Advances in single-cell genomics: spatial genomics

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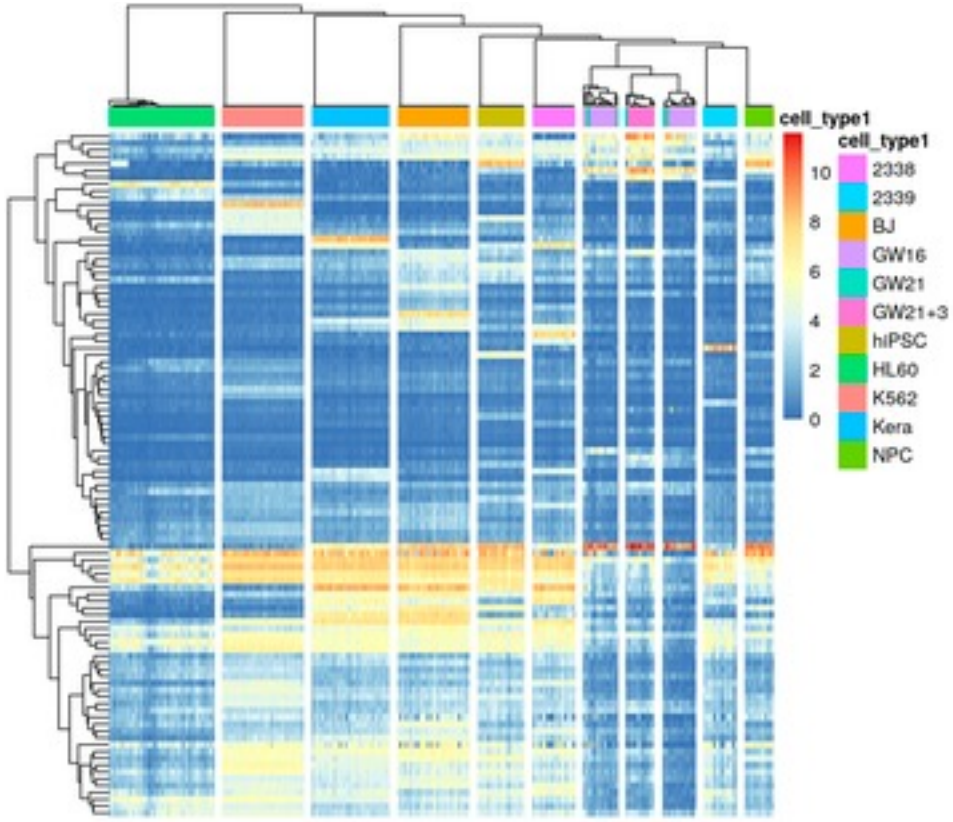
November 8, 2024

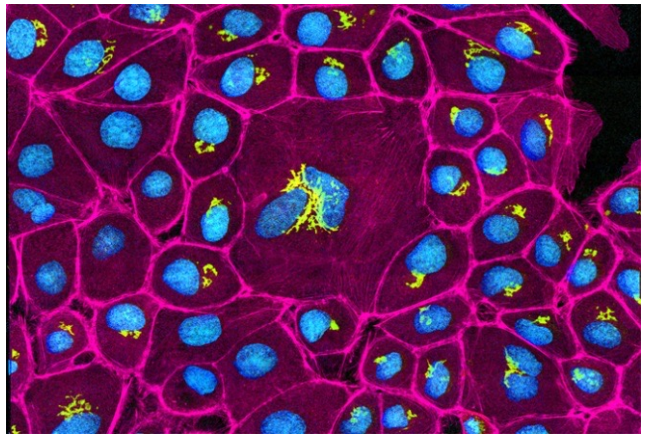
Physalia

Courses

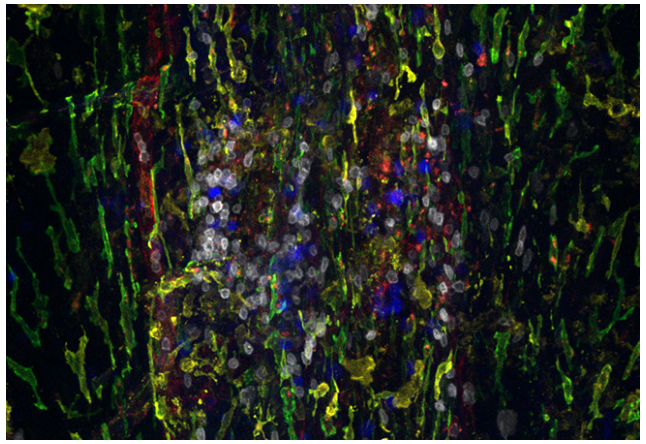


Incredible diversity in cell types, states, and interactions across human tissues

