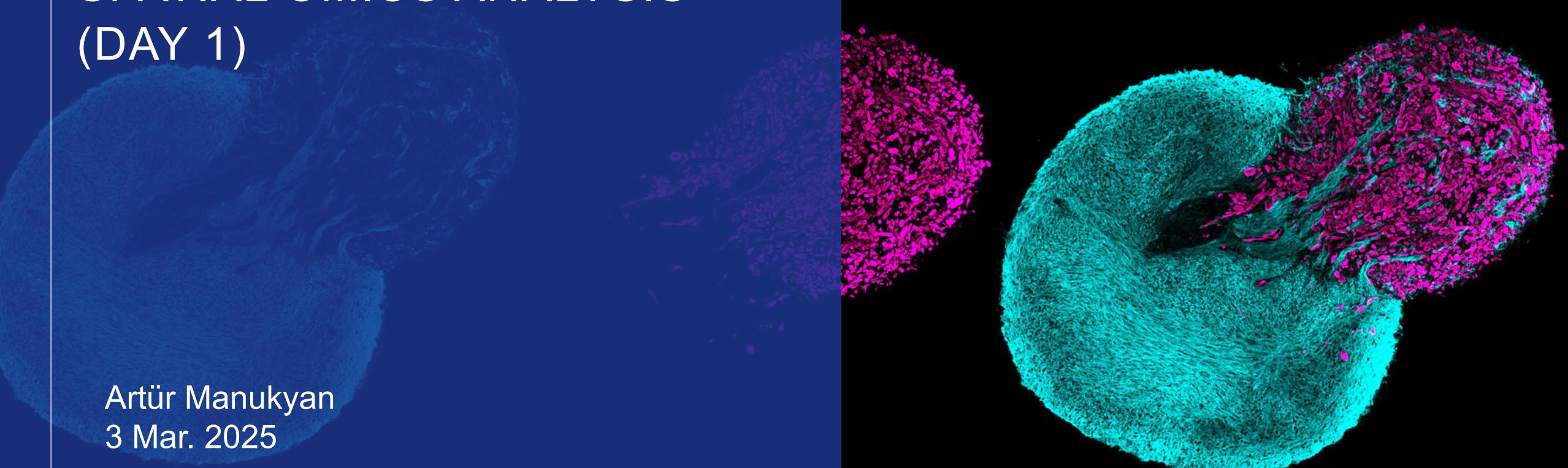


# INTRODUCTION TO SPATIAL OMICS ANALYSIS (DAY 1)

Artür Manukyan  
3 Mar. 2025

BIMSBBioinfo  
CompGen2025 Module 2



## 1. Introduction to Spatial Transcriptomics

### 2. Technology-specific omic datasets

1. Visium
2. Xenium
3. VisiumHD

### 3. Introduction to VoltRon

1. Managing VoltRon objects
2. Image Manipulation
3. Visualization
4. Multilayer Visualization

### 4. Spatially Aware Analysis

1. Relationship to single cell analysis
2. Niche Clustering
3. Hot Spot Analysis
4. Spatial Data Alignment

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# WHAT IS SPATIAL OMICS ?

**Spatial Omics** is a collection of (transcript/prote/gen) omics approaches where **features** and **spatial localization** of omic profiles (of biological observations) are captured and interrogated on a 2D/3D field of view **simultaneously**.

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	Profile 1	Profile 2	...	Profile p
Feature 1	$X_{1,1}$	$X_{1,2}$	...	$X_{1,p}$
Feature 2	$X_{2,1}$	$X_{2,2}$	...	$X_{2,p}$
...	...	...	...	...
Feature m	$X_{m,1}$	$X_{m,2}$	...	$X_{n,p}$

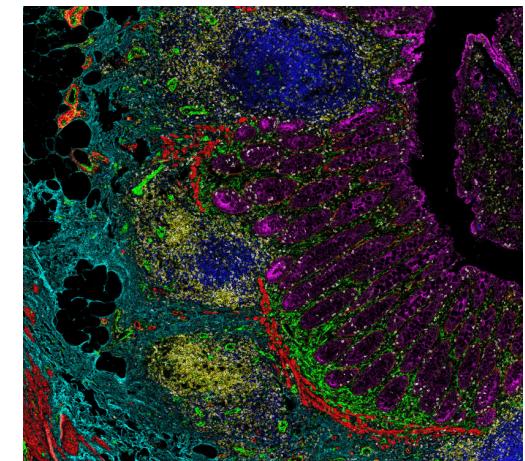
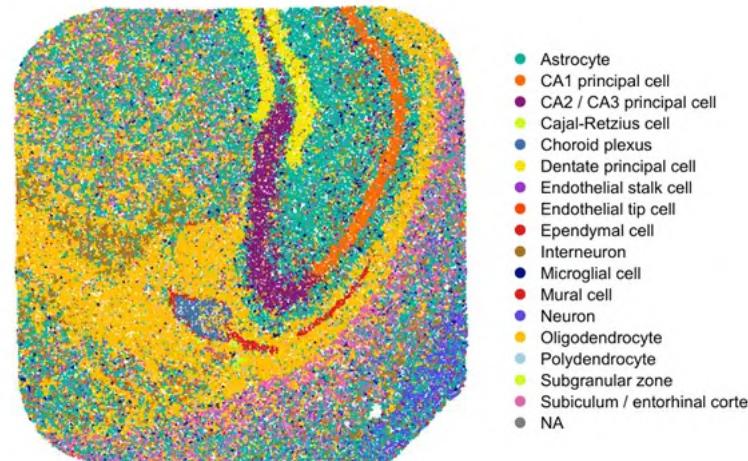
  

Coordinates	x	$C_{x,1}$	$C_{x,2}$	$C_{x,p}$
	y	$C_{y,1}$	$C_{y,2}$	$C_{y,p}$

# WHAT IS SPATIAL OMICS ?

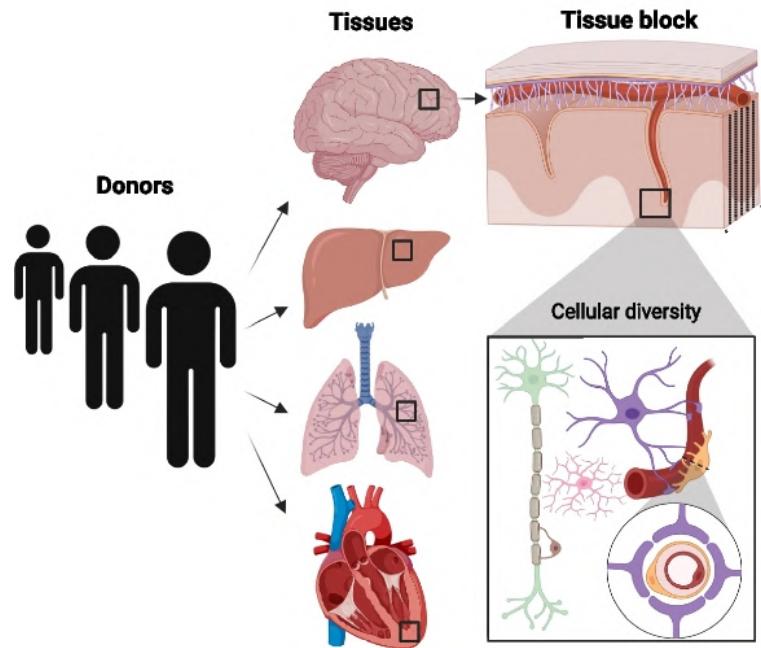
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...	...	...	...	...
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Coordinates	$C_{x,1}$	$C_{x,2}$	$C_{x,p}$	
y	$C_{y,1}$	$C_{y,2}$	$C_{y,p}$	



