Spatial Transcriptomics Technologies

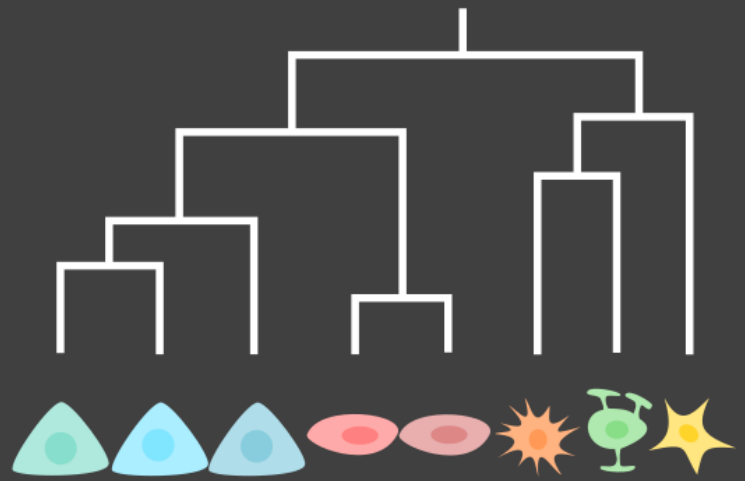
Lars Borm
Lab of Computational Biology lead by Stein Aerts

VIB science meets life

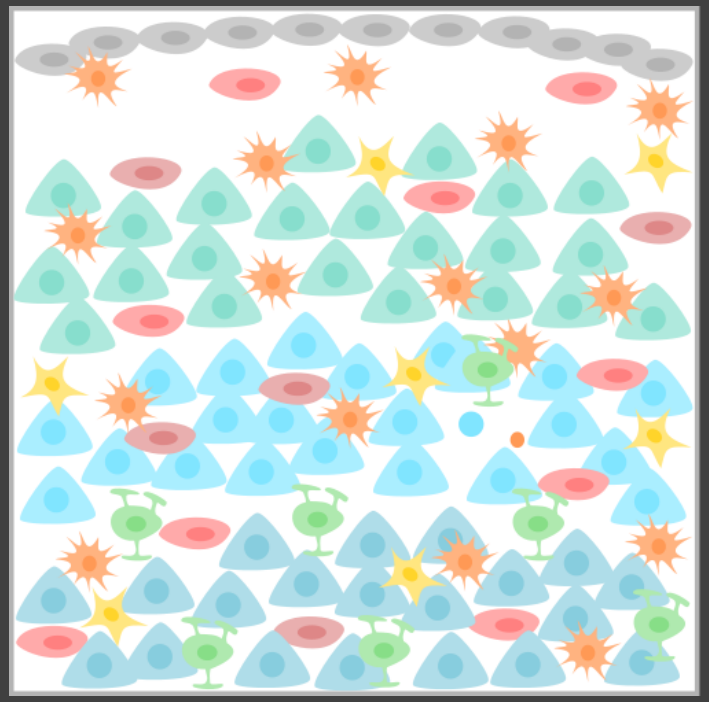
KU LEUVEN

Single Cell RNA sequencing

Cellular complexity

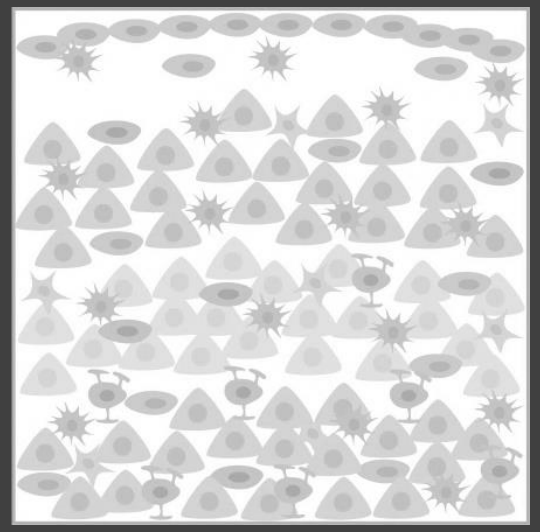


Tissue architecture

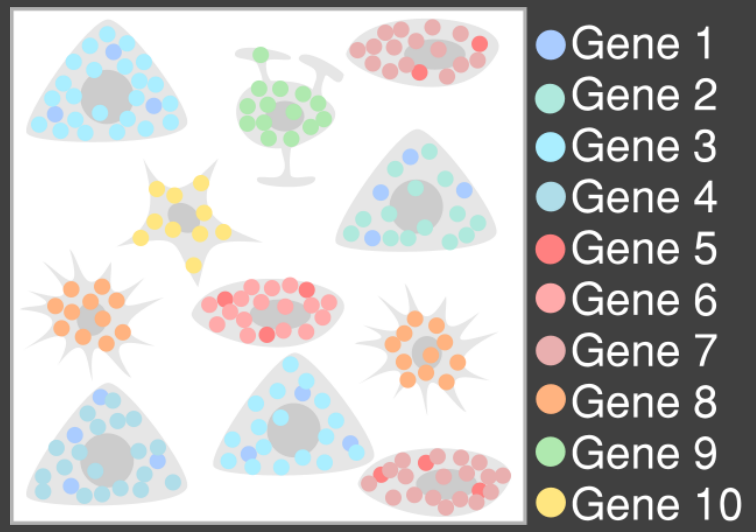


Spatial RNA detection

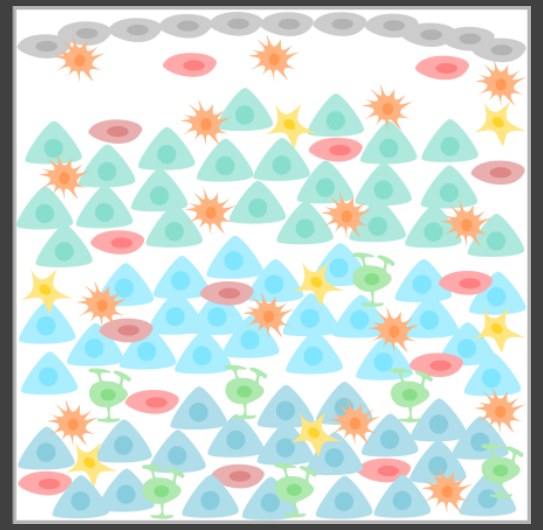
Complex tissue



Spatial measurement



Spatial cellular atlas



Goal

Understanding spatial technologies

Opening the black box

Goal

Understanding spatial technologies

- Choose the right technology
- Know the limitations / biases
- Recognize technical artifacts

Spatial RNA detection

4 Main approaches

(Many other methods not discussed)

Resolution

Sensitivity

Number of targets

Area

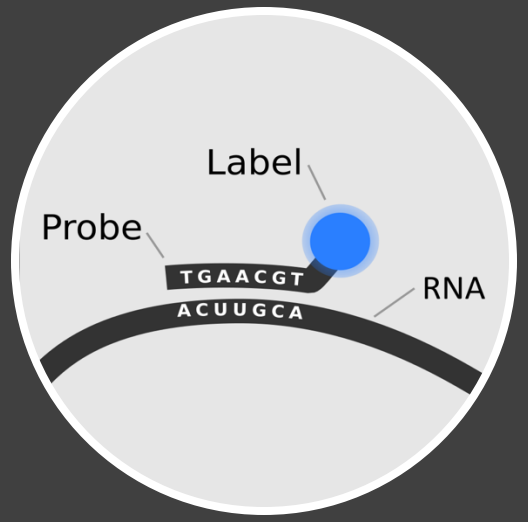
Spatial RNA detection

Microscopy

Sequencing

Spatial RNA detection

Microscopy



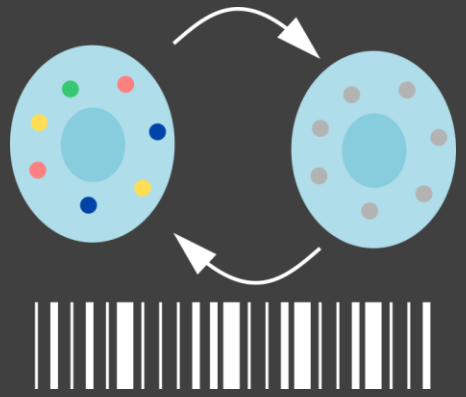
in situ Hybridization

Sequencing

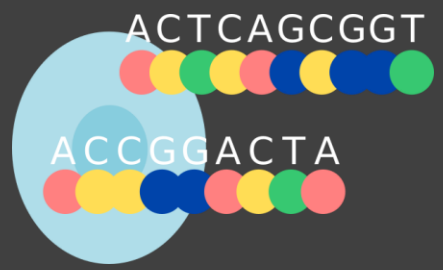
Spatial RNA detection

Microscopy

Barcoded FISH



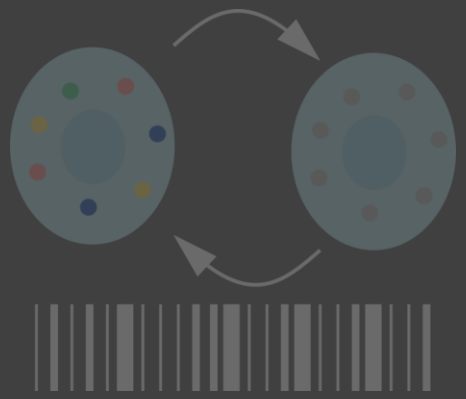
in situ Sequencing



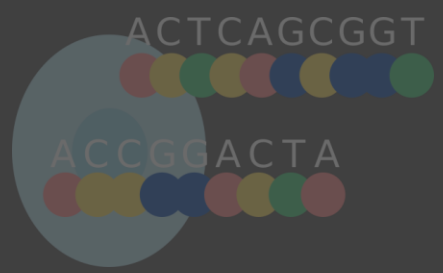
Spatial RNA detection

Microscopy

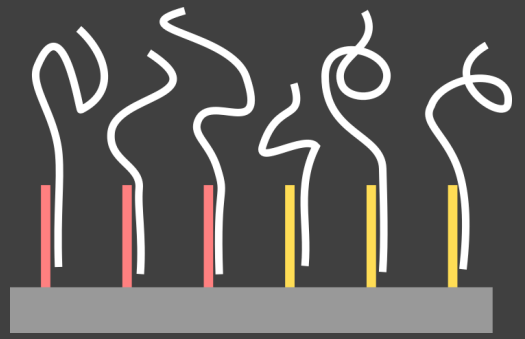
Barcoded FISH



in situ Sequencing



Sequencing

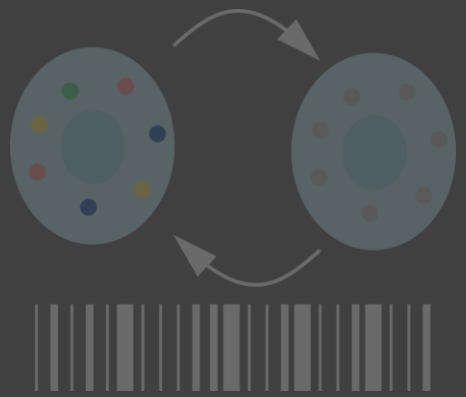


Spatial barcodes

Spatial RNA detection

Microscopy

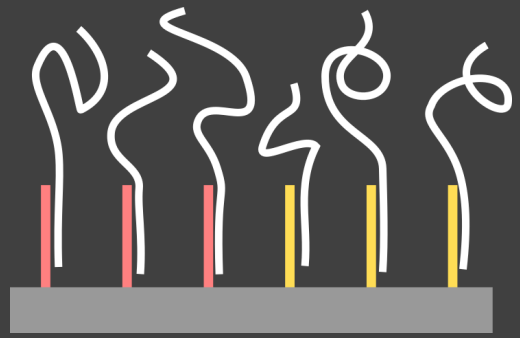
Barcoded FISH



in situ Sequencing

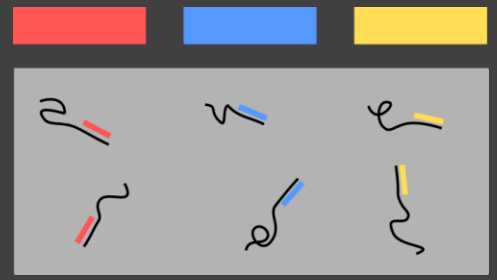
Sequencing

Spatial Sequencing



RNA moves

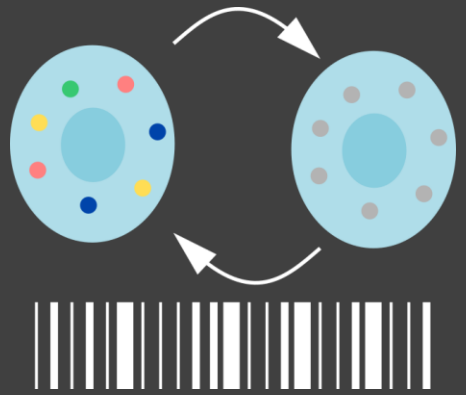
Spatial tagging



Barcodes move

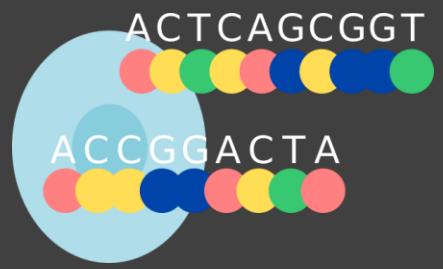
Spatial RNA detection

Barcoded FISH



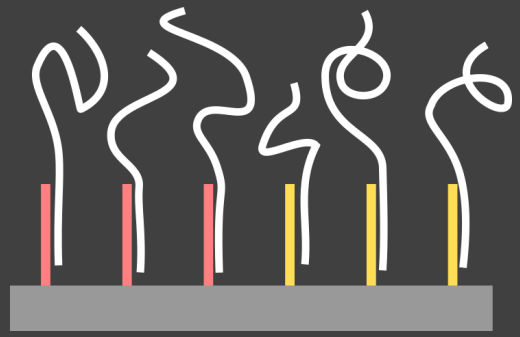
Targeted

in situ Sequencing



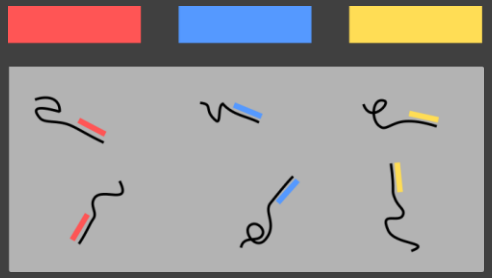
Targeted & Un-targeted

Spatial Sequencing



Targeted & Un-targeted

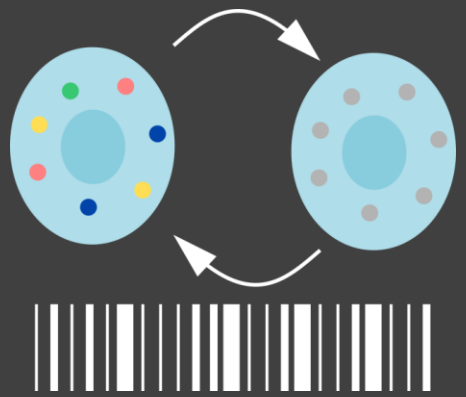
Spatial tagging



Un-targeted

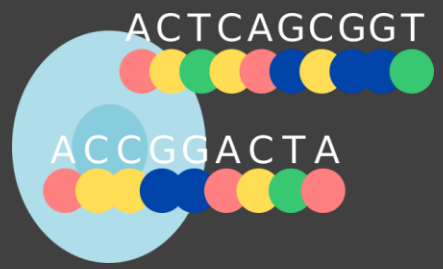
What do you use?

Barcoded FISH



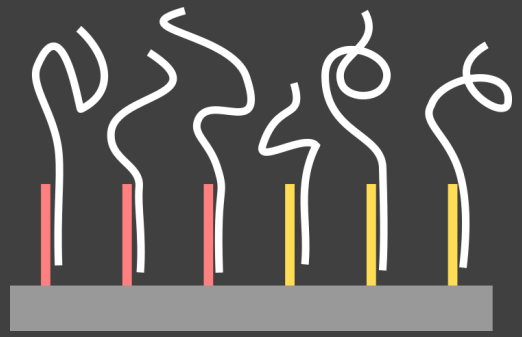
- MERFISH
- Vizgen - MERFISH
- seqFISH
- Spatial Genomics - GenePS
- EEL-FISH
- HybISS
- 10X – Xenium
- Nanostring/Bruker – CosMx
- Resolve – Mol. Cartography

in situ Sequencing



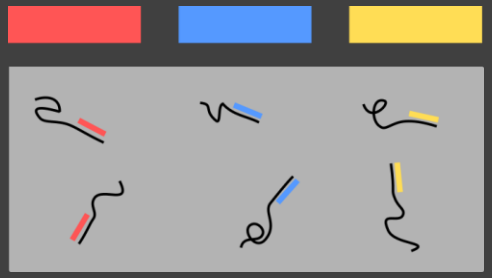
- ISS
- STARmap
- StellarOmics
- Singular genomics – G4X

Spatial Sequencing



- Spatial Transcriptomics
- 10X – Visium (HD)
- Slide-seq
- Curio - Seeker
- Stereo-seq
- BGI STOmics - Stereo-seq
- Seq-Scope, Open-ST, Nova-ST

Spatial tagging

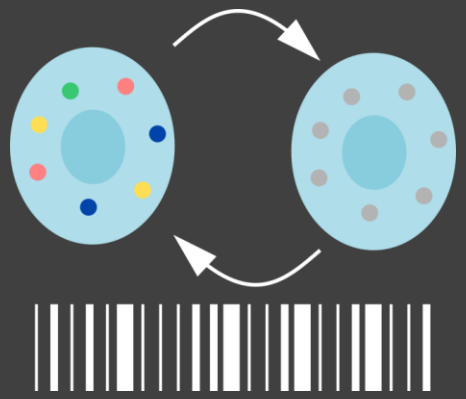


- DBiT-seq
- AtlasXomics
- Slide-tags
- Curio – Trekker

Spatial RNA detection

Microscopy

Barcoded FISH



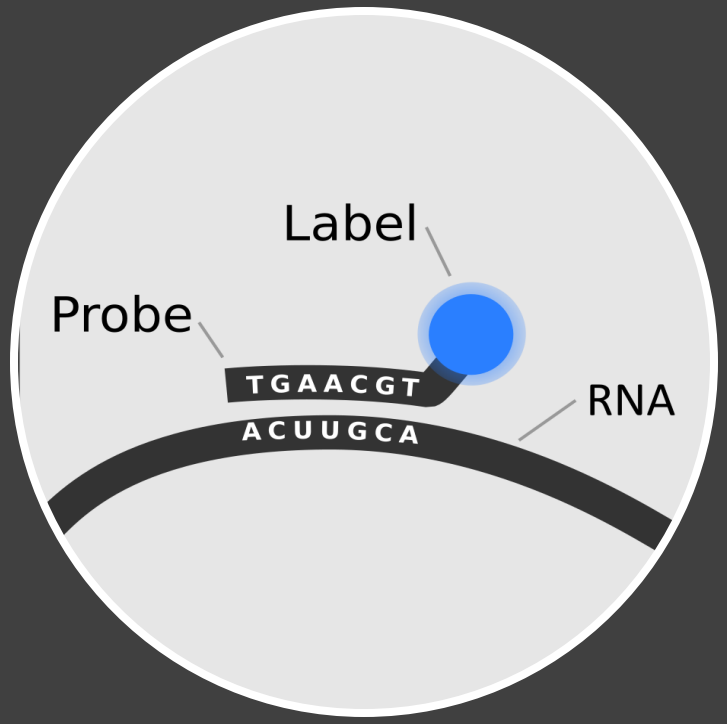
in situ Sequencing

Sequencing

Spatial Sequencing

Spatial tagging

Fluorescent *in situ* Hybridization (FISH)



single molecule FISH (smFISH)

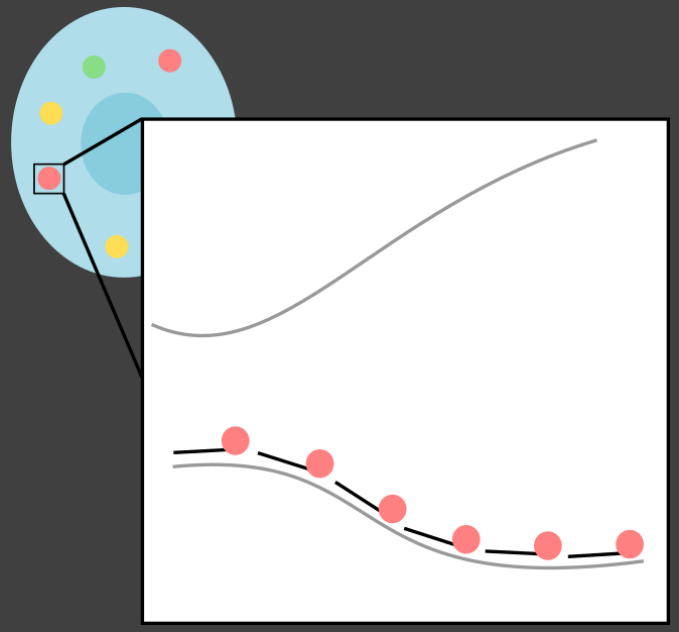
Probes

Gene 1 40X

Gene 2 40X

Gene 3 40X

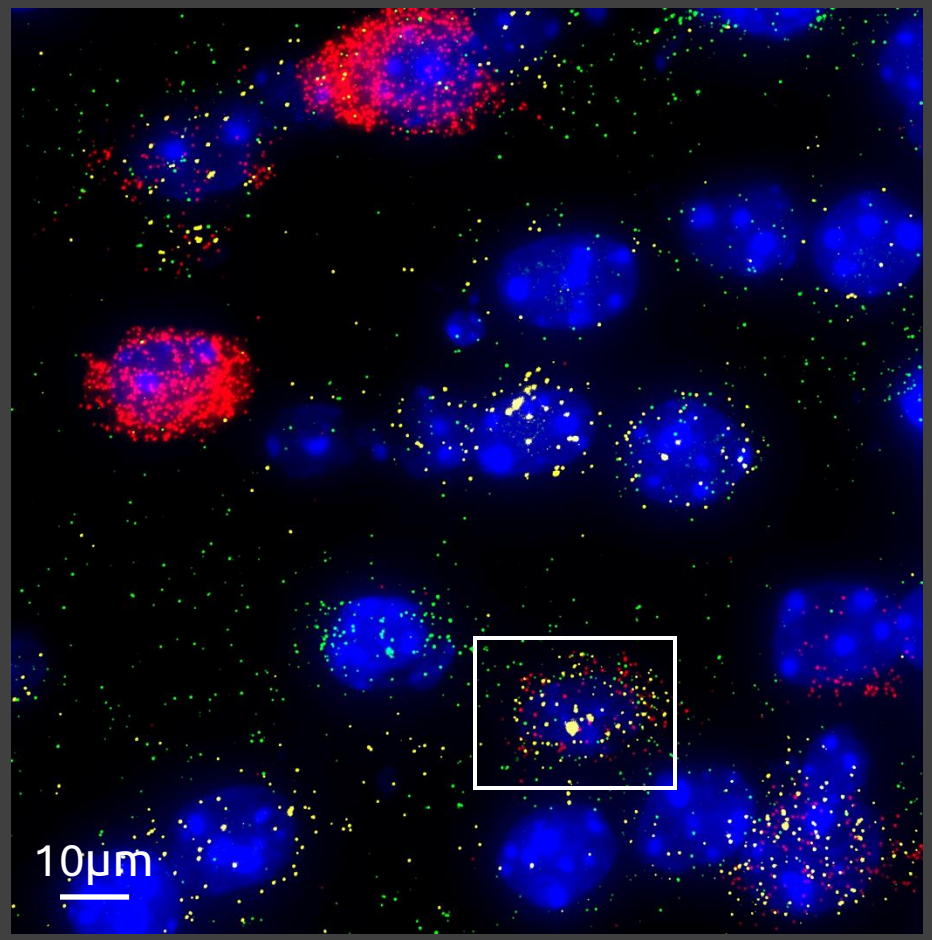
Gene 4 40X

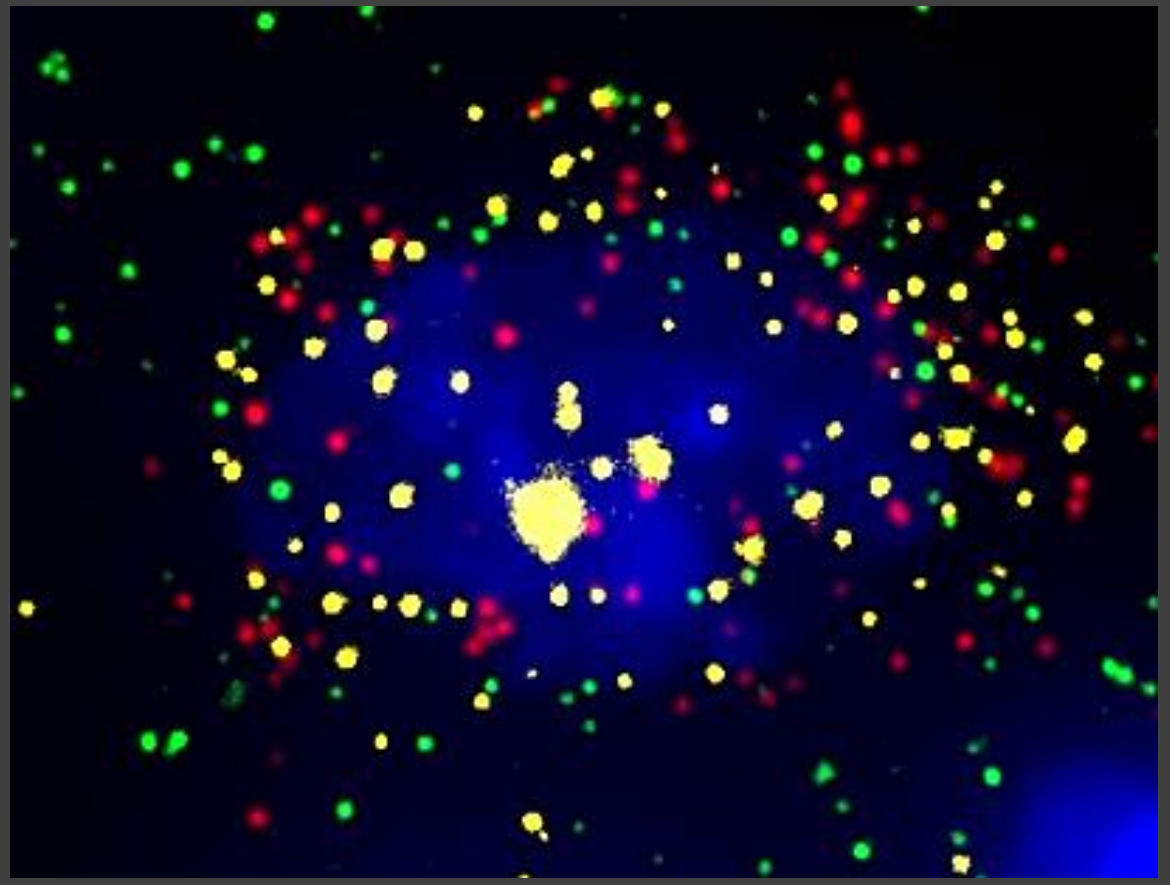


Femino *et al.* Science 1998
Raj *et al.* Nature Methods 2008

single molecule FISH (smFISH)