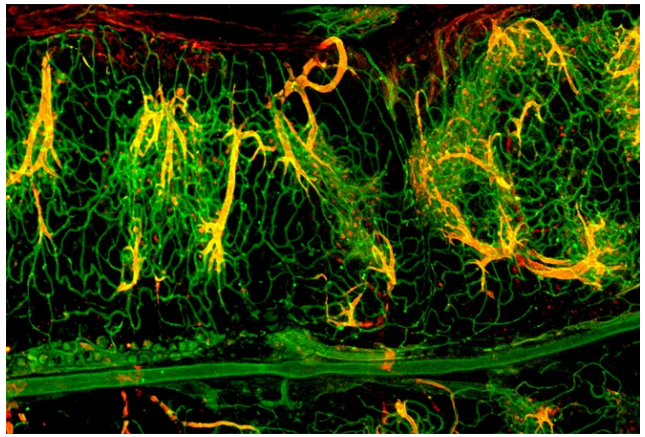
Skin epithelium

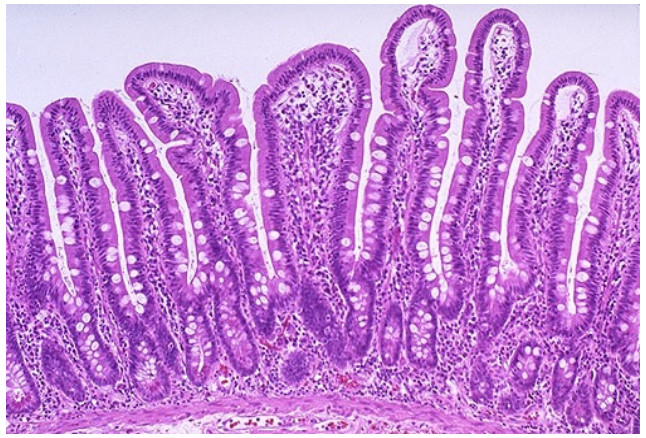


Brain meninges

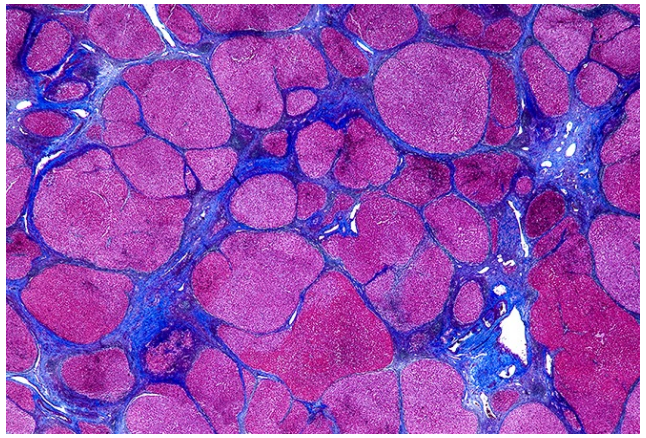


Blood vessels

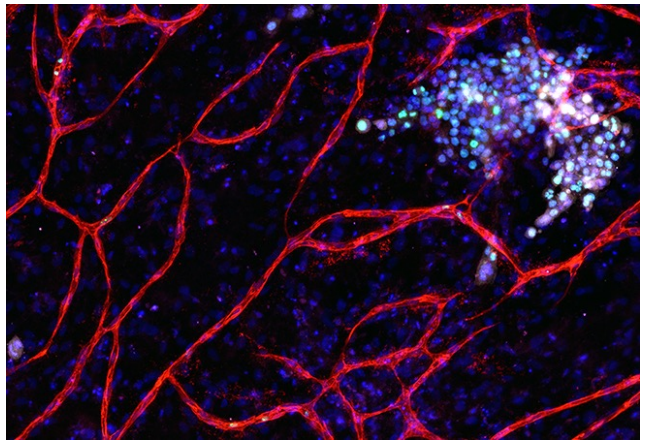




Small intestine



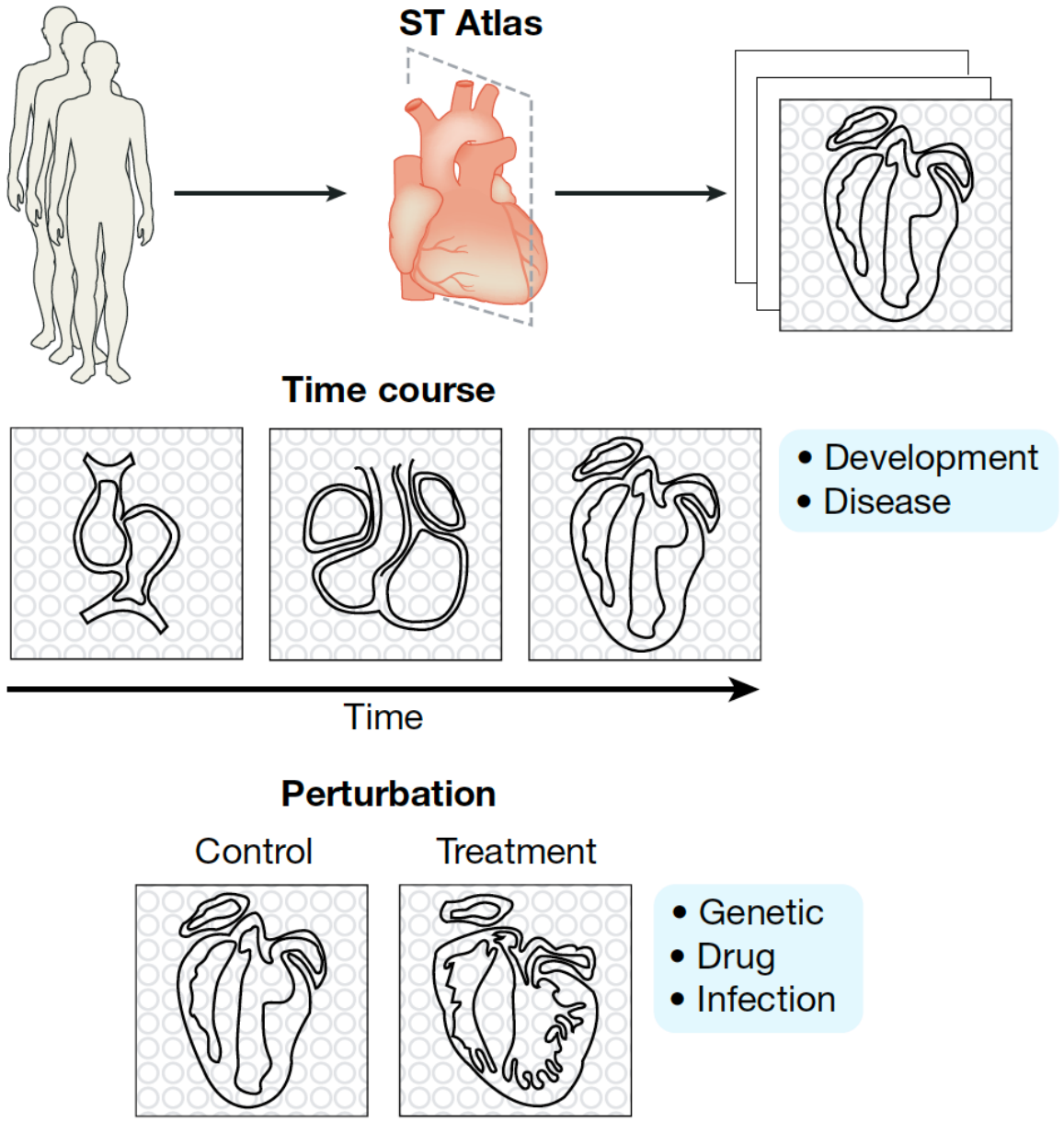
Liver cirrhosis



Breast cancer

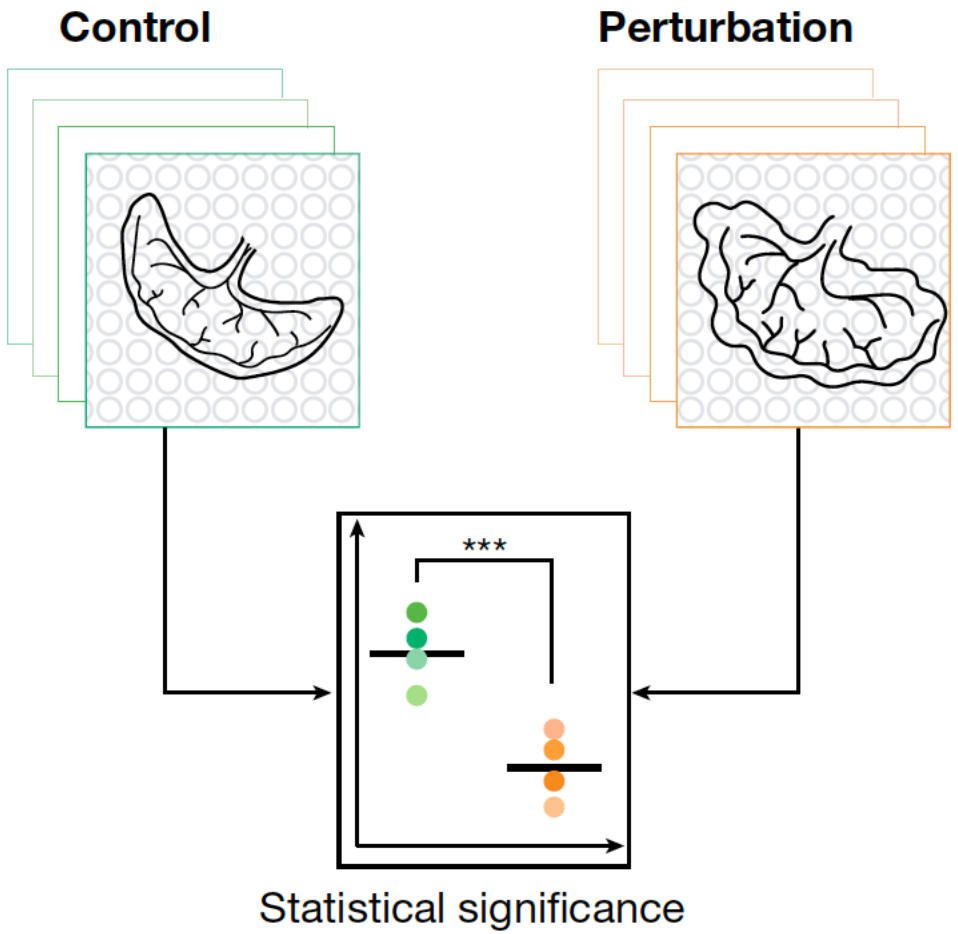
http://www.cell.com/pictureshow/skin
https://library.med.utah.edu/WebPath/webpath.html

Spatial technologies used for hypothesis generation



Rao, A. et al *Nature* (2021).

Spatial technologies used for hypothesis testing

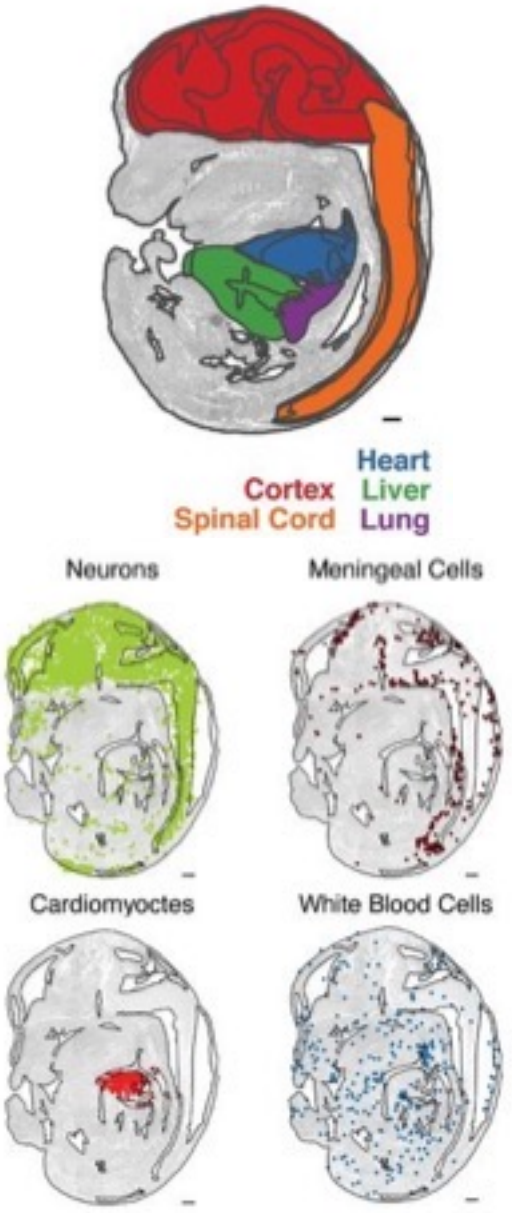


Rao, A. et al *Nature* (2021).

