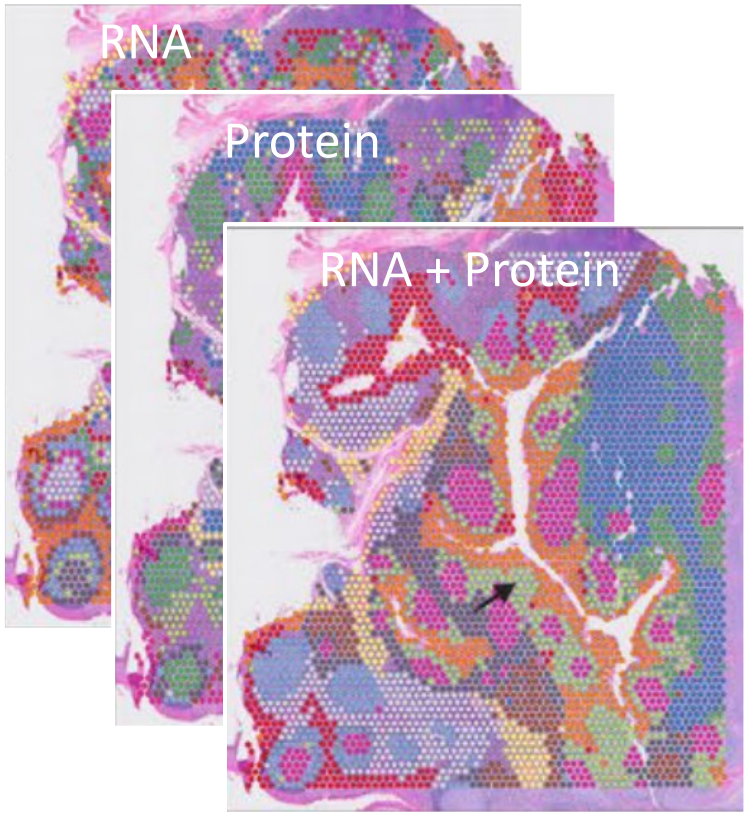
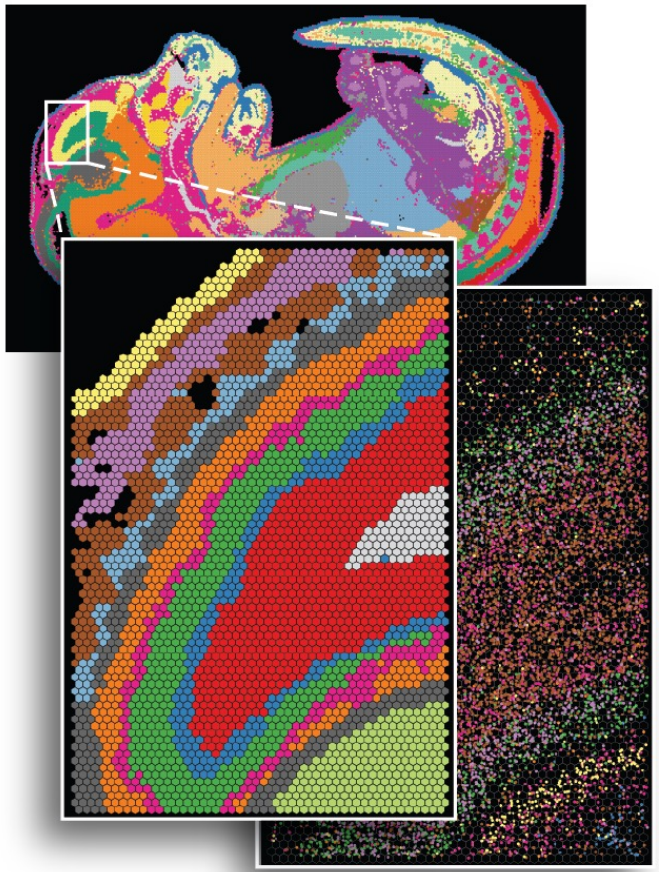
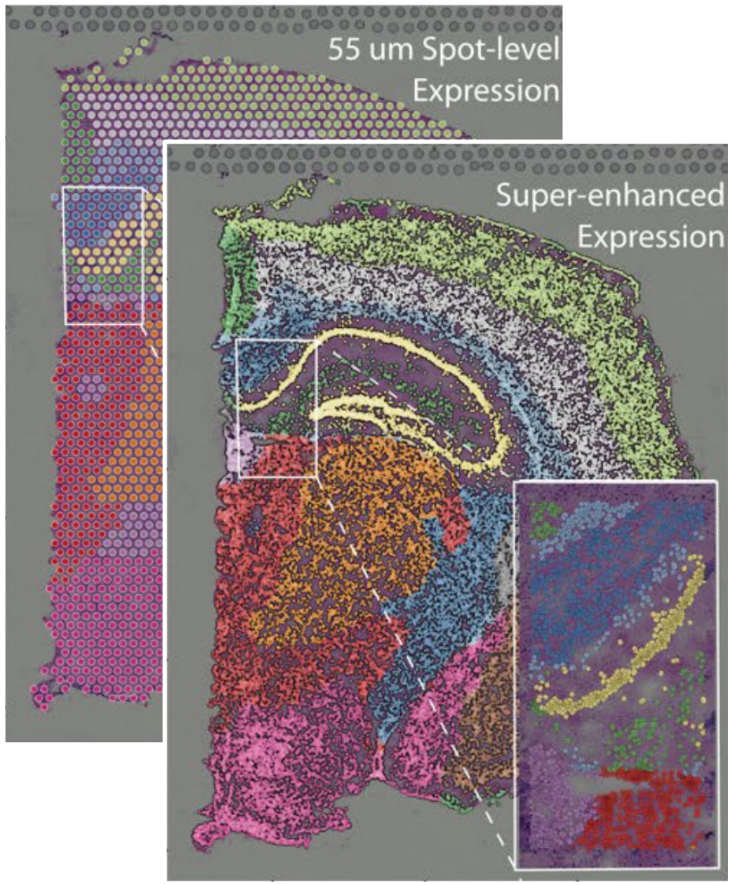
Spatial omics technologies and applications



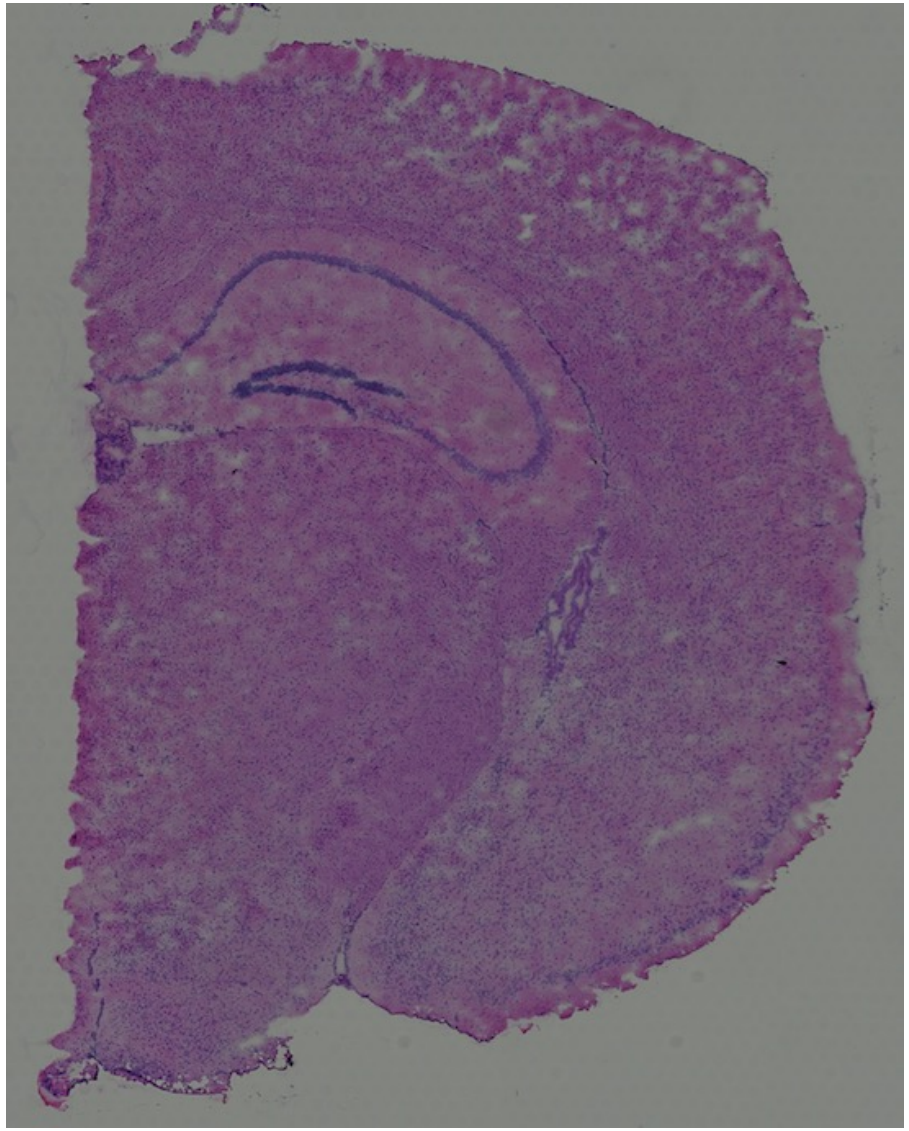




Ruben Dries
GS workshop, 08/05

A spatial transcriptomic example

Mouse brain



H&E stain:

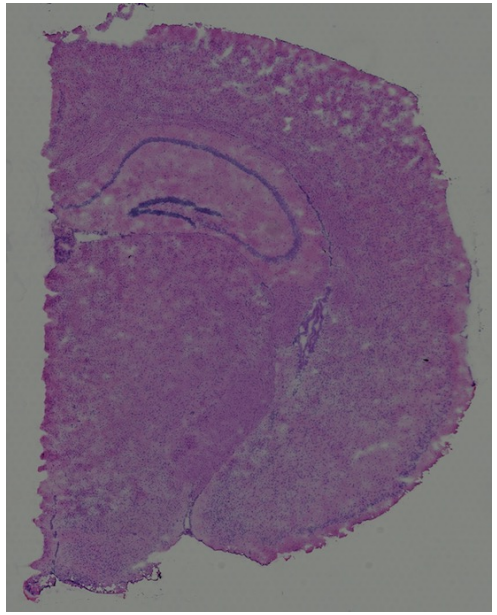
- Haematoxylin = nuclei
- Eosin = extracellular matrix

→ Cornerstone of pathology: study the morphology to assess disease status

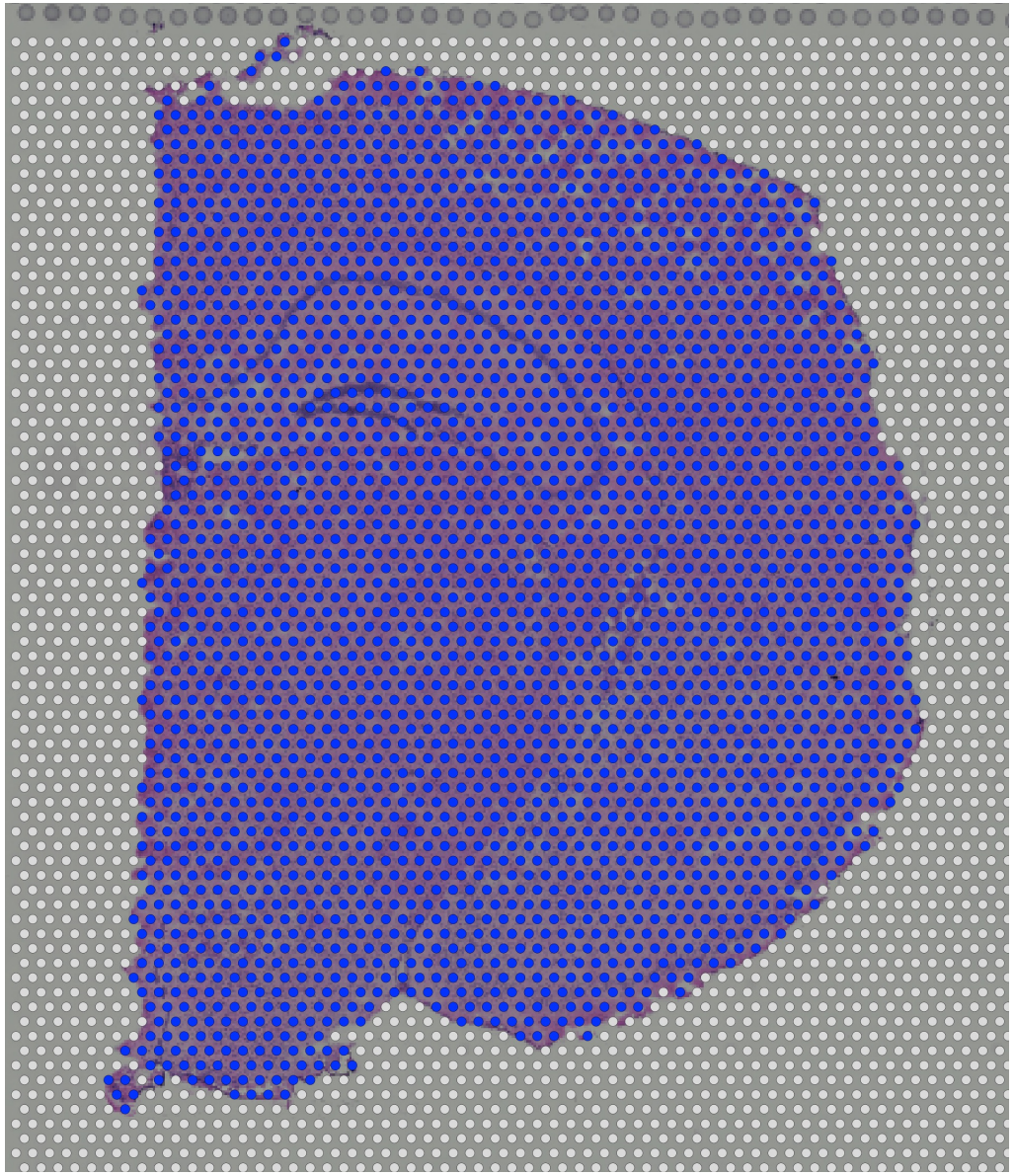
→ What other information is available? 1000's of molecular analytes (RNA, Protein, Metabolites, etc)

A spatial transcriptomic example

Mouse brain



Extract spatial information (e.g. RNA)



A spatial transcriptomic example

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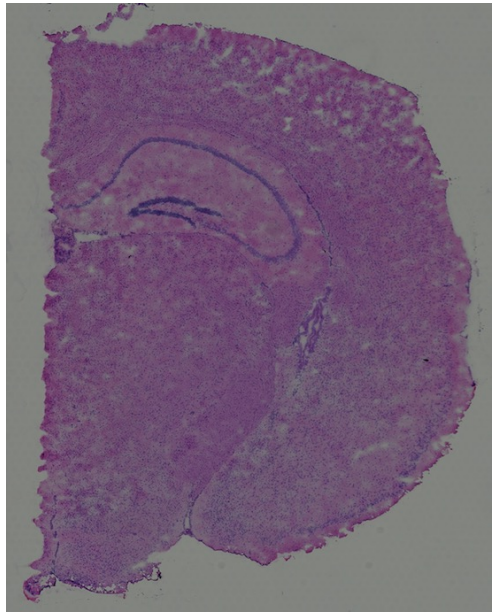

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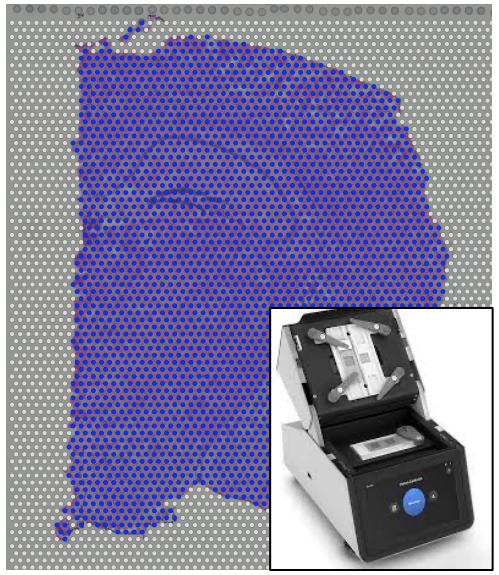
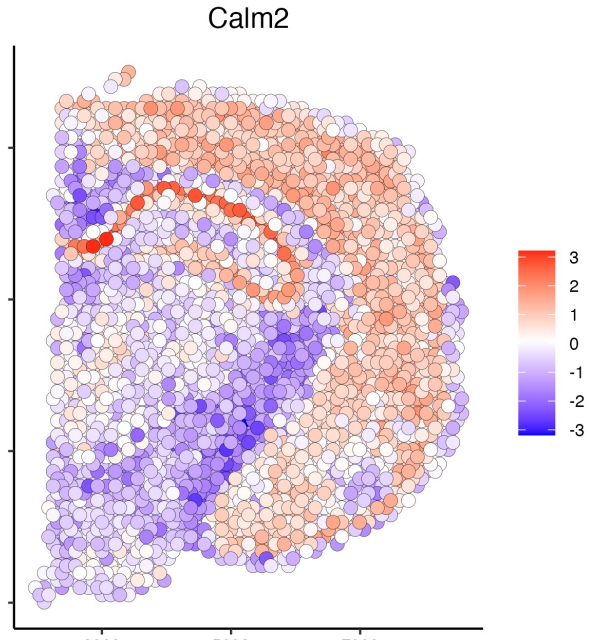
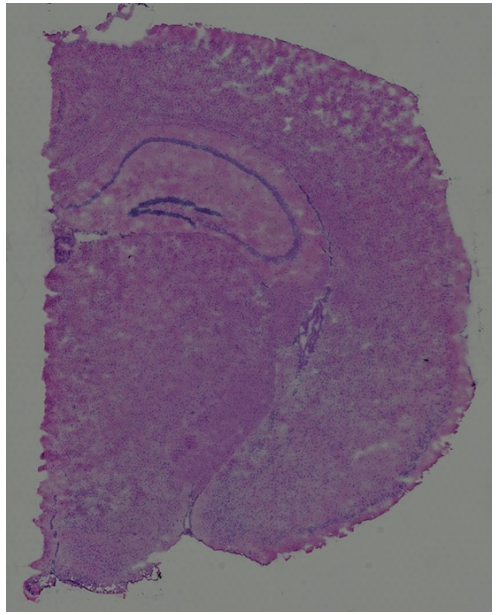


Map spatial information back (e.g. RNA expression levels)



A spatial transcriptomic example

Mouse brain



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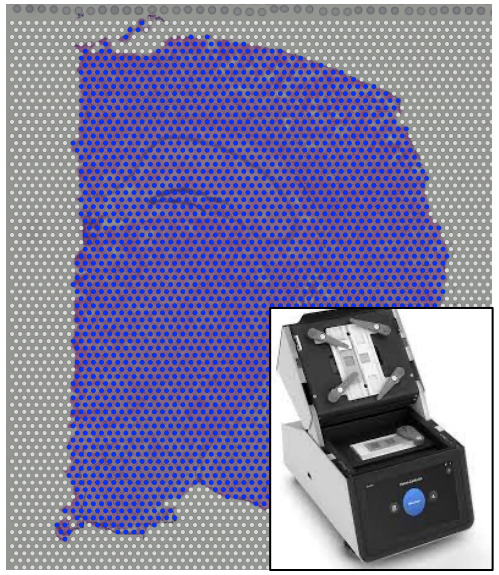

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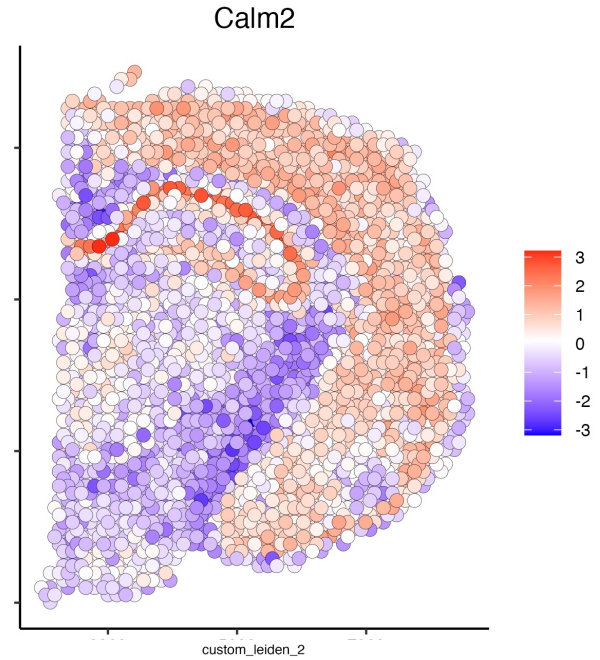
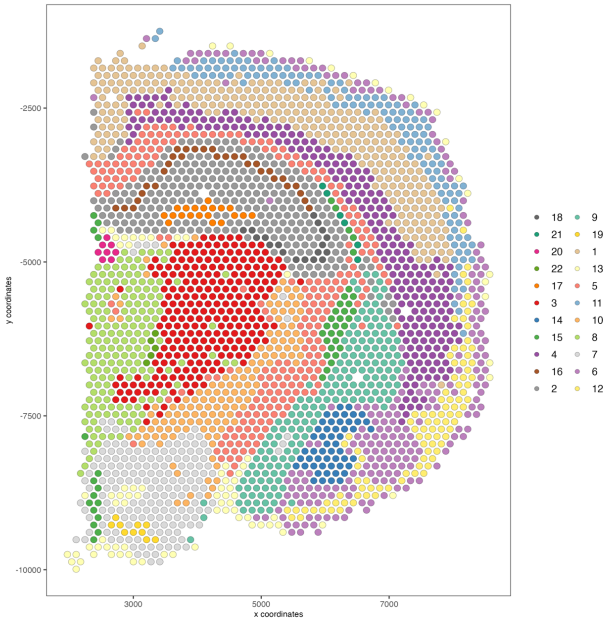
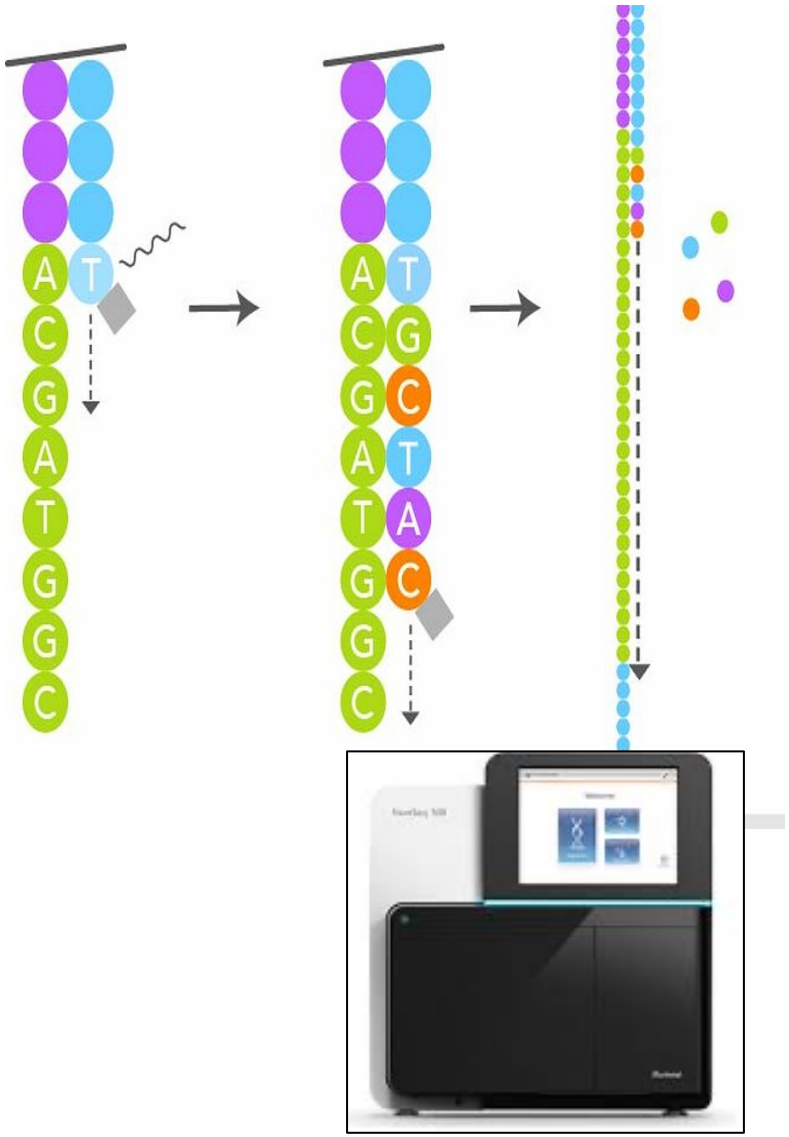


Obtain spatial insights



What is spatial omics?

Sequencing



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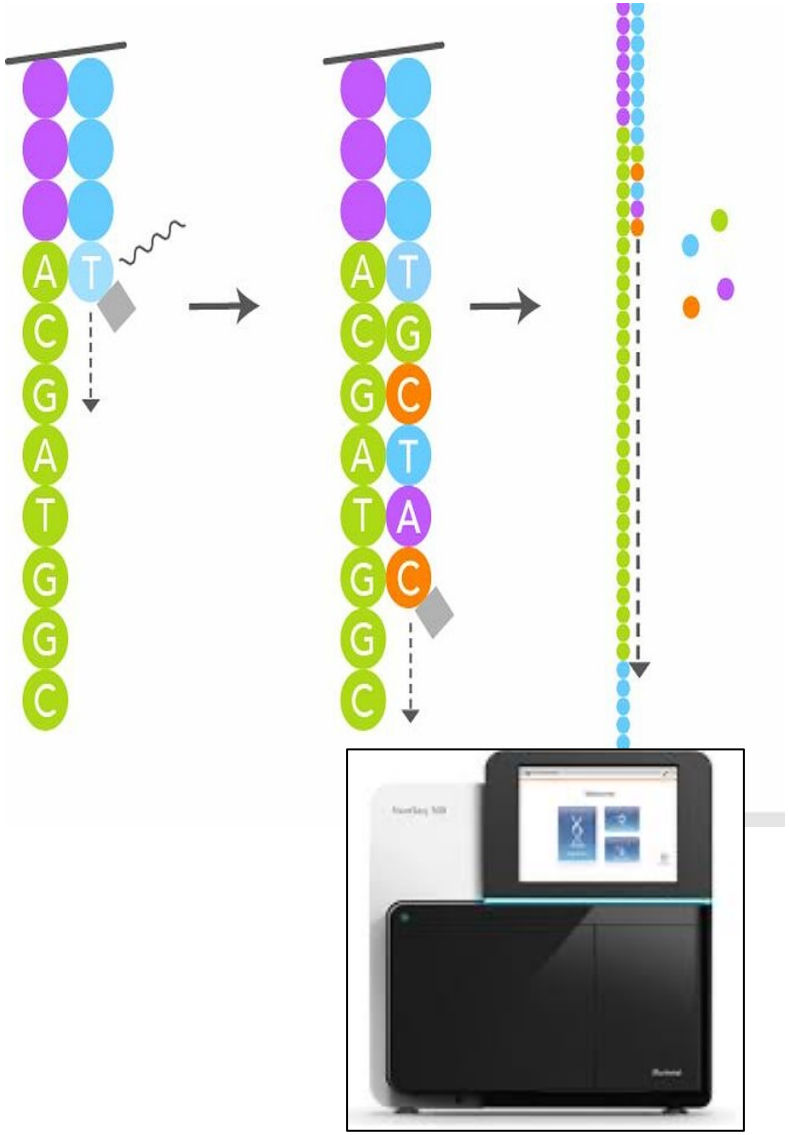
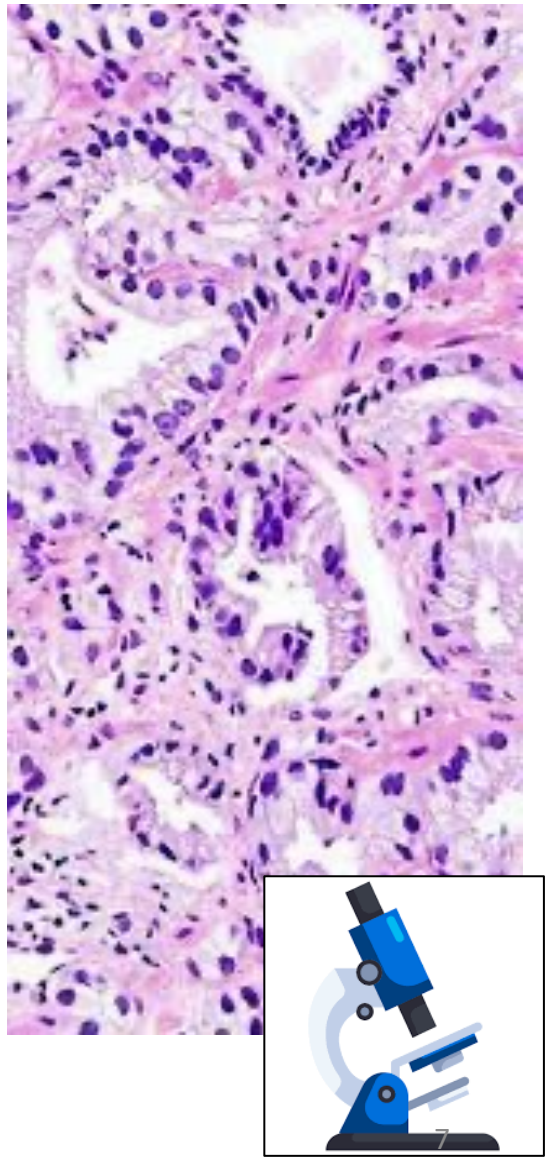