Gene 1 Gene 2 Gene 3 DNA





Breaking the color barrier

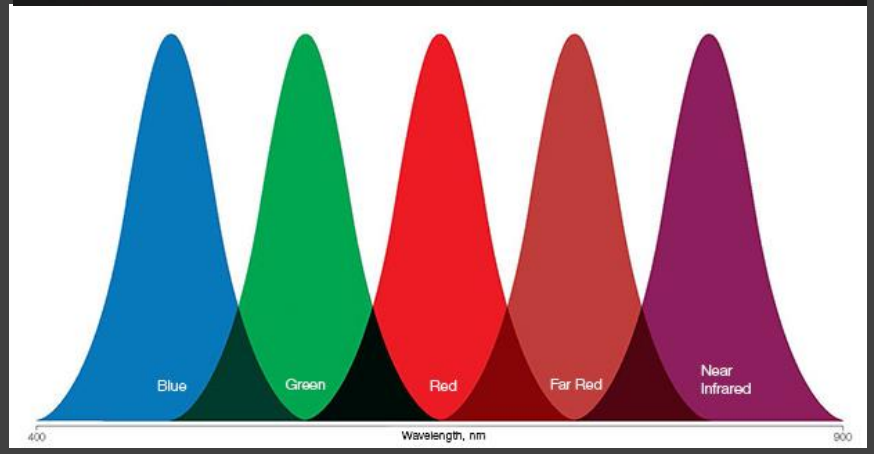
~20.000 genes

4-7 colors

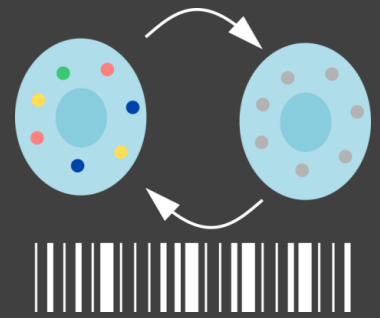
Solution:

- Repeated staining on same sample
- Barcoding

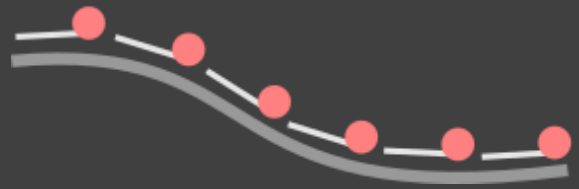
Limited fluorophores



Reprobing same molecule

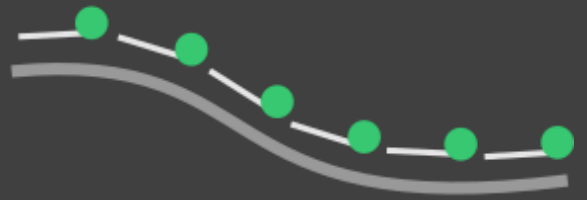


Round 1

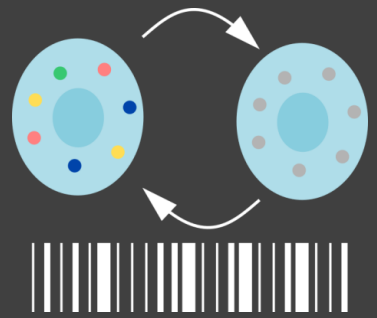


Stripping

Round 2

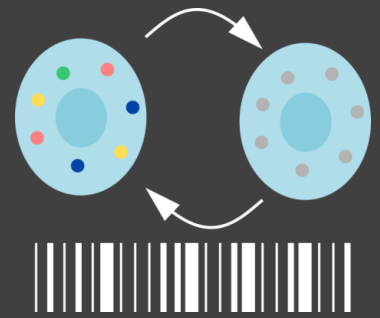


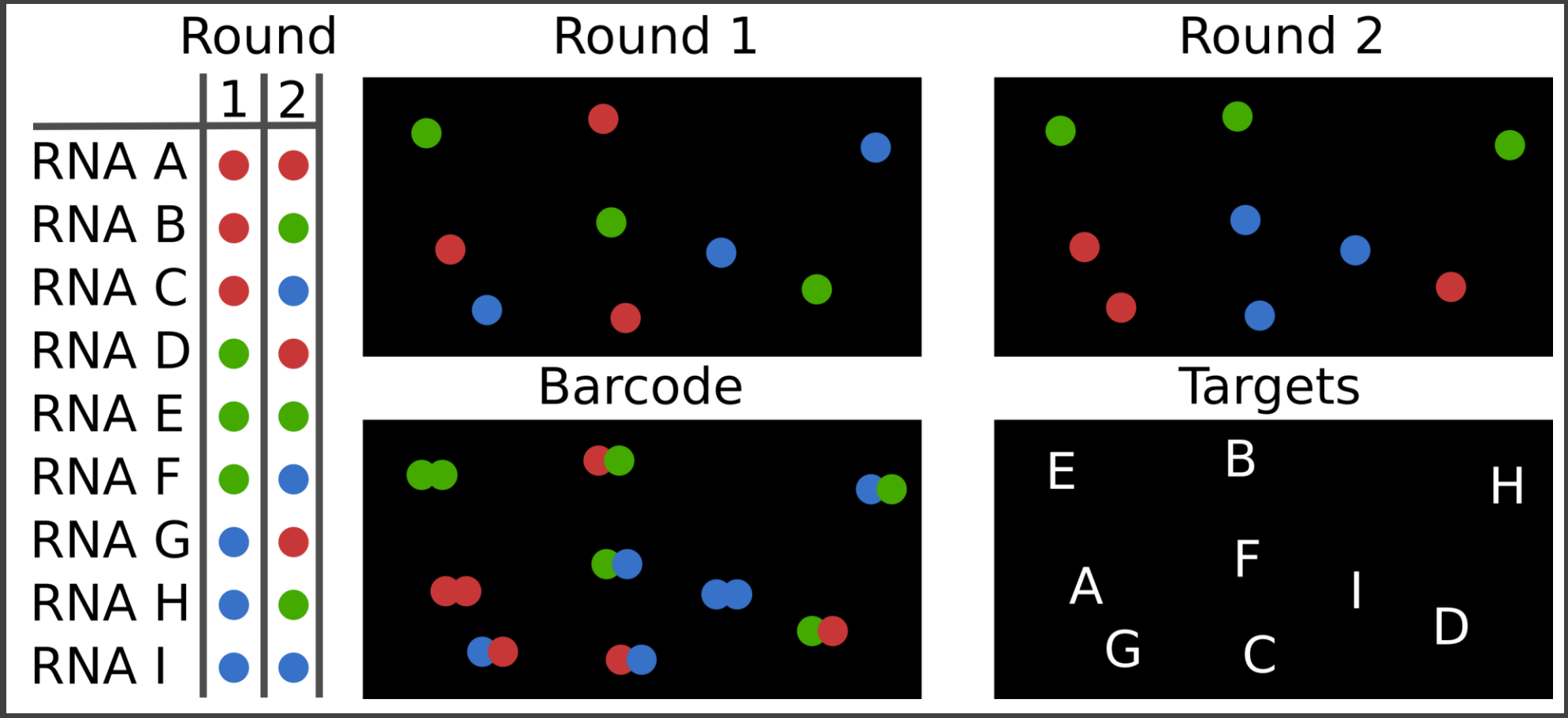
Barcoding



	Round 1	Round 2
RNA A	●	●
RNA B	●	●
RNA C	●	●
RNA D	●	●
RNA E	●	●
RNA F	●	●
RNA G	●	●
RNA H	●	●
RNA I	●	●

Barcoding





Lubeck et al. Nature Methods 2014

Barcoding

Scaling:

$$targets = f^n$$

$4^8 = 65{,}536$

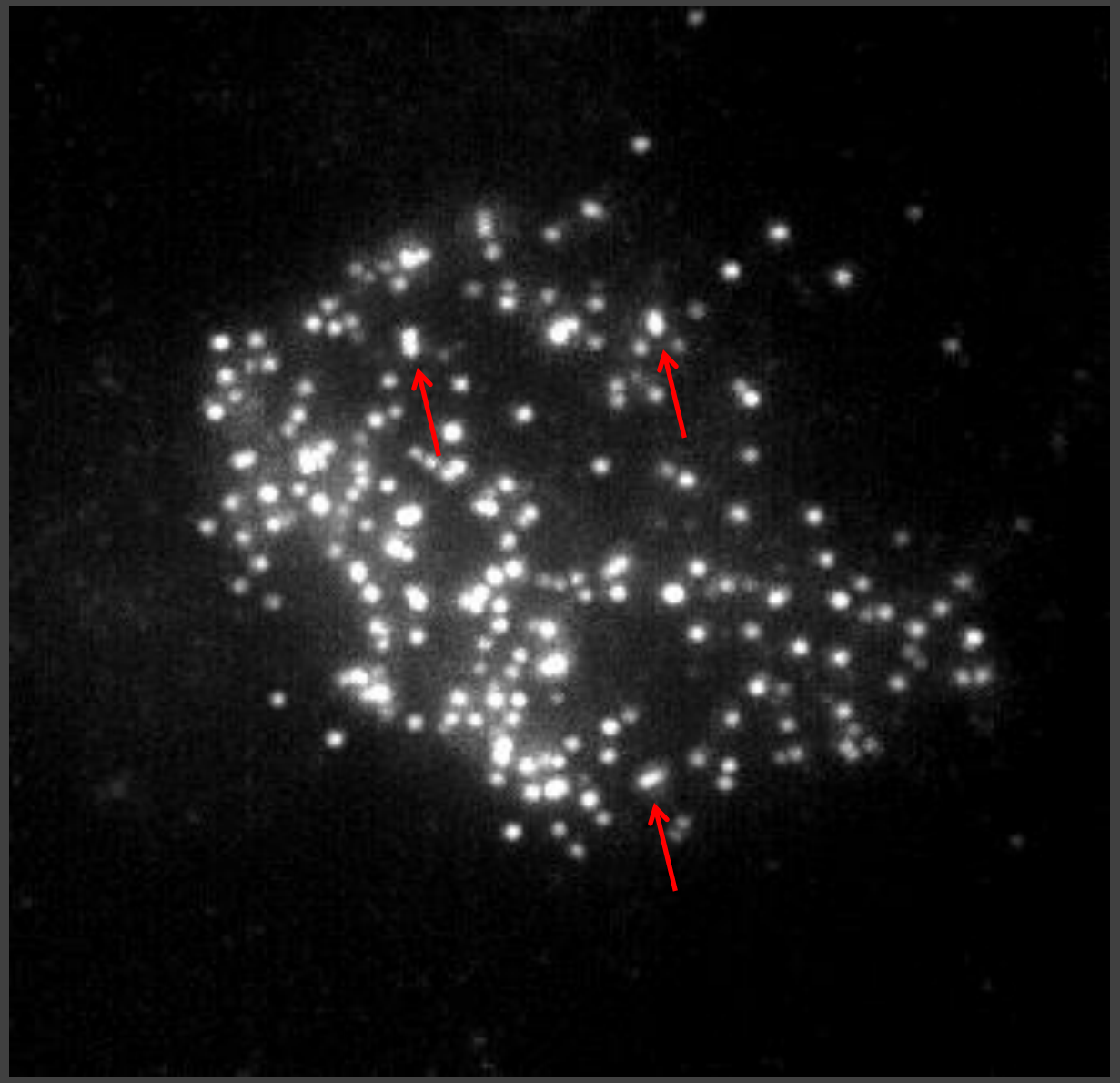
Barcoding

Problems:

- Optical density
- Errors

Solution:

- Sparce barcodes



Barcoding

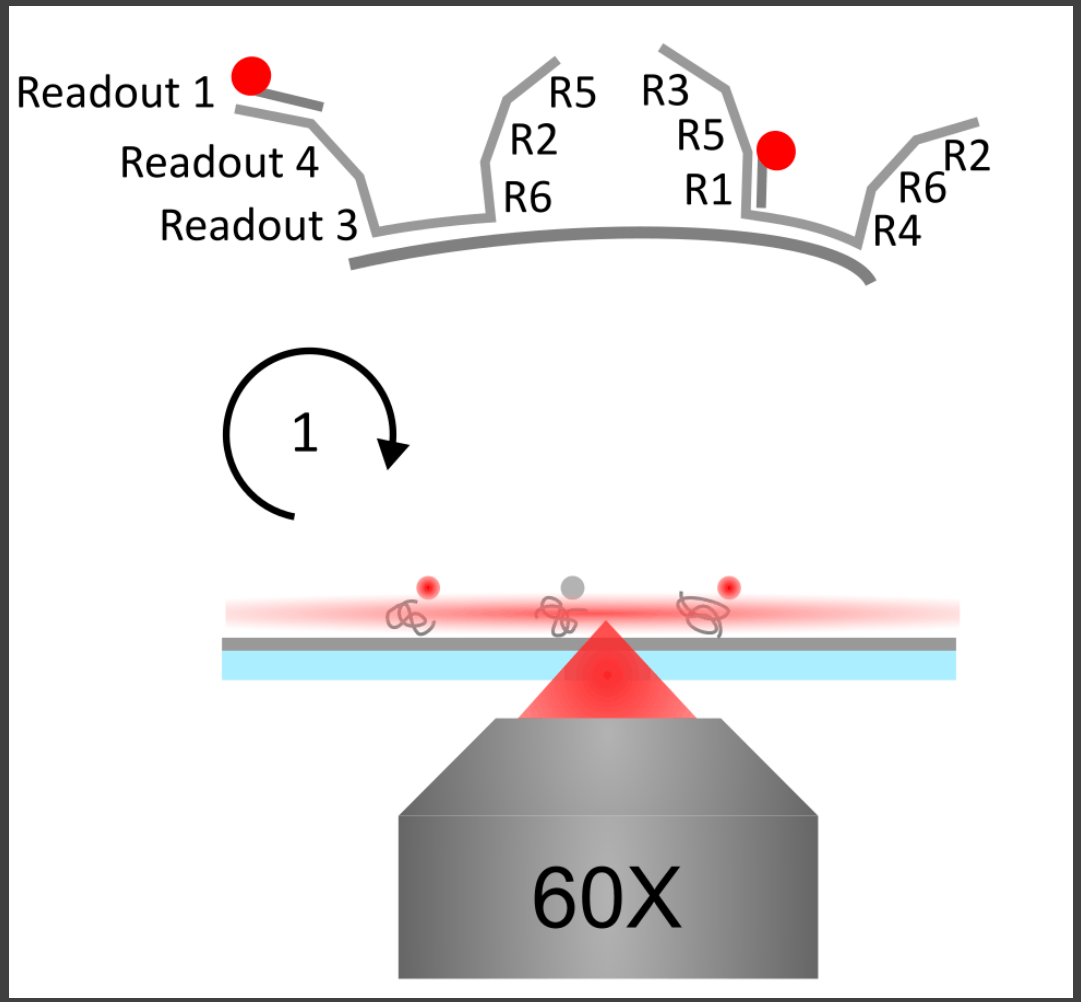
Solution:

