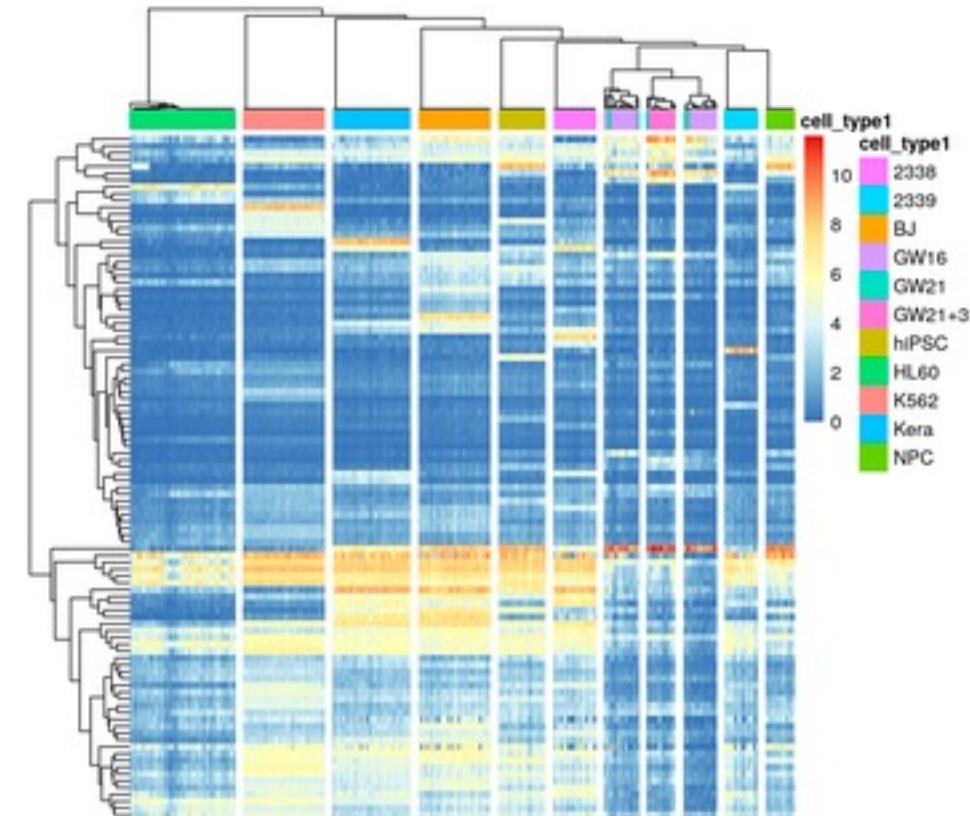
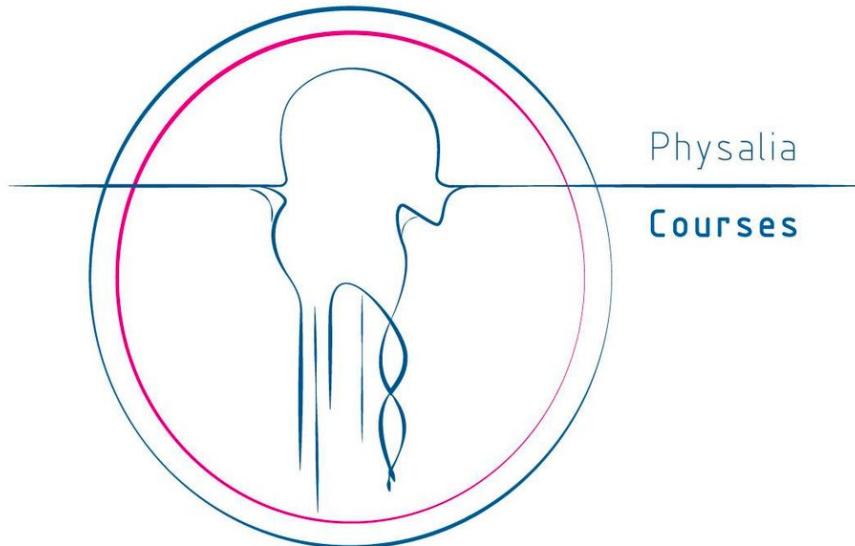


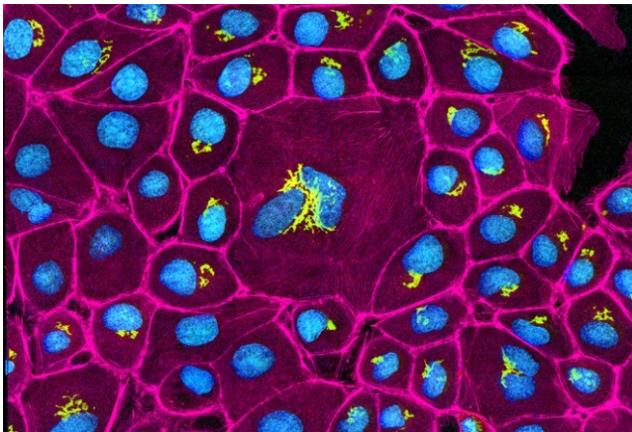
Advances in single-cell genomics: spatial genomics

Orr Ashenberg, Jacques Serizay, Fabrício Almeida-Silva
November, 2024

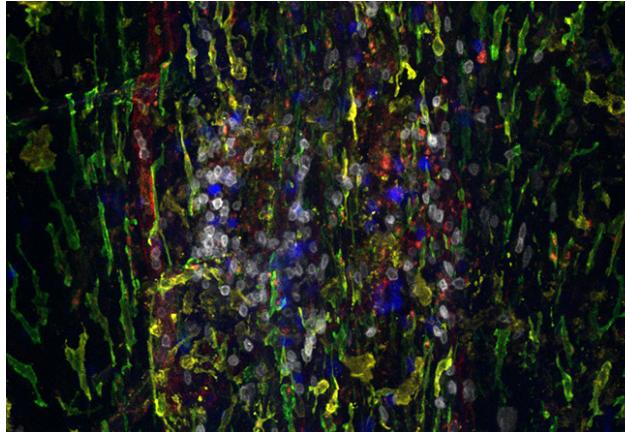


Incredible diversity in cell types, states, and interactions across human tissues

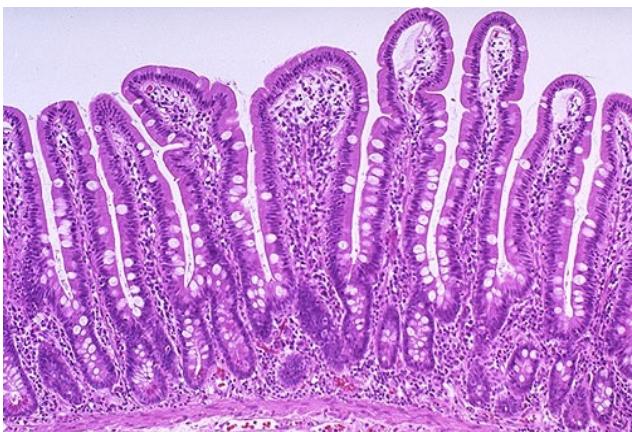
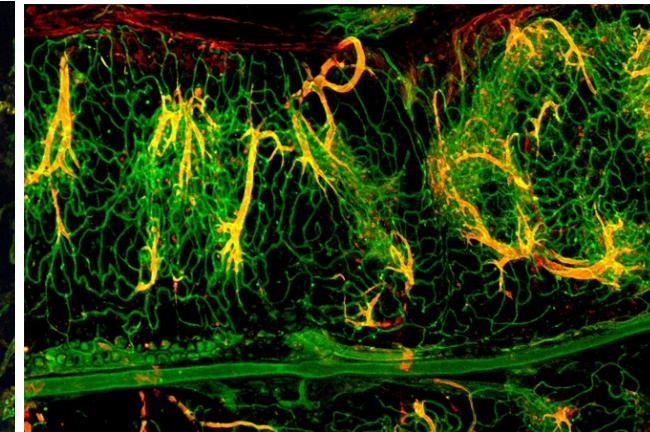
Skin epithelium