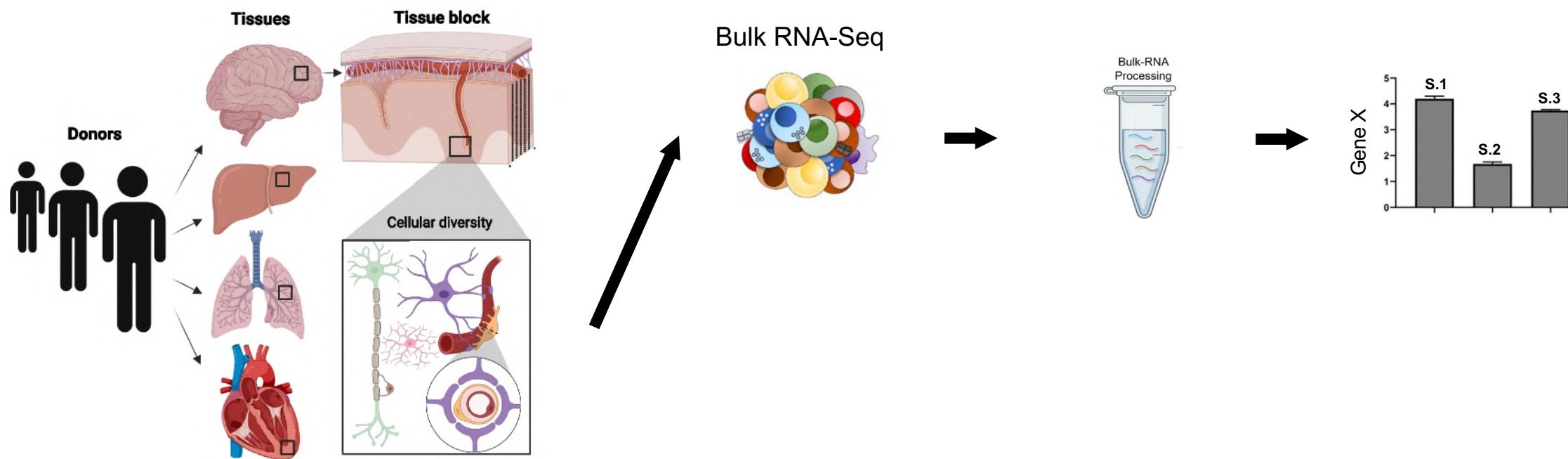
# EVOLUTION OF OMICS TECHNOLOGIES

From Bulk --> to Single Cells --> to Spatial



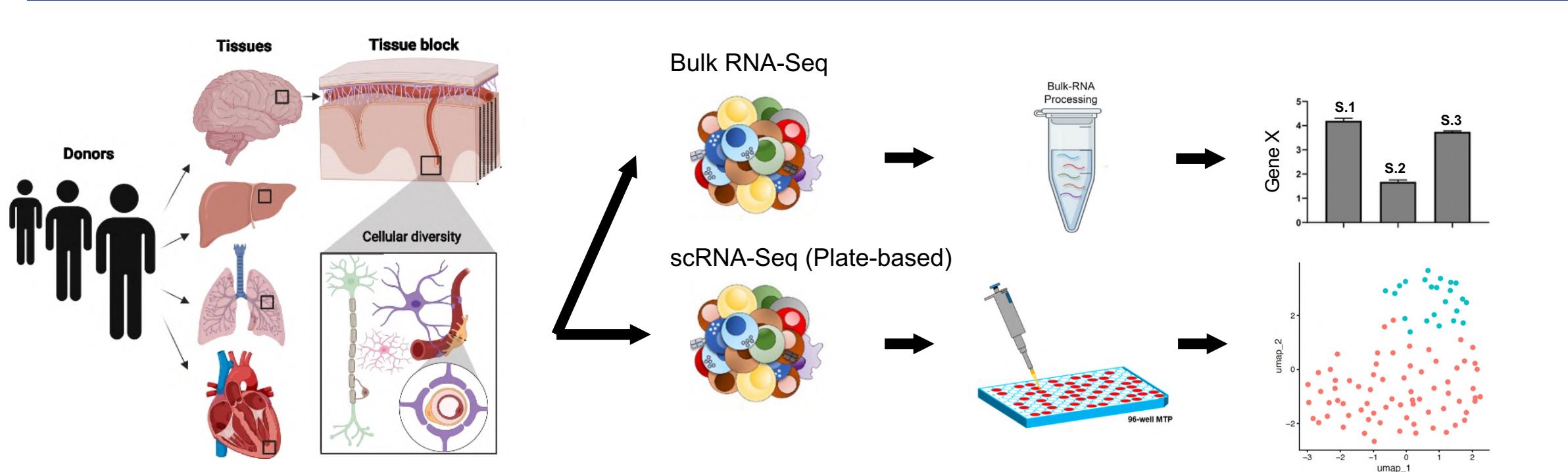
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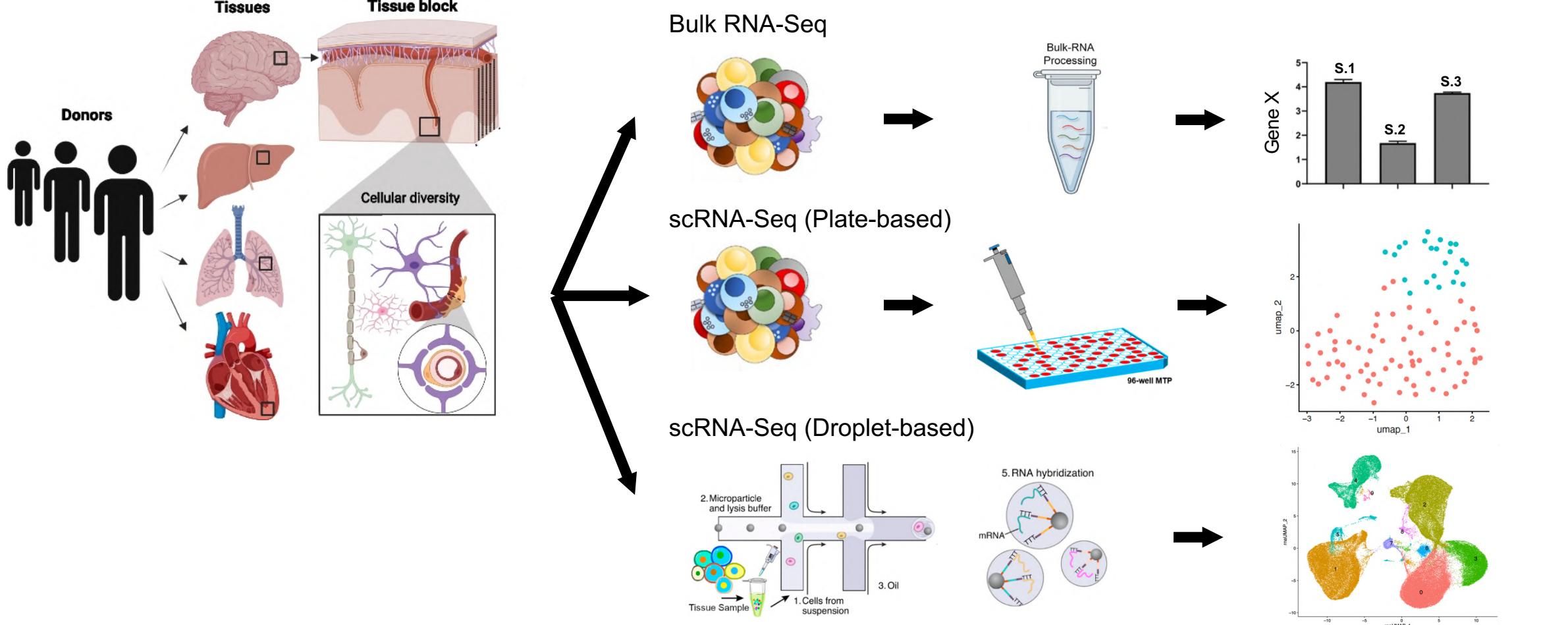
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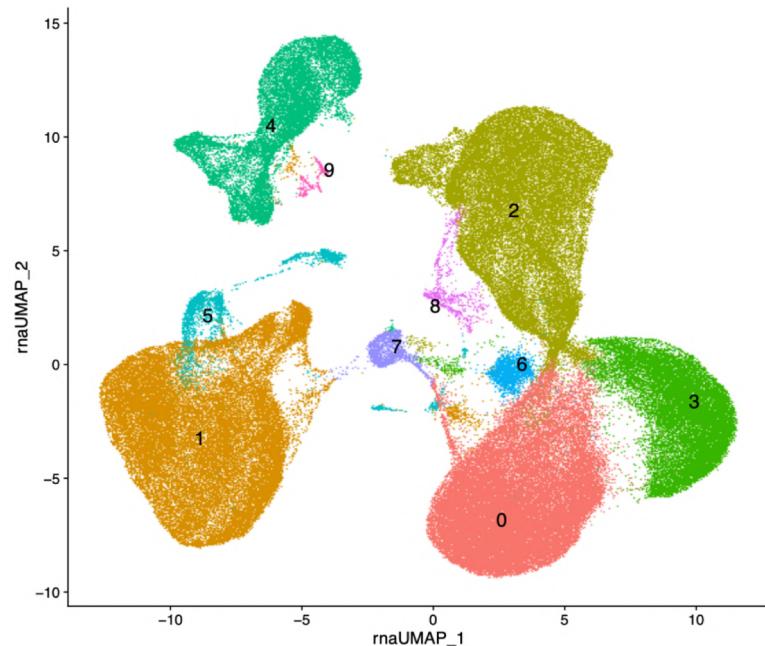
# EVOLUTION OF OMICS TECHNOLOGIES

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# SPATIAL TRANSCRIPTOMICS

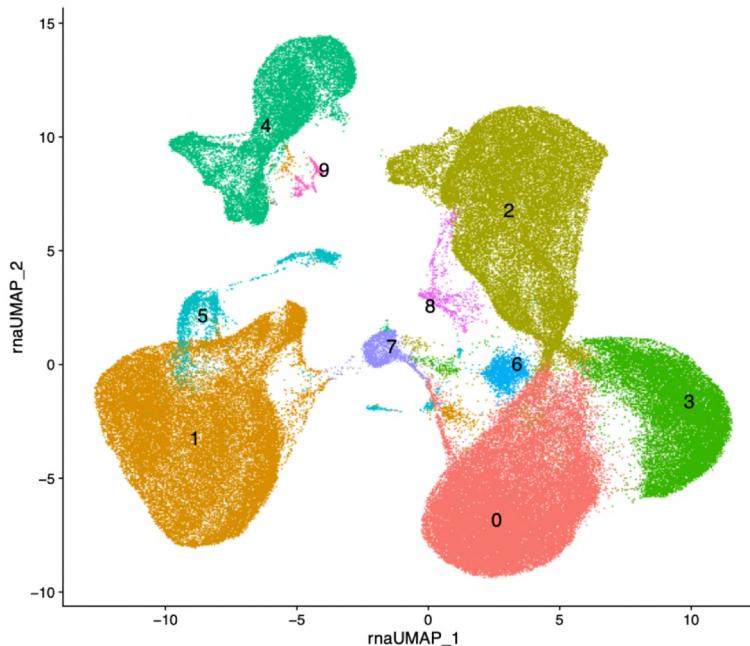
First spot-level spatial omic profiling approach



Where are the cells ?

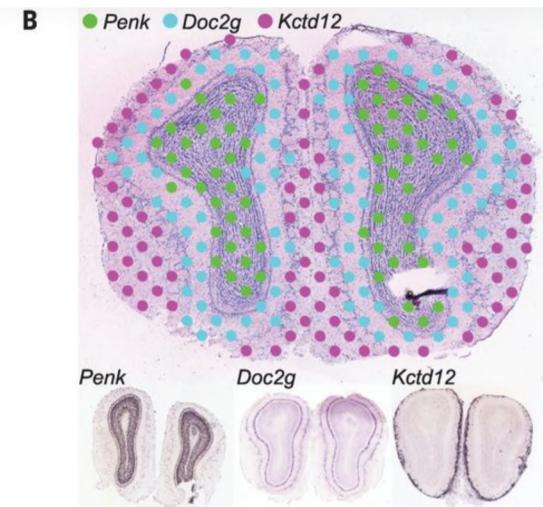
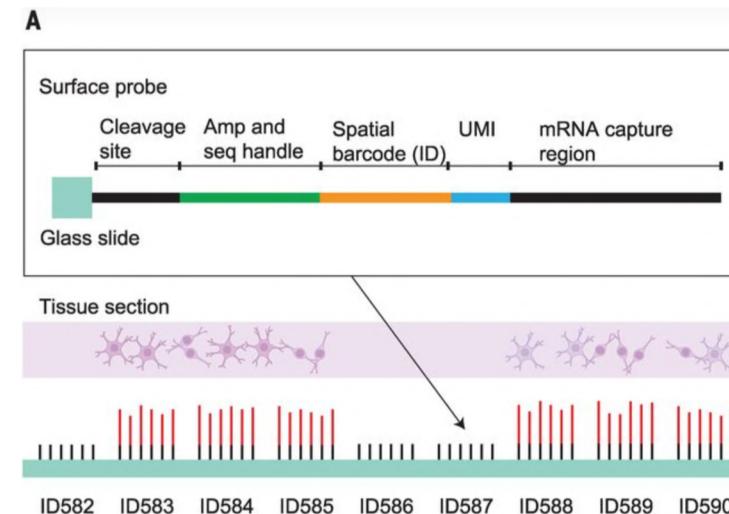
# SPATIAL TRANSCRIPTOMICS

First spot-level spatial omic profiling approach



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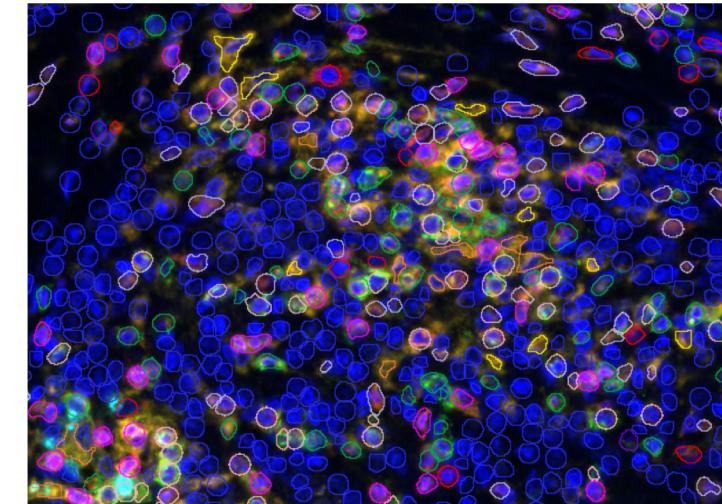
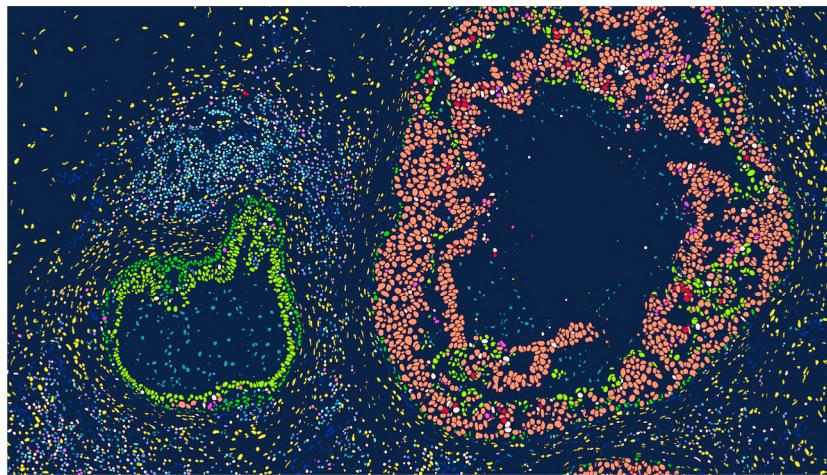
Spatial Transcriptomics (published in *Science*, 2016)



# SPATIAL OMICS TECHNOLOGIES

Categories of different spatial omics methods/approaches

## 1. Cells

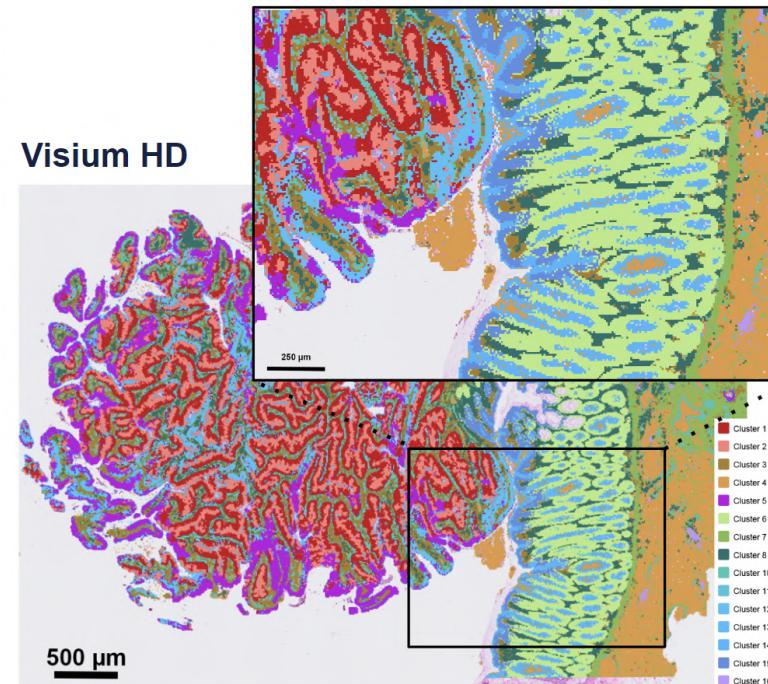
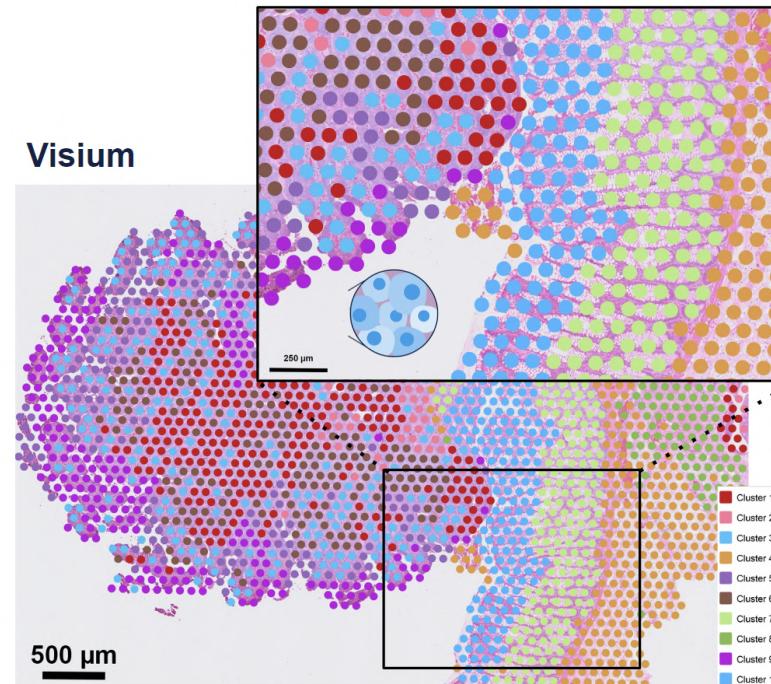


