



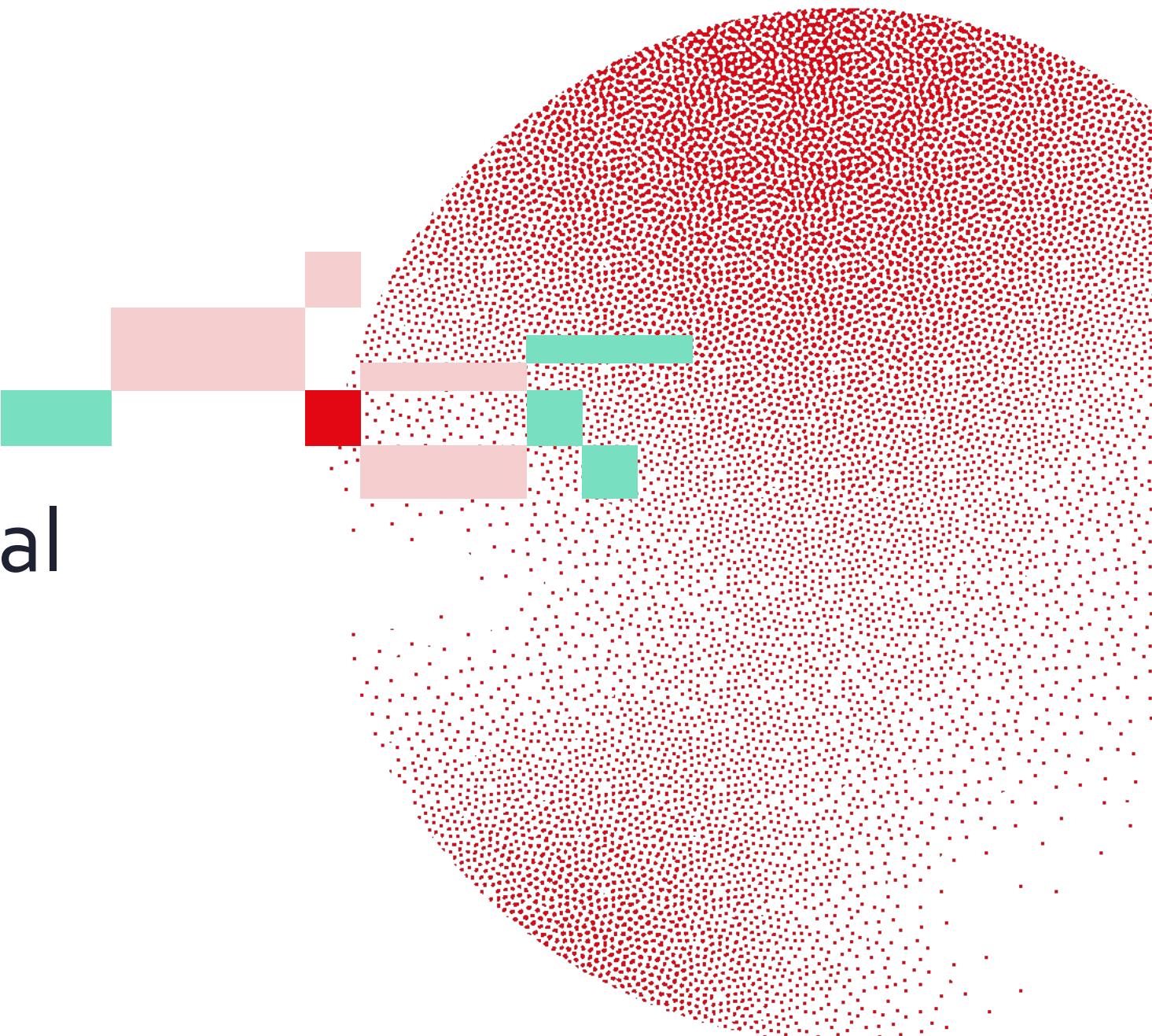
Swiss Institute of  
Bioinformatics

SIB DAYS TUTORIAL

# Introduction to spatial transcriptomics

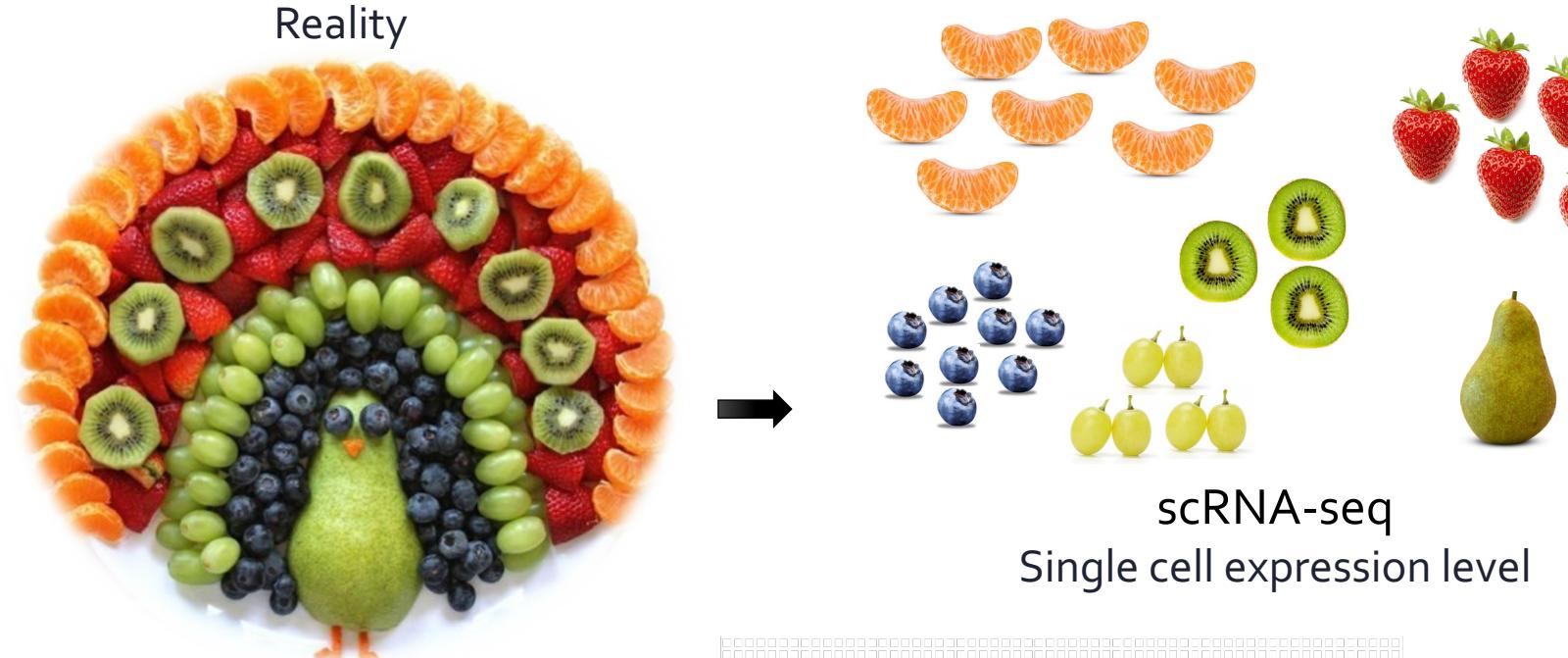
Geert van Geest

24.06.2024 SIB days



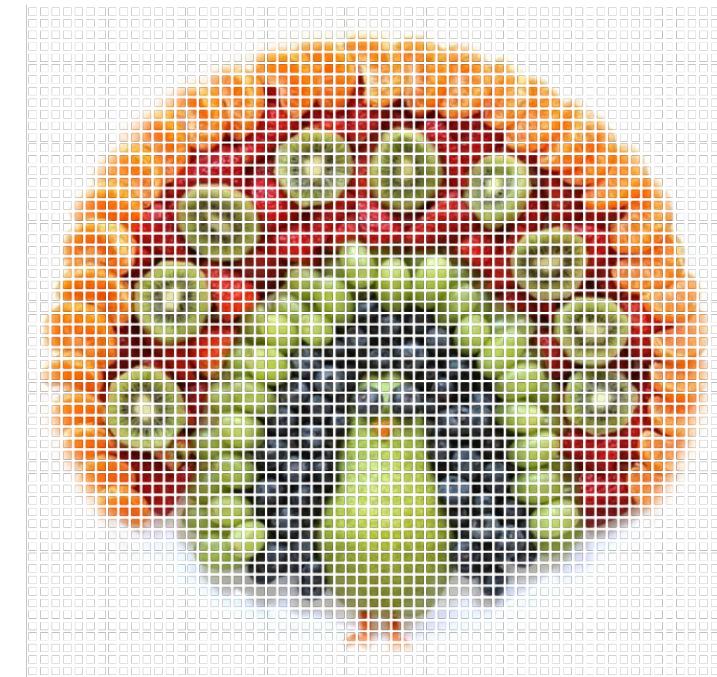


RNA-seq  
Average expression level



scRNA-seq  
Single cell expression level

Different resolutions



# Why spatial transcriptomics?

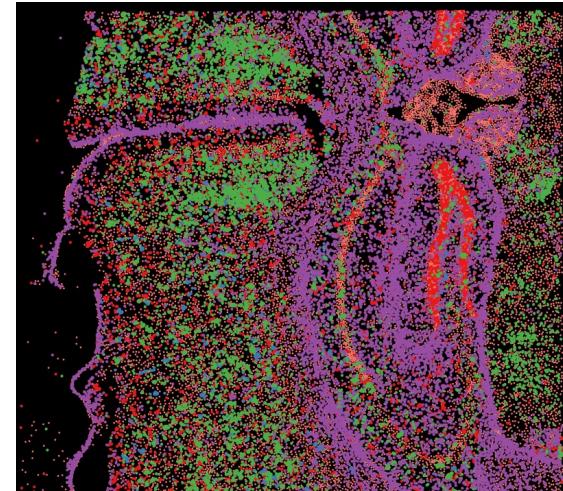