Imaging



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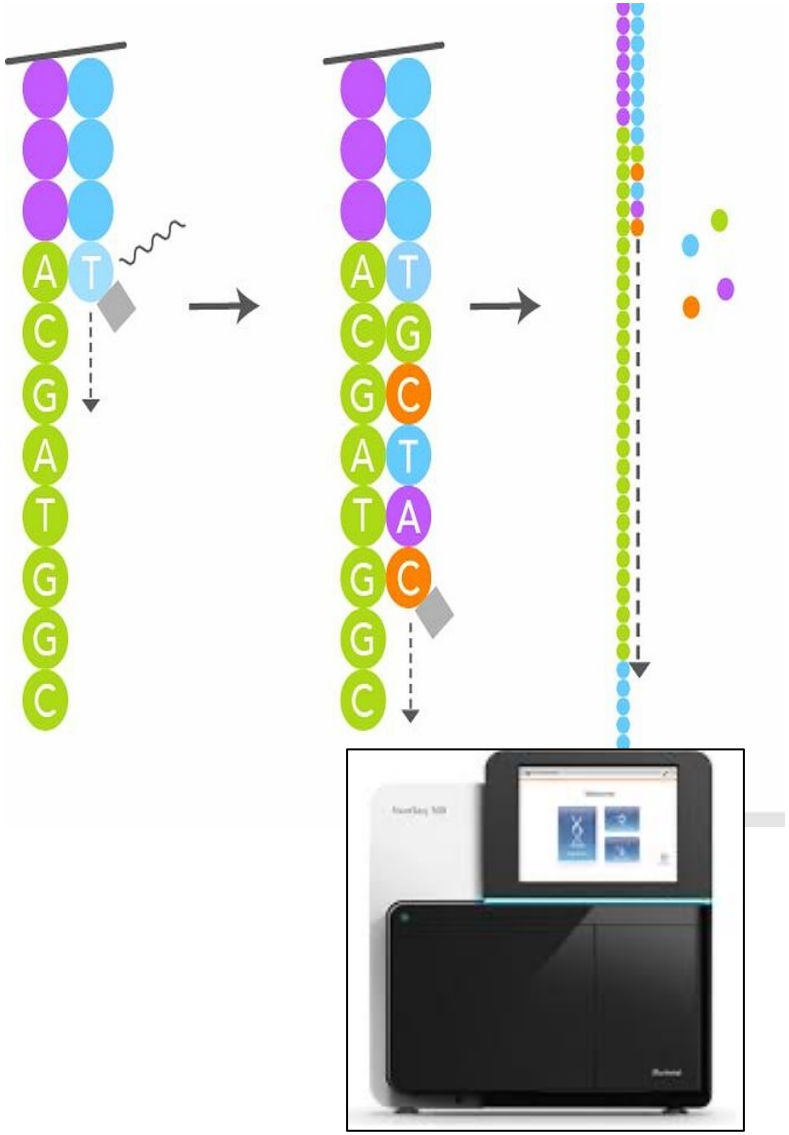
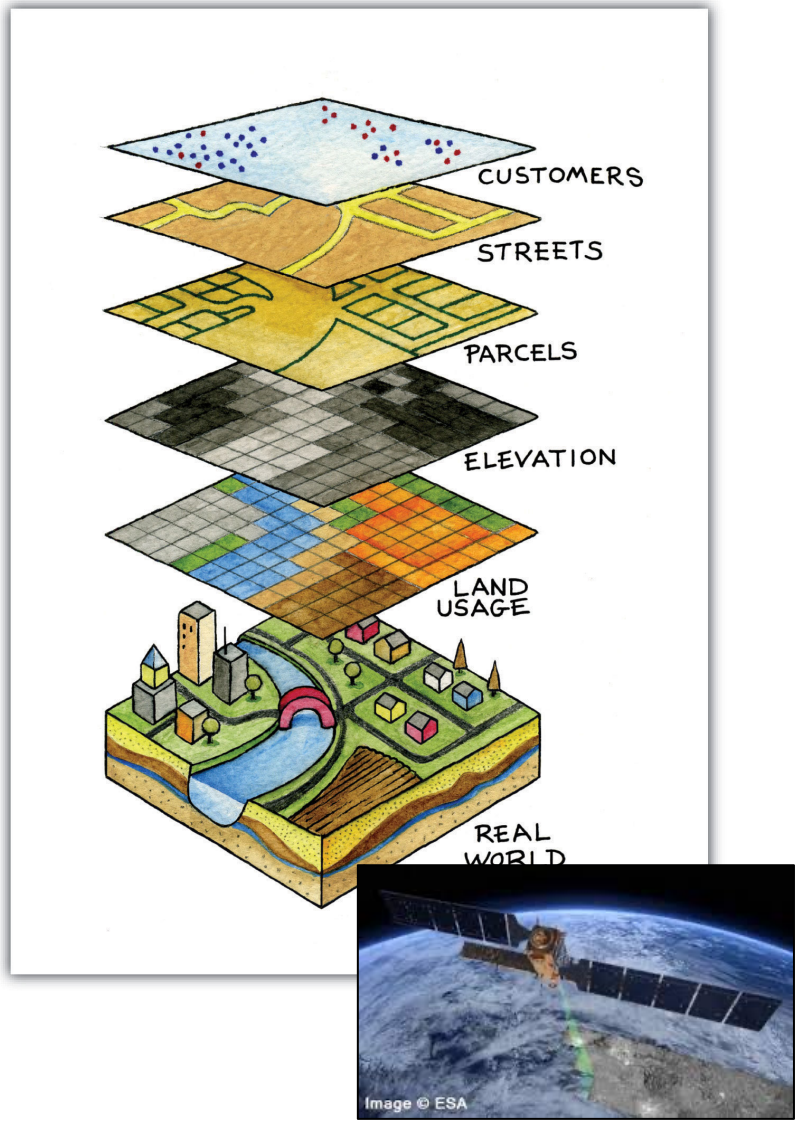


Spatial data science



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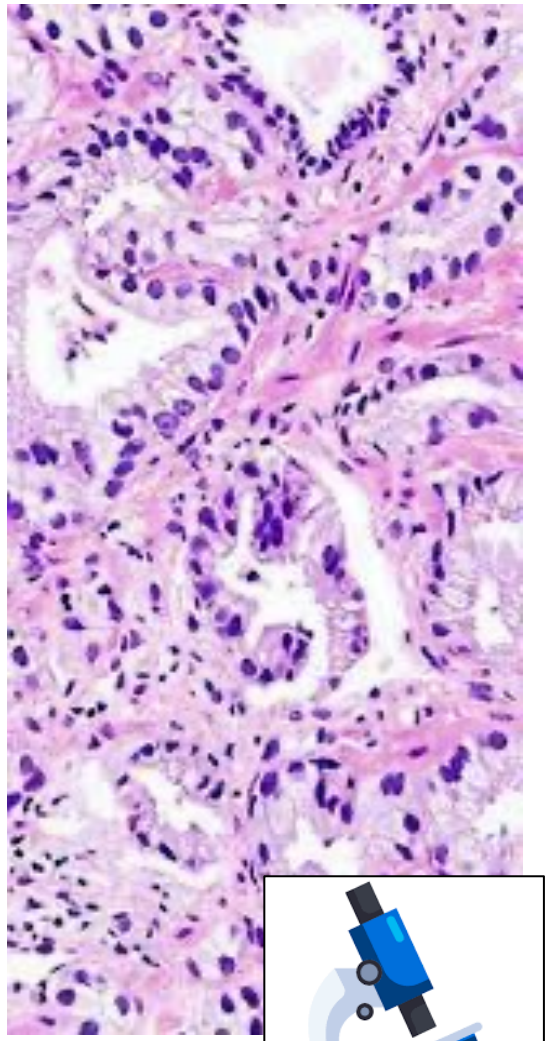
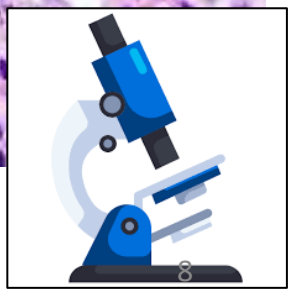


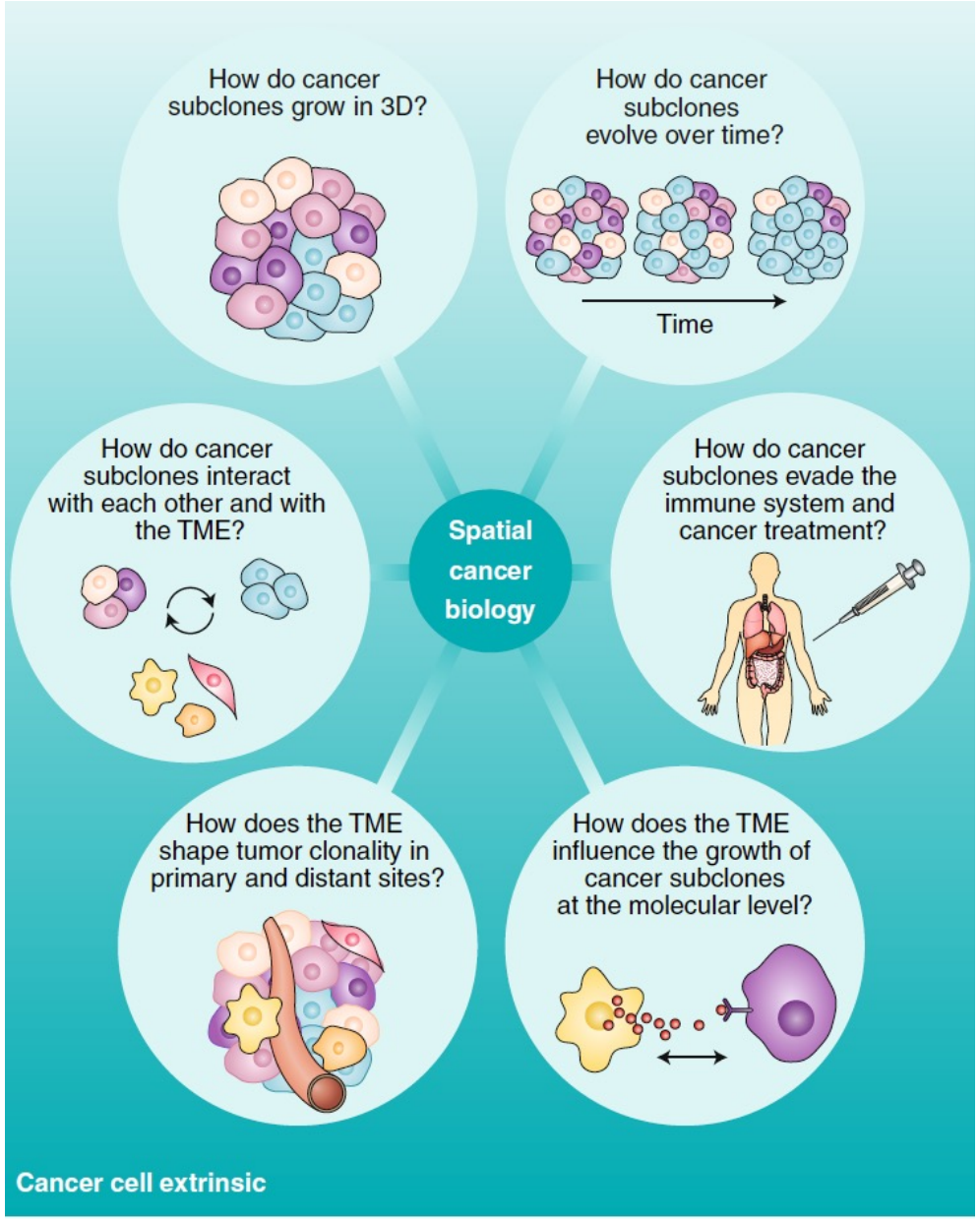


What are the biological questions we're trying to answer?

Examples in the field of cancer biology:

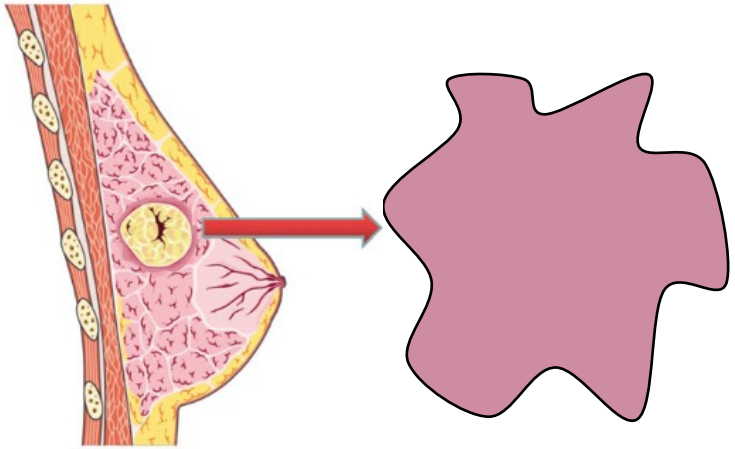
Questions that require us to understand cellular behavior within their normal tissue or environment.

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

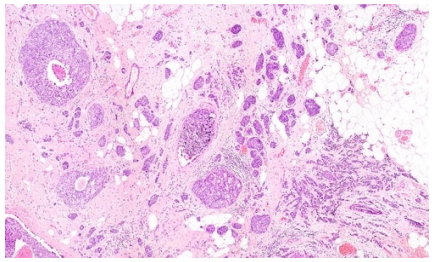


Lewis, S. M. *et al.* Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:10.1038/s41592-021-01203-6.

Historically …



Morphology: Pathology



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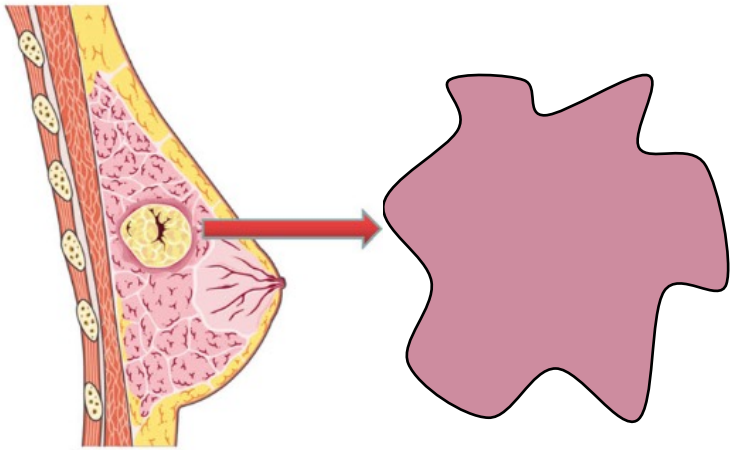

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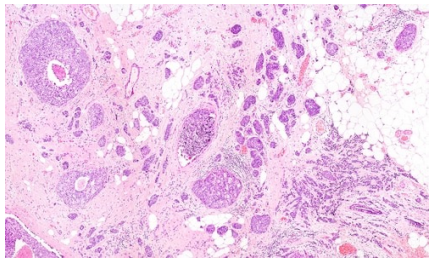
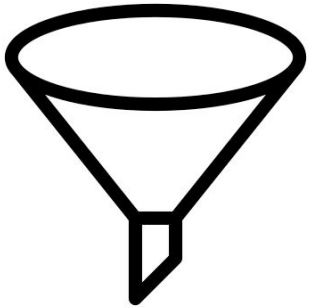
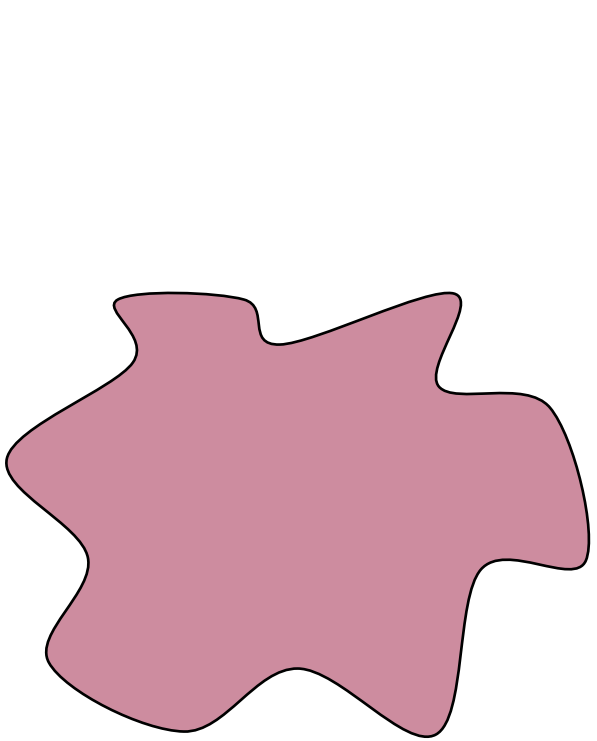


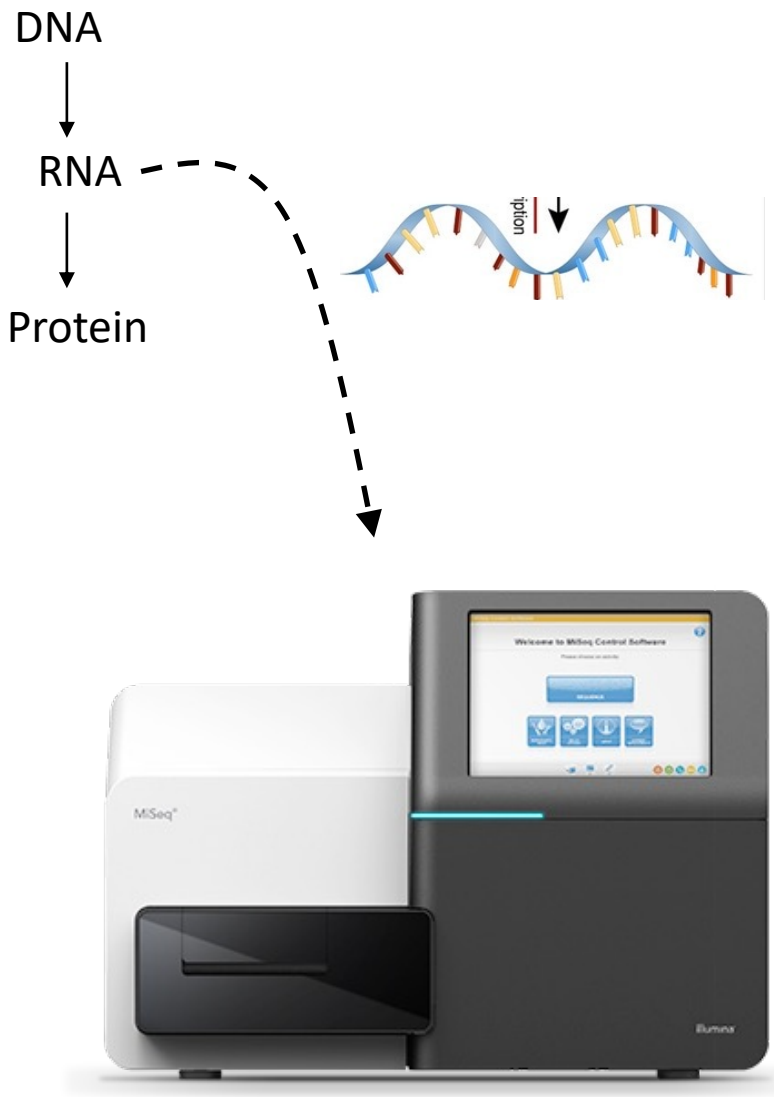
Molecular level



- 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





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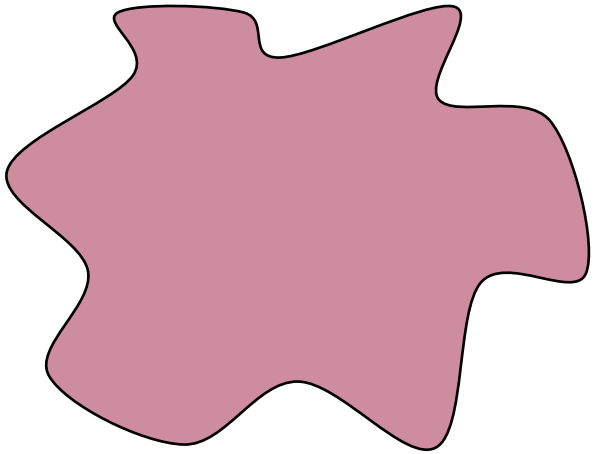
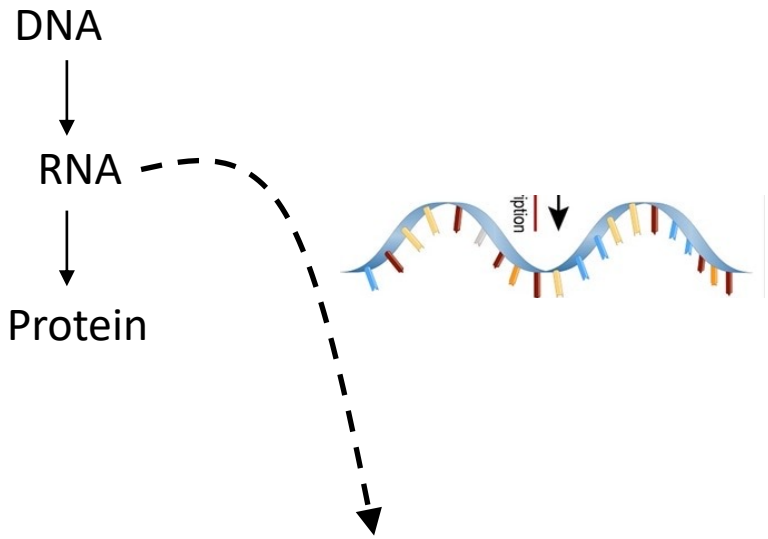
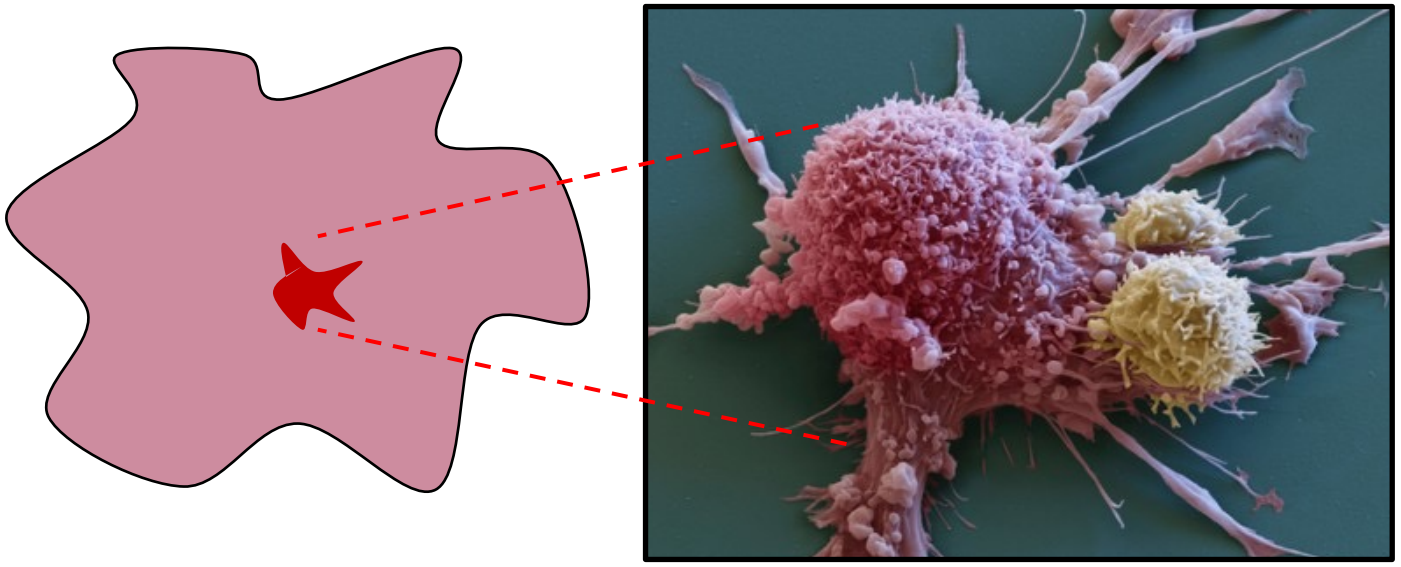




Historically … limitations of RNA-seq



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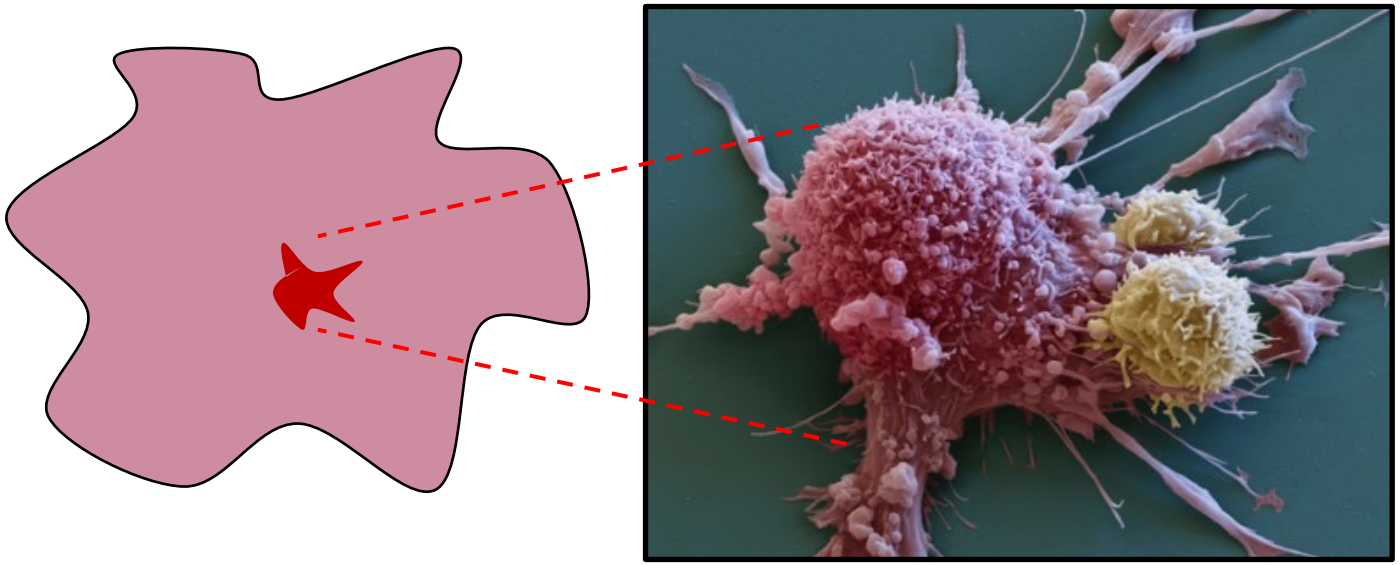
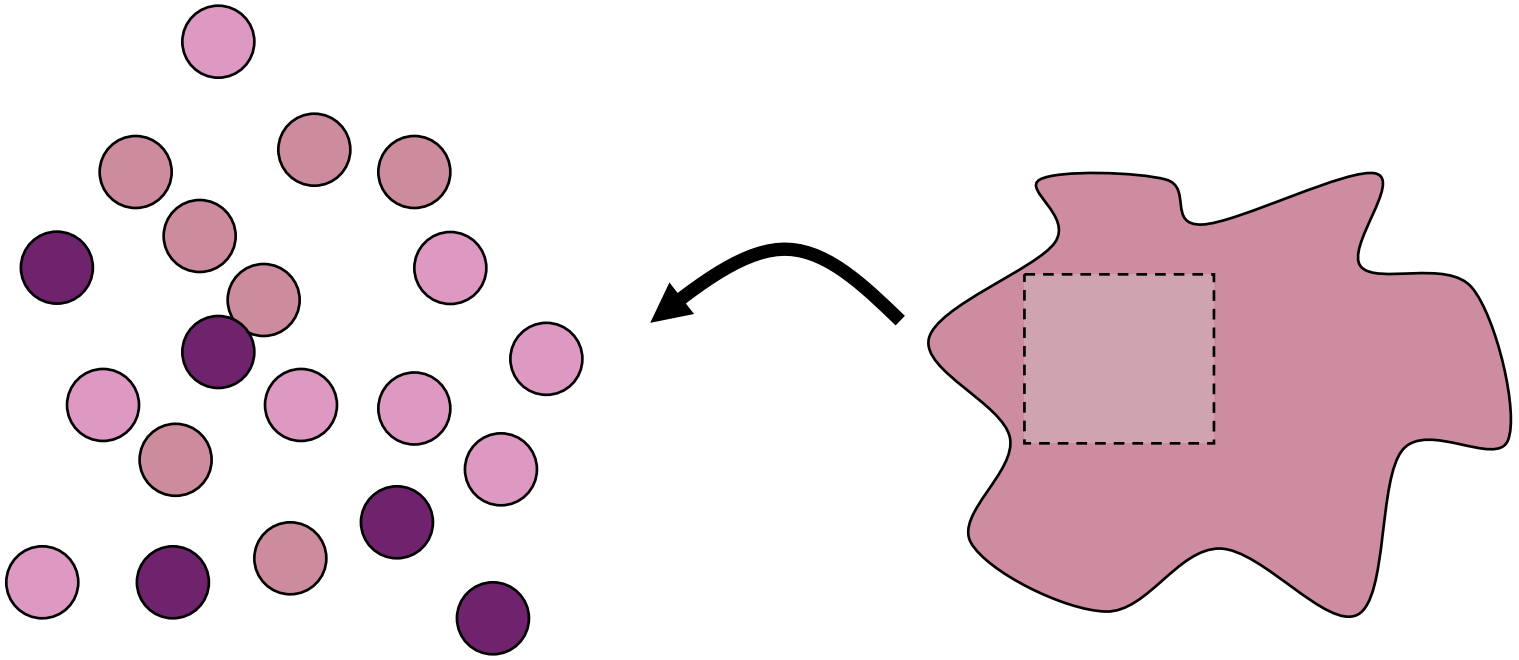


Historically … single-cell RNA-seq



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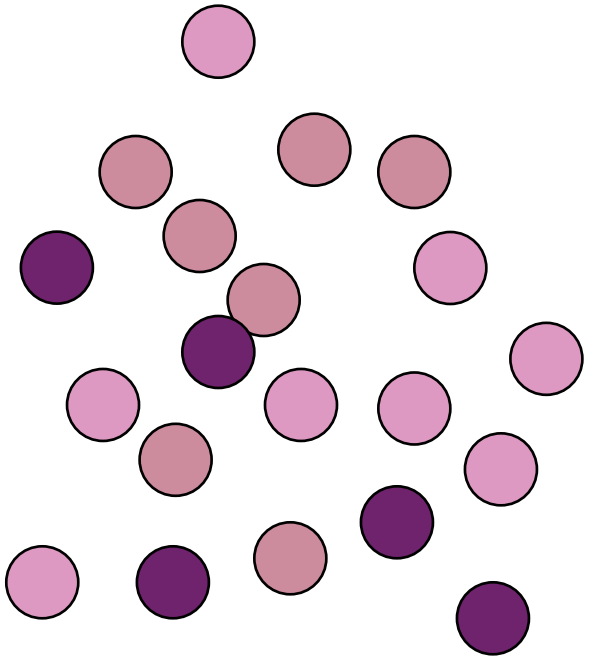

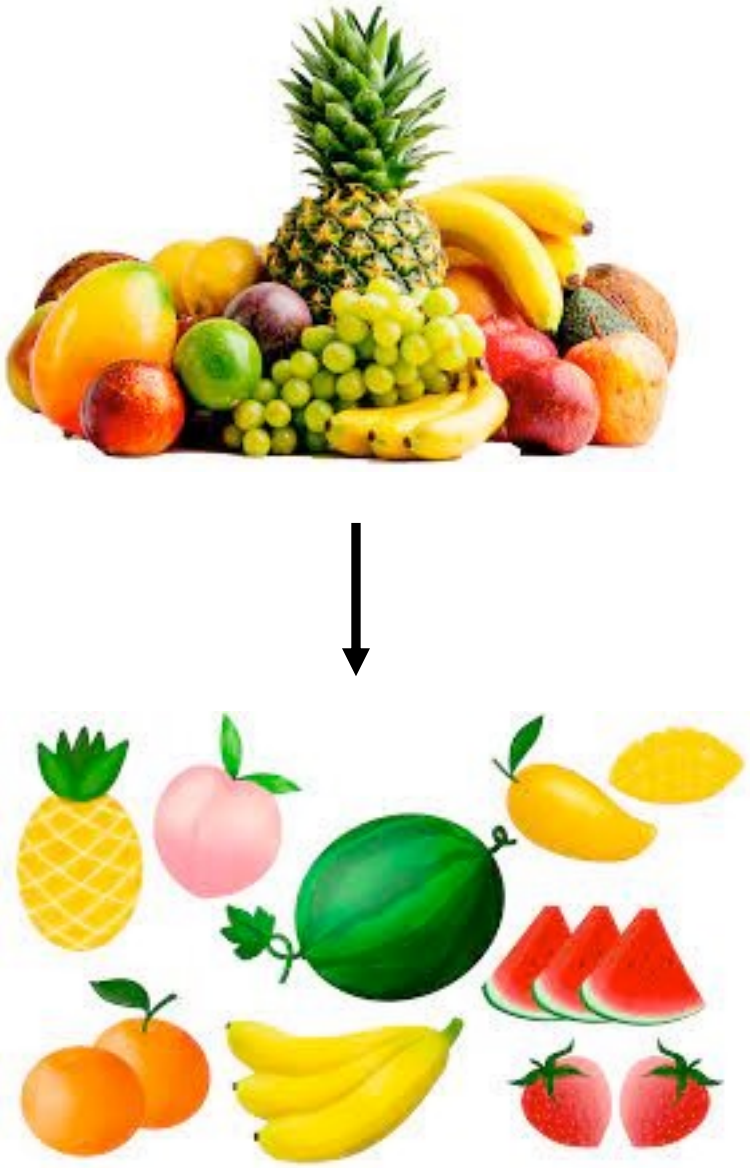

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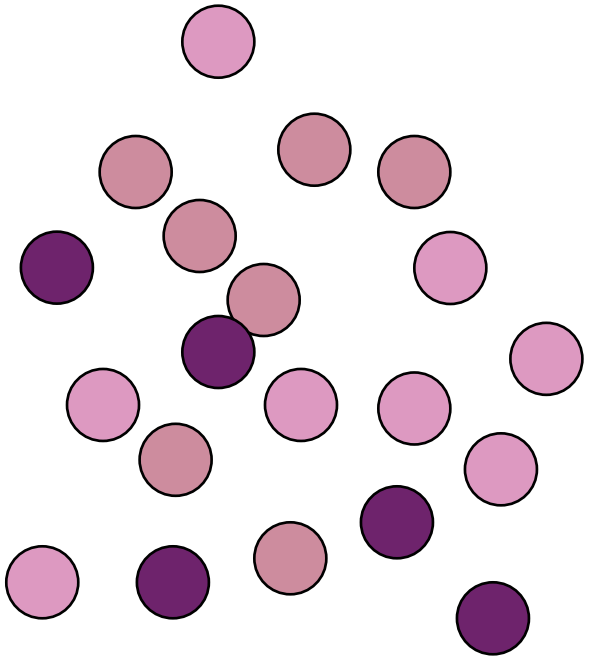
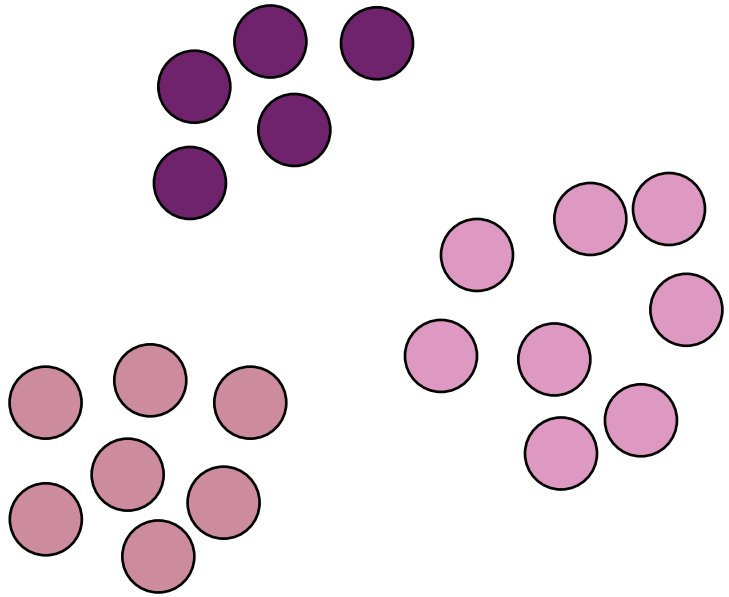