# SPATIAL OMICS TECHNOLOGIES

Categories of different spatial omics methods/approaches

## 1. Cells

## 2. Spots

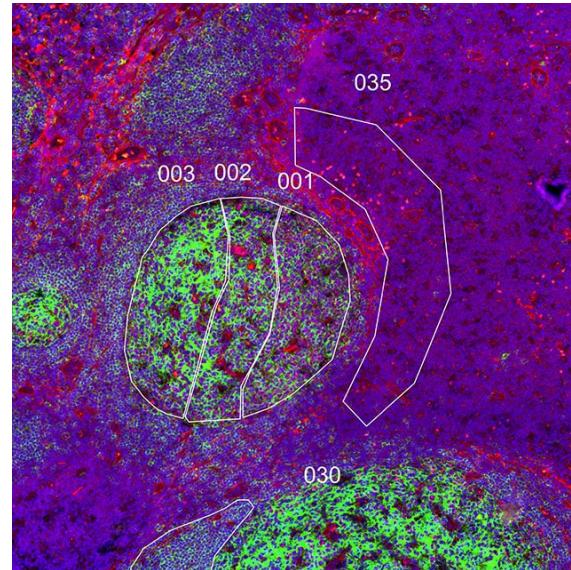


<https://www.10xgenomics.com/blog/your-introduction-to-visium-hd-spatial-biology-in-high-definition>

# SPATIAL OMICS TECHNOLOGIES

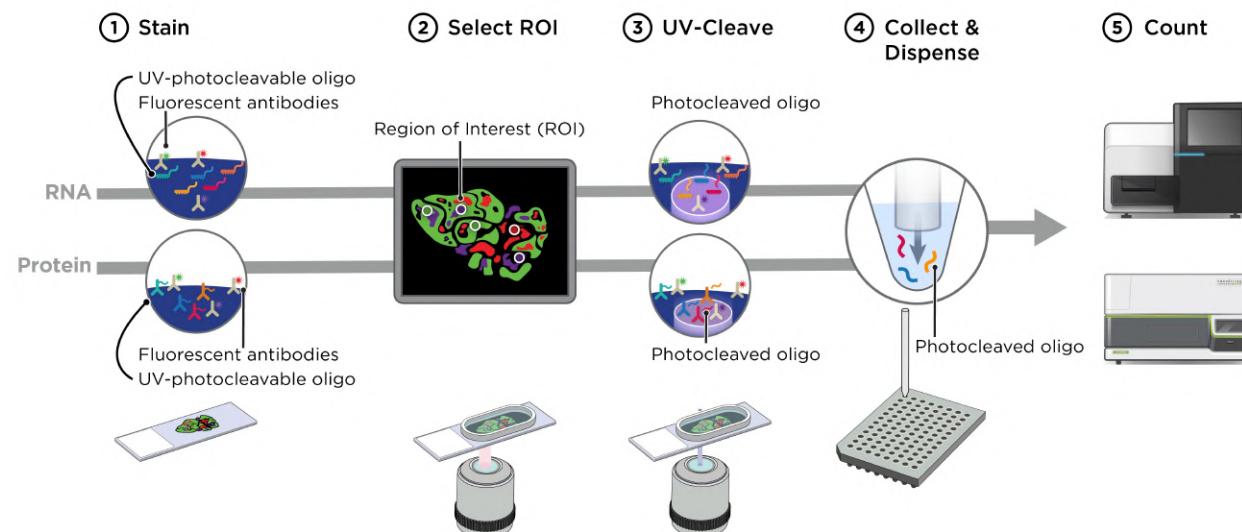
Categories of different spatial omics methods/approaches

1. Cells



2. Spots

3. Regions of Interest (ROIs)



<https://www.biochain.com/nanostring-geomx-digital-spatial-profiling/>

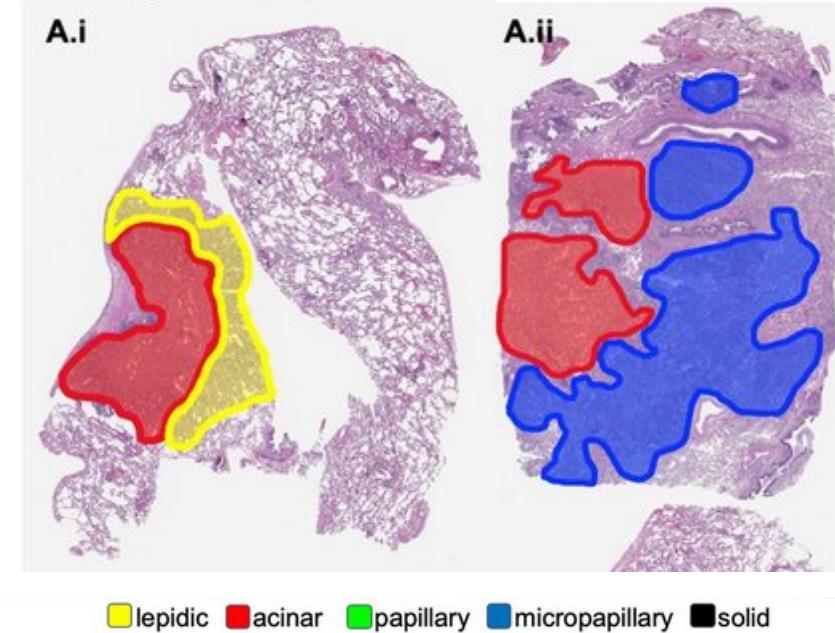
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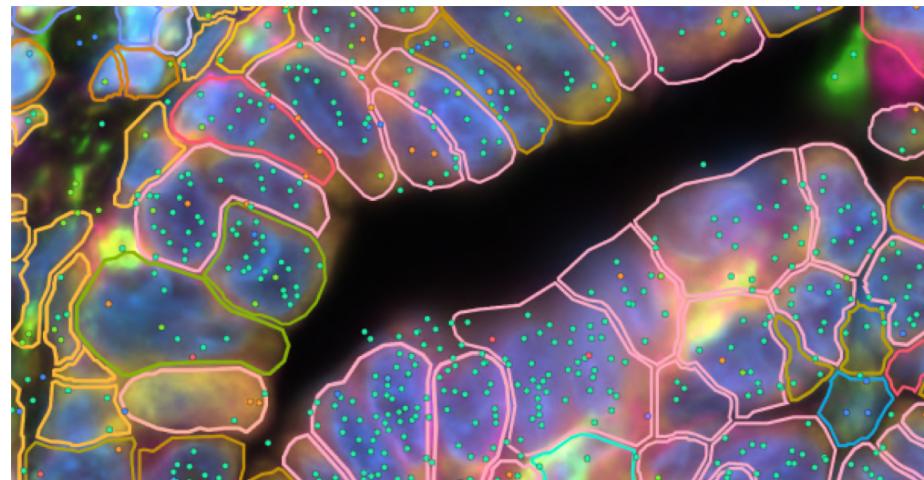
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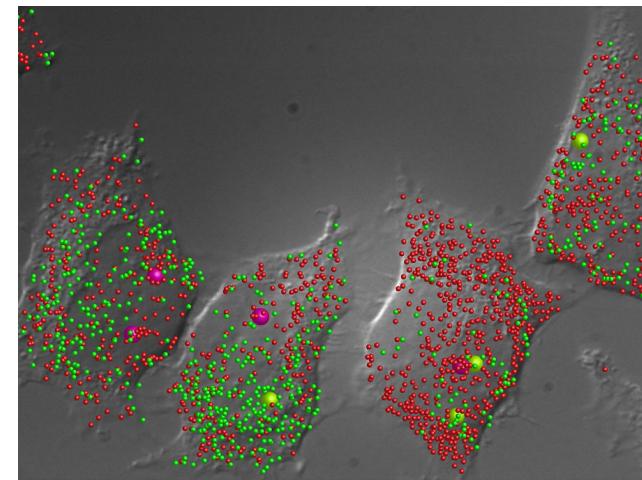


<https://www.10xgenomics.com/>

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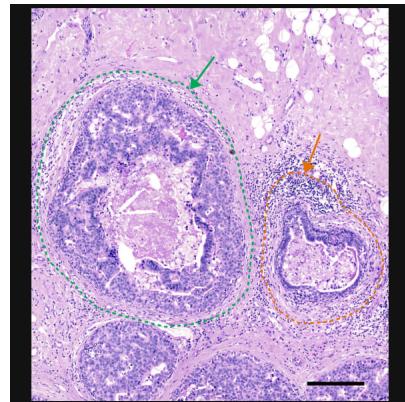
4. Molecules



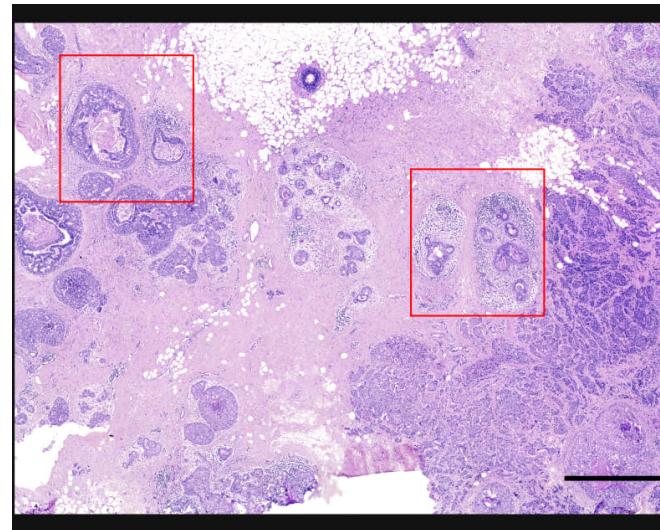
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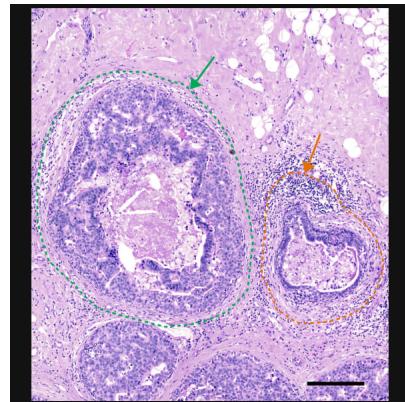
5. Images/Tiles/Pixels

<https://www.10xgenomics.com/products/xenium-in-situ/human-breast-dataset-explorer>

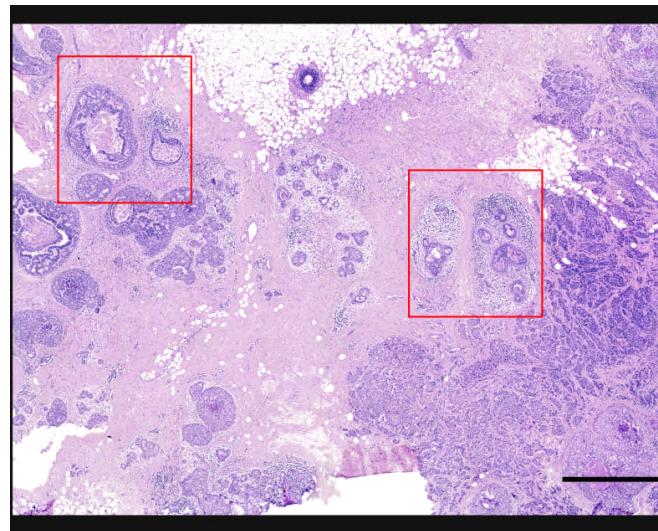
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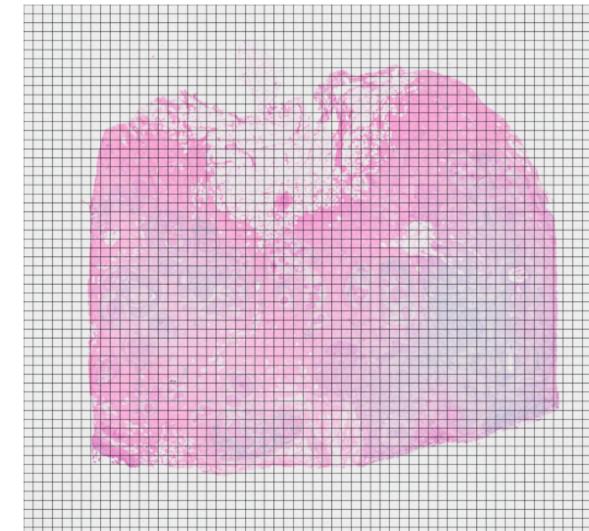


