

Spatial Transcriptome profiling

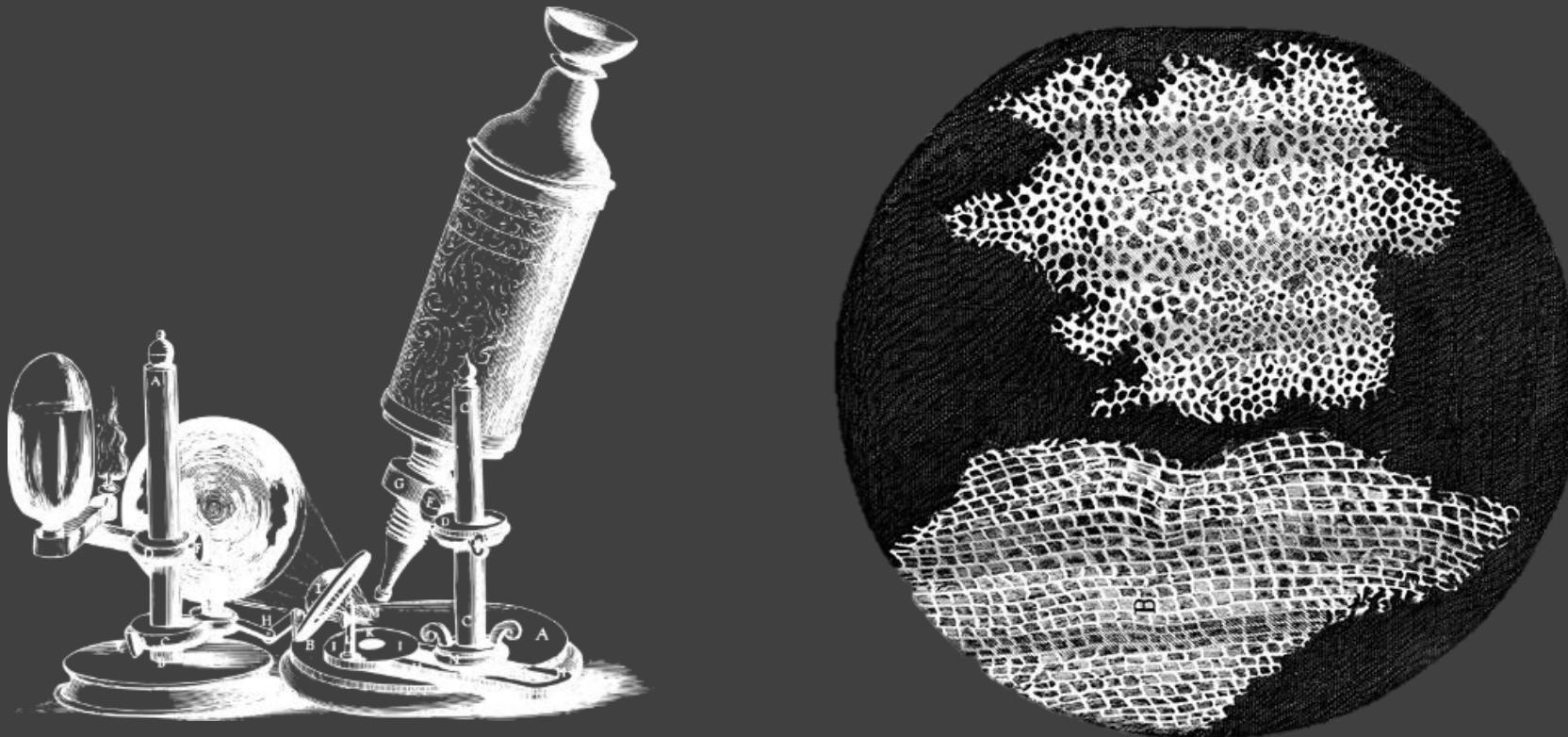
Lars Borm

Linnarsson Lab
karolinska Institutet



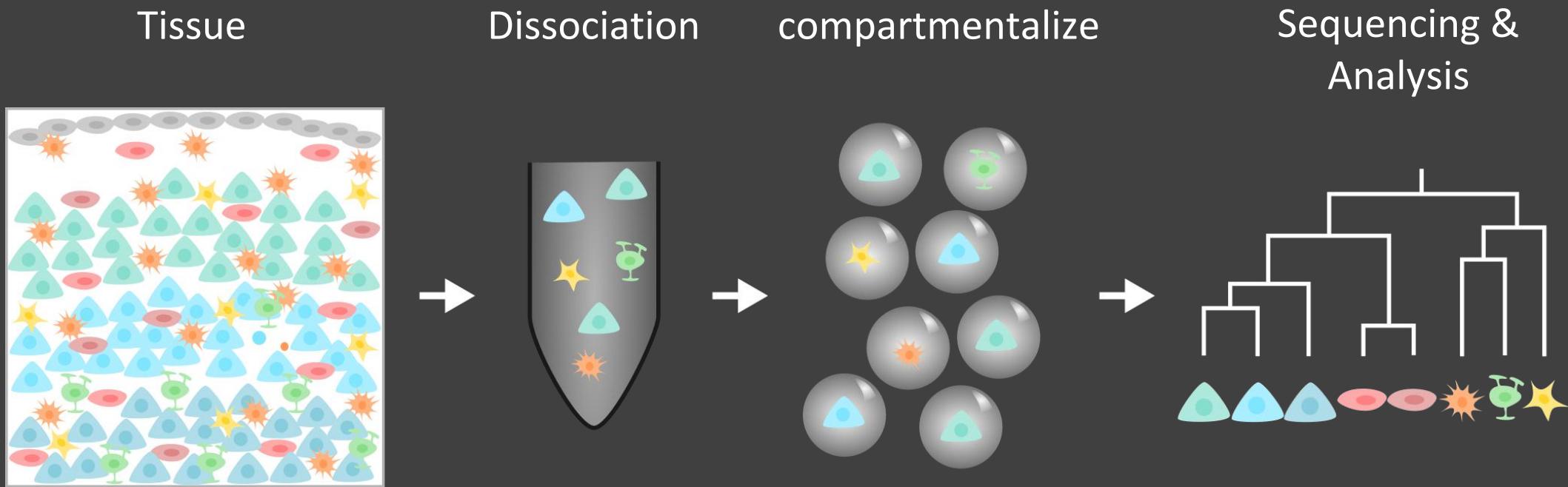
Karolinska
Institutet

Single cell biology



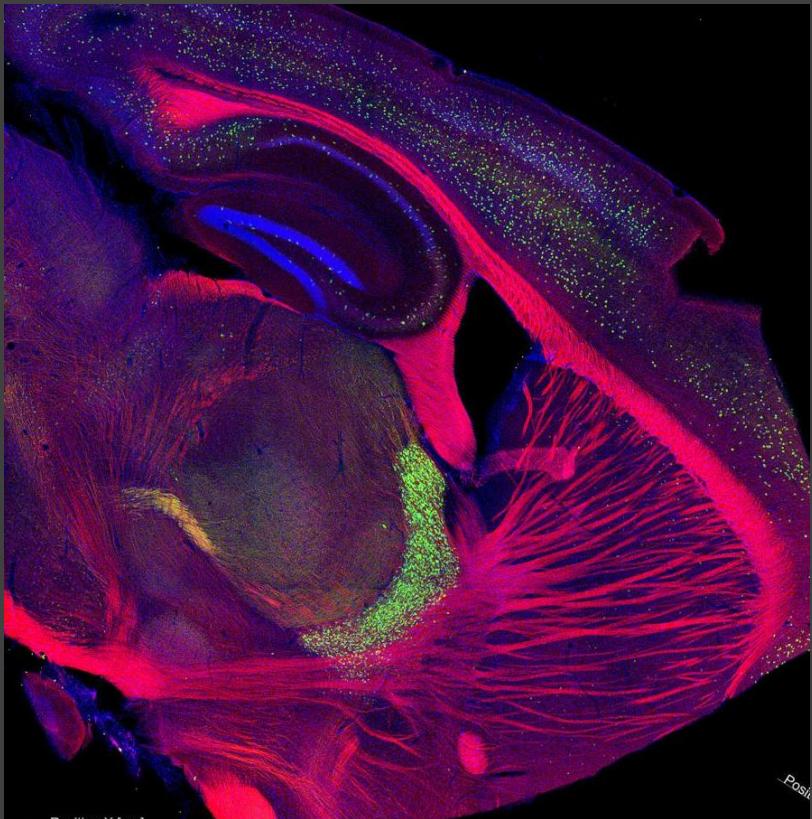
Robert Hooke 1665

Single Cell RNA sequencing



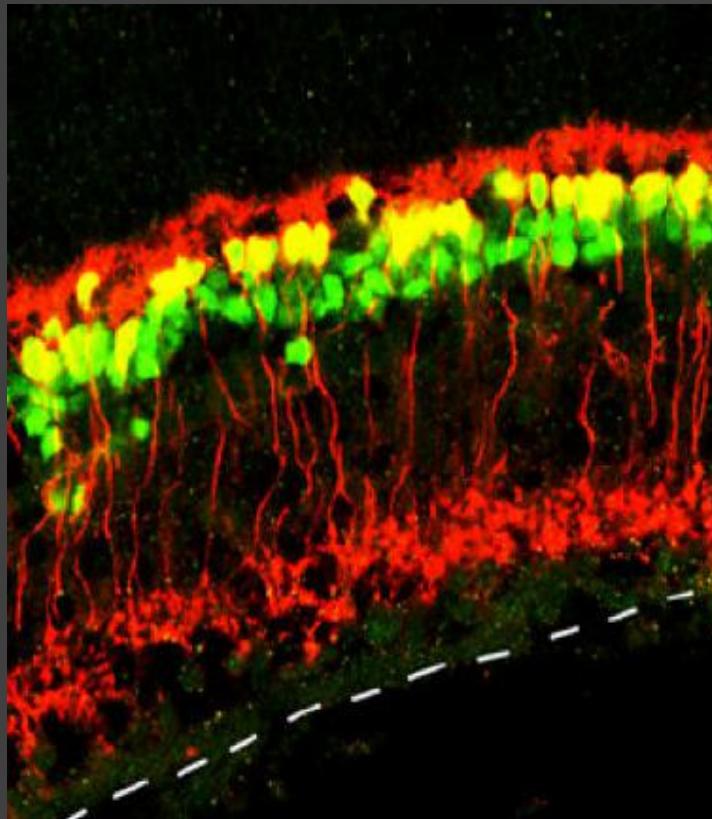
Tissue architecture

Brain



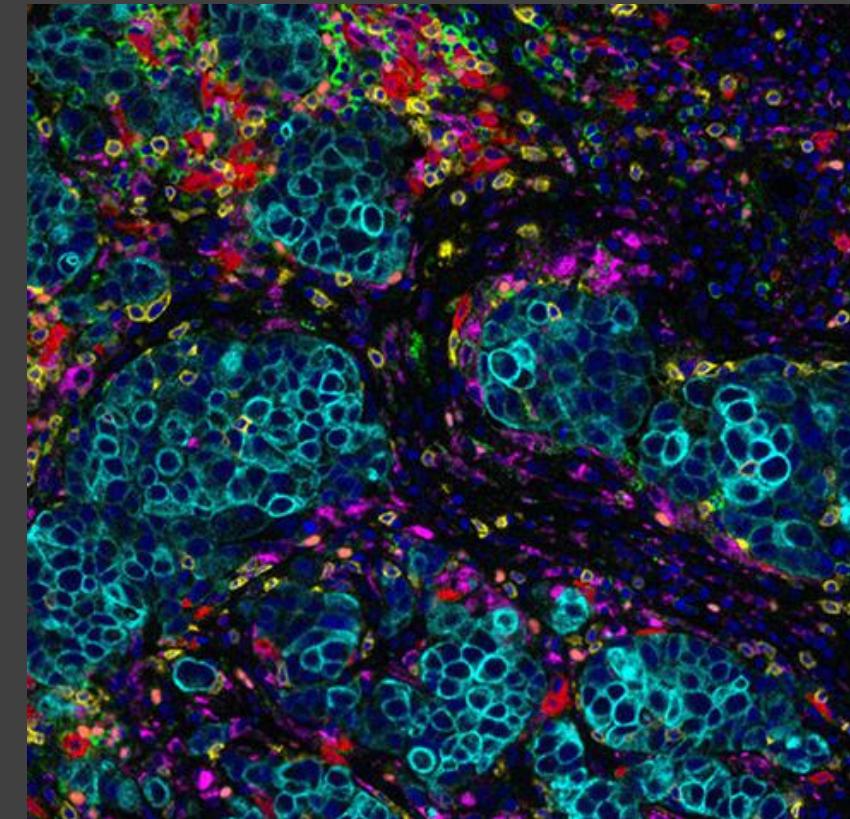
IGB Illinois

Retina



Morrow *et al.* 2008

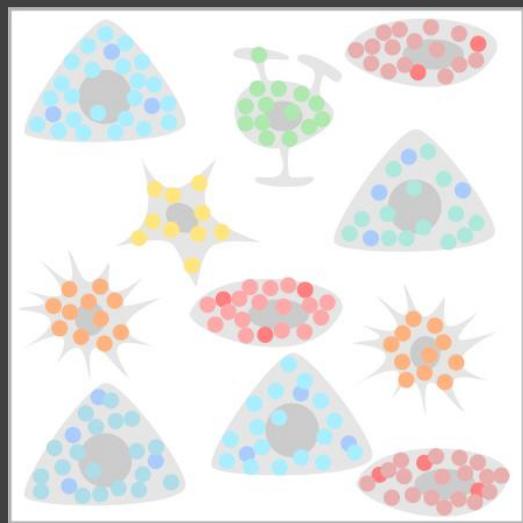
Cancer



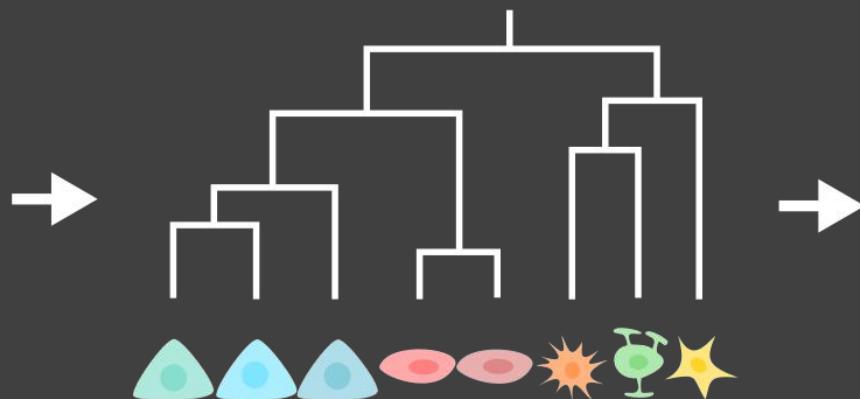
Perkin Elmer

Detecting RNA in tissue

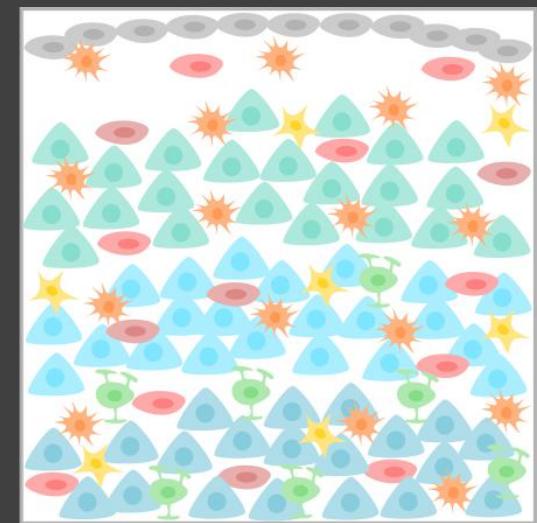
RNA detection *in situ*



Analysis

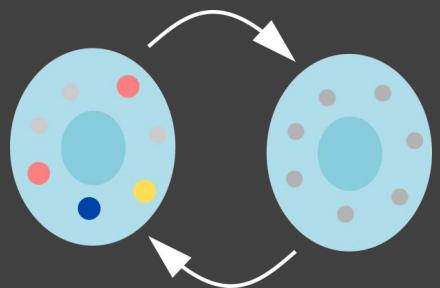


Spatial transcriptomics

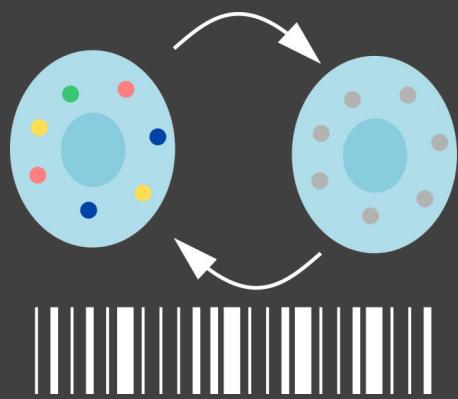


RNA spatial detection

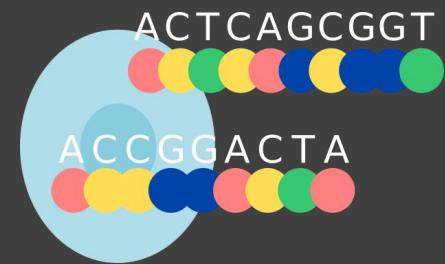
Cyclic FISH



Barcoded FISH



in situ Sequencing

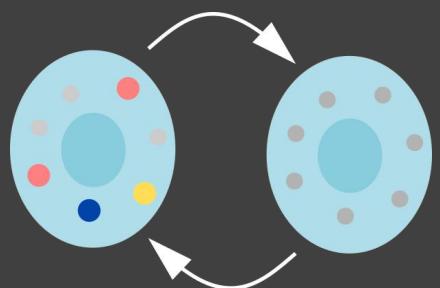


Spatial Sequencing

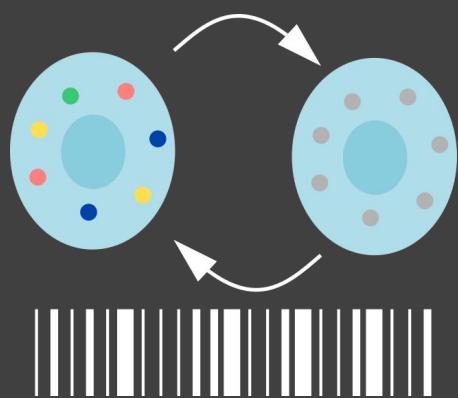


Microscopy methods

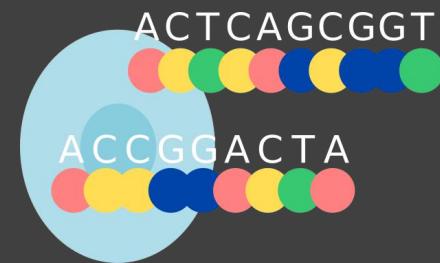
Cyclic FISH



Barcode FISH



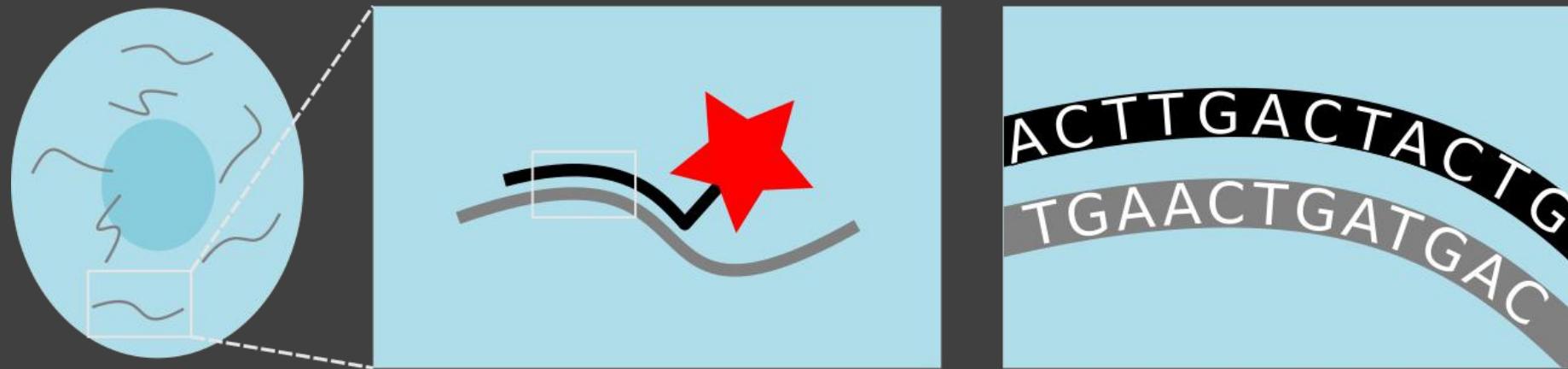
in situ Sequencing



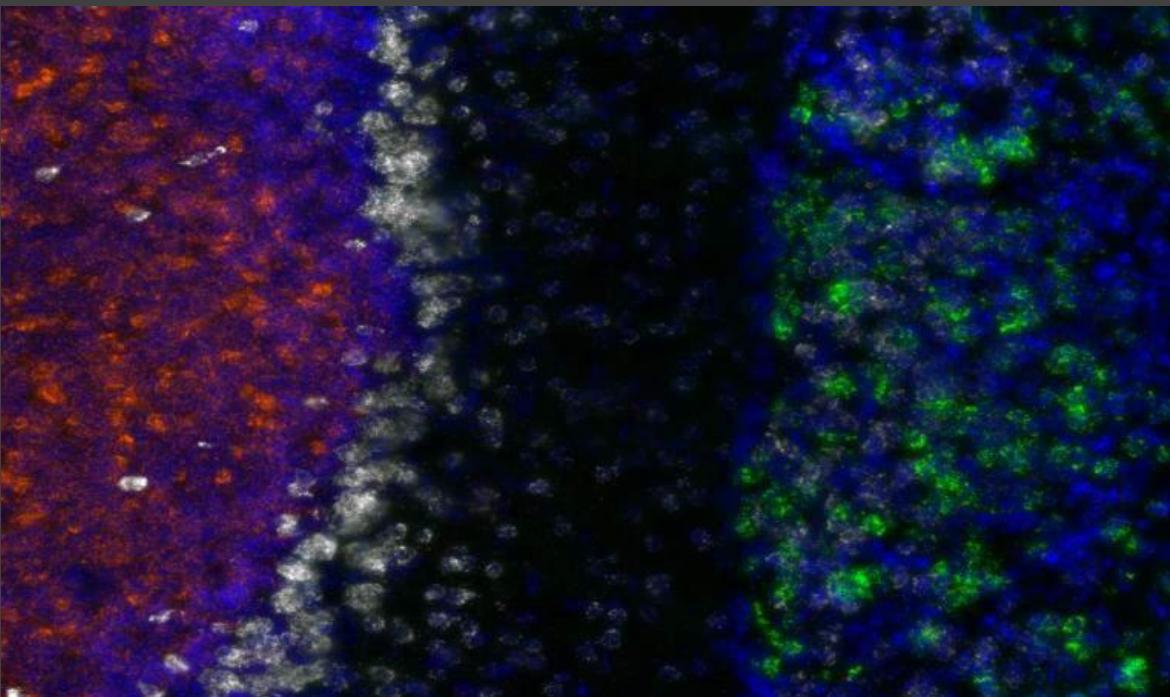
Spatial Sequencing



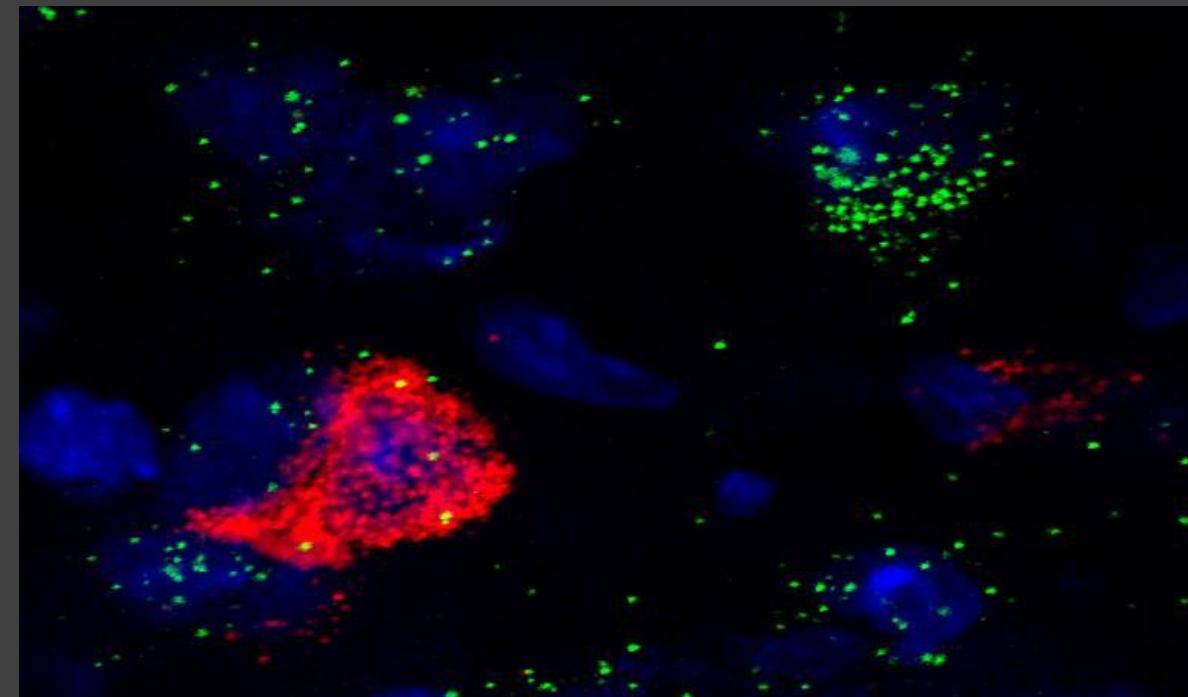
in situ Hybridization



Fluorescent in situ Hybridization (FISH)