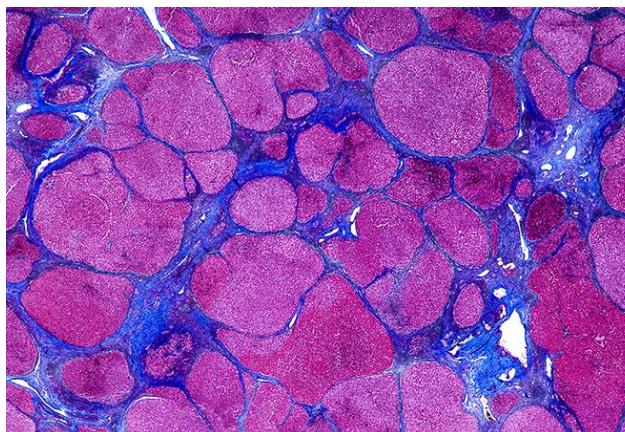
Brain meninges



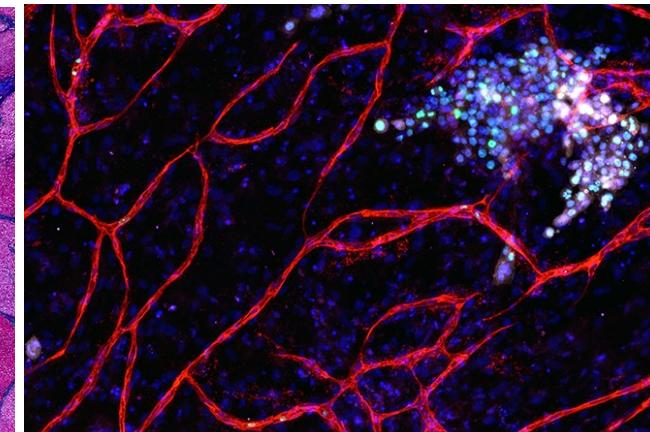
Blood vessels



Small intestine

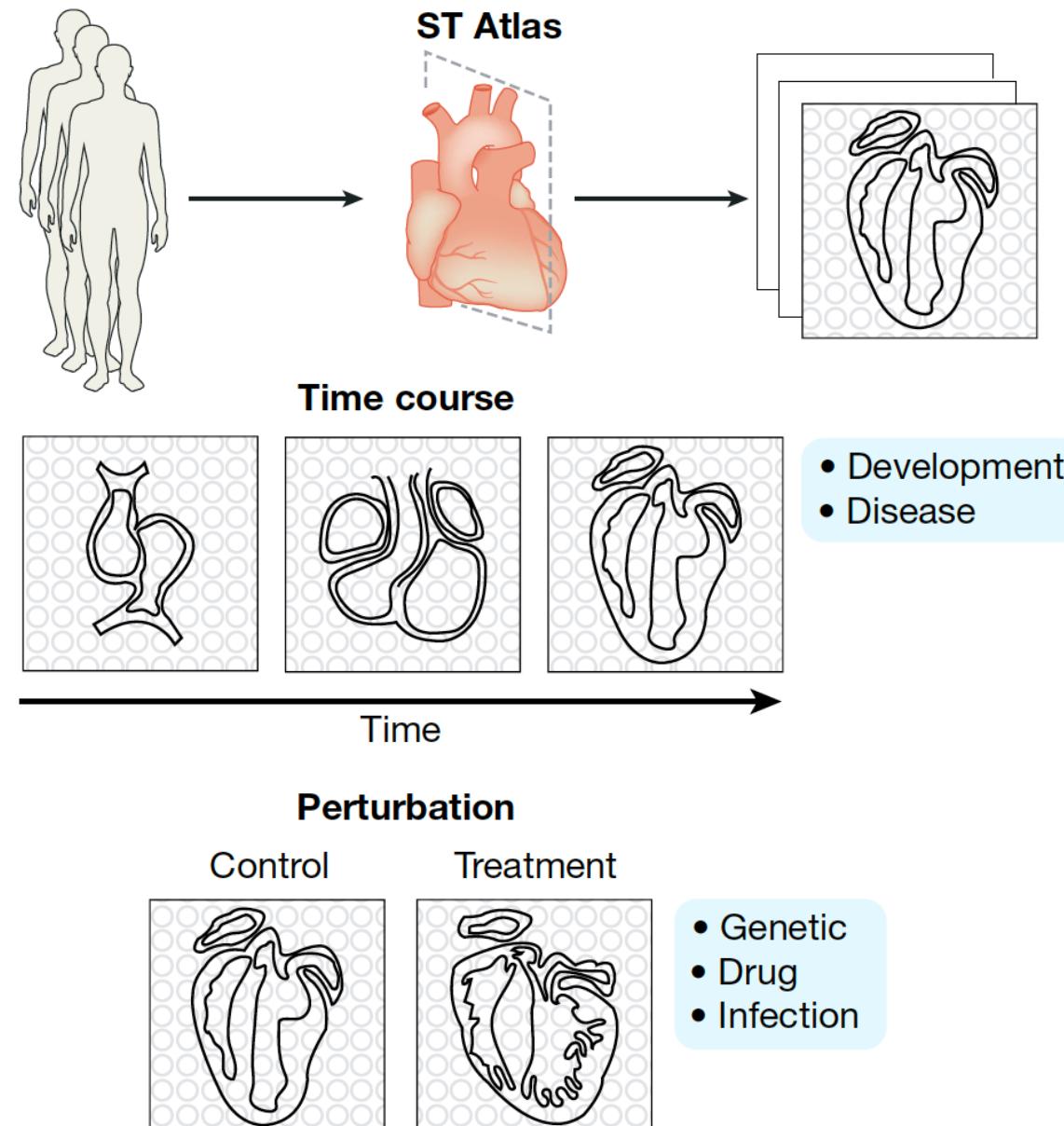


Liver cirrhosis

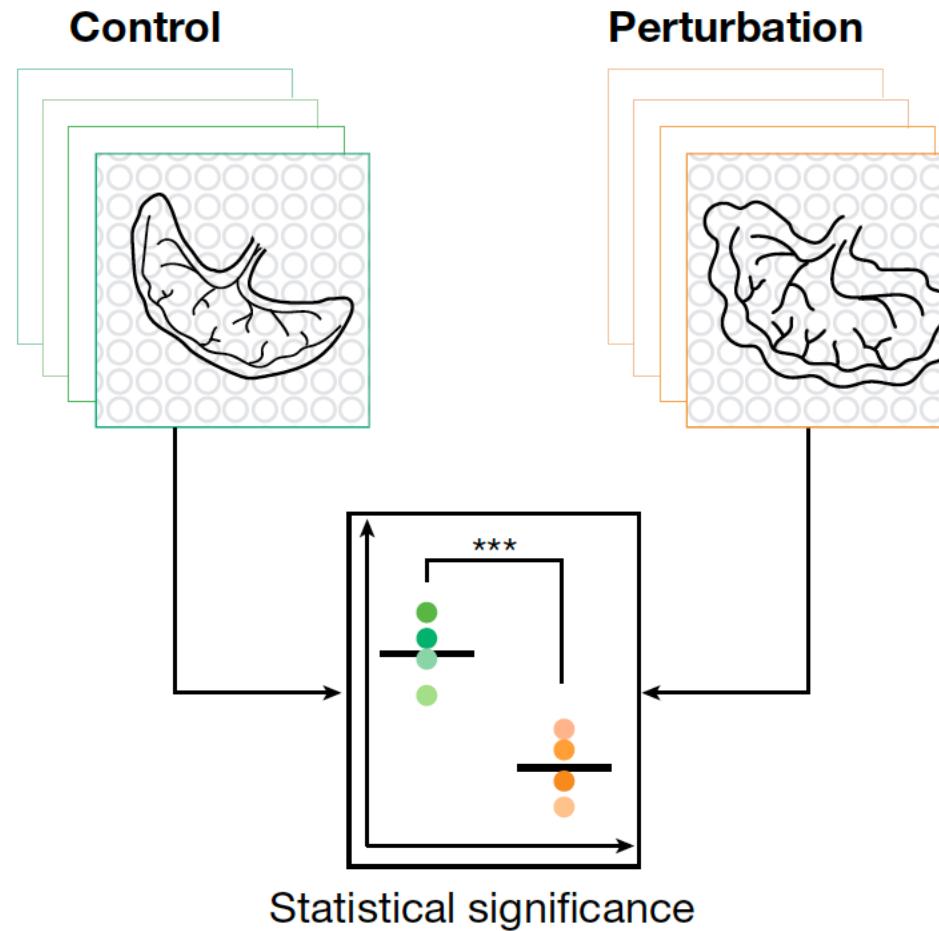


Breast cancer

Spatial technologies used for hypothesis generation



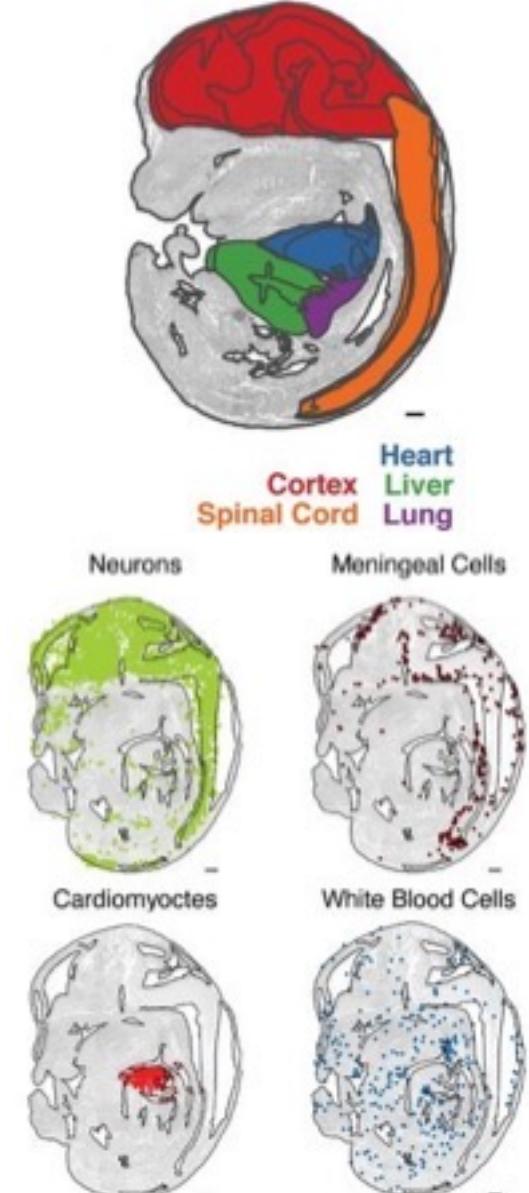
Spatial technologies used for hypothesis testing



Tissue biology questions

- New cell types and states
- How cell interactions with environment drive cell identity
- How cells organize into functional units of tissue structure
- How cells' organization changes during tissue development

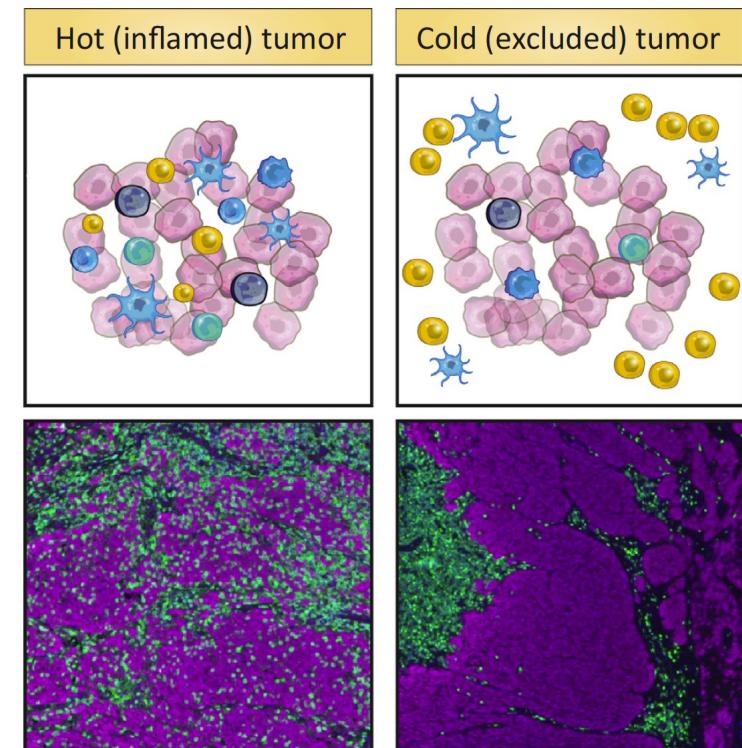
E14.0 mouse embryo



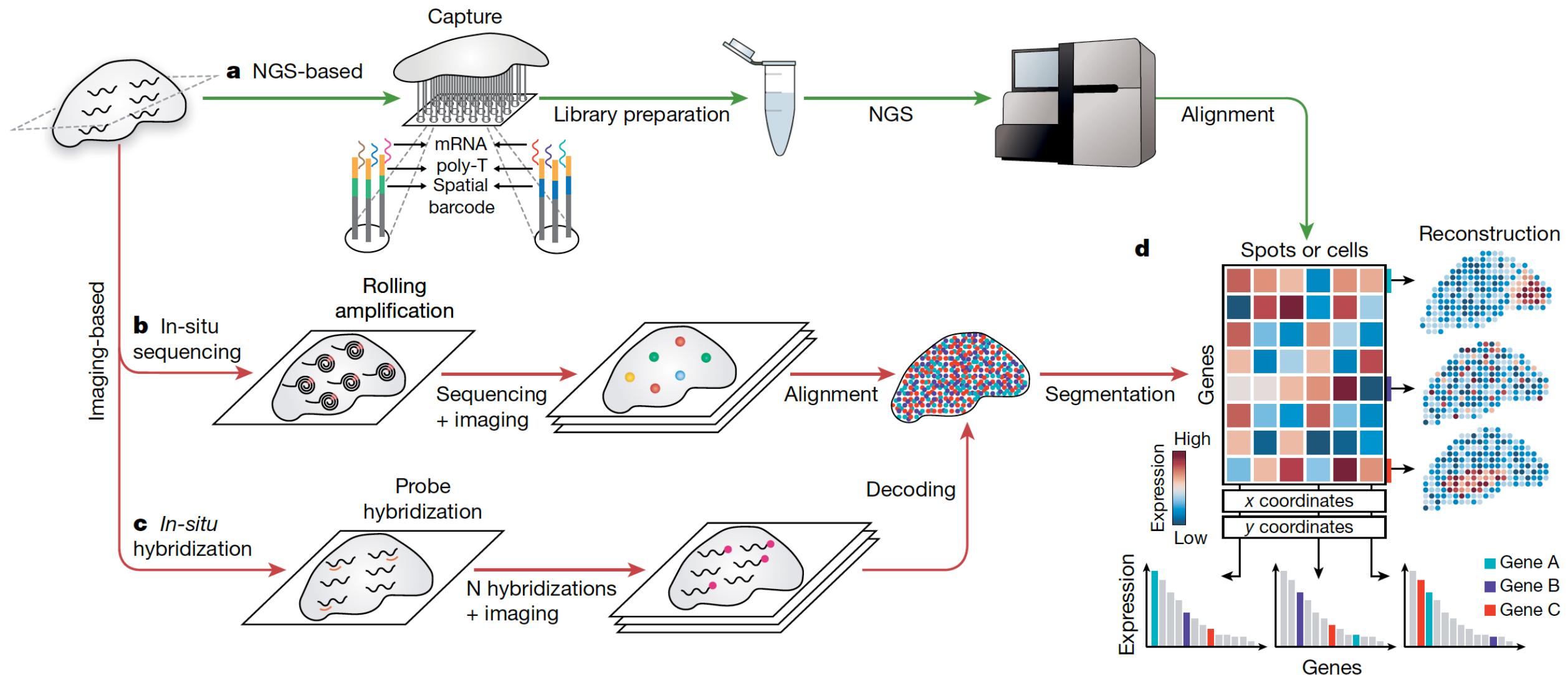
Srivatsan, S. et al Science (2021).

