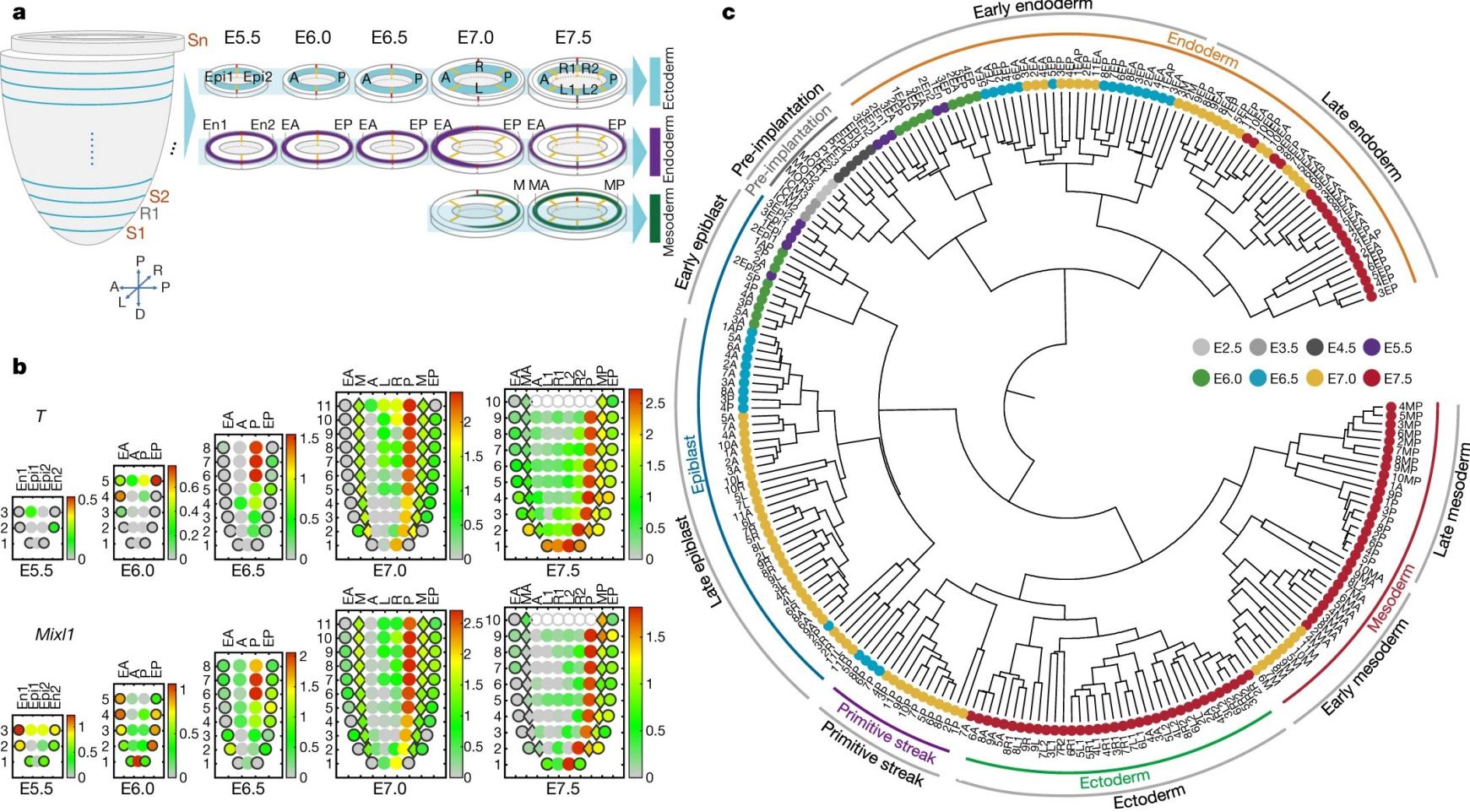
Spatial transcriptomics  
(FISH)



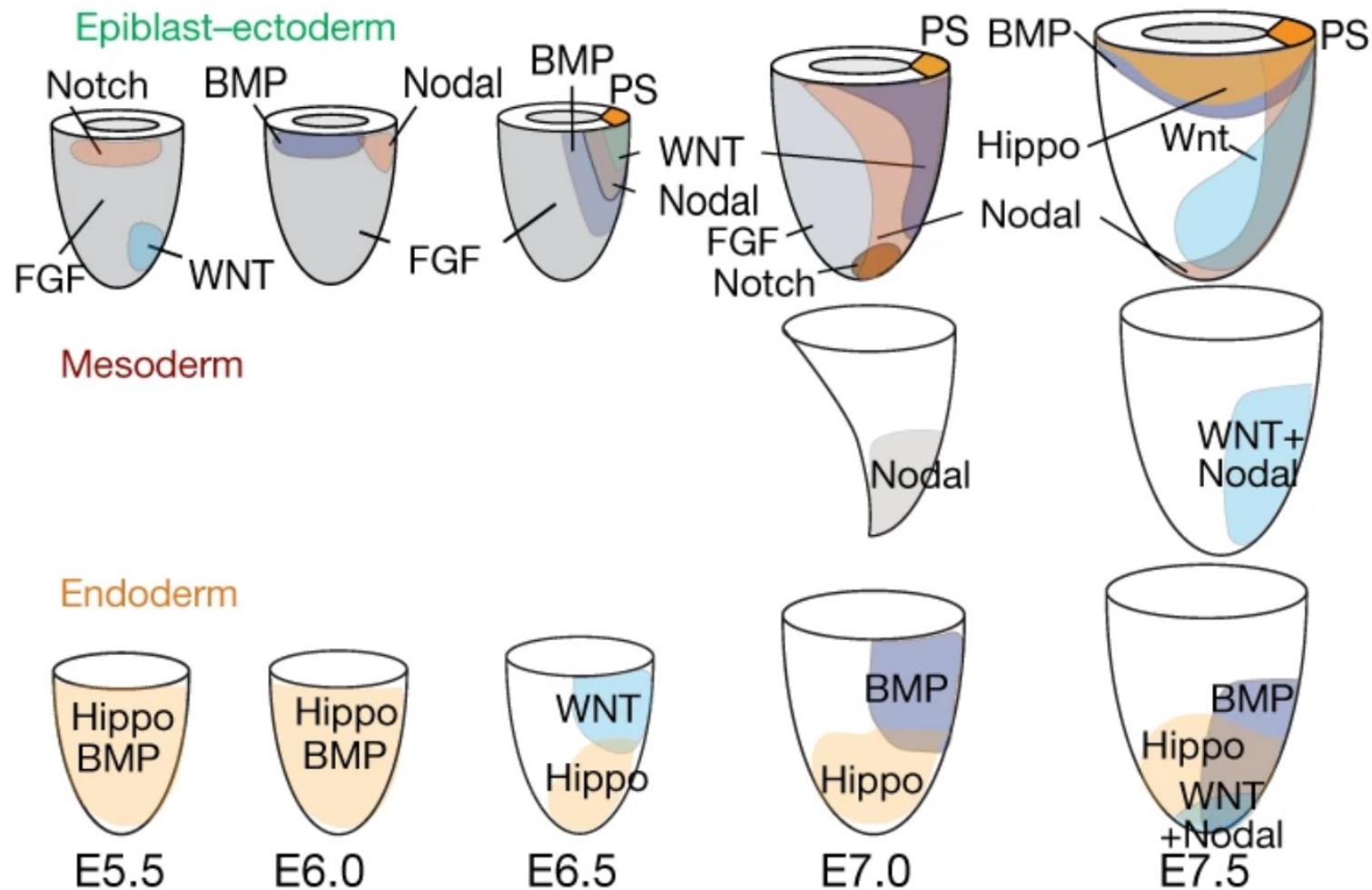
Spatial transcriptomics  
(sequencing)



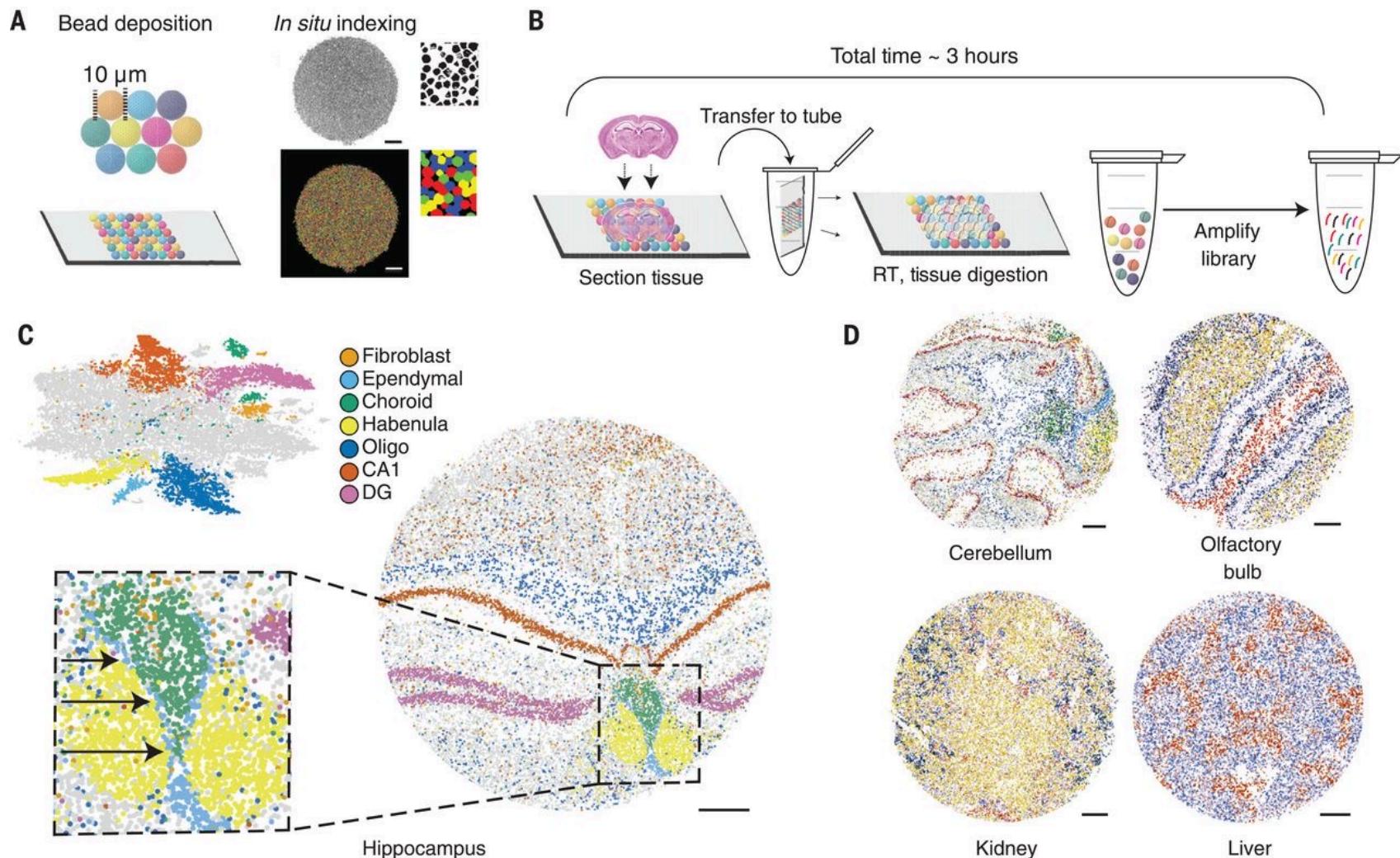
# 基于激光微切割的GEO-seq (2019)



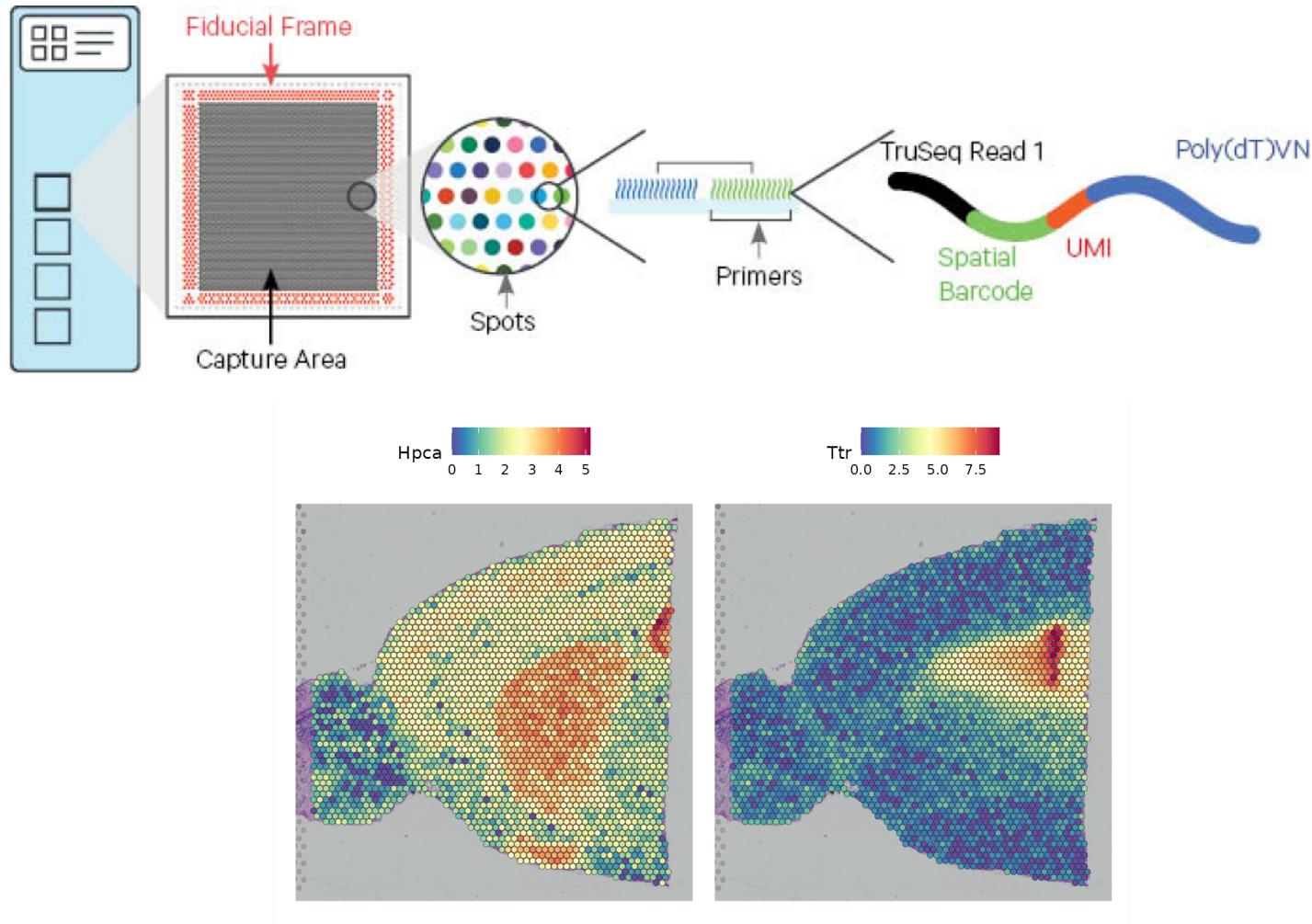
# 多能性状态转变与细胞命运的关系



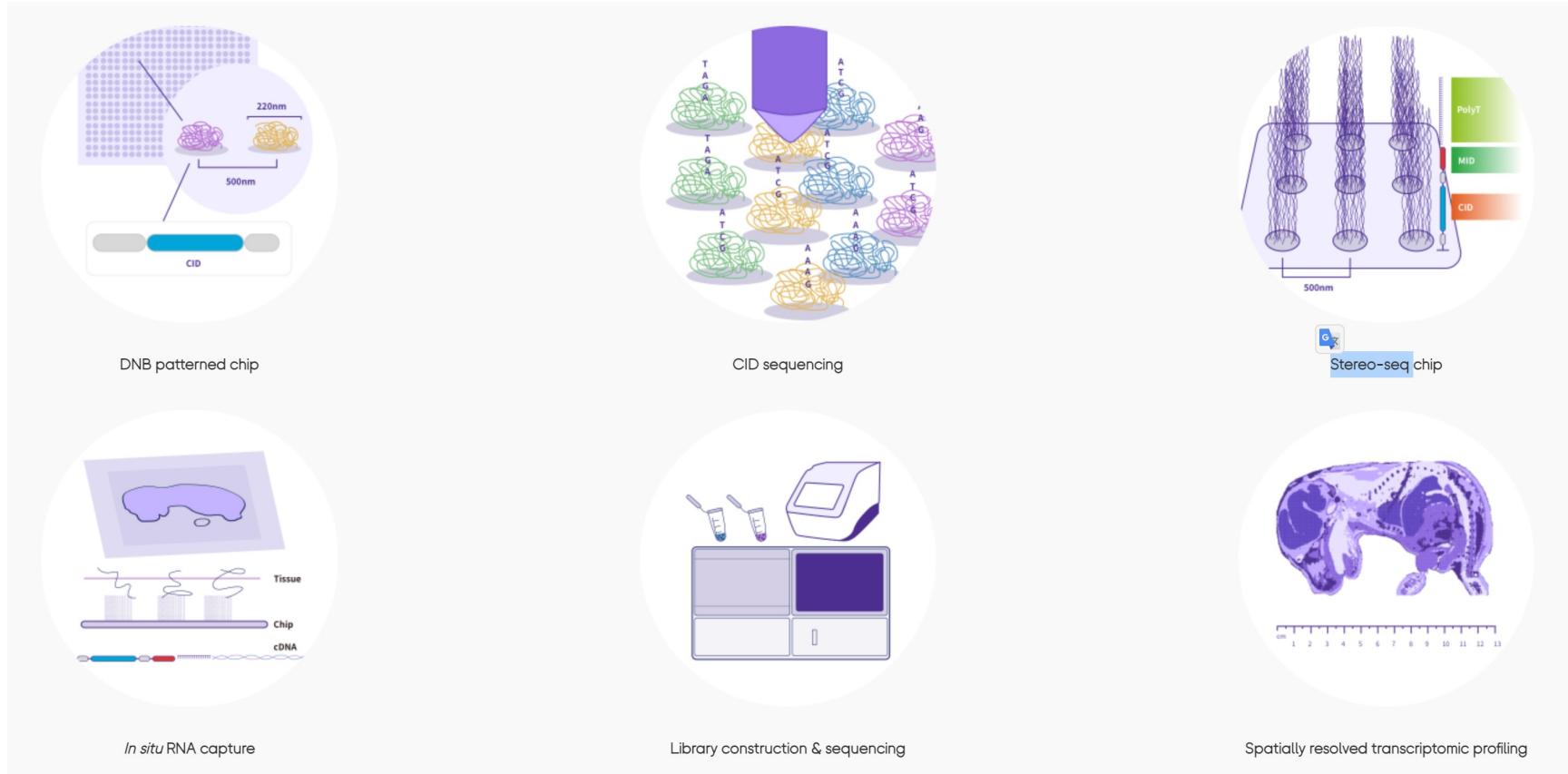
# 空间转录组技术SLIDE-seq (2019)



# 10x Genomics Visium (2019)



# 华大基因 Stereo-seq (2021)

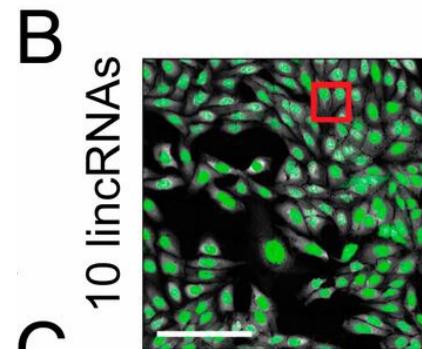
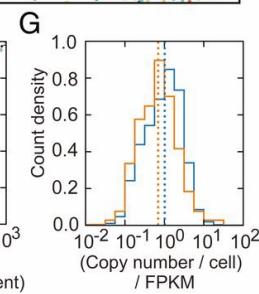
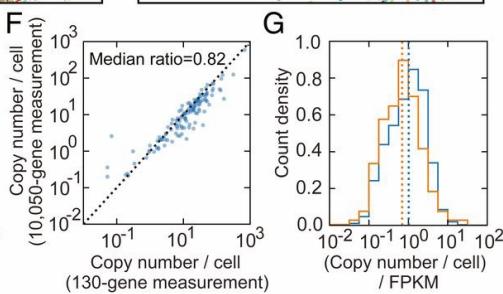
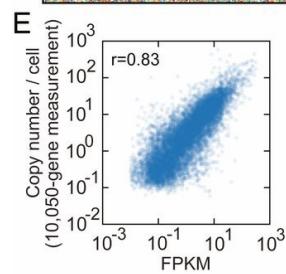
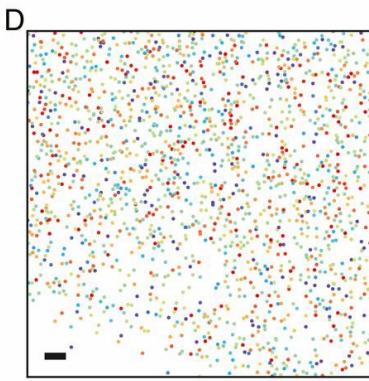
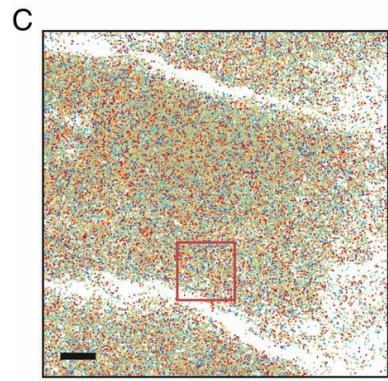
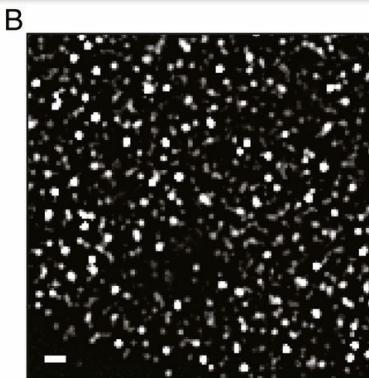
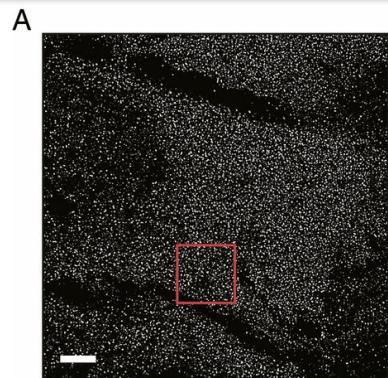


