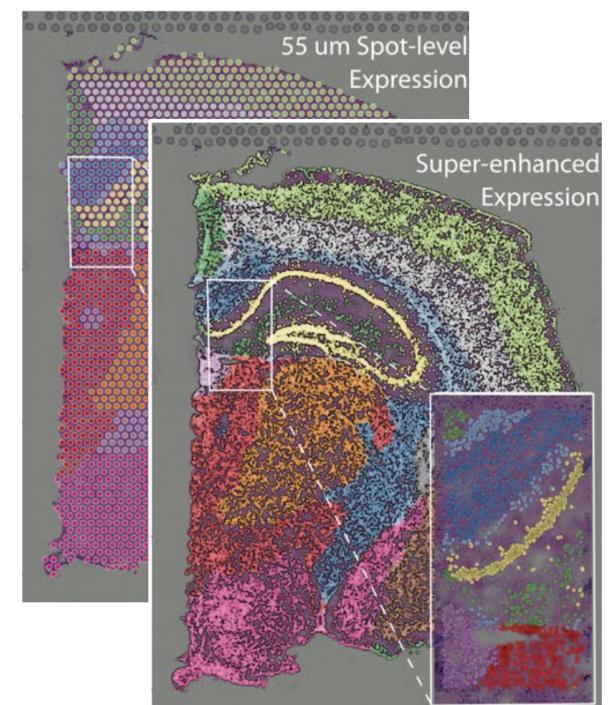
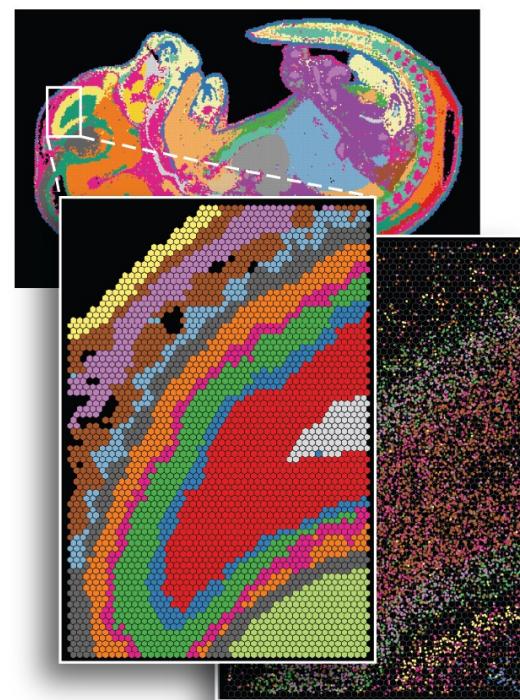
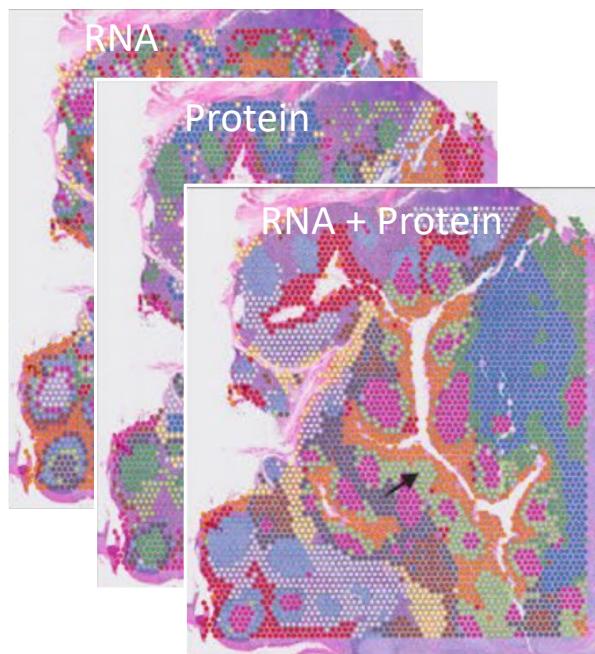


Introduction to Spatial Technologies



A spatial transcriptomic example

Mouse
brain



H&E stain:

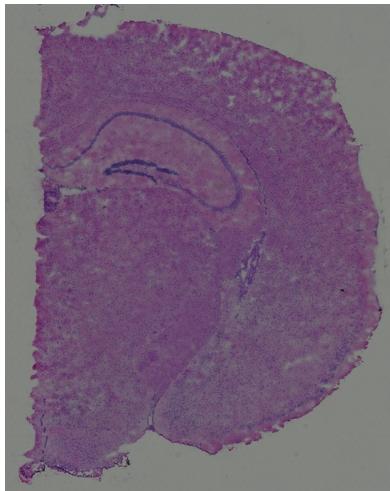
- Haematoxylin = nuclei
- Eosin = extracellular matrix

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

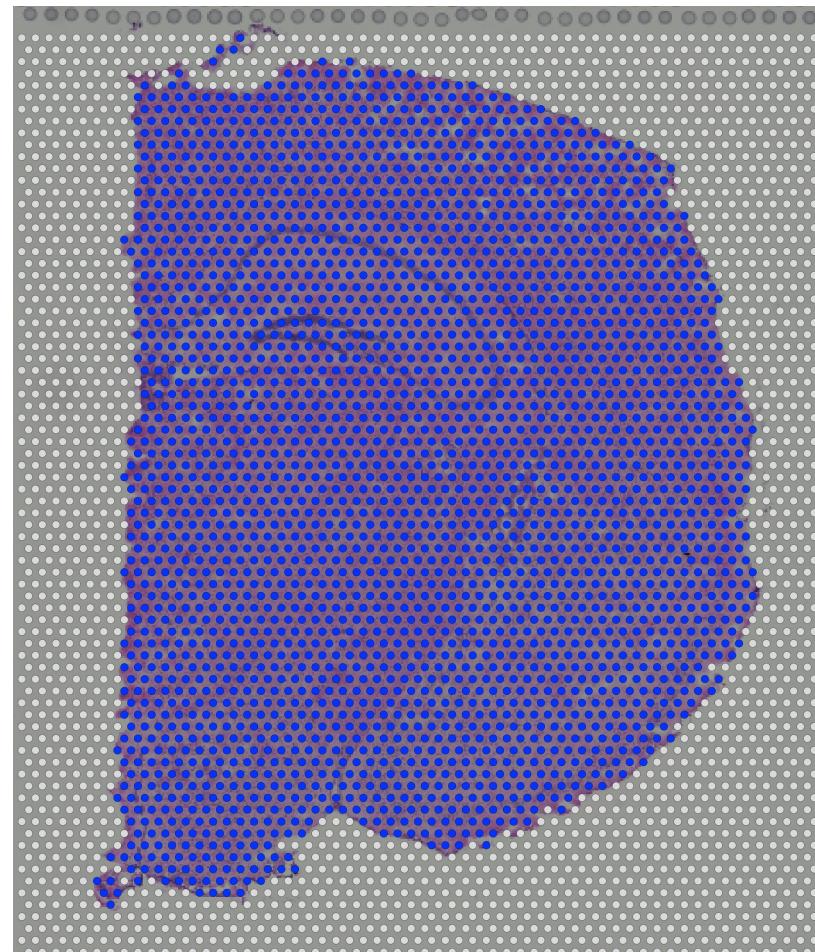
→ What other information is
available? 1000's of molecular
analytes (RNA, Protein,
Metabolites, etc)

A spatial transcriptomic example

Mouse
brain

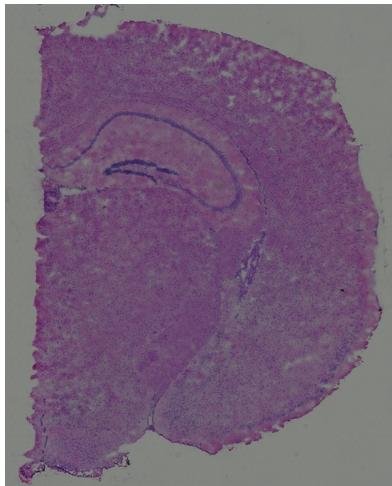


Extract spatial
information
(e.g. RNA)

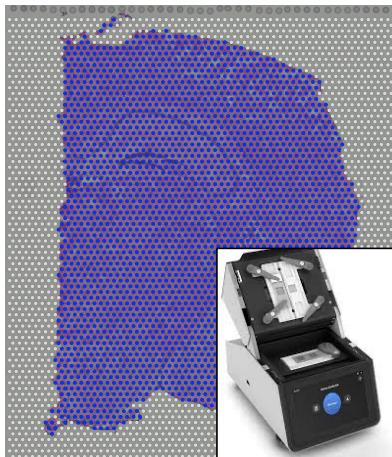


A spatial transcriptomic example

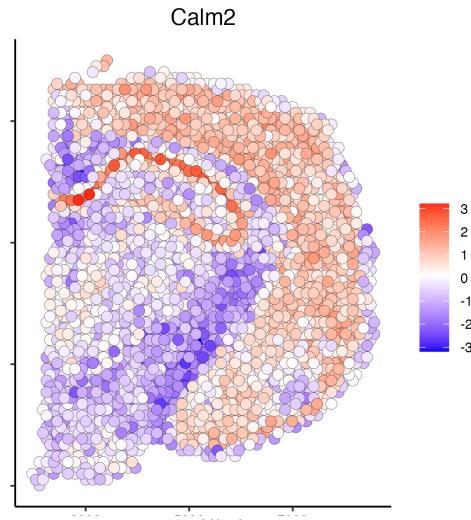
Mouse
brain



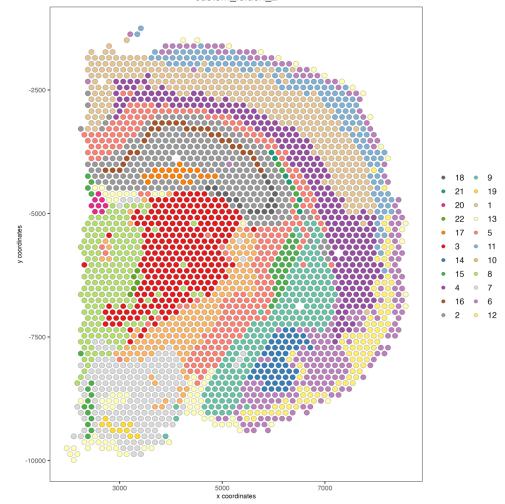
Extract spatial
information
(e.g. RNA)



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

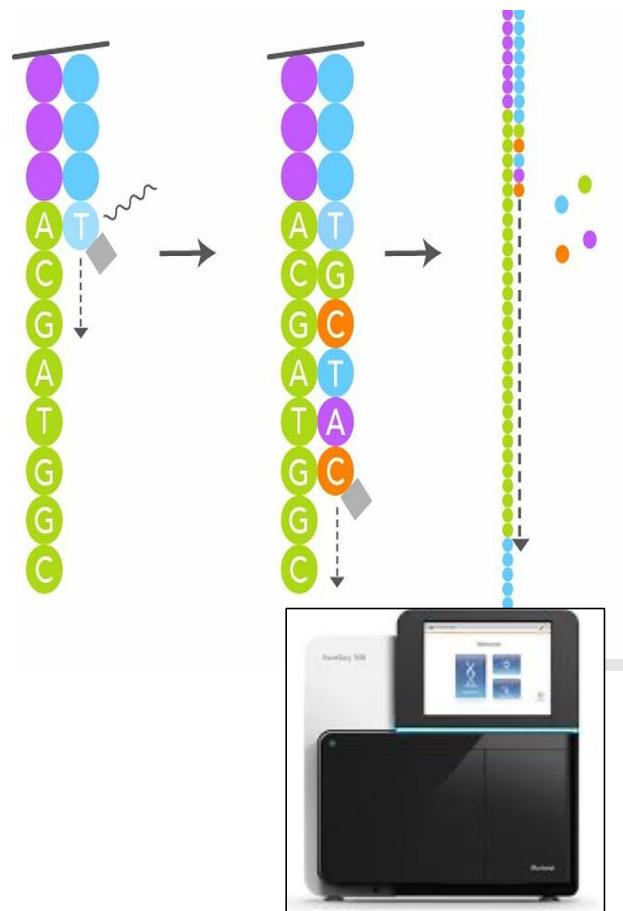


Obtain spatial
insights

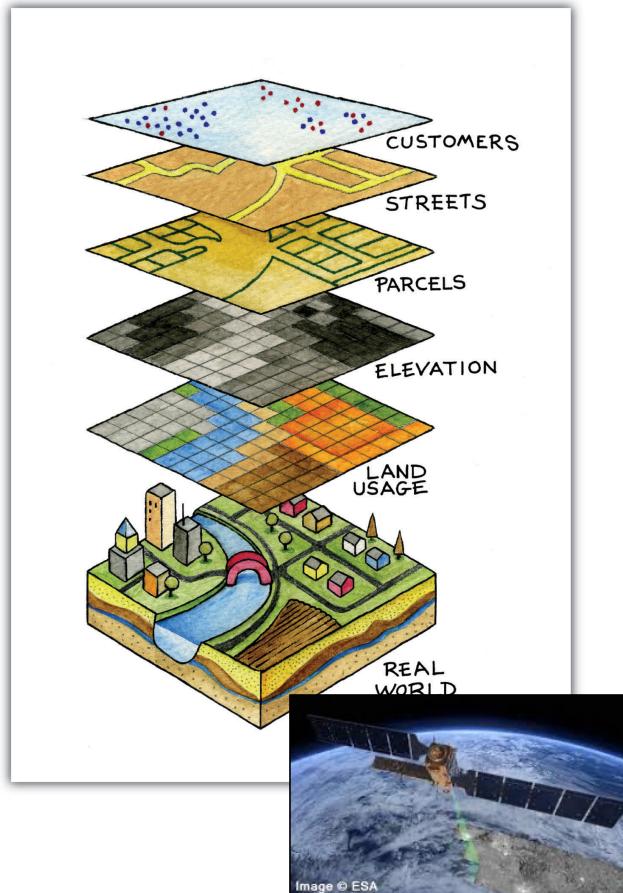


What is spatial omics?

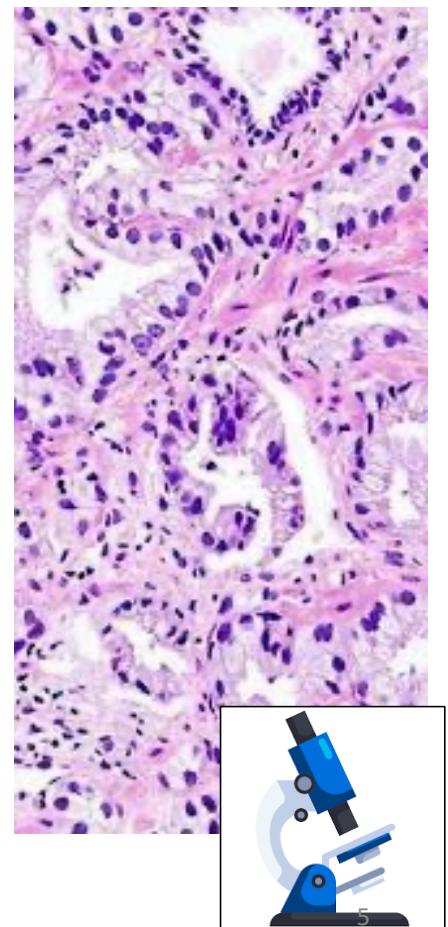
Sequencing



Spatial data science



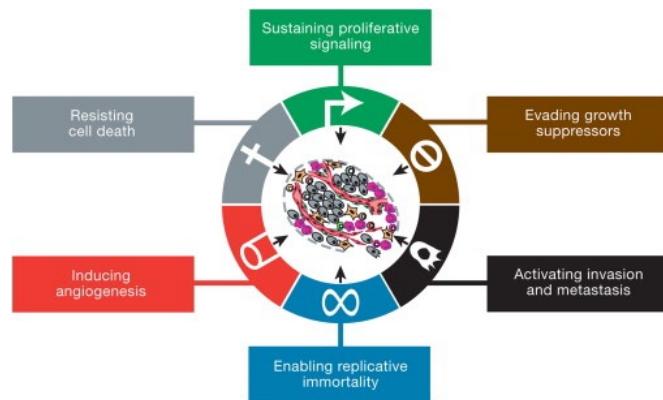
Imaging



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

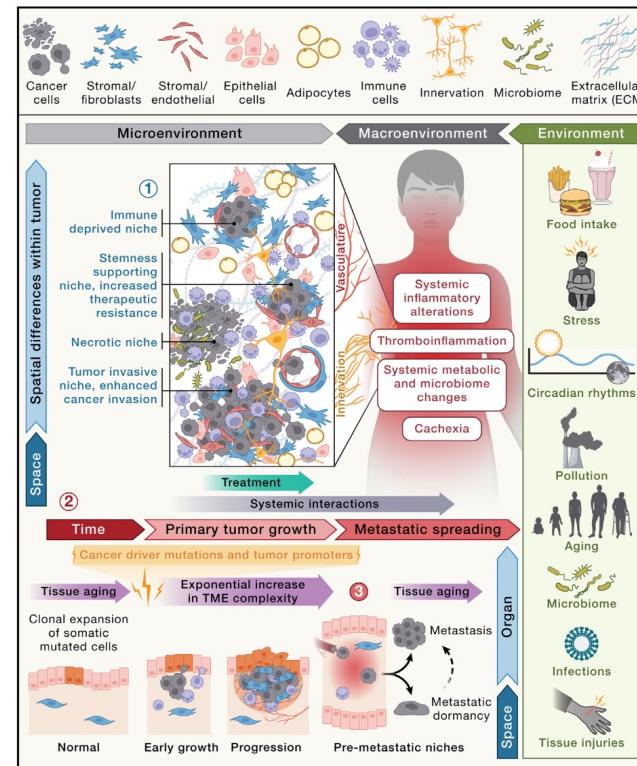
Examples in the field of cancer biology:

The Hallmarks of Cancer (2000)



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](https://doi.org/10.1038/s41592-021-01203-6).

