

Spatial Transcriptome profiling

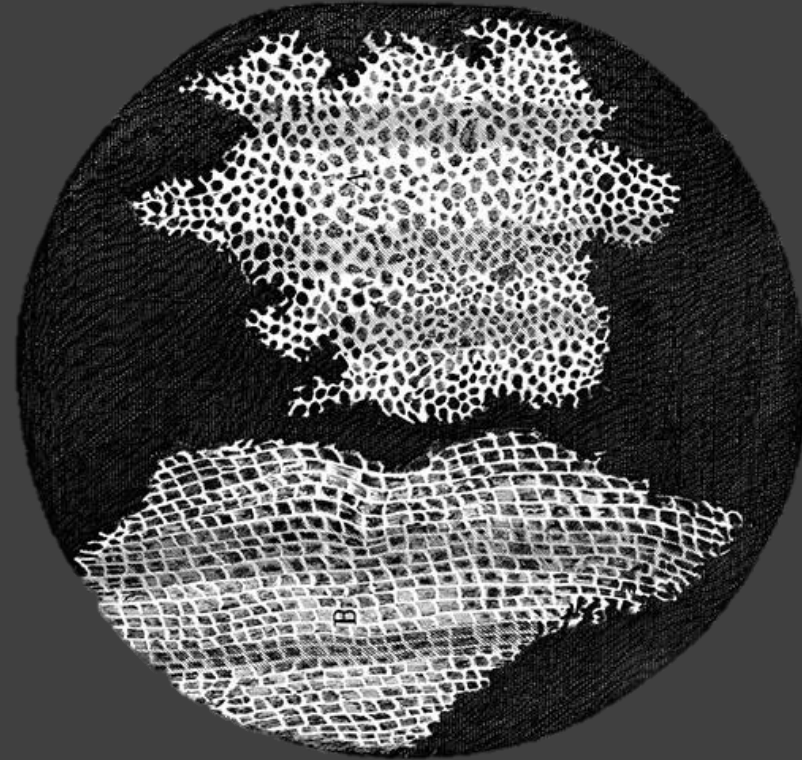
Lars Borm

Linnarsson Lab
karolinska Institutet



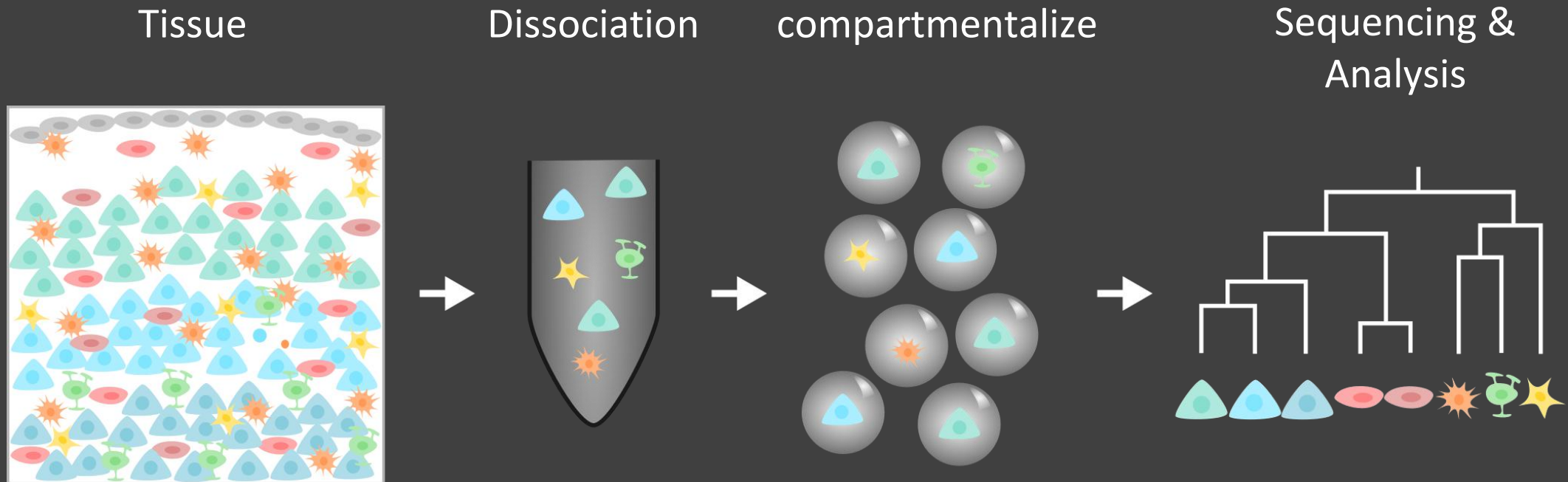
**Karolinska
Institutet**

Single cell biology



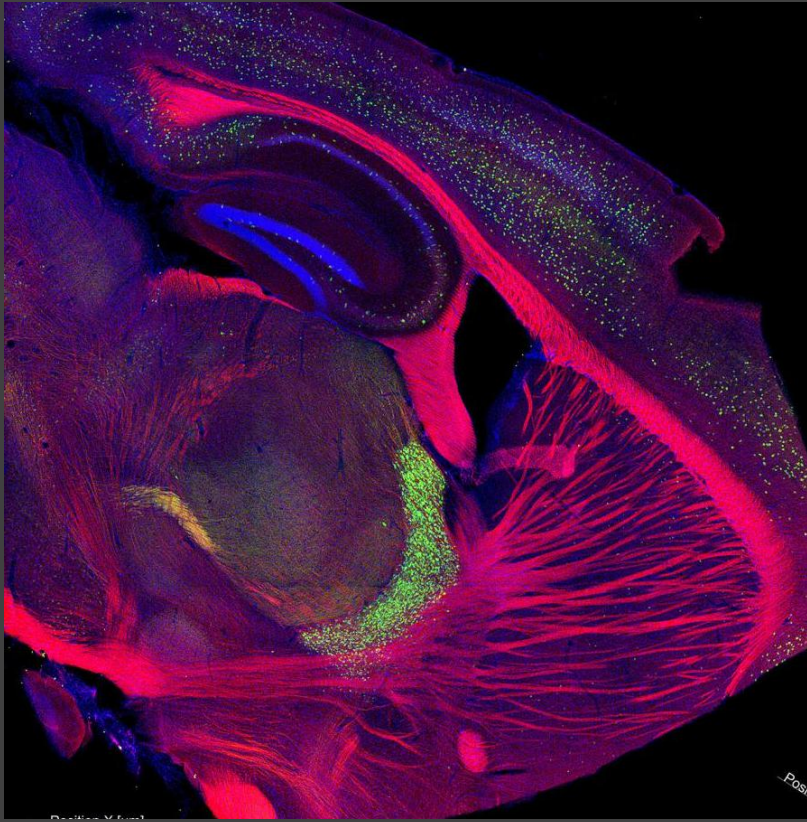
Robert Hooke 1665

Single Cell RNA sequencing



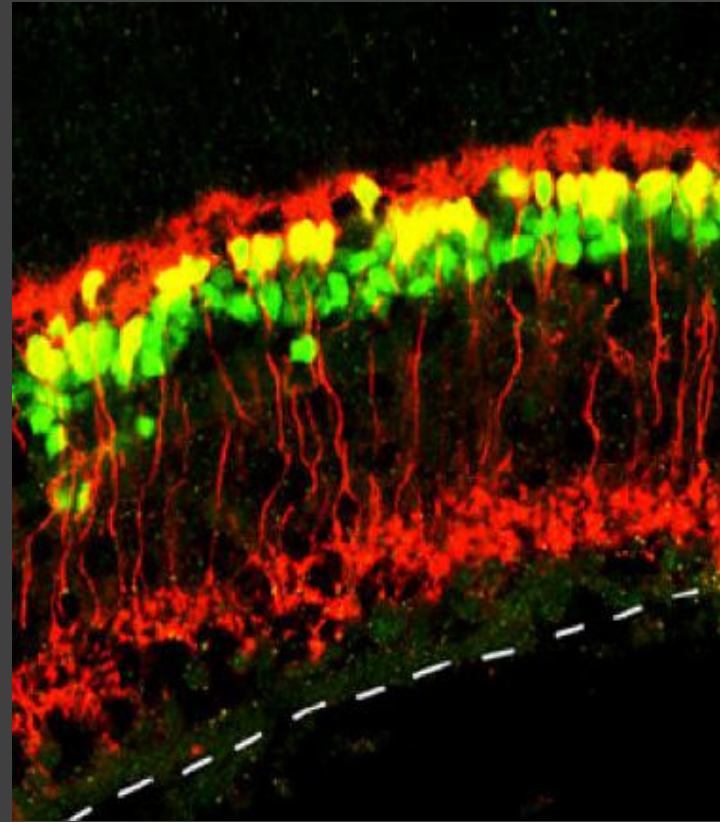
Tissue architecture

Brain



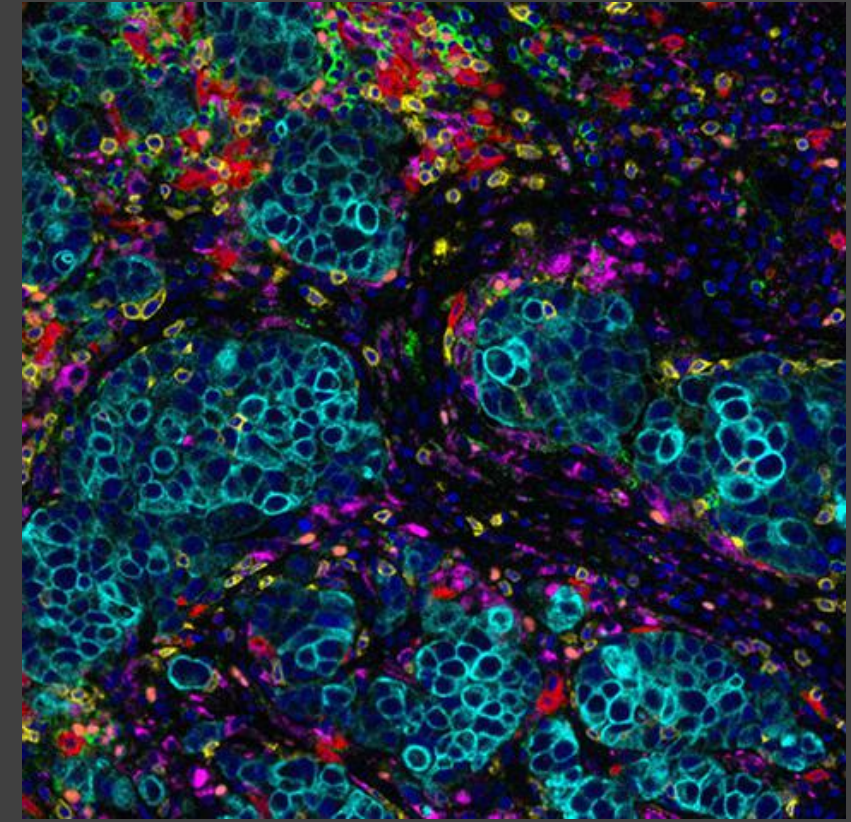
IGB Illinois

Retina



Morrow *et al.* 2008

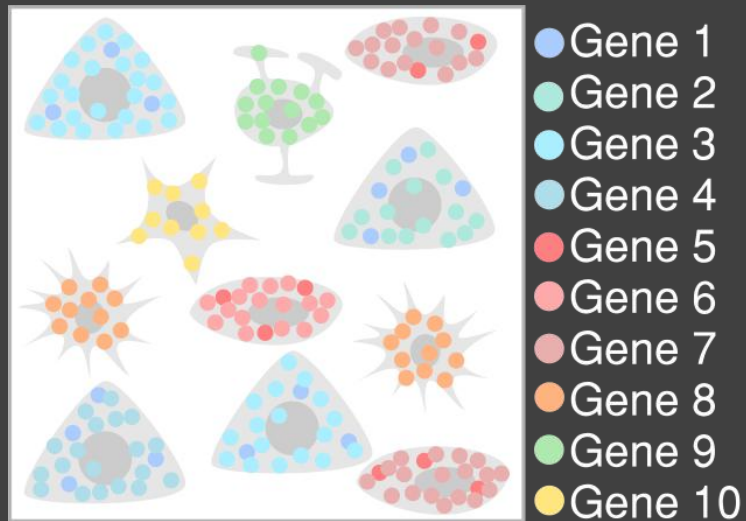
Cancer



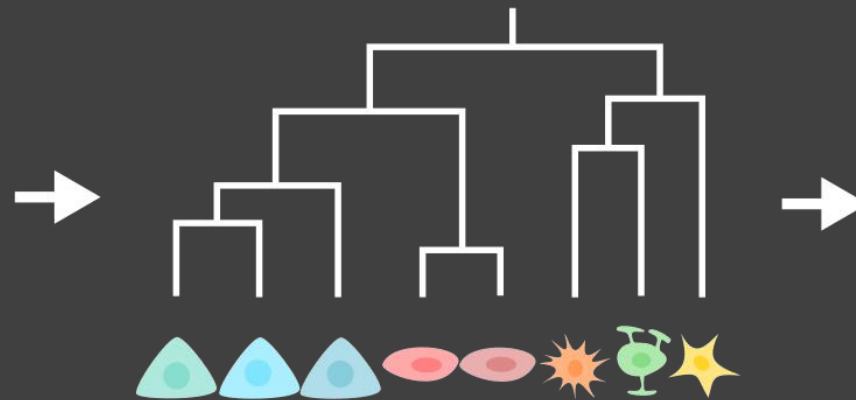
Perkin Elmer

Detecting RNA in tissue

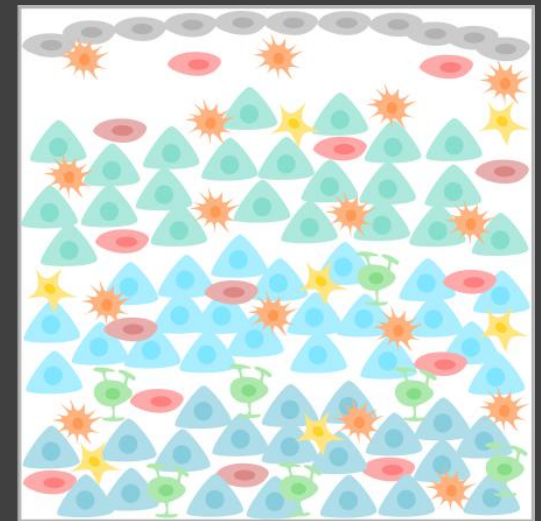
RNA detection *in situ*



Analysis

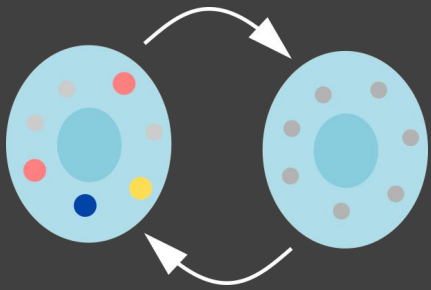


Spatial transcriptomics

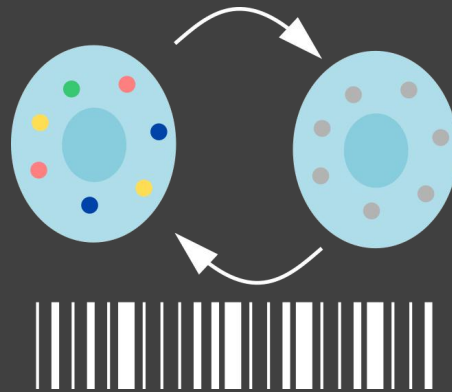


RNA spatial detection

Cyclic FISH



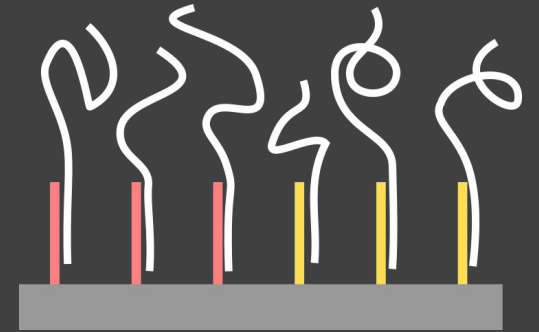
Barcoded FISH