# 基于成像的空间转录组学技术

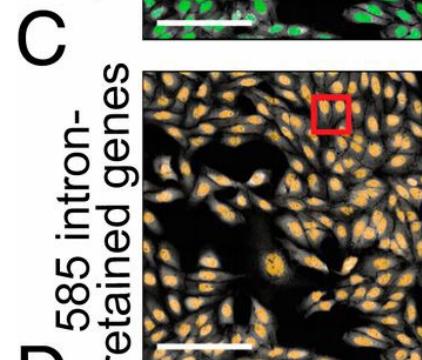
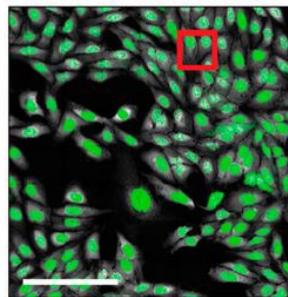
## Spatial transcriptomics (FISH)

	Barcode	No. of targets	Tissue prep.
smFISH	NA	<10	FF FFPE
Spectral barcoding	● + ● + ● = ●	32 (792)	NA
Spatial barcoding	● ● ●	<10	NA
Round 1	Round 2	Round <i>n</i>	
osmFISH	Formamide	Formamide	NA 33 FF
MERFISH	Photobleach	Photobleach	1 0 ... 1 10,000 FF
seqFISH	DNase	DNase	● ● ... ● 249 FF

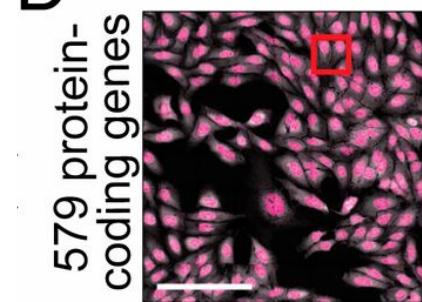
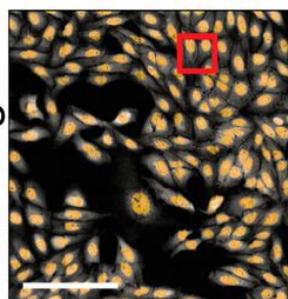
# MERFISH (2019)



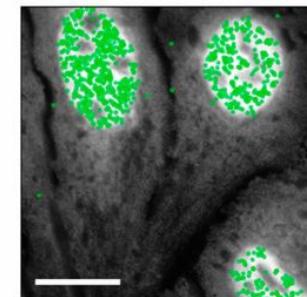
10 lincRNAs



585 intron-retained genes



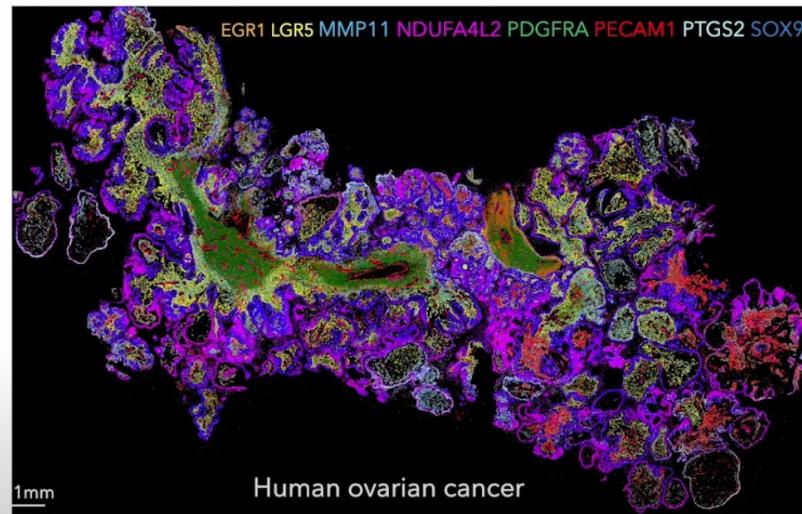
579 protein-coding genes



# MERFISH+MERSCOPE (commercialized by Vizgen)



**Profile gene expression *in situ*, from whole tissue, to sub-cellular**



**500 gene panel, 174 million RNA transcripts detected**

# Pipeline for 10x Visium datasets

First, we load Seurat and the other packages necessary for this vignette.

```
library(Seurat)
library(SeuratData)
library(ggplot2)
library(patchwork)
library(dplyr)
```

## Dataset

Here, we will be using a recently released dataset of sagittal mouse brain slices generated using the Visium v1 chemistry. There are two serial anterior sections, and two (matched) serial posterior sections.

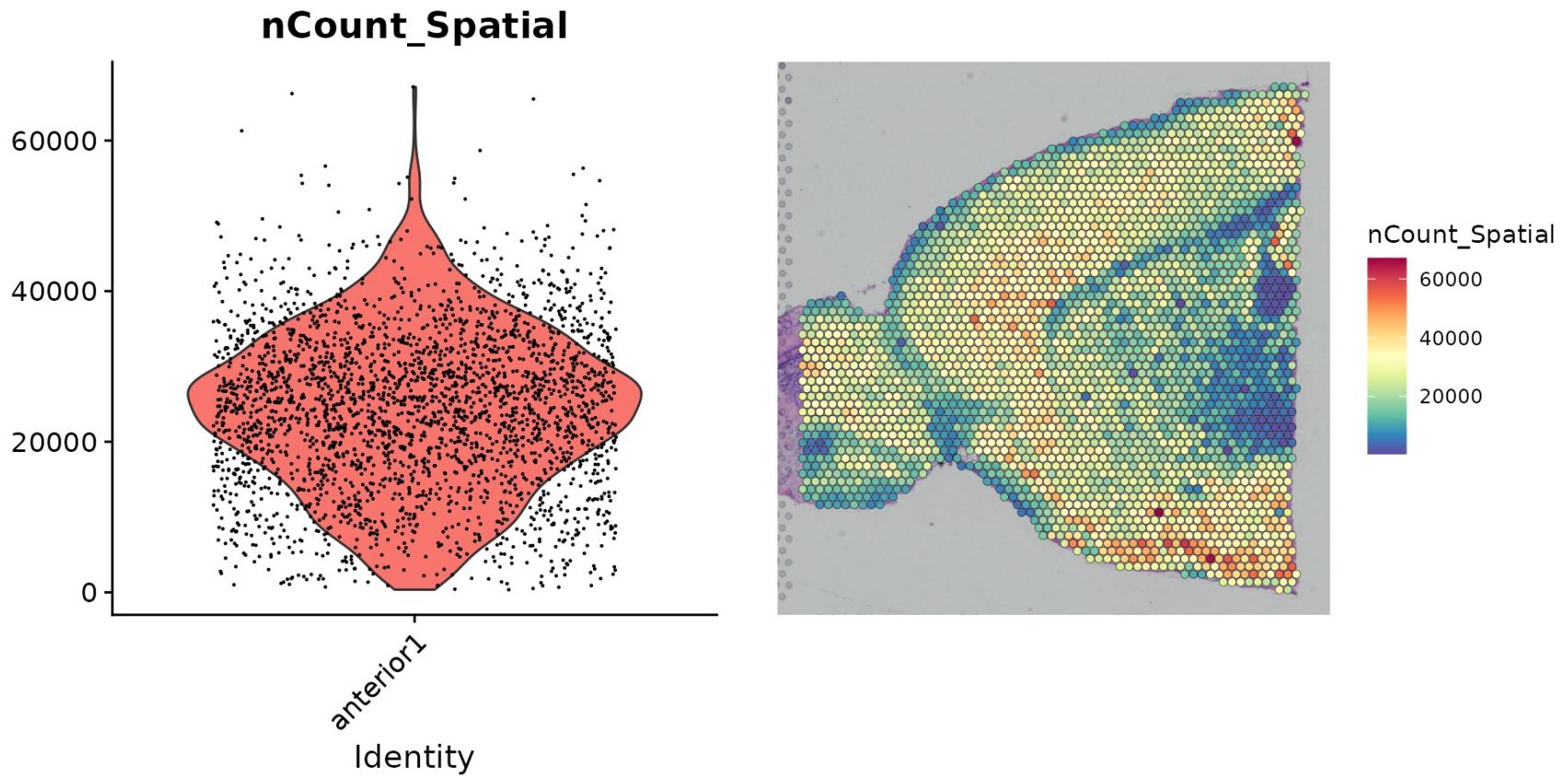
You can download the data [here](#), and load it into Seurat using the `Load10X_Spatial()` function. This reads in the output of the [spaceranger](#) pipeline, and returns a Seurat object that contains both the spot-level expression data along with the associated image of the tissue slice. You can also use our [SeuratData package](#) for easy data access, as demonstrated below. After installing the dataset, you can type `?stxBraint` to learn more.

```
InstallData("stxBraint")
```

```
brain <- LoadData("stxBraint", type = "anterior1")
```

# Data preprocessing

---



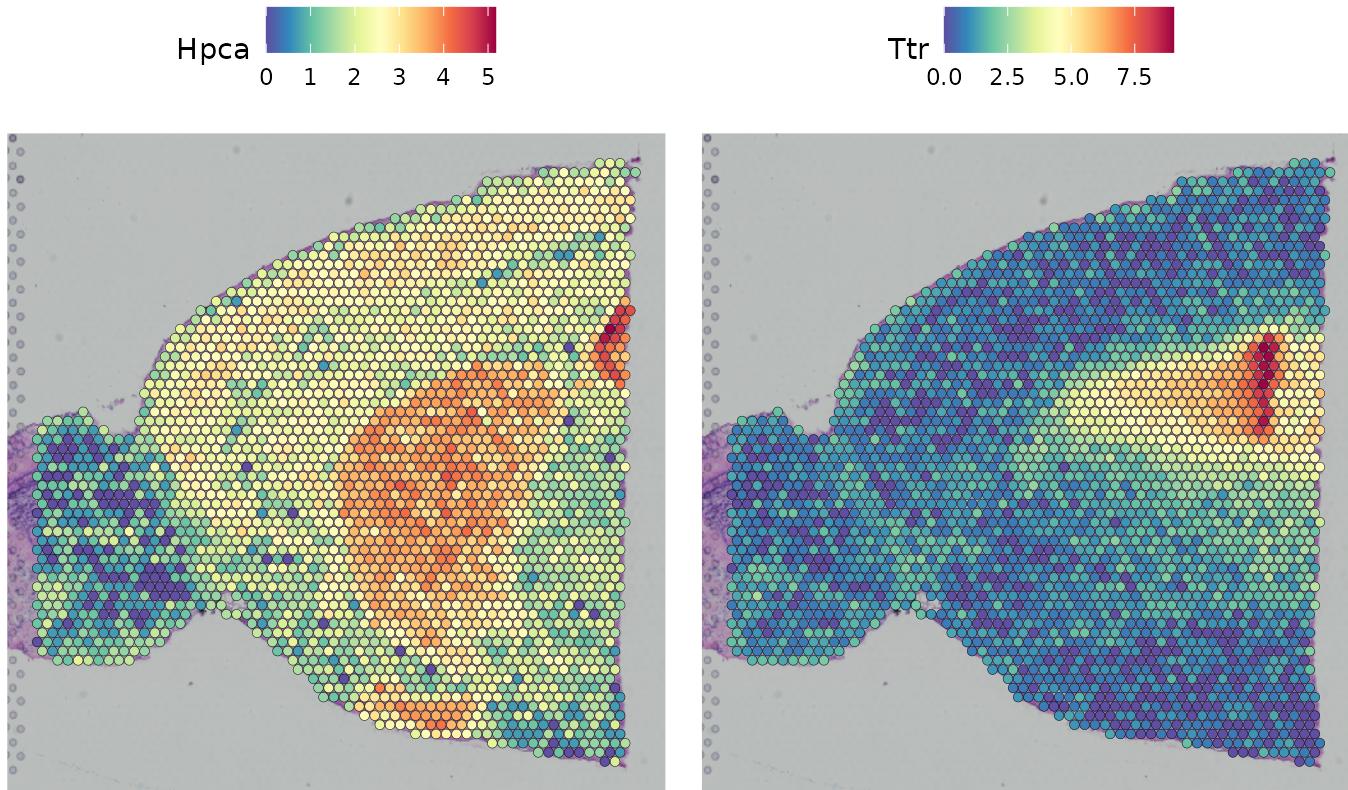
```
brain <- SCTransform(brain, assay = "Spatial", verbose = FALSE)
```

---

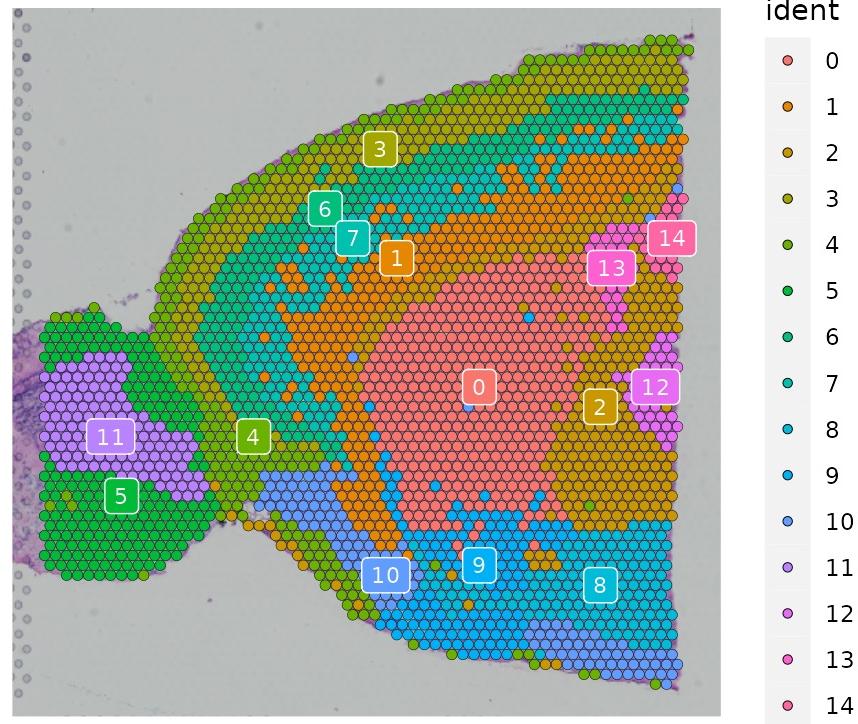
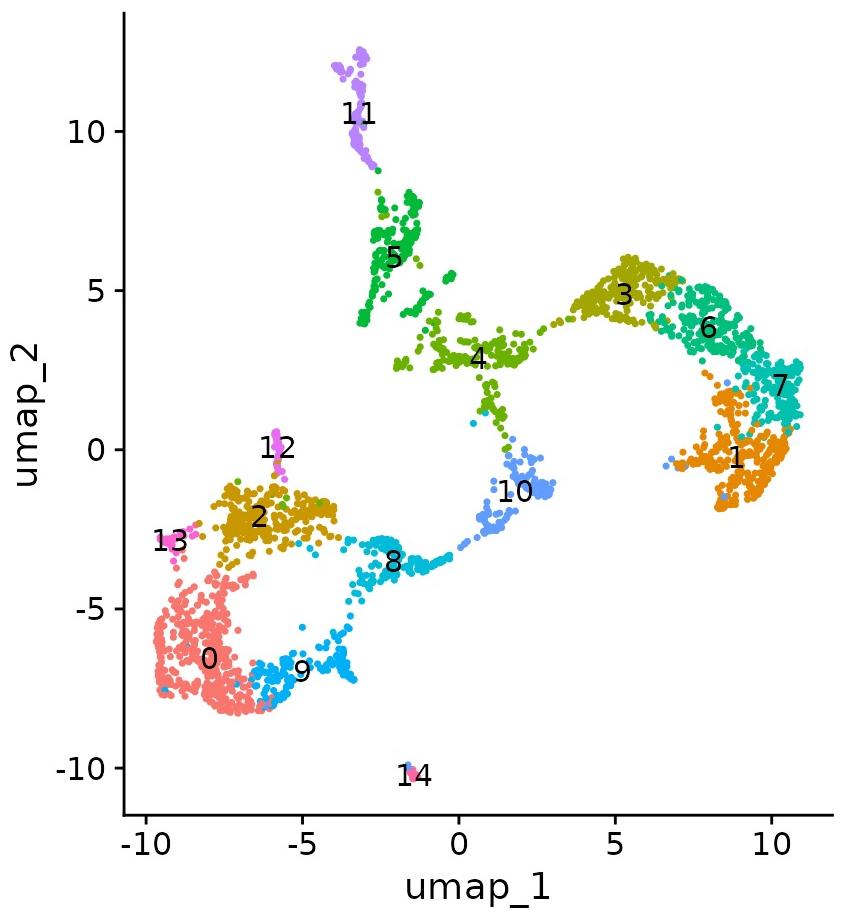
# Gene expression visualization