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

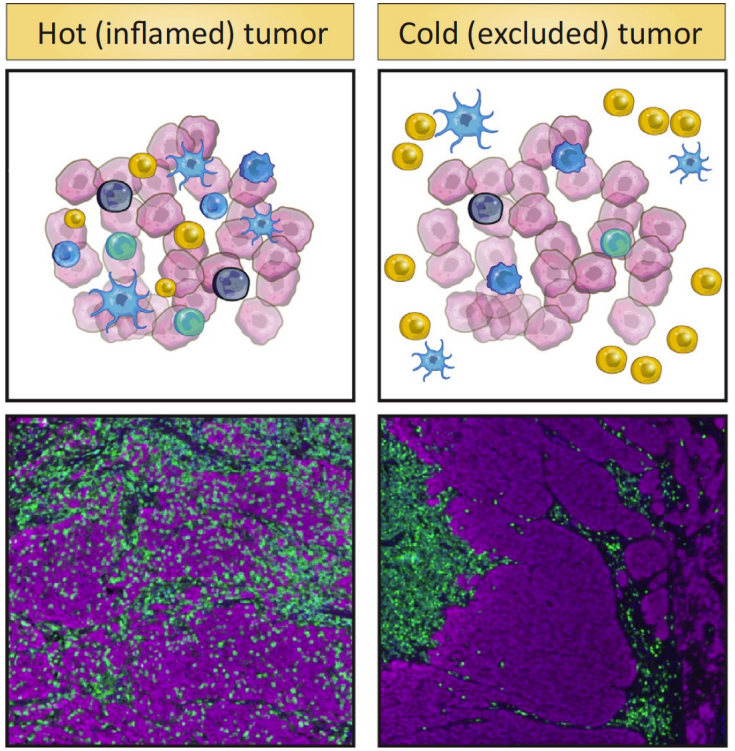
E14.0 mouse embryo



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

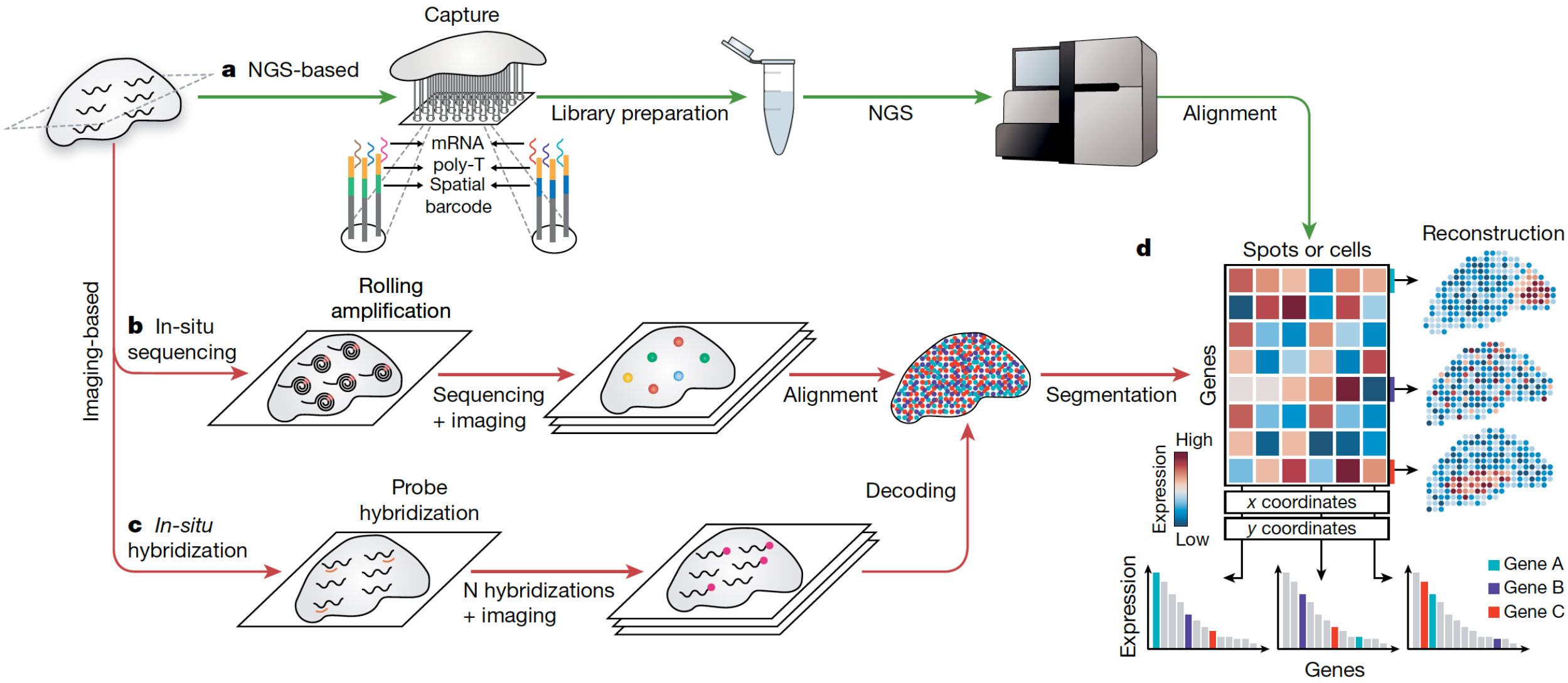
Translational questions

- 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



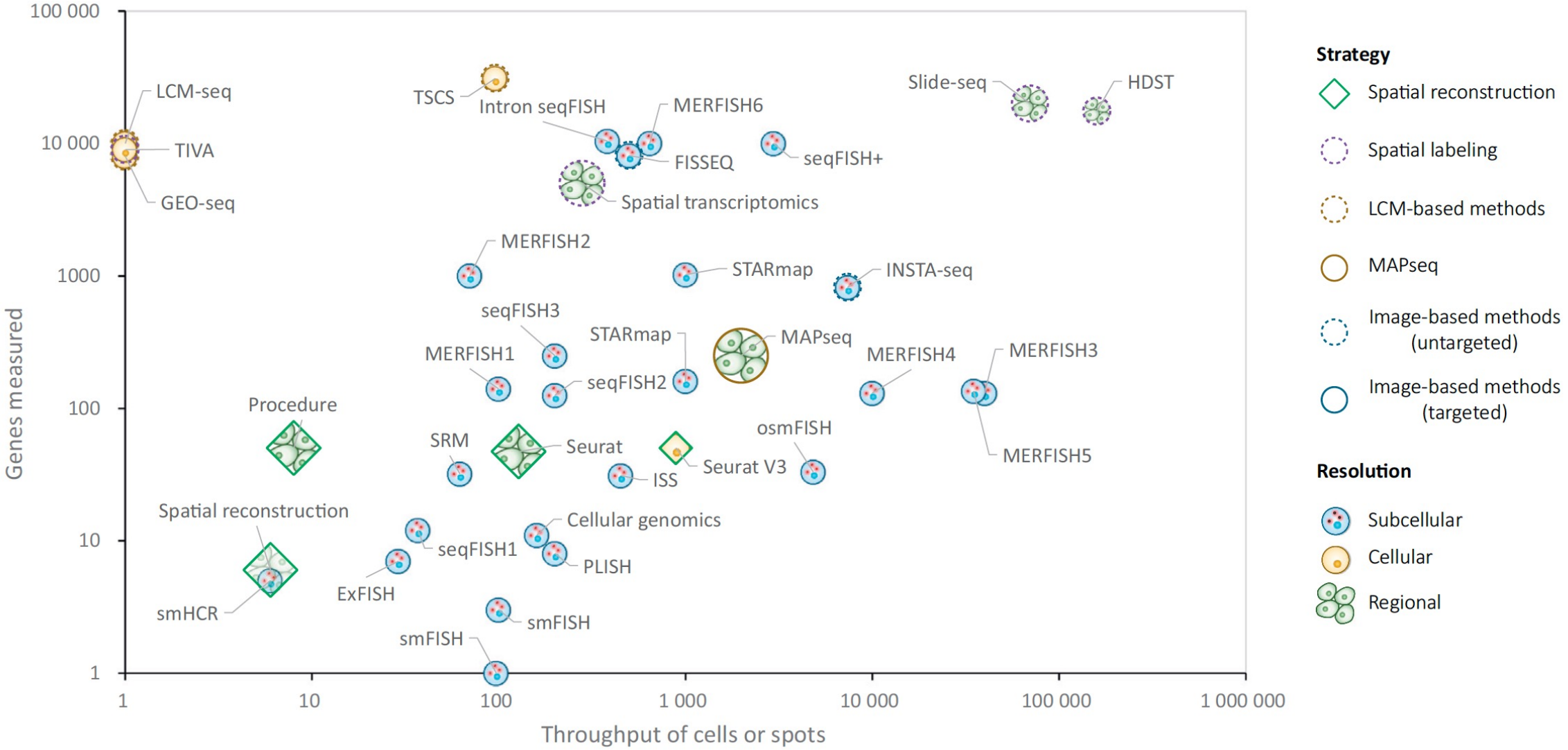
van der Woude, L. L. et al *Trends in Cancer* (2017).

Spatial transcriptomic technologies



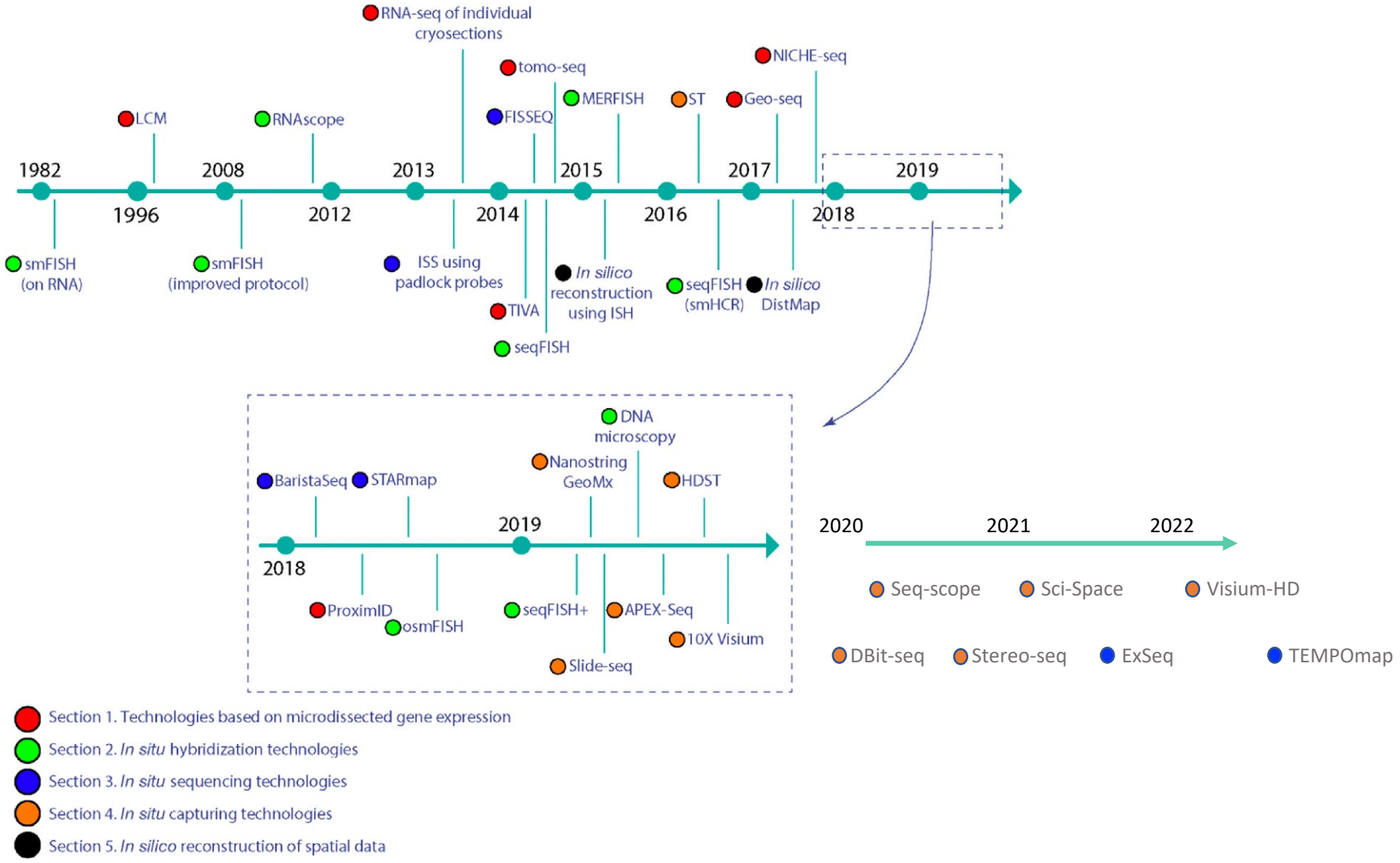
Rao, A. et al *Nature* (2021).

Tradeoffs in gene sensitivity, throughput, and resolution



Liao, J. et al *Trends in Biotechnology* (2021).

Historical timeline of spatial transcriptomics methods



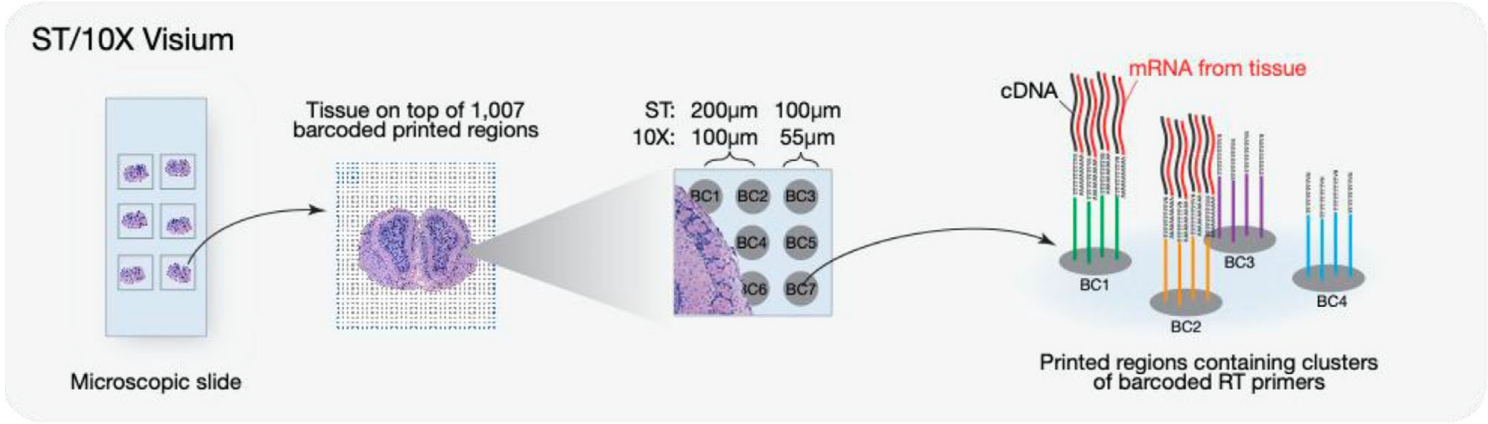
Asp *et al.*, BioEssays 2020

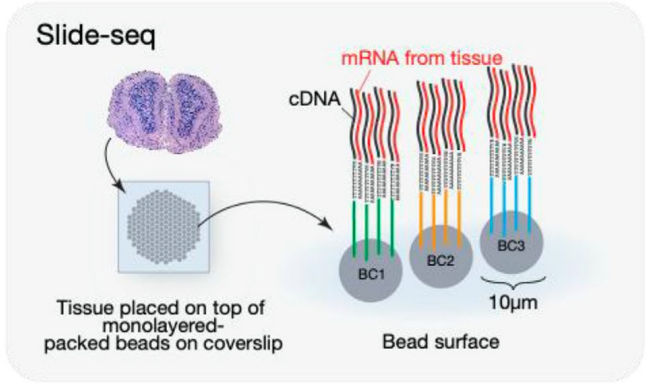
Spatial transcriptomic method categories and examples

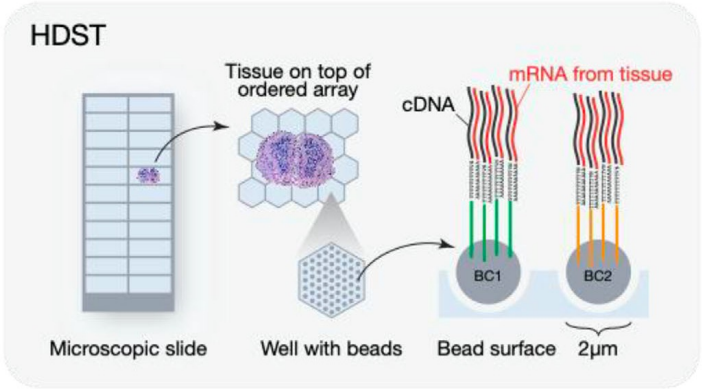
In-situ barcoding

- 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)