2. Spots



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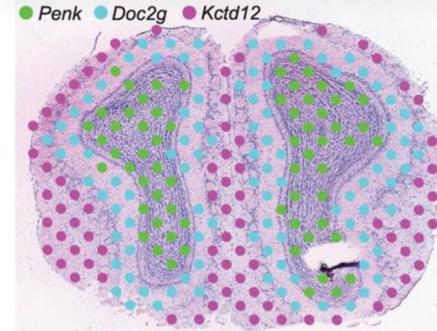
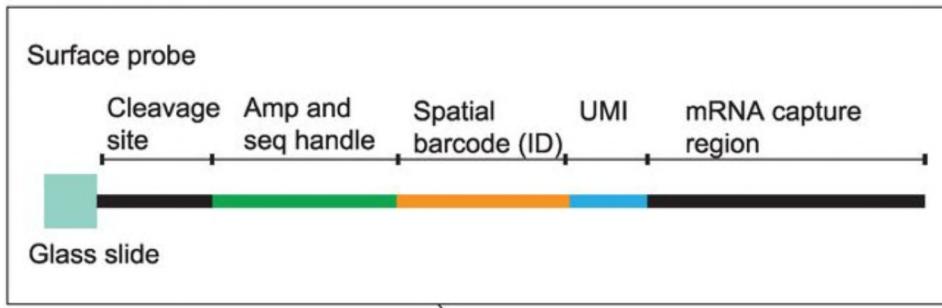
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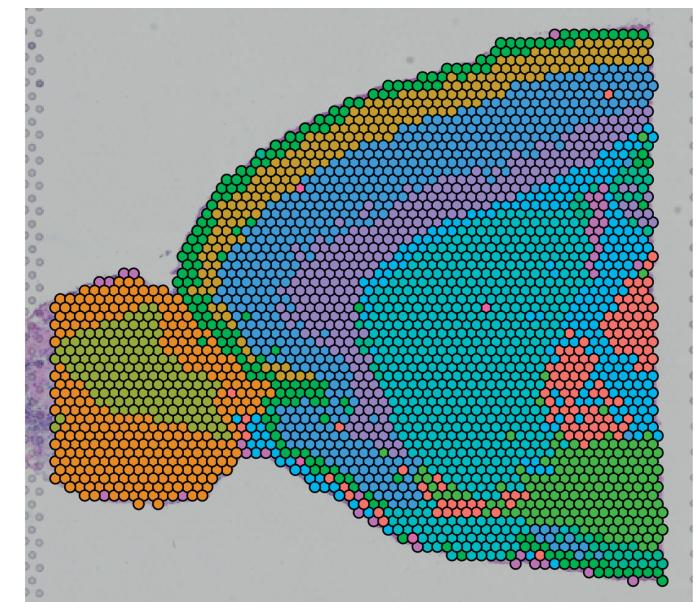
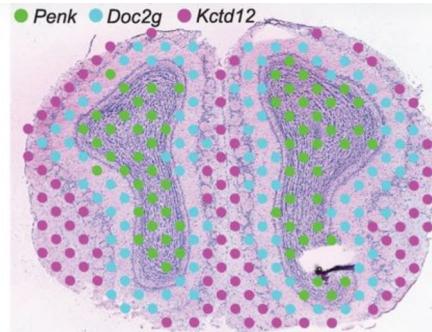
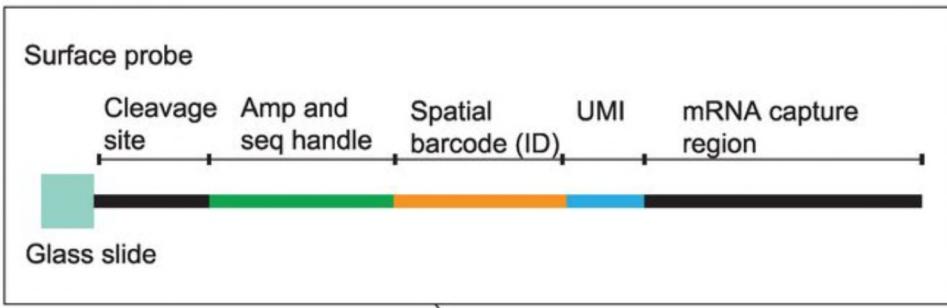
# Spot-level Spatial Omics

## Spatial Transcriptomics Lundeberg

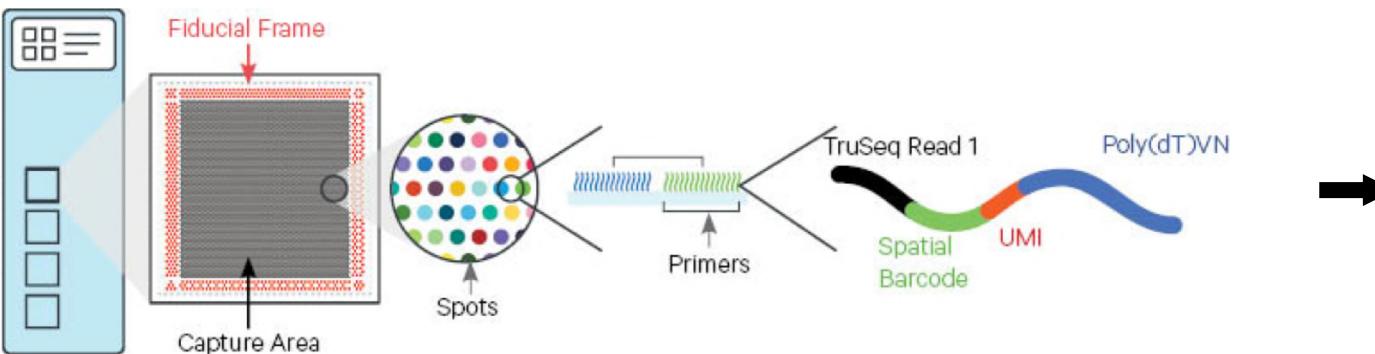


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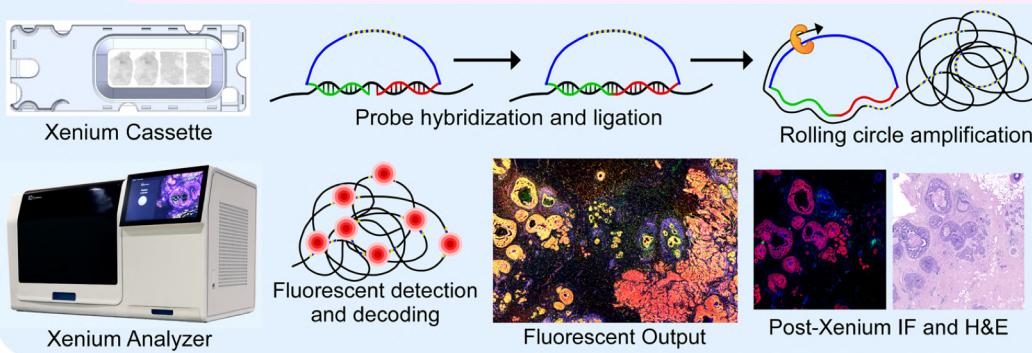
## 10x Visium Gene Expression



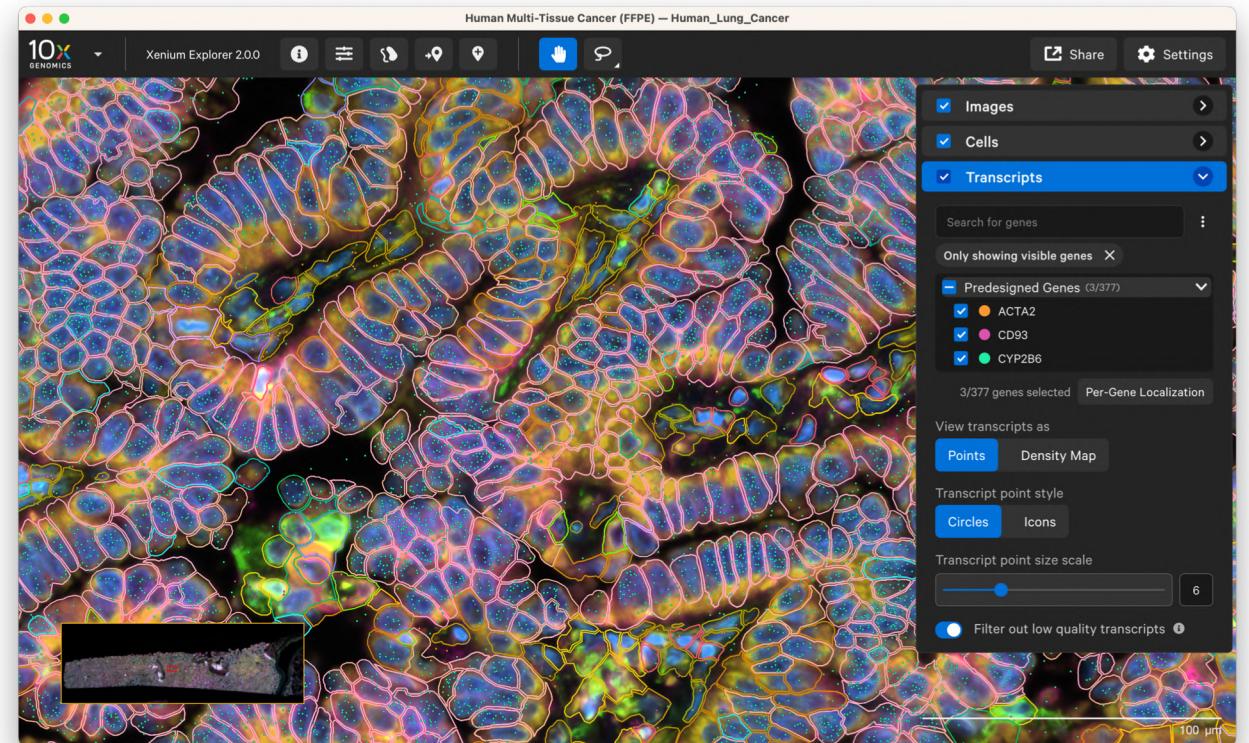
# XENIUM (10X GENOMICS)

## Cell (and subcellular)-level Spatial Omics

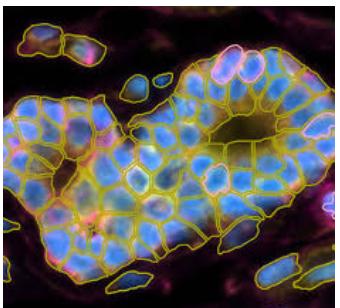
### High-throughput single molecule FISH



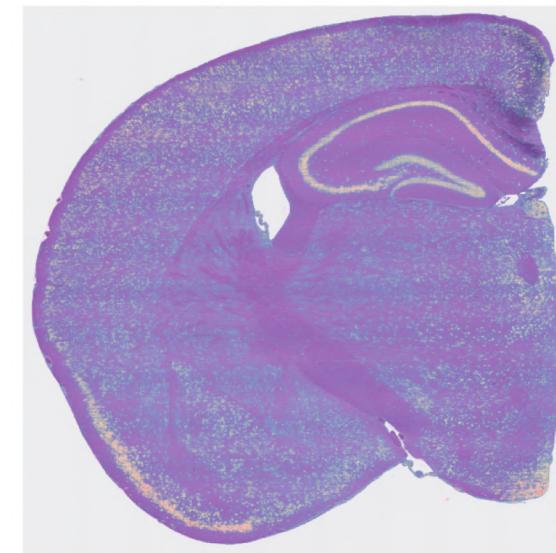
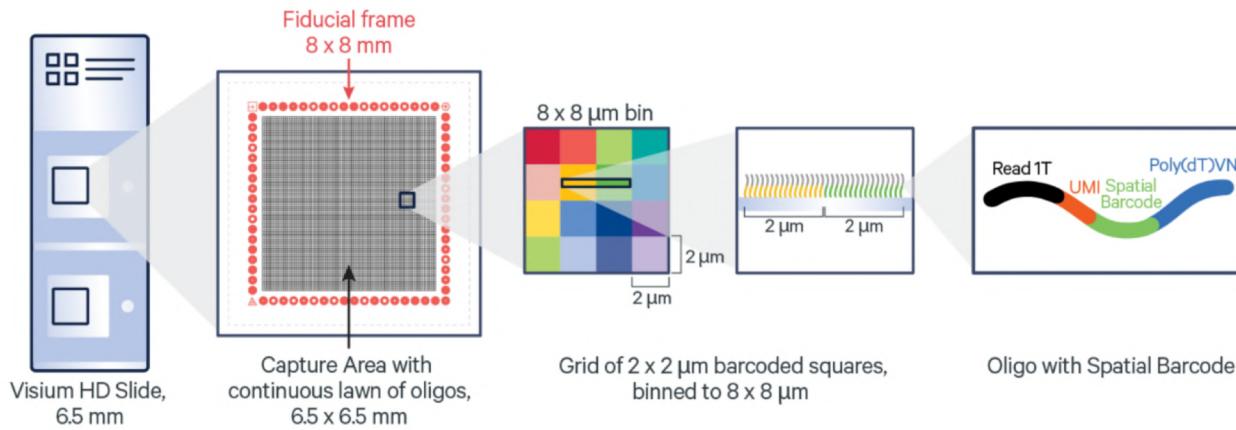
### Single Cell Assay



### Cell Segmentation



# Spot (subcellular)-level Spatial Omics



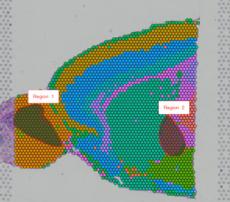
# OTHER SPATIAL TECHNOLOGIES

## VoltRon tutorial for importing datasets

### Other Utilities

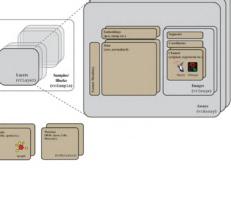
Here, we provide a group of tutorials to use additional features of the VoltRon objects as well as further information on how to use VoltRon. We describe a collection of features that VoltRon package utilizes such as **interactive annotation/visualization** and importing spatially aware data from diverse spatial omic technologies. VoltRon is able to convert its objects to a diverse set of objects/datatypes commonly incorporated spatial data analysis (**Seurat**, **SpatialExperiment**, **Giotto**, **AnnData** etc.). Large VoltRon objects can be saved ondisk and efficiently analyzed without straining memory.