in situ Sequencing

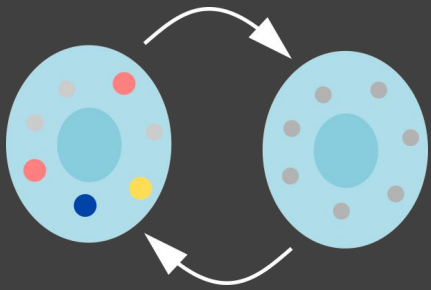


Spatial Sequencing

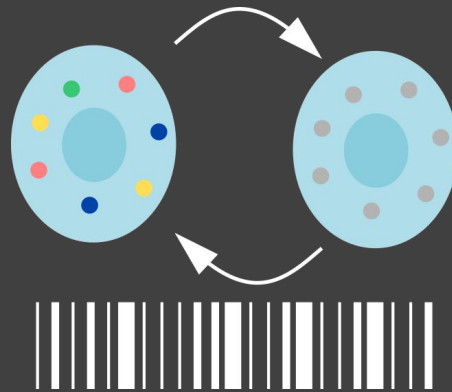


Microscopy methods

Cyclic FISH



Barcoded FISH



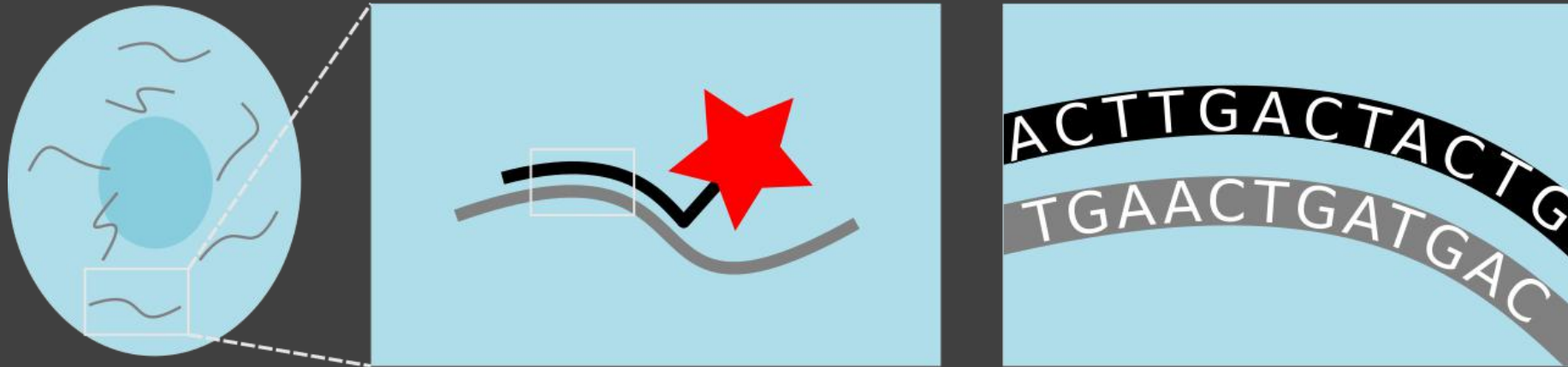
in situ Sequencing



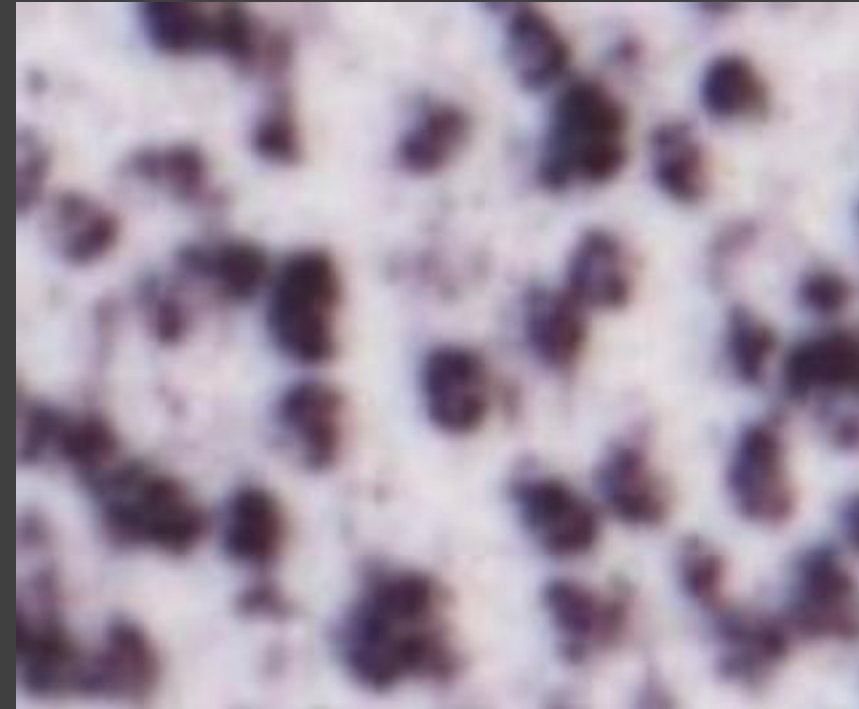
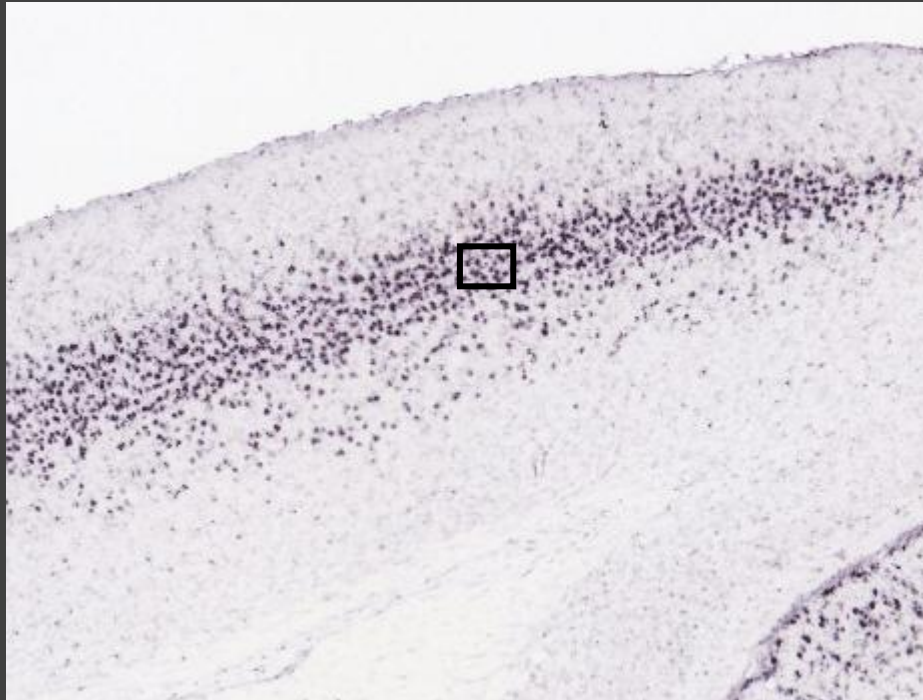
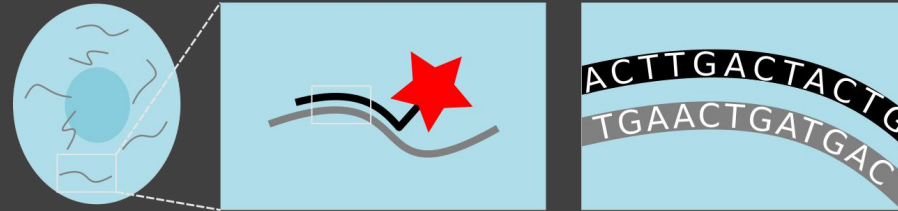
Spatial Sequencing



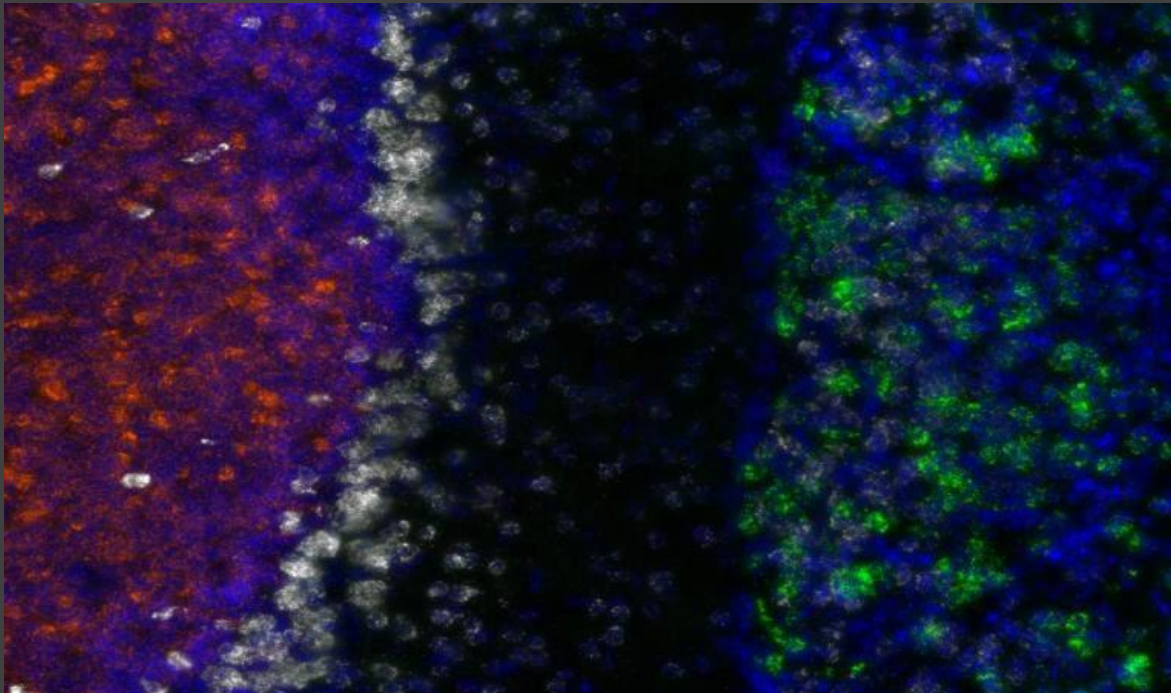
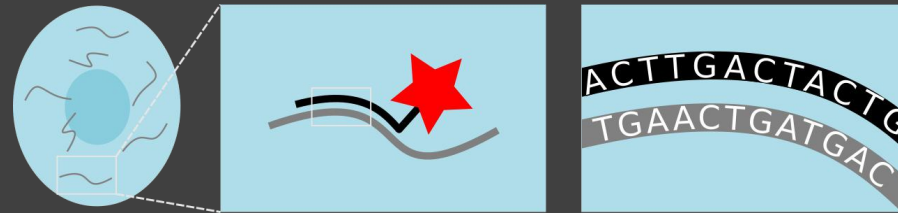
in situ Hybridization



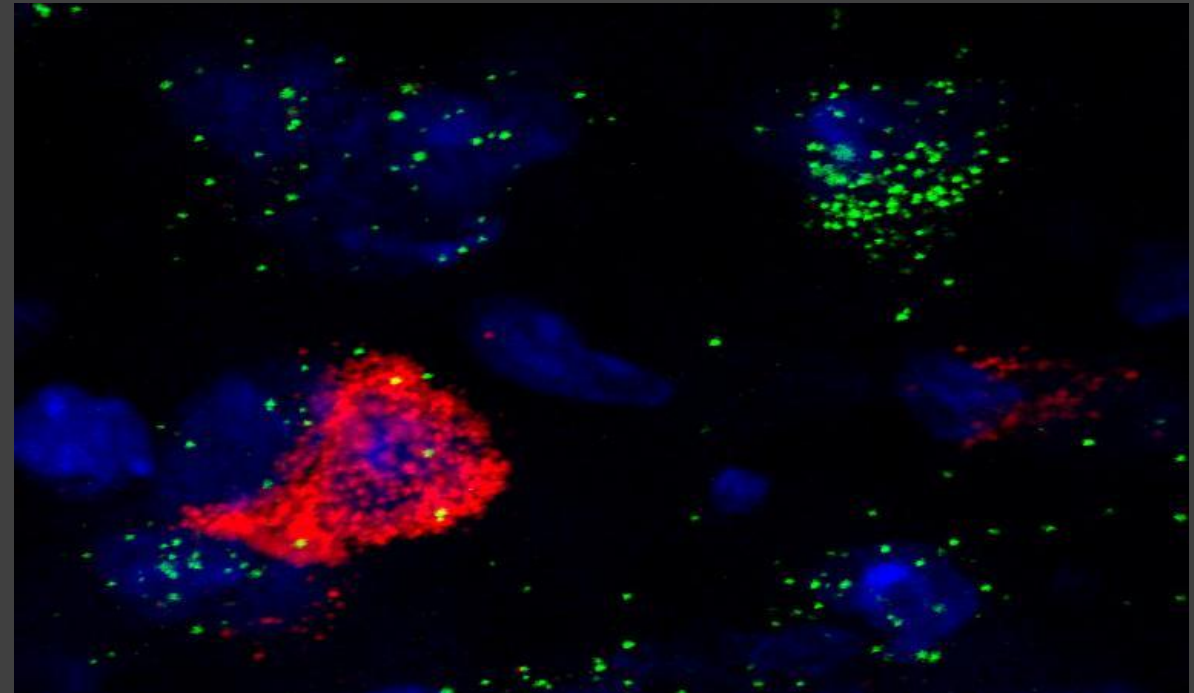
in situ Hybridization (ISH)



Fluorescent in situ Hybridization (FISH)

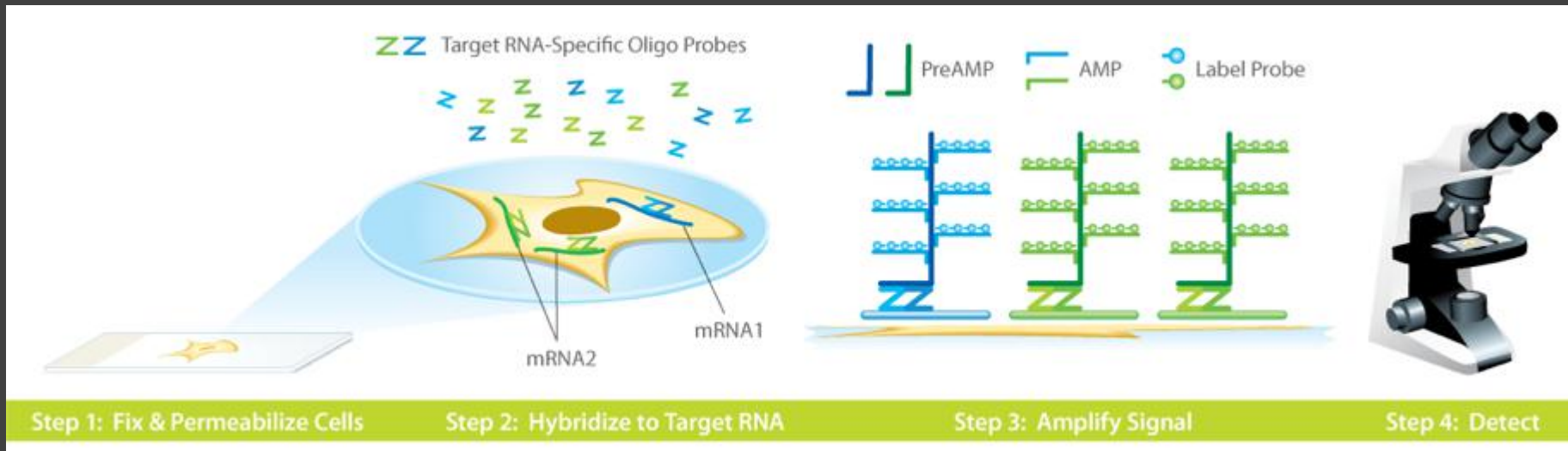


ACD Bio

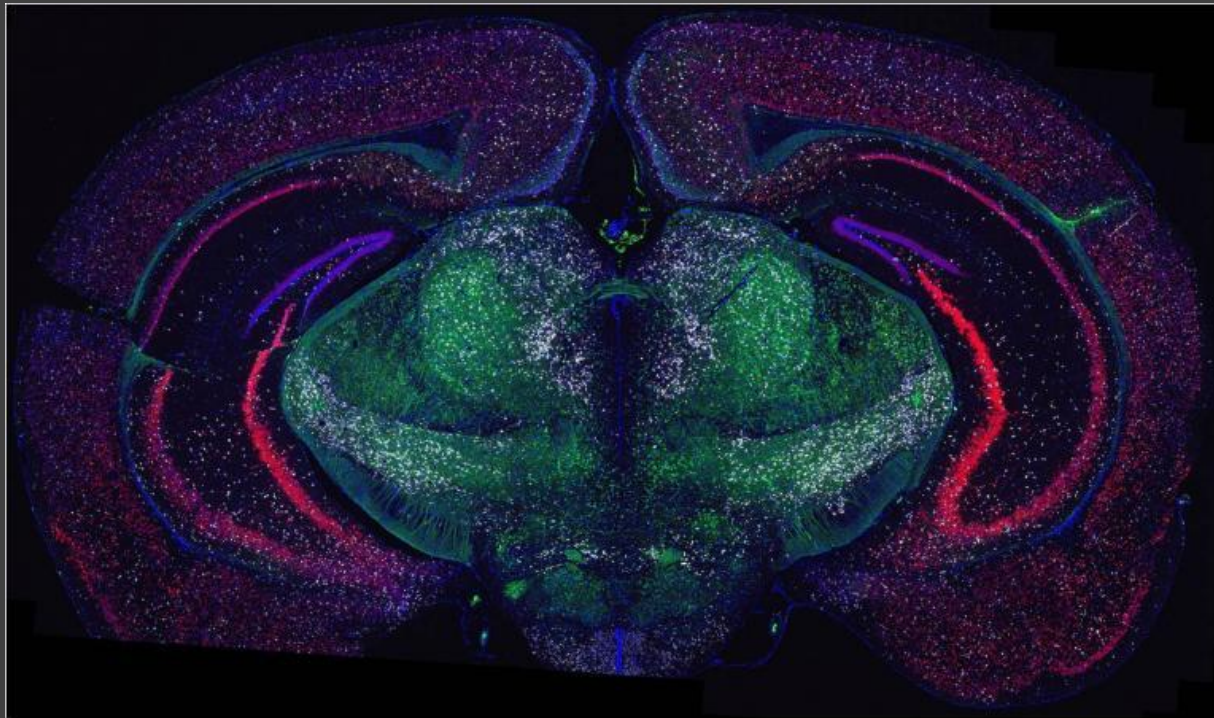
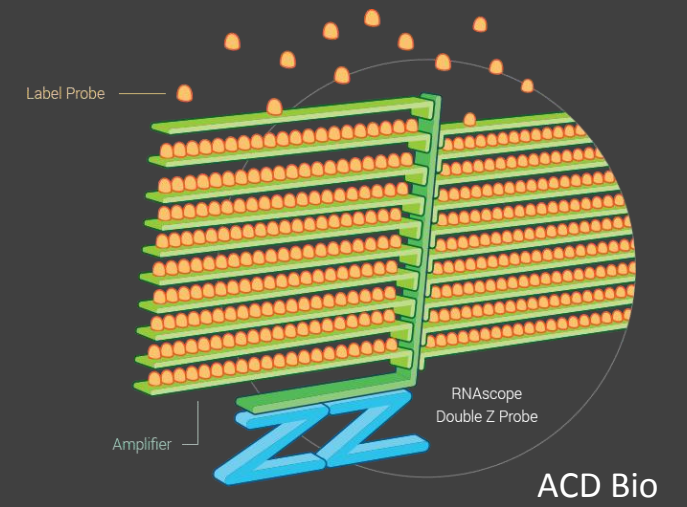


ACD Bio

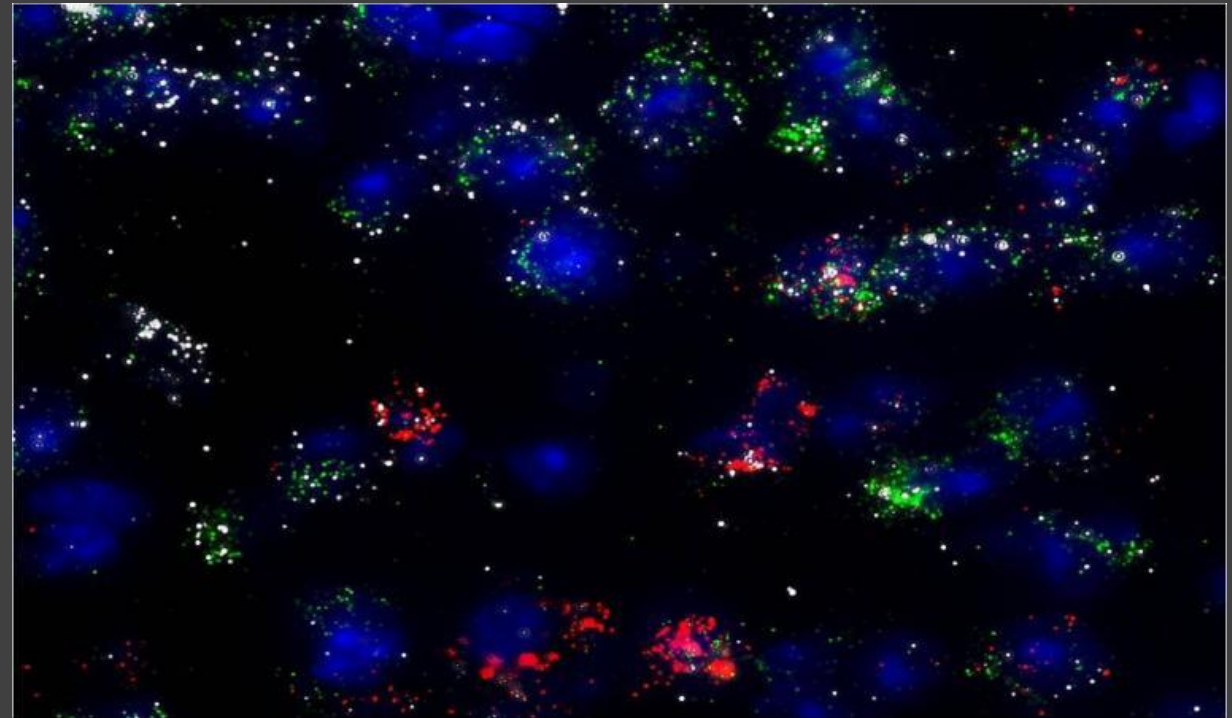
RNAscope



RNAscope

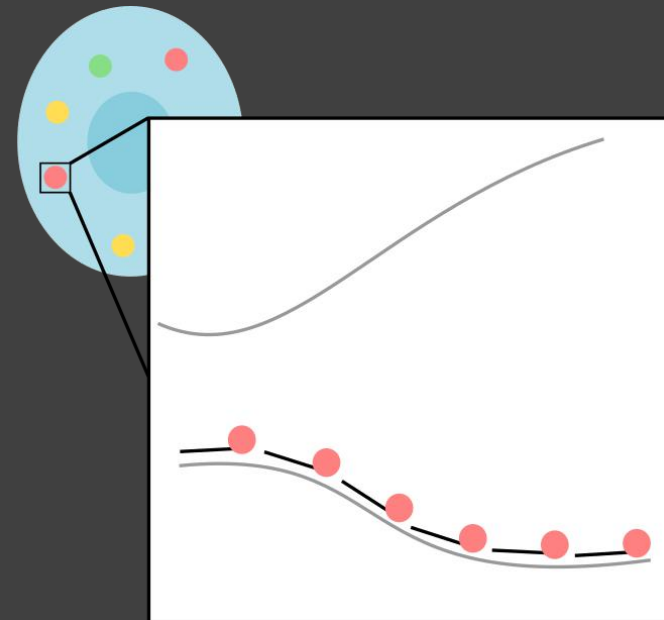
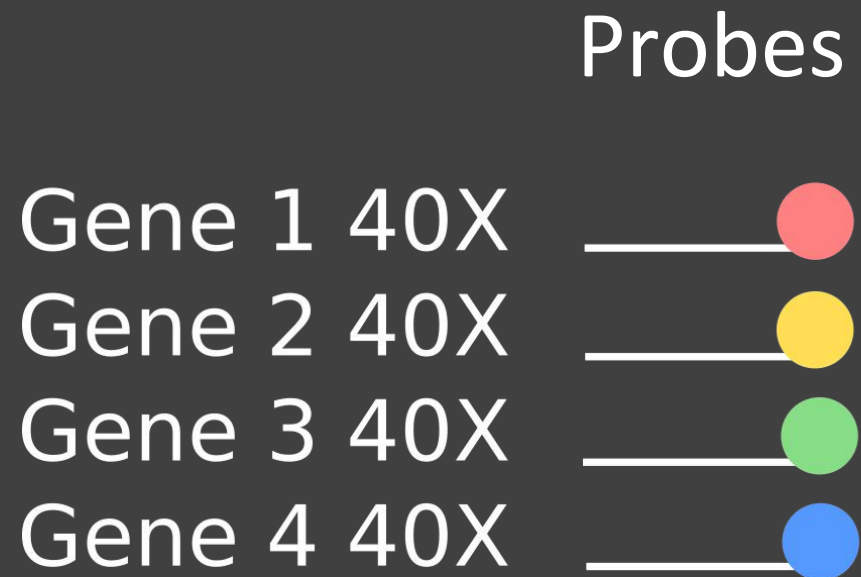