---

```
SpatialFeaturePlot(brain, features = c("H pca", "Ttr"))
```

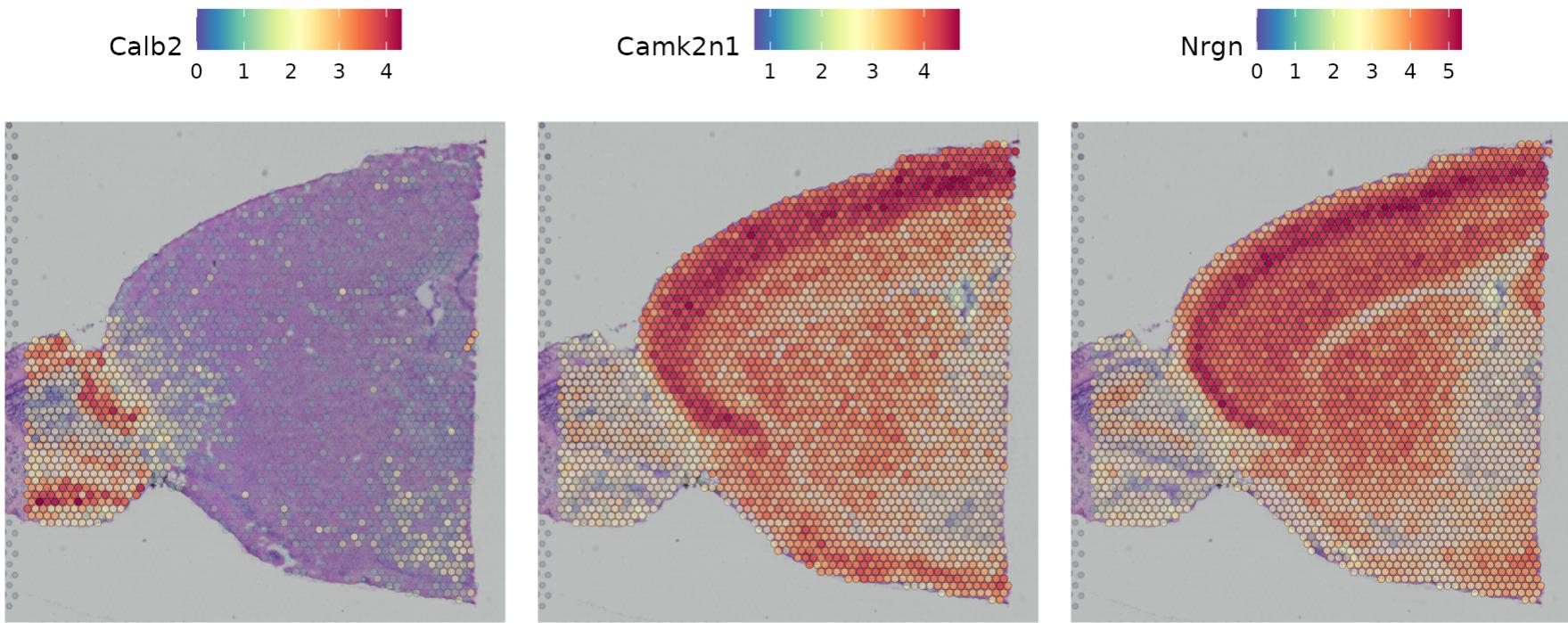


# Dimensionality reduction, clustering, and visualization



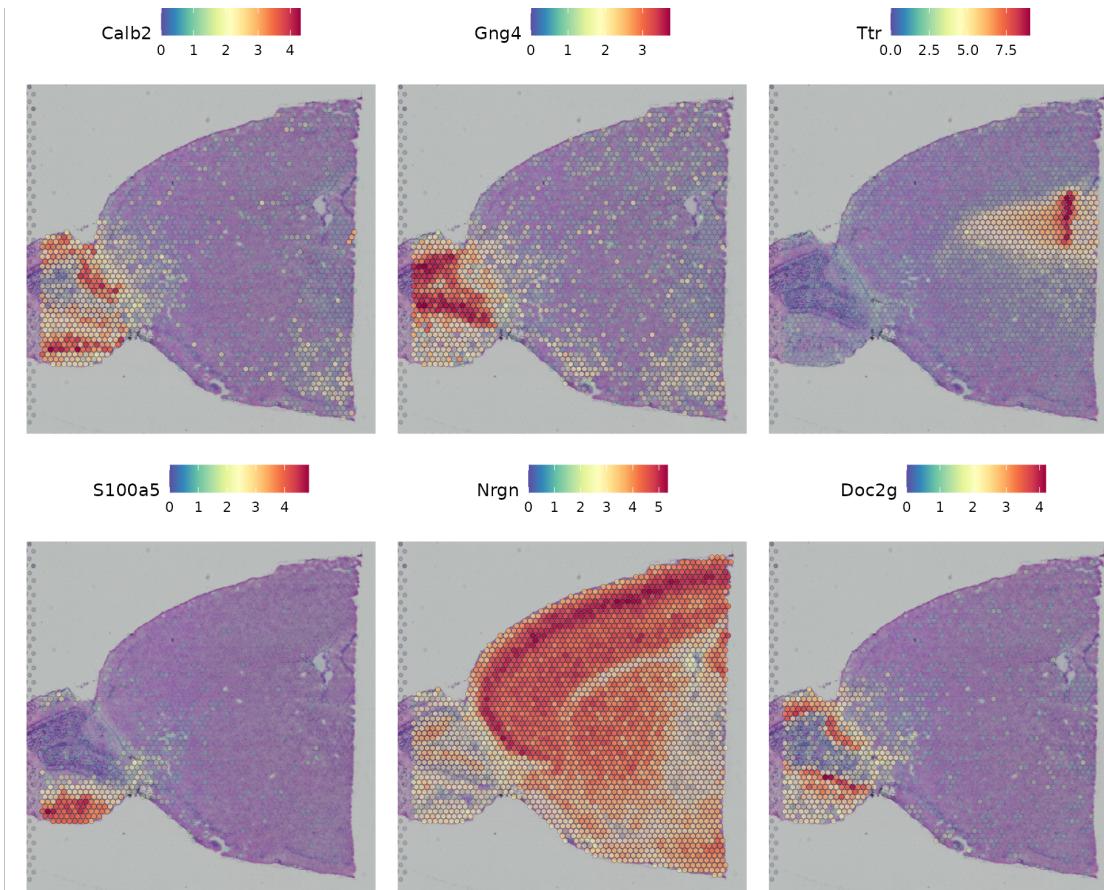
# Identification of Spatially Variable Features

```
de_markers <- FindMarkers(brain, ident.1 = 5, ident.2 = 6)
SpatialFeaturePlot(object = brain, features = rownames(de_markers)[1:3], alpha = c(0.1, 1), ncol = 3)
```



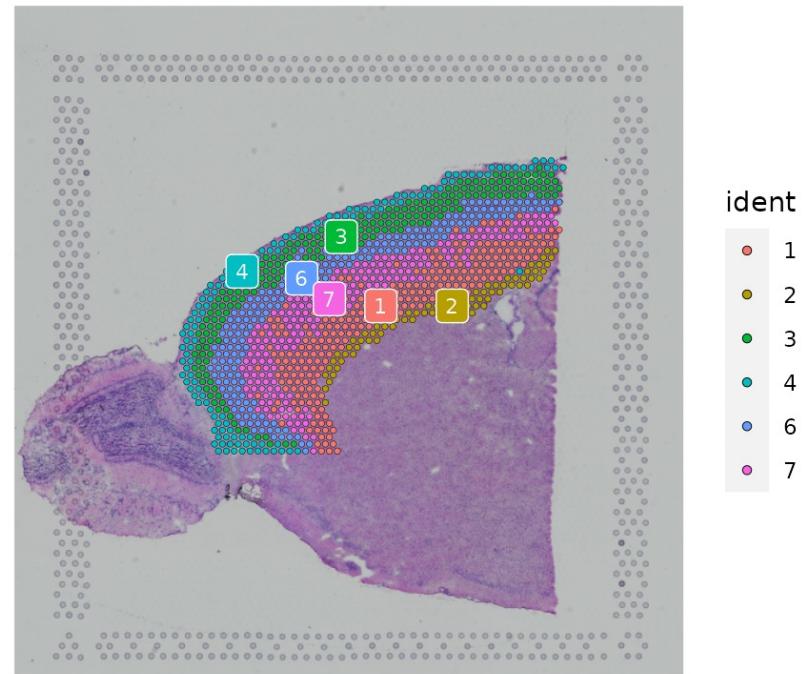
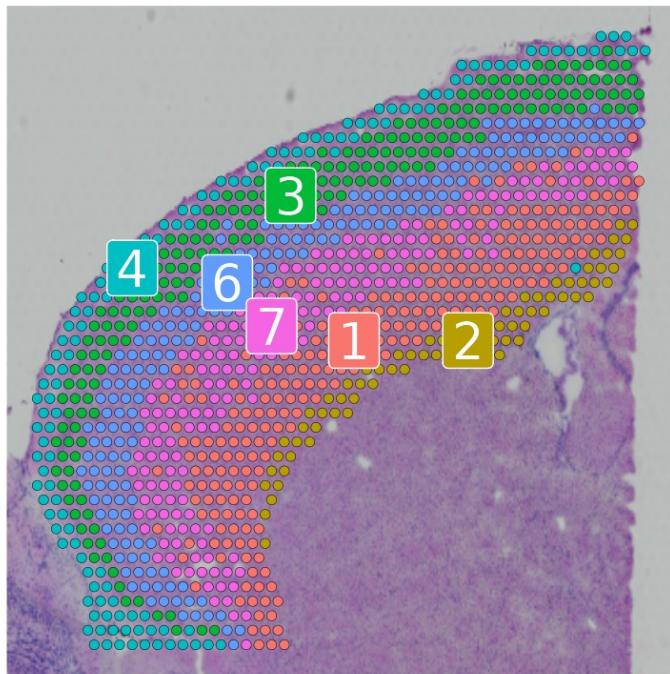
# Identification of Spatially Variable Features

```
top.features <- head(SpatiallyVariableFeatures(brain, selection.method = "moransi"), 6)
SpatialFeaturePlot(brain, features = top.features, ncol = 3, alpha = c(0.1, 1))
```

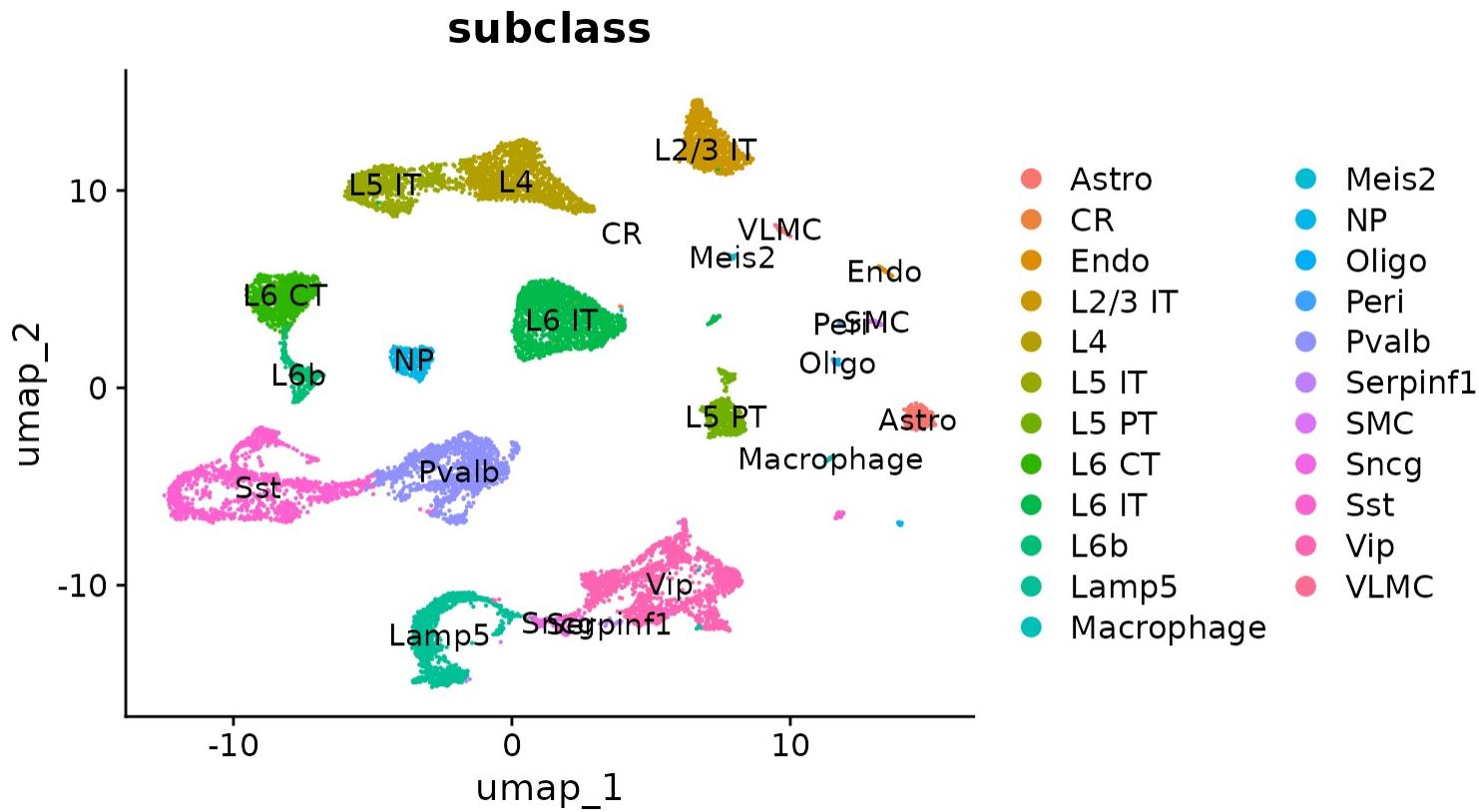


# Subset out anatomical regions

---

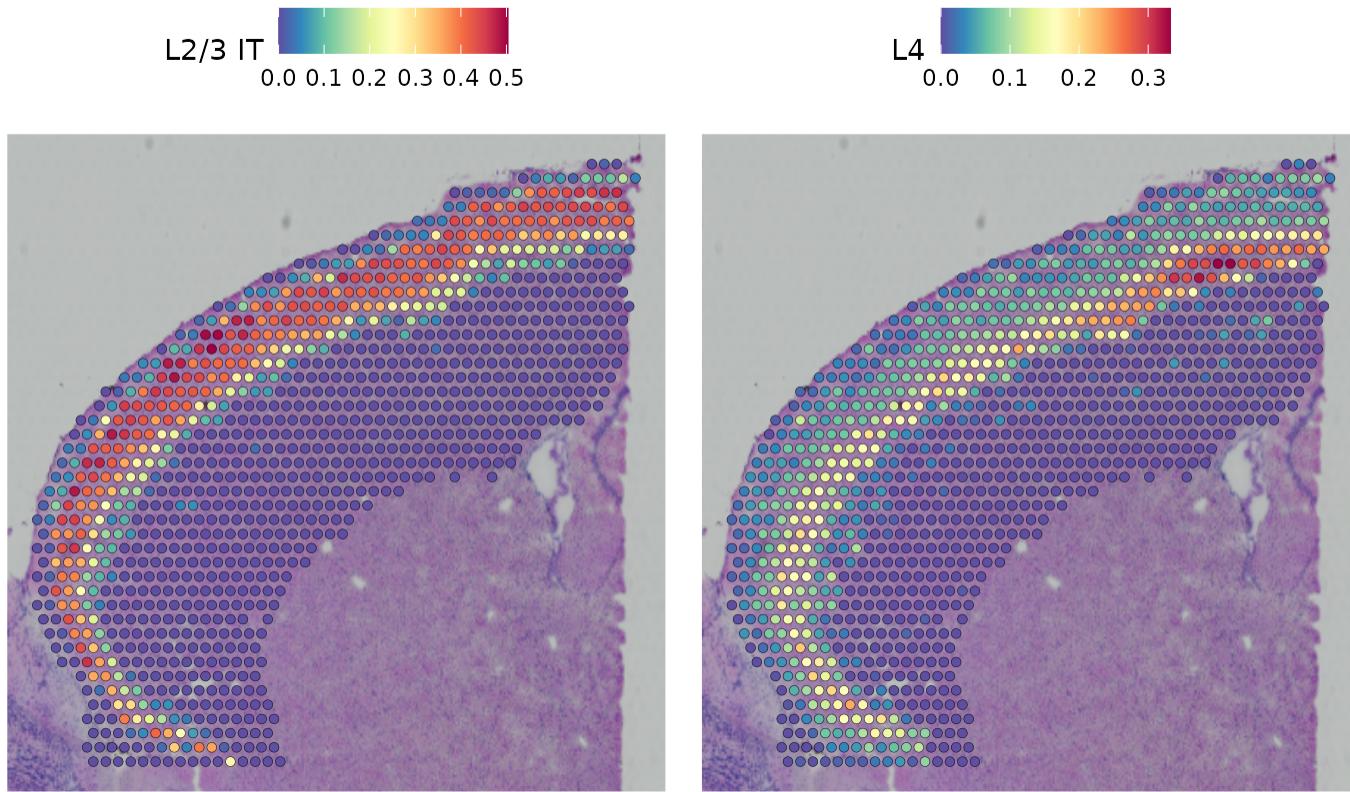


# Integration with single-cell data

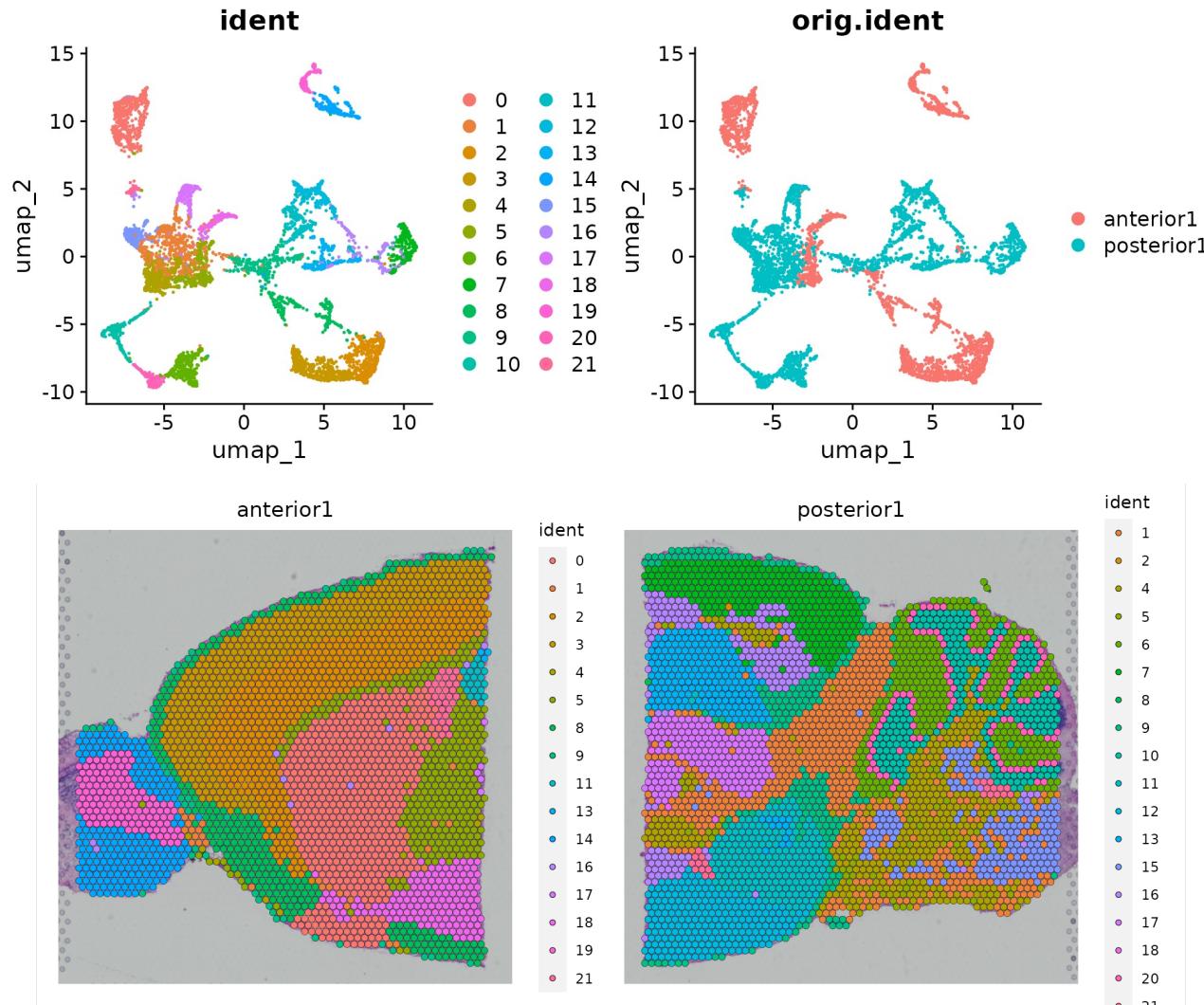


# Integration with single-cell data

---

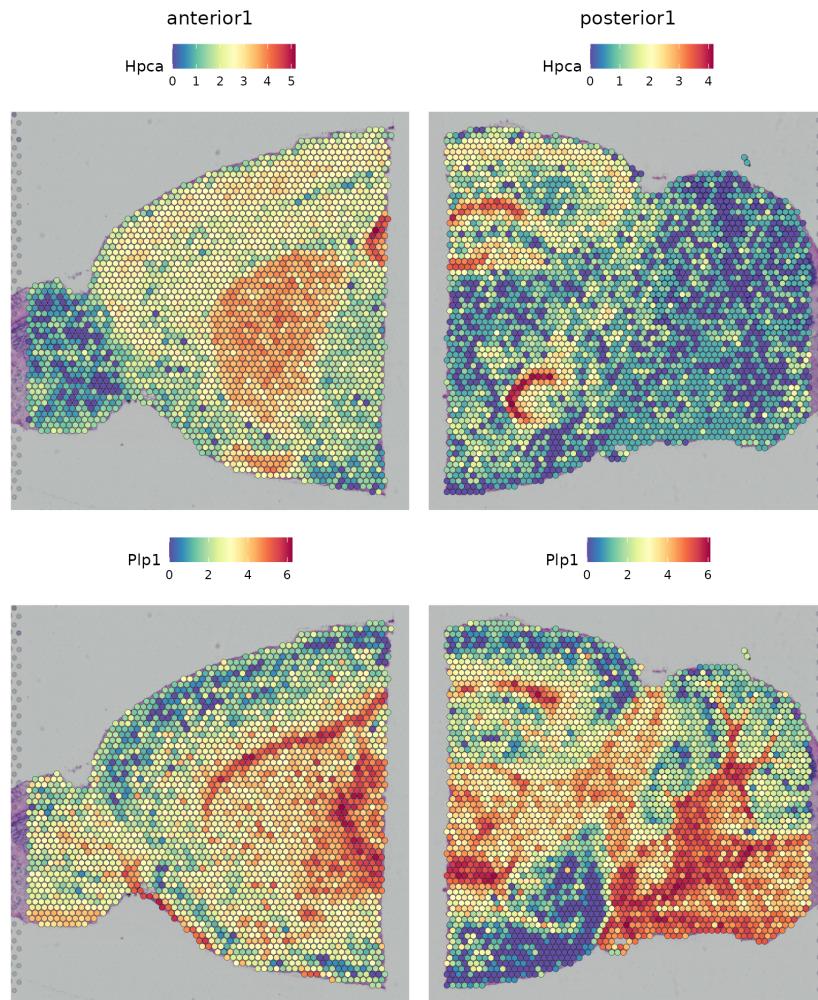


# Working with multiple slices in Seurat



# Working with multiple slices in Seurat

---



# Mouse Brain: Vizgen MERSCOPE

---

The next pieces of information are specific to imaging assays, and is stored in the images slot of the resulting Seurat object:

- ▶ **Cell Centroids:** The spatial coordinates marking the centroid for each cell being profiled
- ▶ **Cell Segmentation Boundaries:** The spatial coordinates that describe the polygon segmentation of each single cell
- ▶ **Molecule positions:** The spatial coordinates for each individual molecule that was detected during the multiplexed smFISH experiment.

