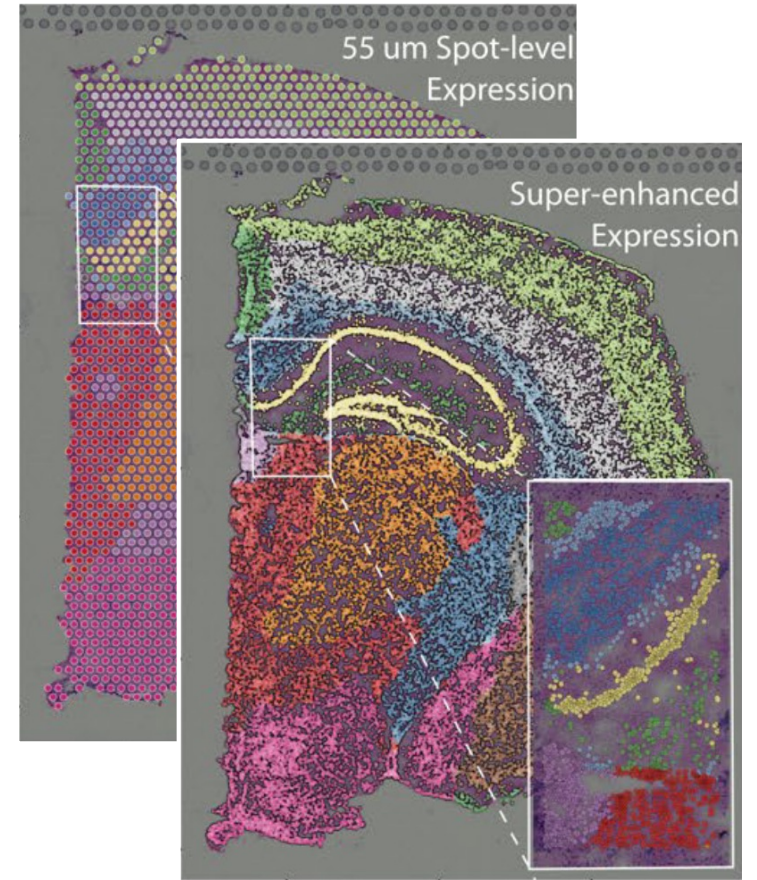
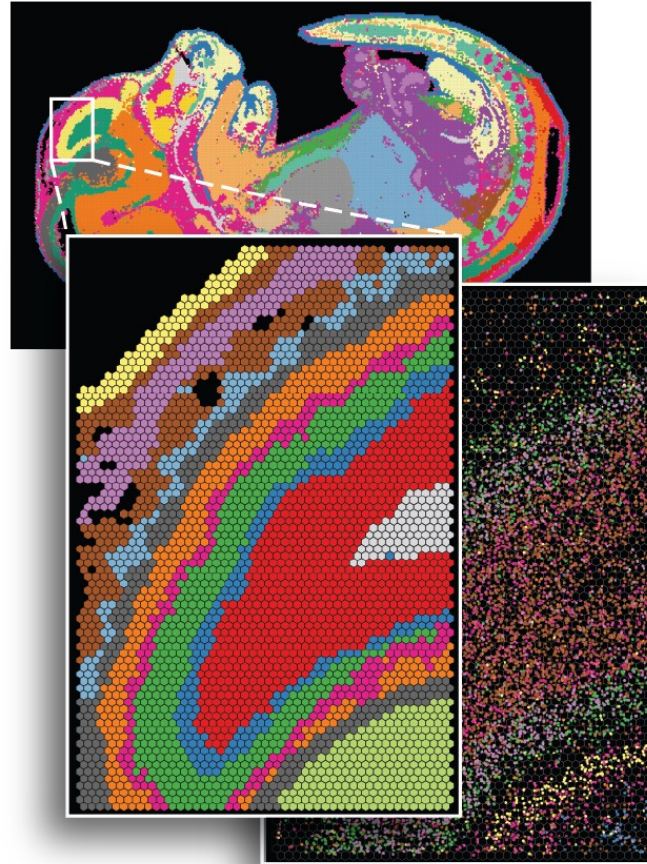
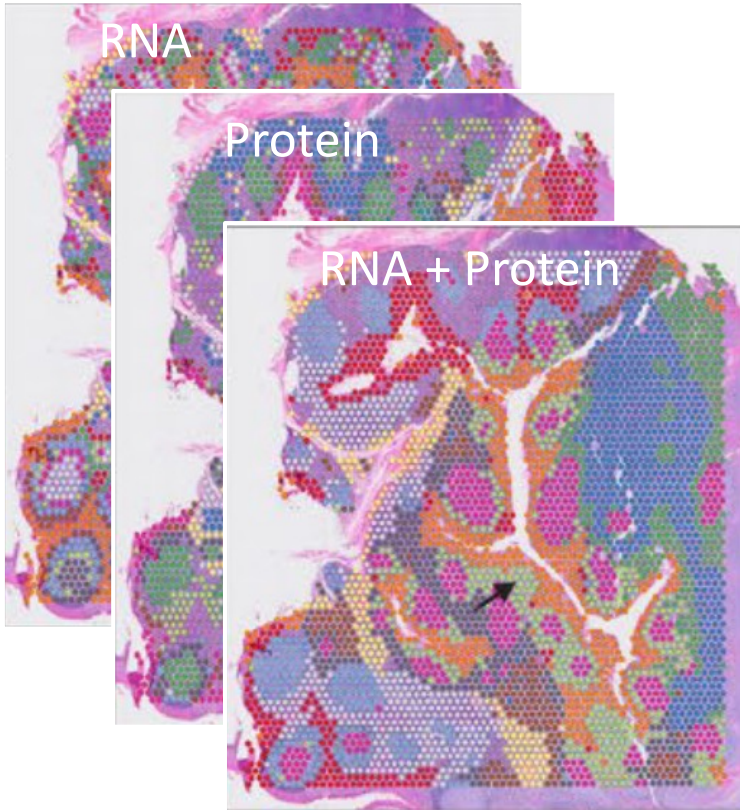


# Spatial omics technologies and applications



Ruben Dries  
GS workshop, 08/05

# A spatial transcriptomic example

Mouse  
brain



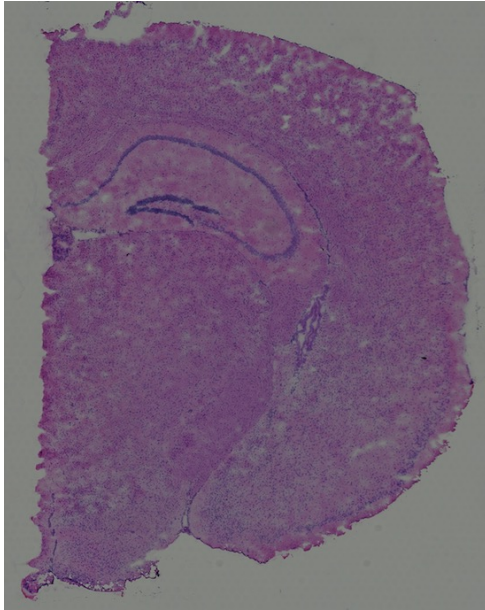
H&E stain:

- Haematoxylin = nuclei
- Eosin = extracellular matrix

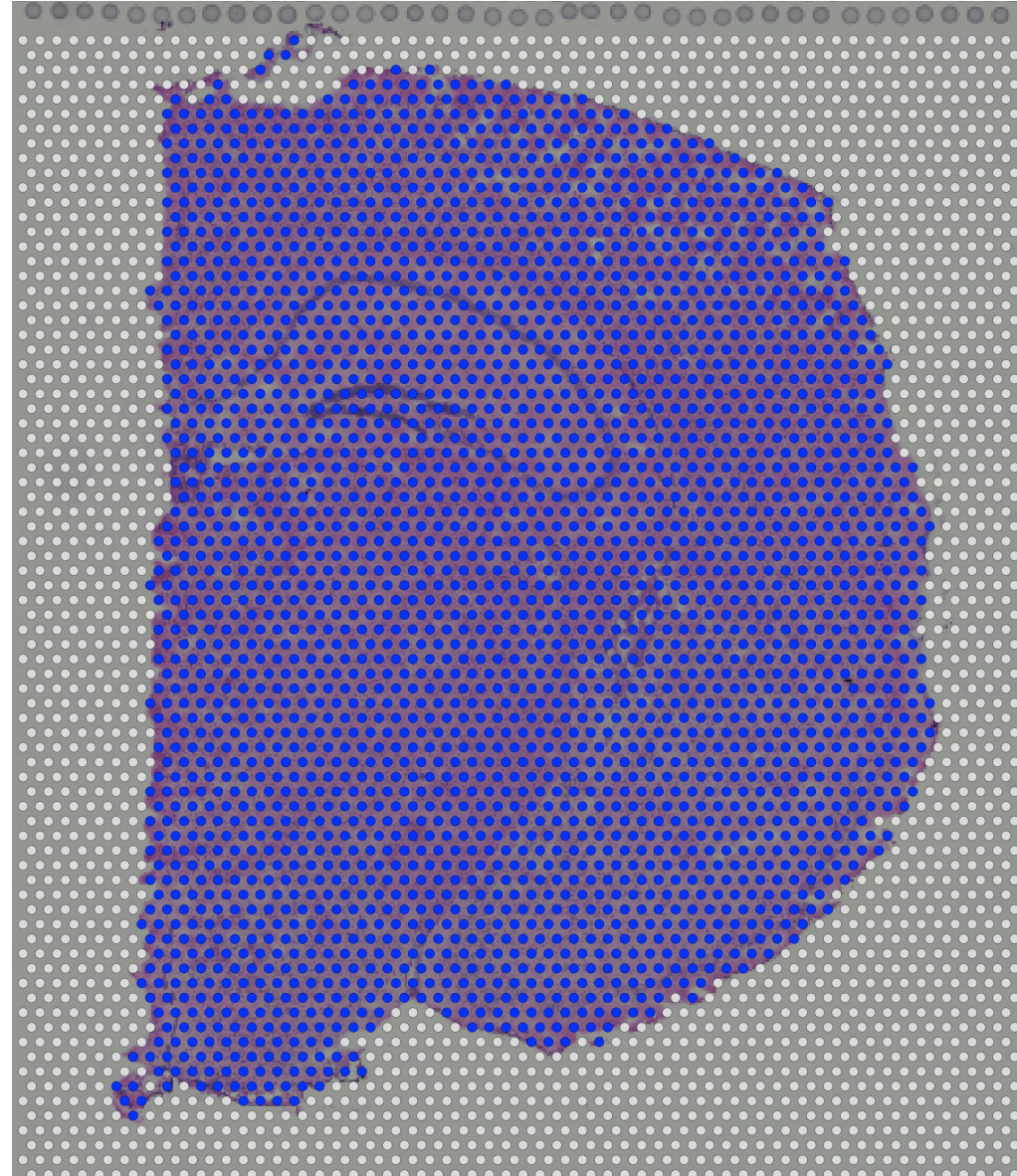
- Cornerstone of pathology: study the morphology to assess disease status
- What other information is available? 1000's of molecular analytes (RNA, Protein, Metabolites, etc)

# A spatial transcriptomic example

Mouse  
brain

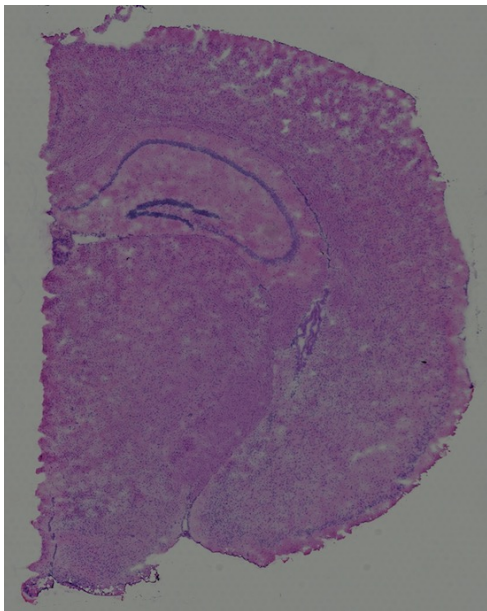


Extract spatial  
information  
(e.g. RNA)

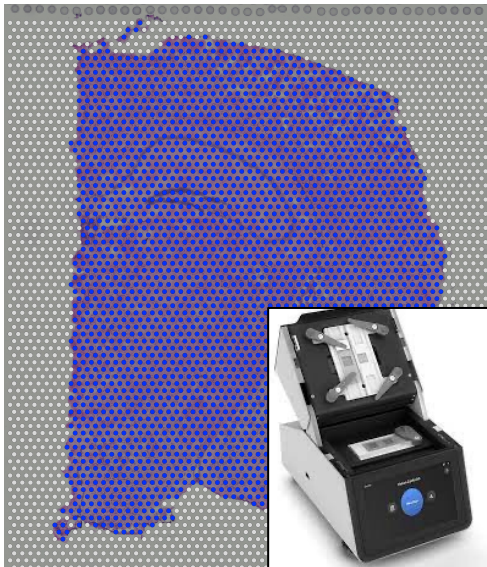


# A spatial transcriptomic example

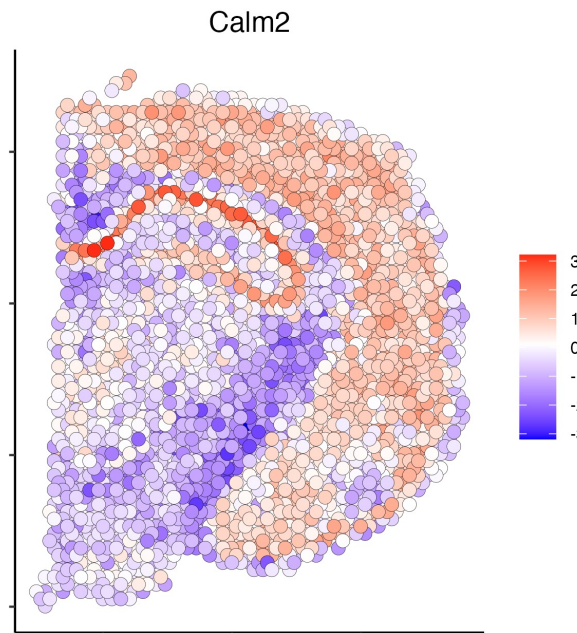
Mouse  
brain



Extract spatial  
information  
(e.g. RNA)

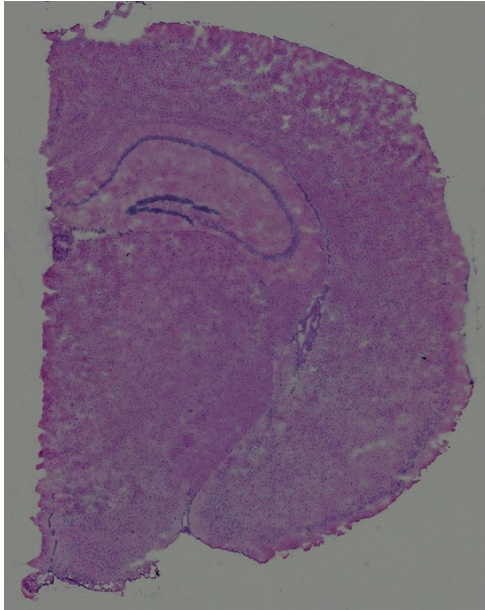


Map spatial  
information back  
(e.g. RNA  
expression levels)

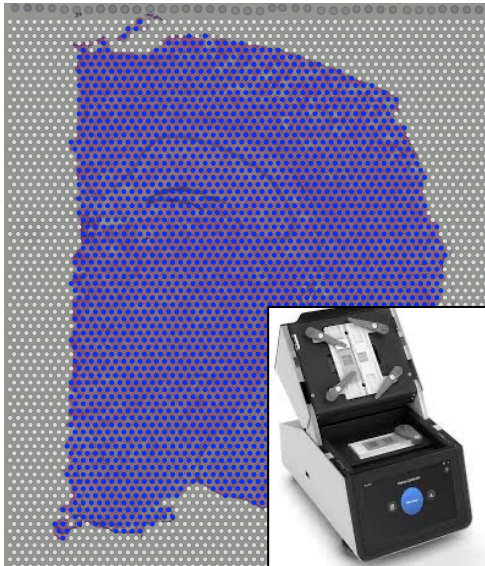


# A spatial transcriptomic example

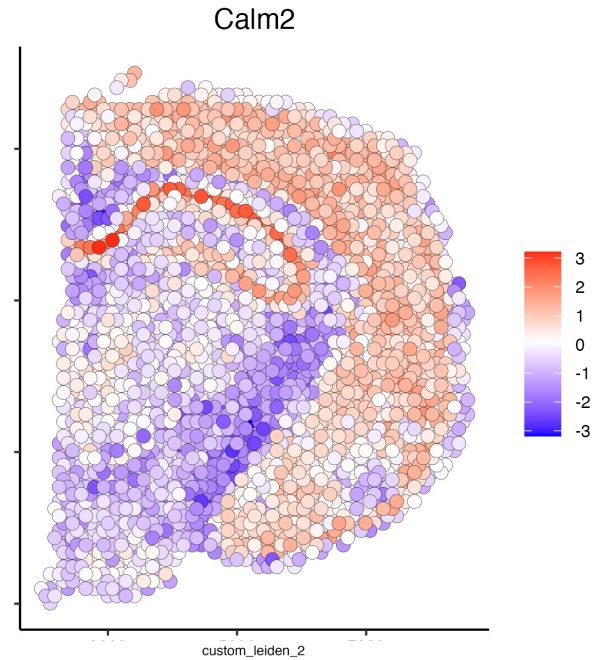
Mouse  
brain



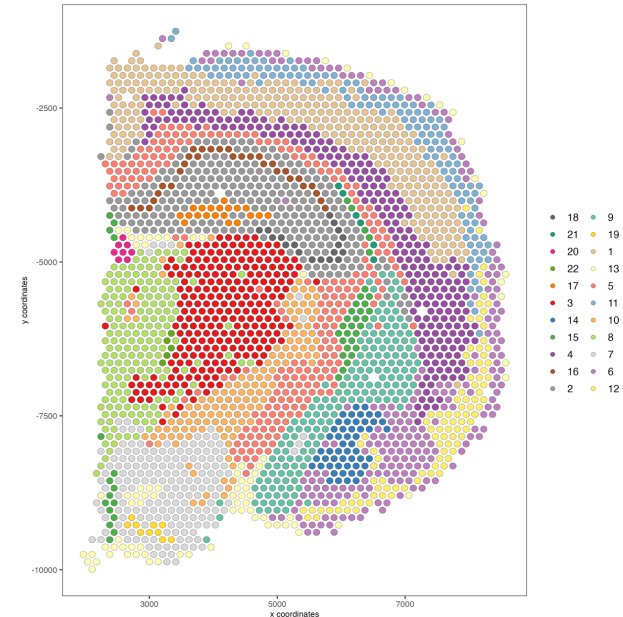
Extract spatial  
information  
(e.g. RNA)



Map spatial  
information back  
(e.g. RNA  
expression levels)



