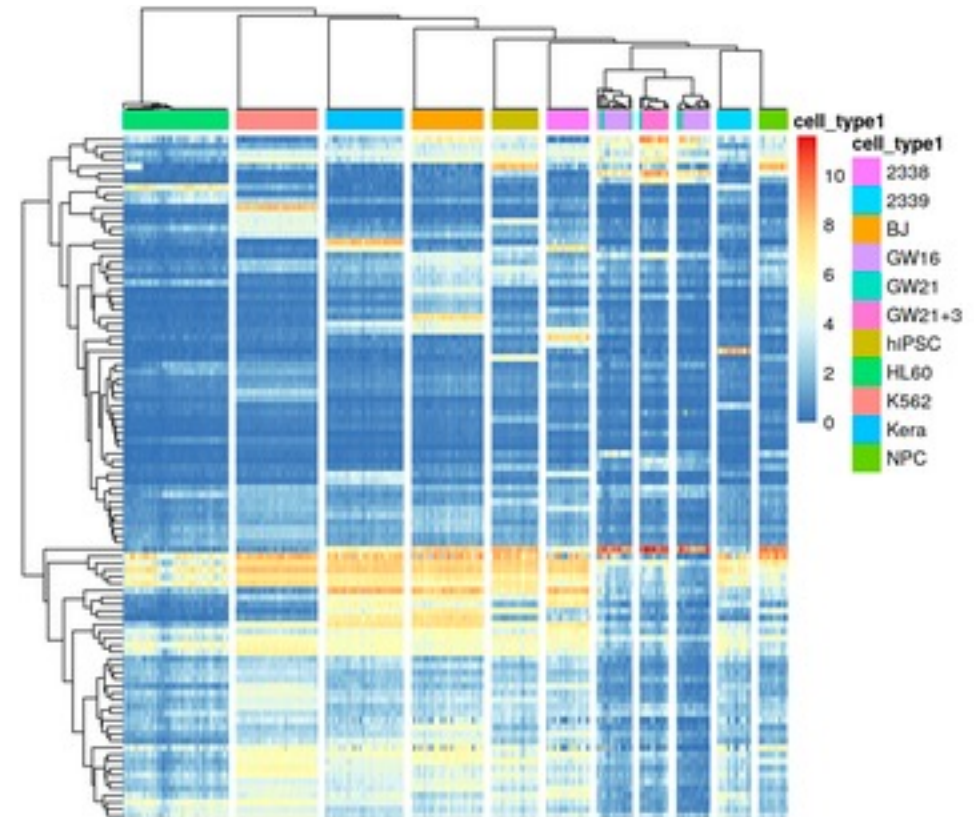
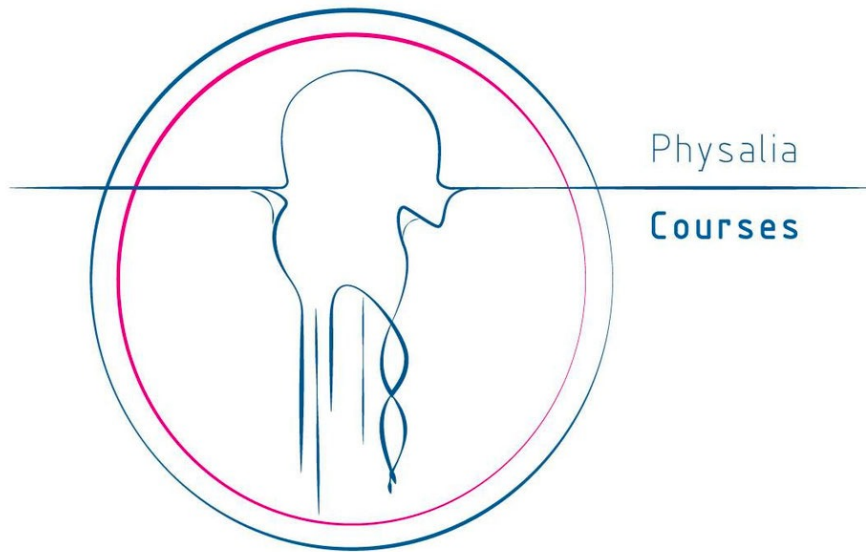


Advances in single-cell genomics: spatial genomics

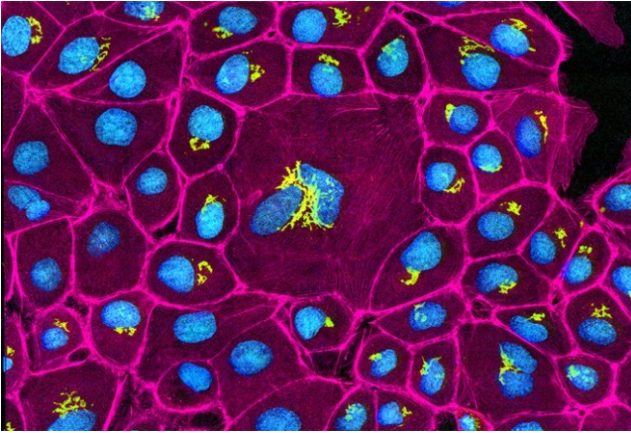
Orr Ashenberg, Jacques Serizay, Arnav Mehta

June 9, 2021

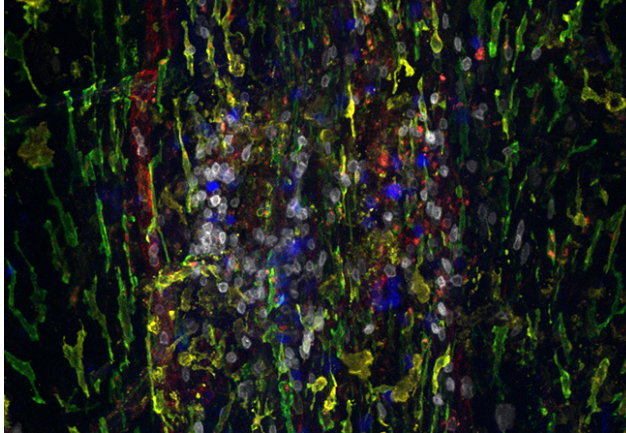


Incredible diversity in cell types, states, and interactions across human tissues