**Interactive Utilities**



Interactive annotation and visualization

**Working with VoltRon Objects**



Manipulating and configuring VoltRon objects

**Importing Spatial Data**



Importing readouts of spatial technologies

**Converting VoltRon Objects**



Converting VoltRon objects into Seurat, SpatialExperiment and SquidPy (annData) etc.

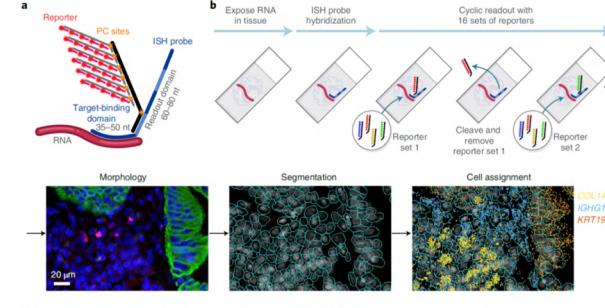
**OnDisk Analysis Utilities**



Efficient access to large VoltRon objects

Importing Spatial Datasets
Visium (10x Genomics)
VisiumHD (10x Genomics)
Xenium (10x Genomics)
GeoMx (NanoString)
<b>CosMx (NanoString)</b>
STOmics (MGI)
GenePS (Spatial Genomics)
PhenoCycler (Akoya)
OpenST
DBIT-Seq
ST data
Custom VoltRon objects
Image-only VoltRon objects

### CosMx (NanoString)



The NanoString's **CosMx Spatial Molecular Imaging** platform is a high-plex spatial multiomics technology that captures the spatial localization of both (i) transcripts from thousands of genes as well as (ii) the single cells with transcriptomic and proteomic profiles.

We will use the readouts from two slides of a single CosMx experiment. You can download the data from the [Nanostring website](#).

We use the `importCosMx` function to import the CosMx readouts and create a VoltRon object. Here, we point to the folder of the `TileDB` array that stores feature matrix as well as the transcript metadata.

```
CosMxR1 <- importCosMx(tiledbURI = "MuBrainDataRelease/")
```

```
VoltRon Object
Slide1:
  Layers: Section1
Slide2:
  Layers: Section1
  Assays: CosMx(Main)
```

You can use the `import_molecules` argument to import positions and features of the transcripts along with the single cell profiles.

```
CosMxR1 <- importCosMx(tiledbURI = "MuBrainDataRelease/", import_molecules = TRUE)
```

### STOmics (MGI)



## 1. Introduction to Spatial Transcriptomics

### 2. Technology-specific omic datasets

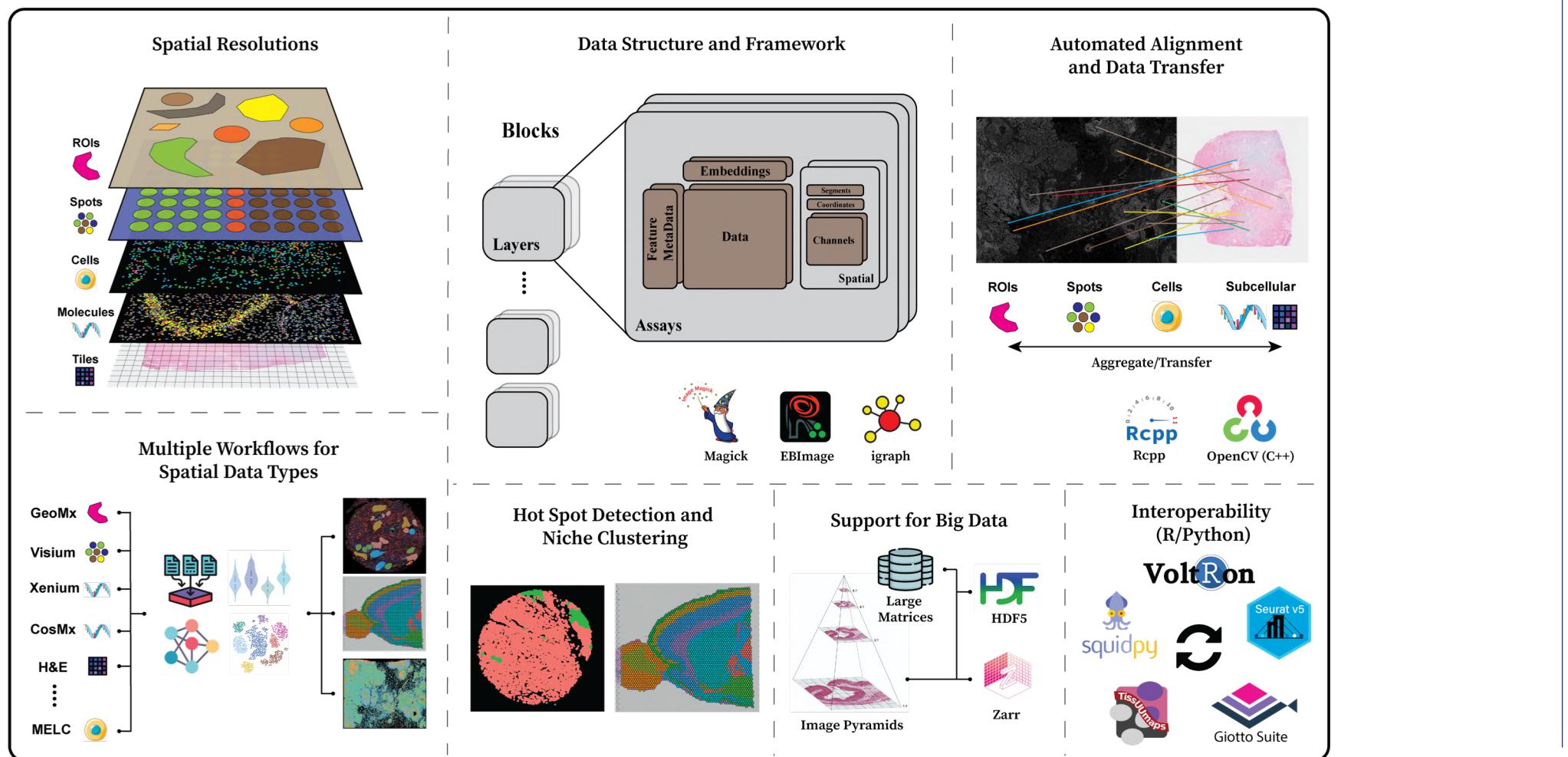
1. Visium
2. Xenium
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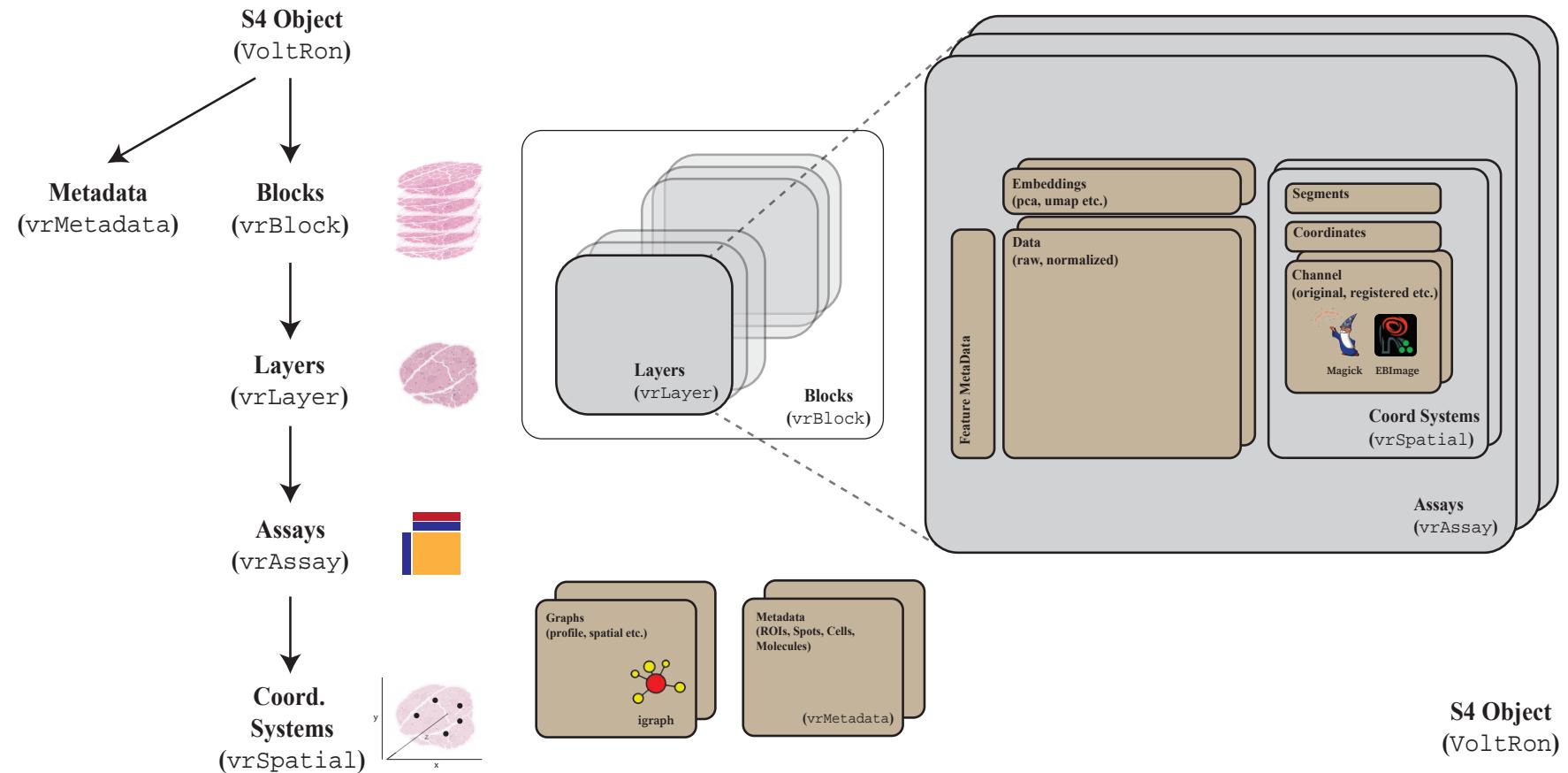
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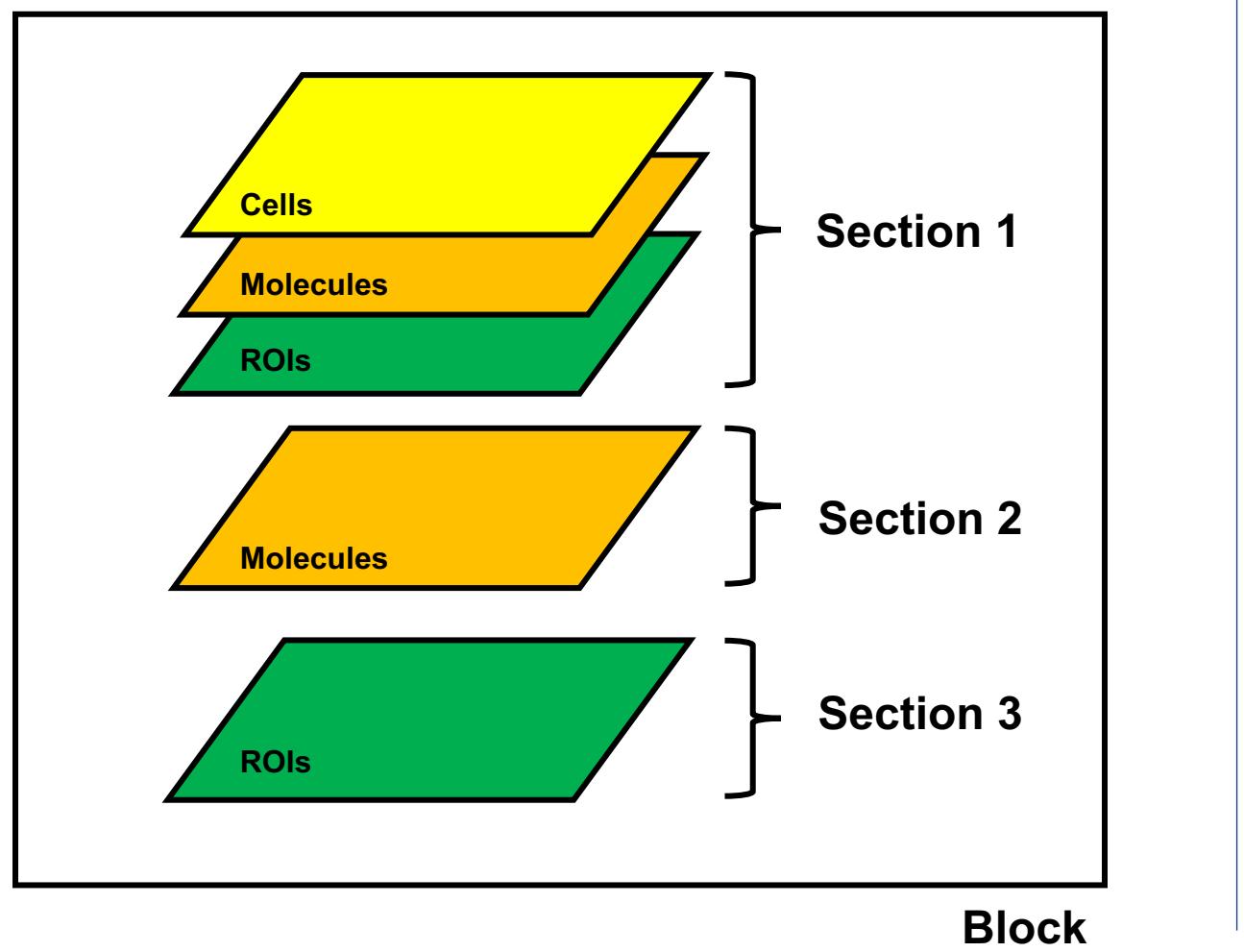


```
Library(VoltRon)
data("merged_object")
merged_object

# VoltRon Object
# Block:
#   Layers: Section1 Section2 Section3
# Assays: CellAssay(Main) MolAssay ROIAssay
# Features: main(Main)

SampleMetadata(merged_object)

#           Assay      Layer Sample
# Assay1  CellAssay  Section1  Block
# Assay2  MolAssay  Section2  Block
# Assay3  ROIAssay  Section3  Block
# Assay4  MolAssay  Section1  Block
# Assay5  ROIAssay  Section1  Block
```