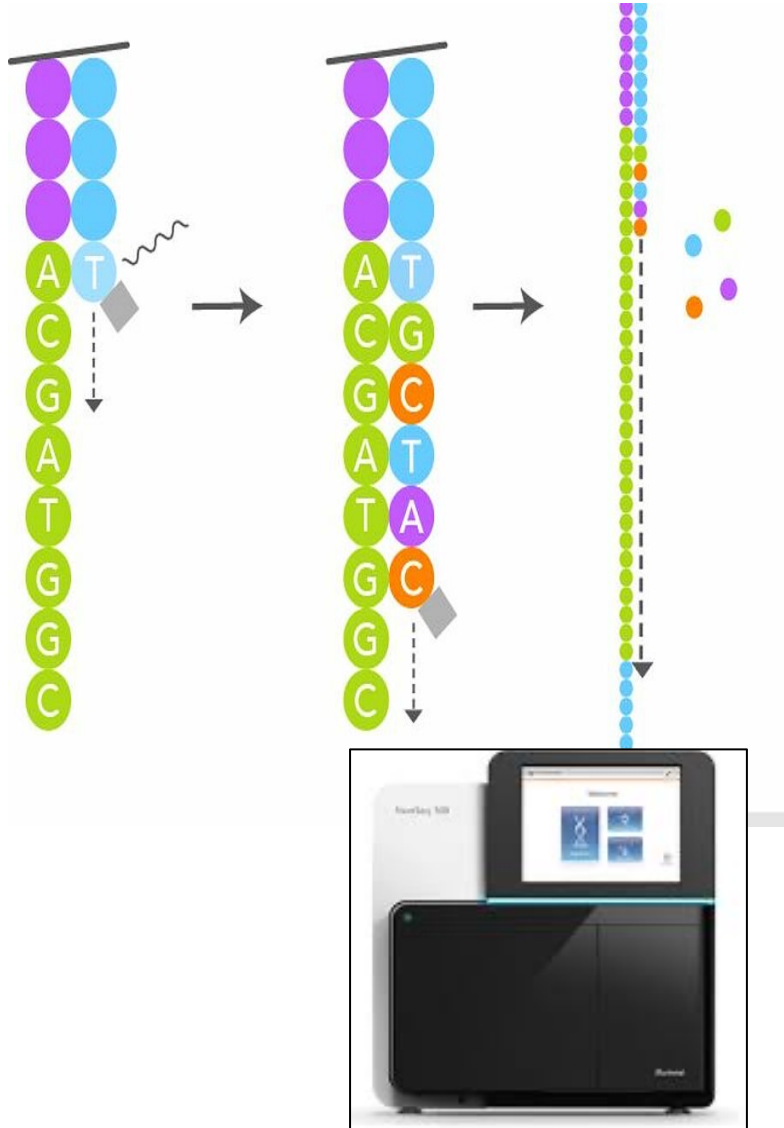
Obtain spatial  
insights



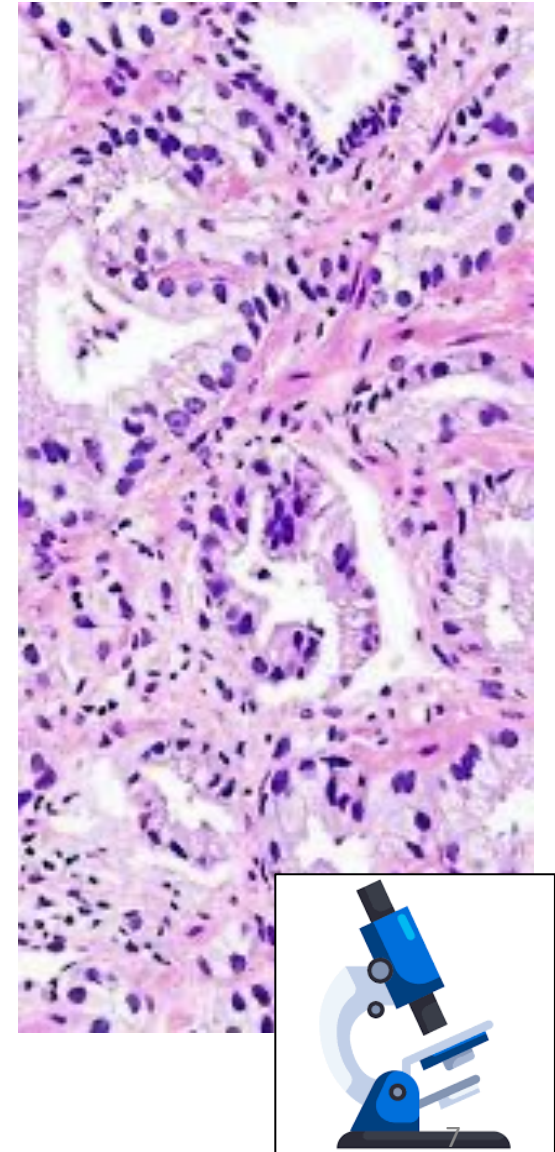


# What is spatial omics?

## Sequencing

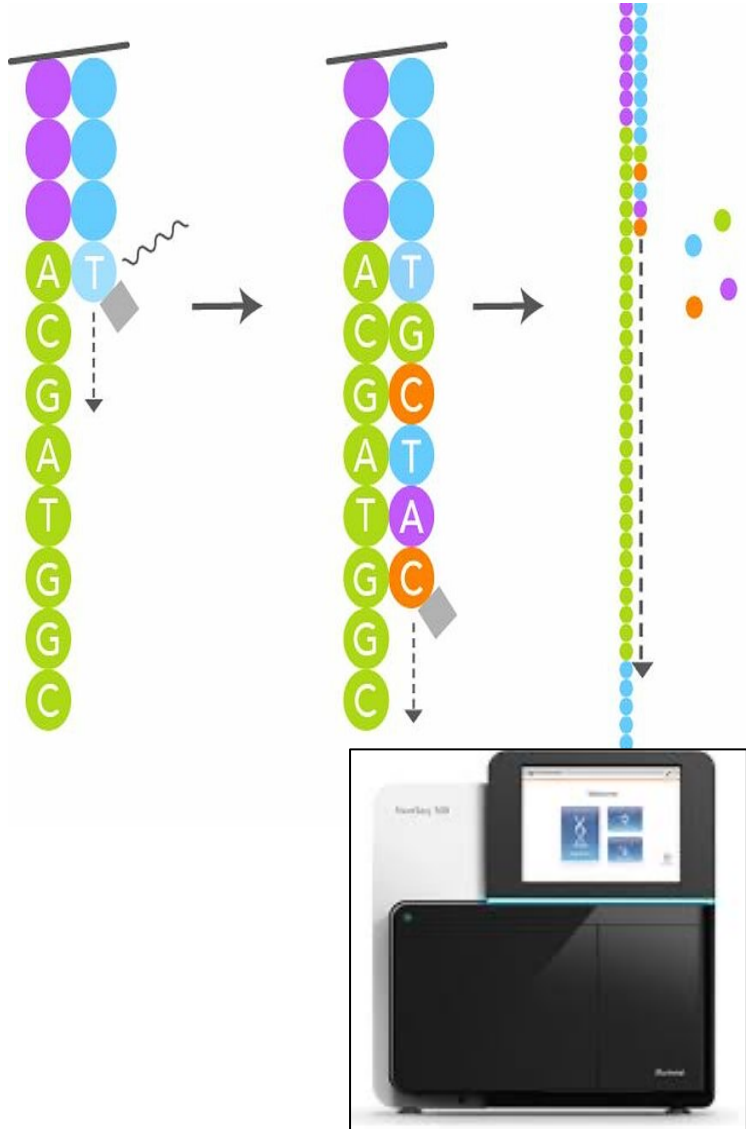


## Imaging

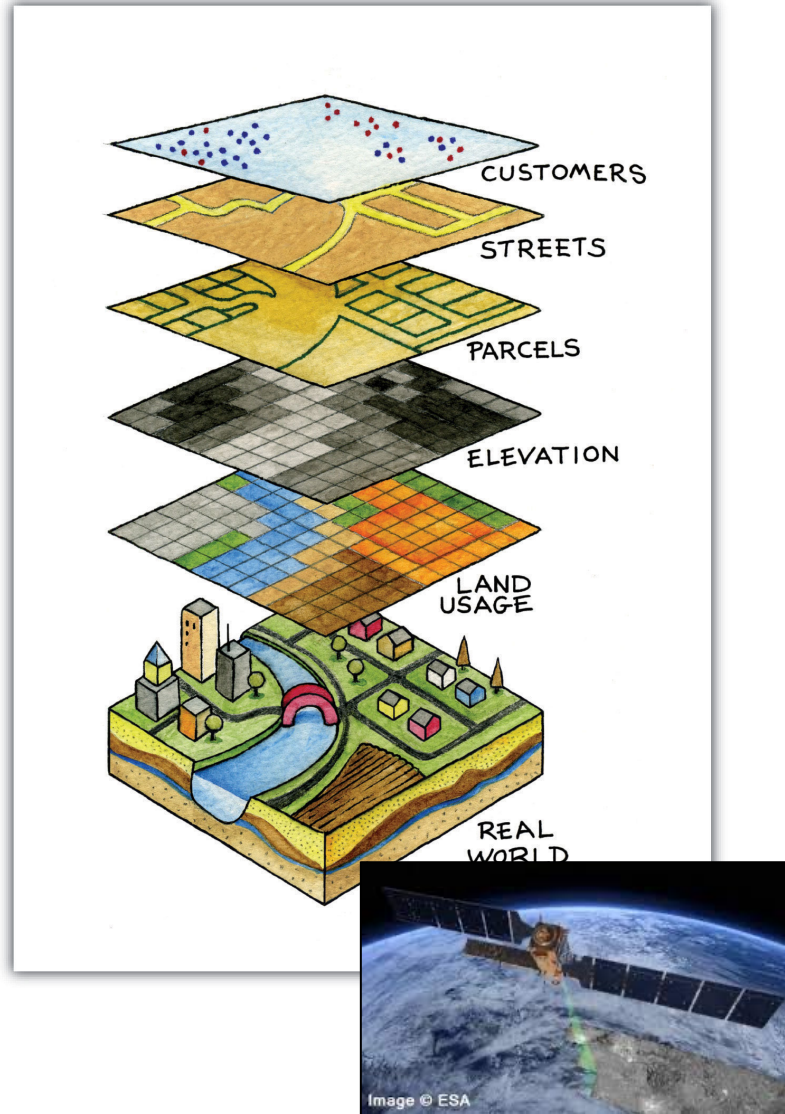


# What is spatial omics?

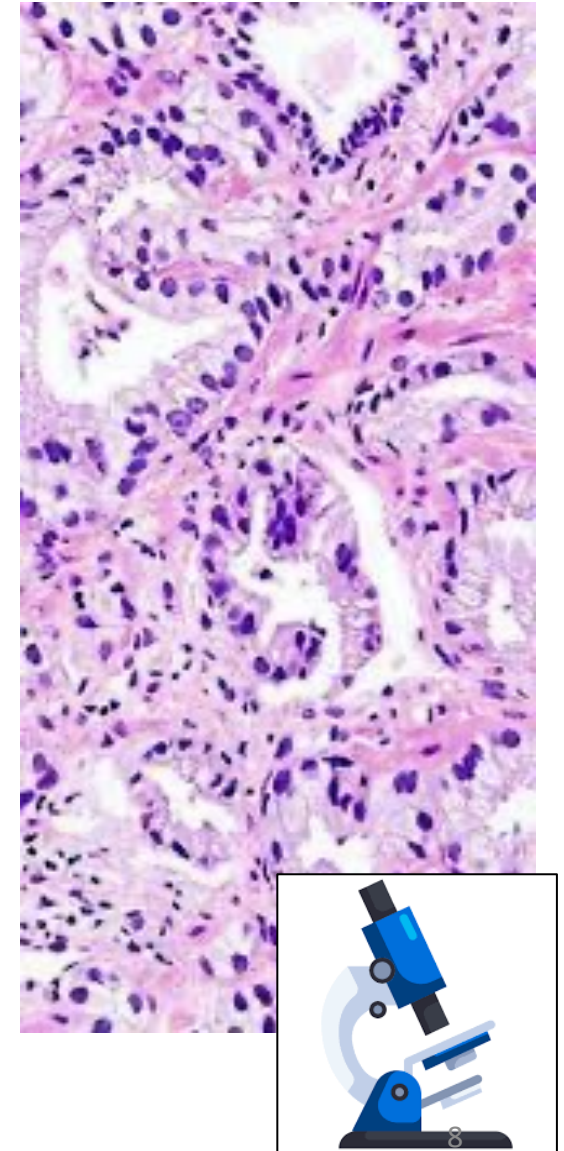
## Sequencing



## Spatial data science



## Imaging



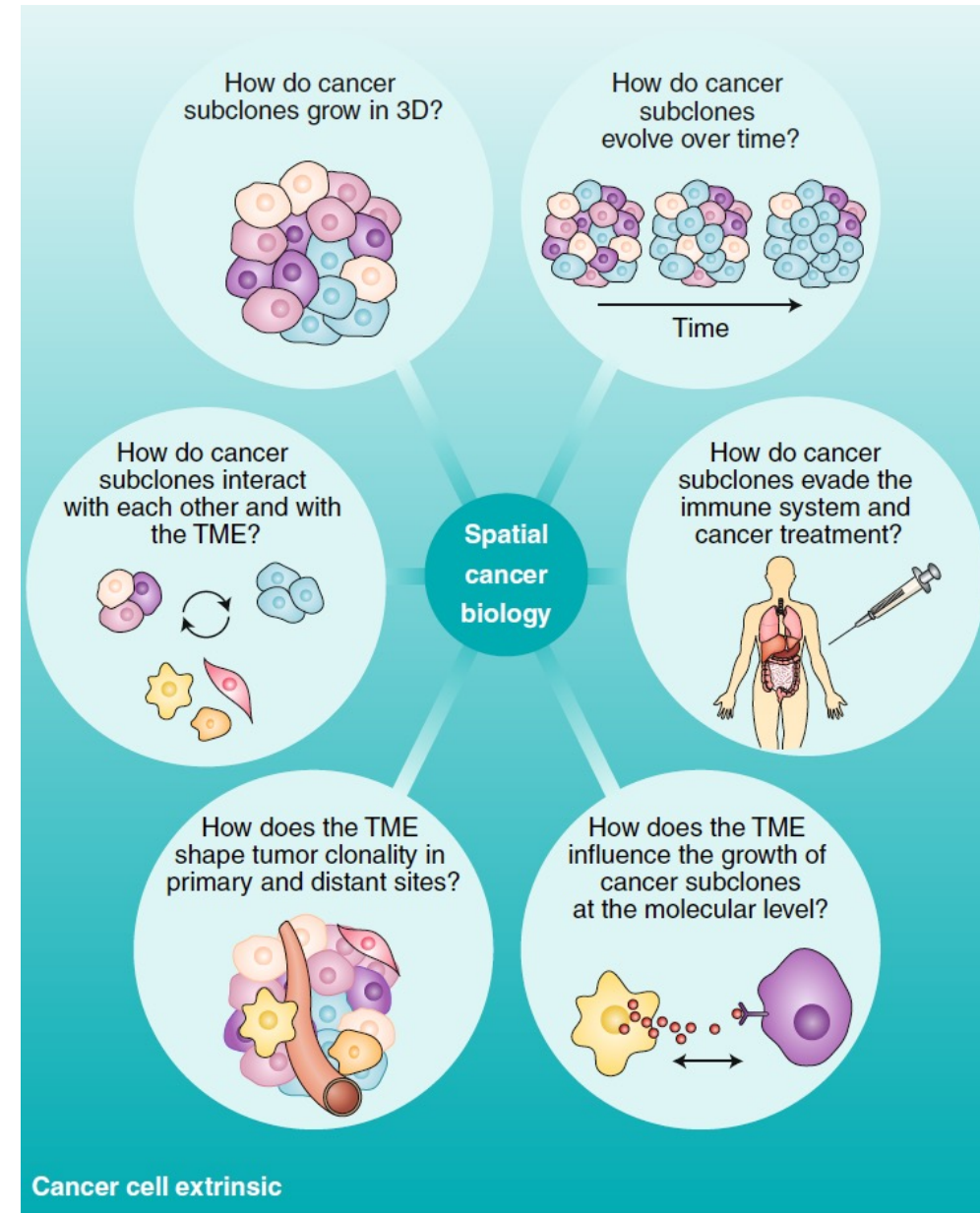


# What are the biological questions we're trying to answer?

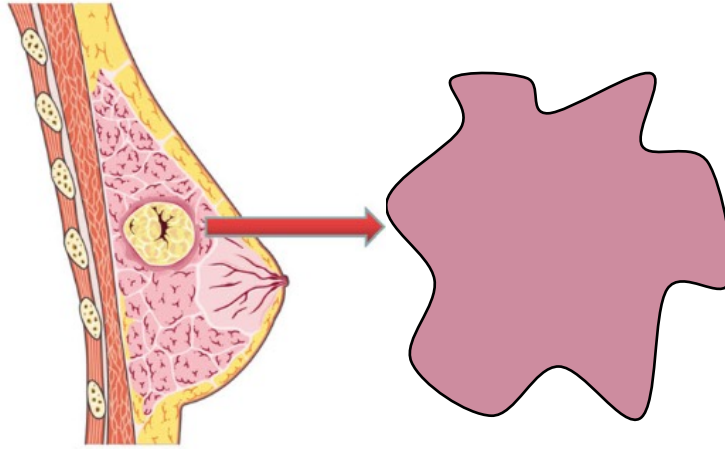
Examples in the field of cancer biology:

*Questions that require us to understand cellular behavior within their normal tissue or environment.*

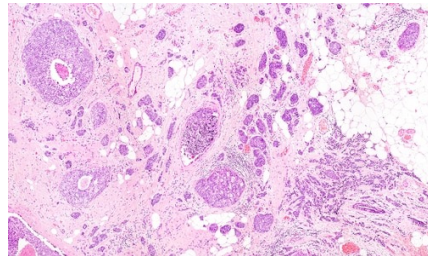
- *Growth of cancer clones*
- *Interaction with tumor microenvironment*
- *Signaling and cellular crosstalk*



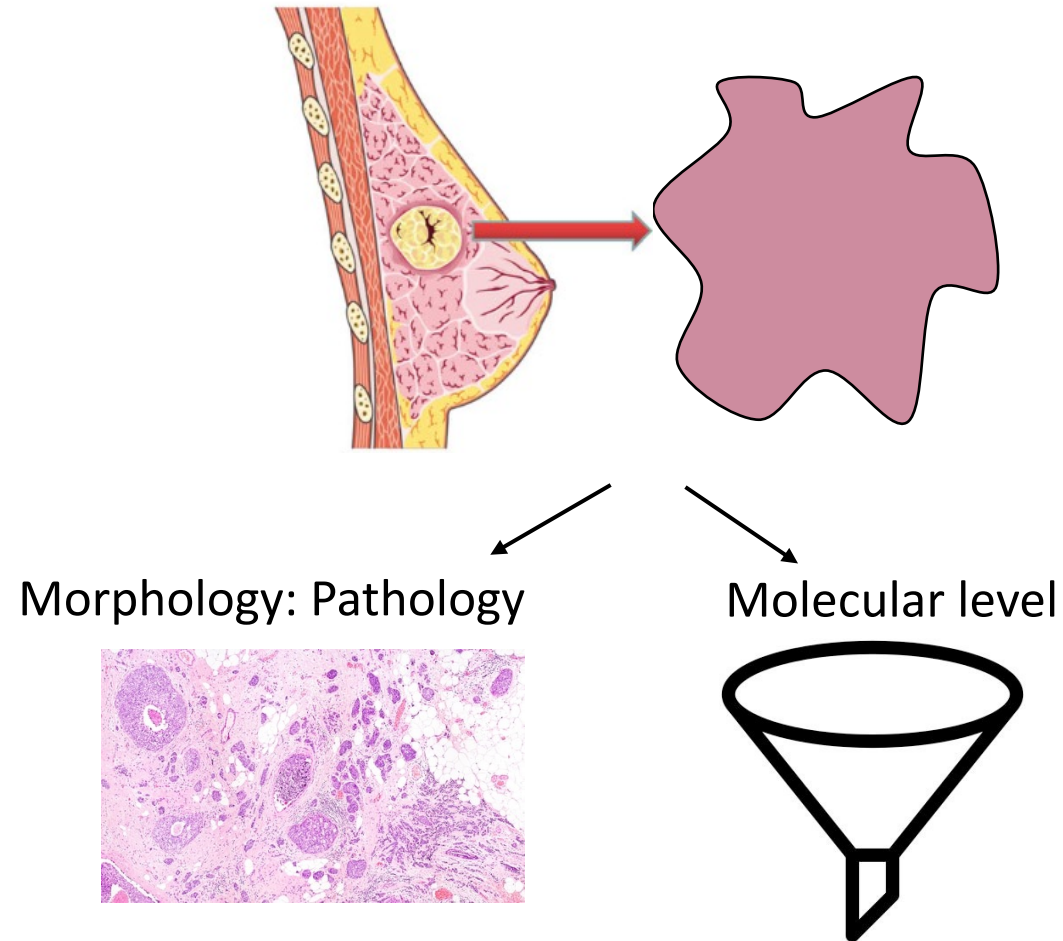
Historically ...



Morphology: Pathology

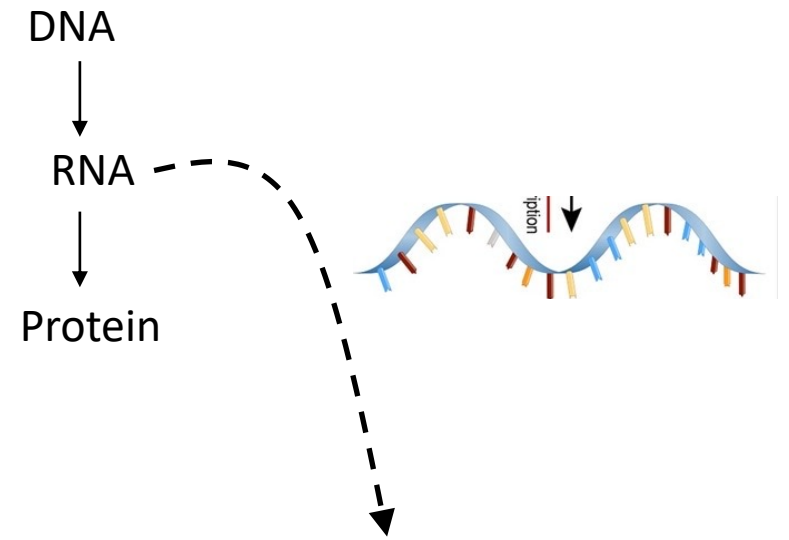
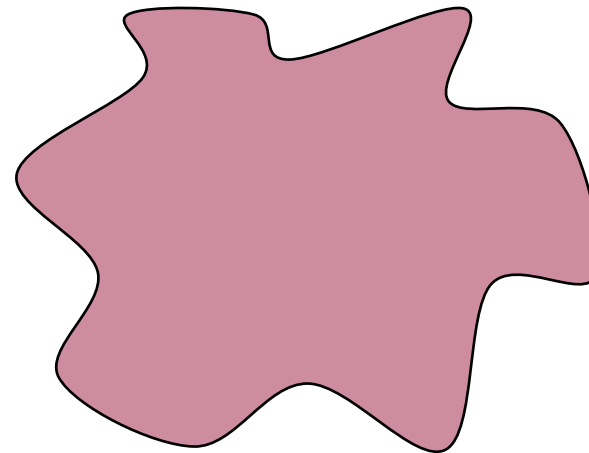


# Historically ...



- Which genes drive treatment resistance?
- Is the immune system activated or suppressed?
- Is there a biomarker that predict response to treatment?
- How do adipocytes or stromal cells communicate with cancer cells?

# Historically ... RNA-seq

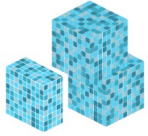


# Historically ... RNA-seq

## NATIONAL CANCER INSTITUTE THE CANCER GENOME ATLAS

### TCGA BY THE NUMBERS

TCGA produced over  
**2.5**  
PETABYTES  
of data



TCGA data describes

**33**  
DIFFERENT  
TUMOR TYPES

...including

**10**  
RARE  
CANCERS

To put this into perspective, 1 petabyte of data is equal to

**212,000**  
DVDs

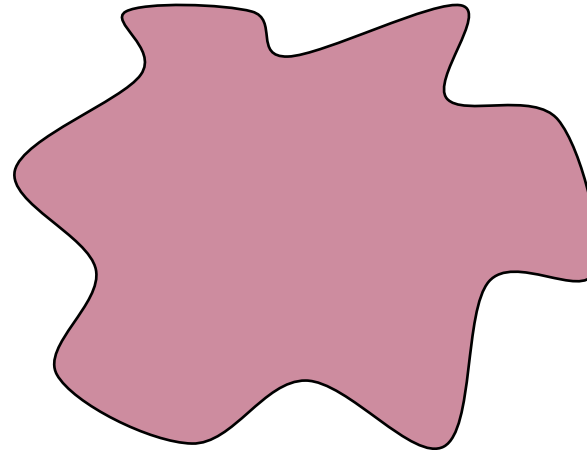


...based on paired tumor and normal tissue sets collected from

**11,000**  
PATIENTS

...using

**7** DIFFERENT  
DATA TYPES



DNA



RNA



Protein



### TCGA RESULTS & FINDINGS



MOLECULAR  
BASIS OF  
CANCER

Improved our understanding of the genomic underpinnings of cancer

For example, a TCGA study found the basal-like subtype of breast cancer to be similar to the serous subtype of ovarian cancer on a molecular level, suggesting that despite arising from different tissues in the body, these subtypes may share a common path of development and respond to similar therapeutic strategies.



TUMOR  
SUBTYPES

Revolutionized how cancer is classified

TCGA revolutionized how cancer is classified by identifying tumor subtypes with distinct sets of genomic alterations.\*



THERAPEUTIC  
TARGETS

Identified genomic characteristics of tumors that can be targeted with currently available therapies or used to help with drug development

TCGA's identification of targetable genomic alterations in lung squamous cell carcinoma led to NCI's Lung-MAP Trial, which will treat patients based on the specific genomic changes in their tumor.

### THE TEAM



**20**

COLLABORATING  
INSTITUTIONS  
across the United States  
and Canada

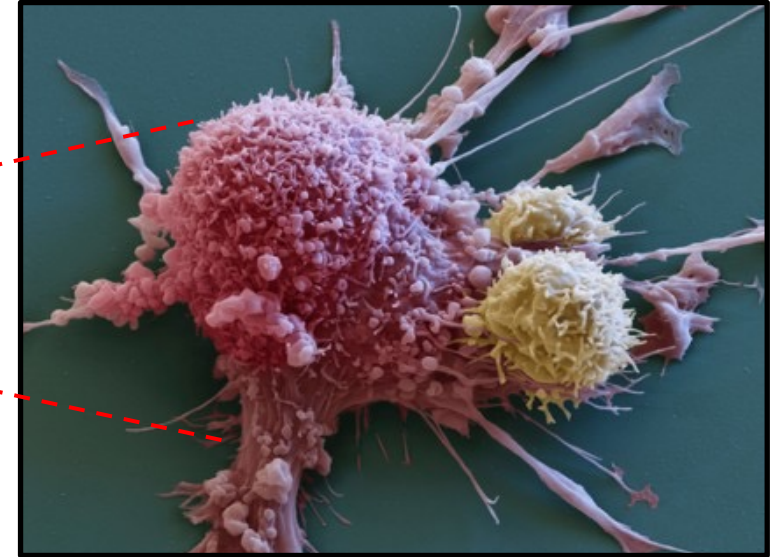
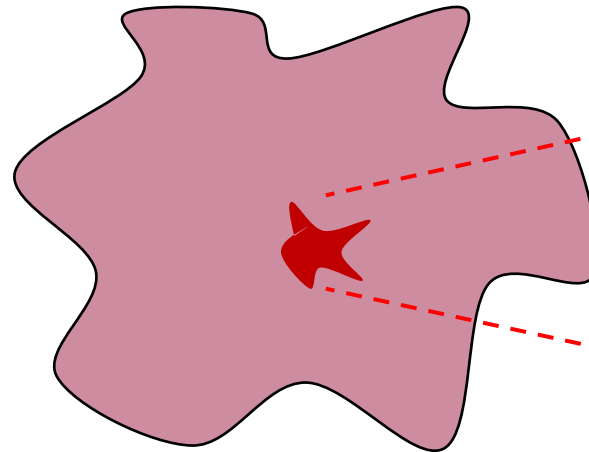
### WHAT'S NEXT?

The Genomic Data Commons (GDC) houses TCGA and other NCI-generated data sets for scientists to access from anywhere. The GDC also has many expanded capabilities that will allow researchers to answer more clinically relevant questions with increased ease.

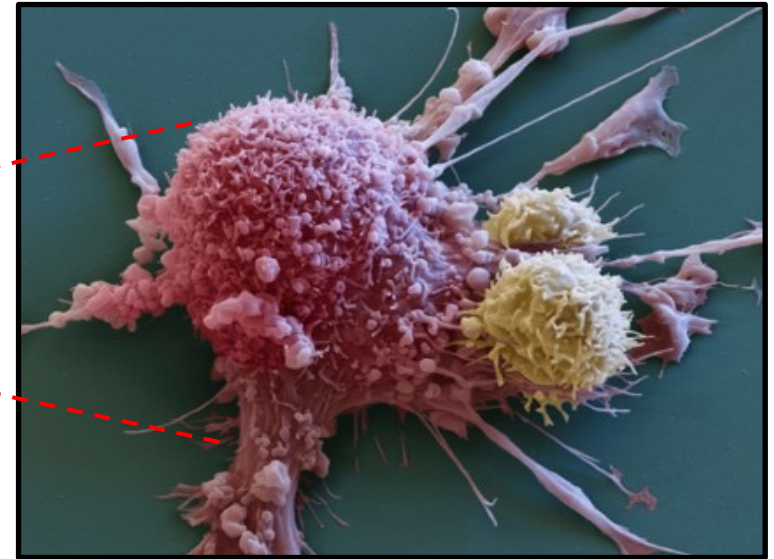
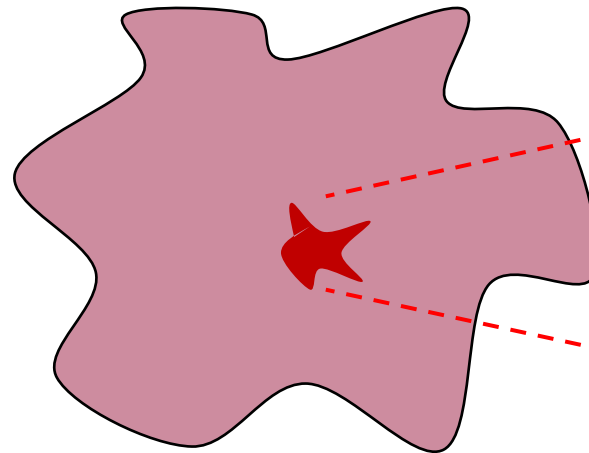


\*TCGA's analysis of stomach cancer revealed that it is not a single disease, but a disease composed of four subtypes, including a new subtype characterized by infection with Epstein-Barr virus.

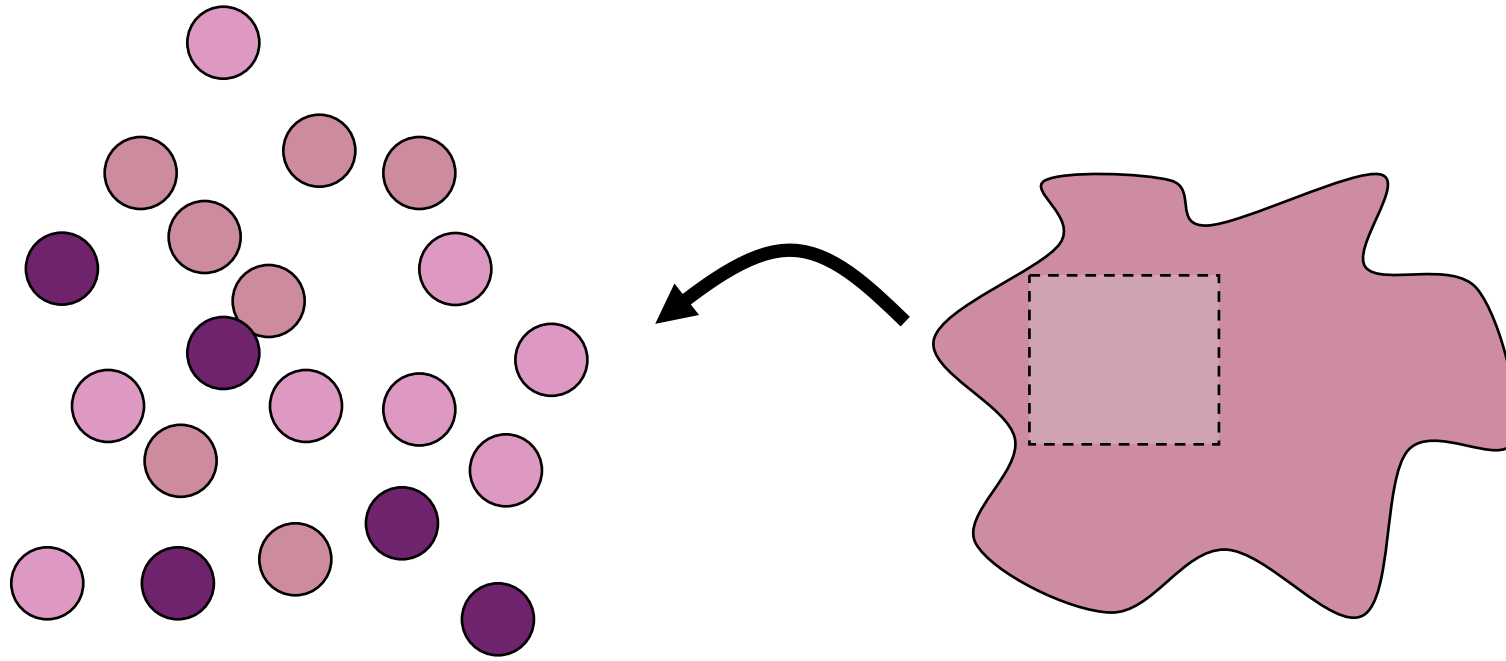
# Historically ... **limitations** of RNA-seq