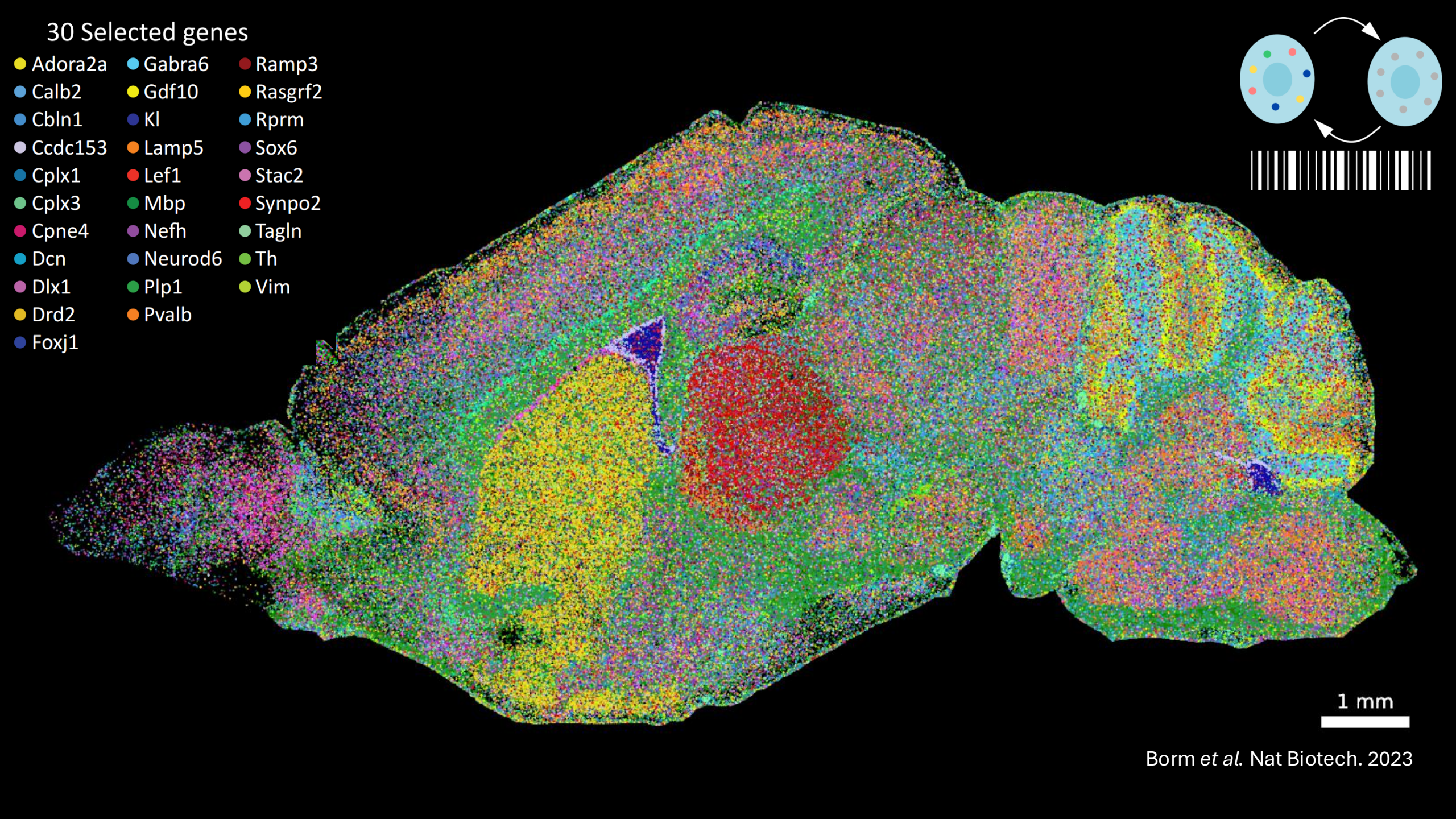
- Sparce barcodes
  100110001

- Error robustness

MERFISH

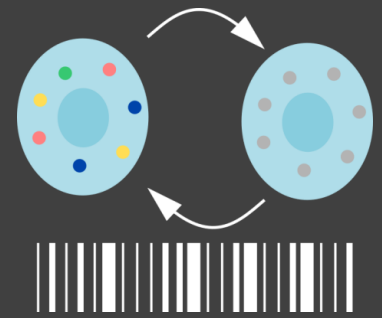
Chen et al. 2015 Science

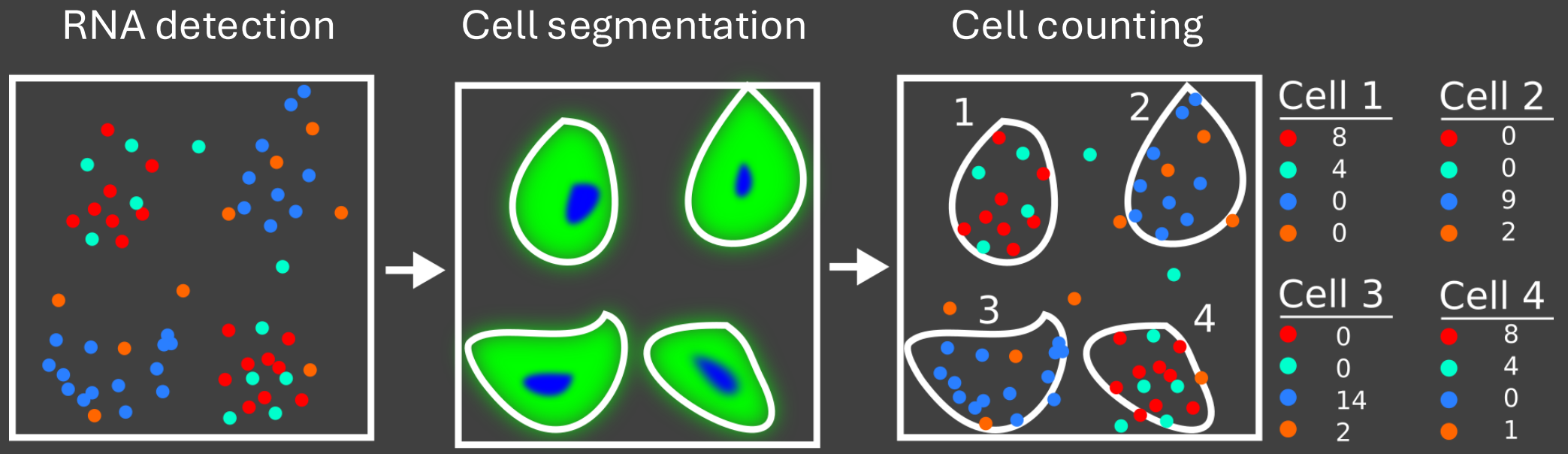




Borm *et al.* Nat Biotech. 2023

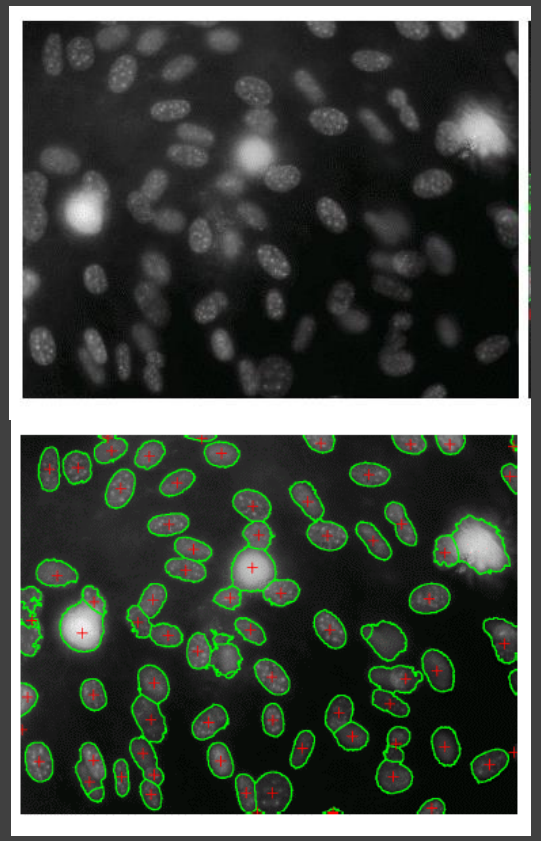
Cell assignment





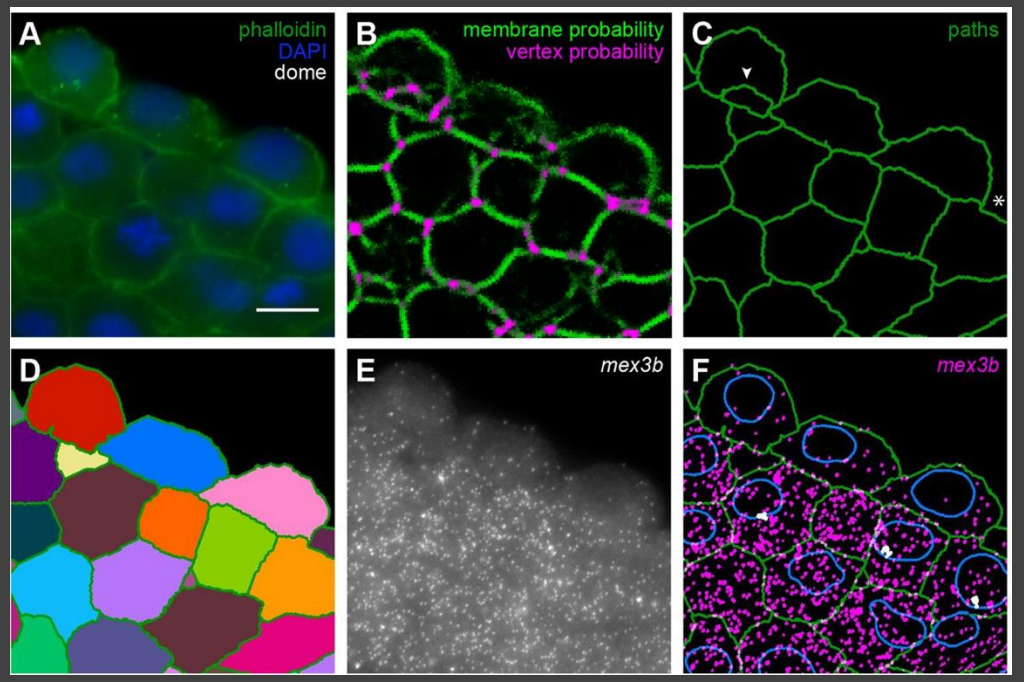
Cell segmentation

Nuclei



Panagiotakis *et al.* IEEE 2018

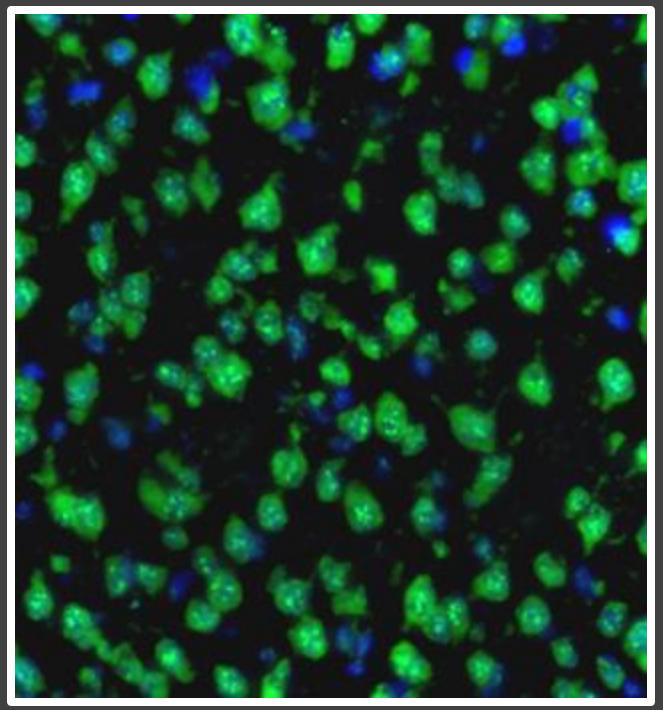
Membrane



Stapel *et al.* Development 2016

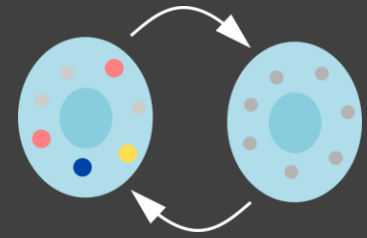
Cell body

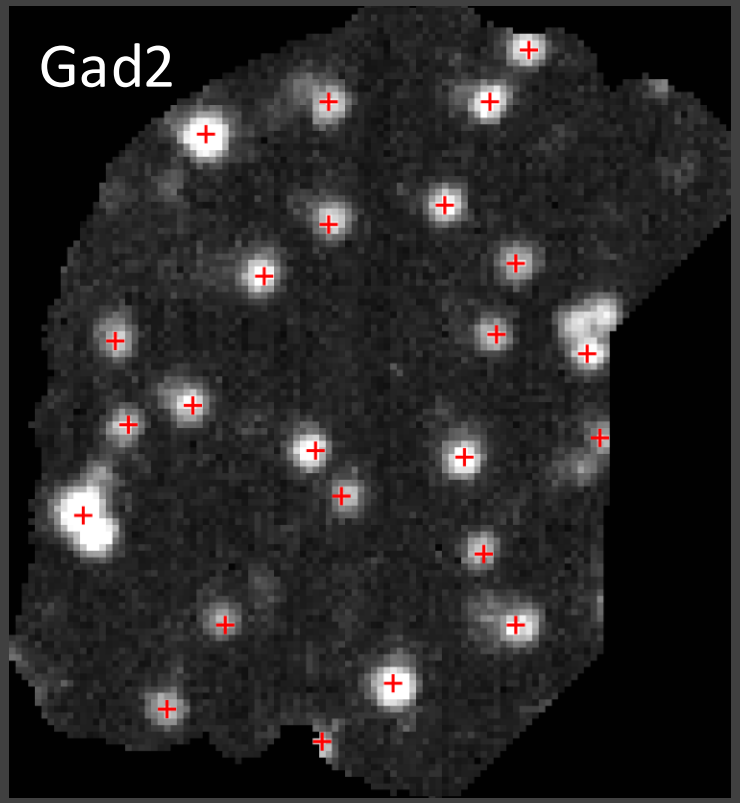
DNA RNA



Codeluppi *et al.* 2018 Nature Methods

Cell segmentation





Terabytes

Genes

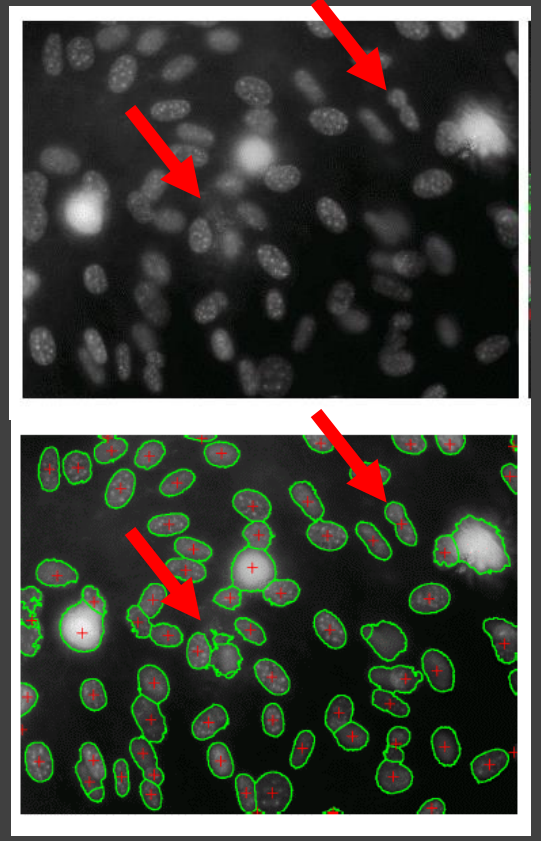
Cells

	1124	2325	2400	241	6248	5992	275	2573	330	1149	...	5162	532	3607	3251	7173	2757	1228	1234	7797	4653
Hybridization1_Tbr1	13	11	28	12	7	6	14	24	5	3	...	57	20	26	8	5	5	0	14	18	5
Hybridization1_Aldoc	38	0	9	5	38	2	4	3	7	10	...	11	10	5	4	10	2	0	2	2	9
Hybridization1_Foxj1	0	0	0	1	5	0	3	1	1	1	...	0	0	0	2	1	8	1	2	0	4
Hybridization6_Bmp4	1	0	0	0	0	0	0	0	1	0	...	0	0	0	0	0	0	1	1	0	0
Hybridization6_Itpr2	4	0	0	1	0	0	1	0	2	1	...	3	0	1	2	3	0	0	0	0	0
Hybridization6_Vip	13	1	2	4	30	1	3	2	1	4	...	0	11	2	5	1	7	2	3	6	1
Hybridization4_Cnr1	0	0	0	0	65	5	0	0	0	0	...	2	0	9	0	17	0	0	0	0	5
Hybridization4_Plp1	16	0	0	0	8	0	0	6	0	0	...	0	0	0	10	1	27	5	1	2	0
Hybridization4_Vtn	0	0	0	2	4	0	2	1	1	0	...	0	3	1	2	2	2	0	0	0	3
Hybridization7_Rorb	4	0	0	1	0	4	0	0	2	3	...	0	27	14	0	0	1	0	1	0	1
Hybridization7_Sox10	52	0	1	1	3	3	13	3	19	33	...	1	4	0	10	12	40	15	32	1	0
Hybridization7_Ctps	6	3	9	15	3	3	3	5	2	1	...	6	4	12	14	1	2	0	2	1	6
Hybridization11_Syt6	1	16	20	0	0	0	3	21	1	2	...	4	0	1	11	1	3	0	0	12	2
Hybridization11_Tbr1	4	13	36	6	2	5	9	12	6	15	...	30	19	30	0	3	2	0	0	10	4
Hybridization11_Tmem6	2	0	0	3	1	1	2	2	1	2	...	4	1	3	1	1	0	0	0	0	4
Hybridization8_Pdgfra	1	1	2	0	1	0	2	1	20	1	...	1	1	1	2	26	0	0	0	1	6
Hybridization8_Serpinf1	13	1	2	6	2	4	2	1	10	2	...	0	5	10	8	6	5	6	2	2	2
Hybridization8_Pthlh	2	0	0	0	8	0	1	1	0	0	...	0	0	1	1	0	0	0	0	1	0
Hybridization10_Crhbp	2	0	1	0	0	0	0	0	0	3	...	0	0	0	0	0	0	0	0	0	0
Hybridization10_Crh	2	0	2	0	3	0	6	1	0	2	...	1	1	1	1	0	0	0	3	1	0
Hybridization10_Apln	3	5	2	31	0	2	3	4	8	5	...	0	2	3	1	2	3	1	3	5	1
Hybridization9_Lamp5	6	38	51	126	0	1	52	44	51	0	...	4	1	168	5	5	0	0	1	3	90
Hybridization9_Lum	1	0	0	3	0	0	0	0	3	6	...	0	0	0	1	1	0	0	0	0	0
Hybridization9_Anln	19	1	1	1	2	2	2	6	3	23	...	0	1	10	3	8	14	8	11	3	0
Hybridization12_Kcnip	1	25	50	14	6	3	20	14	7	0	...	25	23	64	0	2	2	0	0	3	22
Hybridization12_Slc32a1	2	1	2	2	22	0	1	0	2	0	...	0	1	1	0	0	4	0	0	0	0
Hybridization12_Vtn	2	2	0	1	2	0	0	0	0	2	...	0	1	0	0	2	0	0	0	0	1
Hybridization5_Acta2	3	1	1	1	1	0	1	4	0	2	...	0	0	2	0	7	6	0	4	1	0
Hybridization5_Cpne5	0	4	1	1	1	0	2	9	2	0	...	3	0	10	0	3	0	0	0	3	16
Hybridization5_Klk6	0	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	2	0	1	0	0
Hybridization3_Mfge8	6	0	1	2	0	2	2	3	2	13	...	2	2	2	1	7	4	0	2	0	7
Hybridization3_Mrc1	14	2	2	3	2	0	0	6	1	19	...	2	2	26	4	9	0	1	6	2	5
Hybridization3_Hexb	10	0	3	6	1	0	3	4	1	3	...	9	2	4	3	6	2	0	0	1	2
Hybridization2_Gad2	7	4	3	5	65	1	7	1	2	12	...	2	6	9	2	2	2	1	9	3	11
Hybridization2_Flt1	0	0	0	0	0	0	0	0	0	0	...	0	3	0	0	0	0	0	0	0	0
Hybridization2_Gfap	57	0	1	0	1	0	3	3	0	32	...	3	1	4	5	0	0	1	2	0	1
Hybridization13_Cnr1	1	1	2	14	56	3	6	7	5	3	...	3	3	25	1	2	3	0	0	0	6
Hybridization13_Ttr	2	0	0	1	1	0	1	0	1	13	...	0	2	0	3	3	1	1	1	3	0
Hybridization13_Plp1	10	5	3	0	7	0	0	8	5	33	...	3	1	2	35	0	4	7	33	4	1

Megabytes

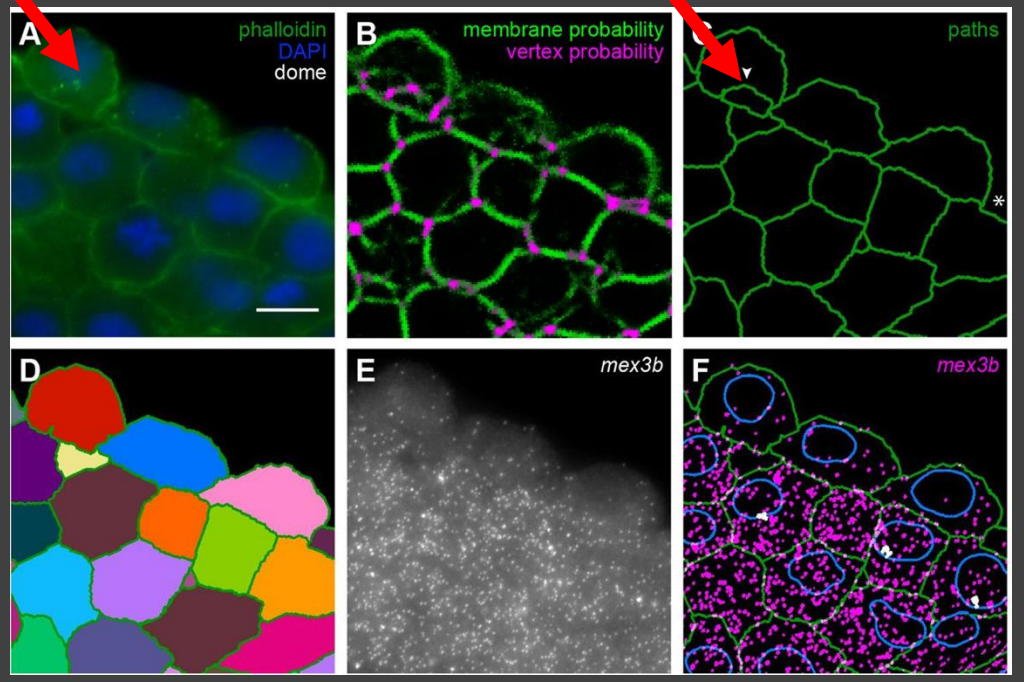
Cell segmentation

Nuclei



Panagiotakis *et al.* IEEE 2018

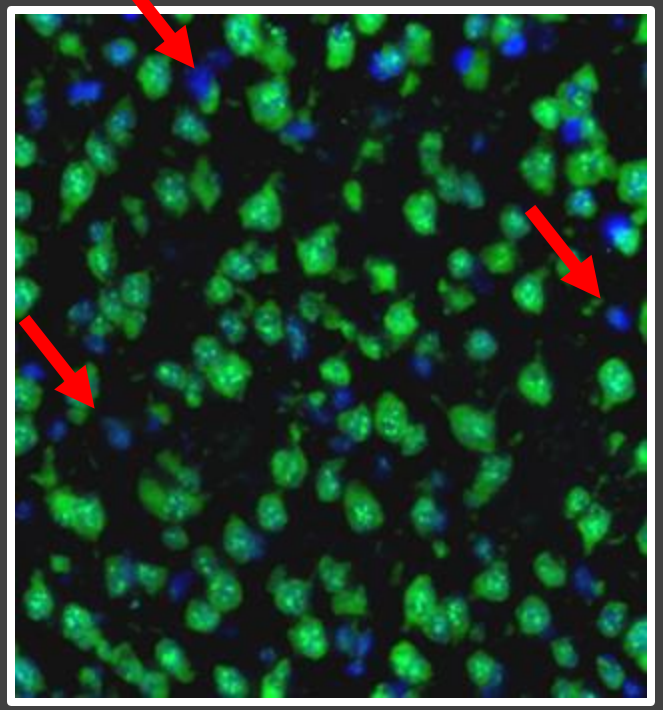
Membrane



Stapel *et al.* Development 2016

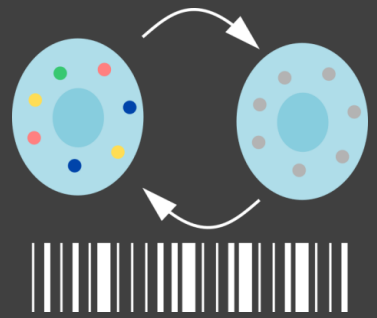
Cell body

DNA RNA



Codeluppi *et al.* 2018 Nature Methods

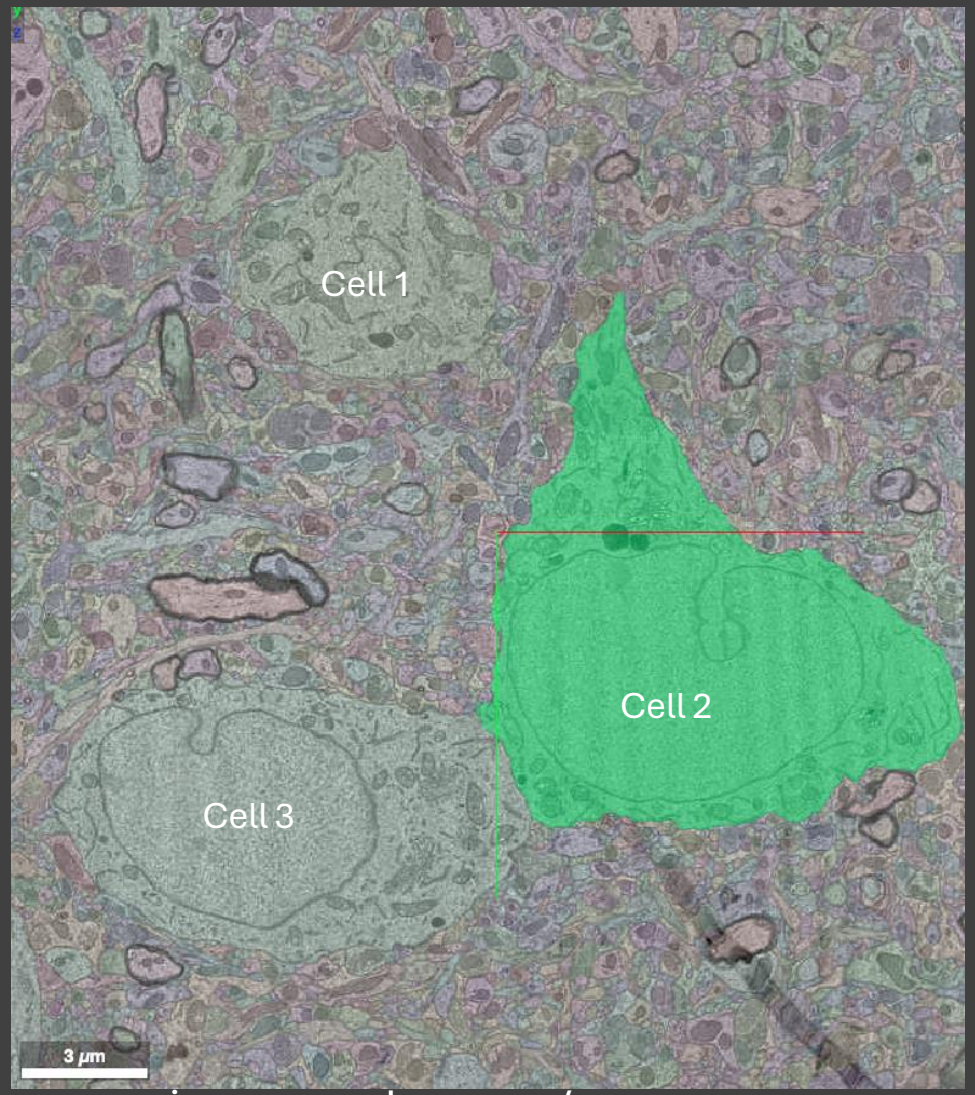
Segmentation challenge



Limitations:

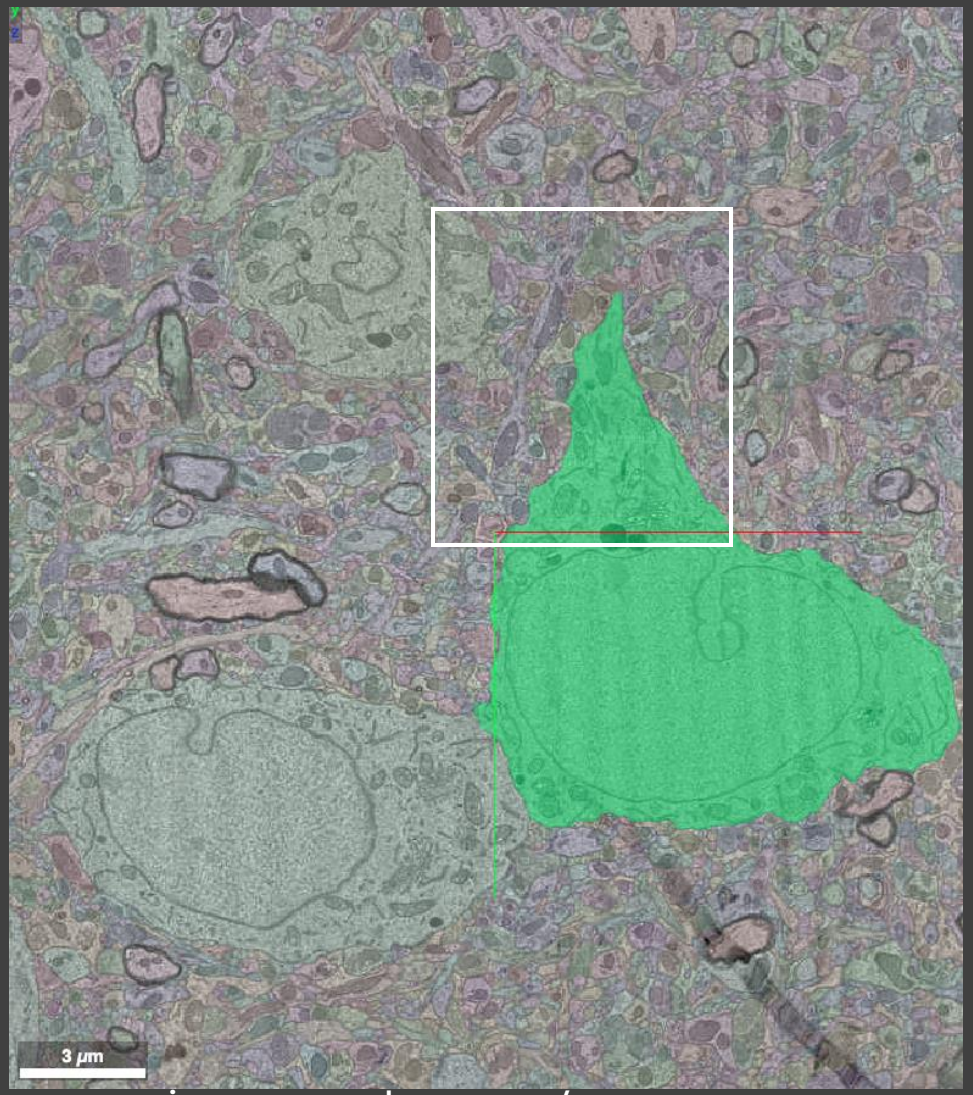
- Counter stains (membrane, nuclei, organelles, cell fill)
- Unclear ground truth
- Resolution