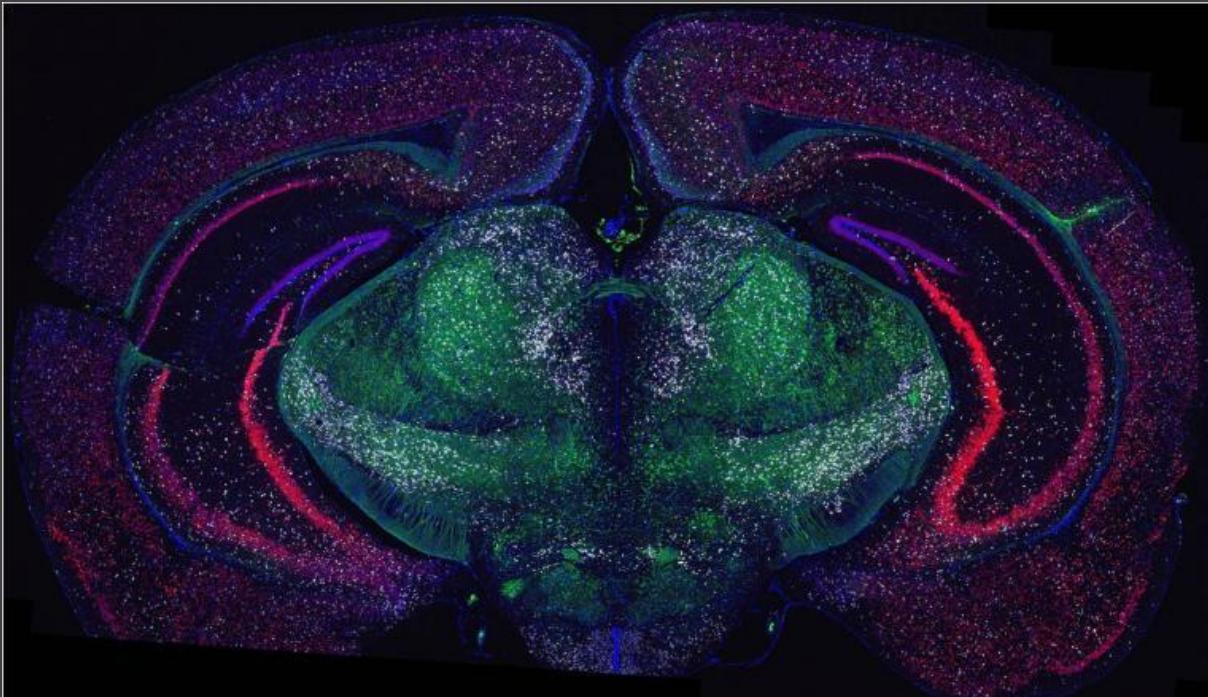
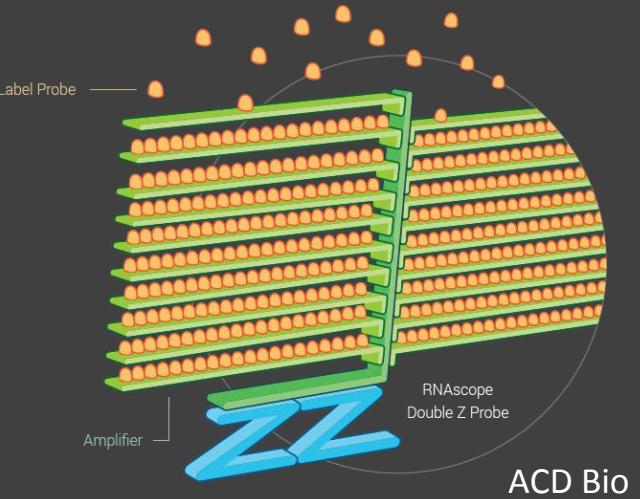


ACD Bio

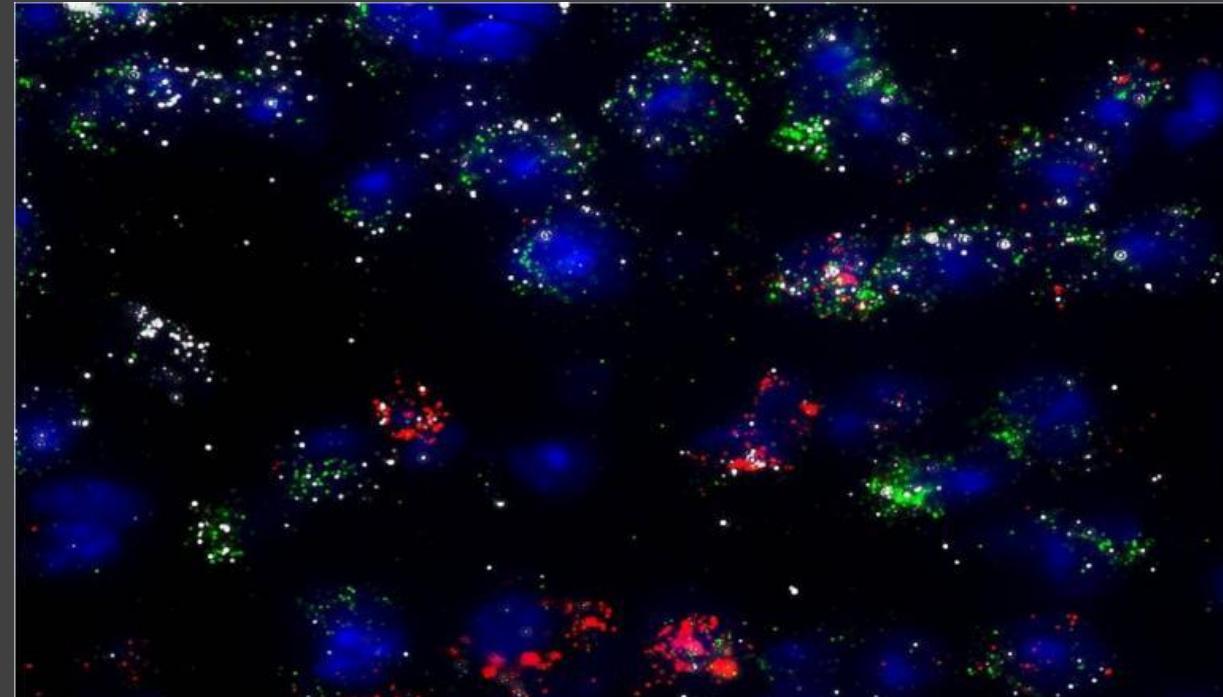


ACD Bio

RNAscope



ACD Bio

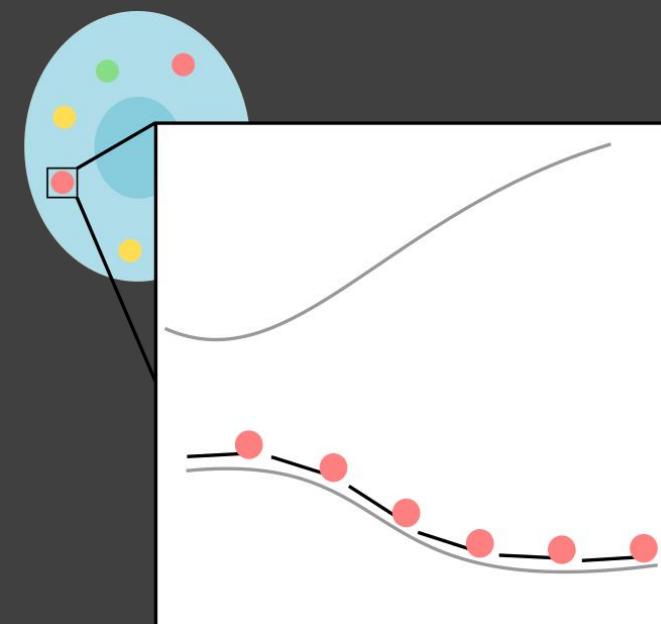


ACD Bio

single molecule FISH (smFISH)

Probes

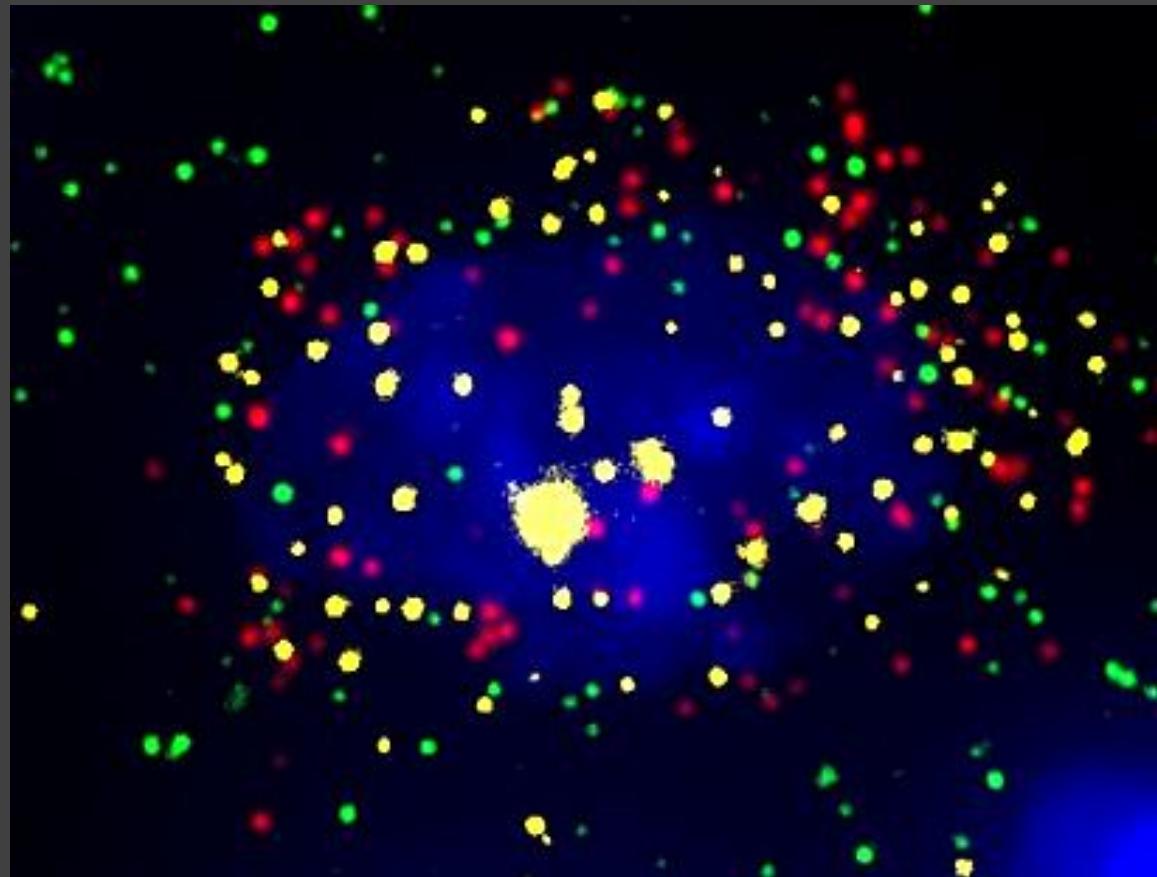
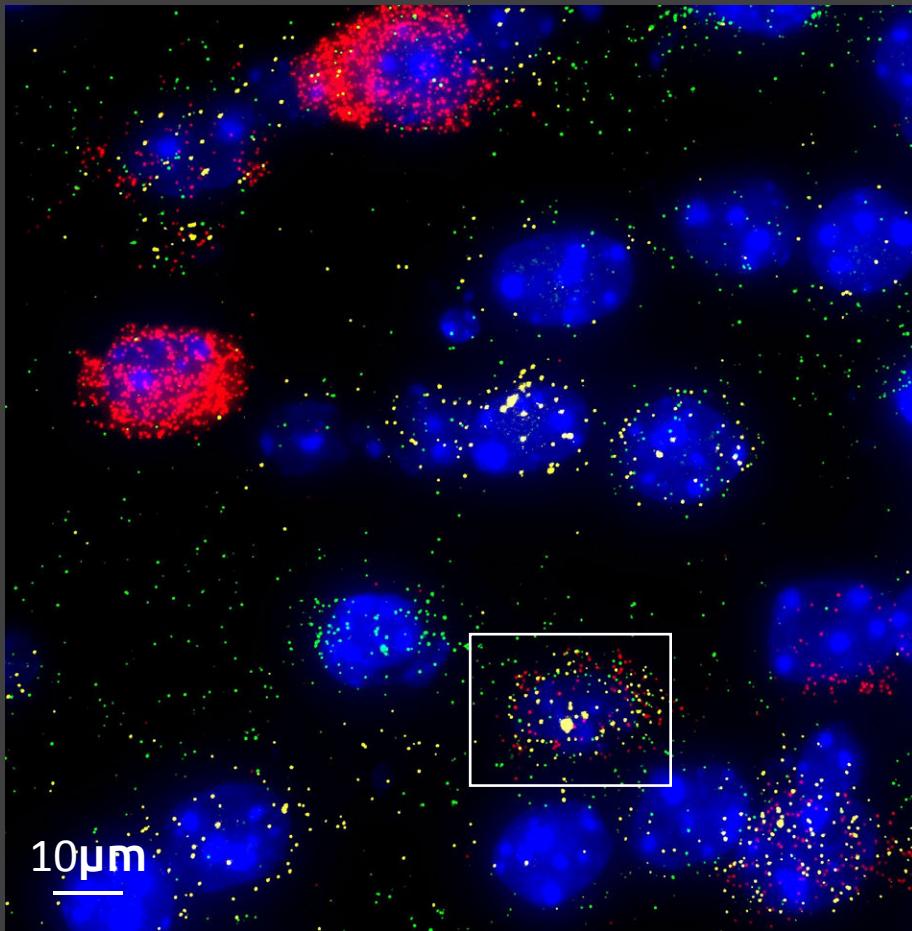
Gene 1 40X	
Gene 2 40X	
Gene 3 40X	
Gene 4 40X	



Raj *et al.* Nature Methods 2008

single molecule FISH (smFISH)

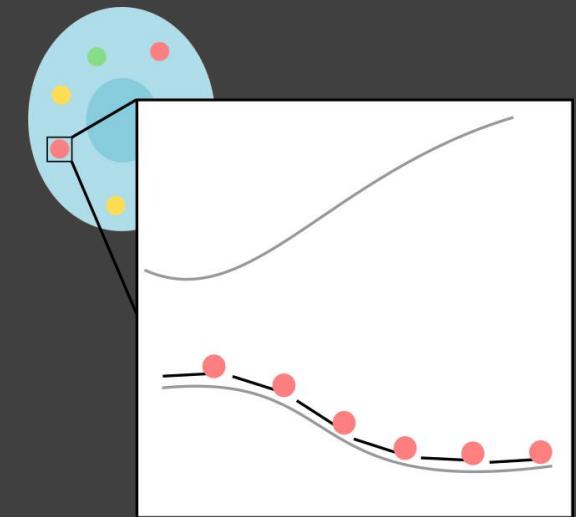
Gene 1 Gene 2 Gene 3 DNA



single molecule FISH (smFISH)

- High sensitivity
- Low false positives
- Low false negatives

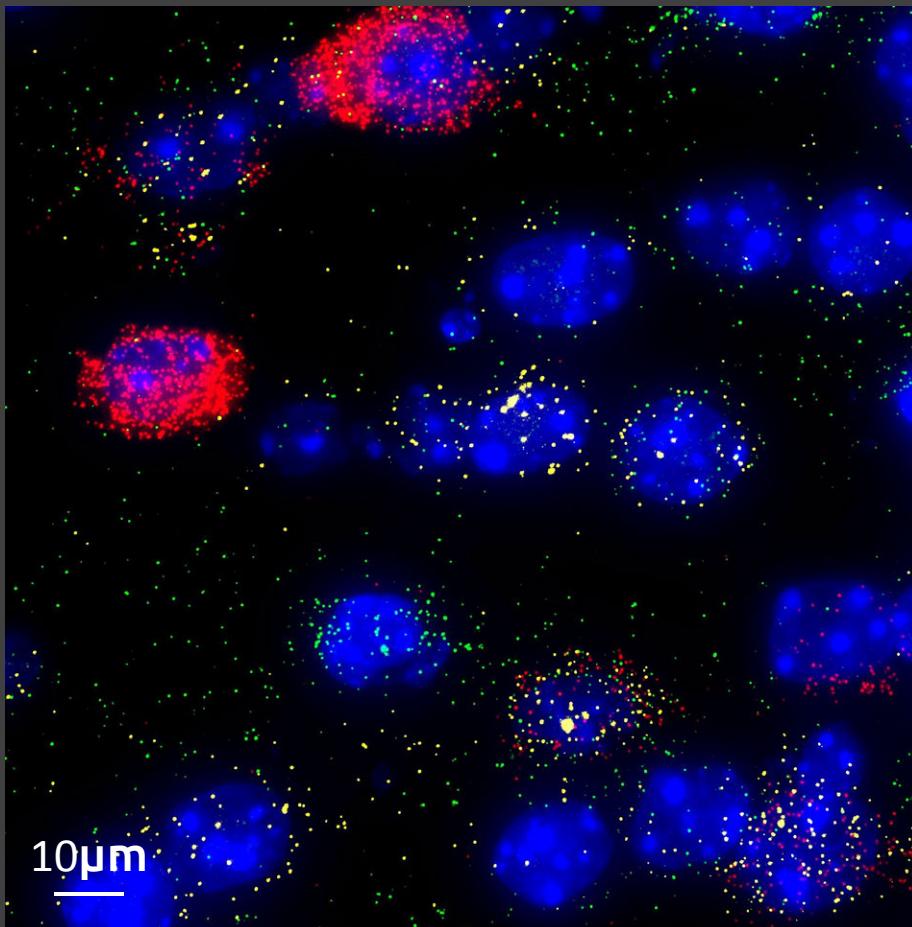
Gene 1 40X 
Gene 2 40X 
Gene 3 40X 
Gene 4 40X 



Raj *et al.* 2008

single molecule FISH (smFISH)