ACD Bio



ACD Bio

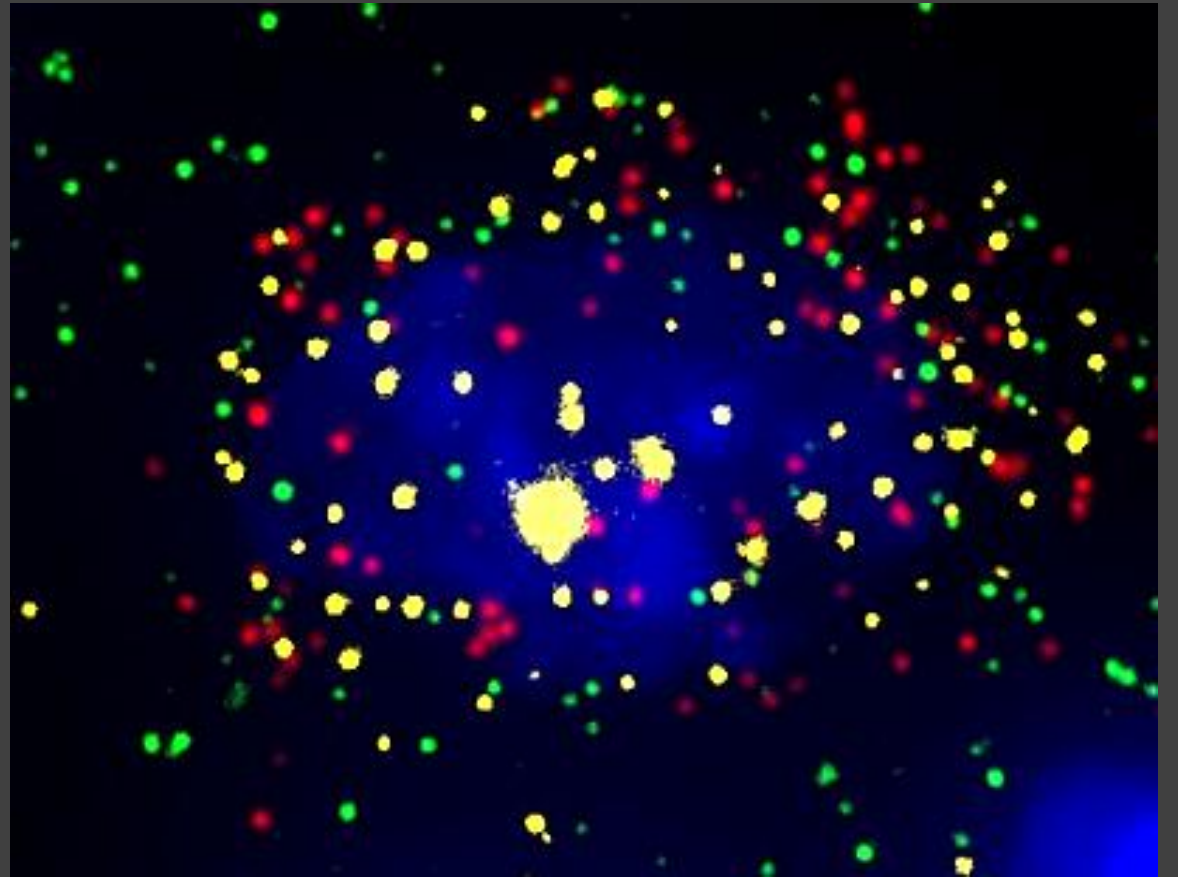
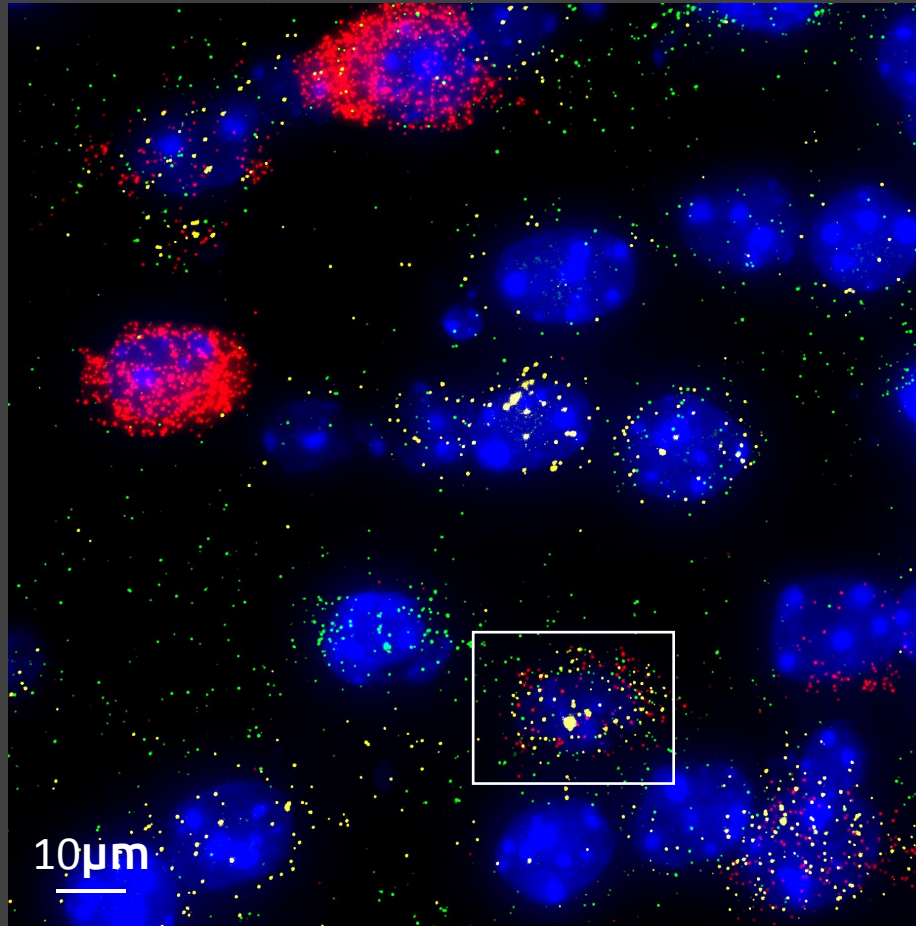
single molecule FISH (smFISH)



Raj *et al.* Nature Methods 2008





single molecule FISH (smFISH)

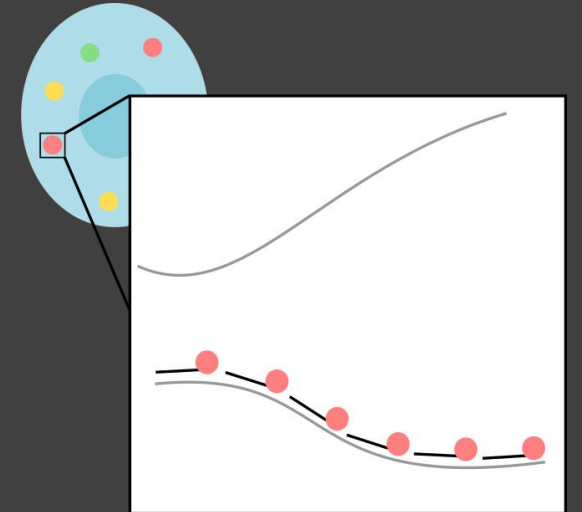
Gene 1 Gene 2 Gene 3 DNA



single molecule FISH (smFISH)

- High sensitivity
- Low false positives
- Low false negatives

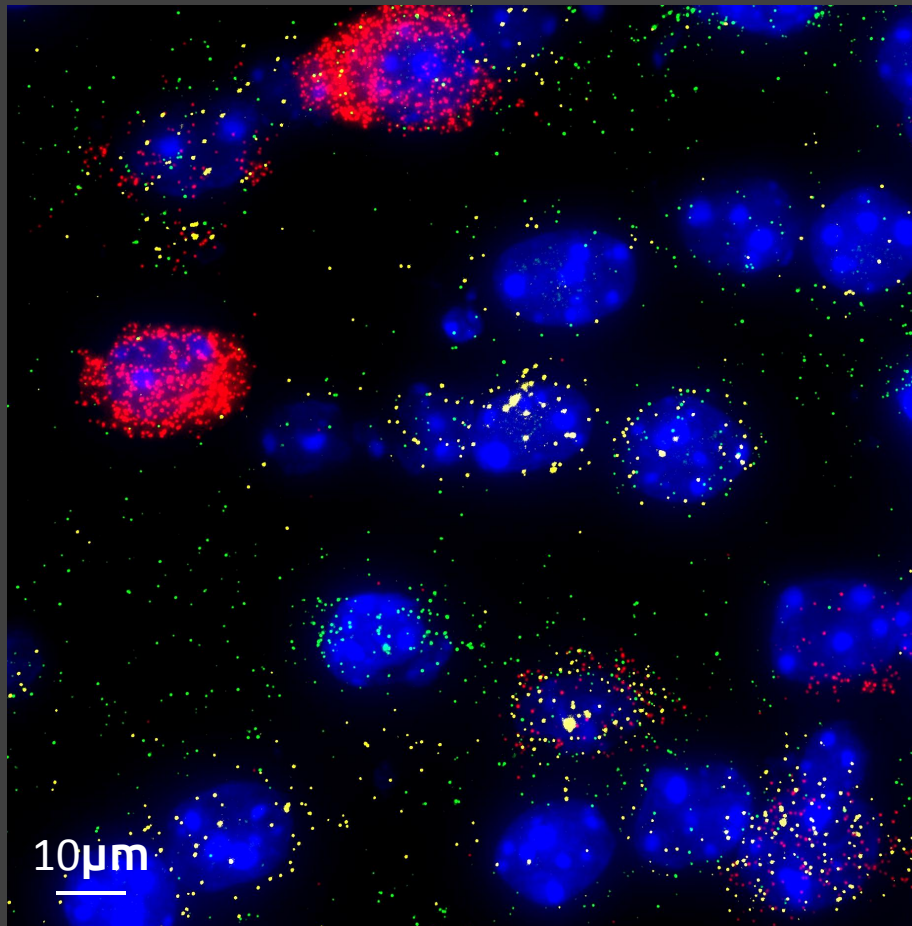
Gene 1 40X 
Gene 2 40X 
Gene 3 40X 
Gene 4 40X 



Raj *et al.* 2008

single molecule FISH (smFISH)

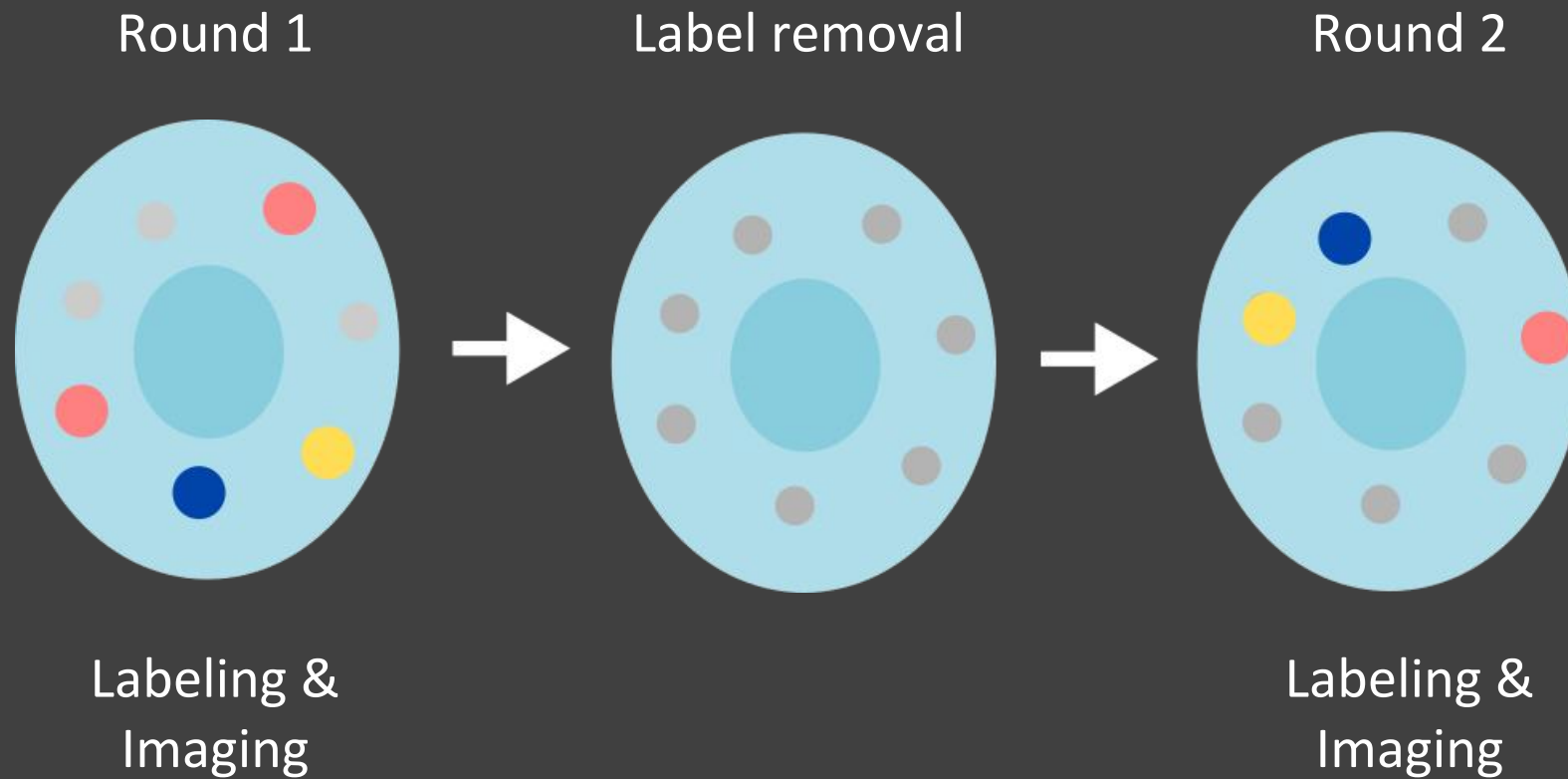
Gene 1 Gene 2 Gene 3 DNA



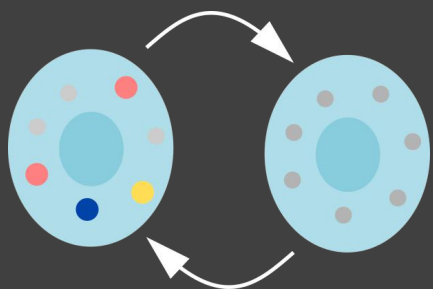
Limited fluorophores



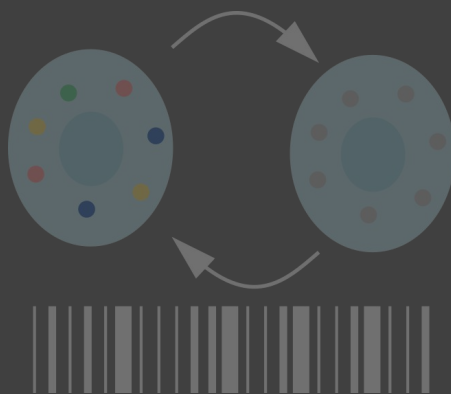
Cyclic labeling



Cyclic FISH



Barcoded FISH



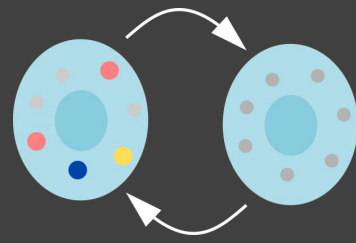
in situ Sequencing



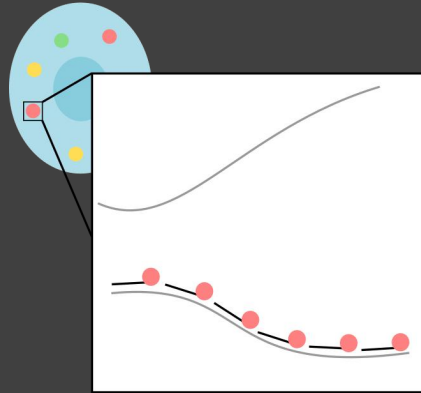
Spatial Sequencing



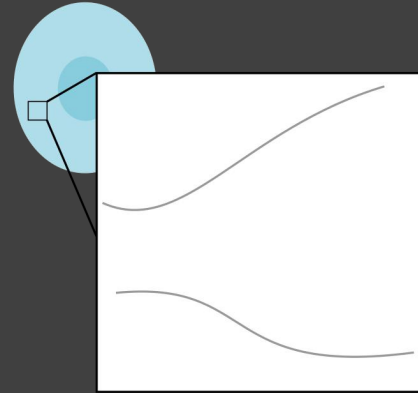
osmFISH



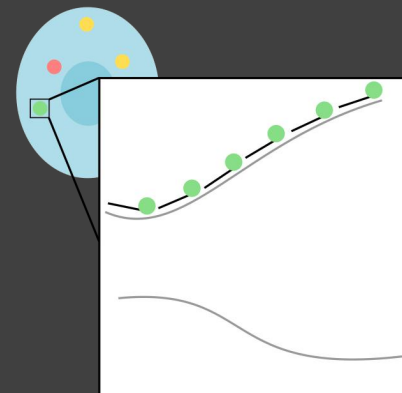
Hybridization 1



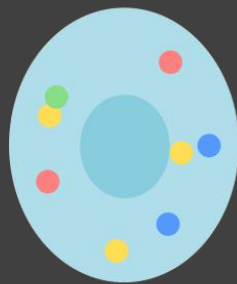
Strip 1



Hybridization 2

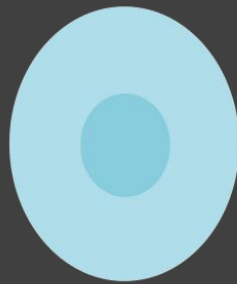


Hyb 1

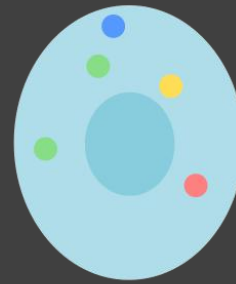


Gene 1 ●
Gene 2 ●
Gene 3 ●
Gene 4 ●

Strip 1



Hyb 2



Gene 5 ● → ●
Gene 6 ● → ●
Gene 7 ● → ●
Gene 8 ● → ●

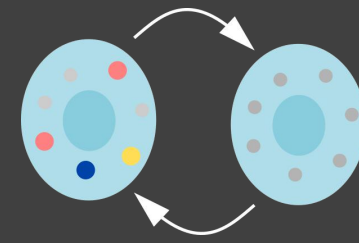
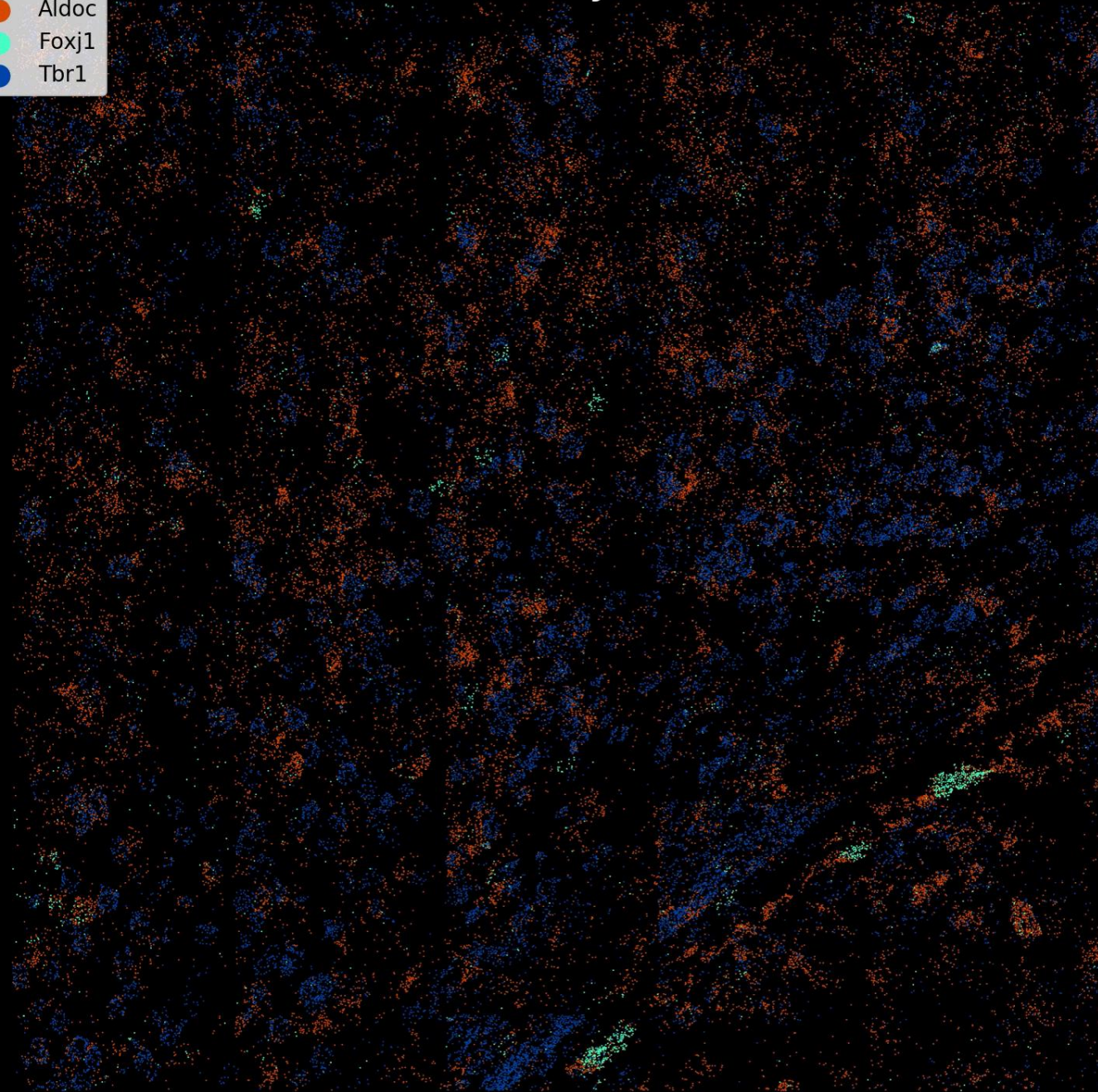
Composite