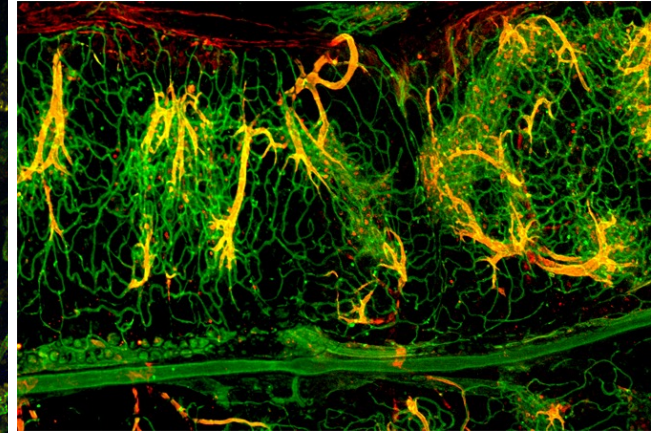
Skin epithelium



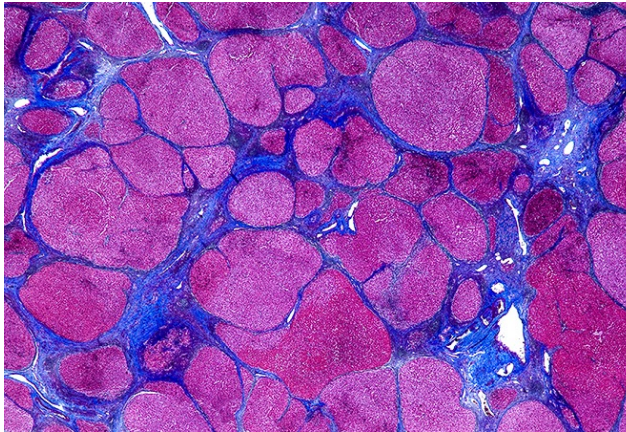
Brain meninges



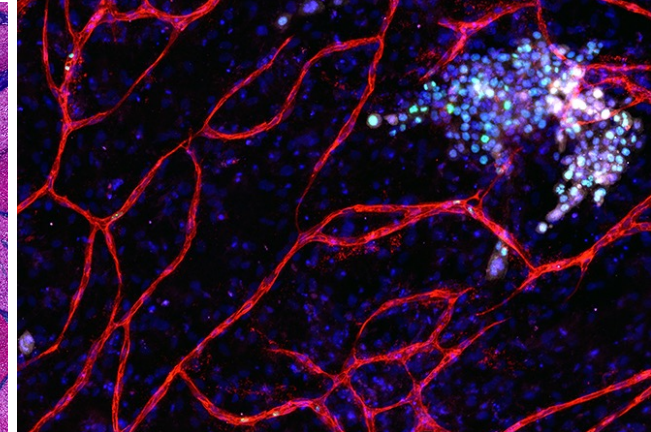
Blood vessels



Small intestine

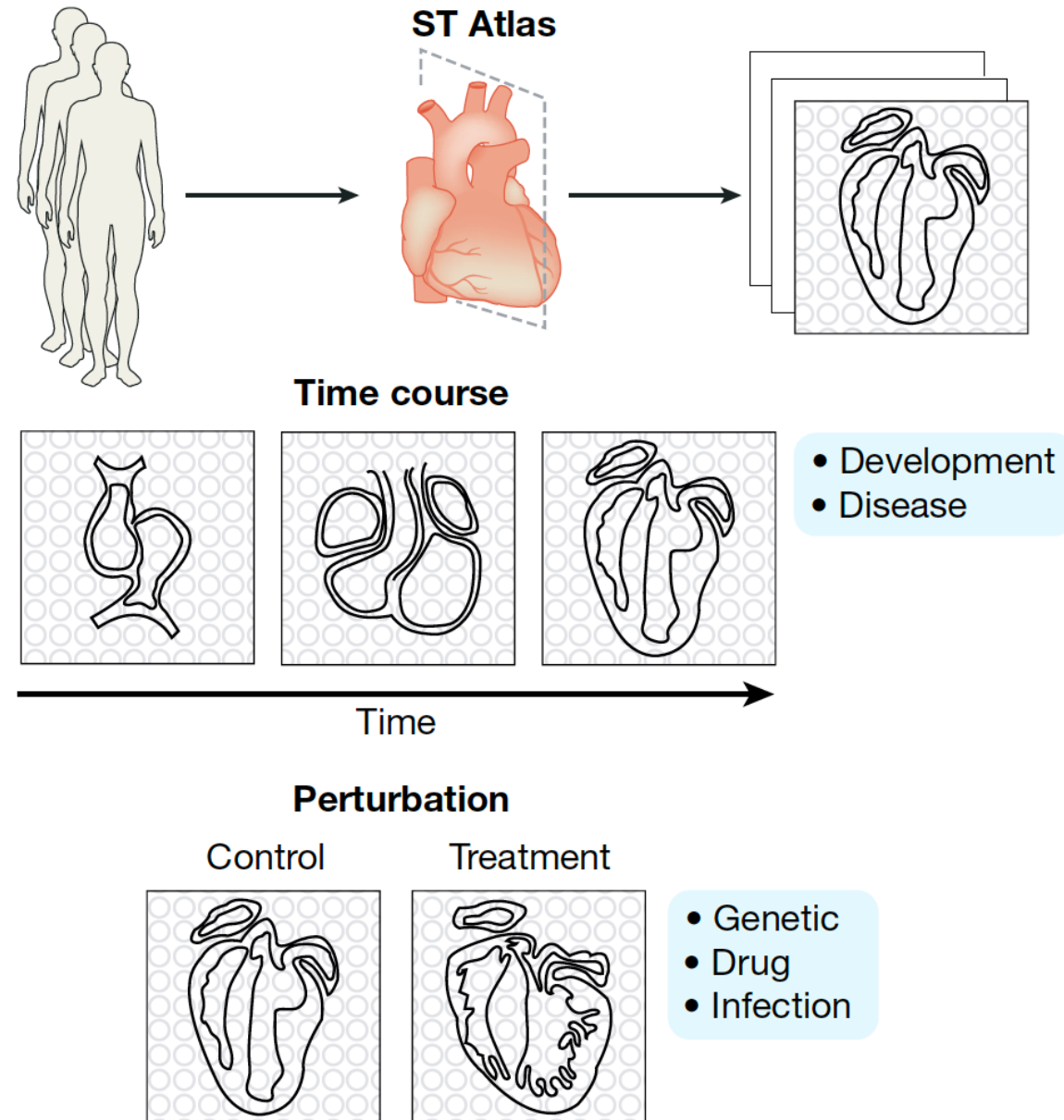


Liver cirrhosis

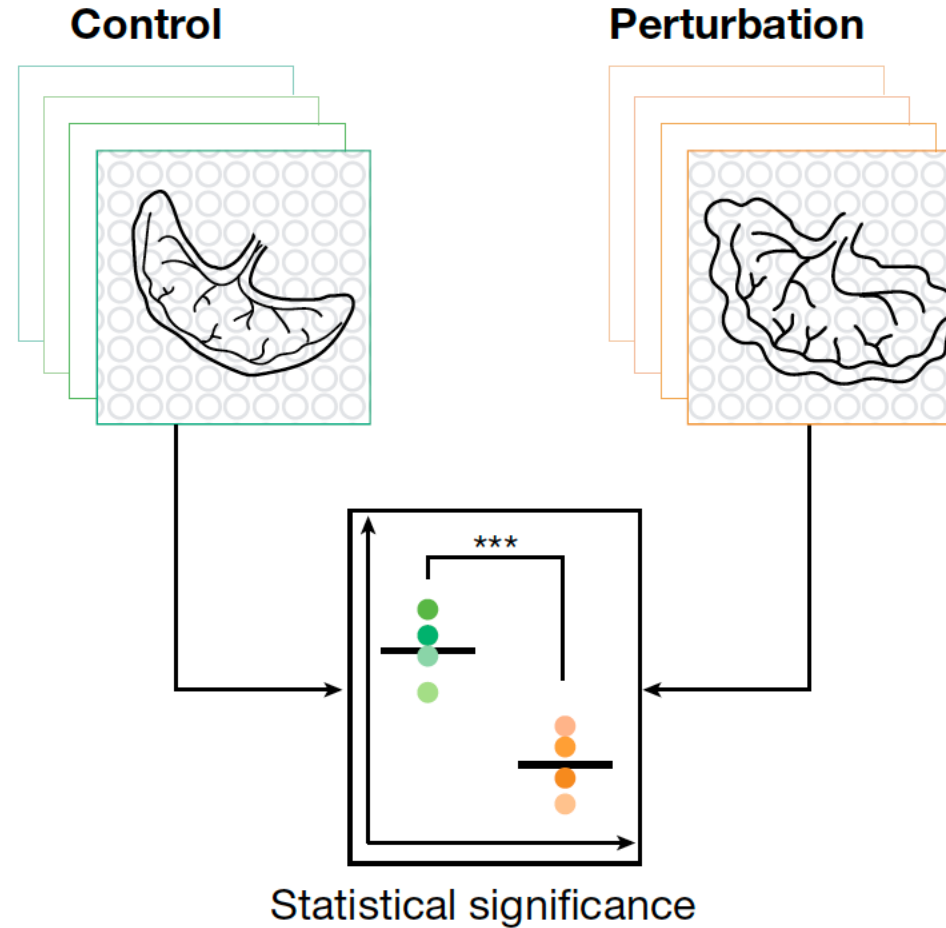


Breast cancer

Spatial technologies used for hypothesis generation



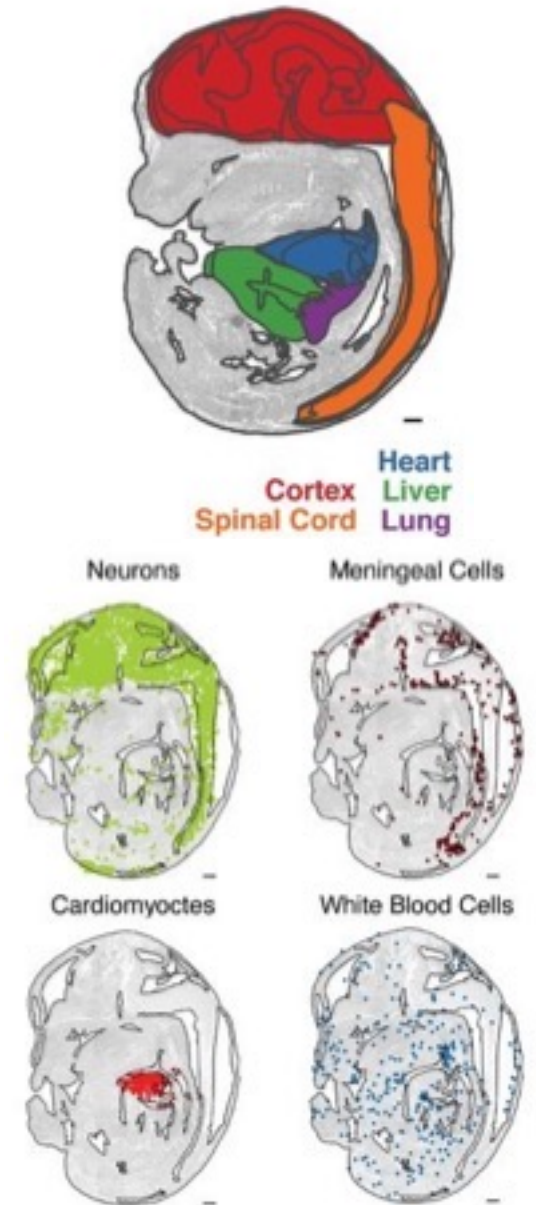
Spatial technologies used for hypothesis testing



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

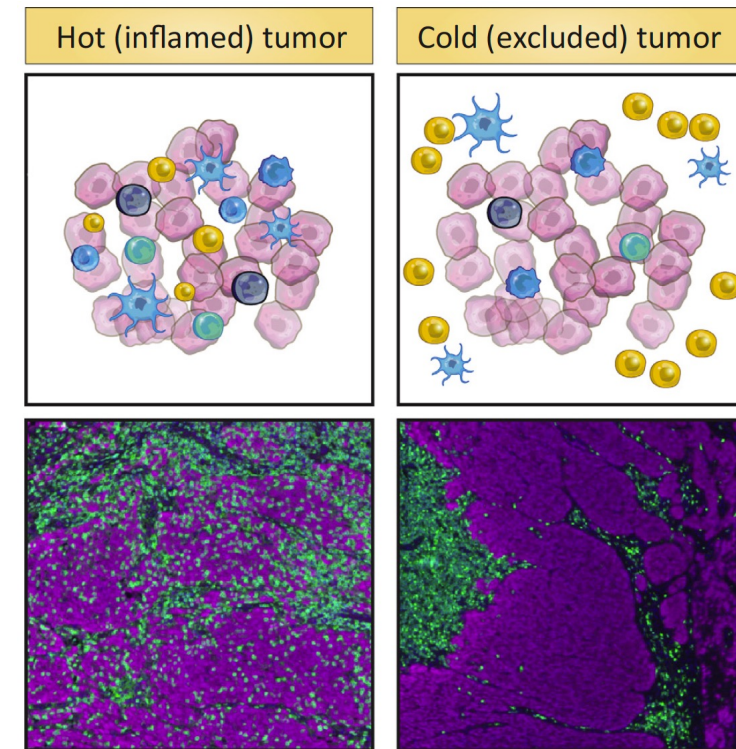
E14.0 mouse embryo



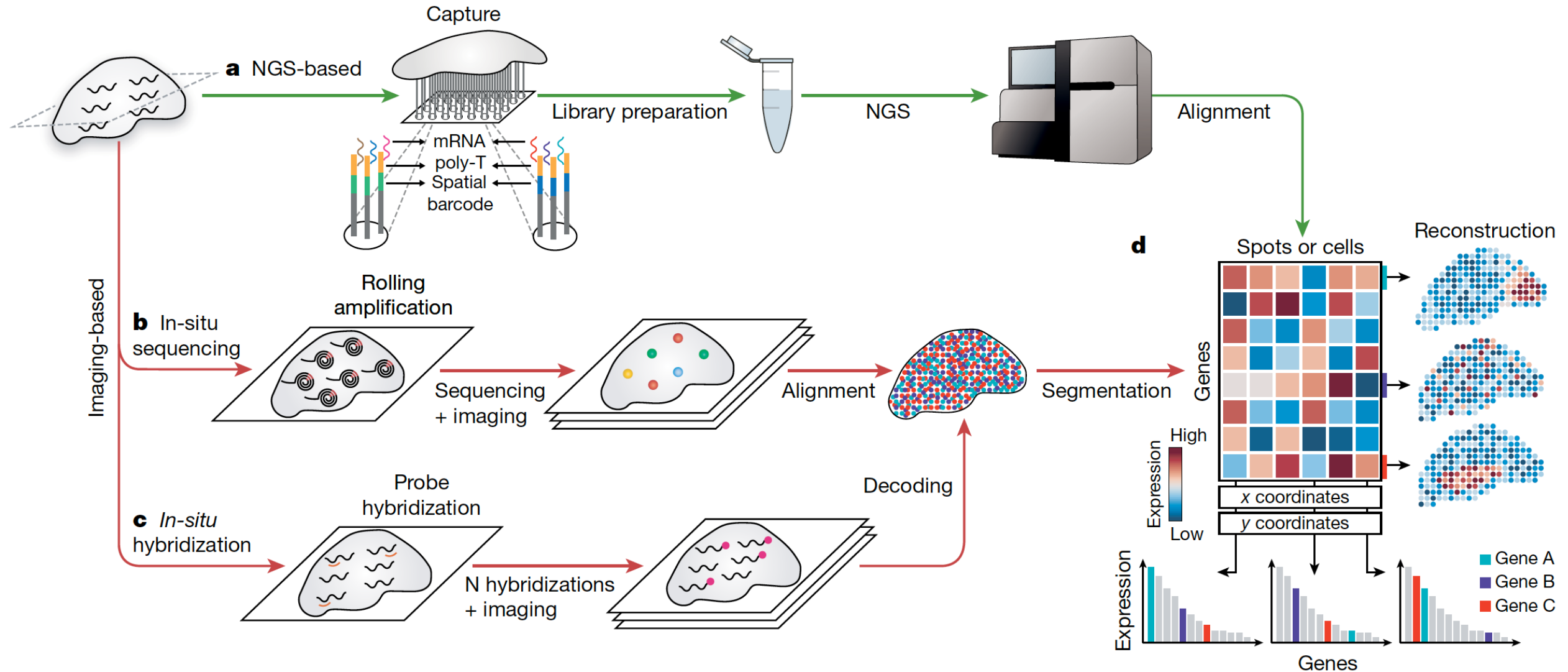
Srivatsan, S. et al *Science* (2021).