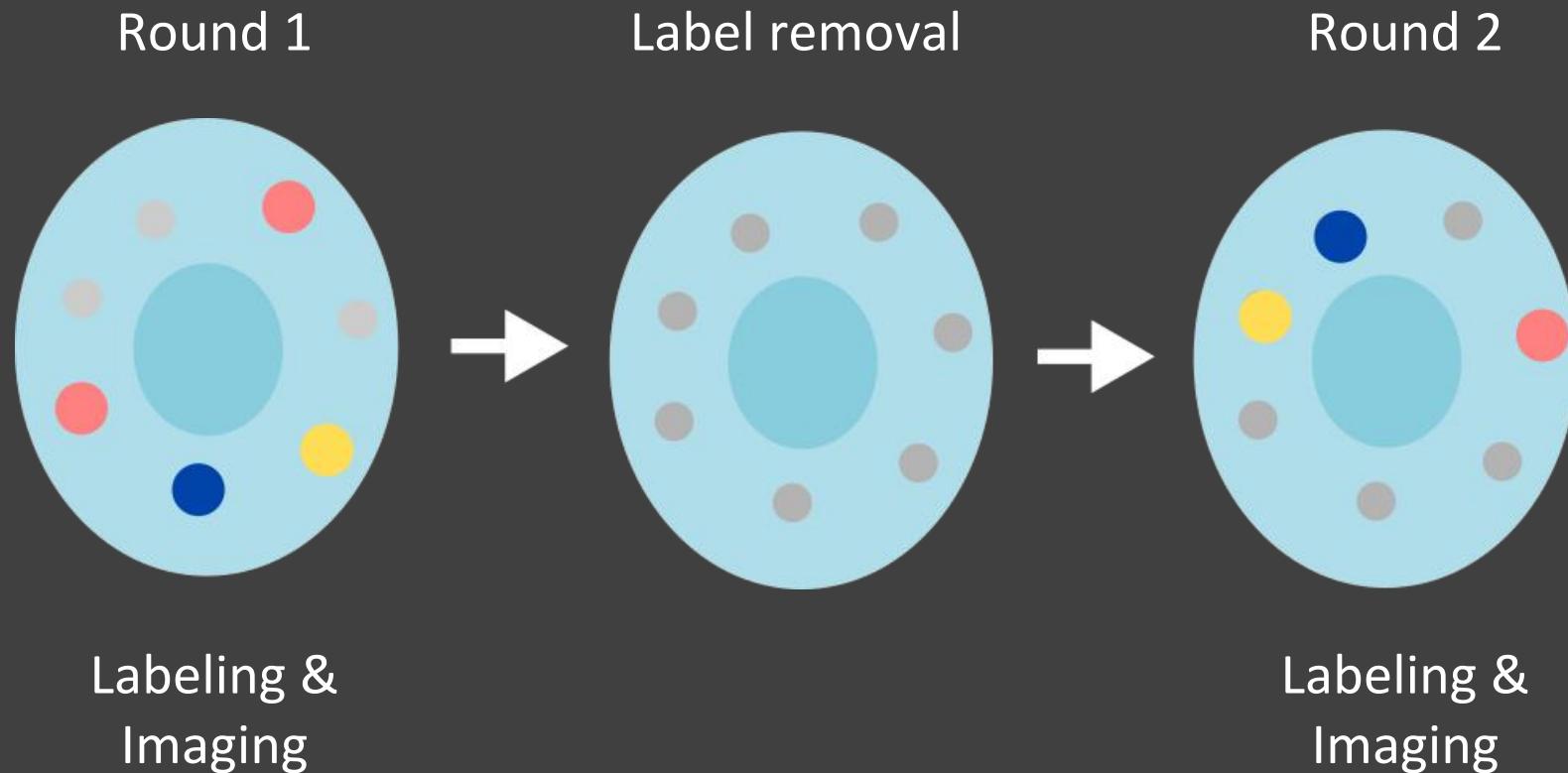
Gene 1 Gene 2 Gene 3 DNA



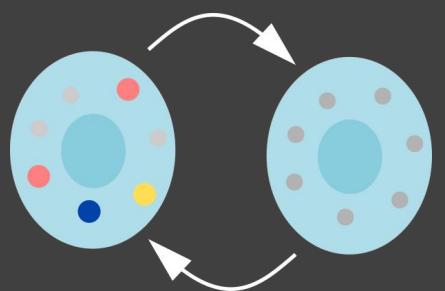
Limited fluorophores



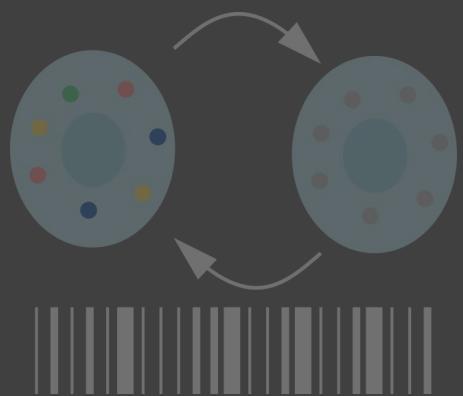
Cyclic labeling



Cyclic FISH



Barcoded FISH



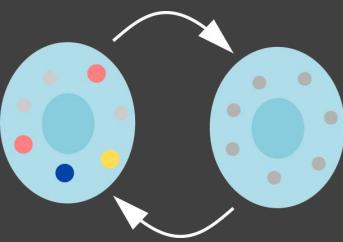
in situ Sequencing



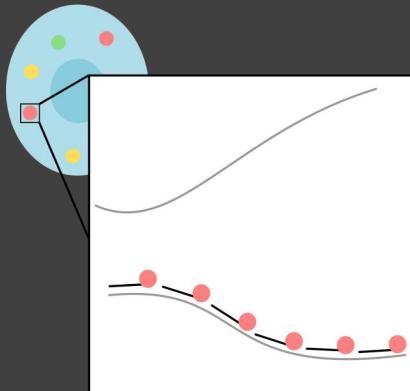
Spatial Sequencing



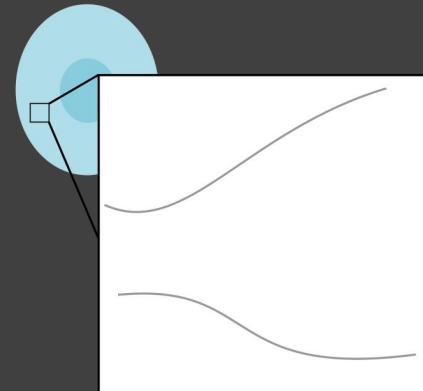
osmFISH



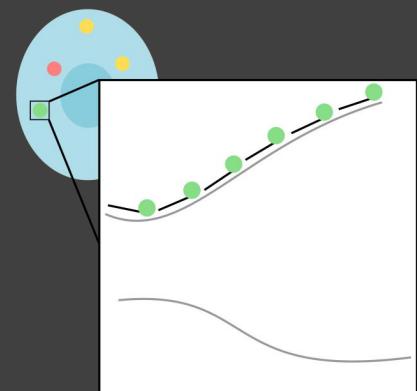
Hybridization 1



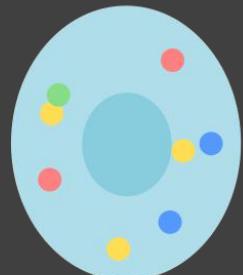
Strip 1



Hybridization 2

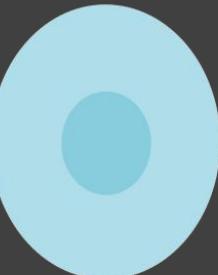


Hyb 1

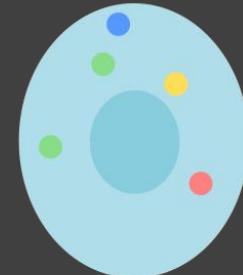


Gene 1 ●
Gene 2 ○
Gene 3 ▲
Gene 4 △

Strip 1

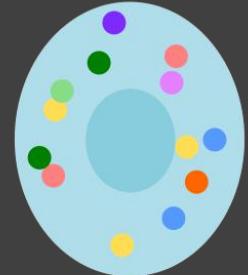


Hyb 2



Gene 5 ● → ○
Gene 6 ○ → ▲
Gene 7 ▲ → △
Gene 8 △ → △

Composite

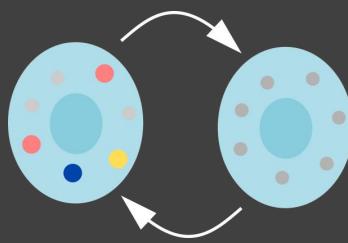
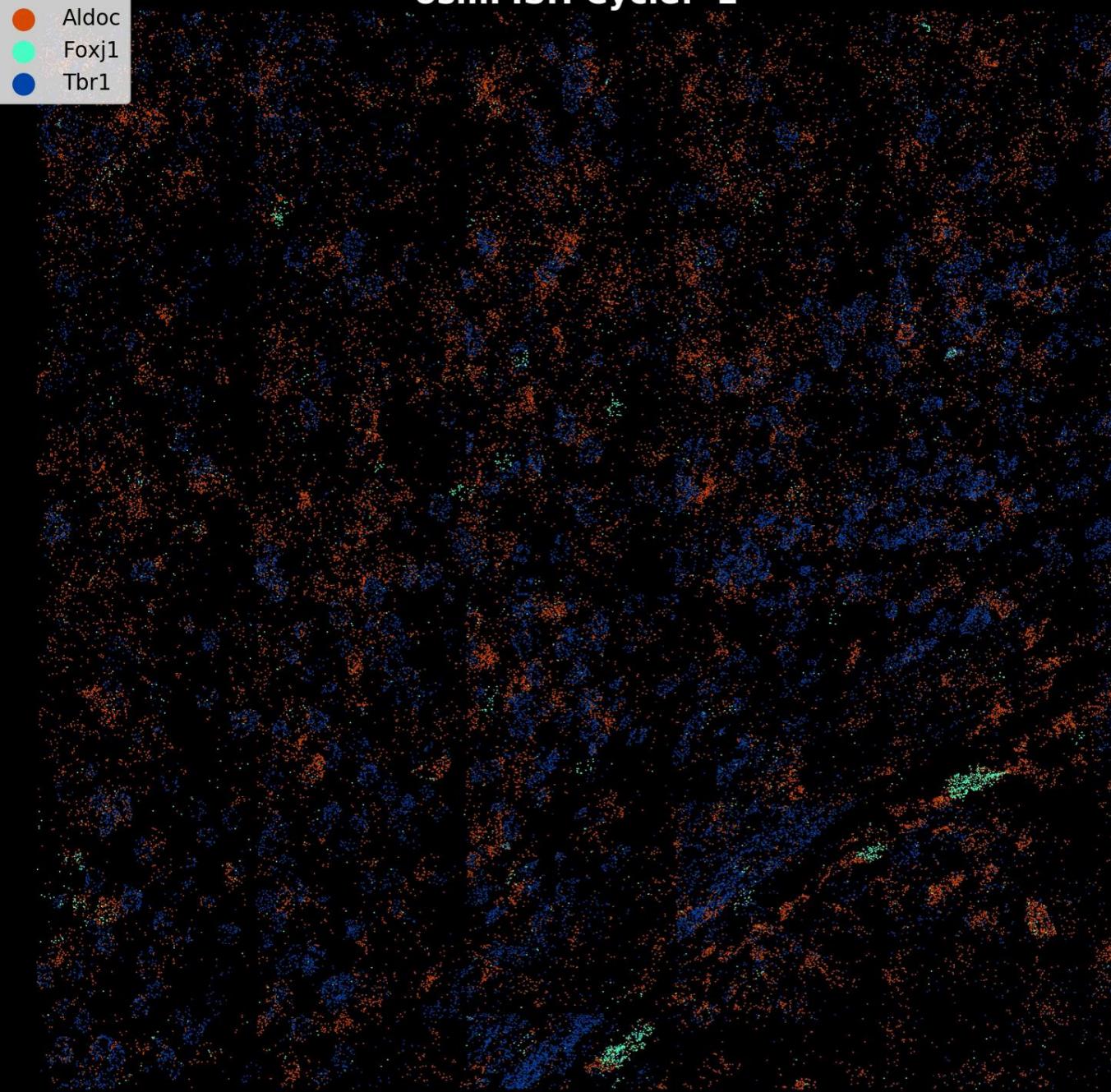


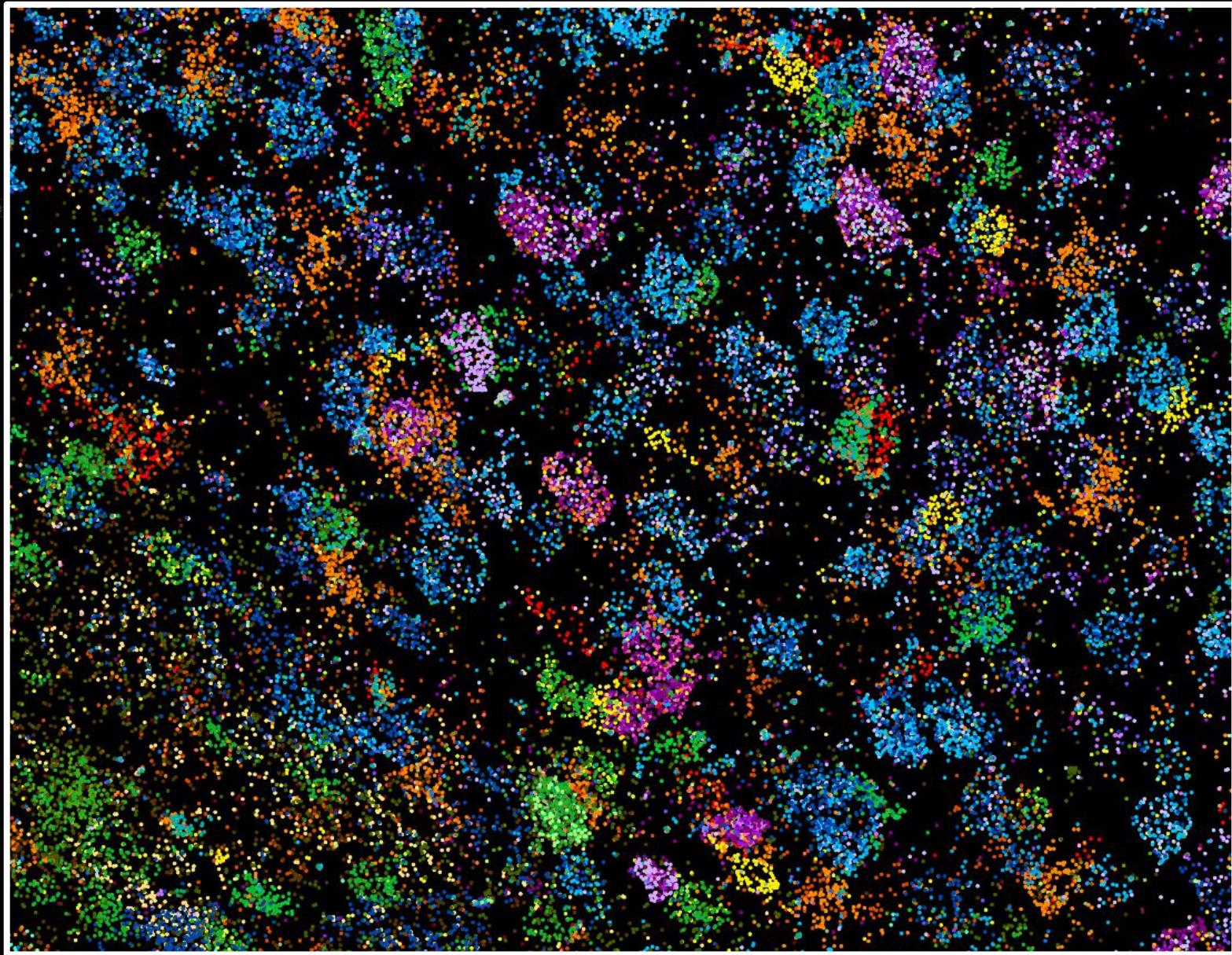
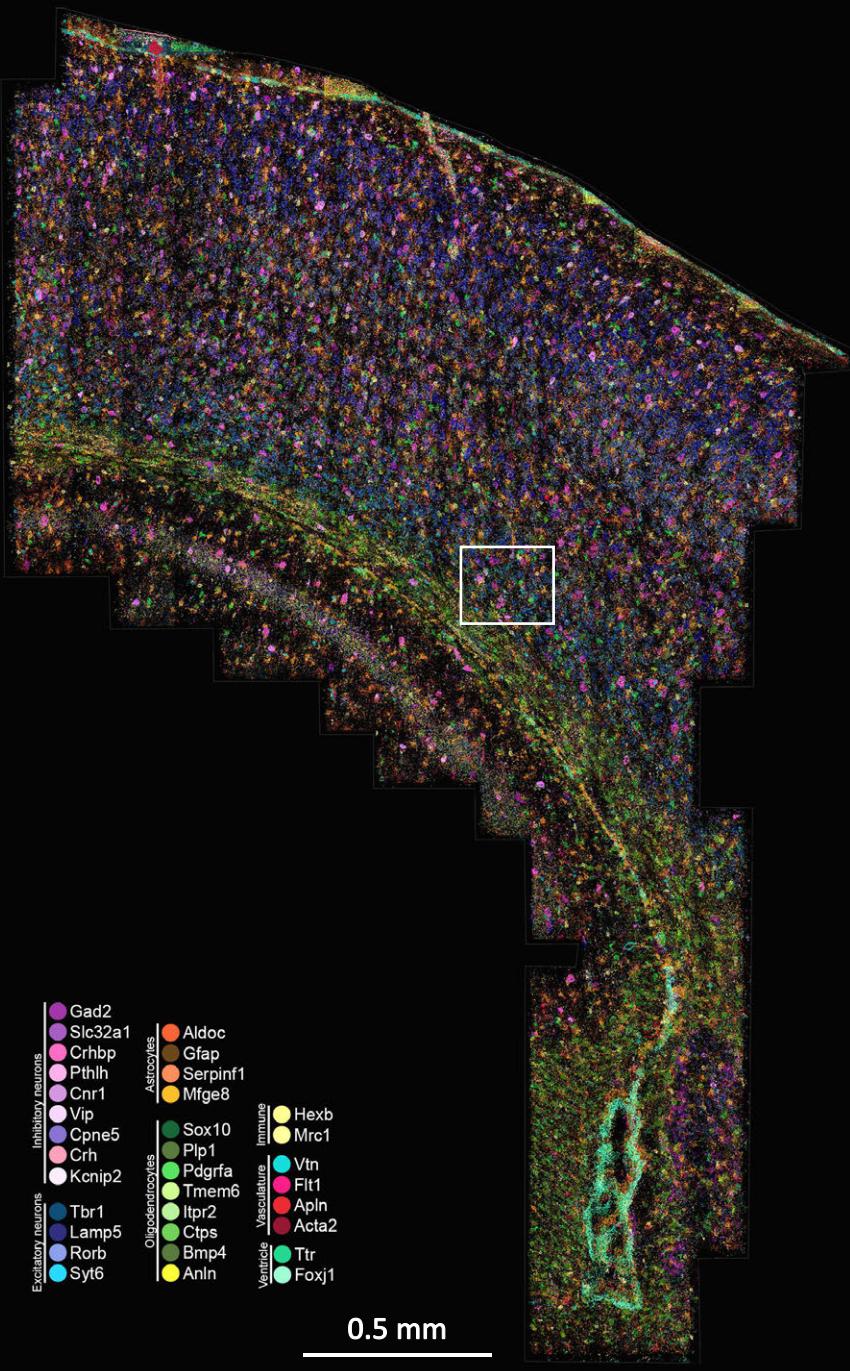
Gene 1 ●
Gene 2 ○
Gene 3 ▲
Gene 4 △
Gene 5 ○
Gene 6 ▲
Gene 7 △
Gene 8 △