Translational questions

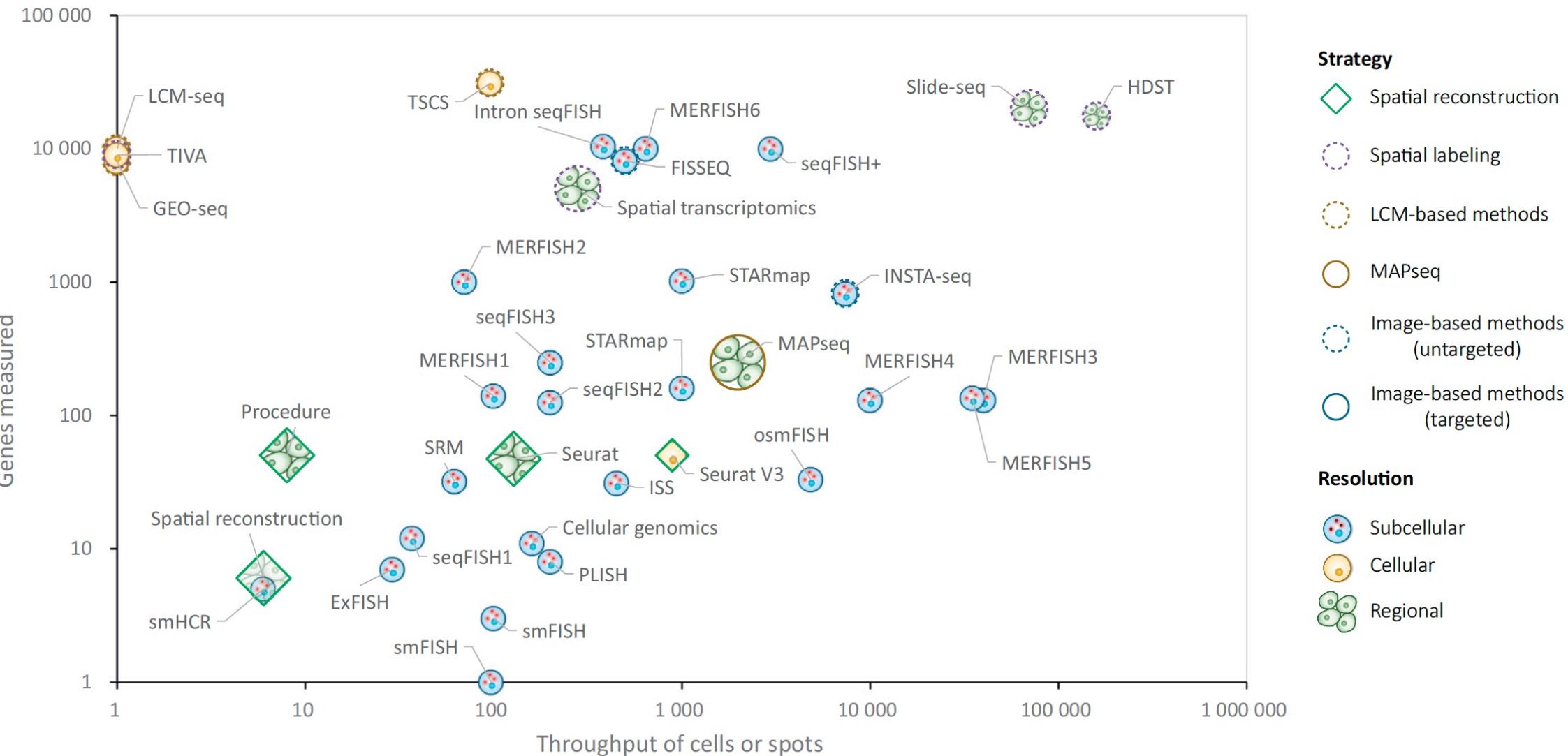
- How cell and tissue organization change during disease progression
- How cell and tissue organization change with treatment response or patient outcomes
- Cell or tissue features that serve as predictive biomarkers



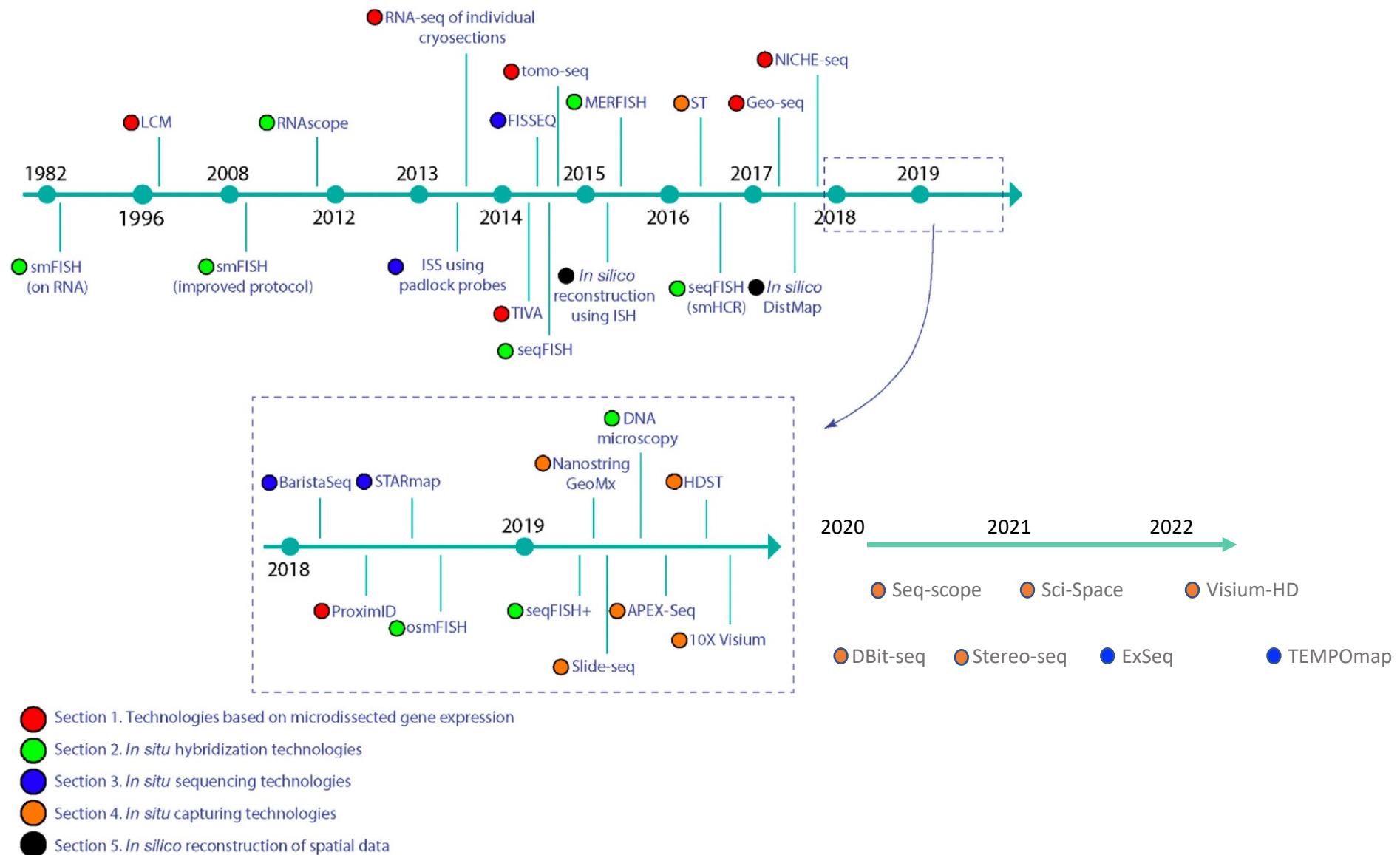
Spatial transcriptomic technologies



Tradeoffs in gene sensitivity, throughput, and resolution



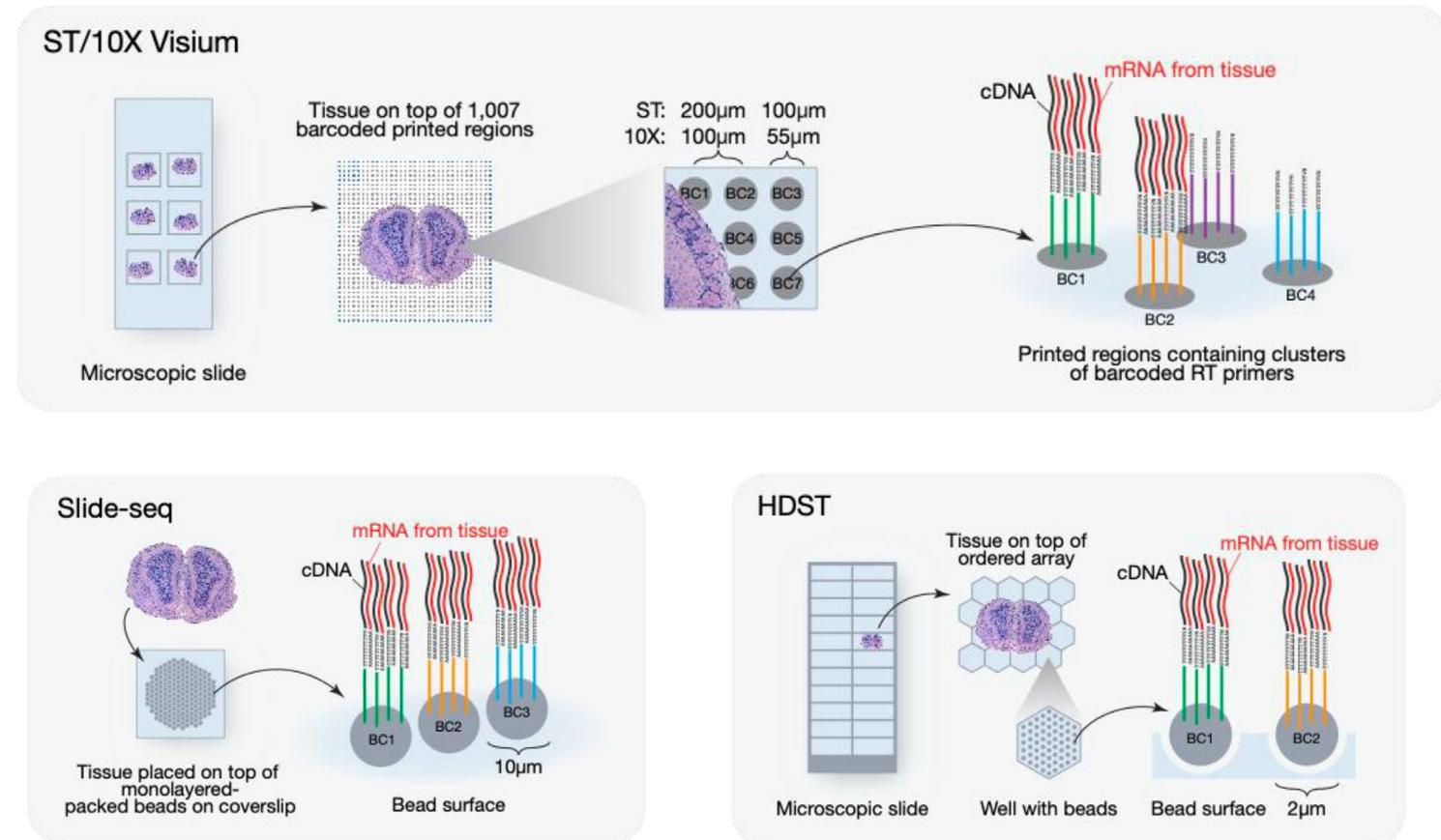
Historical timeline of spatial transcriptomics methods