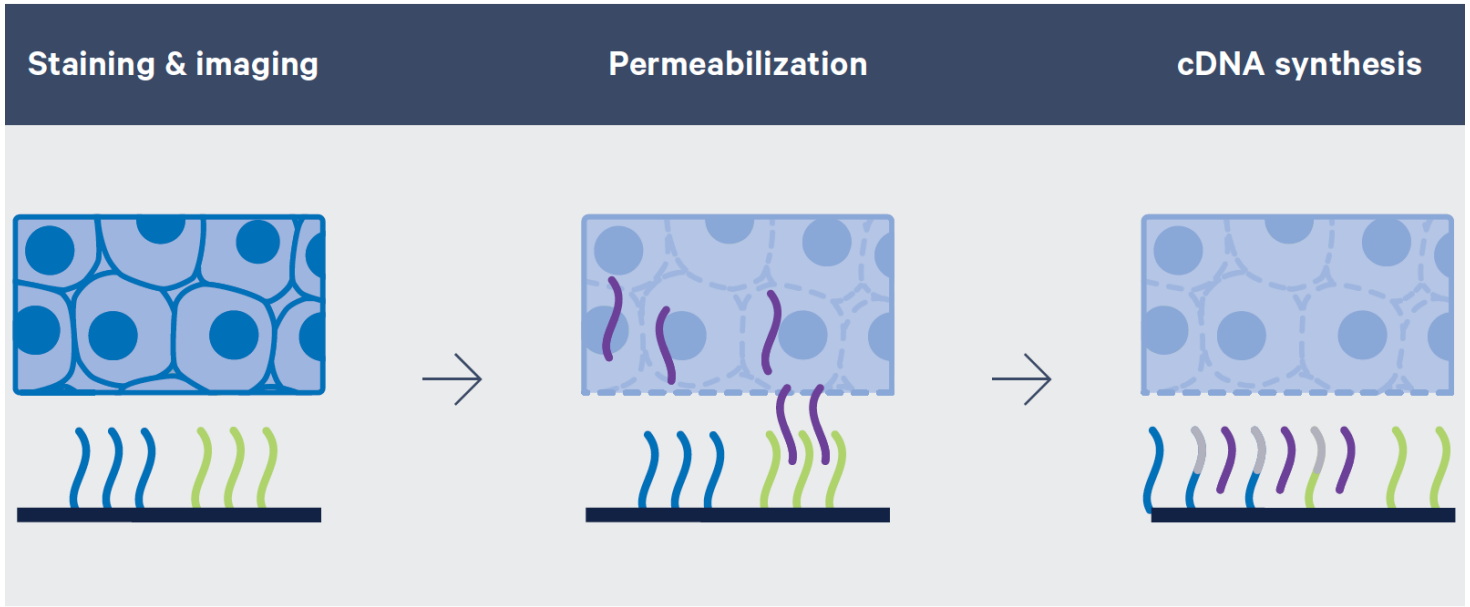


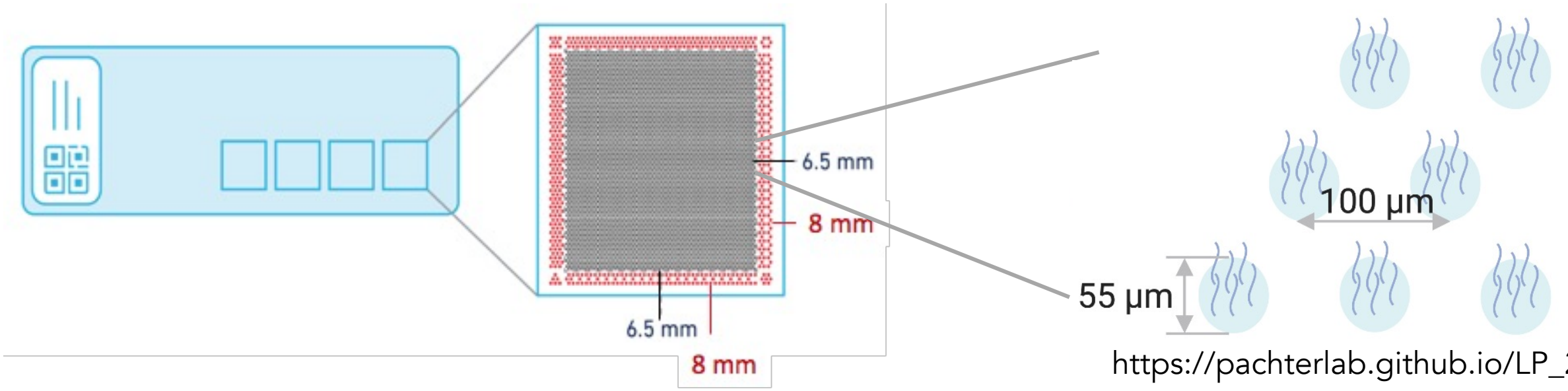




Asp *et al.*, BioEssays 2020

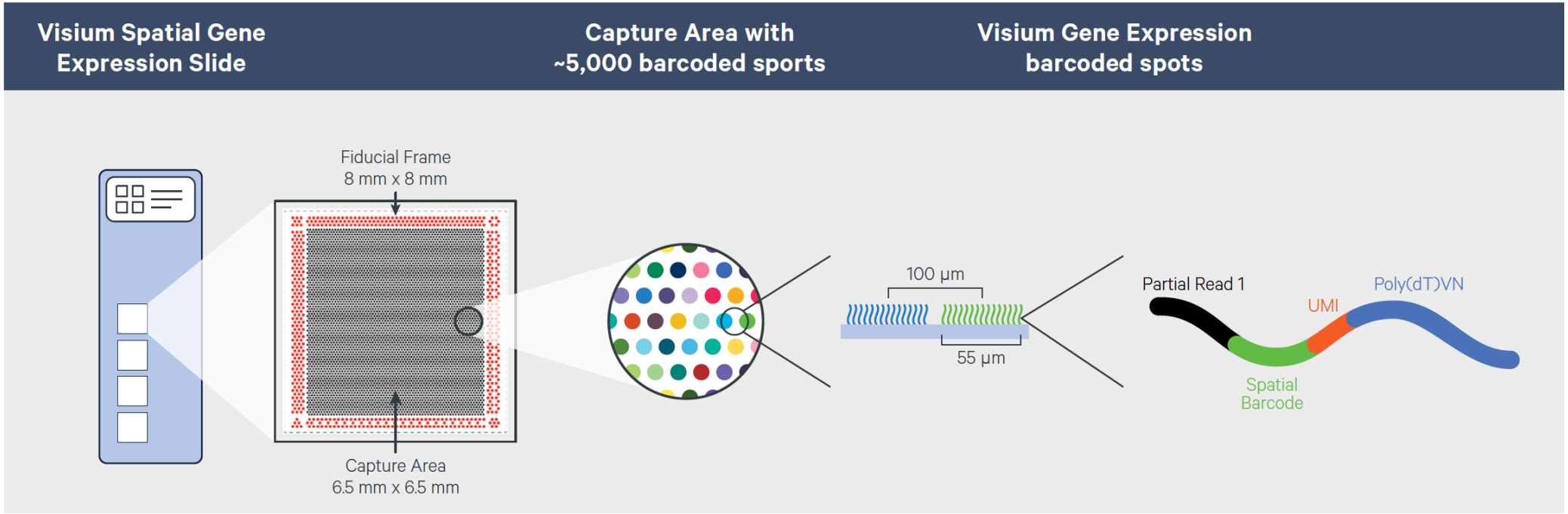
Spatially resolved single-cell transcriptomics: *in situ* barcoding



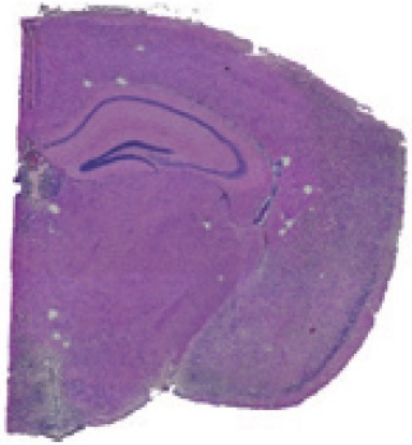


https://pachterlab.github.io/LP_2021/

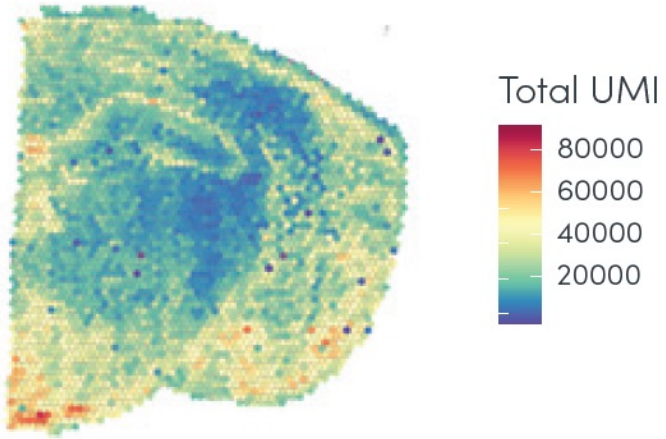
Spatially resolved single-cell transcriptomics: *in situ* barcoding



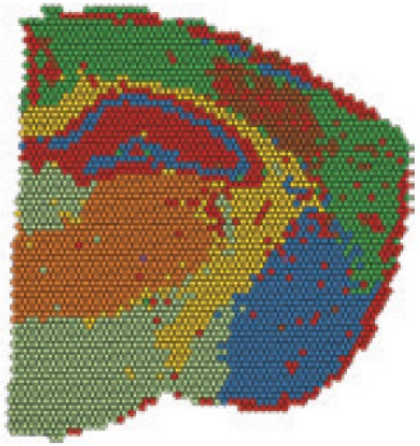
H&E



Visium whole transcriptome



Visium spot clusters

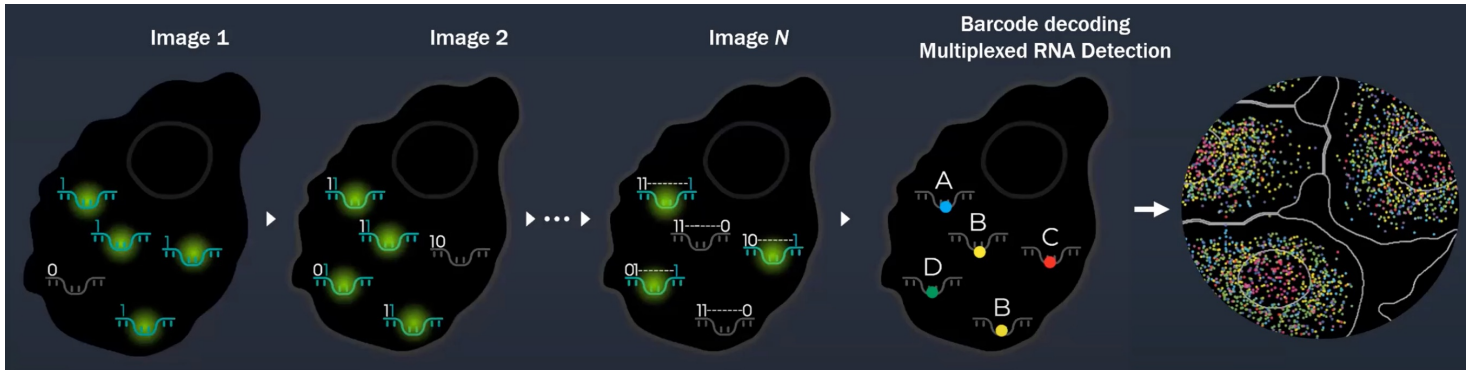


Spatial transcriptomic method categories and examples

Imaging-based methods

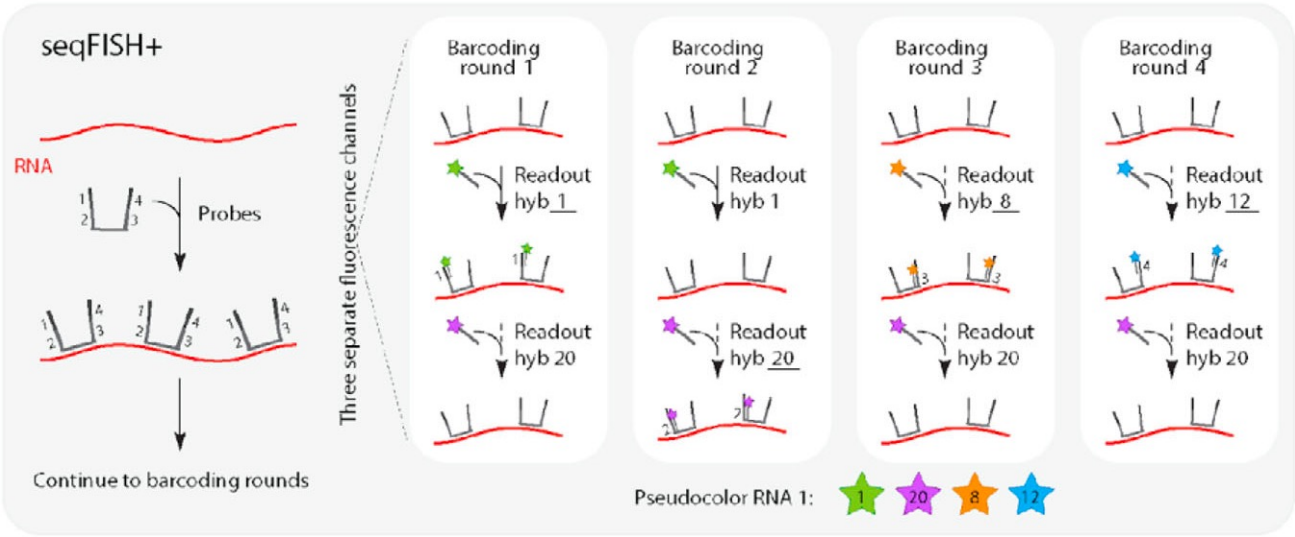
- Fluorescent *in situ* hybridization (FISH)
  - MERFISH/Vizgen (2015, FF)
  - seqFISH+ (2019, FF)
  - SABER-FISH (2019)
  - osmFISH (2018, FF)
- *In situ* sequencing (ISS)
  - FISSEQ/ReadCoor -> 10x
  - Padlock probe based based *in situ* sequencing/Cartana -> 10x
  - STARMap (2018, FF)
- ISS with *ex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