Translational questions

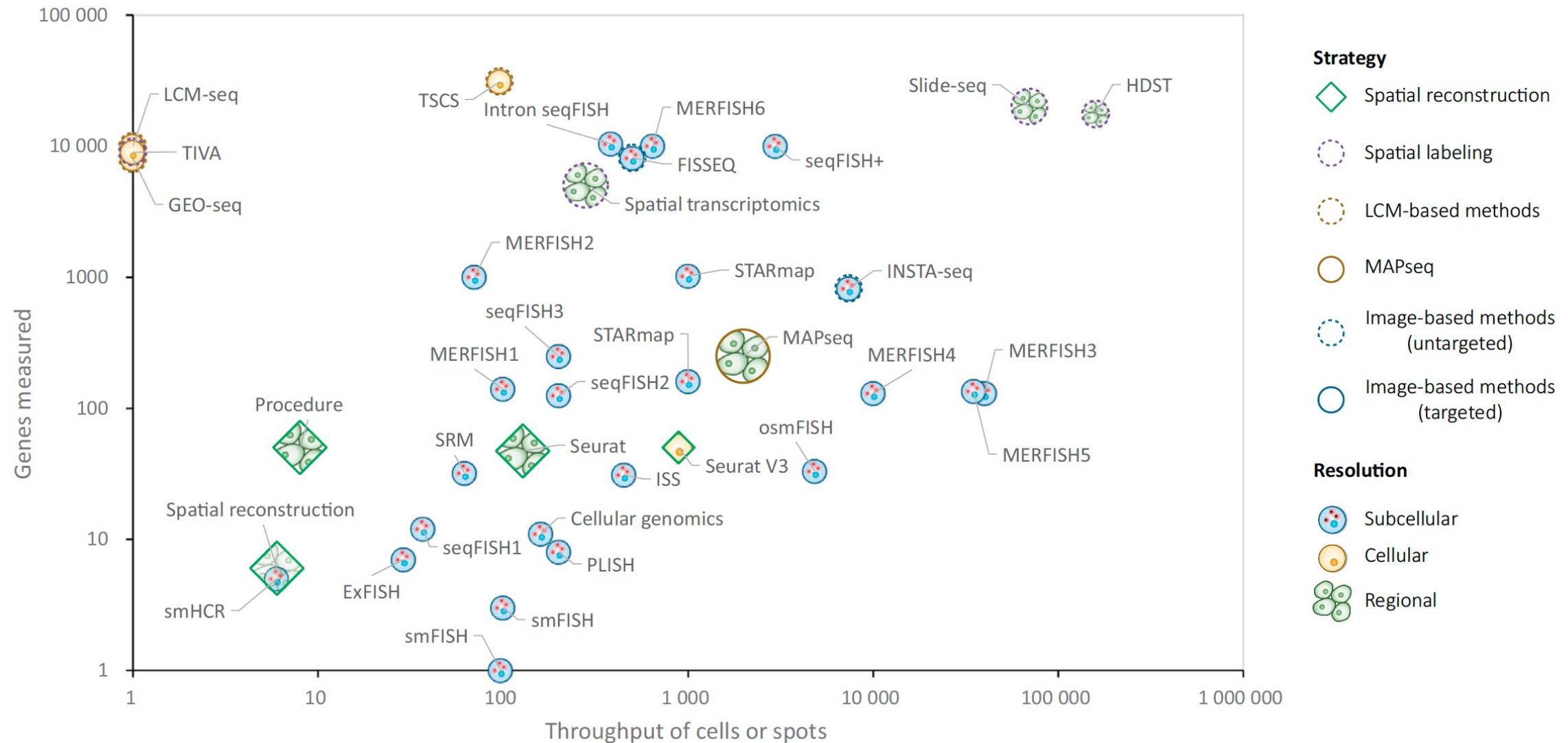
- How cell and tissue organization change during disease progression
- How cell and tissue organization change with treatment response or patient outcomes
- Cell or tissue features that serve as predictive biomarkers



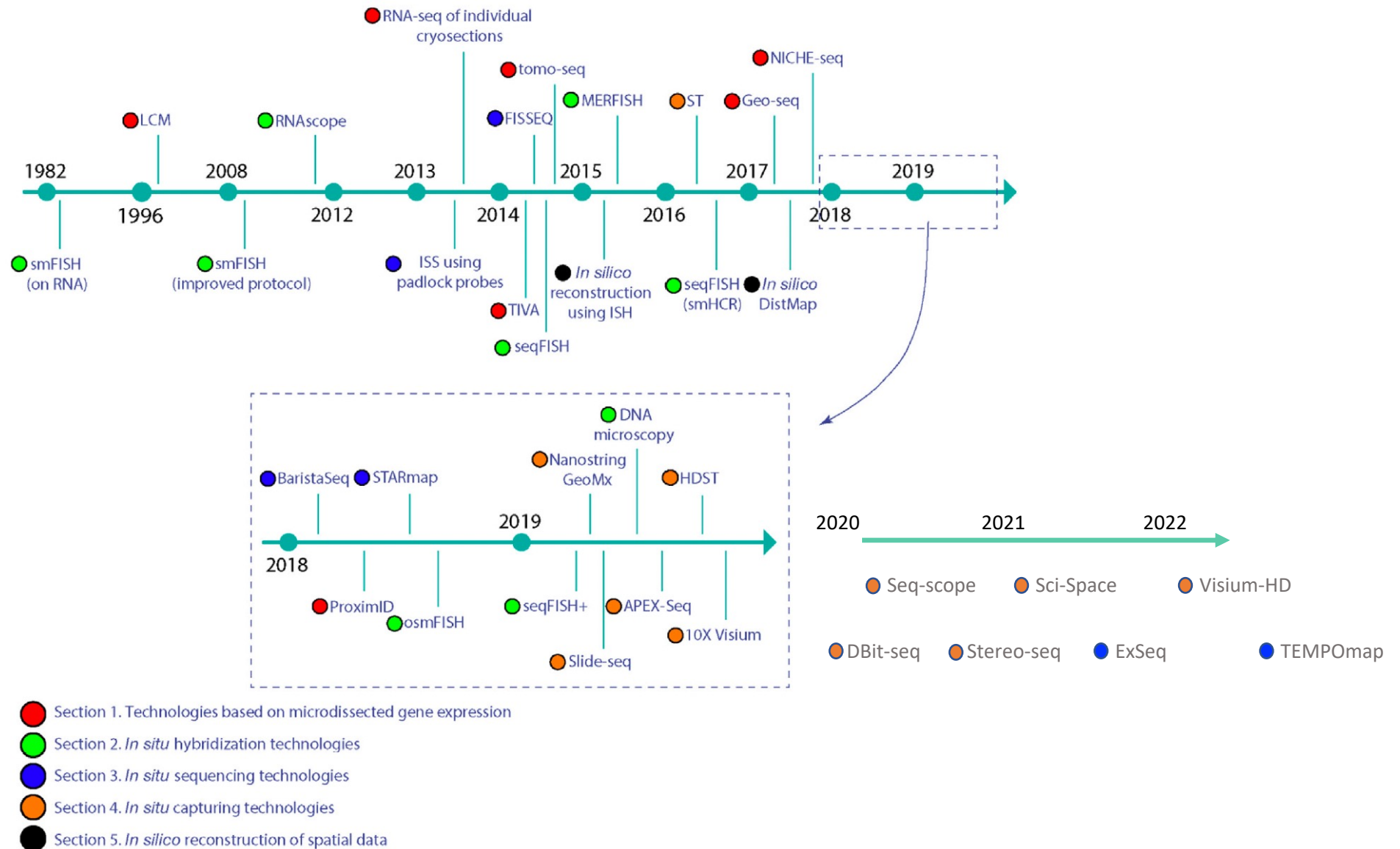
Spatial transcriptomic technologies



Tradeoffs in gene sensitivity, throughput, and resolution



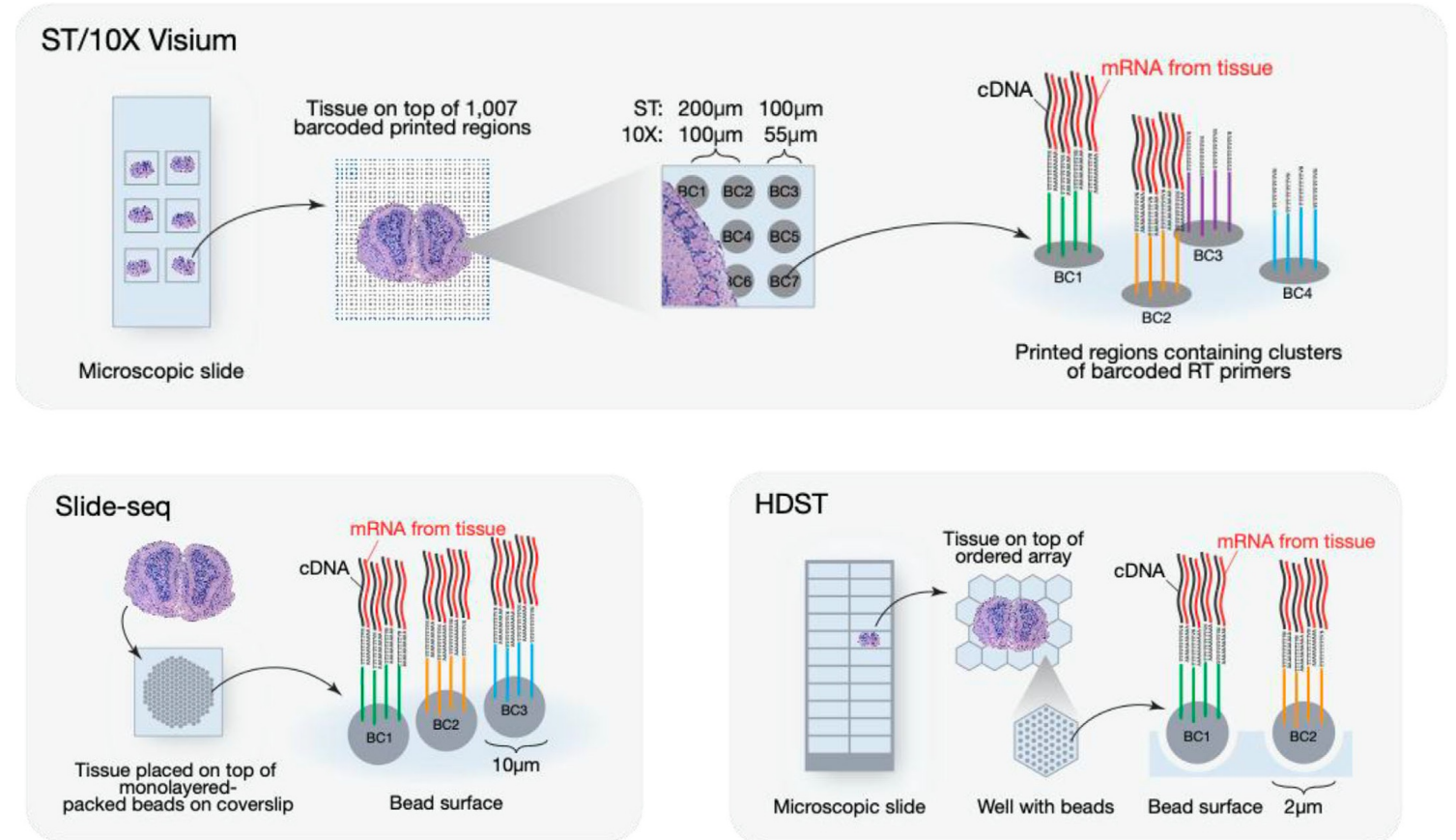
Historical timeline of spatial transcriptomics methods



Spatial transcriptomic method categories and examples

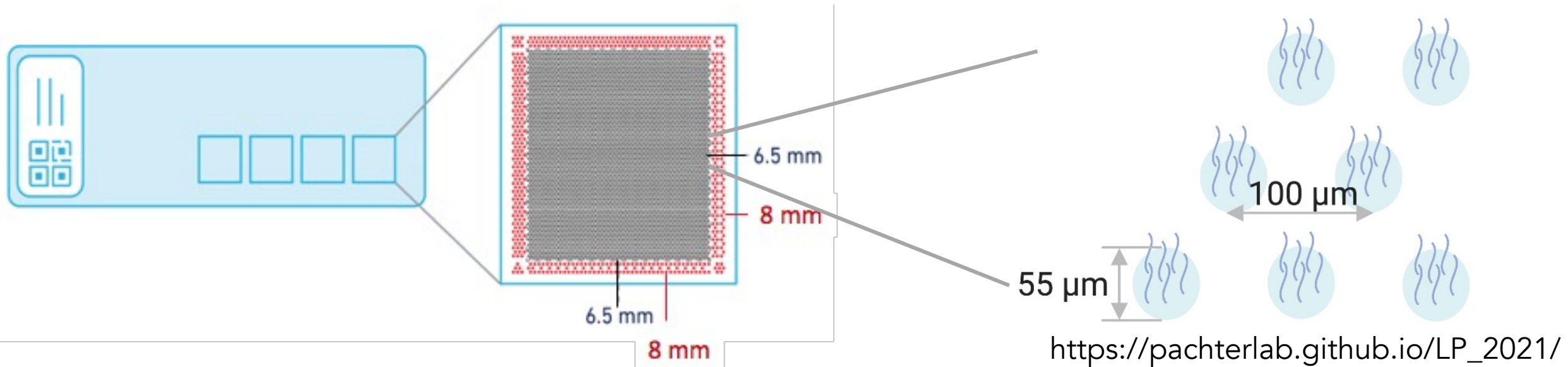
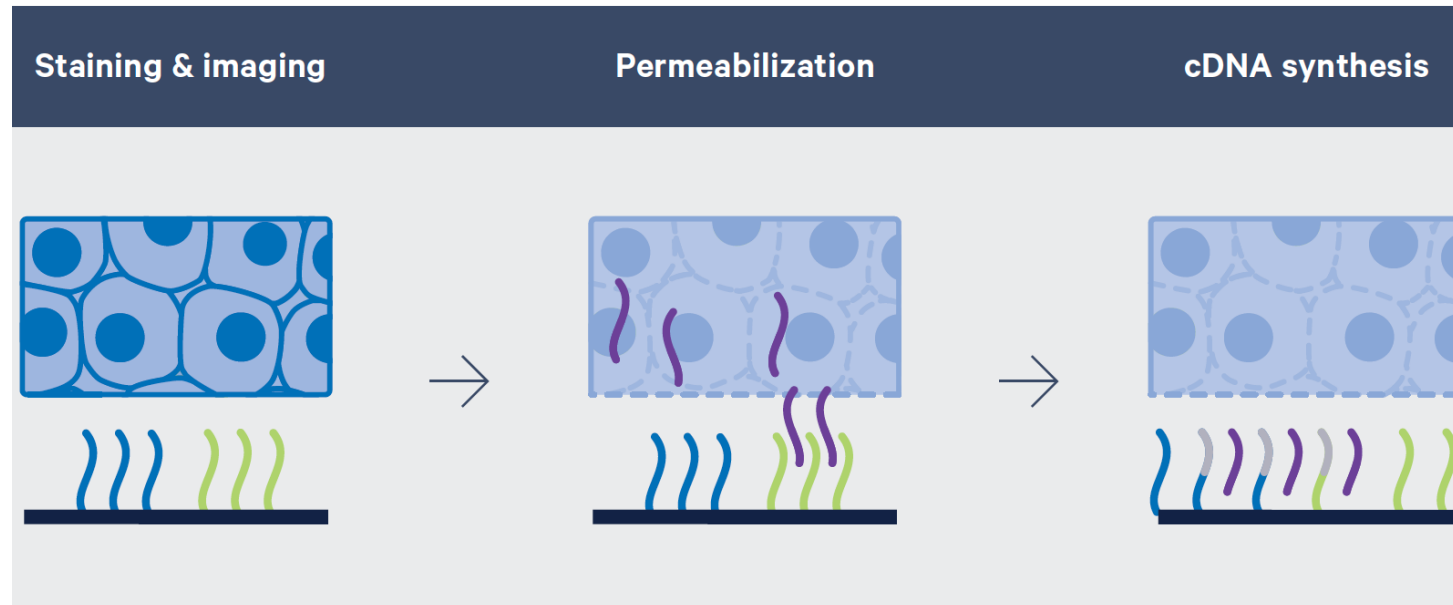
In-situ barcoding