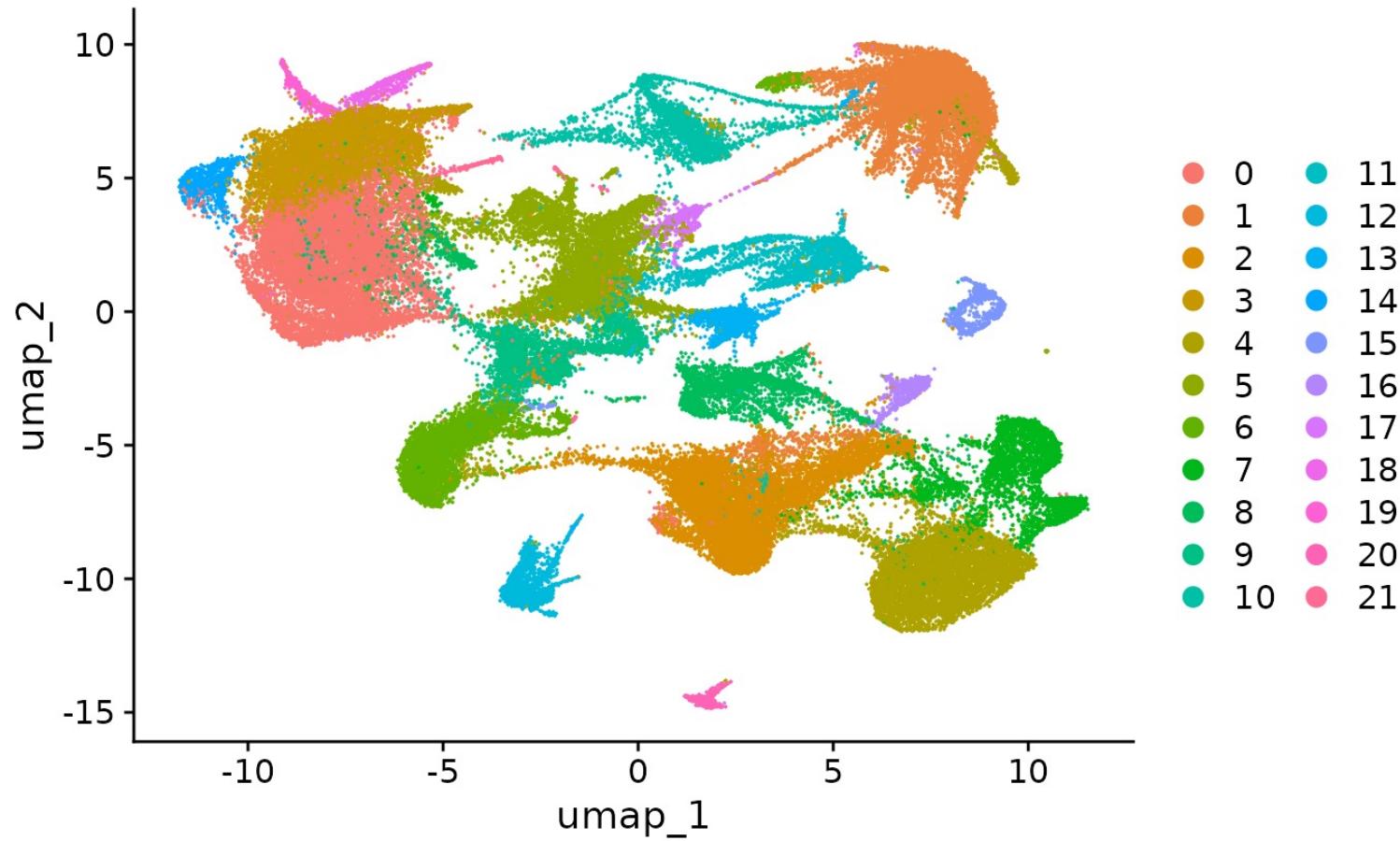
## Preprocessing and unsupervised analysis

We start by performing a standard unsupervised clustering analysis, essentially first treating the dataset as an scRNA-seq experiment. We use SCTransform-based normalization, though we slightly modify the default clipping parameters to mitigate the effect of outliers that we occasionally observe in smFISH experiments. After normalization, we can run dimensional reduction and clustering.

```
vizgen.obj <- SCTransform(vizgen.obj, assay = "Vizgen", clip.range = c(-10, 10))
vizgen.obj <- RunPCA(vizgen.obj, npcs = 30, features = rownames(vizgen.obj))
vizgen.obj <- RunUMAP(vizgen.obj, dims = 1:30)
vizgen.obj <- FindNeighbors(vizgen.obj, reduction = "pca", dims = 1:30)
vizgen.obj <- FindClusters(vizgen.obj, resolution = 0.3)
```

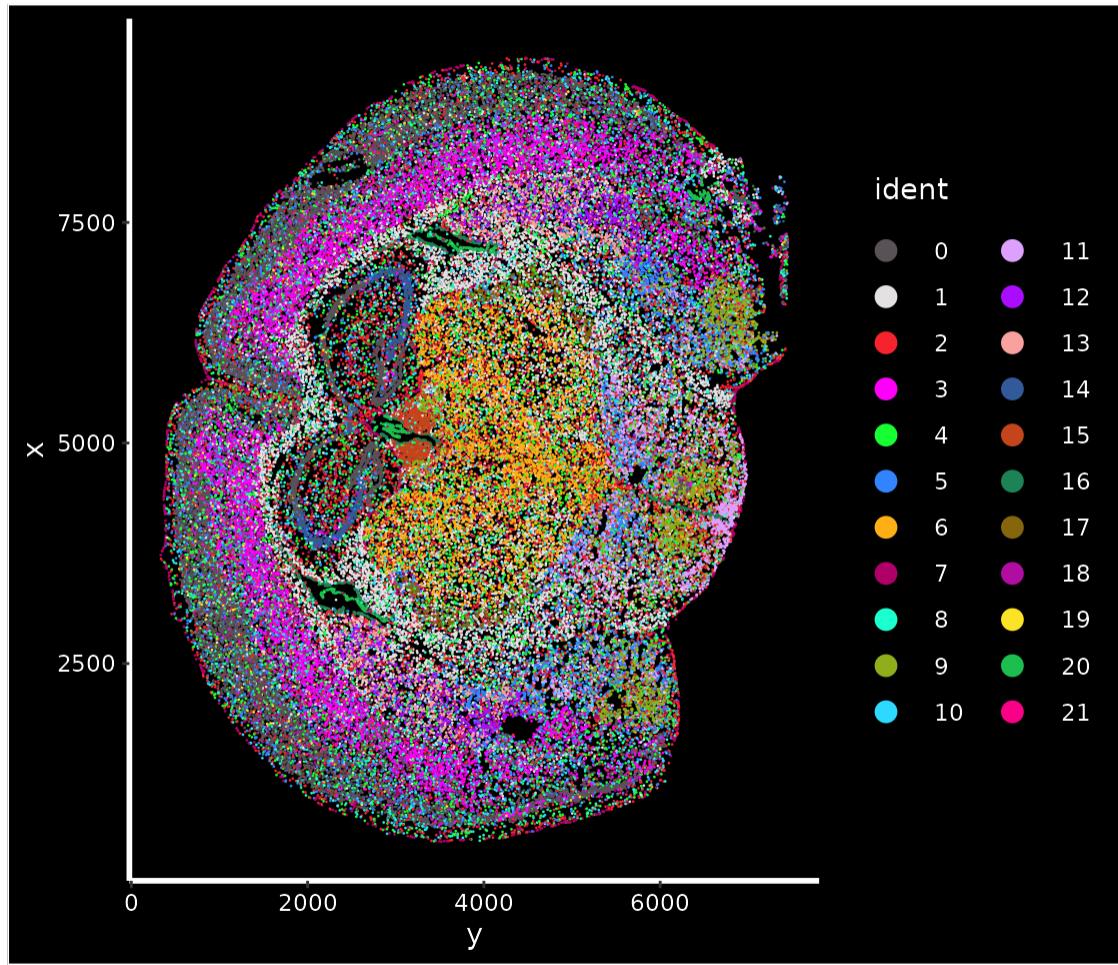
# Visualize the results of the clustering

---



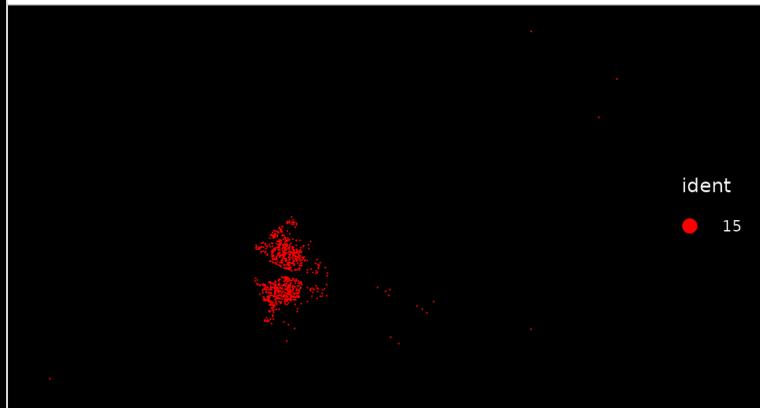
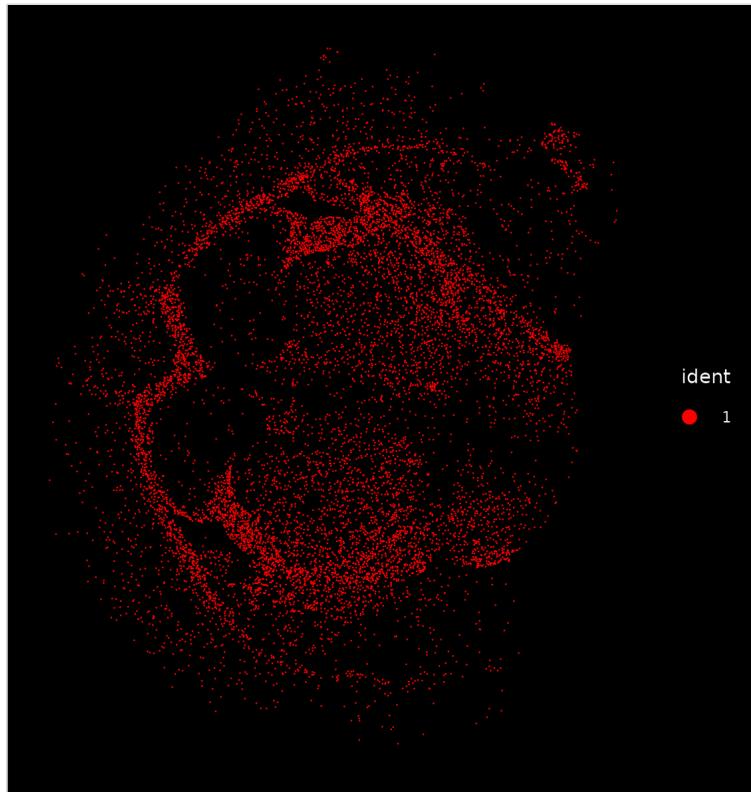
# Visualize the results of the clustering

---

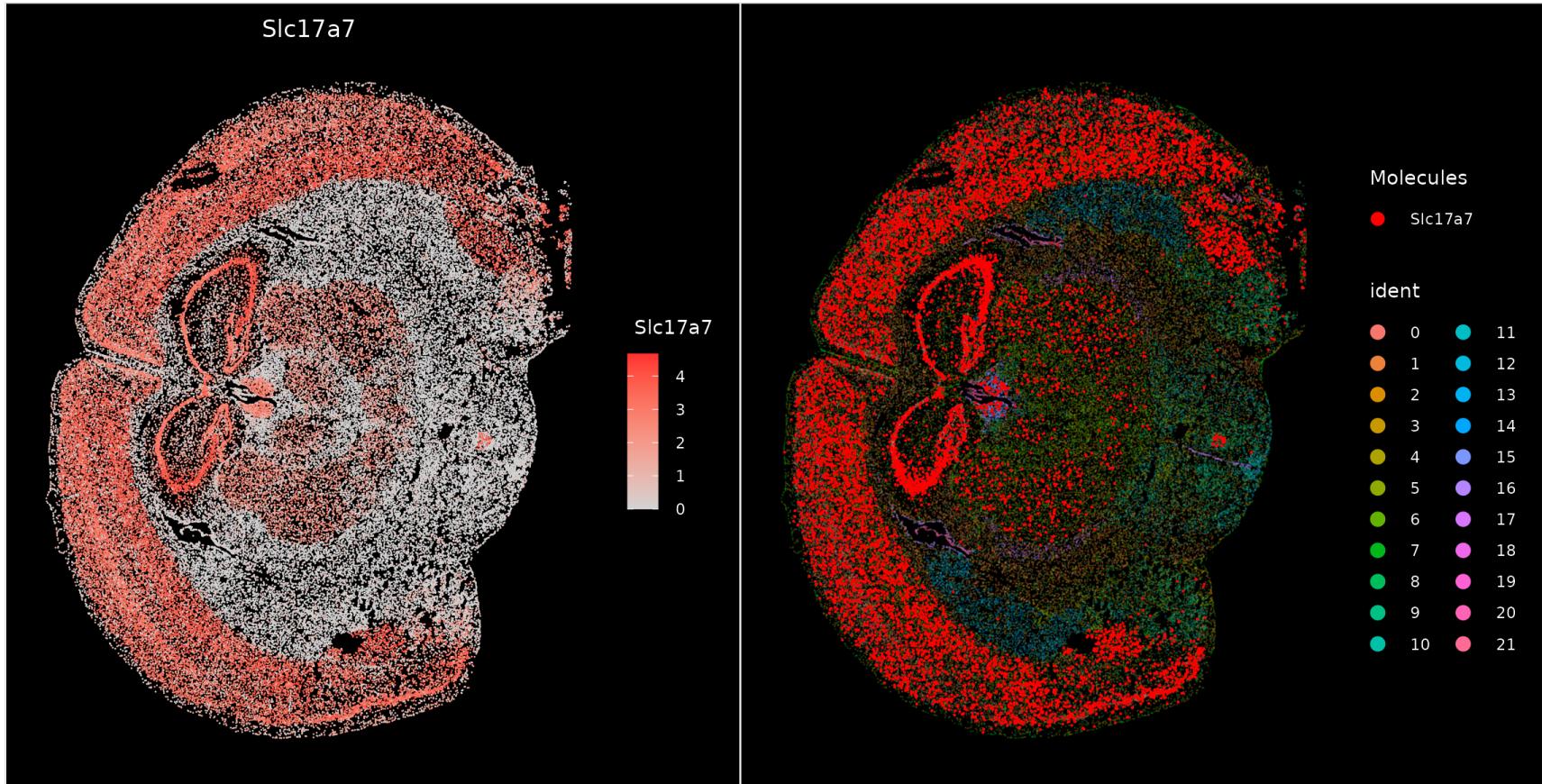


# highlight all cells that belong to a particular cluster

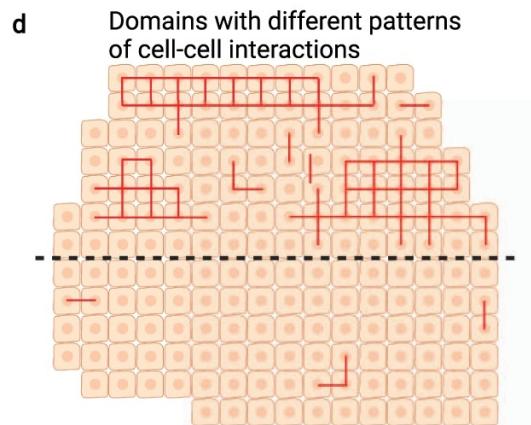
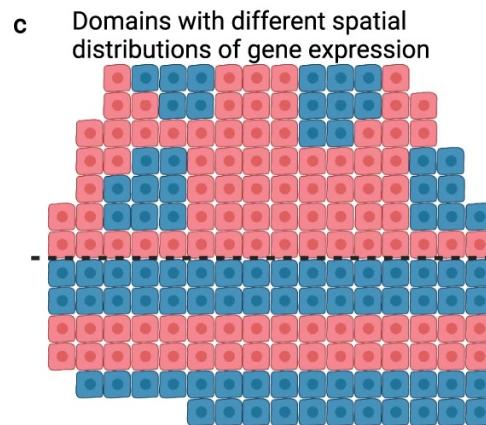
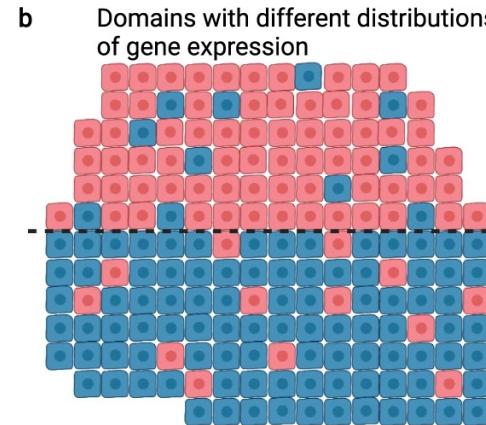
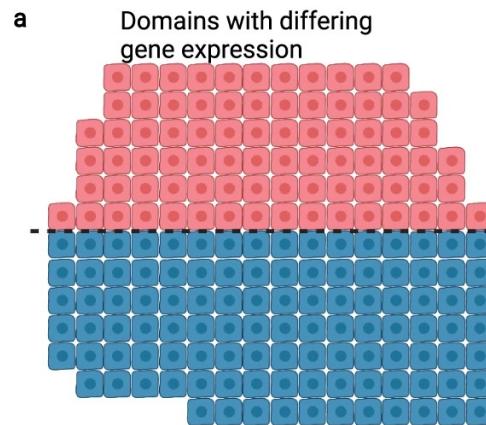
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# Markers of individual clusters and visualize their spatial expression pattern



# Illustration of different traits that can separate spatial regions



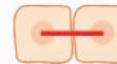
Spatial gene  
expression class 1



Spatial gene  
expression class 2

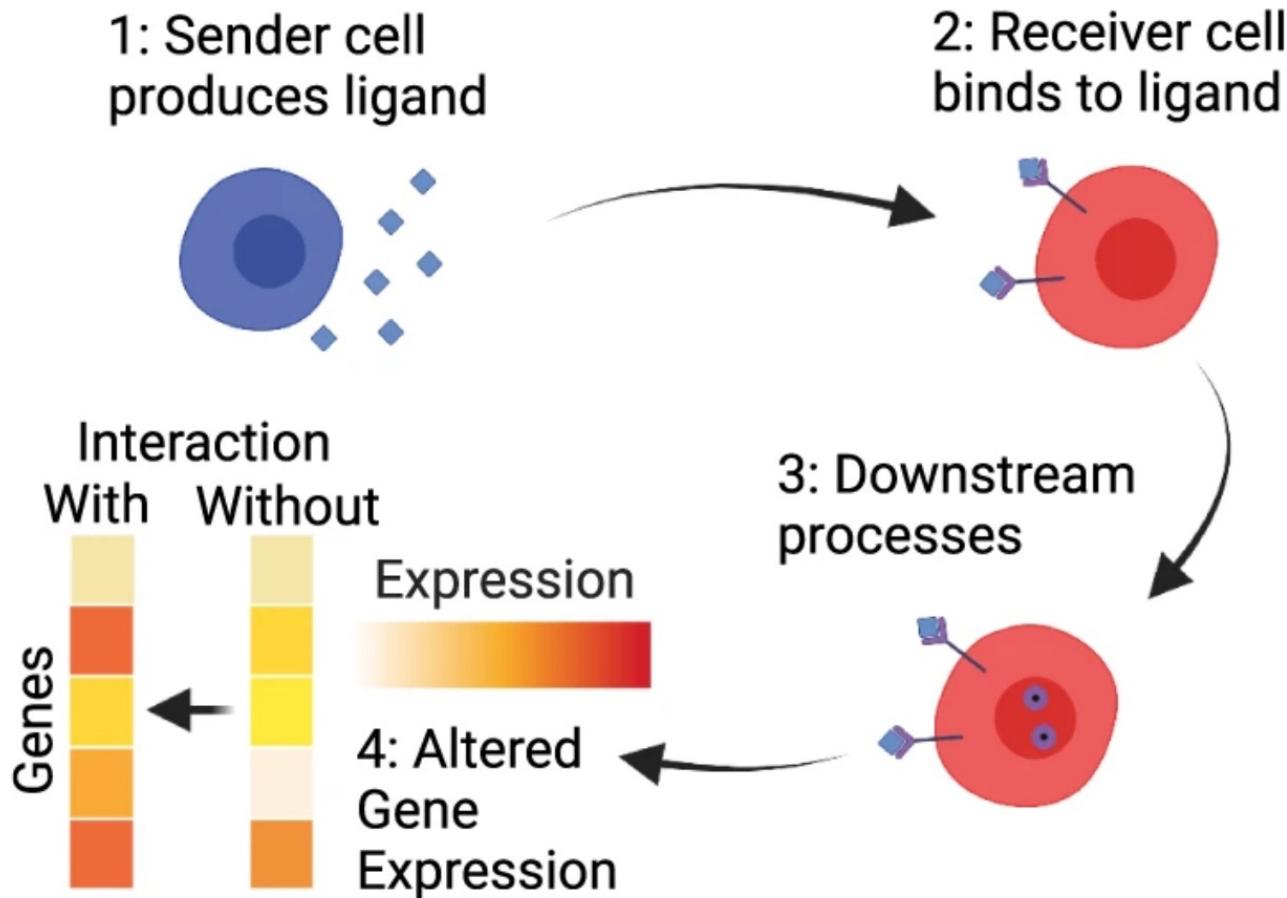


Interacting pair



# 从 ST 数据中提取细胞间相互作用

## a Cell-cell interactions



# 从 ST 数据中提取细胞间相互作用

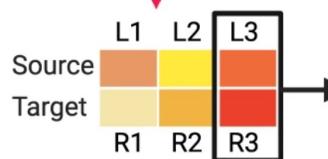
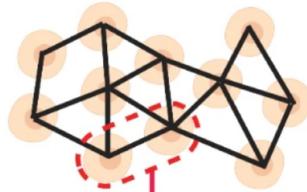
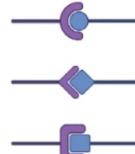
## b Pairwise Co-expression Methods