Embracing cancer complexity: Hallmarks of systemic disease (2024)

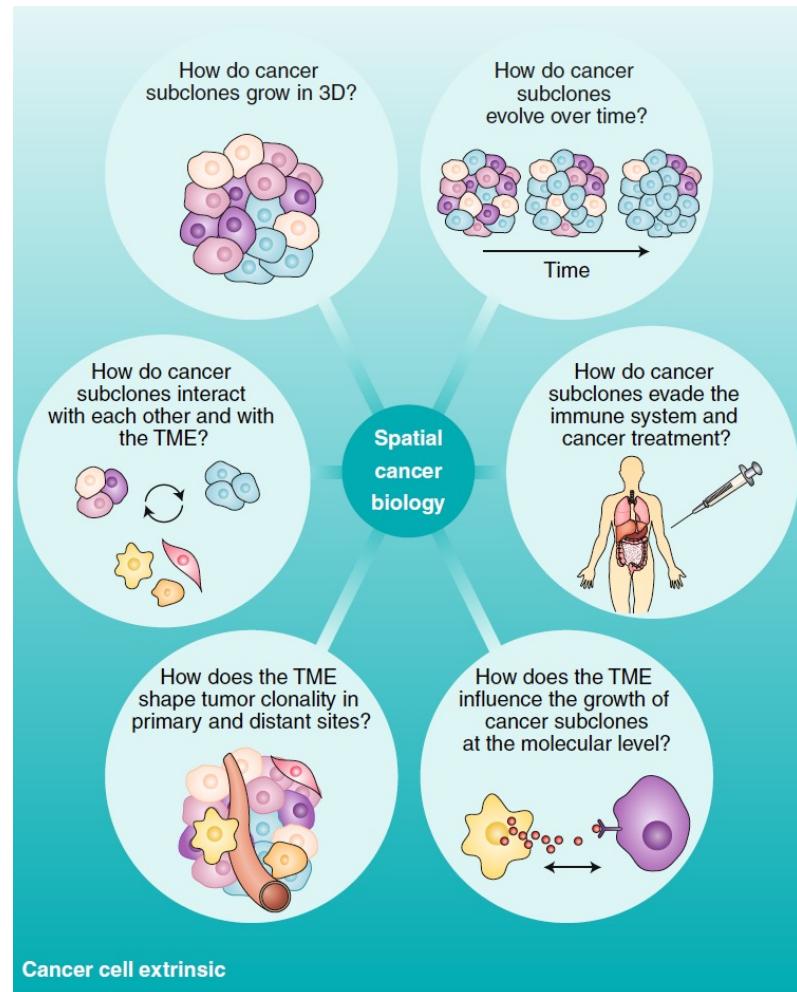


What are the biological questions we're trying to answer?

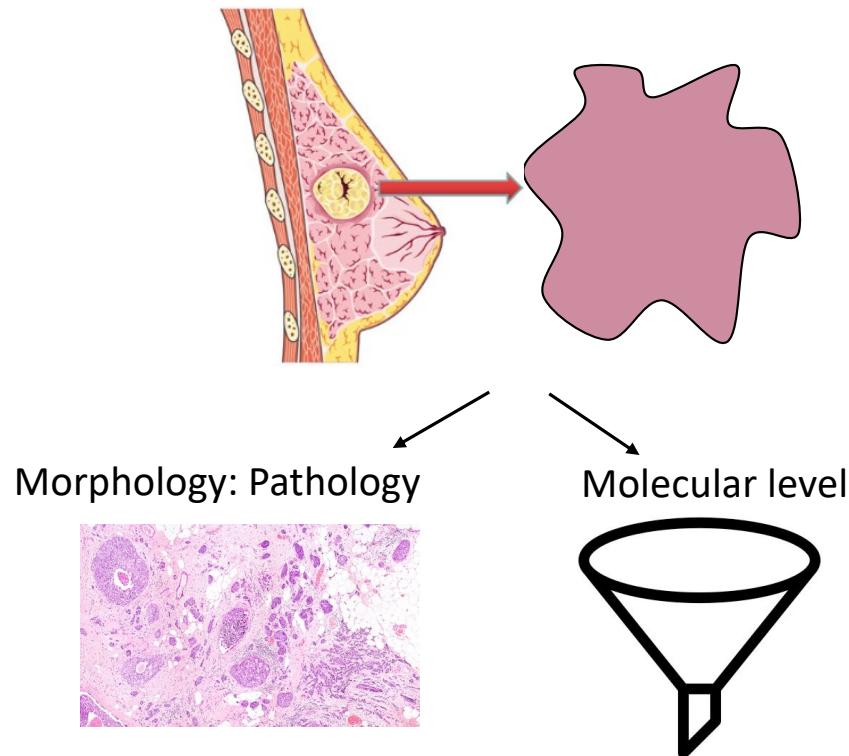
Examples in the field of cancer biology:

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 ...



- 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

NATIONAL CANCER INSTITUTE THE CANCER GENOME ATLAS

TCGA BY THE NUMBERS

TCGA produced over

2.5 PETABYTES of data

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

212,000 DVDs



TCGA data describes

33 DIFFERENT TUMOR TYPES ...including

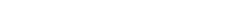
...based on paired tumor and normal tissue sets collected from

11,000 PATIENTS



...using

7 DIFFERENT DATA TYPES



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.*



THE 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

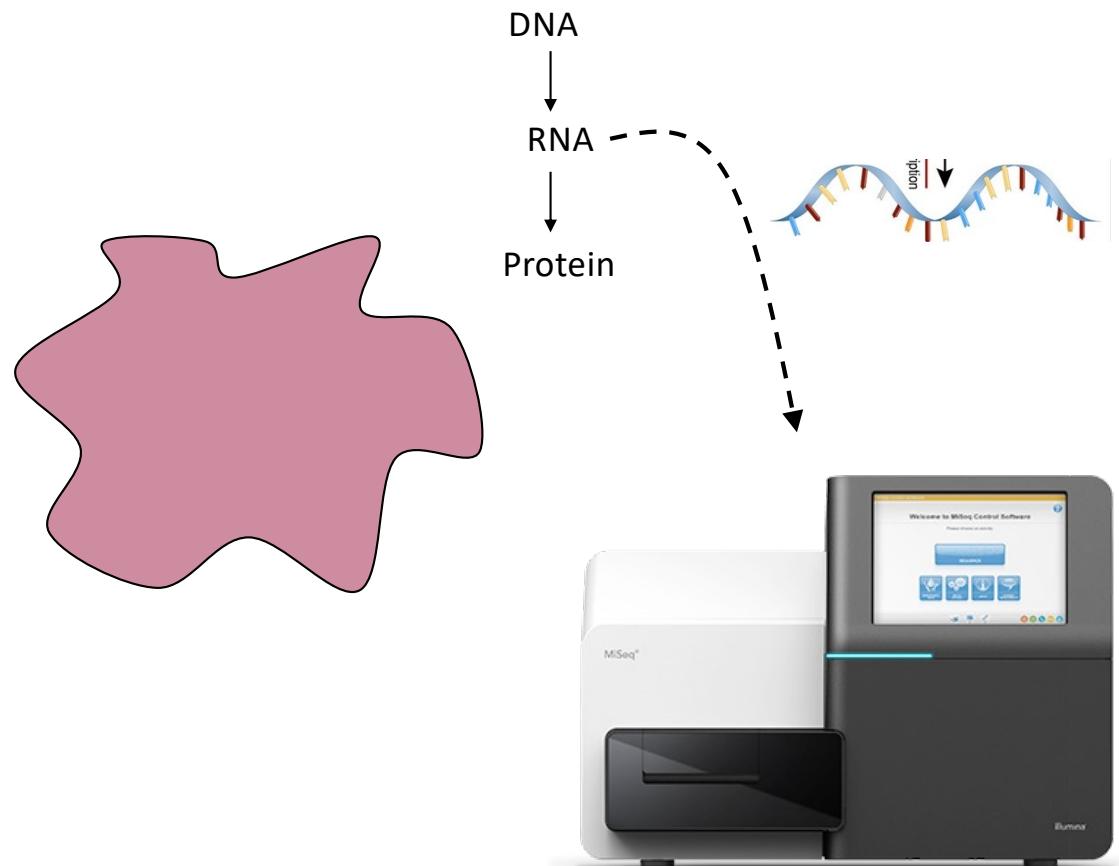


20 COLLABORATING INSTITUTIONS across the United States and Canada

WHAT'S NEXT?

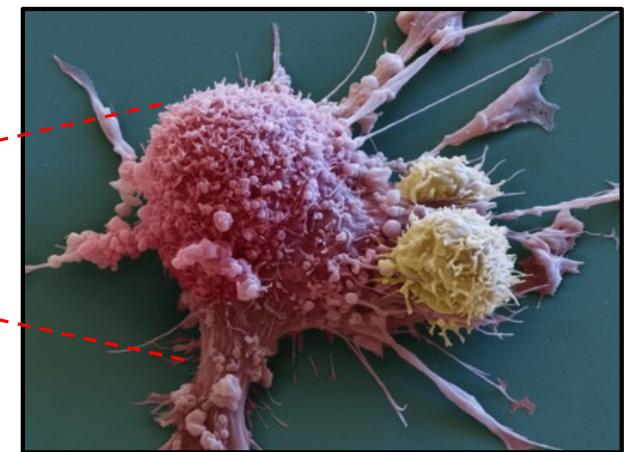
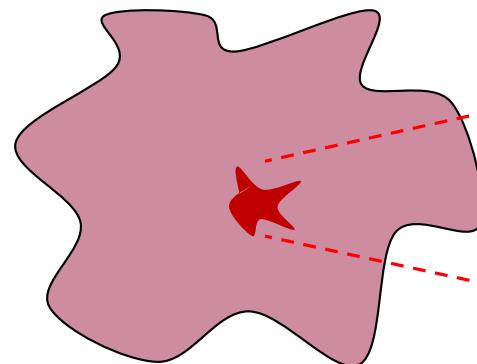
The Genomic Data Commons (GDC) houses TCGA and other NCI-generated data sets for scientists to access anywhere. The GDC also has many expanded capabilities that will allow researchers to answer more clinically relevant questions with increased ease.

www.cancer.gov/ccg

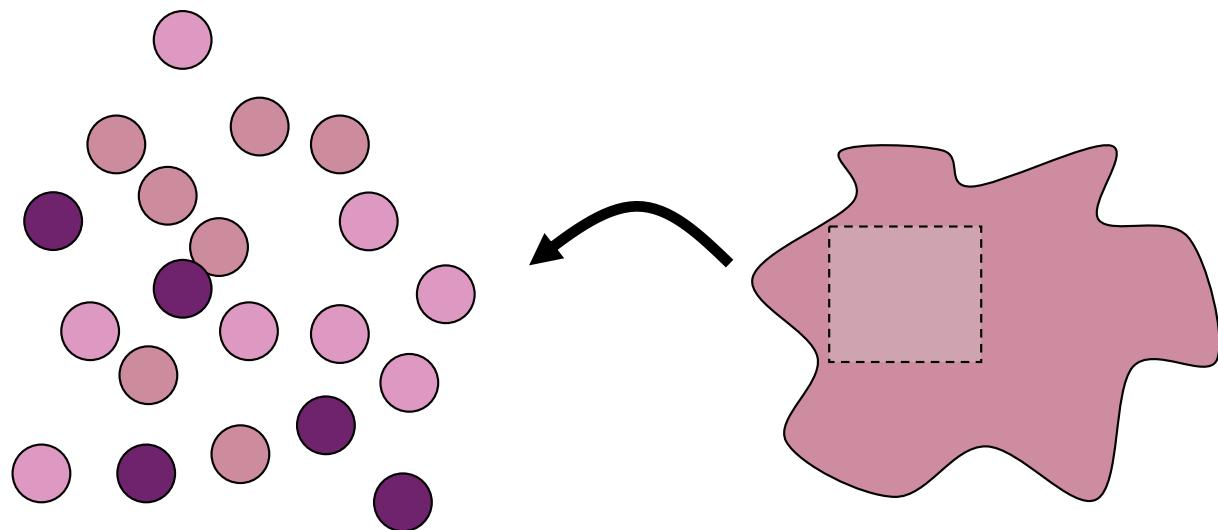


*TCGA's analysis of stomach cancer revealed that it is not a single disease, but a disease composed of four subtypes, including a new subtype characterized by infection with Epstein-Barr virus.

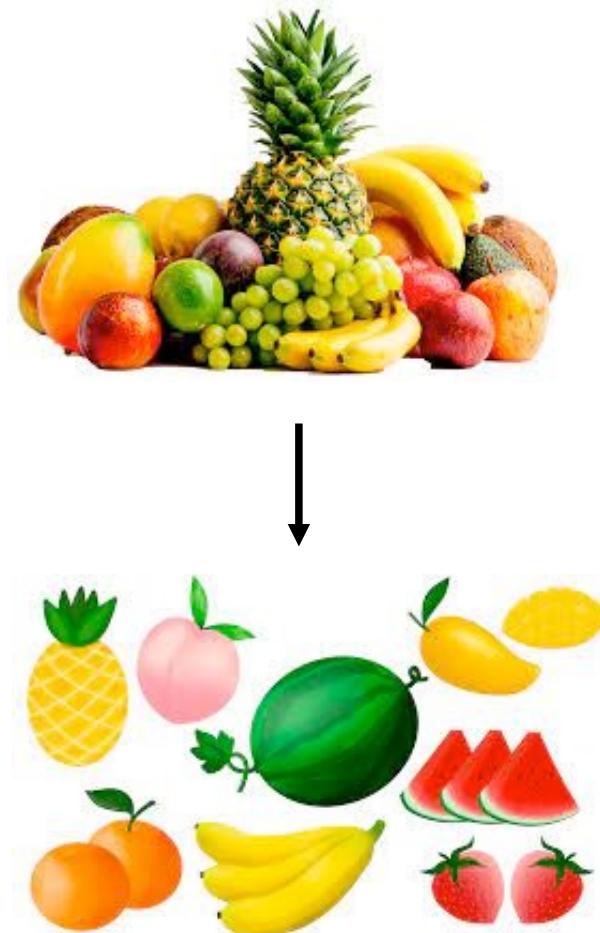
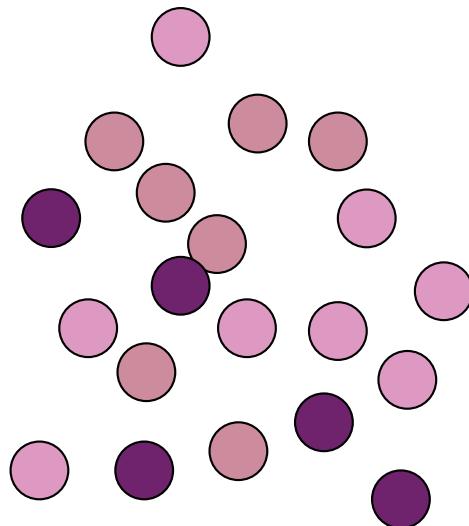
Historically ... **limitations** of RNA-seq