- » **Cells are diverse in shape and size**
  - » Single cell not always possible
  - » How cells interact matters
  
- » **Location, location, location:**
  - » Cell-cell interaction depends on proximity
  - » RNA molecules move



# Methods

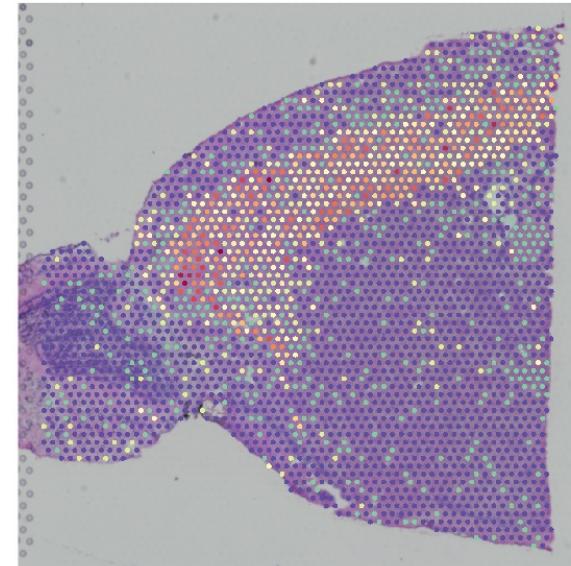
- » **Image based:**

- » Nanostring CosMx
- » vizgen MERFISH
- » 10X Xenium (in situ sequencing)