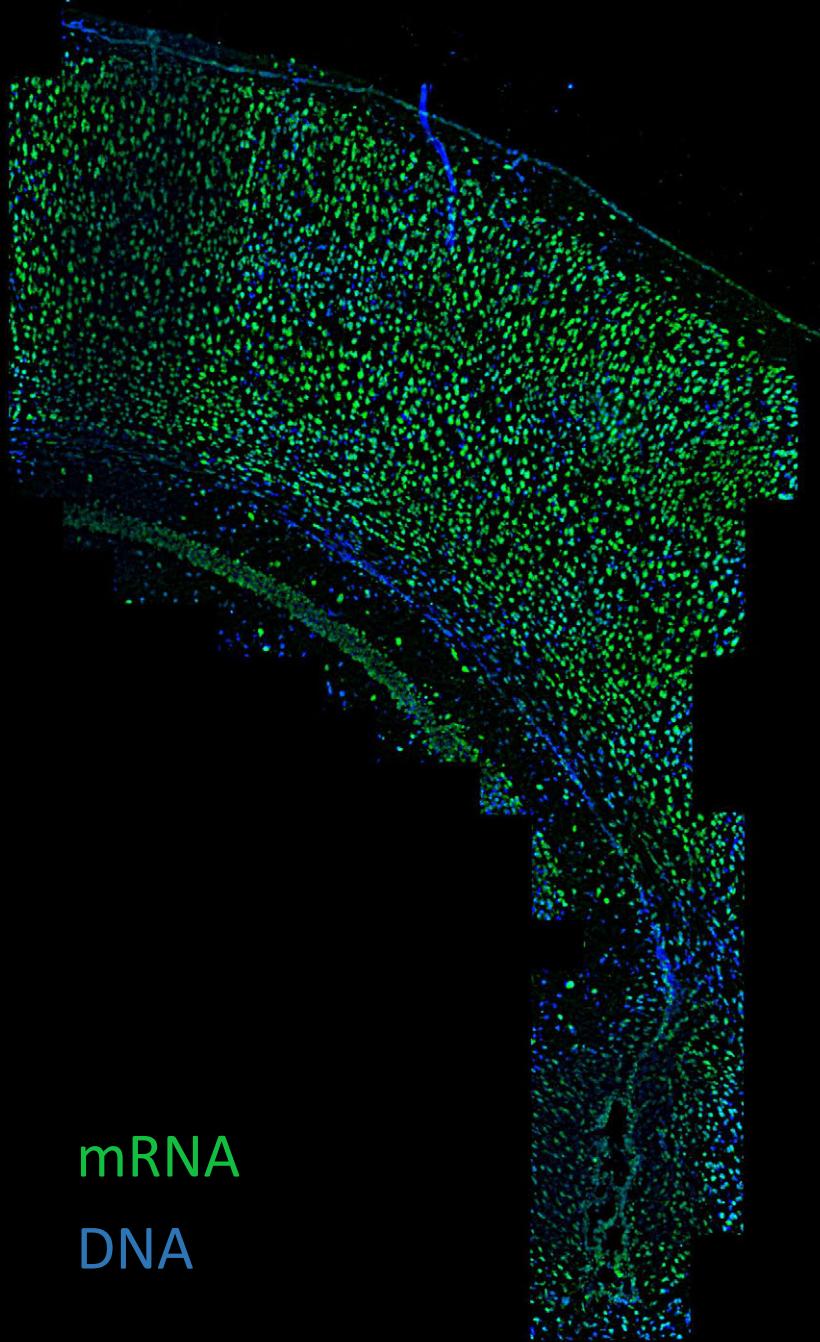
Genes

- Aldoc
- Foxj1
- Tbr1

osmFISH Cycle: 1



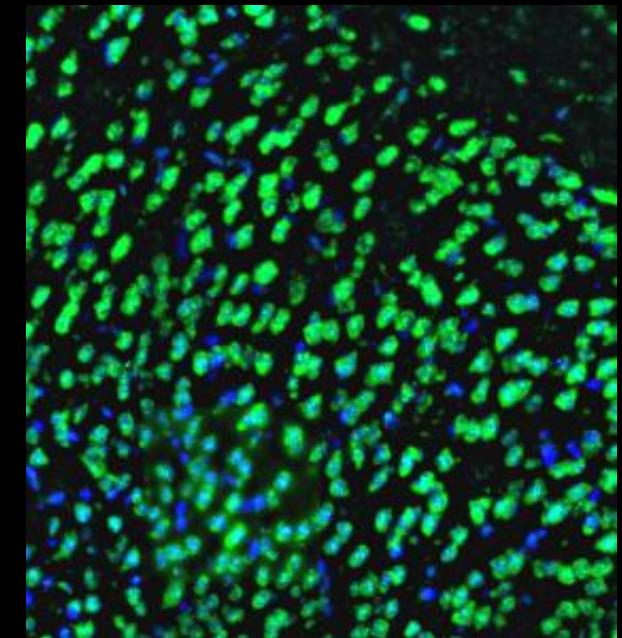




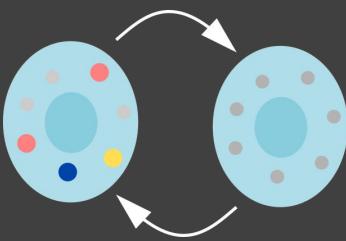
mRNA

DNA

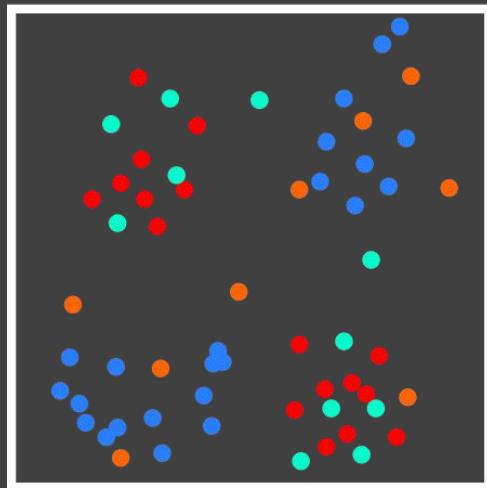
Segmented



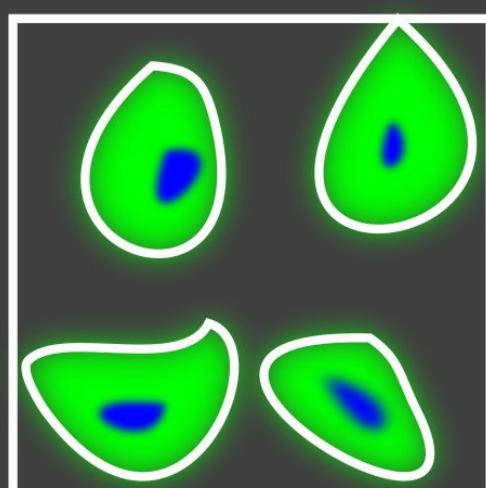
Pipeline



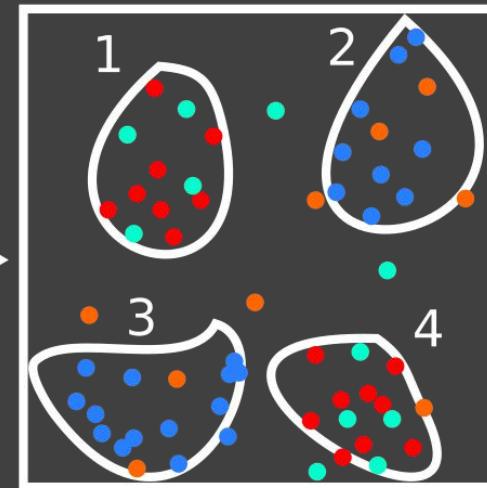
RNA detection



Cell segmentation

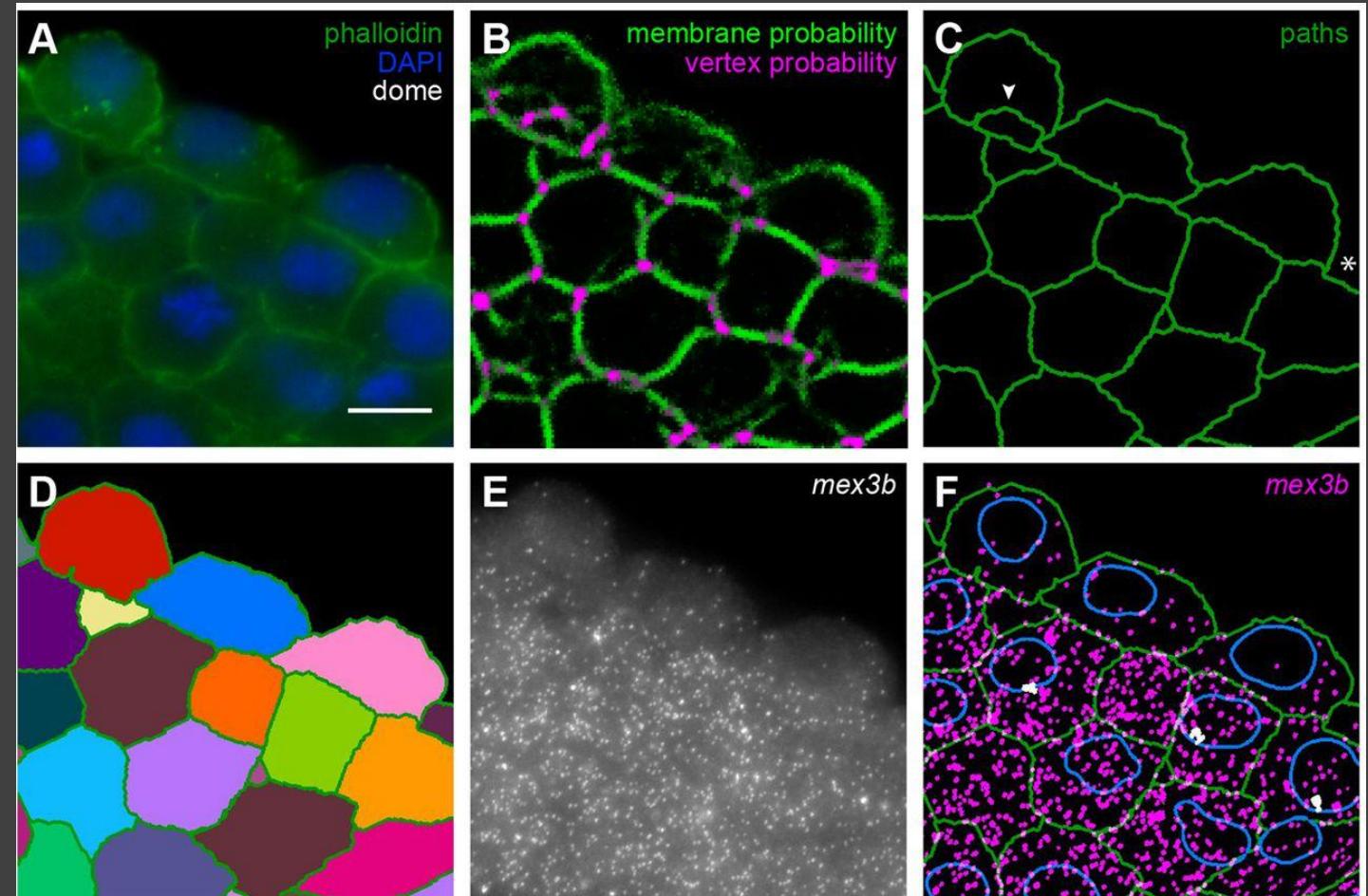
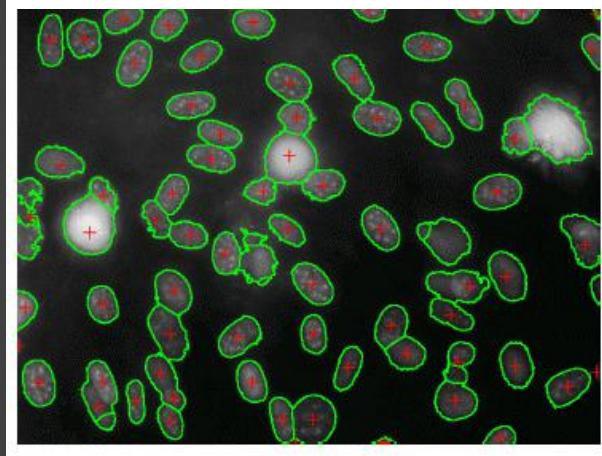
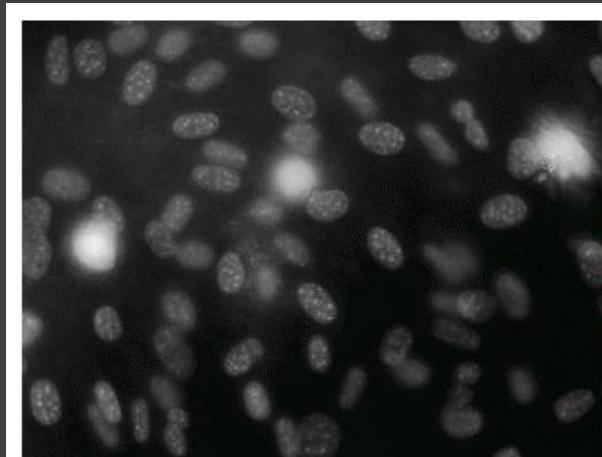
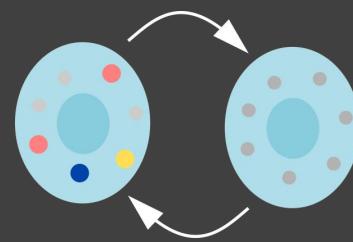


Cell counting

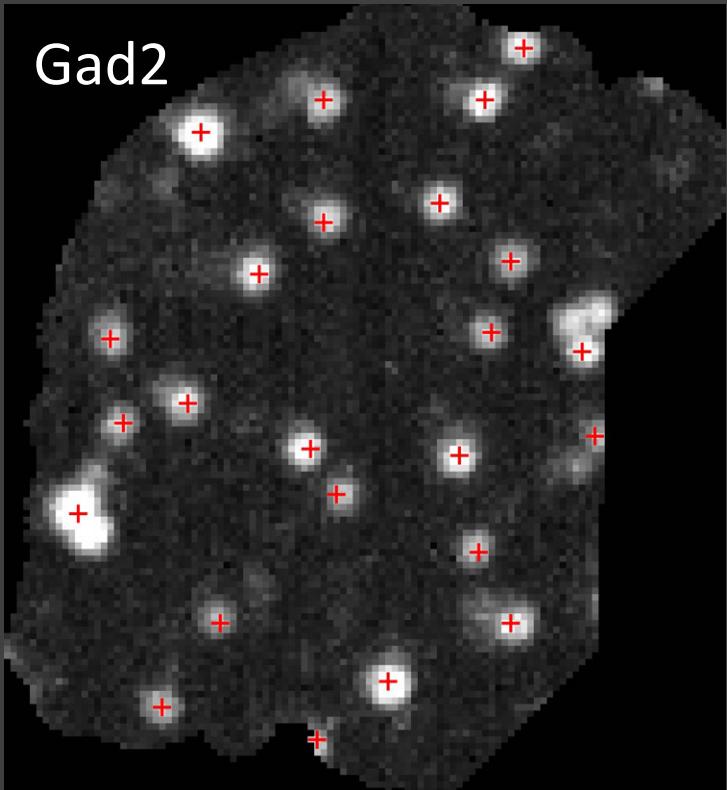
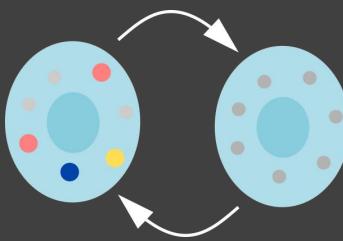


	Cell 1	Cell 2
red	8	0
cyan	4	0
blue	0	9
orange	0	2
	Cell 3	Cell 4
red	0	8
cyan	0	4
blue	14	0
orange	2	1

Cell segmentation



Single cell expression



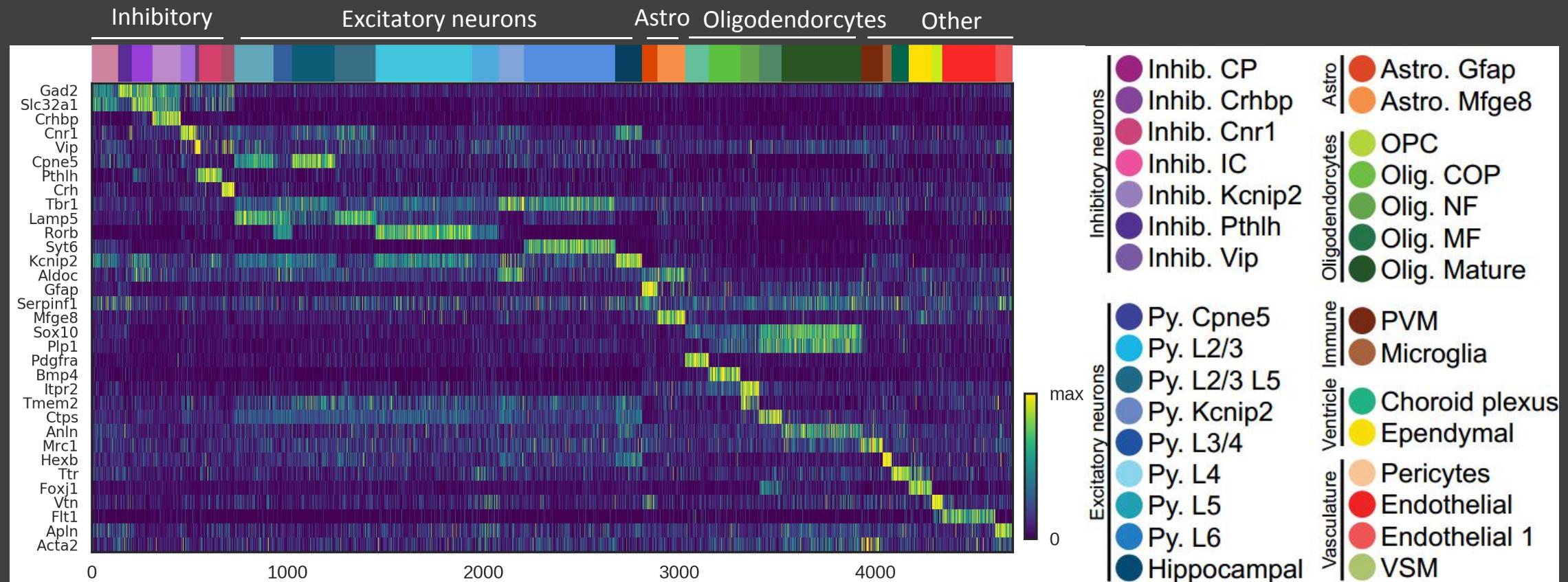
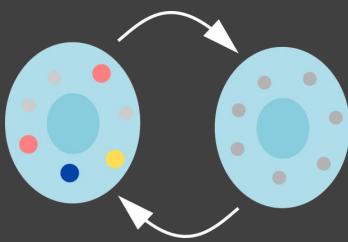
Genes

Cells

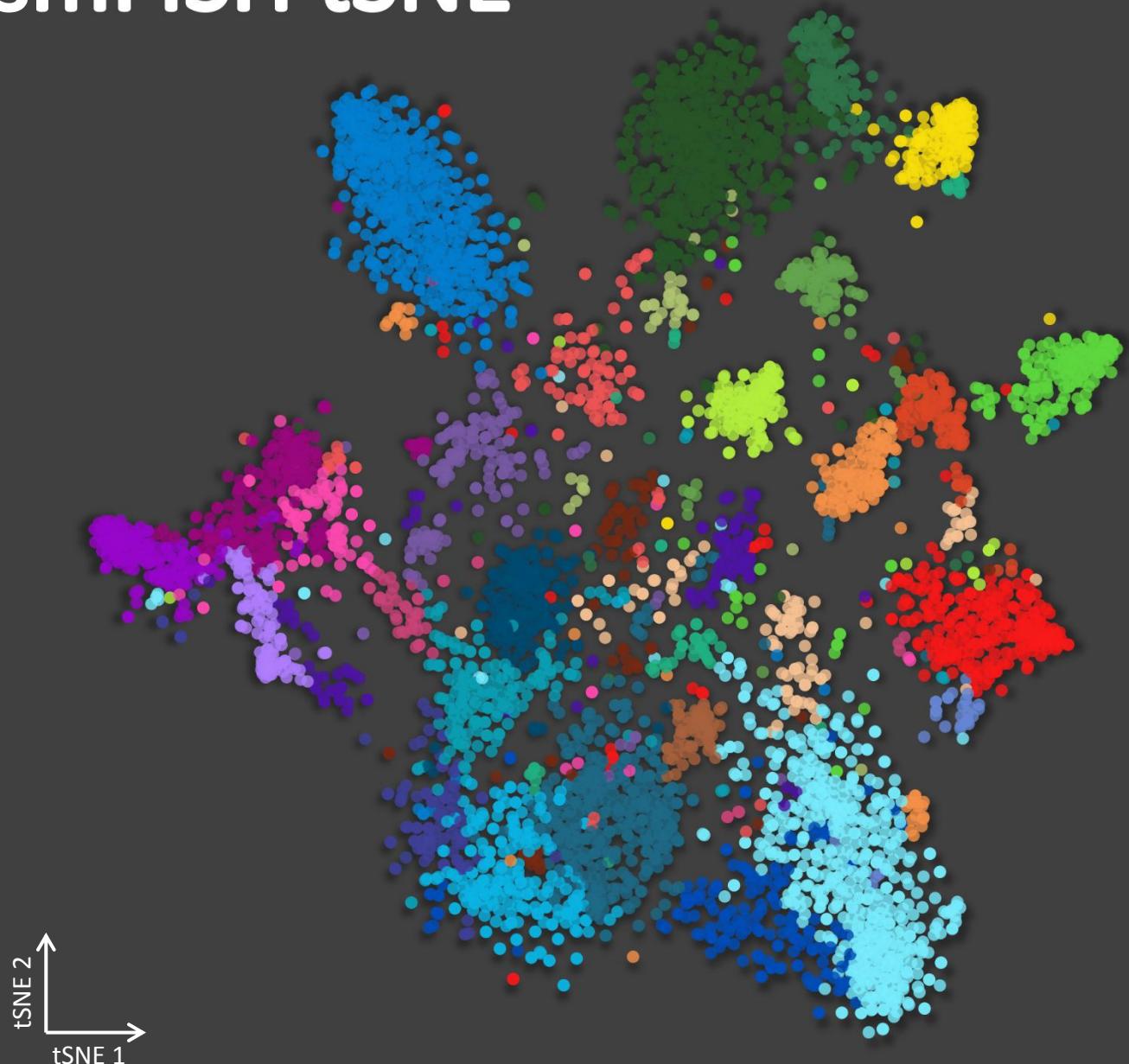
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Hybridization1_Aldoc	38	0	9	5	38	2	4	3	7	10	...	11	10	5	4	10	2	0	2	2	9
Hybridization1_Foxj1	0	0	0	1	5	0	3	1	1	1	...	0	0	0	2	1	8	1	2	0	4
Hybridization6_Bmp4	1	0	0	0	0	0	0	0	1	0	...	0	0	0	0	0	0	1	1	0	0
Hybridization6_Itpk2	4	0	0	1	0	0	1	0	2	1	...	3	0	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_Plip1	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_Ctph	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	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_Cri	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	
Hybridization9_Anln	19	1	1	1	2	2	2	6	3	23	...	0	1	10	3	8	14	8	11	3	0
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Hybridization12_Slc32a1	2	1	2	2	22	0	1	0	2	0	...	0	1	1	0	0	4	0	0	0	0
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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	1	9	3	11	
Hybridization2_Fit1	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
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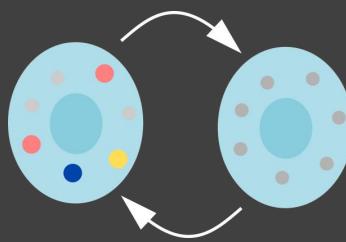
osmFISH clustering



osmFISH tSNE



Inhibitory neurons	Inhib. CP Inhib. Crhbp Inhib. Cnr1 Inhib. IC Inhib. Kcnip2 Inhib. Pthlh Inhib. Vip	Astro	Astro. Gfap Astro. Mfge8
Oligodendrocytes	OPC Olig. COP Olig. NF Olig. MF Olig. Mature		
Excitatory neurons	Py. Cpne5 Py. L2/3 Py. L2/3 L5 Py. Kcnip2 Py. L3/4 Py. L4 Py. L5 Py. L6 Hippocampal	Immune	PVM Microglia
Ventricles			Choroid plexus Ependymal
Vasculature			Pericytes Endothelial Endothelial 1 VSM



Cell type map

Types

- Inhib CP
- Inhib Crhbp
- Inhib Cnr1
- Inhib IC
- Inhib Kcnip2
- Inhib Pthlh
- Inhib Vip

- Py Cpne5
- Py L2/3
- Py L2/3 L5
- Py Kcnip2
- Py L3/4
- Py L4
- Py L5
- Py L6
- Hippocampal

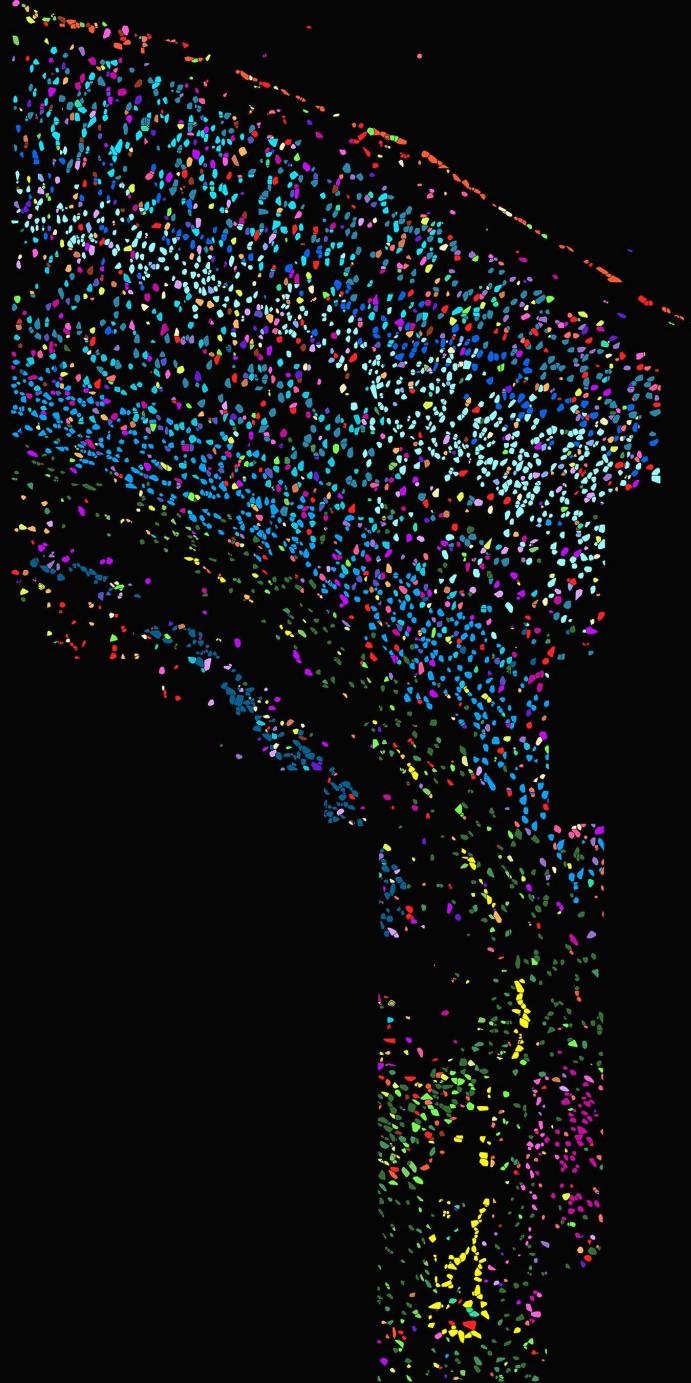
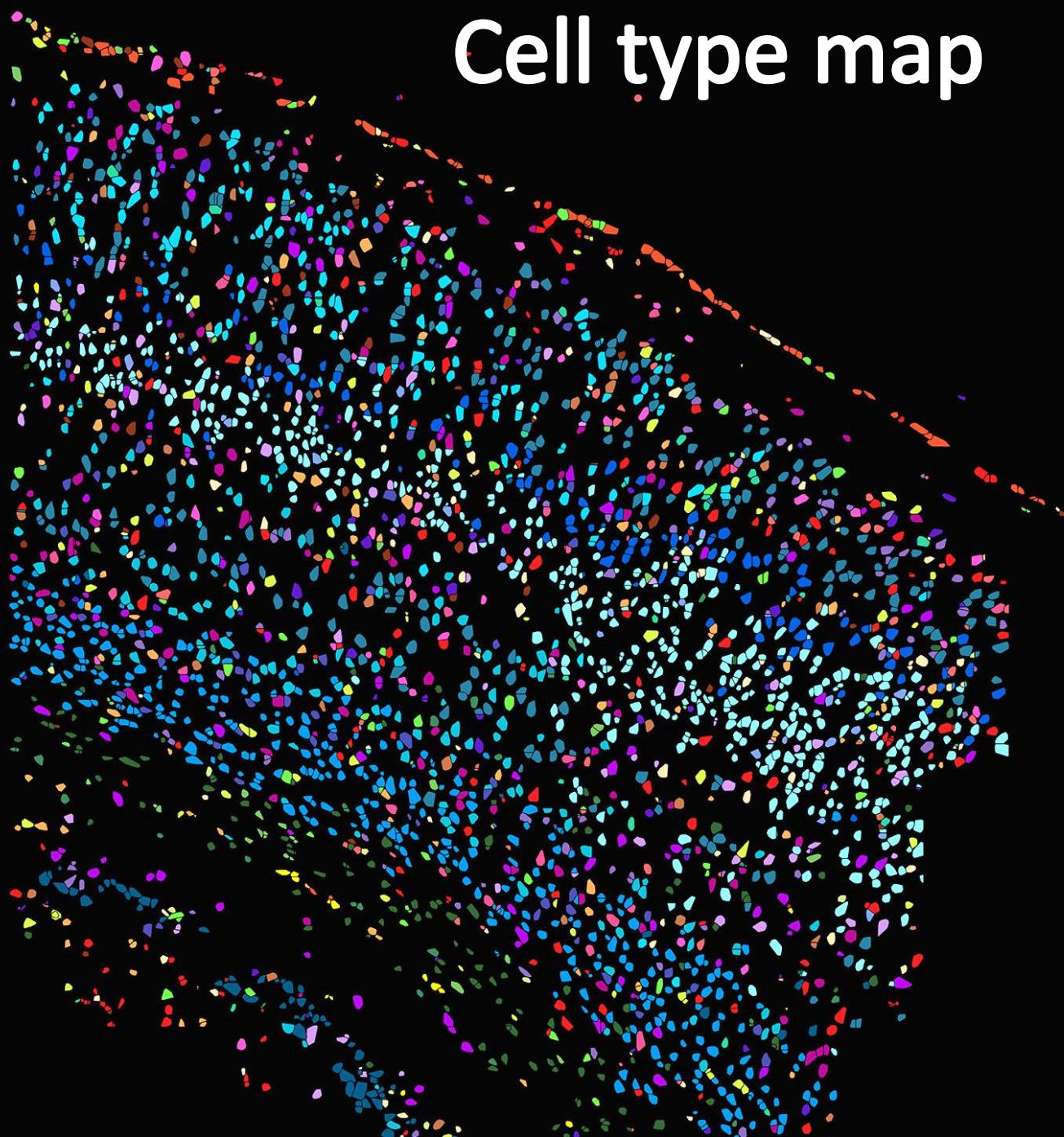
- Astro Gfap
- Astro Mfge8