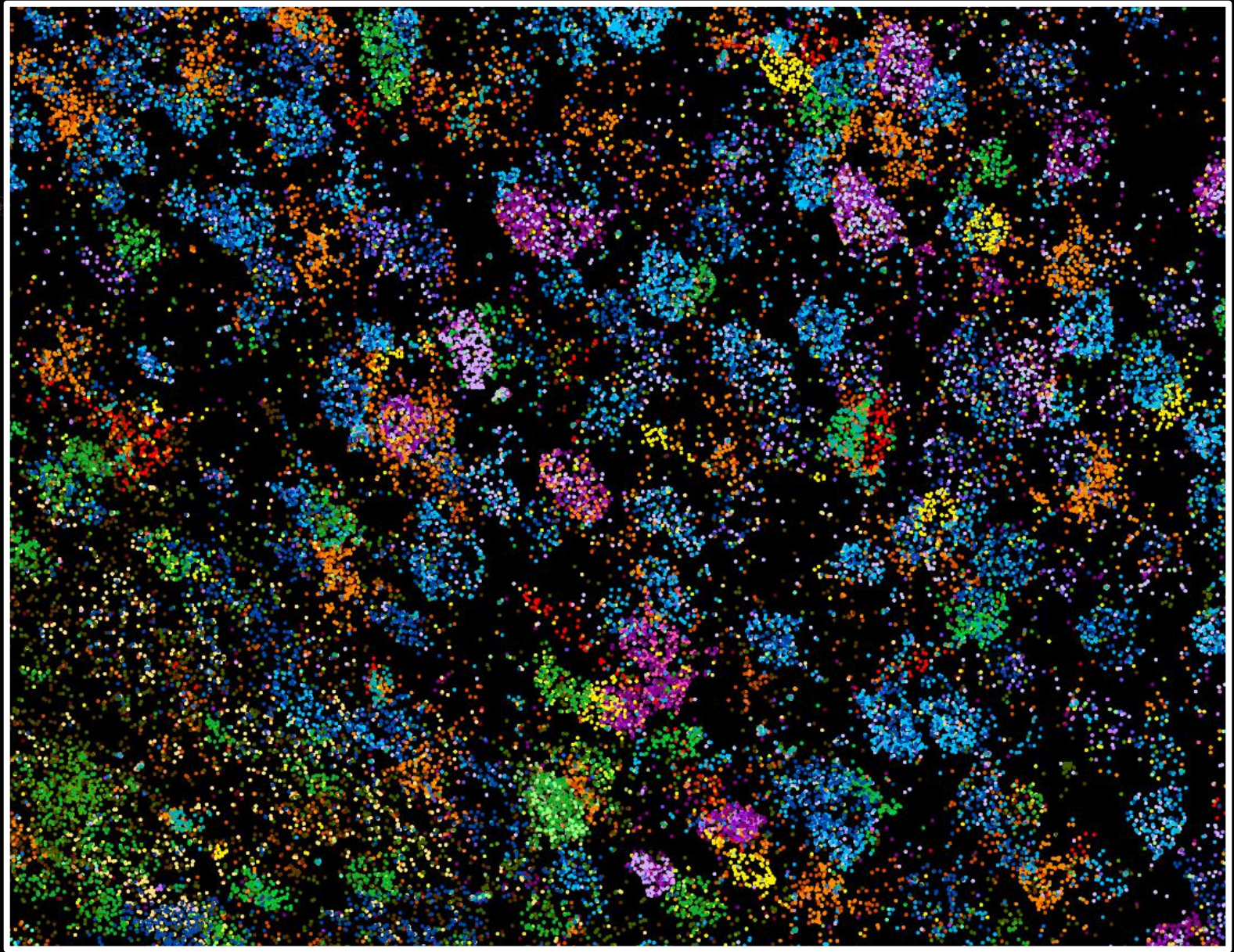
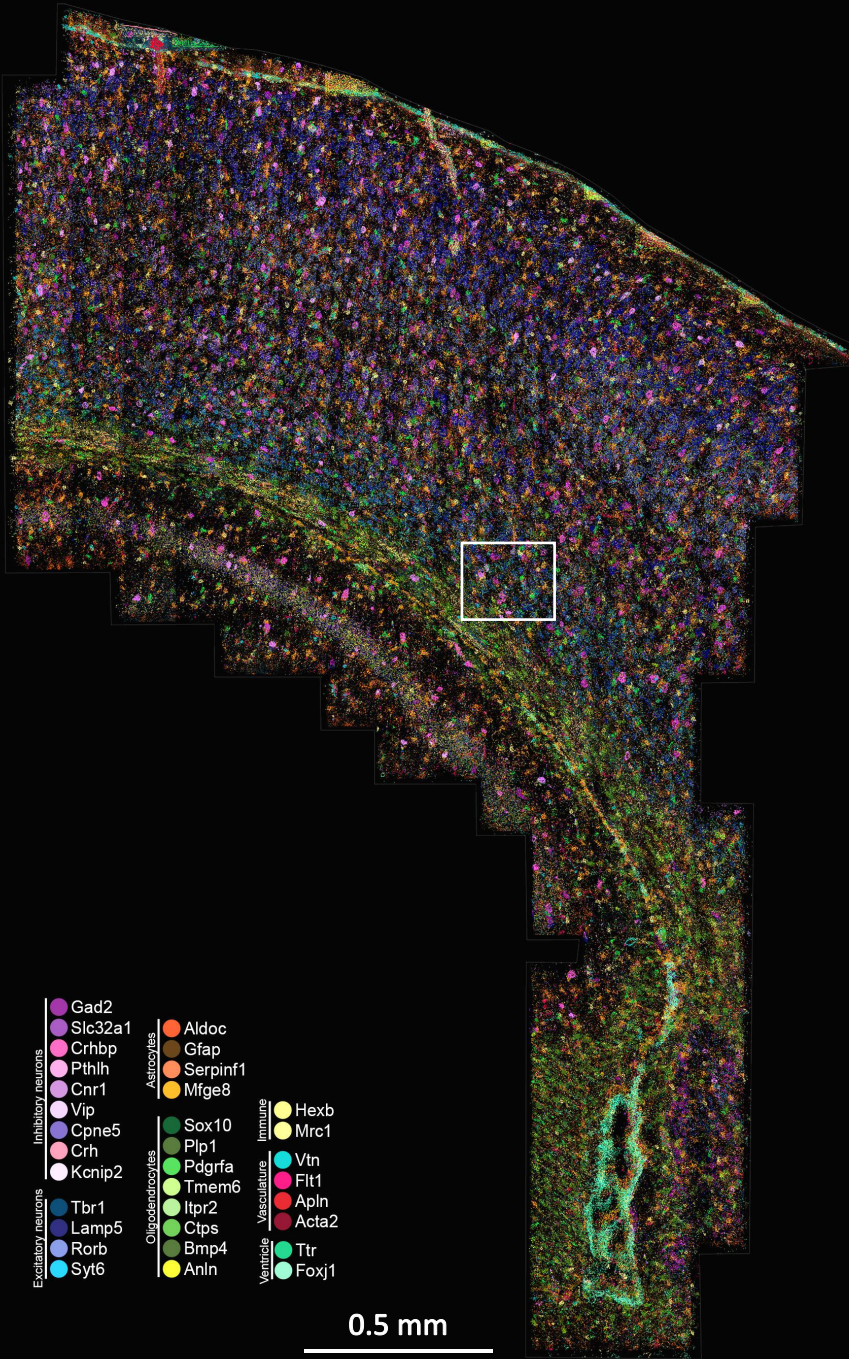


Gene 1 ●
Gene 2 ●
Gene 3 ●
Gene 4 ●
Gene 5 ●
Gene 6 ●
Gene 7 ●
Gene 8 ●

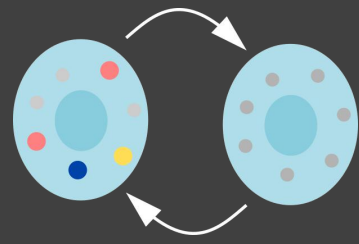
osmFISH Cycle: 1

- Genes
- Aldoc
 - Foxj1
 - Tbr1

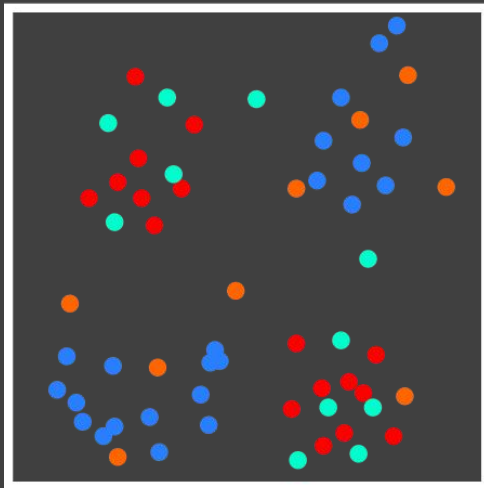




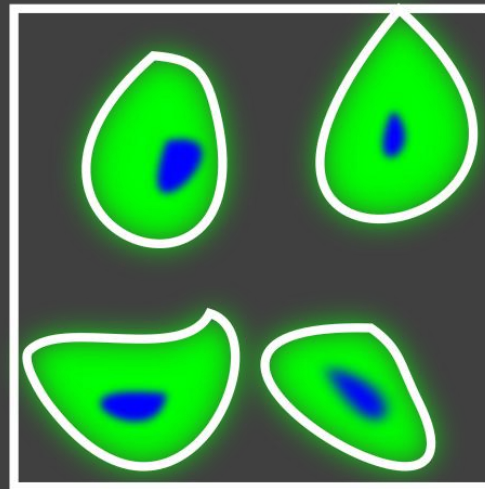
Pipeline



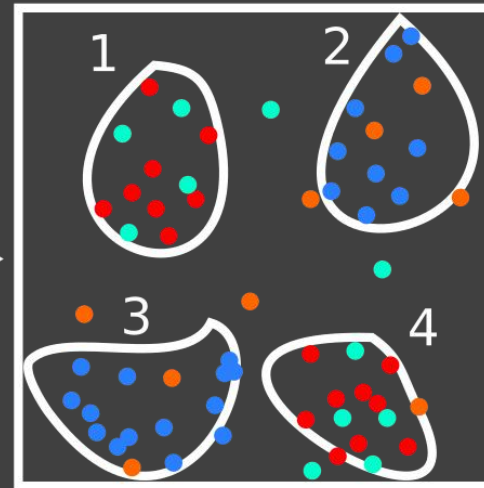
RNA detection



Cell segmentation



Cell counting



Cell 1

●	8
●	4
●	0
●	0

Cell 2

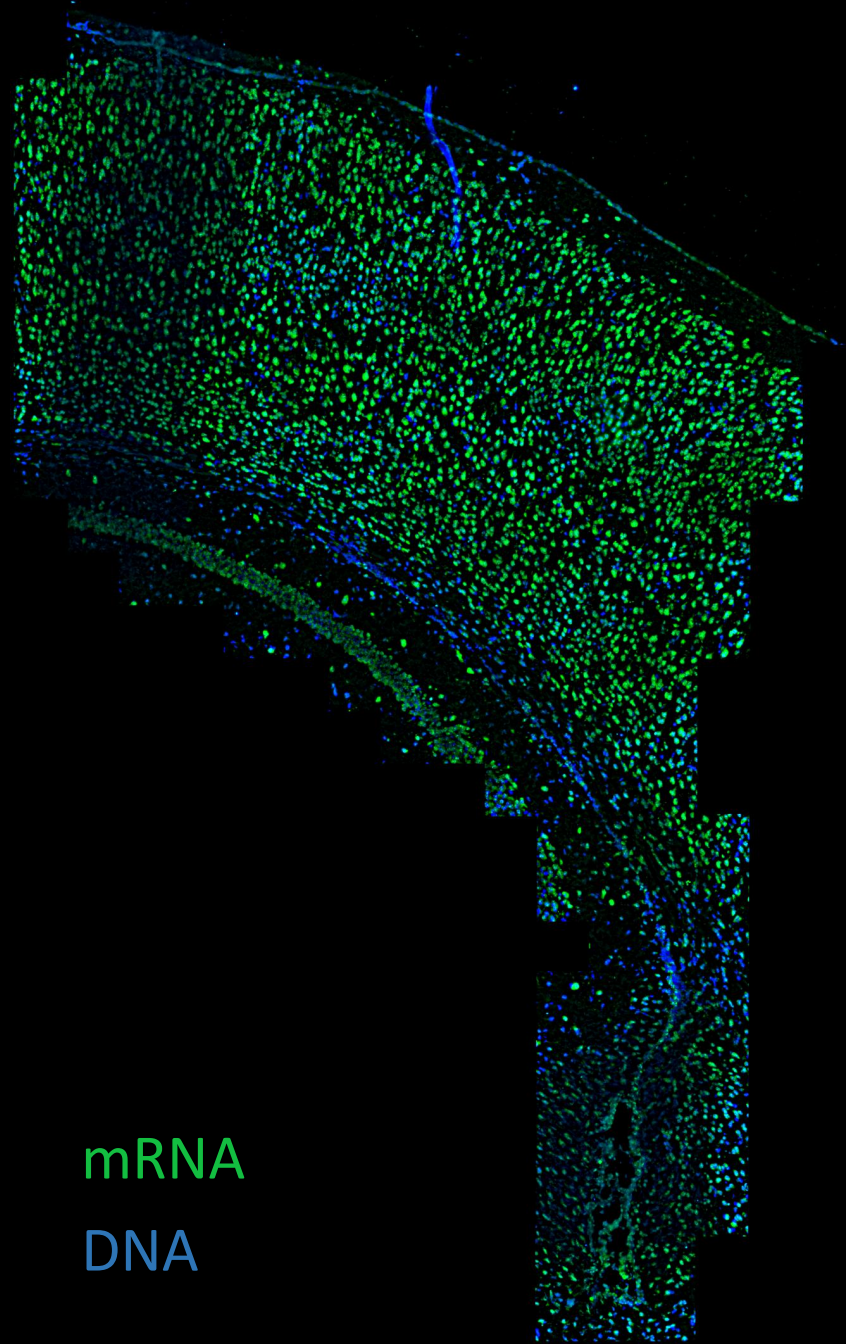
●	0
●	0
●	9
●	2

Cell 3

●	0
●	0
●	14
●	2

Cell 4

●	8
●	4
●	0
●	1

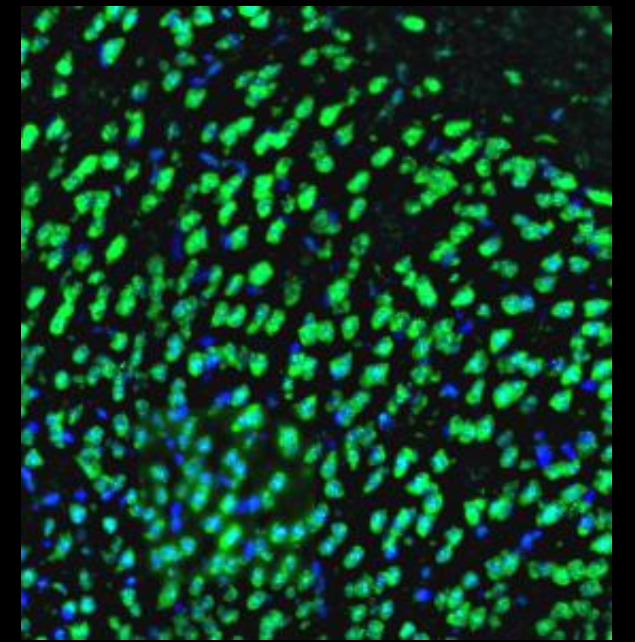


mRNA

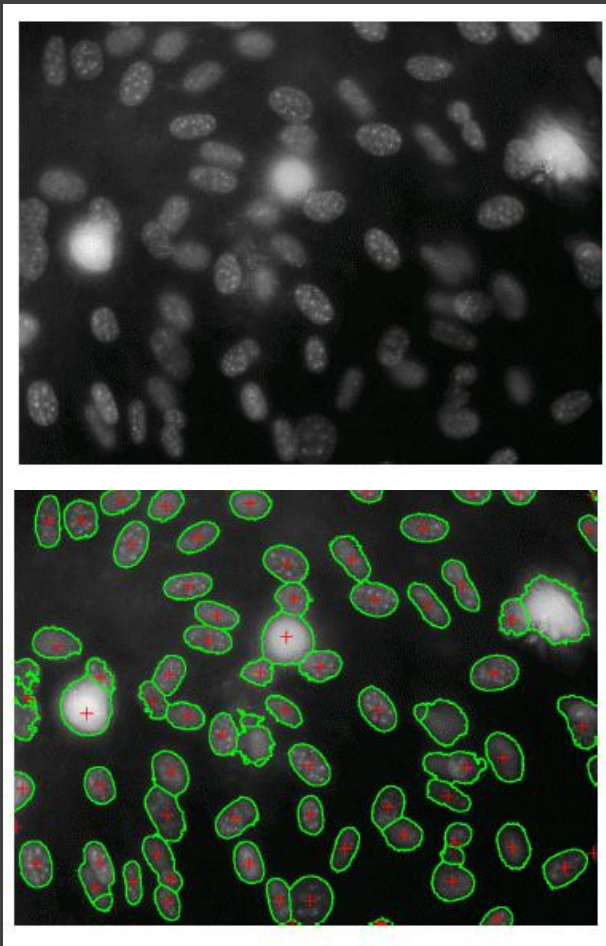
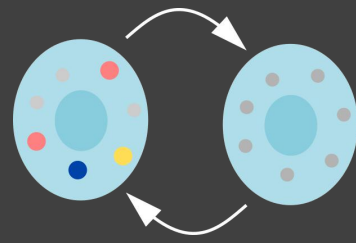
DNA