Cluster cell types



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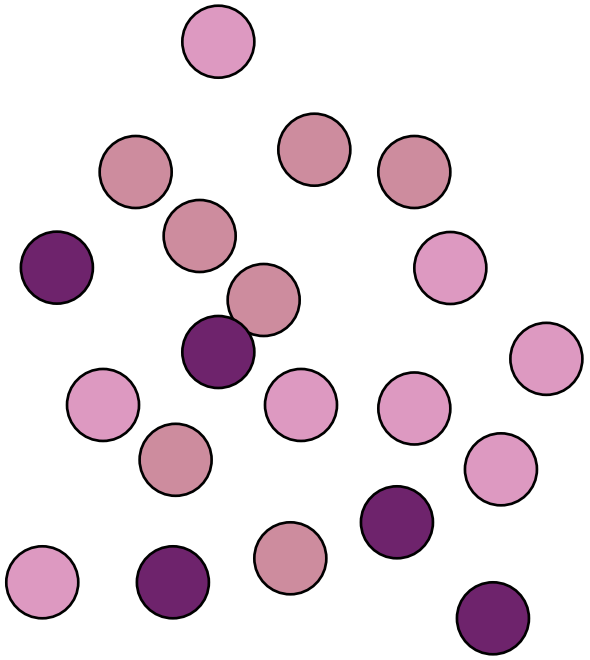
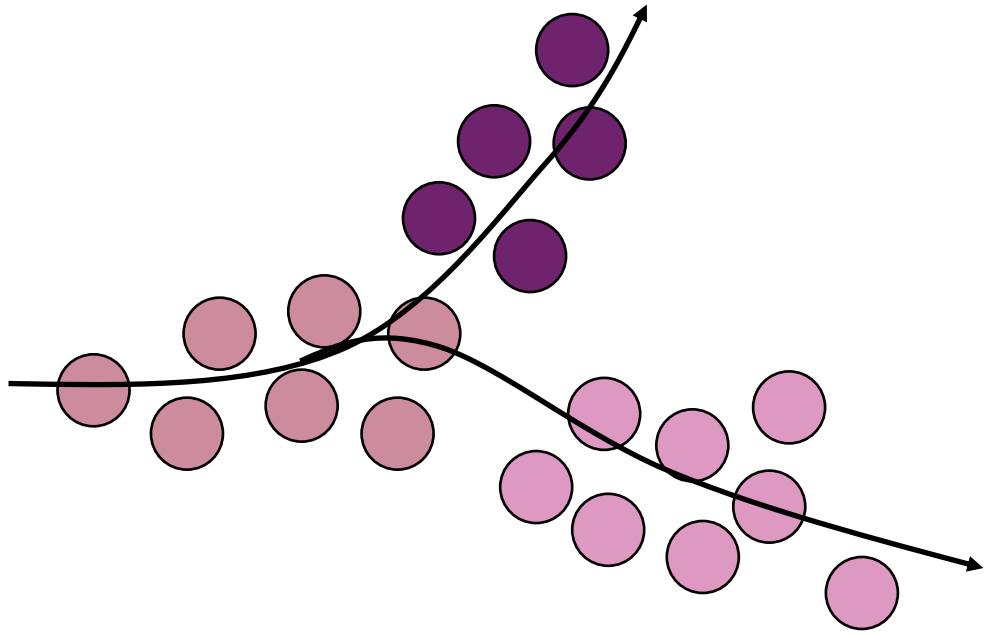


Cell trajectories



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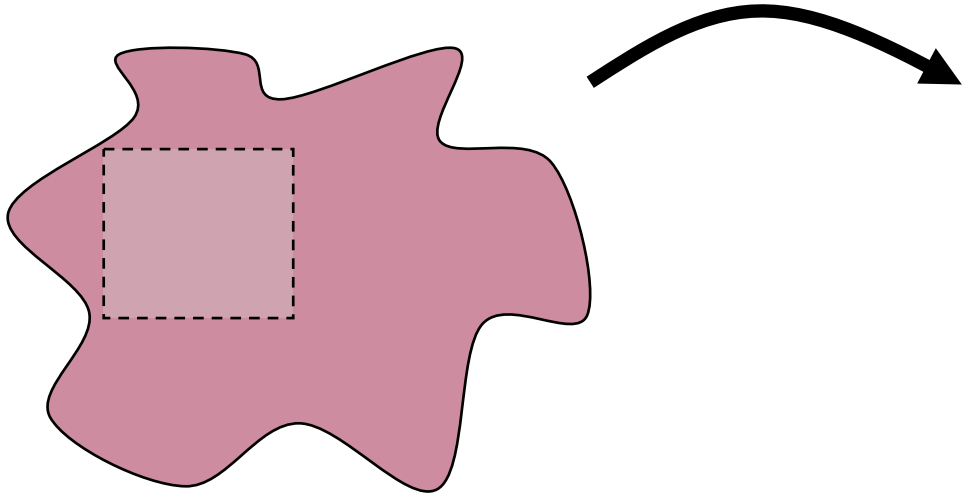
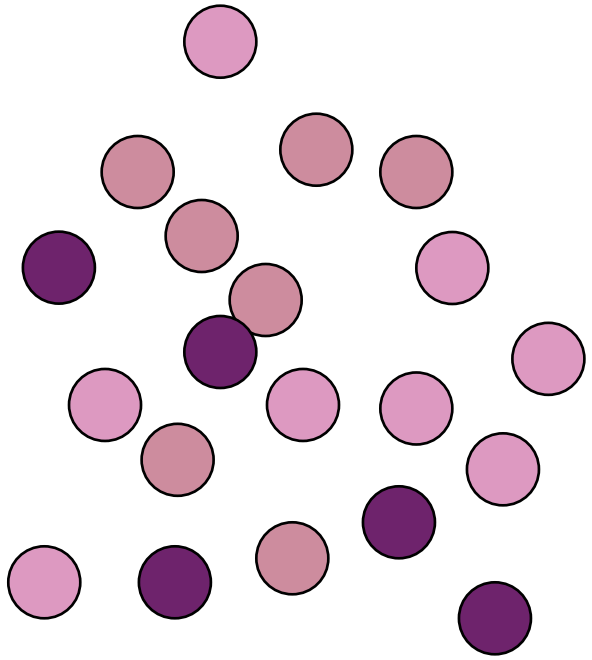


Historically … limitations of single-cell RNA-seq



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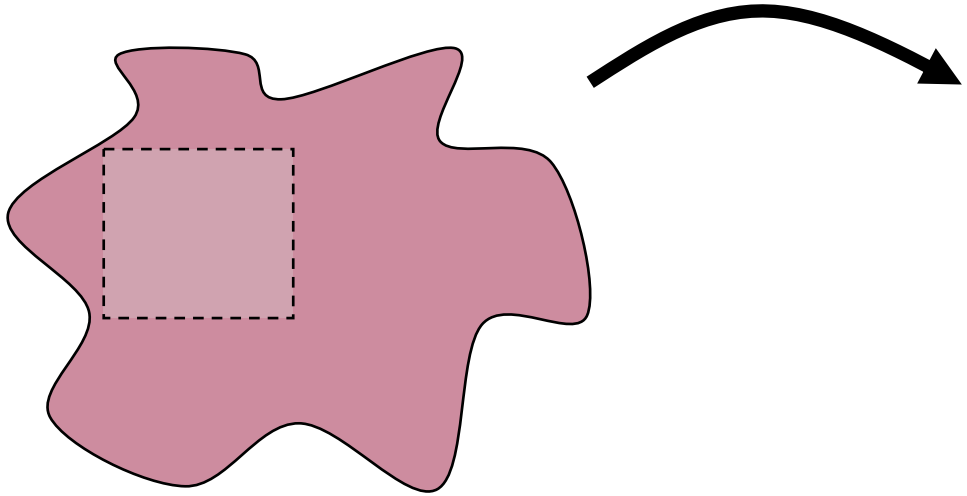
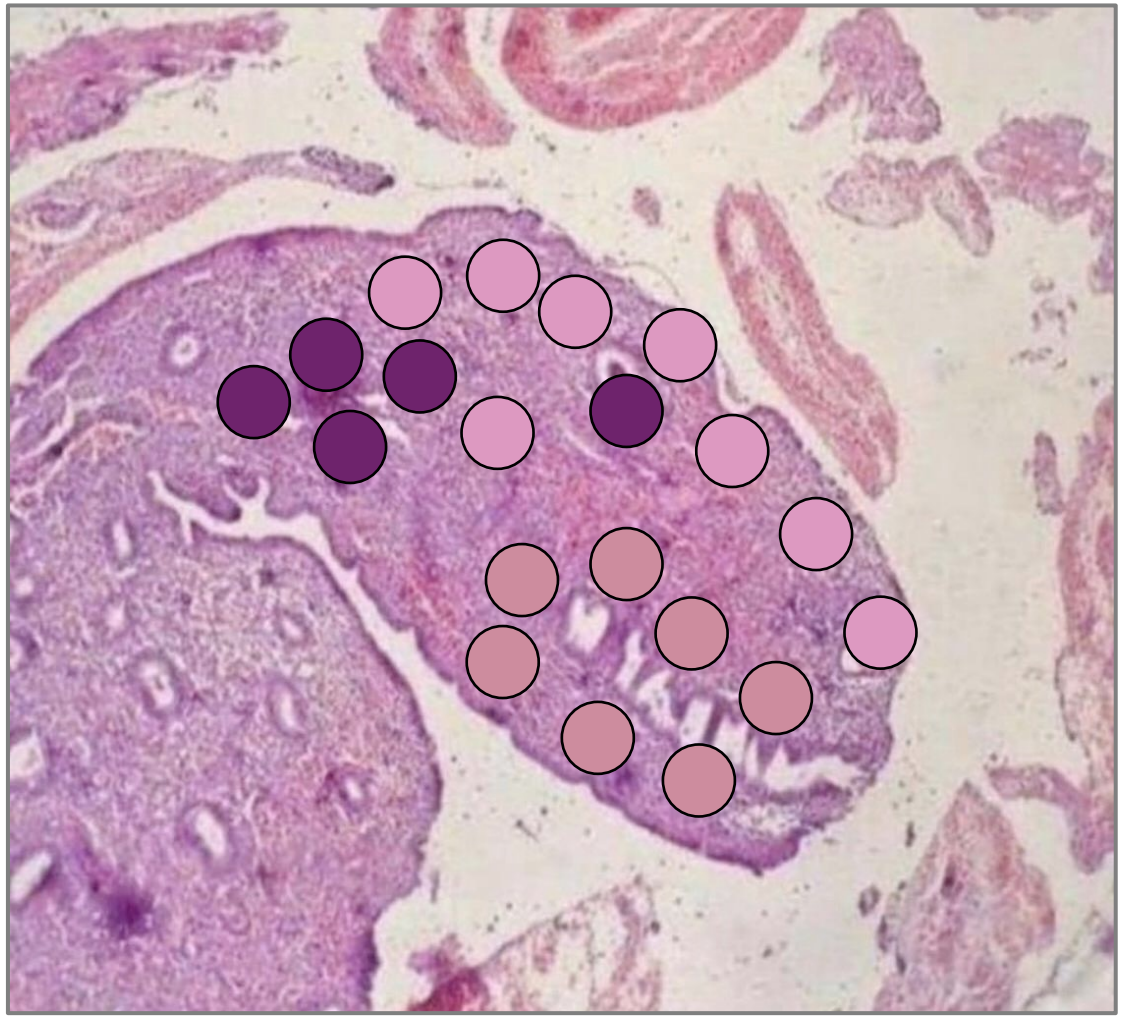
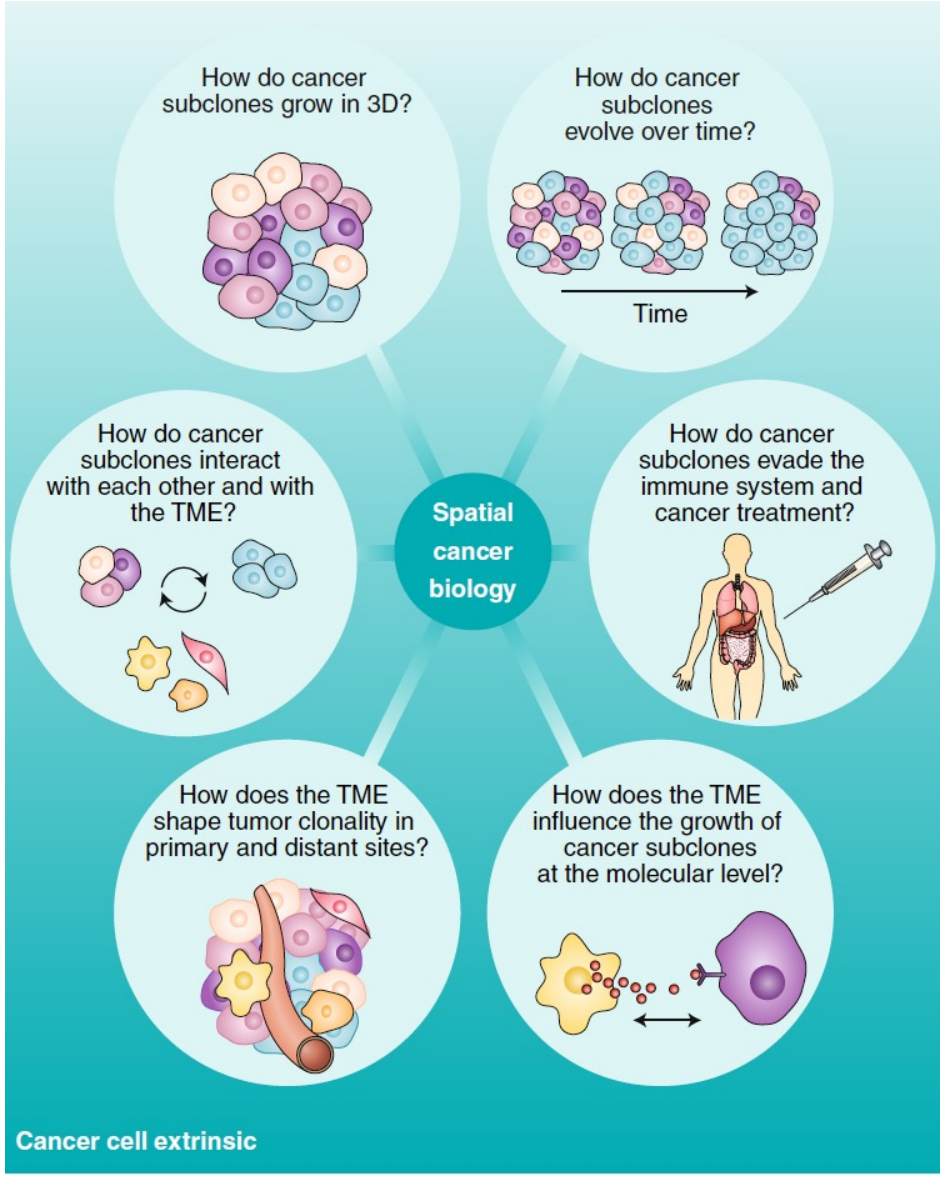
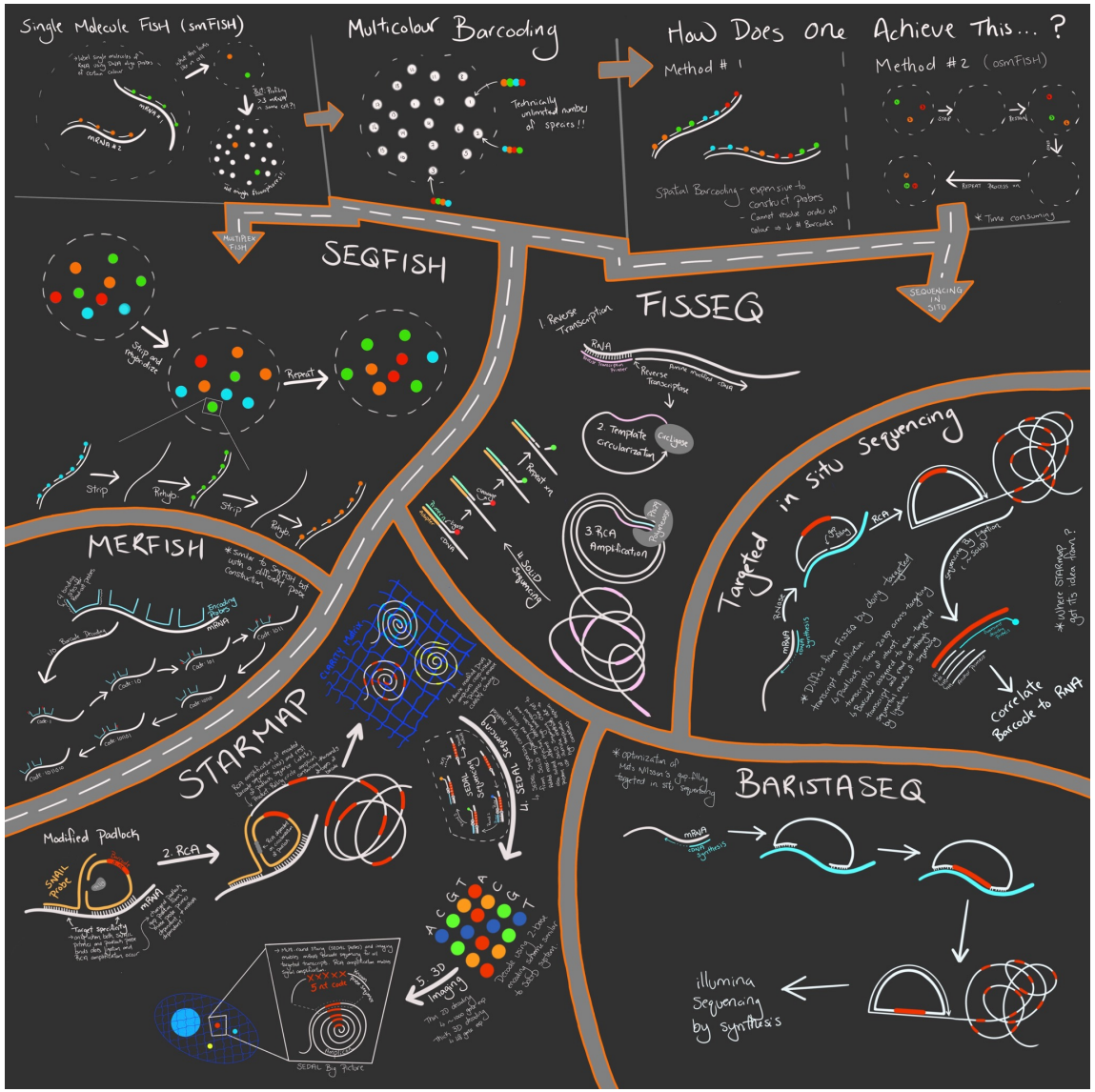




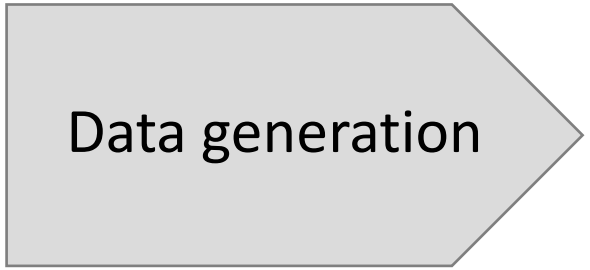
How to choose and create a spatial omics dataset?

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





The different steps in spatial omics research



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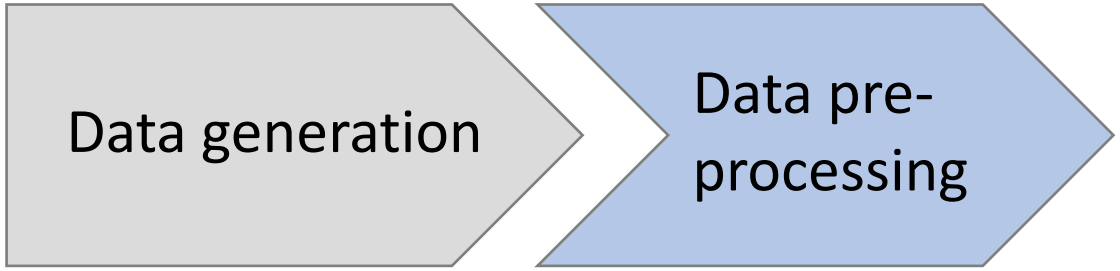

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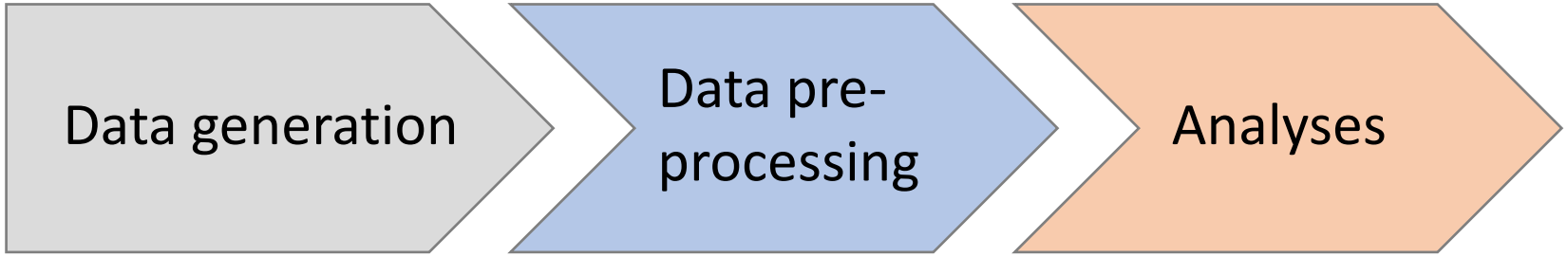

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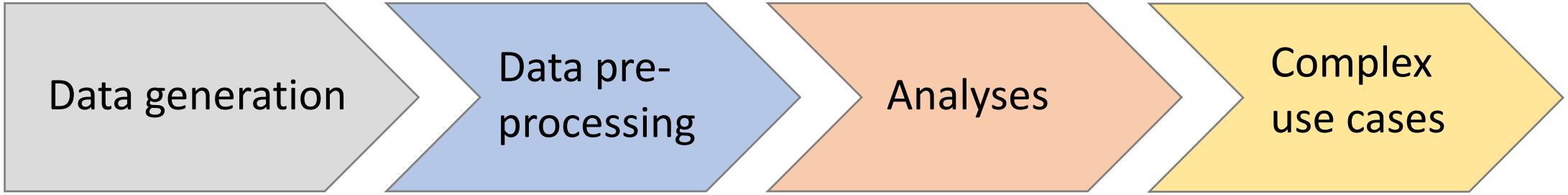

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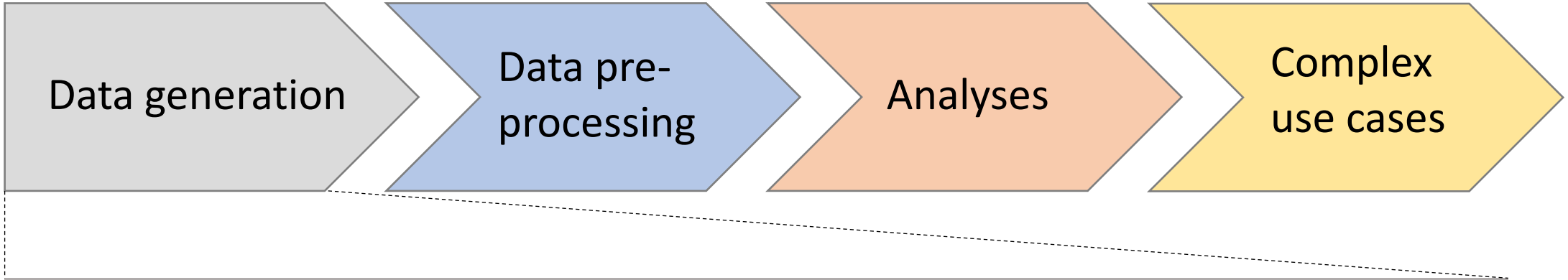


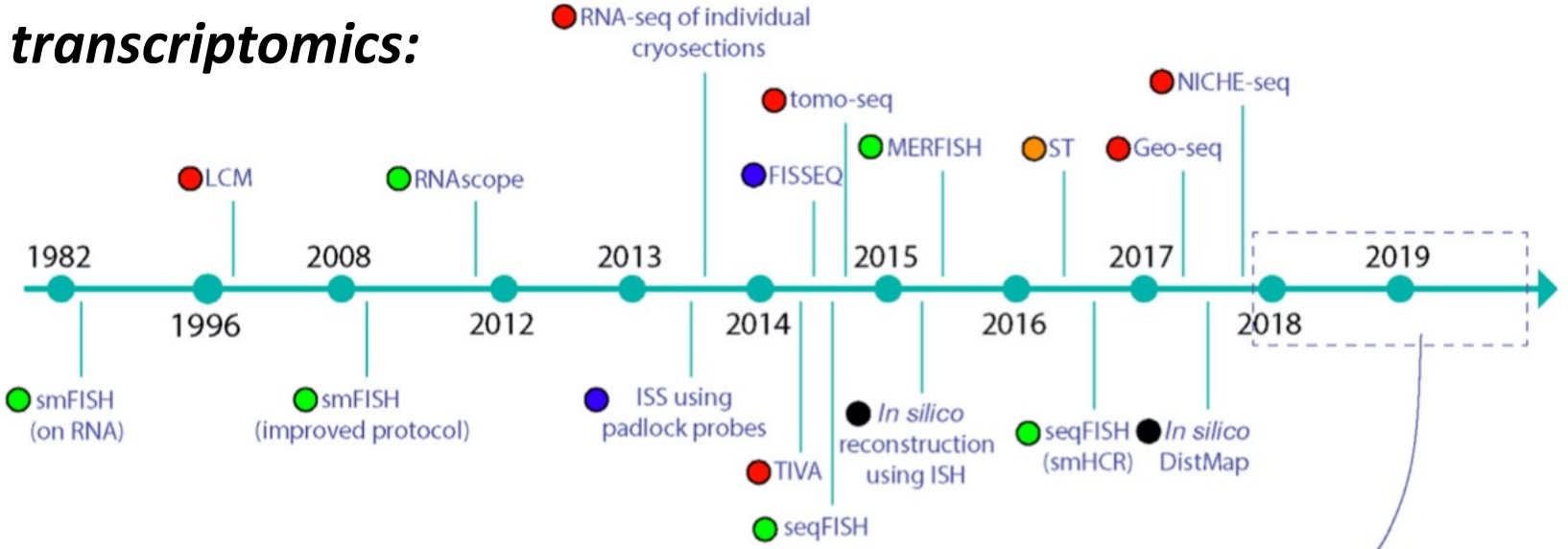
- Variety in data characteristics: each technology is different
- Variety of data outputs: spatial is a multi-modal experiment by default

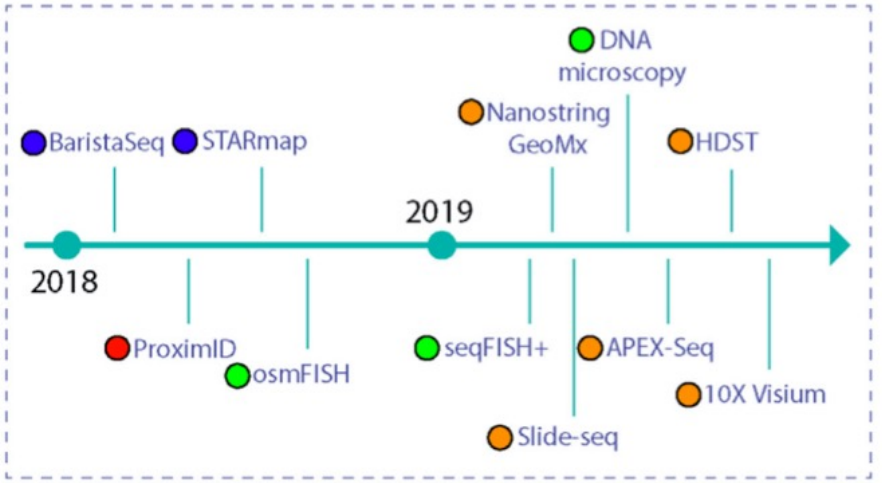
Long history of spatial technology development

Spatial proteomics:

CODEX
CyCIF
MIBI
IMC
seqIF (lunaphore)
…

Spatial transcriptomics:





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

Asp et al. Spatially resolved transcriptomes. *BioEssays 2020*

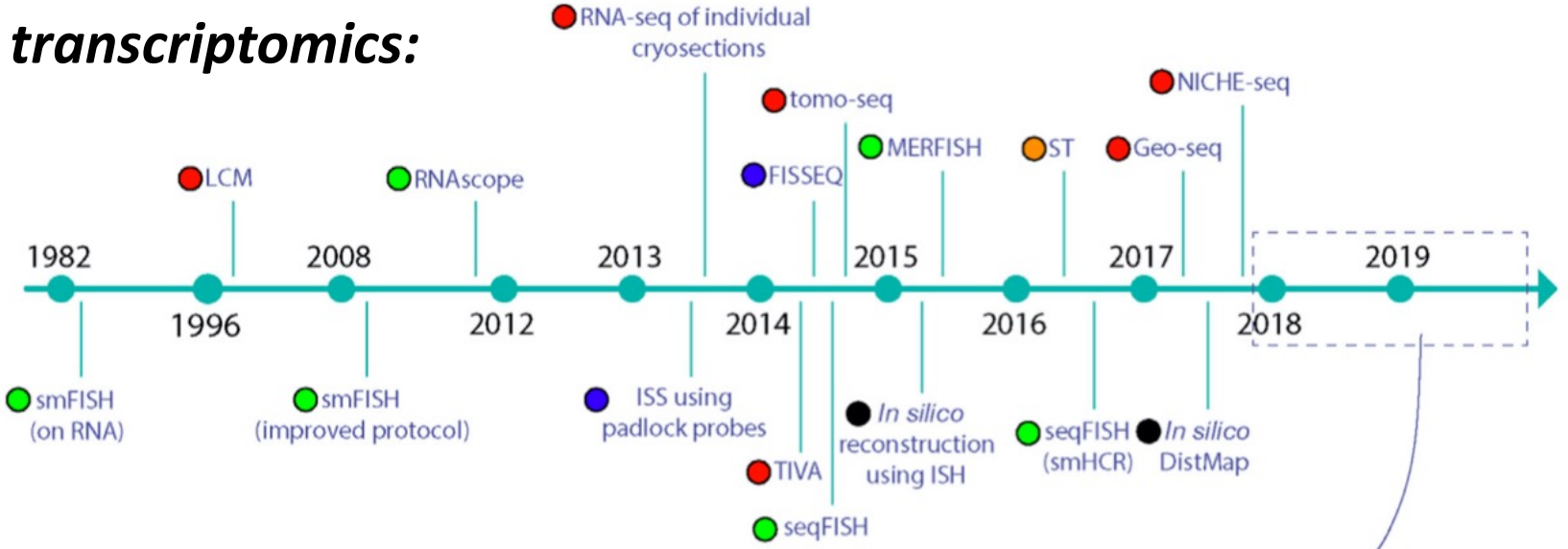
Long history of spatial technology development

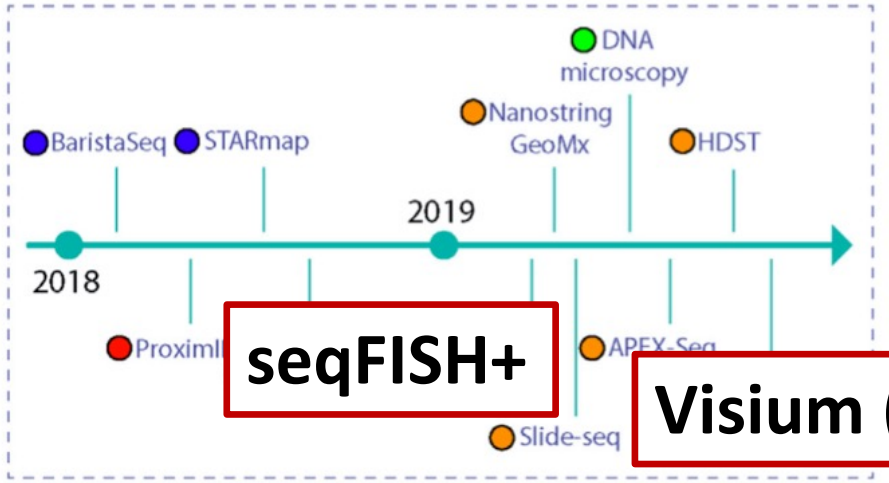
Spatial proteomics:

CODEX
CyCIF
MIBI
IMC

seqIF (lunaphore)

Spatial transcriptomics:





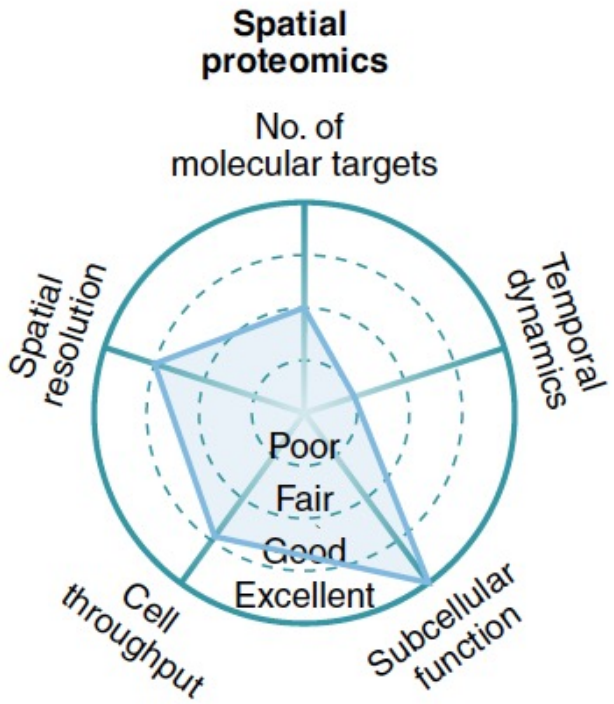
seqFISH+

DBiT-seq
ZipSeq
Split-FISH
seqSCOPE
Pixel-Seq
...