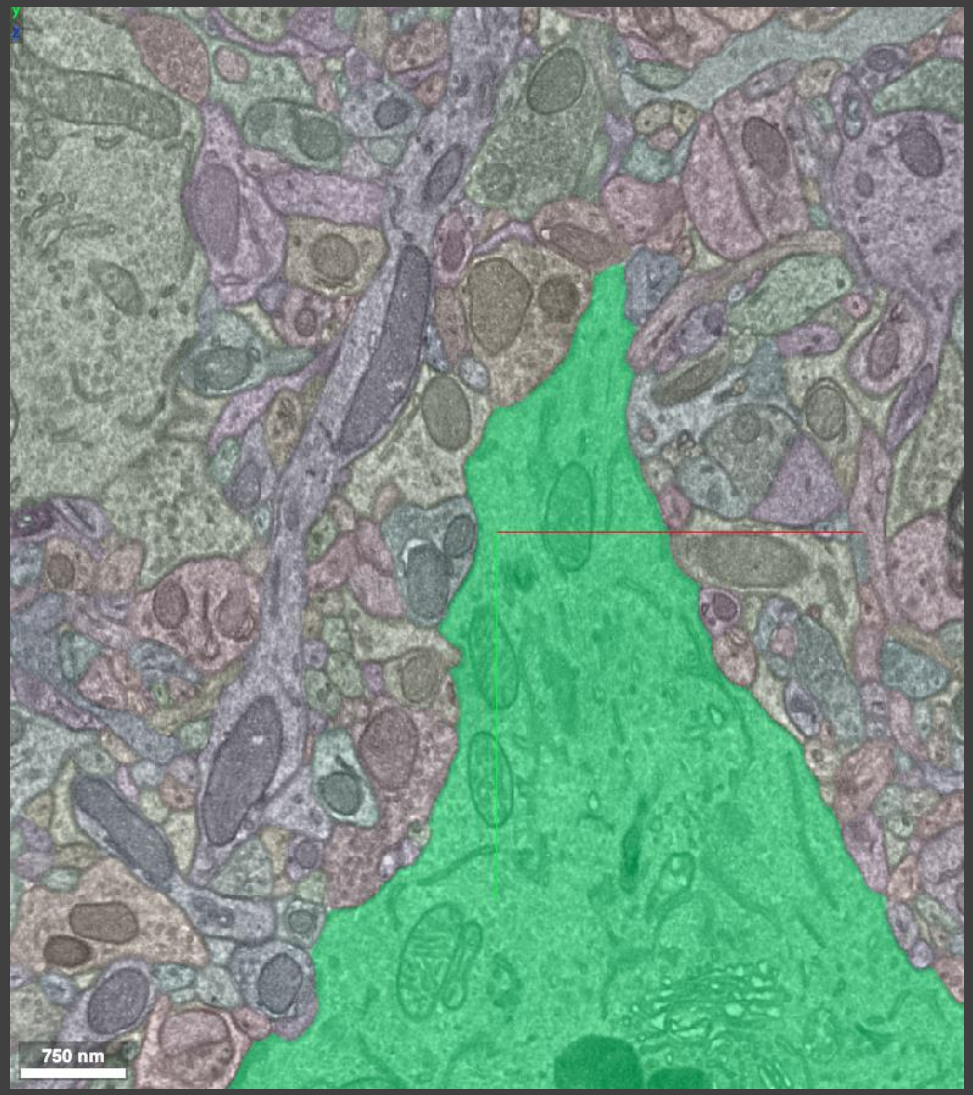
Segmentation challenge



www.microns-explorer.org/

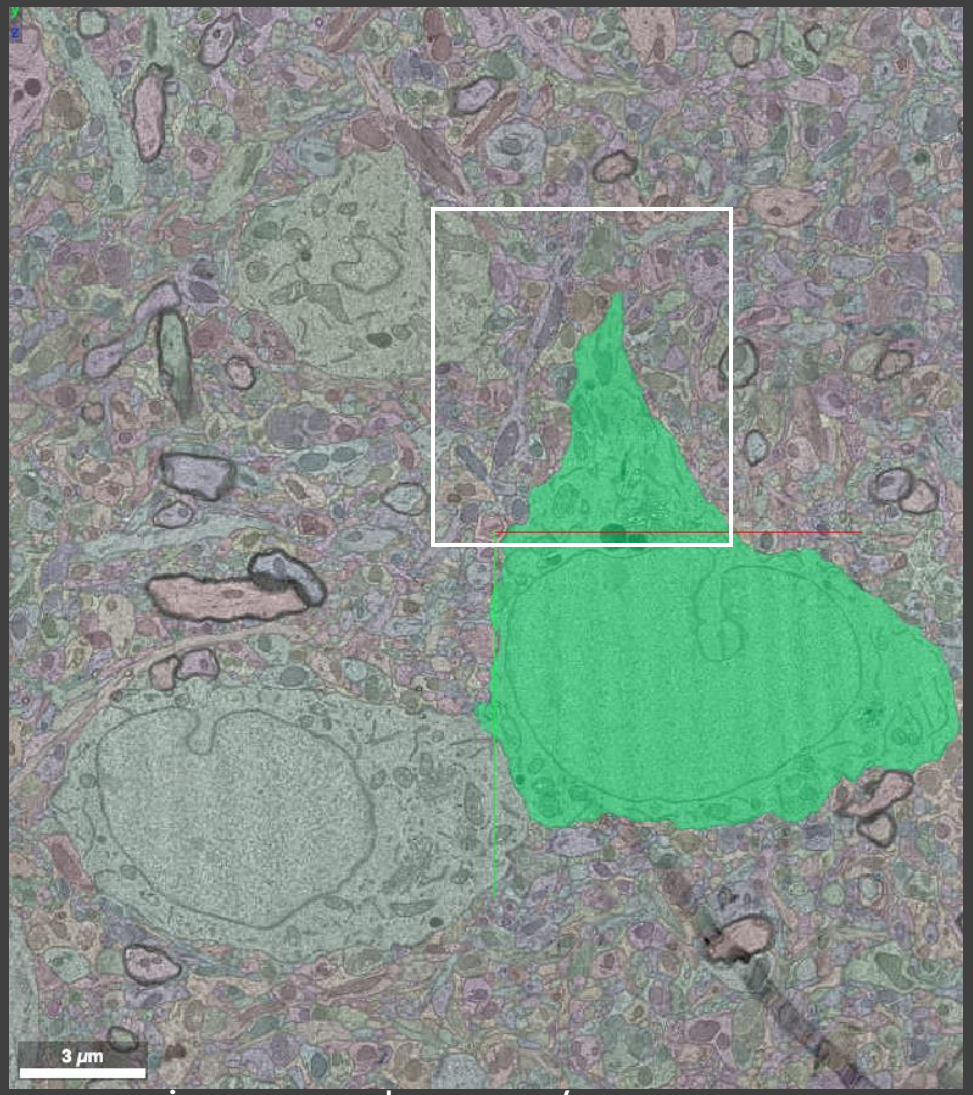
Segmentation challenge

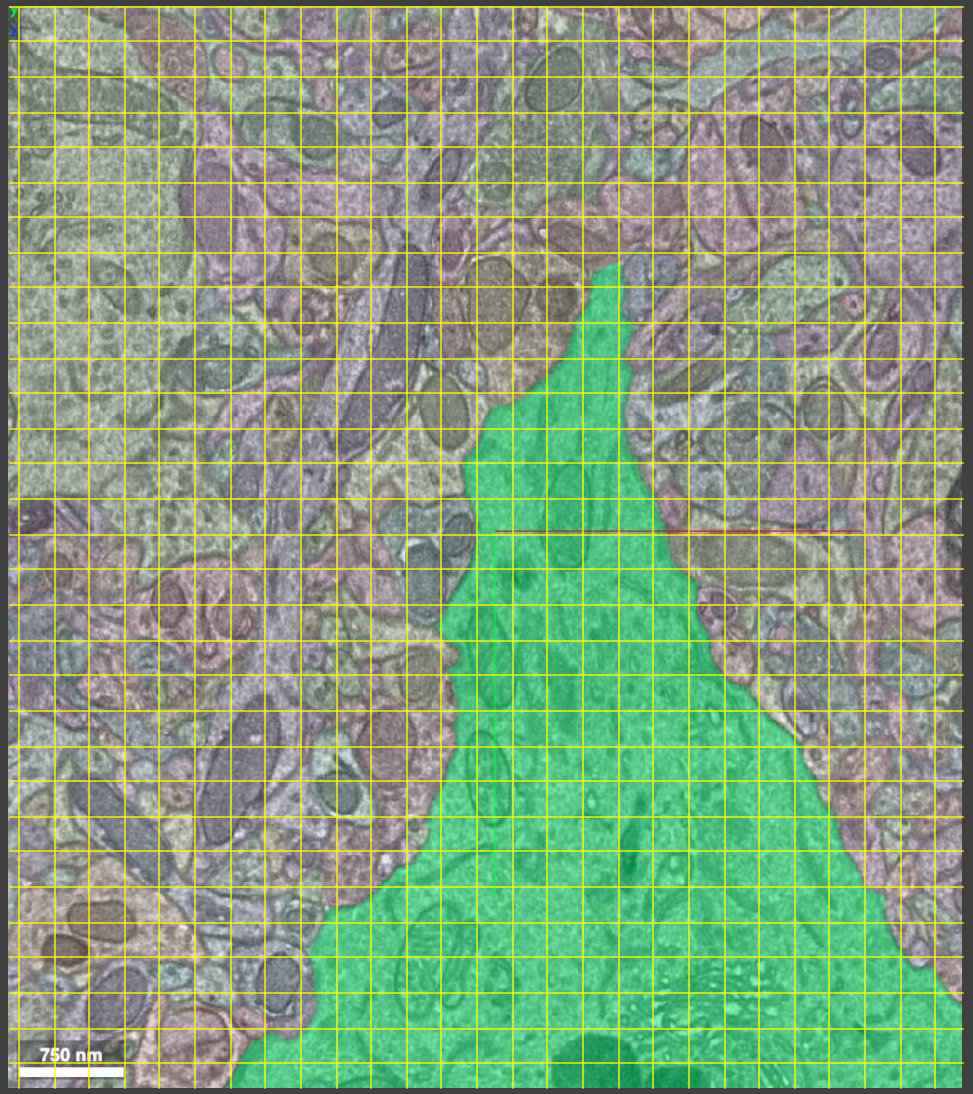




www.microns-explorer.org/

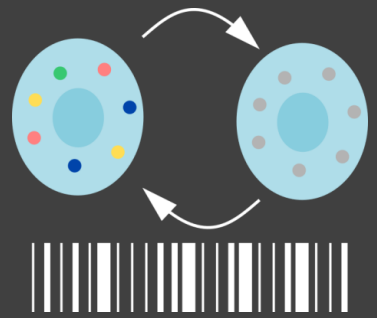
Segmentation challenge





www.microns-explorer.org/

Segmentation challenge



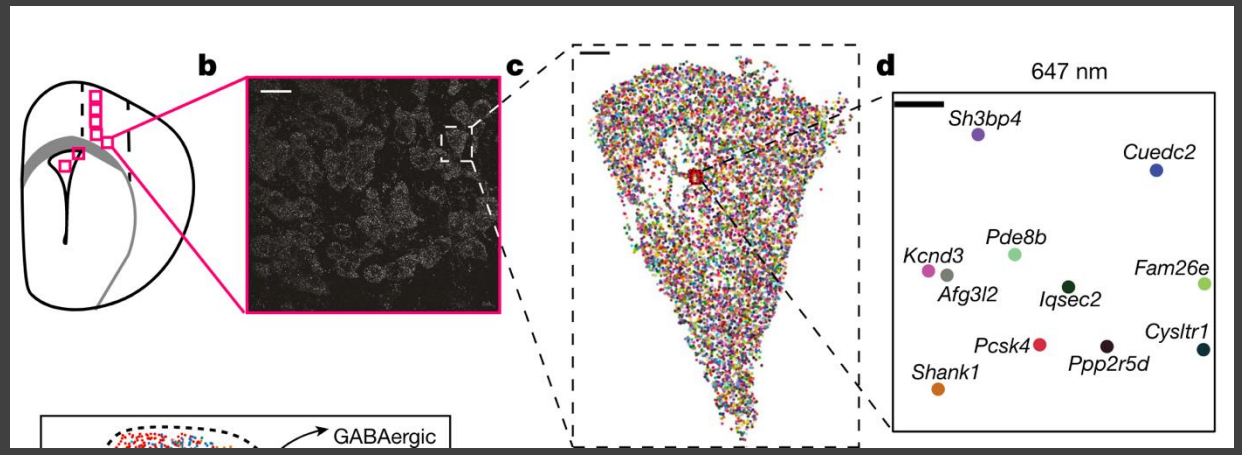
Limitations:

- Counter stains (membrane, nuclei, organelles, cell fill)
- Unclear ground truth
- Resolution

Progress:

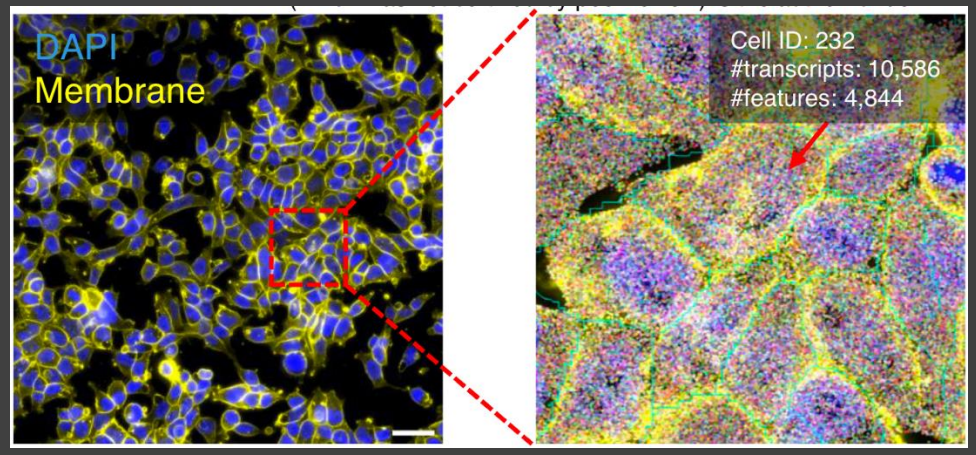
- Counter stains
- Algorithms
- Segmentation free approaches

seqFISH+



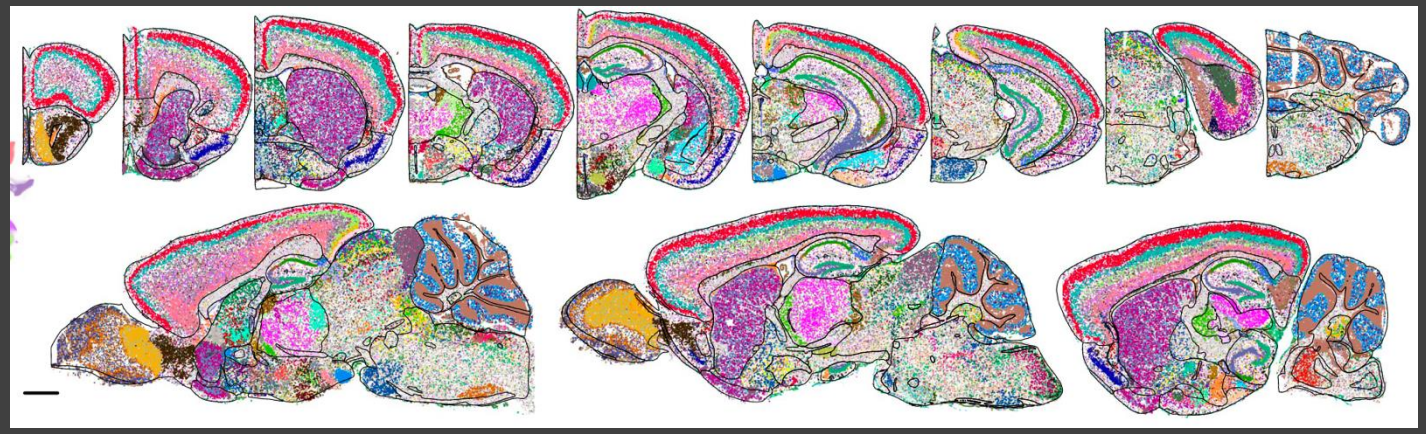
Eng *et al.* 2019 Nature. 10,000 plex

CosMx



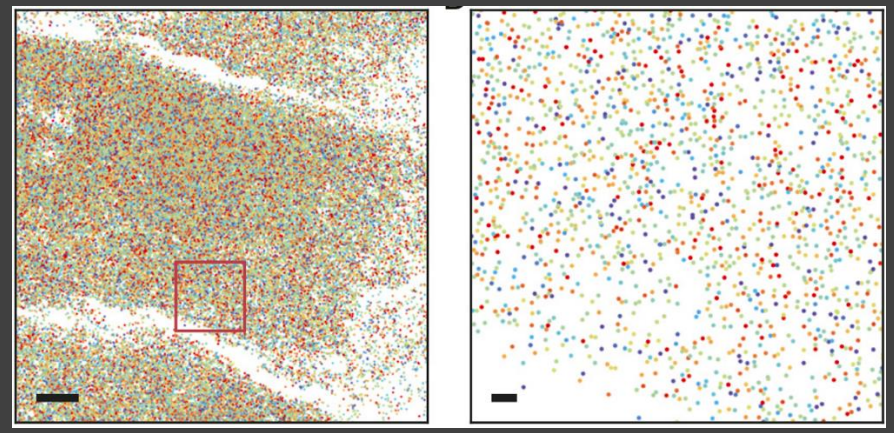
Khafizov *et al.* 2024 BioRxiv. 18,993 plex

MERFISH



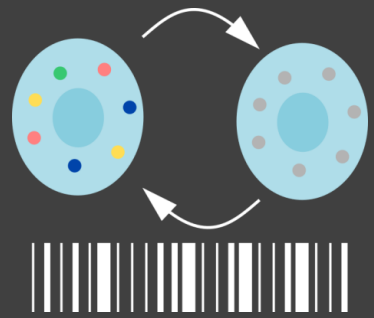
Zhang *et al.* 2023 Nature. Full mouse brain

MERFISH



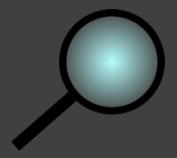
Xia *et al.* 2019 PNAS. 10,000 plex

Barcoded smFISH



Methods: MERFISH, seqFISH, EEL FISH

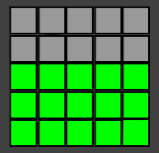
Companies: Vizgen, Spatial Genomics, Nanostring/Bruker, Resolve



Resolution: Diffraction limited (150-300nm)