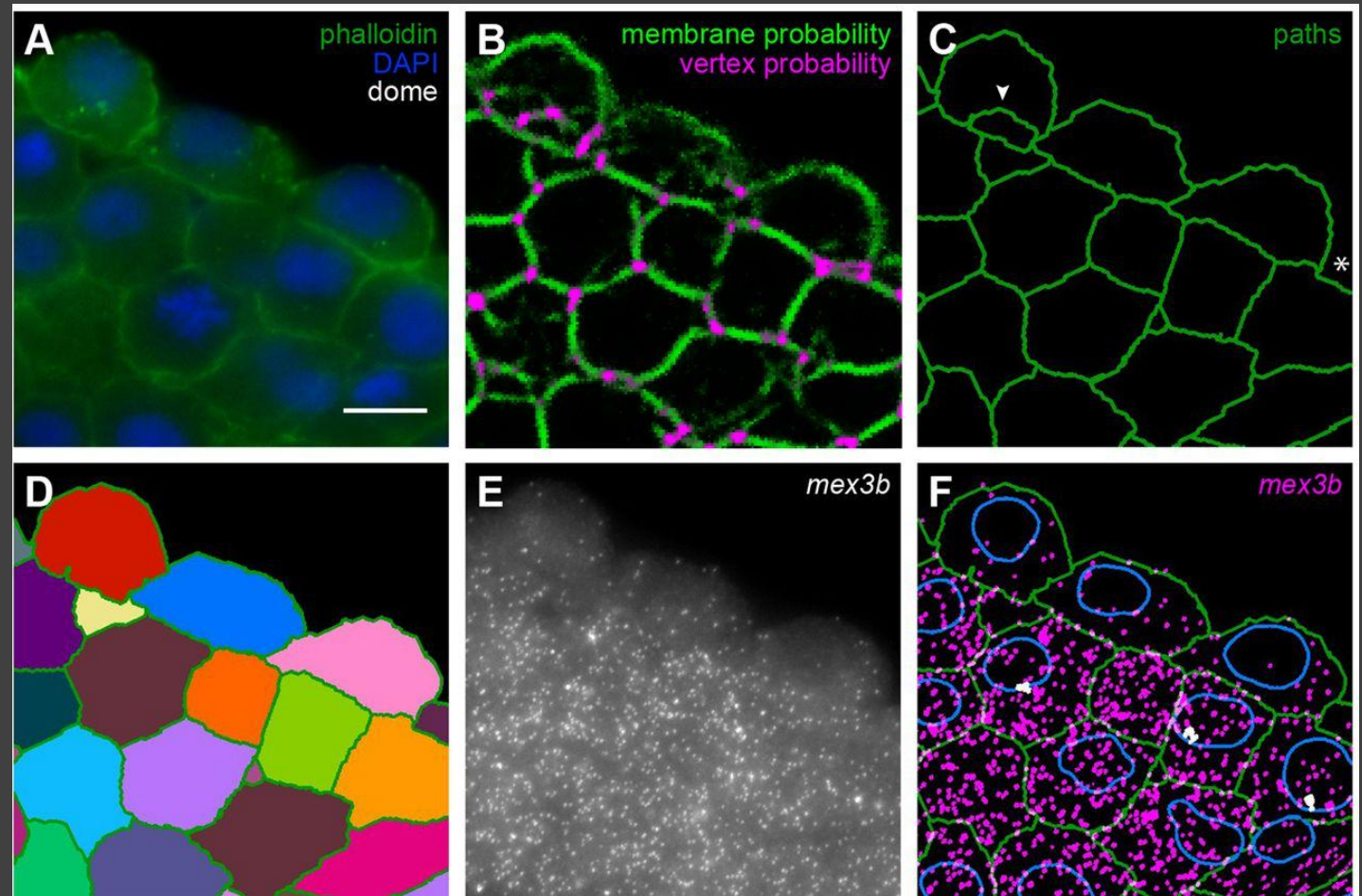
Segmented



Cell segmentation

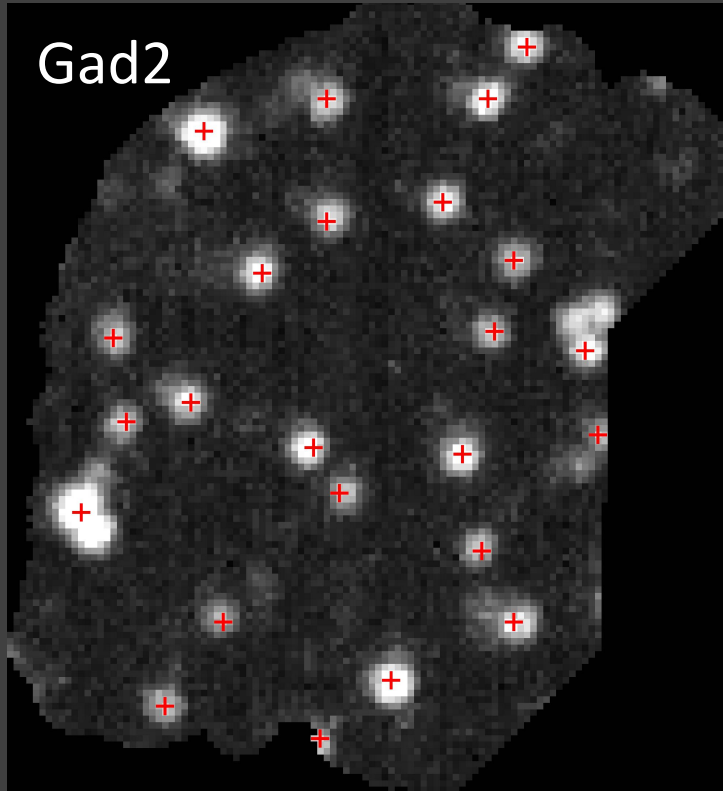
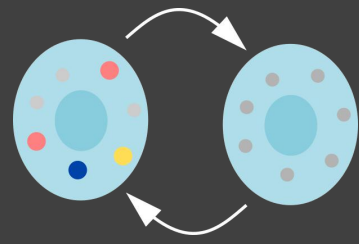


Panagiotakis *et al.* IEEE 2018



Stapel *et al.* Development 2016

Single cell expression



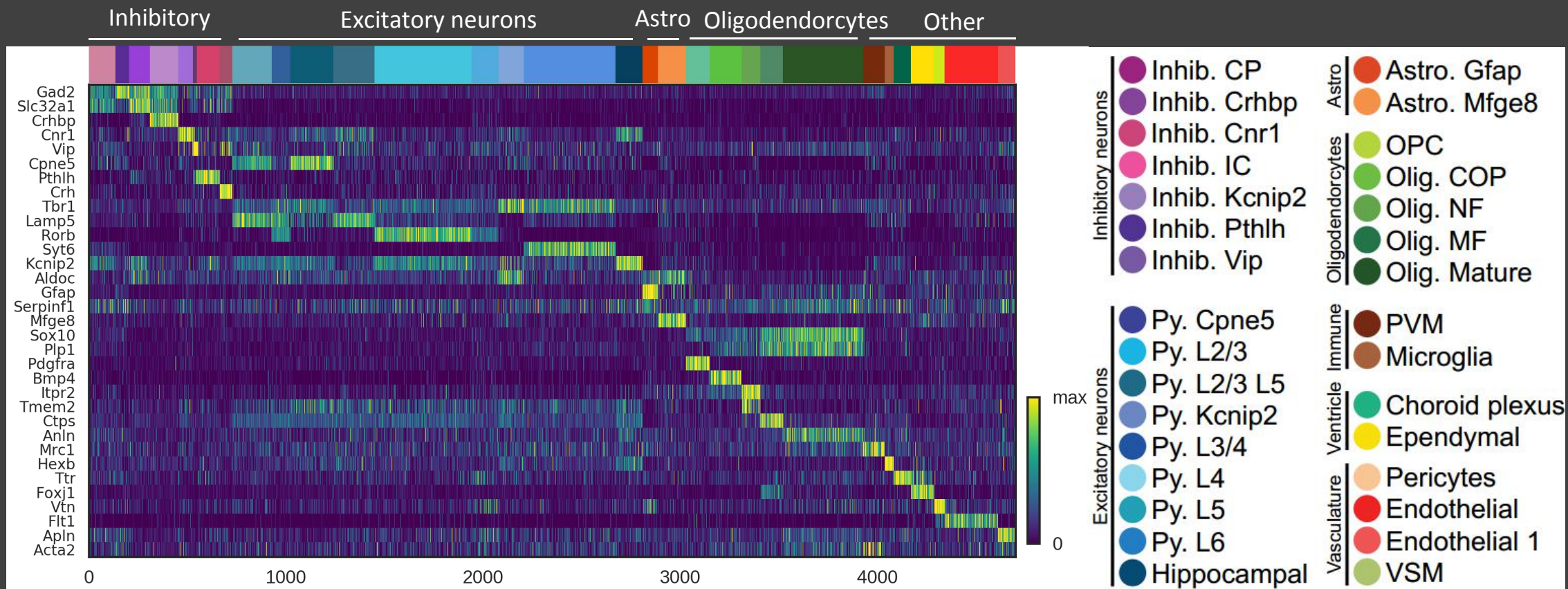
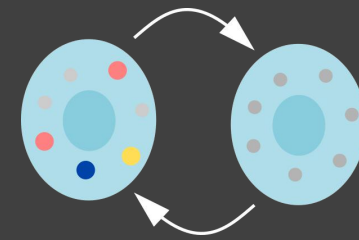
Genes

Cells

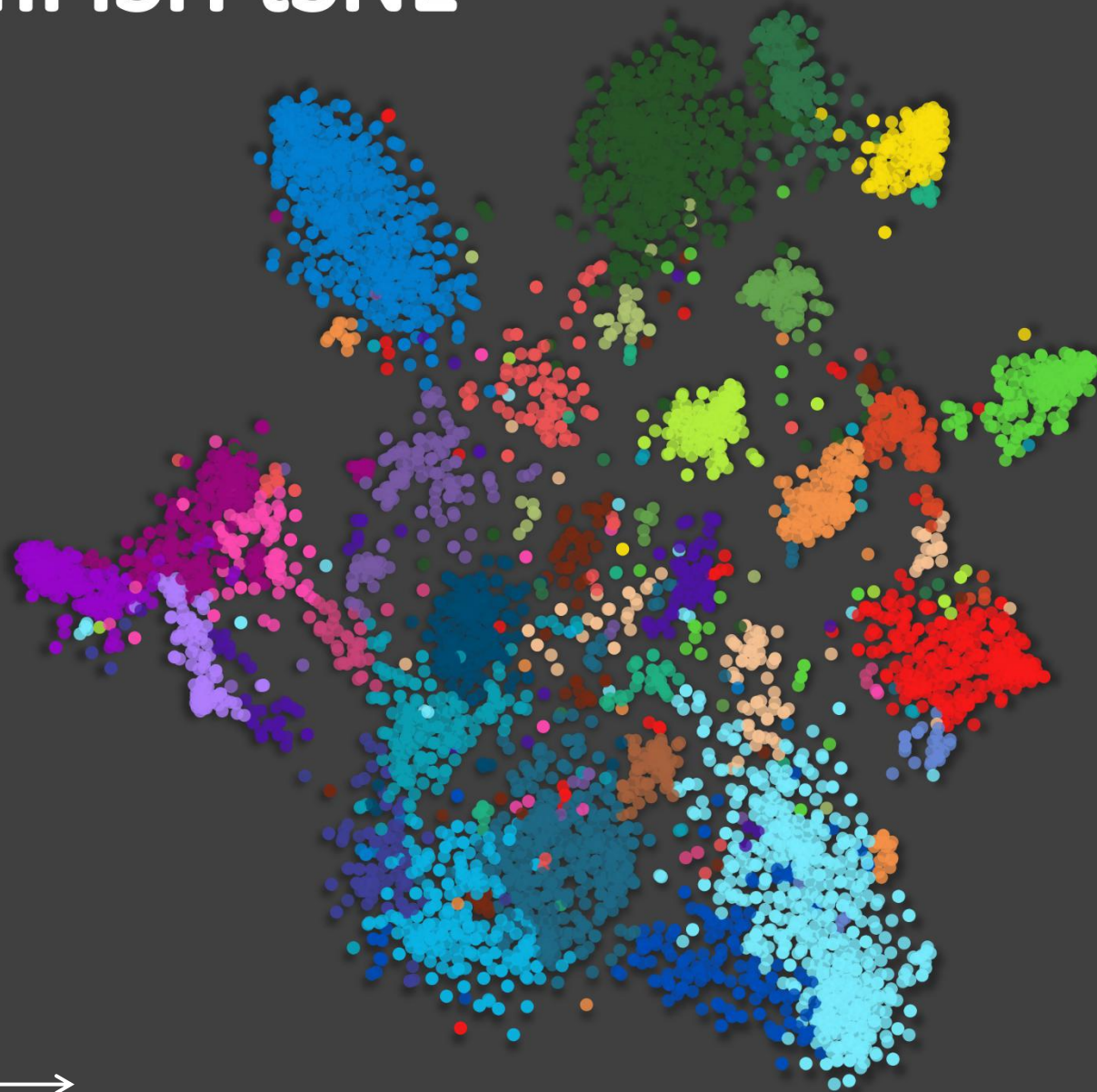
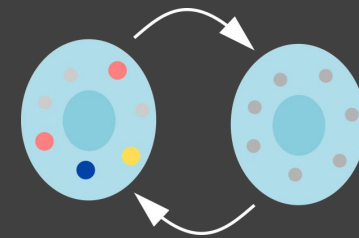
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Hybridization1_Aldoc	38	0	9	5	38	2	4	3	7	10	...	11	10	5	4	10	2	0	2	2	9
Hybridization1_Foxj1	0	0	0	1	5	0	3	1	1	1	...	0	0	0	2	1	8	1	2	0	4
Hybridization6_Bmp4	1	0	0	0	0	0	0	0	1	0	...	0	0	0	0	0	0	1	1	0	0
Hybridization6_Itr2	4	0	0	1	0	0	1	0	2	1	...	3	0	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
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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
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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_Fit1	7	4	3	5	65	1	7	1	2	12	...	2	6	9	2	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_Cfap	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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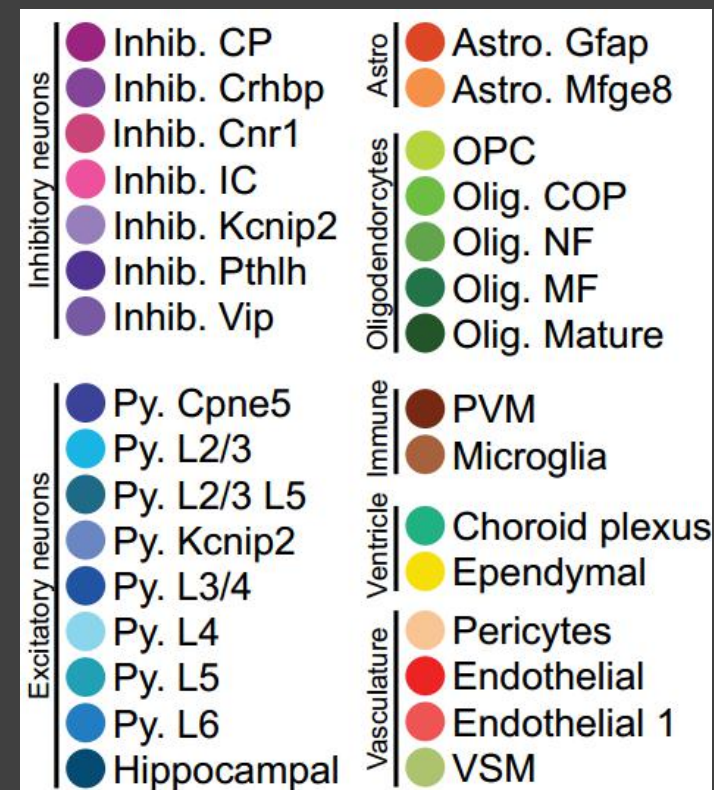
osmFISH clustering



osmFISH tSNE



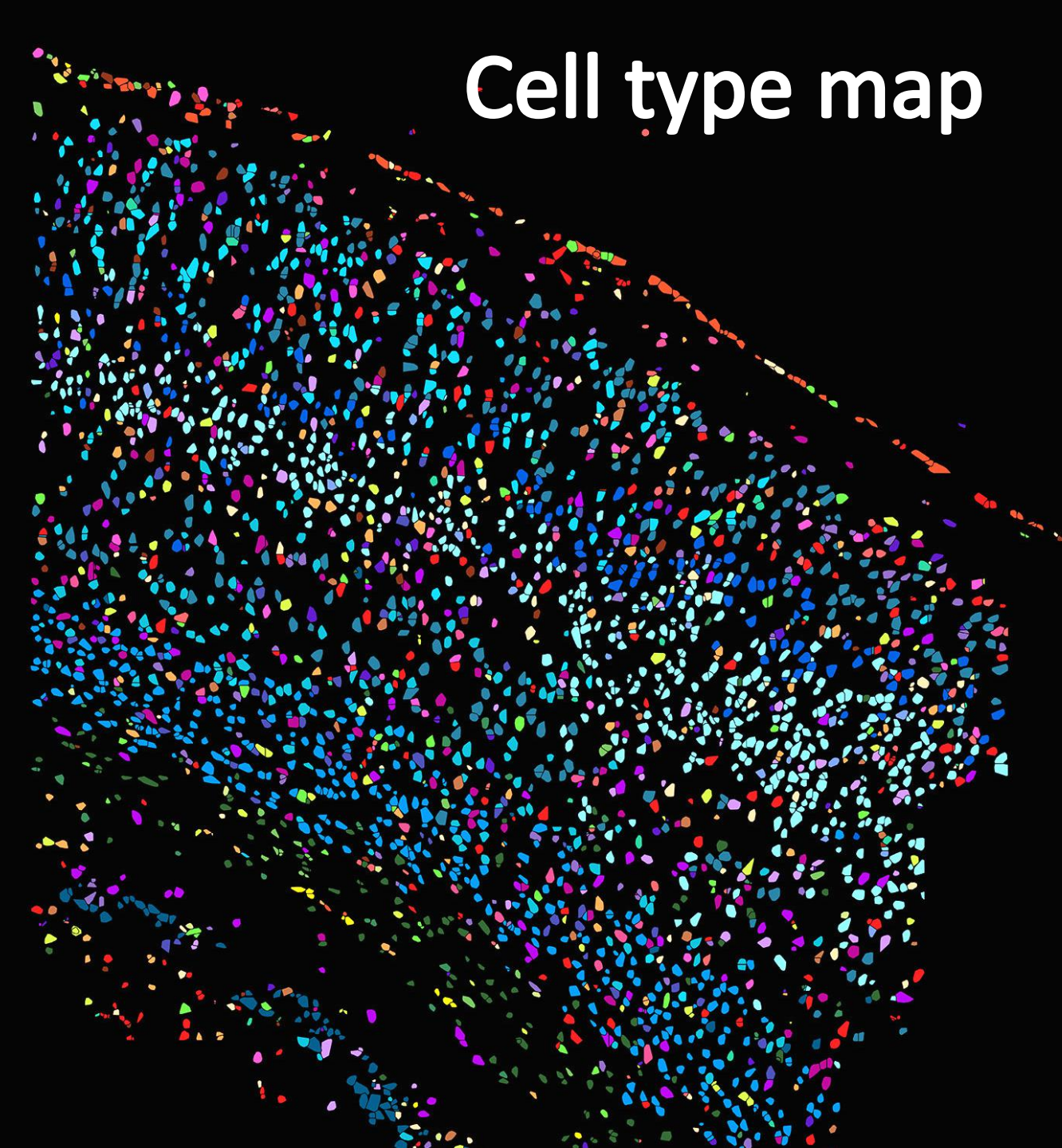
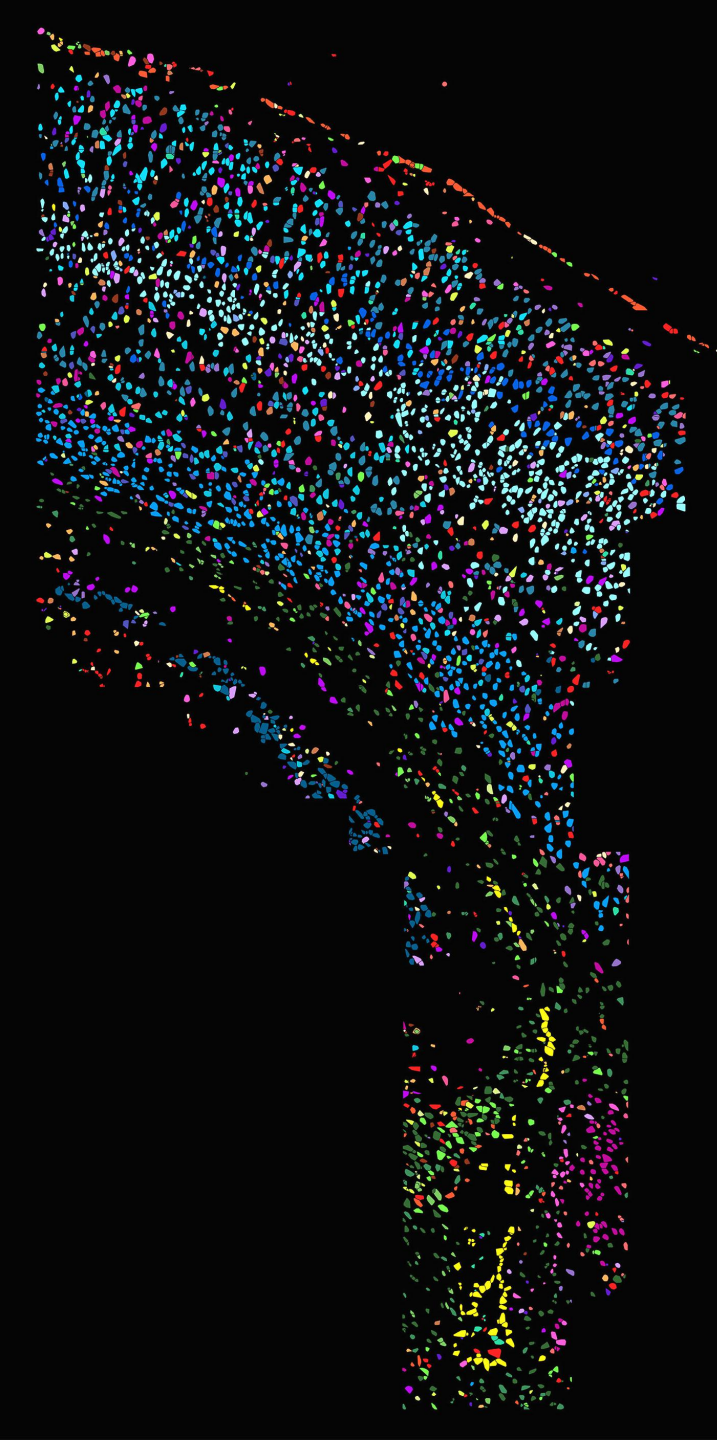
tSNE 2
tSNE 1