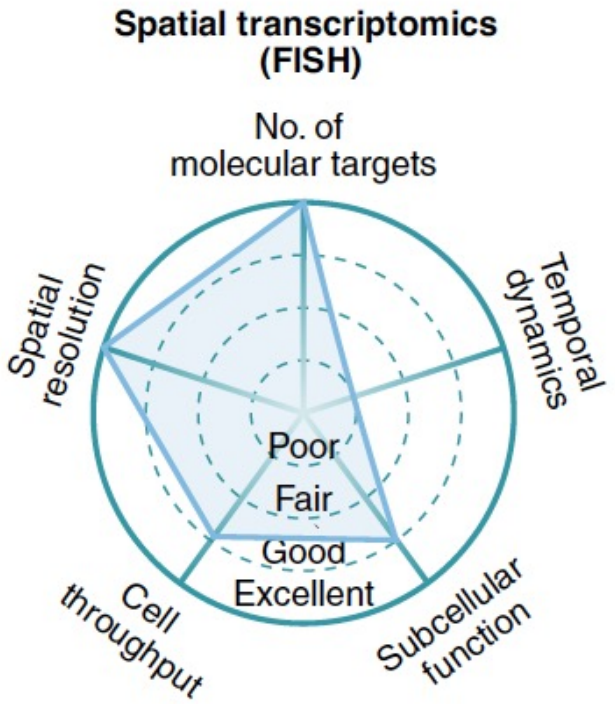
Visium (10X)

Asp et al. Spatially resolved transcriptomes. *BioEssays 2020*

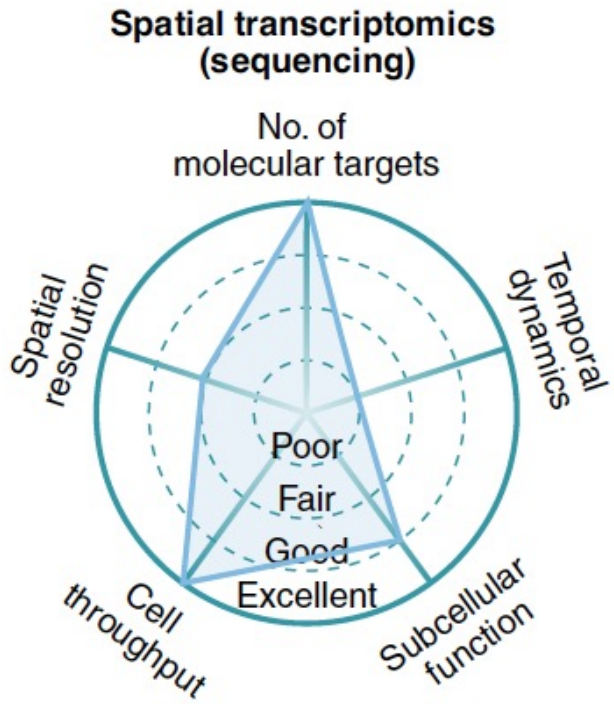
Long history of spatial technology development



seqIF / lunaphore



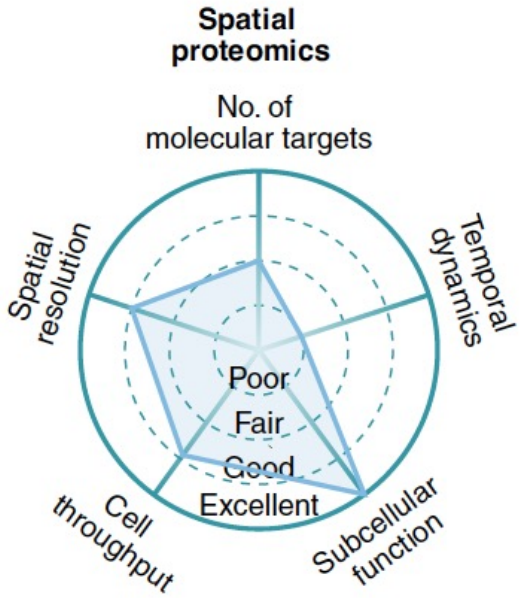
seqFISH+

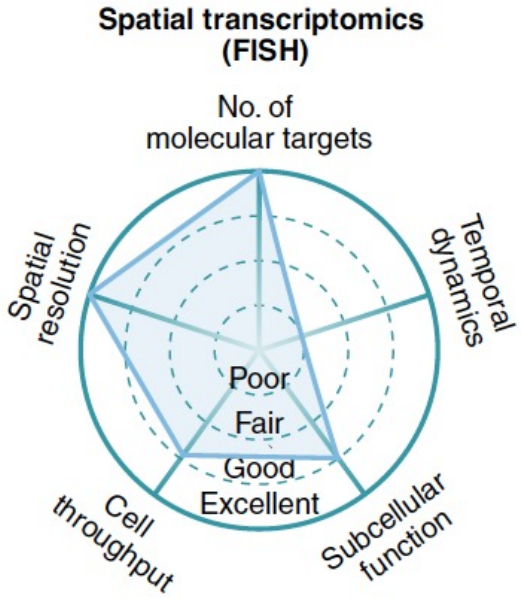


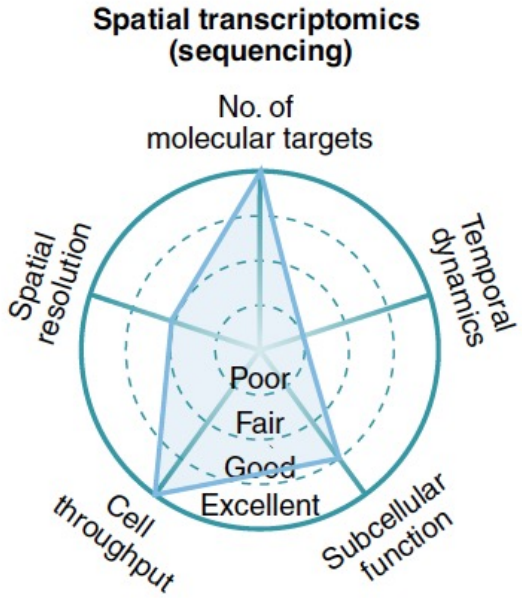
Visium / 10X

Lewis, S. M. *et al.* Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:10.1038/s41592-021-01203-6.

Long history of spatial technology development

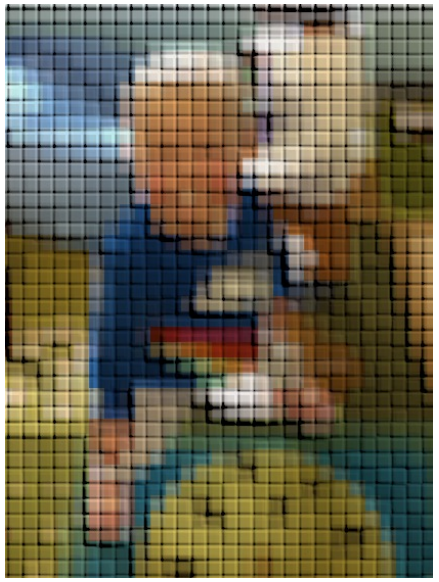




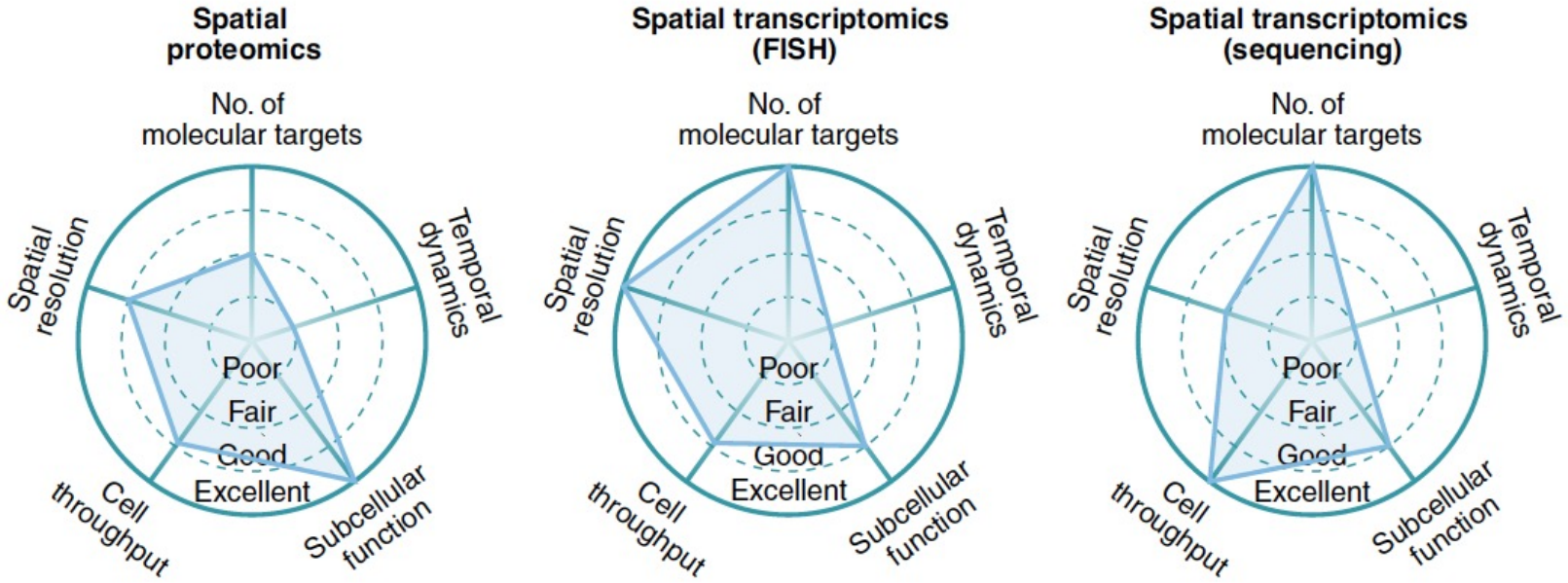


Spatial resolution?

What is the spatial resolution of my picture?



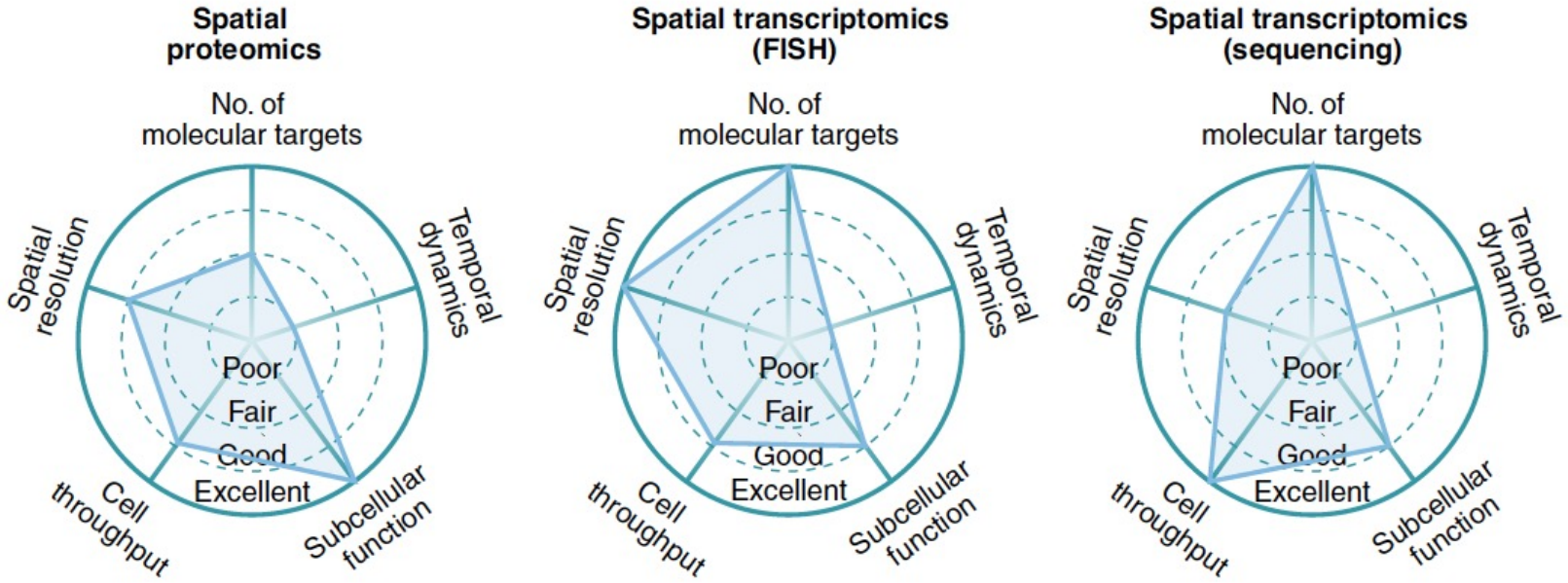
Long history of spatial technology development



Number of molecular targets?

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

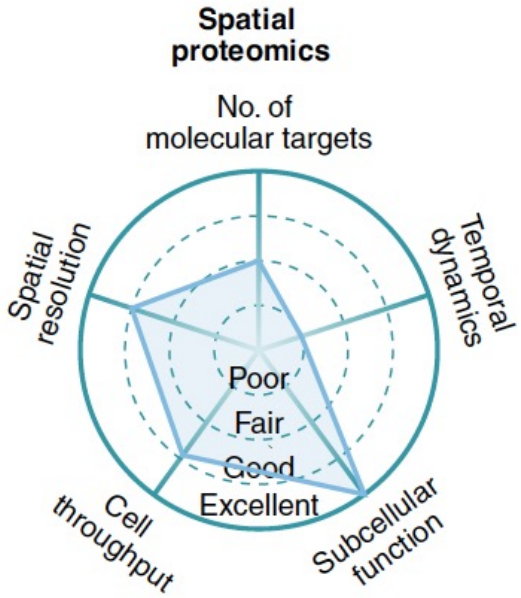
Long history of spatial technology development

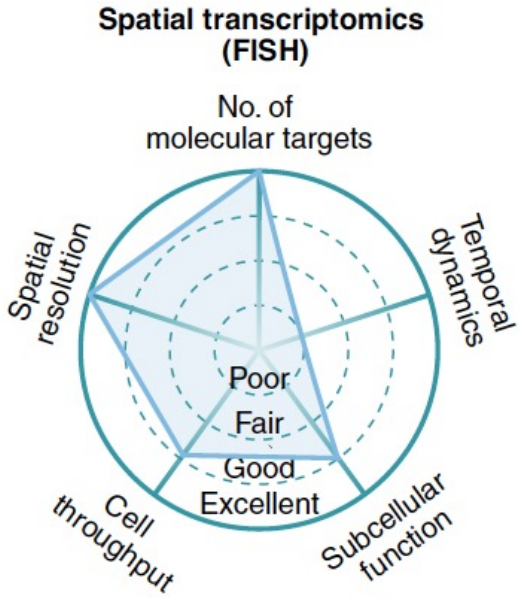


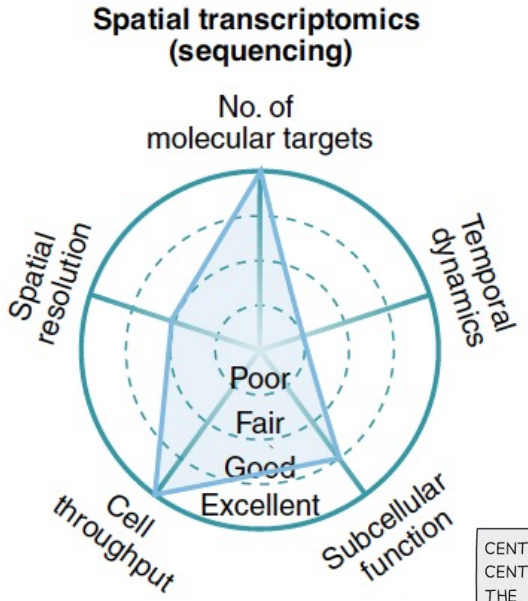
Temporal dynamics?

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

Long history of spatial technology development



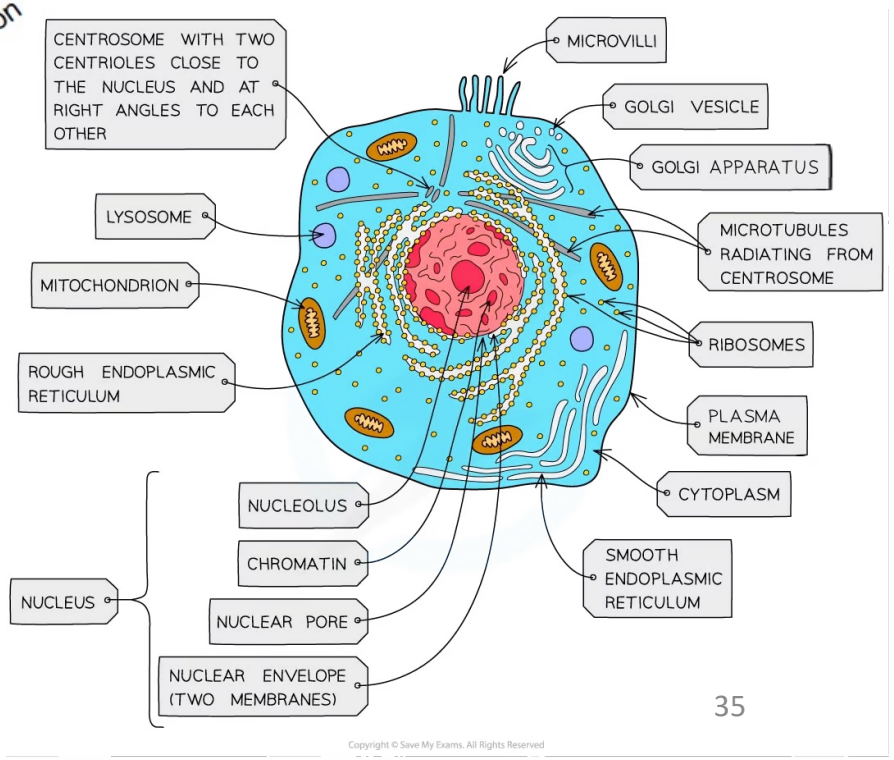




Subcellular function?

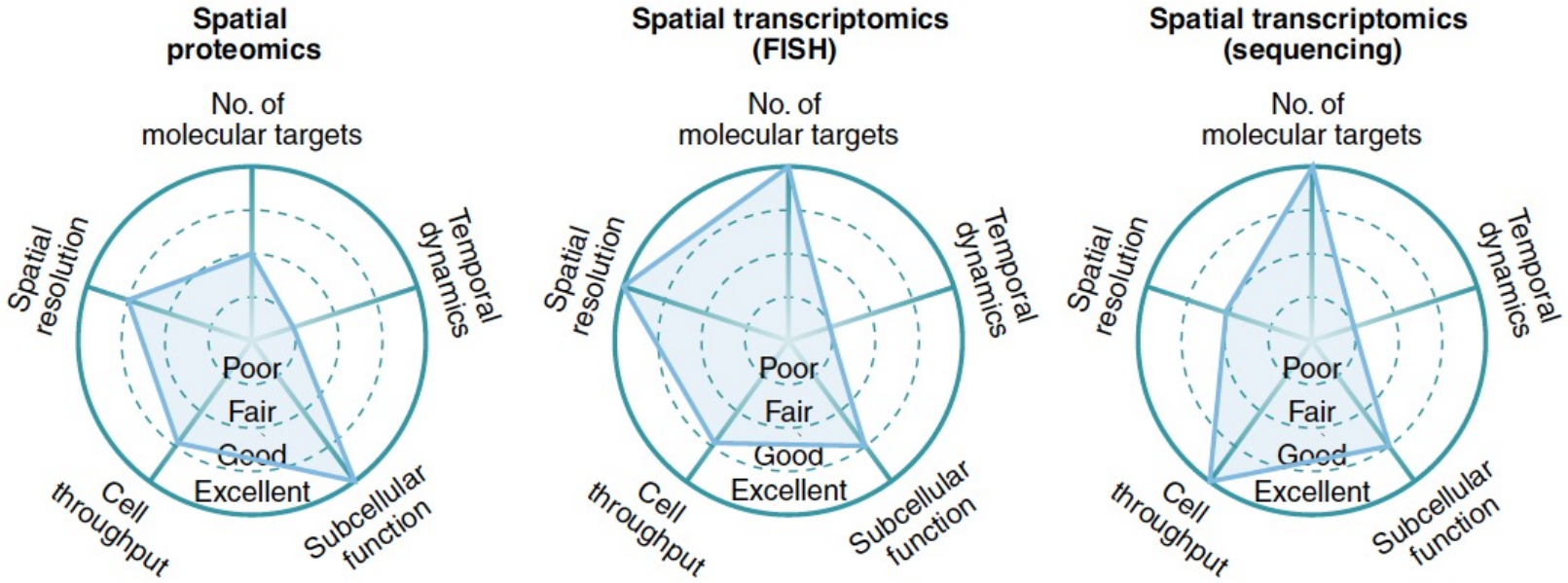
Can the data be used to infer subcellular function or differences?

"The fluid inside a cell's nucleus is 300 times more viscous than honey… " , Zidovska et all



https://physicsworld.com/a/fluid-in-living-cells-is-300-times-more-viscous-than-honey/

Long history of spatial technology development

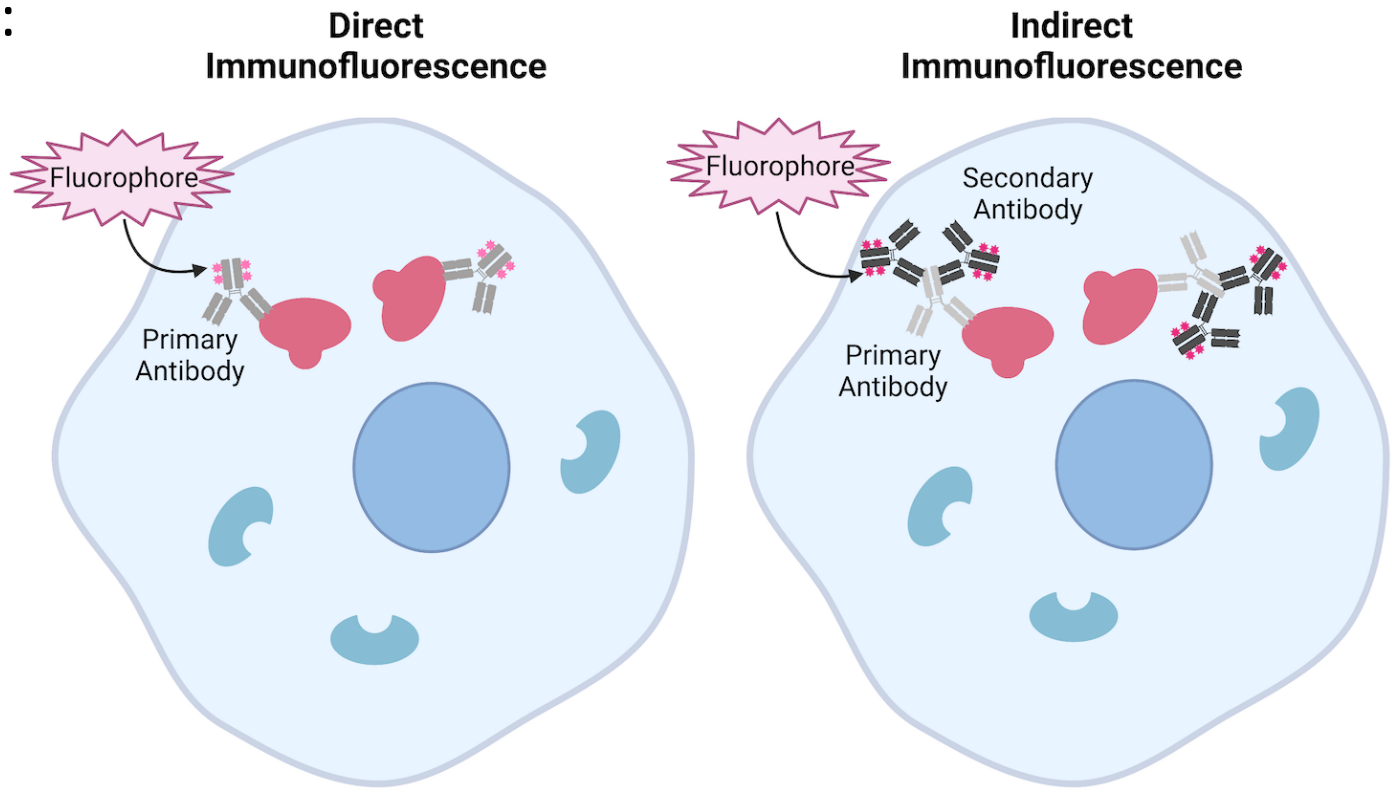


Cell throughput?