# Historically ... **limitations** of RNA-seq

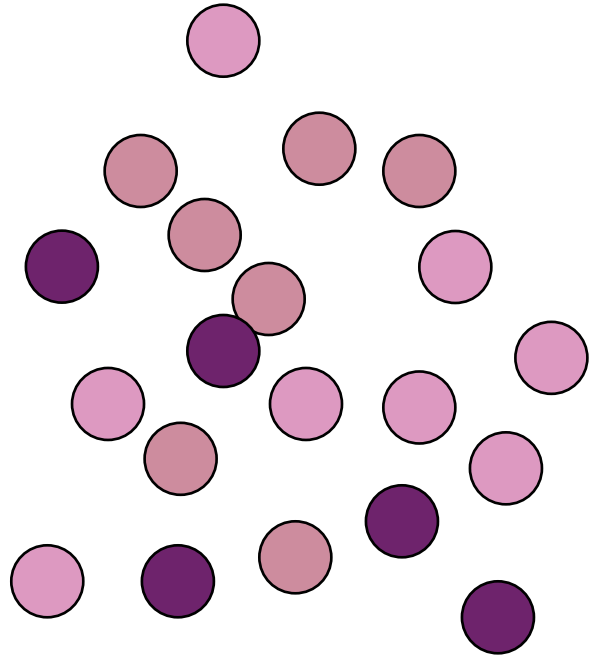


# Historically ... single-cell RNA-seq

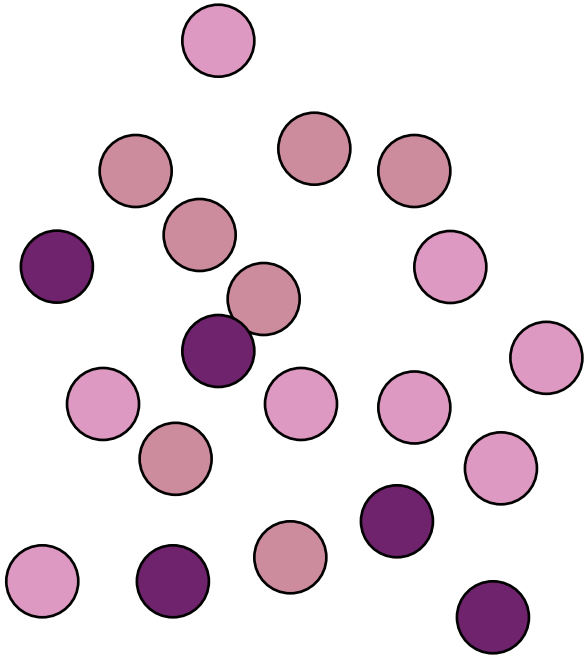




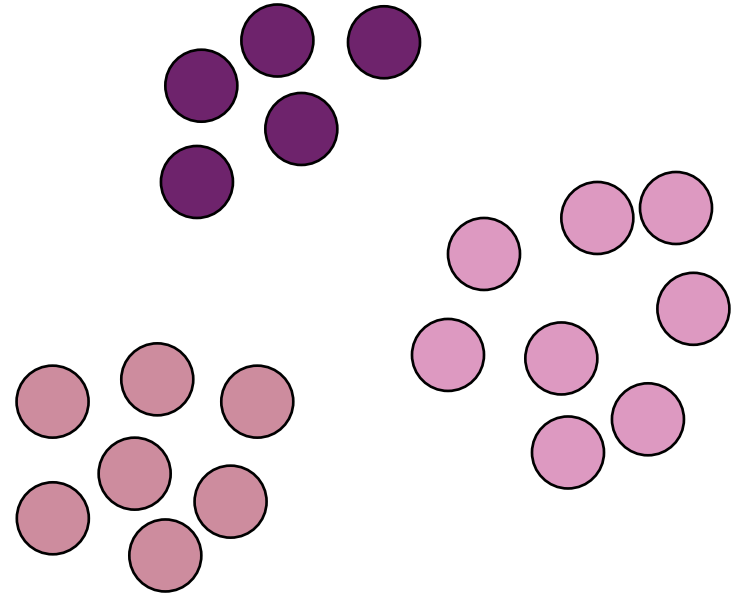
# Historically ... single-cell RNA-seq



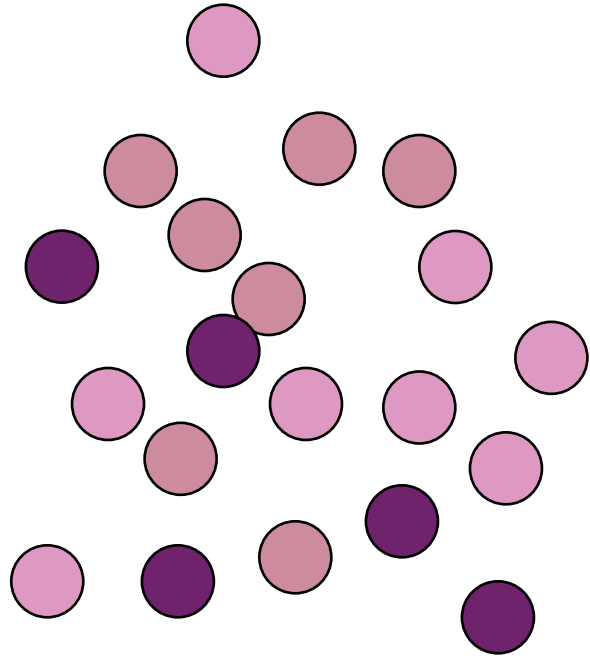
# Historically ... single-cell RNA-seq



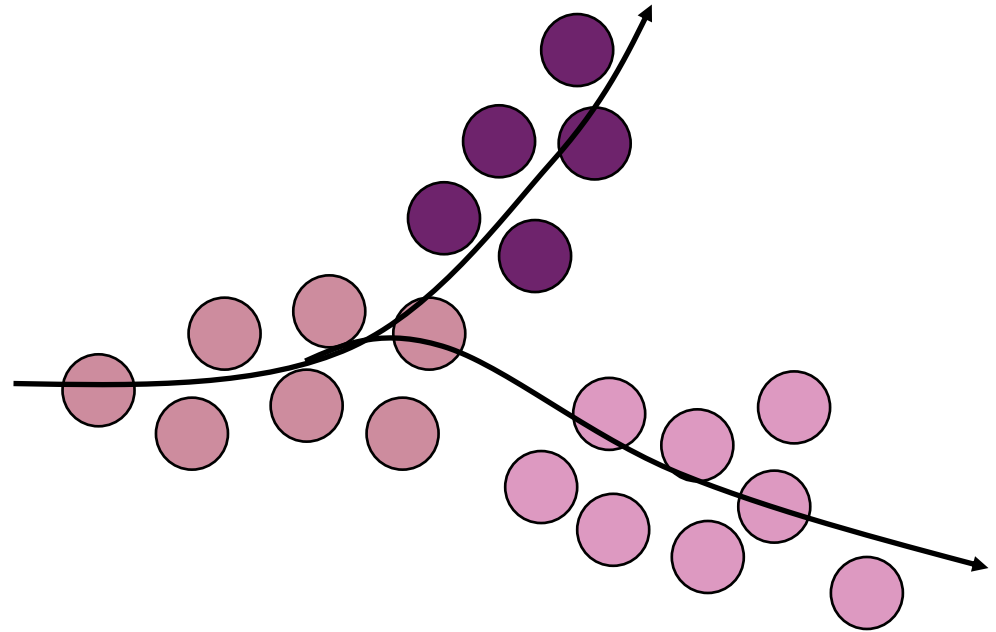
Cluster cell types



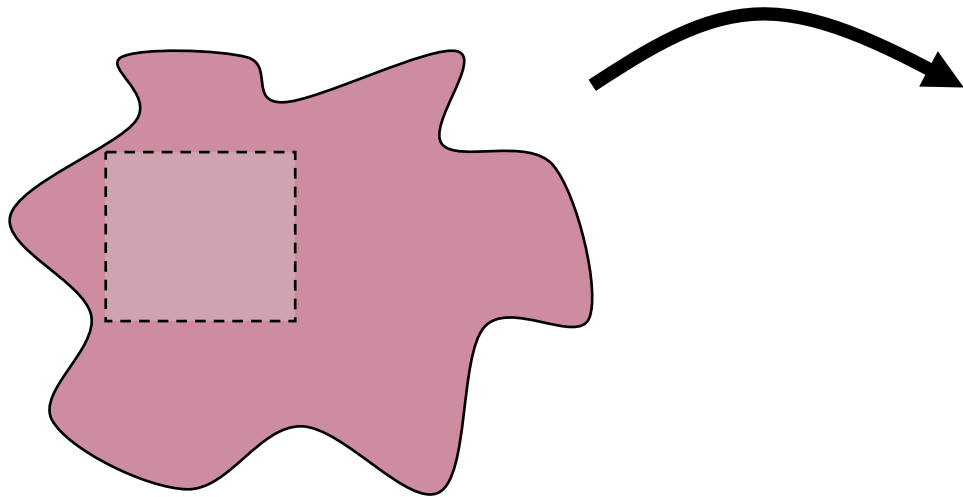
# Historically ... single-cell RNA-seq



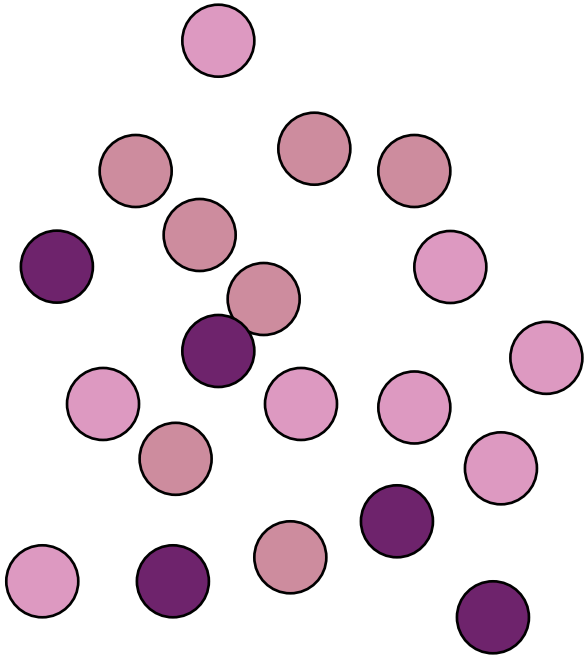
## Cell trajectories



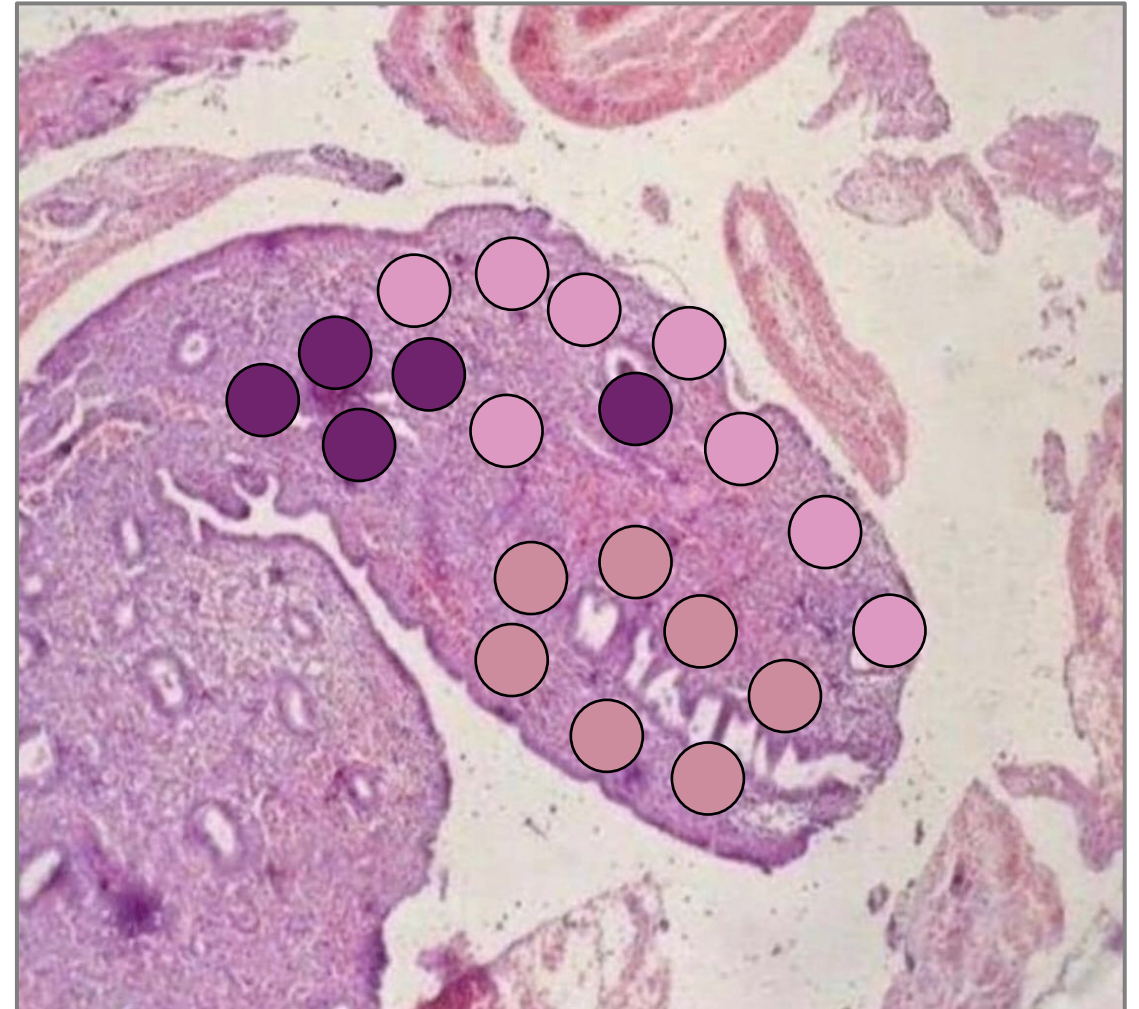
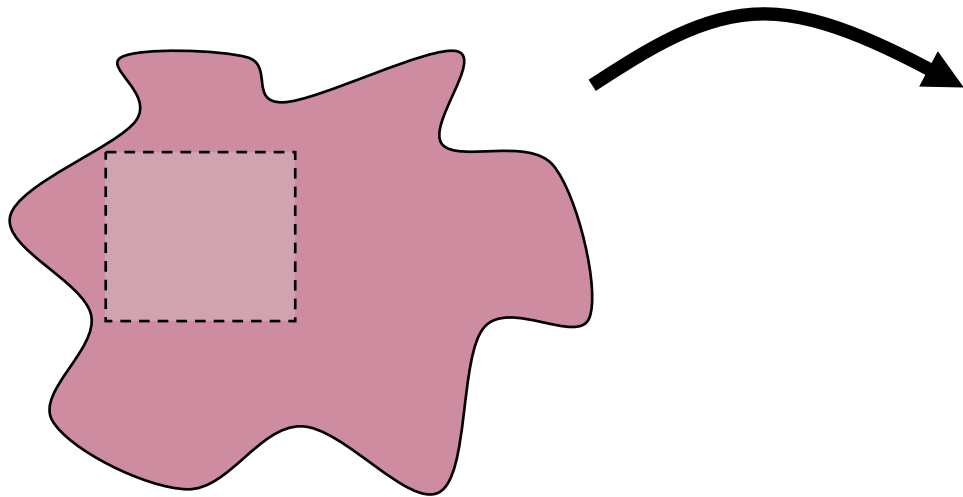
# Historically ... single-cell RNA-seq



# Historically ... **limitations** of single-cell RNA-seq

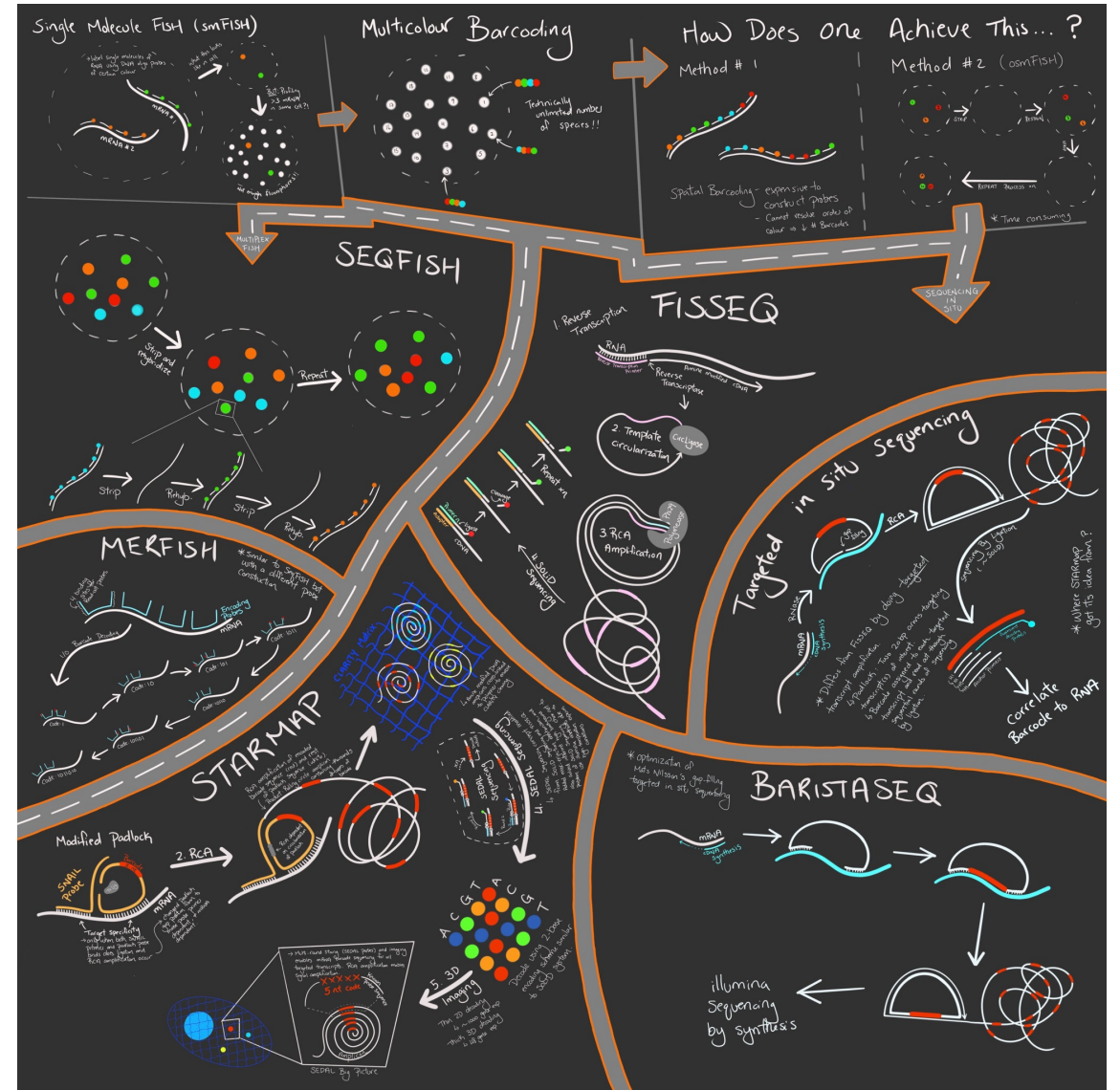
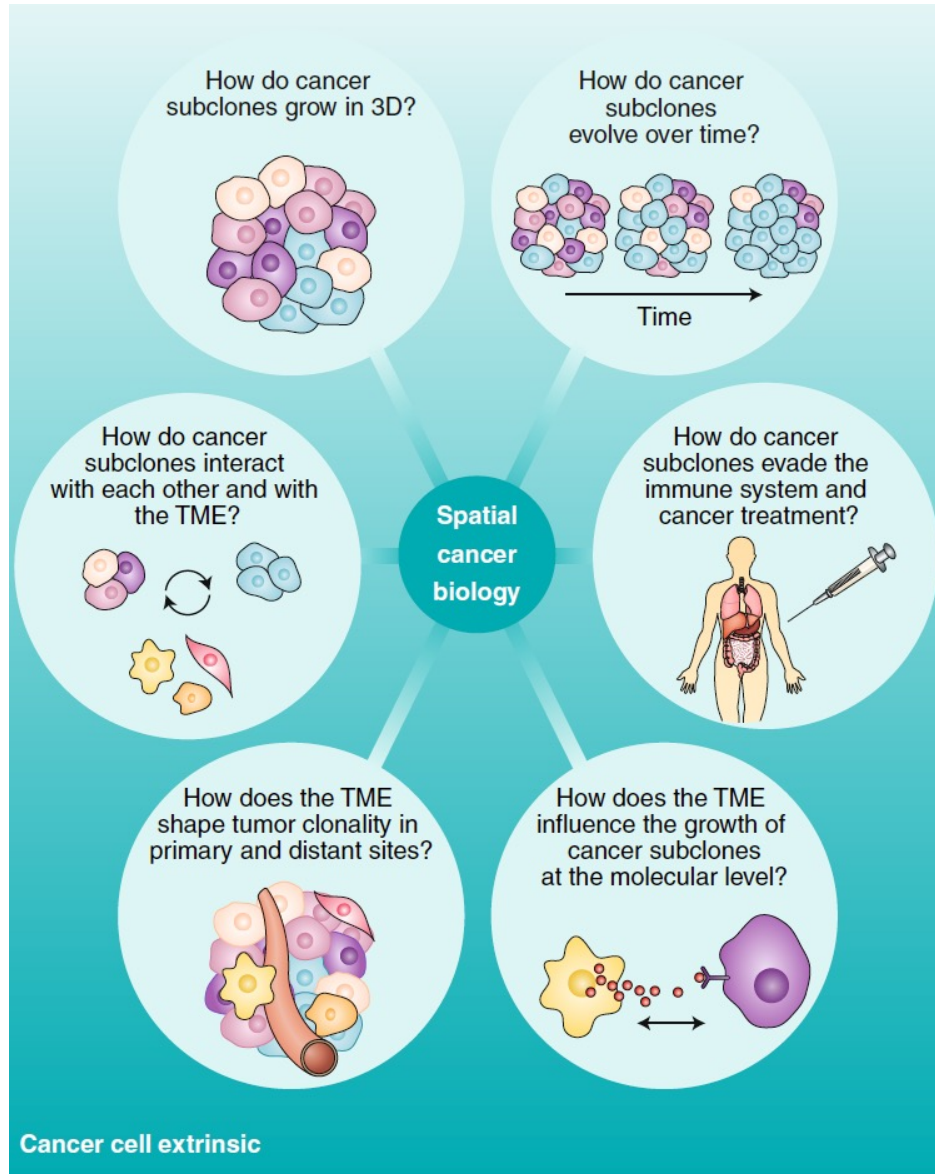


# Historically ... **limitations** of single-cell RNA-seq

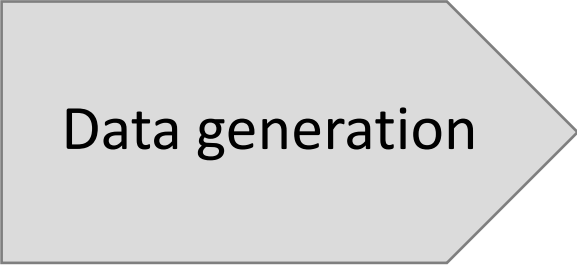


# How to choose and create a spatial omics dataset?

Spatial information is essential to answer these questions, but which method(s) to choose?



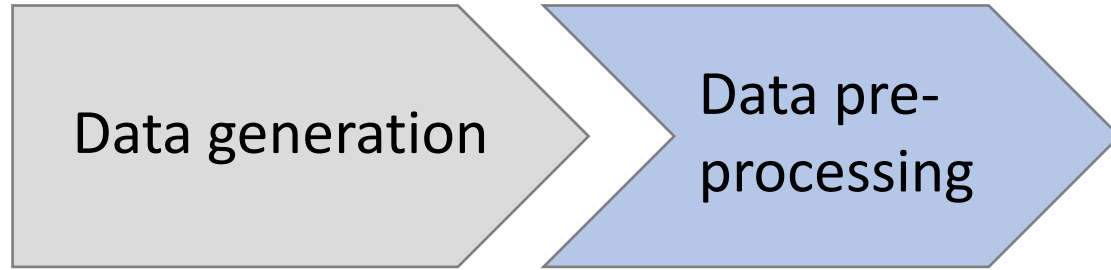
# The different steps in spatial omics research



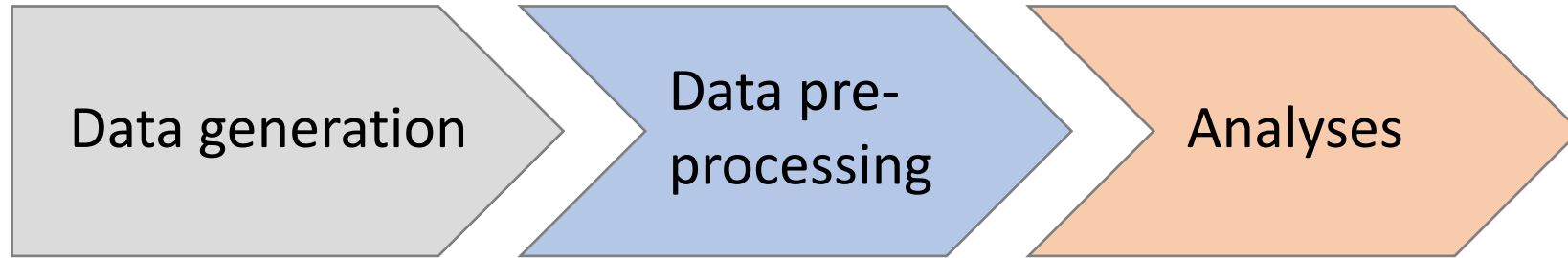
Data generation



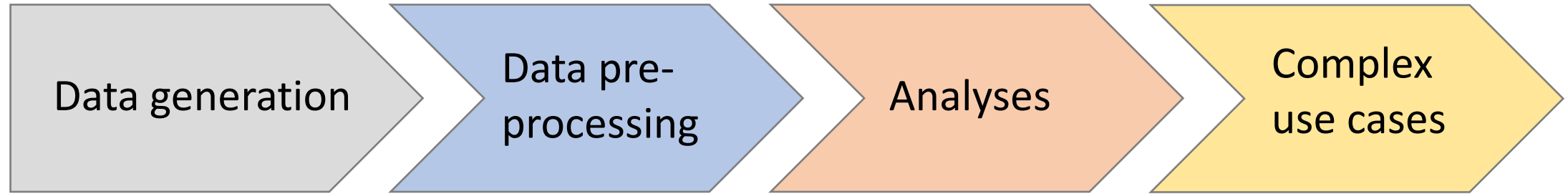
# The different steps in spatial omics research



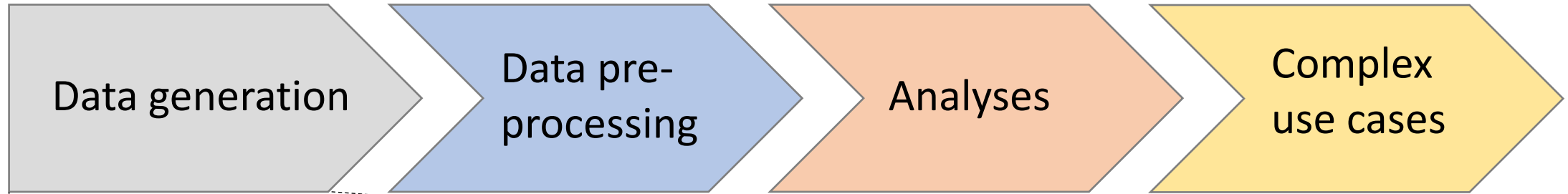
# The different steps in spatial omics research



# The different steps in spatial omics research



# The different steps in spatial omics research



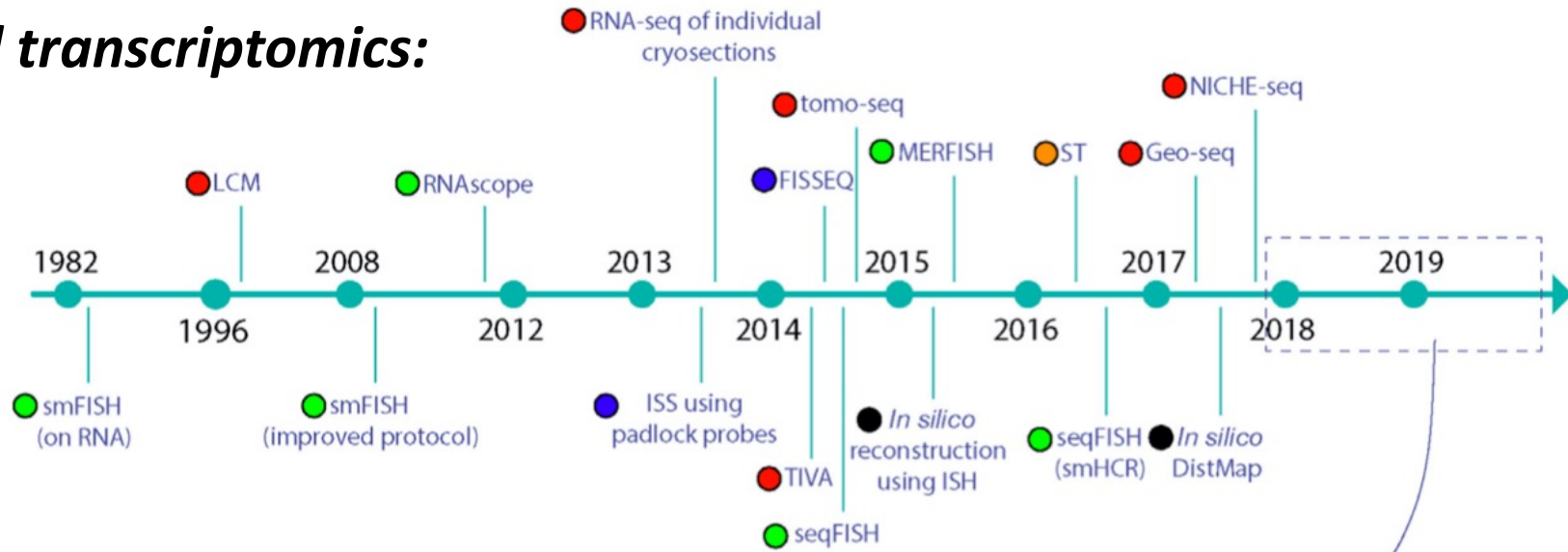
- Variety in data characteristics: each technology is different
- Variety of data outputs: spatial is a multi-modal experiment by default

# Long history of spatial technology development

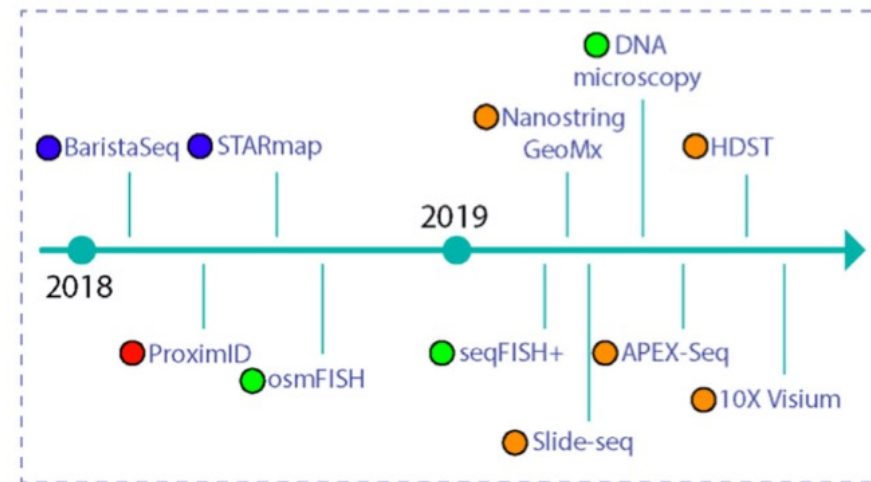
## Spatial proteomics:

CODEX  
 CyCIF  
 MIBI  
 IMC  
 seqIF (lunaphore)  
 ...

## Spatial transcriptomics:



- Section 1. Technologies based on microdissected gene expression
- Section 2. *In situ* hybridization technologies
- Section 3. *In situ* sequencing technologies
- Section 4. *In situ* capturing technologies
- Section 5. *In silico* reconstruction of spatial data



DBiT-seq  
 ZipSeq  
 Split-FISH  
 seqSCOPE  
 Pixel-Seq  
 Stereo-Seq  
 Nanostring  
 Xenium

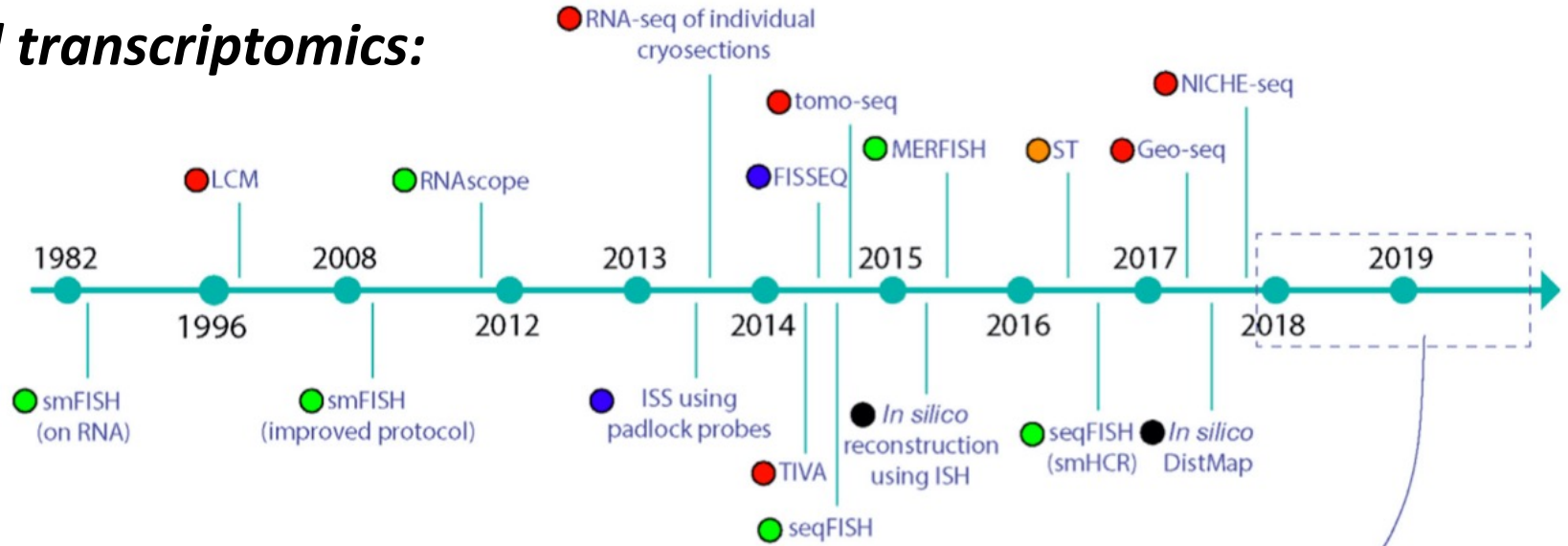
# Long history of spatial technology development