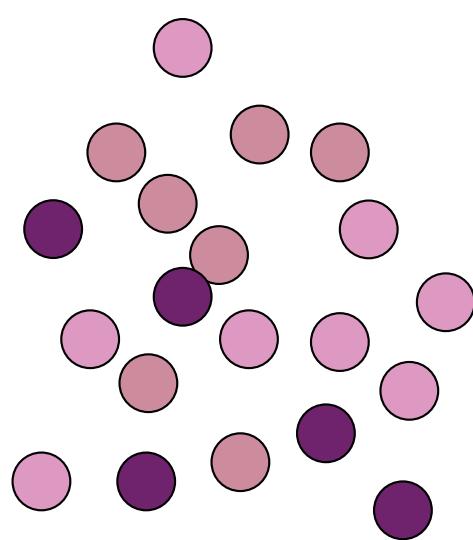
Historically ... single-cell RNA-seq



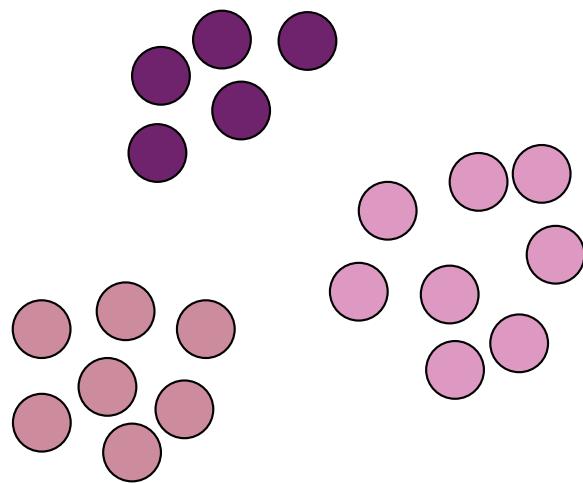
Historically ... single-cell RNA-seq



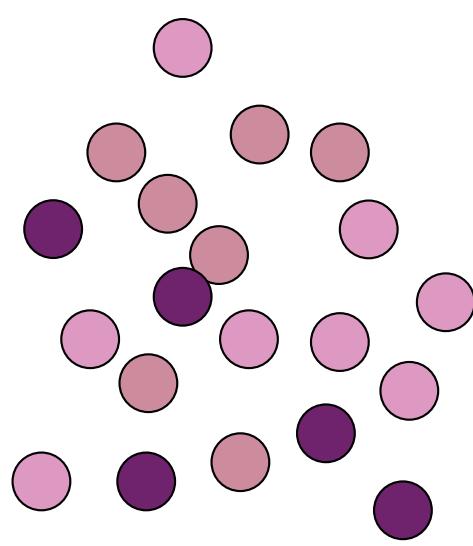
Historically ... single-cell RNA-seq



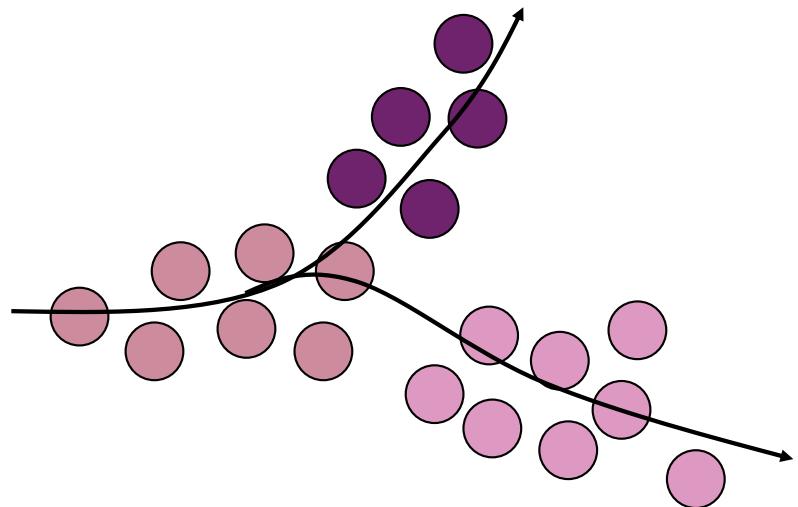
Cluster cell types



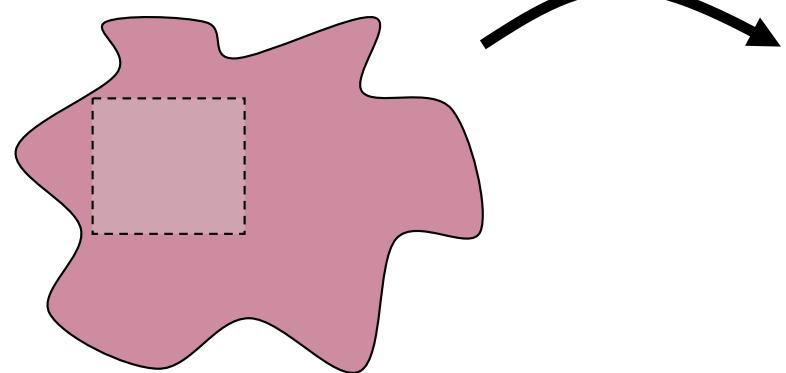
Historically ... single-cell RNA-seq



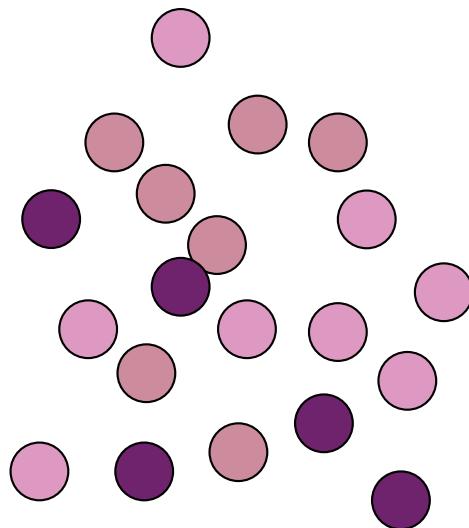
Cell trajectories



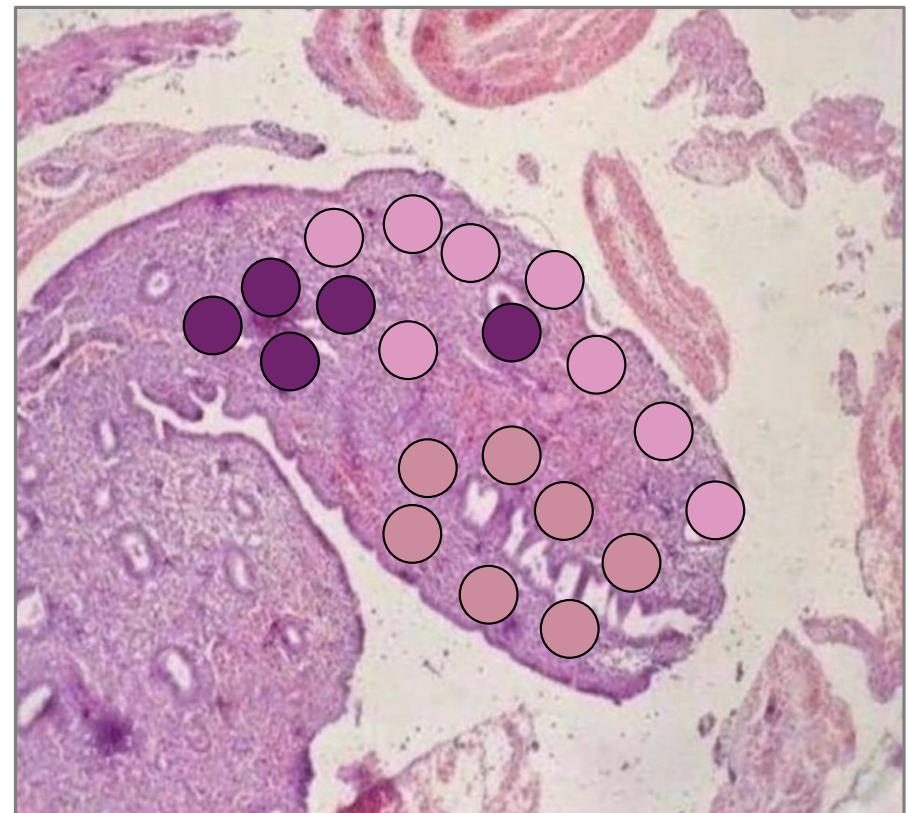
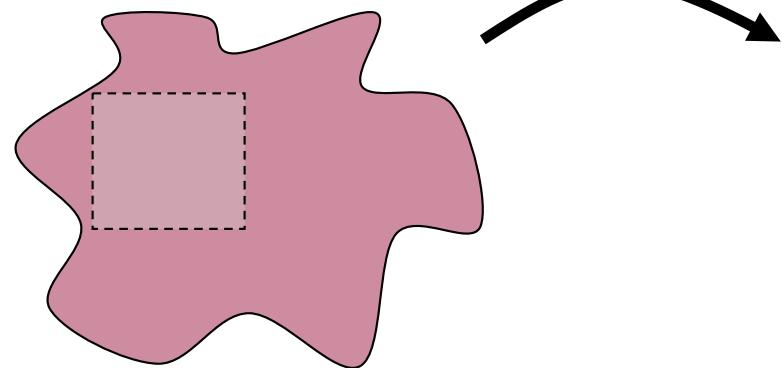
Historically ... single-cell RNA-seq



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

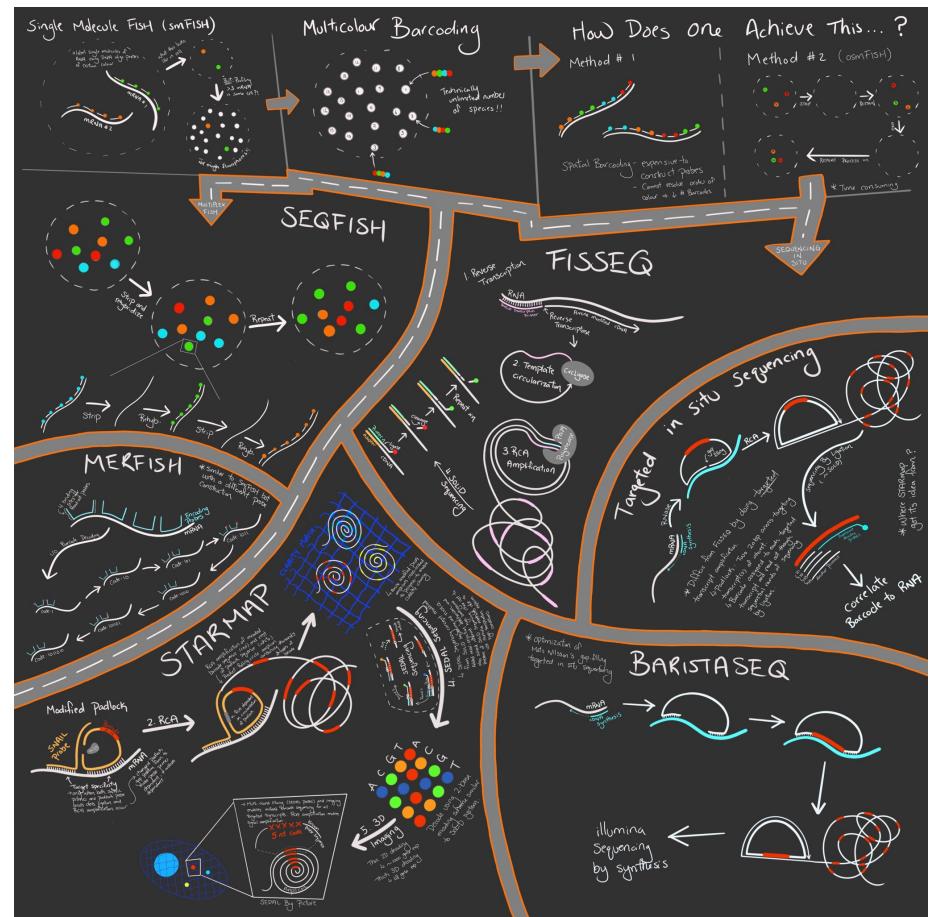
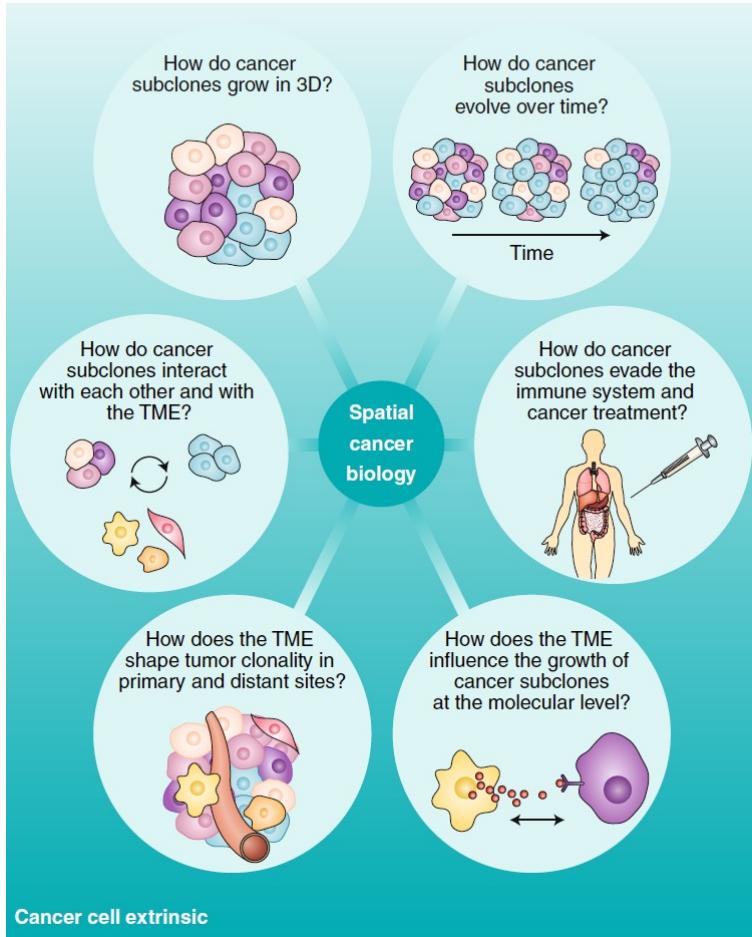


Historically ... **limitations** of single-cell RNA-seq

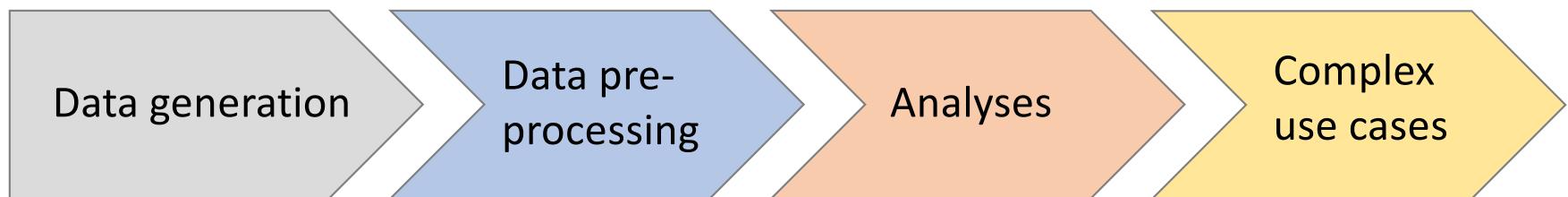


How to choose and create a spatial omics dataset?

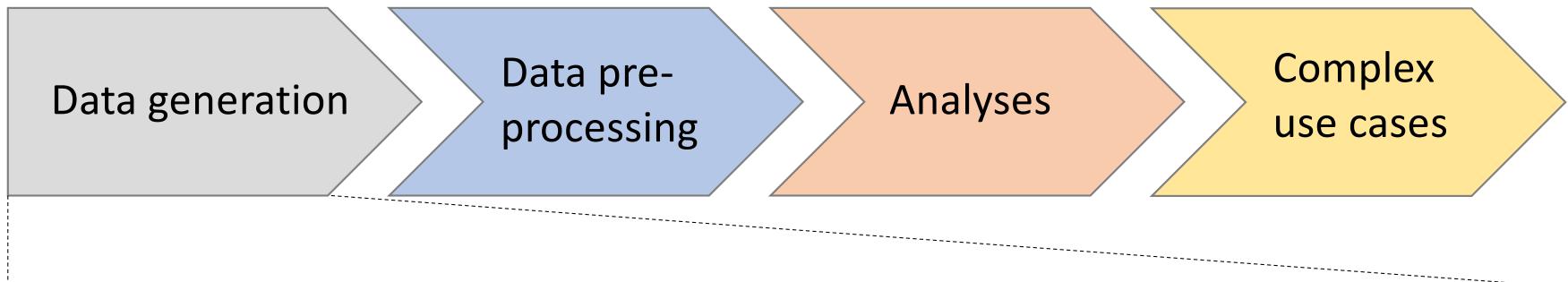
Spatial information is essential to answer these questions, but which method(s) to choose?