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

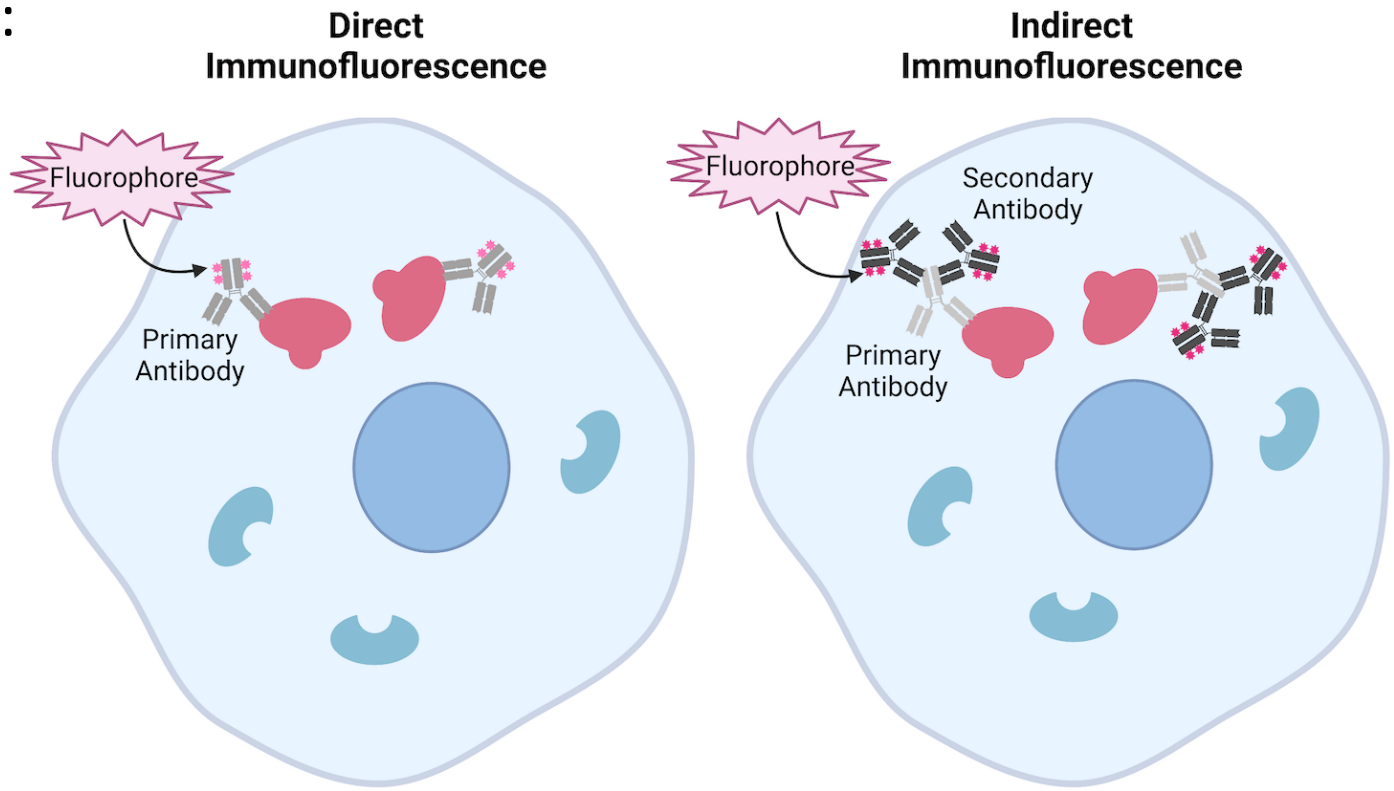
Immunofluorescence:



Problem: limited fluorophores and secondary antibodies

Spatial proteomics

Immunofluorescence:

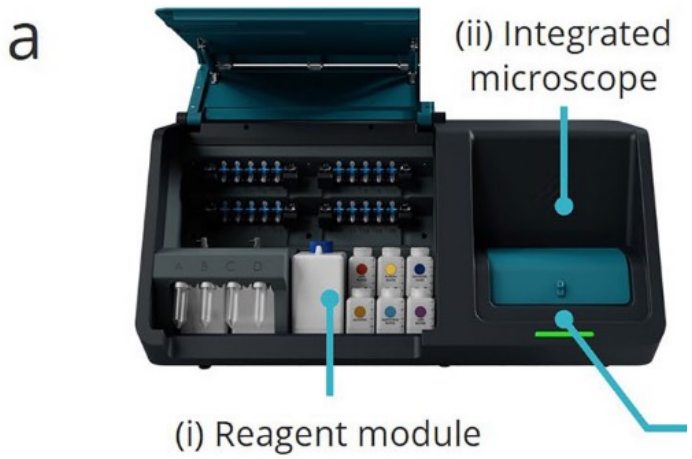


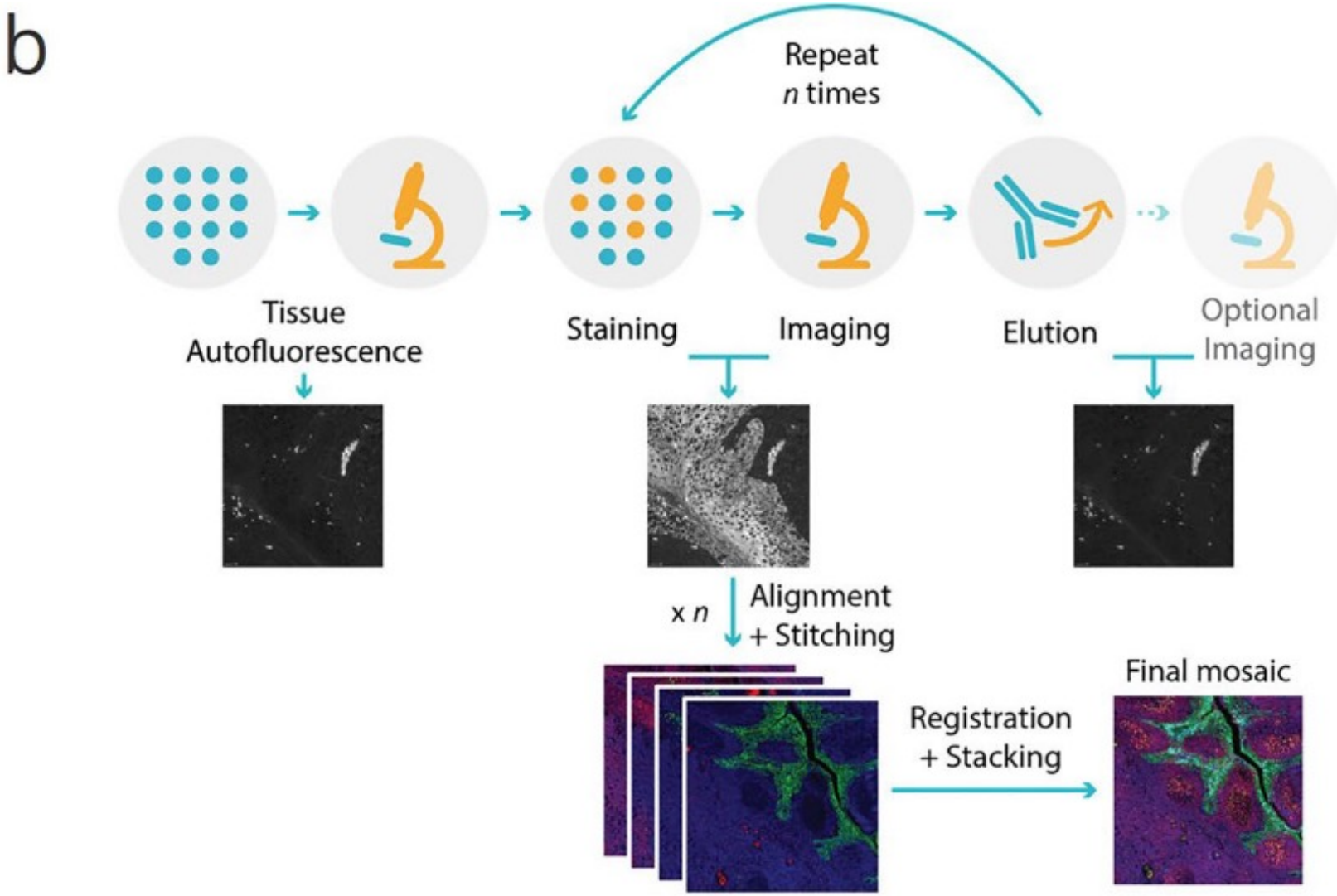
Problem: limited fluorophores and secondary antibodies

Solution: Multiplexing strategies

Spatial proteomics

seqIF / lunaphore

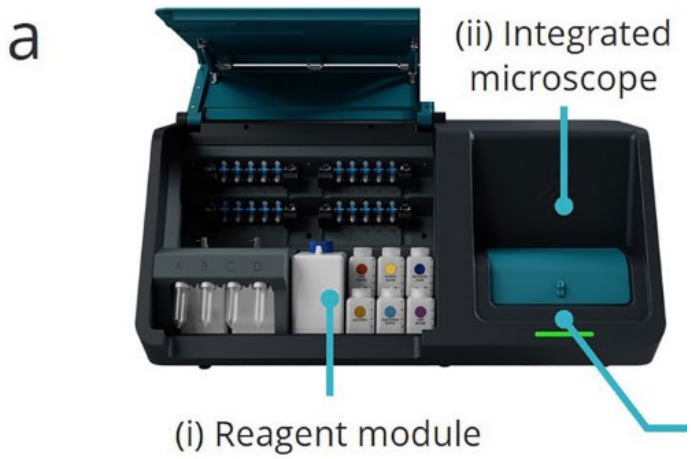


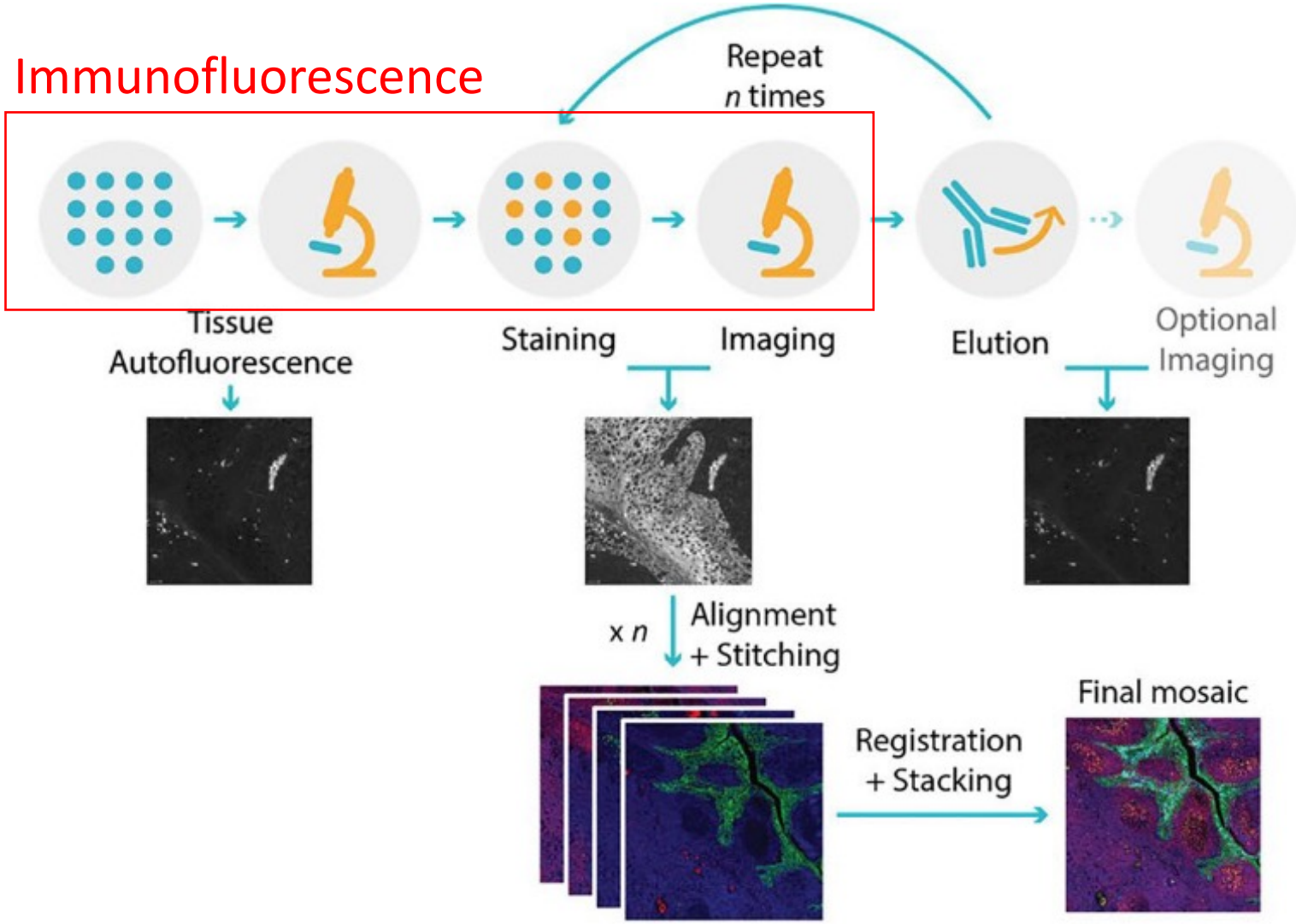


Rivest, F. *et al.* Fully automated sequential immunofluorescence (seqIF) for hyperplex spatial proteomics. *Sci Rep* **13**, 16994 (2023).

Spatial proteomics

seqIF / lunaphore





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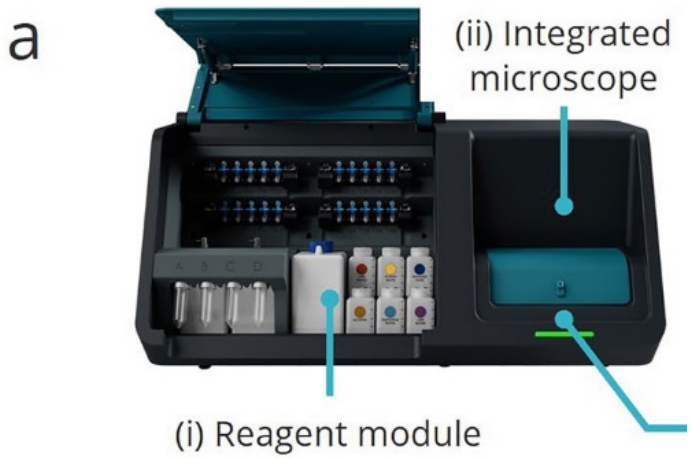
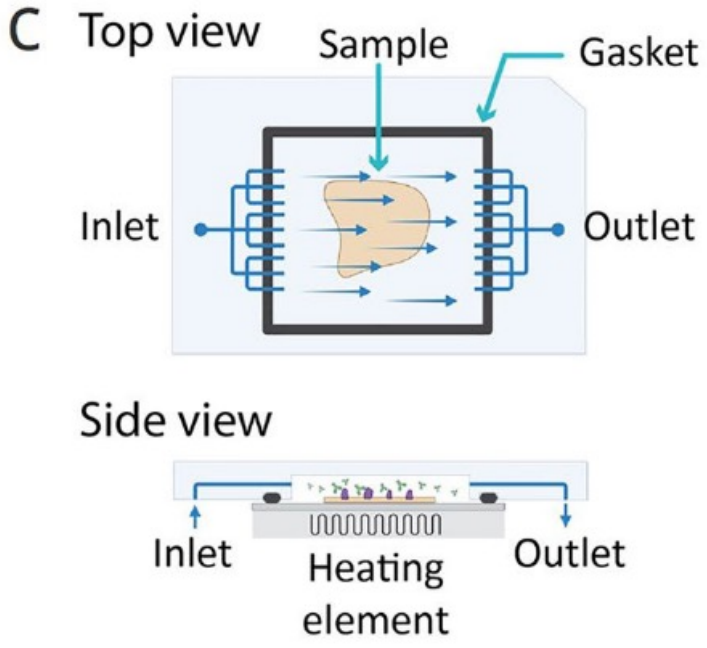
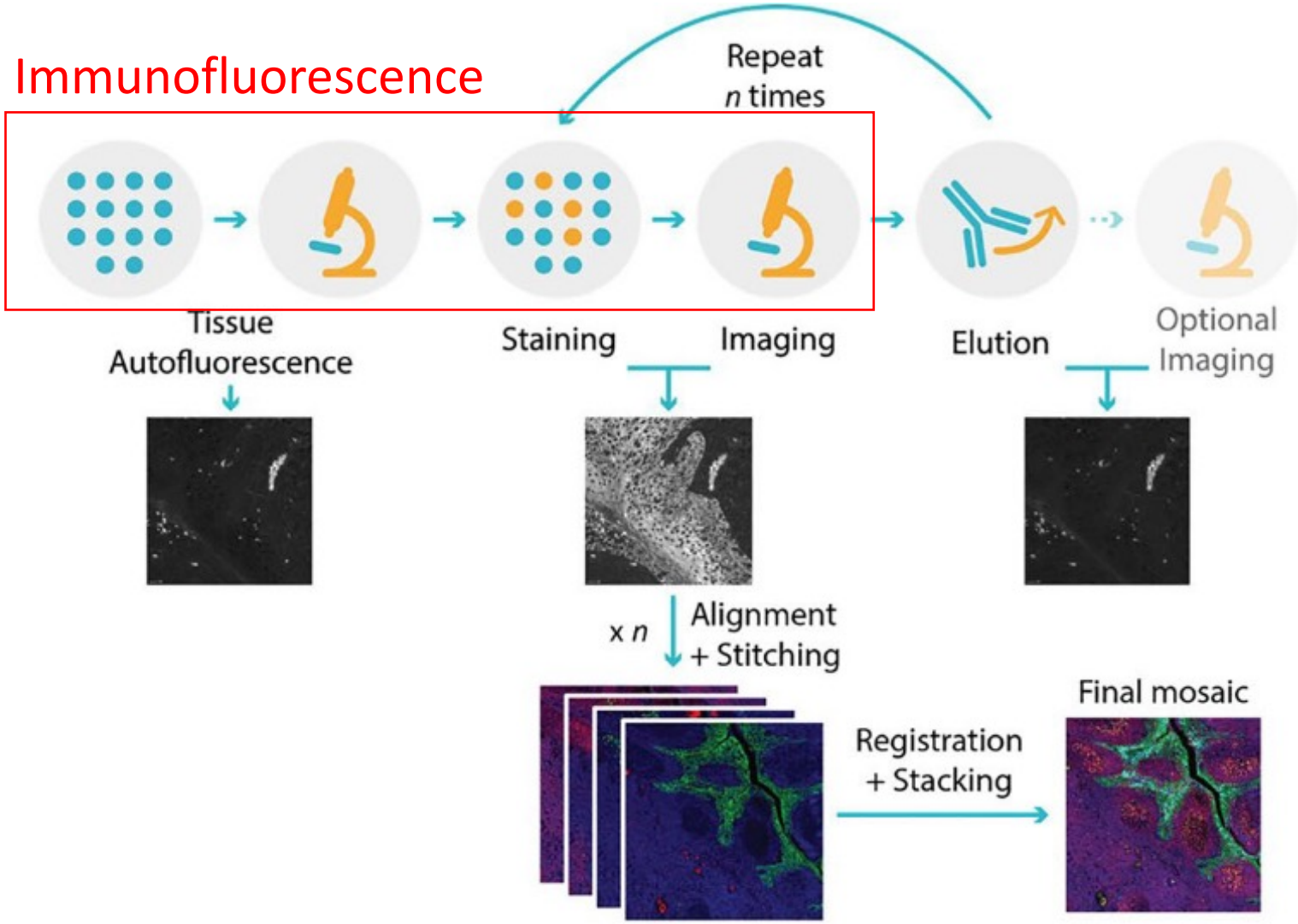
Spatial proteomics

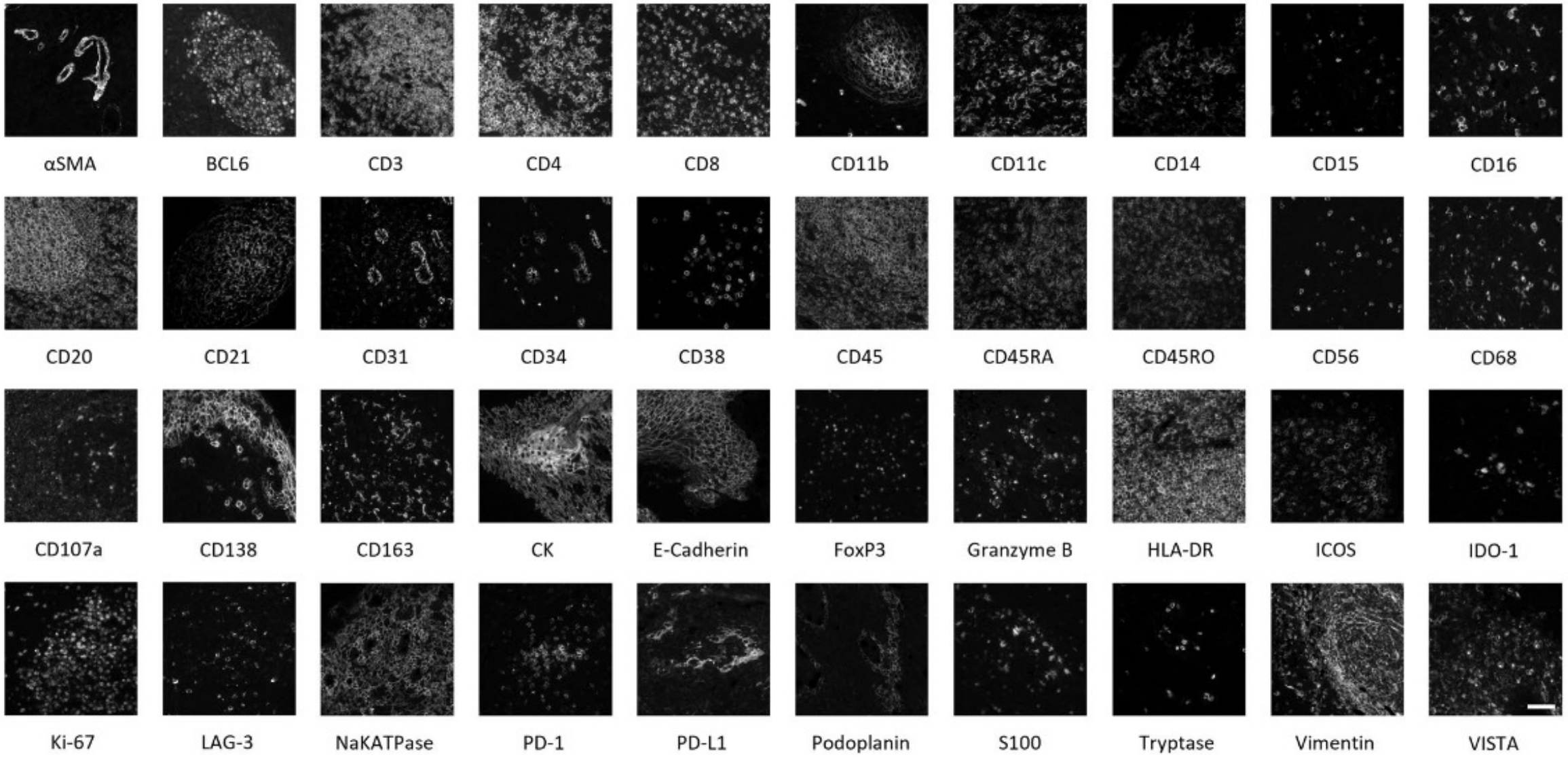
seqIF / lunaphore







Spatial proteomics **seqIF / lunaphore**



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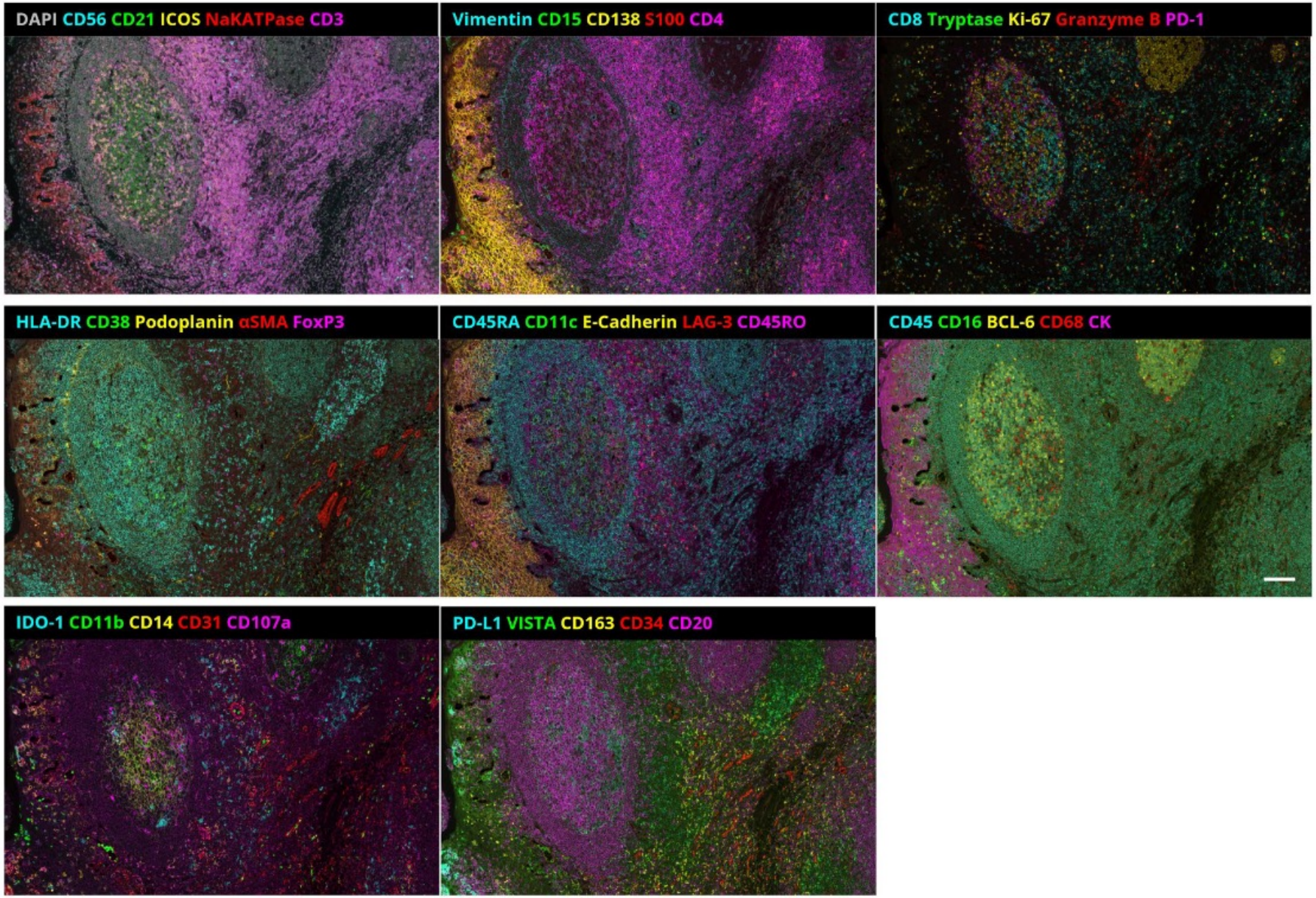
Spatial proteomics

seqIF / lunaphore



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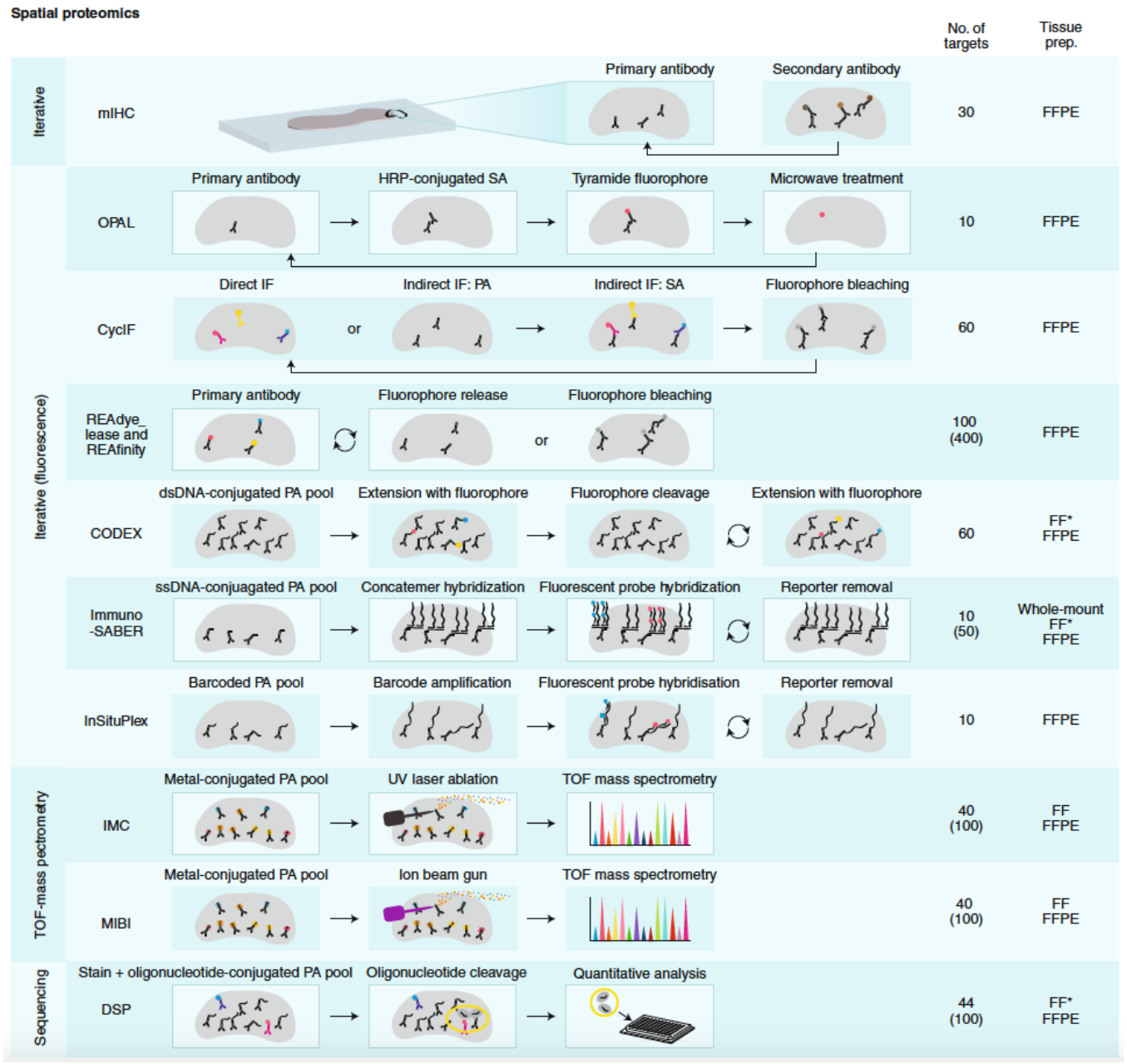
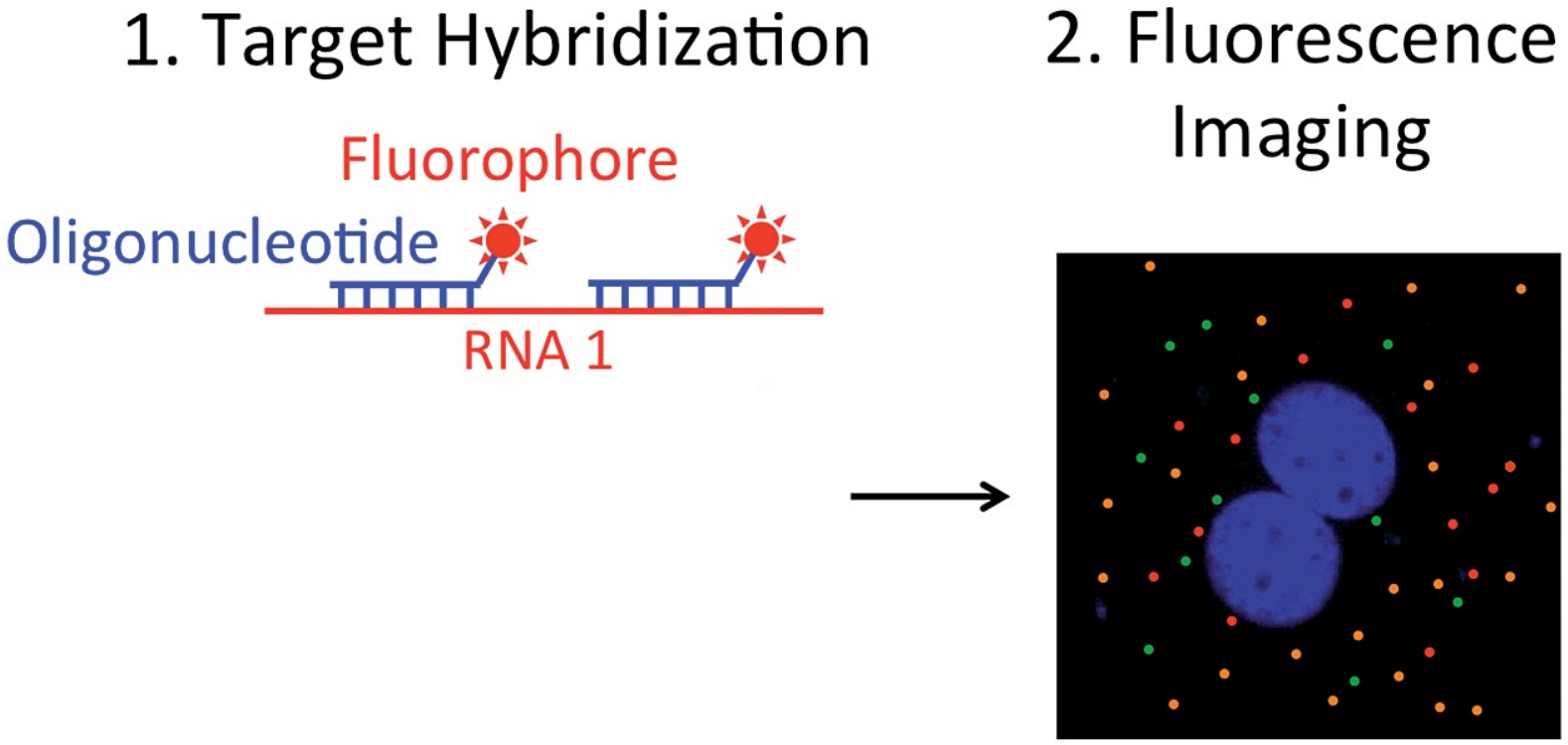
Spatial proteomics

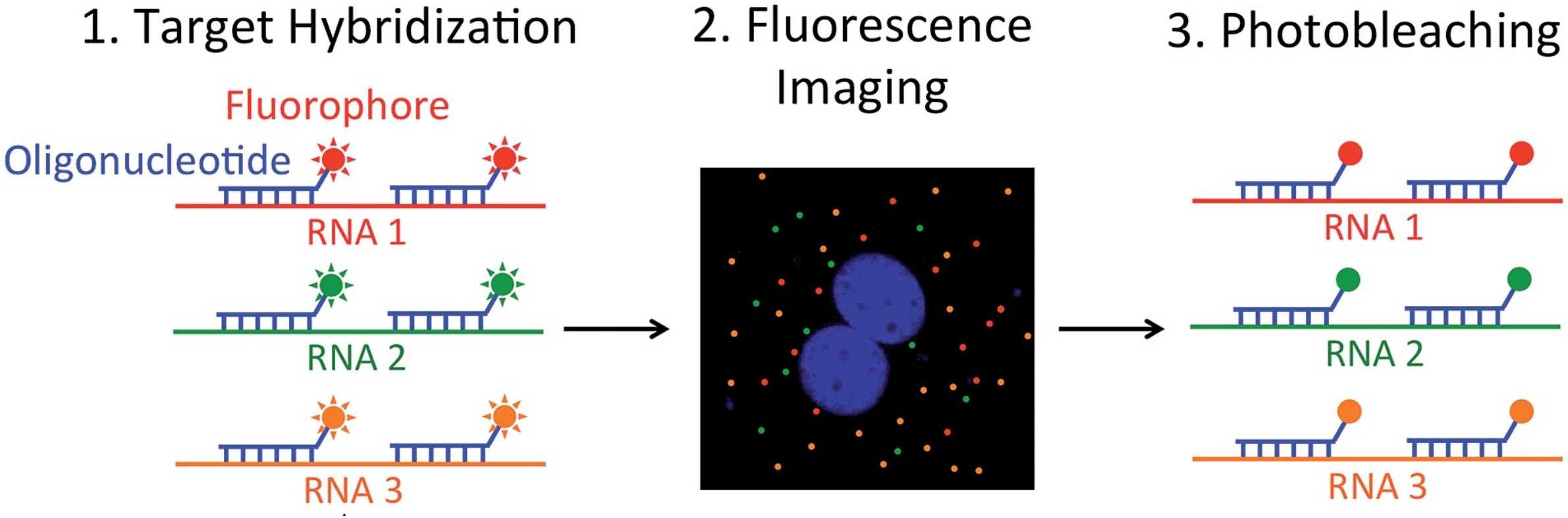


Lewis, S. M. *et al.* Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:10.1038/s41592-021-01203-6.