- OPC
- Olig COP
- Olig NF
- Olig MF
- Olig Mature

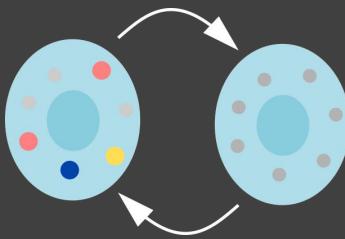
- Pvm
- Microglia

- Choroid plexus
- Ependymal

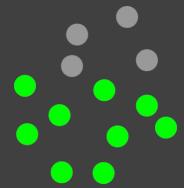
- Pericytes
- Endothelial
- Endothelial 1
- Vsm



Cyclic smFISH



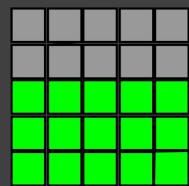
Resolution: Diffraction limited (150-300nm)



Detection efficiency: ~100%

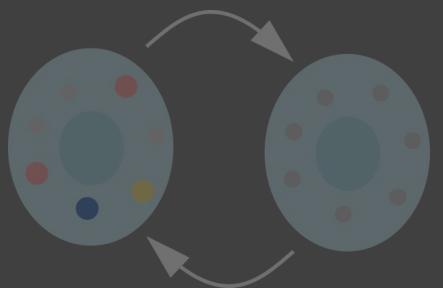


Gene throughput: tens of genes (F x R)

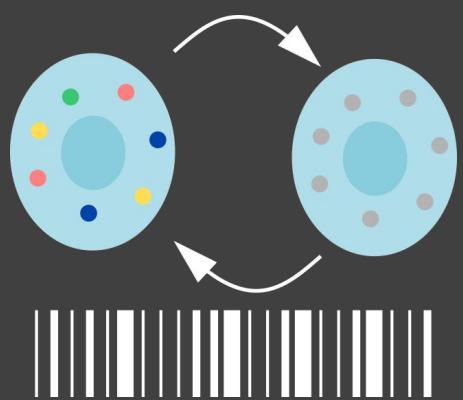


Spatial throughput: several mm²

Cyclic FISH



Barcoded FISH



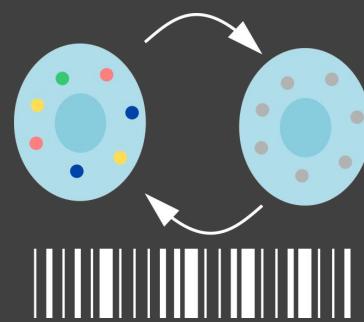
in situ Sequencing



Spatial Sequencing



Reprobing same molecule



Round 1



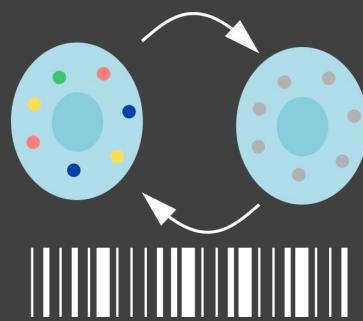
Stripping



Round 2

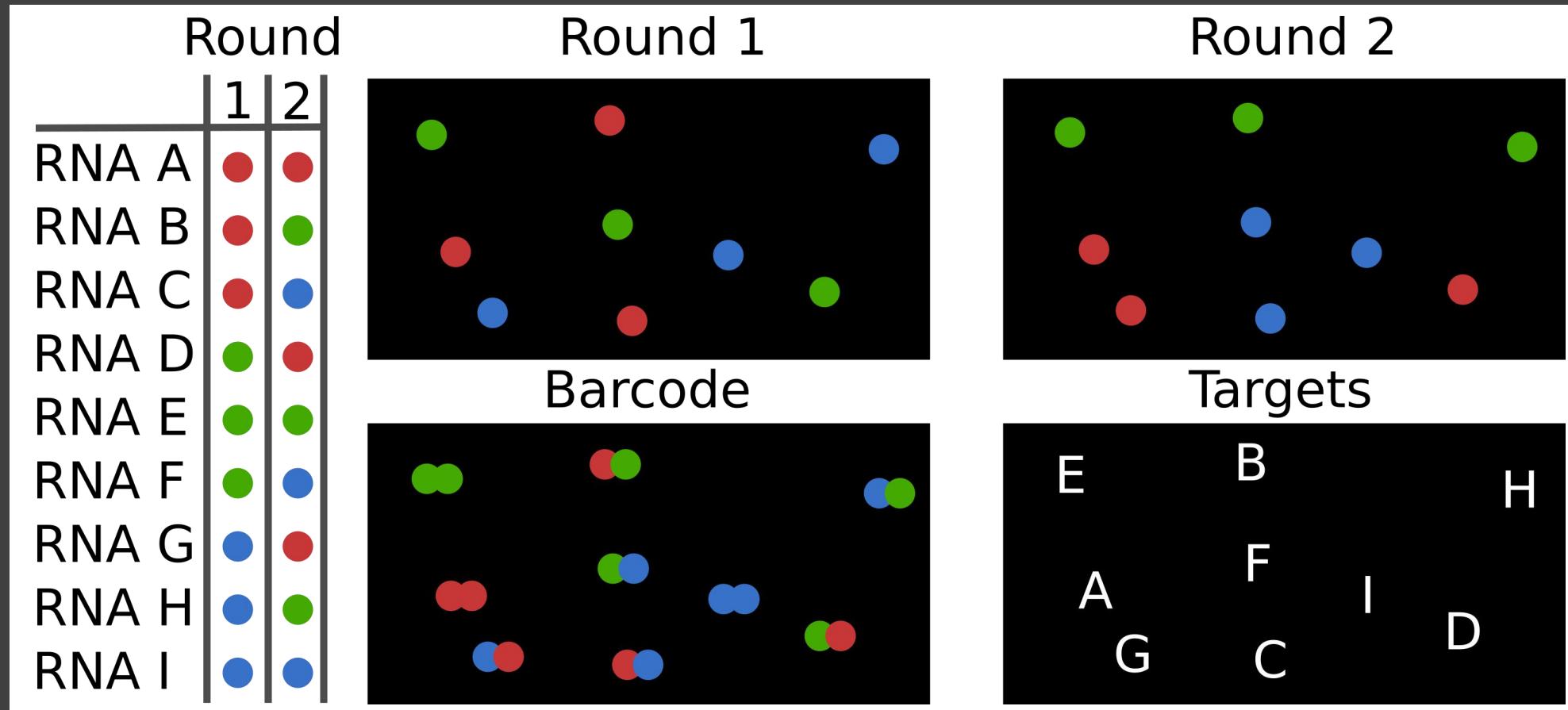
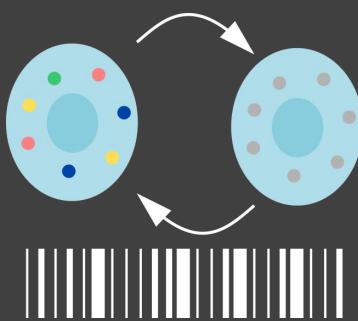


Barcoding

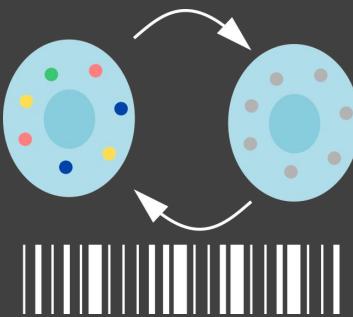


	Round	
	1	2
RNA A	●	●
RNA B	●	●
RNA C	●	●
RNA D	●	●
RNA E	●	●
RNA F	●	●
RNA G	●	●
RNA H	●	●
RNA I	●	●

Barcoding



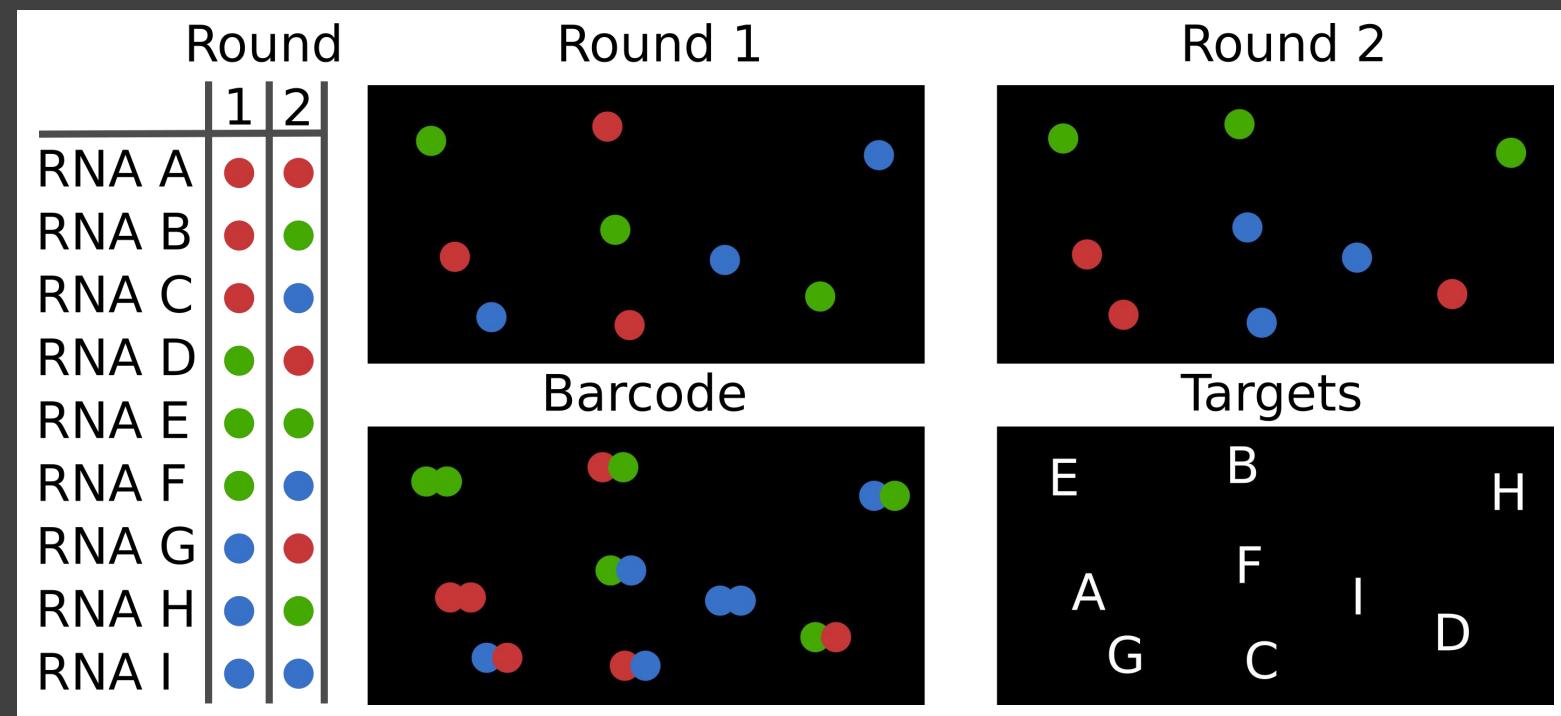
Barcoding



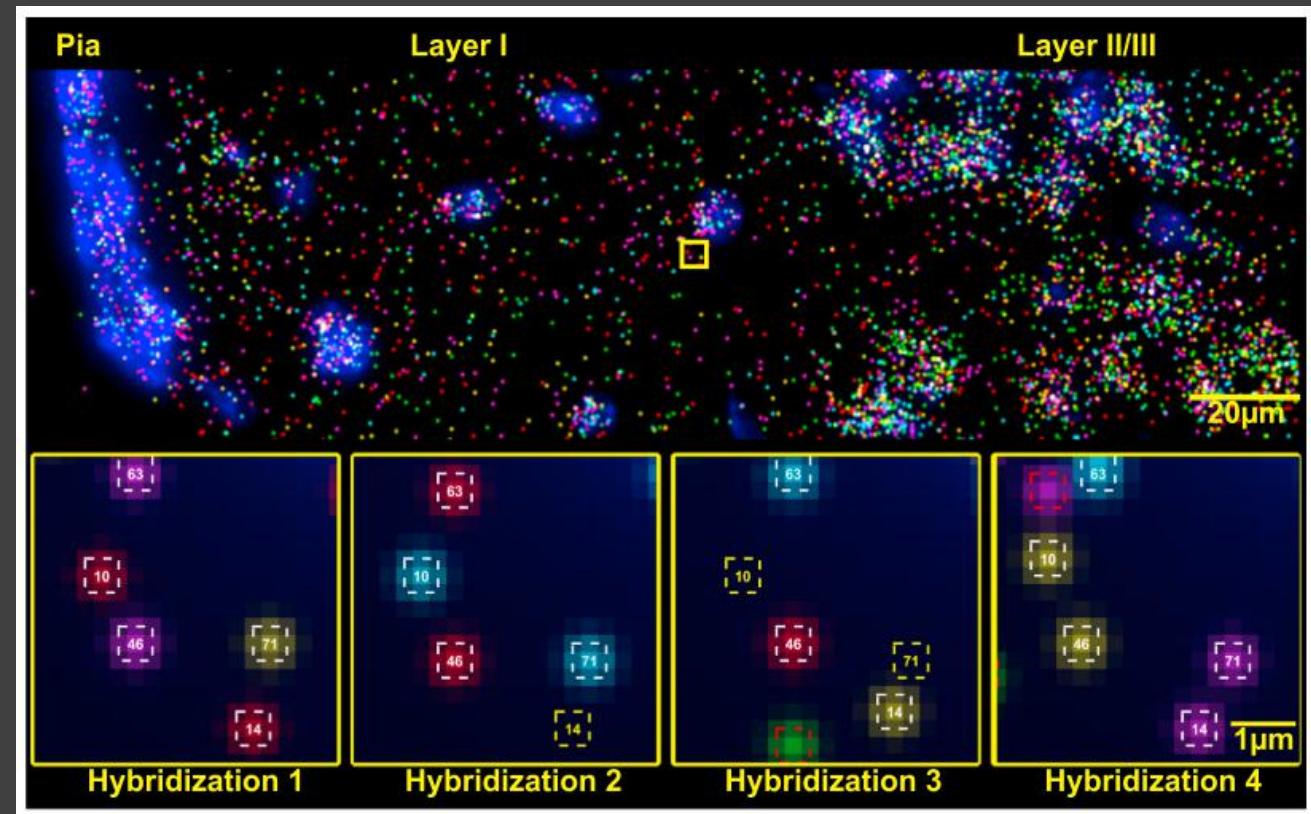
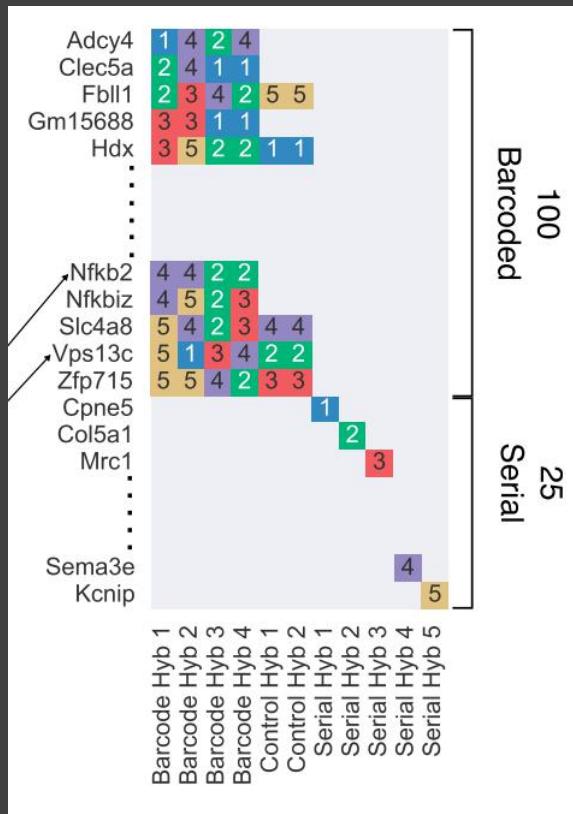
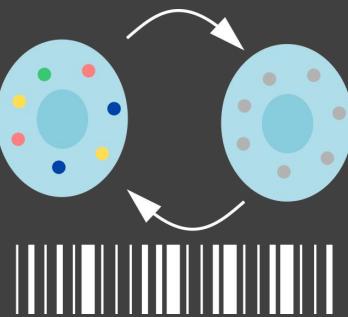
Scaling: Fluo^{Rounds}

$$4^7 = 16,384 \text{ genes}$$

$$4^8 = 65,536 \text{ genes}$$

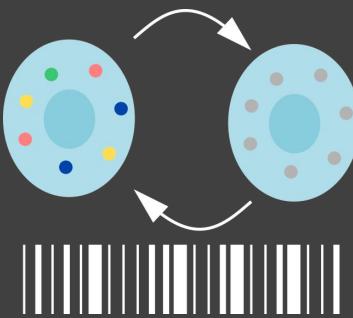


SeqFISH



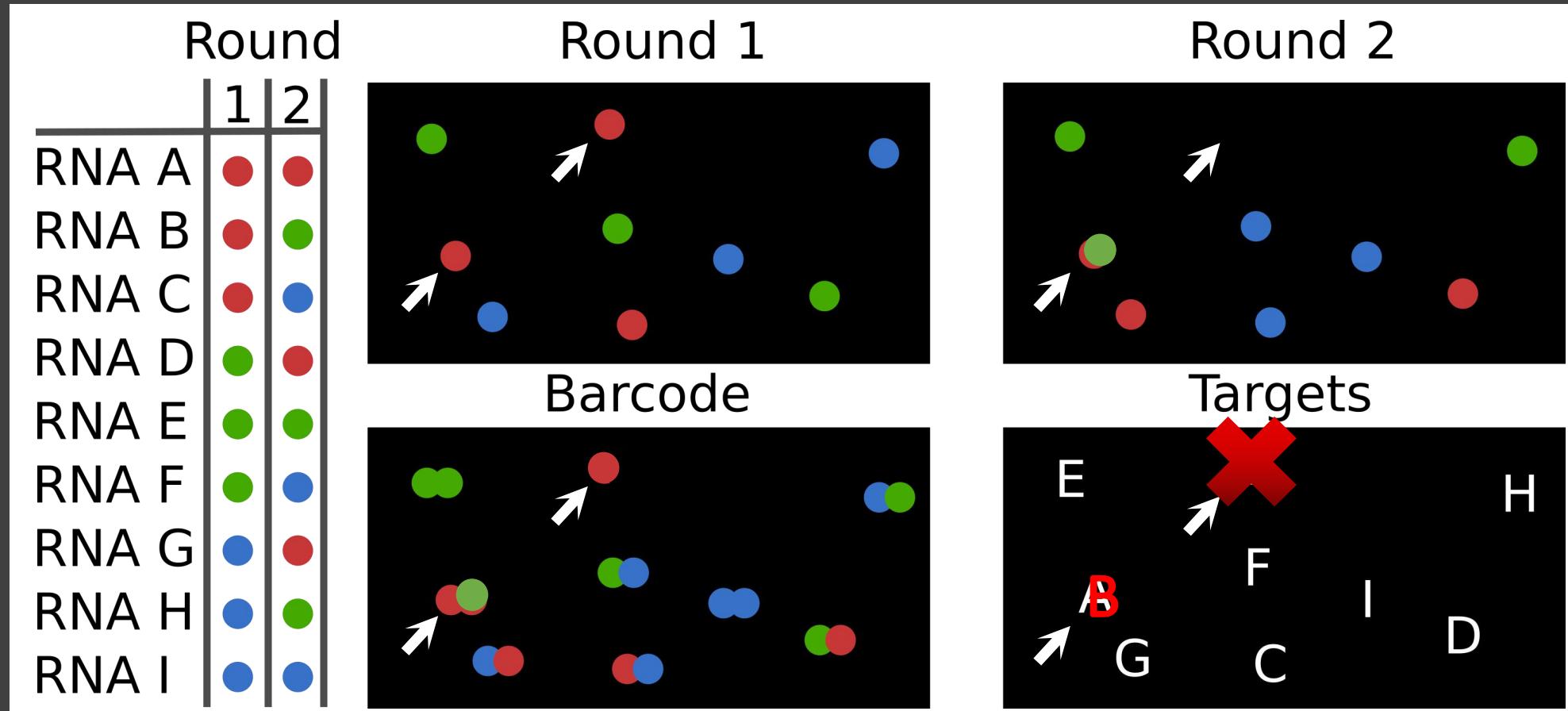
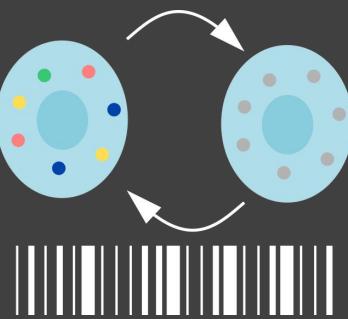
Shah *et al.* Neuron 2016

Challenges

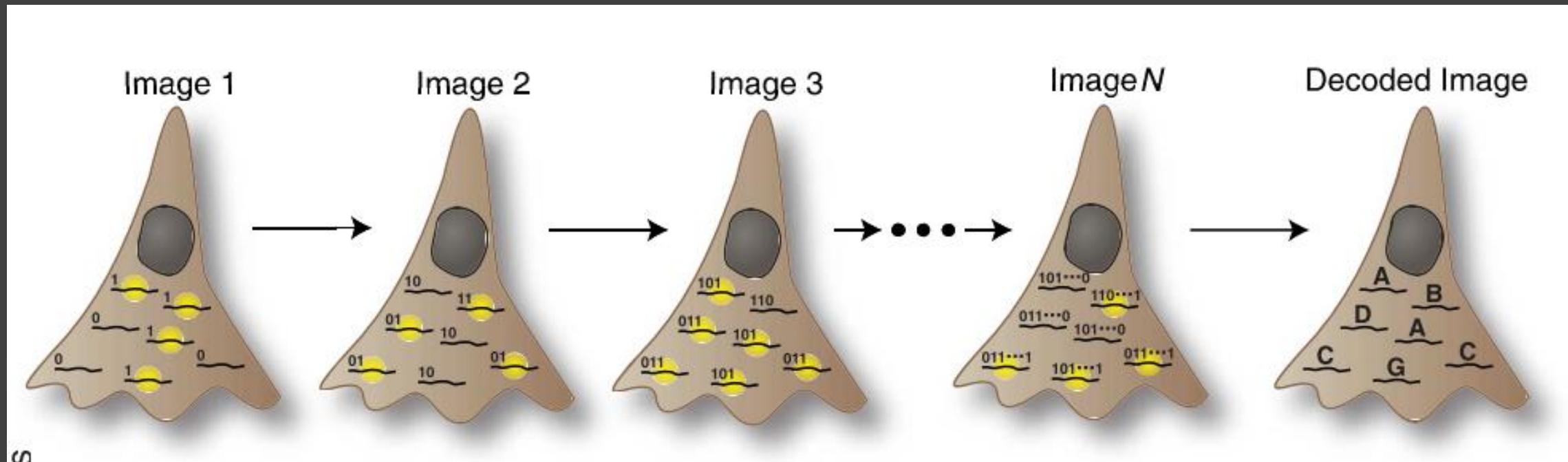
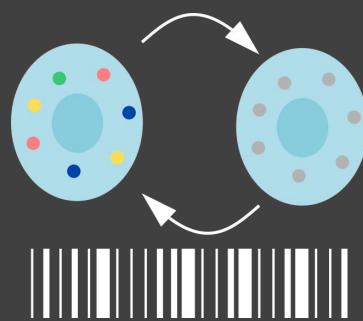