- 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)

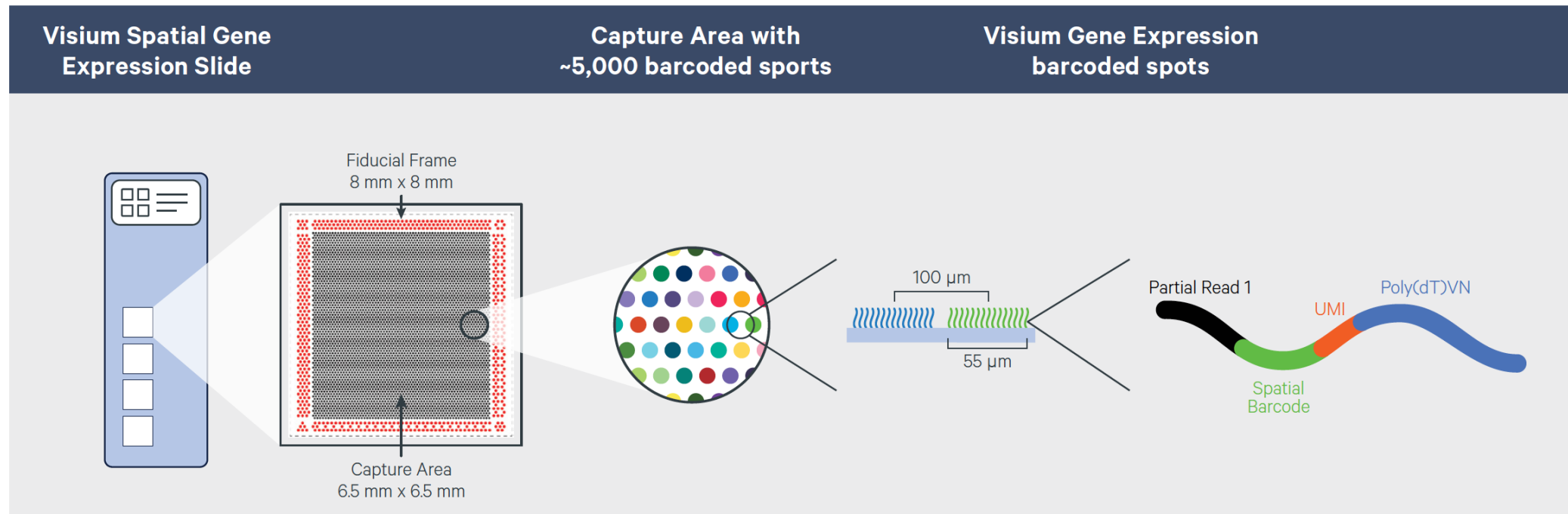


Asp et al., BioEssays 2020

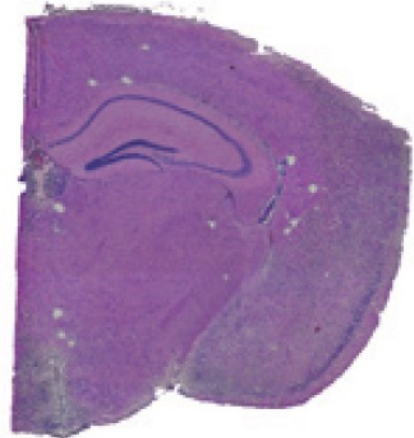
Spatially resolved single-cell transcriptomics: *in situ* barcoding



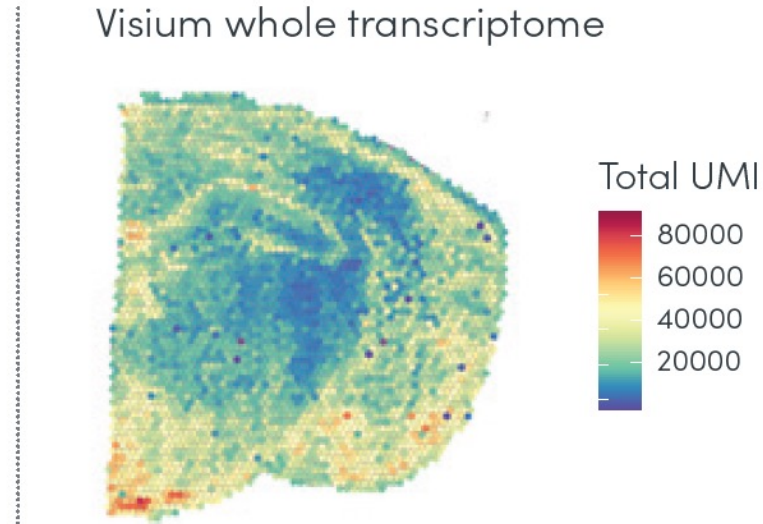
Spatially resolved single-cell transcriptomics: *in situ* barcoding



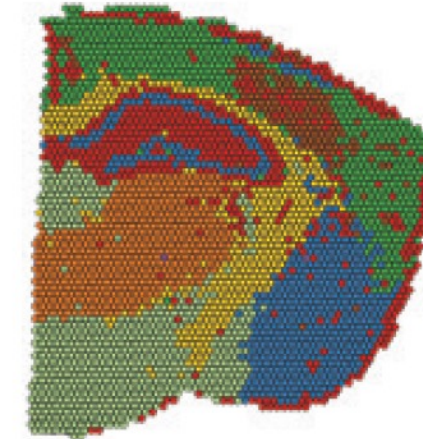
H&E



Visium whole transcriptome



Visium spot clusters



Cluster

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

Cluster 4 mRNAs

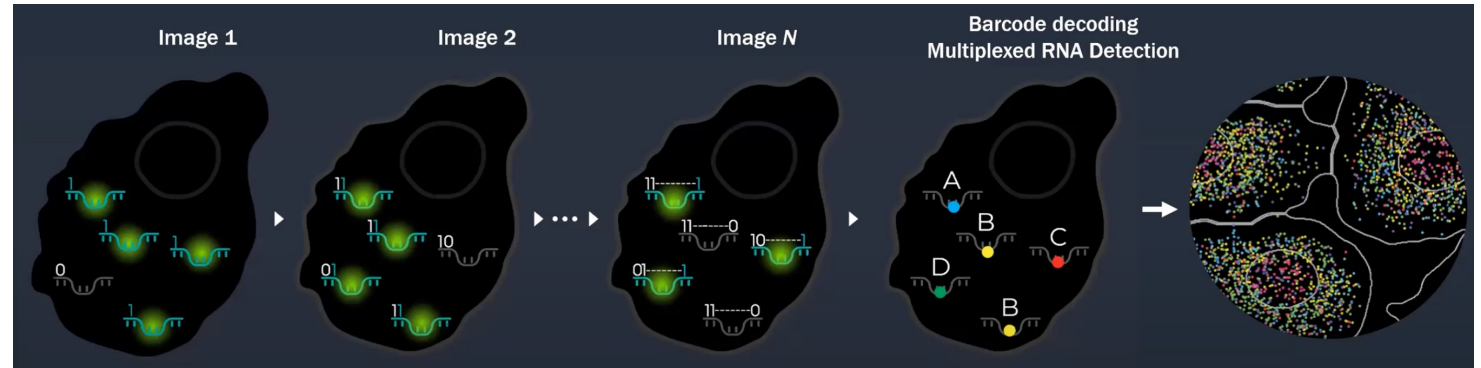
Lamp5
Igfbp6
Tbr1
Ephx4
Lmo4
Vip
Satb1
Egr1
Epop
Ier5

Spatial transcriptomic method categories and examples

Imaging-based methods

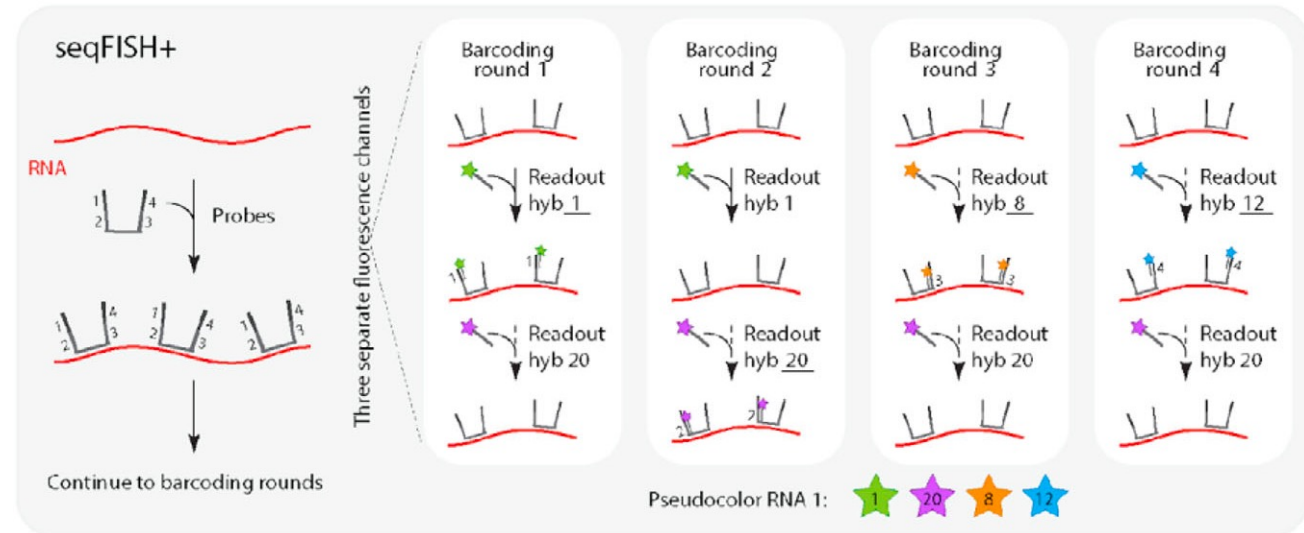
- Fluorescent *in situ* hybridization (FISH)
 - MERFISH/Vizgen (2015, FF)
 - seqFISH+ (2019, FF)
 - SABER-FISH (2019)
 - osmFISH (2018, FF)
- *In situ* sequencing (ISS)
 - FISSEQ/ReadCoor -> 10x
 - Padlock probe based *in situ* sequencing/Cartana -> 10x
 - STARMap (2018, FF)
- ISS with *ex situ* sequencing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