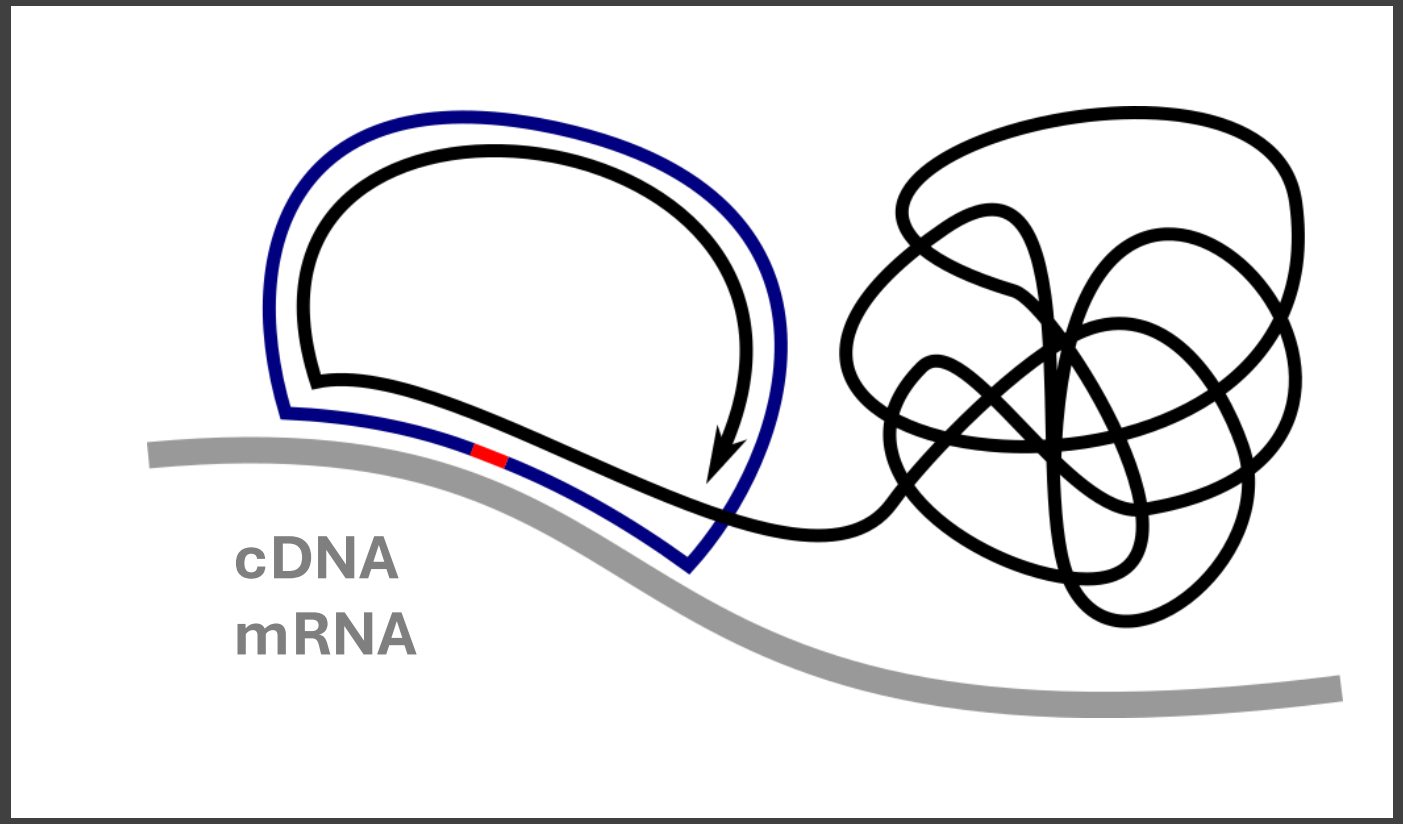
Detection efficiency: 70-90% *

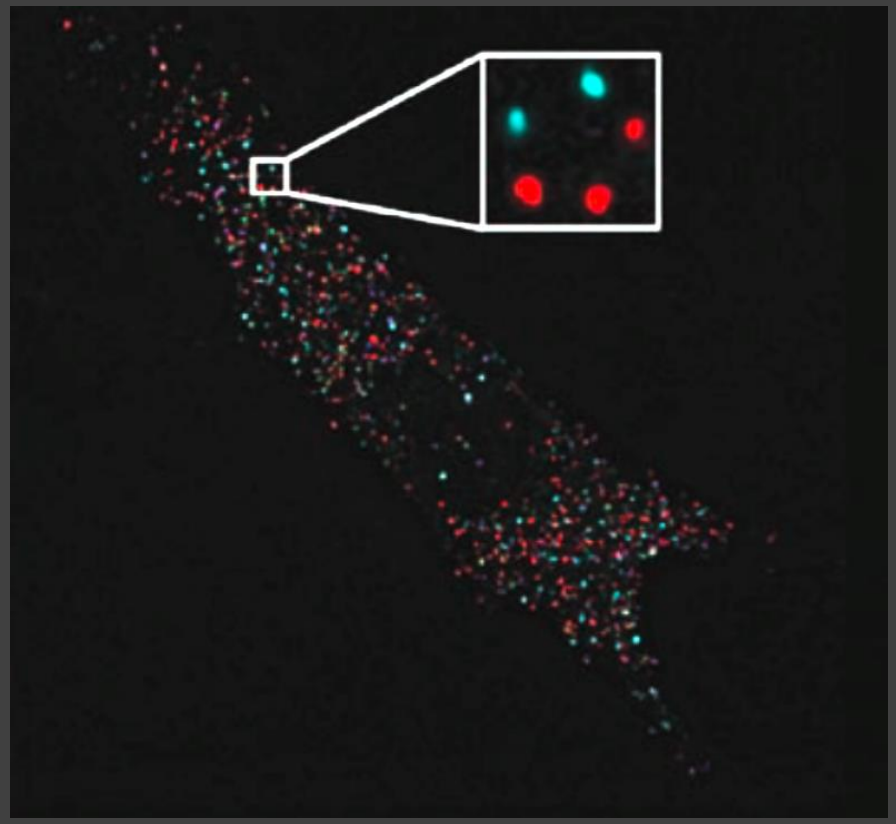
Gene throughput: 100 - 19,000



Spatial throughput: several mm^2 - cm^2

Rolling circle amplification

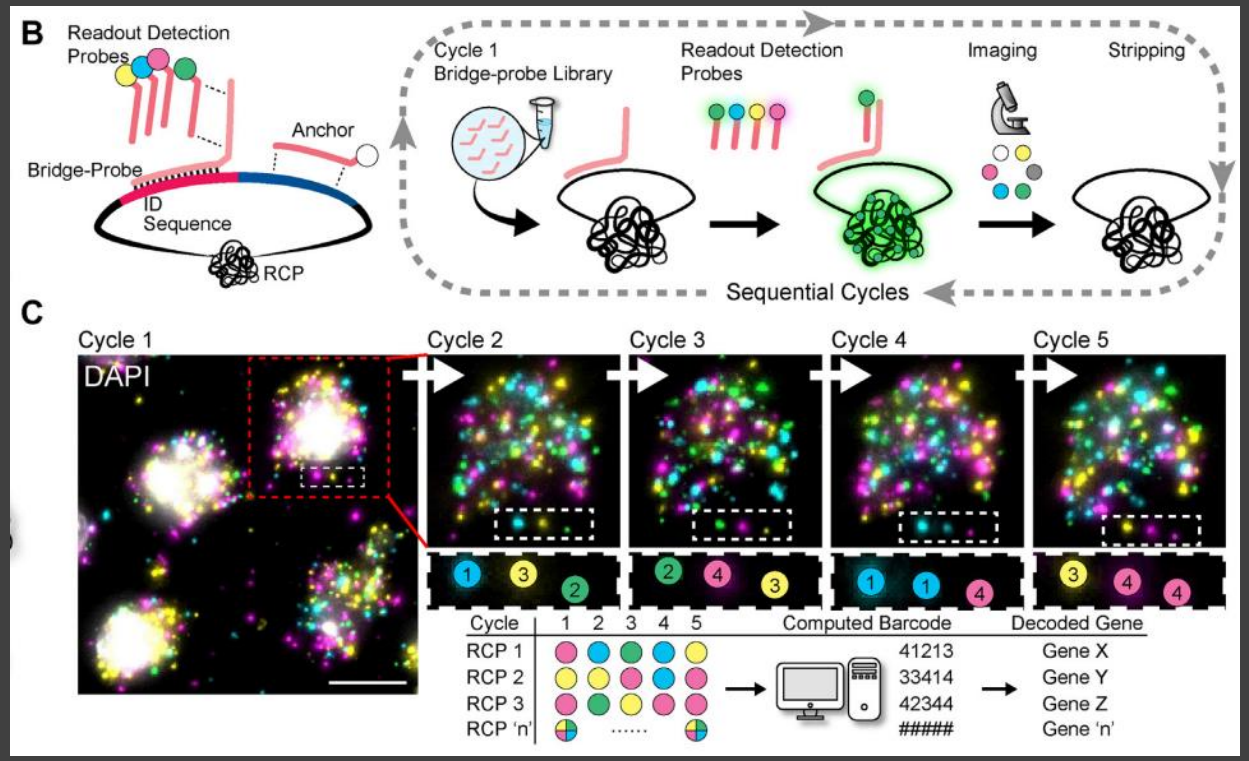




Padlock probe, Targeted, Enzymatic amplification

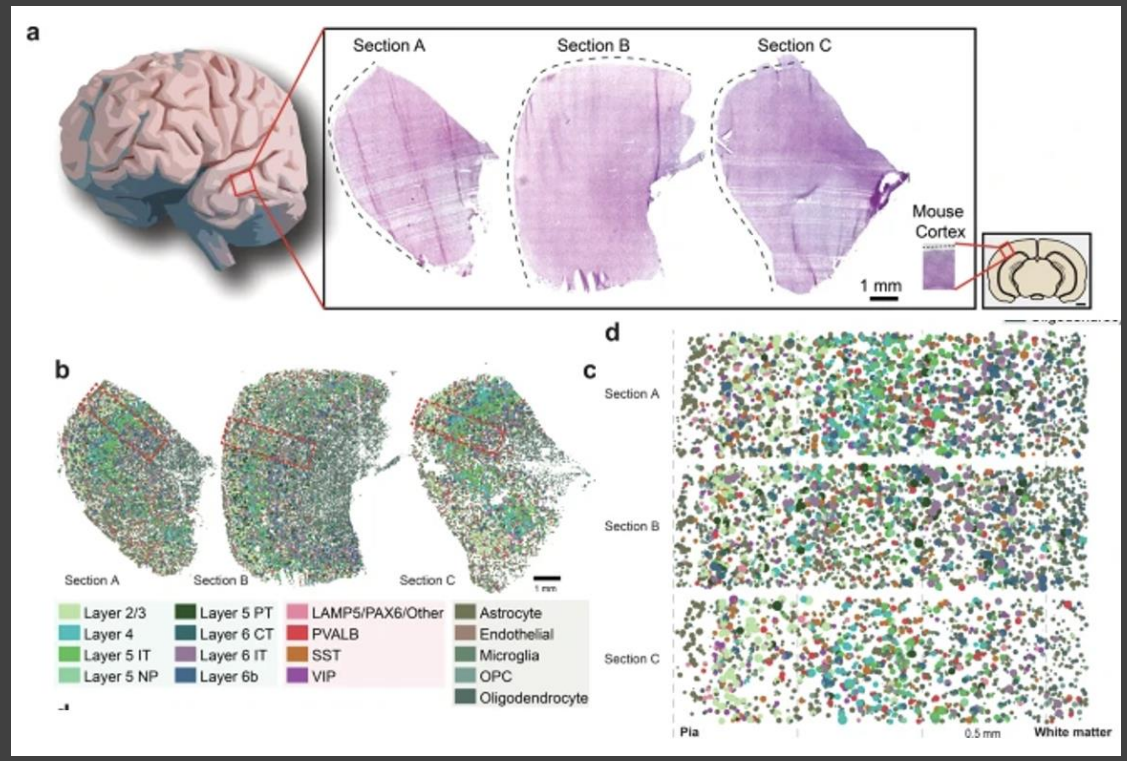
RCA amplified barcoded FISH

HybISS



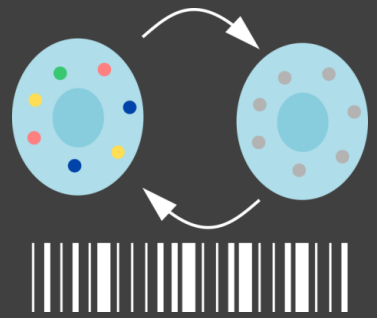
Gylborg *et al.* 2020 Nucleic Acids Research

HybISS



Mattsson Langseth *et al.* 2021 Nature Communications Biology

Amplified barcoded smFISH



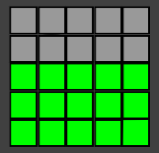
Methods: HybISS, HybRISS

Companies: 10X Xenium

Resolution: Amplicon size ~0.5-1 um

Detection efficiency: 10 - ~50%

Gene throughput: 100 – 1,000

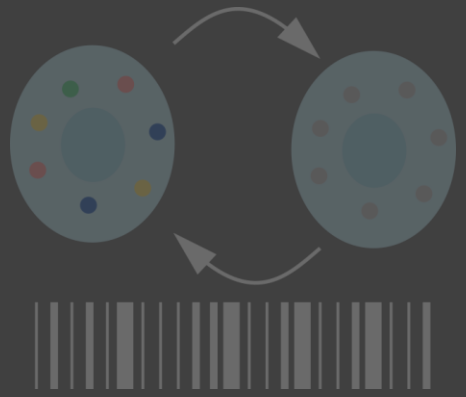


Spatial throughput: cm^2 - several cm^2

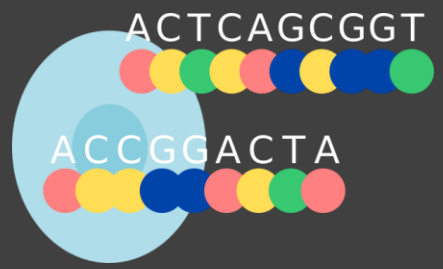
spatial RNA detection

Microscopy

Barcoded FISH



in situ Sequencing



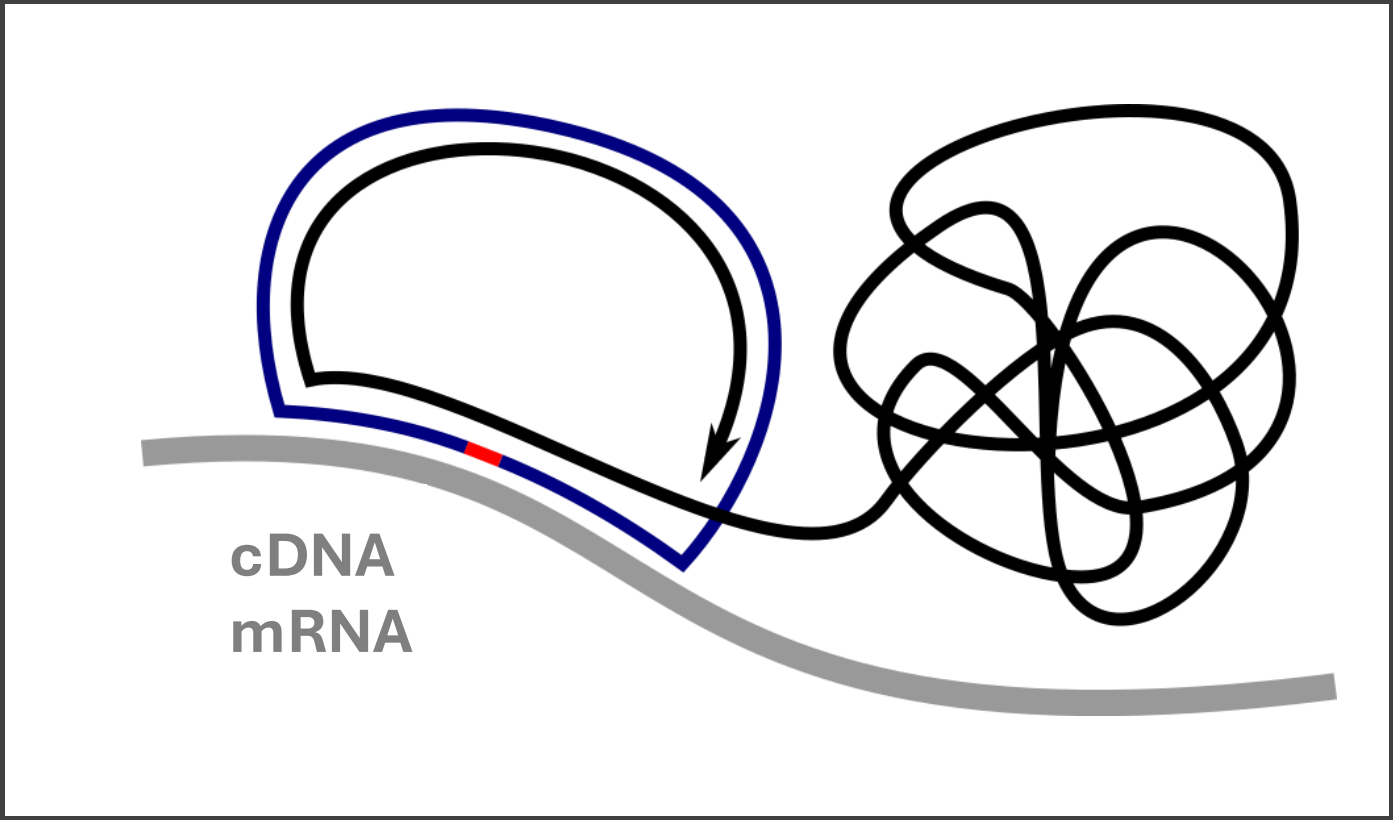
Sequencing

Spatial Sequencing

Spatial tagging

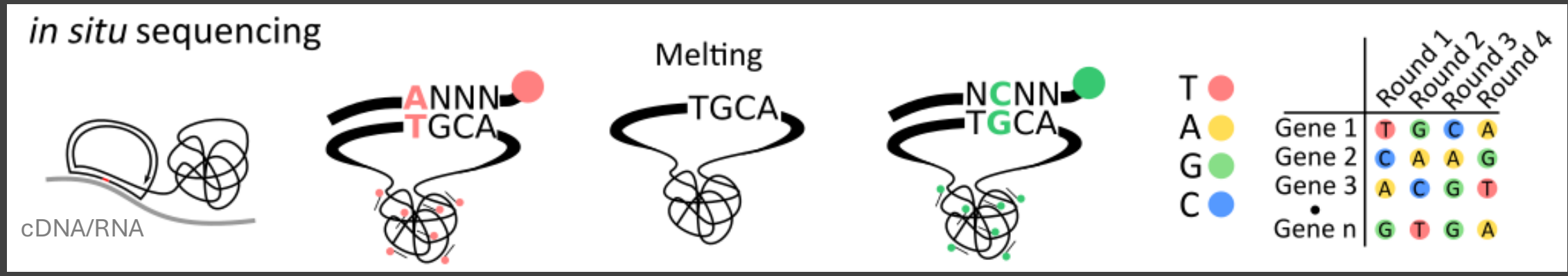
in situ sequencing

Rolling circle amplification



Padlock probe. Targeted

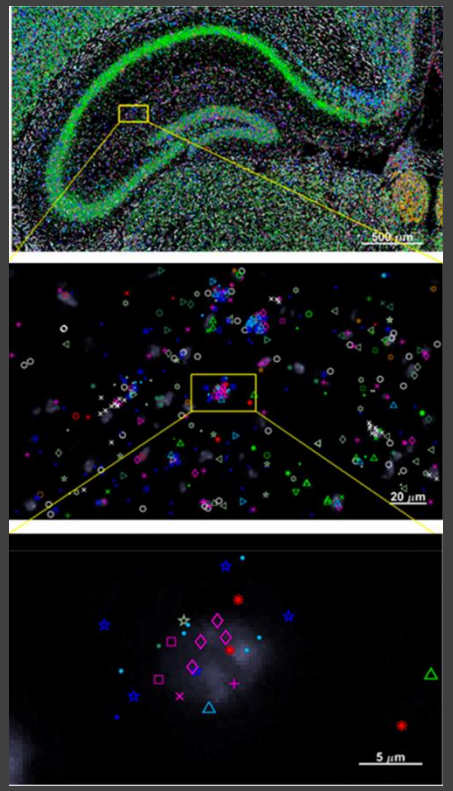
in situ sequencing (ISS)



Sequencing by ligation

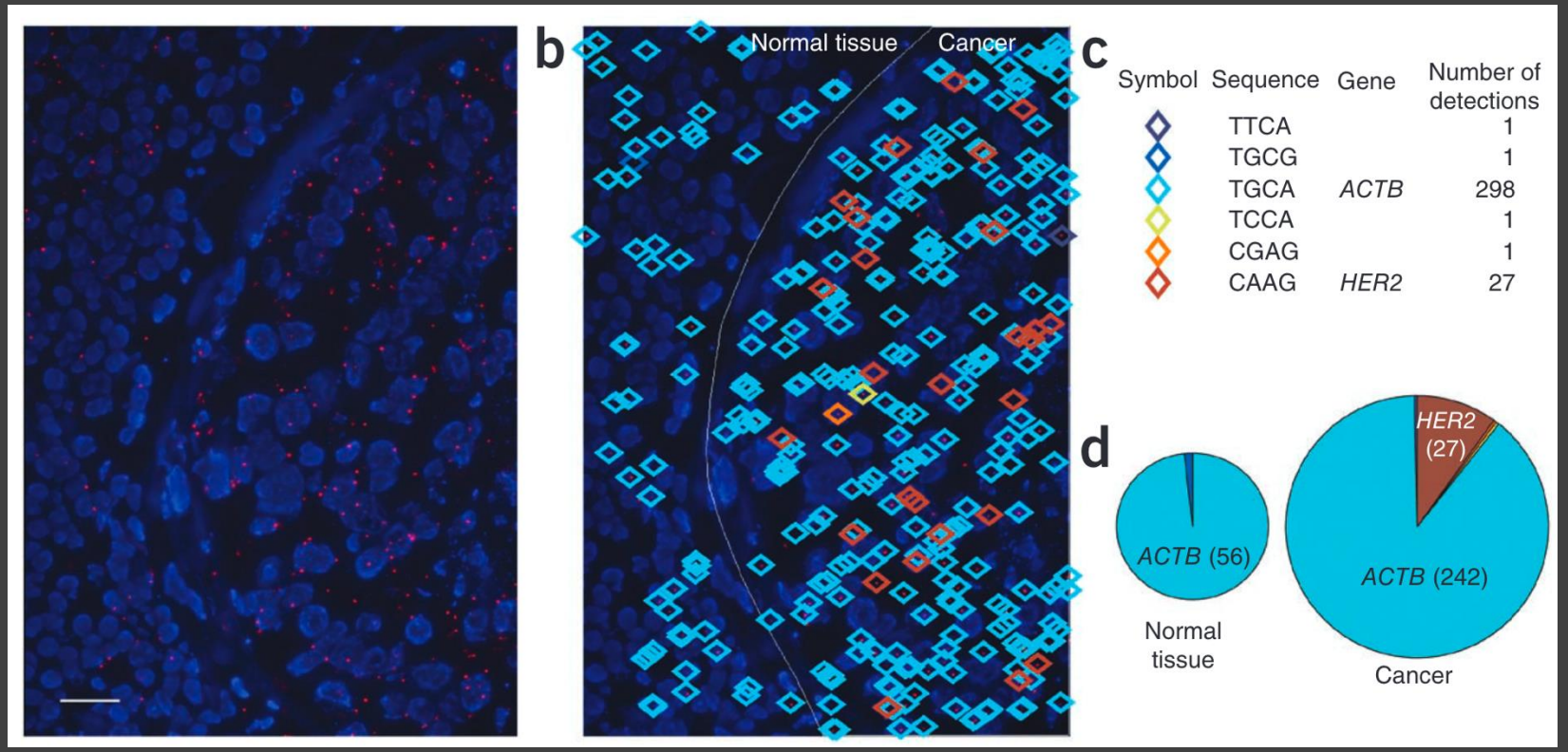
in situ sequencing (ISS)

Barcode sequencing



Qian *et al*. 2020 Nature Methods

De-novo sequencing

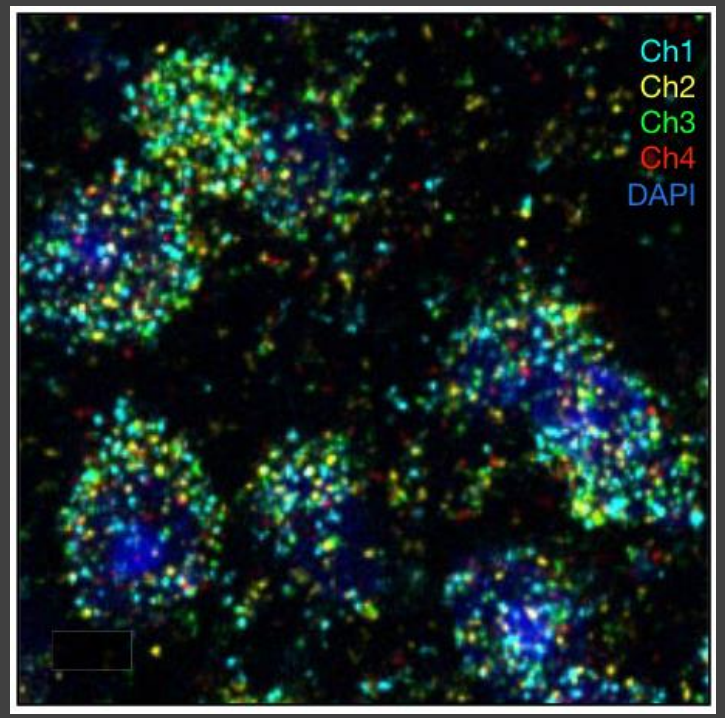


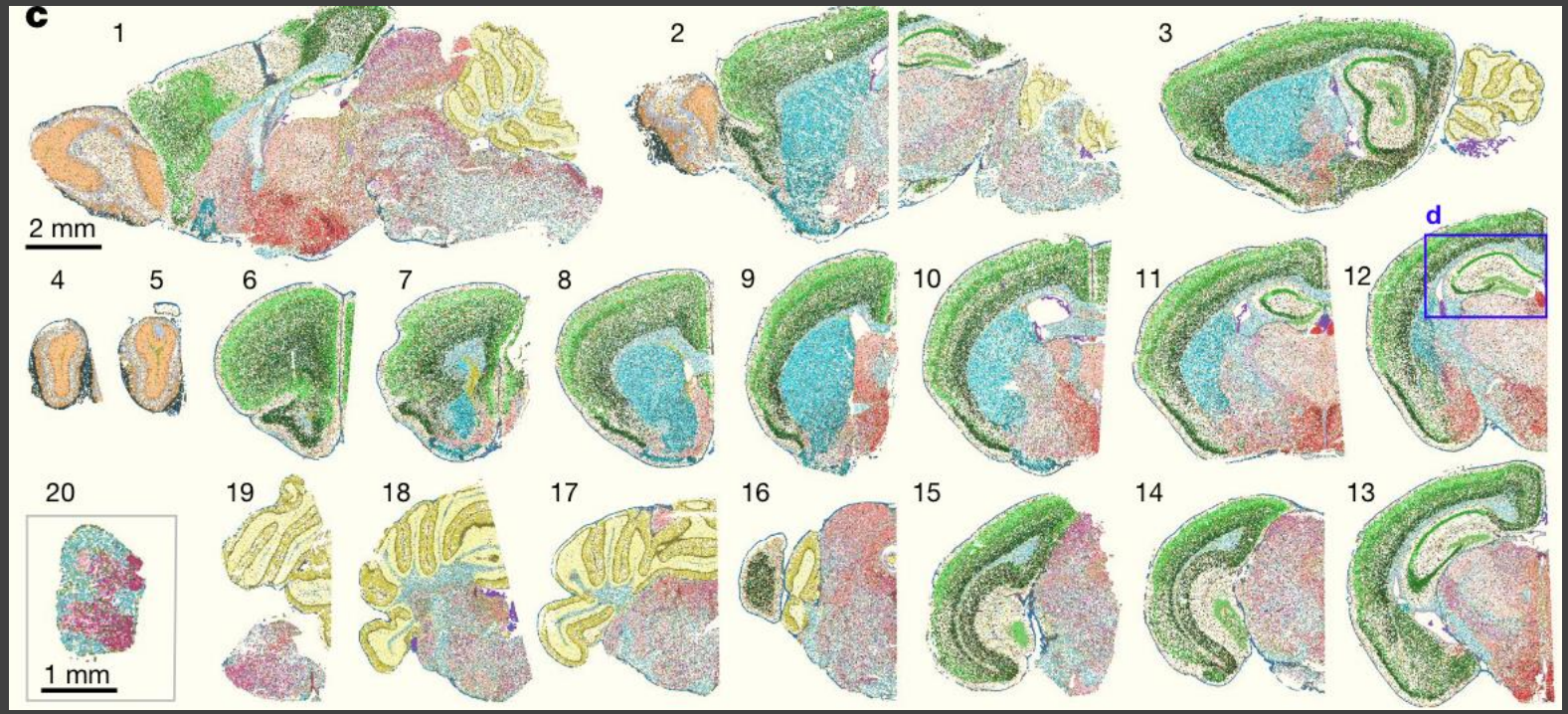
Ke *et al*. 2013 Nature Methods

ISS is the predecessor of Xenium

STARmap

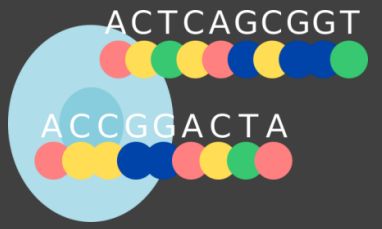
SEDAL sequencing





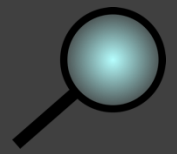
Hailing *et al*. 2023 Nature

Sequencing *in situ*



Methods: ISS, STARmap.