Spatial transcriptomic method categories and examples

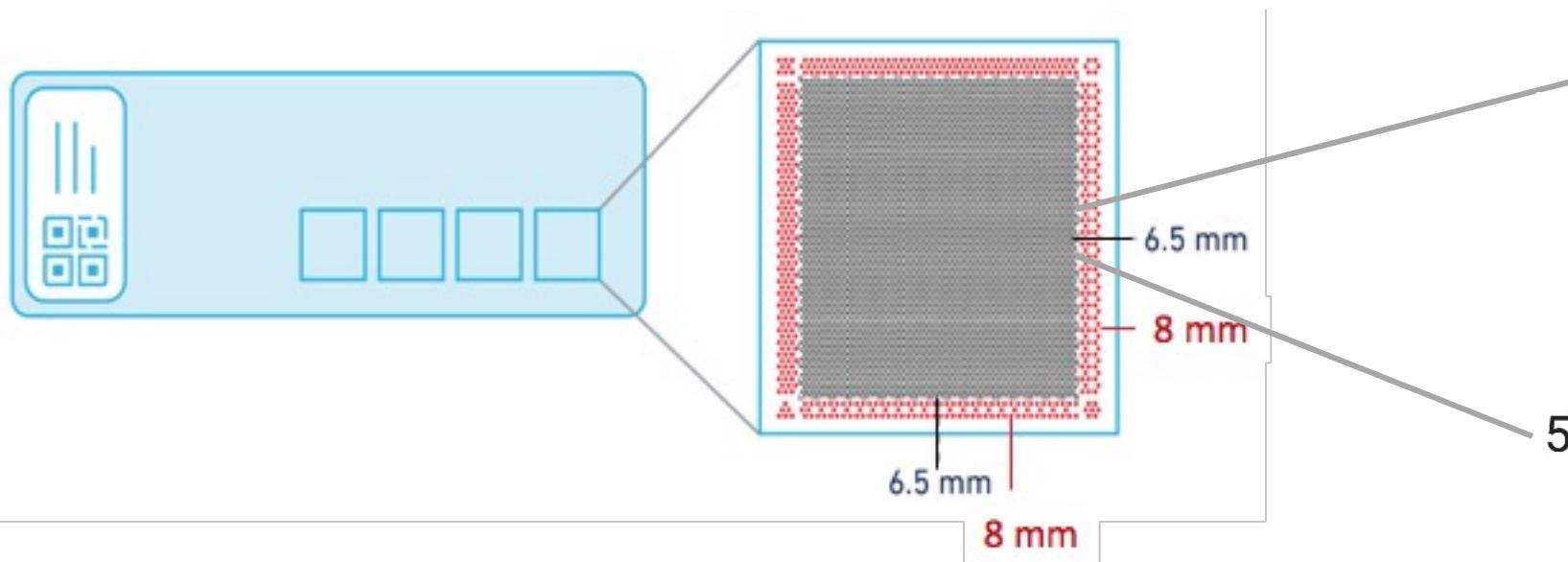
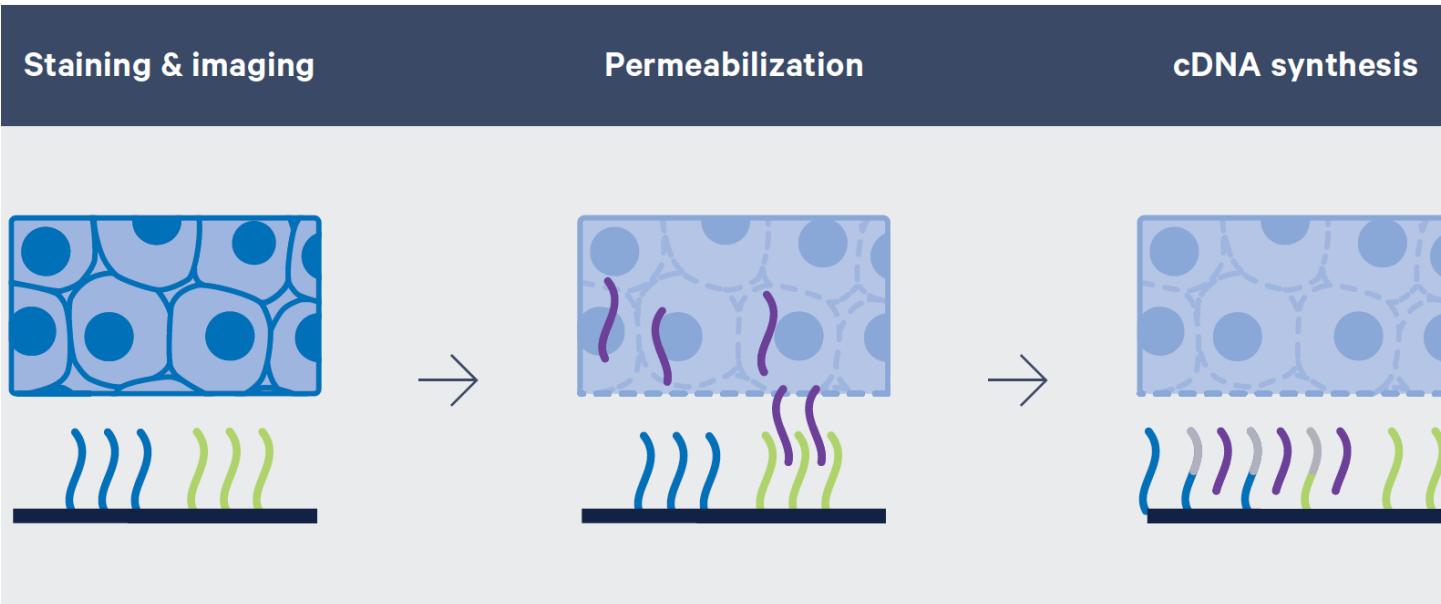
In-situ barcoding

- solid phase-based capture (SPBC)
 - Spatial Transcriptomics (ST) (2016, FF)
 - 10x Visium (2019, FF or FFPE)
 - Slide-seq (2019, FF)
 - HDST (2019, FF)
 - Sci-Space (2021, FF)
 - DBit-seq (2020, FF)
 - Seq-Scope (2021, FF)
 - Stereo-seq (2021, FF)
- selective barcoding (SB)
 - Nanostring DSP/SMI (2020-21, FFPE)
 - ZipSeq (2020, live cells)

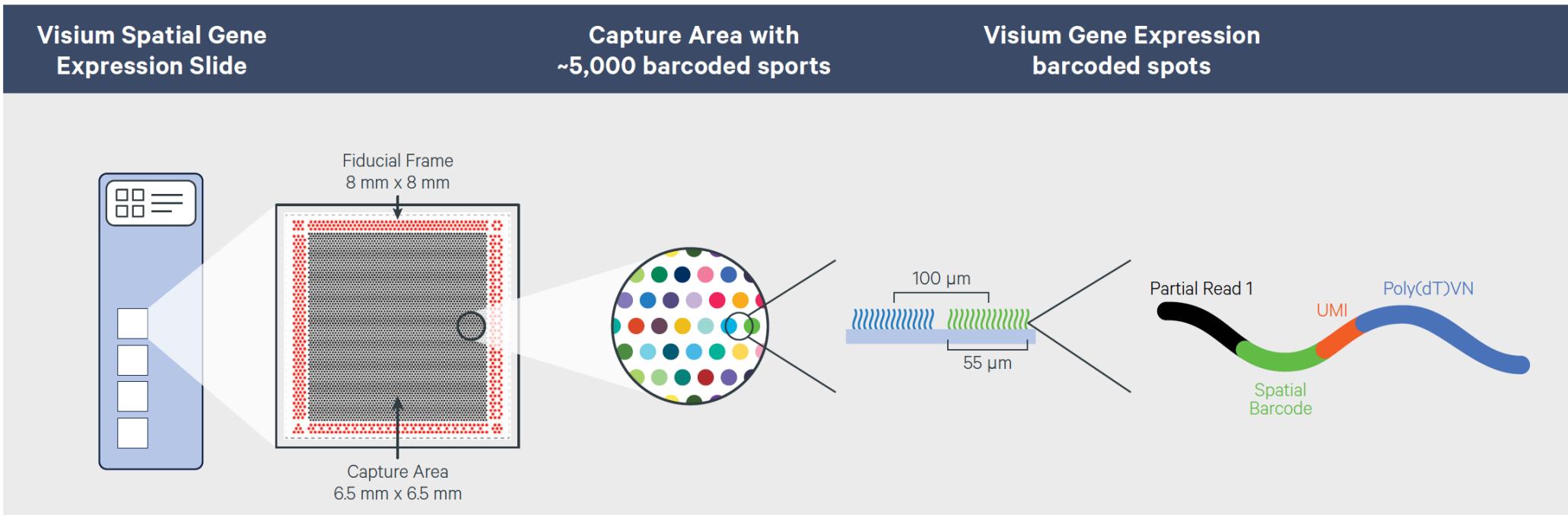


Asp et al., BioEssays 2020

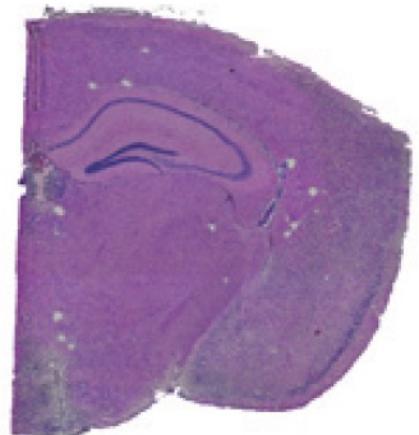
Spatially resolved single-cell transcriptomics: *in situ* barcoding



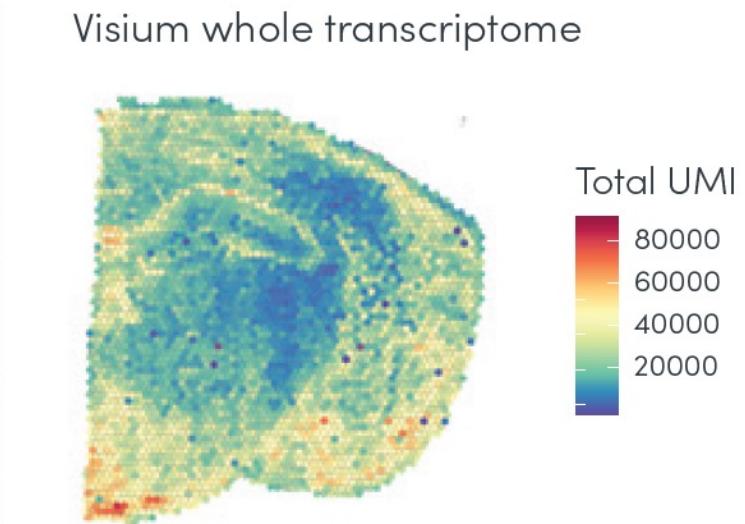
Spatially resolved single-cell transcriptomics: *in situ* barcoding



H&E

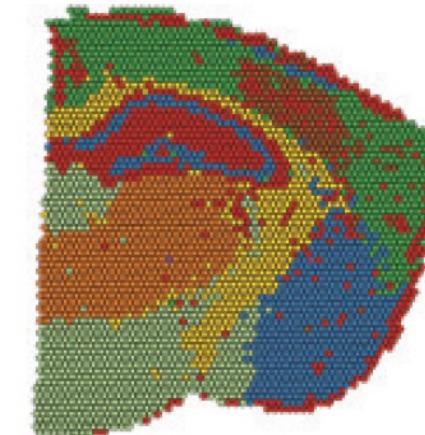


Visium whole transcriptome



Total UMI
80000
60000
40000
20000

Visium spot clusters



Cluster

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8

Cluster 4 mRNAs

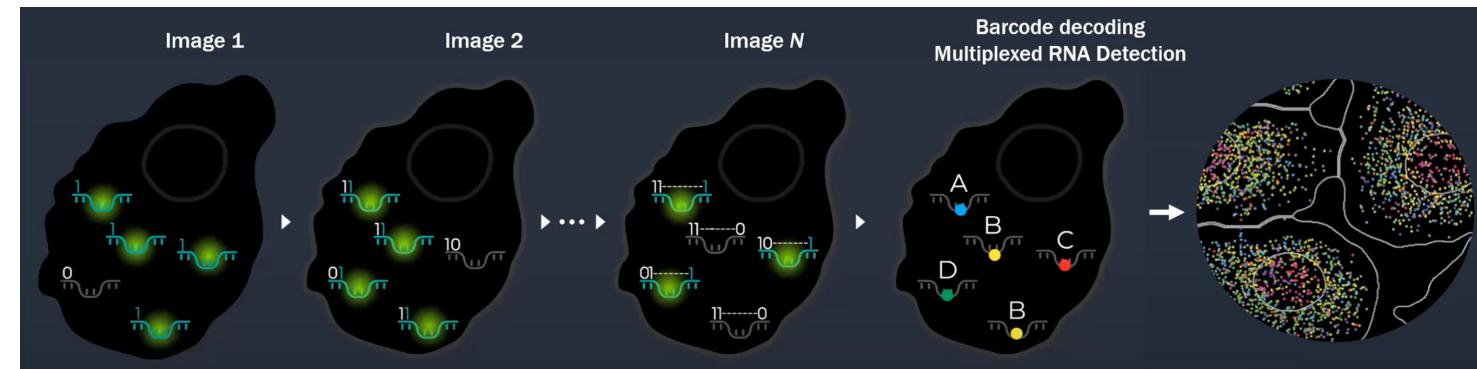
- Lamp5
- Igfbp6
- Tbr1
- Ephx4
- Lmo4
- Vip
- Satb1
- Egr1
- Epop
- ler5