Spatial transcriptomics (FISH)



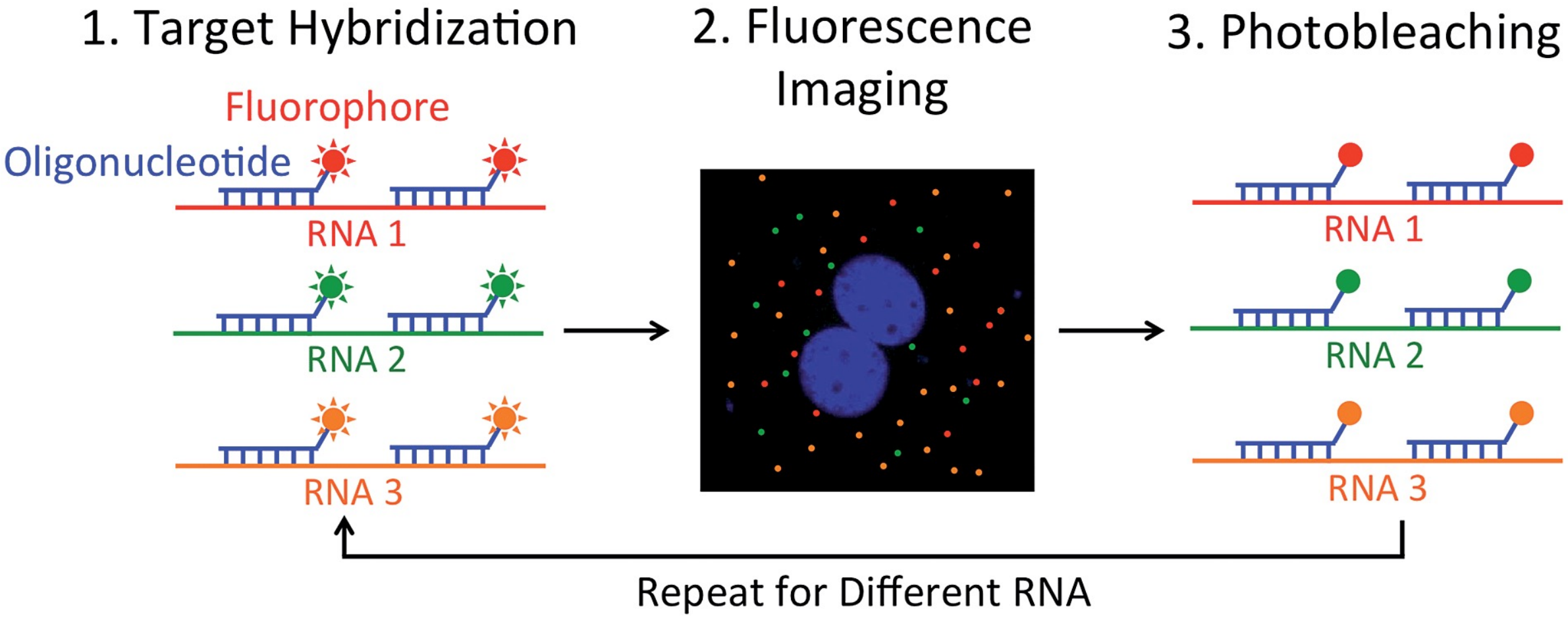
Spatial transcriptomics (FISH)



Problem: limited fluorophores

Solution: Multiplexing strategies

Spatial transcriptomics (FISH)



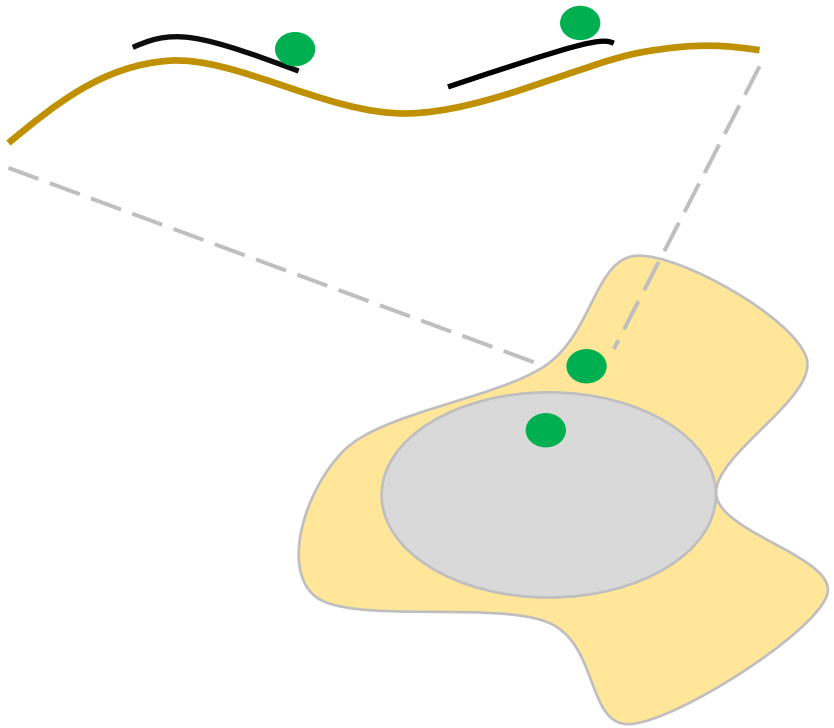
Problem: limited fluorophores

Solution: Multiplexing strategies

Spatial transcriptomics (FISH) **seqFISH+**

in situ hybridizations

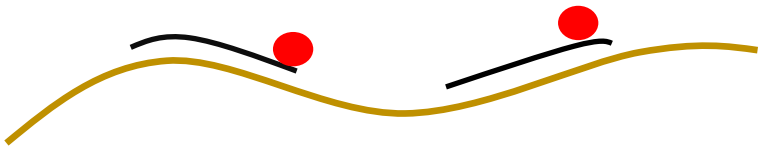
- seqFISH(+)
- merFISH
- osmFISH
- smFISH
- splitFISH
- ...

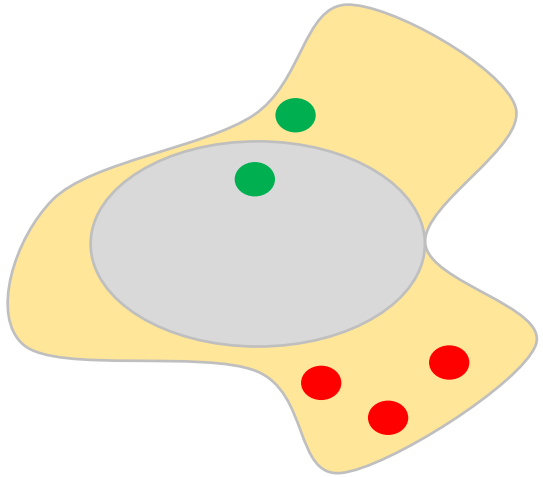


transcript 1 =

Spatial transcriptomics (FISH) **seqFISH+**

in situ hybridizations



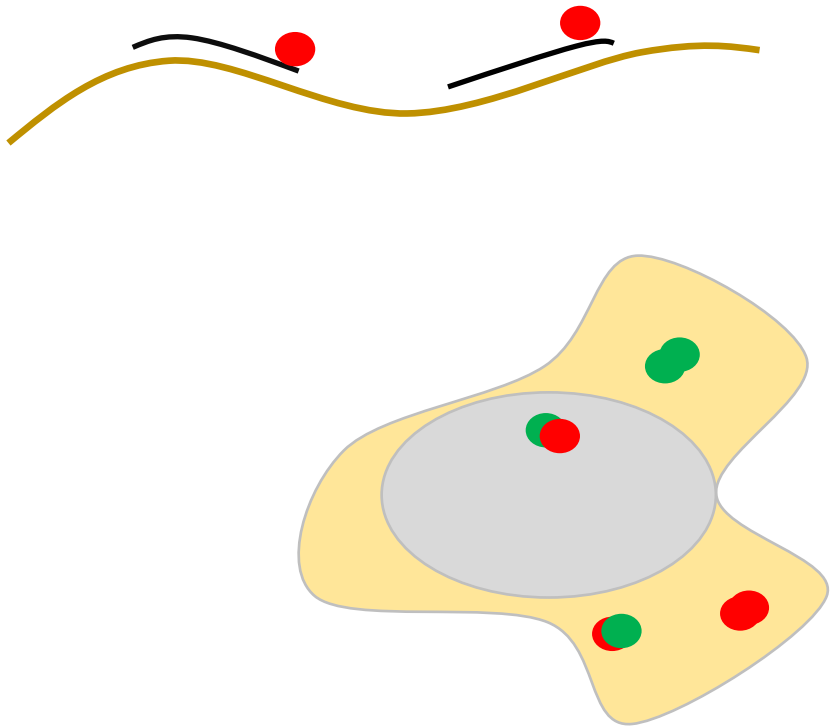


transcript 1 =

transcript 2 =

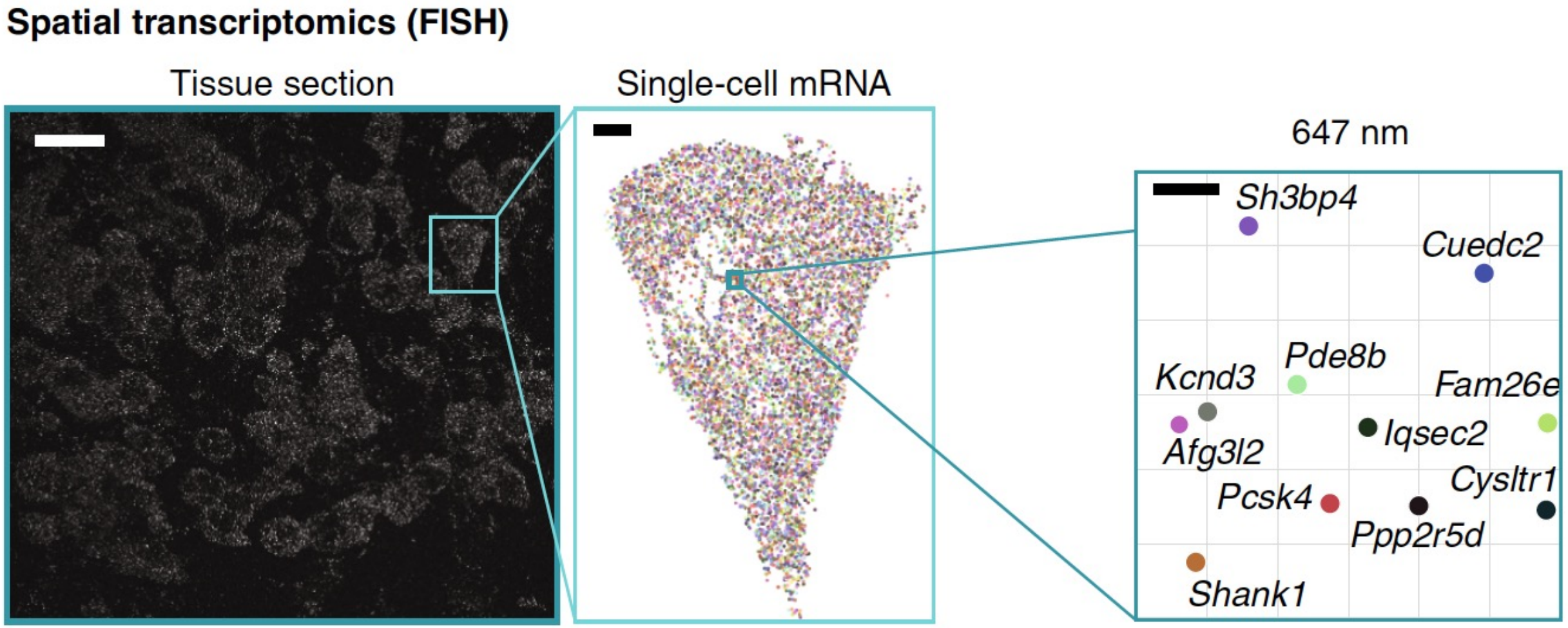
Spatial transcriptomics (FISH) **seqFISH+**

Sequential *in situ* hybridizations



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

Spatial transcriptomics (FISH) **seqFISH+**



Eng, C.-H. L. *et al.* Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH. *Nature* **568**, 235–239 (2019).

Spatial transcriptomics (FISH)

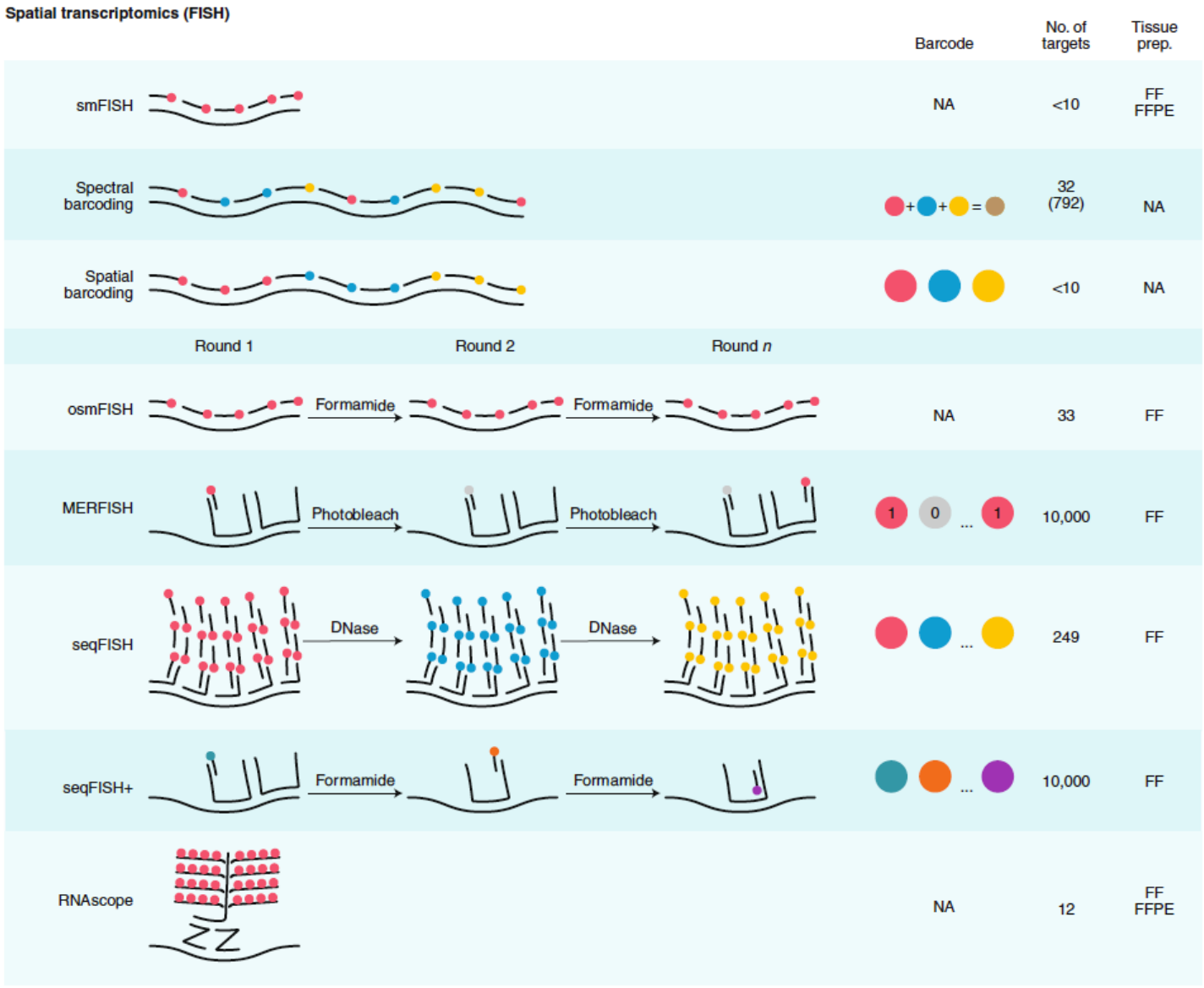
Box 1 | Calculating multiplexing capacity

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

Lewis, S. M. *et al.* Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:10.1038/s41592-021-01203-6.

Spatial transcriptomics (FISH)



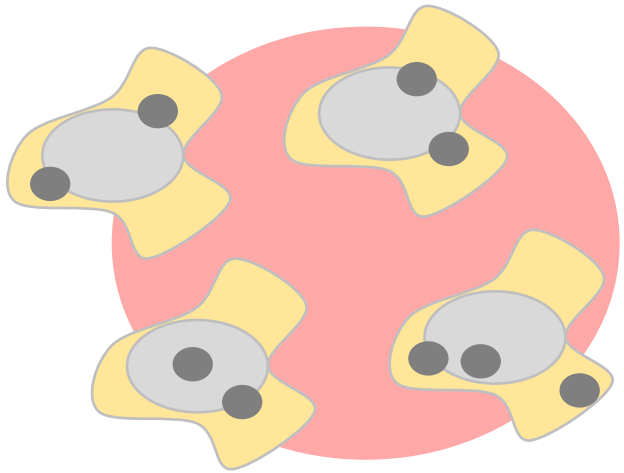
Lewis, S. M. *et al.* Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:10.1038/s41592-021-01203-6.

Spatial transcriptomics (sequencing)

in situ hybridizations

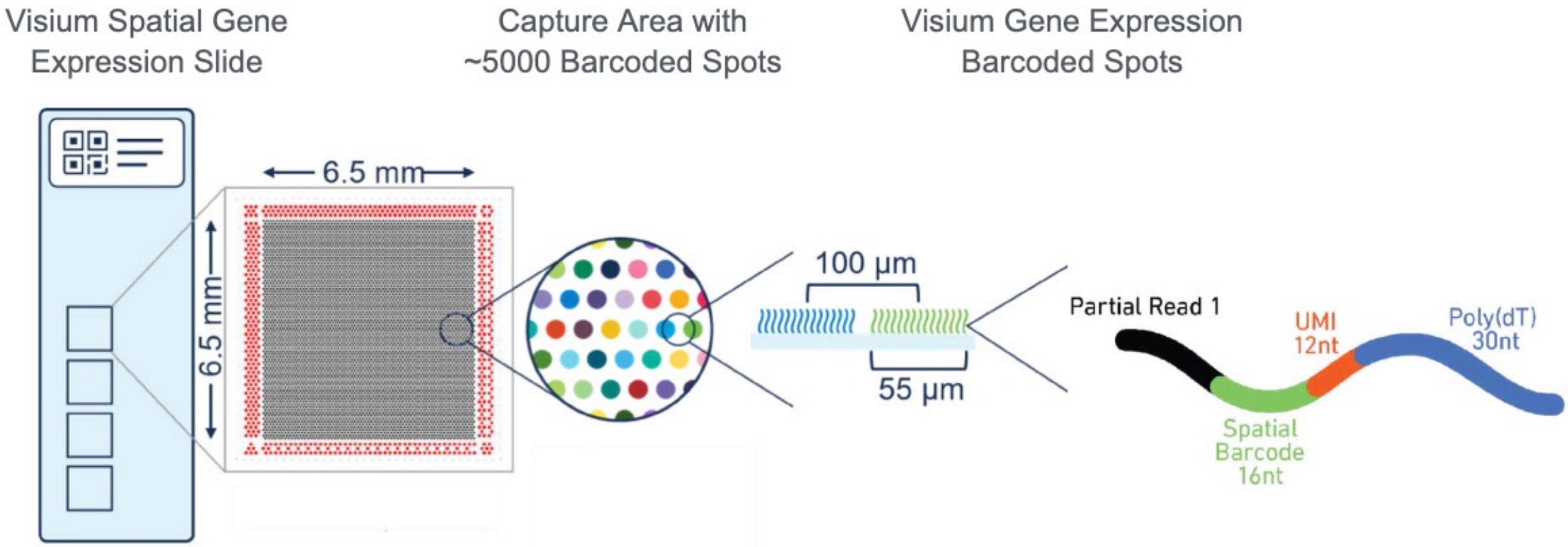
in situ sequencing

in situ capture

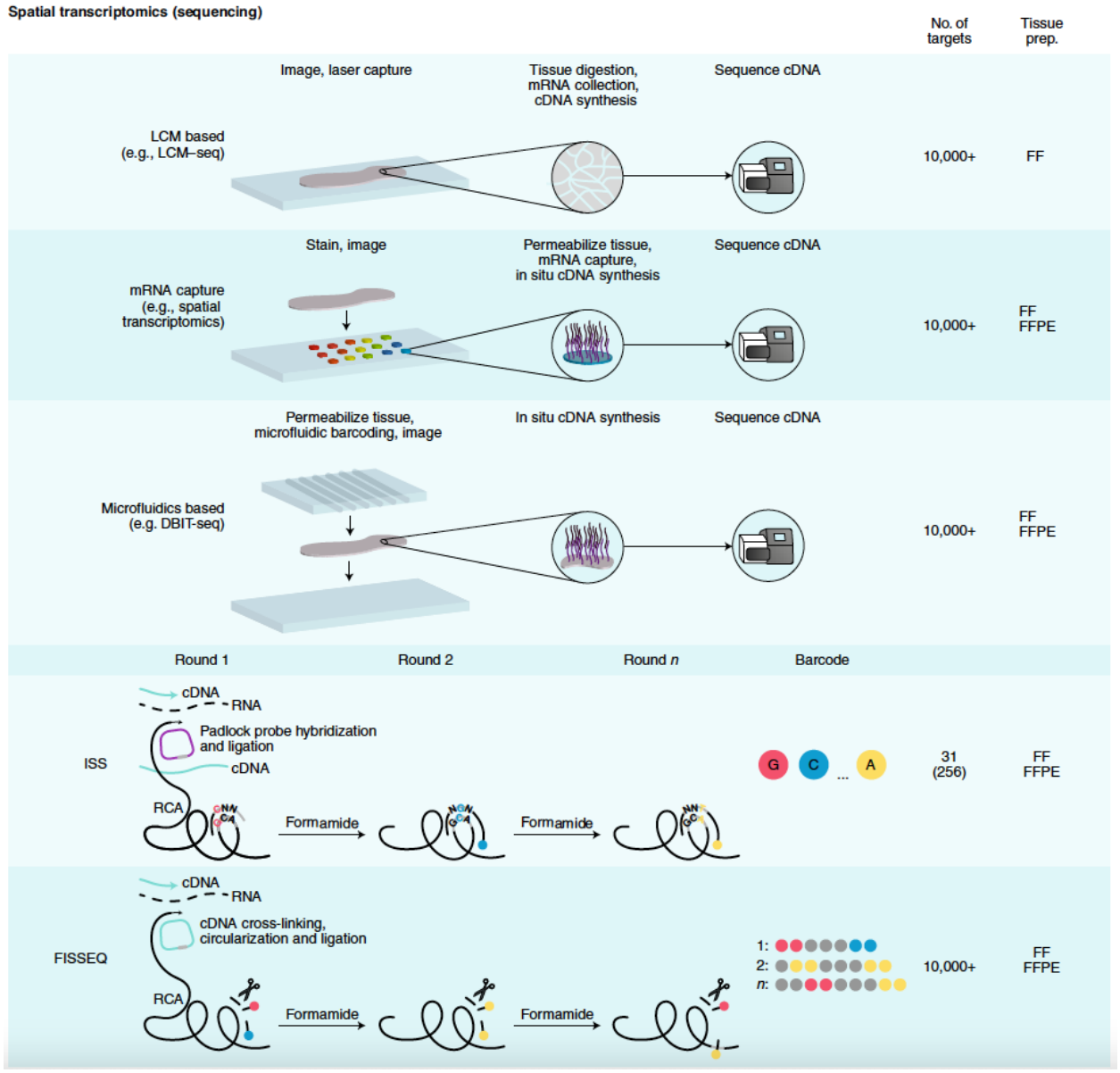


Spatial transcriptomics (sequencing)

Visium / 10X



Spatial transcriptomics (sequencing)



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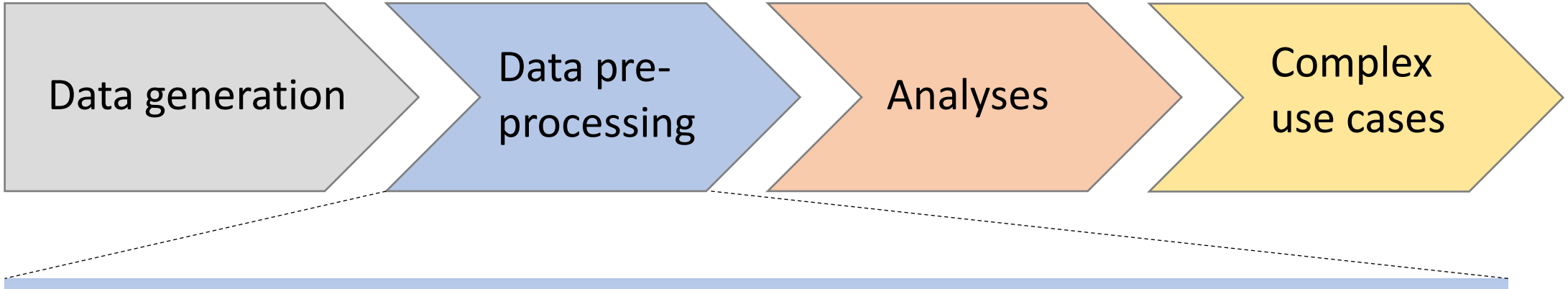
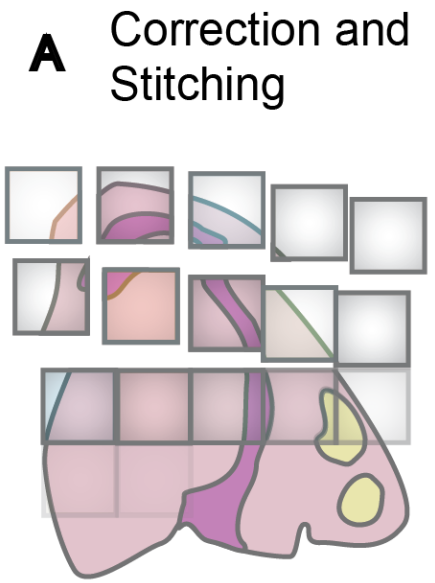
The different steps in spatial omics research



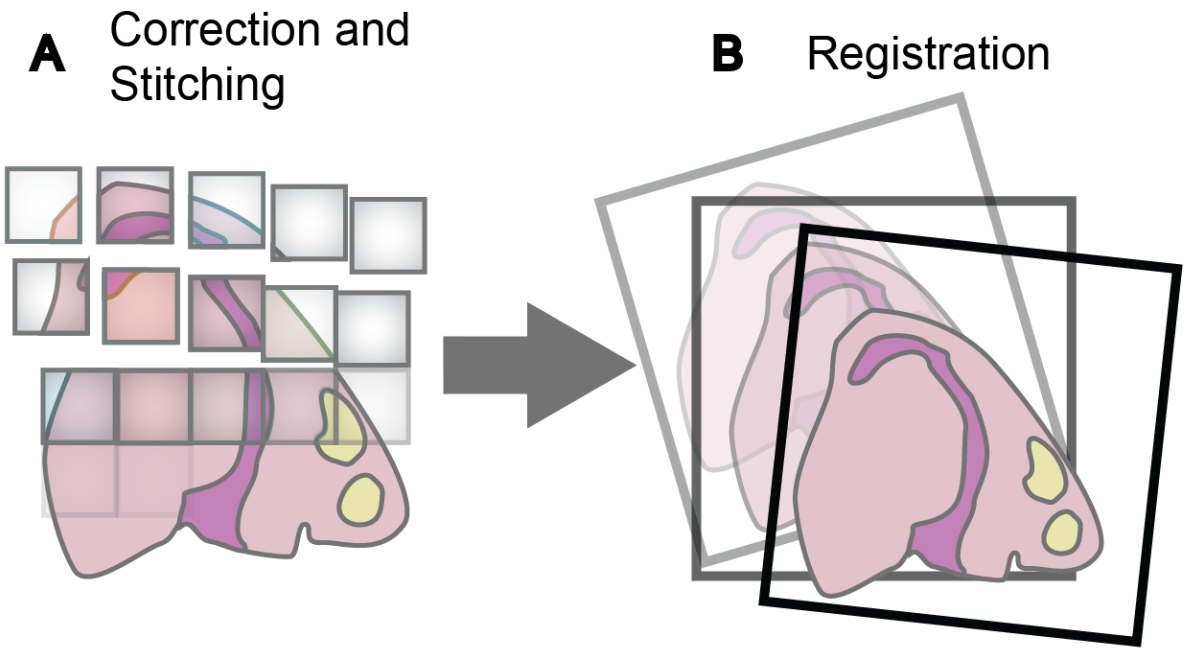
- Image processing
- From raw signal to usable data

Essential pre-processing steps: image stitching



Dries, R. *et al.* Advances in spatial transcriptomic data analysis. *Genome Res* **31**, 1706–1718 (2021).