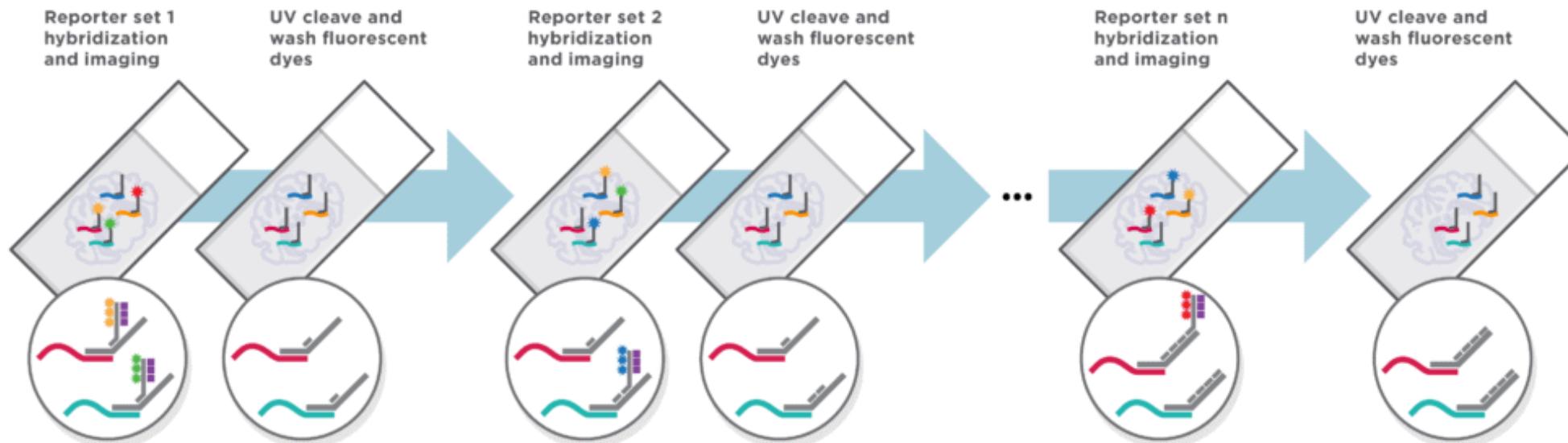
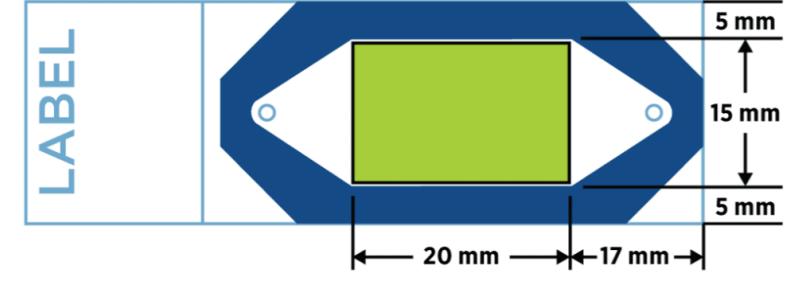
- » **Spot based:**

- » Curio seeker (SlideSeq2)
- » Nanostring GeoMx ('microdissection')
- » 10X Visium



# Nanostring CosMx

- » Single molecule FISH (smFISH)
- » FFPE and FF
- » Combinatorial hybridization
- » Up to 6000 genes



# vizgen MERFISH

- » Single molecule FISH (smFISH)
- » Combinatorial labelling
- » Microscopy imaging
- » Commercial panel: 1000 genes
- » Fresh frozen

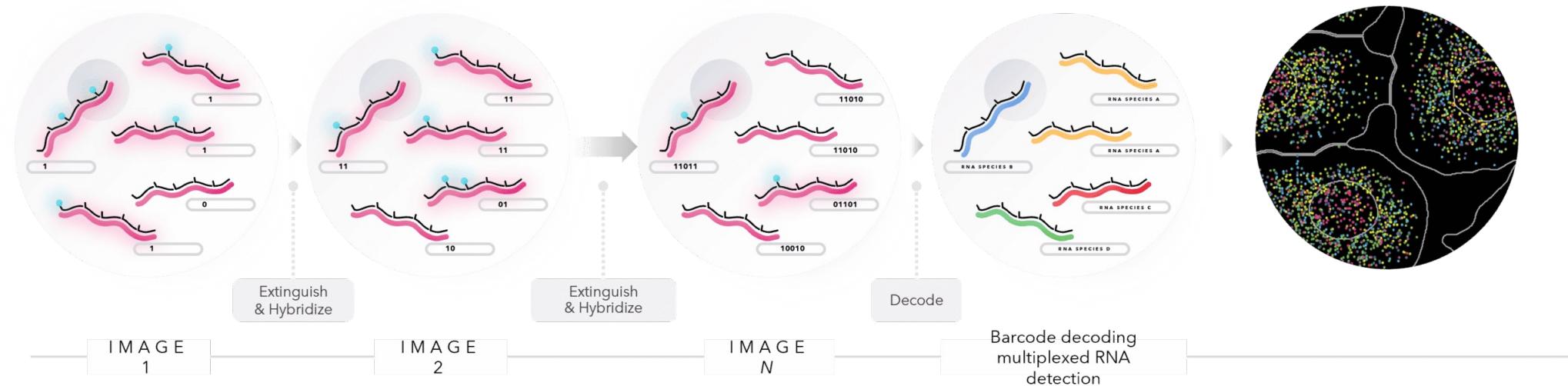
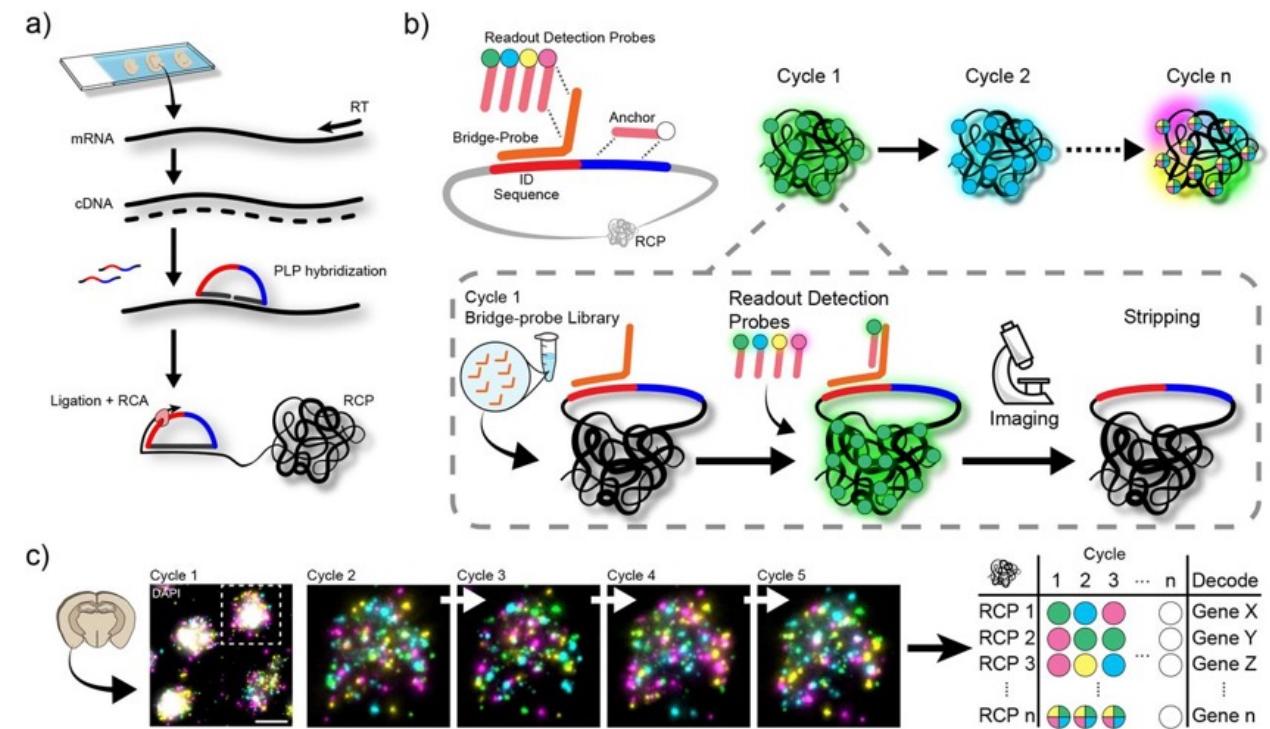