- 1. Introduction to Spatial Transcriptomics**
- 2. Technology-specific omic datasets**
  1. Visium
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## Relationship to Single Cell Analysis

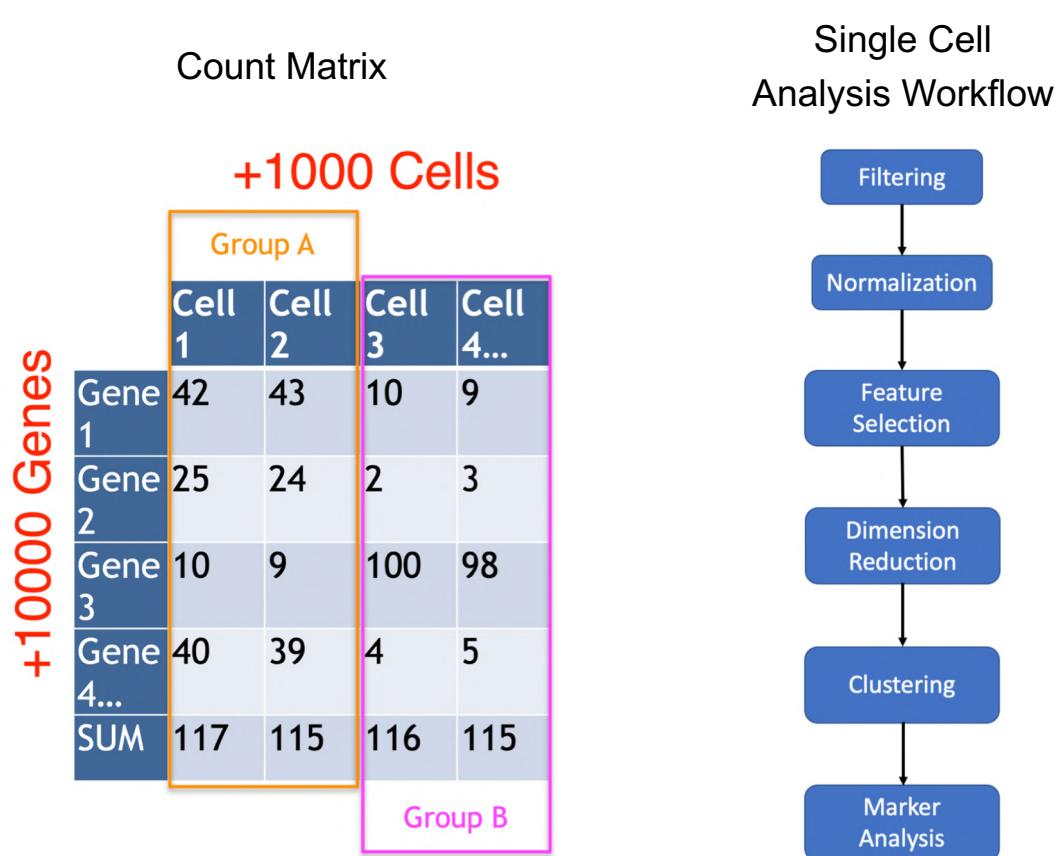
Count Matrix

+1000 Cells

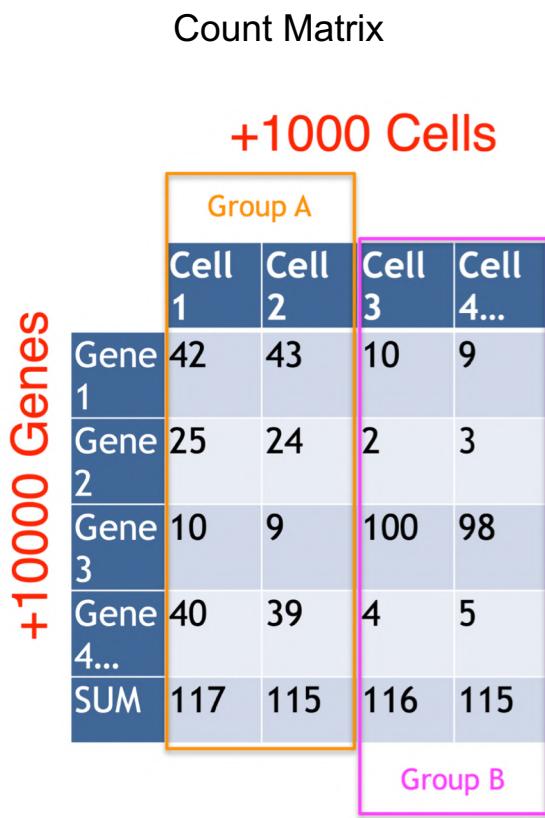
+100000 Genes

		Group A				Group B	
		Cell 1	Cell 2	Cell 3	Cell 4...		
Gene 1	Cell 1	42	43	10	9		
	Cell 2	25	24	2	3		
	Cell 3	10	9	100	98		
	Cell 4...	40	39	4	5		
	SUM	117	115	116	115		

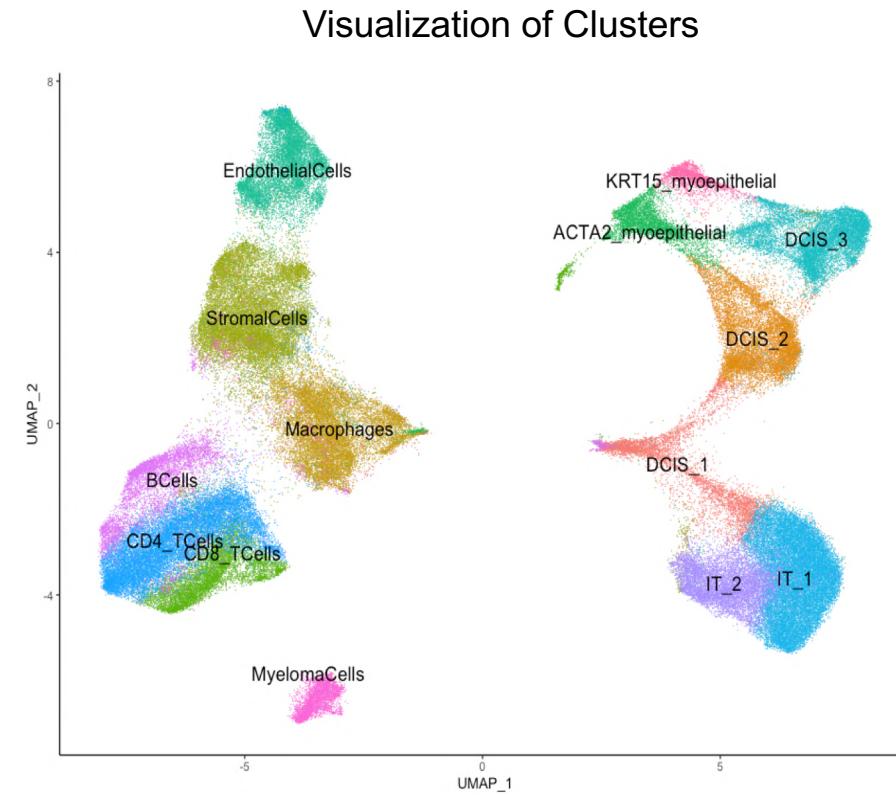
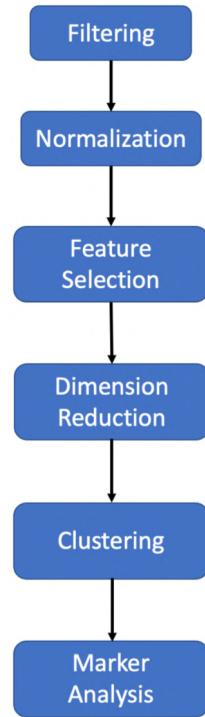
# Relationship to Single Cell Analysis