The different steps in spatial omics research



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

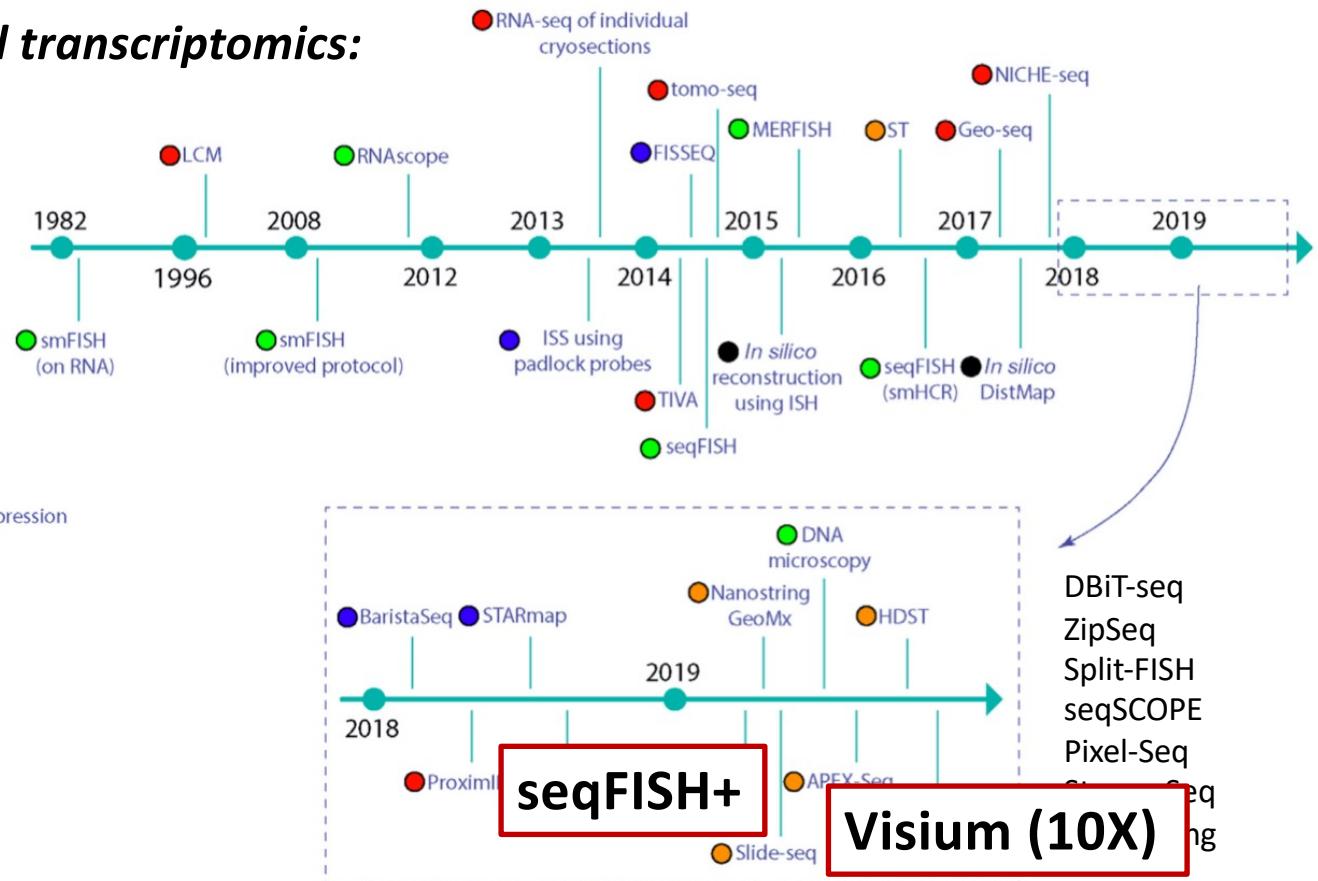
Long history of spatial technology development

Spatial proteomics:

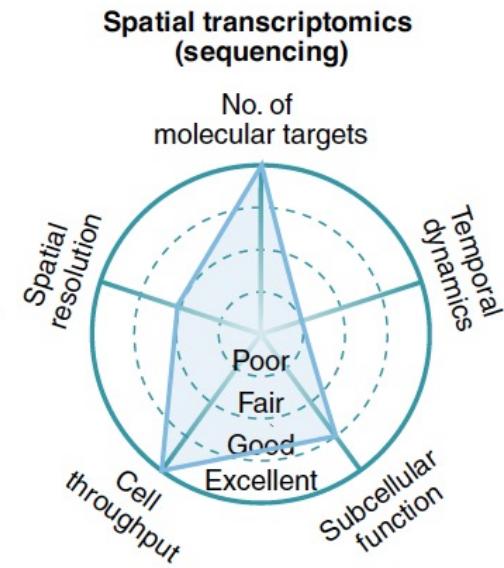
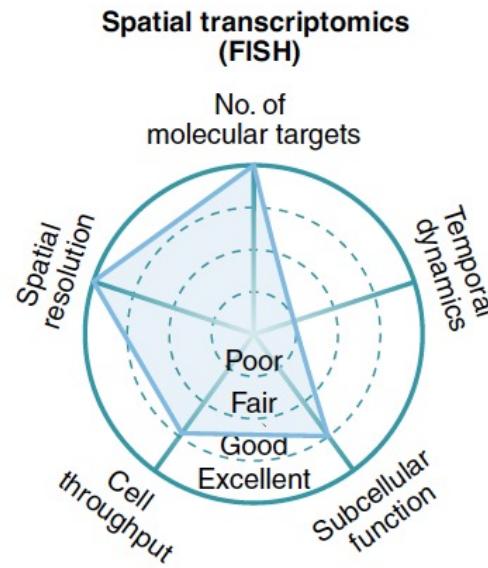
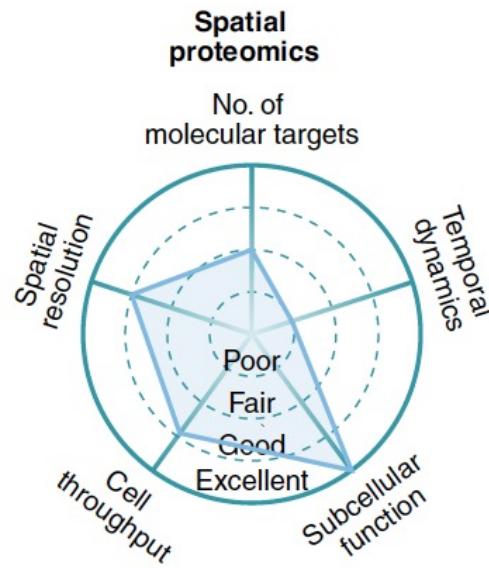
CODEX
CyCIF
MIBI
IMC

seqIF (lunaphore)

Spatial transcriptomics:



Long history of spatial technology development



seqIF / lunaphore

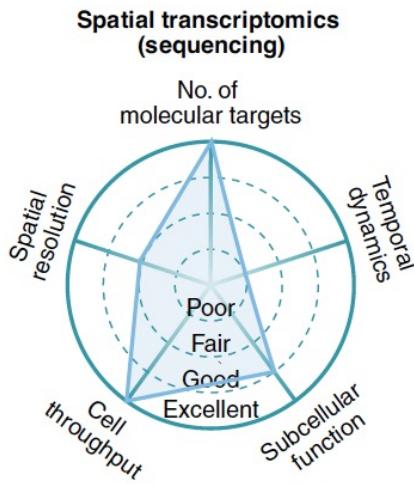
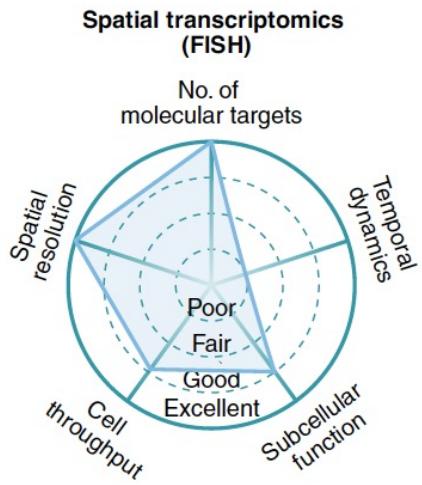
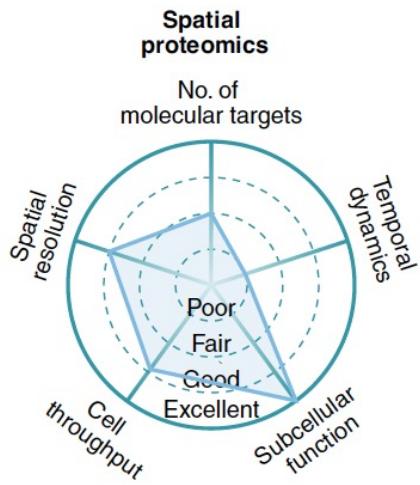
PhenoCycler / Akoya

seqFISH+

**MERFISH
Xenium**

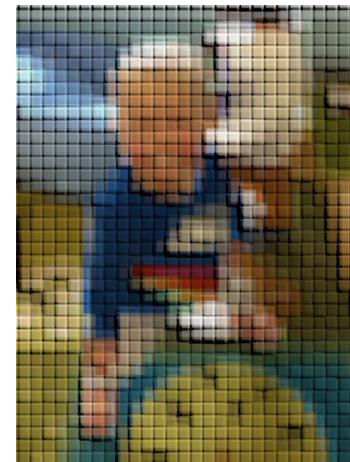
Visium / 10X

Long history of spatial technology development

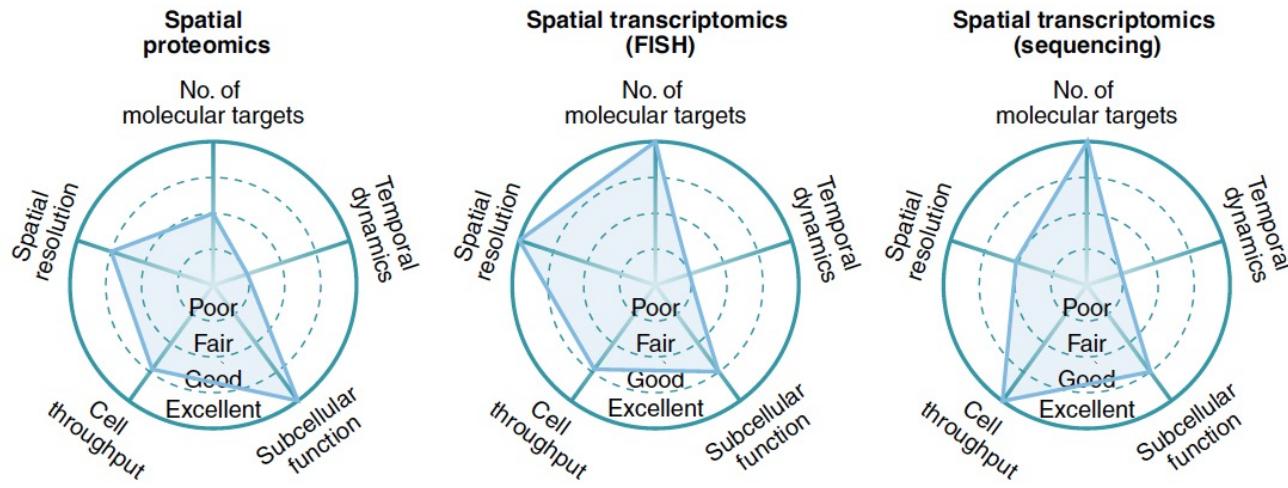


Spatial resolution?

What is the spatial resolution of my picture?



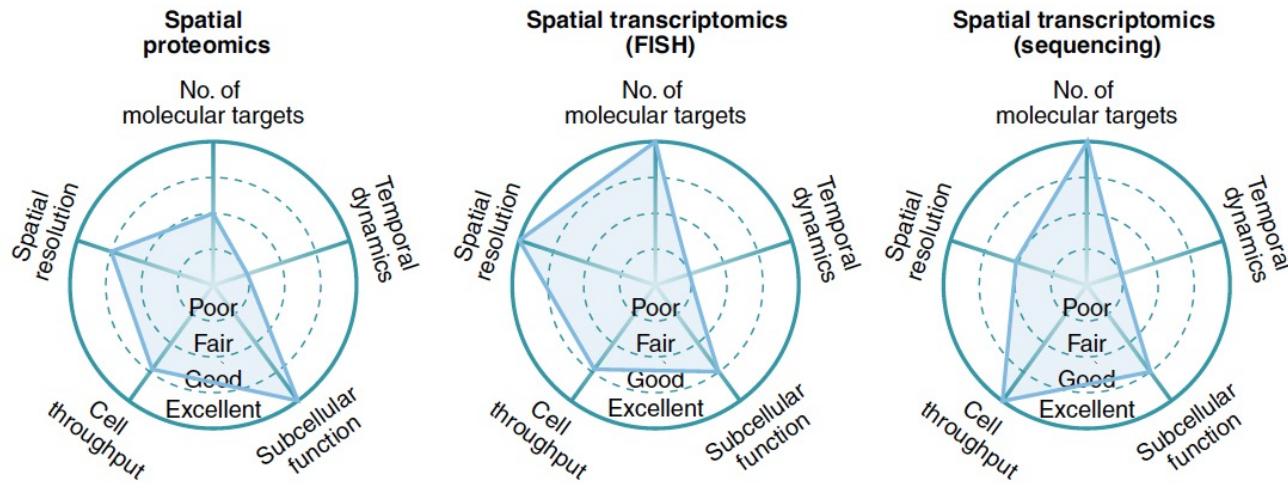
Long history of spatial technology development



Number of molecular targets?

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

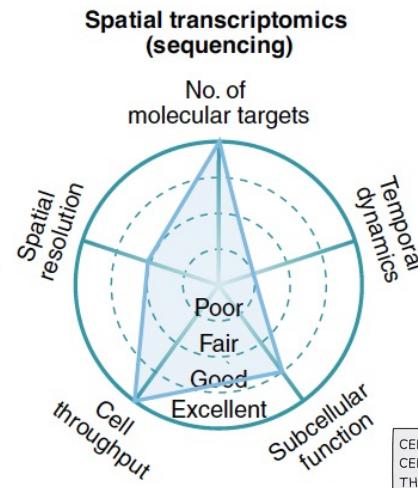
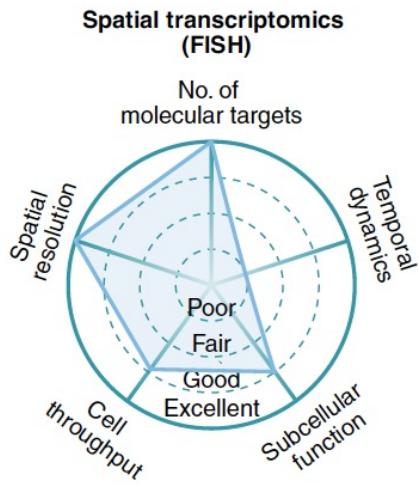
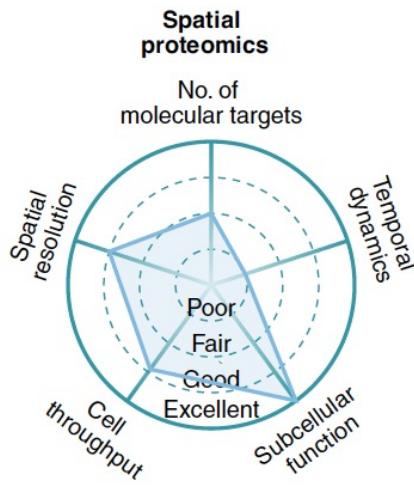
Long history of spatial technology development



Temporal dynamics?

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

Long history of spatial technology development

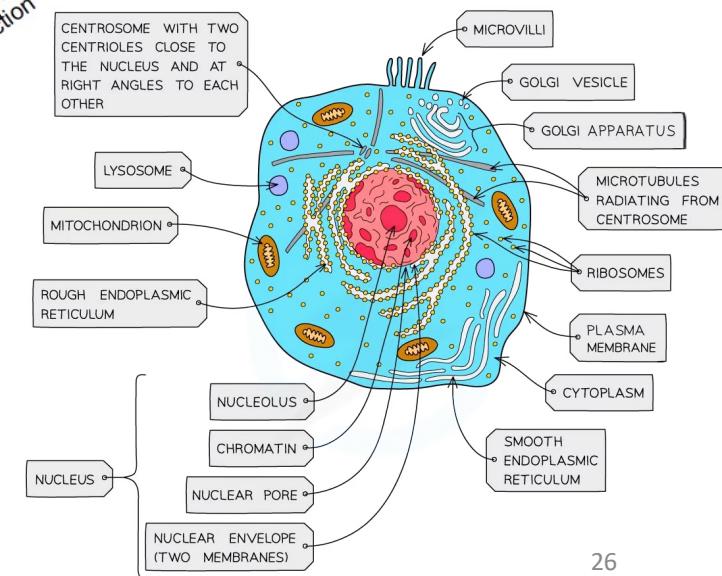


Subcellular function?

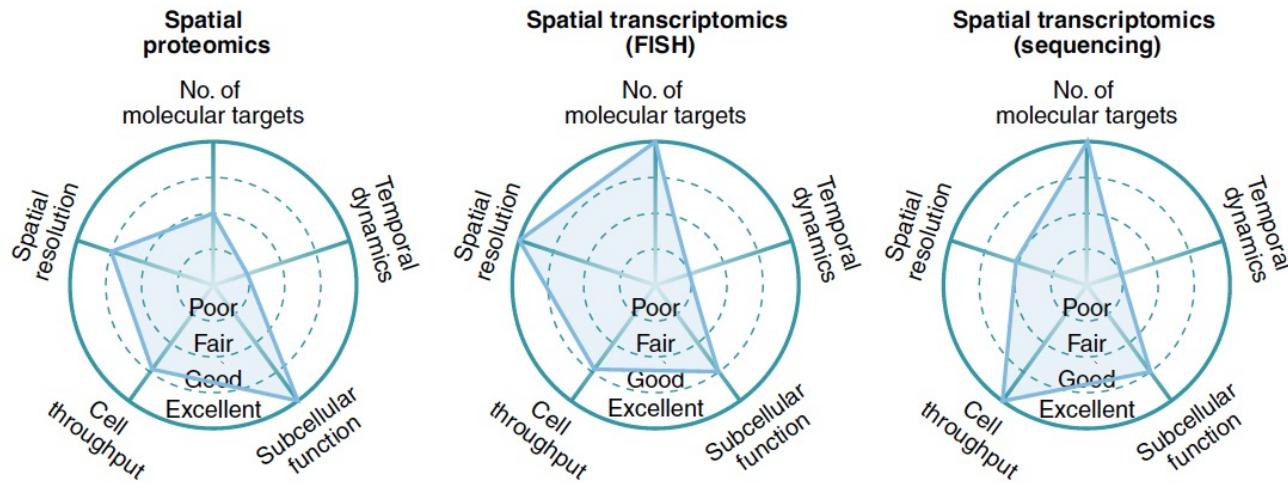
Can the data be used to infer subcellular function or differences?