Identify co-expression of L-R pairs in  
spatially nearby cells

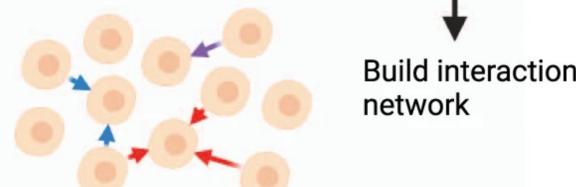
Inputs:

Cell spatial network  
+ expression data

Known L-R Pairs



Identify cell pairs  
co-expressing L+R



Build interaction  
network

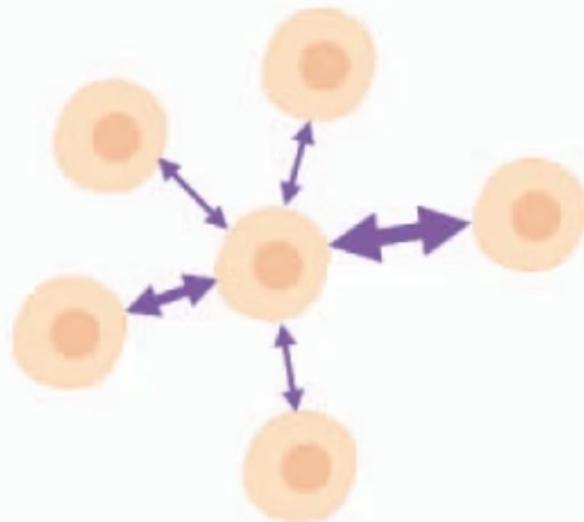
Interactions: → L1-R1   → L2-R2   → L3-R3

# 从 ST 数据中提取细胞间相互作用

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## c Probabilistic Modeling Methods

Identify SVGs explicitly modeling CCI as one source of variance in gene expression

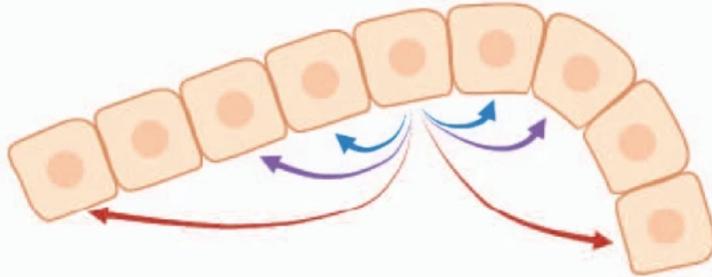


**Inter-cell component**  
represents explanation of variation in expression due to interactions between cells  
**Large inter-cell component**  
identifies cell-cell communication

# 从 ST 数据中提取细胞间相互作用

## d Insights

Distinguishing short-range communication from spurious long-range communication

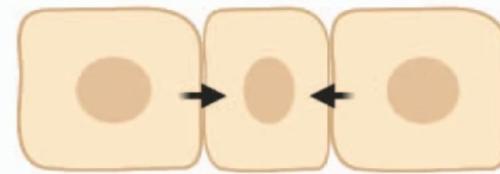


Directly adjacent - membrane bound proteins

Short range - diffusive ligands

Long range - communication spurious

Relationships between CCI and physical contacts and pressure between cells.



High pressure



Low pressure



# 细胞互作-常用软件CellPhoneDB

nature  
protocols

PROTOCOL

<https://doi.org/10.1038/s41596-020-0292-x>

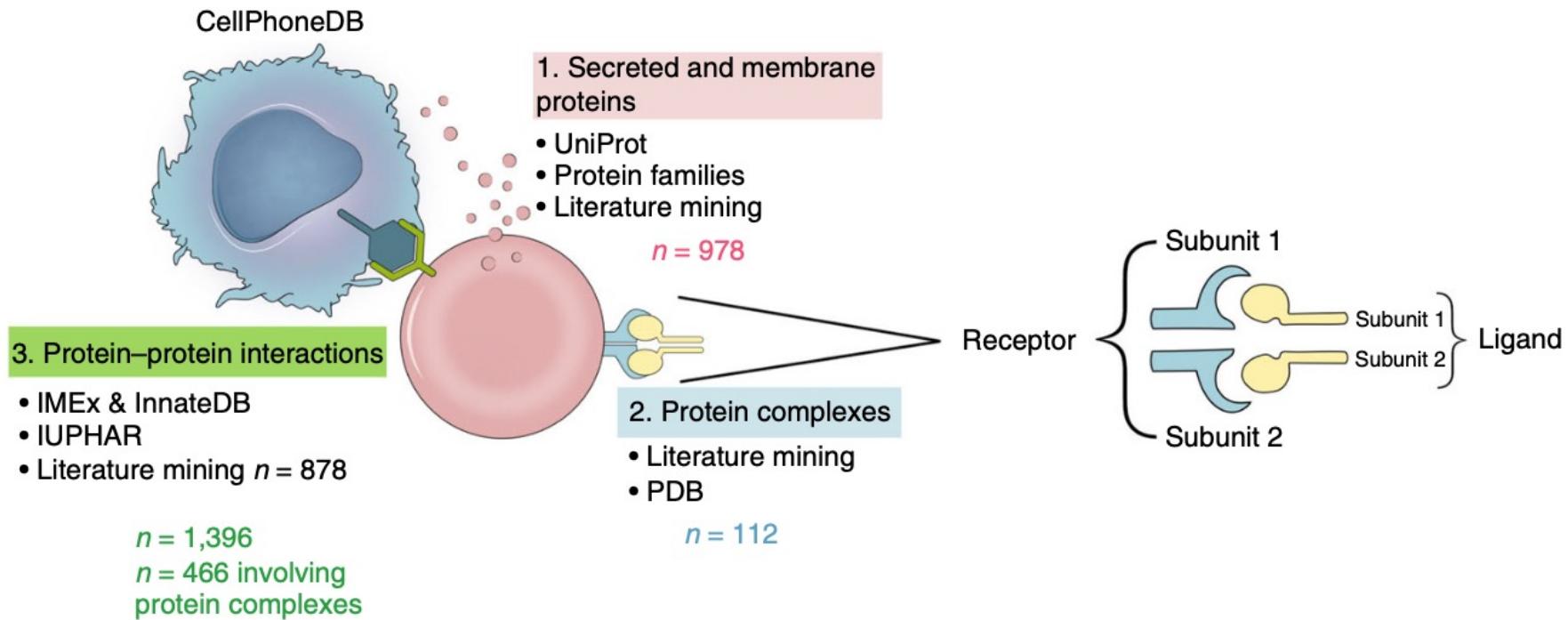
## CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes

Mirjana Efremova<sup>1</sup>, Miquel Vento-Tormo<sup>2</sup>, Sarah A. Teichmann<sup>1,3</sup> and Roser Vento-Tormo<sup>1\*</sup>

- 是公开的人工校正的，储存受体、配体以及两种相互作用的数据库，能够描述异构复合物，可预测多聚体互作，支持空间转录组

# 细胞互作-常用软件CellPhoneDB

## ■ 其包含配体、受体及其相互作用的数据库



# 细胞互作-常用软件CellChat

ARTICLE

 Check for updates

<https://doi.org/10.1038/s41467-021-21246-9>

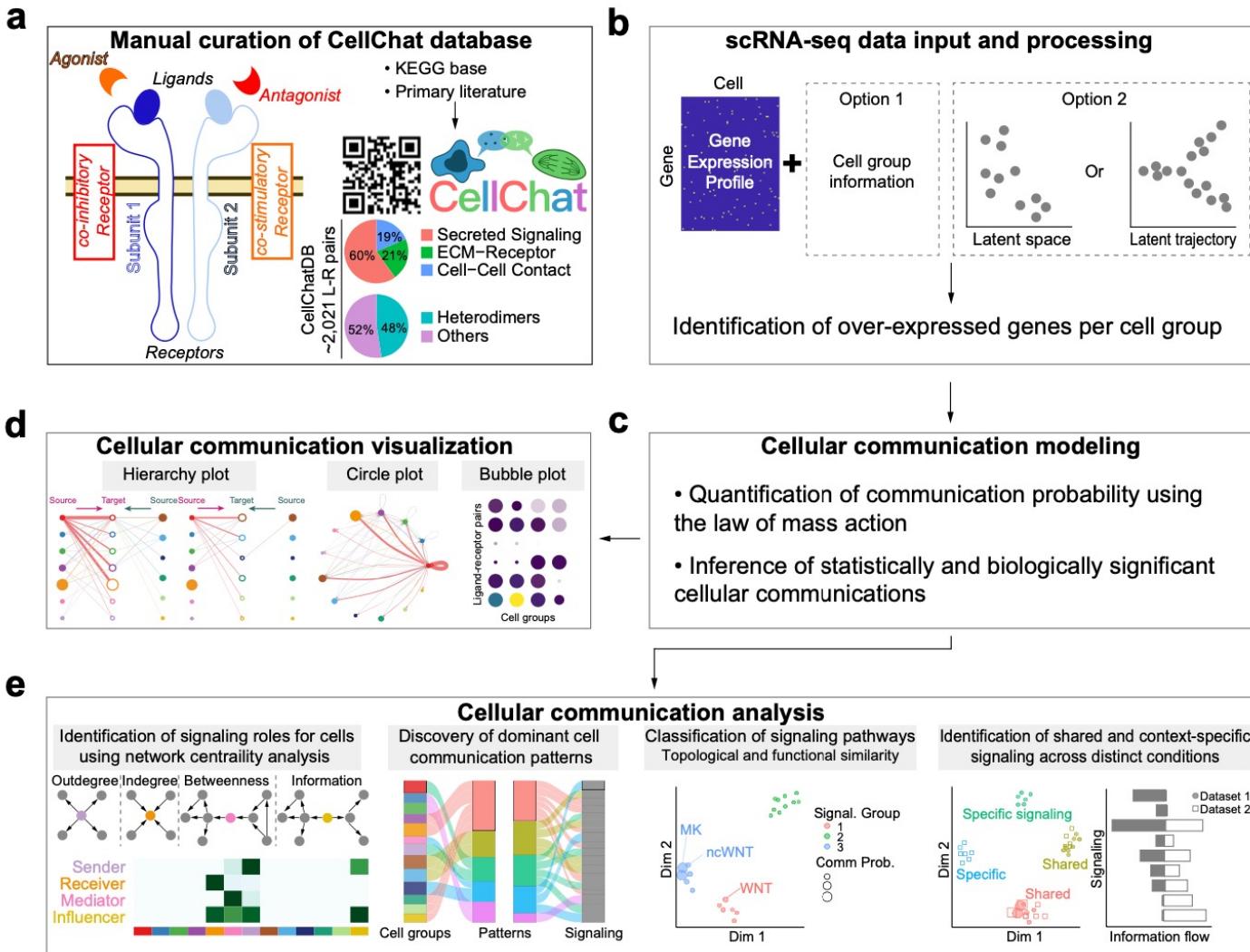
OPEN

## Inference and analysis of cell-cell communication using CellChat

Suoqin Jin  <sup>1,2</sup>, Christian F. Guerrero-Juarez  <sup>1,2,3,4</sup>, Lihua Zhang <sup>1,2</sup>, Ivan Chang <sup>5,6</sup>, Raul Ramos <sup>2,3,4</sup>, Chen-Hsiang Kuan <sup>3,4,7,8</sup>, Peggy Myung  <sup>9,10</sup>, Maksim V. Plikus  <sup>2,3,4</sup>✉ & Qing Nie  <sup>1,2,3</sup>✉

■ 将细胞的基因表达数据作为输入，并结合配体受体及其辅助因子的相互作用来模拟细胞间通讯，可预测辅因子互作，支持空间转录组

# 细胞互作-CellChat



<https://www.nature.com/articles/s41467-021-21246-9>

# 细胞互作-常用软件NicheNet

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Brief Communication | [Published: 09 December 2019](#)

## NicheNet: modeling intercellular communication by linking ligands to target genes

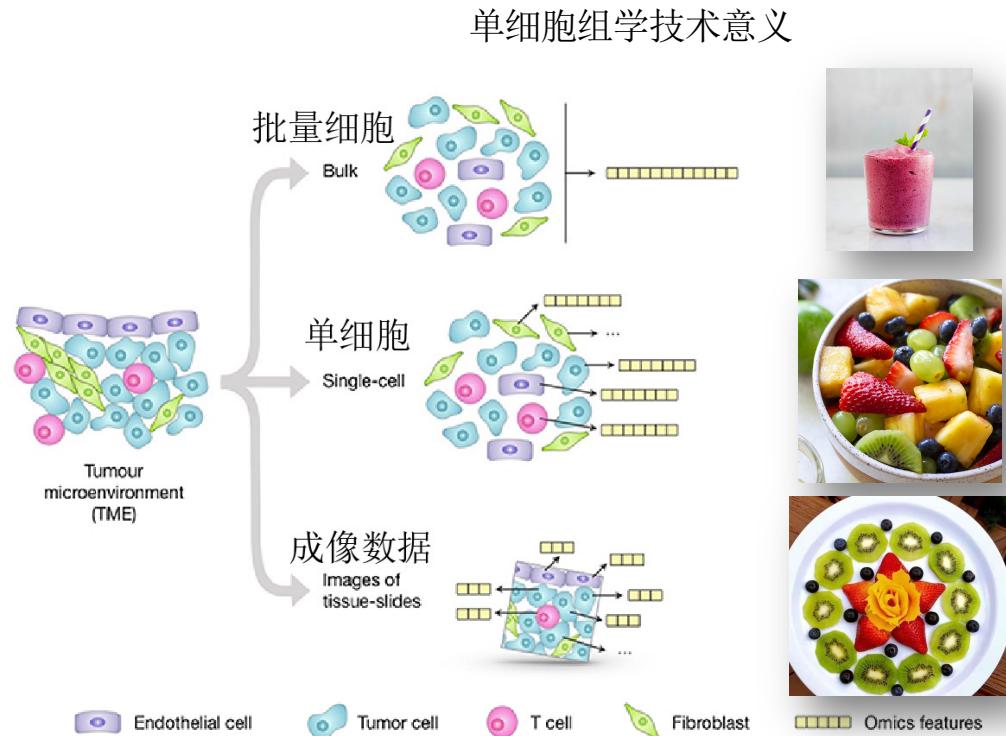
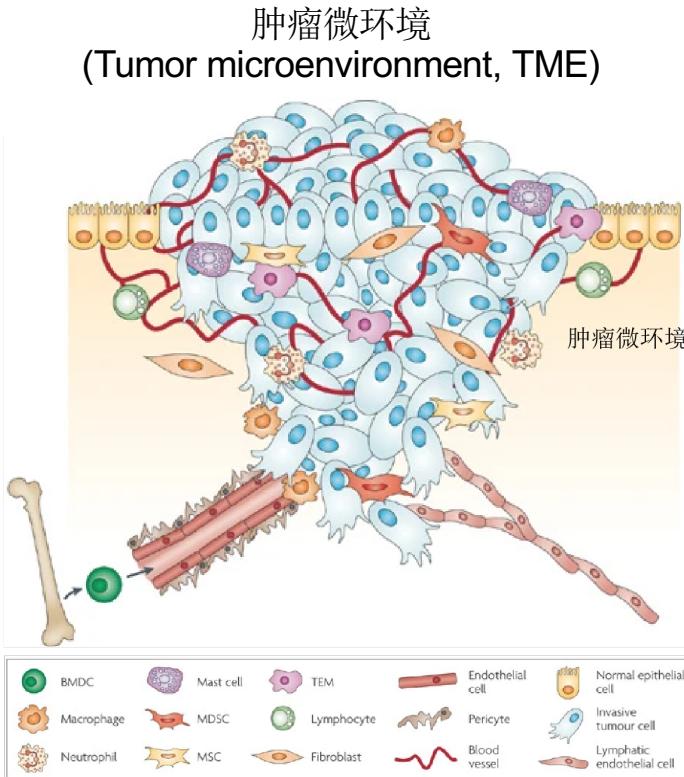
[Robin Browaeys](#), [Wouter Saelens](#) & [Yvan Saeys](#) 

[Nature Methods](#) **17**, 159–162 (2020) | [Cite this article](#)

54k Accesses | 354 Citations | 162 Altmetric | [Metrics](#)

- 通过将相互作用细胞的表达数据与信号和基因调控网络的先验知识相结合来预测相互作用细胞之间的配体-靶标联系的方法

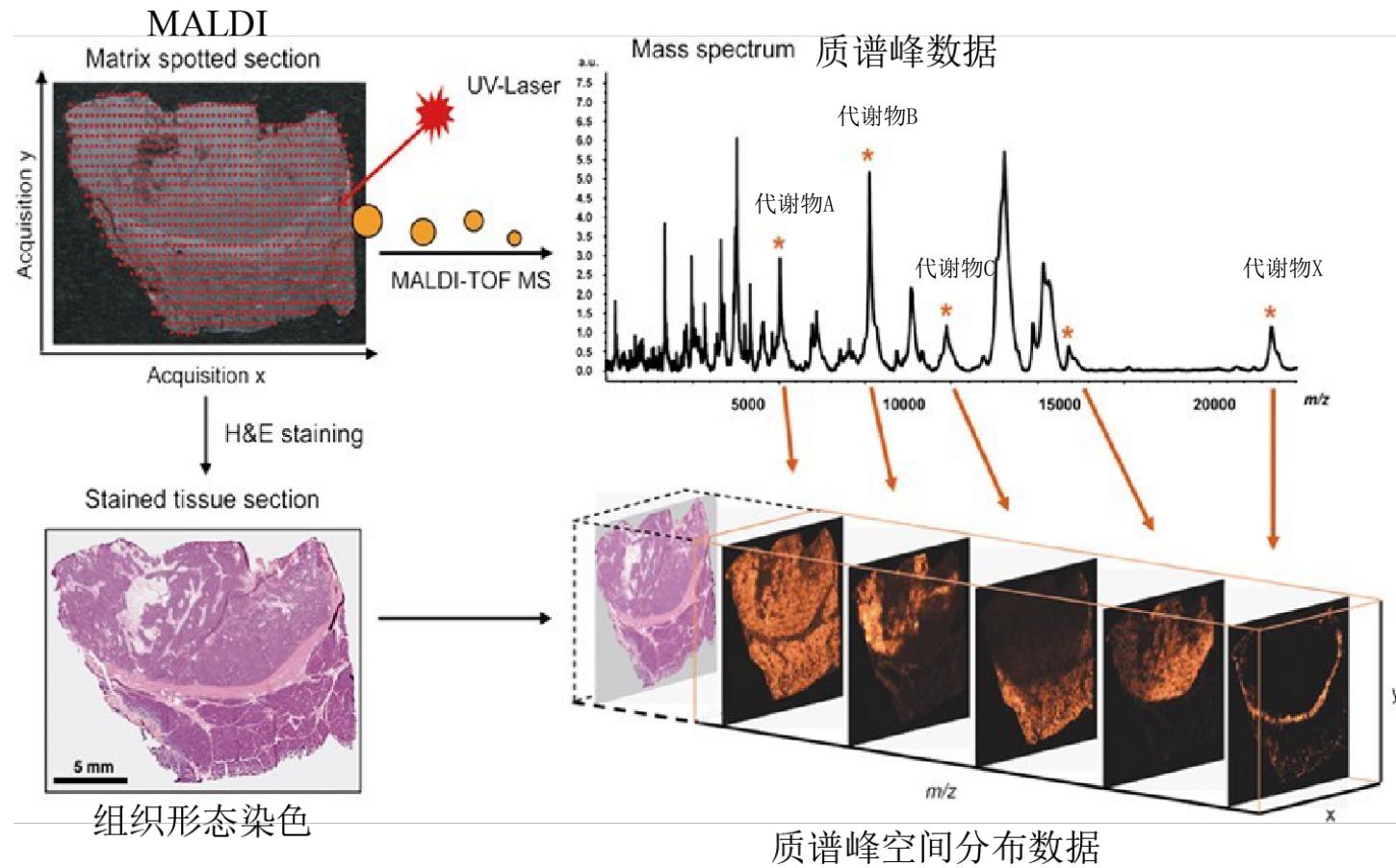
## 背景简介 | 如何在组织水平解析单细胞核中的代谢物质？



不同时空有着不同的代谢微环境

Finotello, F. & Eduati, F. *Frontiers in Oncology* **8**, (2018).  
Joyce, J. A. & Pollard, J. W. *Nature Reviews Cancer* **9**, 239-252 (2009).