image: <https://vizgen.com/technology/>

# 10X Xenium and in situ sequencing

- » in-situ reverse transcription and rolling circle amplification (RCA)
- » Detection by several rounds of combinatorial probe hybridization
- » Up to ~5000 genes



<https://www.scilifelab.se/units/in-situ-sequencing/>

Gyllborg et al, Nucleic Acids Research (2020)

# Overview commercial imaging-based methods

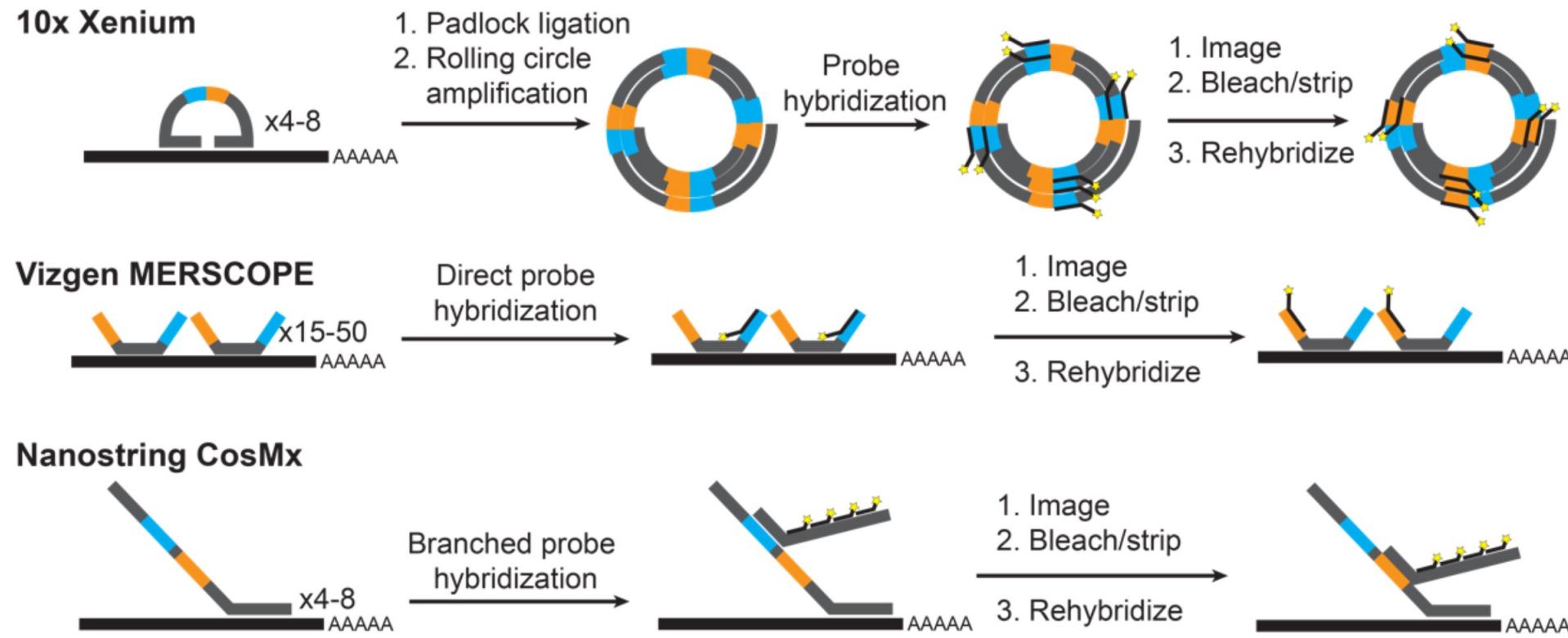
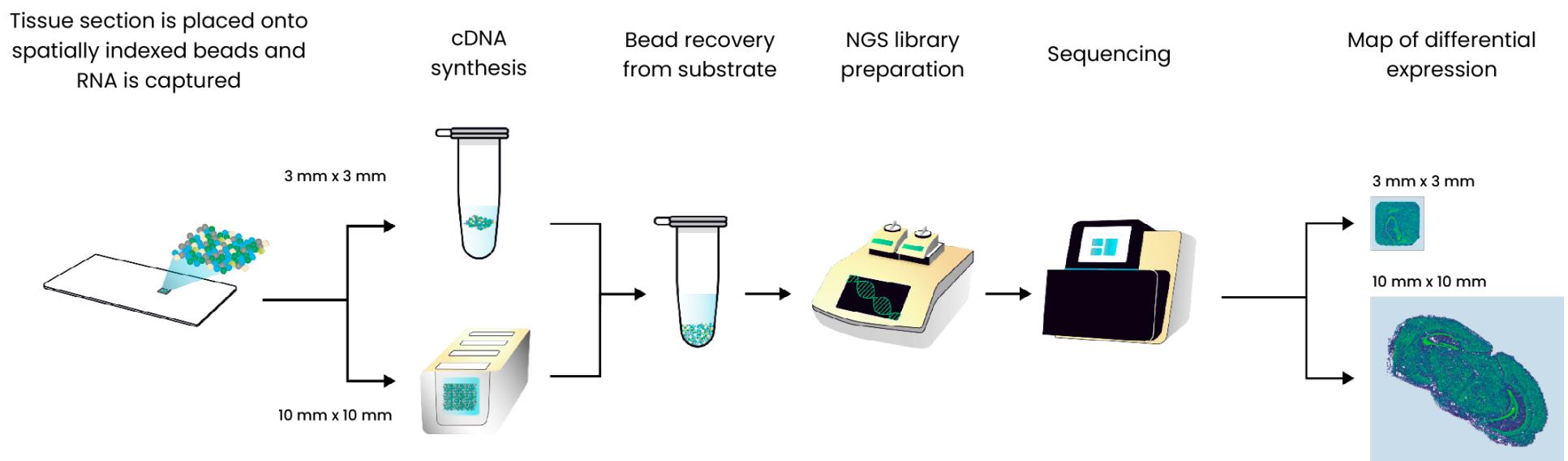