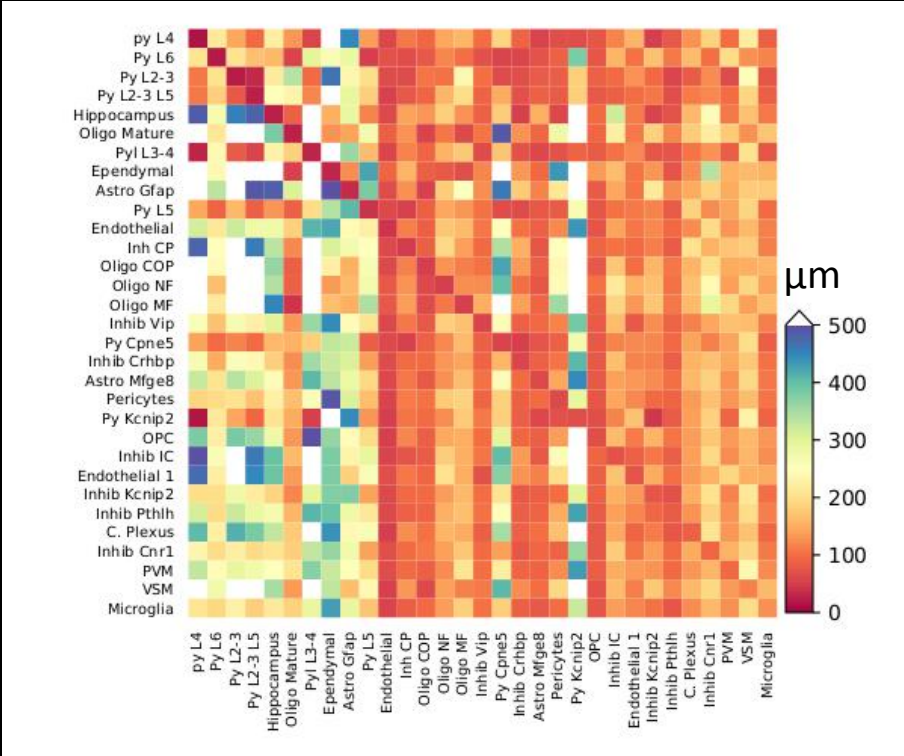
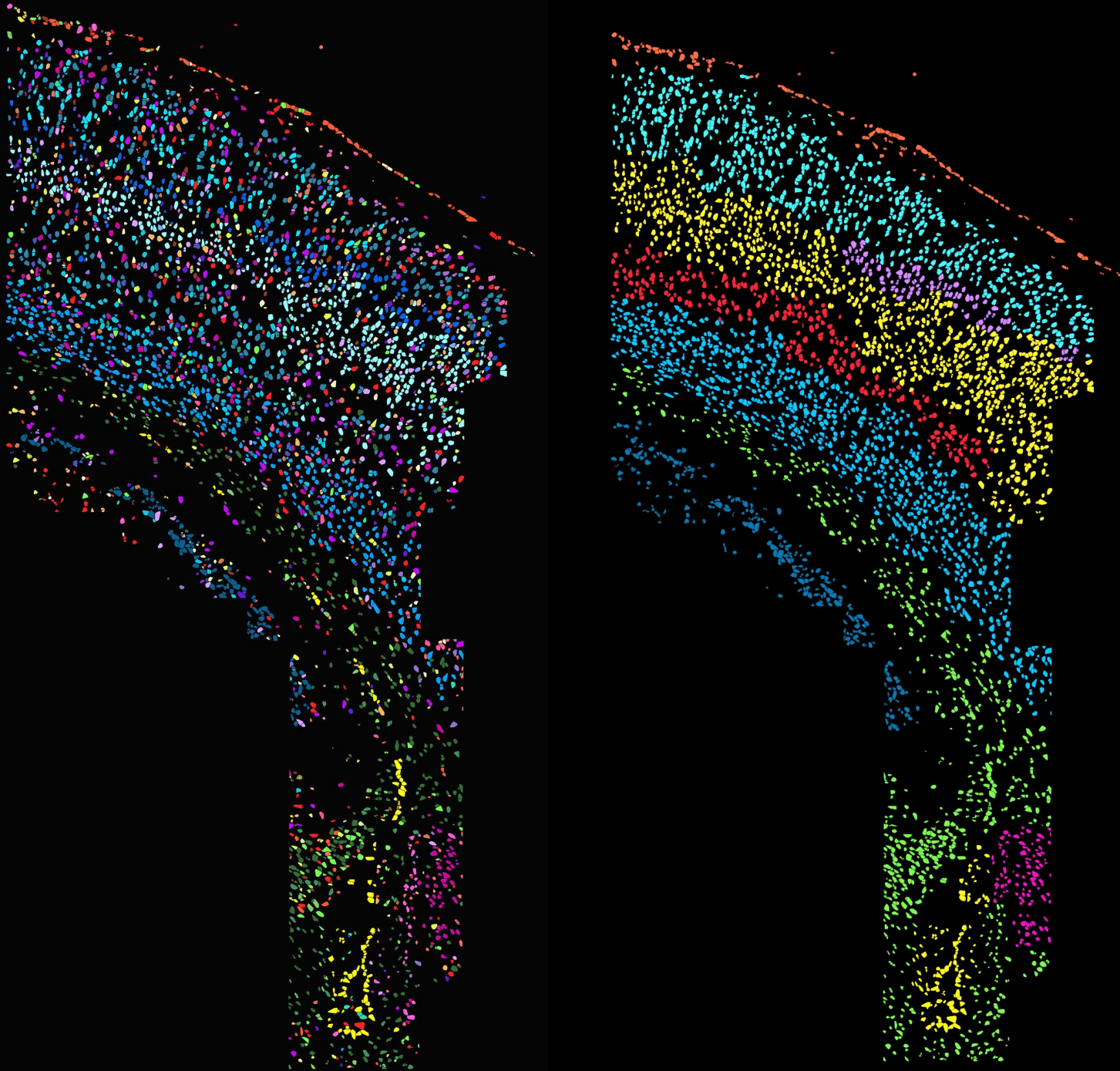
Cell type map

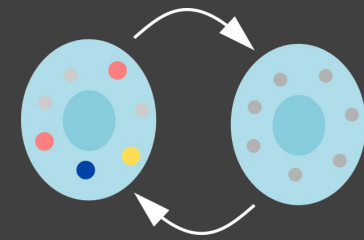
Types

- Inhibitory neurons**
 - Inhib CP
 - Inhib Crhbp
 - Inhib Cnr1
 - Inhib IC
 - Inhib Kcnp2
 - Inhib Pthlh
 - Inhib Vip
- Excitatory neurons**
 - Py Cpne5
 - Py L2/3
 - Py L2/3 L5
 - Py Kcnp2
 - Py L3/4
 - Py L4
 - Py L5
 - Py L6
 - Hippocampal
- Astro**
 - Astro Gfap
 - Astro Mfge8
- Oligodendrocytes**
 - OPC
 - Olig COP
 - Olig NF
 - Olig MF
 - Olig Mature
- Immune**
 - Pvm
 - Microglia
- Ventricle**
 - Choroid plexus
 - Ependymal
- Vasculature**
 - Pericytes
 - Endothelial
 - Endothelial 1
 - Vsm



Spatial analysis

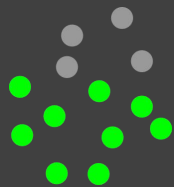




Cyclic smFISH



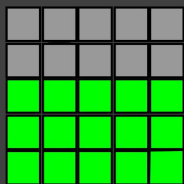
Resolution: Diffraction limited (150-300nm)



Detection efficiency: ~100%

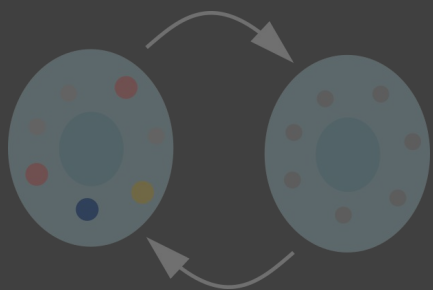


Gene throughput: tens of genes (F x R)

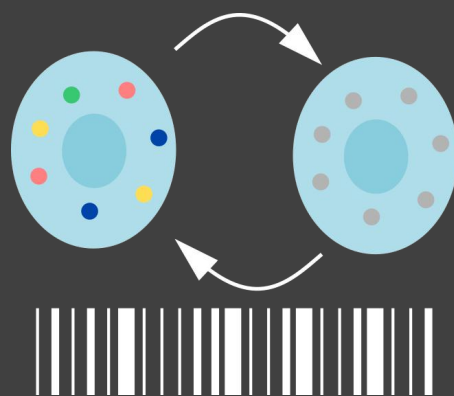


Spatial throughput: several mm²

Cyclic FISH



Barcoded FISH



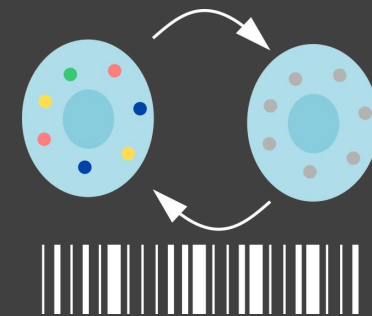
in situ Sequencing



Spatial Sequencing



Reprobing same molecule



Round 1



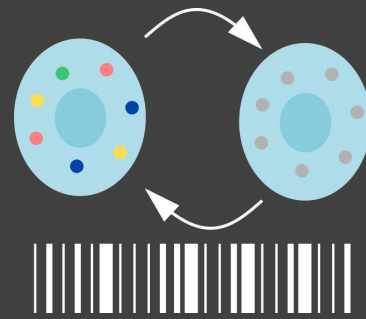
Stripping



Round 2

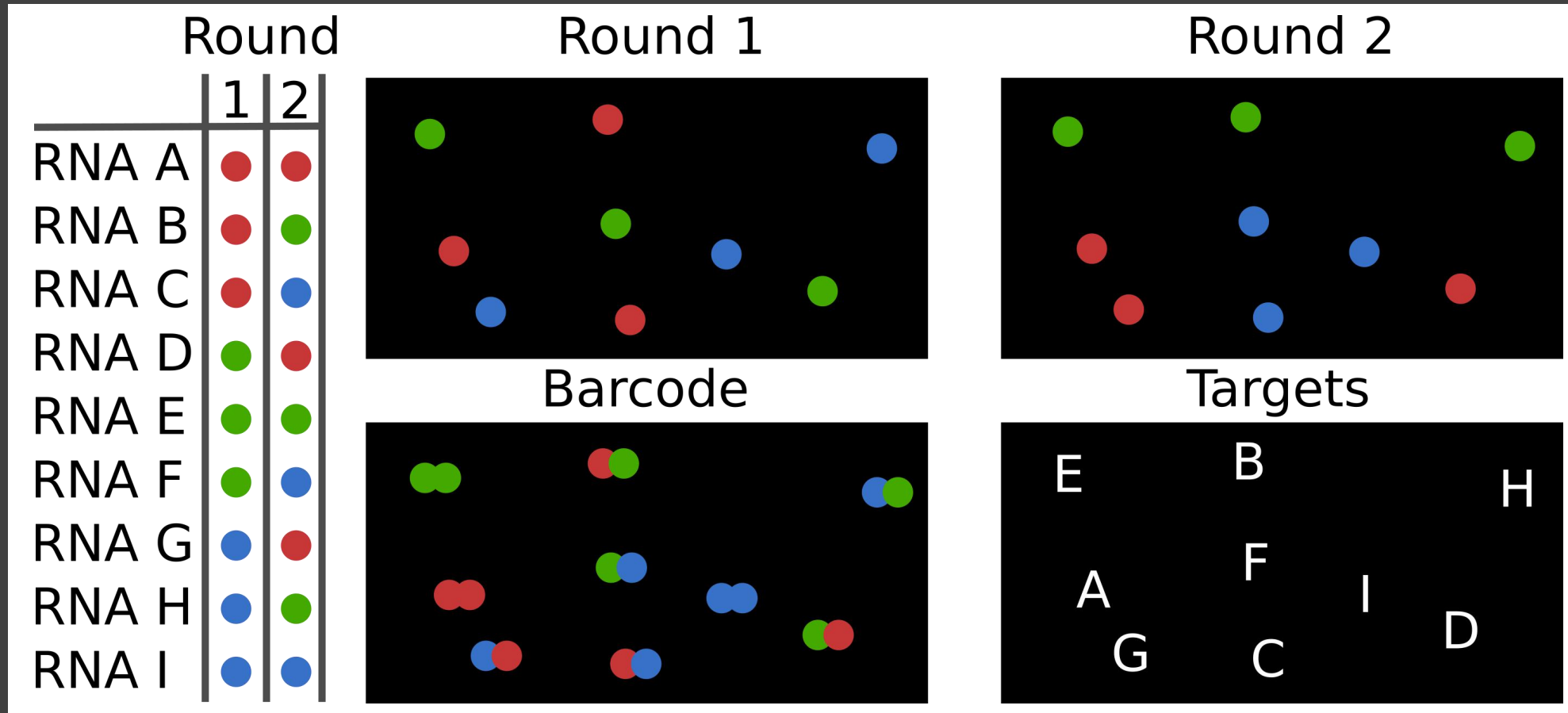
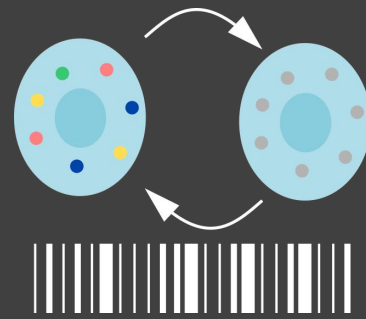


Barcoding

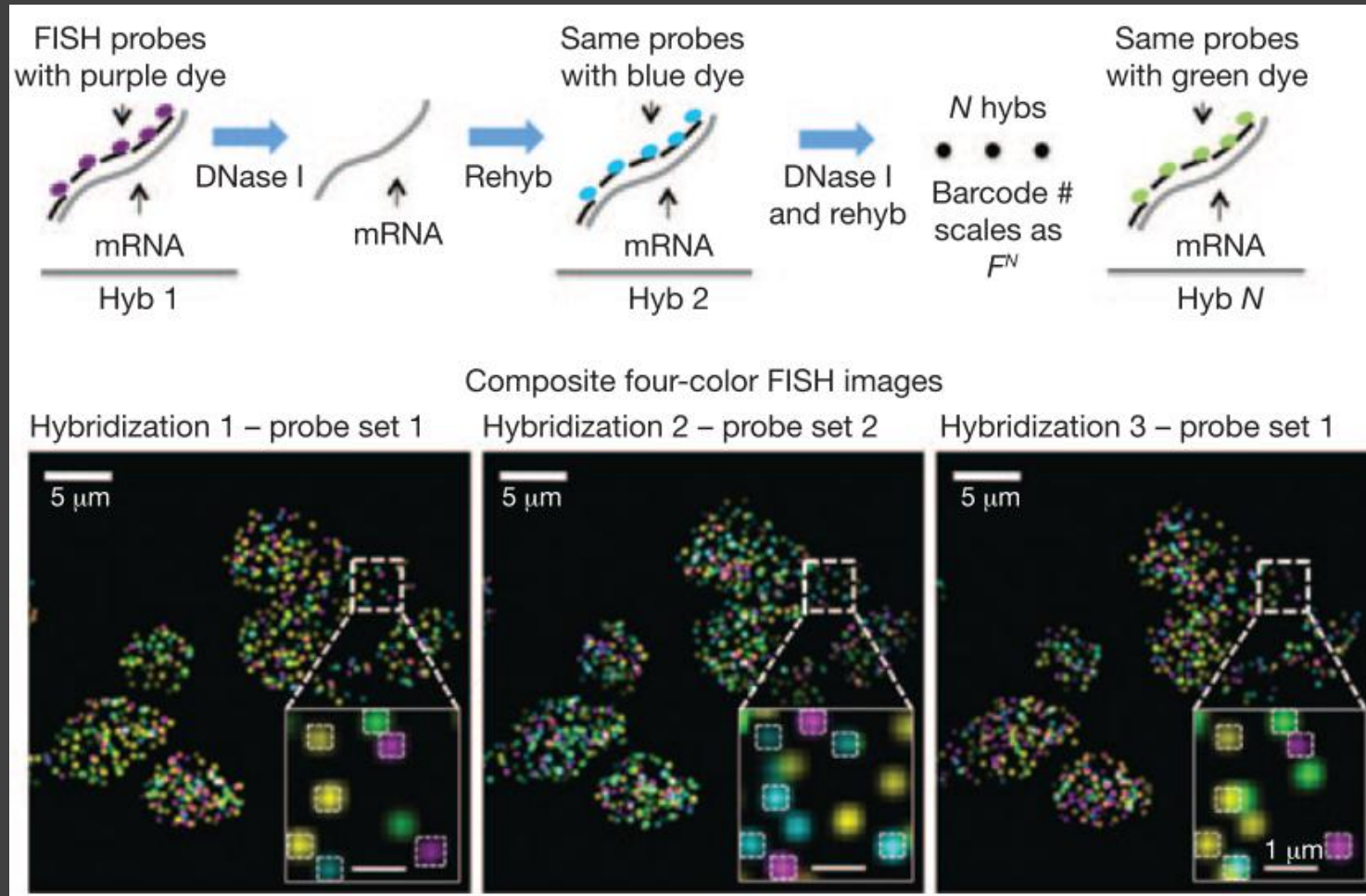
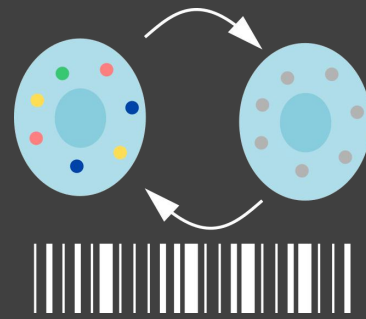