## 背景简介 | 空间代谢组学技术成像原理



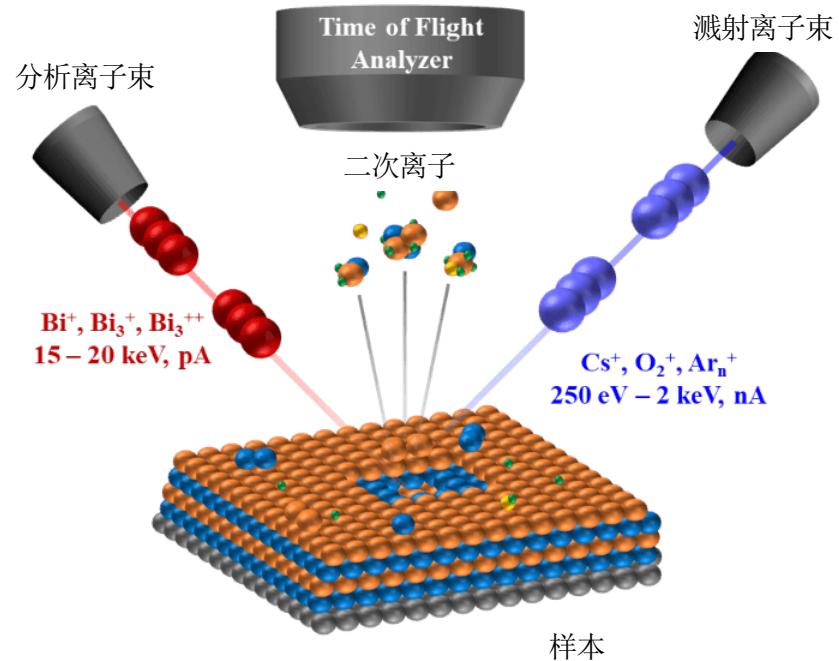
Aichler, M. & Walch, A. *Laboratory Investigation* **95**, 422-431, (2015).

## 背景简介 | 二次离子质谱技术

PHI nanoTOF II TOF-SIMS



二次离子质谱（SIMS）技术原理



实现亚细胞空间分辨率（最高可到~50nm）



## SEAM方法

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Article | [Published: 04 October 2021](#)

## SEAM is a spatial single nuclear metabolomics method for dissecting tissue microenvironment

[Zhiyuan Yuan](#), [Qiming Zhou](#), [Lesi Cai](#), [Lin Pan](#), [Weiliang Sun](#), [Shiwei Qumu](#), [Si Yu](#), [Jiaxin Feng](#), [Hansen Zhao](#), [Yongchang Zheng](#), [Minglei Shi](#), [Shao Li](#), [Yang Chen](#)✉, [Xinrong Zhang](#)✉ & [Michael Q. Zhang](#)✉

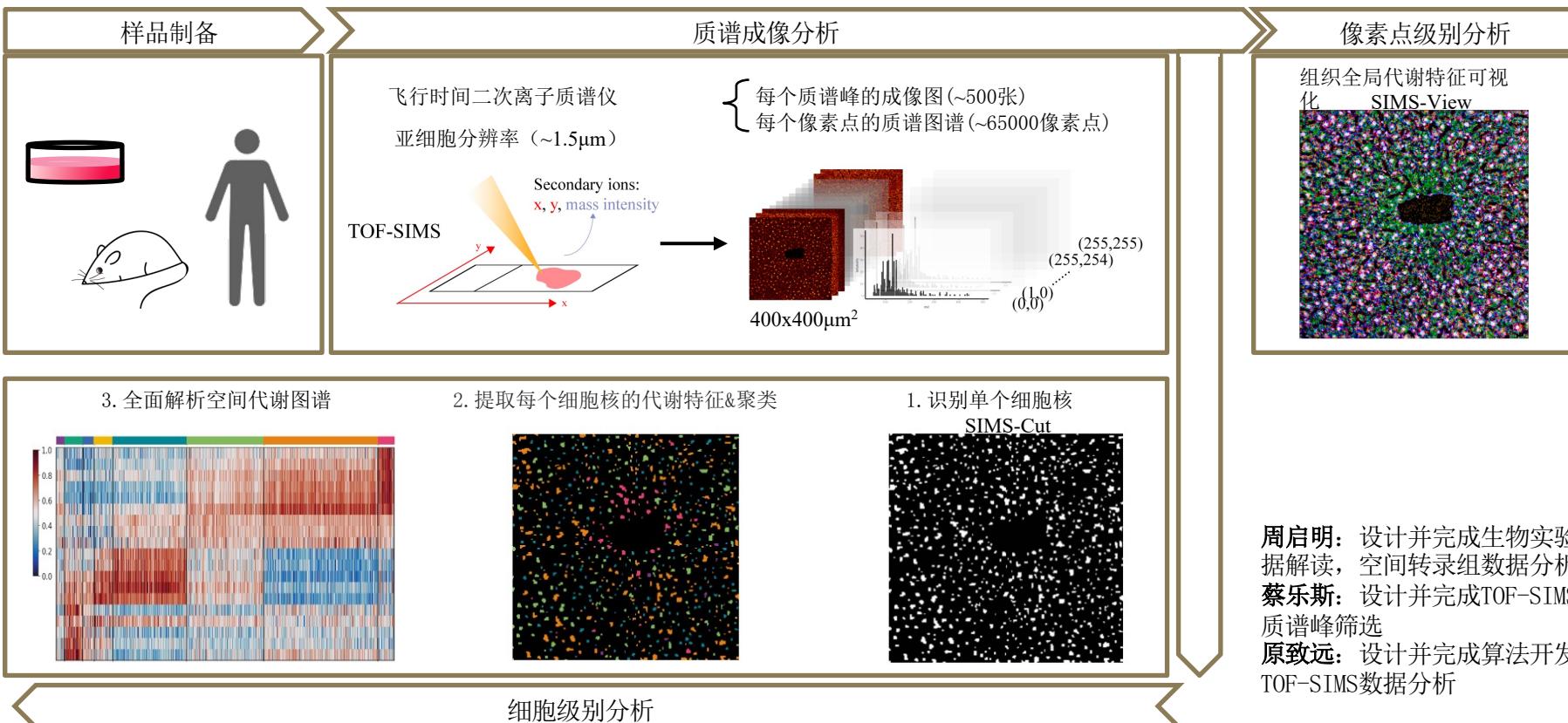
[Nature Methods](#) **18**, 1223–1232 (2021) | [Cite this article](#)

5979 Accesses | 8 Citations | 16 Altmetric | [Metrics](#)

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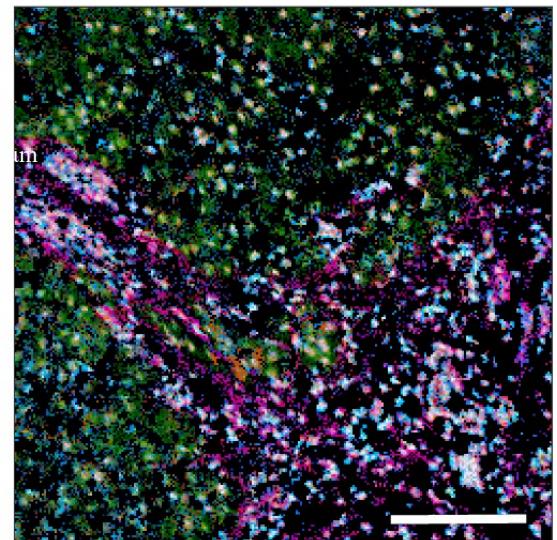
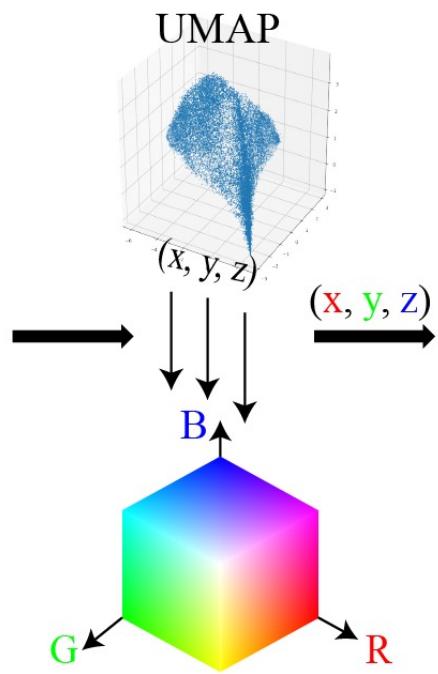
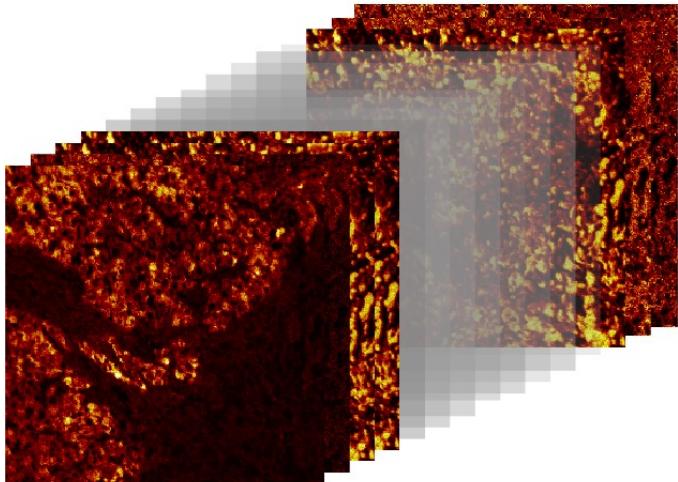
# SEAM方法流程

## Spatial single nuclEar metAboloMics (SEAM)



Zhiyuan Yuan, Qiming Zhou, Lesi Cai, et al. 2021. Nature Methods.

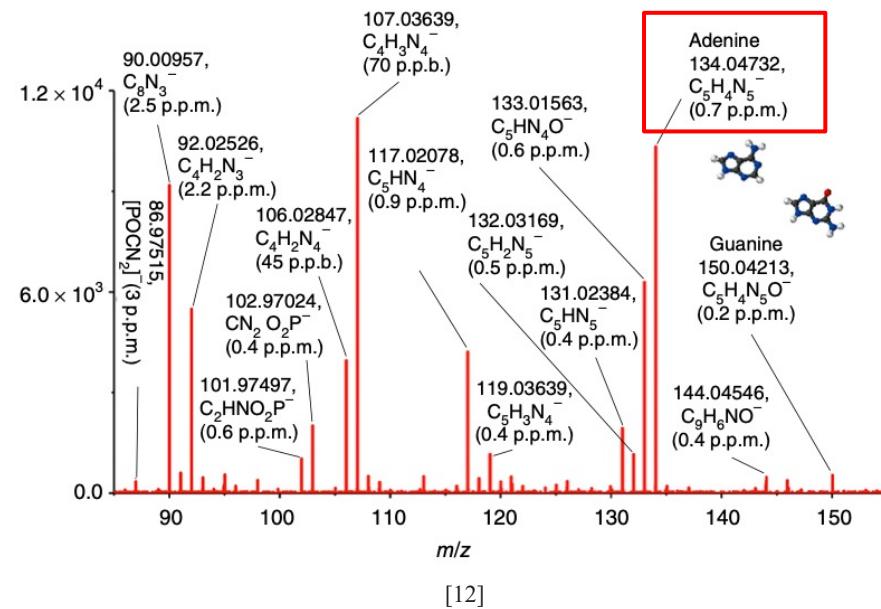
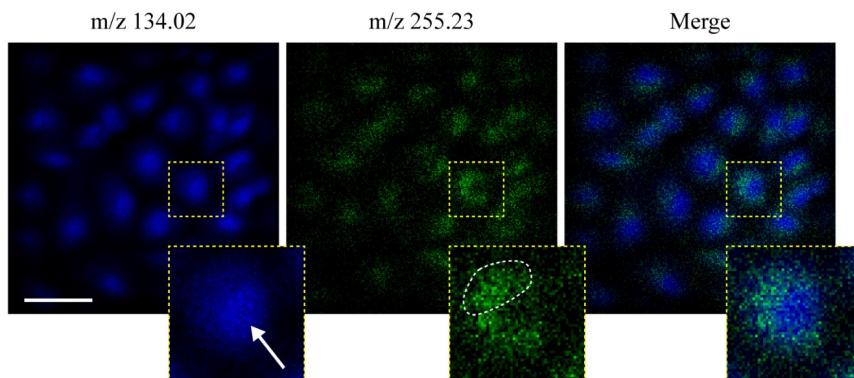
## SIMS-View降维可视化空间代谢组数据



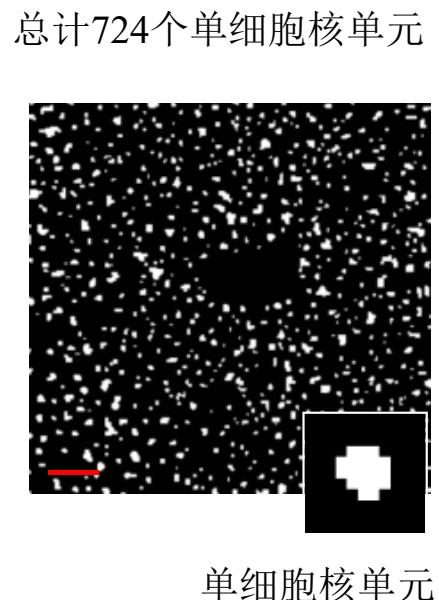
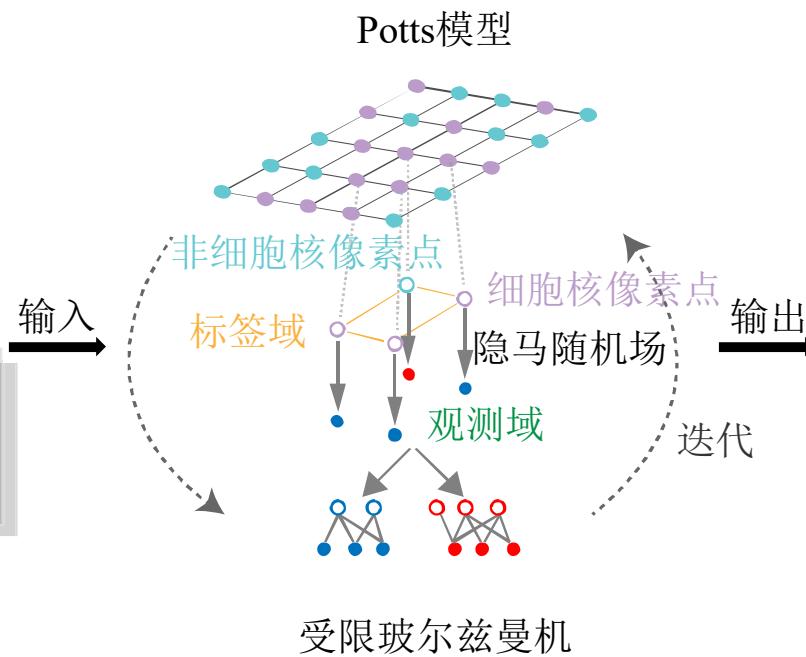
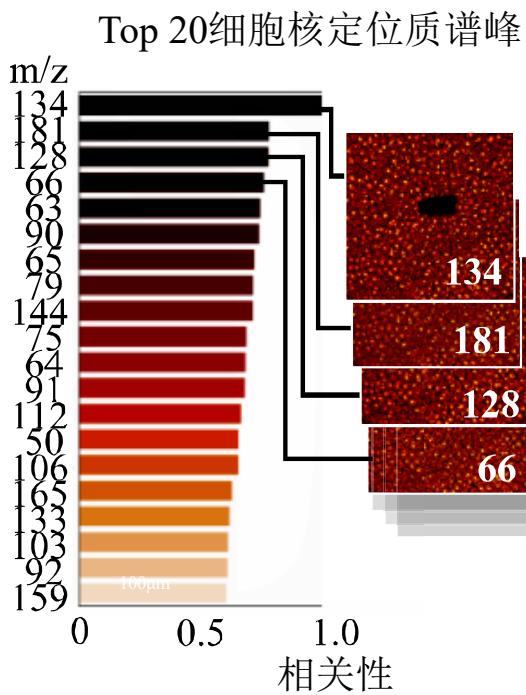
## 细胞核代谢物特征观察

开发一个具有单细胞分辨率的组织原位空间代谢组的系统性研究方法:

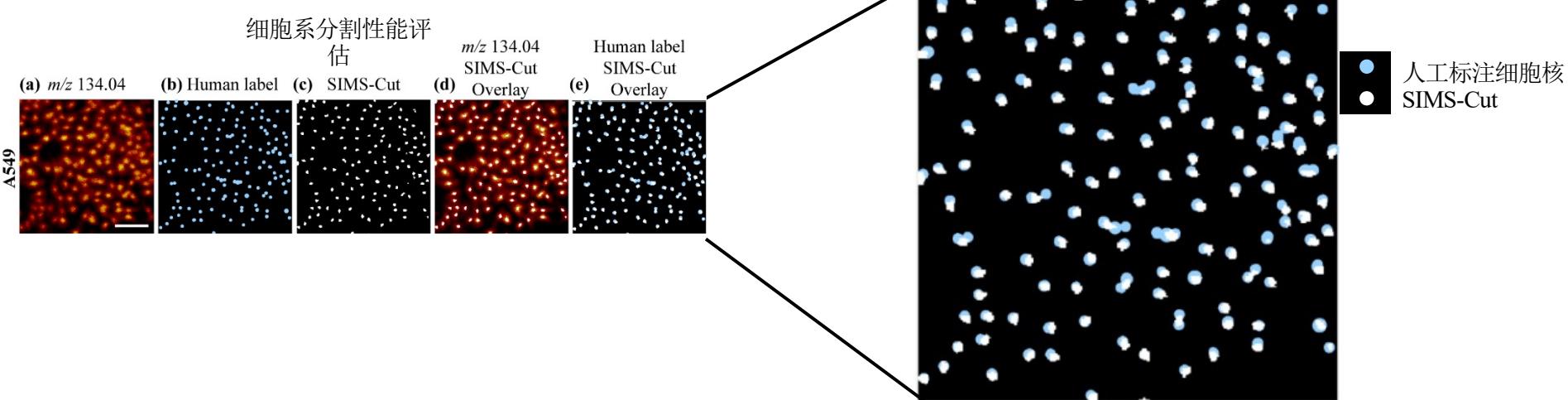
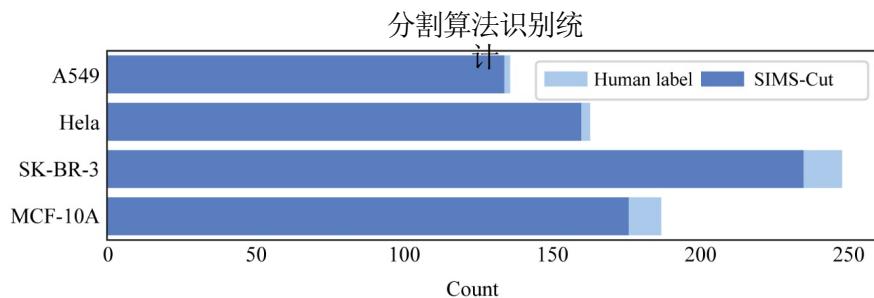
1. 识别单个细胞核
2. 提取每个细胞核的代谢特征
3. 全面解析空间代谢图谱