Image: Wang, Huan, et al. "Systematic Benchmarking of Imaging Spatial Transcriptomics Platforms in FFPE Tissues," 2023. <https://doi.org/10.1101/2023.12.07.570603>. CC-BY-NC-ND 4.0

# Curio seeker (SlideSeq2)

- » Bead based
- » resolution:  $10 \mu\text{m}$
- »  $3 \text{ mm} \times 3 \text{ mm}$  or  $10 \text{ mm} \times 10 \text{ mm}$
- » polyA capture
- » Fresh frozen



# Nanostring GeoMx

- » Probe based
- » ROI 'Microdissection' by UV illumination
- » ROI diameter:  $10 \mu\text{m}$  -  $600 \mu\text{m}$  (can be any shape)
- » Sequencing probe barcodes
- » Full transcriptome possible

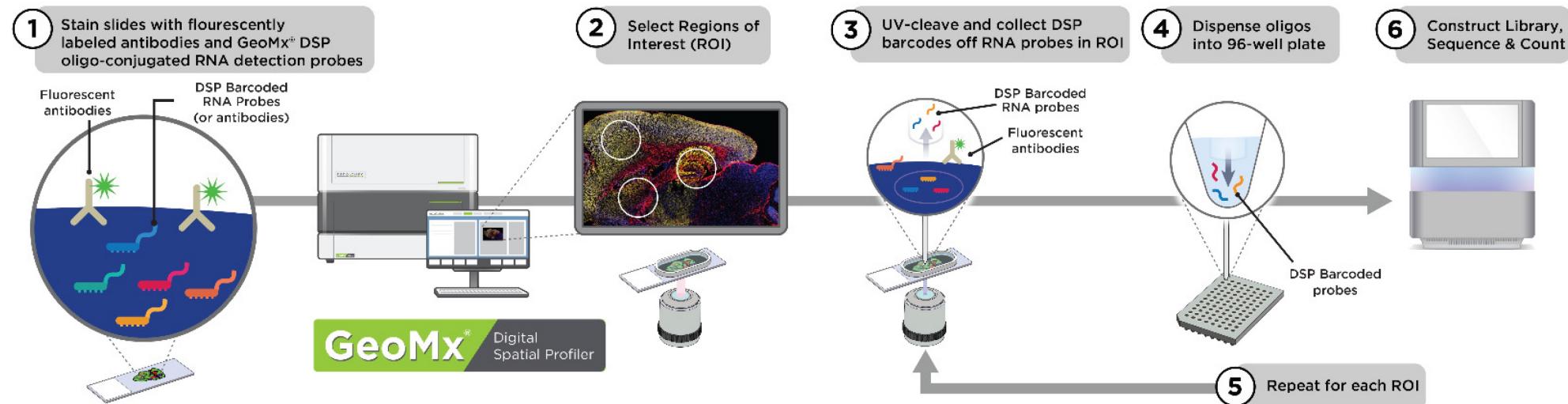
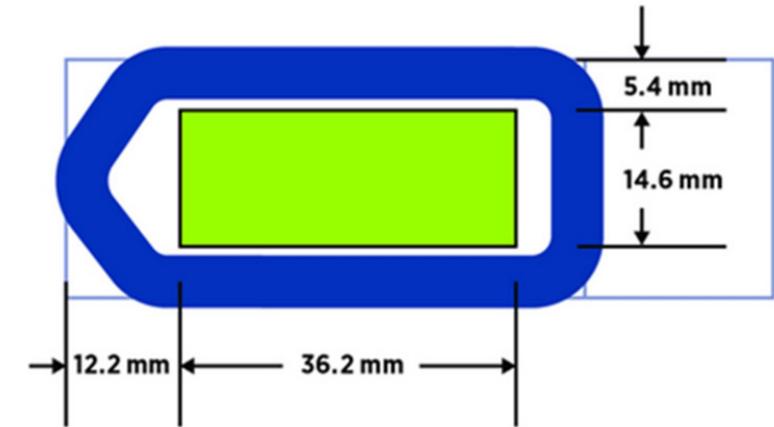
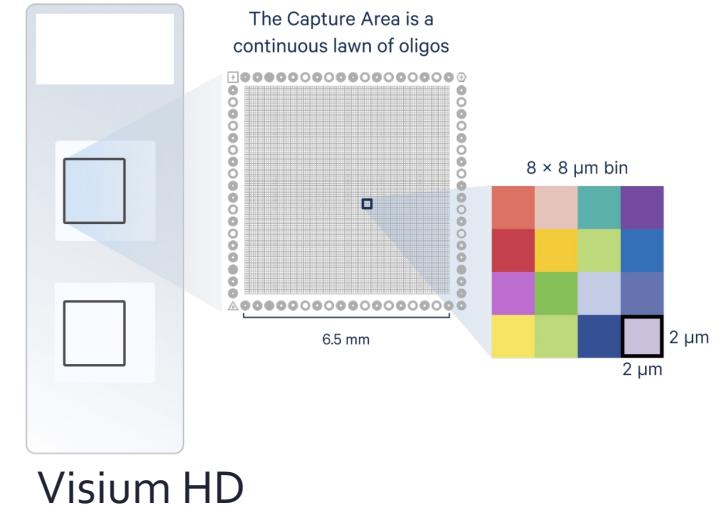