- Errors in barcodes

Errors

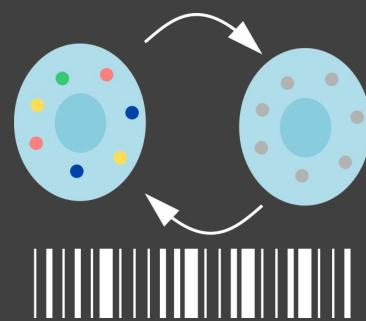


MERFISH

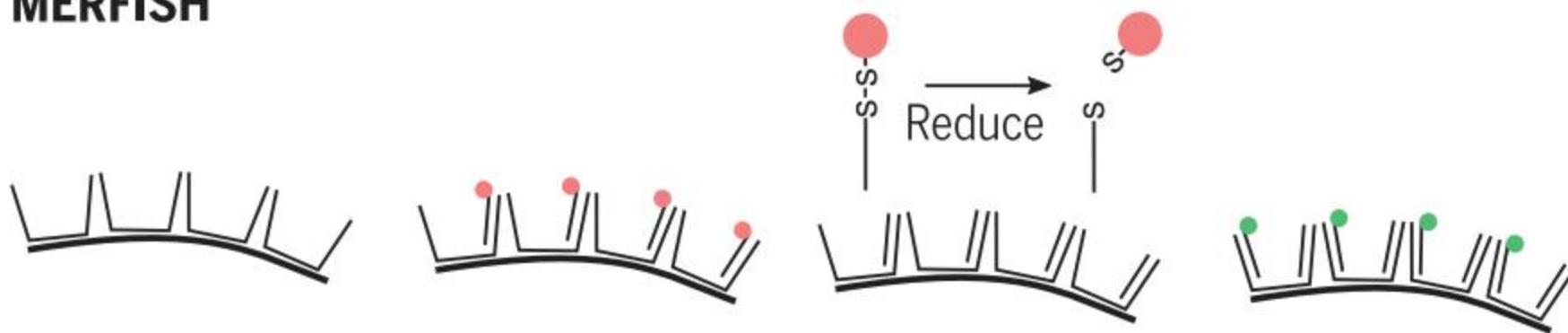


Chen *et al.* Science 2015

MERFISH

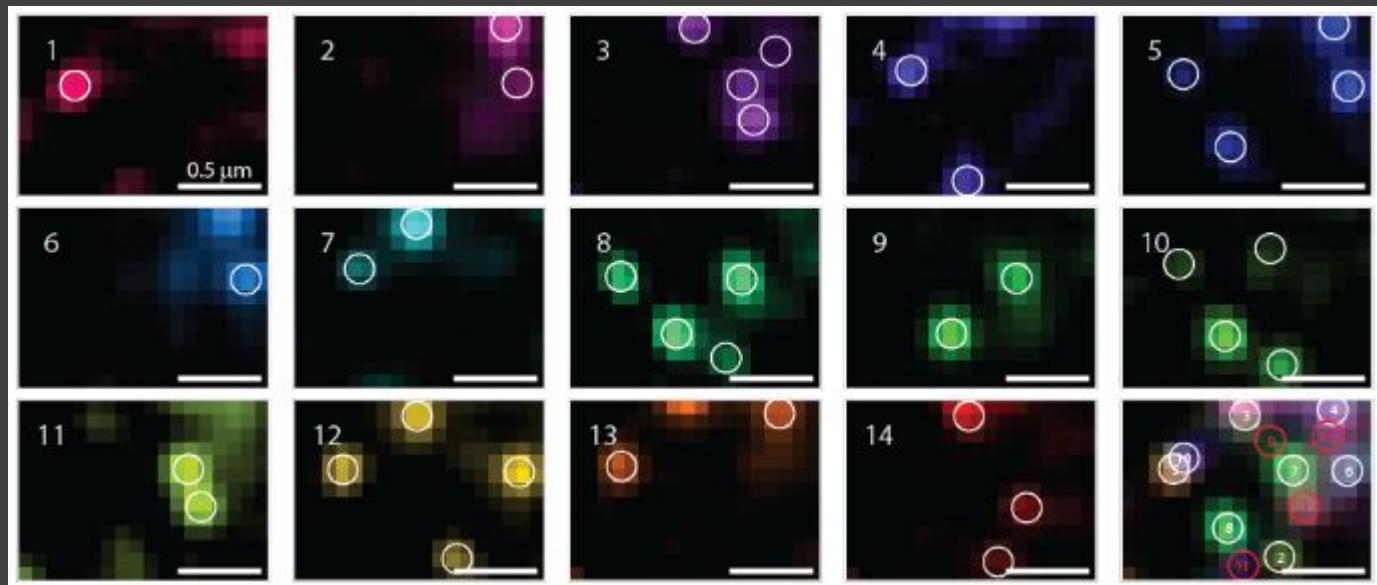
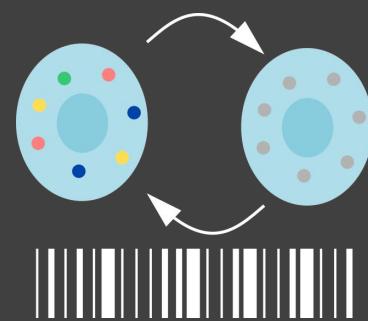


MERFISH



Bit	Round 1	Round 2
1	1	5
2	2	6
3	3	7
4	4	8
Gene 1	1 0 0 0	0 0 1 0
Gene 2	0 0 1 0	1 0 0 0
Gene 3	1 0 0 0	0 1 0 0
Gene n	0 0 0 1	0 0 0 1

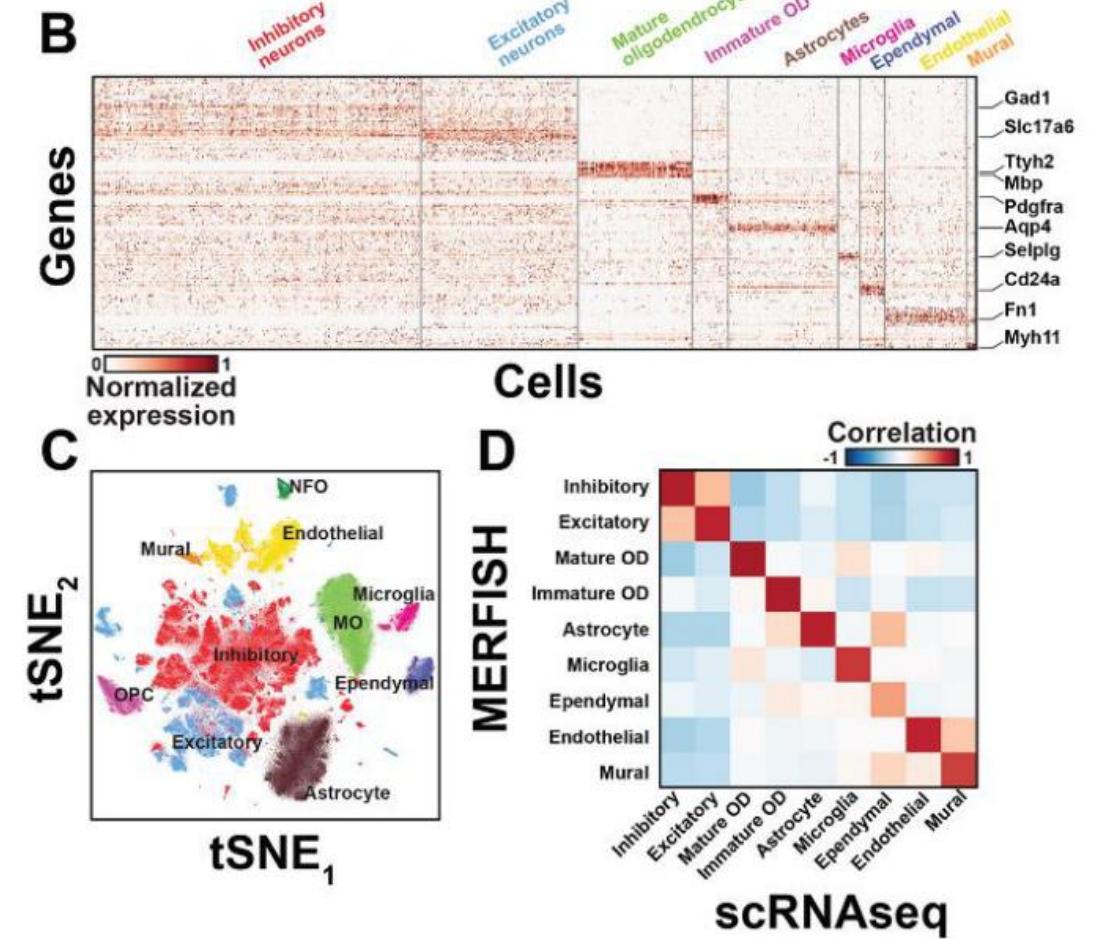
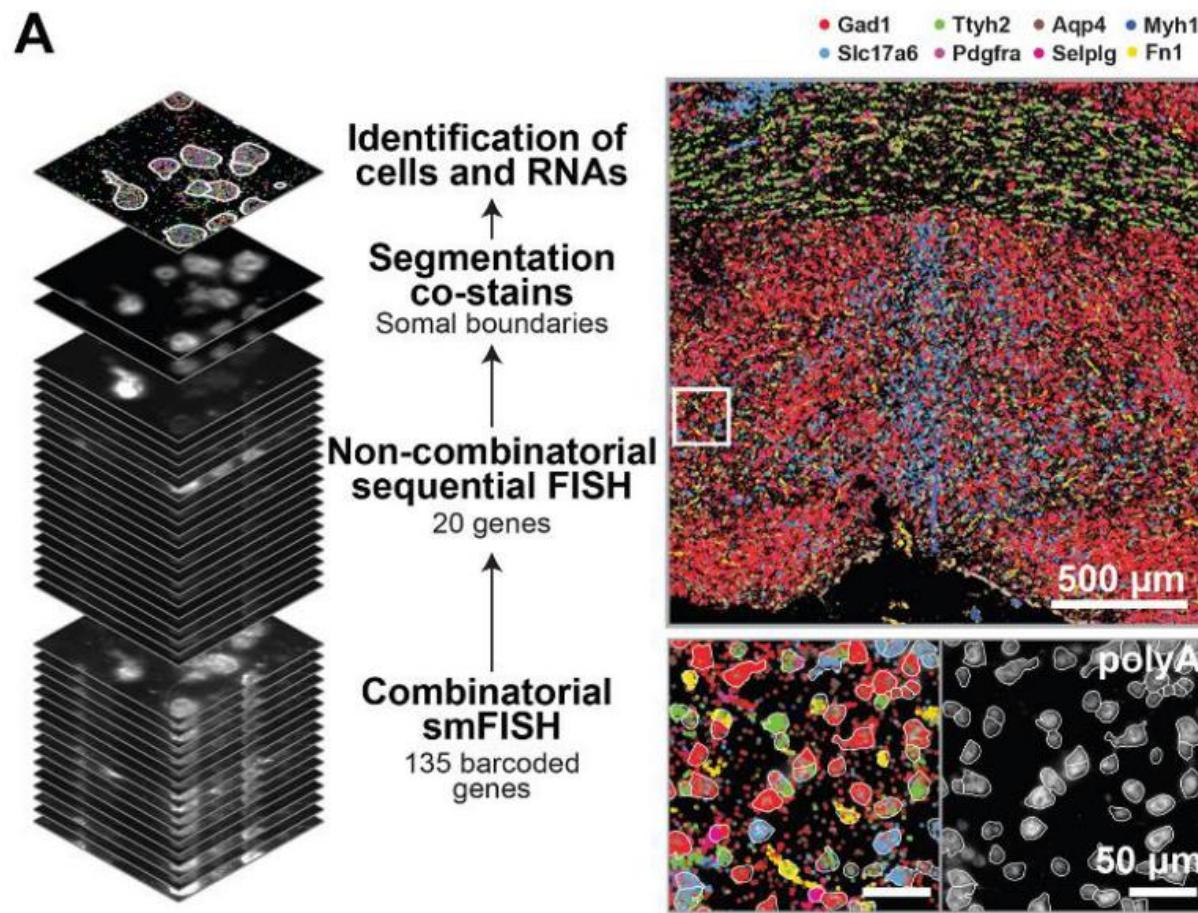
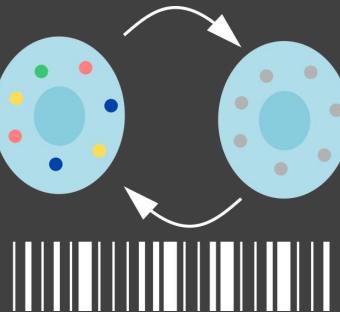
MERFISH



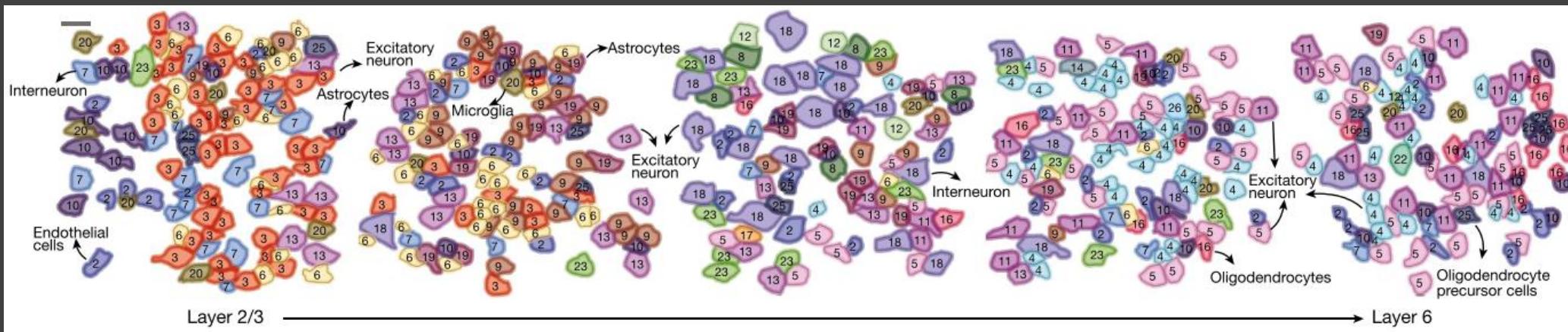
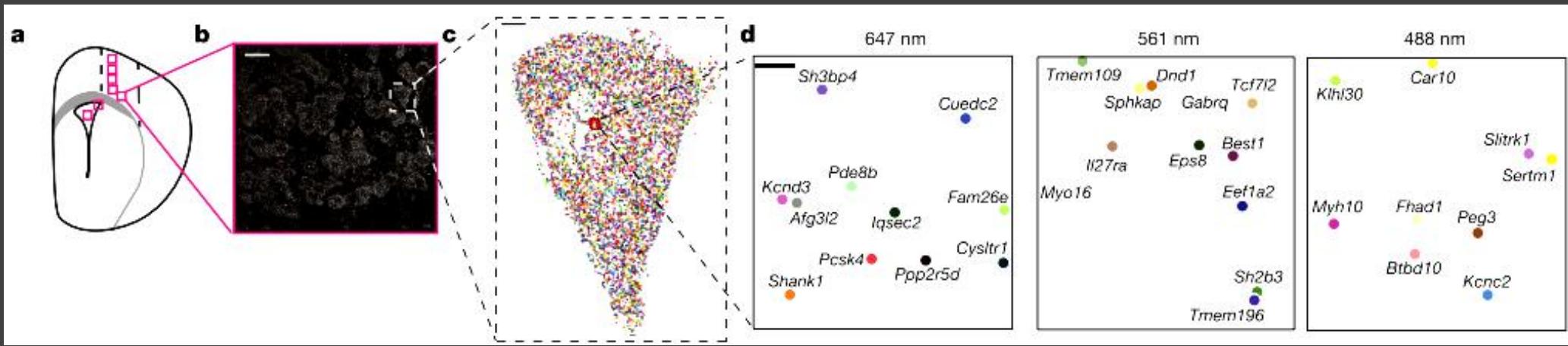
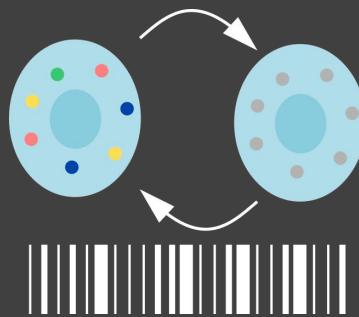
Spot number	Hybridization round													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0	1	0	0	0	0	0	0	0	1	0	0	1
2	0	0	0	0	0	0	0	1	0	1	0	1	0	1
3	0	0	1	0	0	0	1	0	0	0	0	1	0	1
4	0	1	0	0	1	1	0	0	0	0	0	0	1	0
5	1	0	0	0	0	0	0	1	0	0	0	0	1	0
6	0	1	0	0	1	1	0	0	0	0	0	0	1	0
7	0	0	1	0	0	0	0	1	1	0	1	0	0	0
8	0	0	1	0	0	0	0	1	1	1	0	0	0	0
9	0	0	0	1	0	0	0	1	1	1	0	0	0	0
10	0	0	0	0	1	0	0	0	0	0	1	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Chen *et al.* Science 2015

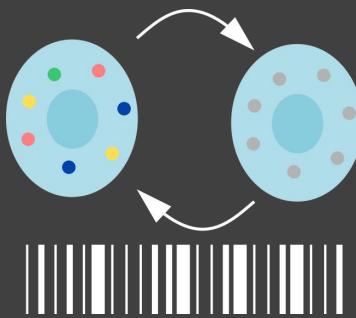
MERFISH



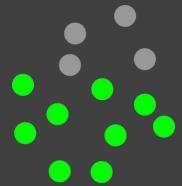
SeqFISH+



Barcoded smFISH



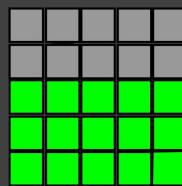
Resolution: Diffraction limited (150-300nm)



Detection efficiency: 70-90%

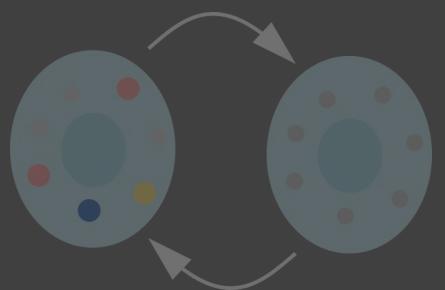


Gene throughput: 100 - 10,000

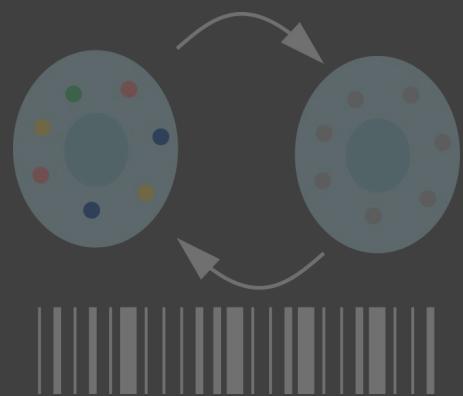


Spatial throughput: several mm²

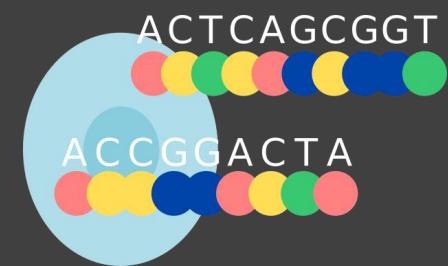
Cyclic FISH



Barcoded FISH



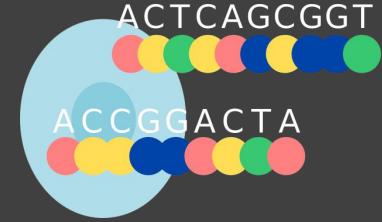
in situ Sequencing



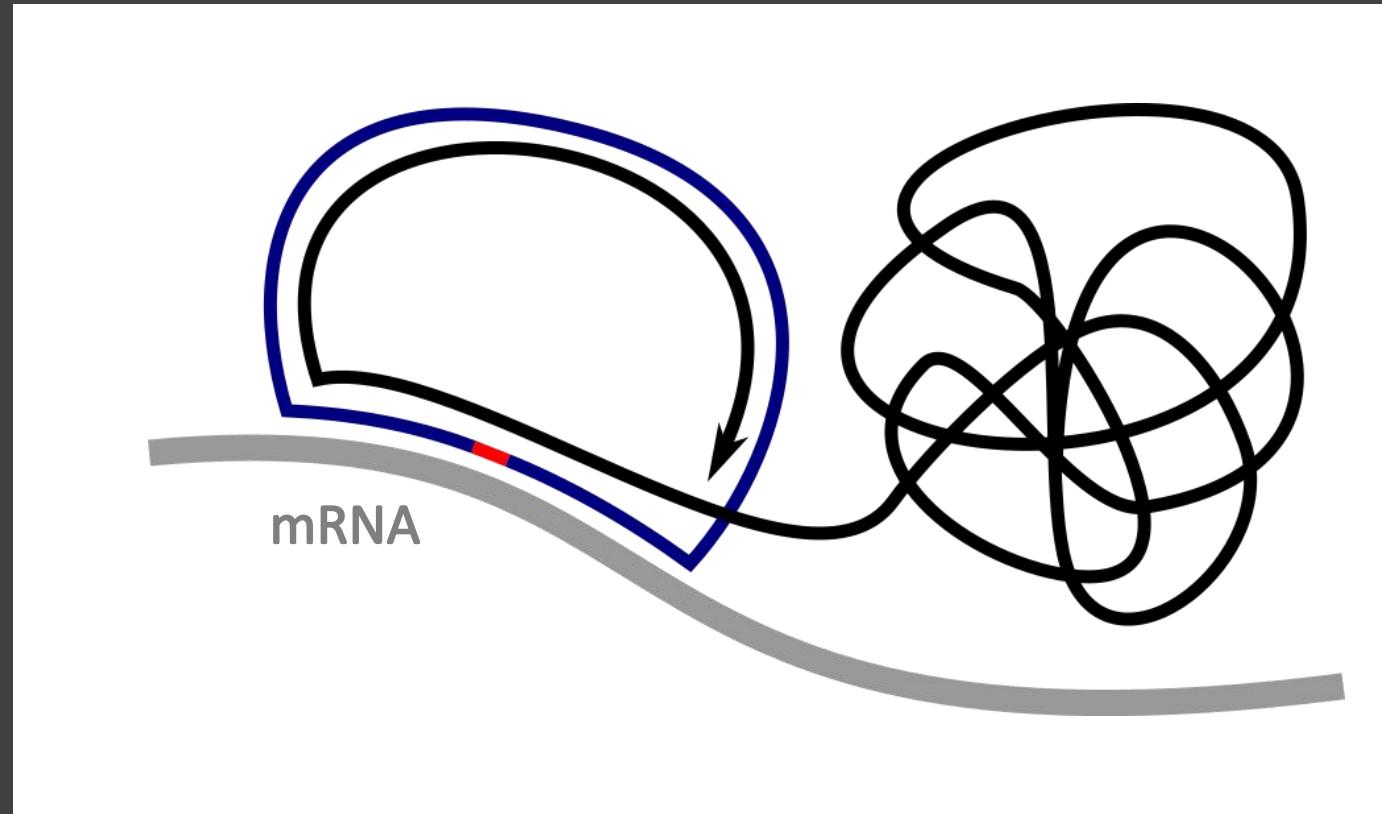
Spatial Sequencing



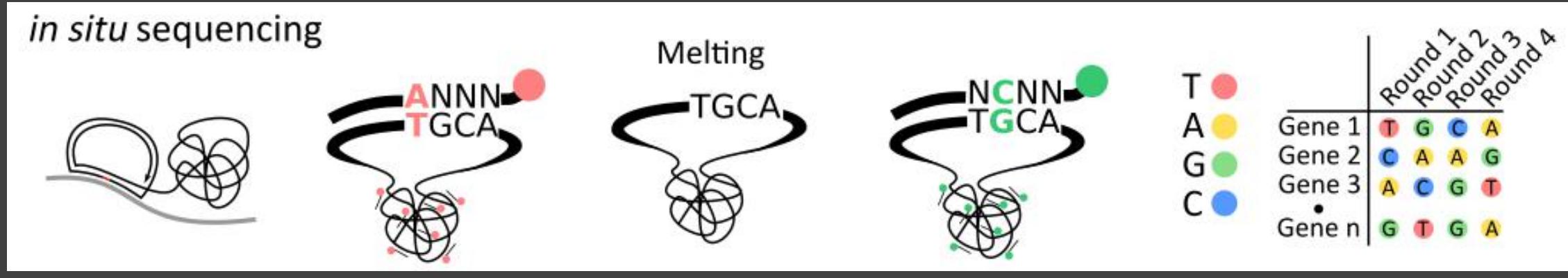
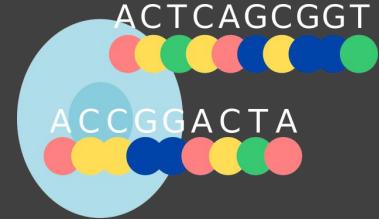
in situ sequencing