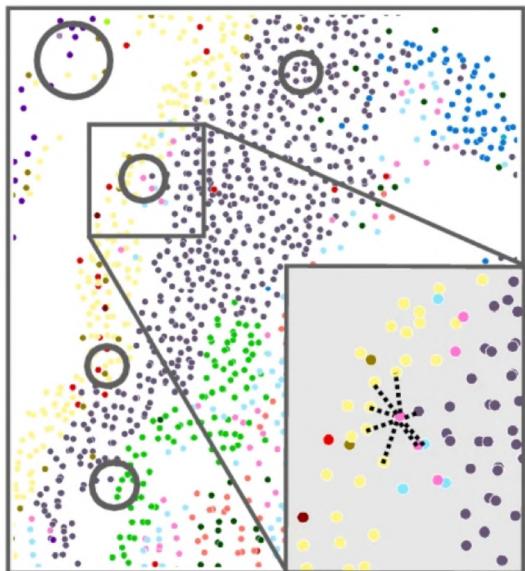
# Relationship to Single Cell Analysis



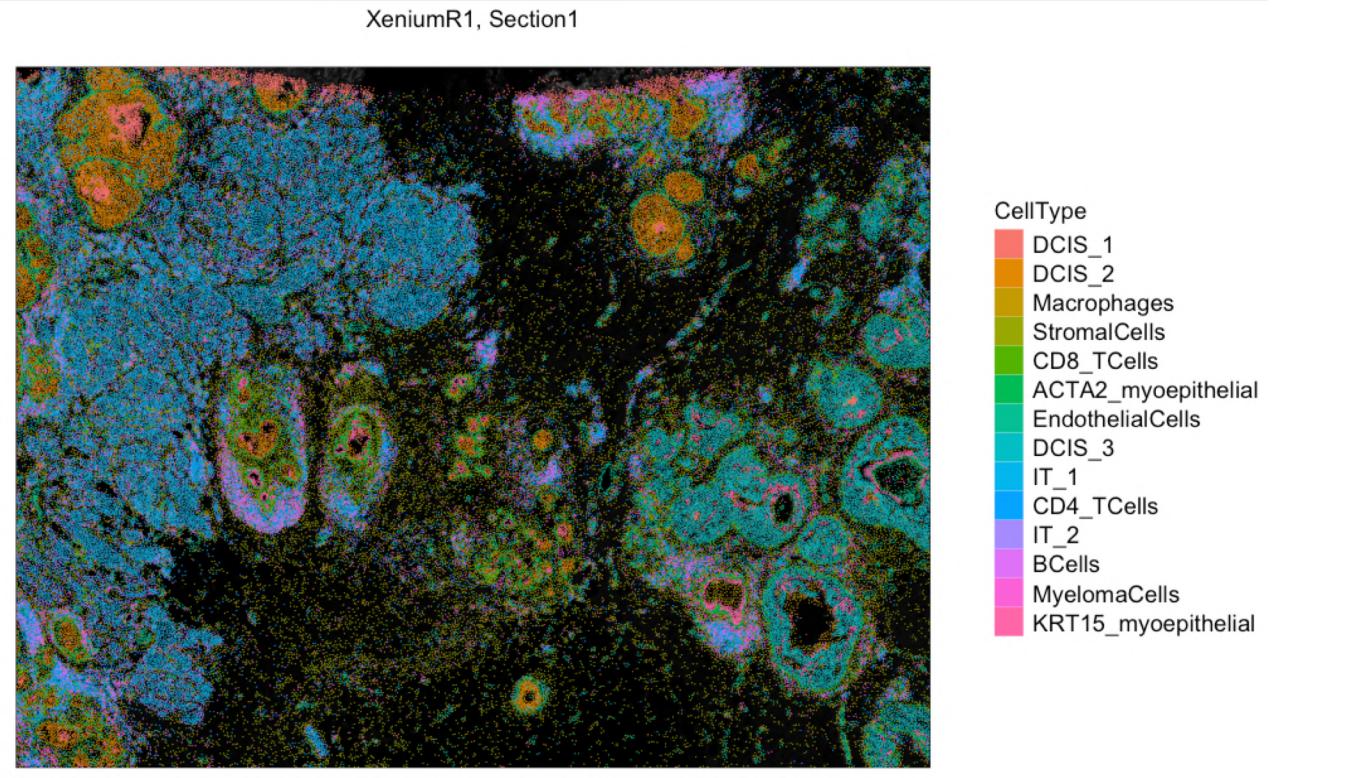
## Single Cell Analysis Workflow



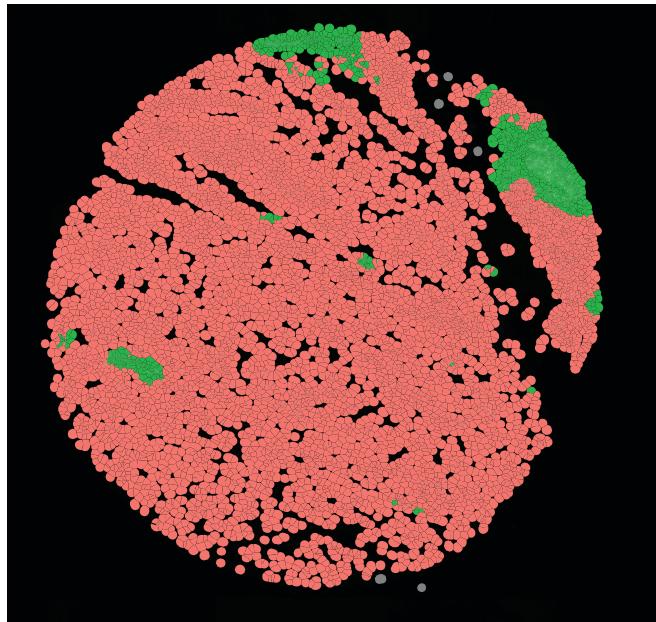
## Niche Clustering



Define cellular niches  
( $k$  spatial nearest neighbors)



# Hot Spot Analysis



Hot Spot Analysis

## ≡ Getis–Ord statistics

### Local statistics [edit]

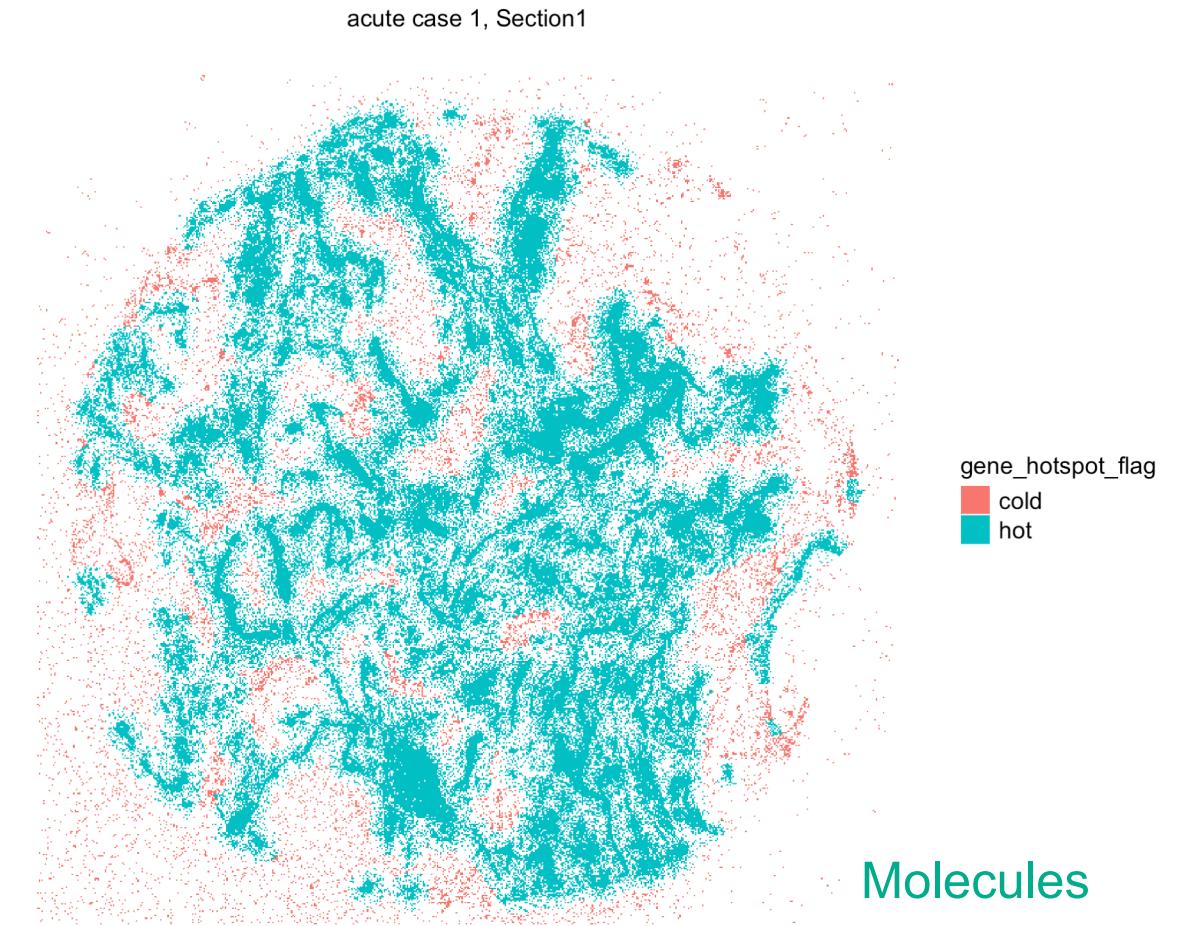
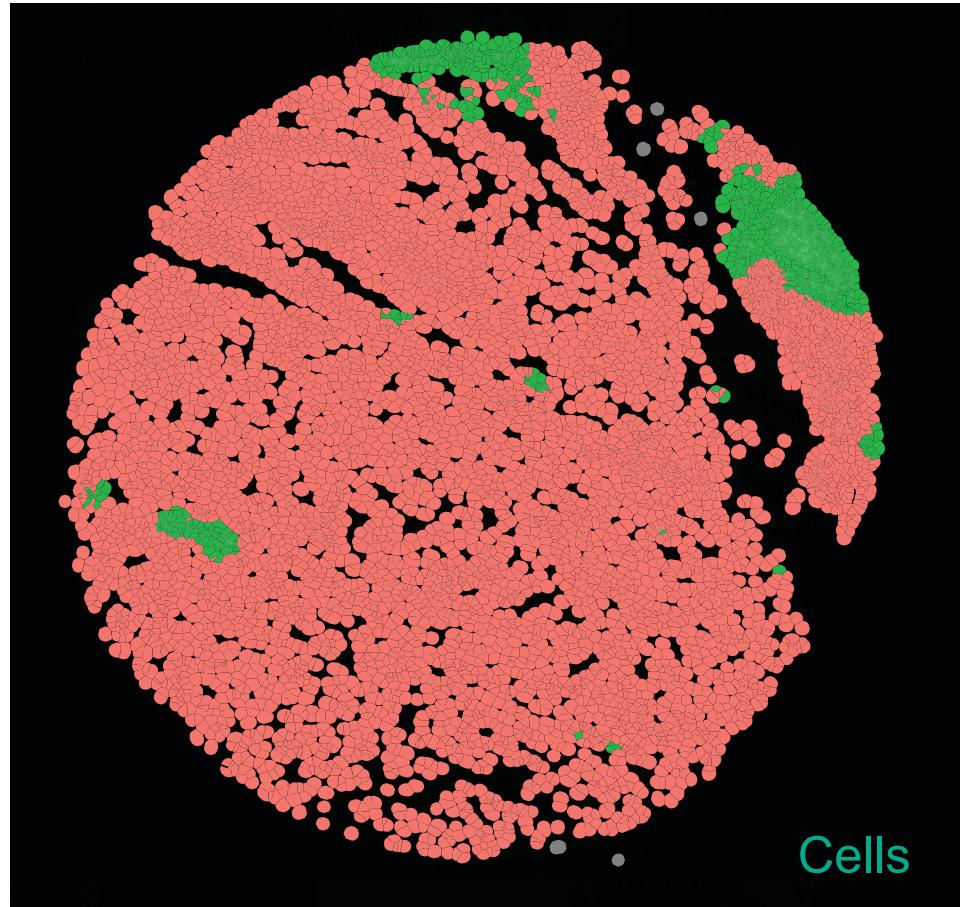
There are two different versions of the statistic, depending on whether the data point at the target location  $i$  is included or not<sup>[6]</sup>

$$G_i = \frac{\sum_{j \neq i} w_{ij} x_j}{\sum_{j \neq i} x_j}$$

$$G_i^* = \frac{\sum_j w_{ij} x_j}{\sum_j x_j}$$

Here  $x_i$  is the value observed at the  $i^{th}$  spatial site and  $w_{ij}$  is the spatial weight matrix which constrains which sites are connected to one another. For  $G_i^*$  the denominator is constant across all observations.

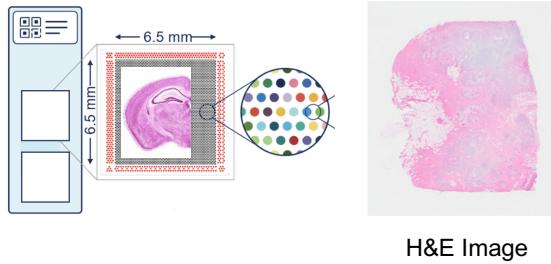
## Hot Spot Analysis



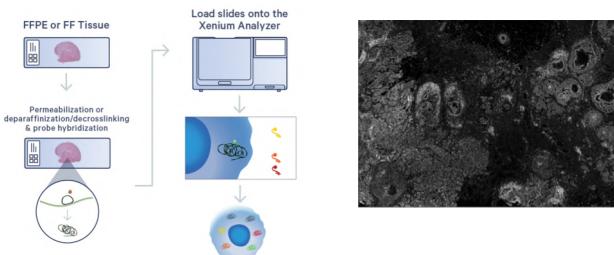
## SPATIAL DATA ALIGNMENT

Aligning adjacent tissue sections

Visium Cytassist Platform (10X Genomics)  
**Spot Resolution, Whole Transcriptome**



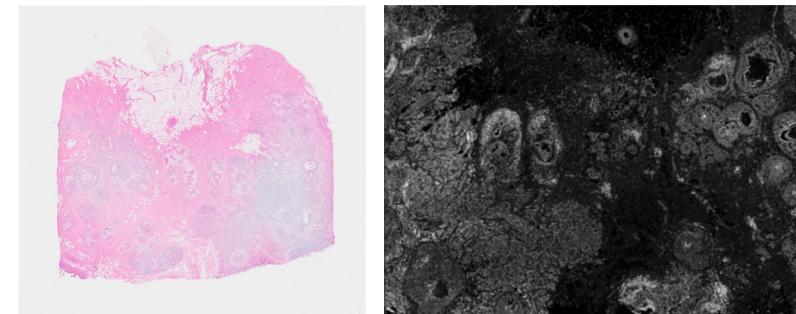
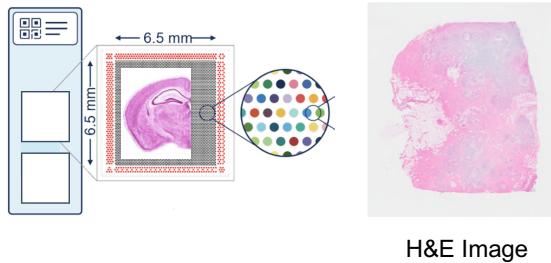
Xenium In Situ Platform (10X Genomics)  
**(Sub)Cellular Resolution, Gene Panel**



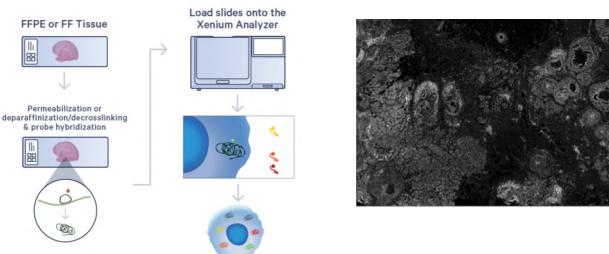
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Aligning adjacent tissue sections

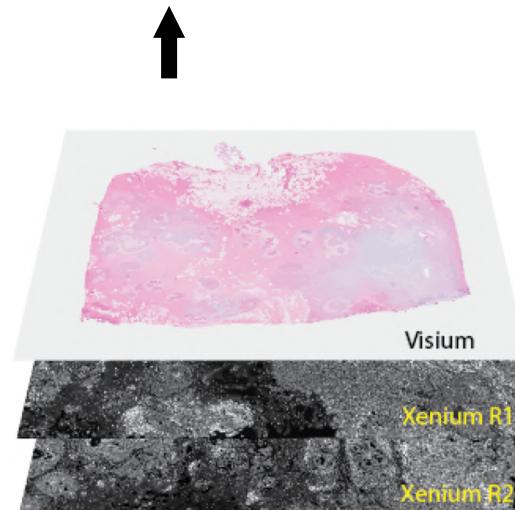
Visium Cytassyst Platform (10X Genomics)  
**Spot Resolution, Whole Transcriptome**