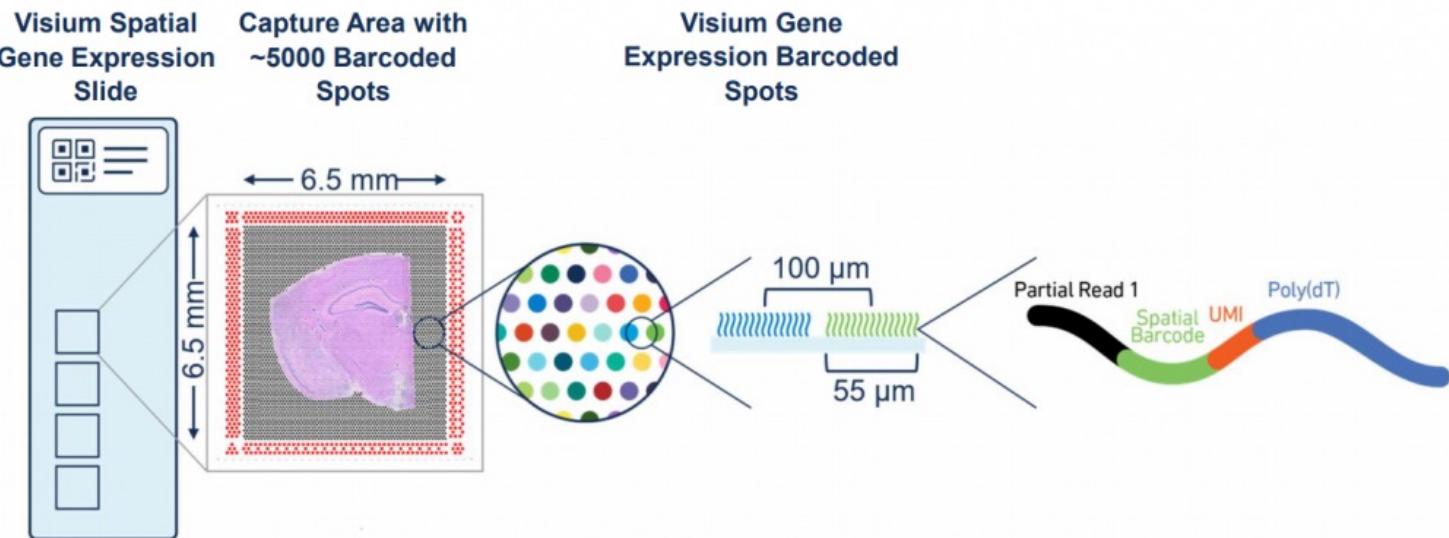
Image: Bergholtz, et al *Cancers* 13, no. 17 (2021): 4456. <https://doi.org/10.3390/cancers13174456>. CC-BY-4.0

# 10X Visium

- » Array based
- » resolution
  - » Standard:  $55\text{ }\mu\text{m}$ ;  $100\text{ }\mu\text{m}$  center-to-center
  - » HD:  $2\text{ }\mu\text{m}$ ; no gaps
- »  $6.5\text{ mm} \times 6.5\text{ mm}$  or  $11\text{ mm} \times 11\text{ mm}$  (standard only)
- » Fresh frozen or FFPE
- » Direct placement or CytAssist (= probes)



Visium HD



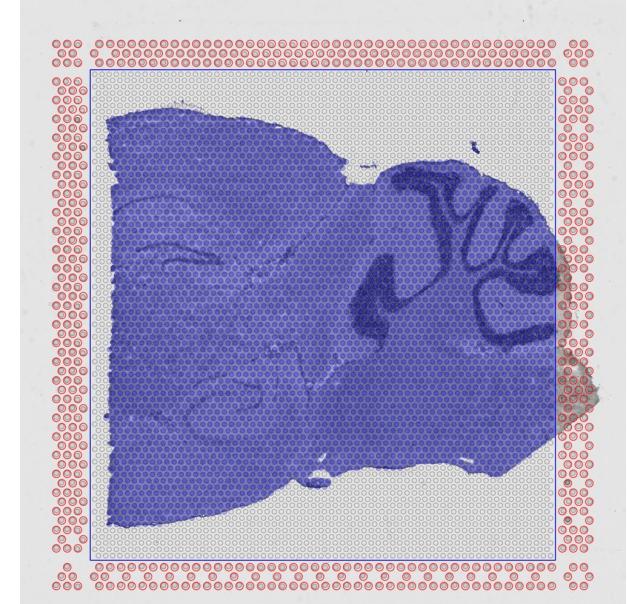
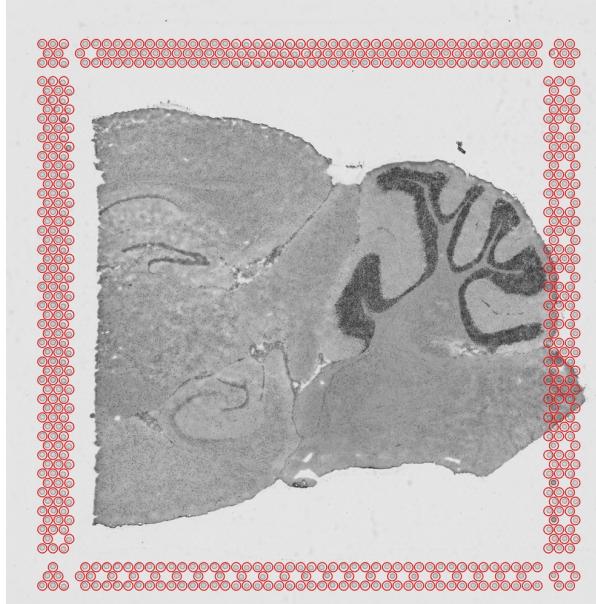
# Spaceranger count – 10X visium data