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



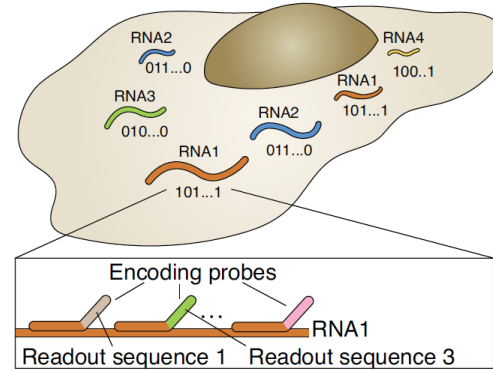
<https://vizgen.com/technology/#merfish>

seqFISH+ - subcellular resolution, >10 000 genes



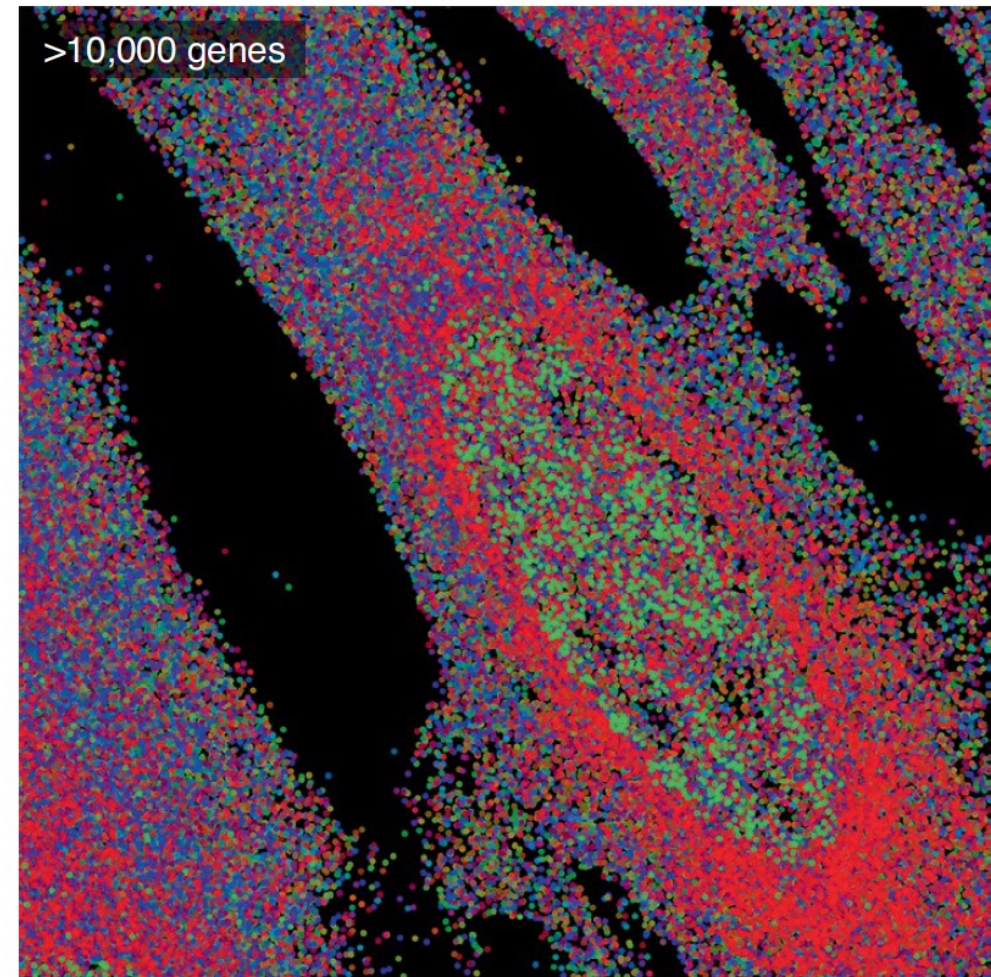
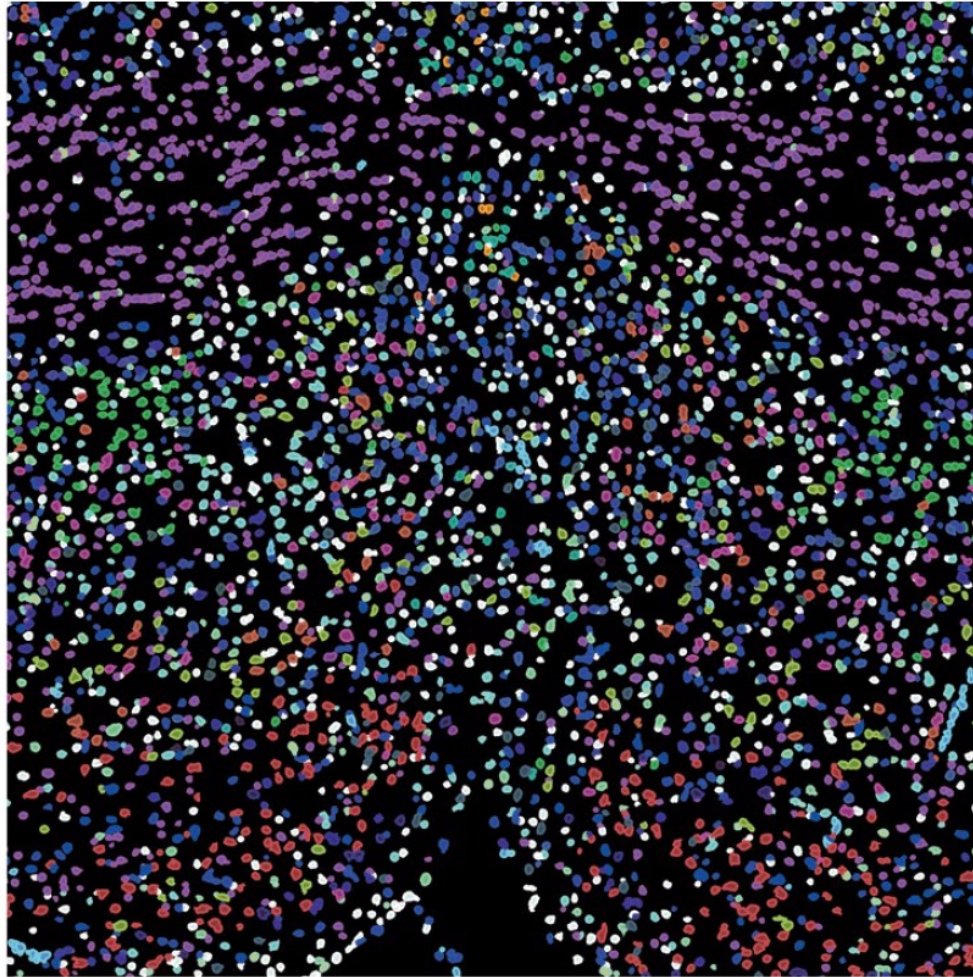
Spatially resolved single-cell transcriptomics by imaging

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

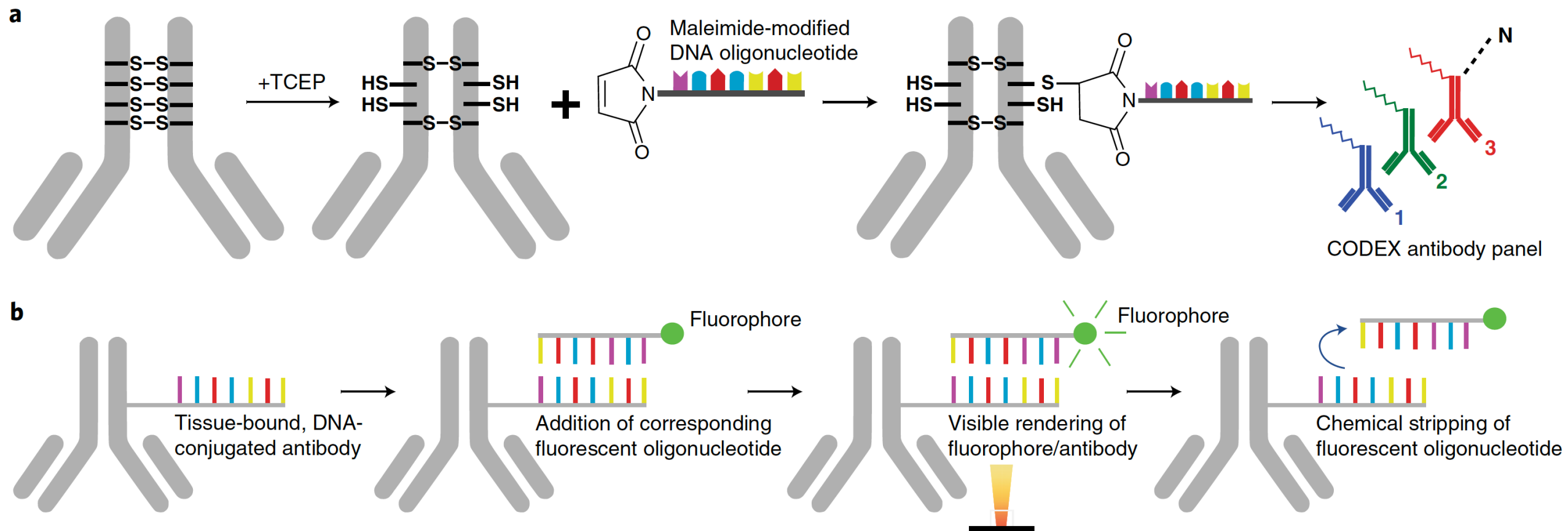


Interact with MERFISH human tumor datasets online:
<https://info.vizgen.com/ffpe-showcase>