Xenium In Situ Platform (10X Genomics)  
**(Sub)Cellular Resolution, Gene Panel**



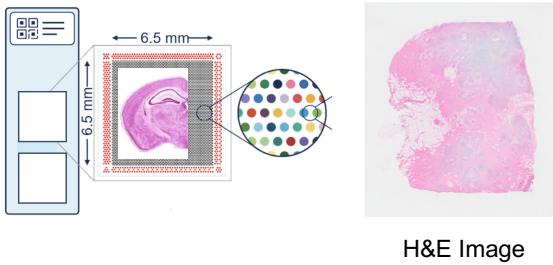
**10X GENOMICS**  
FFPE-preserved  
Breast Cancer Block



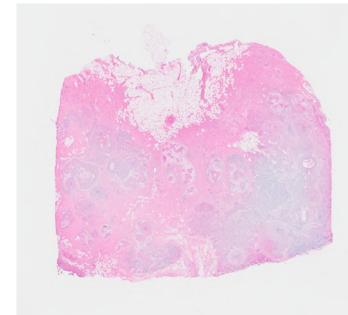
## SPATIAL DATA ALIGNMENT

Aligning adjacent tissue sections

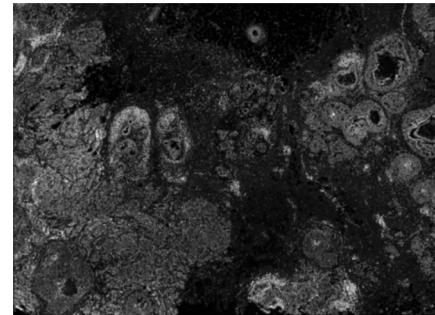
Visium Cytassist Platform (10X Genomics)  
**Spot Resolution, Whole Transcriptome**



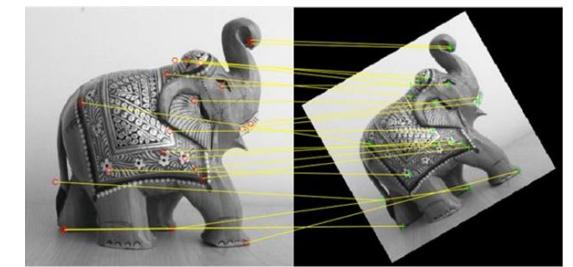
(Reference)



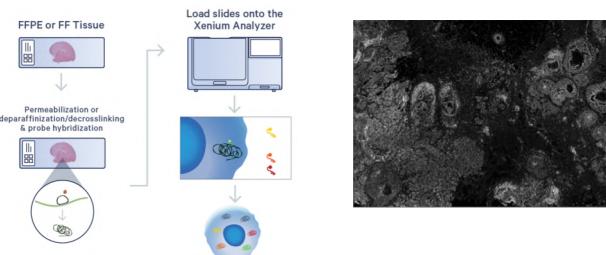
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Automated/Manual Image Registration

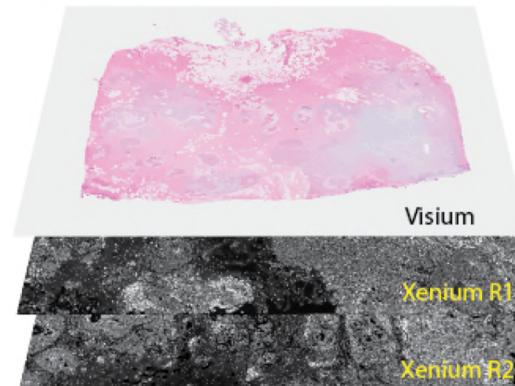


Xenium In Situ Platform (10X Genomics)  
**(Sub)Cellular Resolution, Gene Panel**



**10X GENOMICS**

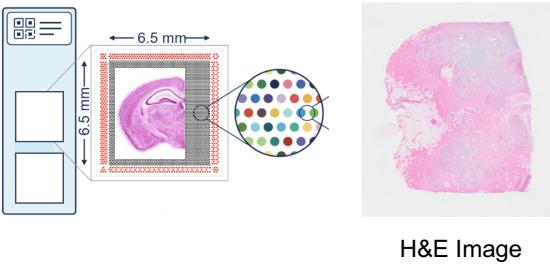
FFPE-preserved  
Breast Cancer Block



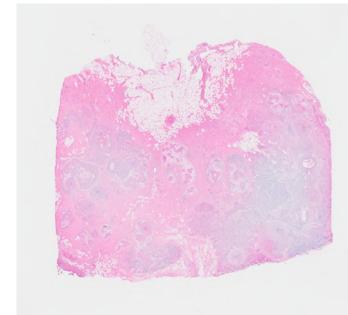
# SPATIAL DATA ALIGNMENT

Aligning adjacent tissue sections

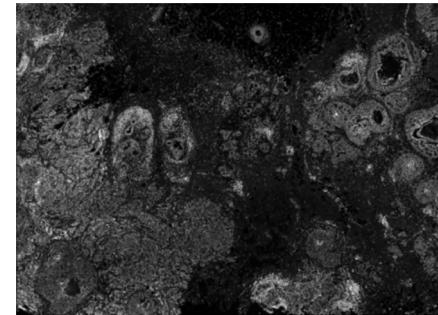
Visium Cytassist Platform (10X Genomics)  
**Spot Resolution, Whole Transcriptome**



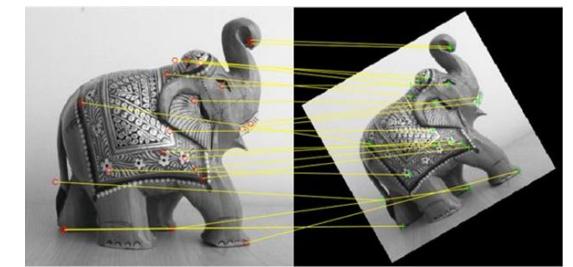
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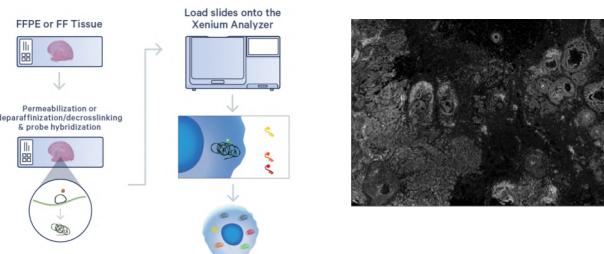
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Automated/Manual Image Registration

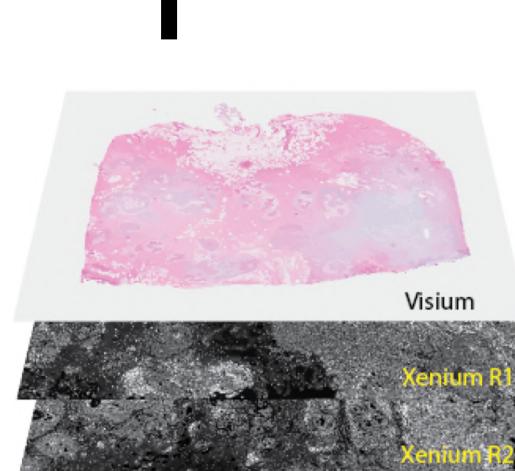


Xenium In Situ Platform (10X Genomics)  
**(Sub)Cellular Resolution, Gene Panel**



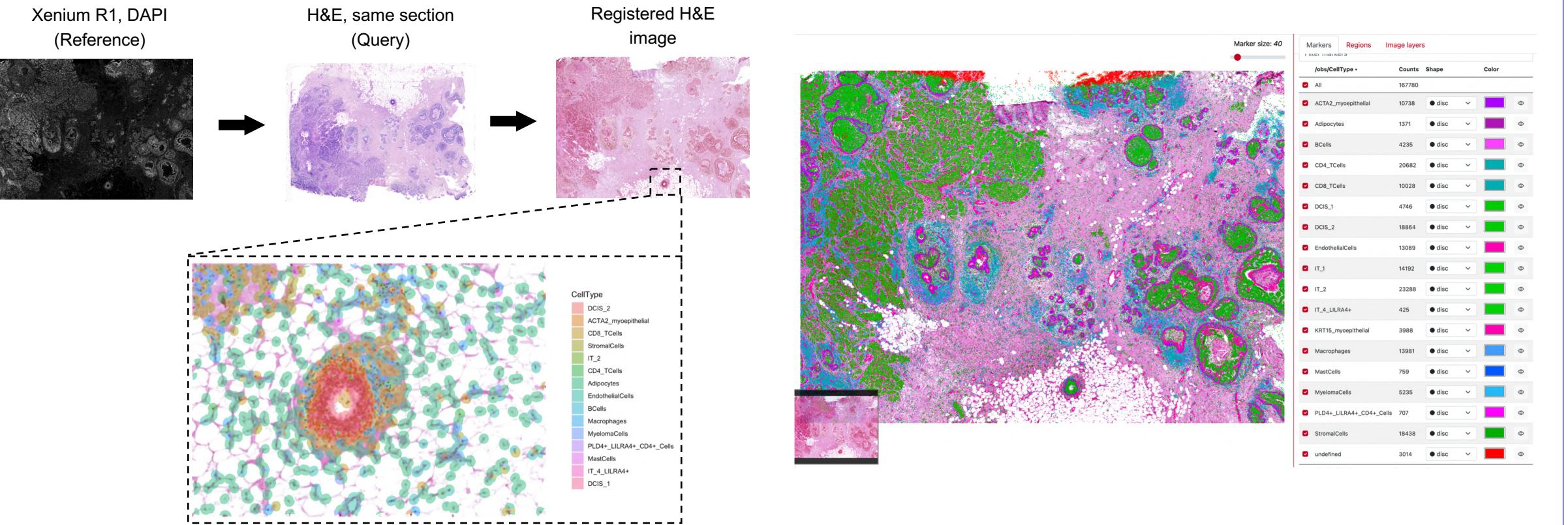
**10X GENOMICS**

FFPE-preserved  
Breast Cancer Block



## SPATIAL DATA ALIGNMENT

Aligning H&E images and interactive visualization of spatial localization



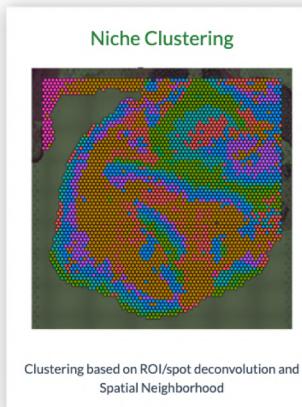
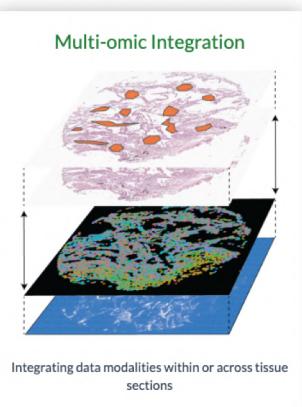
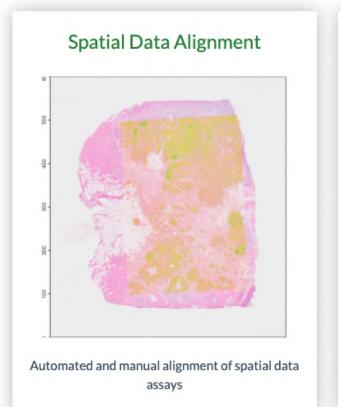
# VOLTRON WEBSITE AND TUTORIALS

<https://bioinformatics.mdc-berlin.de/VoltRon/tutorials.html>

## Spatially Aware Data Integration and Analysis

VoltRon incorporates multiple data integration utilities to achieve data transfer across a diverse set of spatial data modalities and types. VoltRon utilizes OpenCV to align and synchronize spatial omic datasets using computer vision and image registration. Users can automatically or manually align a list of microscopy images (H&E, DAPI etc) using a Shiny App incorporated within our analysis workflow. Once aligned, feature data and metadata level information can be transferred across aligned tissue sections.

In addition, VoltRon provides a number of spatially aware data analysis methods to detecting niches (i.e. Niche Clustering) within tissues. VoltRon allows estimating niches associated with each cell by incorporating the cell type level information around each cell or spot. We either detect cellular populations/compositions within a spatial neighborhood of a cell to create these niche level information (e.g. Xenium) or we estimate the cell type abundances of spots (e.g. Visium, DBIT-Seq) from a reference single cell data (Seurat, SingleCellExperiment etc.) with already annotated cell types. VoltRon can also use these spatial neighborhood to detect hot spots (i.e. Hot Spot Analysis) of features, cell types and even molecular densities.



## Additional Downstream Analysis

VoltRon is also capable of end-to-end analysis of diverse set of spatial data types (or spatial entities) such as ROIs (regions of interest), spots, single cells, molecules and even images. Users can set any data type as default at any time where VoltRon provides minimal set of functions to analyze, process and visualize each of these modalities.