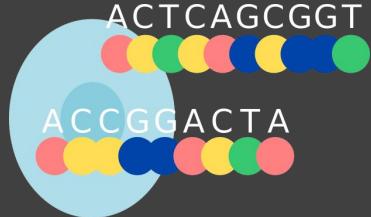
Rolling circle amplification



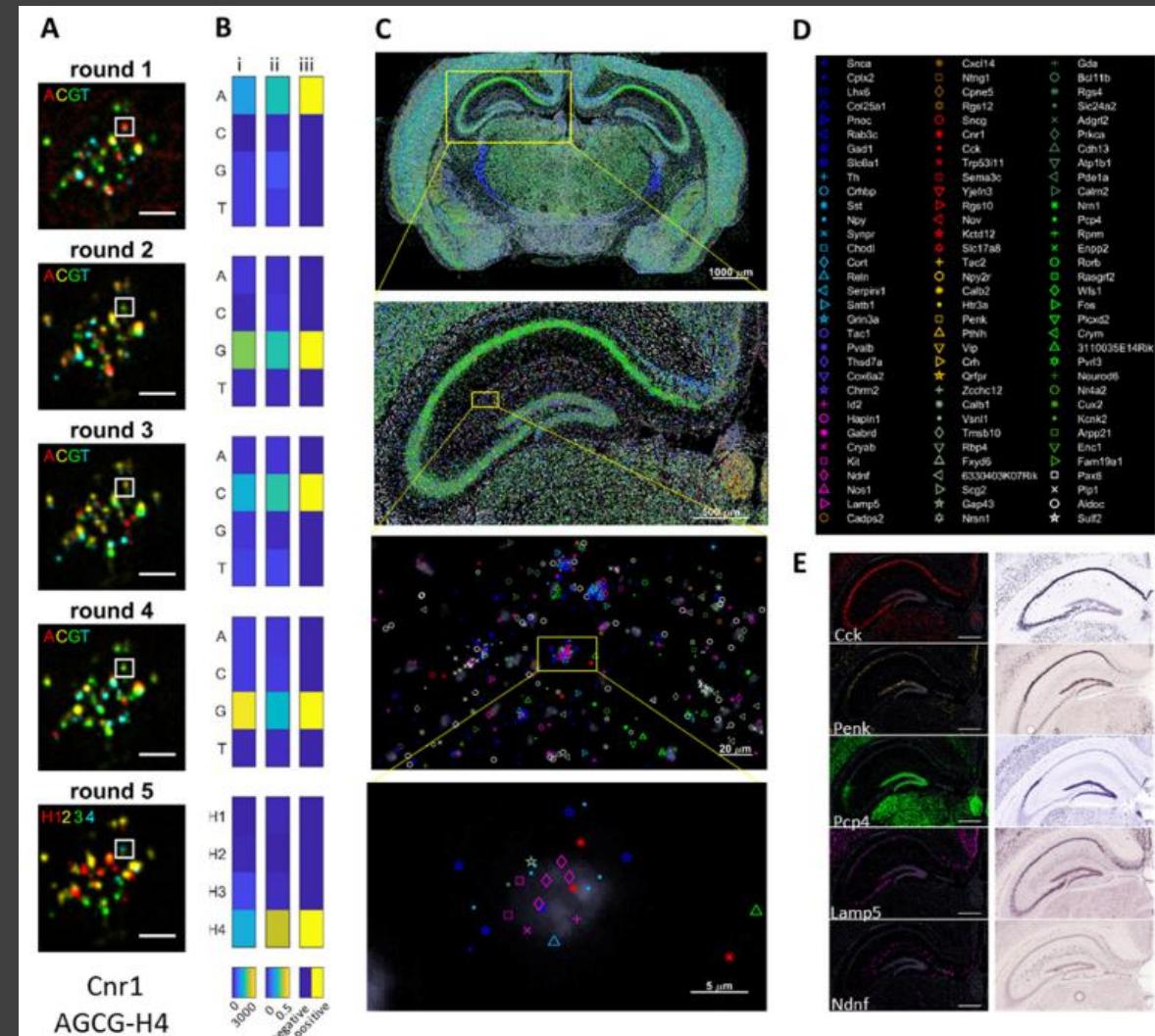
in situ sequencing



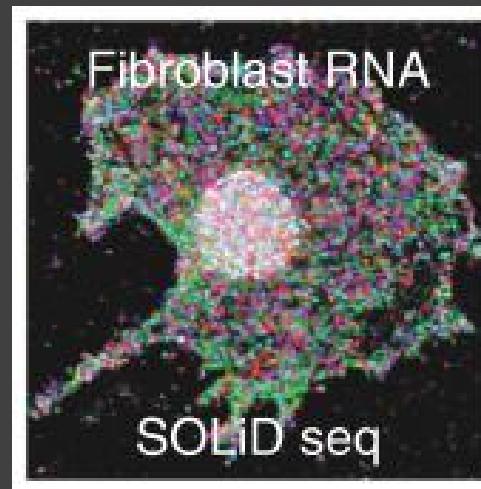
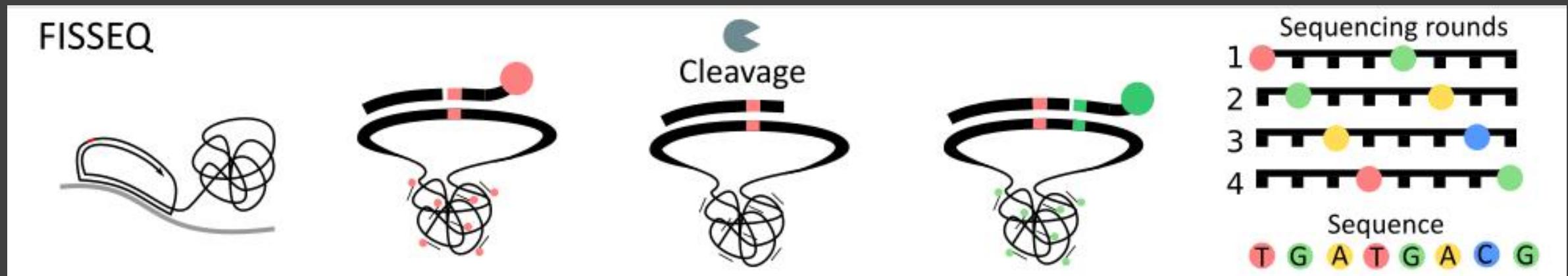
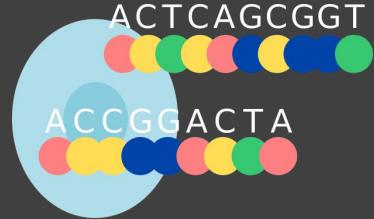
Sequencing by ligation



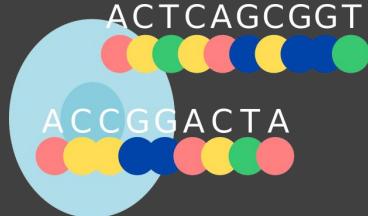
in situ sequencing



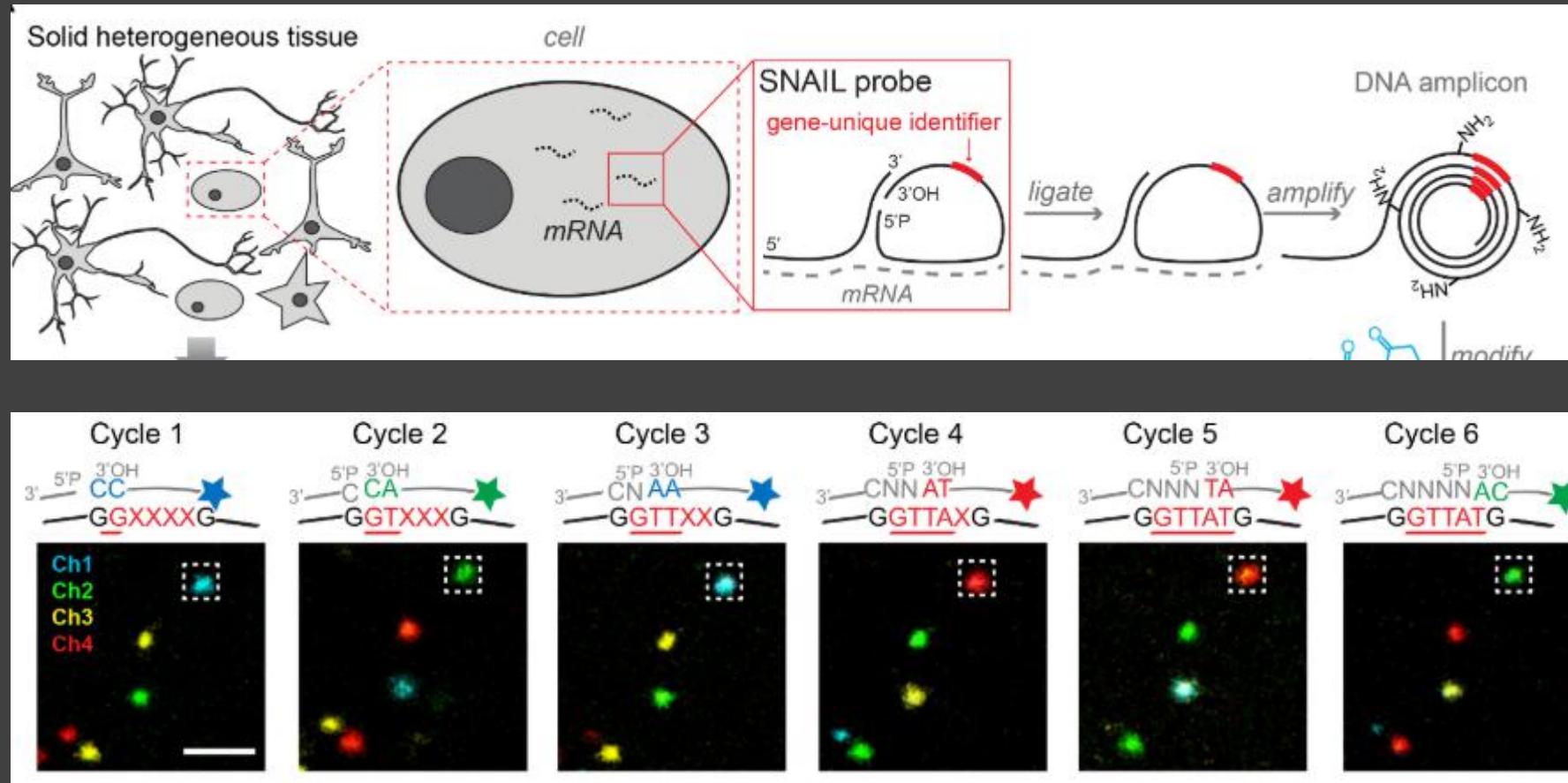
FISSEQ



Lee *et al.* Science 2014

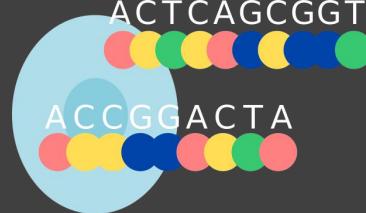
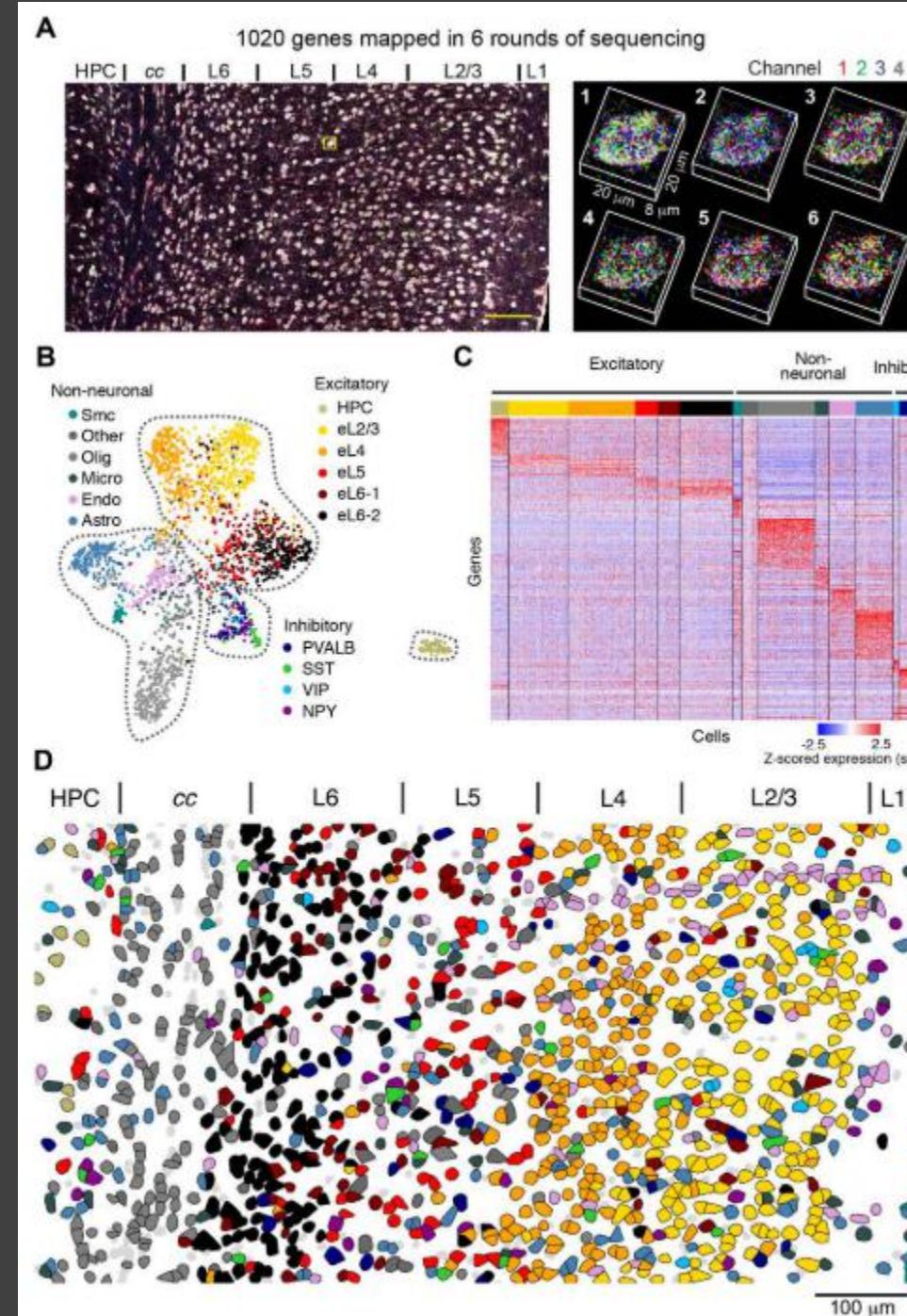


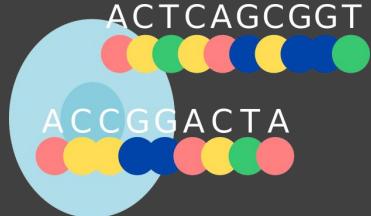
STAR MAP



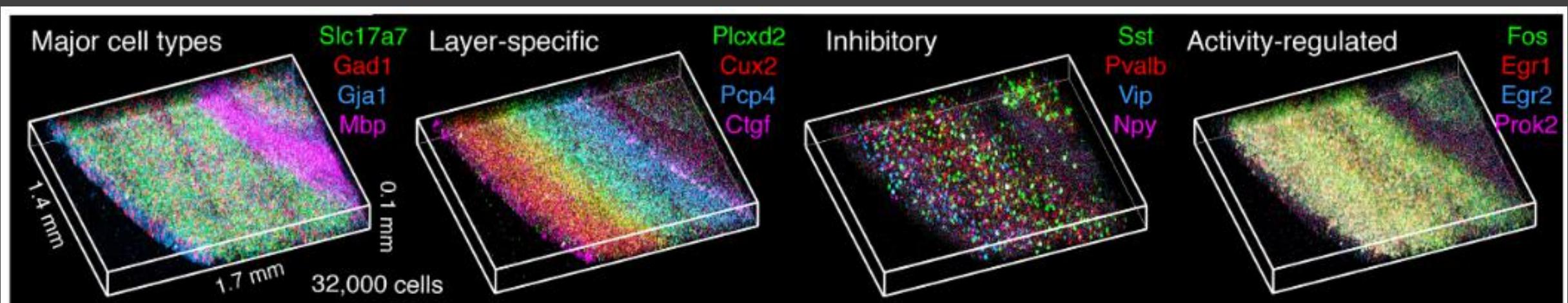
Wang *et al.* Science 2018

STAR MAP

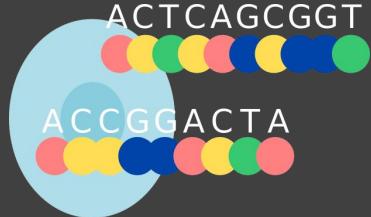




STAR MAP



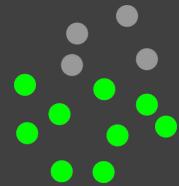
Wang *et al.* Science 2018



Sequencing *in situ*



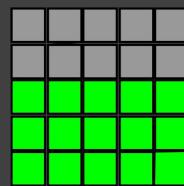
Resolution: Amplicon size (0.5 - 1um)



Detection efficiency: 0.01 - ~50%

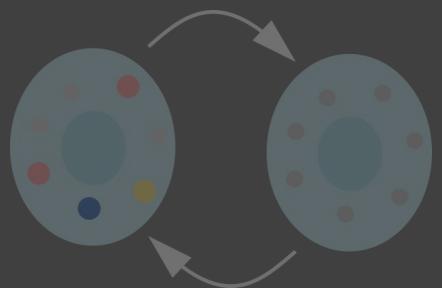


Gene throughput: 10 - 1,000

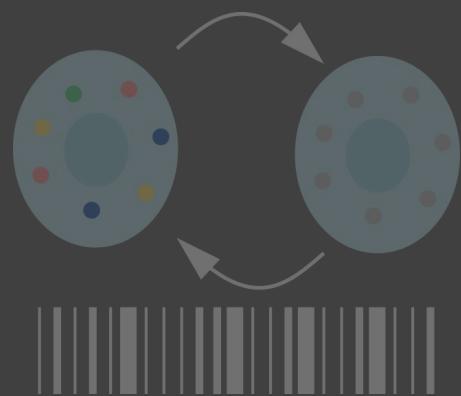


Spatial throughput: several mm² - several cm²

Cyclic FISH



Barcoded FISH



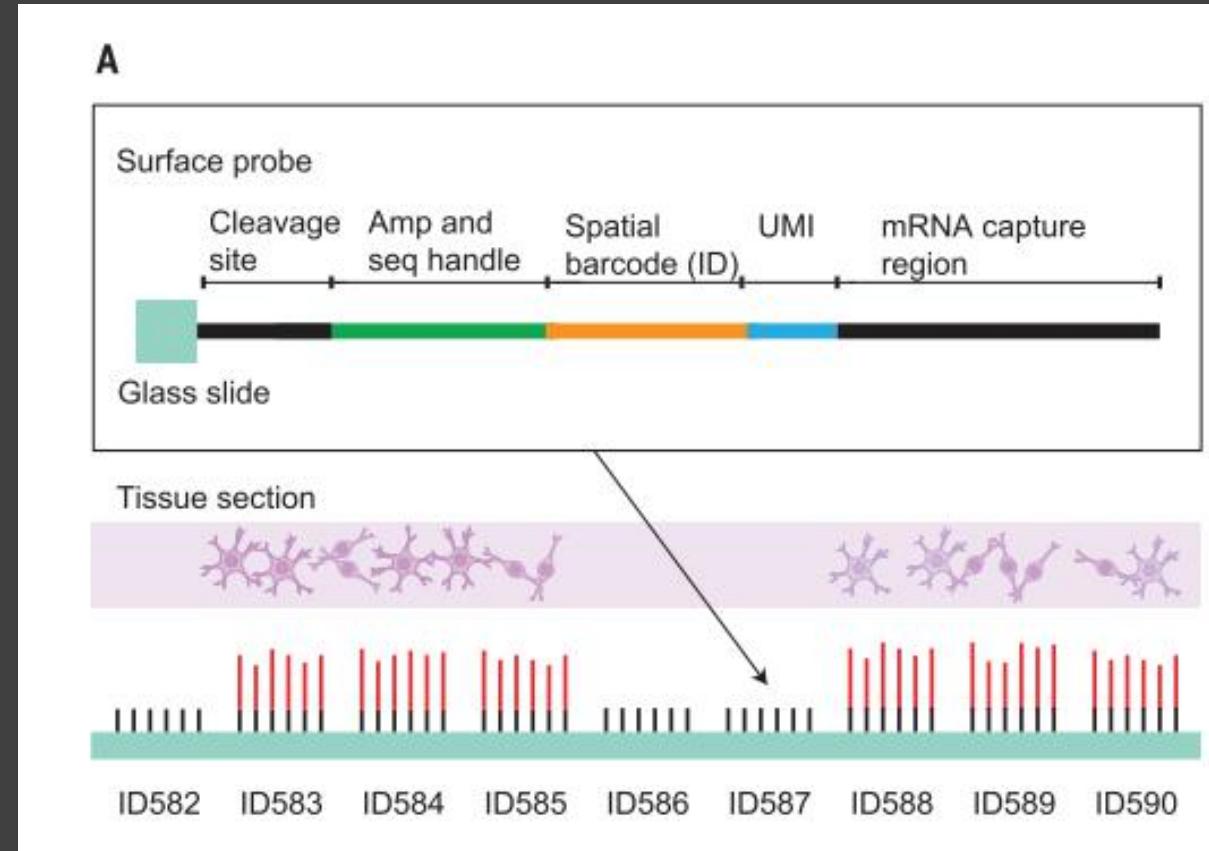
in situ Sequencing



Spatial Sequencing

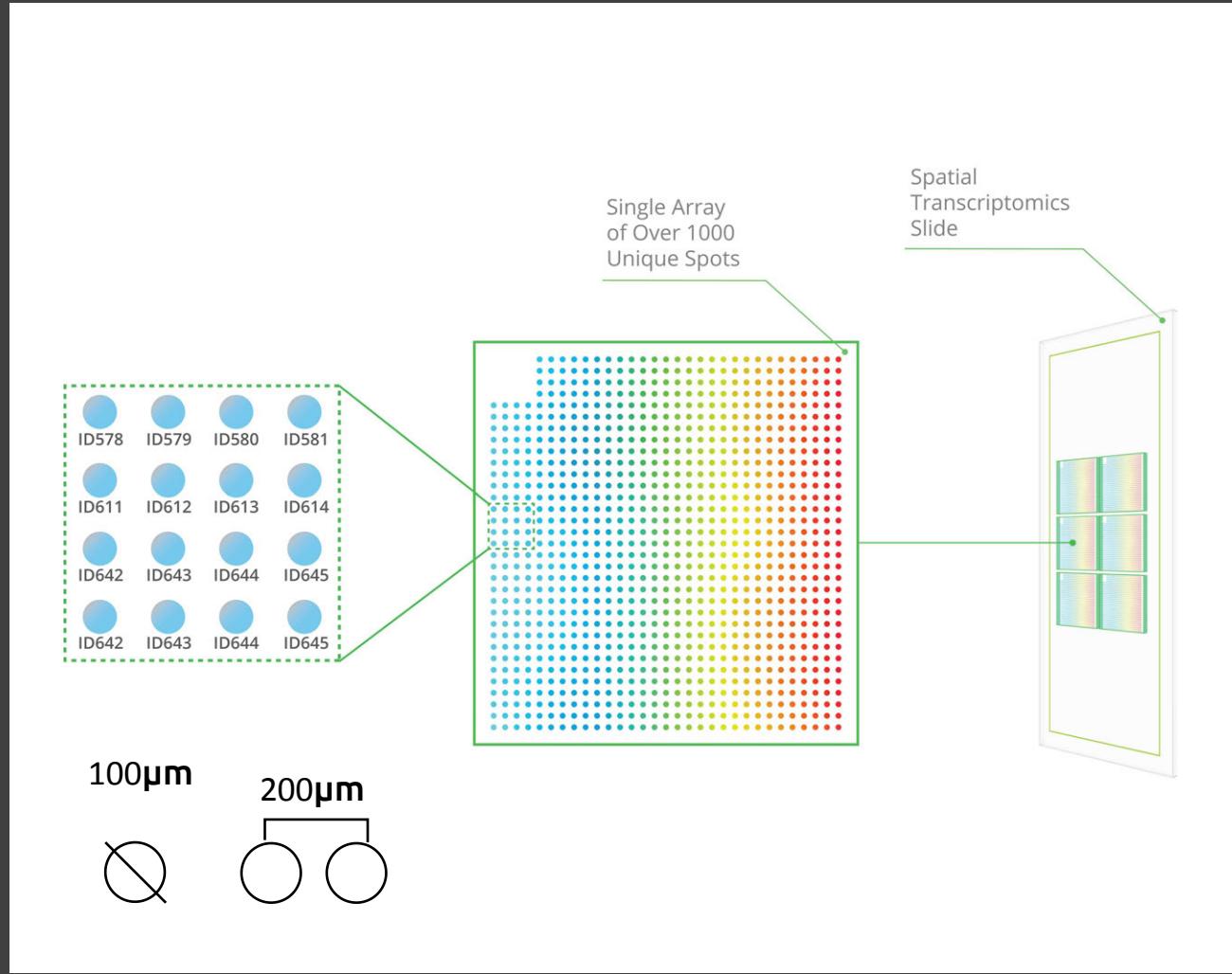
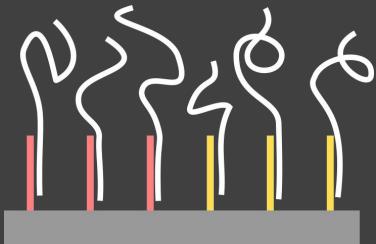