	Round	
	1	2
RNA A	●	●
RNA B	●	●
RNA C	●	●
RNA D	●	●
RNA E	●	●
RNA F	●	●
RNA G	●	●
RNA H	●	●
RNA I	●	●

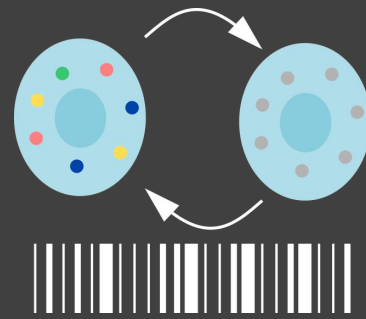
Barcoding



SeqFISH



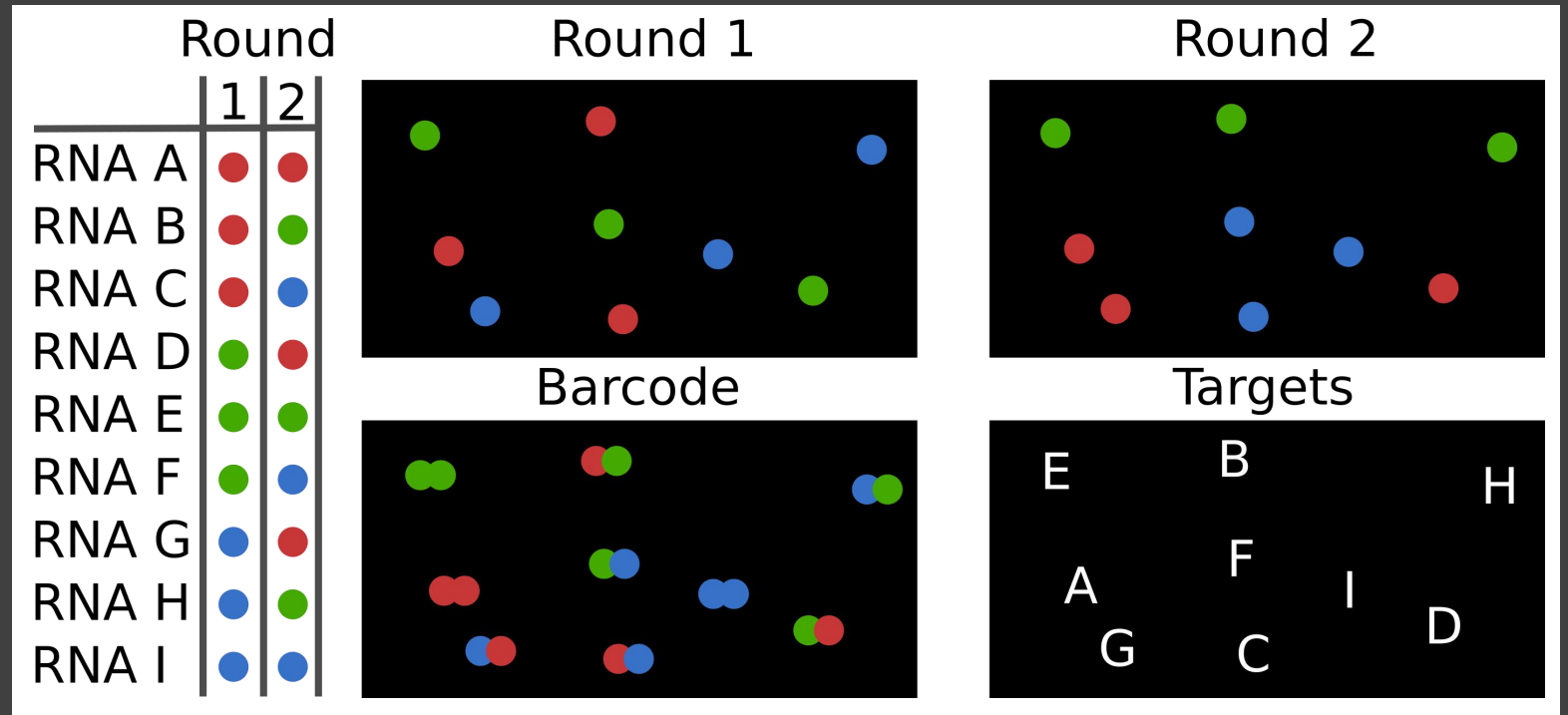
Barcoding



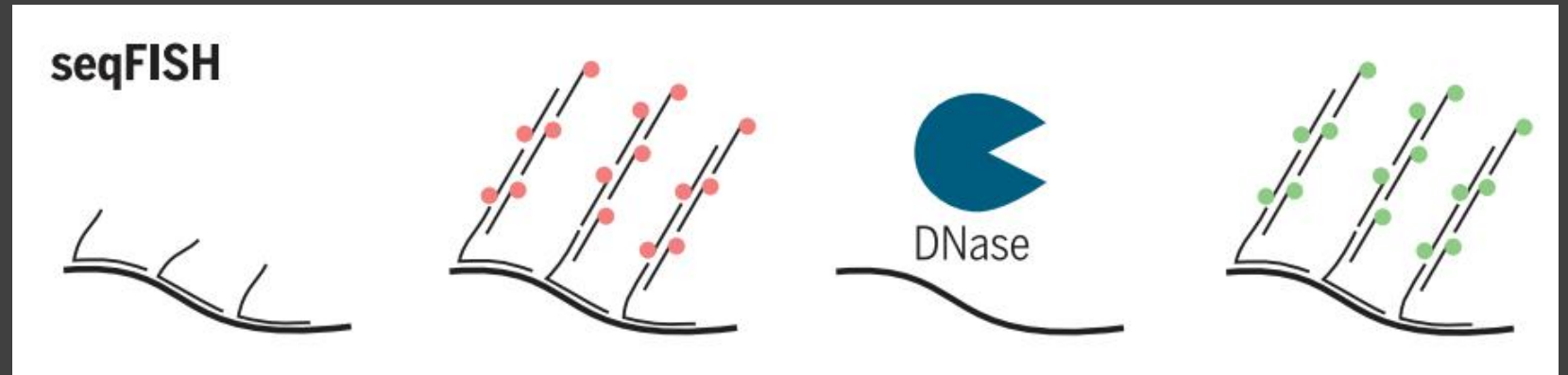
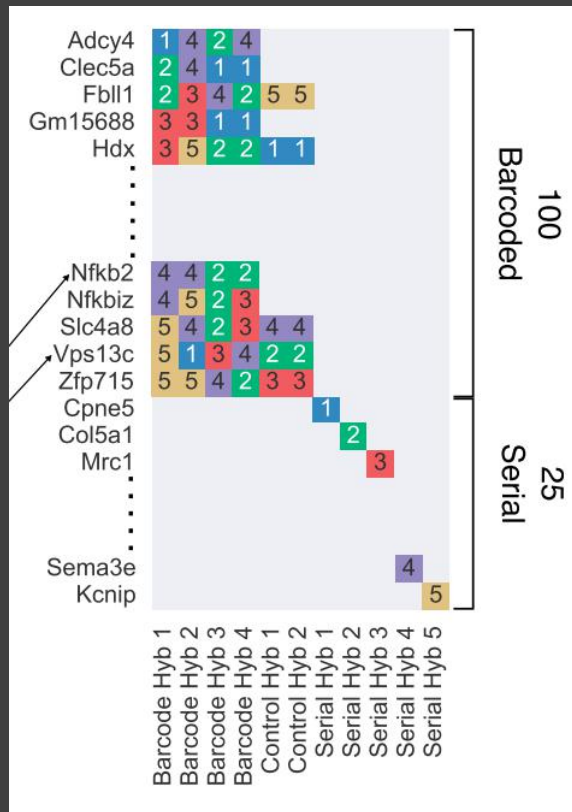
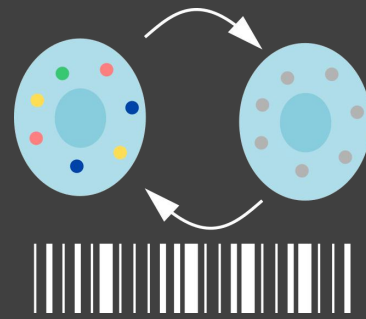
Scaling: Fluo^{Rounds}

$4^7 = 16,384$ genes

$4^8 = 65,536$ genes

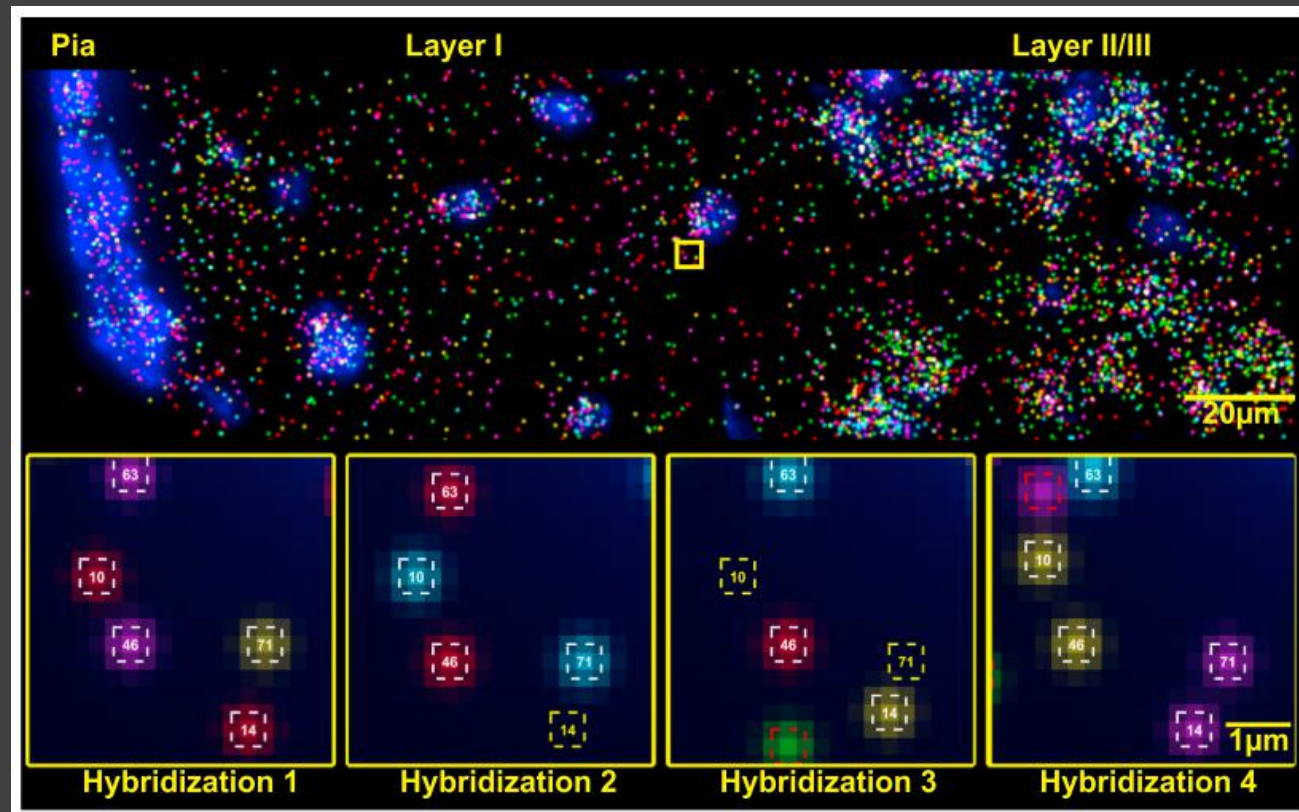
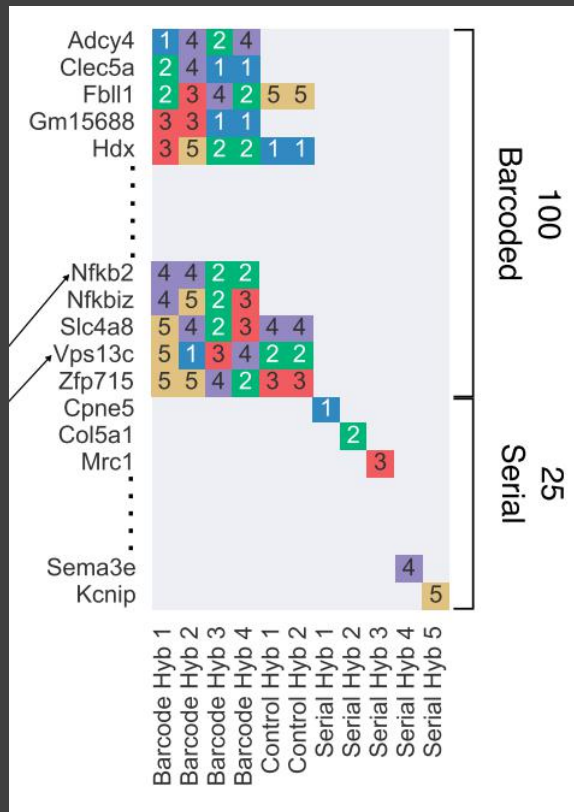
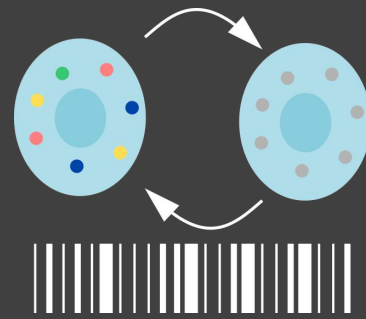


SeqFISH

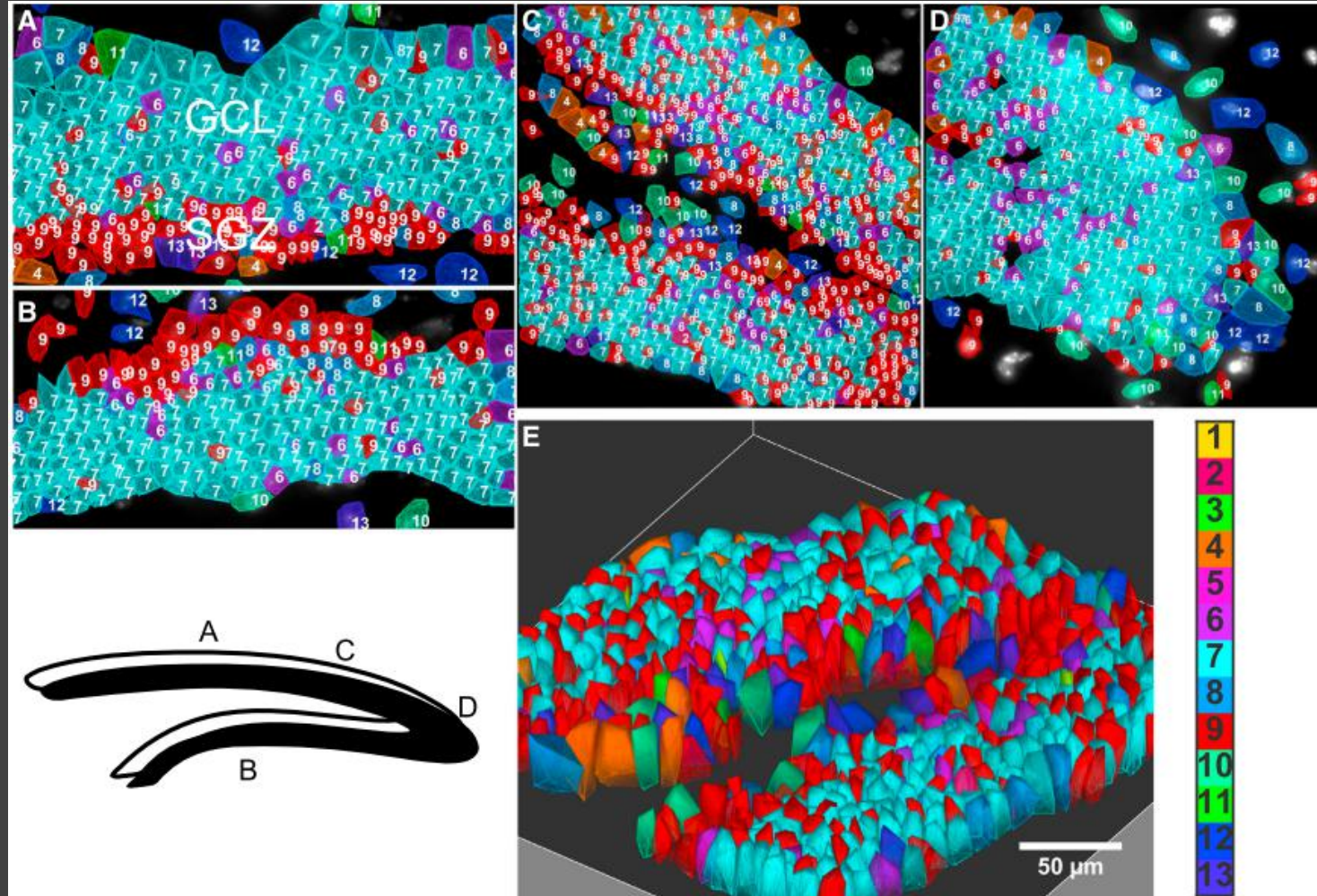
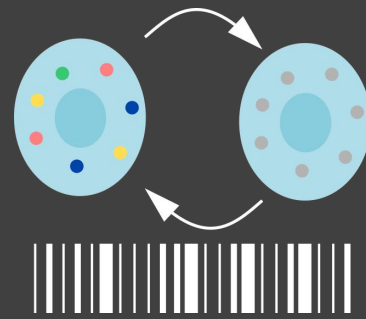


Shah *et al.* Neuron 2016

SeqFISH

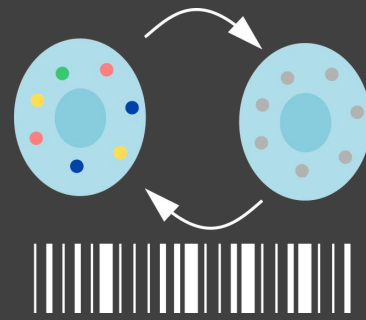


SeqFISH

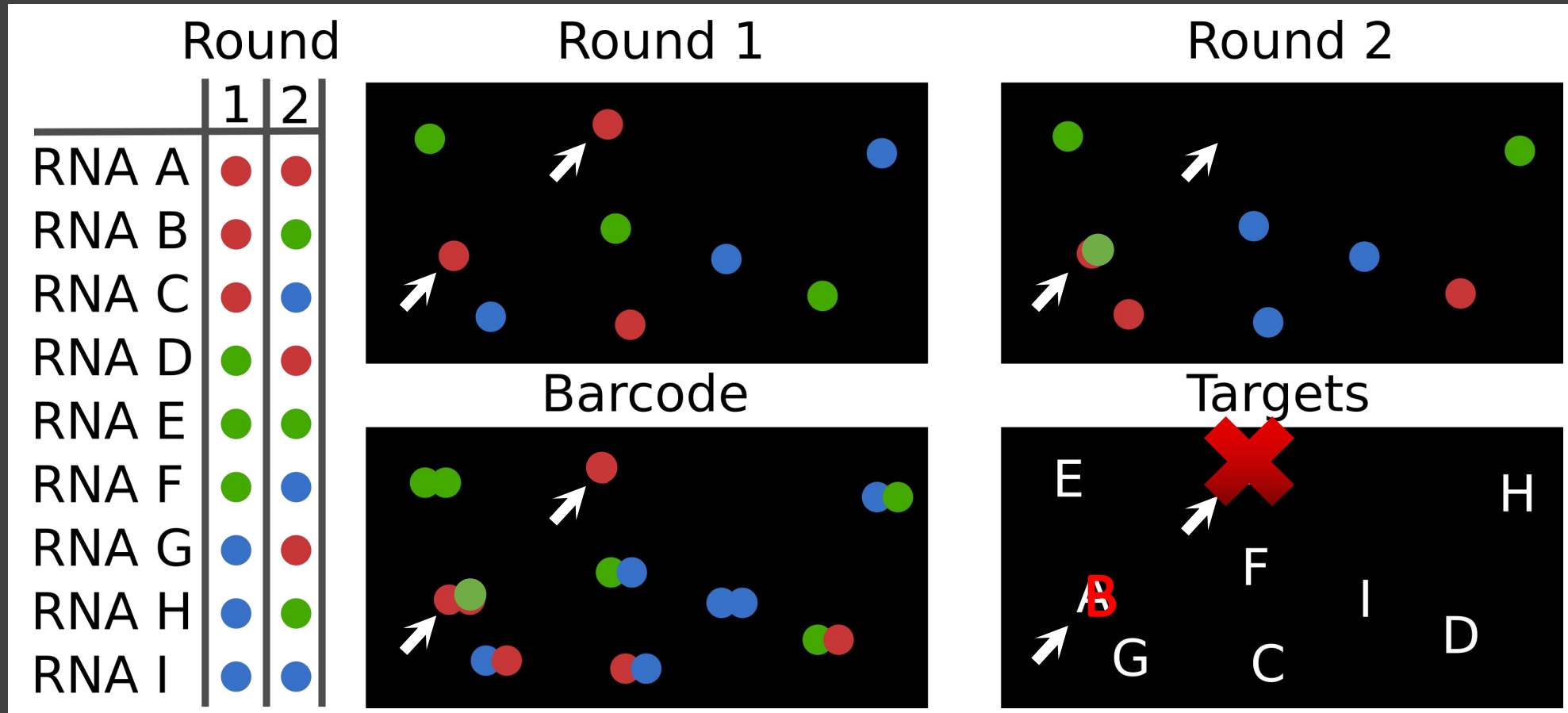
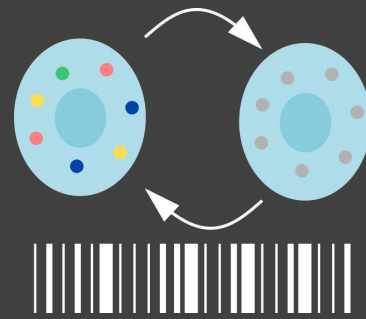