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



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

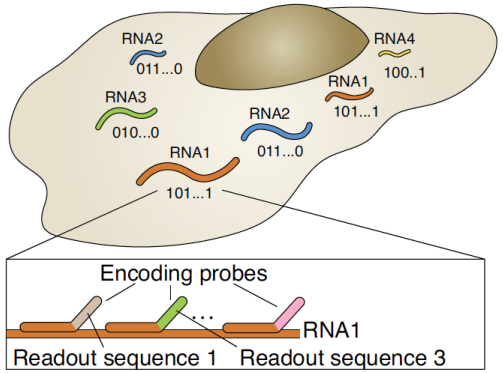
seqFISH+ - subcellular resolution, >10 000 genes



Asp *et al.*, BioEssays 2020

Spatially resolved single-cell transcriptomics by imaging

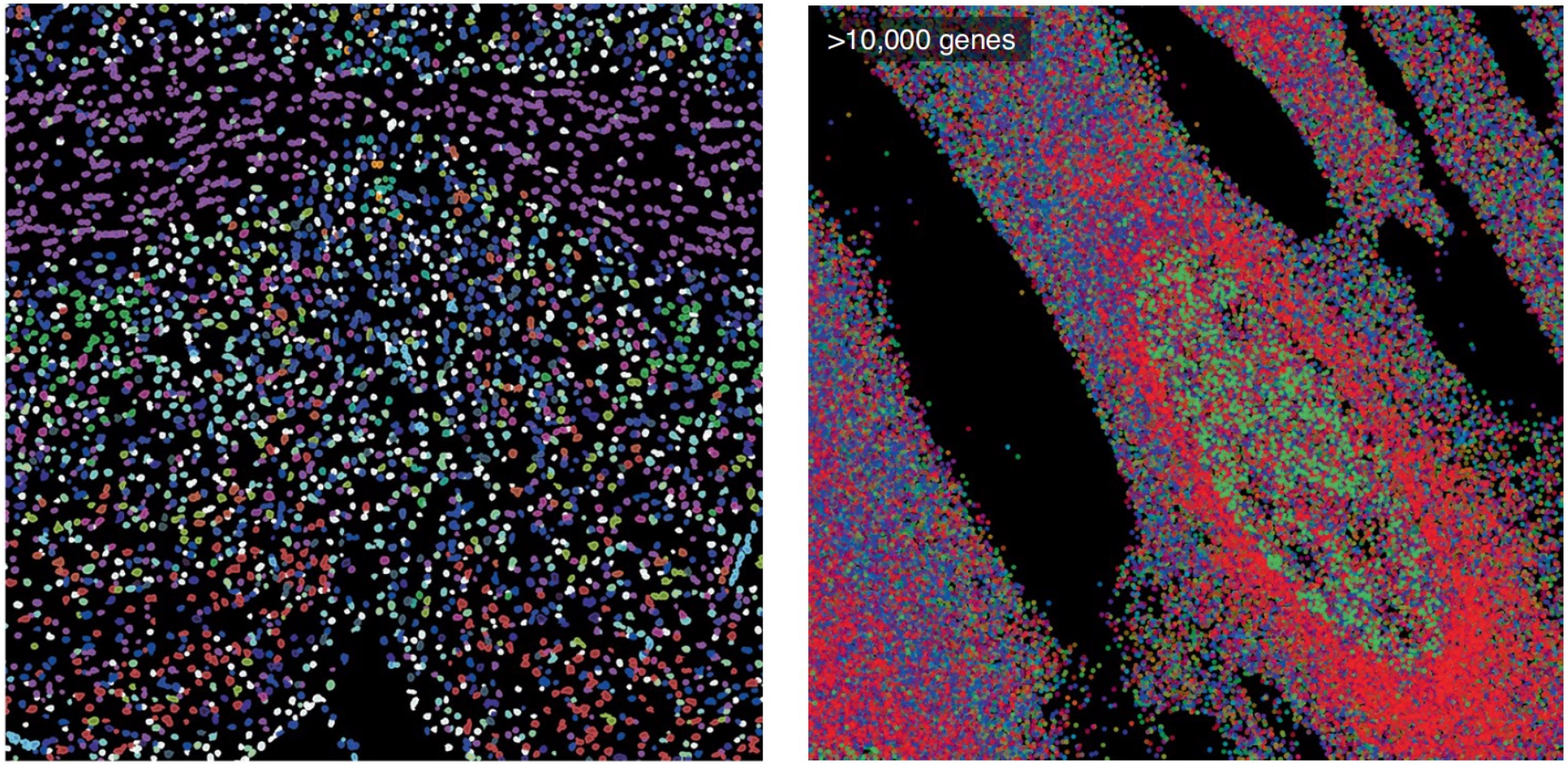
Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH)



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

Spatially resolved single-cell transcriptomics by imaging

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

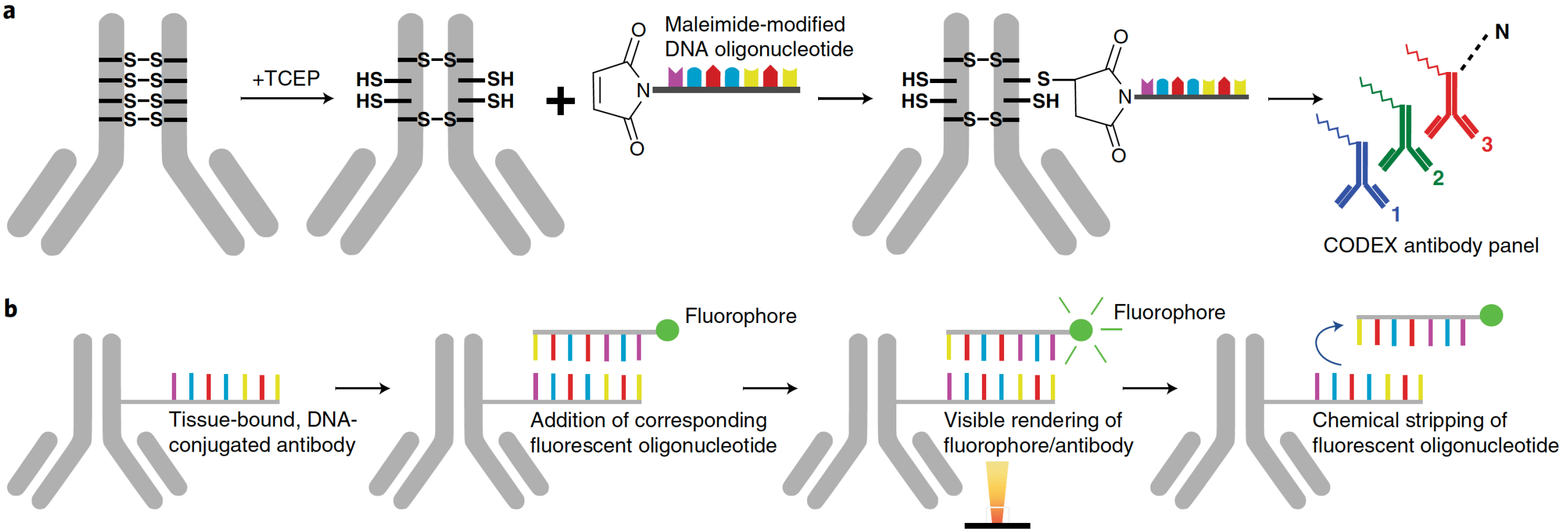


Interact with MERFISH human tumor datasets online:
https://info.vizgen.com/ffpe-showcase