Spatial transcriptomic method categories and examples

Imaging-based methods

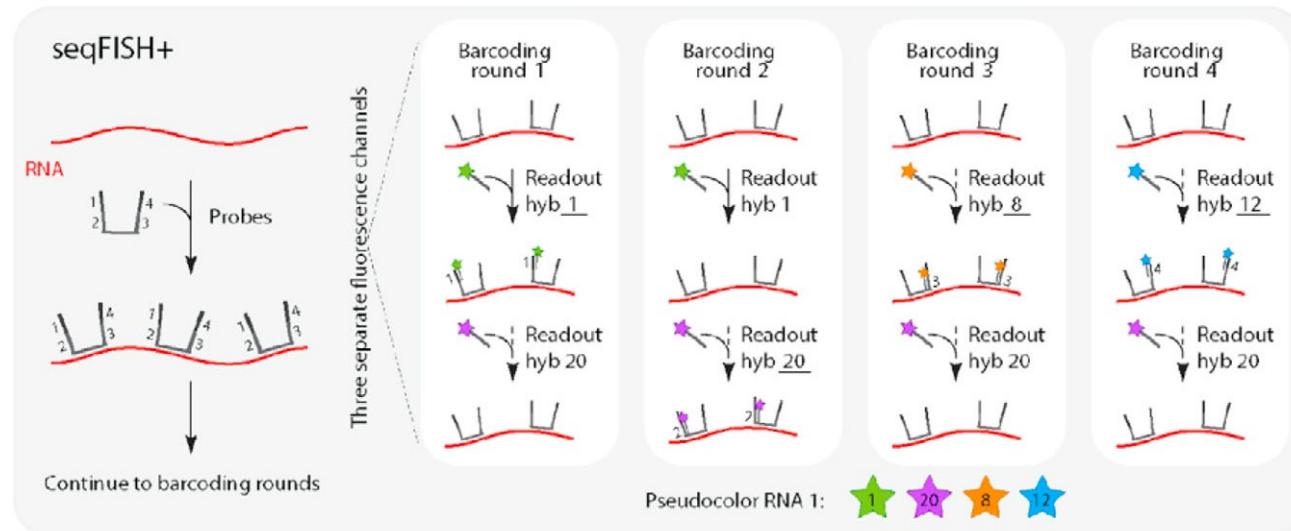
- Fluorescent *in situ* hybridization (FISH)
 - MERFISH/Vizgen (2015, FF)
 - seqFISH+ (2019, FF)
 - SABER-FISH (2019)
 - osmFISH (2018, FF)
- *In situ* sequencing (ISS)
 - FISSEQ/ReadCoor -> 10x
 - Padlock probe based *in situ* sequencing/Cartana -> 10x
 - STARMap (2018, FF)
- ISS with *ex situ* sequencing
 - ExSeq (2021, FF)
 - INSTA-seq (2019)

MERFISH/Vizgen – subcellular resolution, 300-10 000 genes



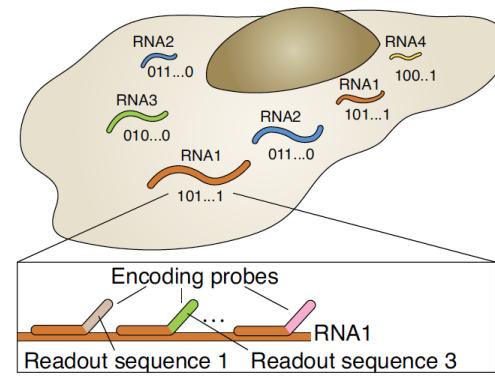
<https://vizgen.com/technology/#merfish>

seqFISH+ - subcellular resolution, >10 000 genes



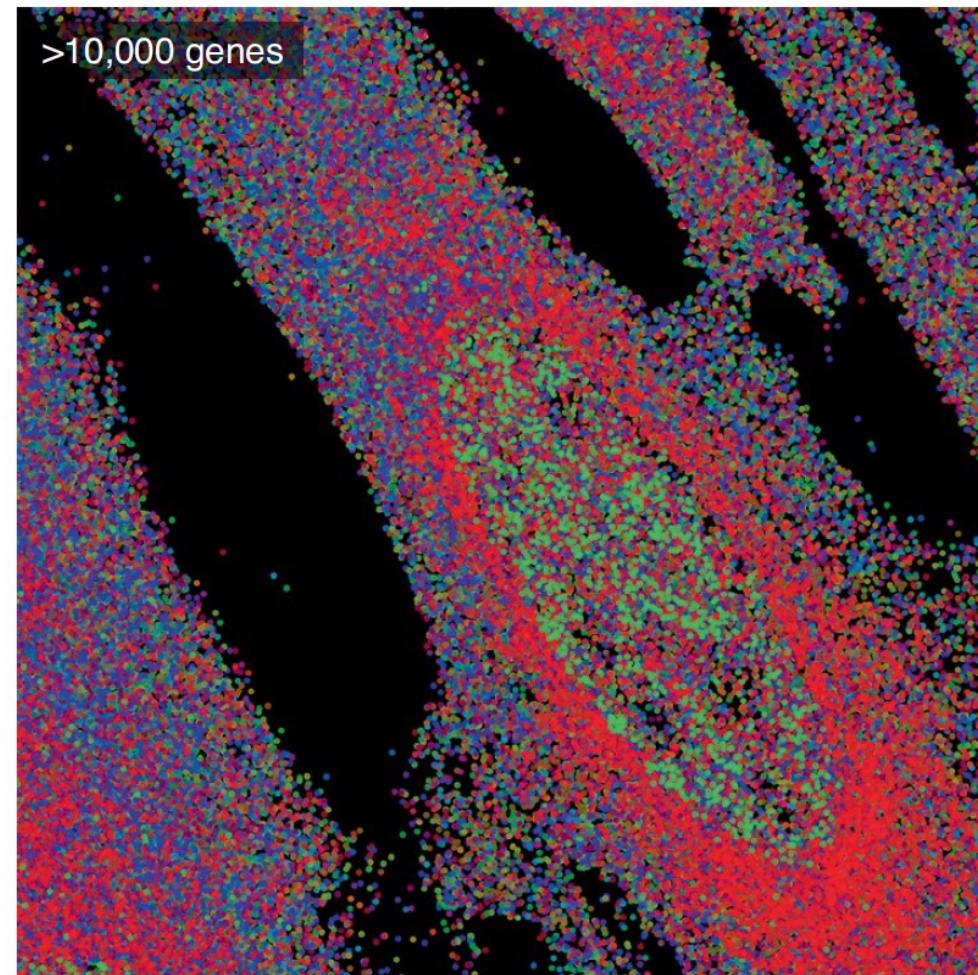
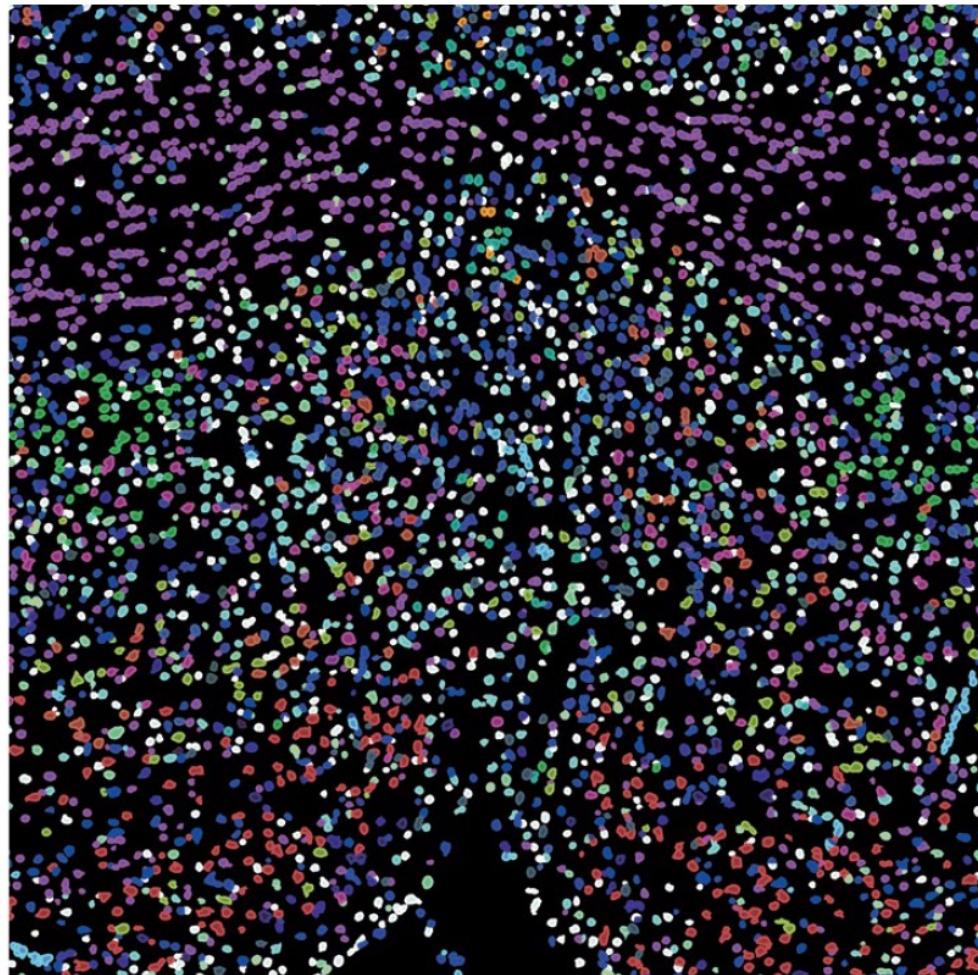
Spatially resolved single-cell transcriptomics by imaging

Multiplexed Error-Robust
Fluorescence In Situ
Hybridization (MERFISH)



Spatially resolved single-cell transcriptomics by imaging

Mouse hypothalamus colored by cell type (left) and individual transcripts (right) in a 10,000 gene panel

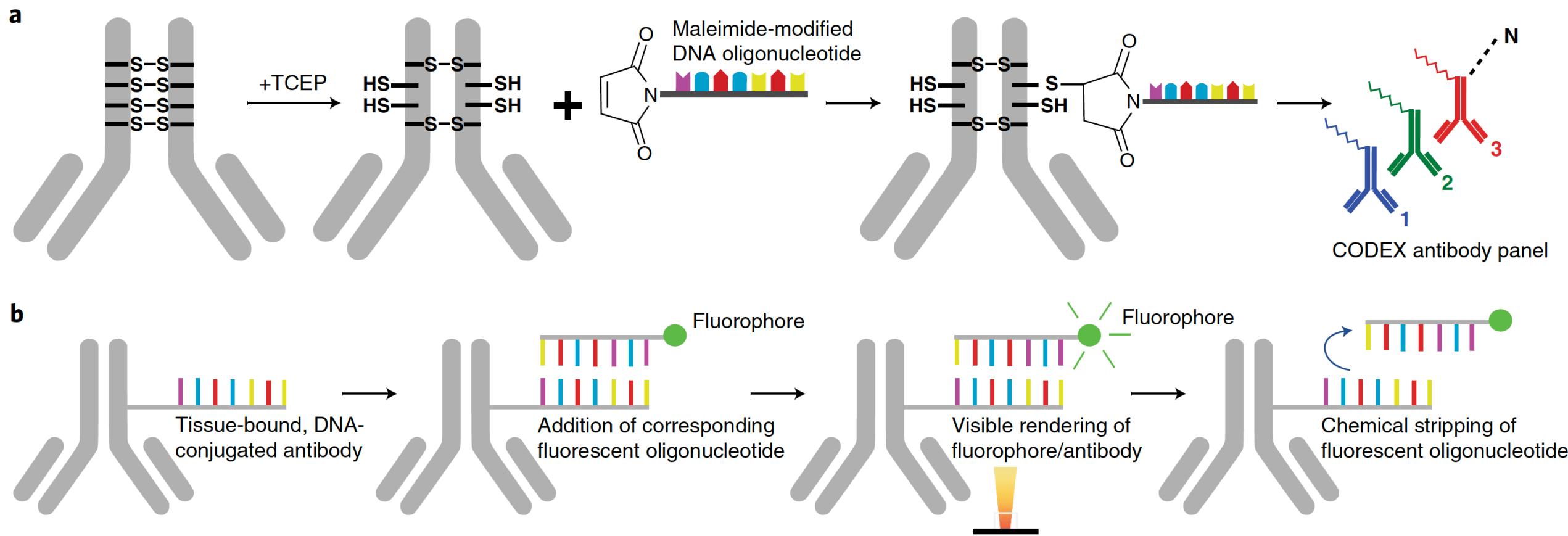


Interact with MERFISH human tumor datasets online:
<https://info.vizgen.com/ffpe-showcase>

Zhuang, X. *Nature Methods* (2021).