- » **Performs:**

- » image alignment
- » read alignment
- » spot on slice identification
- » UMI counting

- » **Input:**

- » Fastq files
- » Reference
- » Probe sets (if probes were used)
- » Slide information (ID, area)
- » Images: H&E or fluorescence



- » **Output:**

- » Images – including aligned fudicials
- » Filtered and unfiltered matrices (spot x gene)
- » Report: web\_summary.html

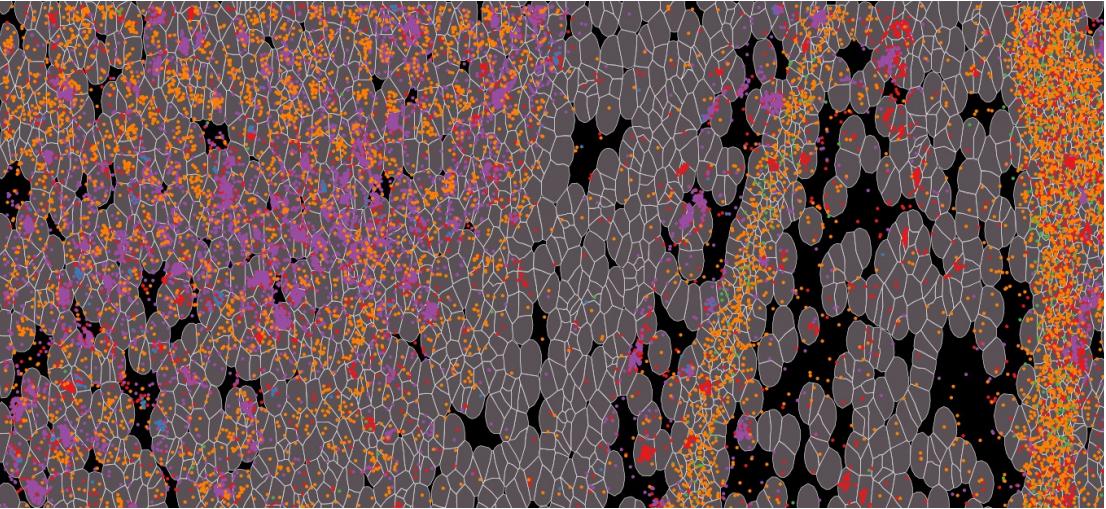
# Spatial transcriptomics data

- » **Matrix:**

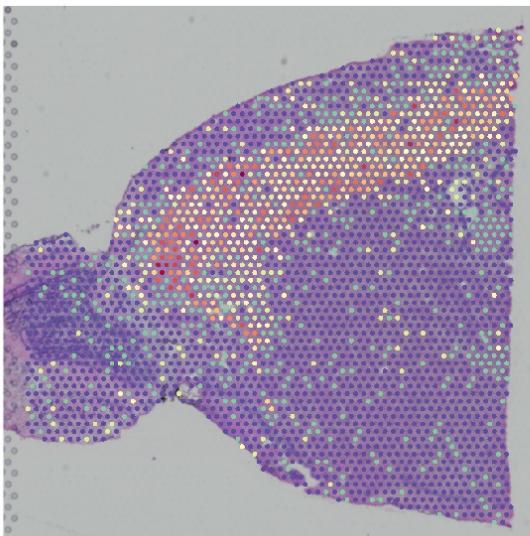
- » rows: genes or transcripts
- » columns: spots or cells

- » **Spatial information:**

- » Sequencing based: Spot position
- » Imaging based: cell centroids, cell boundaries, molecule positions