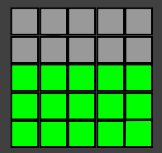
Commercial: (Xenium), StellarOmics



Resolution: Amplicon size (0.5 - 1um)

Detection efficiency: 10 - ~50%

Gene throughput: 10 - 1,000

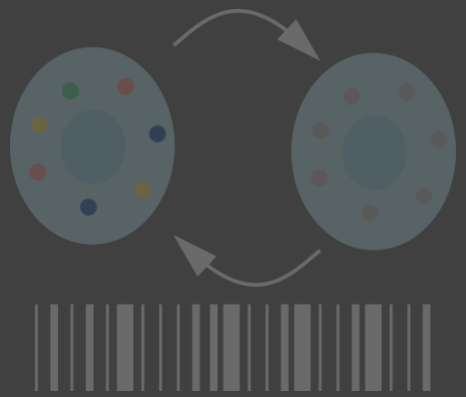


Spatial throughput: several mm^2 - several cm^2

spatial RNA detection

Microscopy

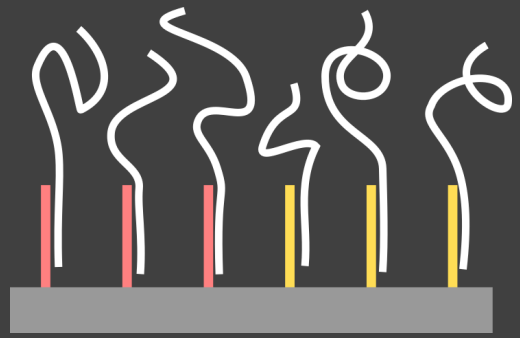
Barcoded FISH



in situ Sequencing

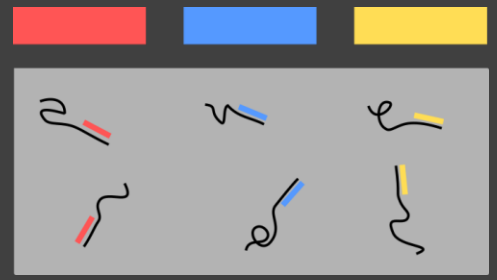
Sequencing

Spatial Sequencing



RNA moves

Spatial tagging

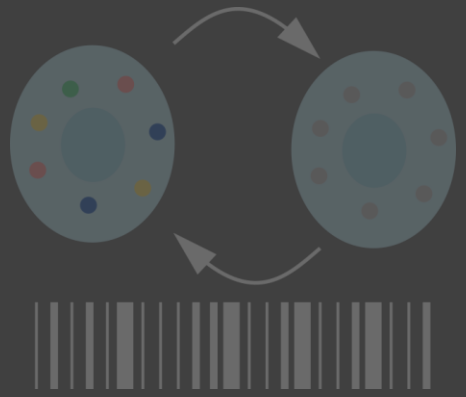


Barcodes move

spatial RNA detection

Microscopy

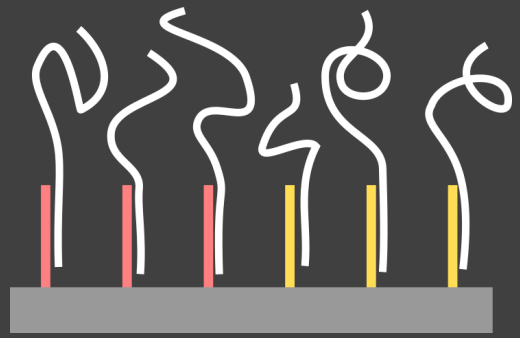
Barcoded FISH



in situ Sequencing

Sequencing

Spatial Sequencing

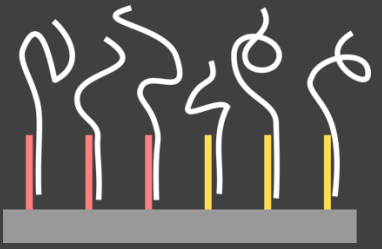


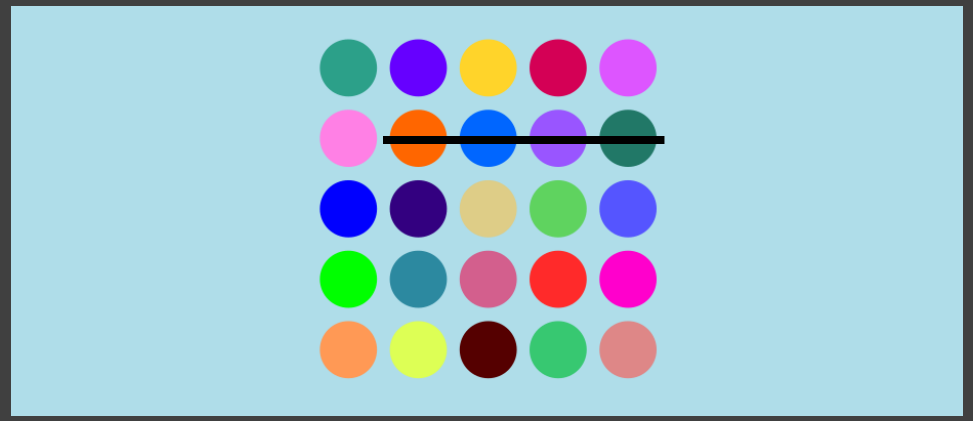
RNA moves

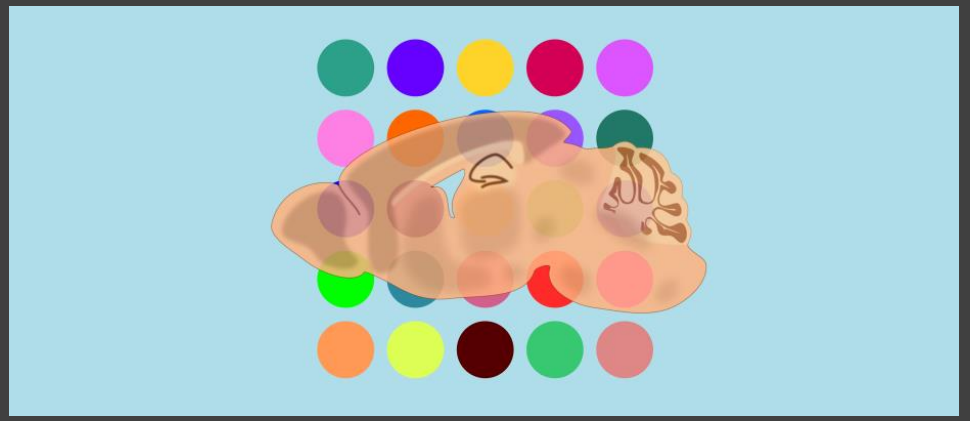
Spatial tagging

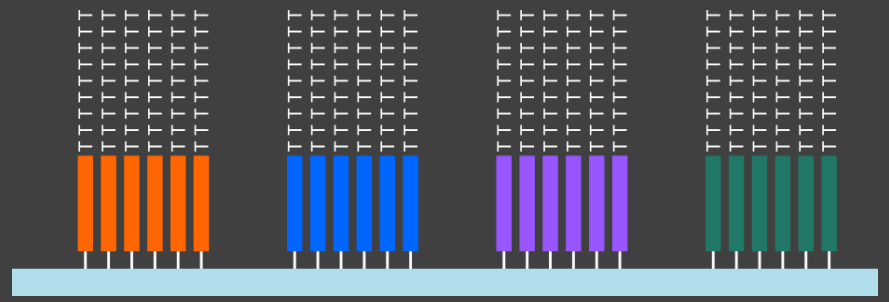
Barcodes move

Spatial transcriptomics

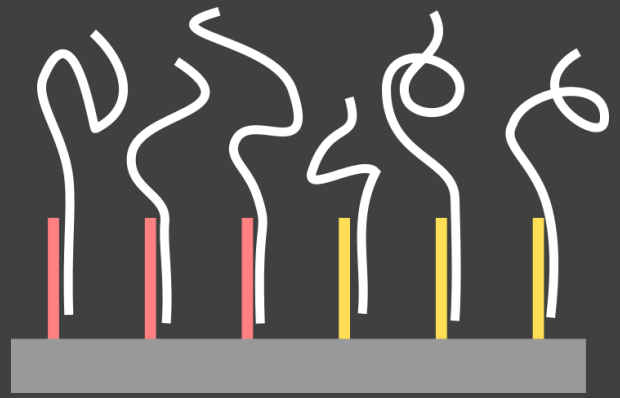






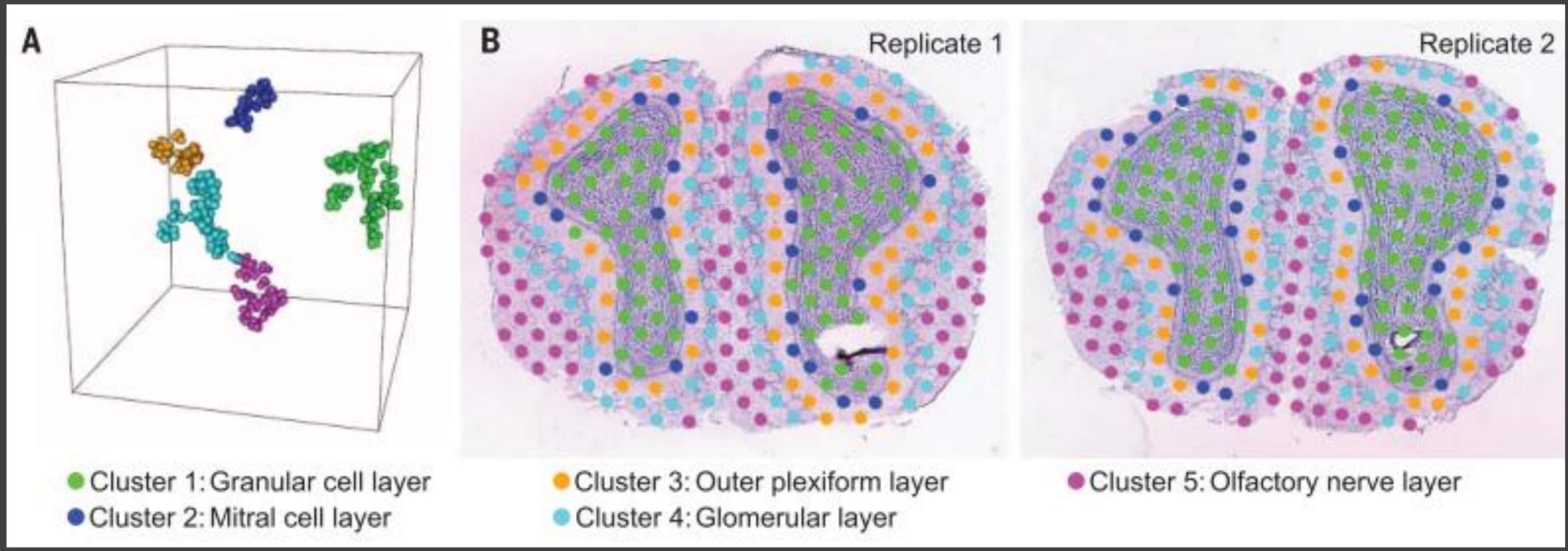


Microarray with spatial barcodes



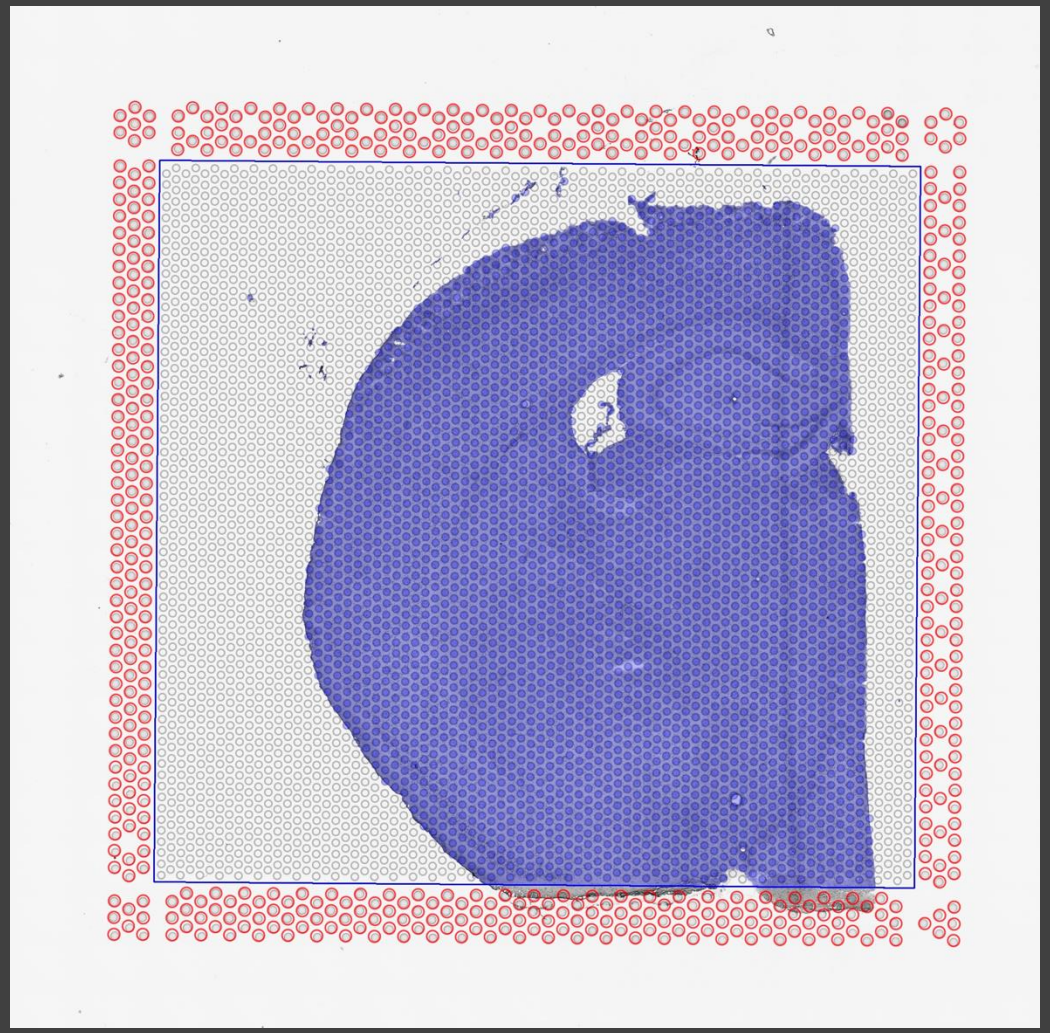
Stahl *et al.* Science 2016

Spatial transcriptomics

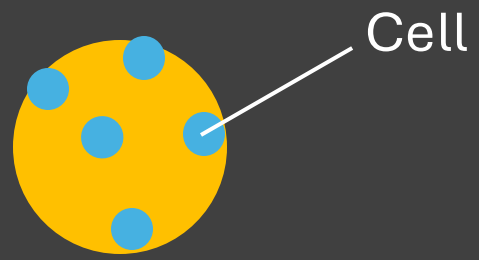