Essential pre-processing steps: image co-registration



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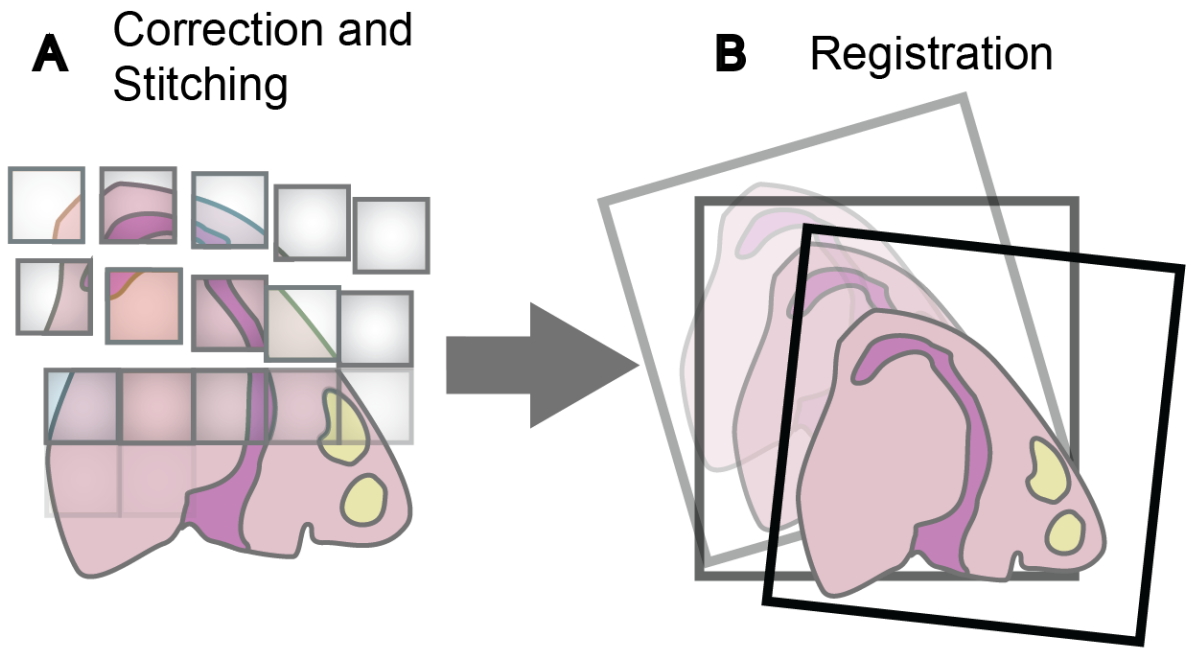

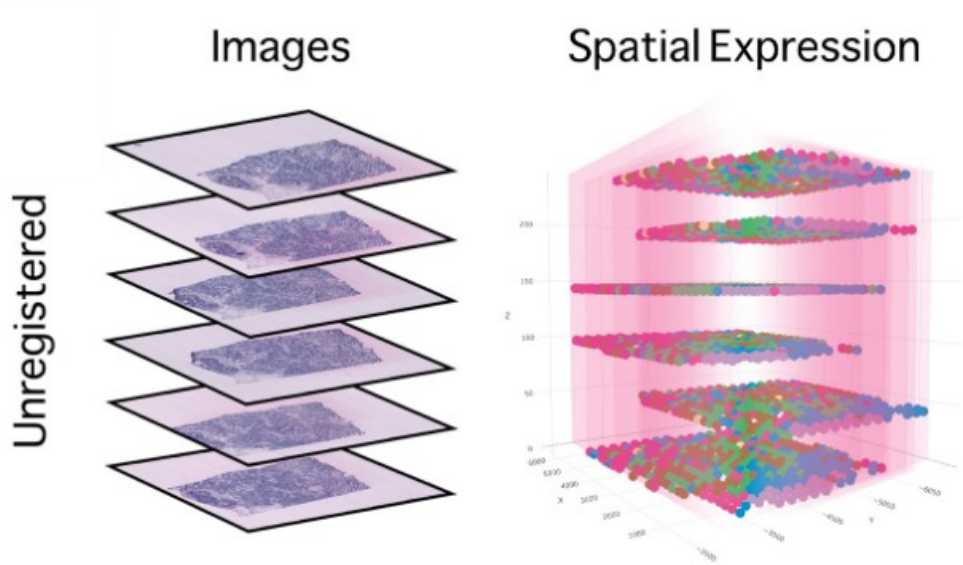

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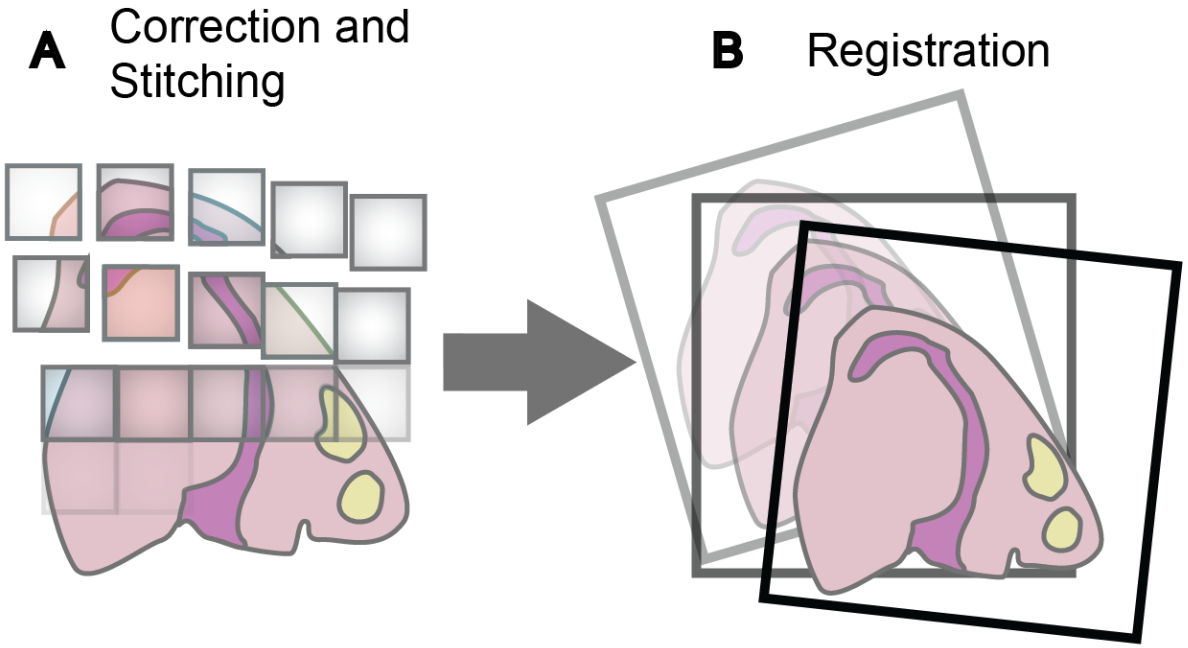
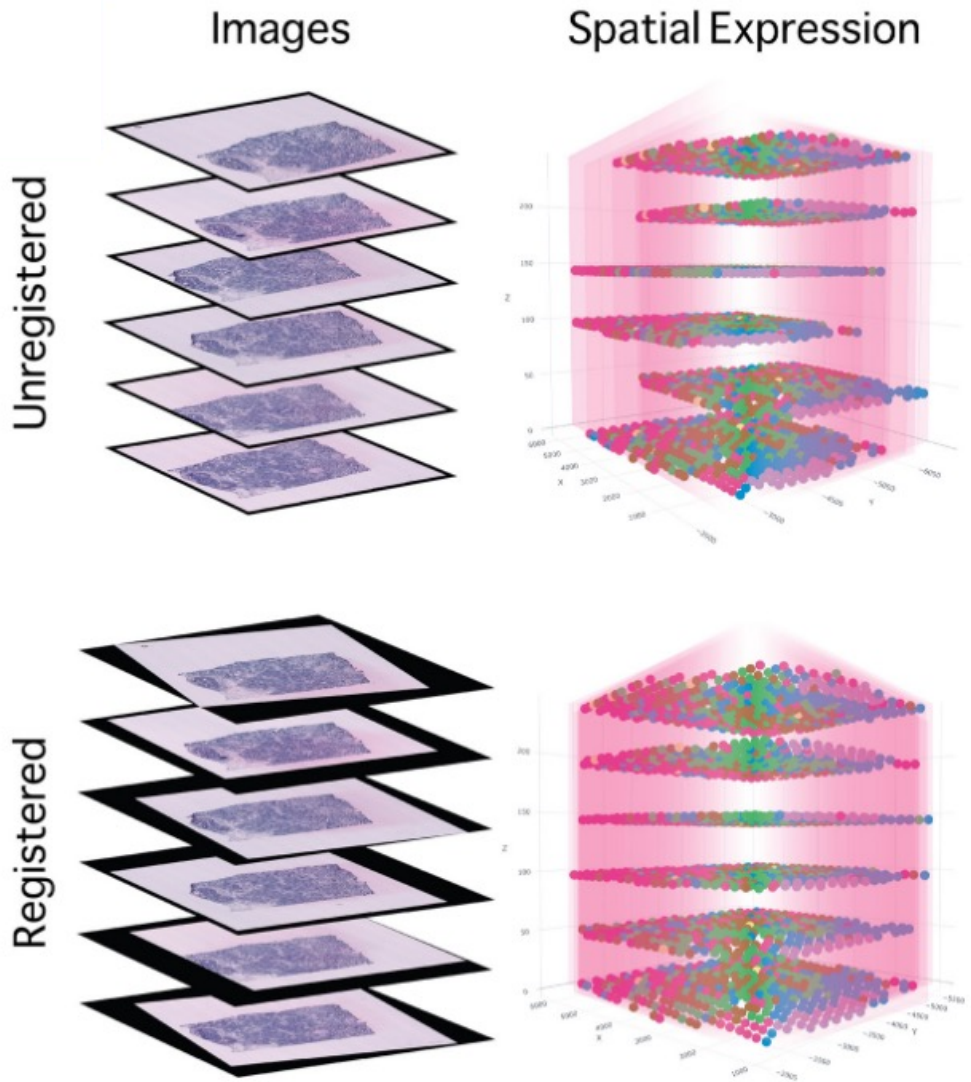
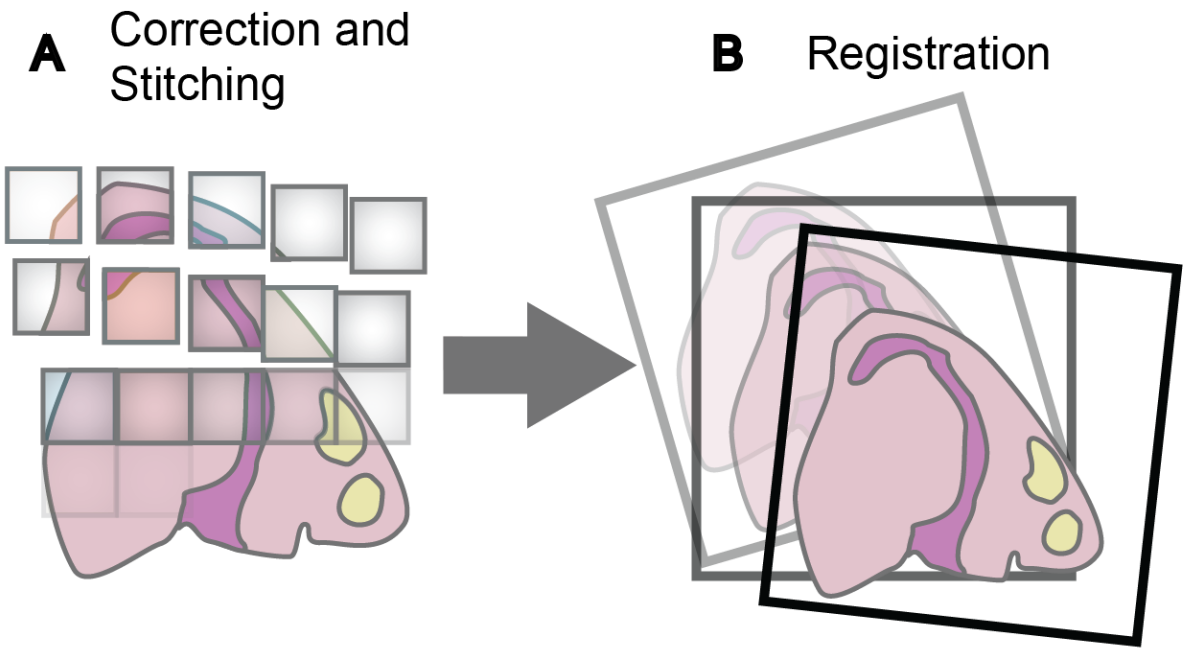
Essential pre-processing steps: image co-registration





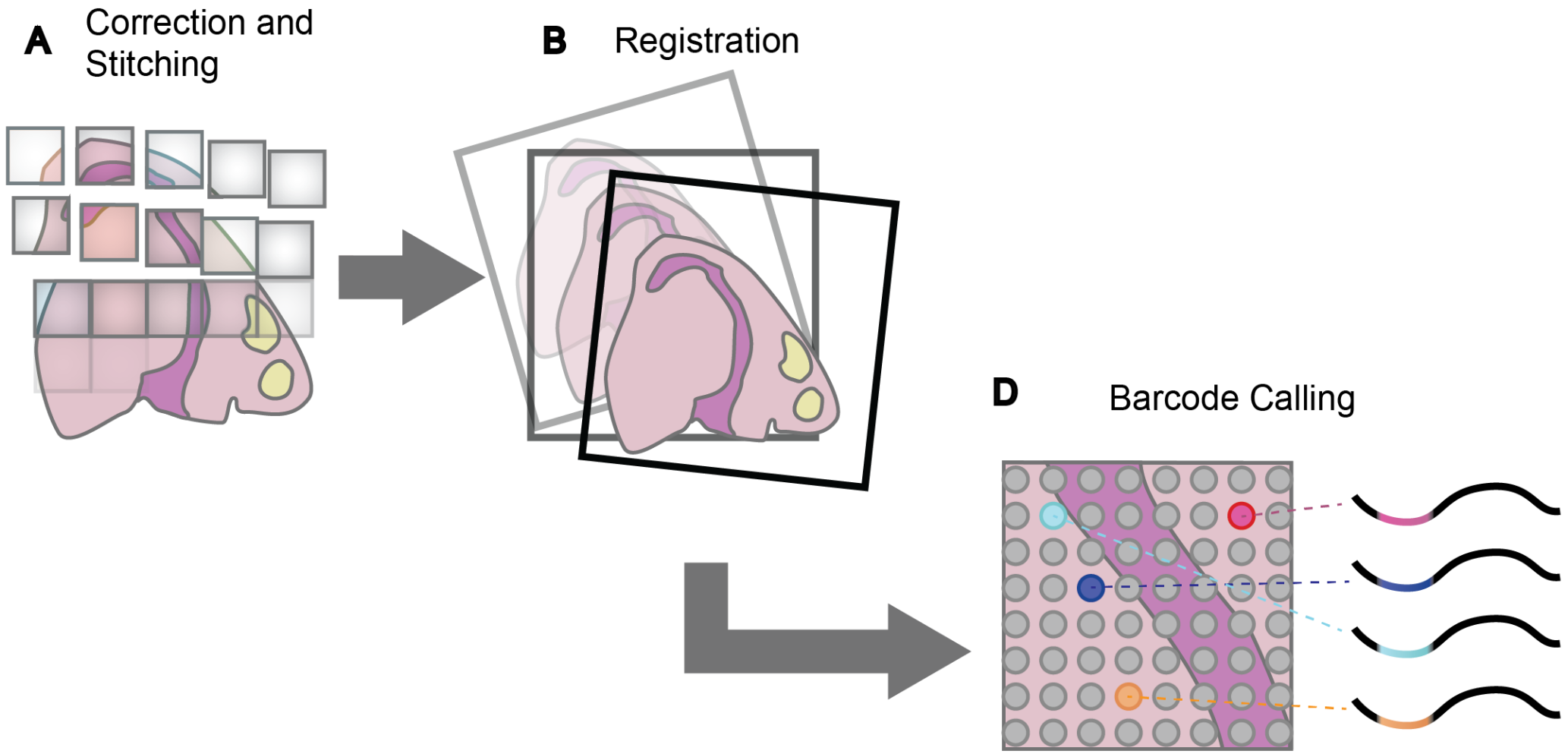
Dries, R. *et al.* Advances in spatial transcriptomic data analysis. *Genome Res* **31**, 1706–1718 (2021).

Essential pre-processing steps:



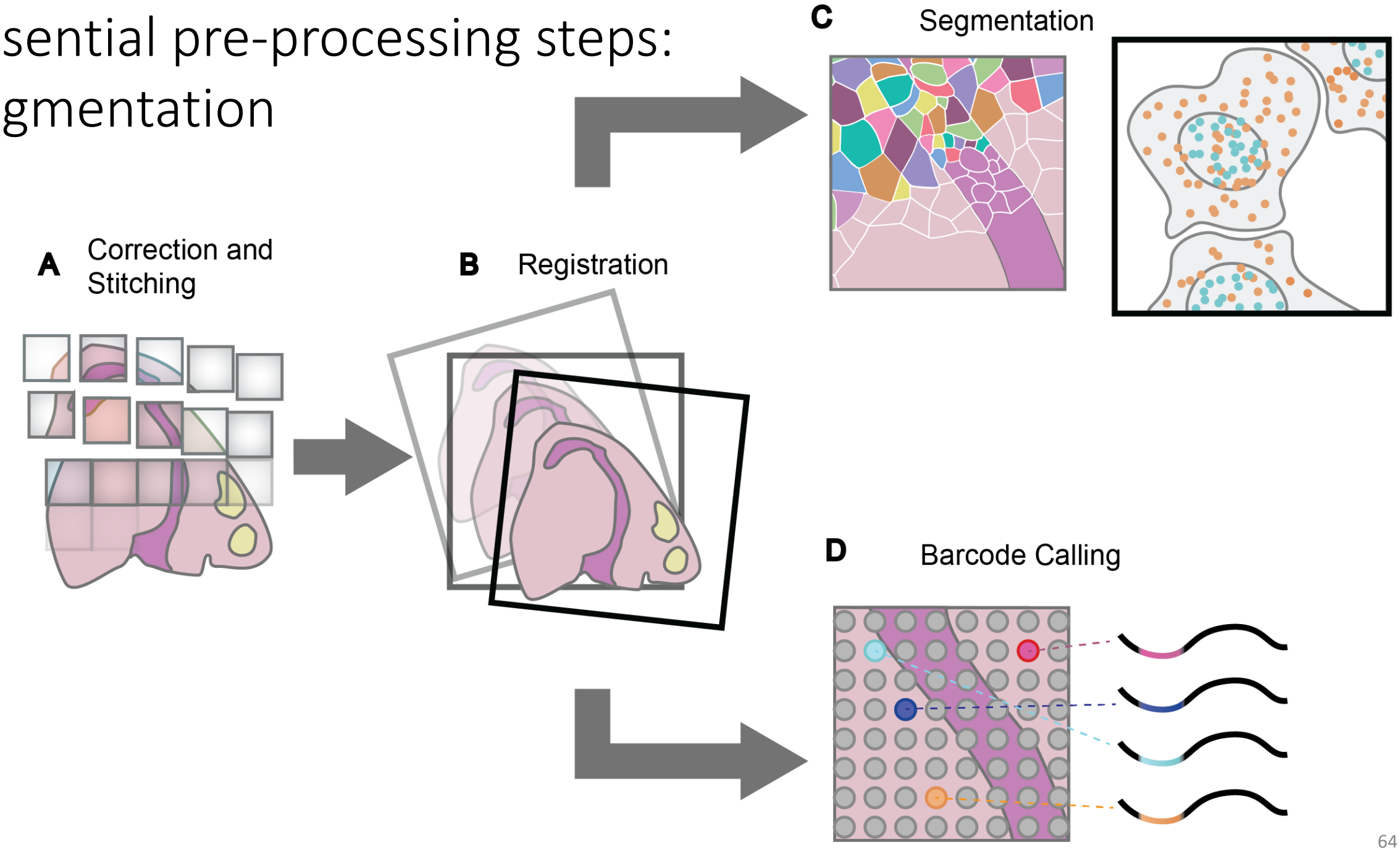
Dries, R. *et al.* Advances in spatial transcriptomic data analysis. *Genome Res* **31**, 1706–1718 (2021).

Essential pre-processing steps: barcode calling



Dries, R. *et al.* Advances in spatial transcriptomic data analysis. *Genome Res* **31**, 1706–1718 (2021).

Essential pre-processing steps: segmentation



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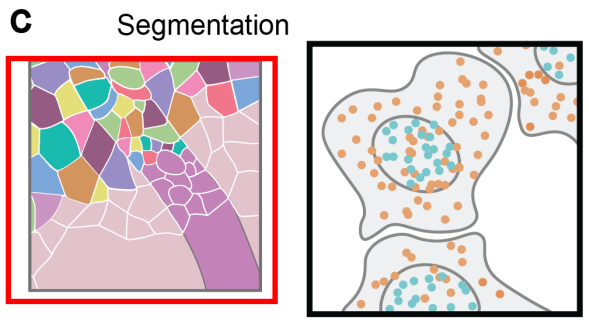
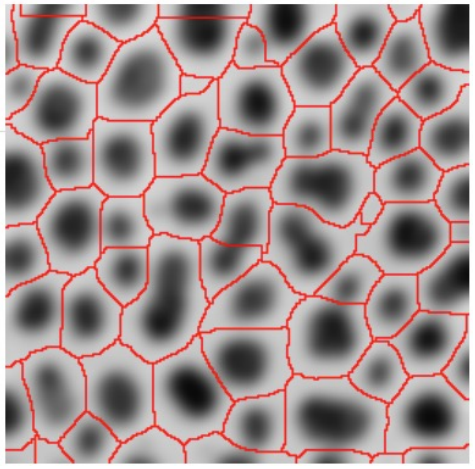
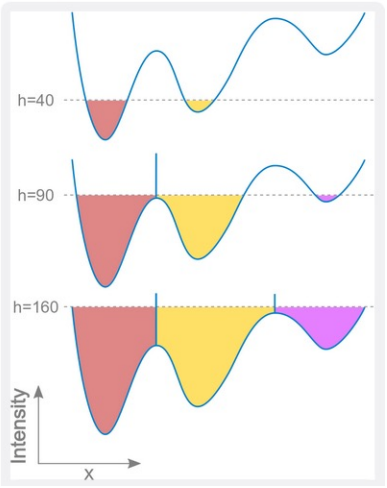


Classic approaches



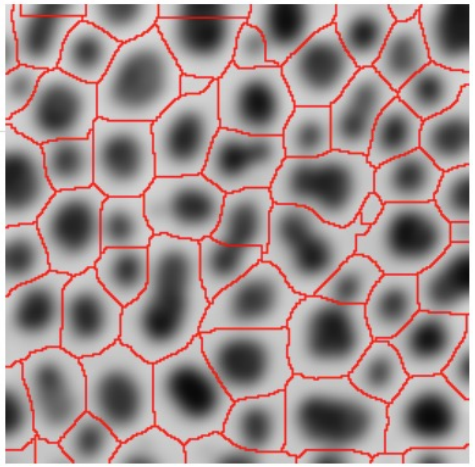


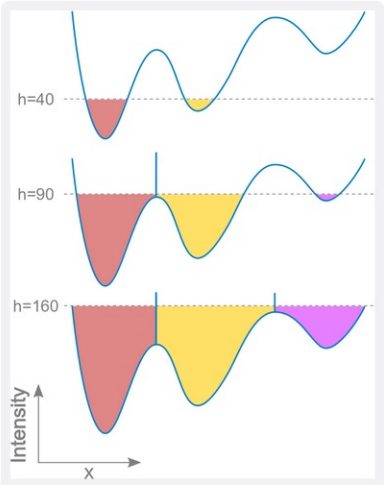
Fiji

QuPath

Essential pre-processing steps: segmentation

Classic approaches





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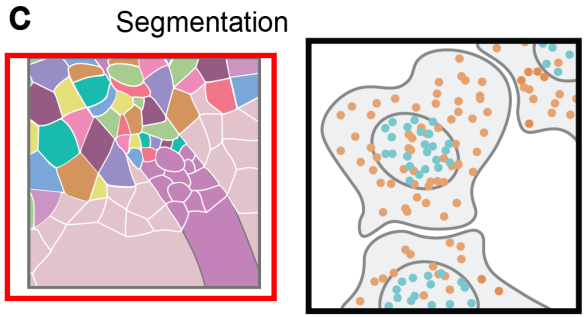
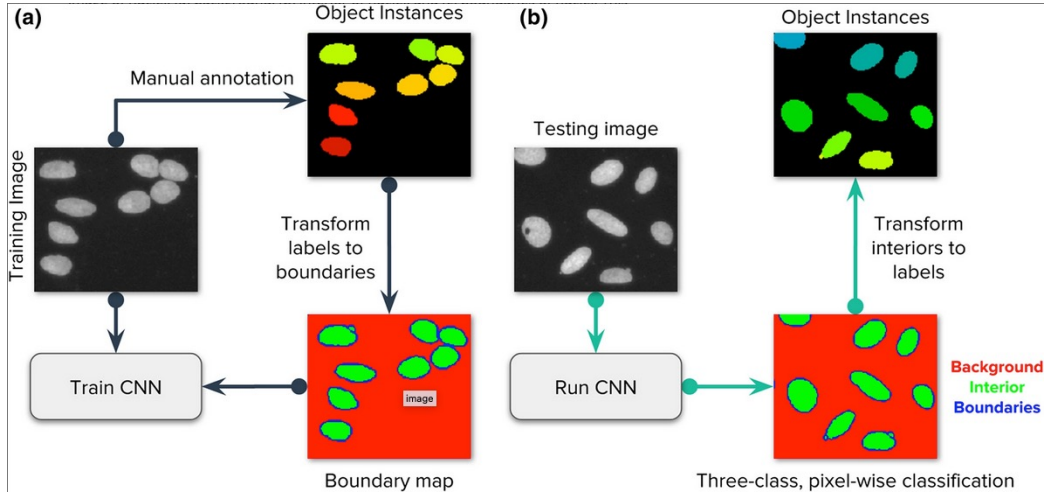


New Deep-learning approaches



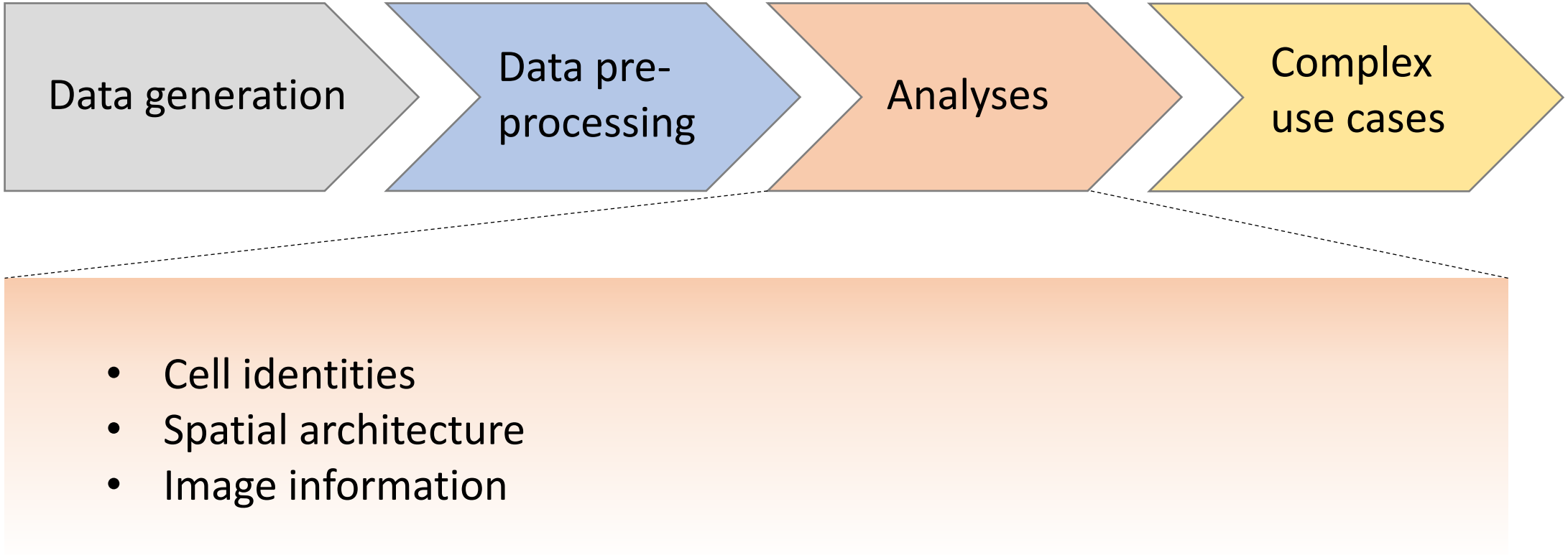
ilastik

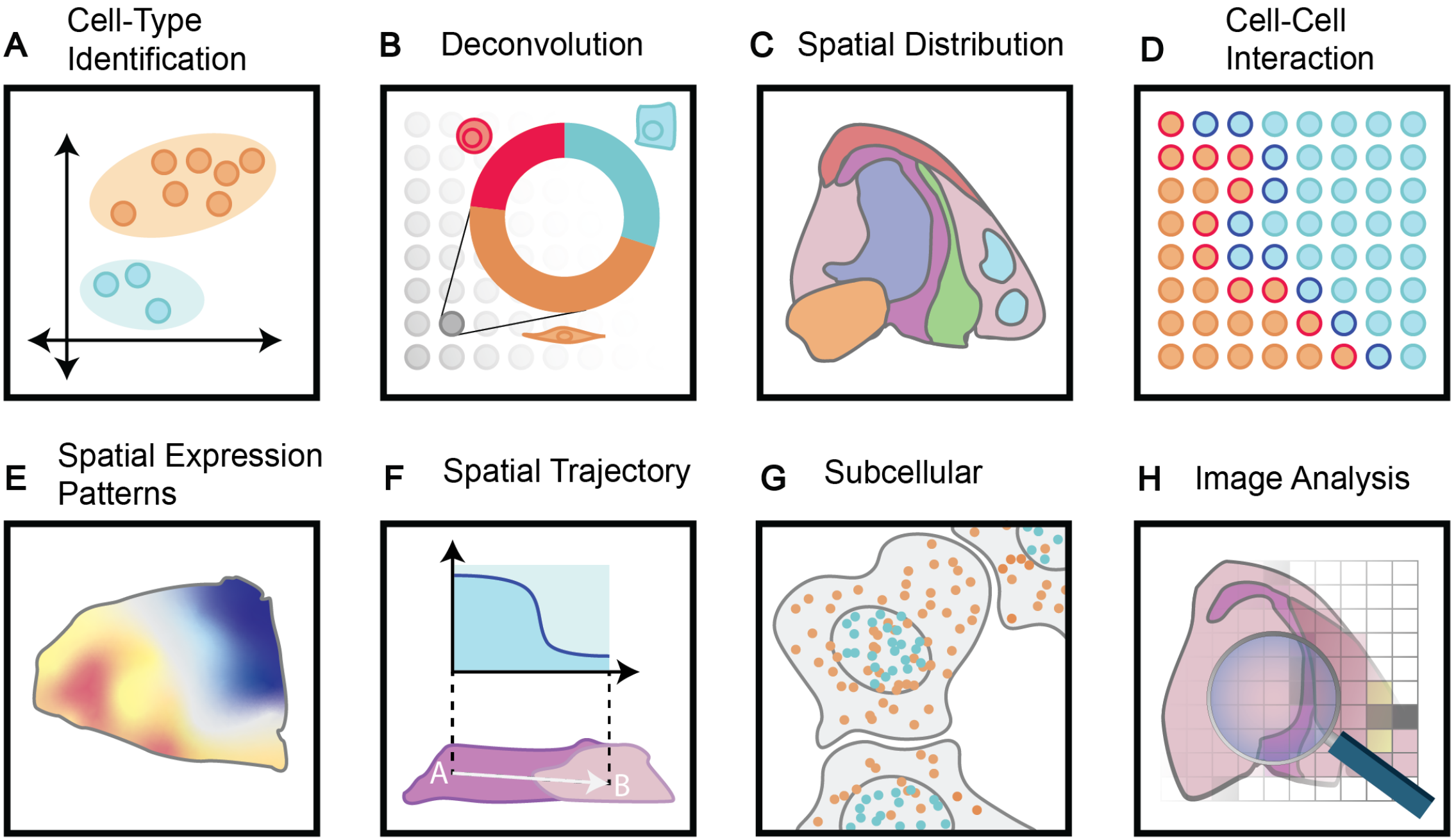
DeepCell

cellpose

CellProfiler™ cell image analysis software

The different steps in spatial omics research





Dries, R., Chen, J., Rossi, N. del, Khan, M.M., Sistig, A., Yuan, G.-C., 2021. Advances in spatial transcriptomic data analysis. Genome Res. 31, 1706–1718.