## 单细胞核识别算法SIMS-Cut

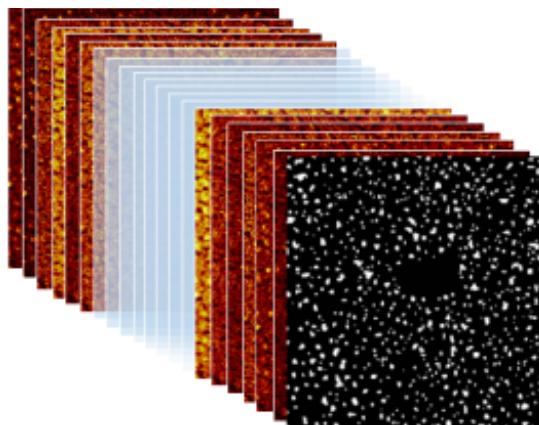


## 单细胞核识别算法SIMS-Cut

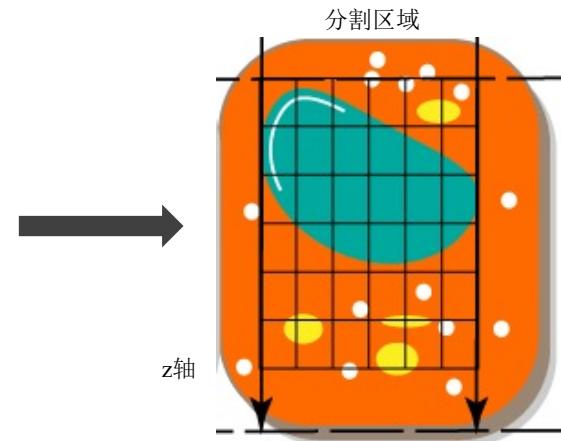


## 细胞核信息提取和代谢离子差异分析

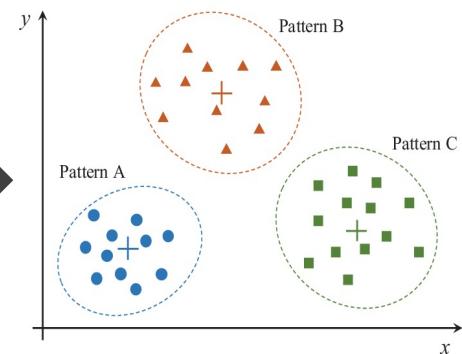
结合细胞分割和质谱信息



提取每个细胞信息



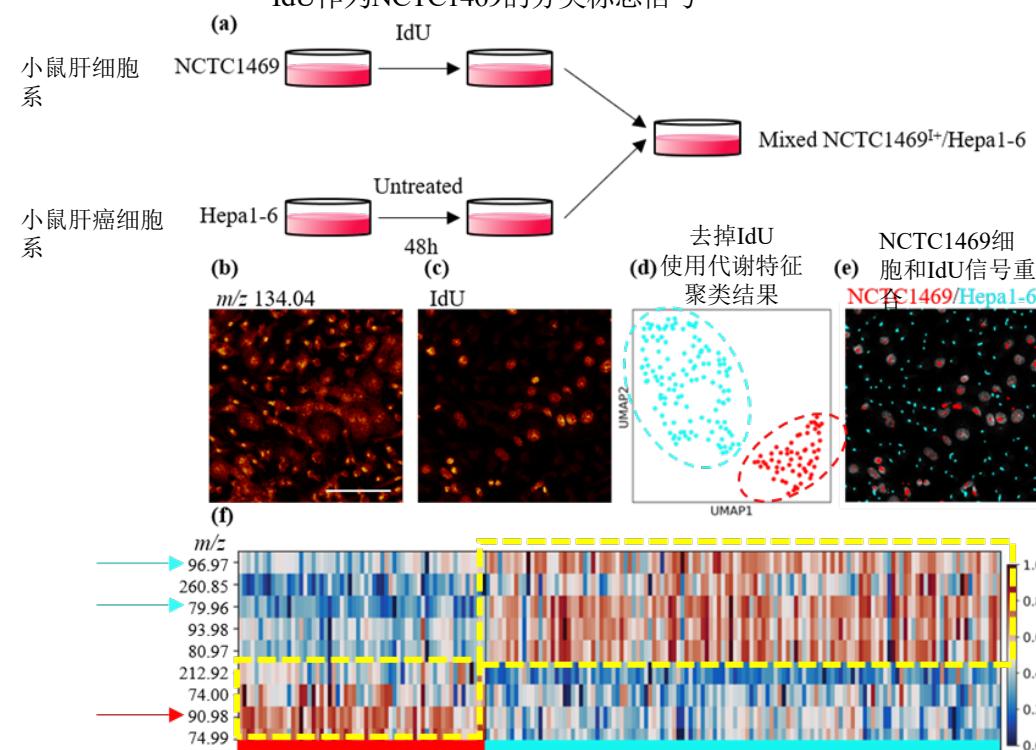
聚类和差异分析



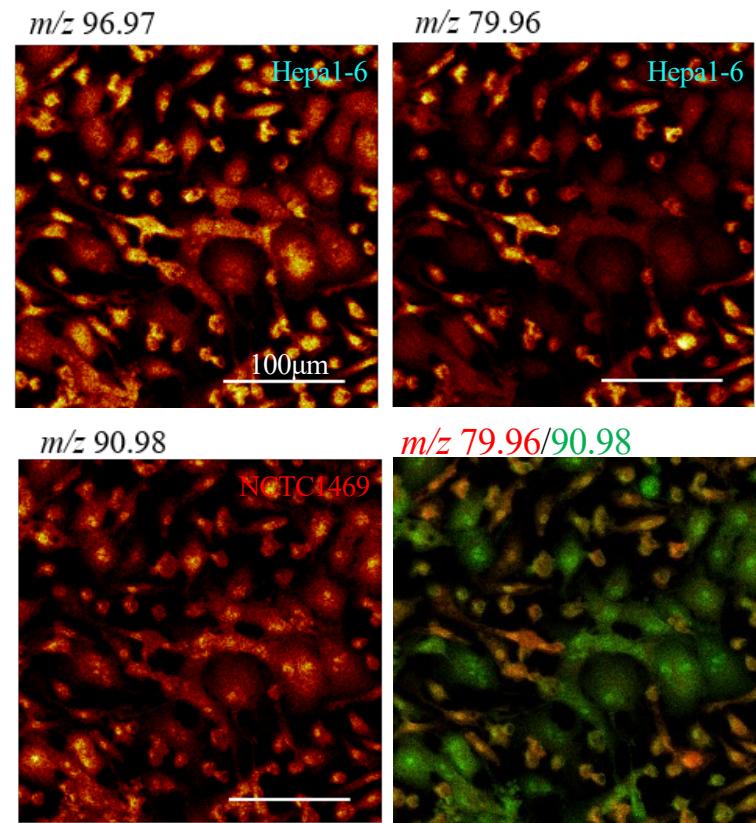
## SEAM在细胞系中聚类真实性验证

### ➤ SEAM在细胞系中聚类真实性验证

IdU作为NCTC1469的分类标志信号

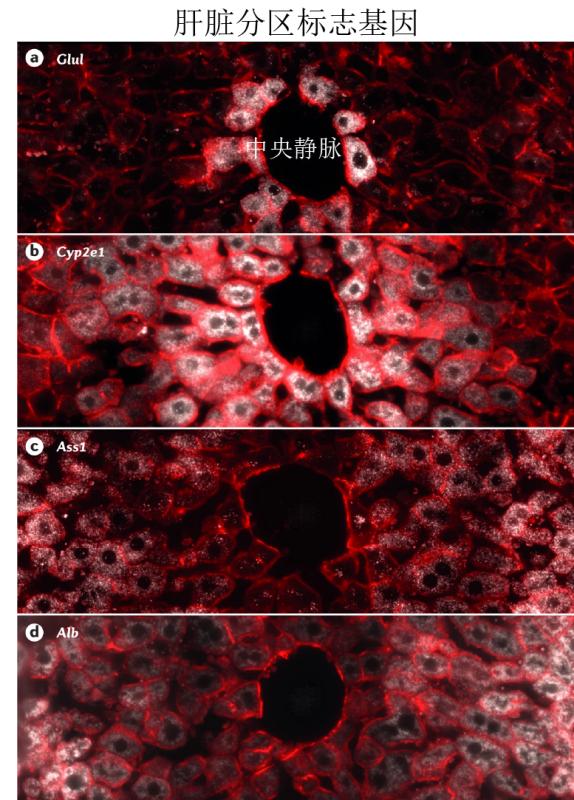
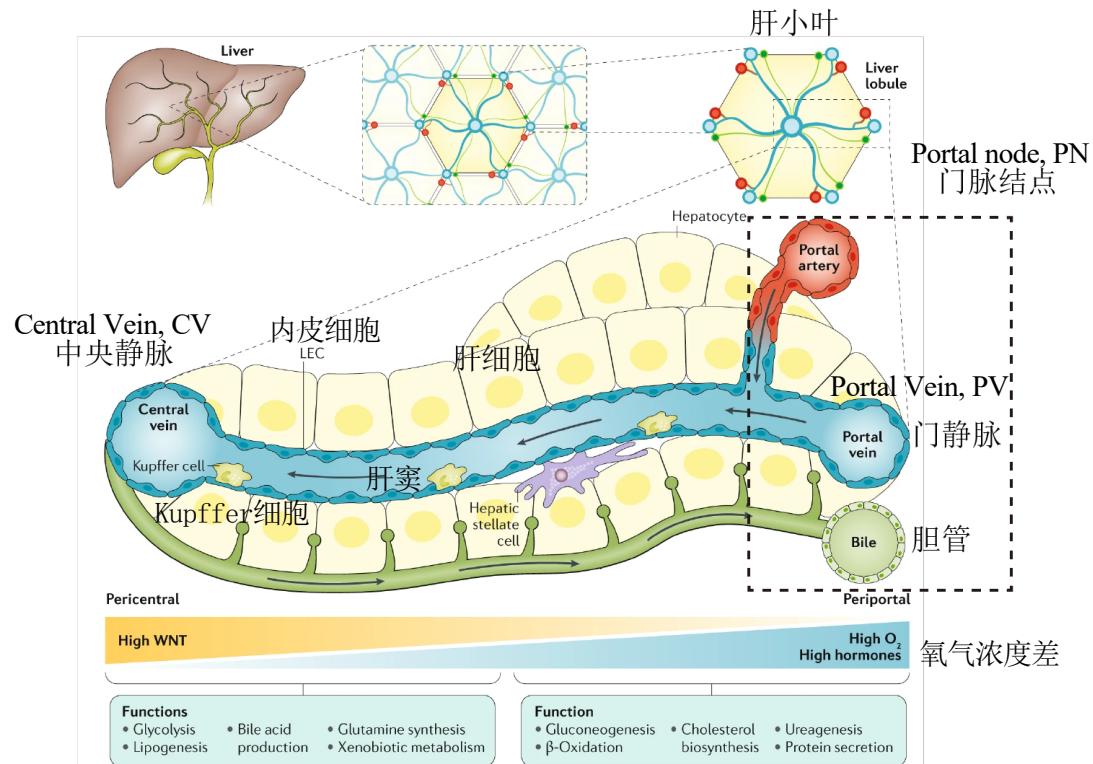


验证细胞分类的真实性

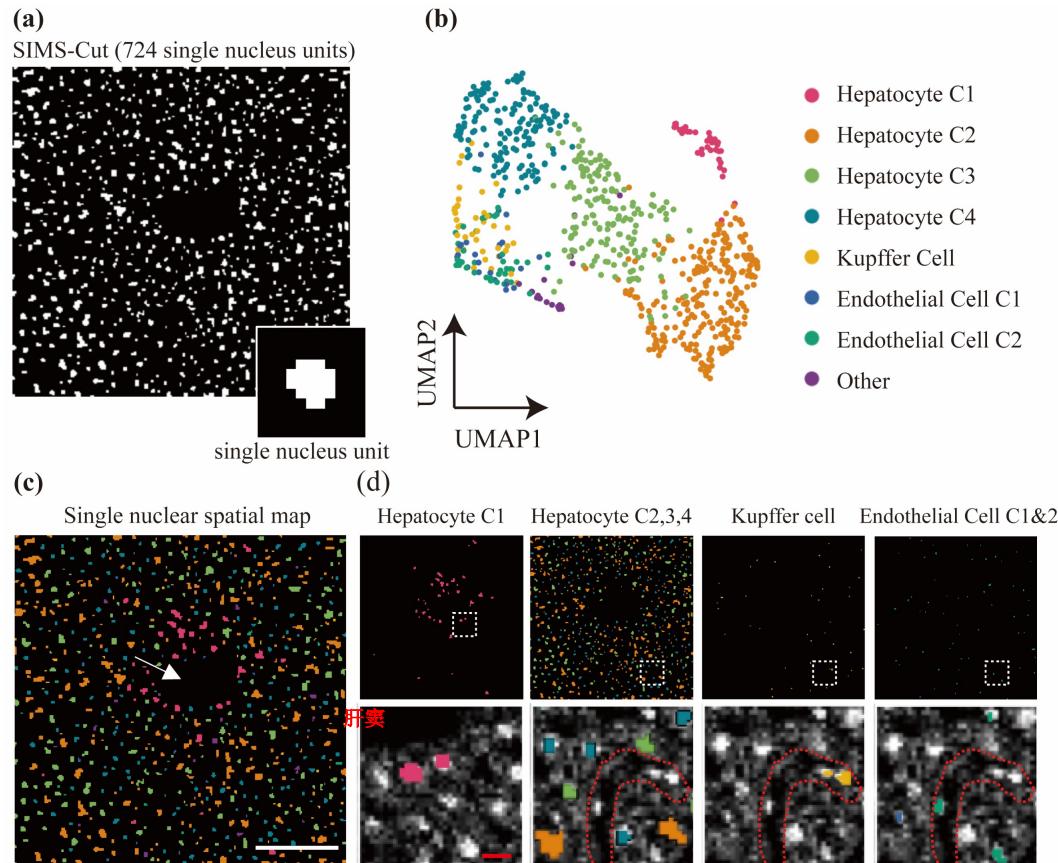


# SEAM方法在小鼠组织中的测试

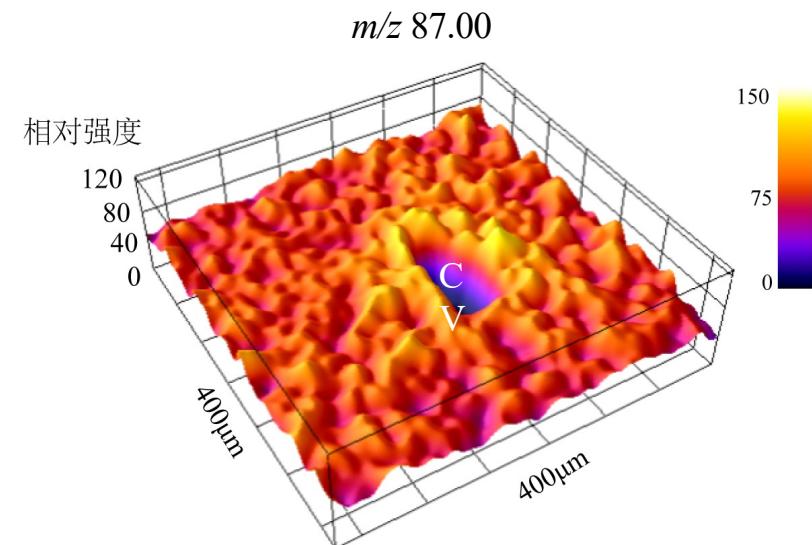
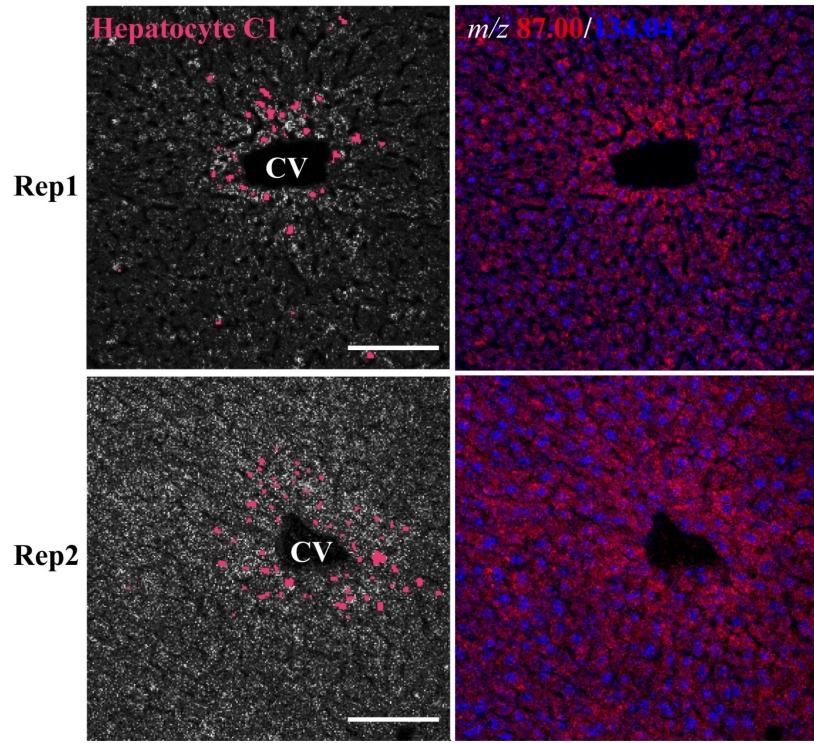
正常肝组织具有肝脏分区化(Liver zonation)现象