Stahl *et al.* Science 2016

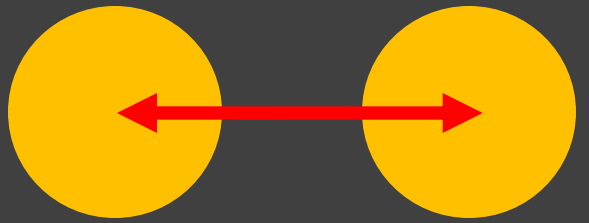
10X Visium



10X Visium

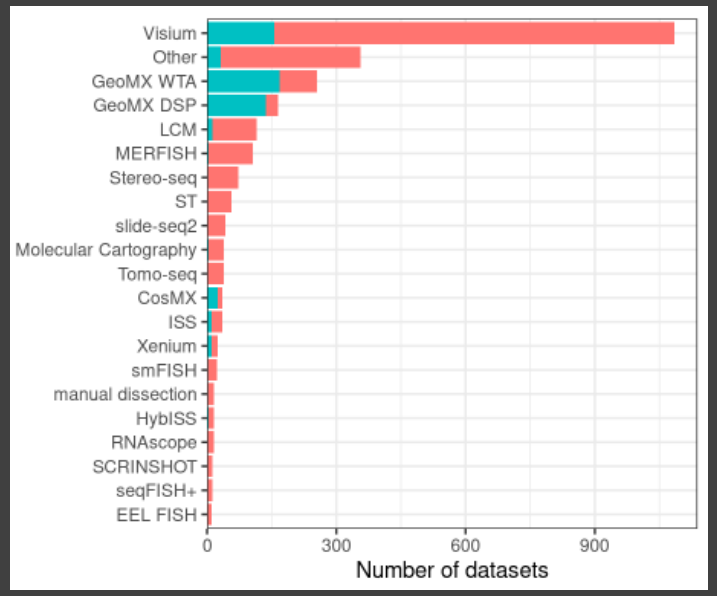


Spot size: 55 µm

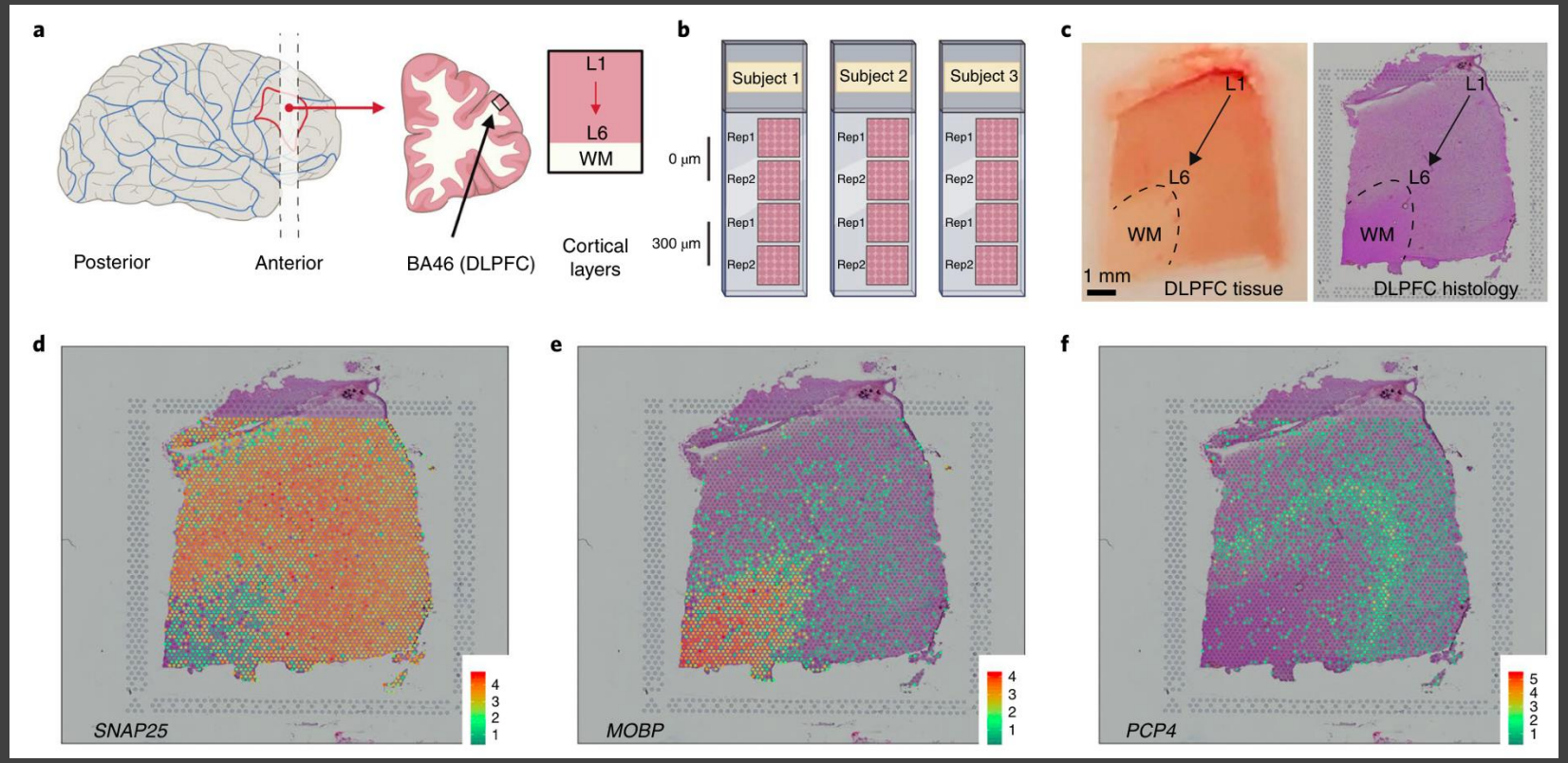


Spot spacing: 100 µm

10X Visium

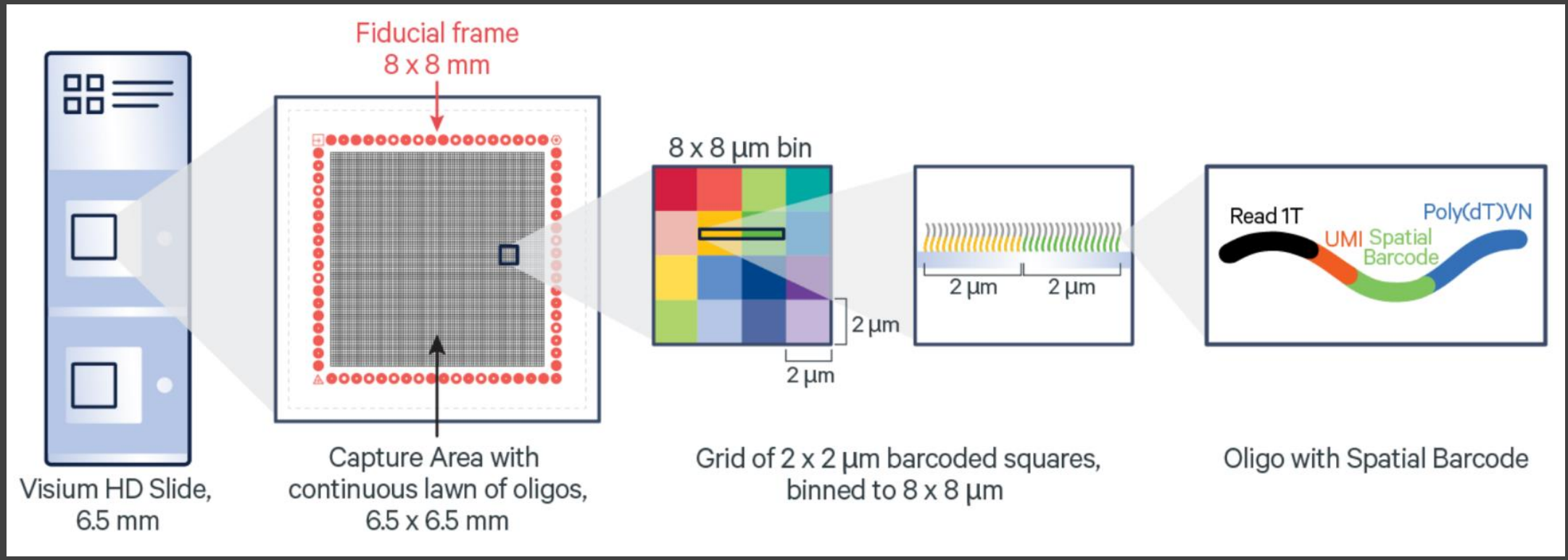


pachterlab.github.io/LP_2021/



Maynard *et al.* 2021 Nature Neuroscience

10X Visium HD



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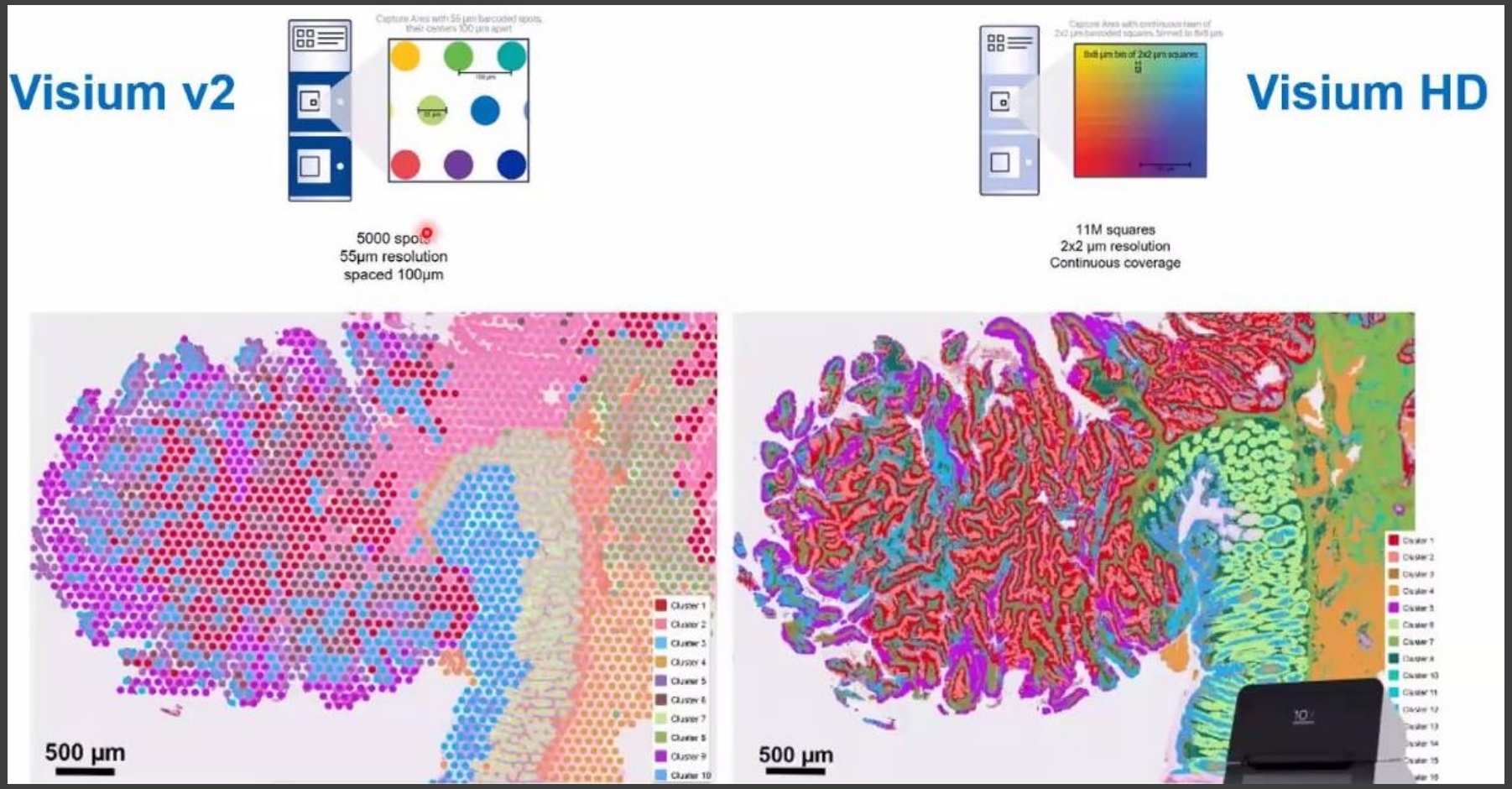
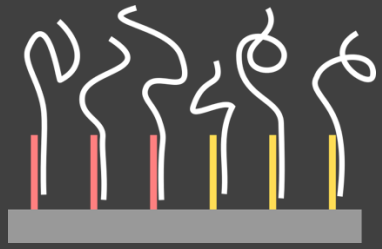
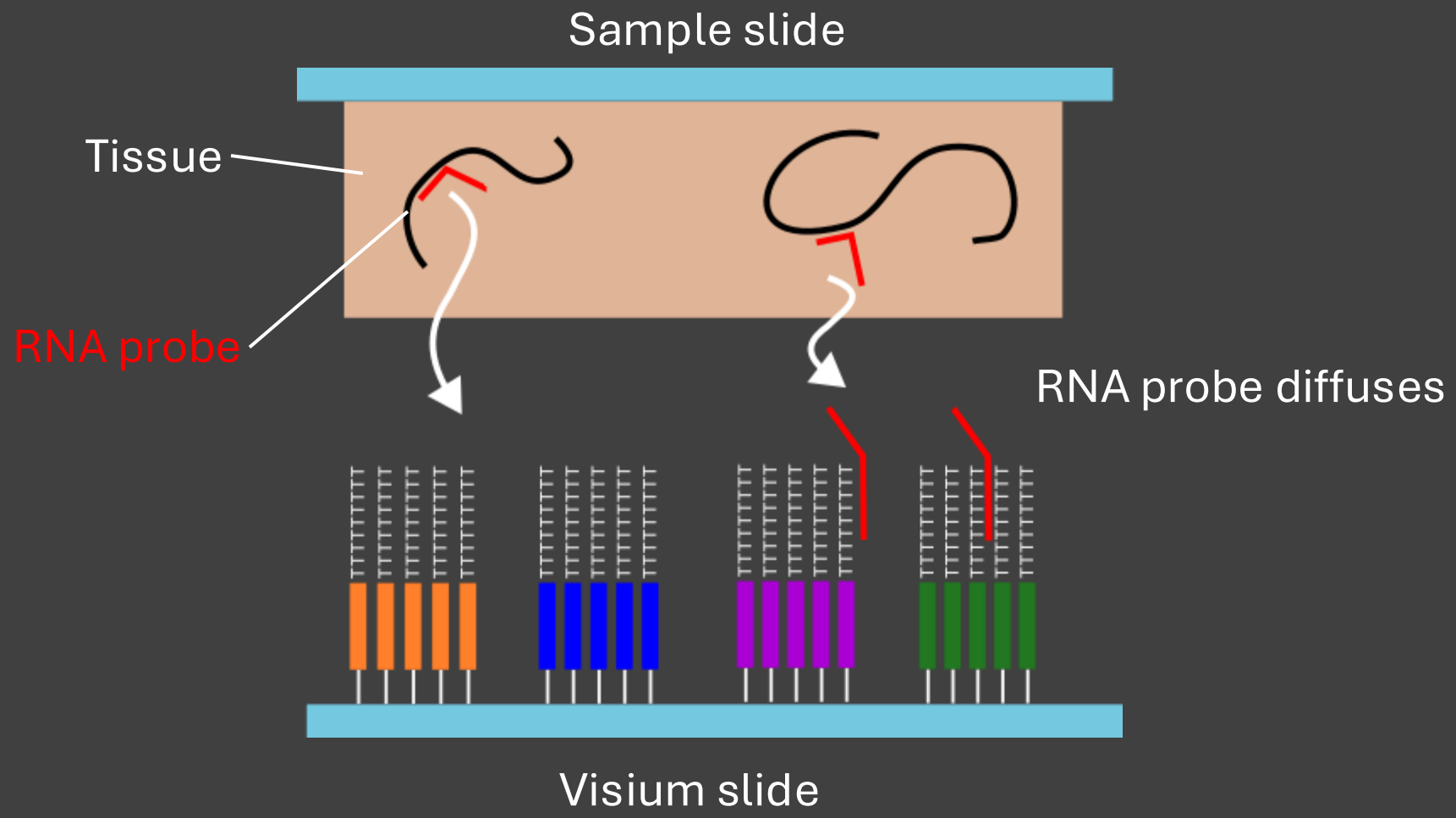


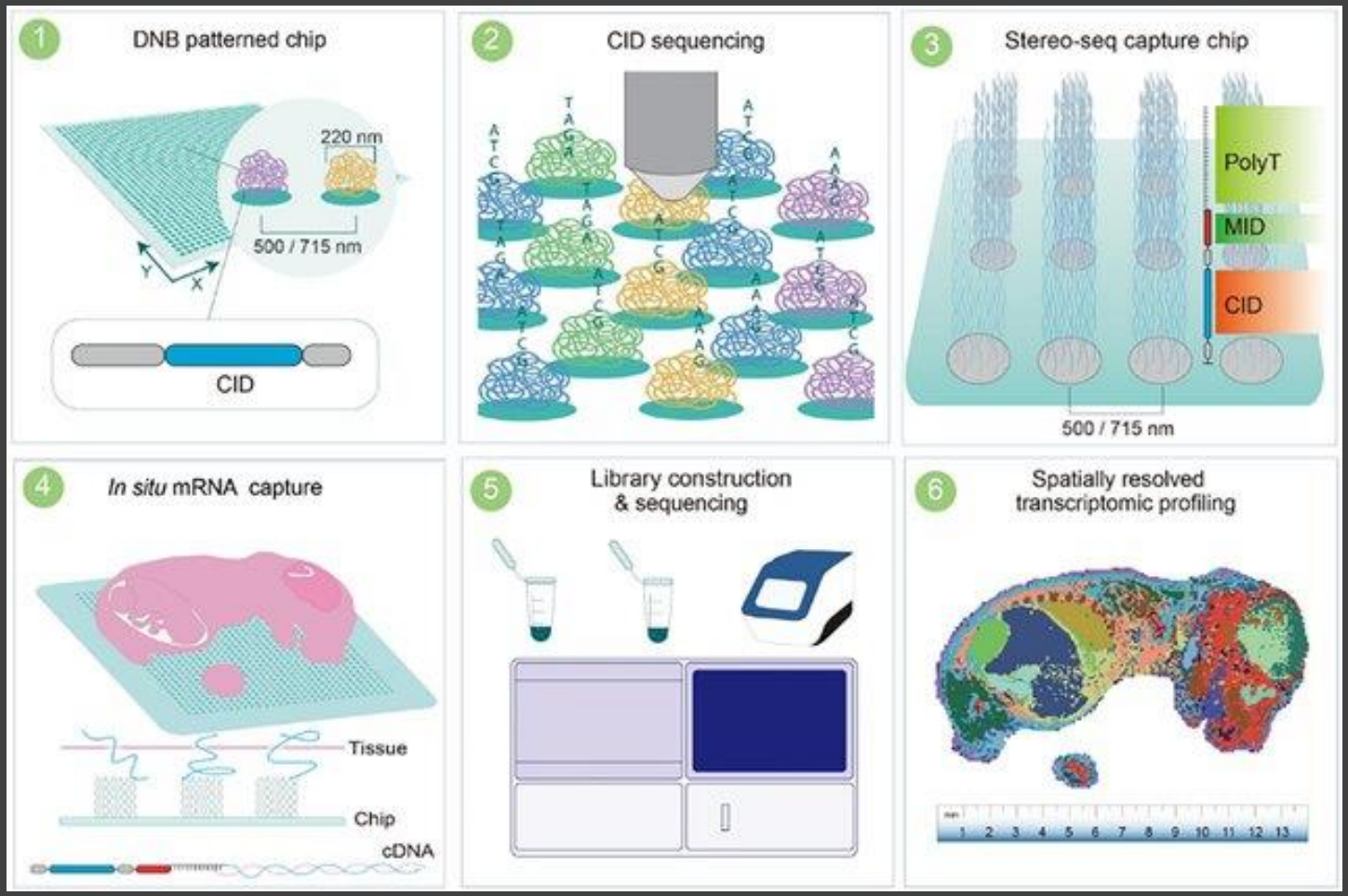
10X Visium HD

10X CytAssist





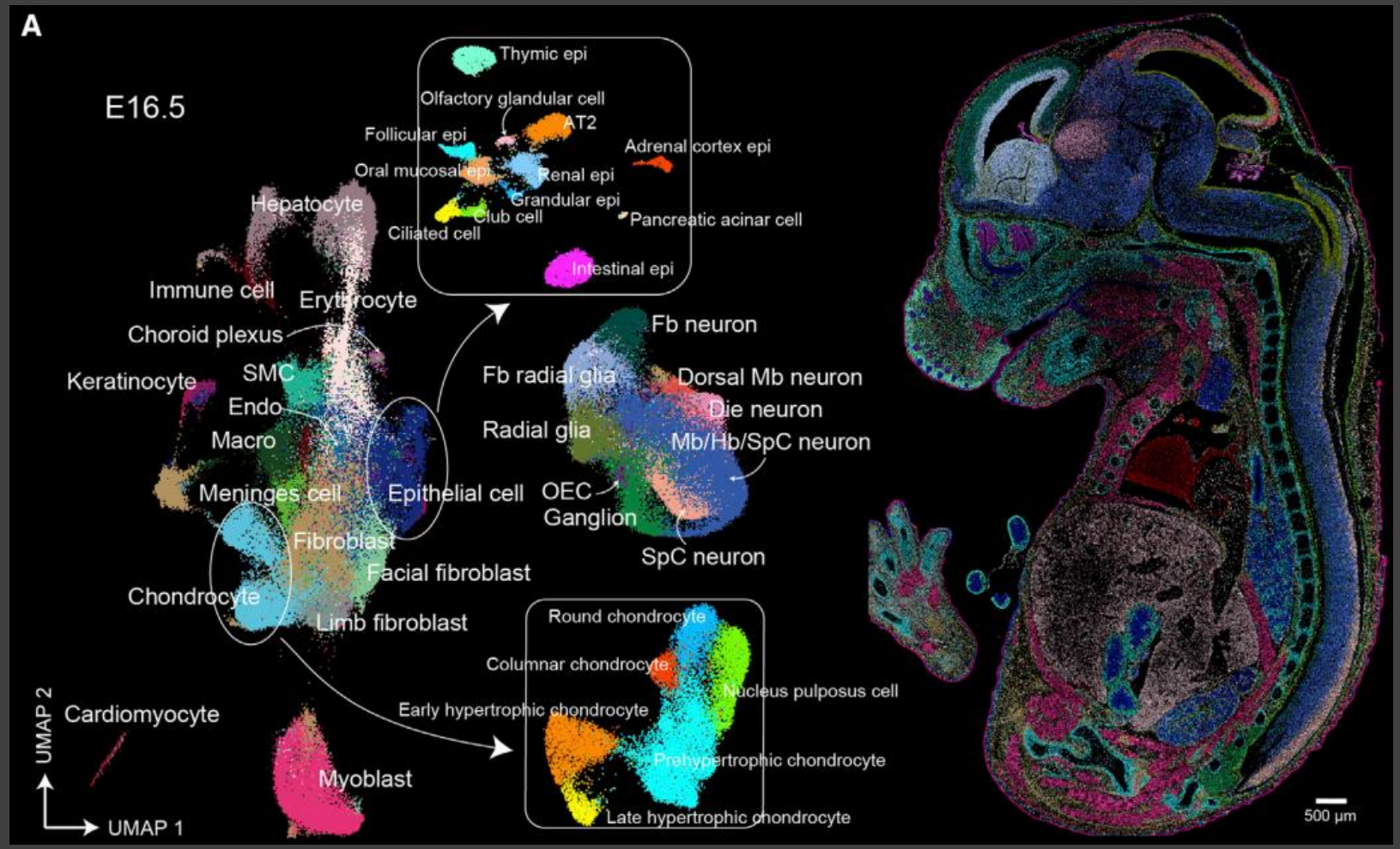
Stereo-seq



220nm DNA nanoballs

Chen *et al*. Cell 2022

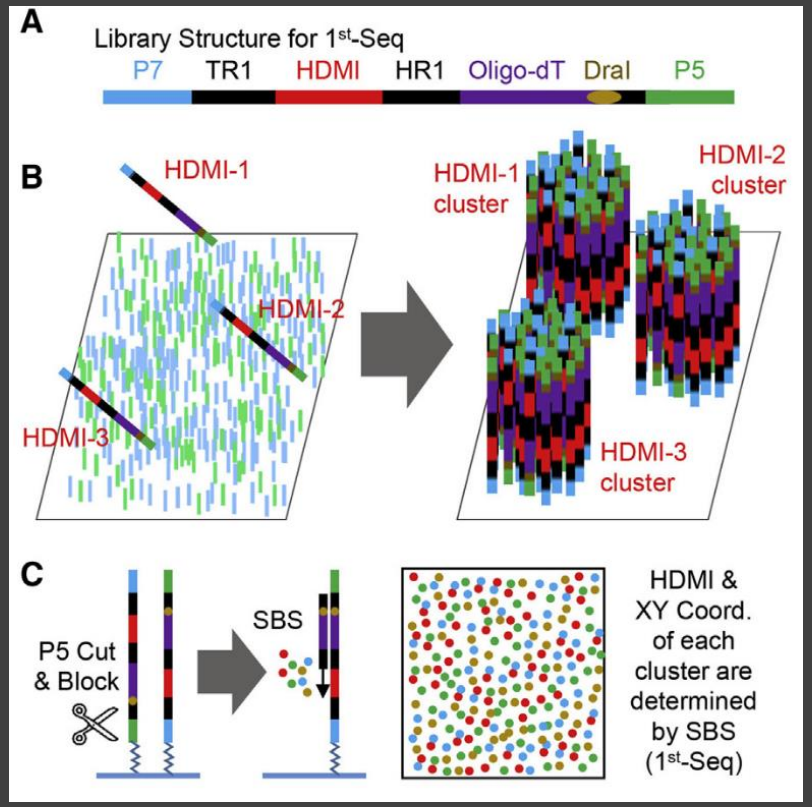
Stereo-seq



Chen *et al*. Cell 2022

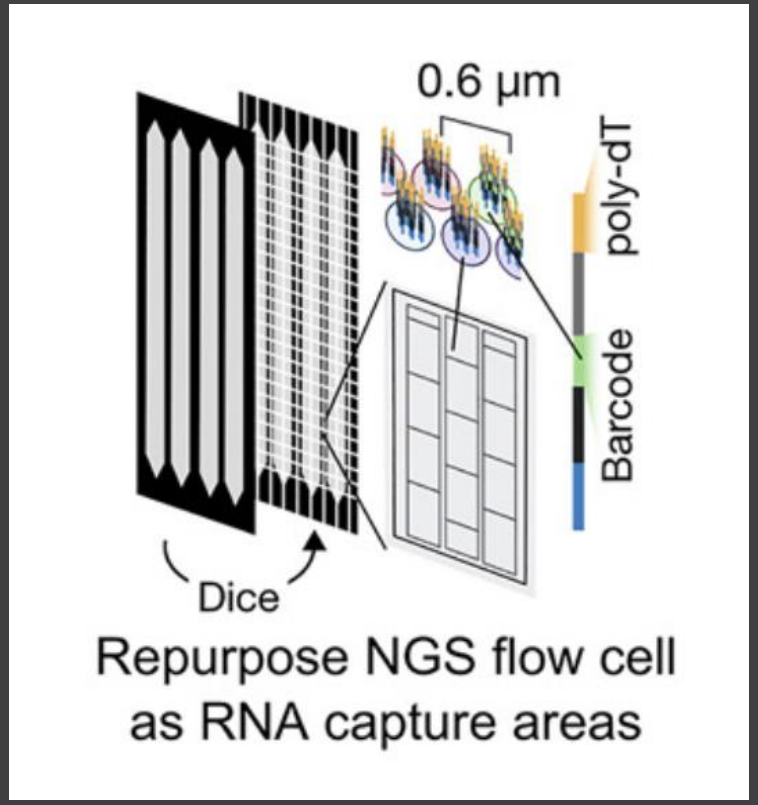
Seq-Scope, Open-ST, Nova-ST

Seq-Scope



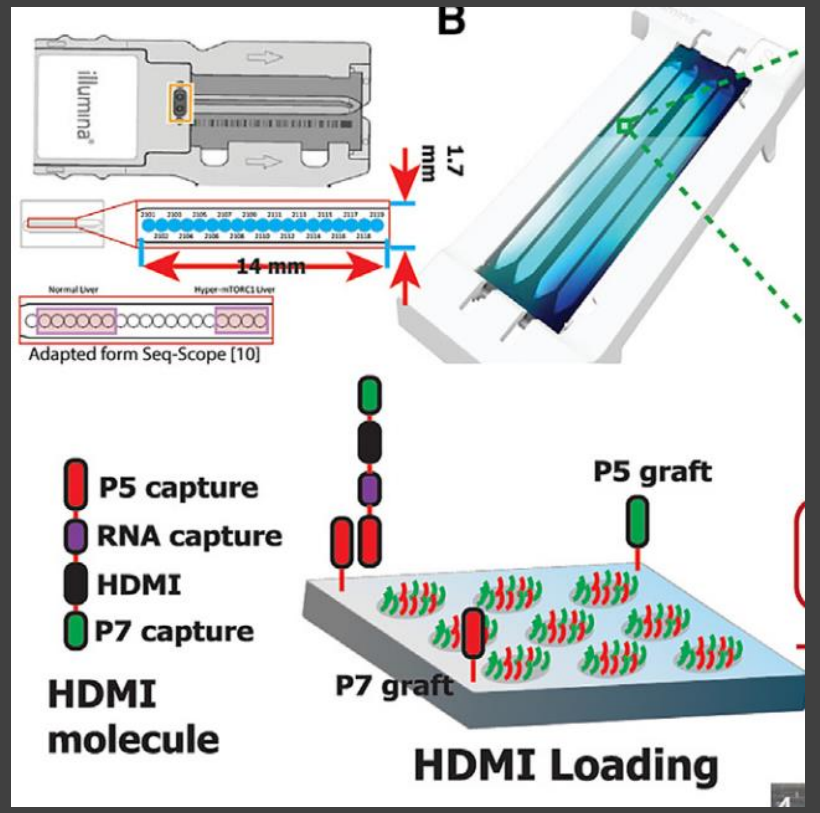
Cho *et al*. 2021 Cell

Open-ST



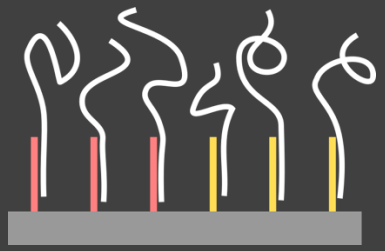
Schott *et al*. 2024 Cell Reports Methods

Nova-ST



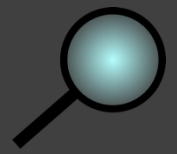
Poovathingal *et al*. 2024 Cell Reports Methods

Spatial Sequencing



Methods: ST, Slide-seq, Stereo-seq.