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

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



Long history of spatial technology development

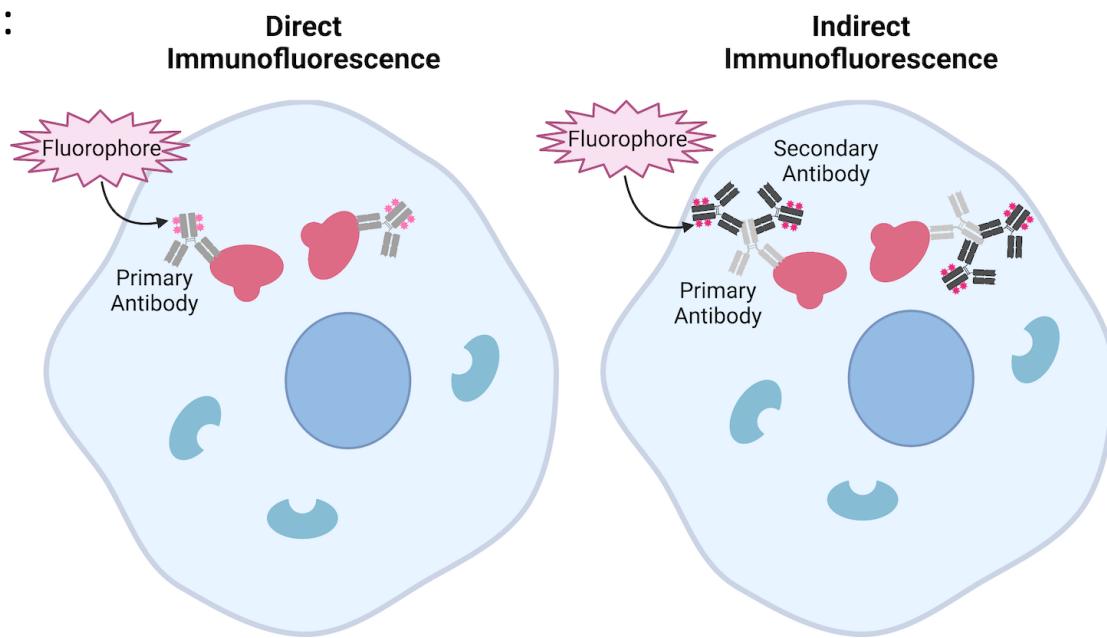


Cell throughput?

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:



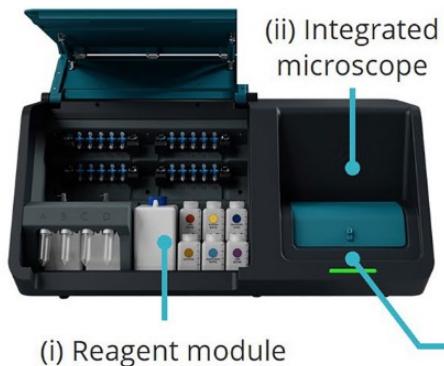
Problem: limited fluorophores and secondary antibodies