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

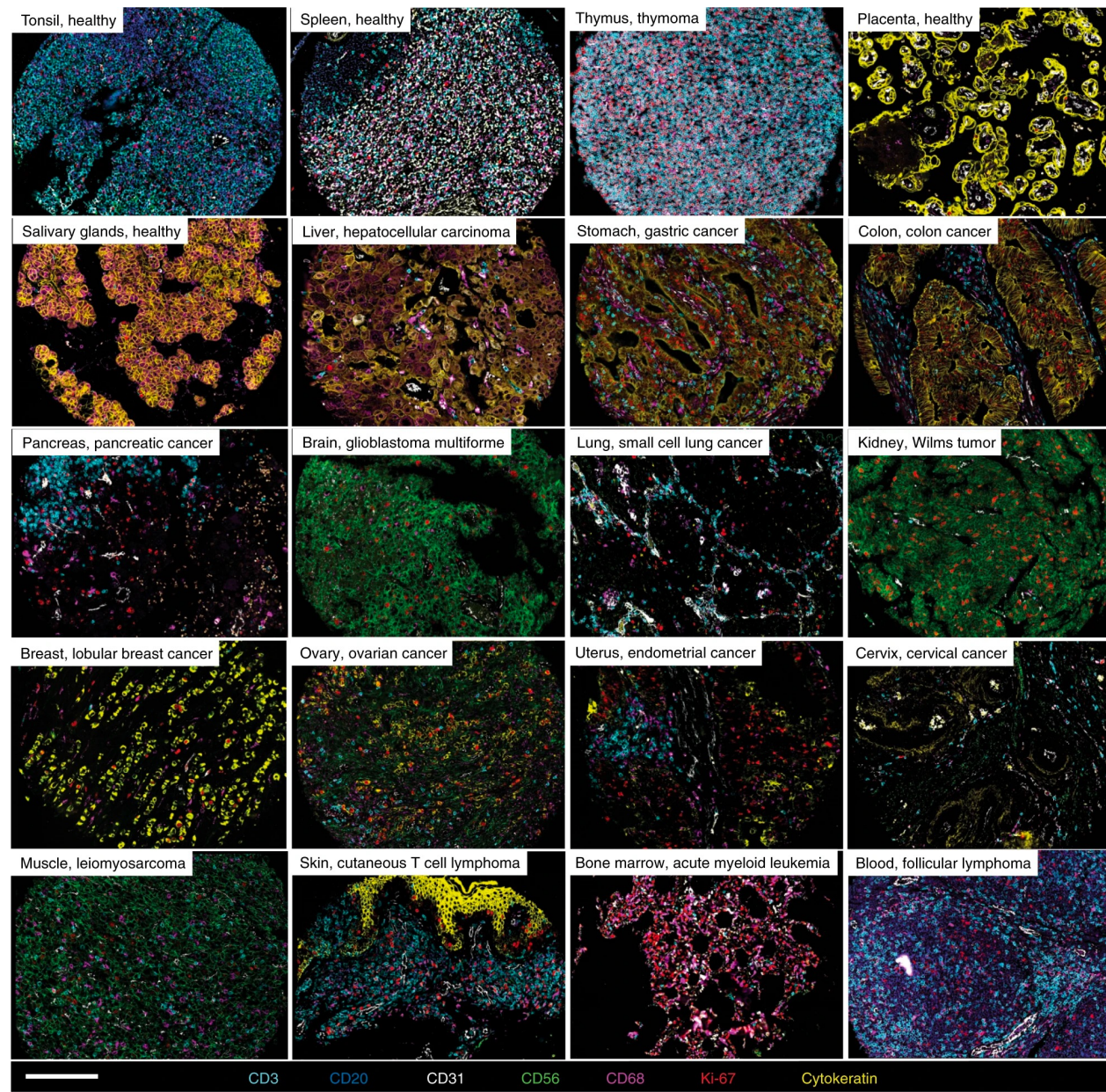
Spatial proteomic technologies

CODEX



Spatial proteomic technologies

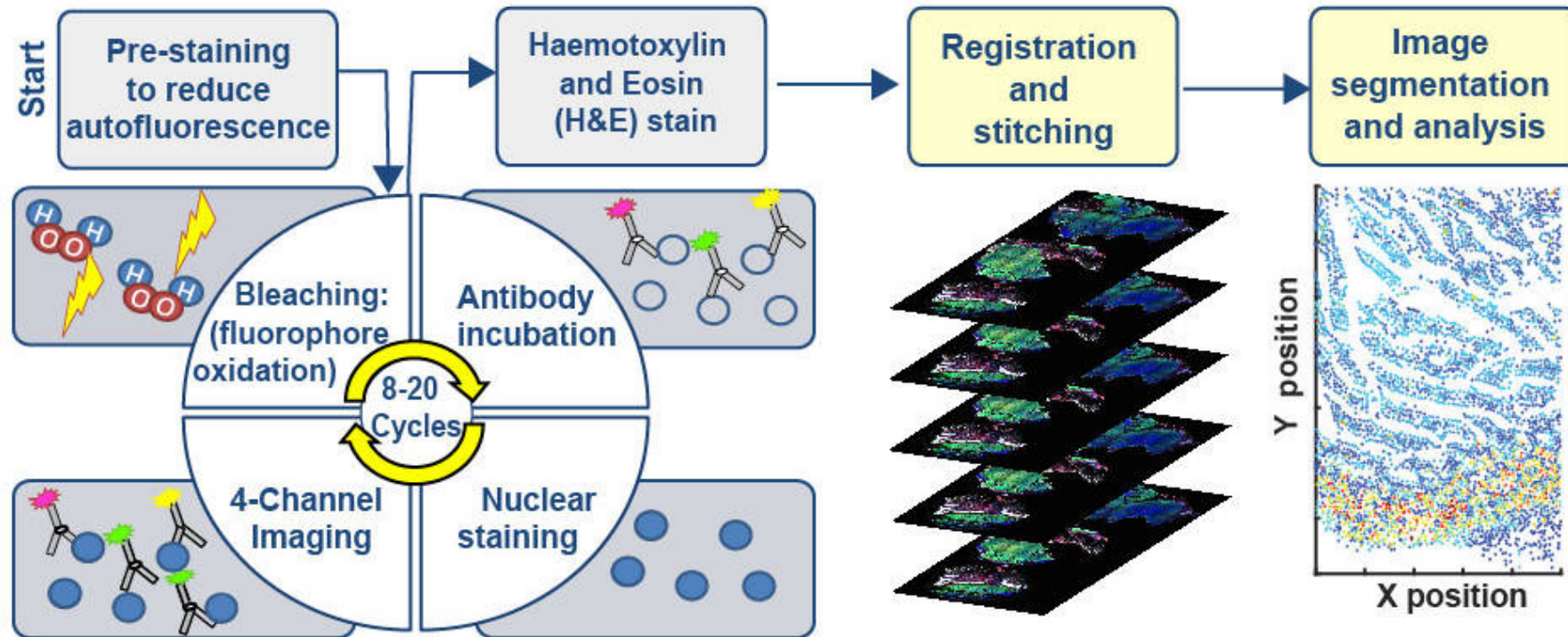
CODEX



Black, S. et al *Nature Protocols* (2021).

Spatial proteomic technologies: Cyclic immunofluorescence (CyclF)

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



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

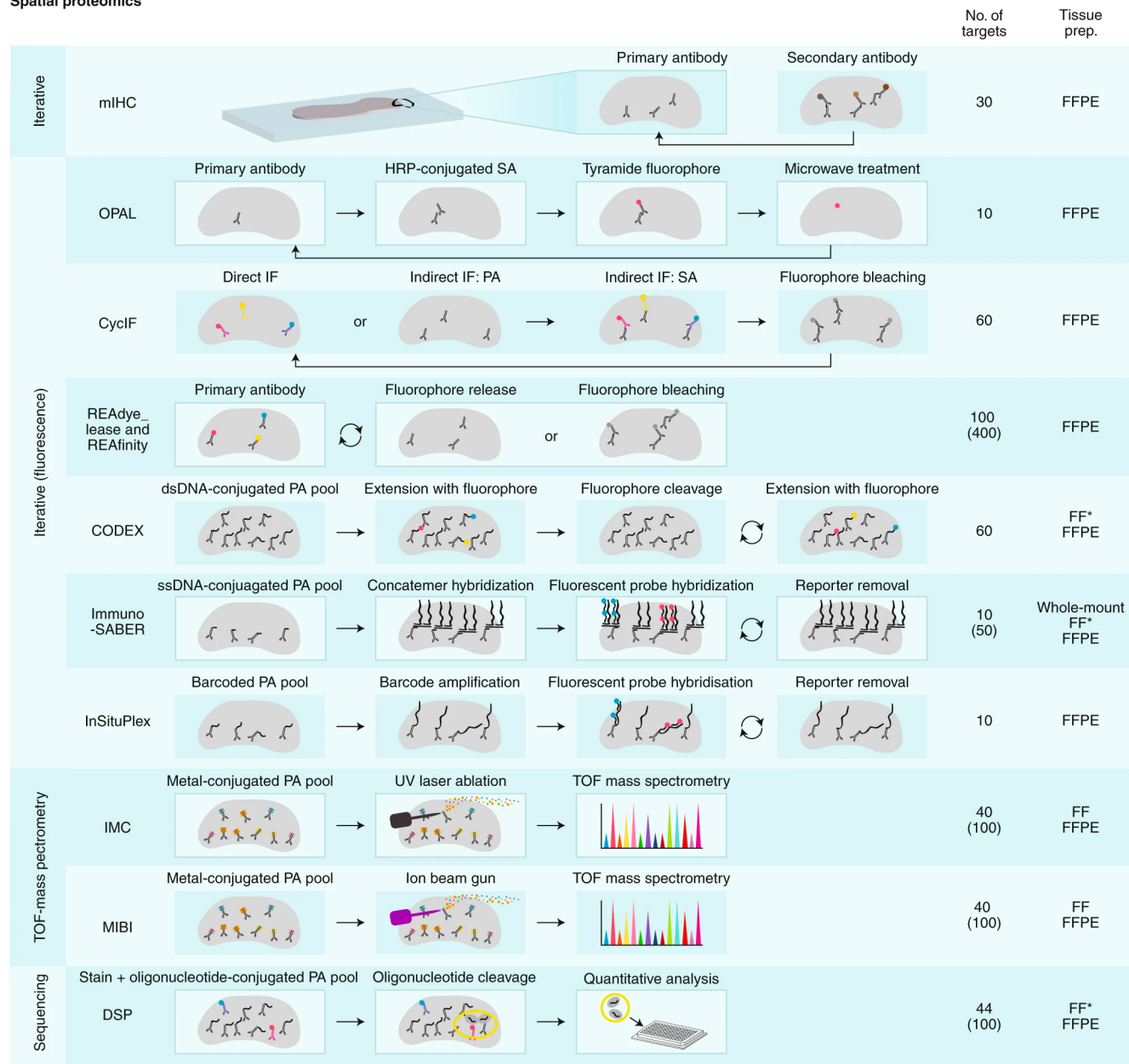
Primary lung cancer analysis: https://www.cycif.org/data/du-lin-rashid-nat-protoc-2019/osd-LUNG_3_DATA.html

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

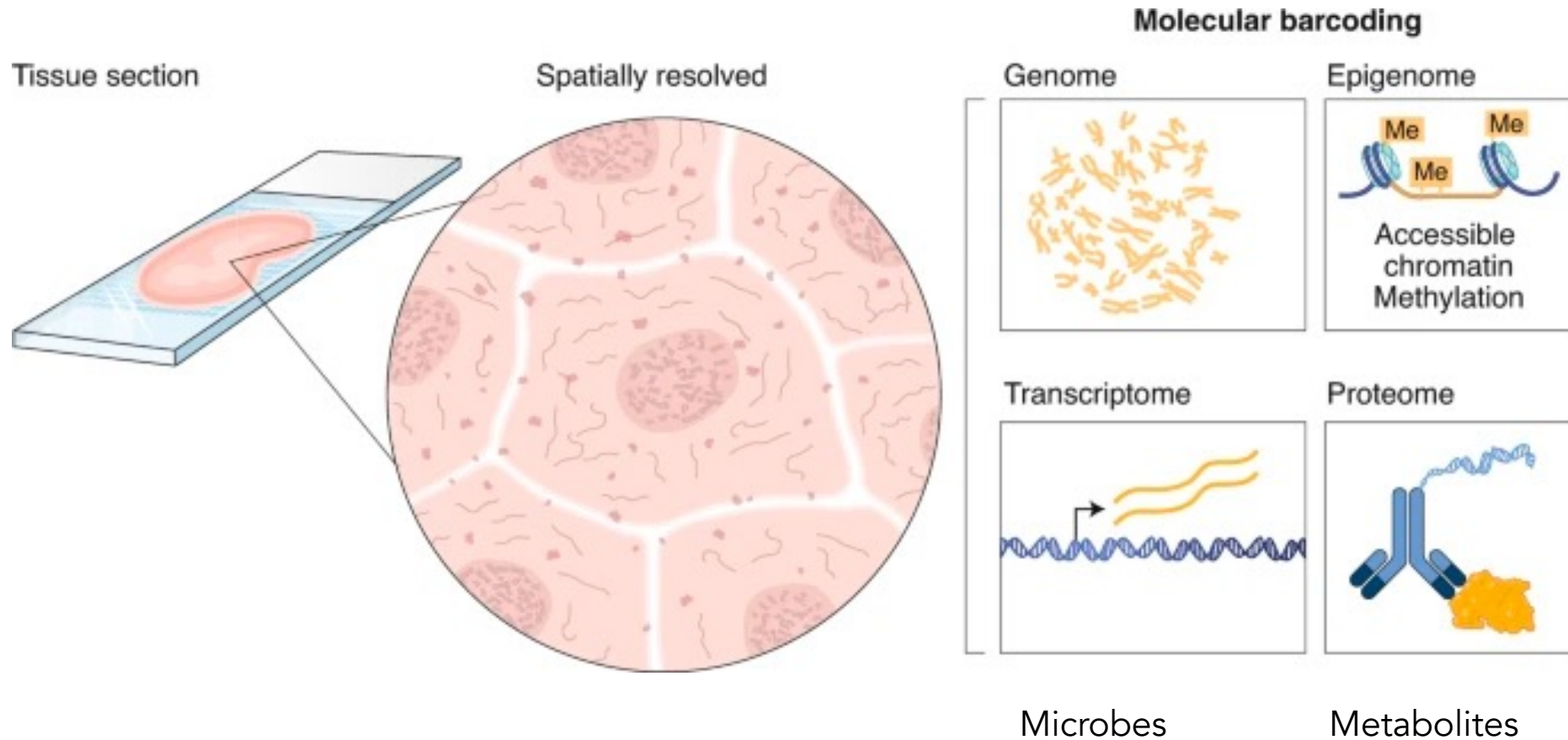
Metastatic melanoma: <https://www.cycif.org/osd-exhibit>