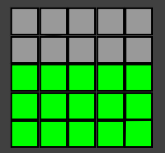
Commercial: 10X Visium, Curio Seeker



Resolution: Spot size 220nm - 100um (but RNA diffuses)

Detection efficiency: 0.1 - 5%

Gene throughput: Full transcriptome

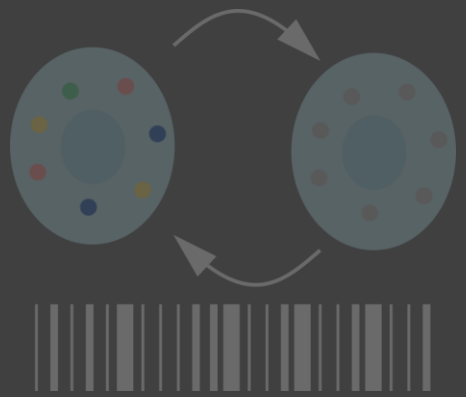


Spatial throughput: several mm^2 – several cm^2

spatial RNA detection

Microscopy

Barcoded FISH



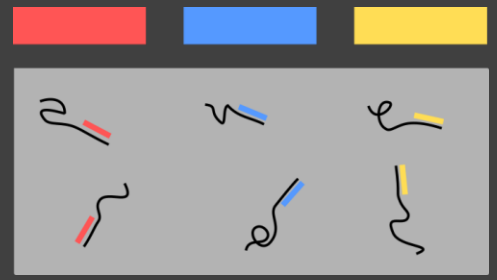
in situ Sequencing

Sequencing

Spatial Sequencing

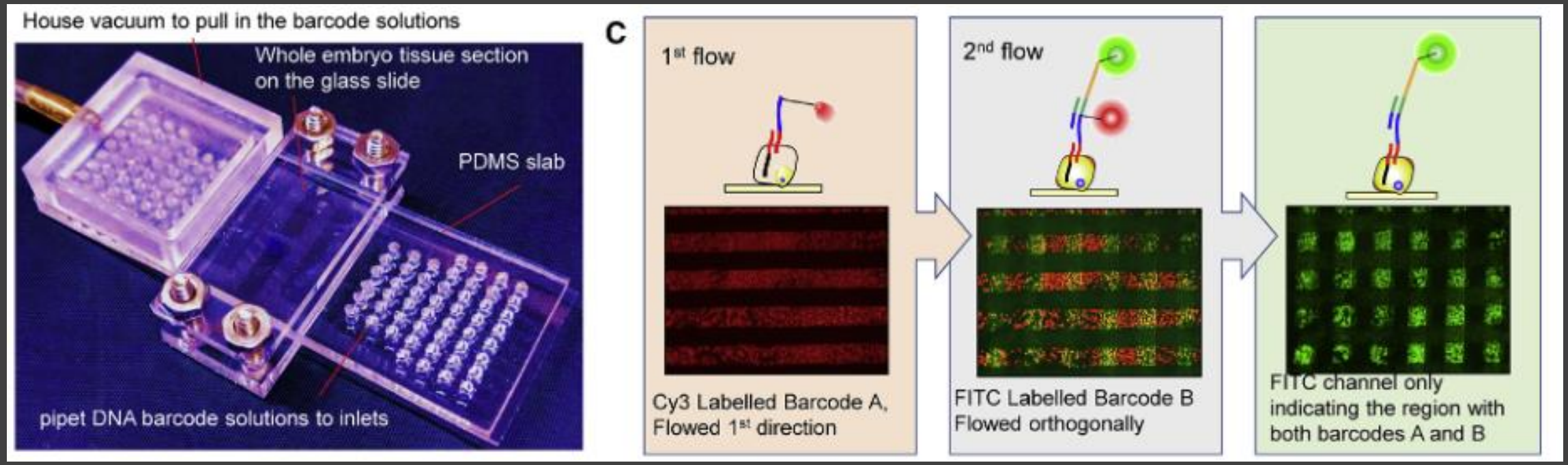
RNA moves

Spatial tagging

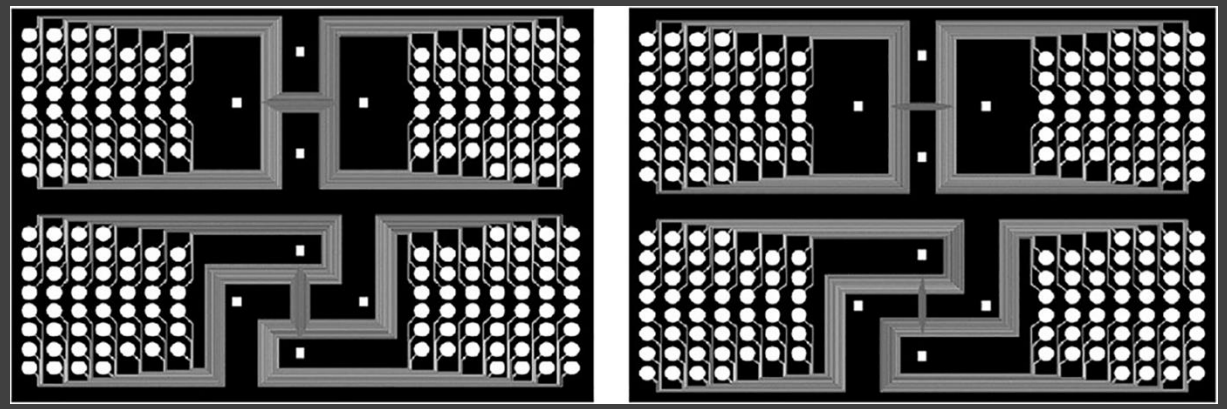


Barcodes move

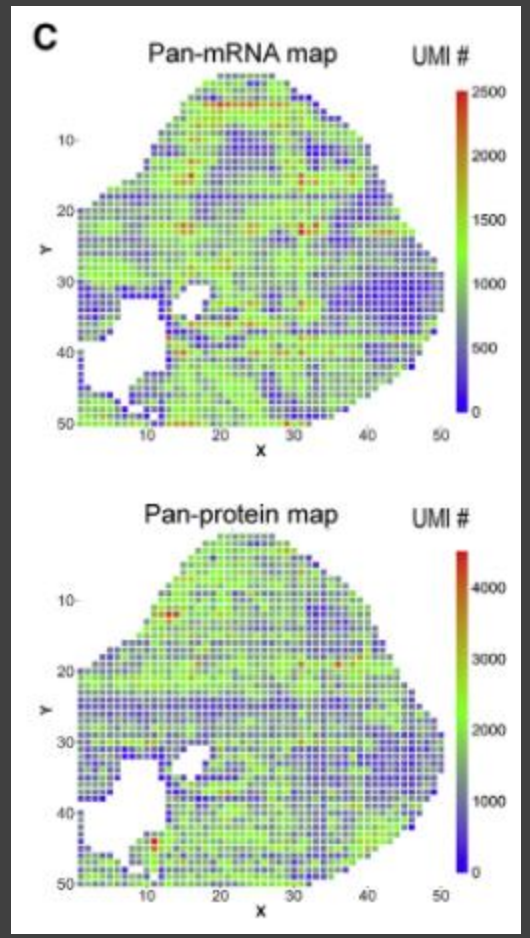
DBiT-seq



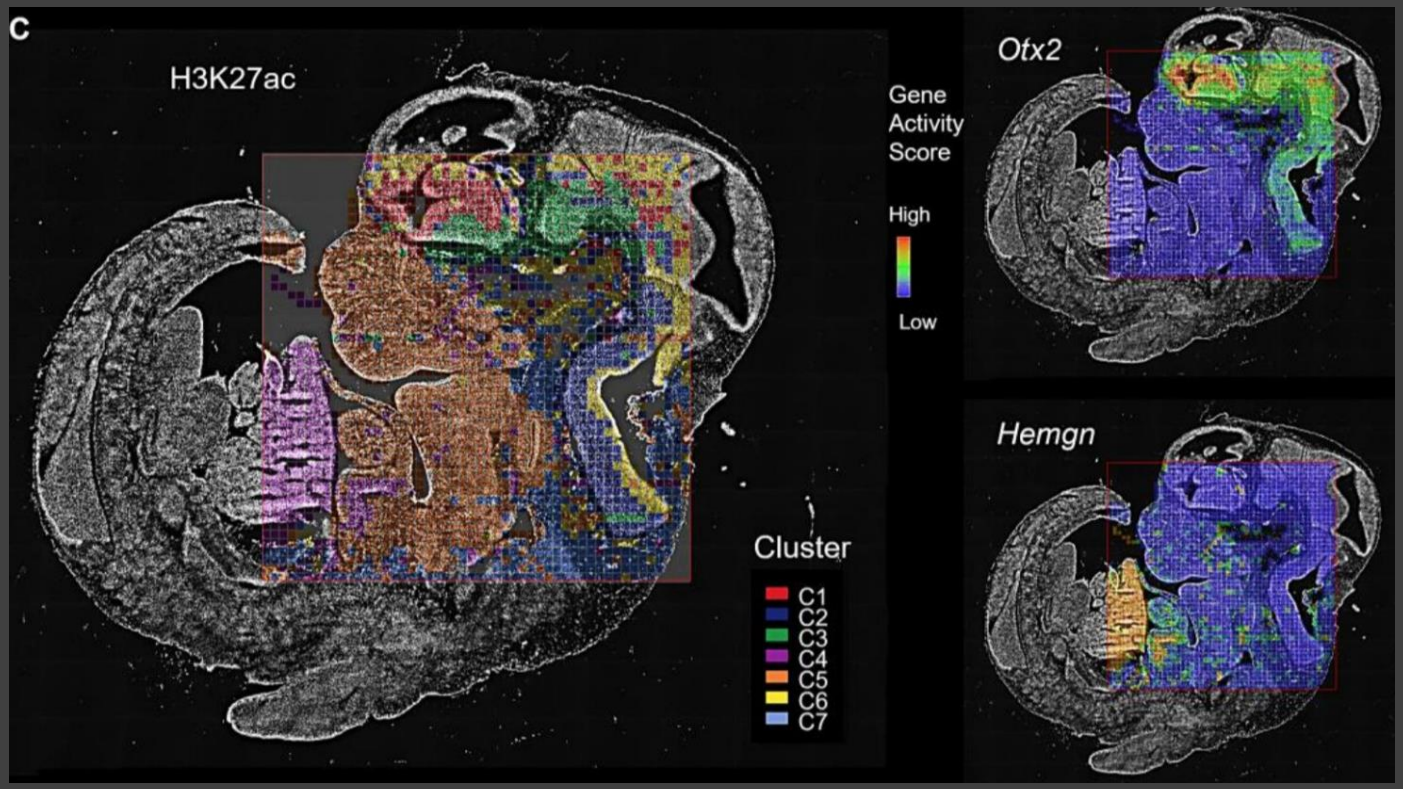
Liu *et al*. Cell 2022



DBiT-seq

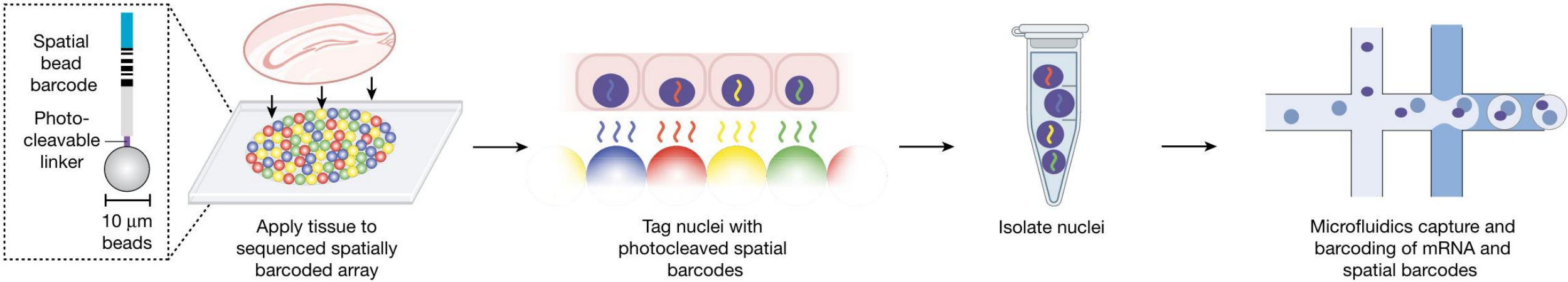


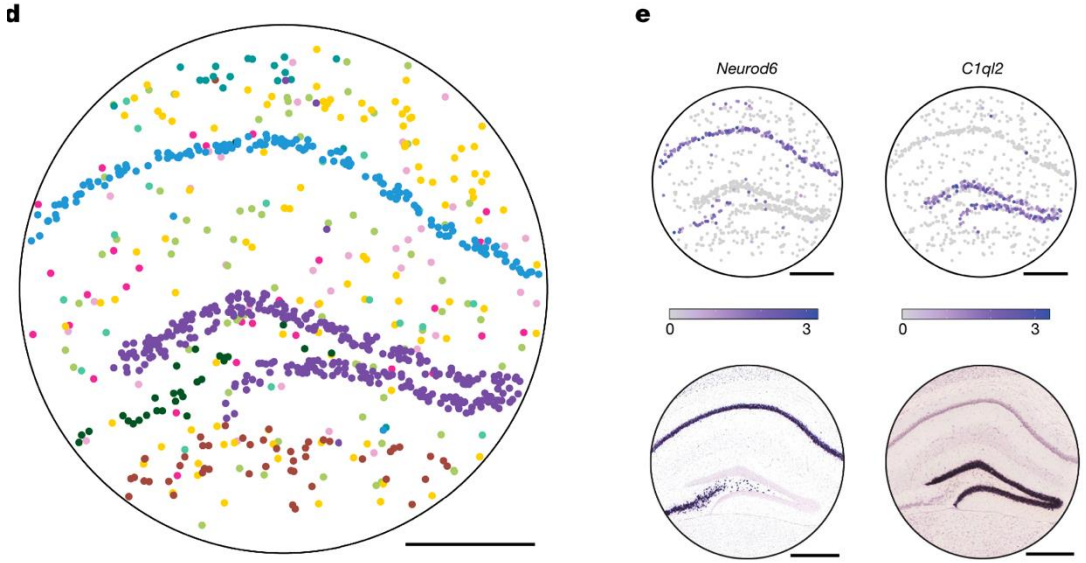
Liu *et al*. Cell 2022



Deng *et al*. Science 2022

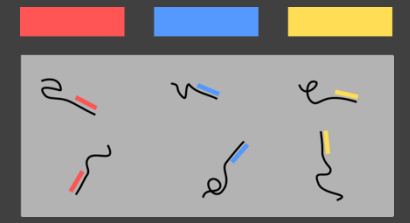
Slide-tags

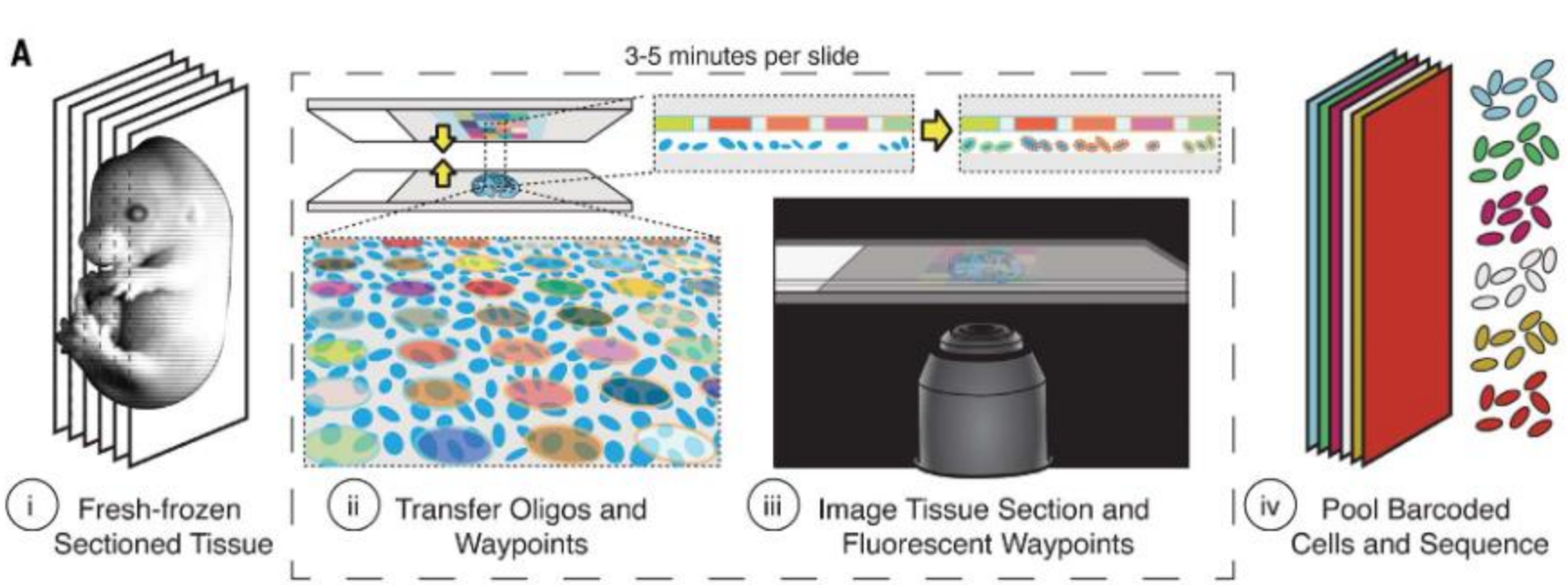


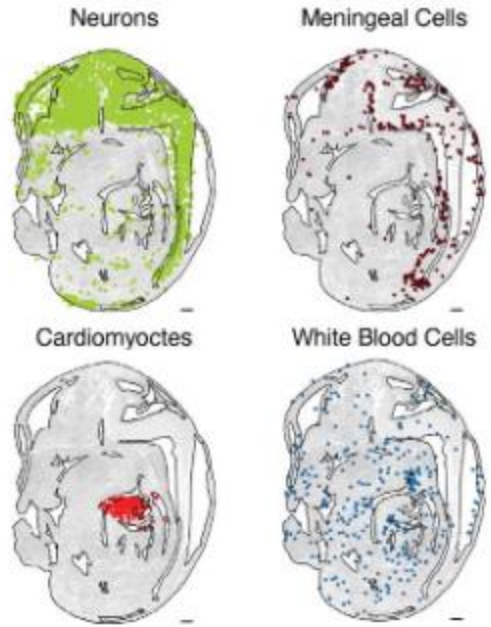


Russel *et al*. Nature 2024

sci-Space



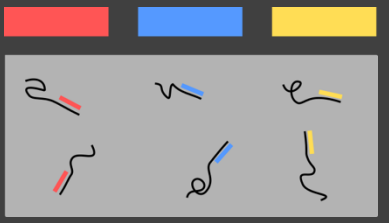




73um spots, 222um between spots, 2.2% of nuclei sampled

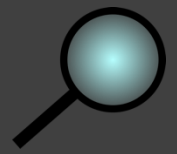
Srivatsan *et al*. Science 2022

Spatial Tagging



Methods: DBiT-seq, Slide-tags, sci-Space

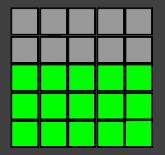
Commercial: DBiT-seq, Curio Trecker



Resolution: 10 – 100µm

Detection efficiency: 1 - 30%

Gene throughput: Full transcriptome

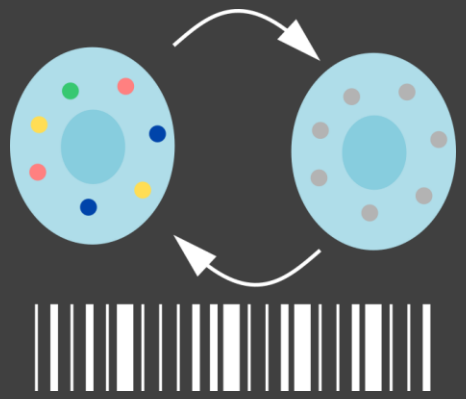


Spatial throughput: several mm^2 – cm^2

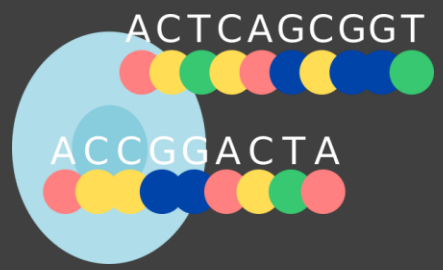
spatial RNA detection

Microscopy

Barcoded FISH

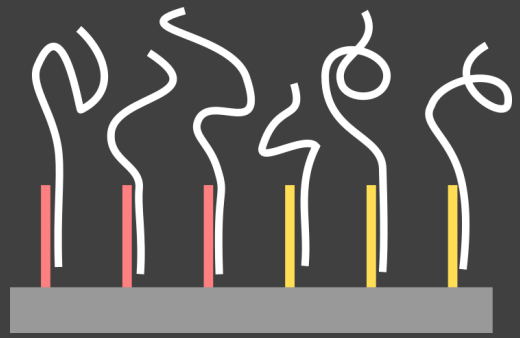


in situ Sequencing



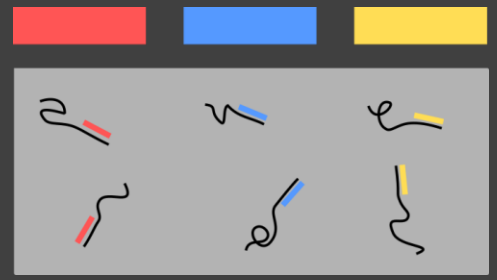
Sequencing

Spatial Sequencing



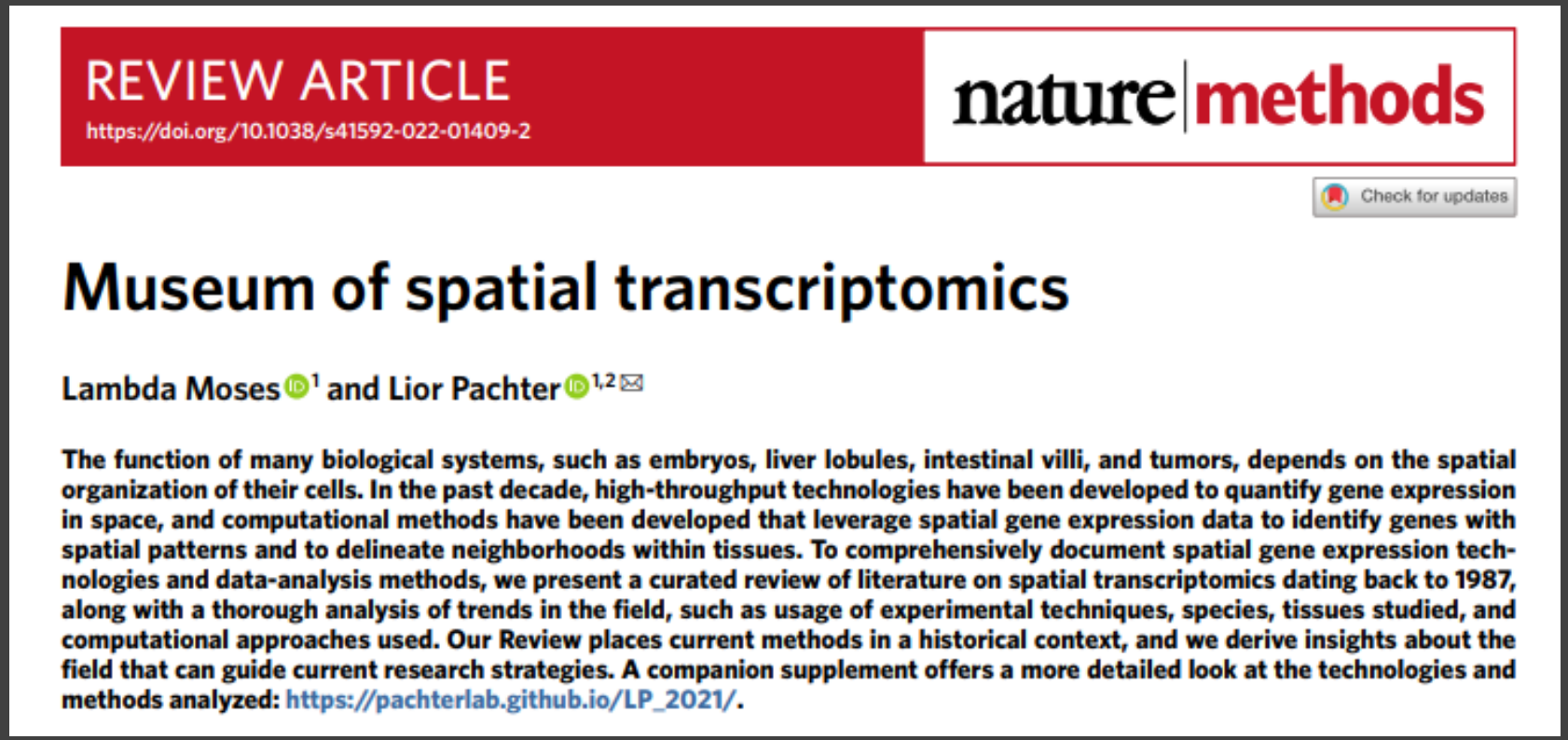
RNA moves

Spatial tagging



Barcodes move

Further reading



REVIEW ARTICLE

https://doi.org/10.1038/s41592-022-01409-2

nature methods

Check for updates

Museum of spatial transcriptomics

Lambda Moses [1] and Lior Pachter [1,2] ✉

The function of many biological systems, such as embryos, liver lobules, intestinal villi, and tumors, depends on the spatial organization of their cells. In the past decade, high-throughput technologies have been developed to quantify gene expression in space, and computational methods have been developed that leverage spatial gene expression data to identify genes with spatial patterns and to delineate neighborhoods within tissues. To comprehensively document spatial gene expression technologies and data-analysis methods, we present a curated review of literature on spatial transcriptomics dating back to 1987, along with a thorough analysis of trends in the field, such as usage of experimental techniques, species, tissues studied, and computational approaches used. Our Review places current methods in a historical context, and we derive insights about the field that can guide current research strategies. A companion supplement offers a more detailed look at the technologies and methods analyzed: https://pachterlab.github.io/LP_2021/.

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