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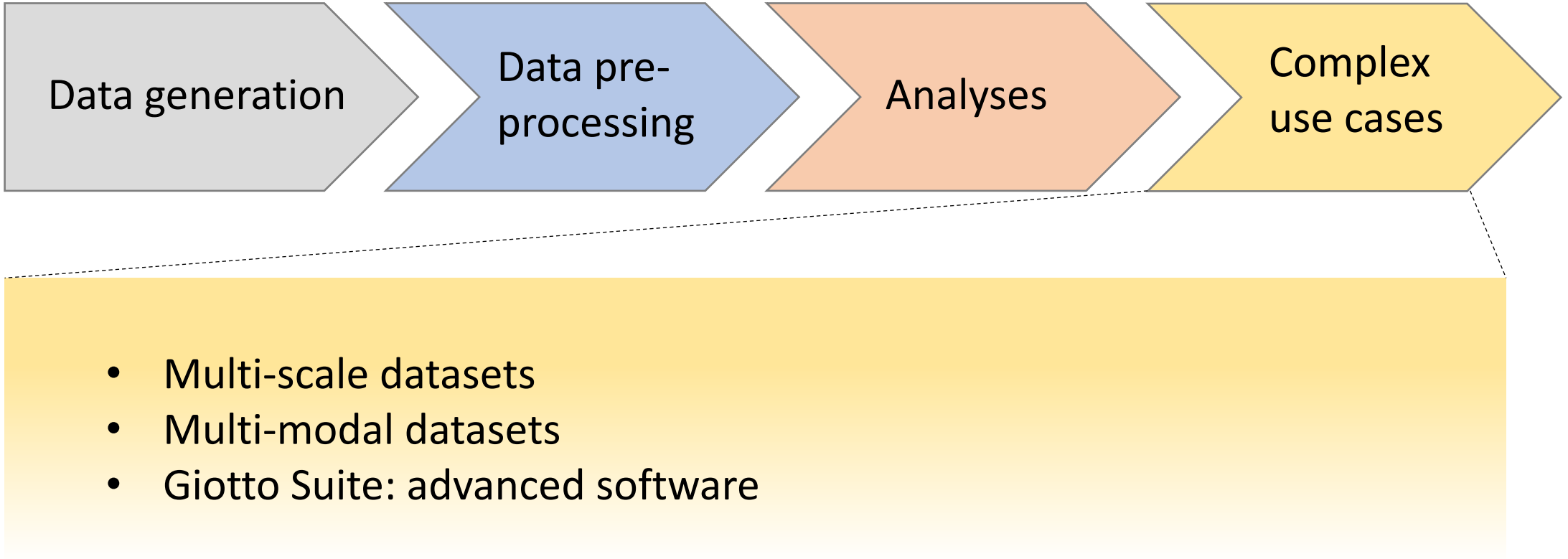


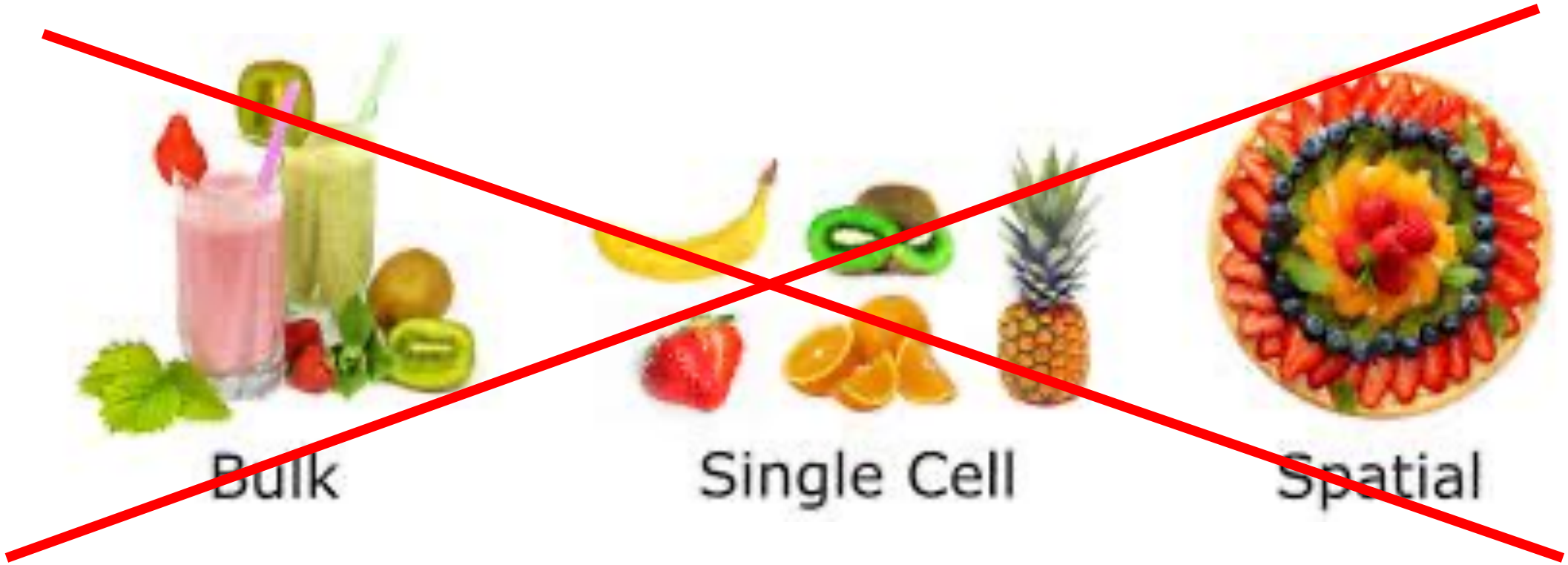
The emerging field of spatial omics

Bulk

Single Cell

Spatial

The emerging field of spatial omics



The emerging field of spatial omics

RNA-seq

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RNA-seq

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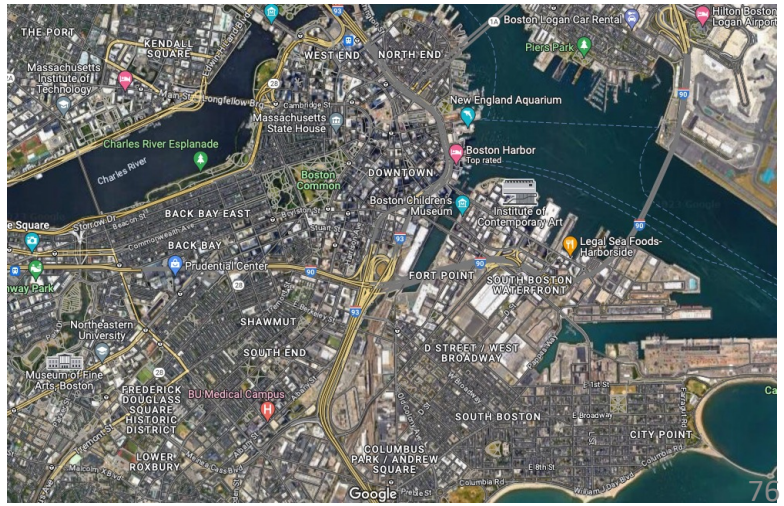
RNA-seq

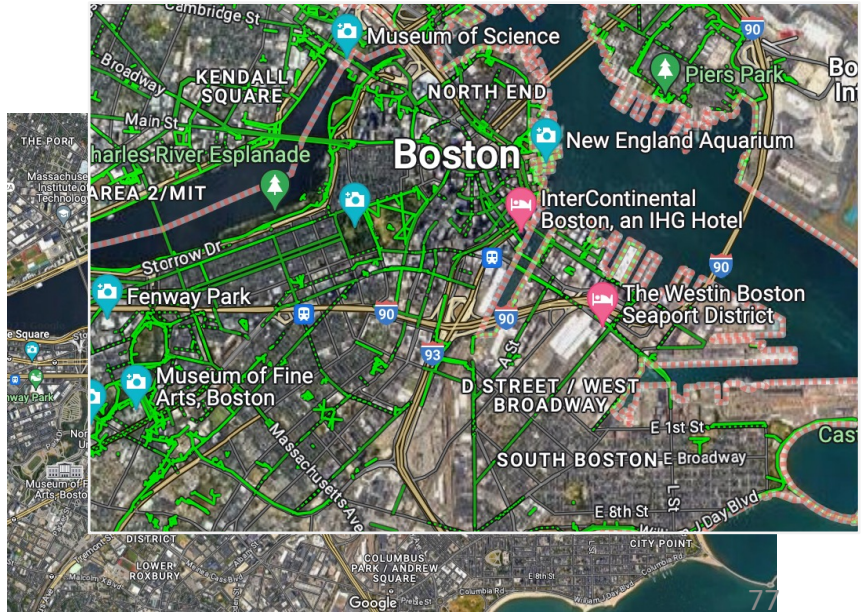
Single-cell RNA-seq

The emerging field of spatial omics

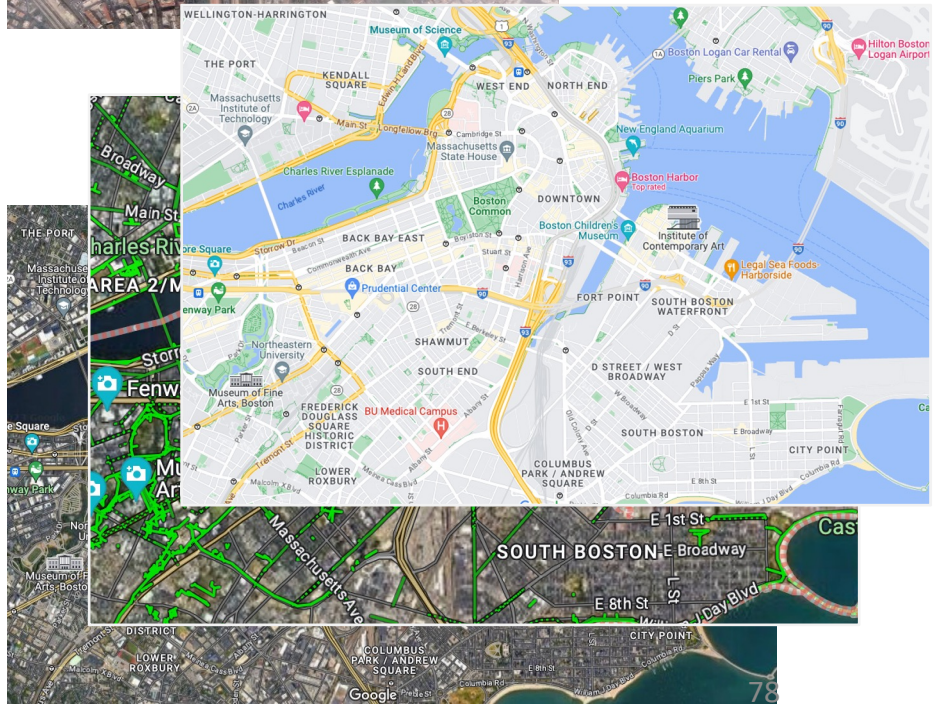
The emerging field of spatial omics



The emerging field of spatial omics



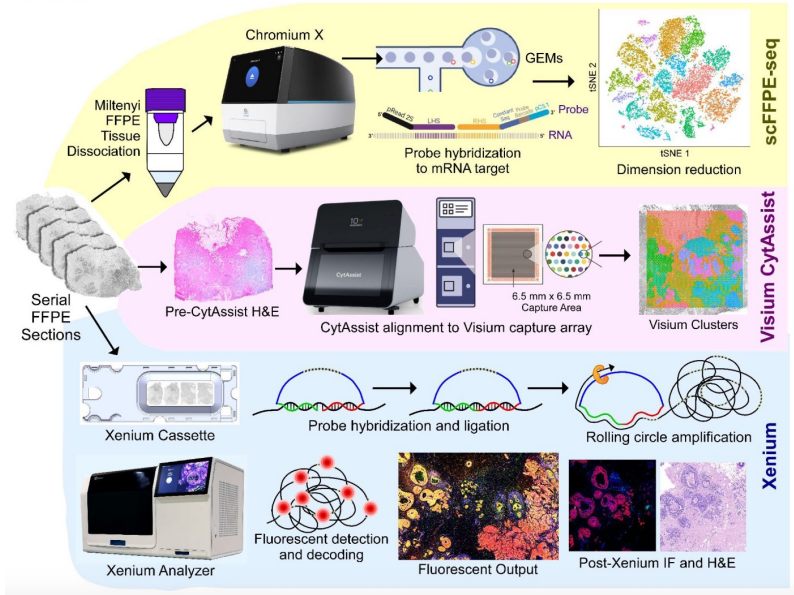
The emerging field of spatial omics



Datasets of the future

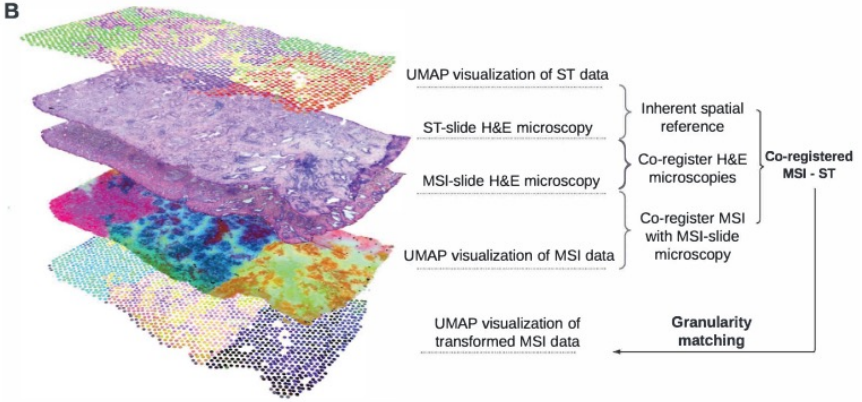
High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis of FFPE tissue

Amanda Janesick, Robert Shelansky, Andrew D. Gottscho, Florian Wagner, Morgane Rouault, Ghezal Beliakoff, Michelli Faria de Oliveira, Andrew Kohlway, Jawad Abousoud, 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 Socciarelli, 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



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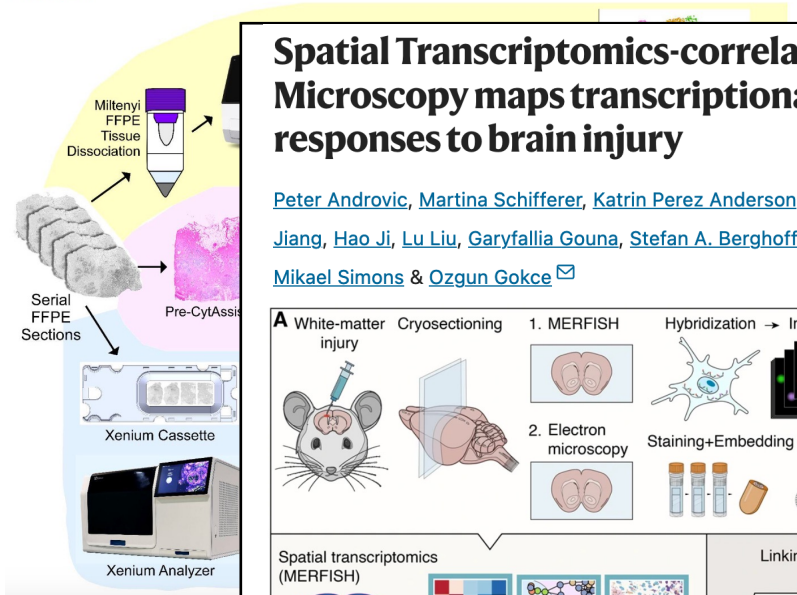
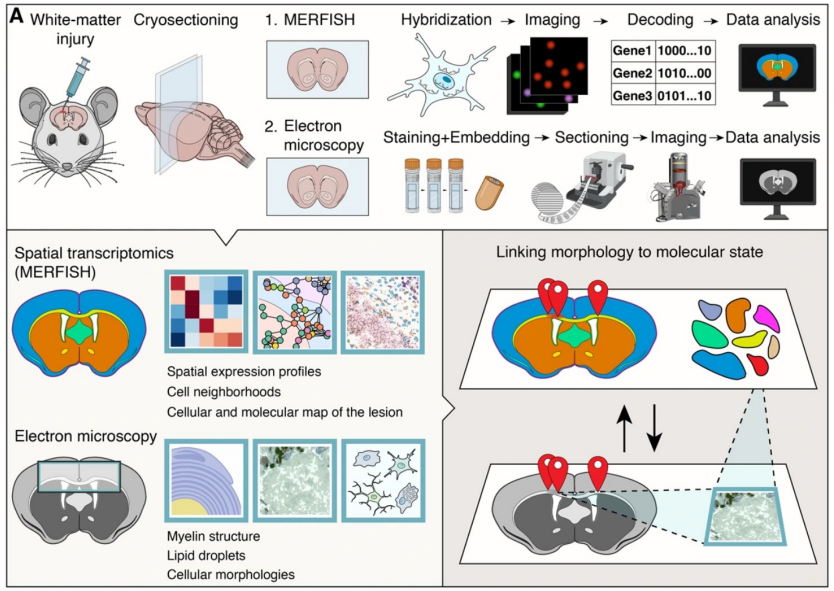


Spatial Transcriptomics-correlated Electron Microscopy maps transcriptional and ultrastructural responses to brain injury

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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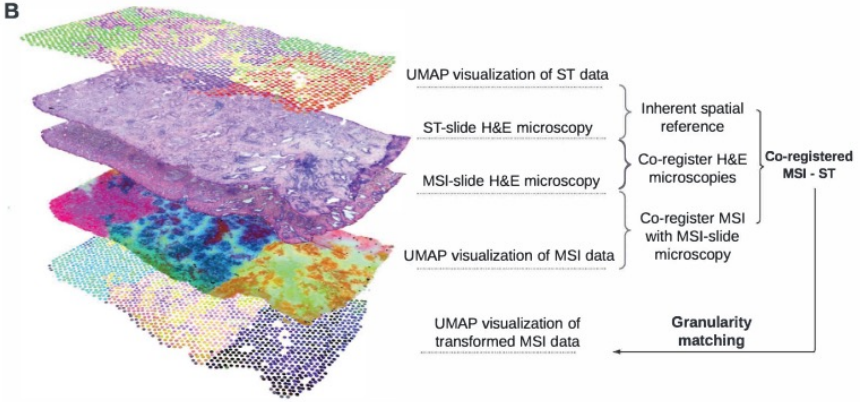

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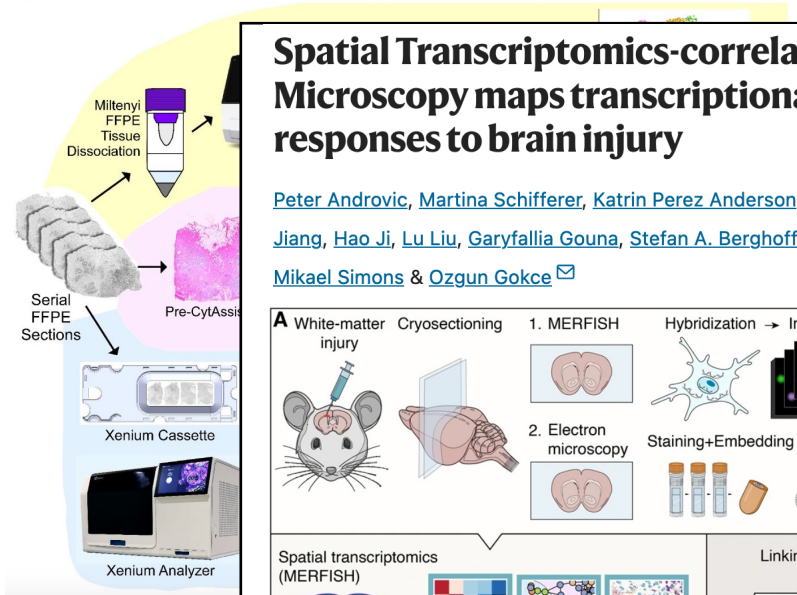

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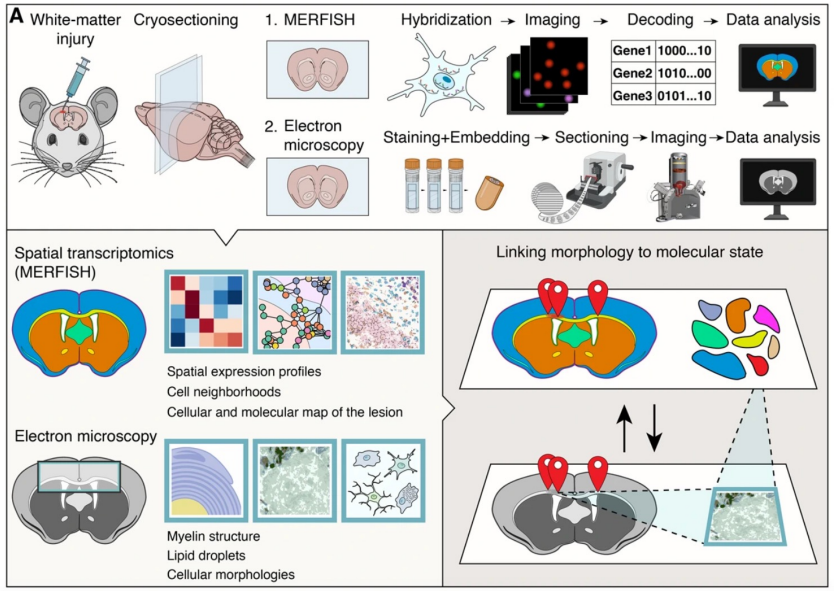

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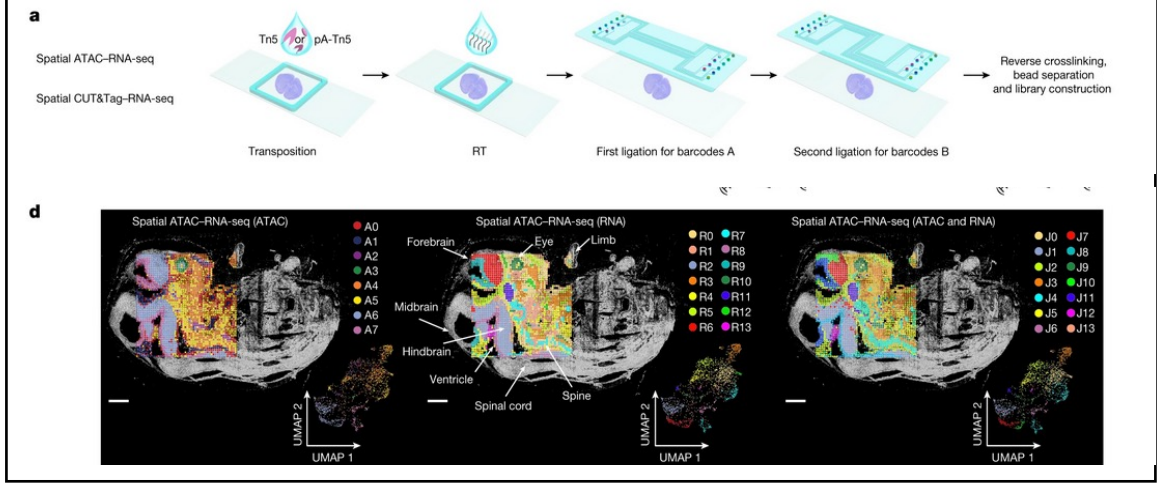
Spatial epigenome–transcriptome co-profiling of mammalian tissues

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



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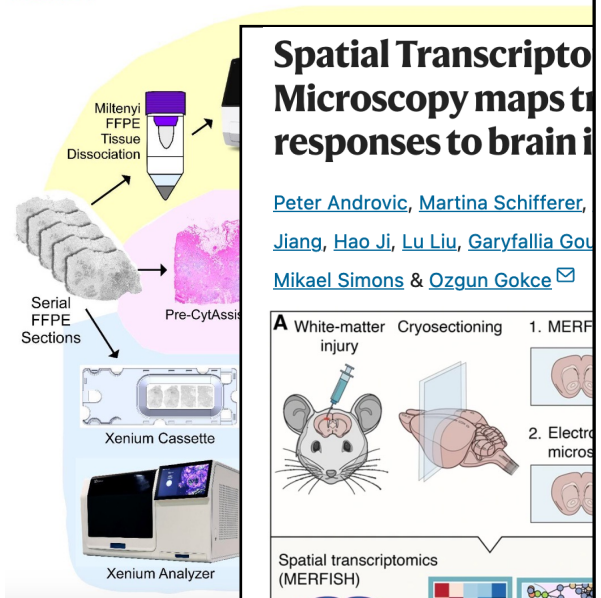

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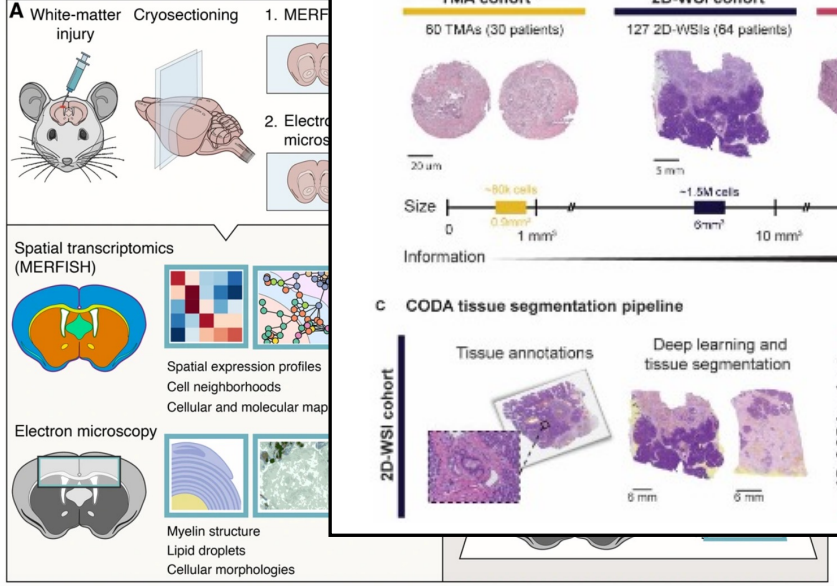
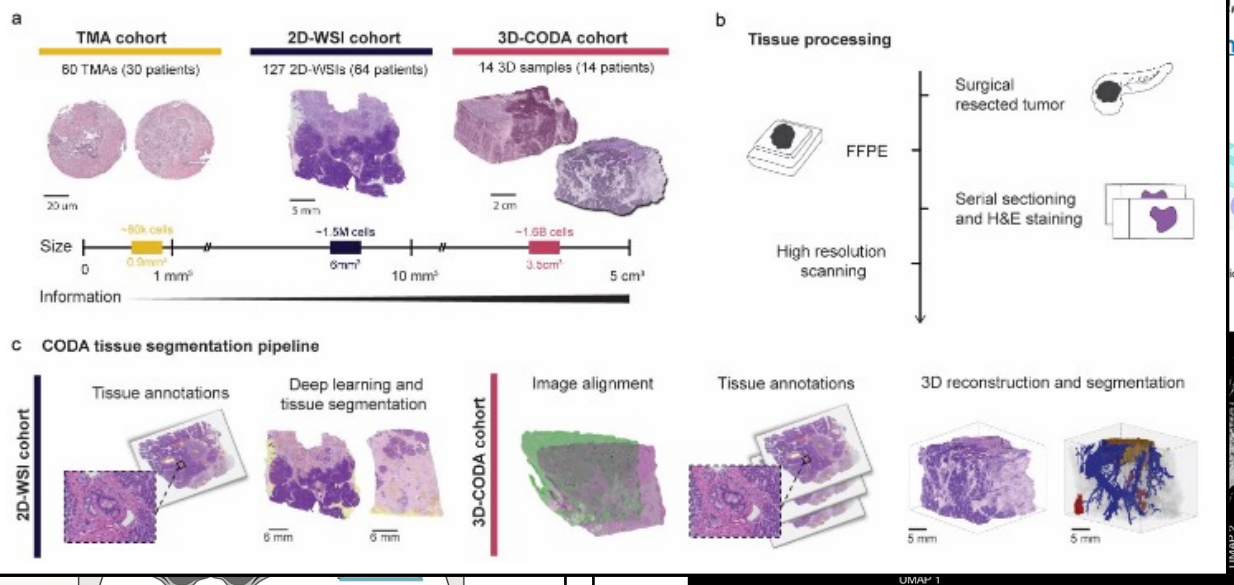


Three-dimensional assessments are necessary to determine the true, spatially-resolved composition of tissues

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



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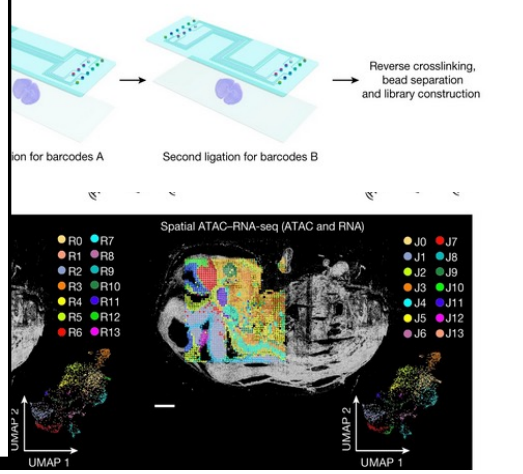
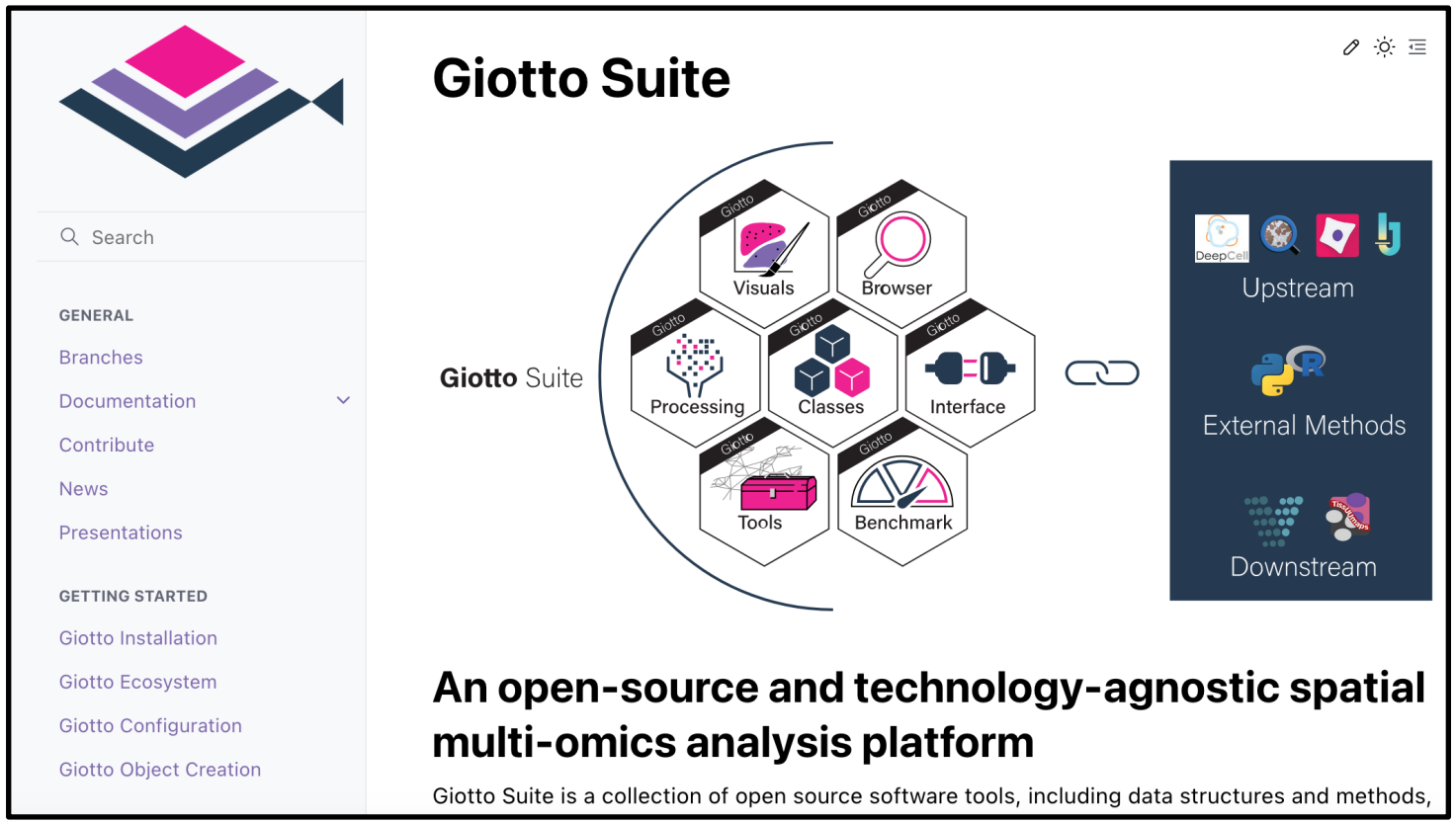


Giotto Suite: A Multi-scale and Technology-agnostic Spatial Multi-omics Analysis Ecosystem

Giotto Suite

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