Spatial transcriptomics

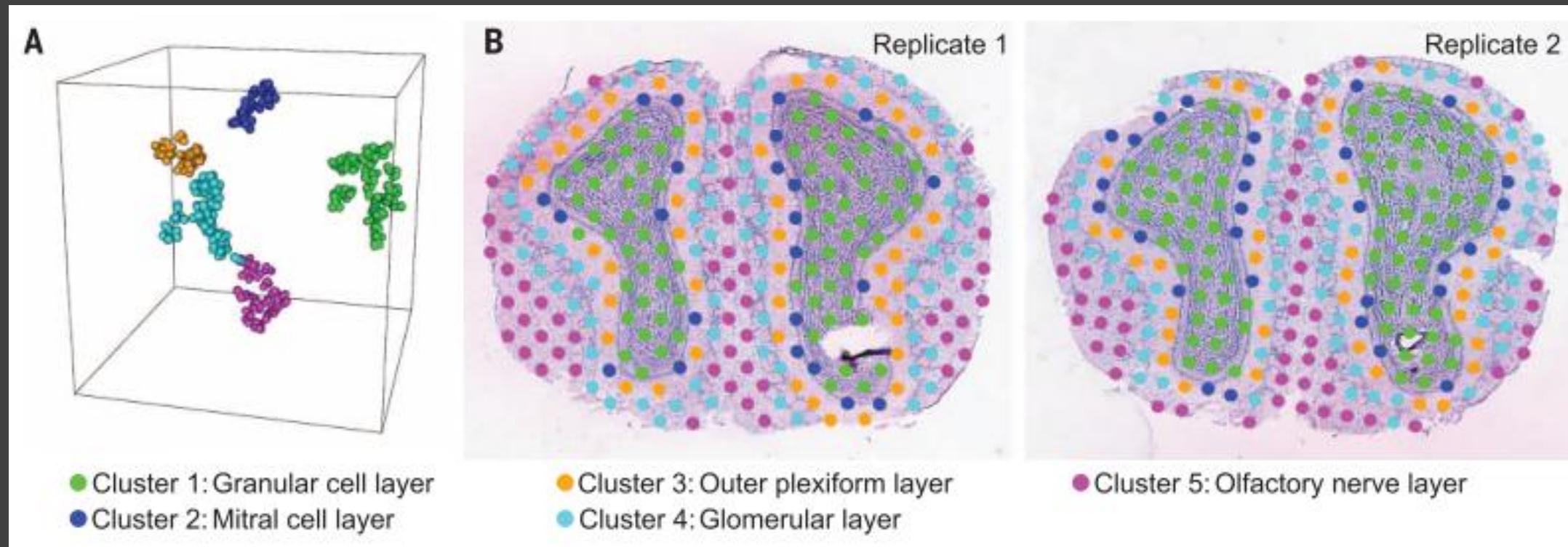
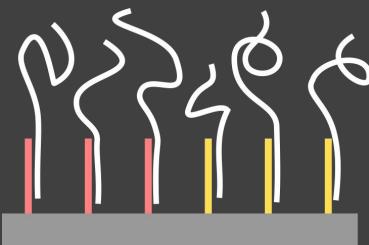


Stahl *et al.* Science 2016

Spatial transcriptomics

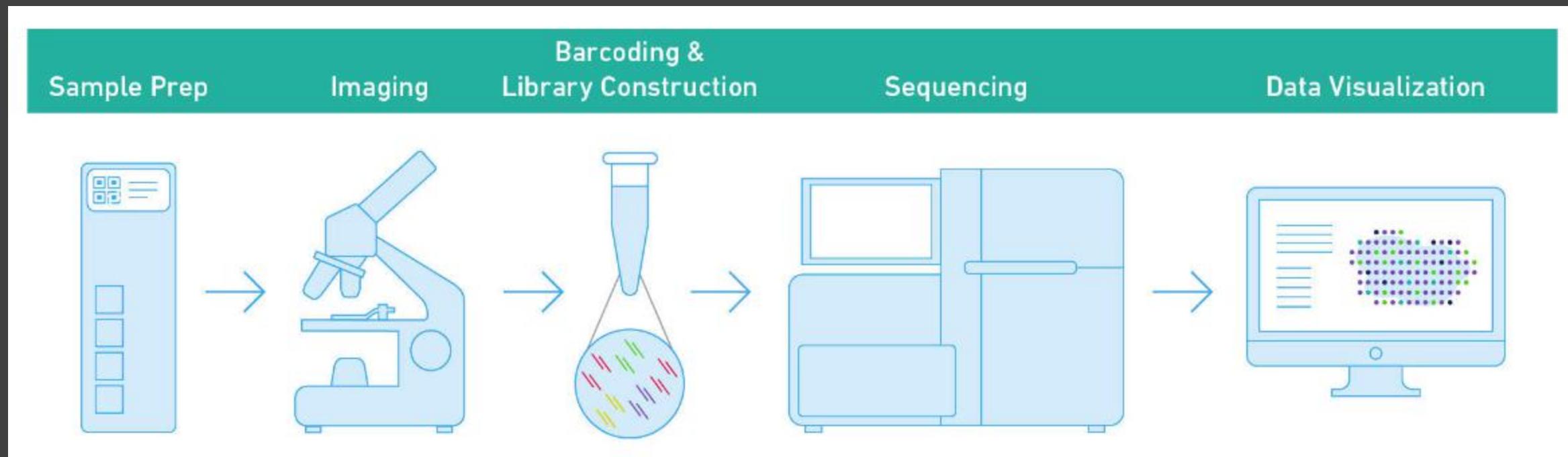


Spatial transcriptomics



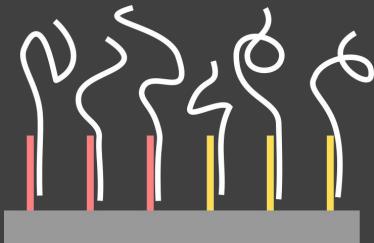
Stahl *et al.* Science 2016

10X Visium

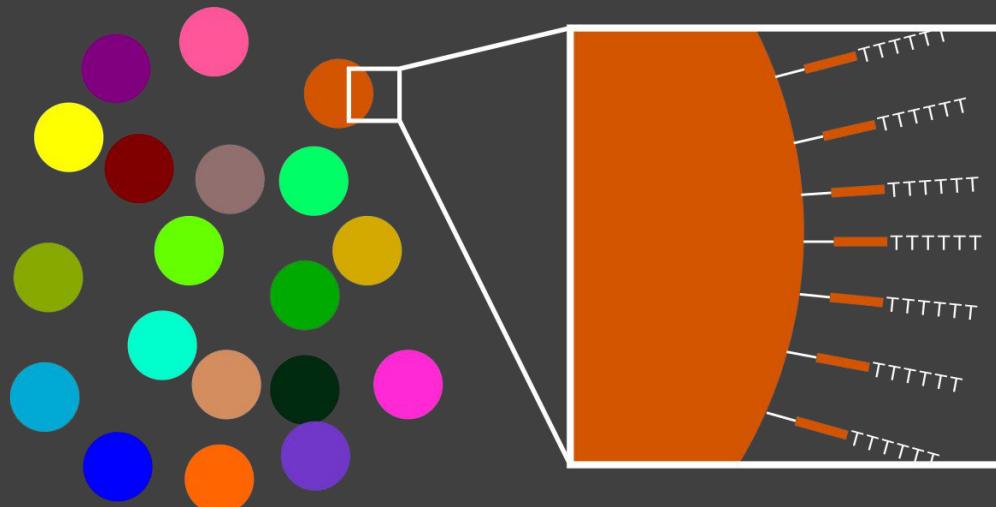


10X Visium

Array of barcoded beads



Random barcoded beads



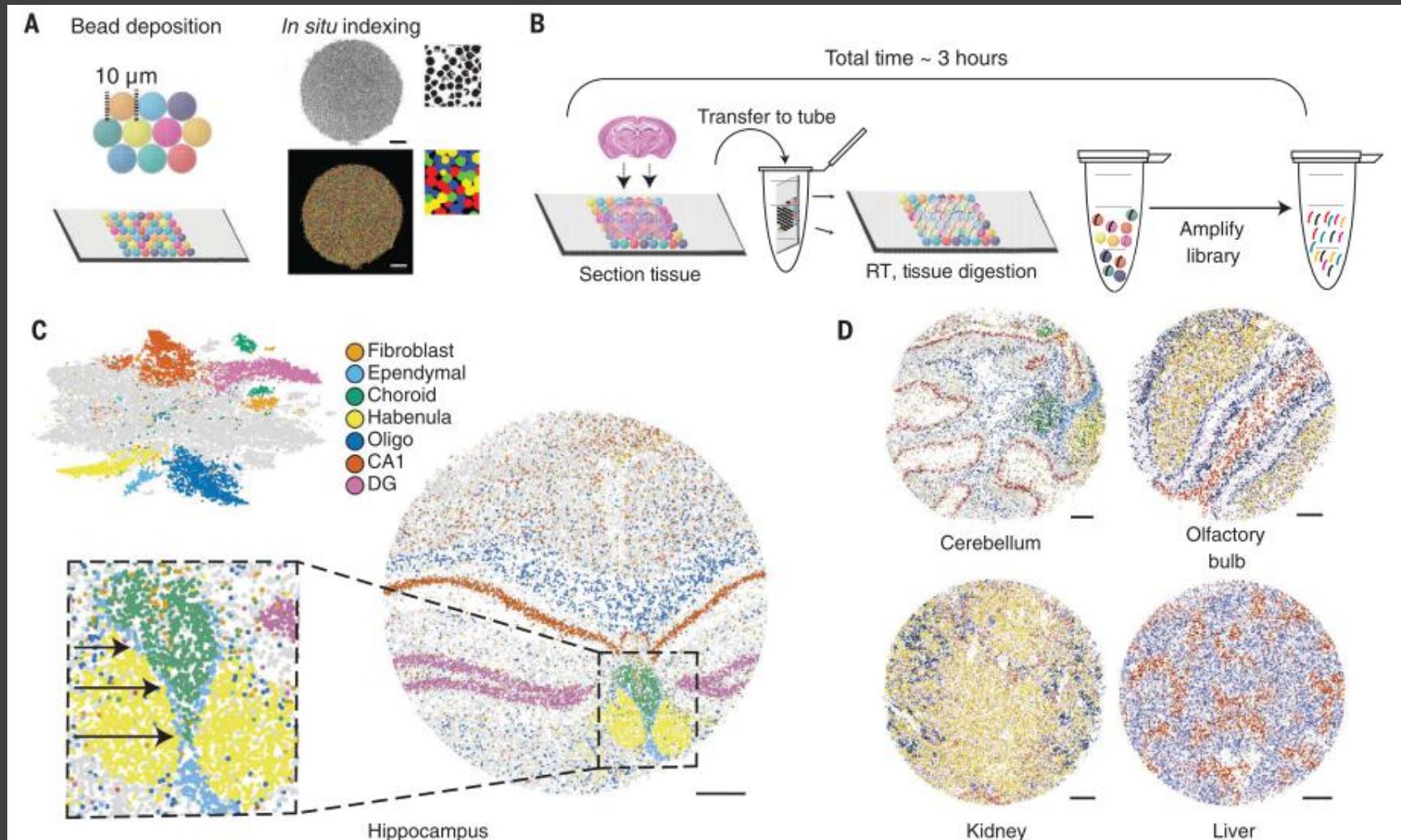
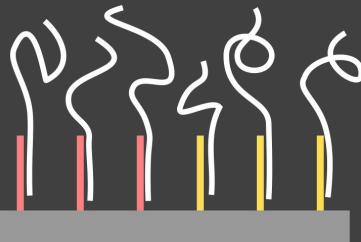
Array



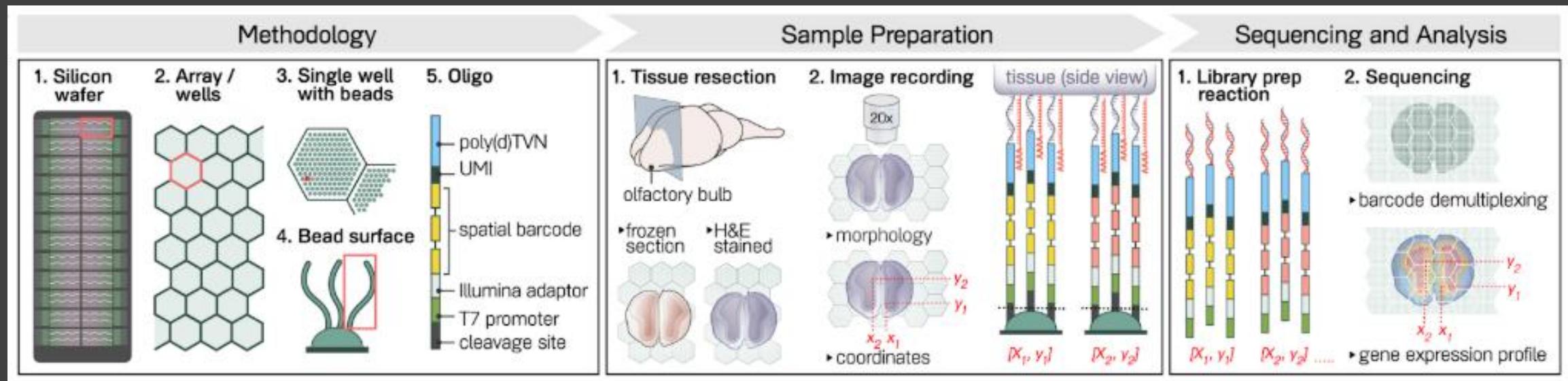
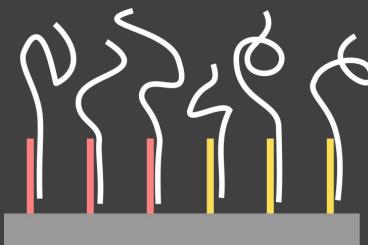
Decoded array

Barcode	X	Y
AACGTC	1	2
CGTTCA	2	5
TCGATC	1	6
GGTACT	5	3
TACCGA	2	3

Slide-Seq

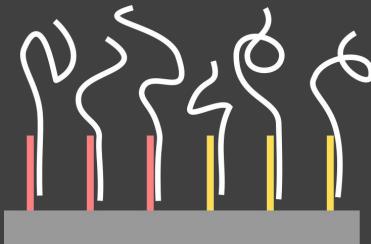


High density Spatial transcriptomics

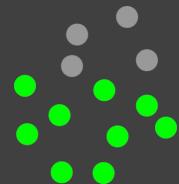


Vickovic *et al.* BioRxiv 2019

Spatial Sequencing



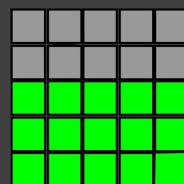
Resolution: Spot size 2um - 100um



Detection efficiency: 0.1 - 1%



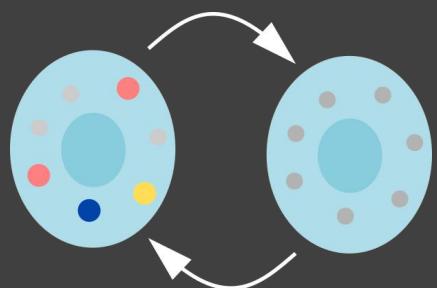
Gene throughput: Full transcriptome



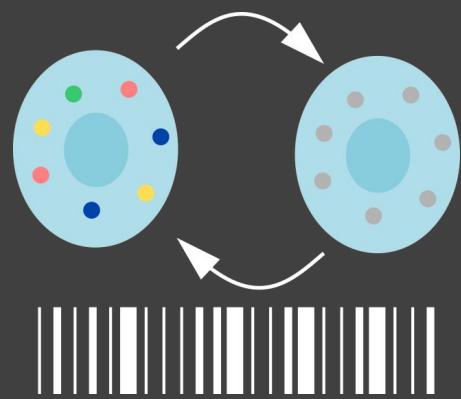
Spatial throughput: several cm²

RNA spatial detection

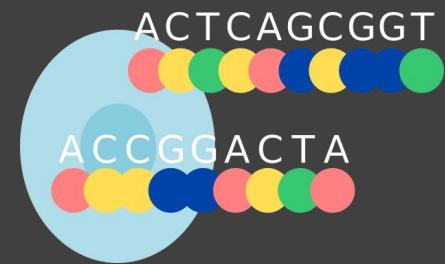
Cyclic FISH



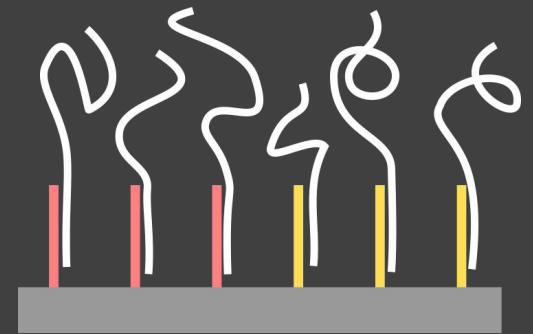
Barcoded FISH



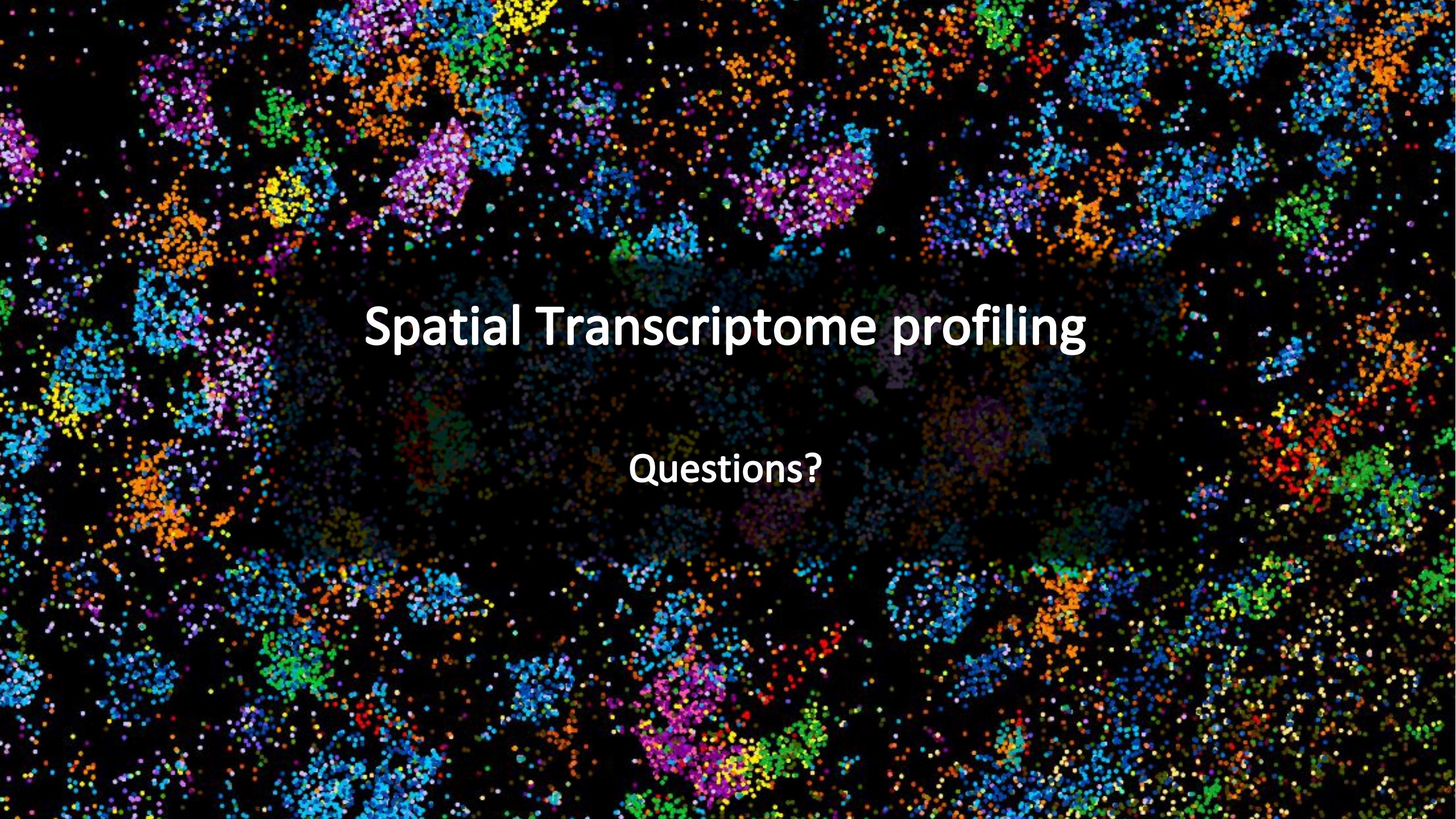
in situ Sequencing



Spatial Sequencing



But there is more: sc reconstruction, Tomography, Array microdissection, Grid barcoding



Spatial Transcriptome profiling

Questions?