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

Spatial proteomic technologies

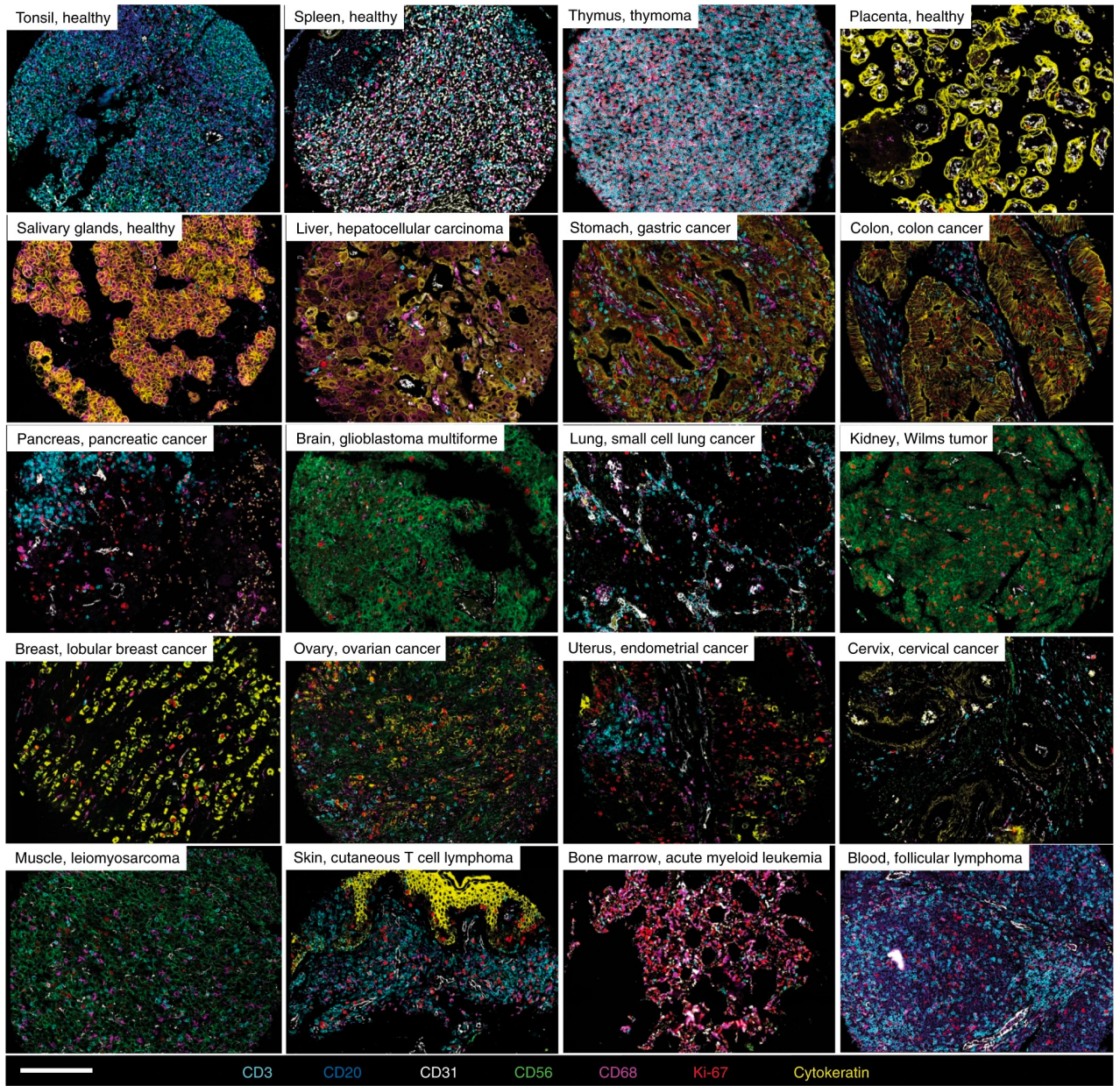
CODEX



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

Spatial proteomic technologies

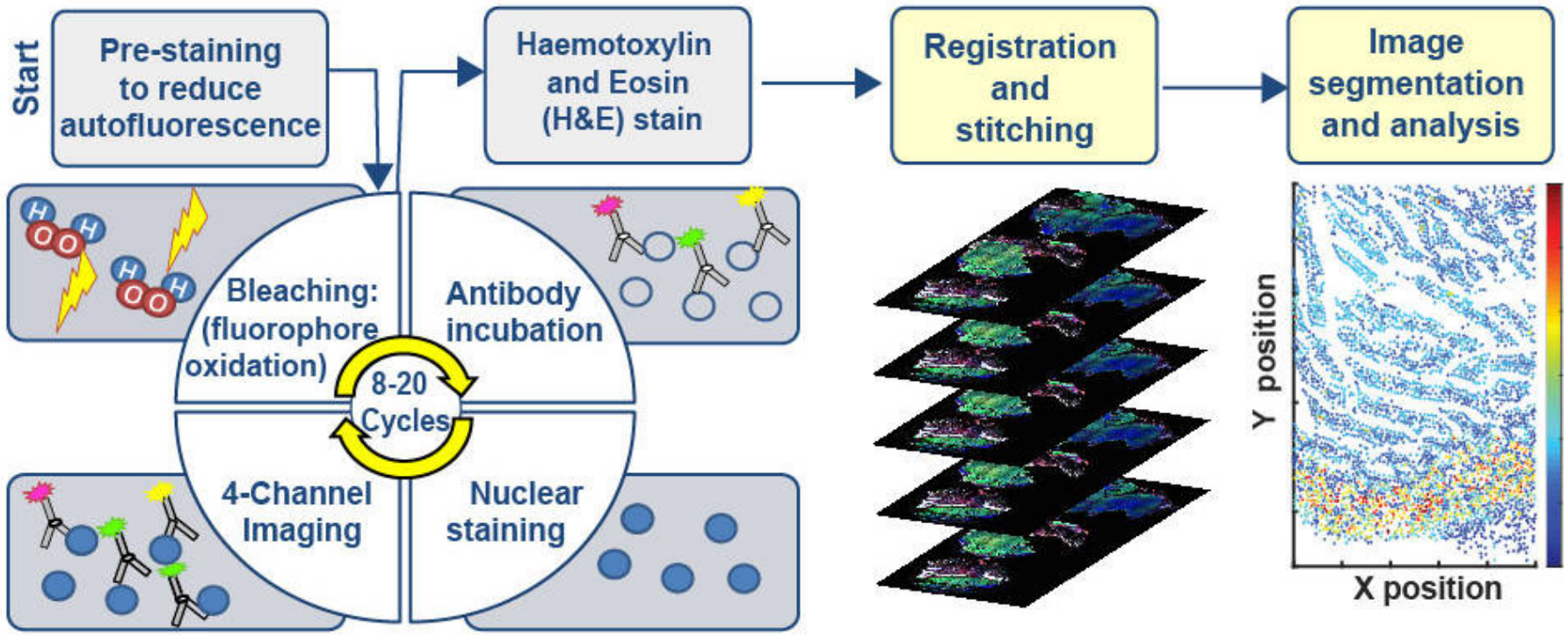
CODEX



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

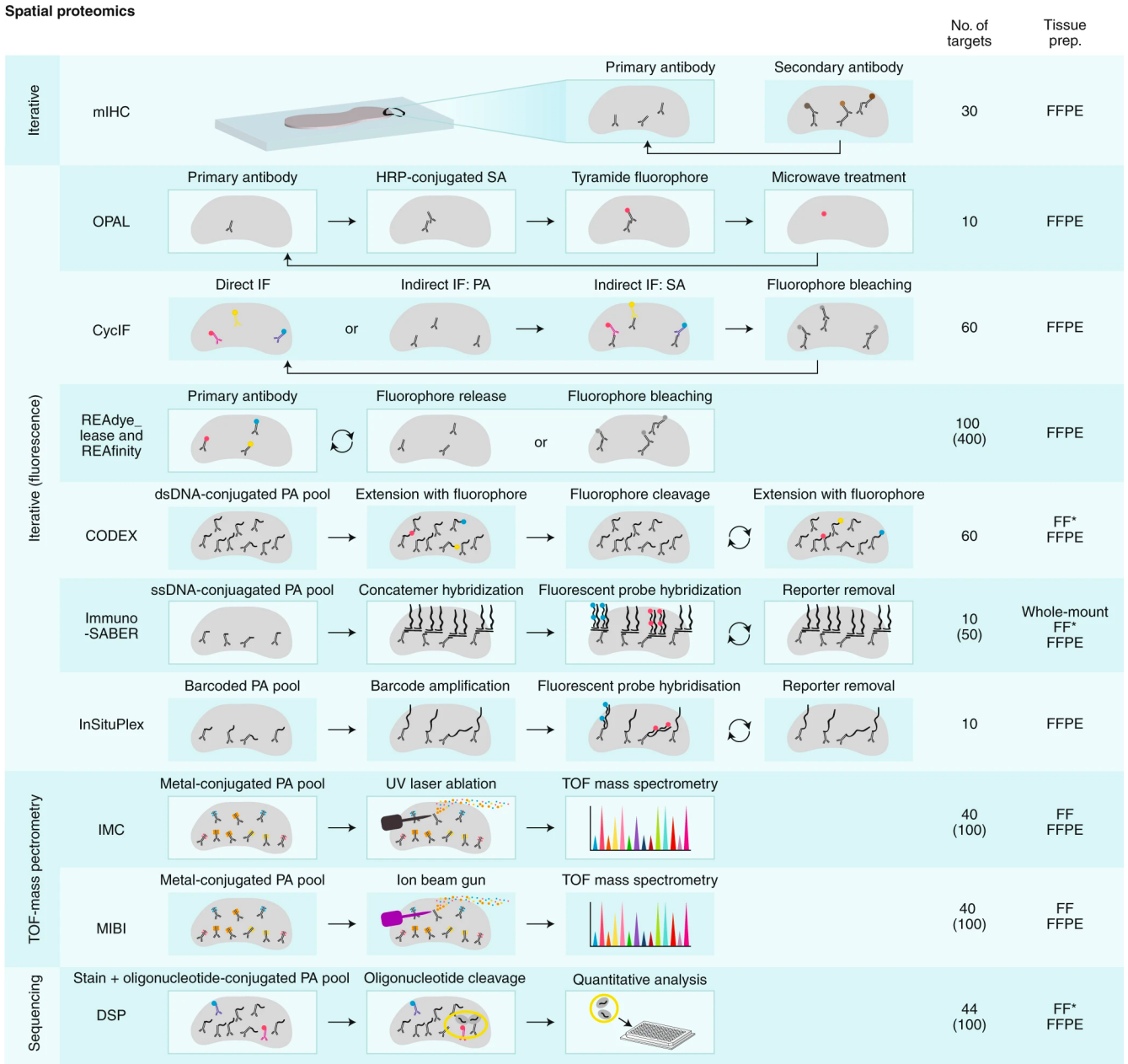
Spatial proteomic technologies: Cyclic immunofluorescence (CycIF)

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



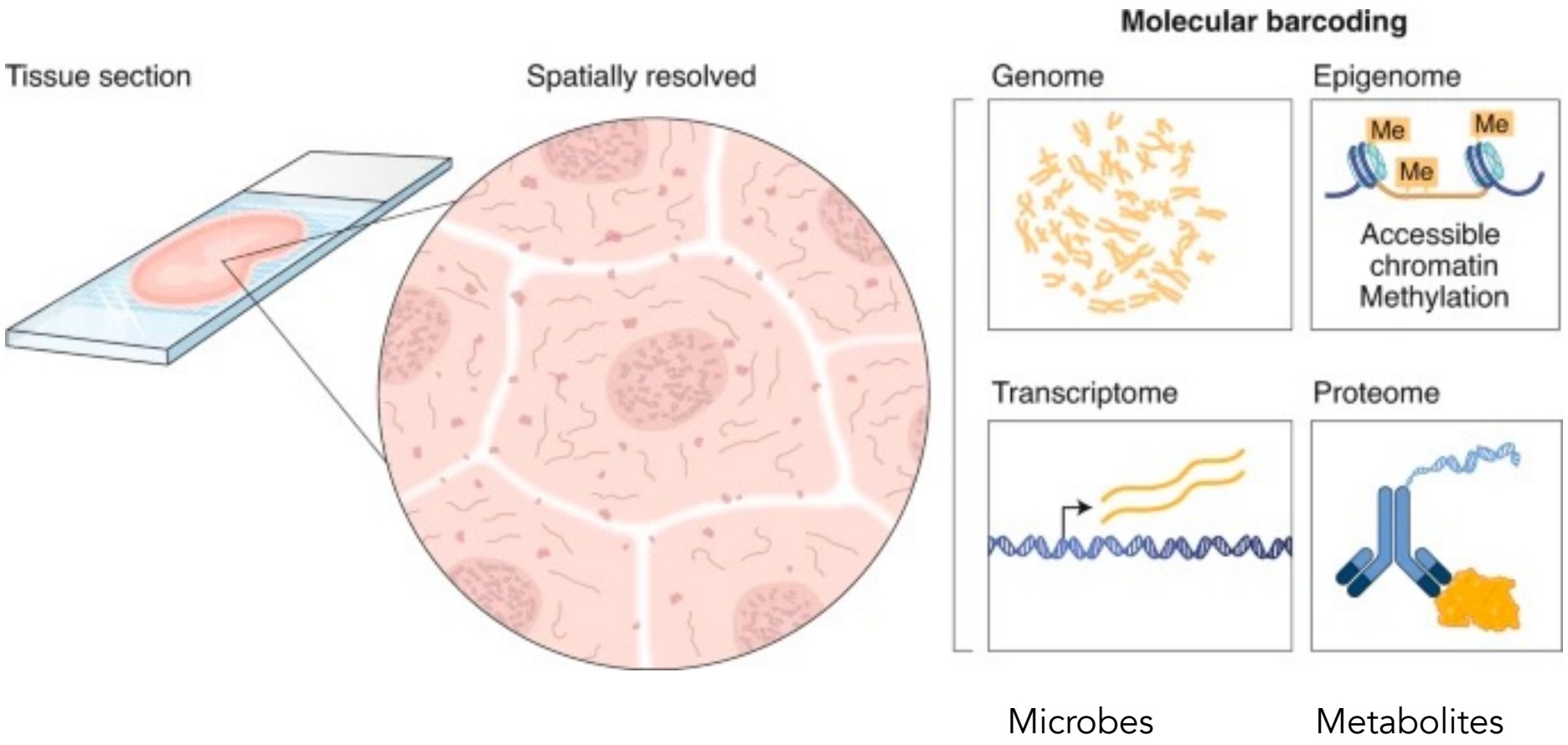
CycIF overview: https://vimeo.com/269904895
Primary lung cancer analysis: https://www.cycif.org/data/du-lin-rashid-nat-protoc-2019/osd-LUNG_3_DATA.html
Primary lung cancer regions: https://www.cycif.org/data/du-lin-rashid-nat-protoc-2019/osd-LUNG_3.html
Metastatic melanoma: https://www.cycif.org/osd-exhibit

Spatial proteomic technologies



Lewis, S. M. et al *Nature Methods* (2021).