Solution: Multiplexing strategies

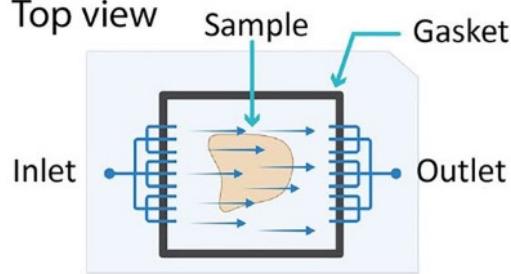
Spatial proteomics

seqIF / lunaphore

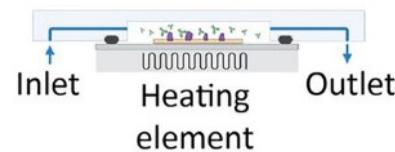
a



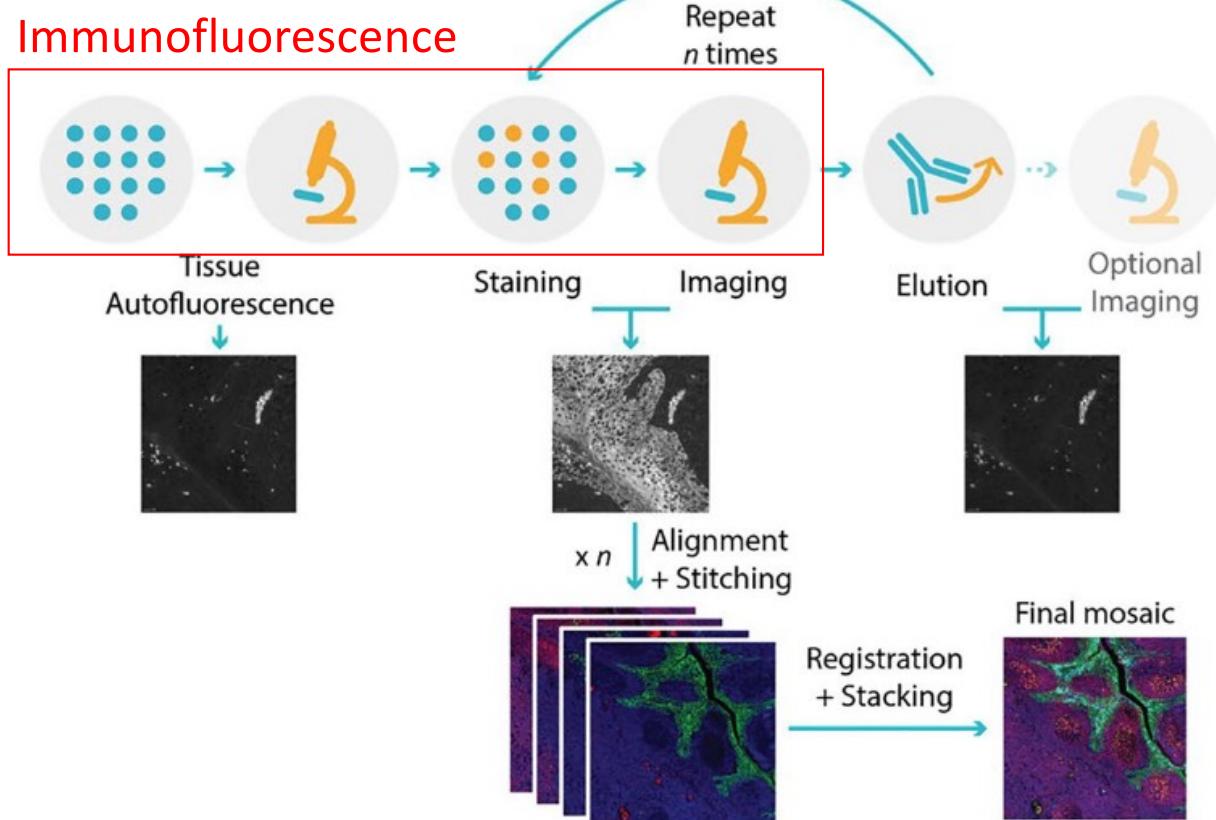
C Top view



Side view

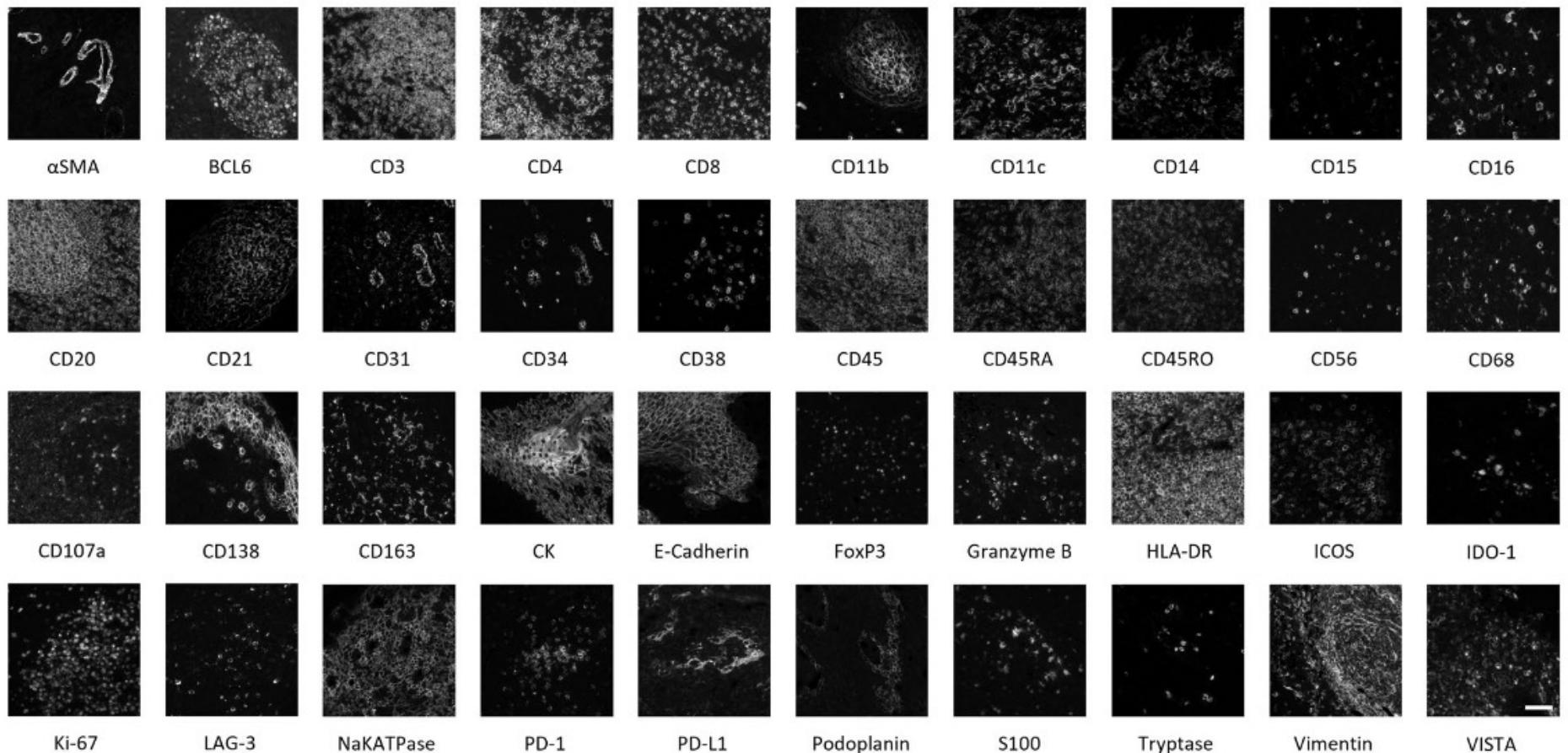


b



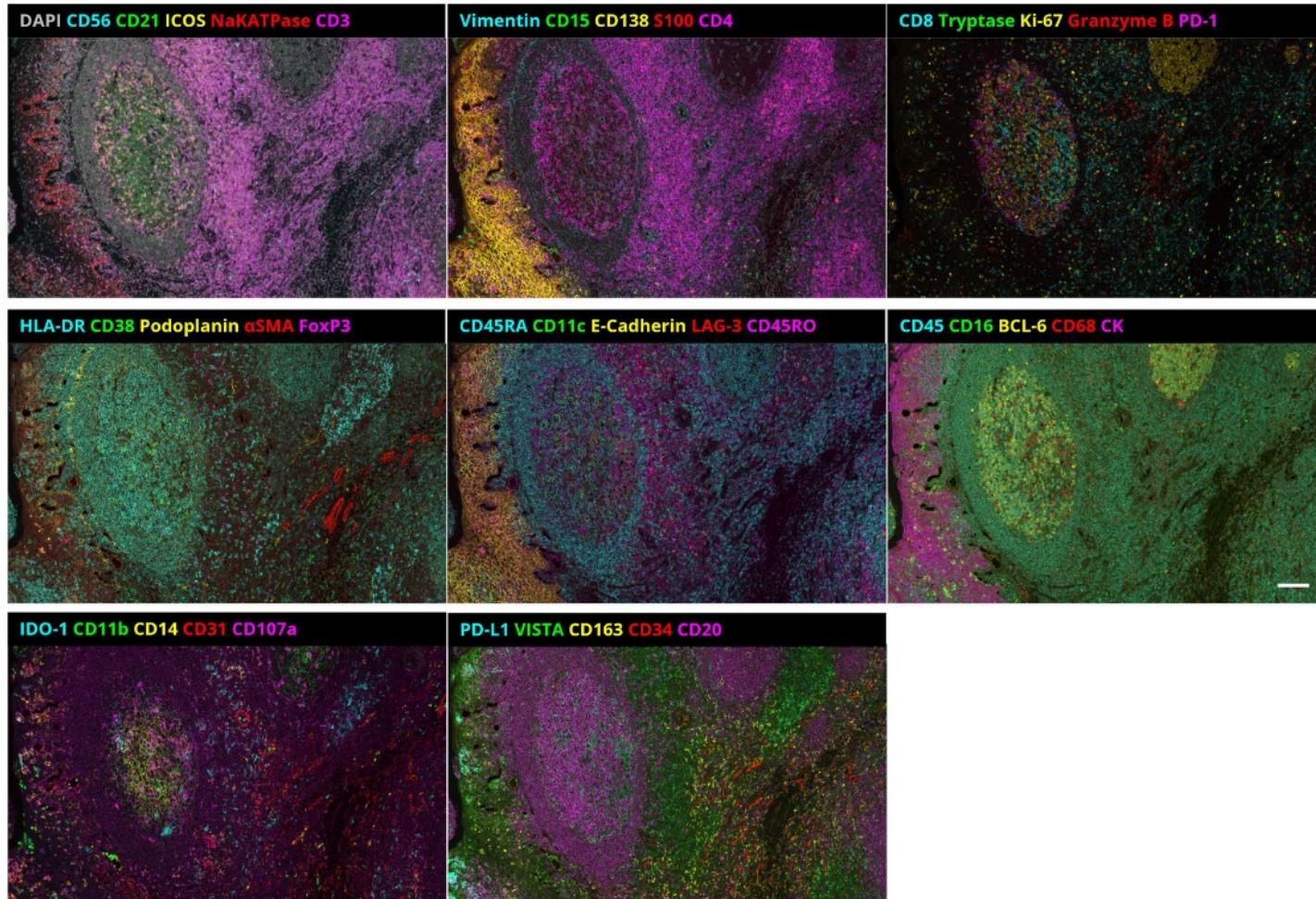
Spatial proteomics

seqIF / lunaphore

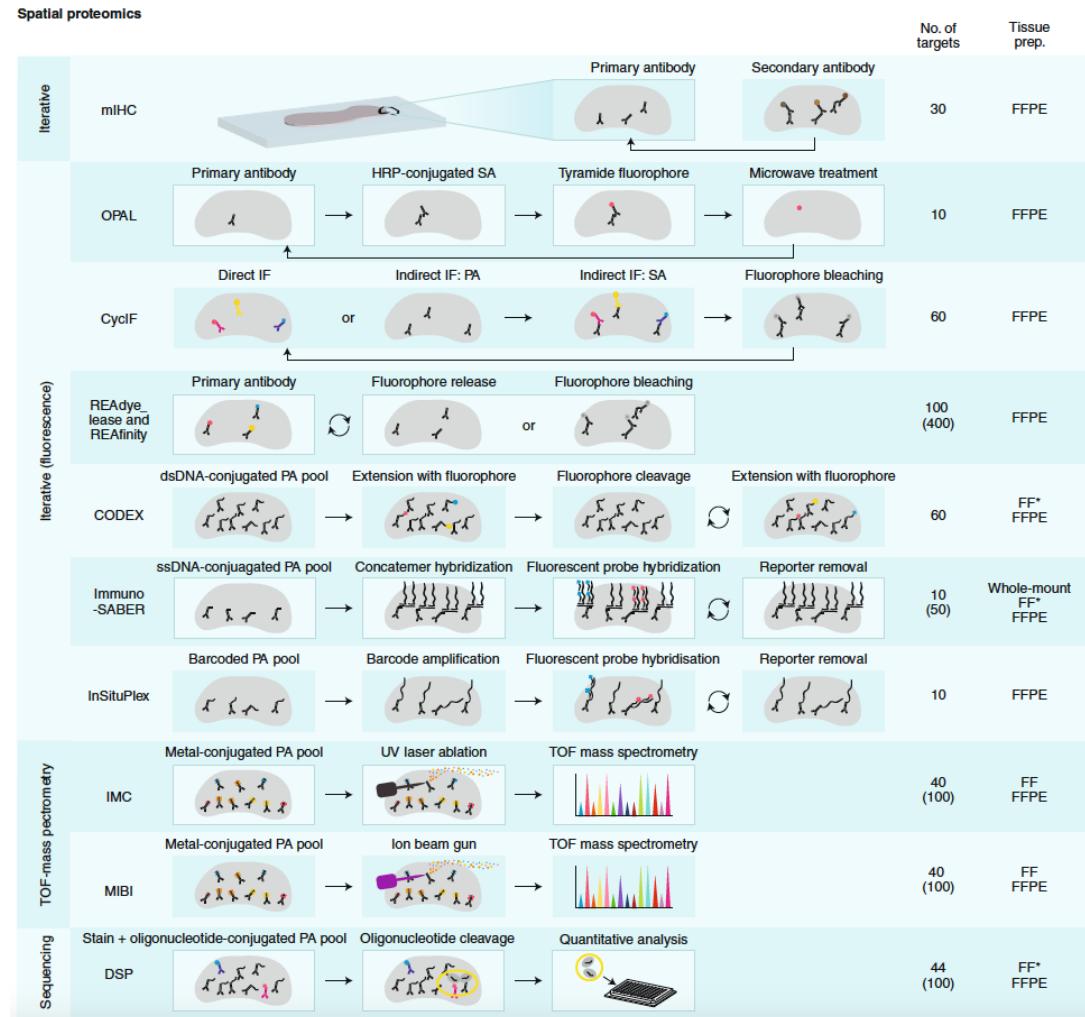


Spatial proteomics

seqIF / lunaphore



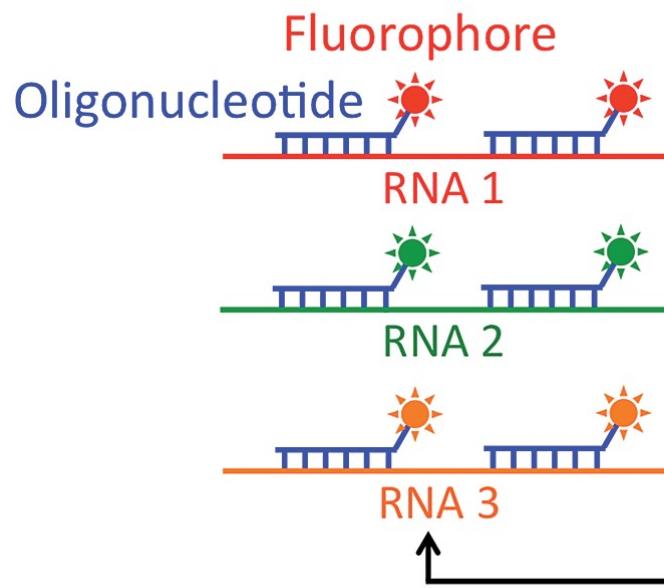
Spatial proteomics



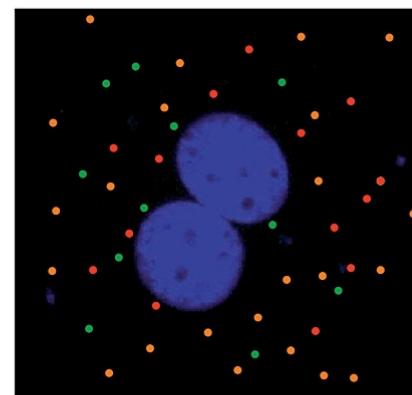
Lewis, S. M. et al. Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:[10.1038/s41592-021-01203-6](https://doi.org/10.1038/s41592-021-01203-6).

Spatial transcriptomics (FISH)

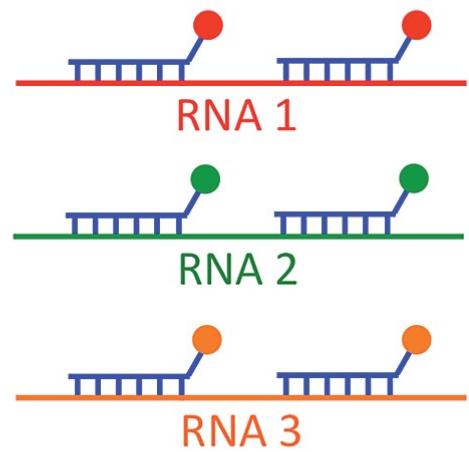
1. Target Hybridization



2. Fluorescence Imaging



3. Photobleaching



Repeat for Different RNA

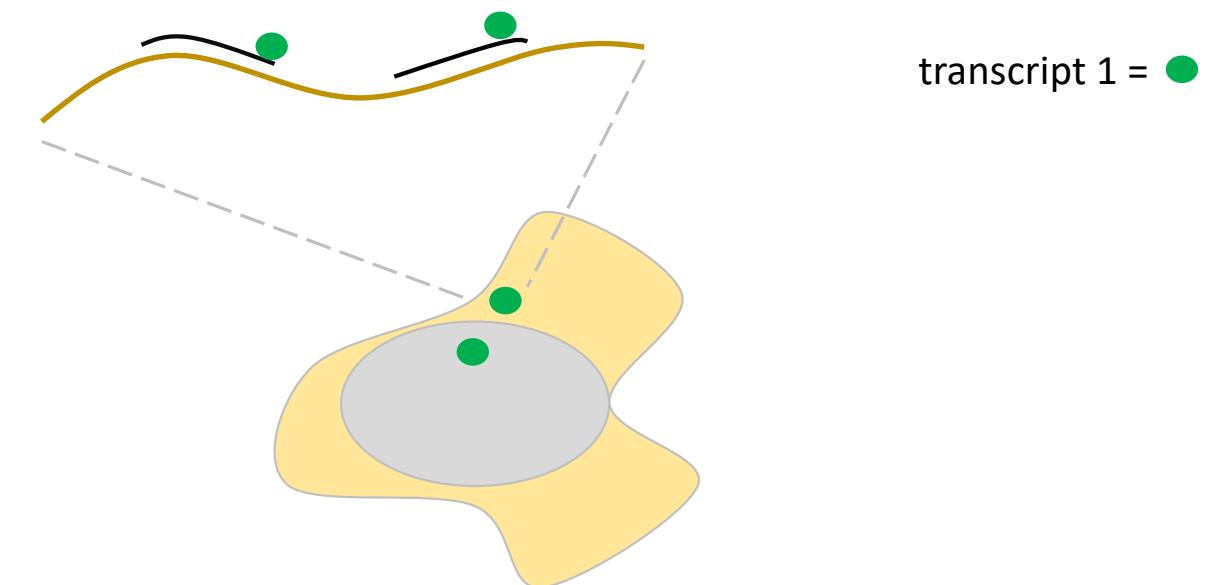
Problem: limited fluorophores

Solution: Multiplexing strategies

Spatial transcriptomics (FISH) **seqFISH+**

in situ hybridizations

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



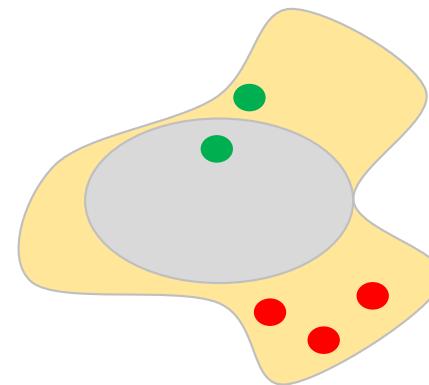
Spatial transcriptomics (FISH)

seqFISH+

in situ hybridizations

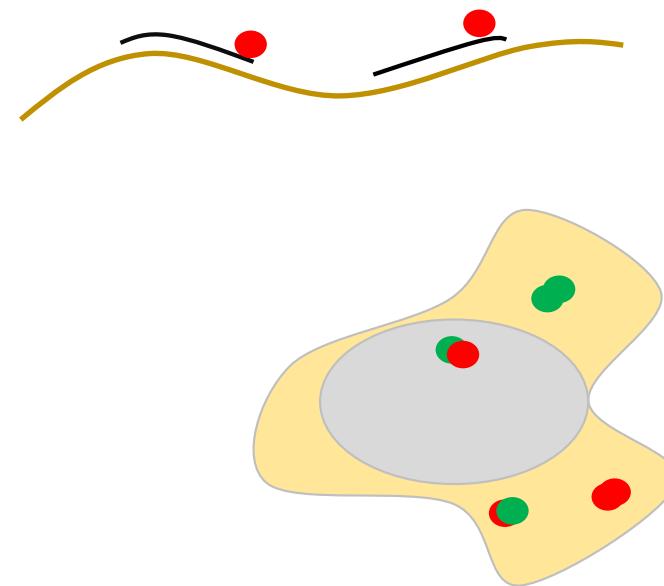


transcript 1 = ●
transcript 2 = ●



Spatial transcriptomics (FISH) **seqFISH+**

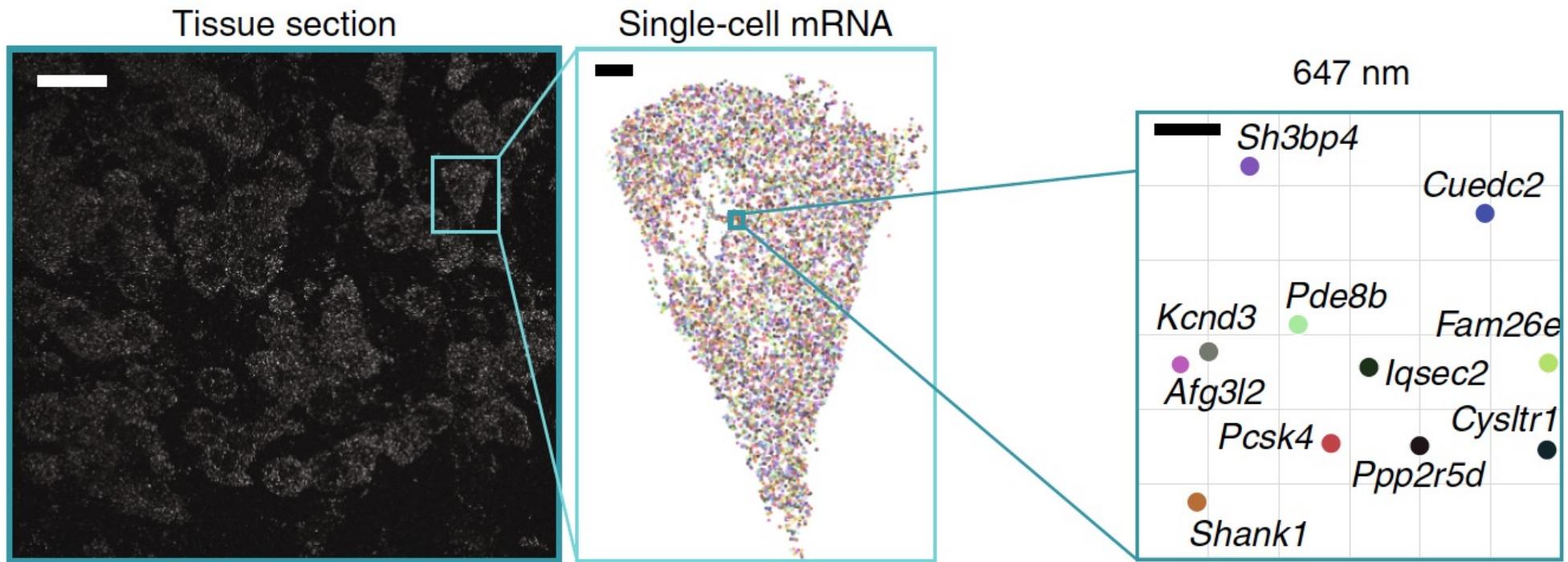
Sequential *in situ* hybridizations



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

Spatial transcriptomics (FISH) **seqFISH+**

Spatial transcriptomics (FISH)



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: $F \times H$

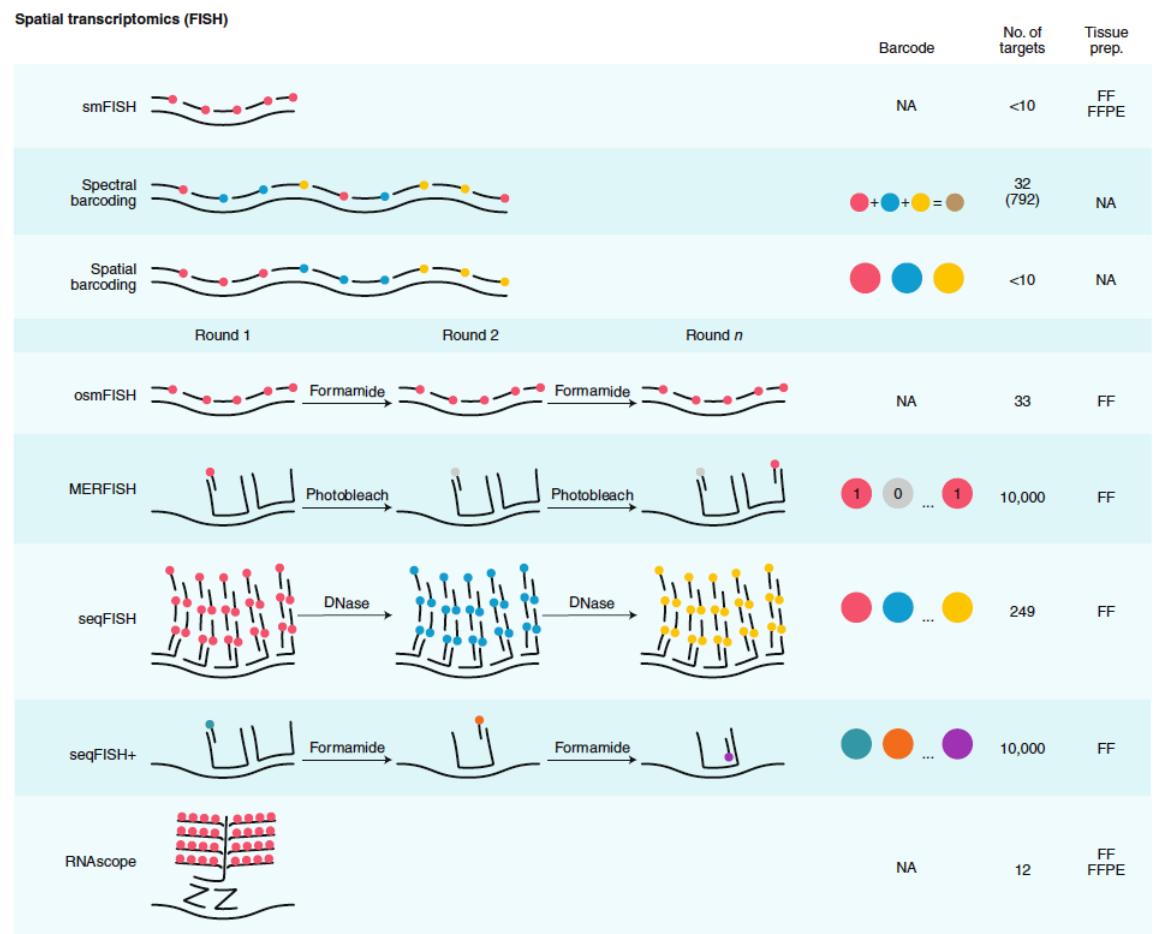
MERFISH: $2^H - 1$

seqFISH: F^H

ISS: 4^B

FISSEQ: 4^B

Spatial transcriptomics (FISH)



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](https://doi.org/10.1038/s41592-021-01203-6).