## Spatial proteomics:

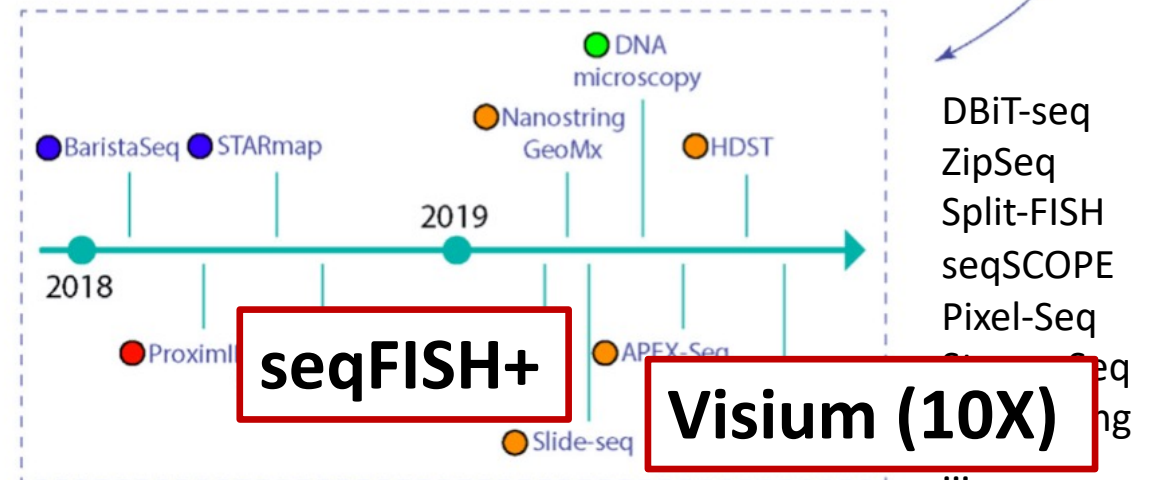
CODEX  
CyCIF  
MIBI  
IMC

**seqIF (lunaphore)**

## Spatial transcriptomics:

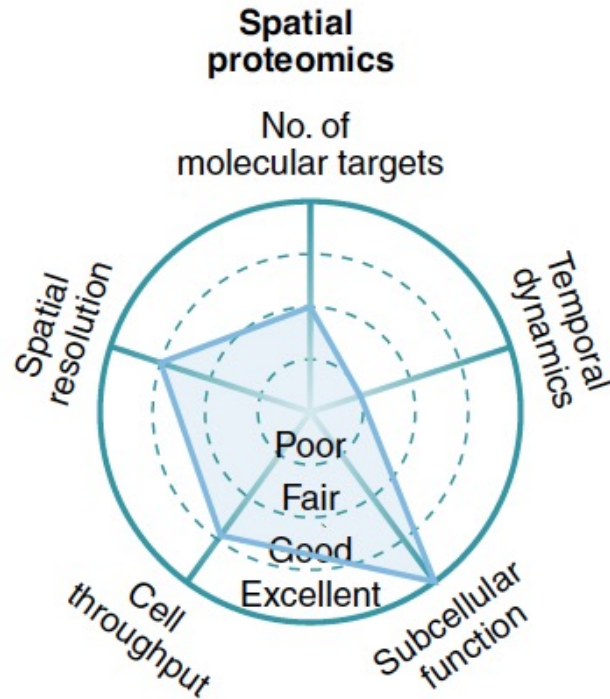


- Section 1. Technologies based on microdissected gene expression
- Section 2. *In situ* hybridization technologies
- Section 3. *In situ* sequencing technologies
- Section 4. *In situ* capturing technologies
- Section 5. *In silico* reconstruction of spatial data

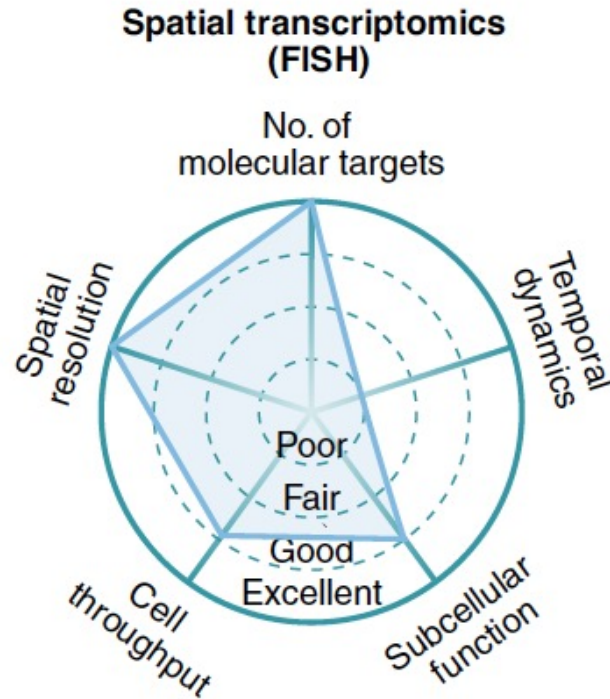


DBiT-seq  
ZipSeq  
Split-FISH  
seqSCOPE  
Pixel-Seq

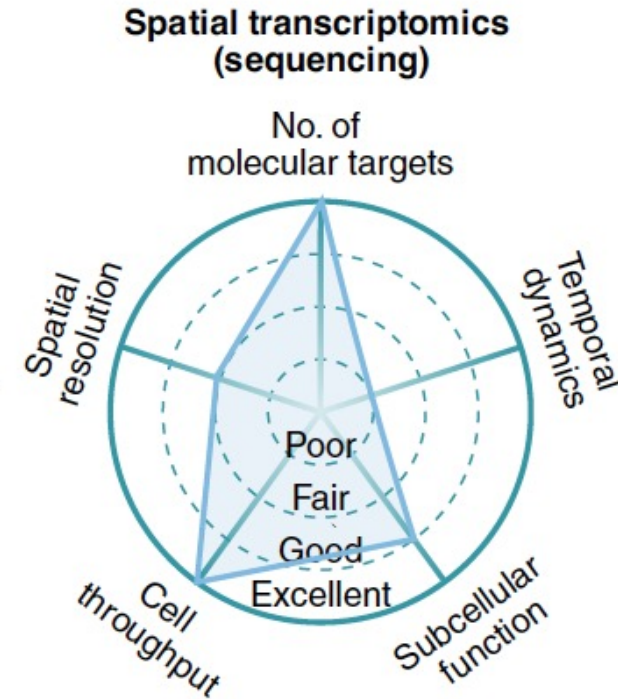
# Long history of spatial technology development



**seqIF / lunaphore**

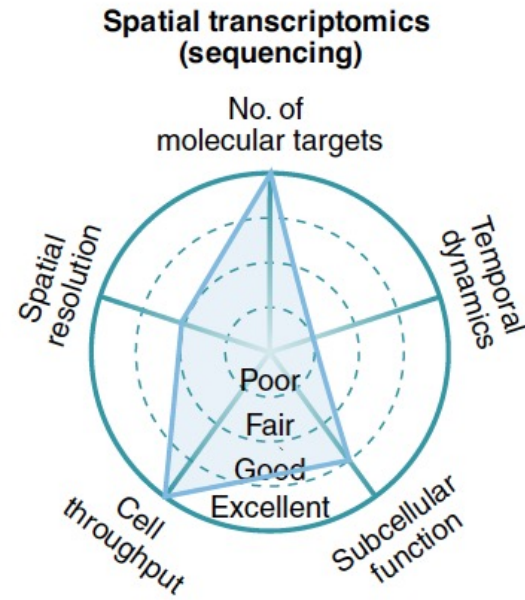
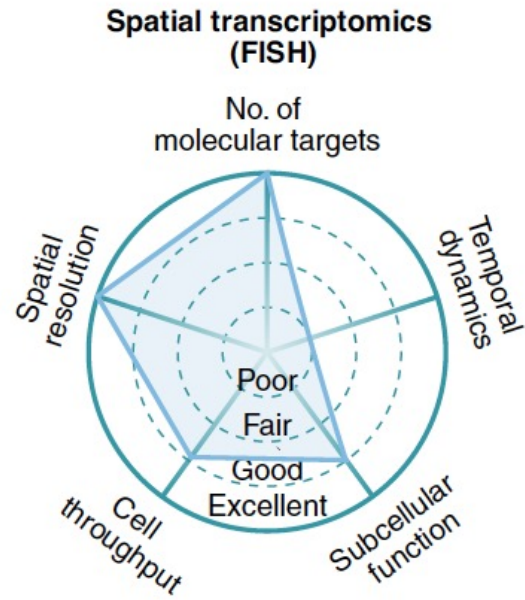
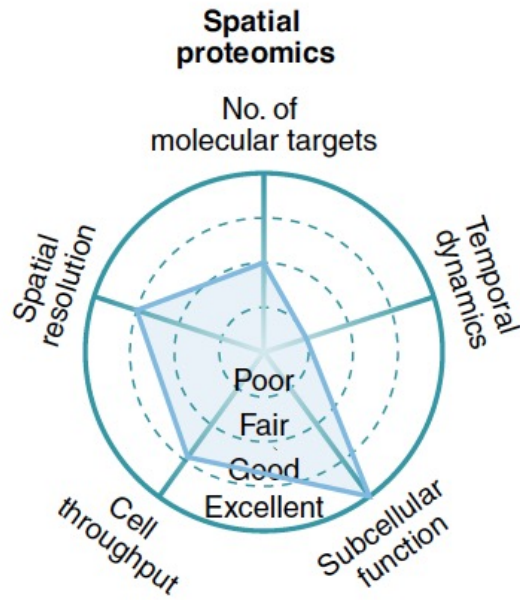


**seqFISH+**



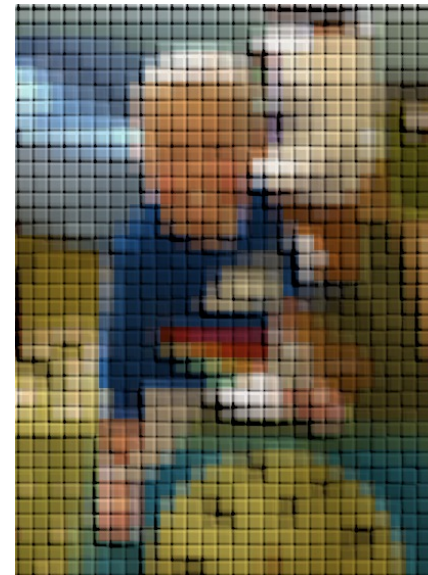
**Visium / 10X**

# Long history of spatial technology development



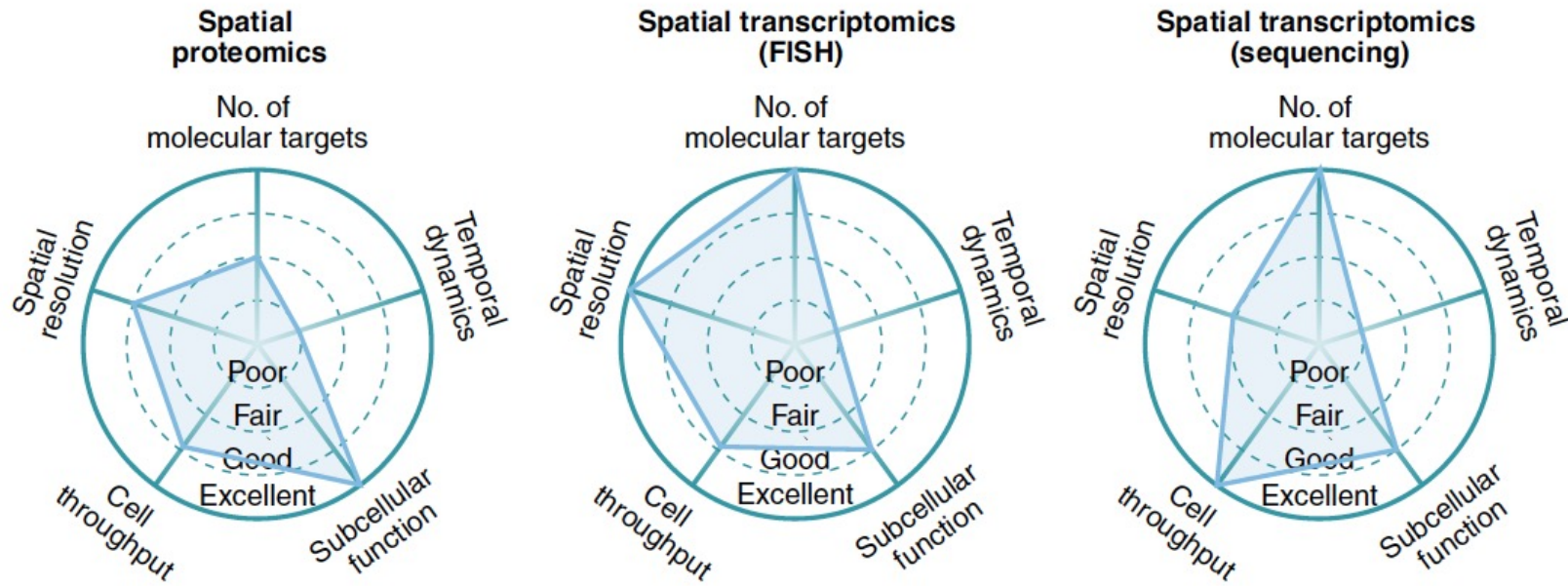
Spatial resolution?

What is the spatial resolution of my picture?





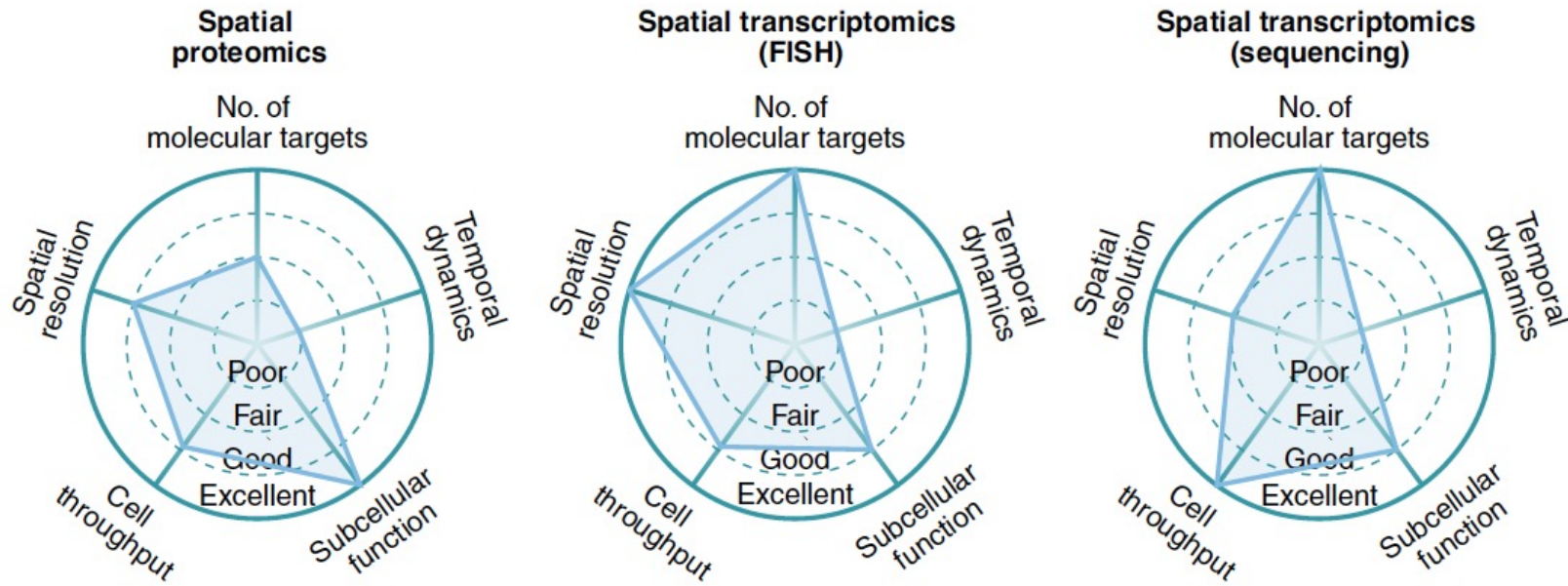
# Long history of spatial technology development



Number of molecular targets?

Unbiased (e.g. polyA enrichment) or targeted (e.g. antibodies or probes)?

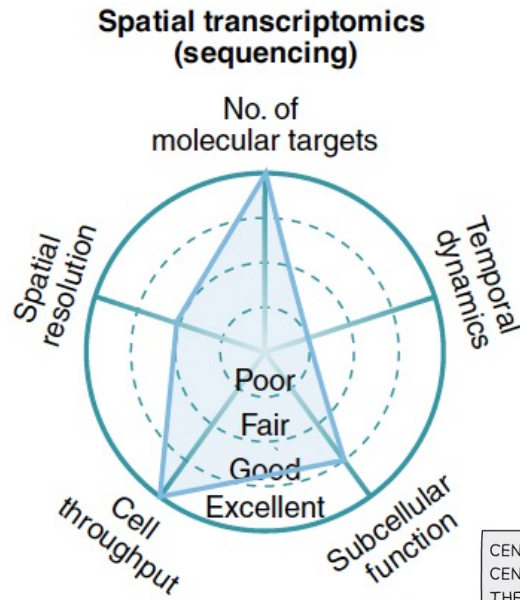
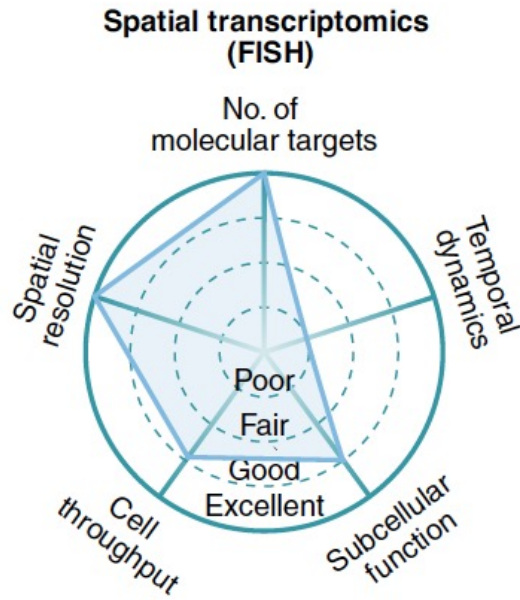
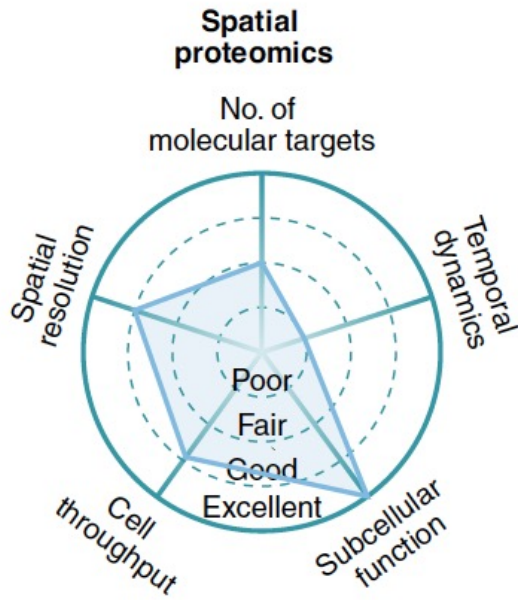
# Long history of spatial technology development



Temporal dynamics?

Live or fixed cells? Virtually all technologies use fixed cells (snap frozen or formalin fixed)

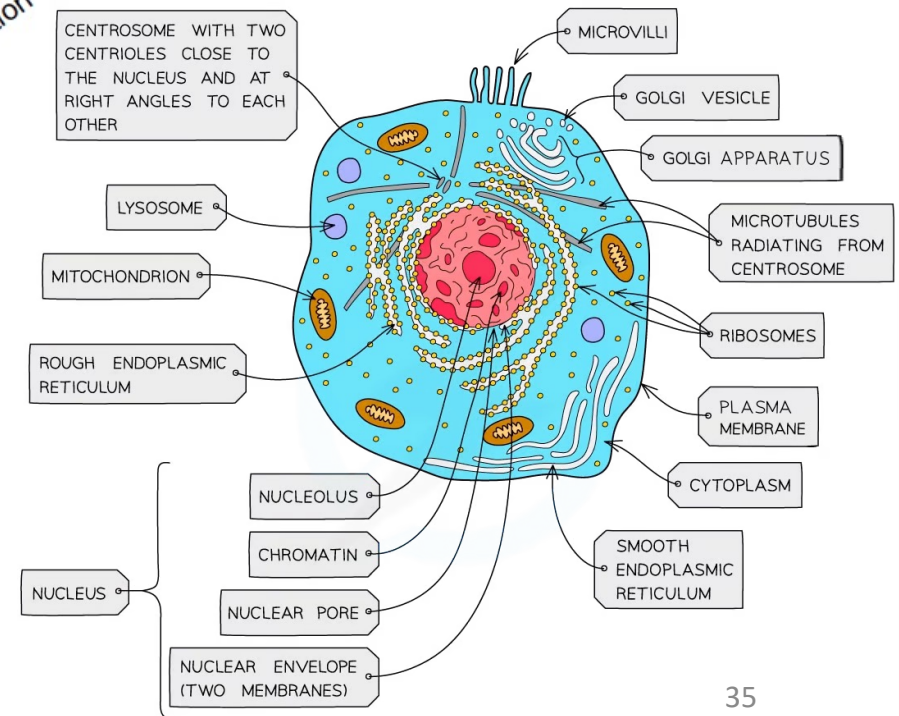
# Long history of spatial technology development



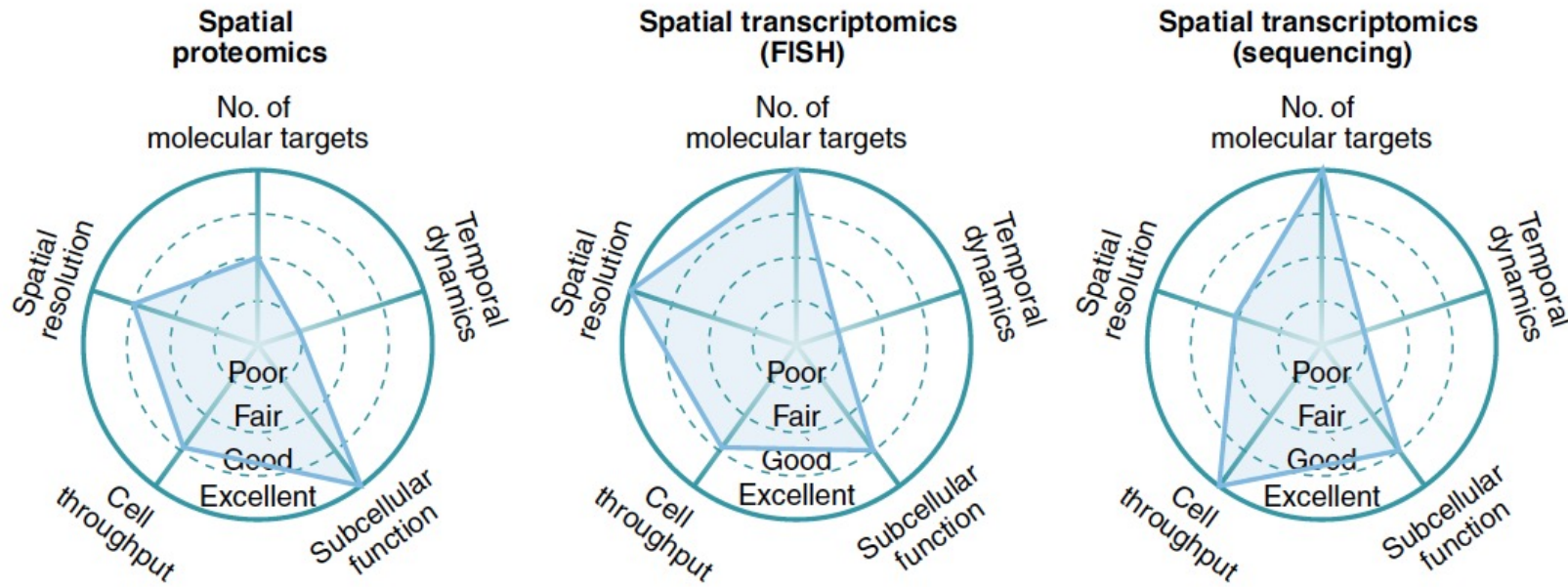
Subcellular function?

Can the data be used to infer subcellular function or differences?

*“The fluid inside a cell’s nucleus is 300 times more viscous than honey... ”, Zidovska et al*



# Long history of spatial technology development

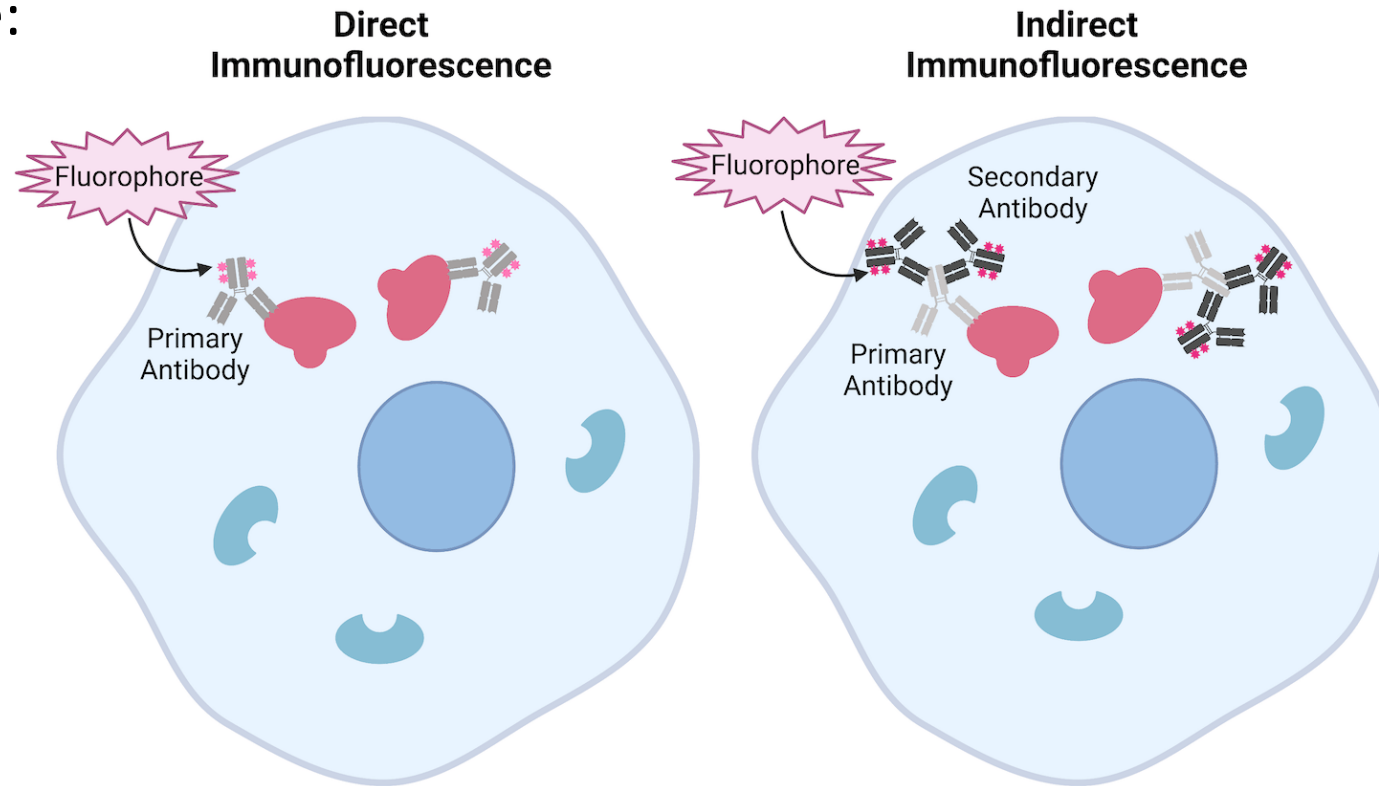


## Cell throughput?

How many cells can be analyzed? Very dependent on technology, but typically 100s of thousands and hence **10 to 100x times more** than scRNA-seq

# Spatial proteomics

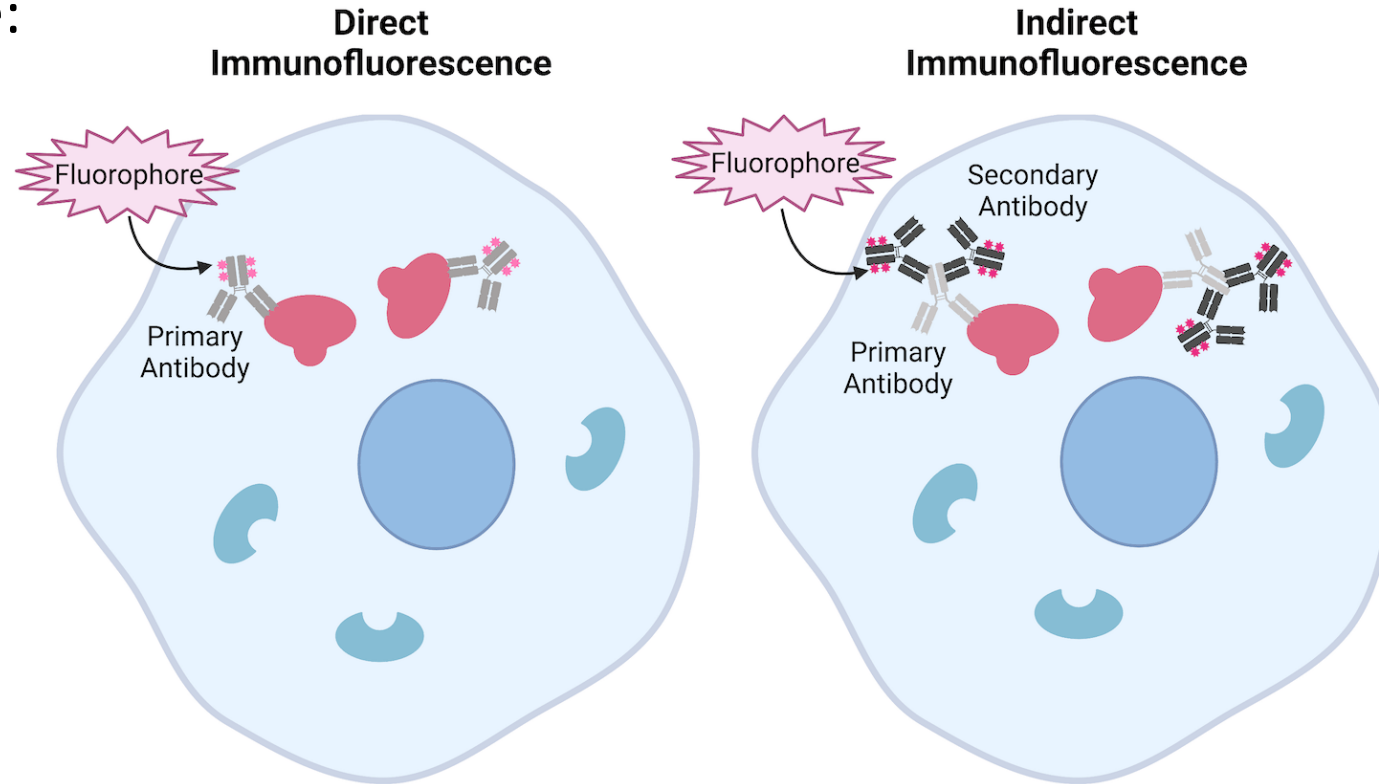
Immunofluorescence:



Problem: limited fluorophores and secondary antibodies

# Spatial proteomics

Immunofluorescence:

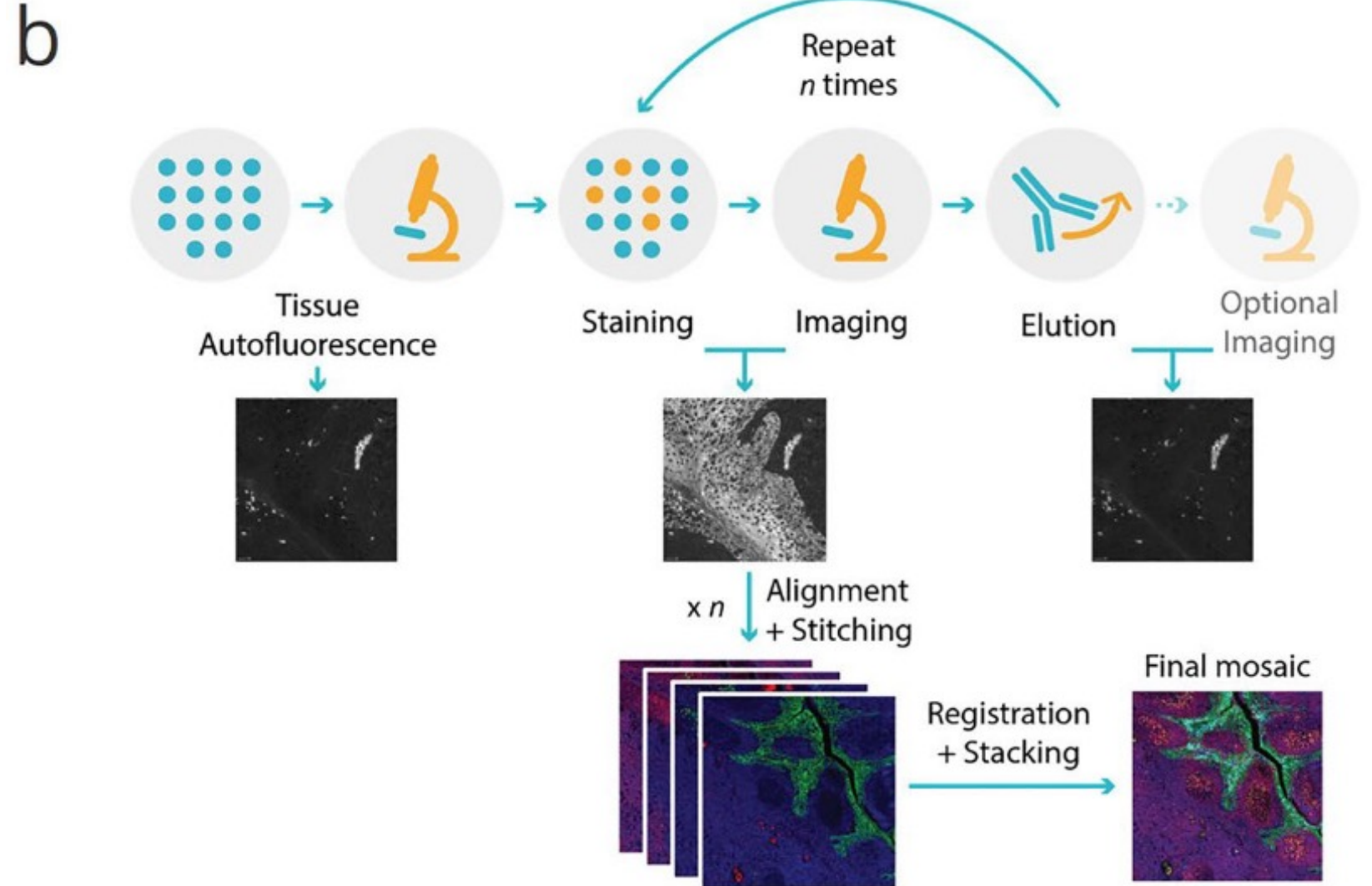
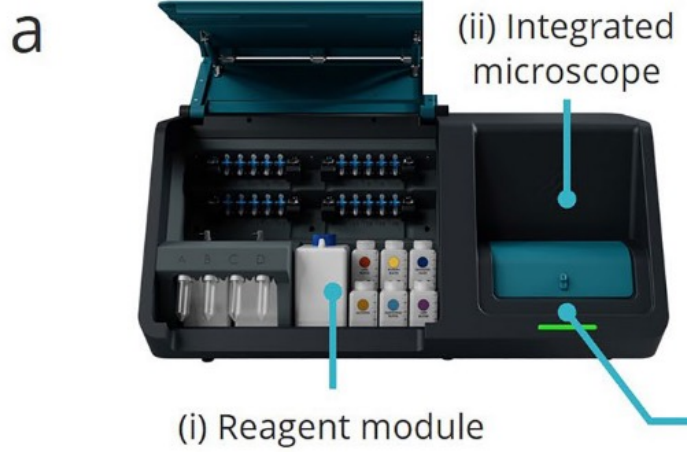


Problem: limited fluorophores and secondary antibodies

Solution: Multiplexing strategies

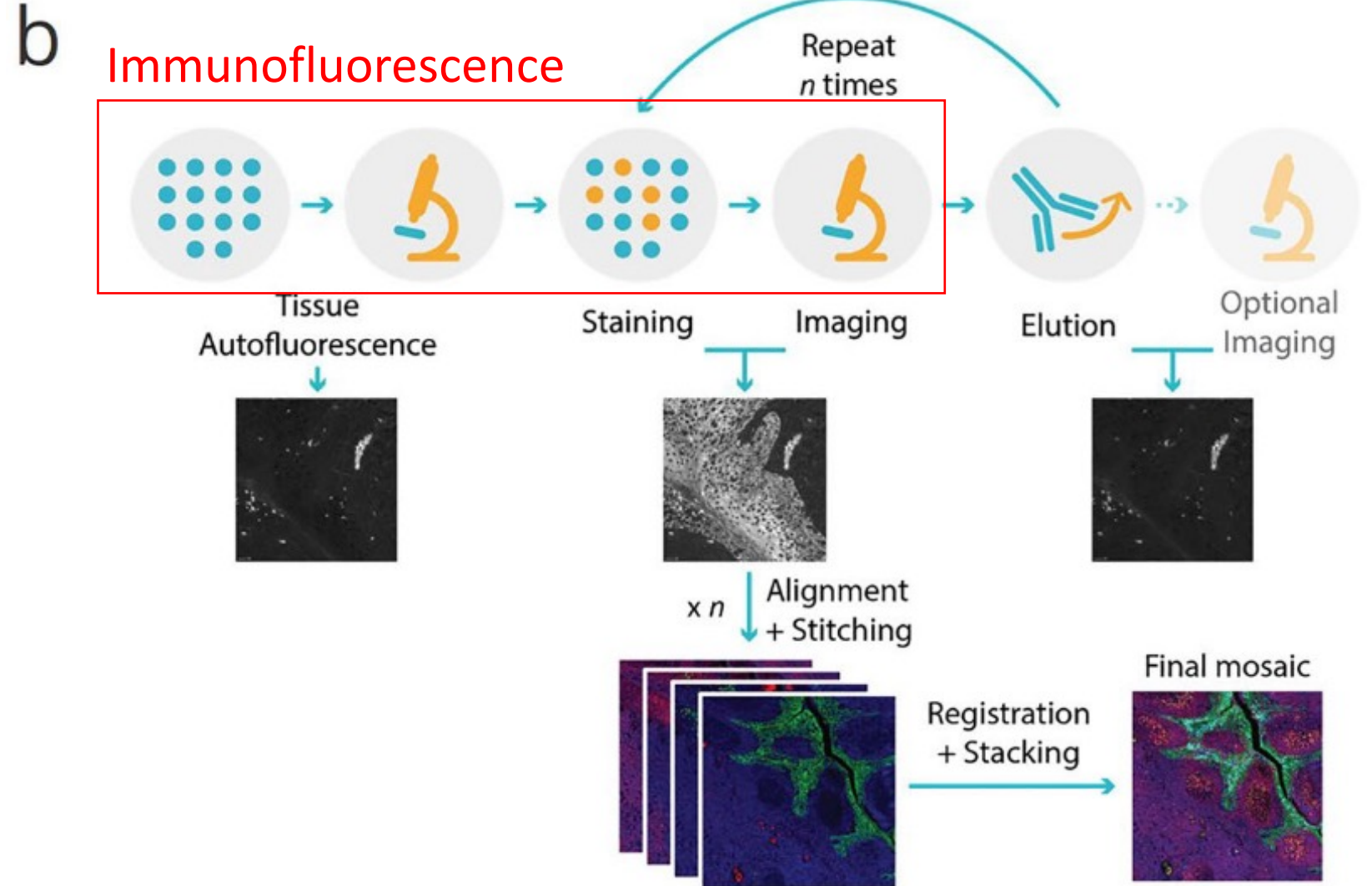
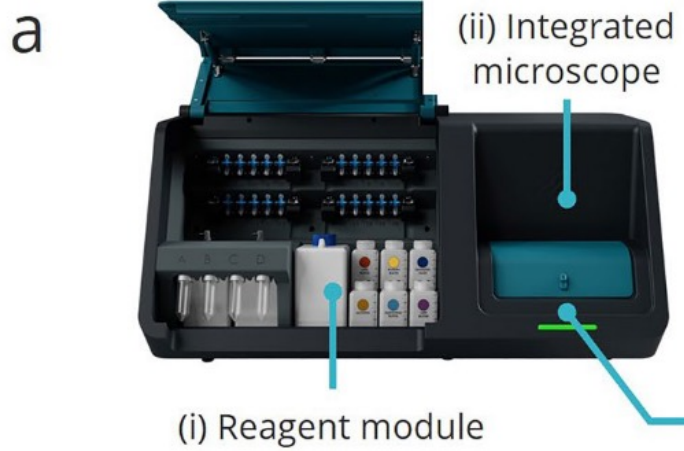
# Spatial proteomics