Towards multimodal spatial measurements



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

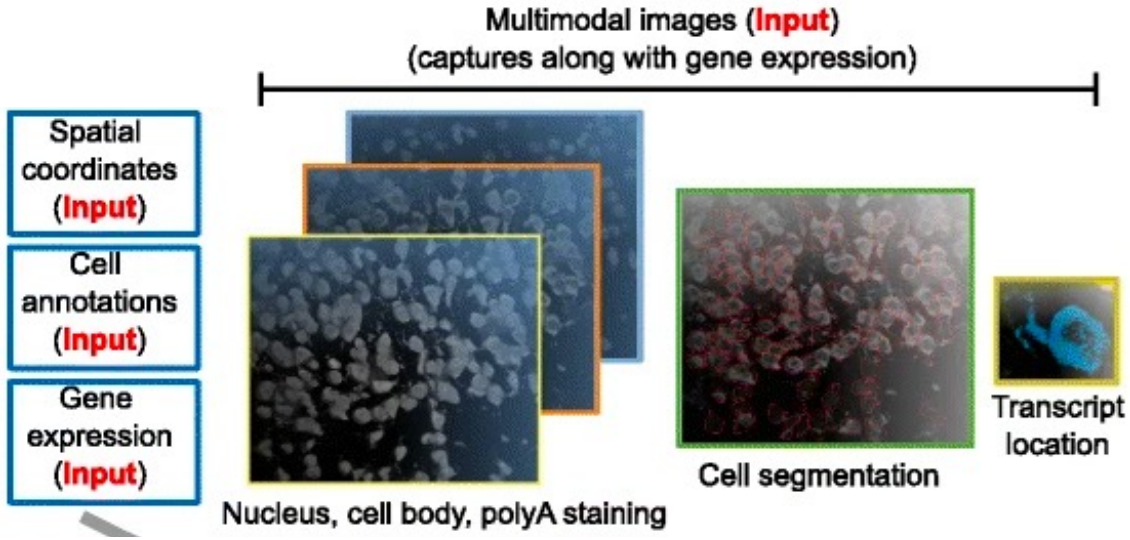
- What samples
  - sufficient power to sample relevant tissue heterogeneity (e.g. cell type composition, cell-cell interactions, transcriptional programs)
  - # biological conditions, # individuals, # sections / individual, # FOVs / individual, FOV size
  - intra- (multiple sections / individual) vs inter- (multiple individuals) individual variability

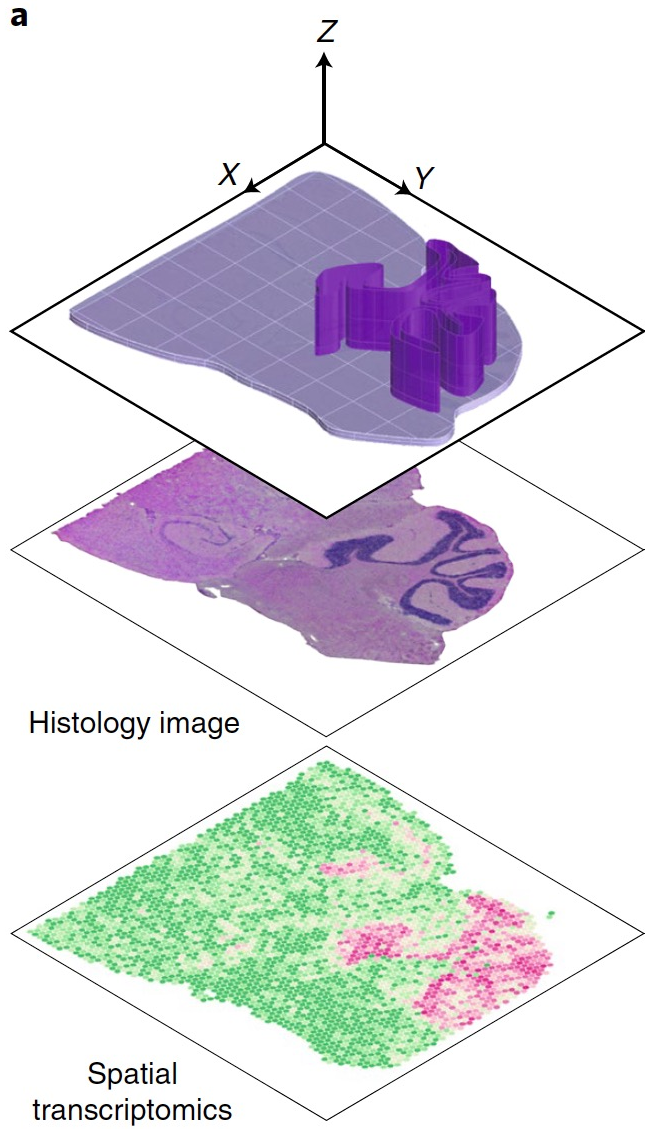
- Design
  - ≥3 samples per biological condition
  - process biological conditions in parallel to minimize batch effects
  - multiple samples from same individual (e.g. time course, pre- vs post- treatment) reduce variability issues

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

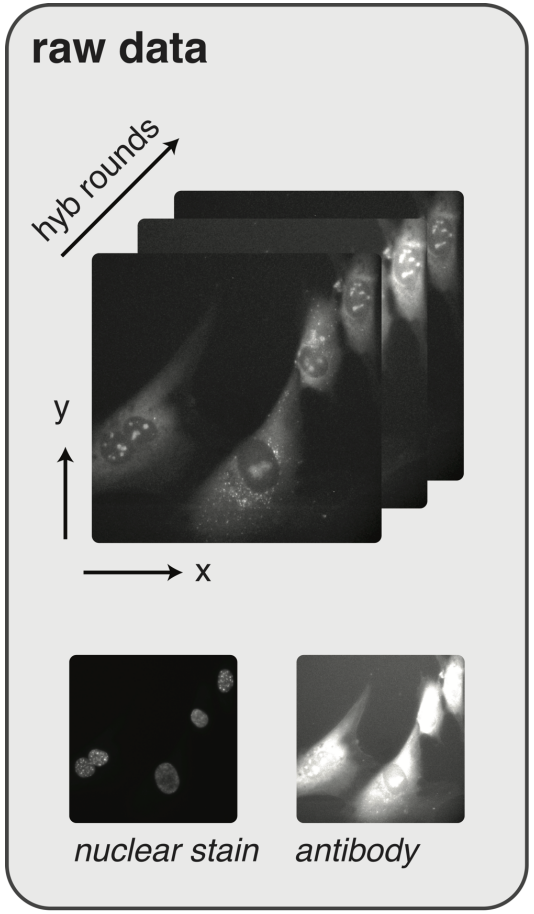


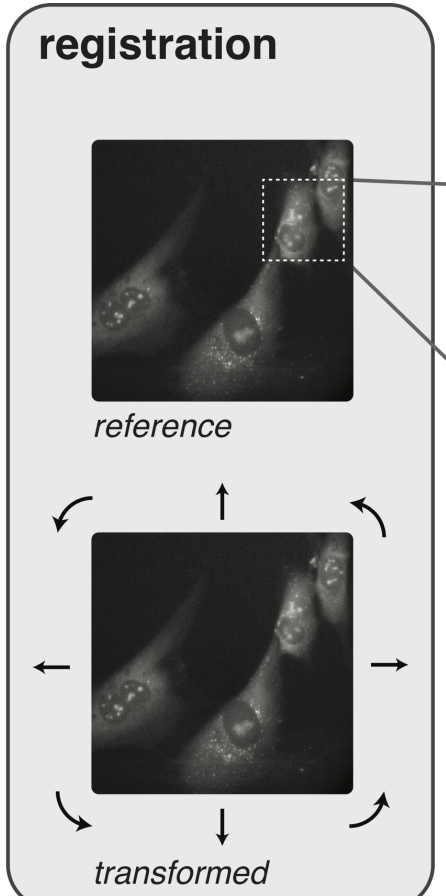


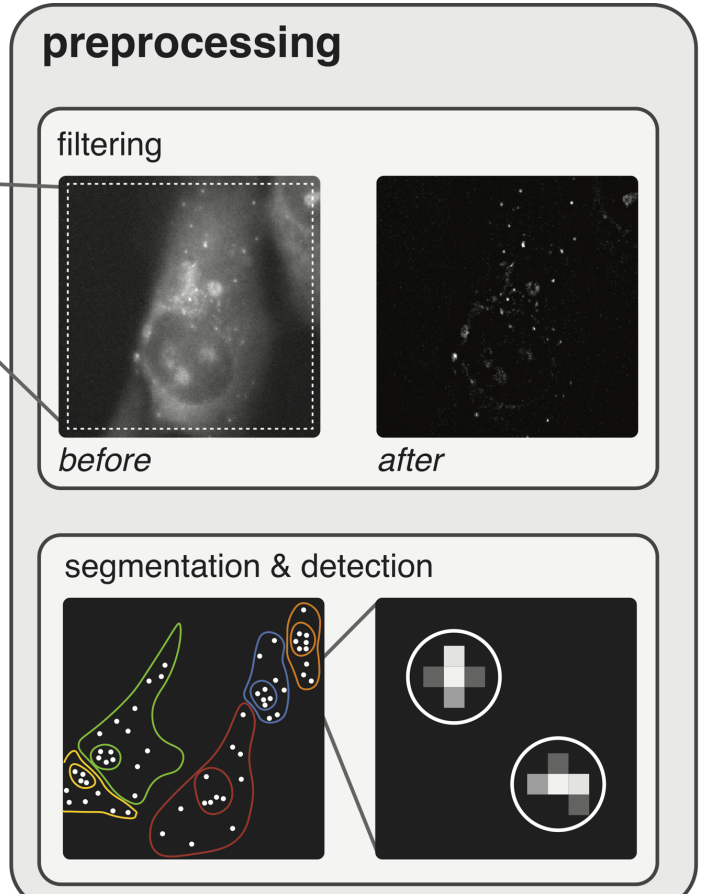
Hu, J. et al *Nature Methods* (2021).
Dries, R. et al *Genome Biology* (2021).

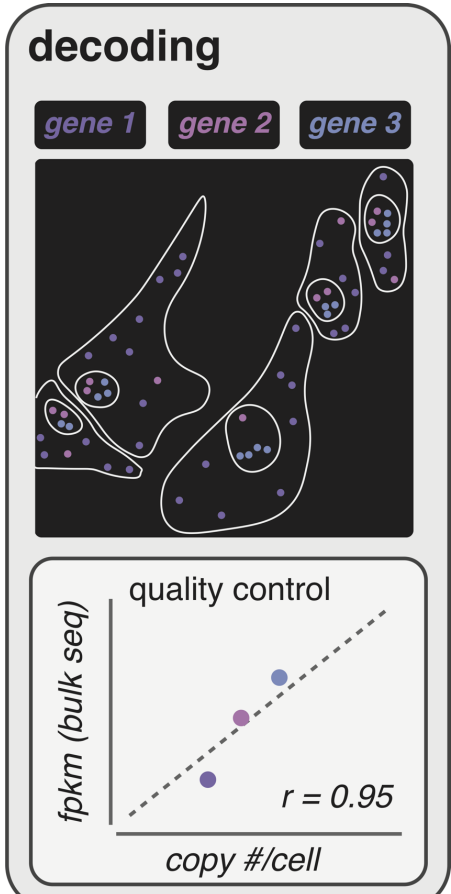
Imaging data has complex preprocessing

starfish
Imaging based
transcriptomics










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

codebook  schemas
hyb | bit position
gene | bit-string
```

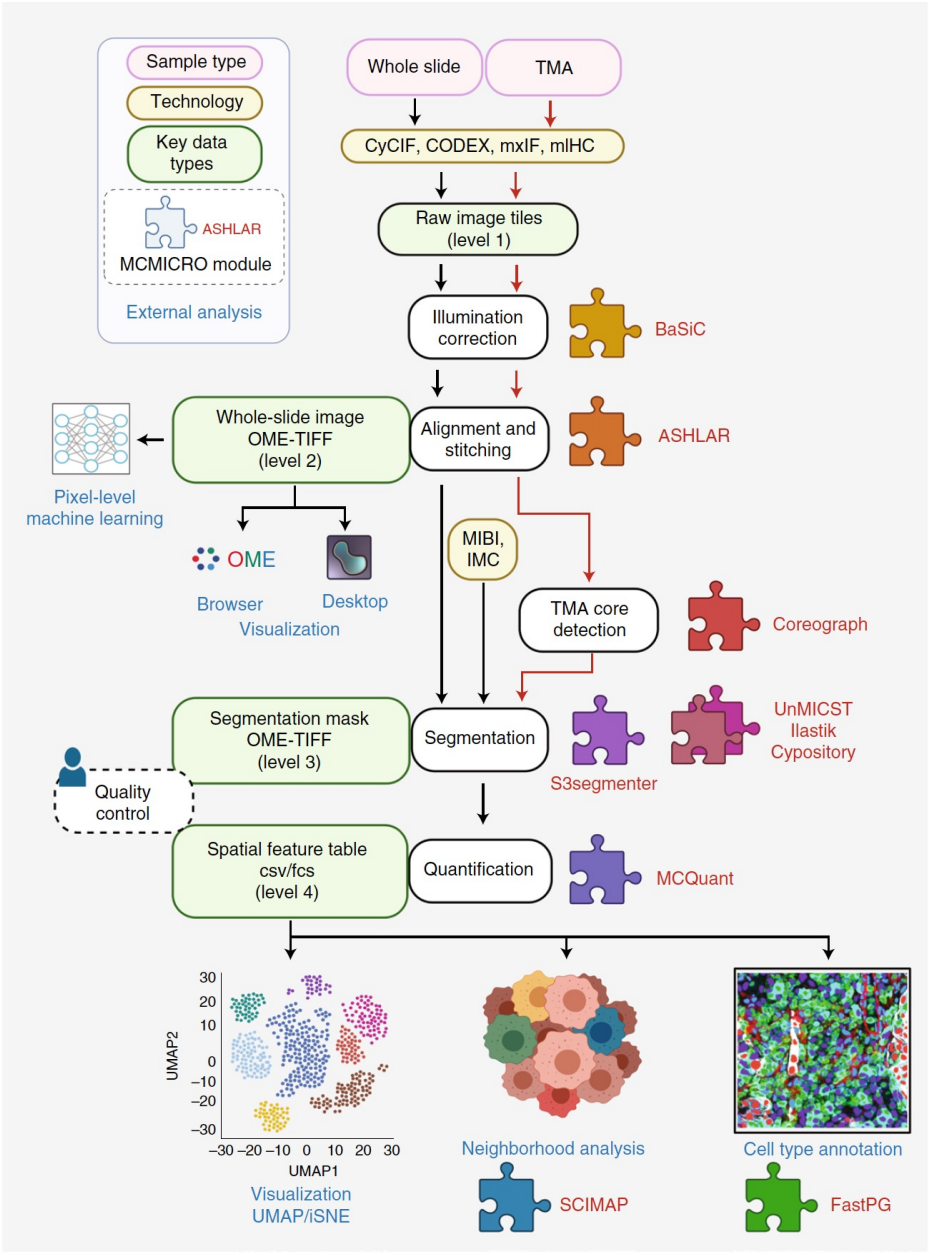
```
per field of view
reg_stack.tif
affine_transform.mat