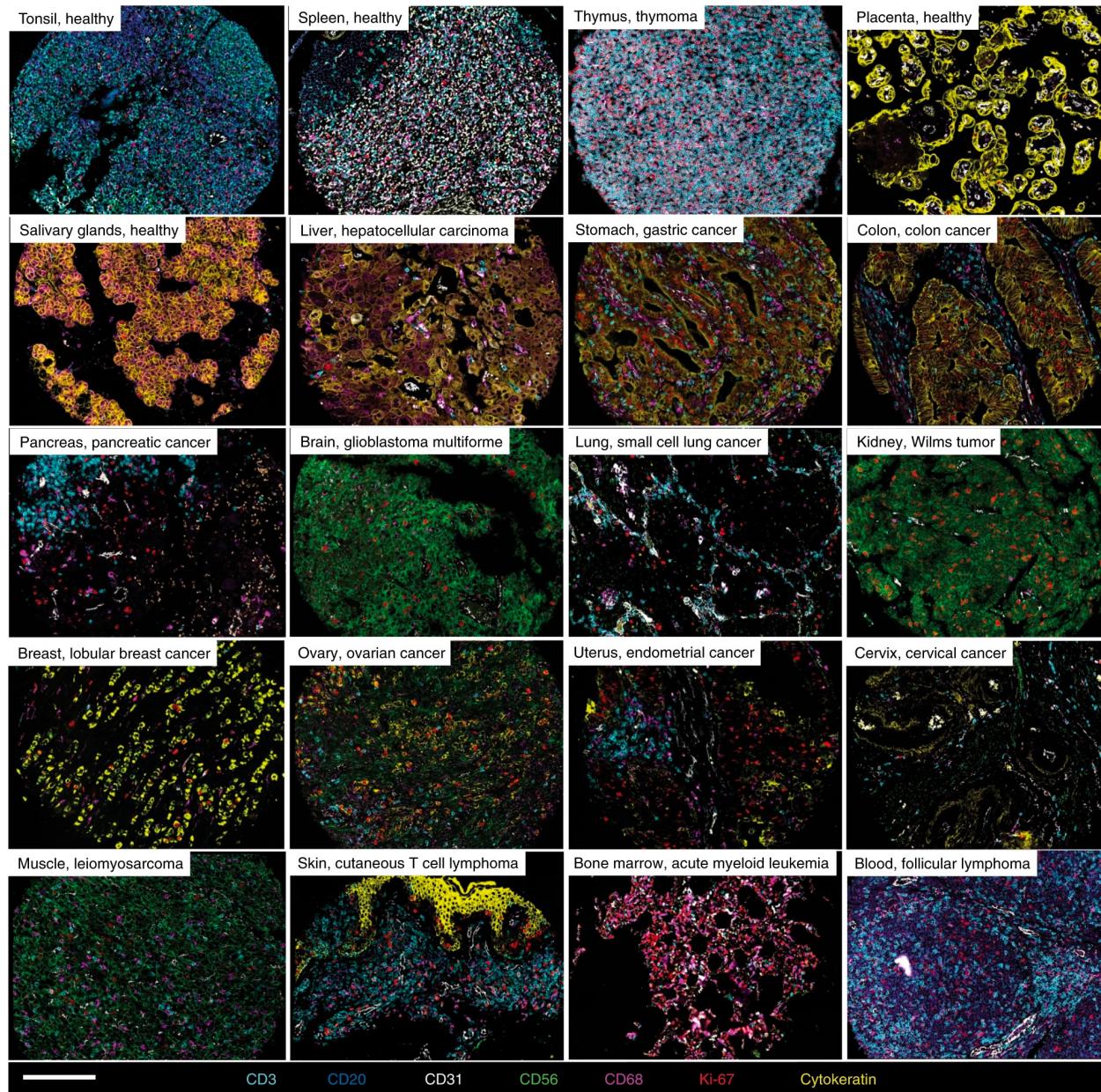
Spatial proteomic technologies

CODEX



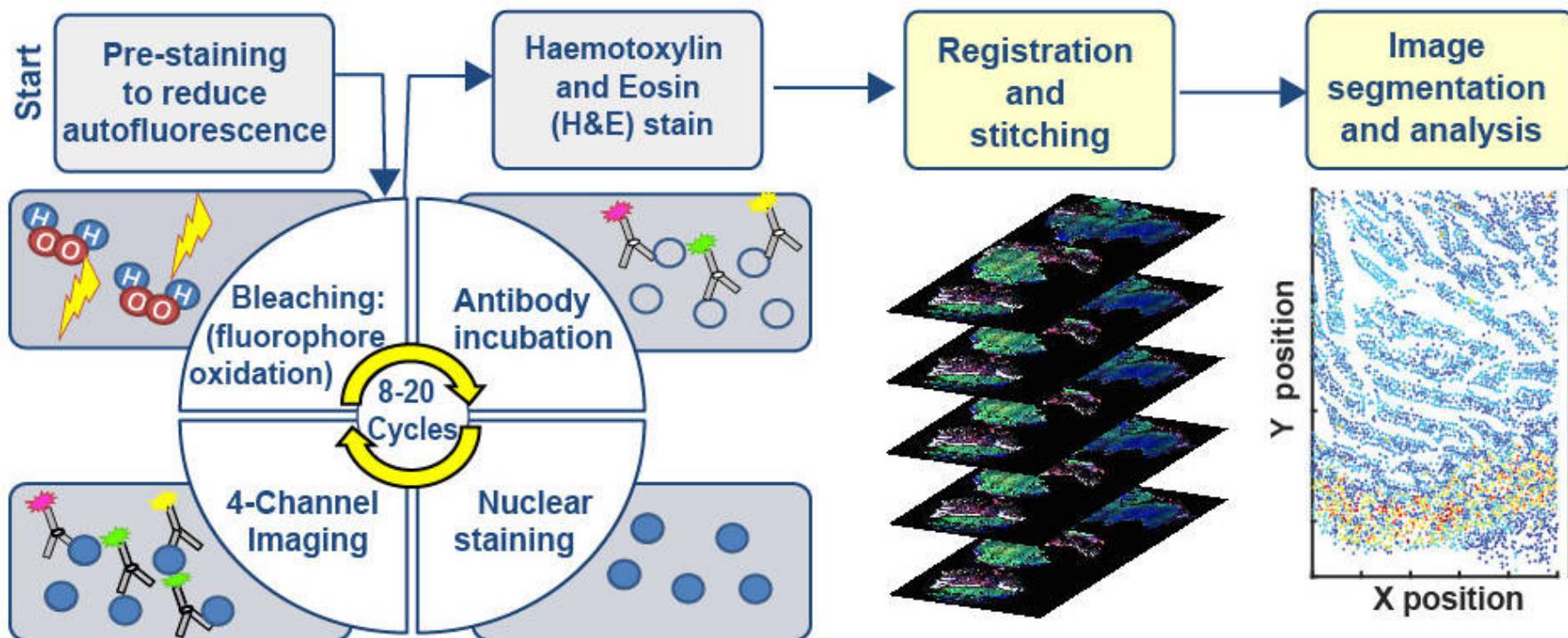
CODEX

Spatial proteomic technologies



Spatial proteomic technologies: Cyclic immunofluorescence (CycIF)

Multi-round multiplex tissue immunofluorescence using
~5 µm thick sections cut from FFPE blocks



CycIF overview: <https://vimeo.com/269904895>

Primary lung cancer analysis: https://www.cycif.org/data/du-lin-rashid-nat-protoc-2019/osd-LUNG_3_DATA.html

Primary lung cancer regions: https://www.cycif.org/data/du-lin-rashid-nat-protoc-2019/osd-LUNG_3.html

Metastatic melanoma: <https://www.cycif.org/osd-exhibit>

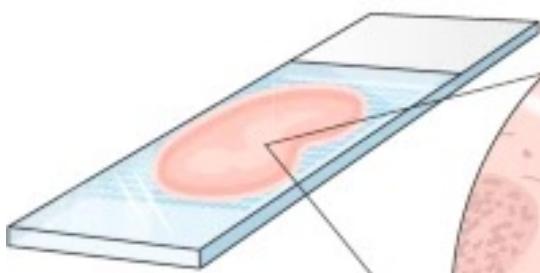
Spatial proteomic technologies

Spatial proteomics

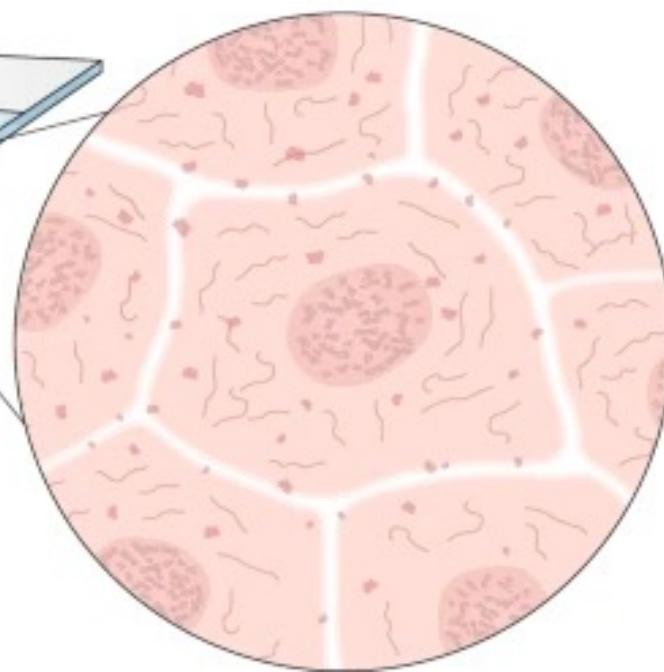
			No. of targets	Tissue prep.
Iterative	miHIC	Primary antibody Secondary antibody	30	FFPE
OPAL	Primary antibody → HRP-conjugated SA → Tyramide fluorophore → Microwave treatment		10	FFPE
CyclIF	Direct IF Indirect IF: PA Indirect IF: SA Fluorophore bleaching		60	FFPE
REAdye-lease and REAfinity	Primary antibody → Fluorophore release → Fluorophore bleaching		100 (400)	FFPE
CODEX	dsDNA-conjugated PA pool → Extension with fluorophore → Fluorophore cleavage → Extension with fluorophore		60	FF* FFPE
Immuno-SABER	ssDNA-conjugated PA pool → Concatemer hybridization → Fluorescent probe hybridization → Reporter removal		10 (50)	Whole-mount FF* FFPE
InSituPlex	Barcoded PA pool → Barcode amplification → Fluorescent probe hybridisation → Reporter removal		10	FFPE
TOF-mass spectrometry	IMC	Metal-conjugated PA pool → UV laser ablation → TOF mass spectrometry	40 (100)	FF FFPE
	MIBI	Metal-conjugated PA pool → Ion beam gun → TOF mass spectrometry	40 (100)	FF FFPE
Sequencing	DSP	Stain + oligonucleotide-conjugated PA pool → Oligonucleotide cleavage → Quantitative analysis	44 (100)	FF* FFPE