Spatial transcriptomics (sequencing)

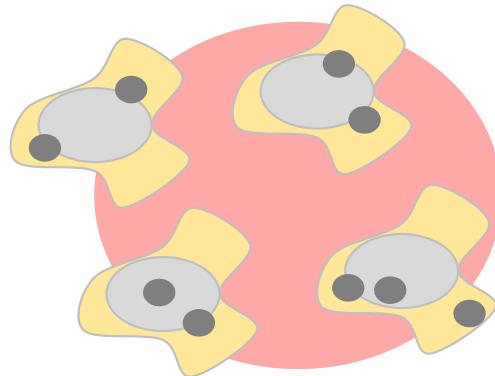
in situ hybridizations



in situ sequencing



in situ capture



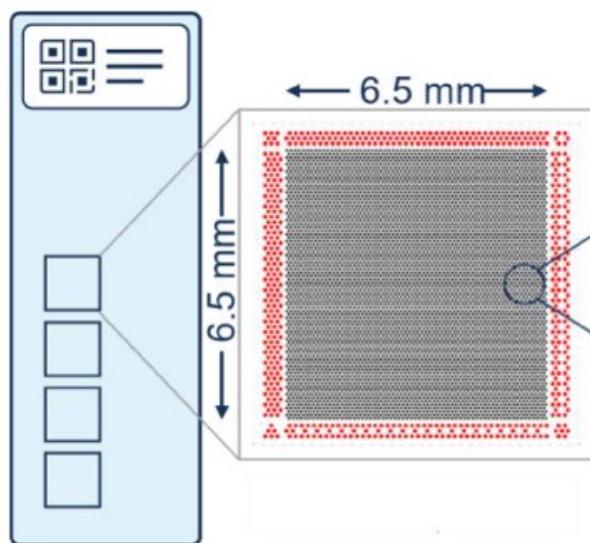
spatial barcode



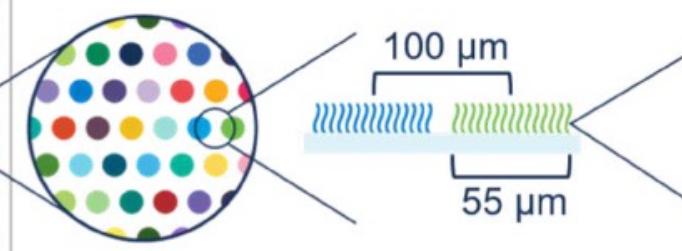
Spatial transcriptomics (sequencing)

Visium / 10X

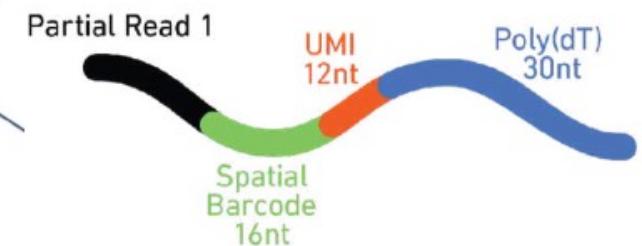
Visium Spatial Gene Expression Slide



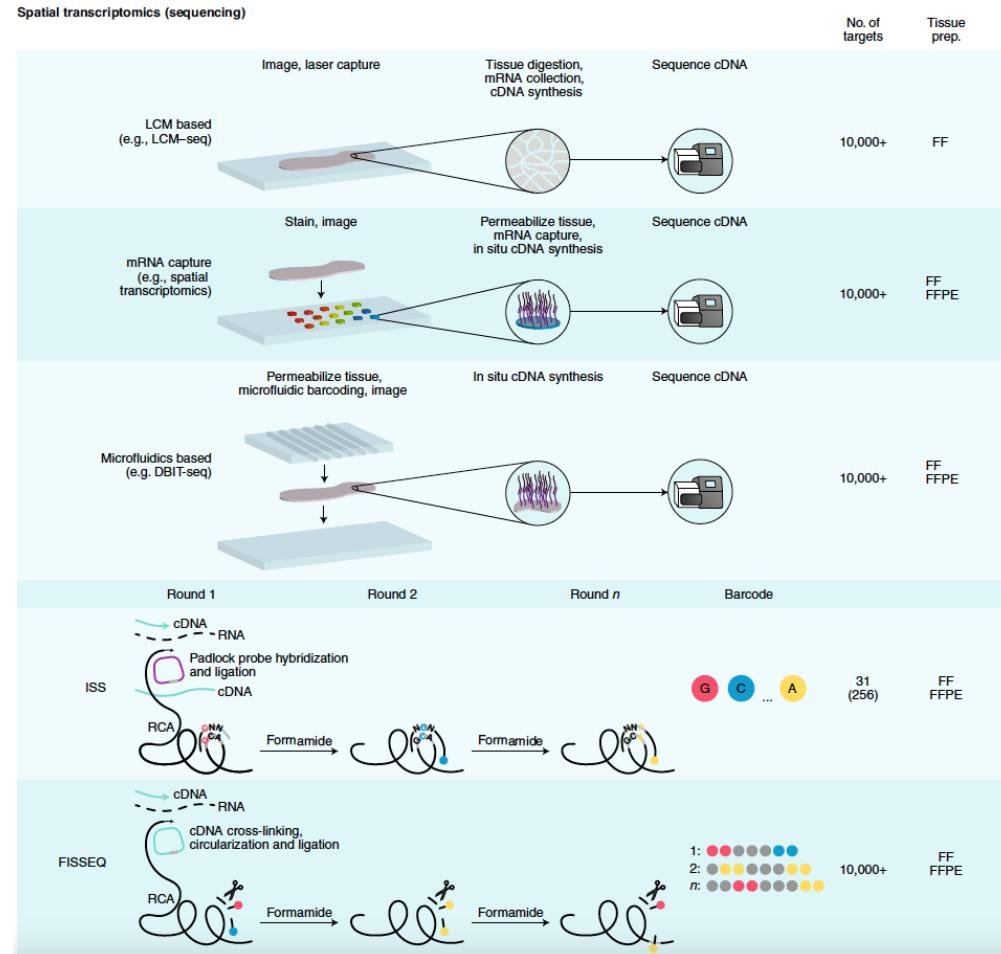
Capture Area with ~5000 Barcoded Spots



Visium Gene Expression Barcoded Spots

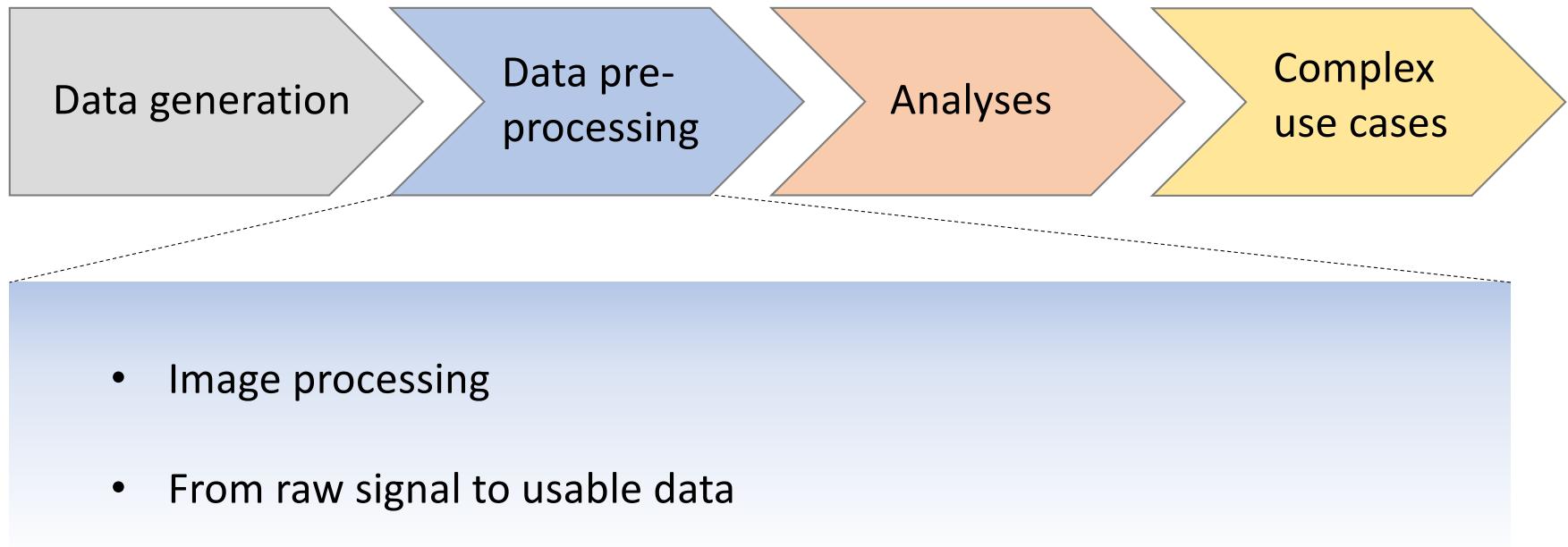


Spatial transcriptomics (sequencing)

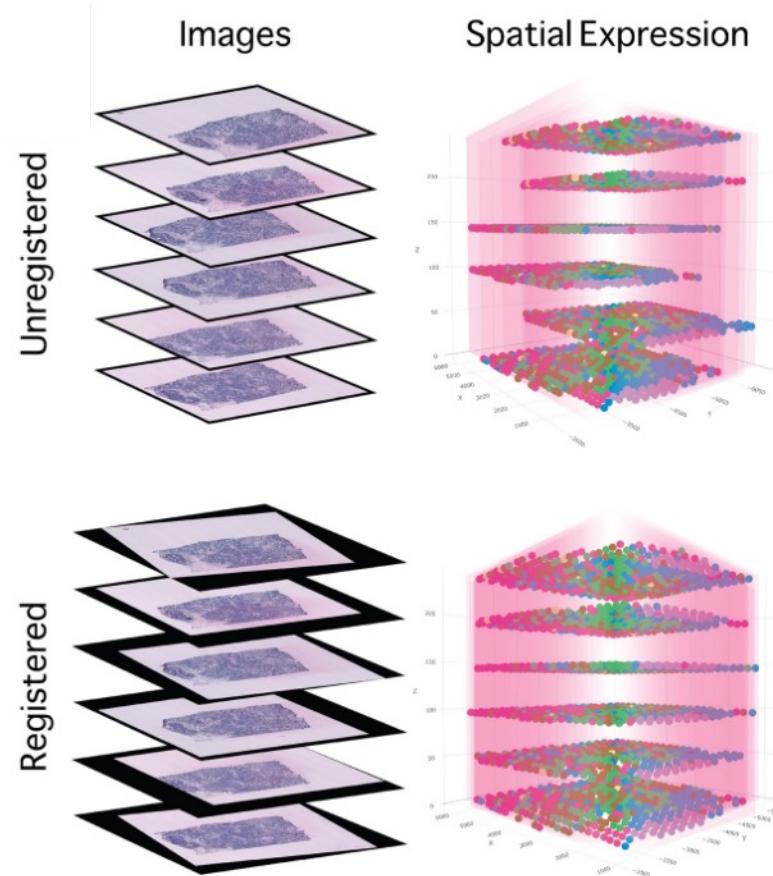
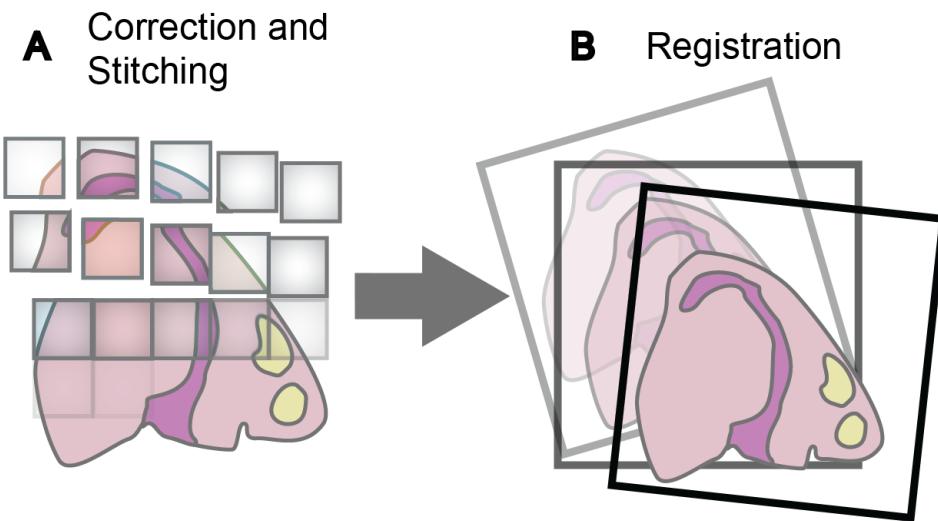


Lewis, S. M. et al. Spatial omics and multiplexed imaging to explore cancer biology. *Nat Methods* 1–16 (2021) doi:[10.1038/s41592-021-01203-6](https://doi.org/10.1038/s41592-021-01203-6).

The different steps in spatial omics research

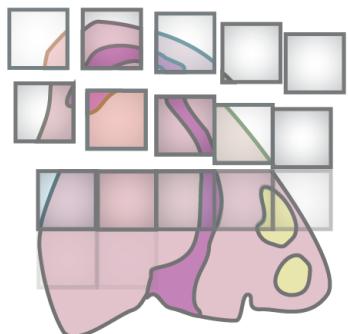


Essential pre-processing steps: image co-registration

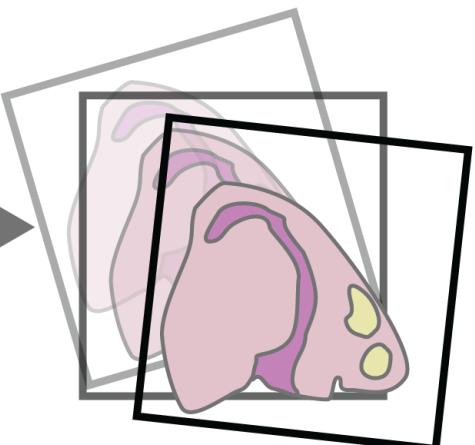


Essential pre-processing steps: segmentation

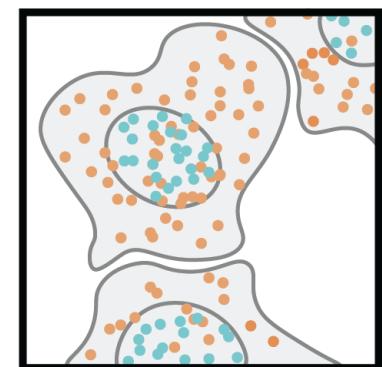
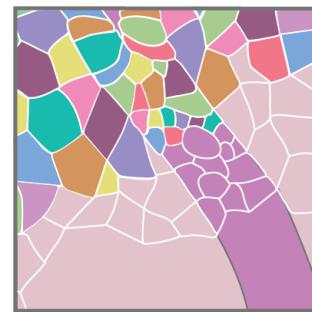
A Correction and
Stitching