## seqIF / lunaphore



# Spatial proteomics

## seqIF / lunaphore

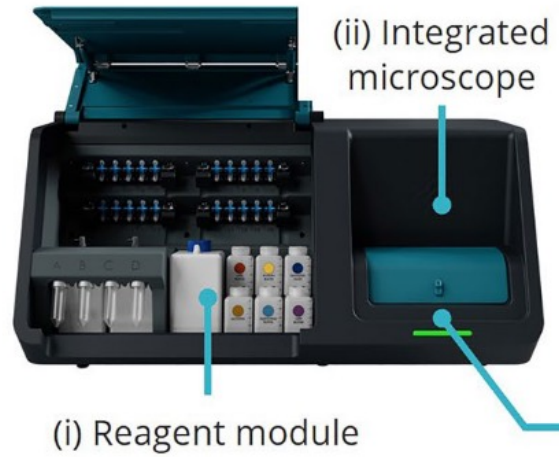




# Spatial proteomics

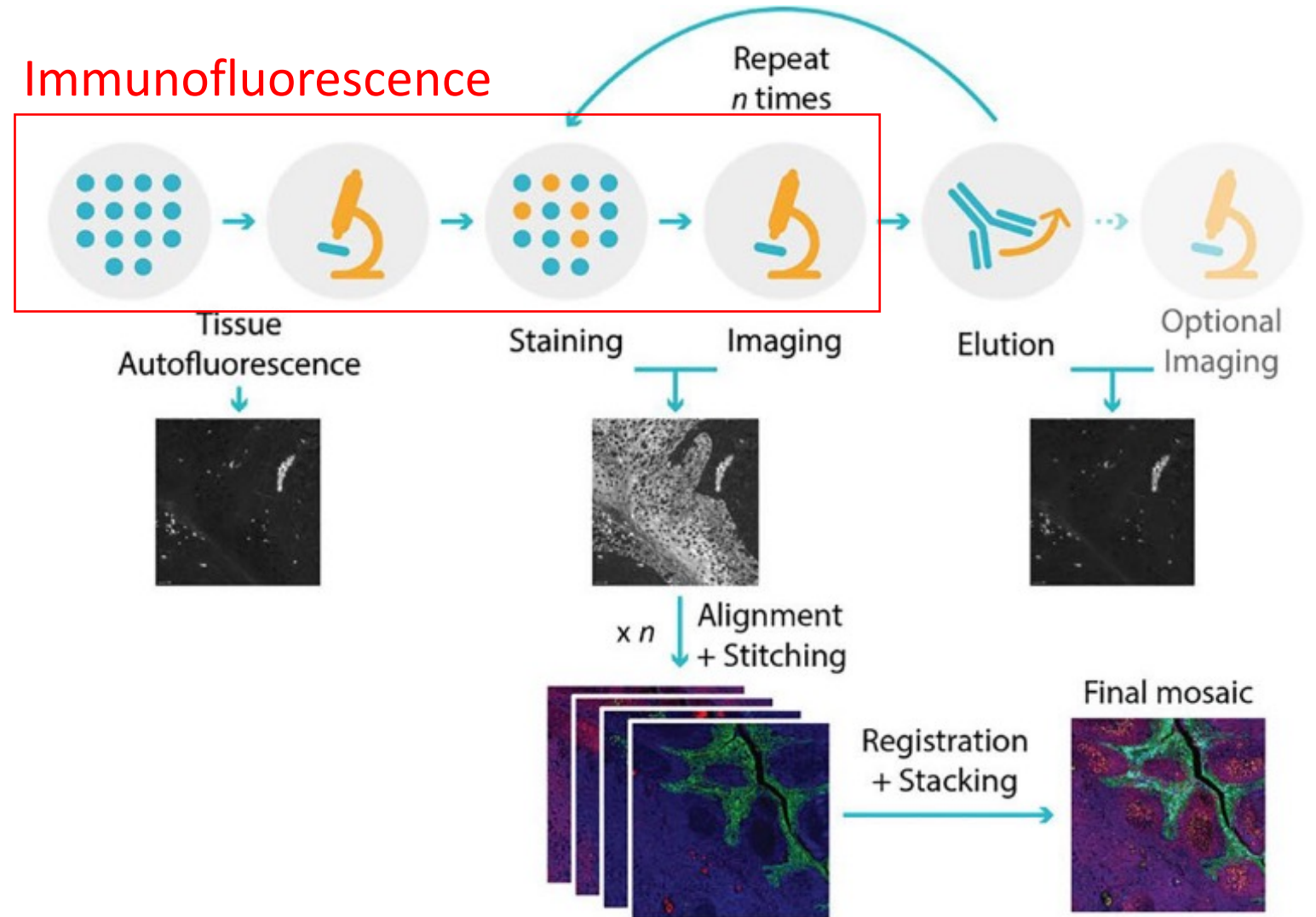
## seqIF / lunaphore

a

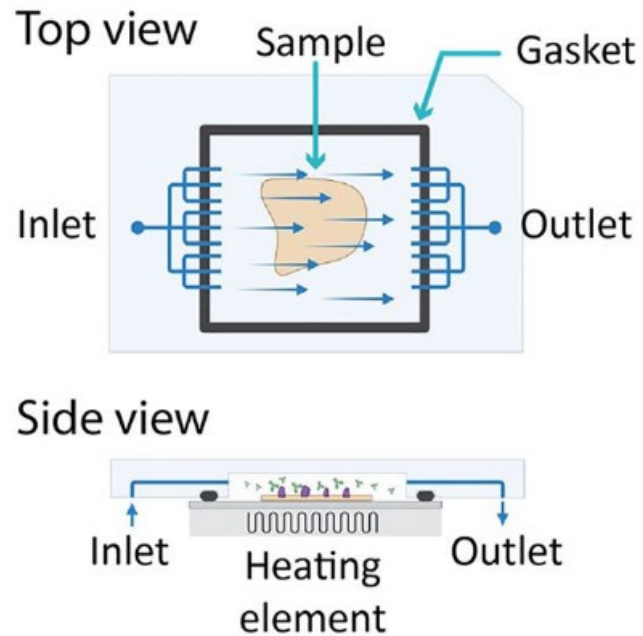


b

### Immunofluorescence

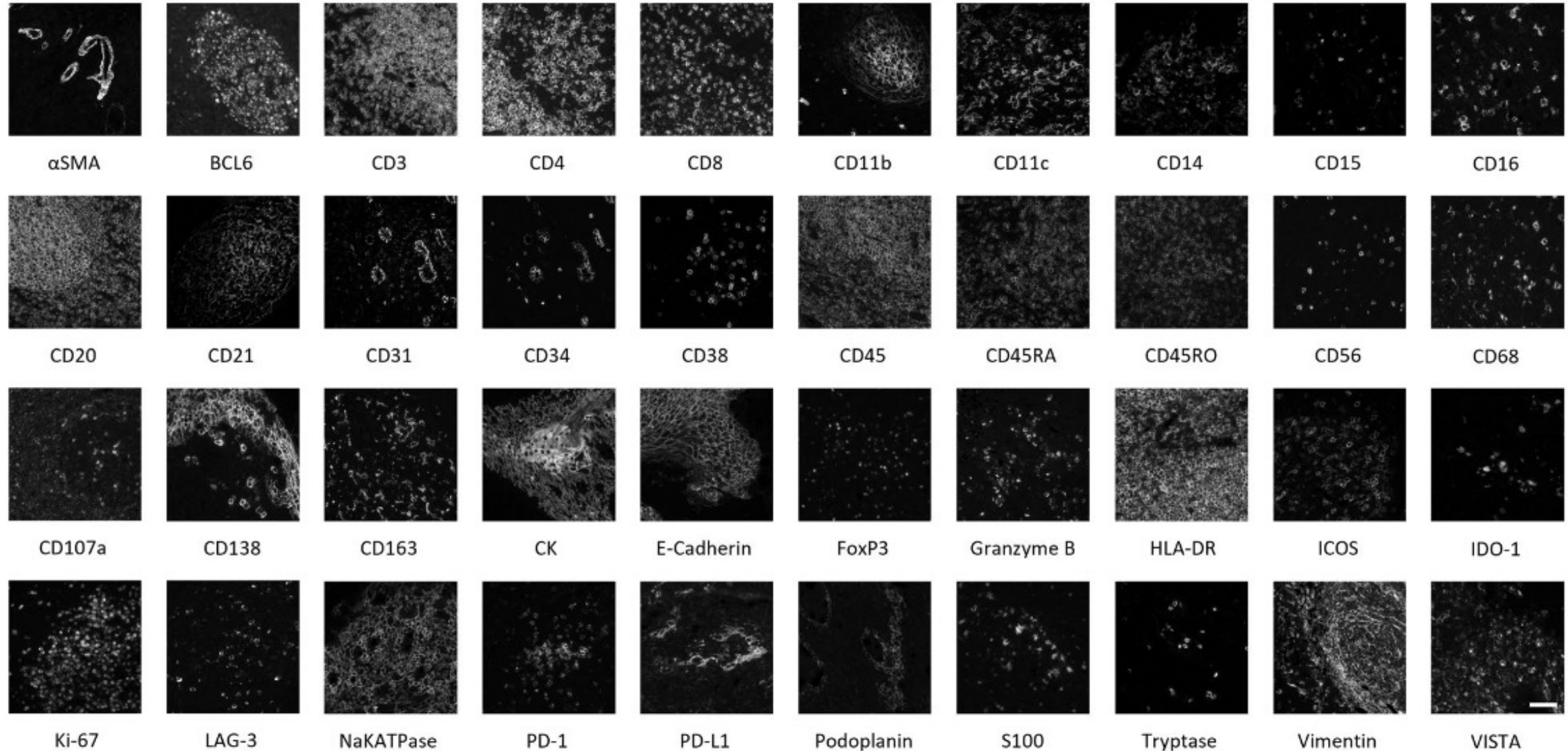


c



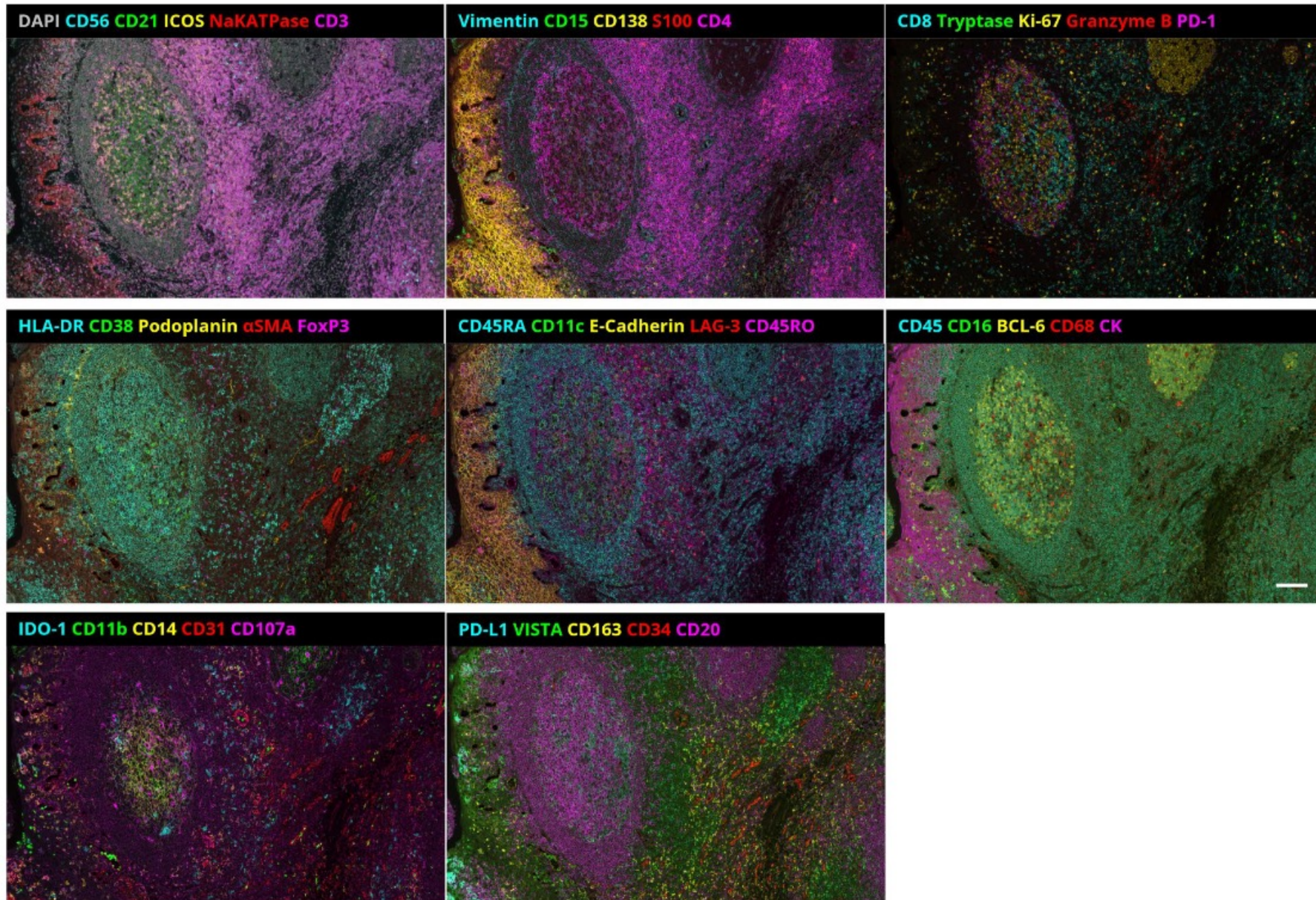
# Spatial proteomics

## seqIF / lunaphore



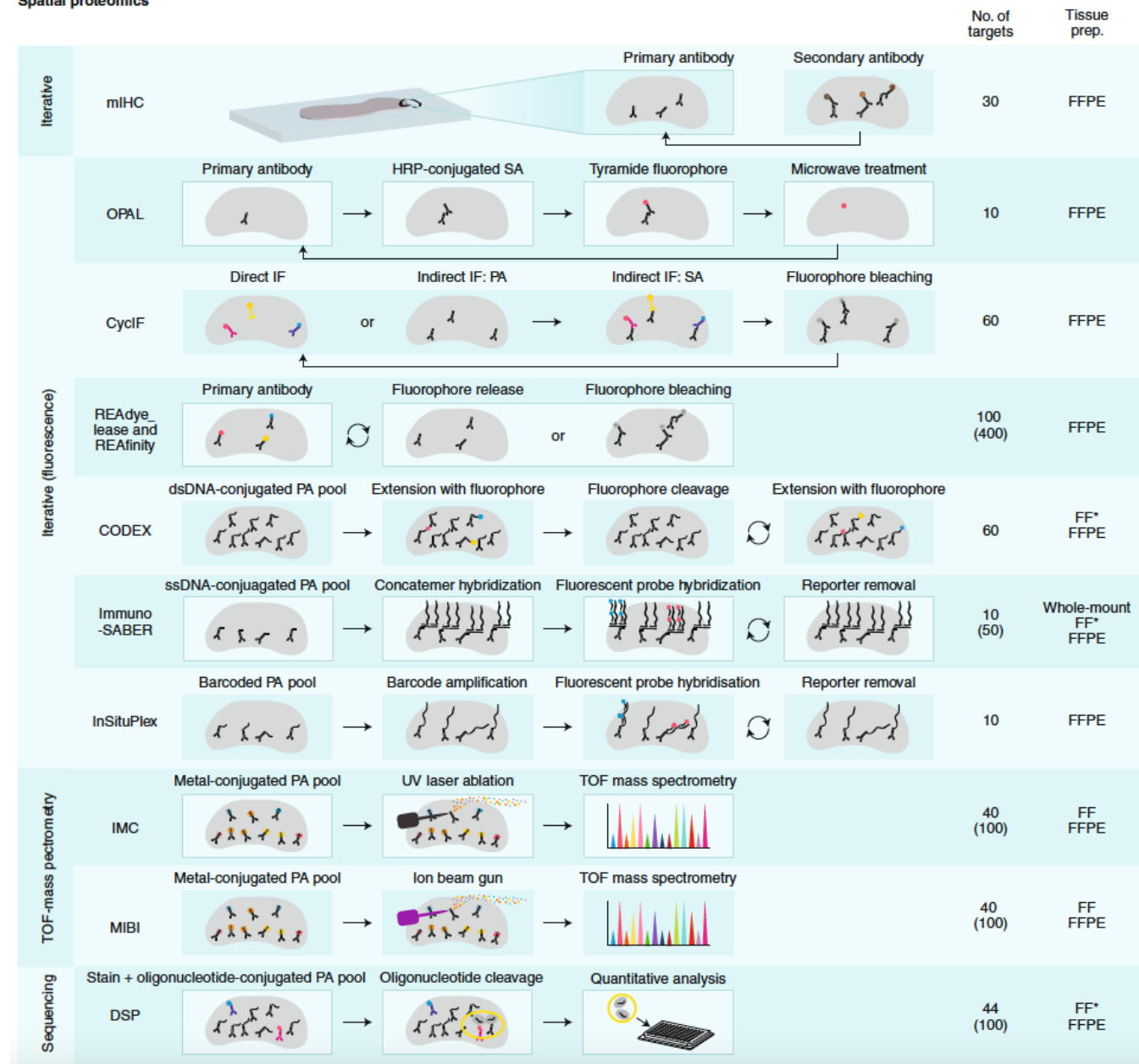
# Spatial proteomics

## seqIF / lunaphore



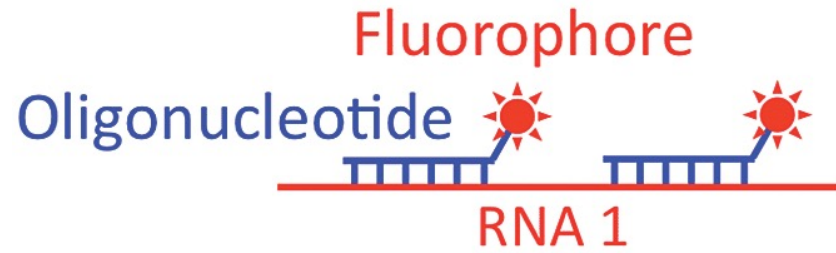
# Spatial proteomics

## Spatial proteomics

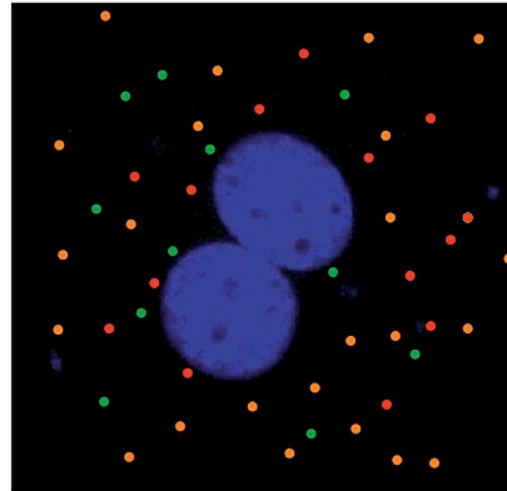


# Spatial transcriptomics (FISH)

## 1. Target Hybridization

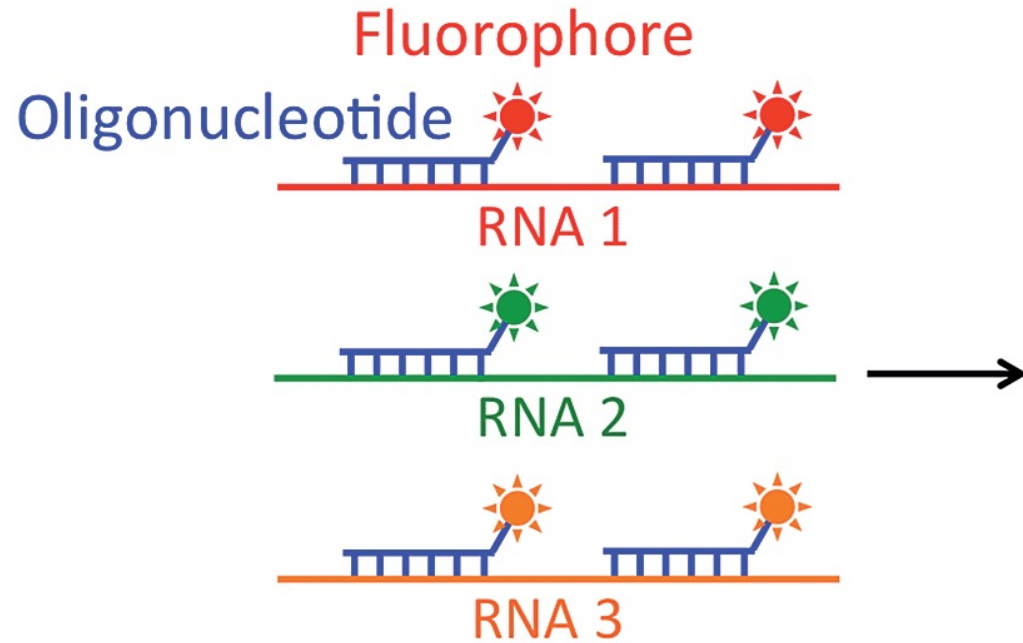


## 2. Fluorescence Imaging

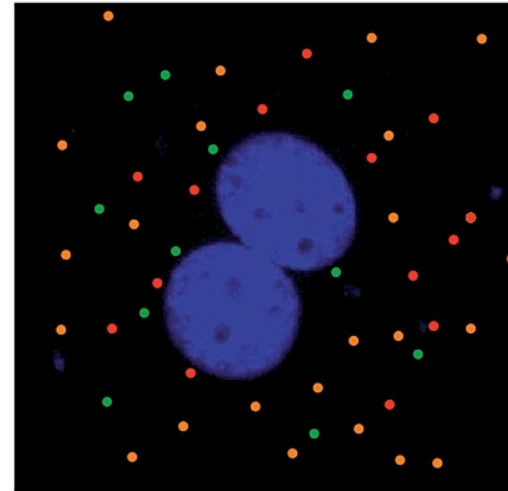


# Spatial transcriptomics (FISH)

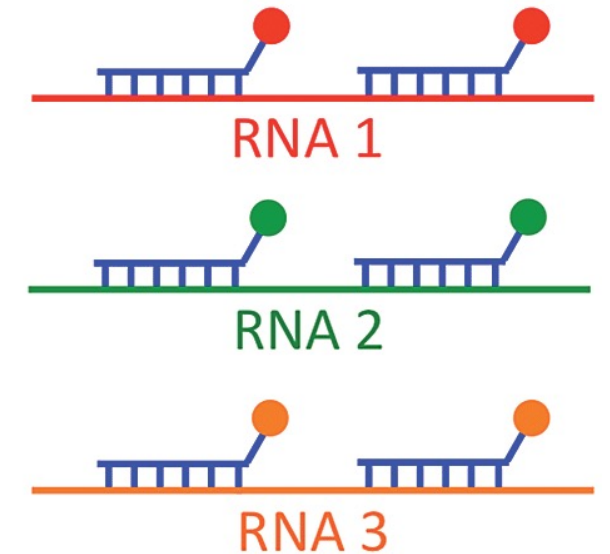
## 1. Target Hybridization



## 2. Fluorescence Imaging



## 3. Photobleaching

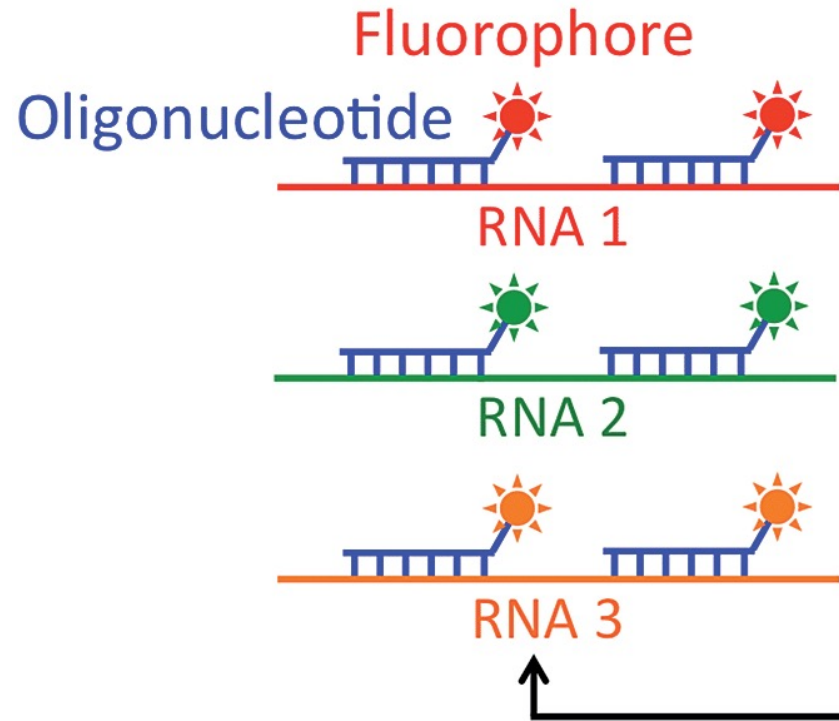


Problem: limited fluorophores

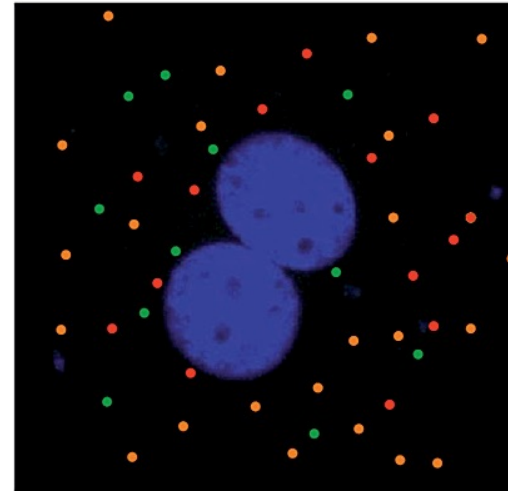
Solution: Multiplexing strategies

# Spatial transcriptomics (FISH)

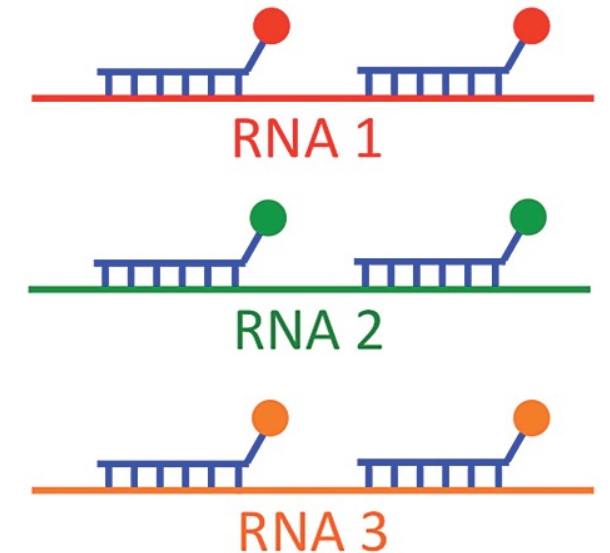
## 1. Target Hybridization



## 2. Fluorescence Imaging



## 3. Photobleaching



Repeat for Different RNA

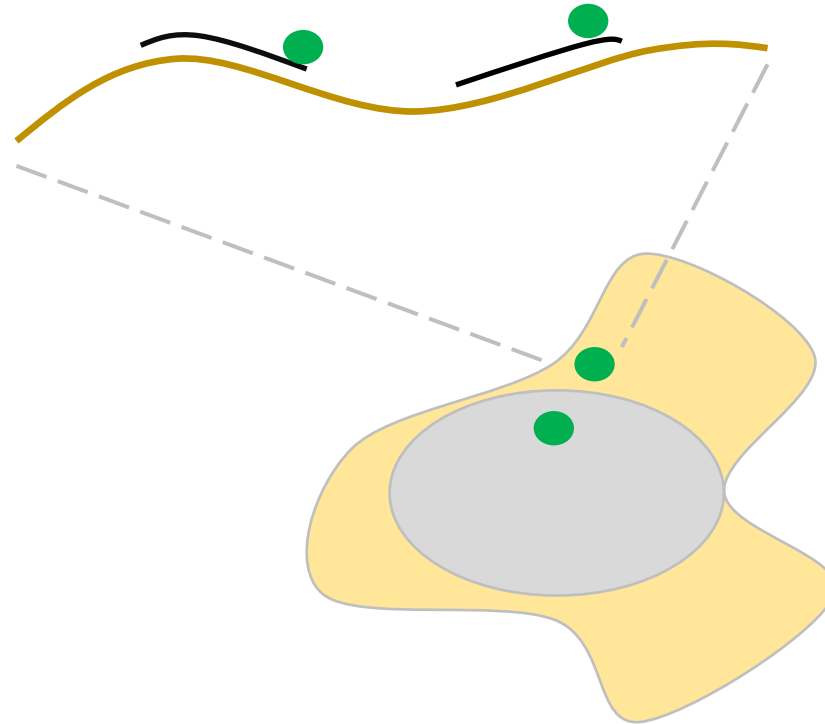
Problem: limited fluorophores

Solution: Multiplexing strategies

# Spatial transcriptomics (FISH) **seqFISH+**

*in situ* hybridizations

- seqFISH(+)
- merFISH
- osmFISH
- smFISH
- splitFISH
- ...

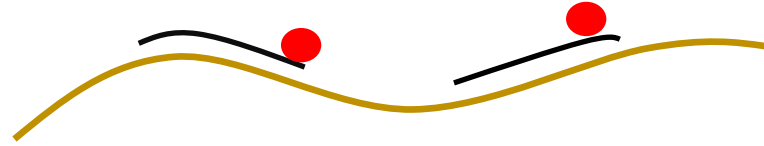


transcript 1 = ●

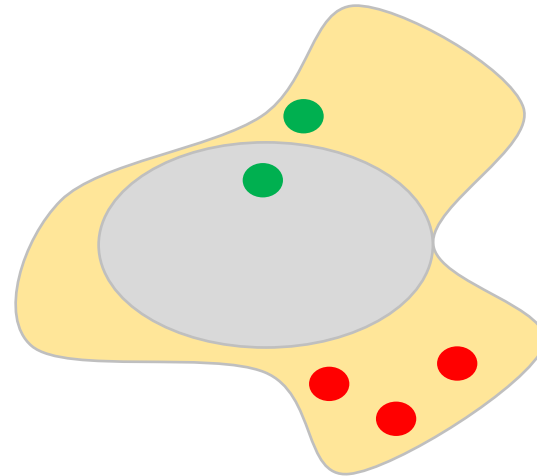


# Spatial transcriptomics (FISH) **seqFISH+**

*in situ* hybridizations

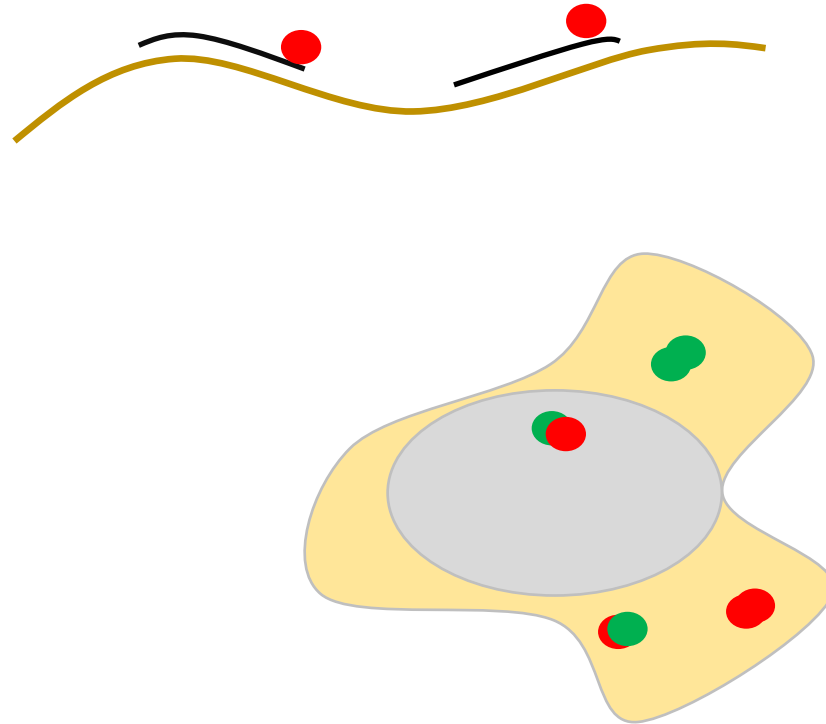


transcript 1 = ●  
transcript 2 = ●



# Spatial transcriptomics (FISH) **seqFISH+**

**Sequential *in situ***  
hybridizations

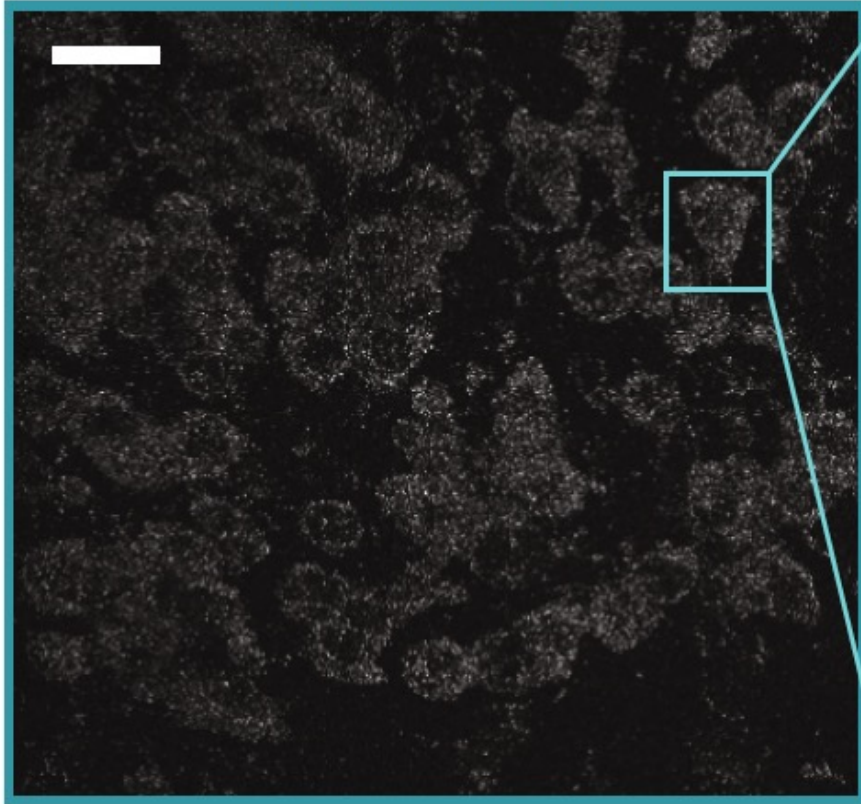


transcript 1 = ● ●  
transcript 2 = ● ●  
transcript 3 = ● ●  
transcript 4 = ● ●

# Spatial transcriptomics (FISH) **seqFISH+**

## Spatial transcriptomics (FISH)

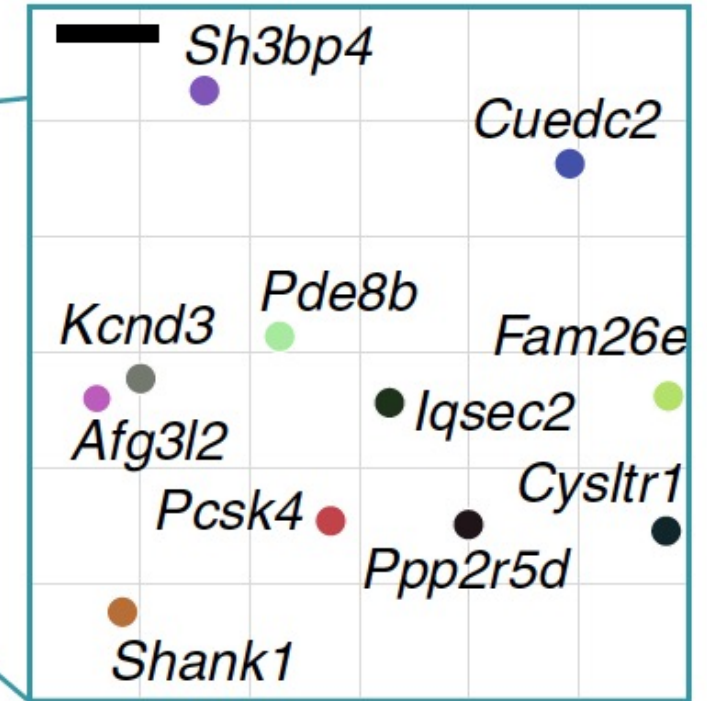
Tissue section



Single-cell mRNA



647 nm



# Spatial transcriptomics (FISH)

## Box 1 | Calculating multiplexing capacity

The list below includes the equations used to calculate the multiplexing capacity of various clonal barcoding, smFISH and sequencing-based methods. Here  $F$  is the number of fluorophores,  $N$  is the number of probe-binding positions,  $H$  is the number of hybridization rounds and  $B$  is the number of nucleotide bases in the readout sequence.

Confetti: 4 (1 allele), up to 10 (2 alleles)

LeGO:  $2^F - 1$

Spectral barcoding:  $\frac{F!}{(F-N)!N!}$

Spatial barcoding:  $\frac{F!}{(F-N)!2}$

osmFISH:  $F \times H$

MERFISH:  $2^H - 1$









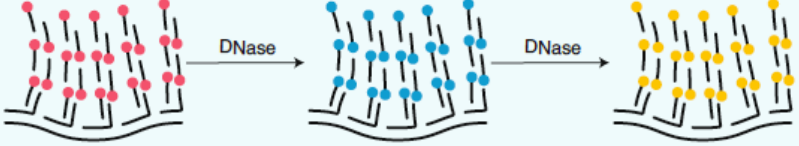



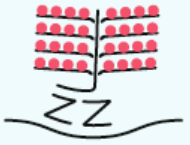
seqFISH:  $F^H$

ISS:  $4^B$

FISSEQ:  $4^B$

# Spatial transcriptomics (FISH)

## Spatial transcriptomics (FISH)

	Barcode	No. of targets	Tissue prep.
 <p>smFISH</p>	NA	<10	FF FFPE
 <p>Spectral barcoding</p>		32 (792)	NA
 <p>Spatial barcoding</p>		<10	NA
	Round 1	Round 2	Round <i>n</i>
 <p>osmFISH</p>	NA	33	FF
 <p>MERFISH</p>		10,000	FF
 <p>seqFISH</p>		249	FF
 <p>seqFISH+</p>		10,000	FF
 <p>RNAscope</p>	NA	12	FF FFPE

# Spatial transcriptomics (sequencing)

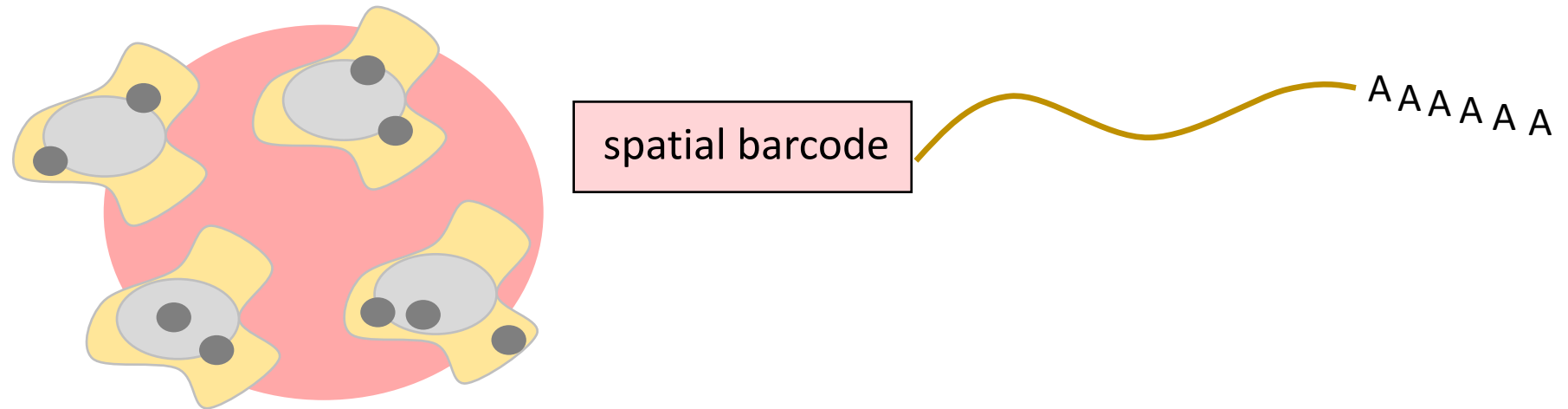
*in situ* hybridizations



*in situ* sequencing



*in situ* capture



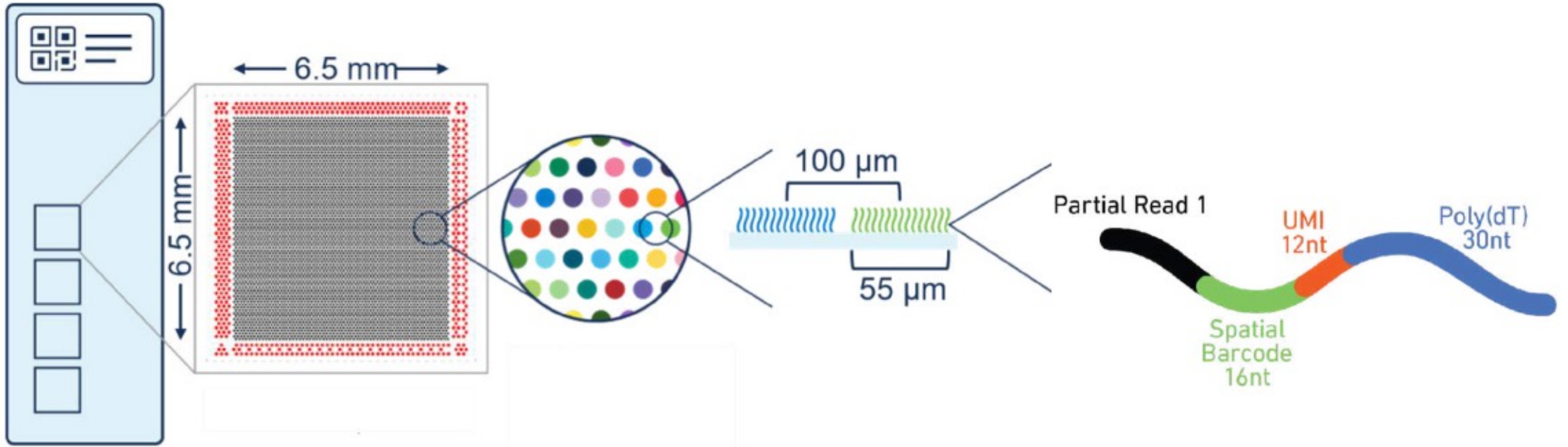
# Spatial transcriptomics (sequencing)

**Visium / 10X**

Visium Spatial Gene  
Expression Slide

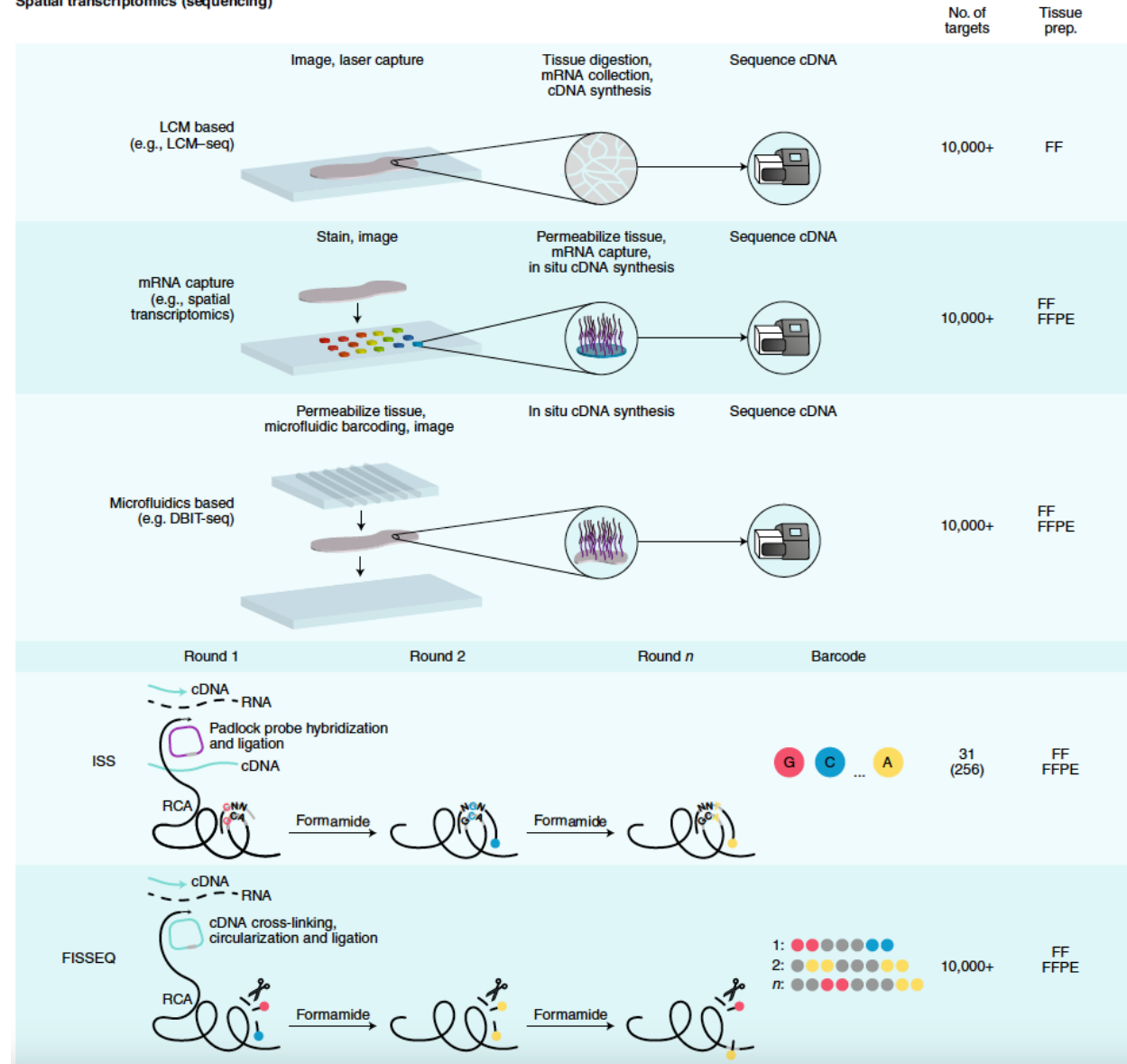
Capture Area with  
~5000 Barcoded Spots

Visium Gene Expression  
Barcoded Spots



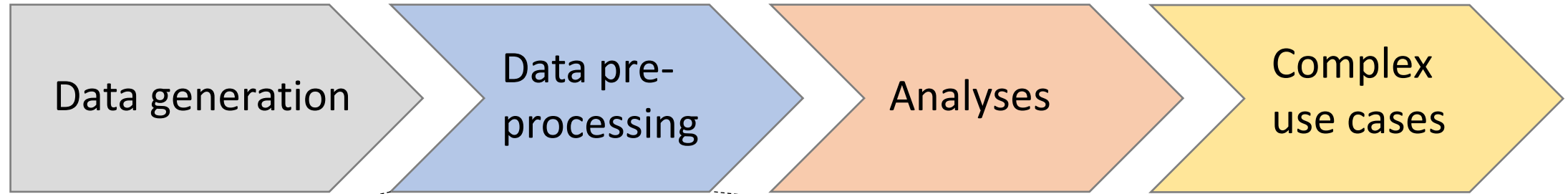
# Spatial transcriptomics (sequencing)

Spatial transcriptomics (sequencing)





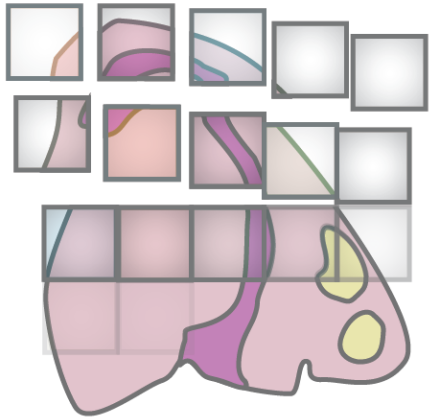
# The different steps in spatial omics research



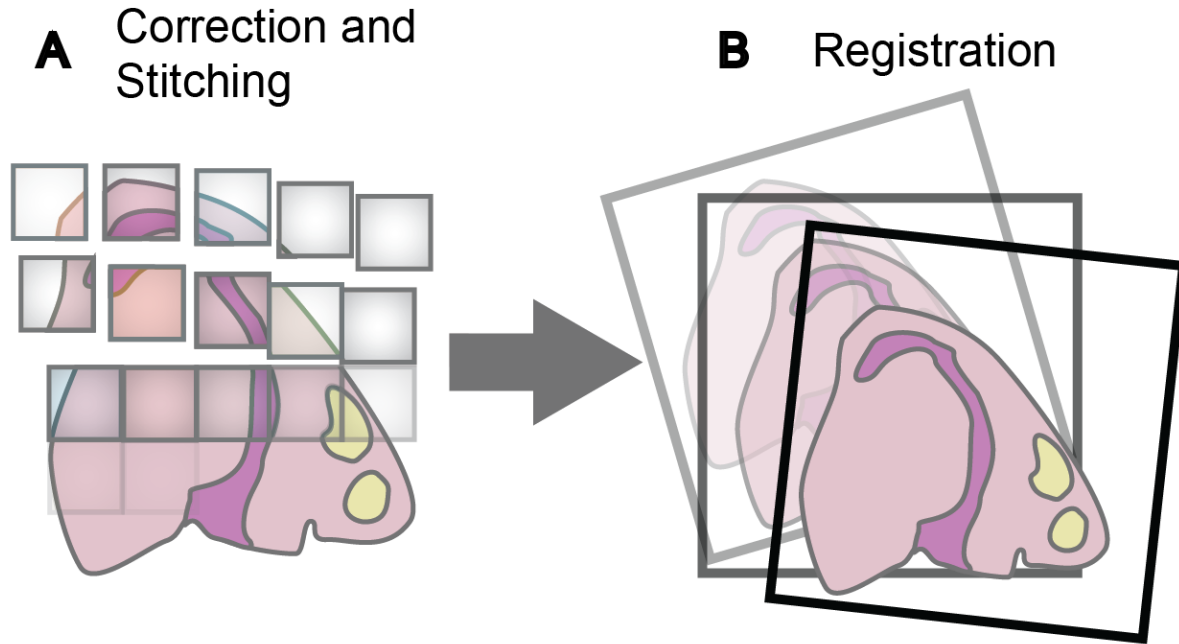
- Image processing
- From raw signal to usable data