Towards multimodal spatial measurements

Tissue section



Spatially resolved

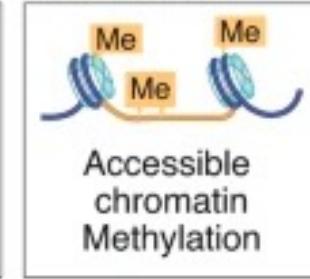


Molecular barcoding

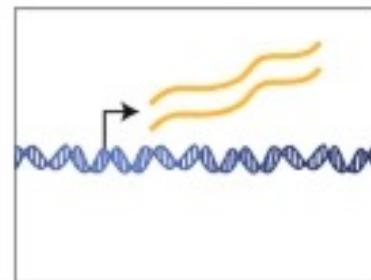
Genome



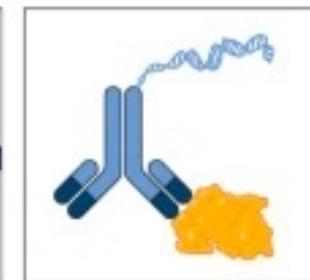
Epigenome



Transcriptome



Proteome



Microbes

Metabolites

Experimental design: technology

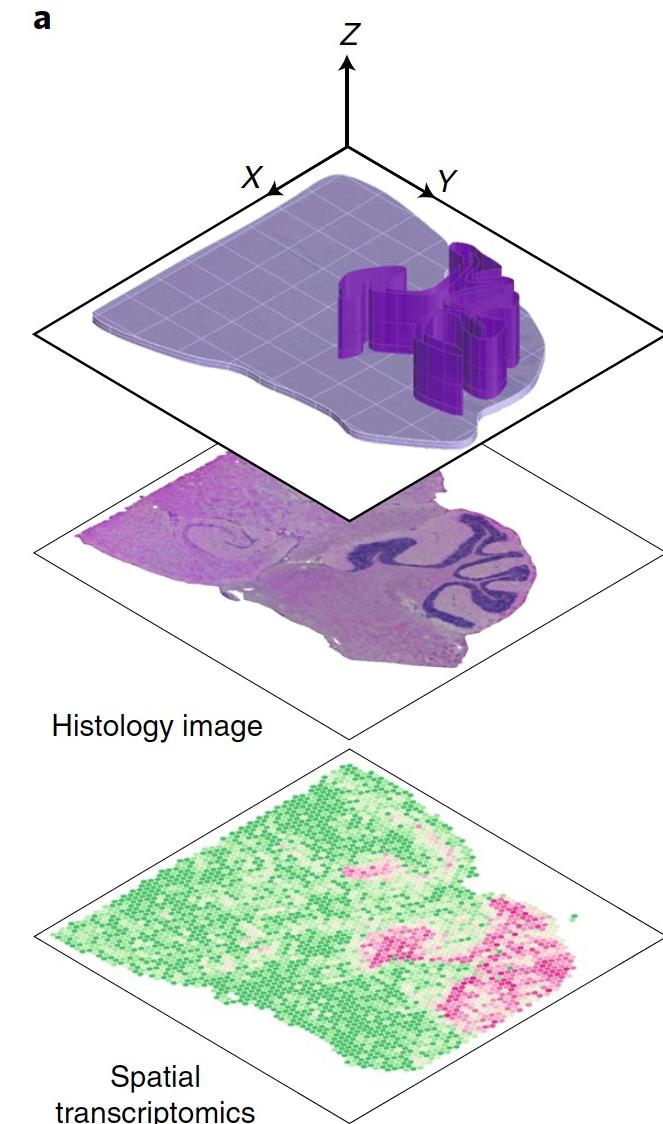
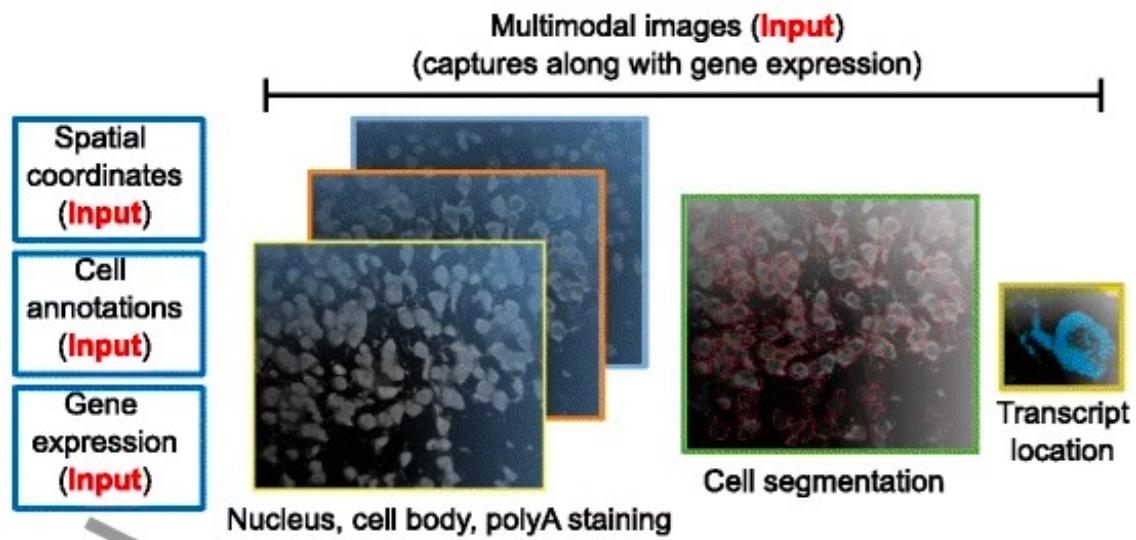
- Which technology
 - tissue source: fresh-frozen or FFPE fixed
 - resolution: 55 µm - single cell and sub-cellular
 - gene throughput: 100s genes / proteins - 20 K genes
 - gene detection sensitivity
 - tissue size and field-of-view (FOV) size
 - focus on specific cell subset? Design gene panel (e.g. immune cells, cancer cells, fibroblasts...) or validated antibody panel
 - histology possible on same tissue section
 - commercially supported

Experimental design: samples

- Consult pathologist
 - annotation of interesting regions
 - register to common coordinate framework
- What samples
 - sufficient power to sample relevant tissue heterogeneity (e.g. cell type composition, cell-cell interactions, transcriptional programs)
 - # biological conditions, # individuals, # sections / individual, # FOVs / individual, FOV size
 - intra- (multiple sections / individual) vs inter- (multiple individuals) individual variability
- Design
 - ≥ 3 samples per biological condition
 - process biological conditions in parallel to minimize batch effects
 - multiple samples from same individual (e.g. time course, pre- vs post- treatment) reduce variability issues

Experimental design: imaging

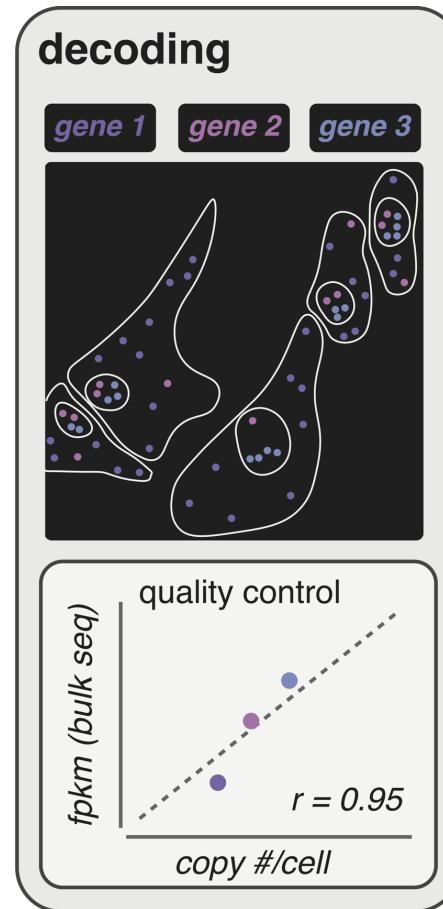
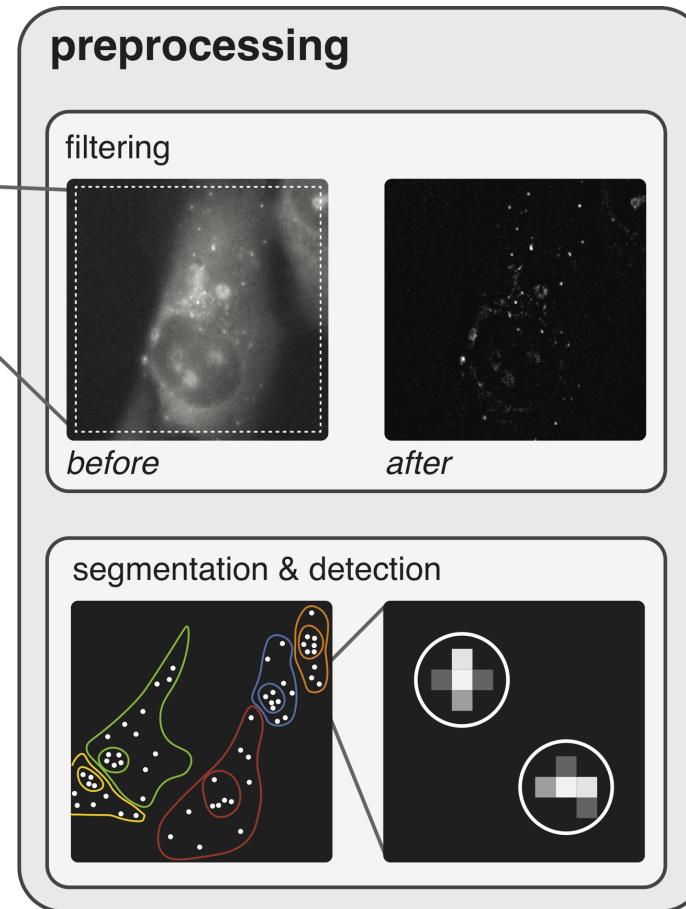
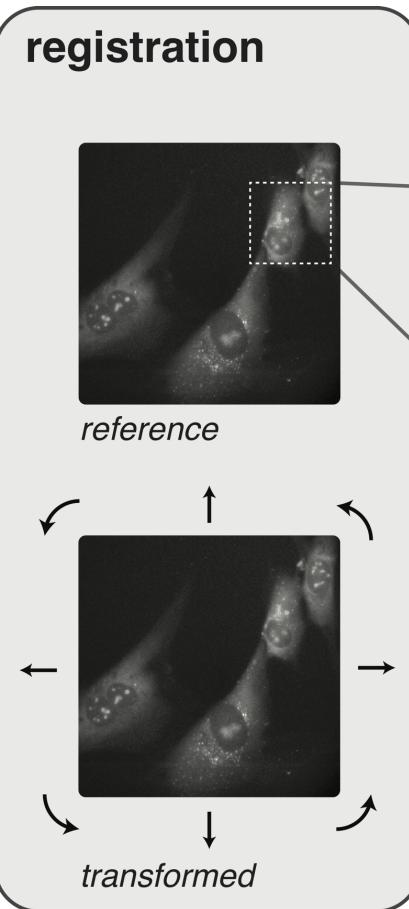
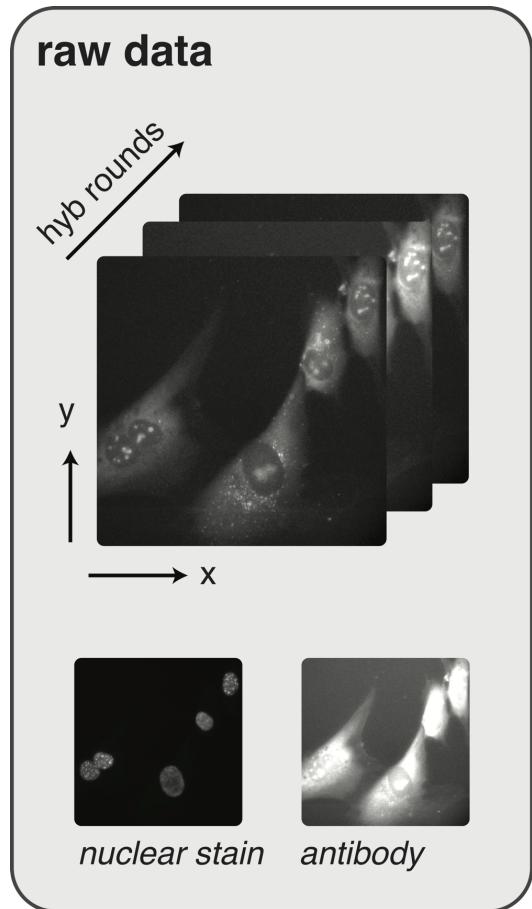
- Dyes for cell segmentation (nuclei, membrane)
- Banking serial sections for imaging and snRNA-Seq
 - Histology (cell morphology)
 - Imaging (ie immunofluorescence, collagen, phosphorylation, histone modifications...)
 - Later validation experiments



Hu, J. et al *Nature Methods* (2021).
Dries, R. et al *Genome Biology* (2021).

Imaging data has complex preprocessing

starfish
Imaging based
transcriptomics



per field of view
raw_stack.tif
dapi.tif, antibody.tif

codebook schemas
hyb | bit position
gene | bit-string

per field of view
reg_stack.tif
affine_transform.mat

global
chromatic_correc.mat
references.csv

per field of view
proc_stack.tif
cells_geo.json
spots_geo.json

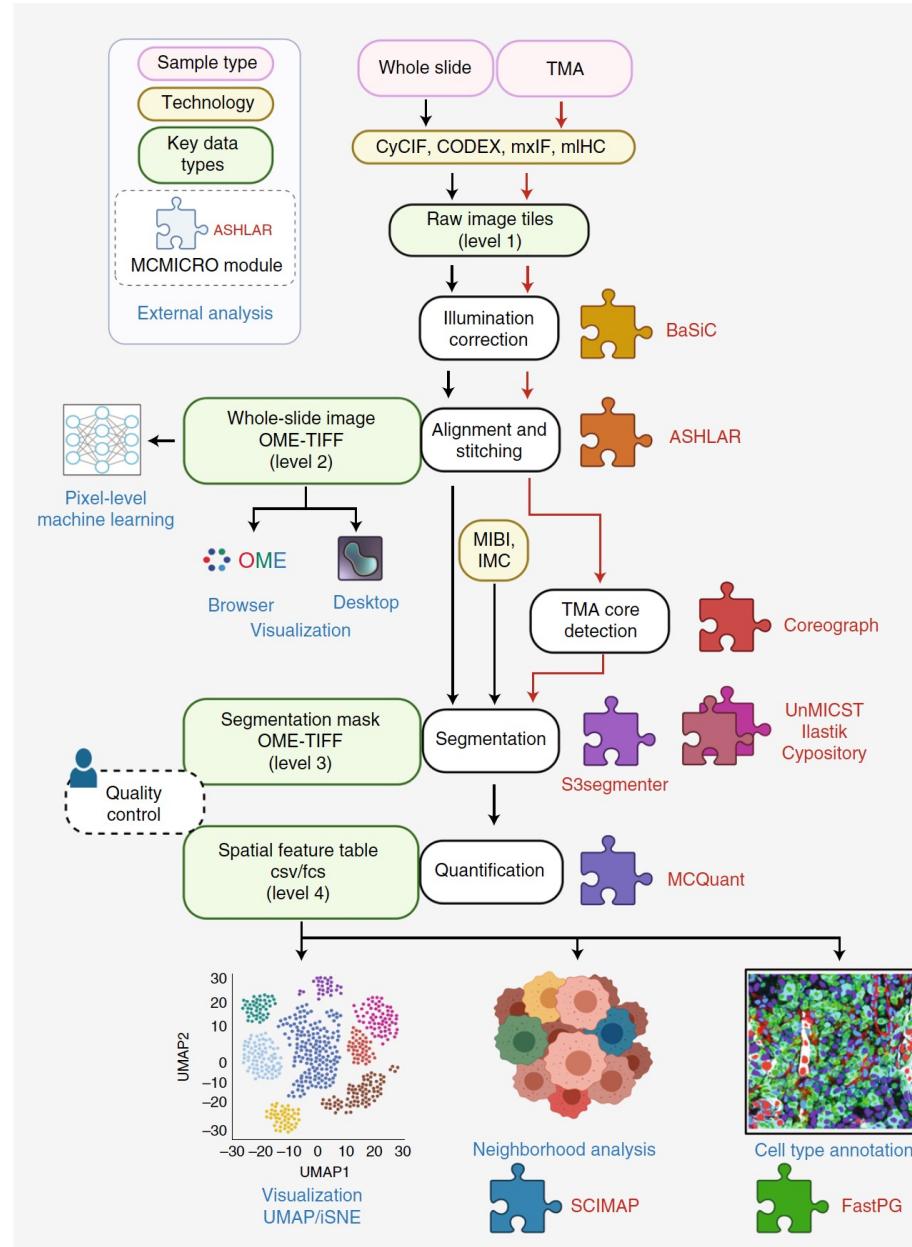
encoder table schema
cell_id | spot_id | x | y | z | hyb | val