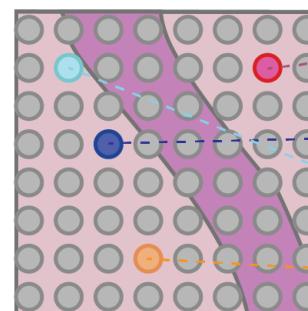
B Registration



C Segmentation

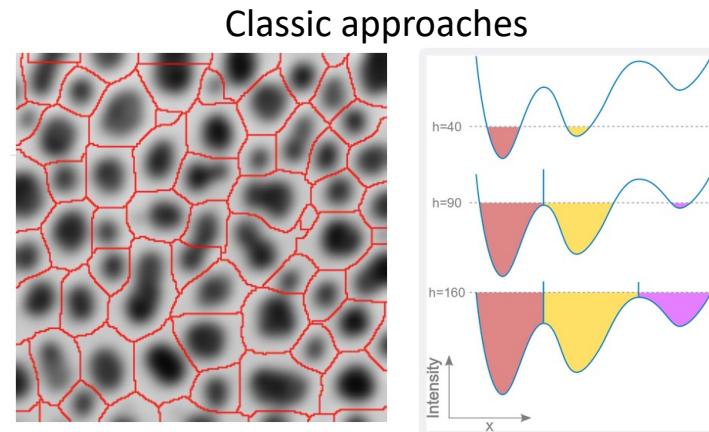


D Barcode Calling

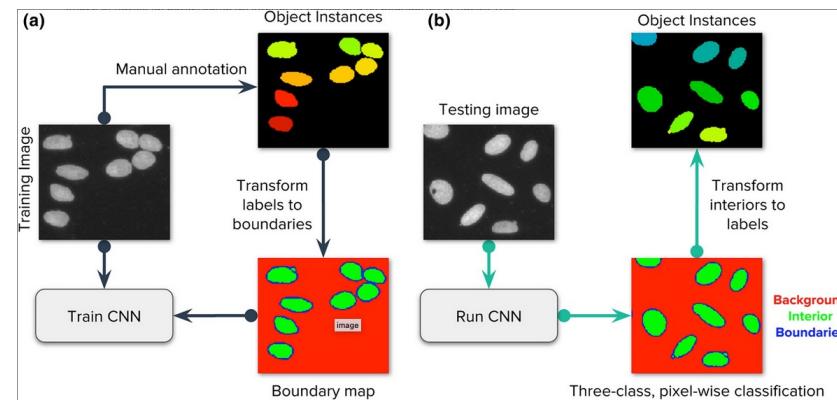


Essential pre-processing steps: segmentation

C Segmentation



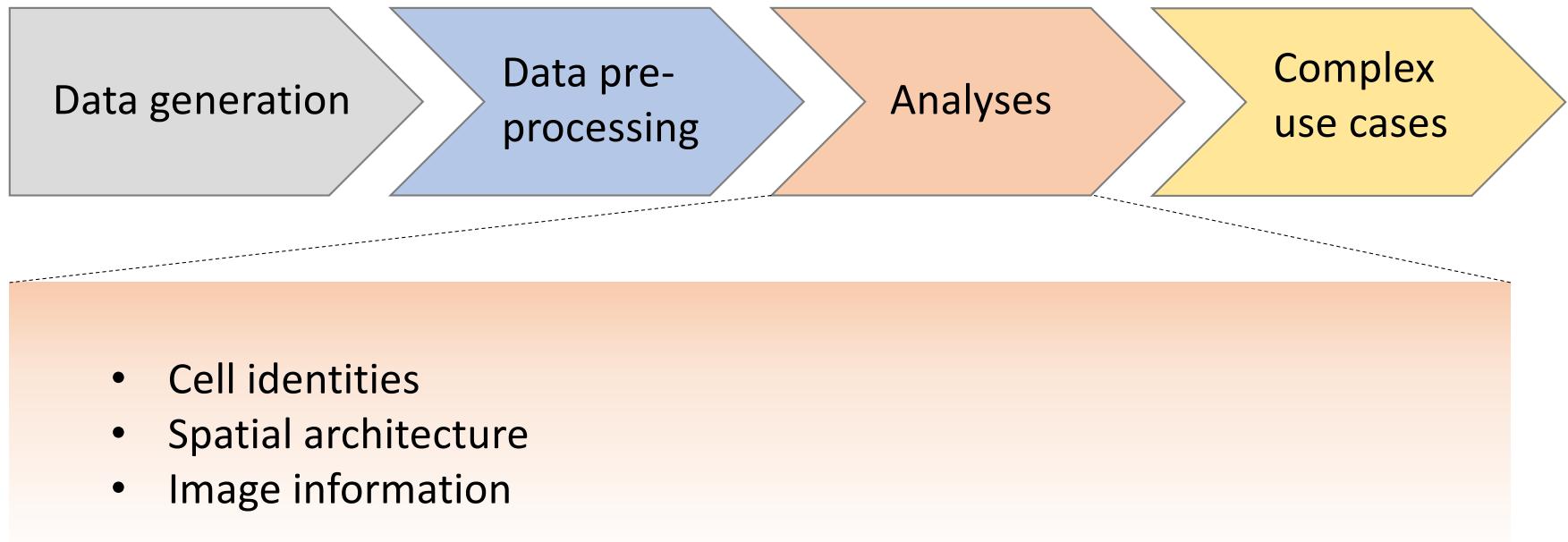
New Deep-learning approaches

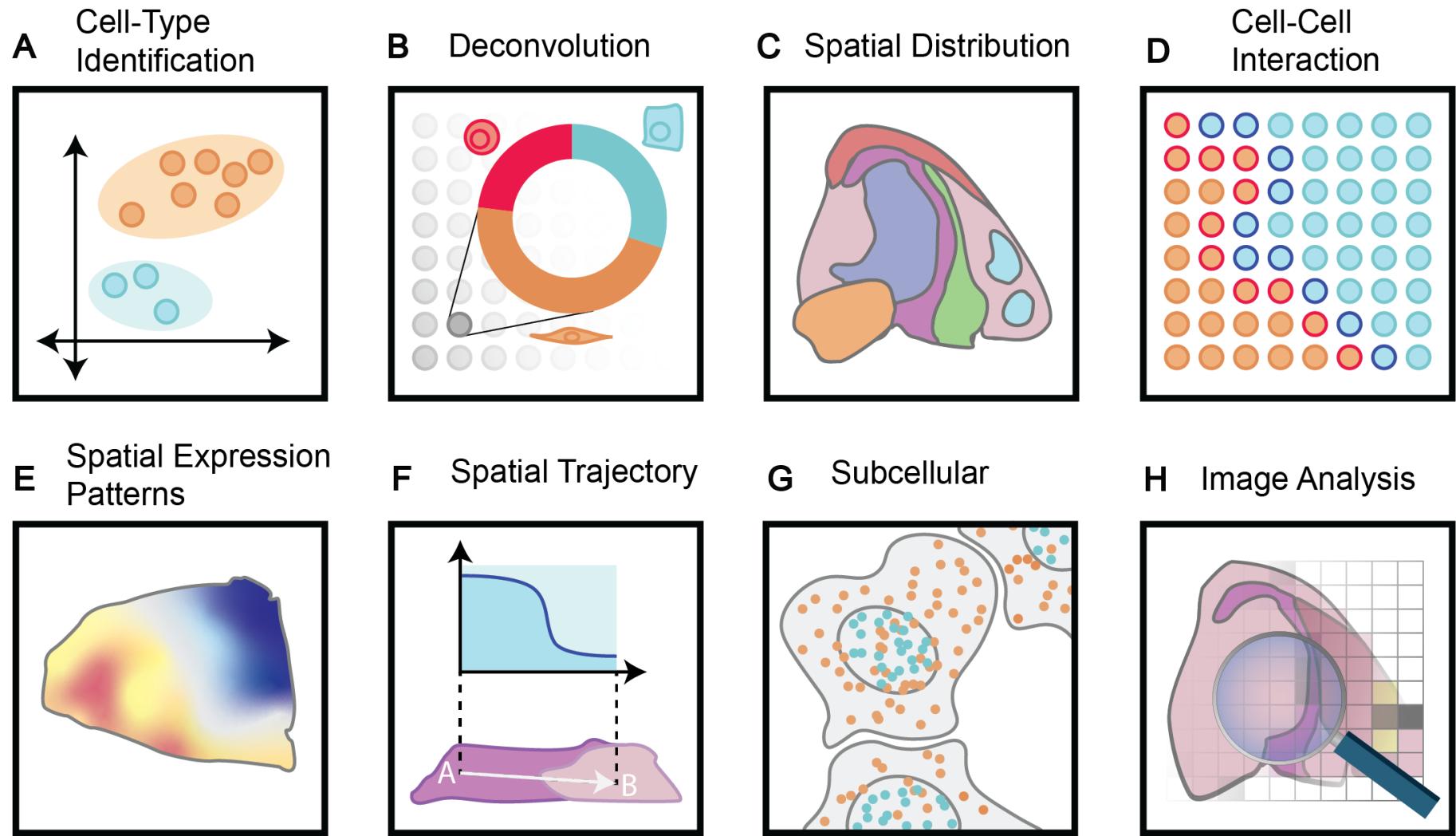


ilastik

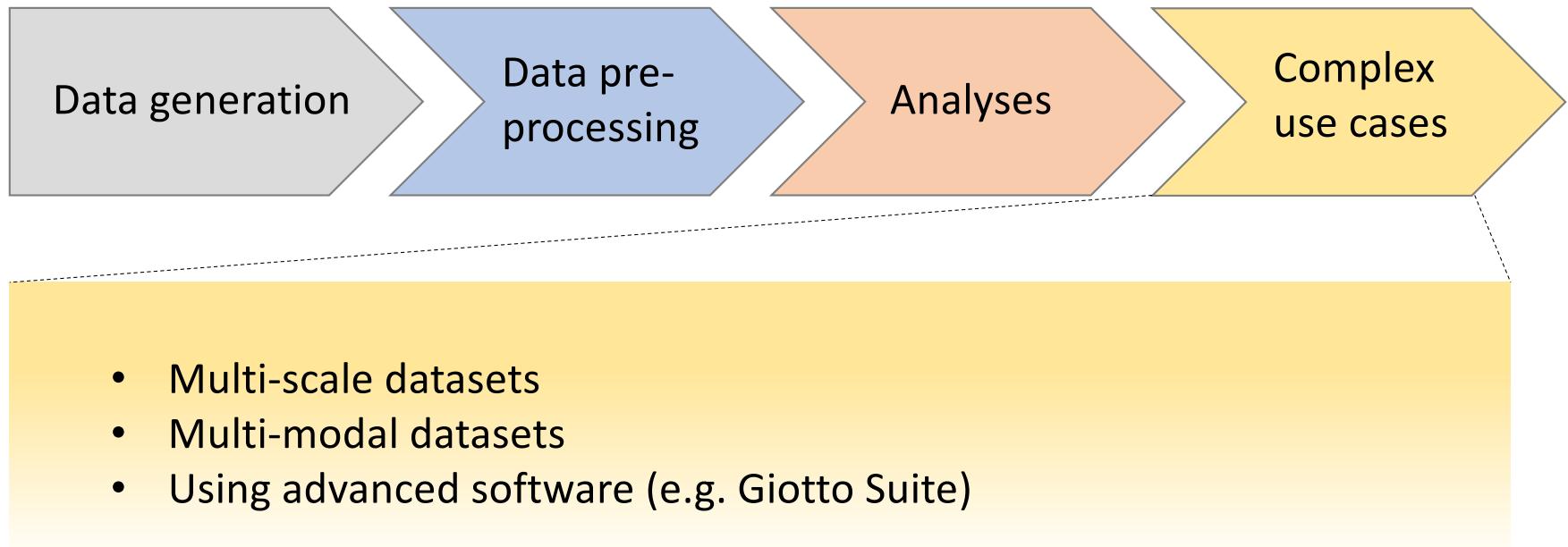


The different steps in spatial omics research

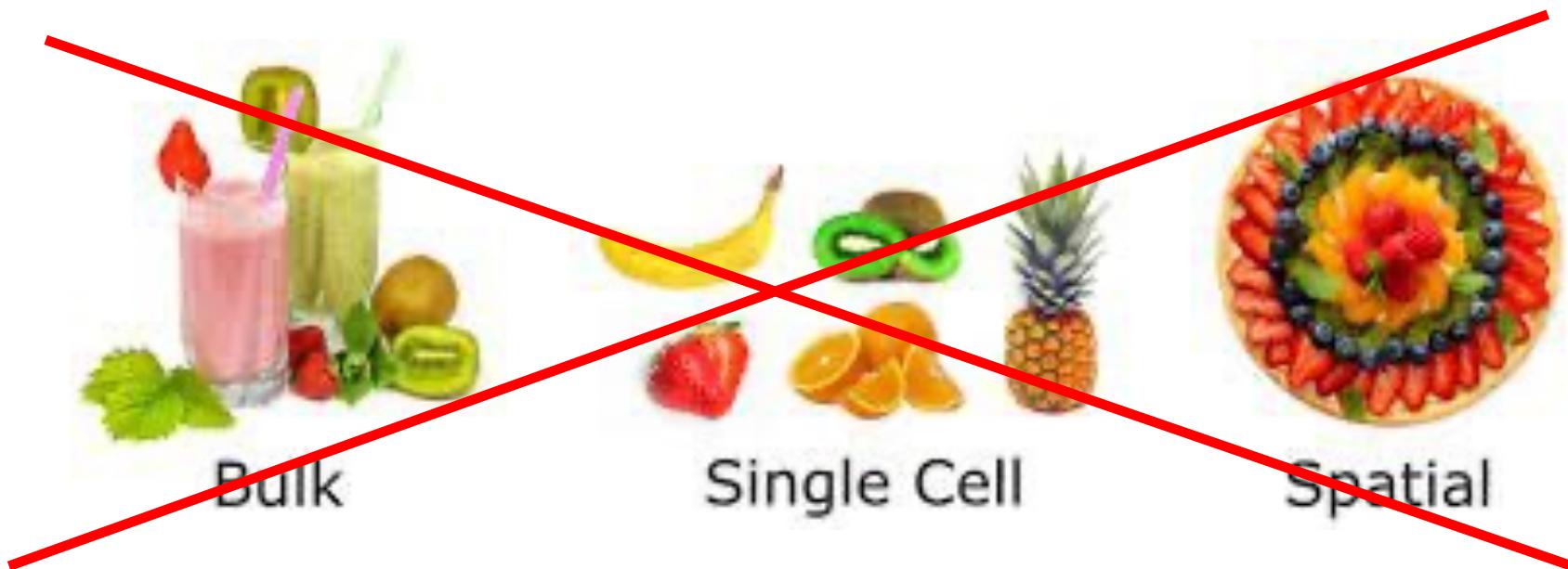




The different steps in spatial omics research



The emerging field of spatial omics



The emerging field of spatial omics



RNA-seq



The emerging field of spatial omics



RNA-seq



Single-cell RNA-seq

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

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

Spatial Transcript Microscopy maps tissue responses to brain injury

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

A White-matter injury → Cryosectioning → 1. MERFISH → 2. Electron microscopy → Spatial transcriptomics (MERFISH) → Electron microscopy

B Miltenyi FFPE Tissue Dissociation → Serial FFPE Sections → Pre-CytoAssay → Xenium Cassette → Xenium Analyzer

Integration of Multiple Spatial Omics Modalities Reveals Unique Insights into Cancer

Sam Kint, Tassiani Sarreto, Idkowiak, David Wouters, Ly, Vincent de Laat, Maria José Q Mantas, M Butler, Massimo Loda, Steven Joniau, Thierry Voet, 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>

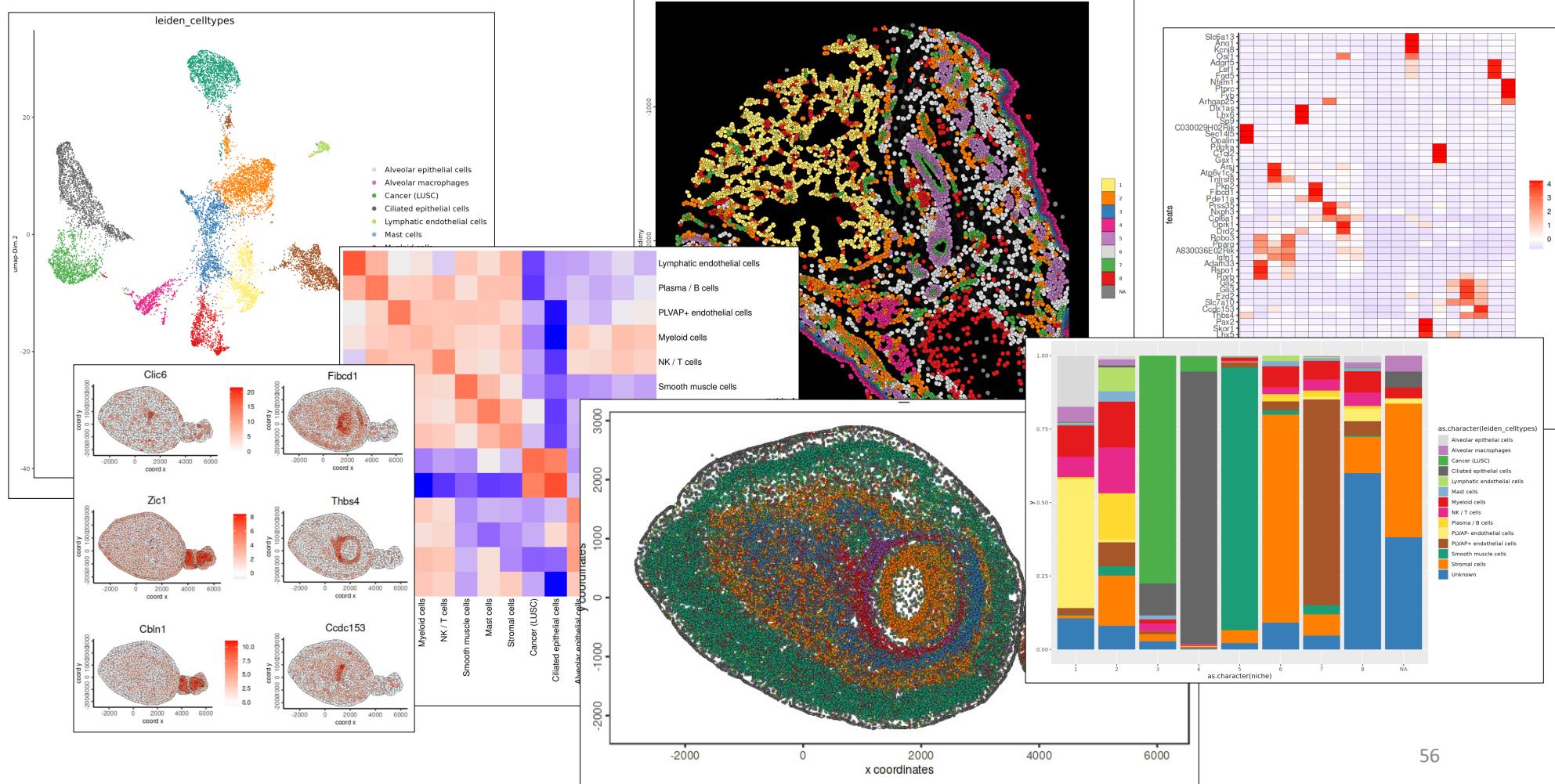
a TMA cohort: 80 TMAs (30 patients), 2D-WSI cohort: 127 2D-WSIs (64 patients), 3D-CODA cohort: 14 3D samples (14 patients). Scale bars: 20 μm, 5 mm, 2 cm. Information: Size (0 to 5 cm³) and Number of cells (~80k cells, ~1.5M cells, ~1.6B cells).

b Tissue processing: Surgical resected tumor → FFPE → Serial sectioning and H&E staining → High resolution scanning.

c CODA tissue segmentation pipeline: 2D-WSI cohort → Tissue annotations (6 mm), Deep learning and tissue segmentation (6 mm) → 3D-CODA cohort → Image alignment, Tissue annotations, 3D reconstruction and segmentation (5 mm, UMAP 1, UMAP 2).

d Spatial ATAC-RNA-seq (ATAC and RNA): UMAP 1, UMAP 2. Legend: R0, R7, J1, J8, R1, R8, J2, J9, R2, R9, R3, R10, R4, R11, R5, R12, J3, J4, J11, J5, J12, J6, R6, R13.

Demonstration with BIL & HuBMAP datasets



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



The screenshot shows the Giotto Suite website interface. At the top, there is a navigation bar with icons for search, general settings, and a menu. The main header reads "Giotto Suite". On the left, a sidebar lists "GENERAL" (Search, Branches, Documentation, Contribute, News, Presentations) and "GETTING STARTED" (Giotto Installation, Giotto Ecosystem, Giotto Configuration, Giotto Object Creation). The central content area displays the Giotto Suite logo and a circular diagram showing the interconnected components: Visuals, Browser, Processing, Classes, Tools, and Benchmark. To the right, a sidebar titled "Upstream" lists "DeepCell", "Cellpose", "CellProfiler", and "Jupyter Notebook". Below it, "External Methods" lists "Python" and "R". At the bottom, "Downstream" lists "CellX" and "CellMiner". A descriptive text box at the bottom states: "An open-source and technology-agnostic spatial multi-omics analysis platform" and "Giotto Suite is a collection of open source software tools, including data structures and methods," followed by a link to "www.giottosuite.com".