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

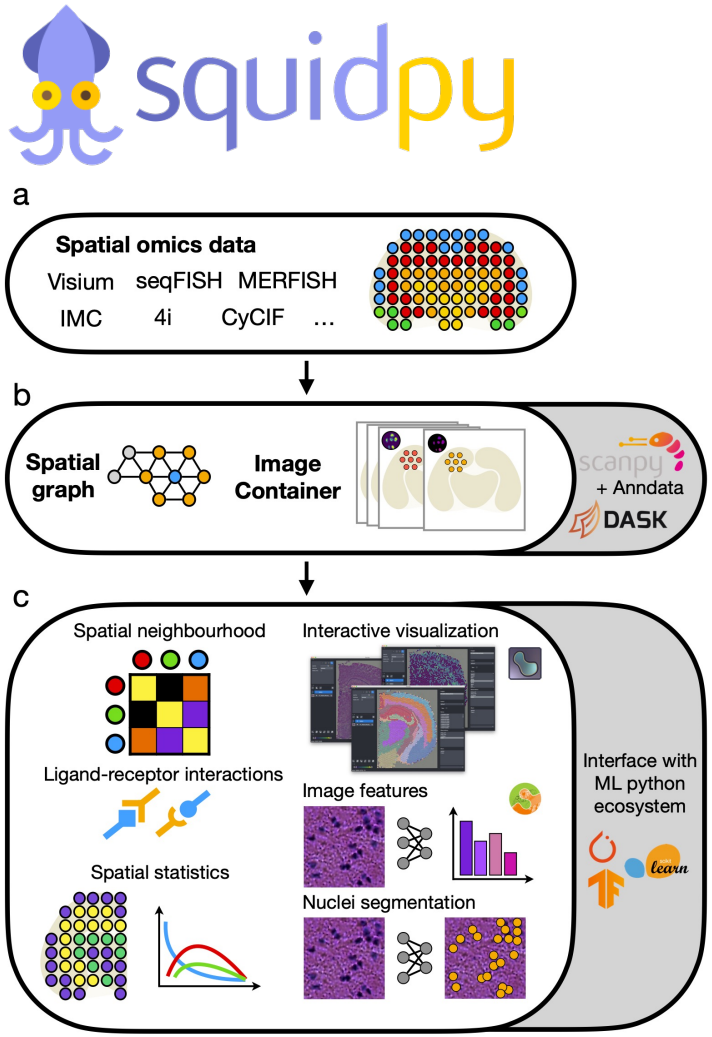
MCMICRO
Multiplex protein imaging



Schapiro, D. et al *Nature Methods* (2021).

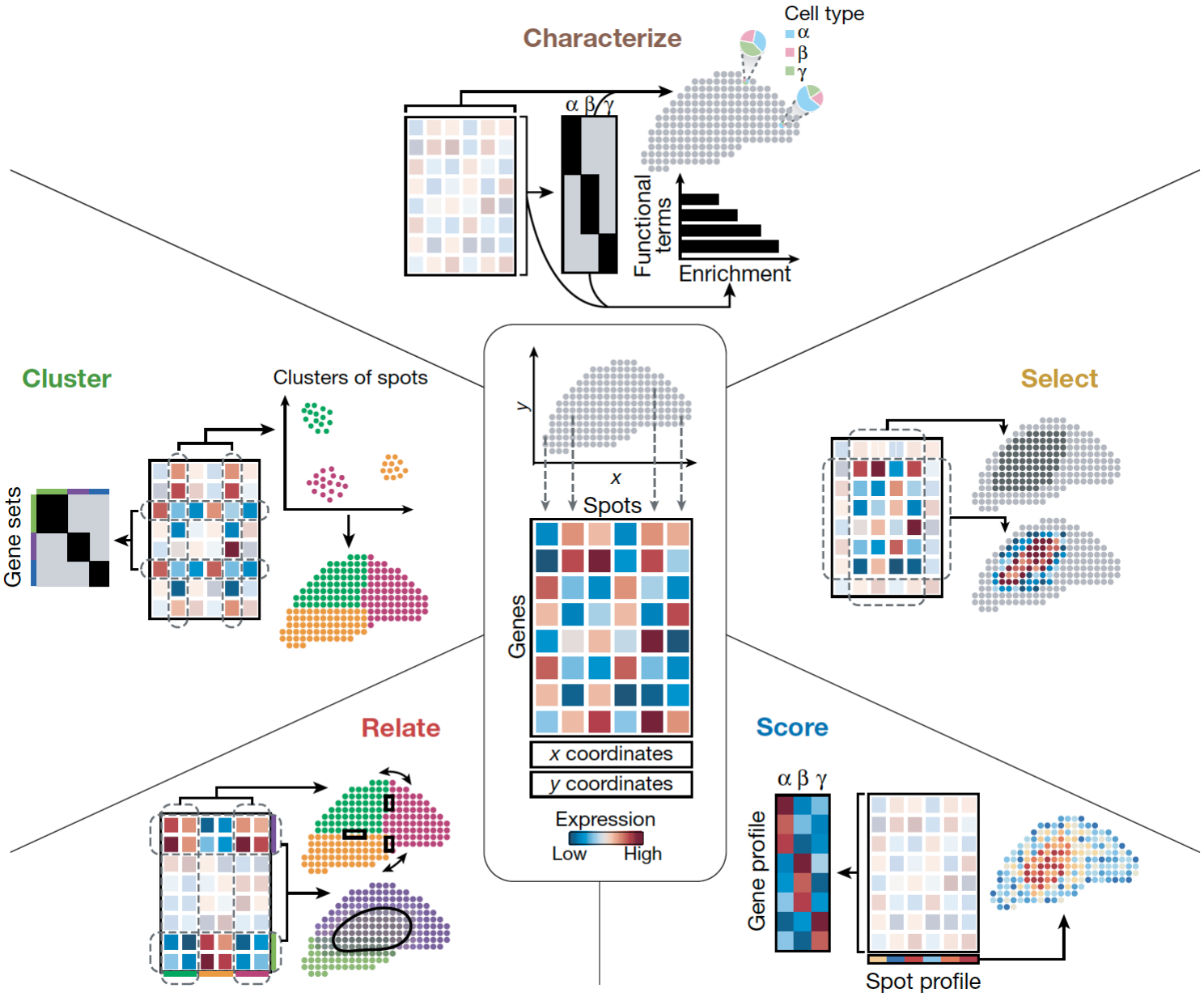
Data analysis frameworks

- Spatial transcriptomics
  - Seurat
  - Giotto
  - STUtility
  - SPATA
  - Meringue
  - …
  - Scanpy
  - Squidpy
  - Starfish
  - stLearn
  - …
- Spatial proteomics
  - MCMICRO
  - Steinbock
  - …



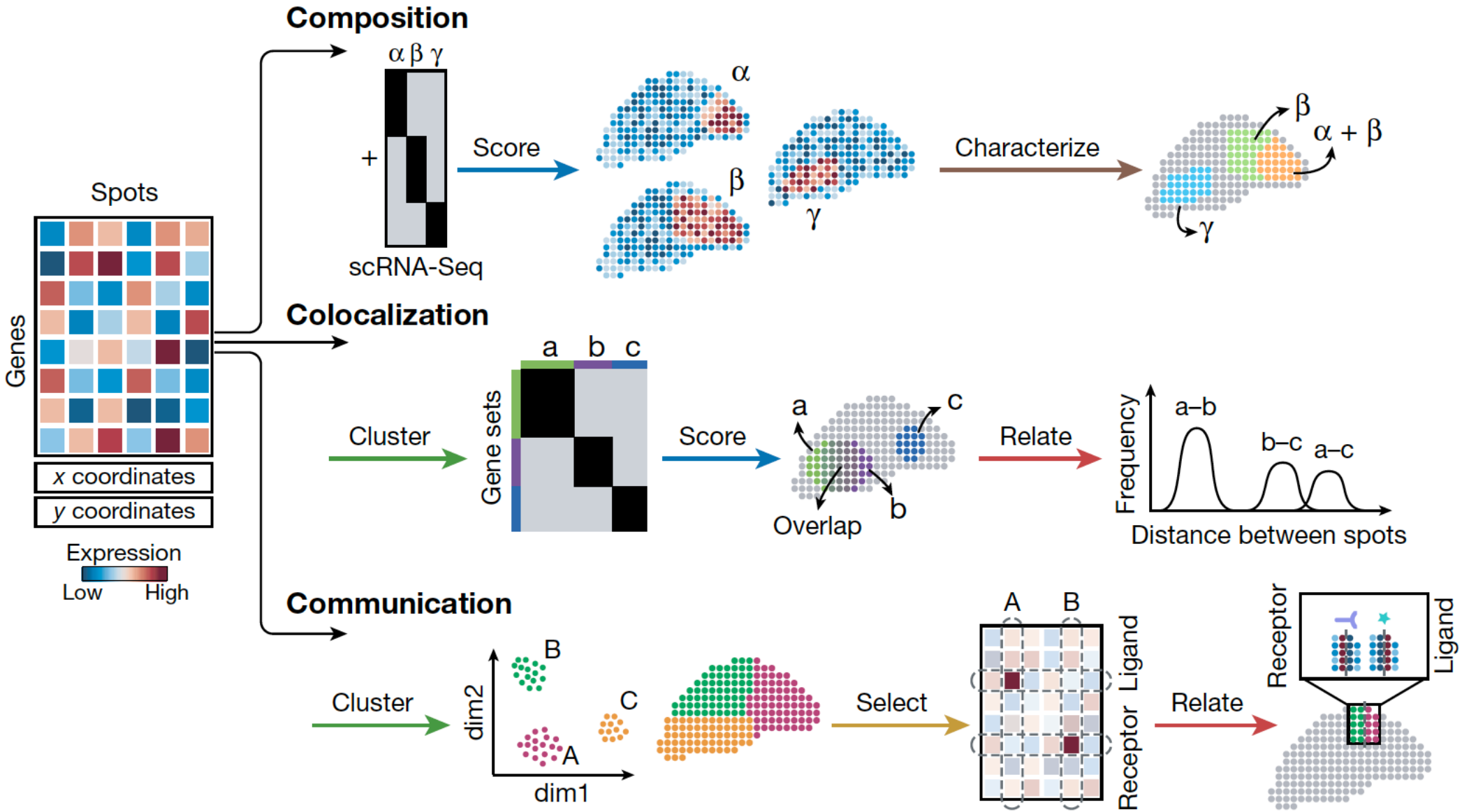
- Data visualization and exploration
  - Napari
  - OMERO
  - TissUUmaps
  - Minerva
  - Loupe Browser
  - …

Identifying cell subsets



Rao, A. et al *Nature* (2021).

Identifying higher-order tissue features



Rao, A. et al *Nature* (2021).

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