Spatial proteomic technologies

Spatial proteomics



Towards multimodal spatial measurements



Experimental design: technology

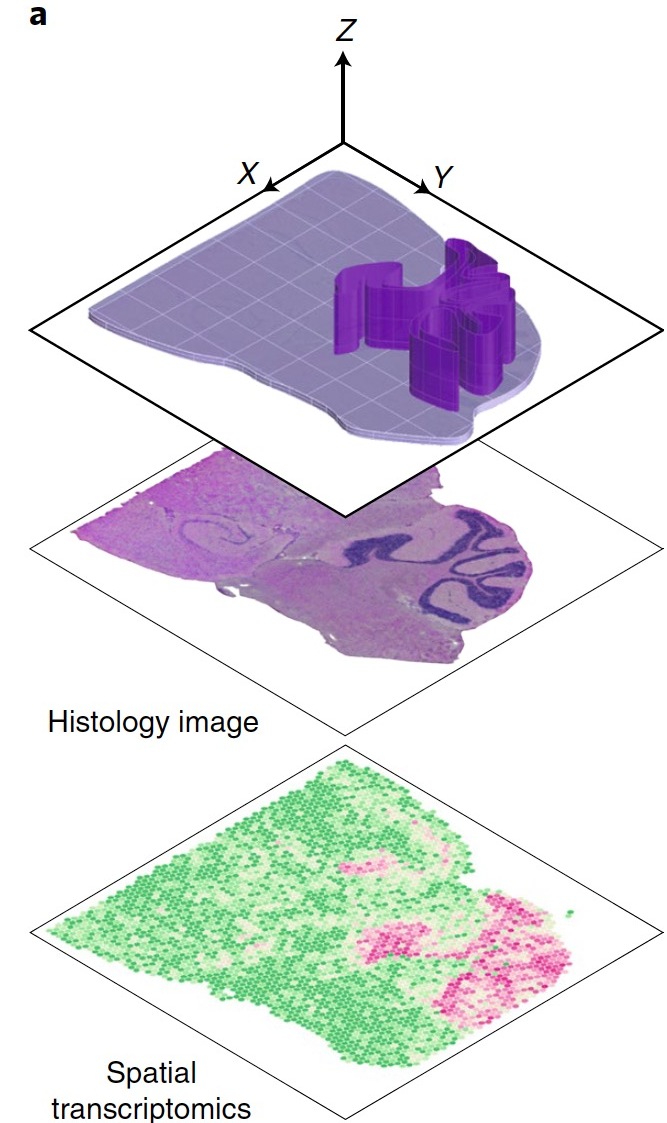
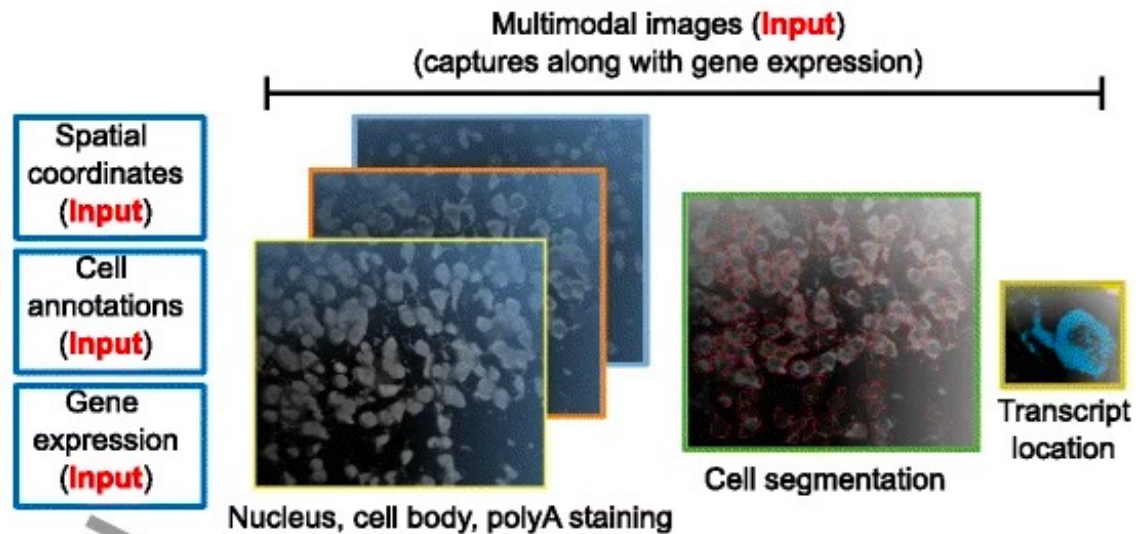
- Which technology
 - tissue source: fresh-frozen or FFPE fixed
 - resolution: 55 μm - single cell and sub-cellular
 - gene throughput: 100s genes / proteins - 20 K genes
 - gene detection sensitivity
 - tissue size and field-of-view (FOV) size
 - focus on specific cell subset? Design gene panel (e.g. 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

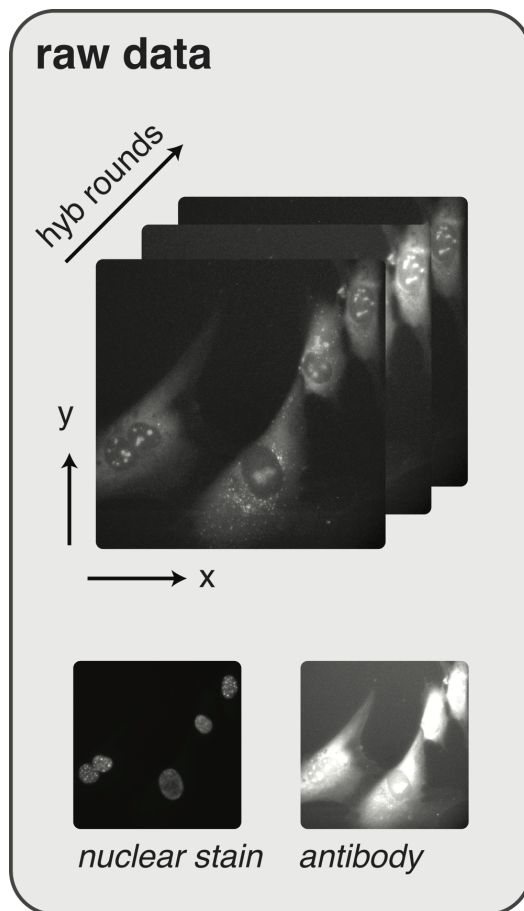
- Dyes for cell segmentation (nuclei, membrane)
- Banking serial sections for imaging and snRNA-Seq
 - Histology (cell morphology)
 - Imaging (ie immunofluorescence, collagen, phosphorylation, histone modifications...)
 - Later validation experiments



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

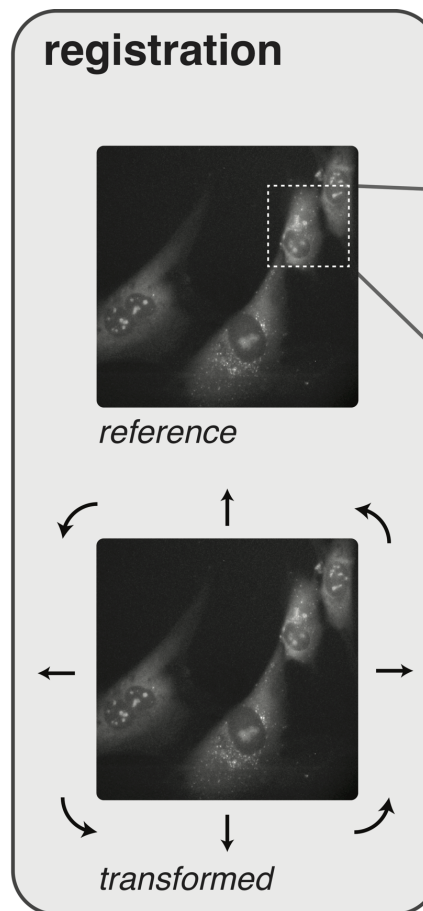
Imaging data has complex preprocessing

starfish
Imaging based
transcriptomics



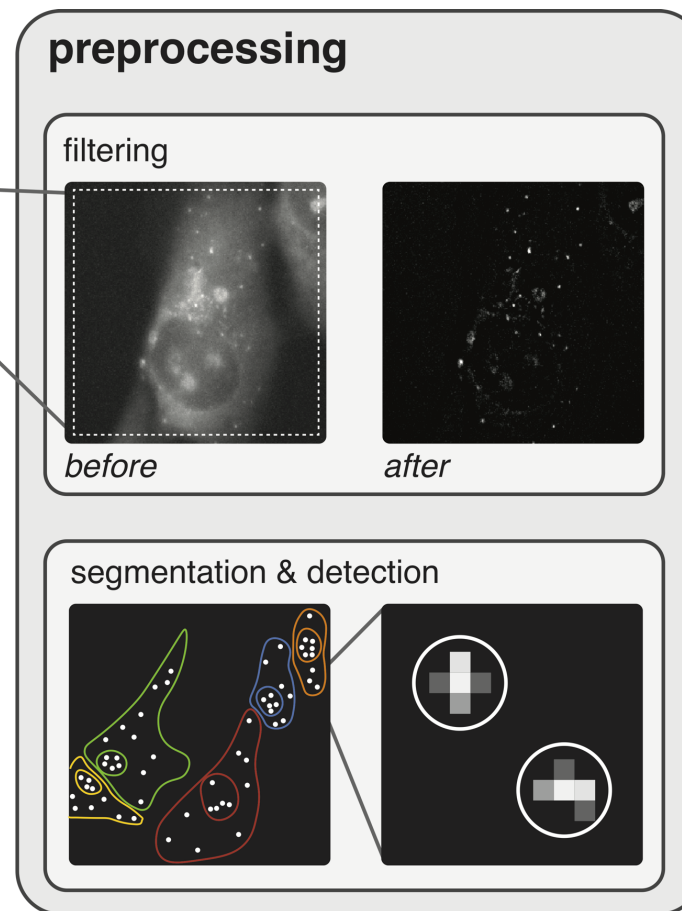
per field of view
raw_stack.tif
dapi.tif, antibody.tif

codebook schemas
hyb | bit position
gene | bit-string



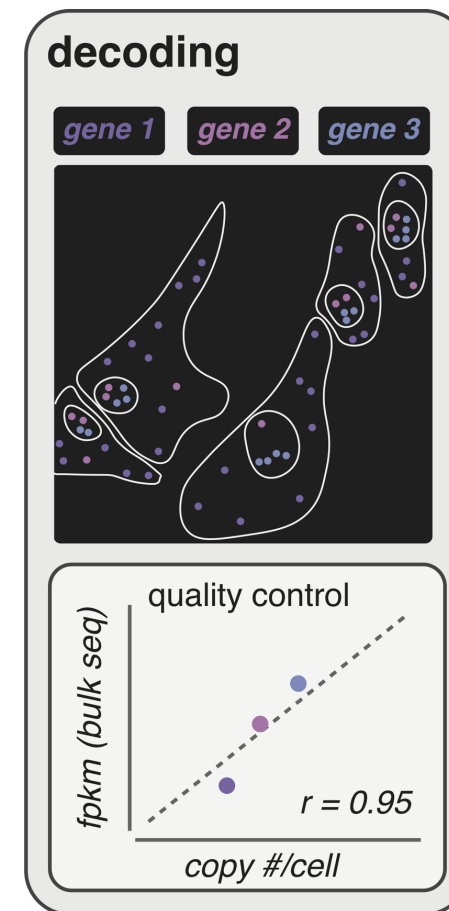
per field of view
reg_stack.tif
affine_transform.mat