# Essential pre-processing steps: image stitching

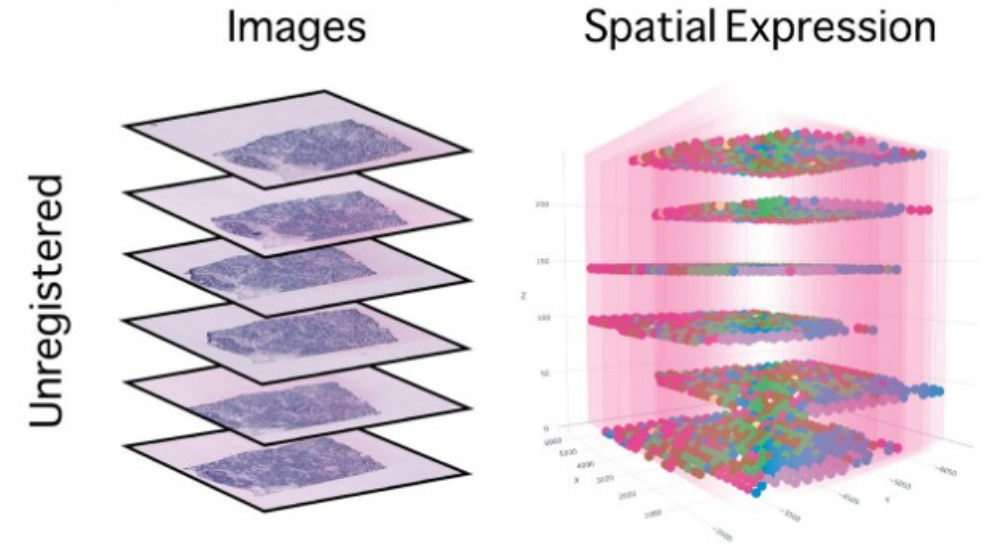
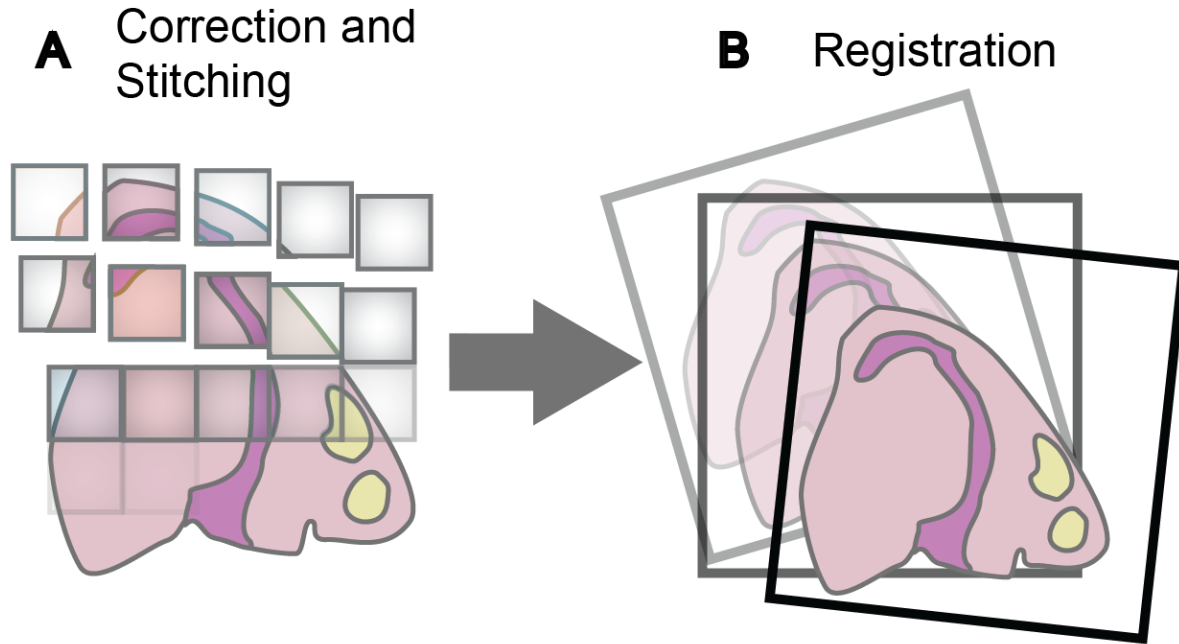
## A Correction and Stitching



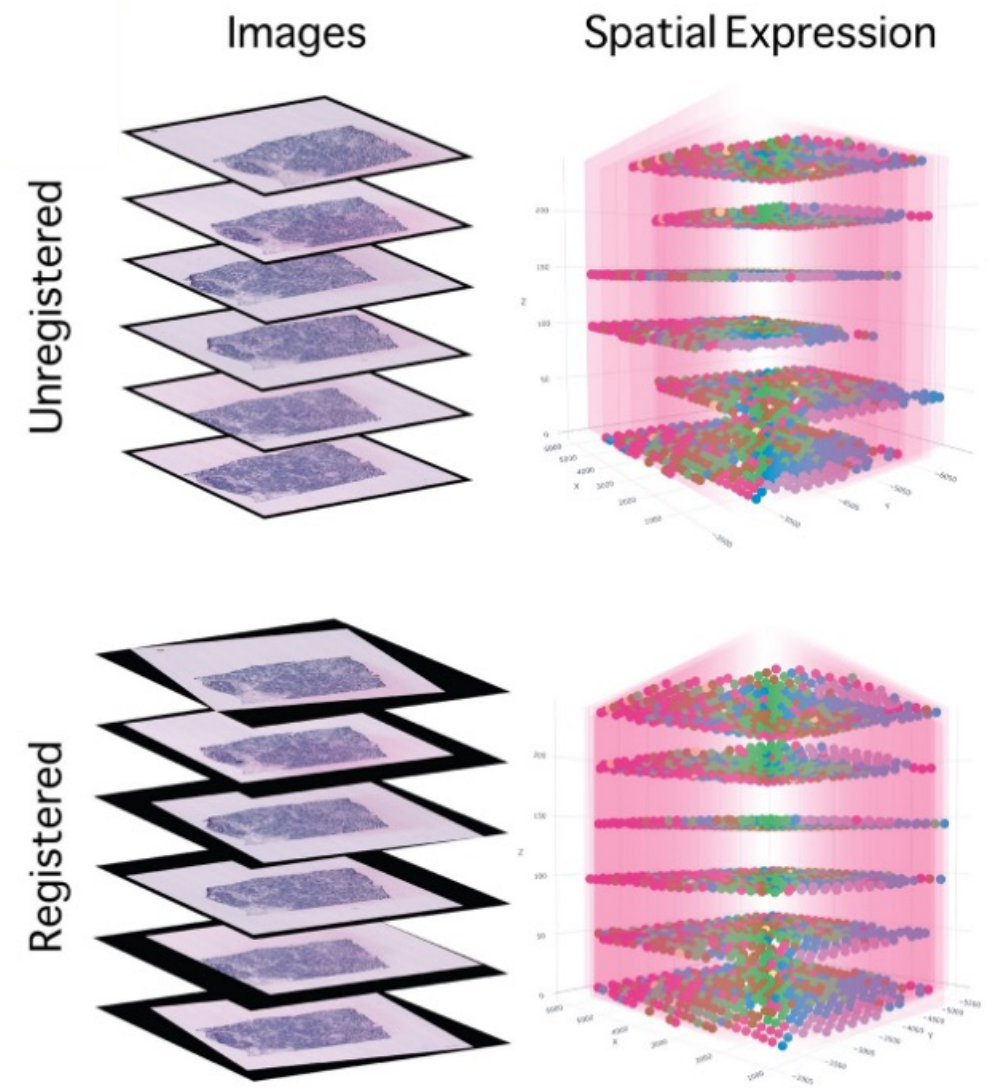
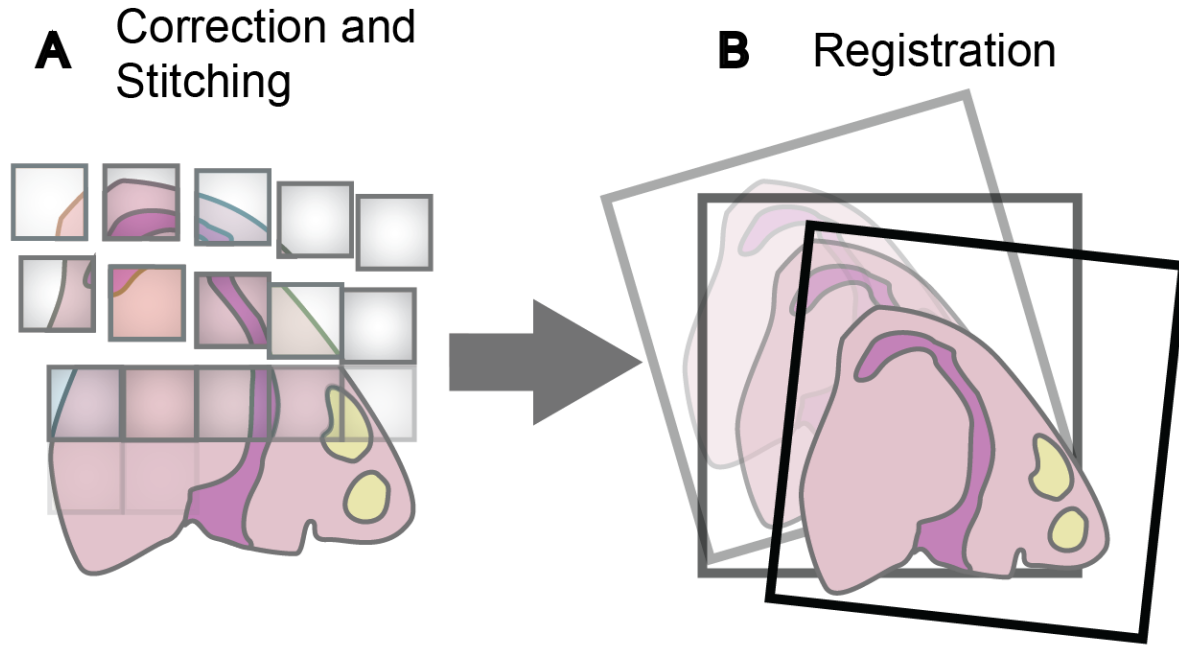
# Essential pre-processing steps: image co-registration



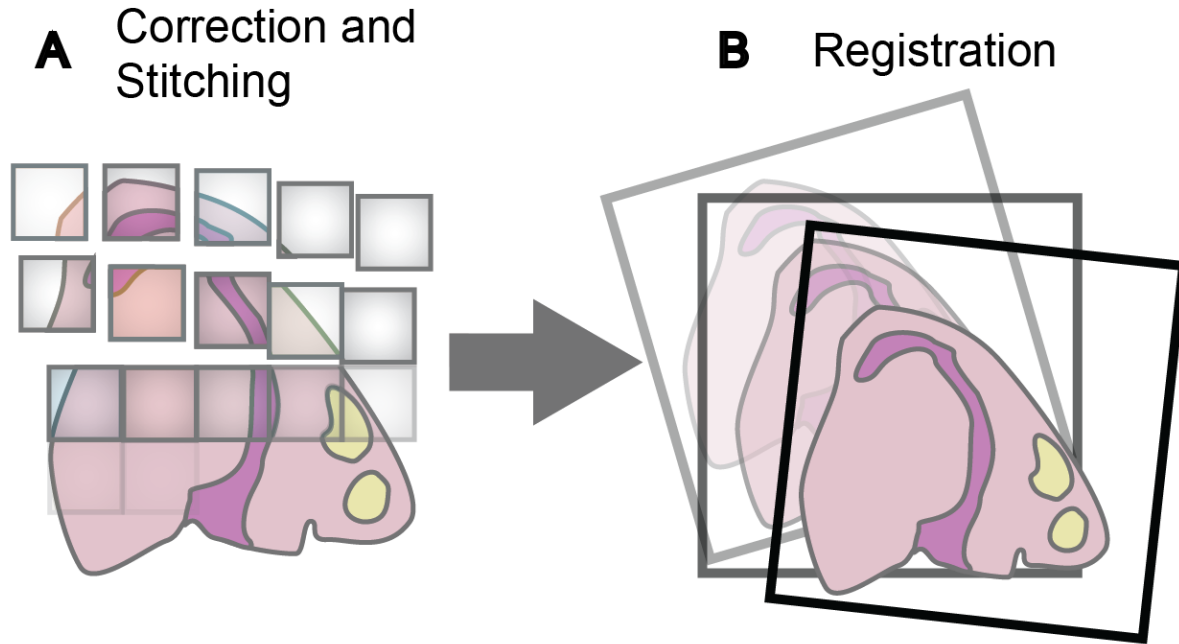
# Essential pre-processing steps: image co-registration



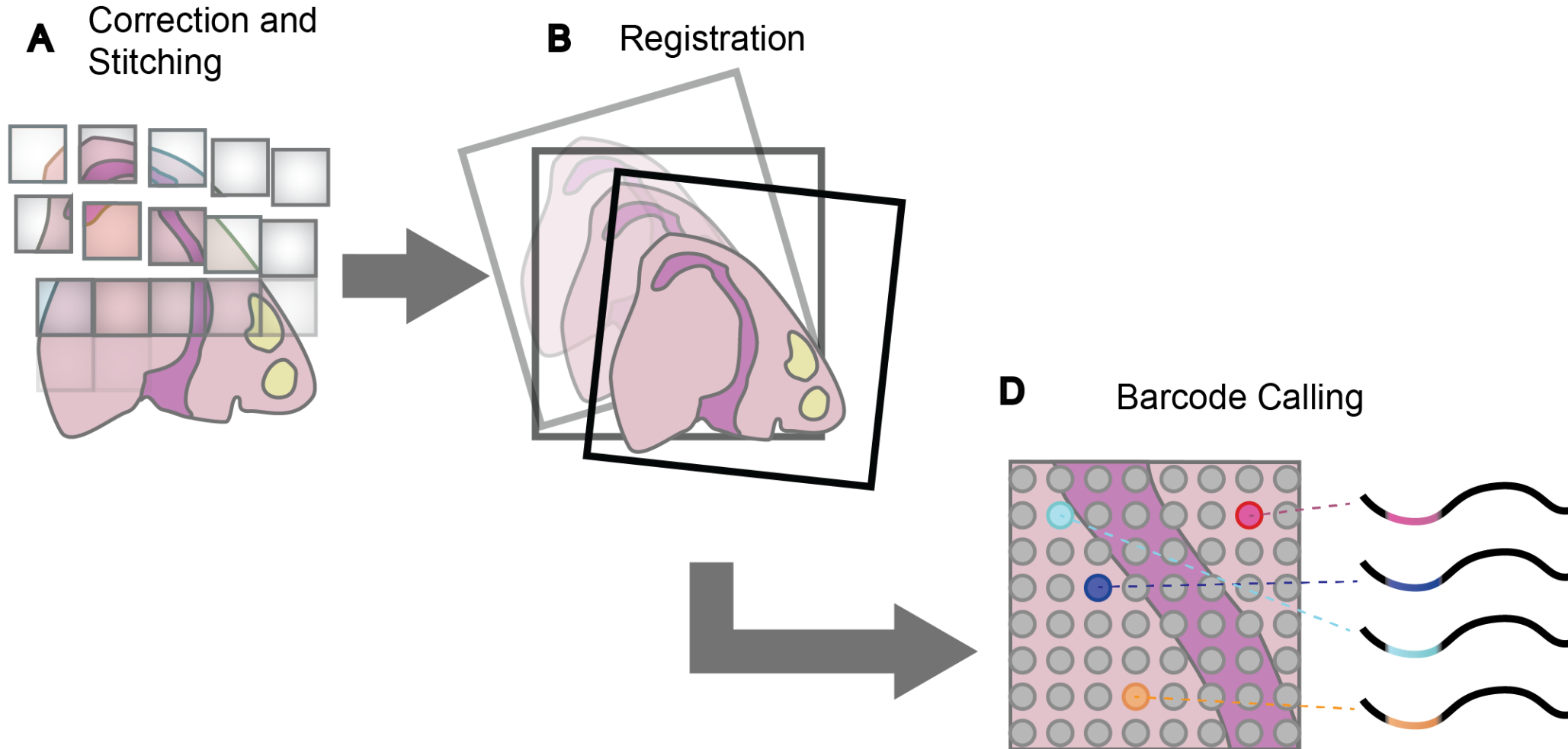
# Essential pre-processing steps: image co-registration



# Essential pre-processing steps:

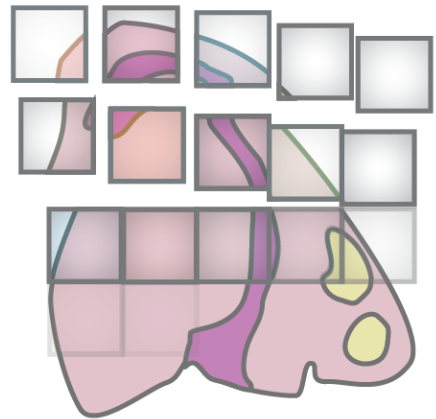


# Essential pre-processing steps: barcode calling

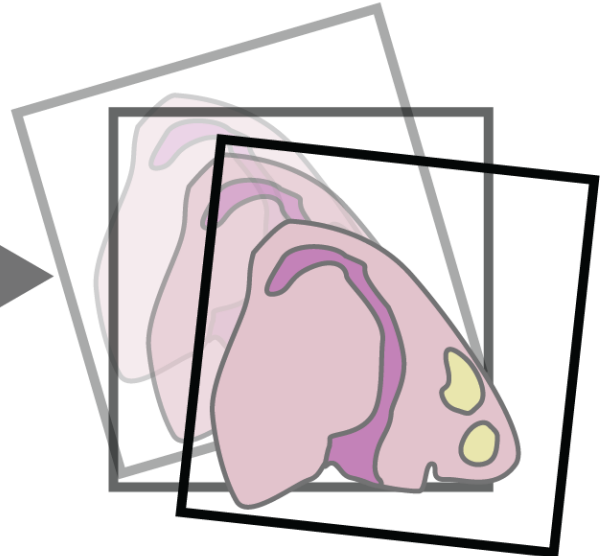


# Essential pre-processing steps: segmentation

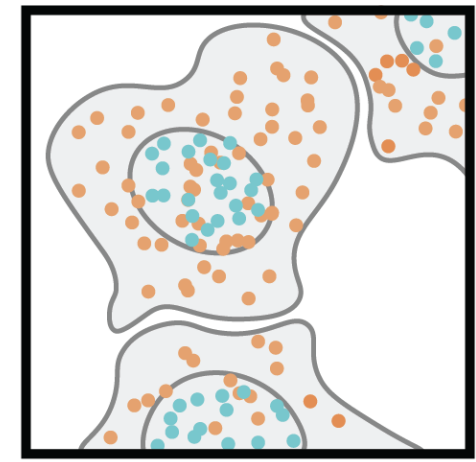
**A** Correction and  
Stitching



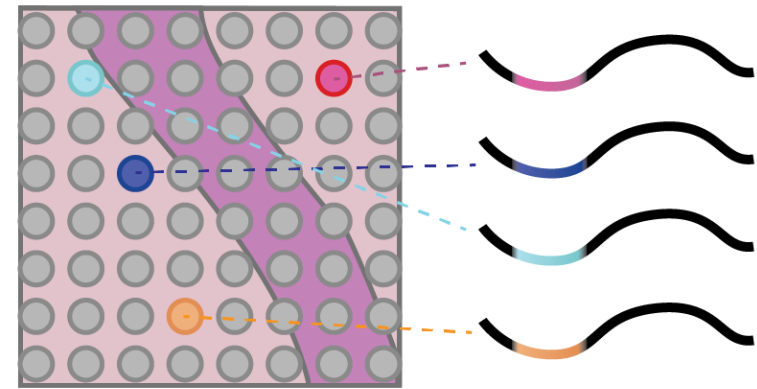
**B** Registration



**C** Segmentation



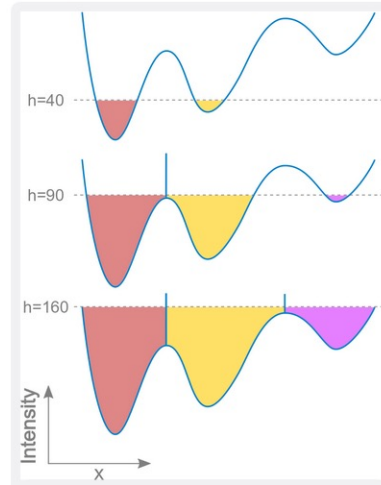
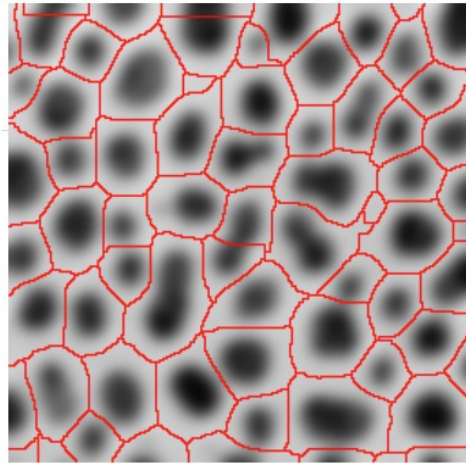
**D** Barcode Calling