10x xenium - cells

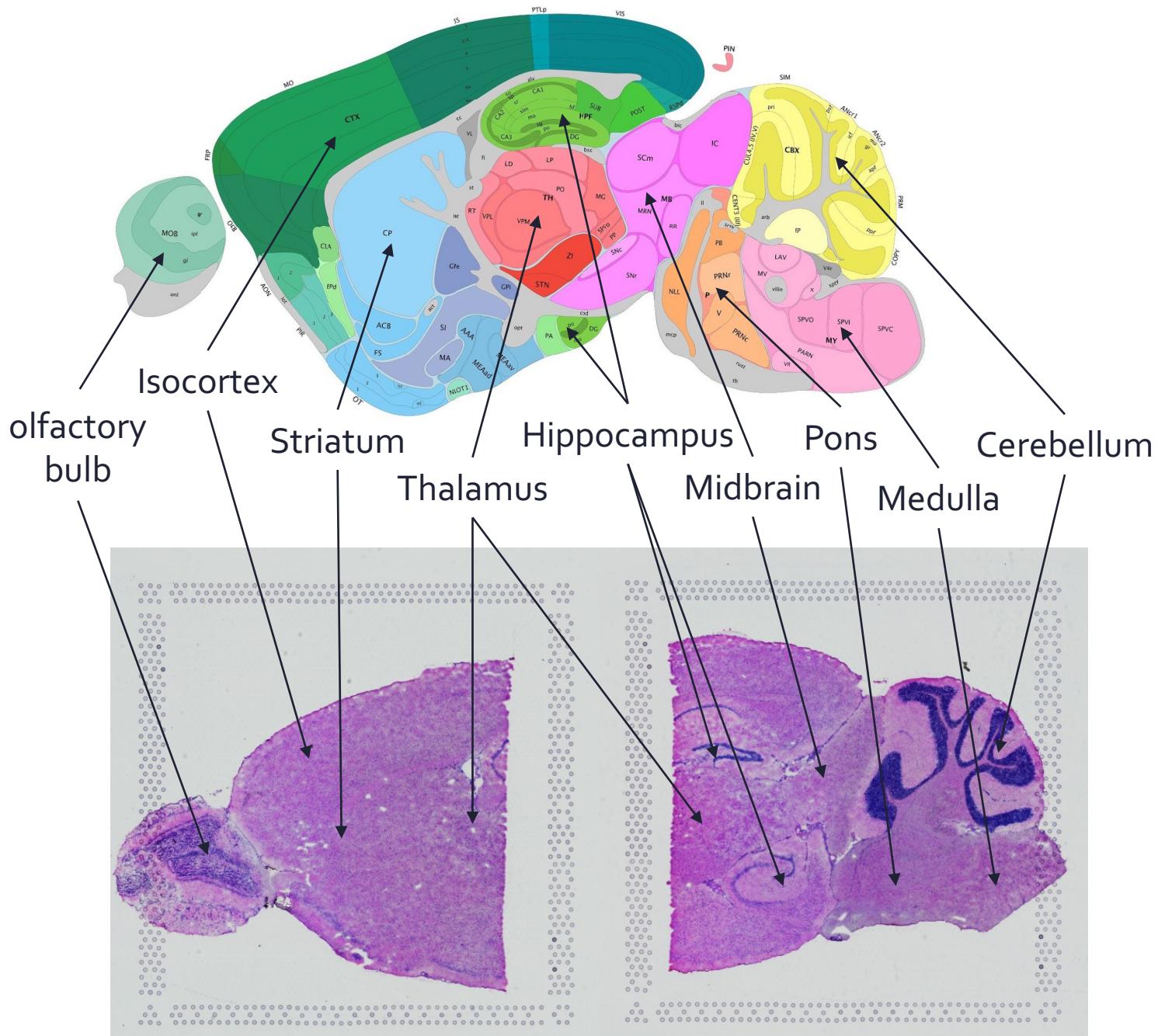


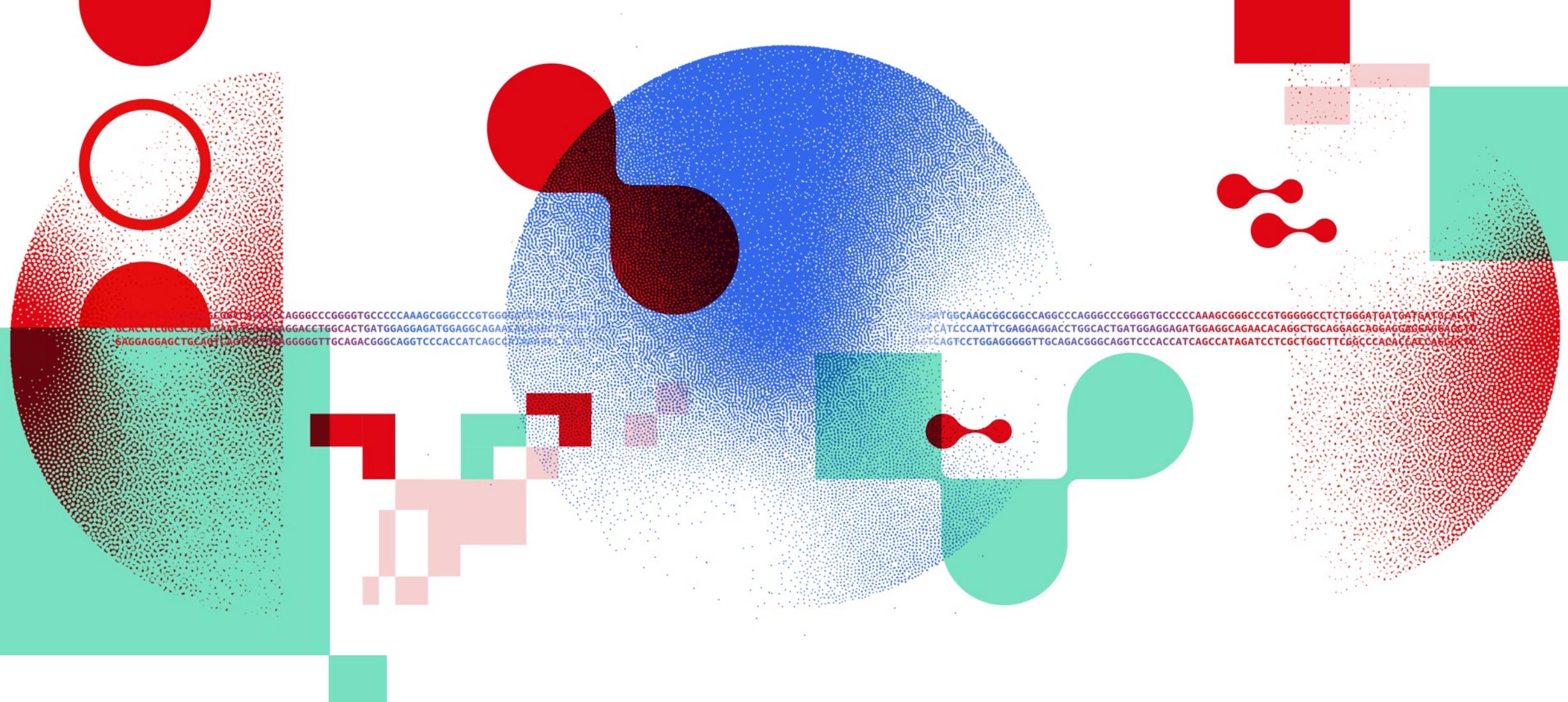
10x visium - spots



# Our dataset

- » 10X visium v1
- » Direct placement
- » Mouse brain
- » Two slices - sagittal
  
- » Perform quality control
- » Integration and clustering
- » Marker gene identification
- » Basic annotation





# Time for exercises!

DATA SCIENTISTS FOR LIFE

[sib.swiss](http://sib.swiss)