qualiy metrics
assay_comp.json
control_barcodes.json
metrics.json

decoder table schema
spot_id | gene

Imaging data has complex preprocessing

MCMICRO
Multiplex protein imaging



Data analysis frameworks

- Spatial transcriptomics

- Seurat
- Giotto
- STUtility
- SPATA
- Meringue

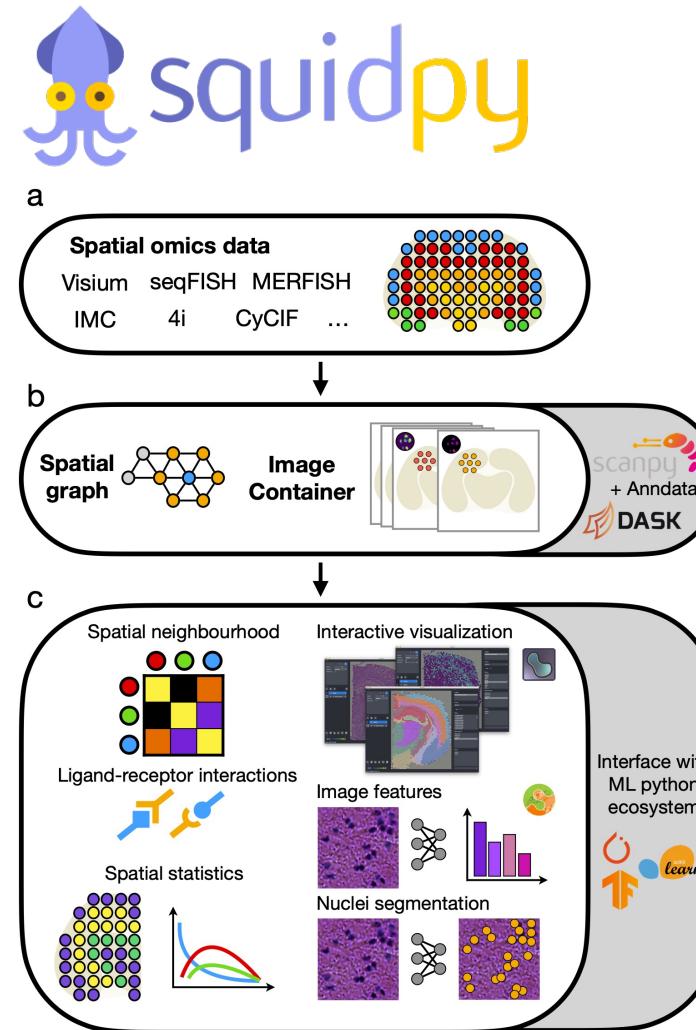
- ...
- Scanpy
- Squidpy
- Starfish
- stLearn

- ...

- Spatial proteomics

- MCMICRO
- Steinbock

- ...

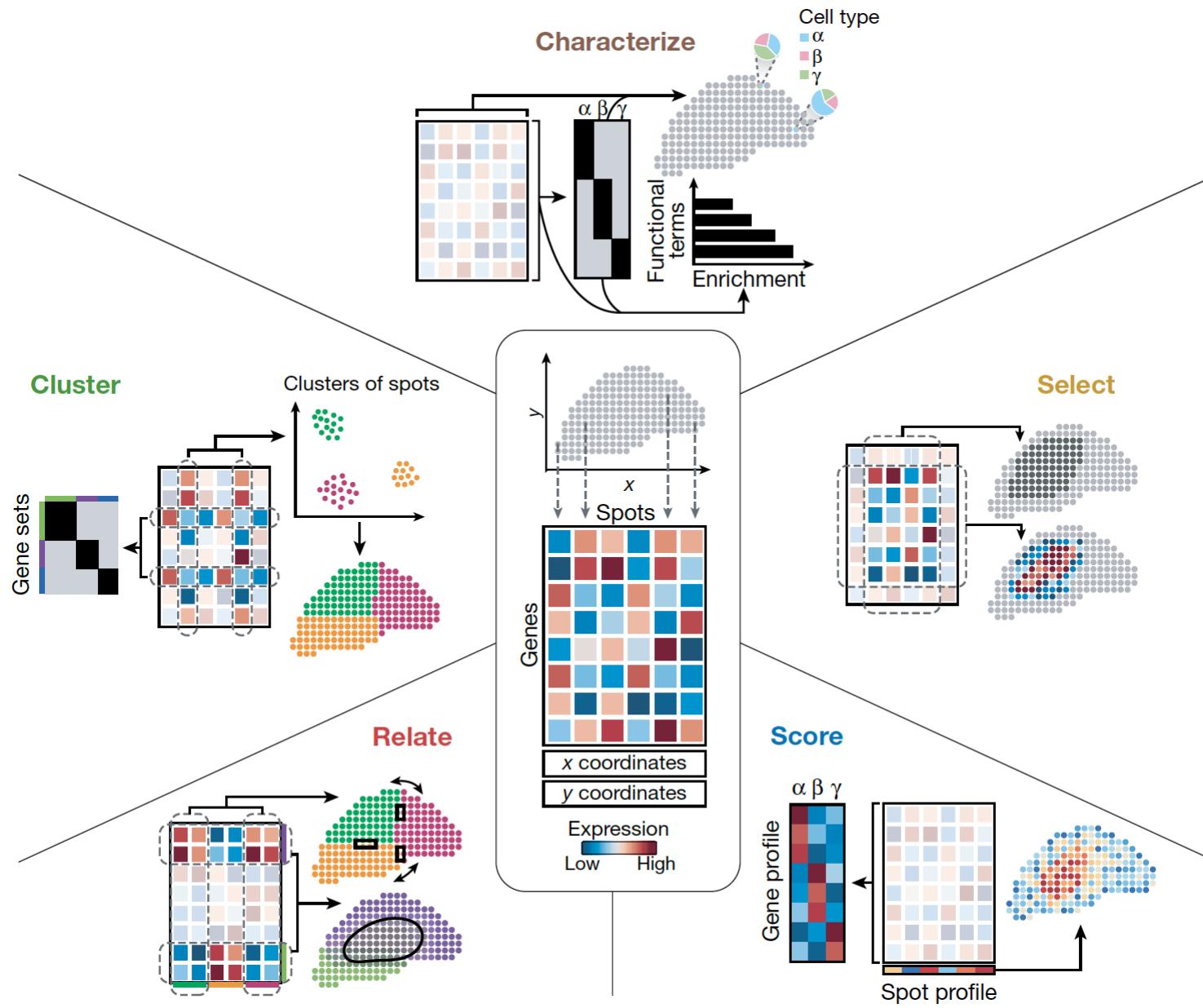


- Data visualization and exploration

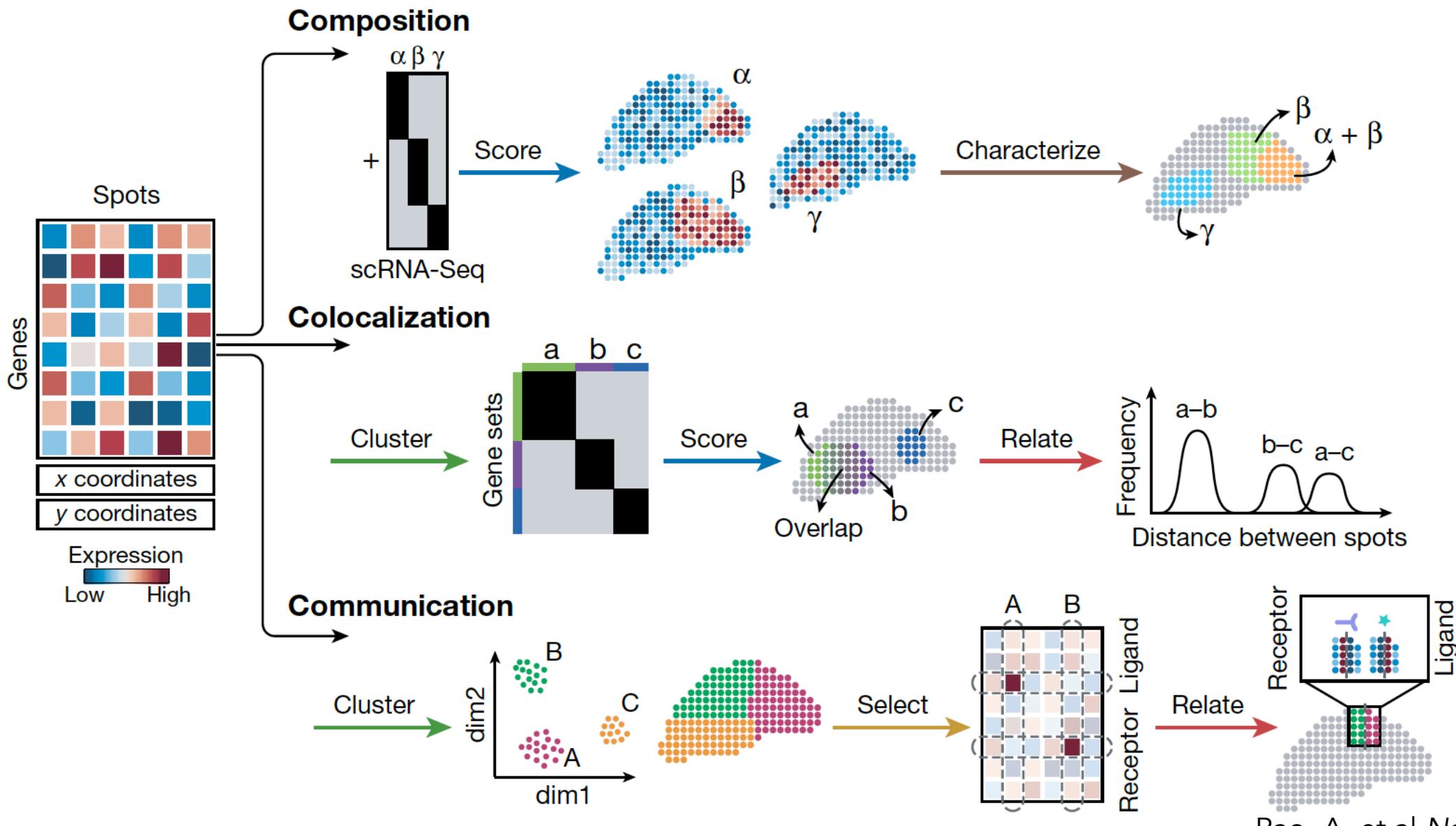
- Napari
- OMERO
- TissUUmaps
- Minerva
- Loupe Browser

- ...

Identifying cell subsets



Identifying higher-order tissue features



A few spatial resources

Computational packages for spatial analysis

<https://satijalab.org/seurat/>

<https://scanpy.readthedocs.io/>

<https://squidpy.readthedocs.io/>

Review articles

Exploring tissue architecture using spatial transcriptomics <https://pubmed.ncbi.nlm.nih.gov/34381231/>

Spatial components of molecular tissue biology <https://pubmed.ncbi.nlm.nih.gov/35132261/>

Museum of spatial transcriptomics <https://pubmed.ncbi.nlm.nih.gov/35273392/>

Online courses

<https://lmweber.org/OSTA-book/>

<https://bioimagebook.github.io/README.html>

https://pachterlab.github.io/LP_2021