# Essential pre-processing steps: segmentation

Classic approaches



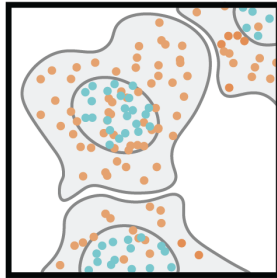
Fiji



QuPath

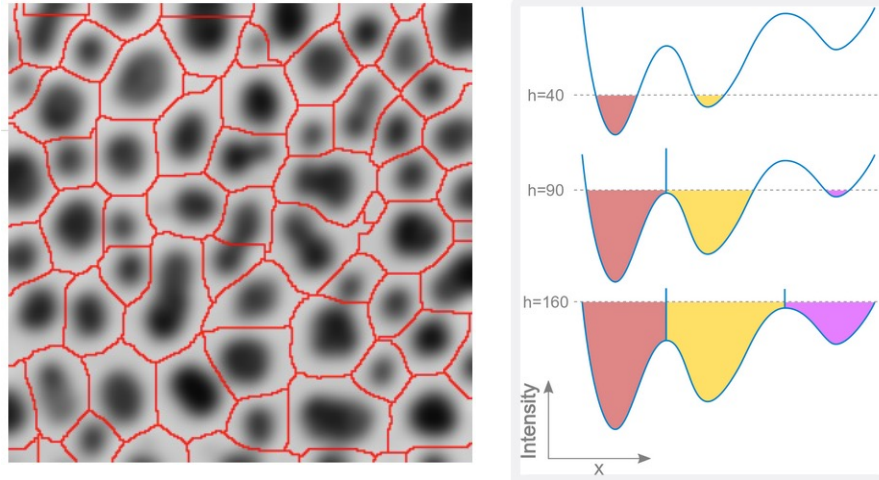


**C** Segmentation



# Essential pre-processing steps: segmentation

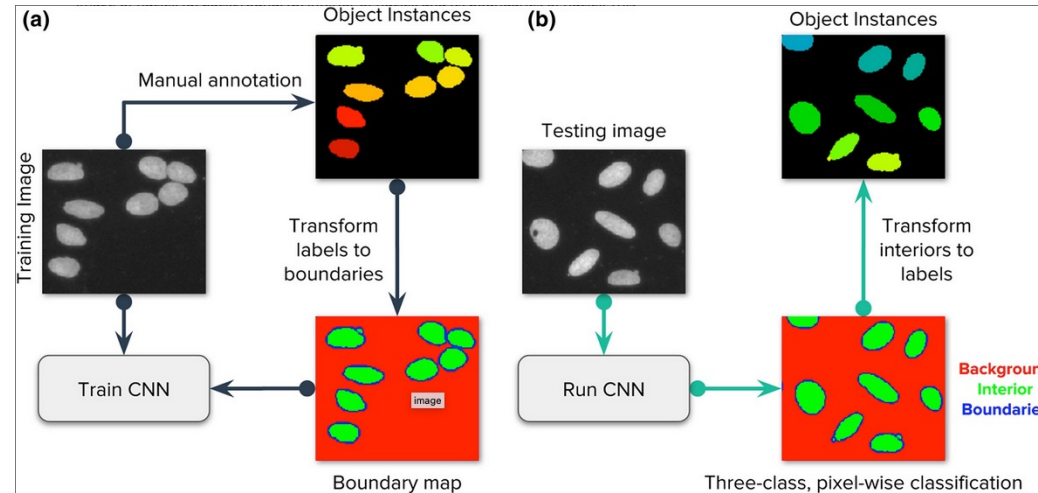
## Classic approaches



**C** Segmentation



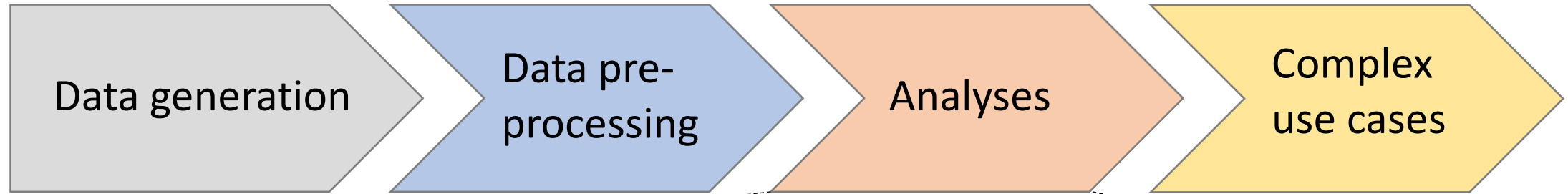
## New Deep-learning approaches



ilastik

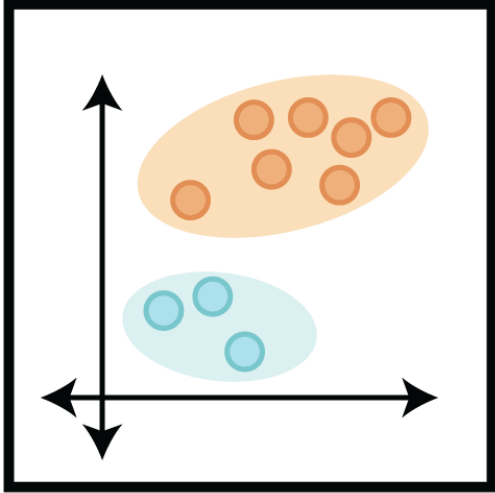


# The different steps in spatial omics research

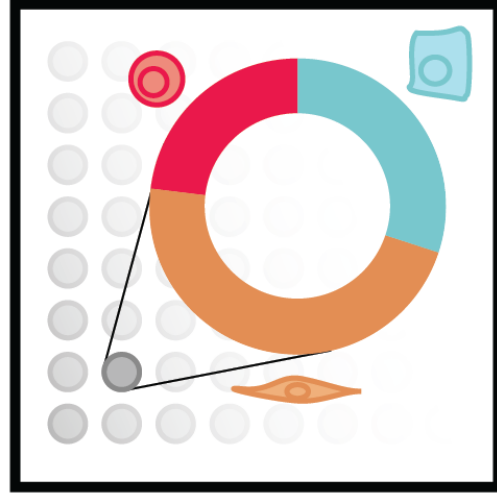


- Cell identities
- Spatial architecture
- Image information

**A** Cell-Type Identification



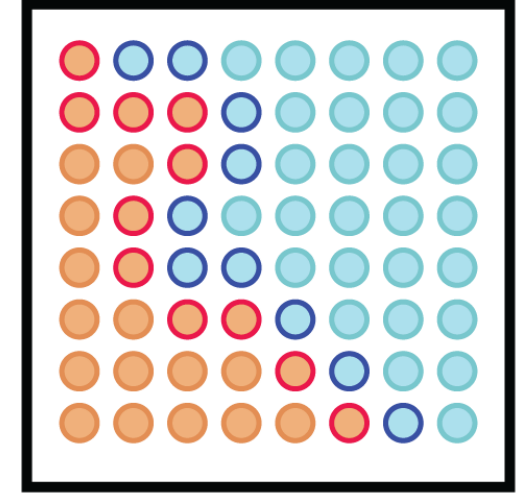
**B** Deconvolution



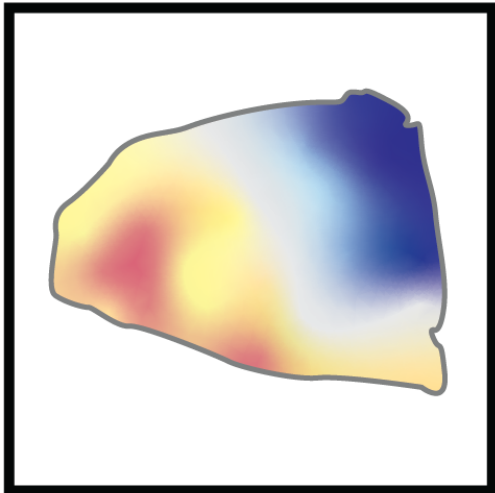
**C** Spatial Distribution



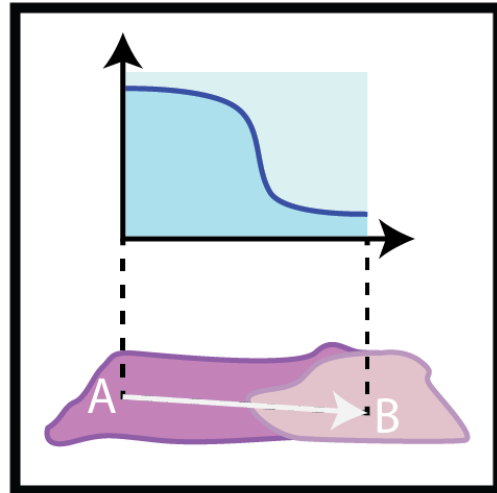
**D** Cell-Cell Interaction



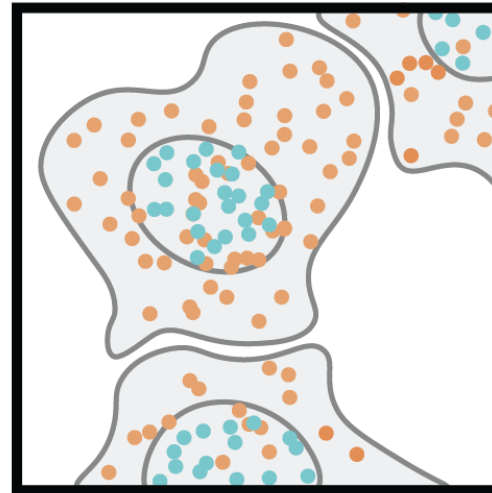
**E** Spatial Expression Patterns



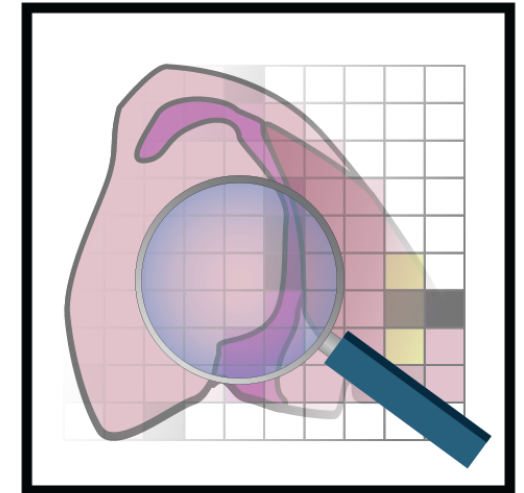
**F** Spatial Trajectory



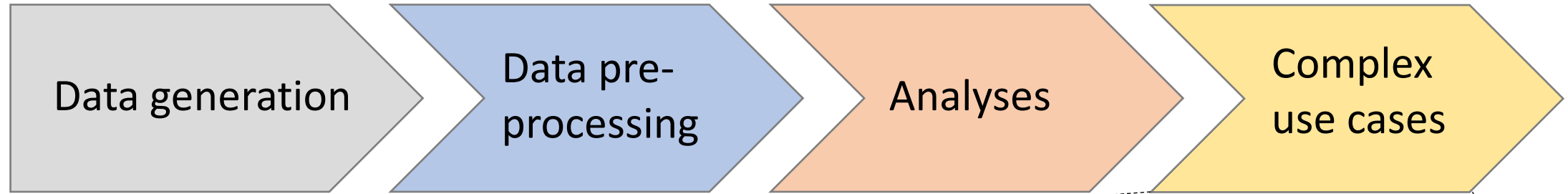
**G** Subcellular



**H** Image Analysis



# The different steps in spatial omics research



- Multi-scale datasets
- Multi-modal datasets
- Giotto Suite: advanced software

# The emerging field of spatial omics



Bulk



Single Cell



Spatial

# The emerging field of spatial omics



Bulk



Single Cell



Spatial

# The emerging field of spatial omics



RNA-seq



# The emerging field of spatial omics



RNA-seq



# The emerging field of spatial omics



RNA-seq



Bricks



Cement



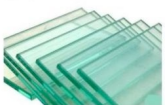
Concrete



Sand



Reinforcement



Glass



Plastic



Wood



Tiles

Single-cell RNA-seq

# The emerging field of spatial omics



Bricks



Cement



Concrete



Sand



Reinforcement



Glass



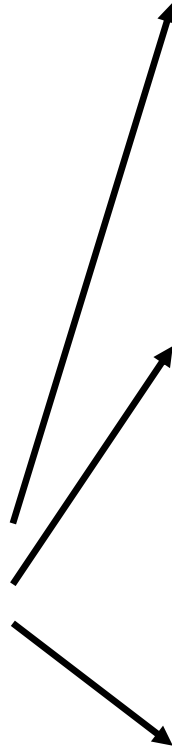
Plastic



Wood



Tiles



# The emerging field of spatial omics



Bricks



Cement



Concrete



Sand



Reinforcement



Glass



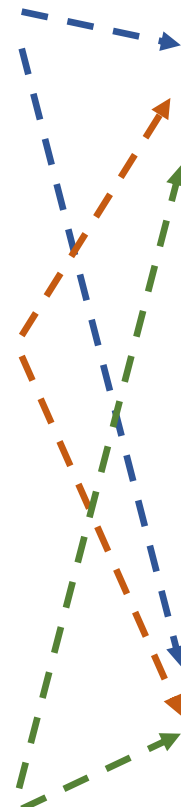
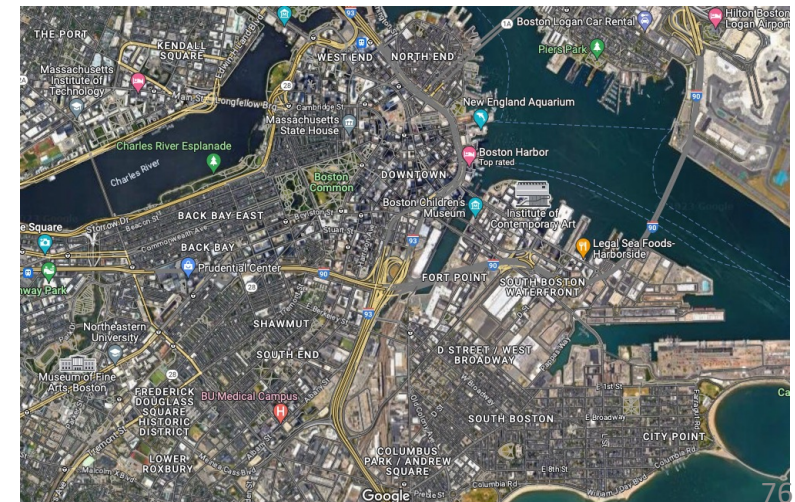
Plastic



Wood



Tiles



# The emerging field of spatial omics



Bricks



Cement



Concrete



Sand



Reinforcement



Glass



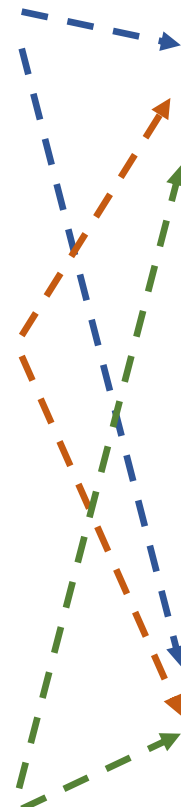
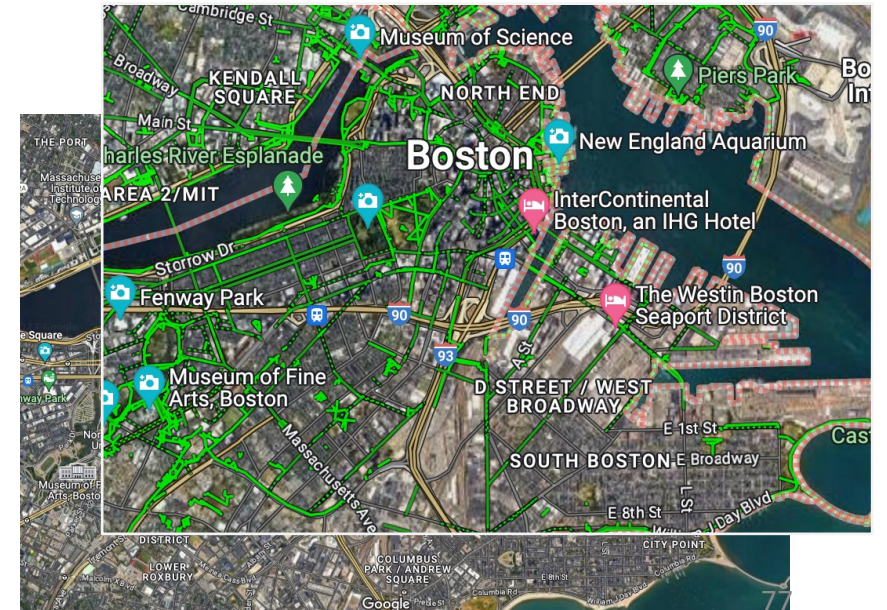
Plastic



Wood



Tiles



# The emerging field of spatial omics



Bricks



Cement



Concrete



Sand



Reinforcement



Glass



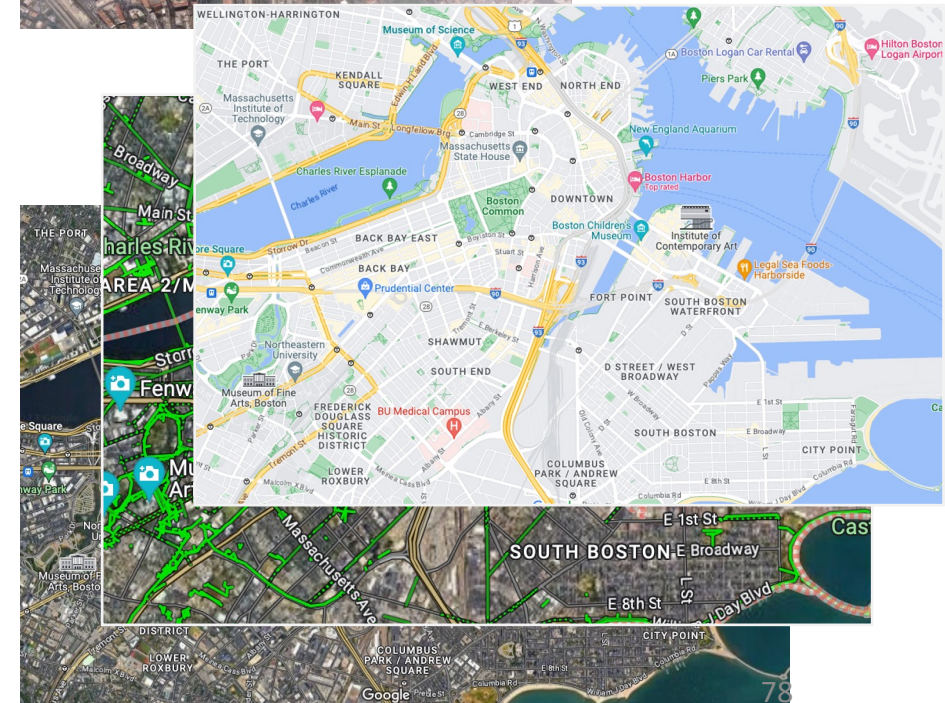
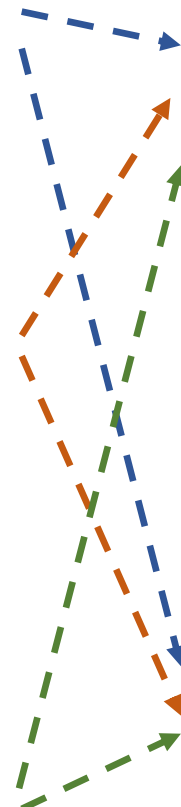
Plastic



Wood



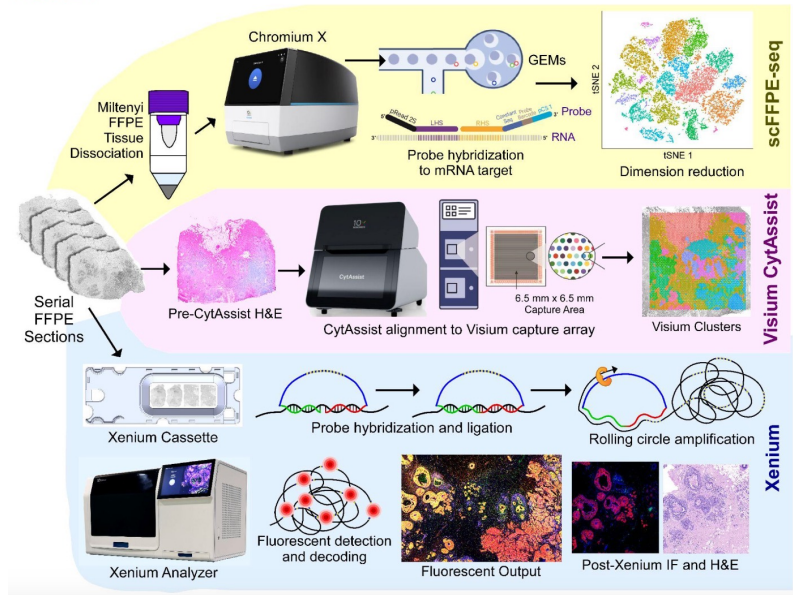
Tiles



# Datasets of the future

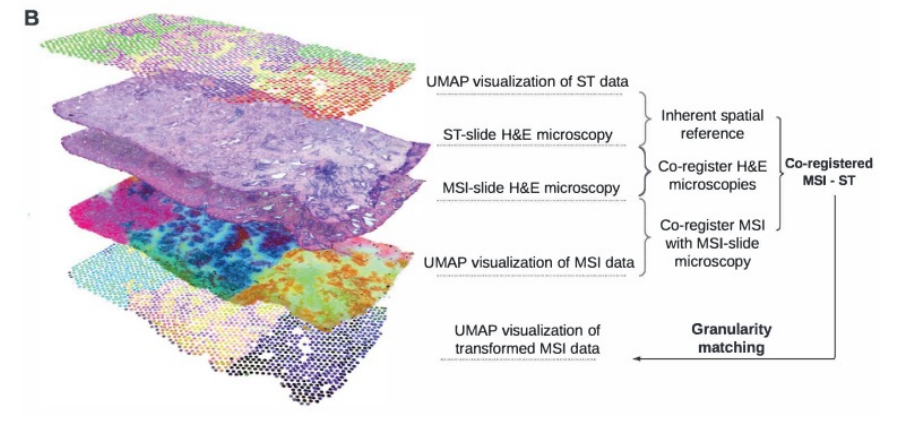
## High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis of FFPE tissue

Amanda Janesick, Robert Shelansky, Andrew D. Gottscho, Florian Wagner, Morgane Rouault, Ghezal Beliakoff, Michelli Faria de Oliveira, Andrew Kohlway, Jawad Arousoud, Carolyn A. Morrison, Tingsheng Yu Drennon, Seayar H. Mohabbat, Stephen R. Williams, 10x Development Teams, Sarah E.B. Taylor



## Integration of Multiple Spatial Omics Modalities Reveals Unique Insights into Molecular Heterogeneity of Prostate Cancer

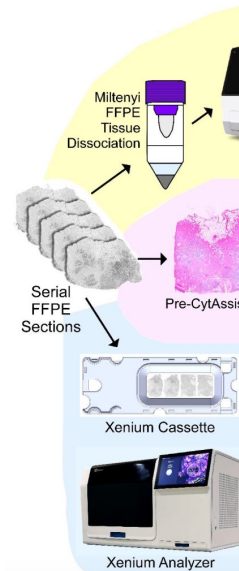
Wanqiu Zhang, Xander Spotbeen, Sebastiaan Vanuytven, Sam Kint, Tassiani Sarretto, Fabio Socciairelli, Katy Vandereyken, Jonas Dehairs, Jakub Idkowiak, David Wouters, Jose Ignacio Alvira Larizgoitia, Gabriele Partel, Alice Ly, Vincent de Laat, Maria José Q Mantas, Thomas Gevaert, Wout Devlies, Chui Yan Mah, Lisa M Butler, Massimo Loda, Steven Joniau, Bart De Moor, Alejandro Sifrim, Shane R. Ellis, Thierry Voet, Marc Claesen, Nico Verbeeck, Johannes V. Swinnen



# Datasets of the future

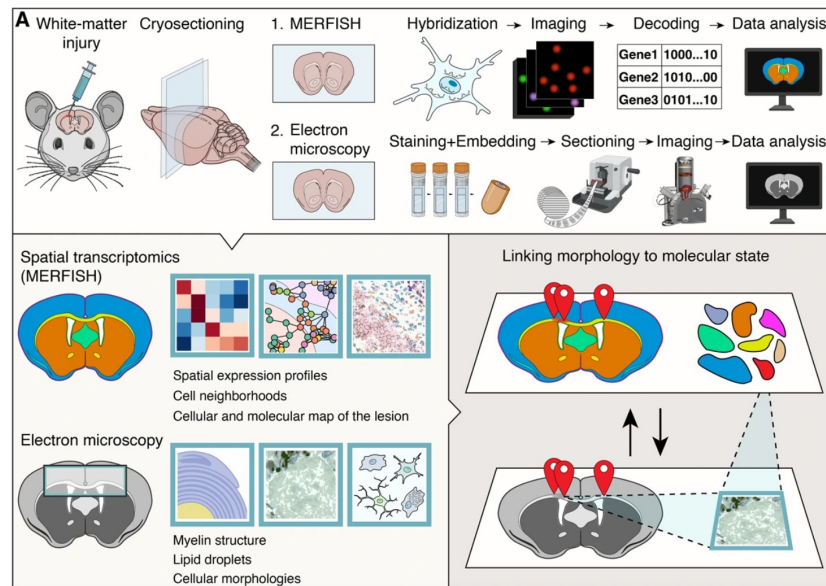
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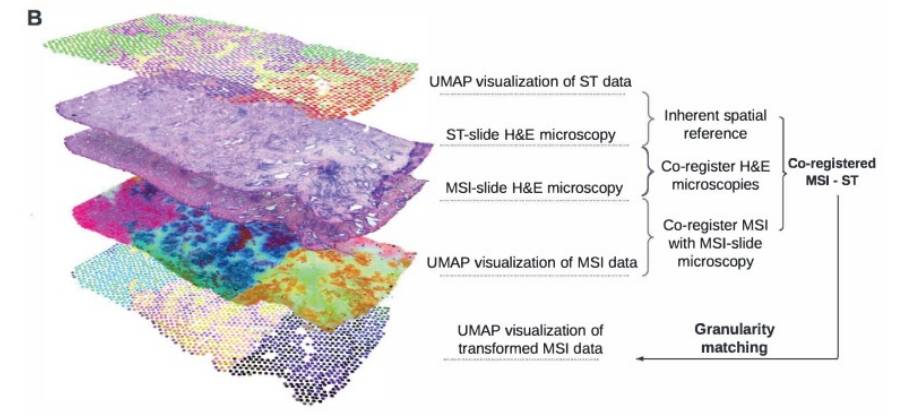
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Peter Androvic, Martina Schifferer, Katrin Perez Anderson, Ludovico Cantuti-Castelvetri, Hanyi Jiang, Hao Ji, Lu Liu, Garyfallia Gouna, Stefan A. Berghoff, Simon Besson-Girard, Johanna Knoferle, Mikael Simons & Ozgun Gokce



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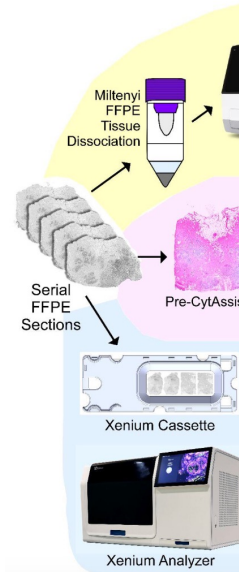




# Datasets of the future

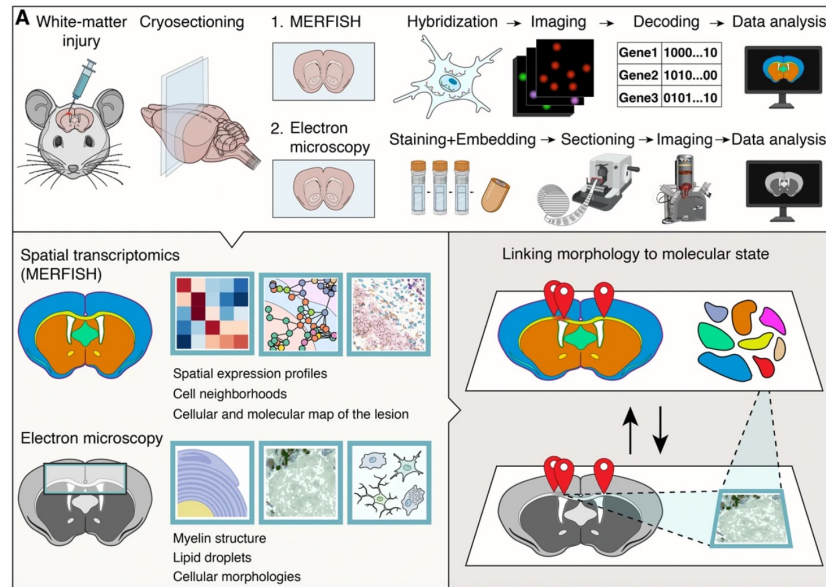
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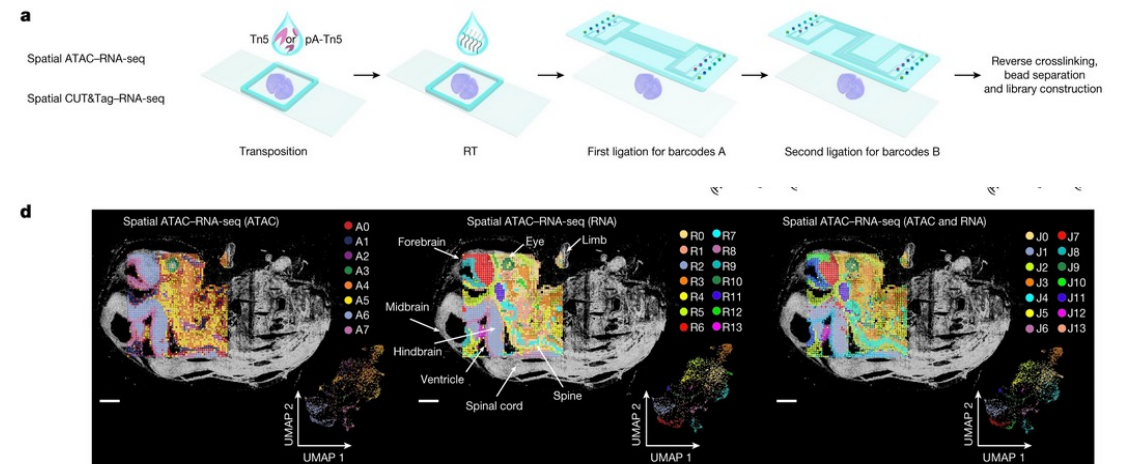


## Integration of Multiple Spatial Omics Modalities Reveals Unique Insights into Molecular Heterogeneity of Prostate Cancer

Wanqiu Zhang, Xander Spotbeen, Sebastiaan Vanuytven, Sam Kint, Tassiani Sarretto, Fabio Socciairelli, Katy Vandereyken, Jonas Dehairs, Jakub Idkowiak, David Wouters, Jose Ignacio Alvira Larizgoitia, Gabriele Partel, Alice Ly, Vincent de Laat, Maria José Q Mantas, Thomas Gevaert, Wout Devlies, Chui Yan Mah, Lisa M Butler, Massimo Loda, Steven Joniau, Bart De Moor, Alejandro Sifrim, Shane R. Ellis, Thierry Voet, Marc Claesen, Nico Verbeeck, Johannes V. Swinnen

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

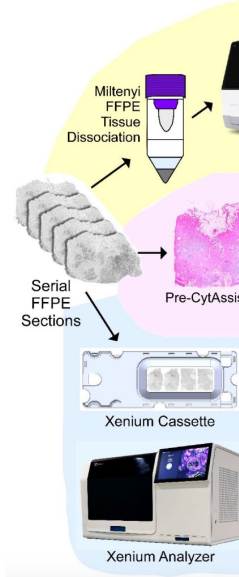
Di Zhang, Yanxiang Deng, Petra Kukanja, Eneritz Agirre, Marek Bartosovic, Mingze Dong, Cong Ma, Sai Ma, Graham Su, Shuozhen Bao, Yang Liu, Yang Xiao, Gorazd B. Rosoklija, Andrew J. Dwork, J. John Mann, Kam W. Leong, Maura Boldrini, Liya Wang, Maximilian Haeussler, Benjamin J. Raphael, Yuval Kluger, Gonçalo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



# Datasets of the future

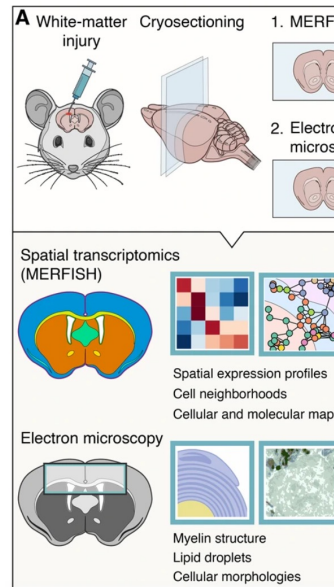
## High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis

Amanda Janesick, Robert Shelansky, Andrew D. Gottscho, Florian Wagner, M. Ghezal Beliakoff, Michelli Faria de Oliveira, Andrew Kohlway, Jawad Arousoud, Tingsheng Yu Drennon, Seayar H. Mohabbat, Stephen R. Williams, 10x Development



## Spatial Transcriptomics maps responses to brain injury

Peter Androvic, Martina Schifferer, Jiang, Hao Ji, Lu Liu, Garyfallia Gouk, Mikael Simons & Ozgun Gokce



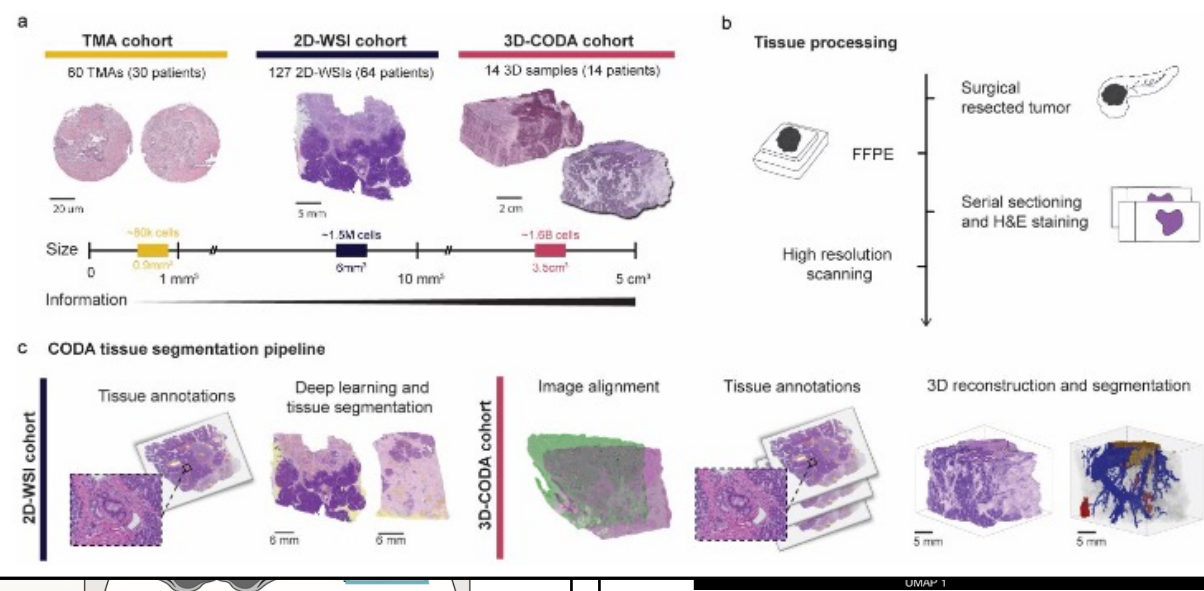
## Integration of Multiple Spatial Omics Modalities Reveals Unique Insights

Sam Kint, Tassiani Sarretto, David Wouters, Vincent de Laat, Maria José Q Mantas, Massimo Loda, Steven Joniau, Marc Claesen, Nico Verbeeck

## Three-dimensional assessments are necessary to determine the true, spatially-resolved composition of tissues

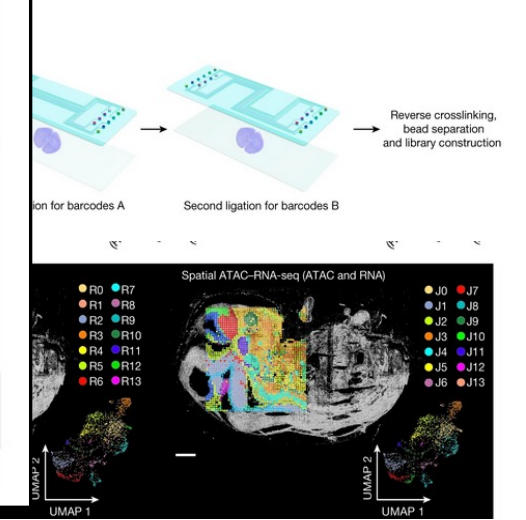
André Forjaz, Eduarda Vaz, Valentina Matos Romero, Saurabh Joshi, Alicia M. Braxton, Ann C. Jiang, Kohei Fujikura, Toby Cornish, Seung-Mo Hong, Ralph H. Hruban, Pei-Hsun Wu, Laura D. Wood, Ashley L. Kiemen, Denis Wirtz

doi: <https://doi.org/10.1101/2023.12.04.569986>



## Genome co-profiling of

Marek Bartosovic, Mingze Dong, Cong Ma, Gorazd B. Rosoklija, Andrew J. Dwork, J. John, Benjamin J. Raphael, Yuval



# Giotto Suite: A Multi-scale and Technology-agnostic Spatial Multi-omics Analysis Ecosystem



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