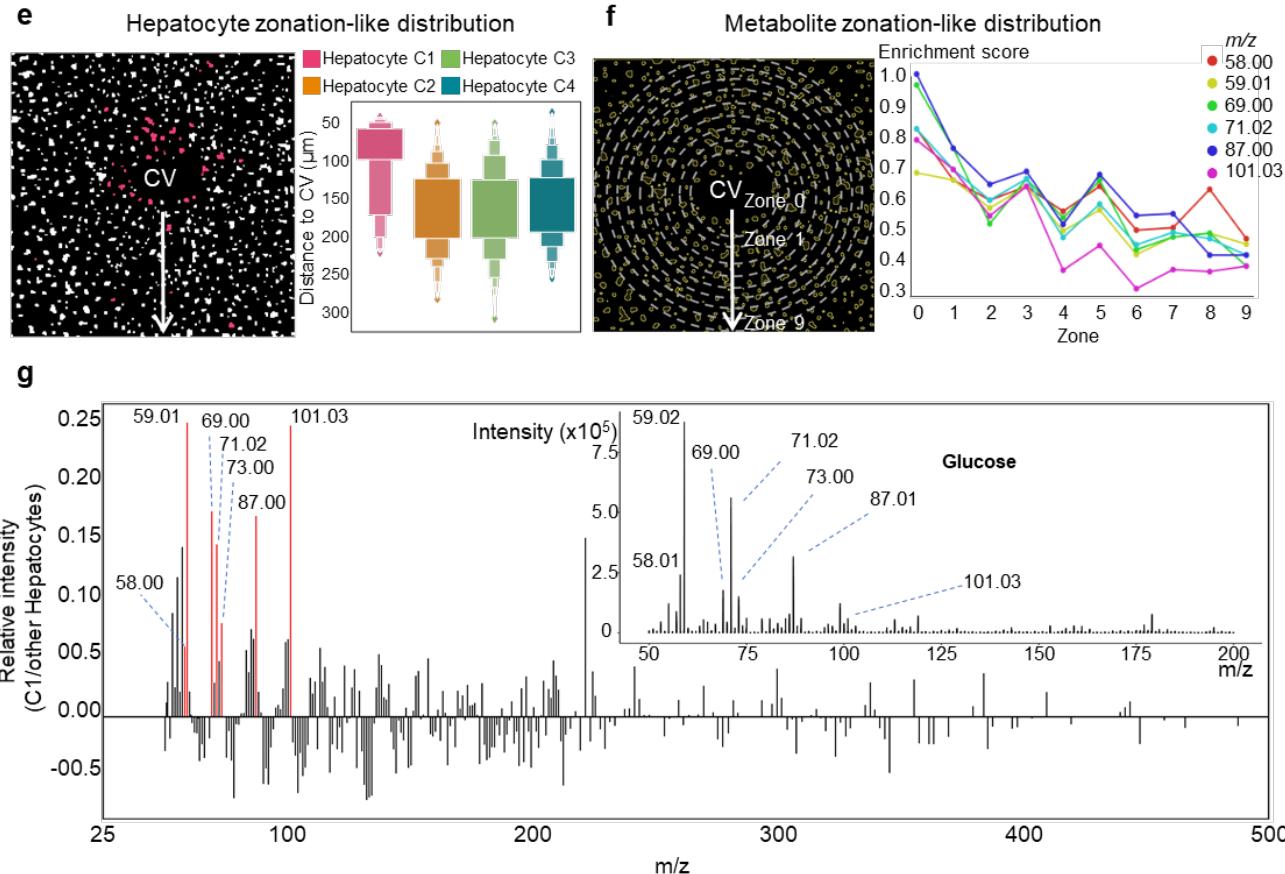
# SEAM鉴定小鼠肝组织不同细胞类型



## SEAM在小鼠肝中发现和肝分区相关的代谢特异性肝细胞亚群



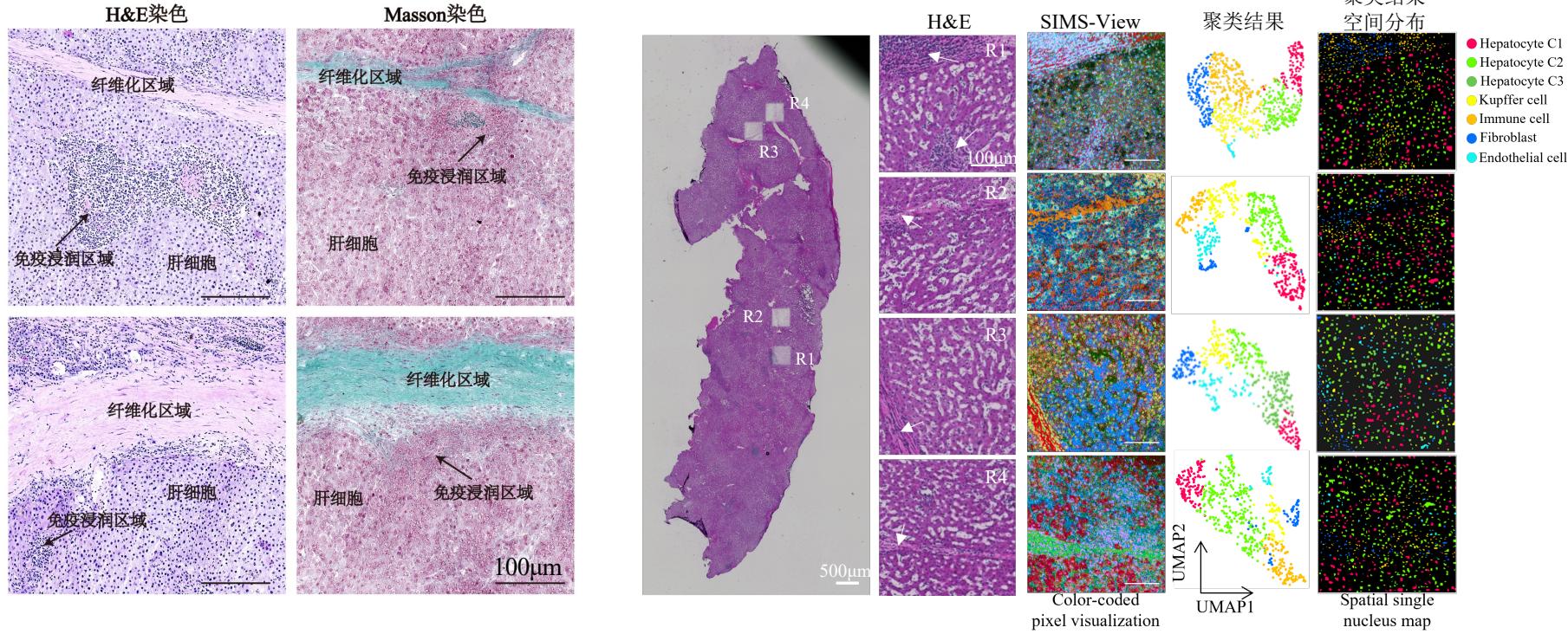
## SEAM在小鼠肝中发现和肝分区相关的代谢特异性肝细胞亚群



SEAM发现的肝脏分区代谢指纹图谱很大可能为葡萄糖

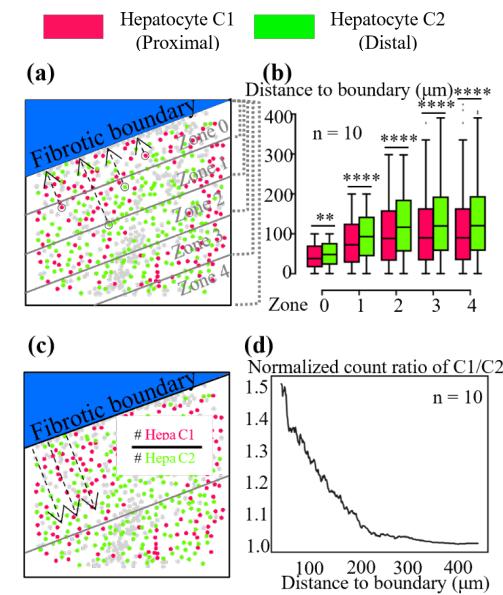
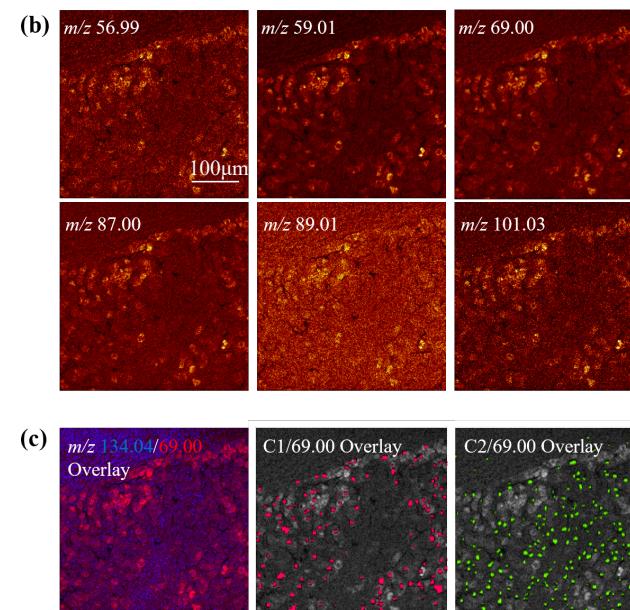
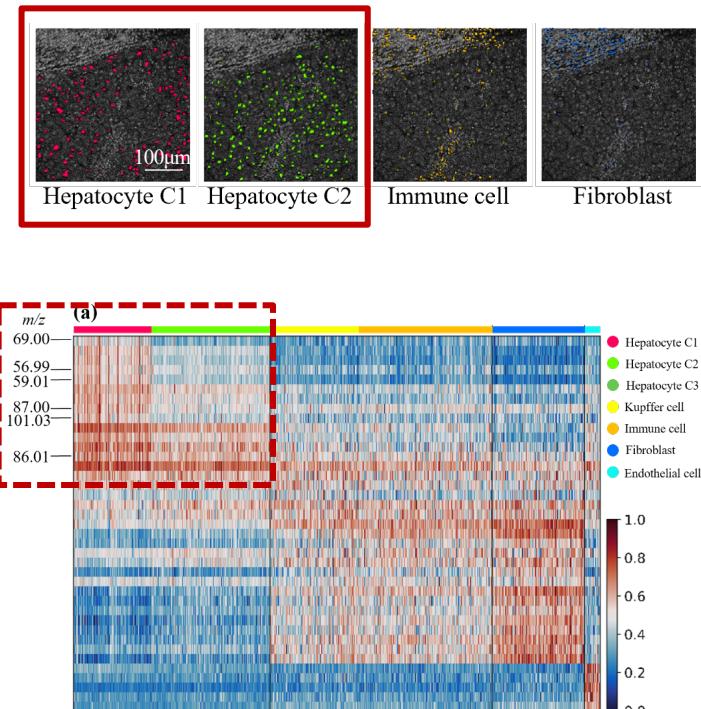
# SEAM方法在人纤维化肝组织中的应用

➤ SEAM发现了与肝纤维化微环境相关的不同肝细胞亚群的代谢异质性



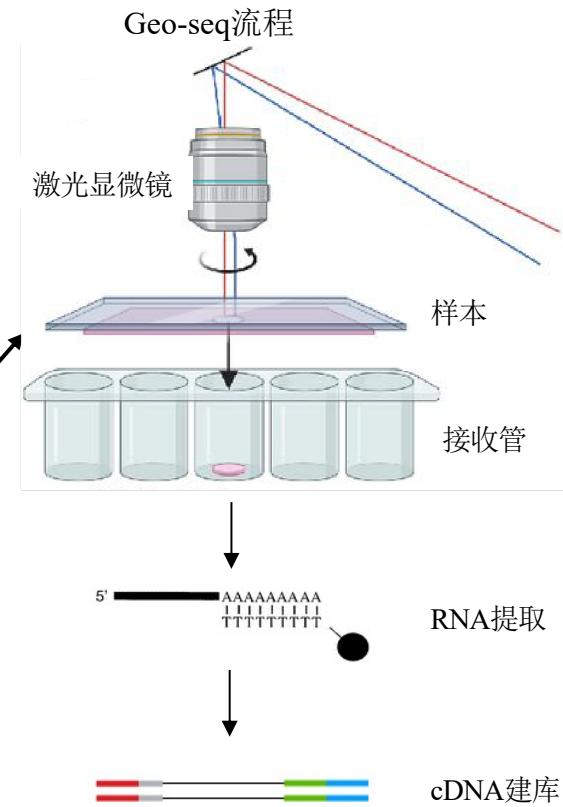
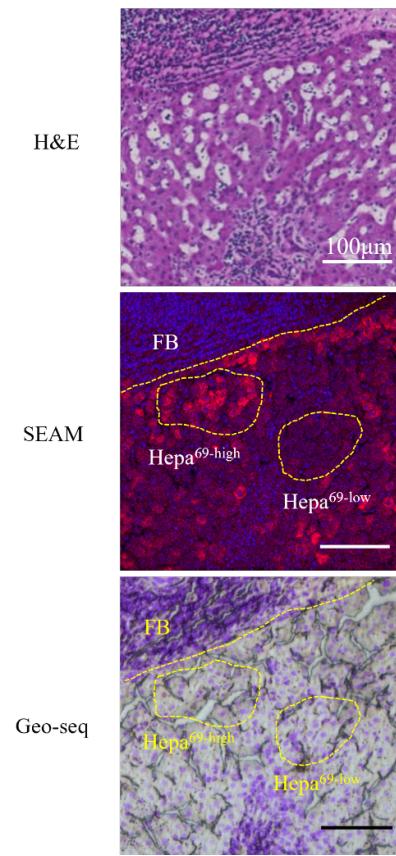
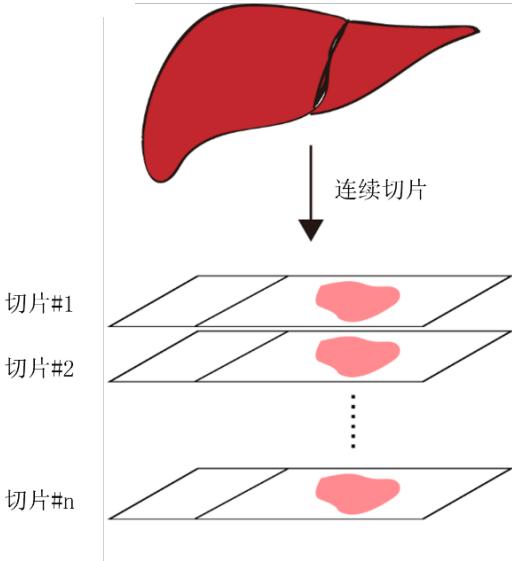
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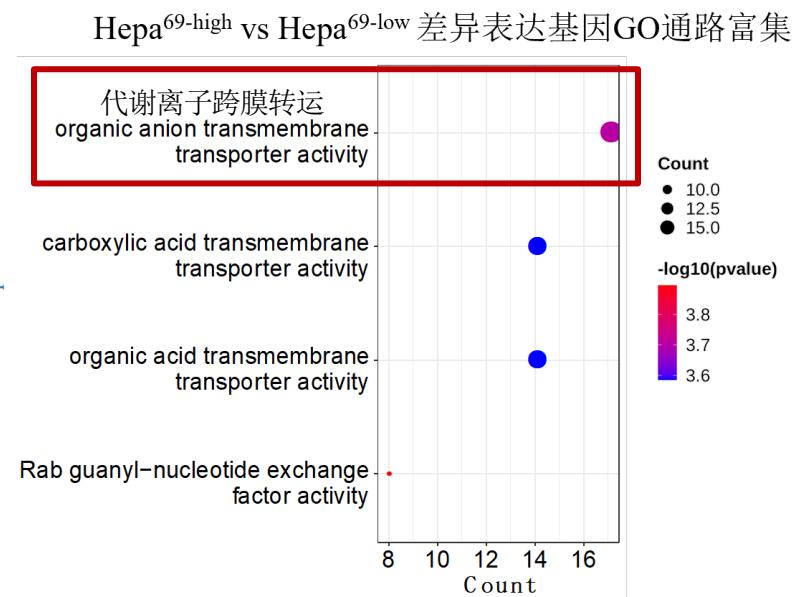
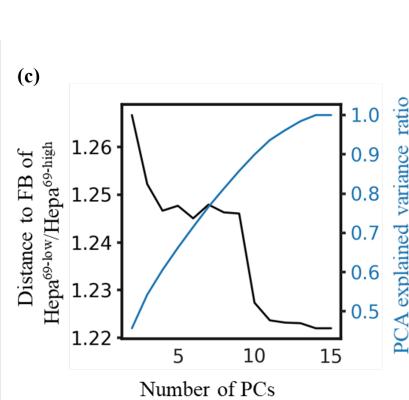
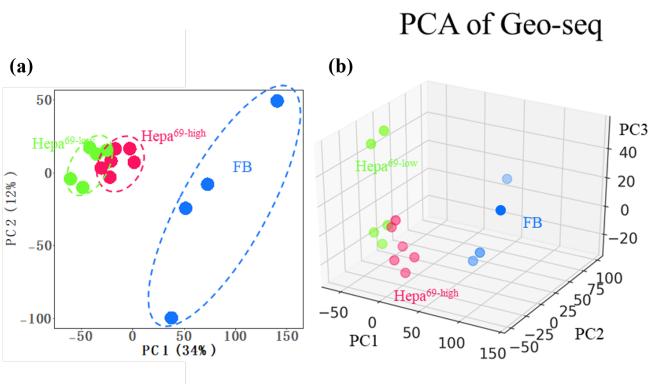


肝细胞亚群的空间分布差异和纤维化微环境之间具有关联性

# 联用空间转录组验证肝细胞亚群存在代谢相关基因差异表达

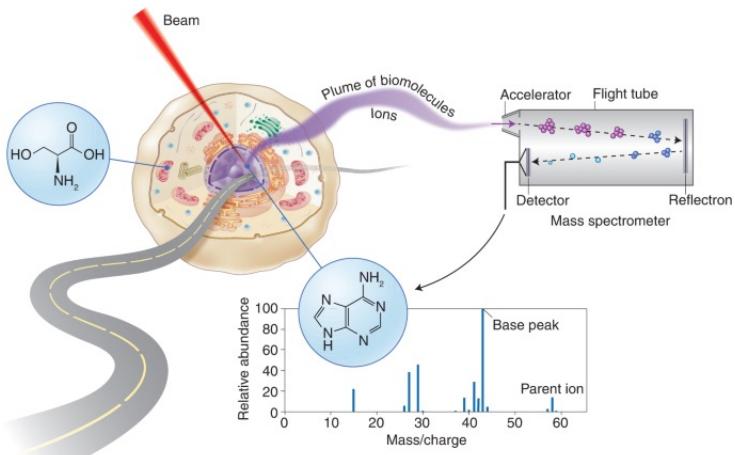


# 空间转录组验证肝细胞亚群有代谢相关基因差异表达

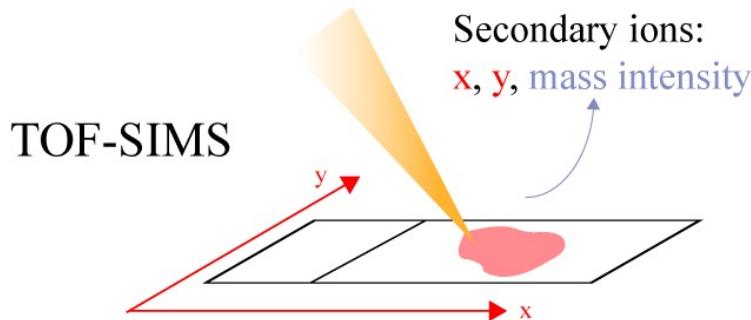


## SEAM算法在其他组学方法上的扩展应用

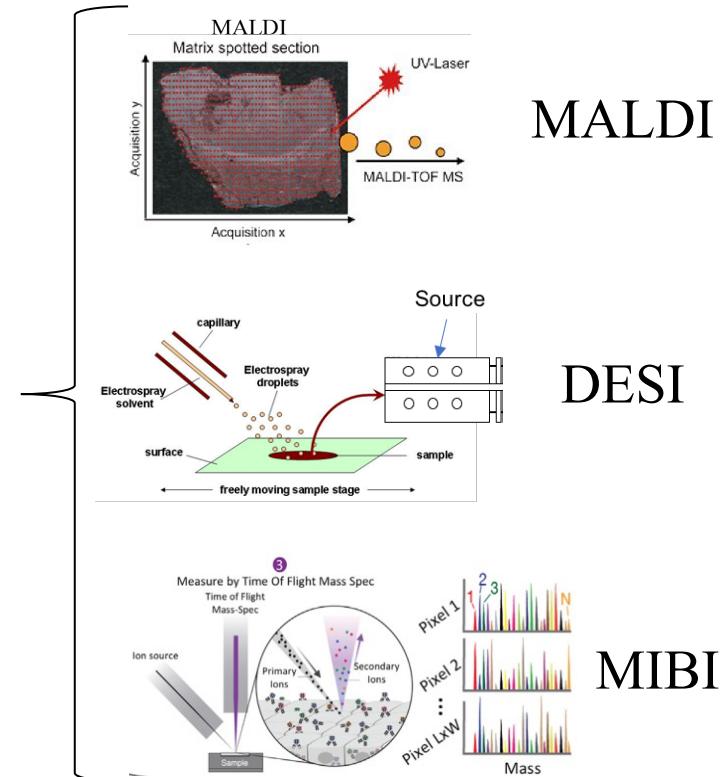
Mass spectrometry comes of age for subcellular organelles. *Nature Methods, New & Views, 2021*



Interchangeable  
to any feature



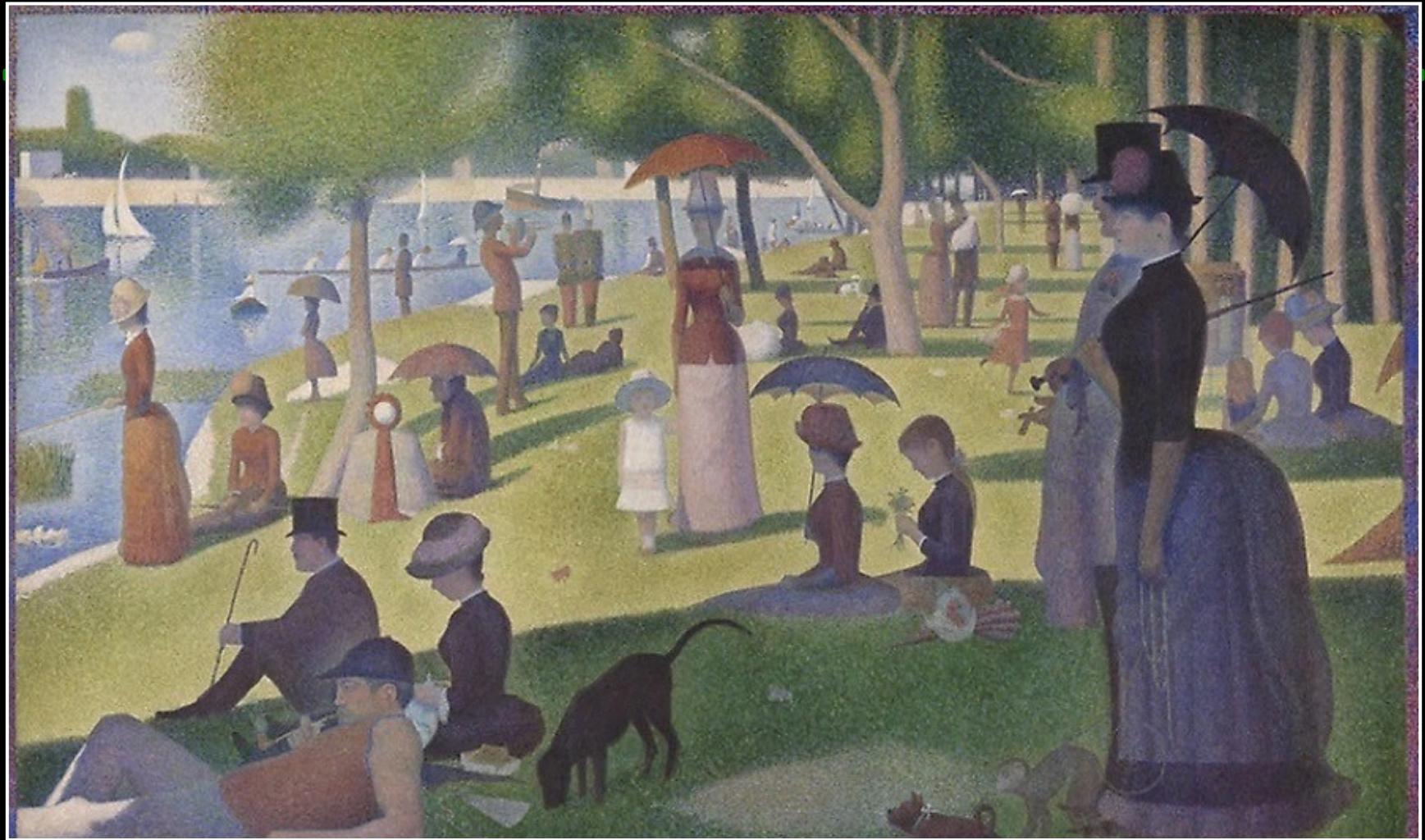
TOF-SIMS



MALDI

DESI

MIBI



A Sunday Afternoon on the Island of La Grande Jatte

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**■ Thanks for your attention!**

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