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

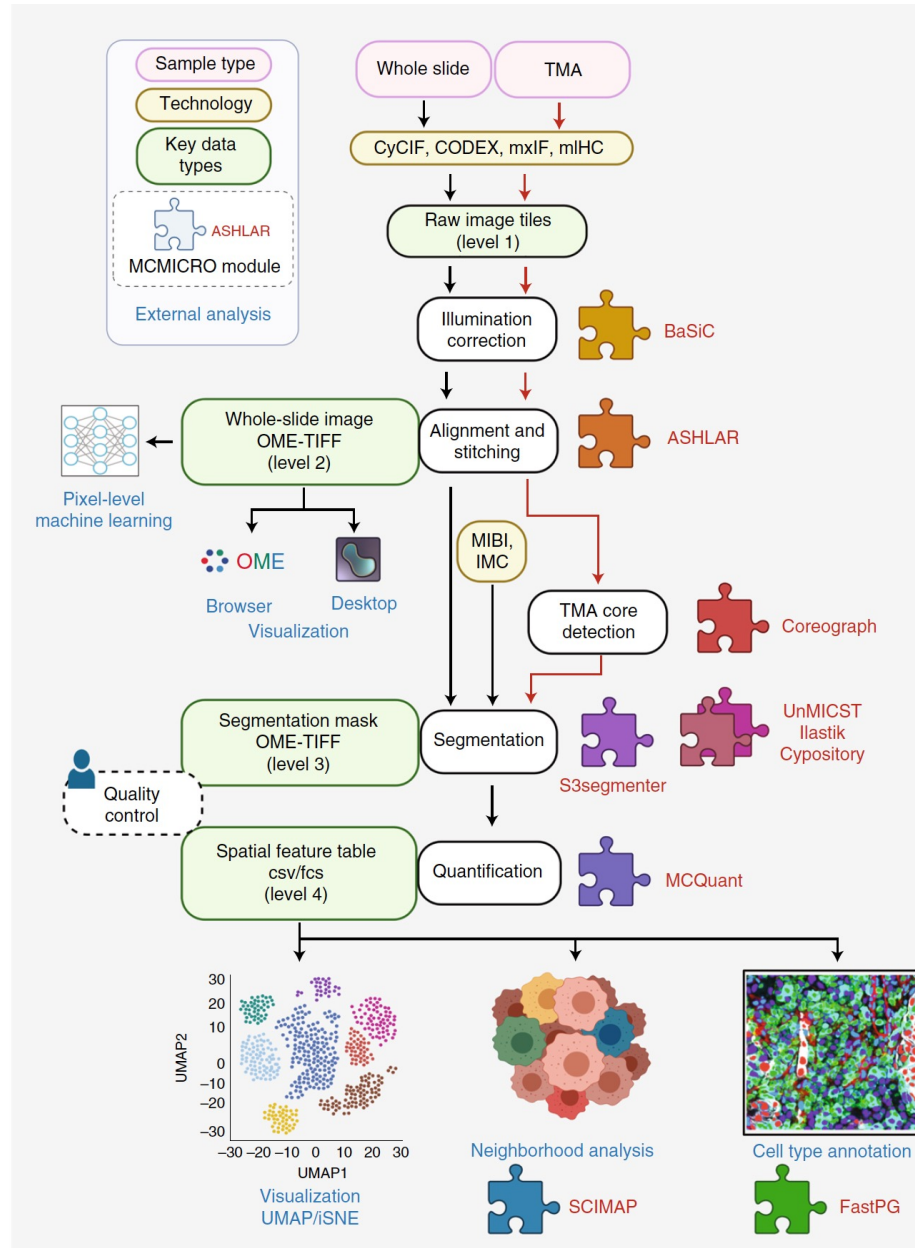


quality metrics
assay_comp.json
control_barcodes.json
metrics.json

decoder table schema
spot_id | gene

Imaging data has complex preprocessing

MCMICRO Multiplex protein imaging



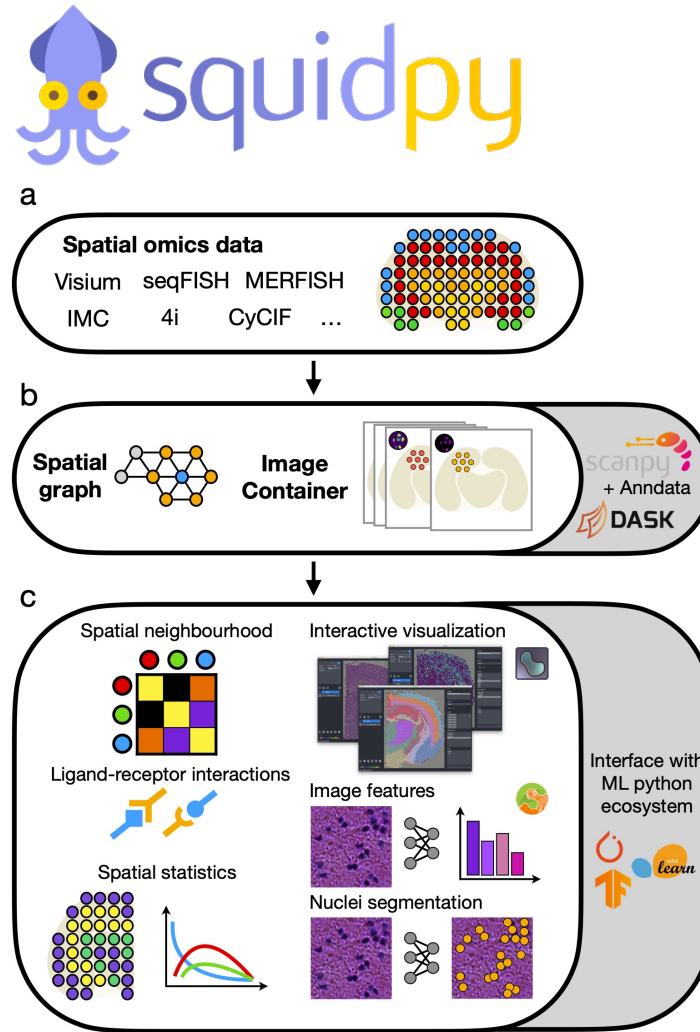
Data analysis frameworks

- Spatial transcriptomics

Seurat
Giotto
STUtility
SPATA
Meringue
...
Scanpy
Squidpy
Starfish
stLearn
...

- Spatial proteomics

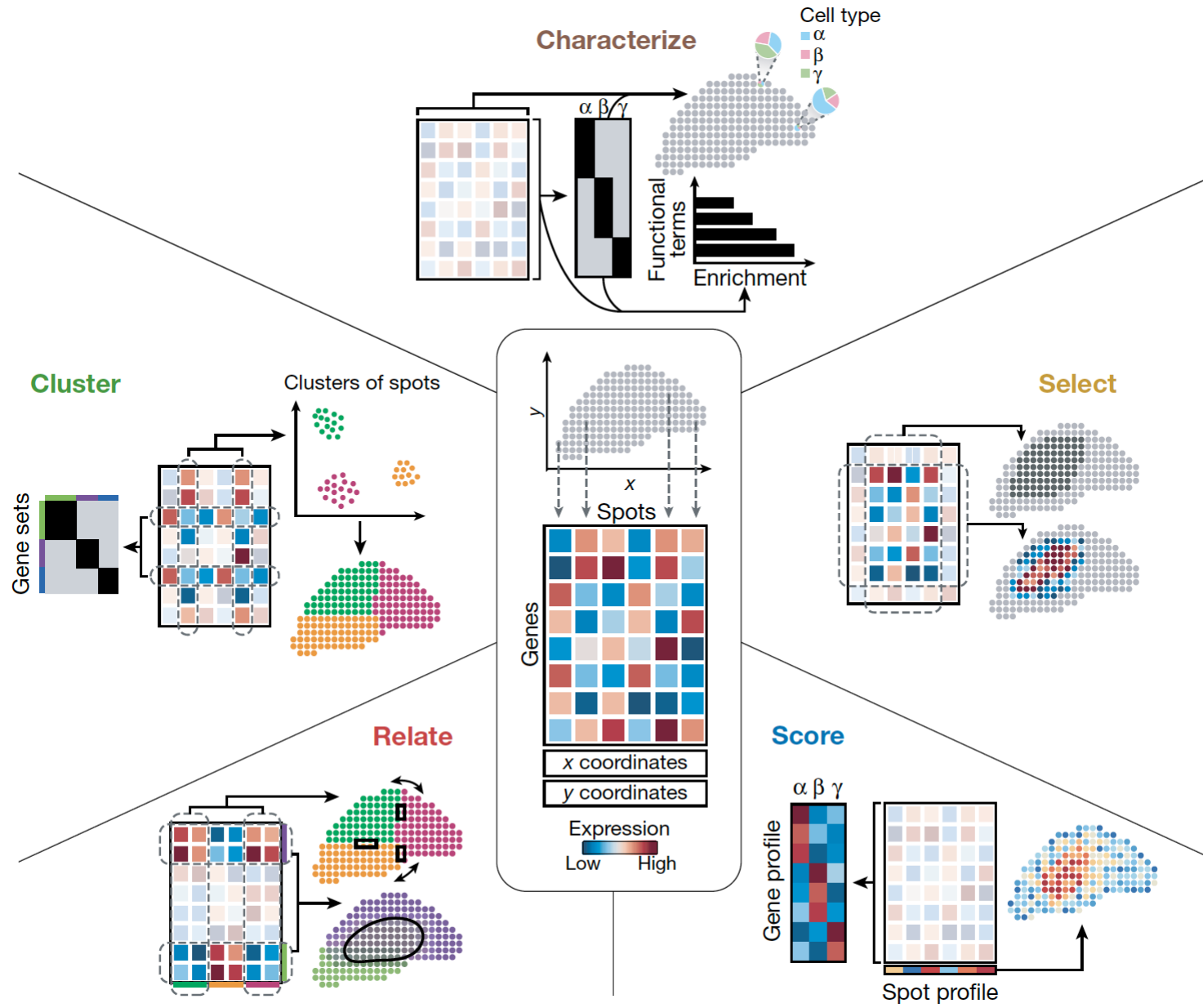
MCMICRO
Steinbock
...



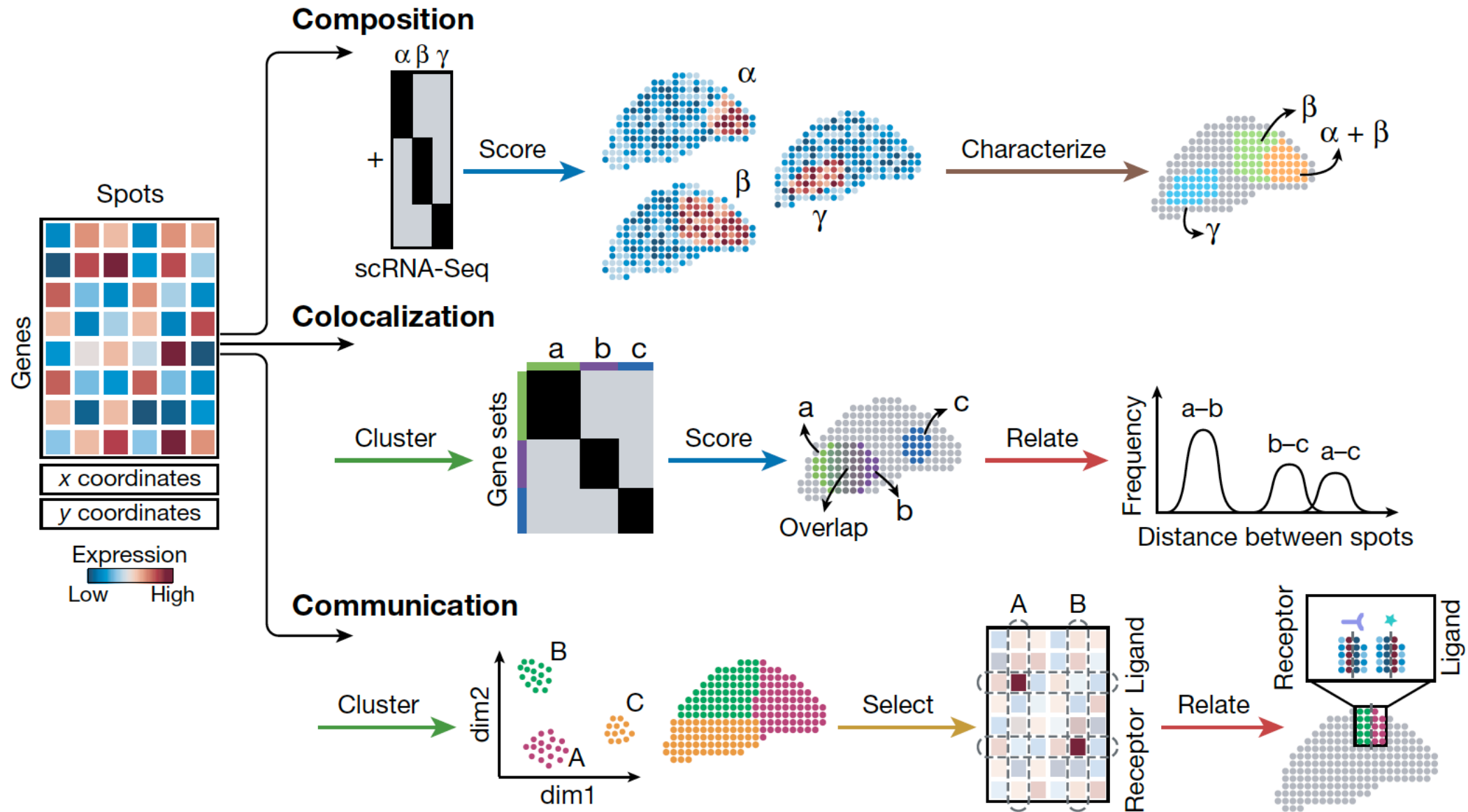
- Data visualization and exploration

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