Challenges

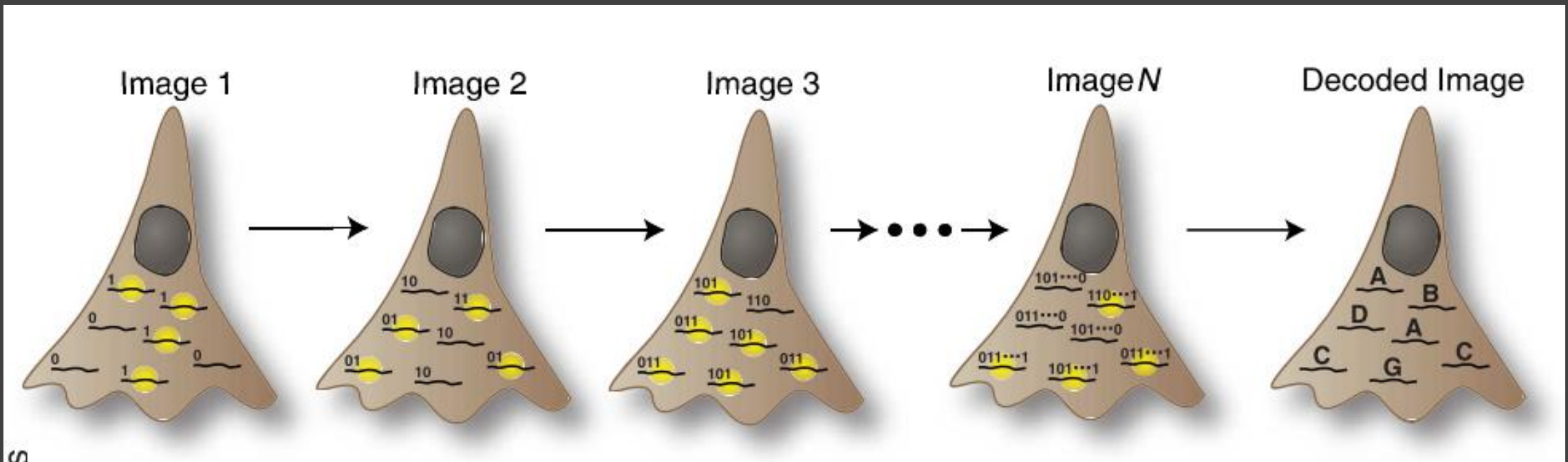
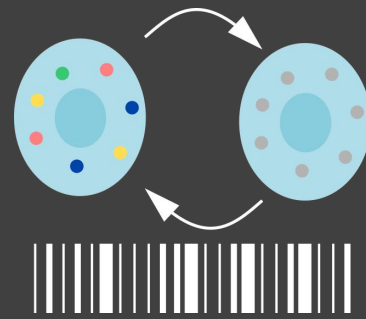
- Errors in barcodes
- Optical density



Errors

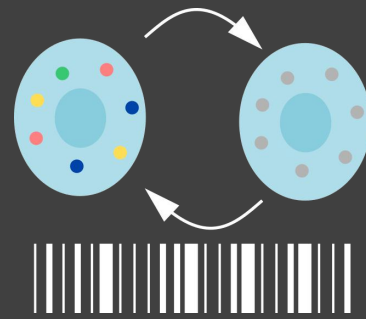


MERFISH



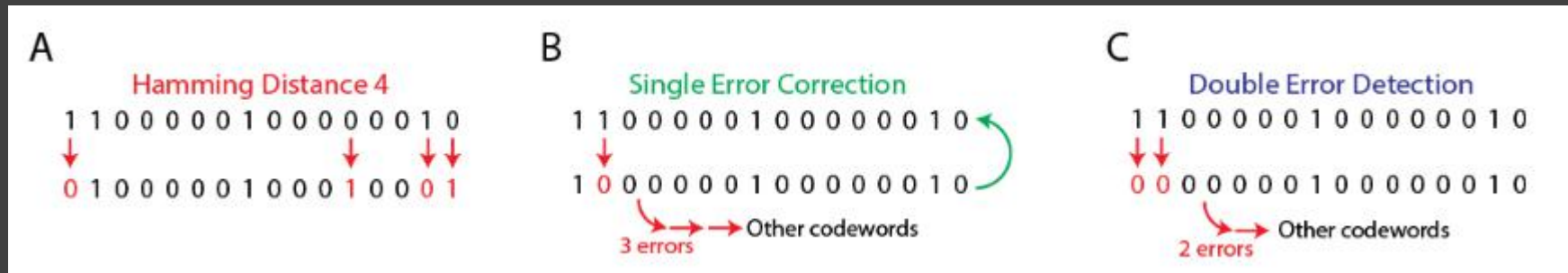
Chen *et al.* Science 2015

MERFISH



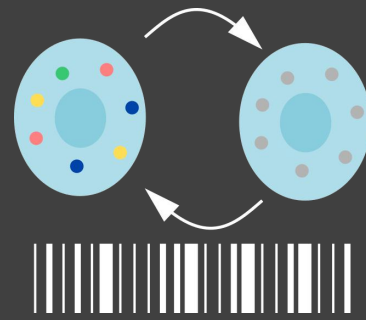
Barcode 1: 1 1 0 0 0 1 0 0 1 0

Barcode 2: 0 1 0 0 0 1 0 0 1 0

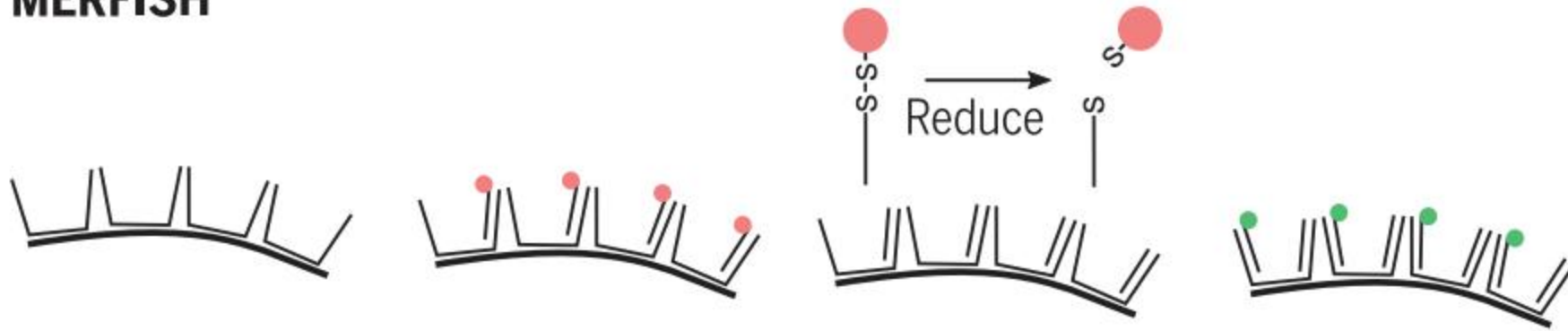


Chen *et al.* Science 2015

MERFISH

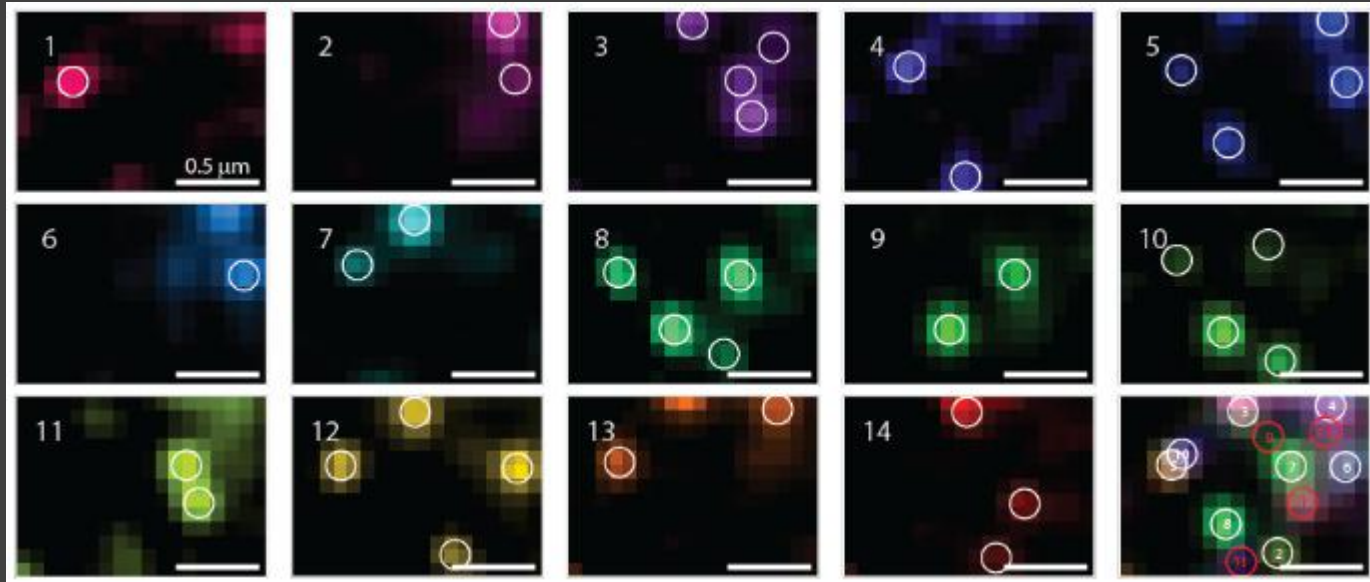
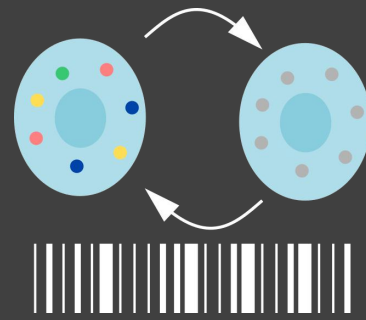


MERFISH



	Round 1				Round 2			
Bit	1	2	3	4	5	6	7	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

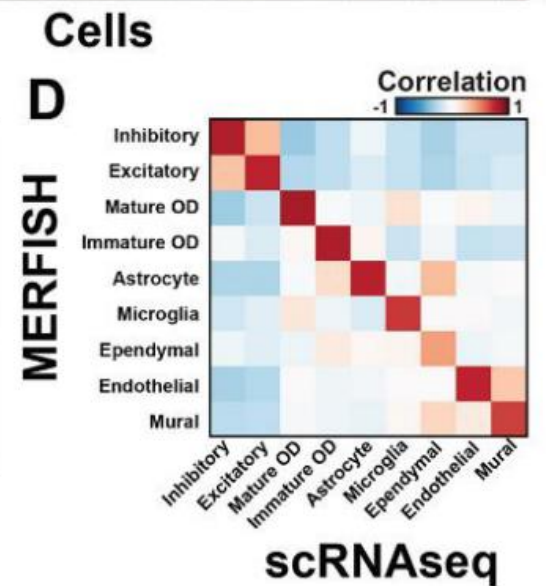
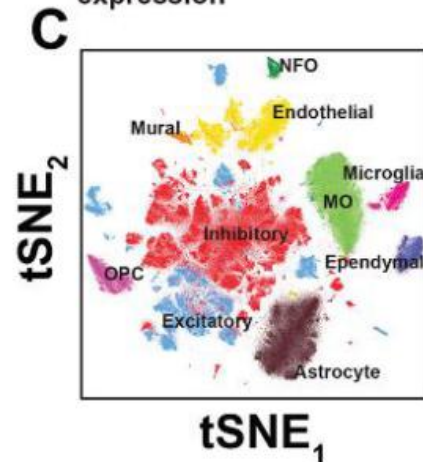
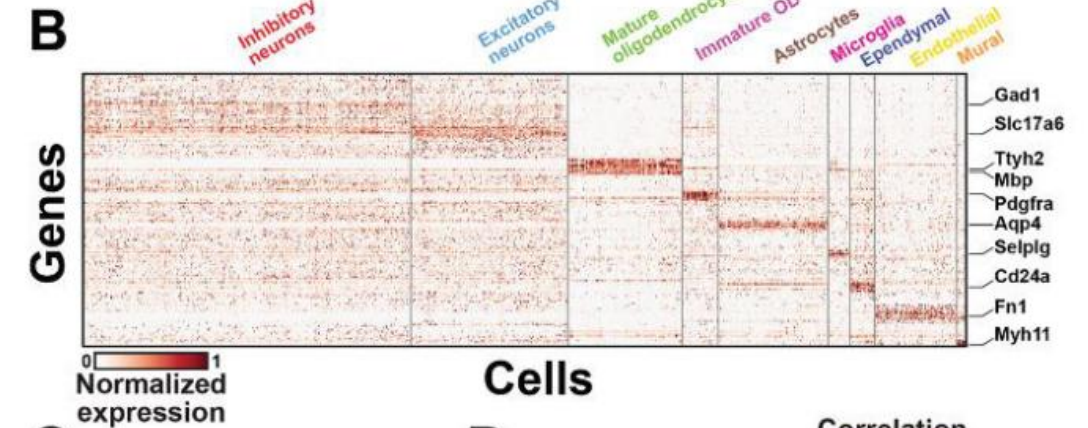
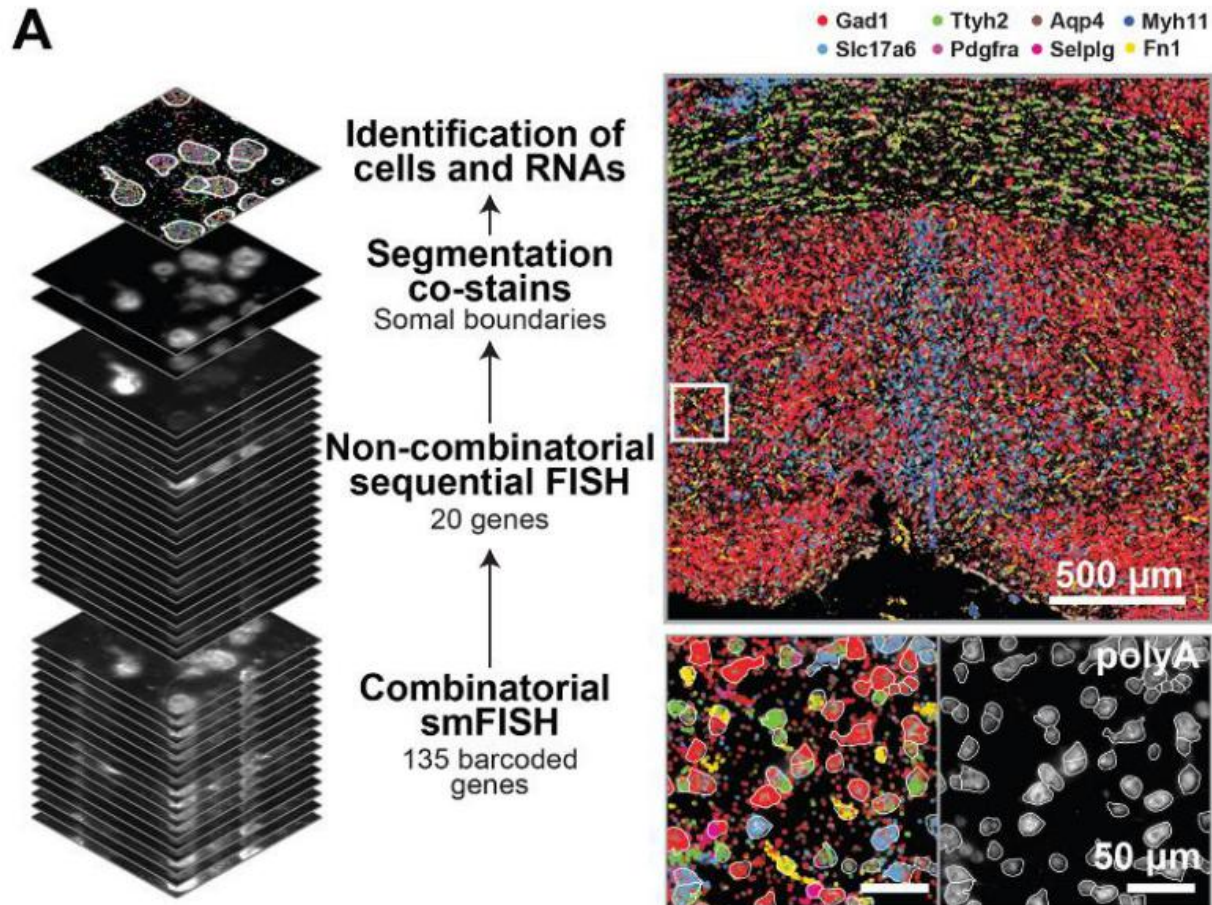
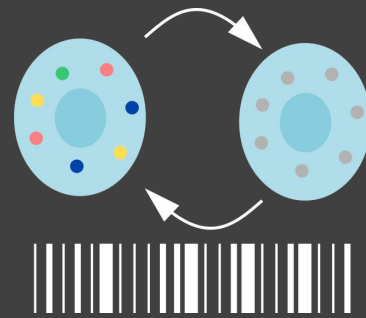
MERFISH



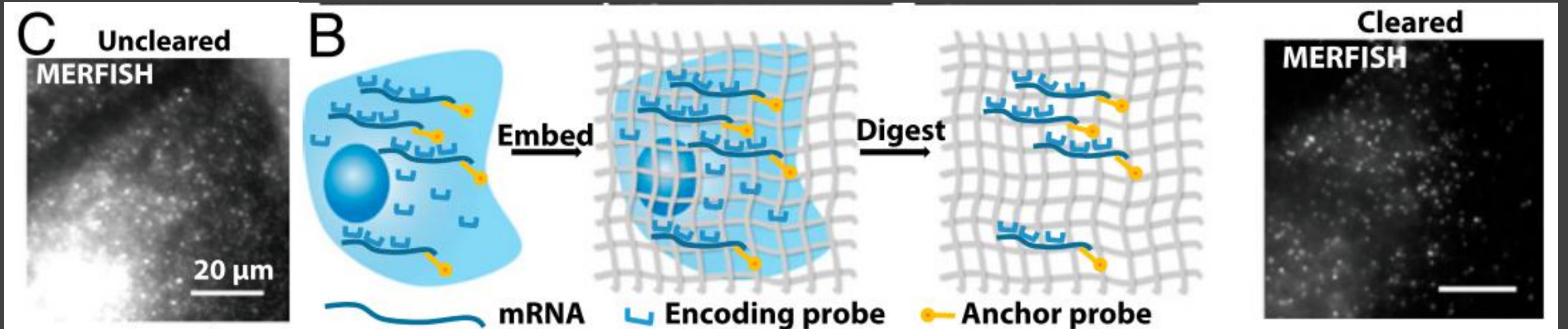
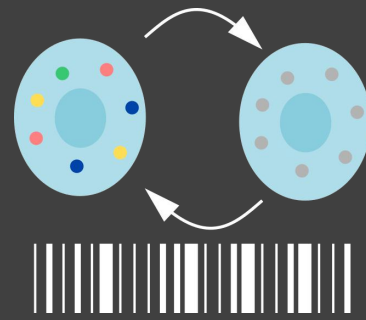
Spot number	Hybridization round														Gene
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	unidentified
2	0	0	0	0	0	0	0	1	0	1	0	1	0	1	FLNC
3	0	0	1	0	0	0	1	0	0	0	0	1	0	1	ZFPM2
4	0	1	0	0	1	1	0	0	0	0	0	0	1	0	LTBP2
5	1	0	0	0	0	0	0	1	0	0	0	1	1	0	TP53BP1
6	0	1	0	0	1	1	0	0	0	0	0	1	0	0	INTS1
7	0	0	1	0	0	0	0	1	1	0	1	0	0	0	PINK1-AS
8	0	0	0	0	1	0	0	1	1	1	0	0	0	0	MLLT4
9	0	0	0	0	0	0	0	0	0	1	0	0	0	0	unidentified
10	0	0	0	1	1	0	1	0	0	1	0	0	0	0	TRIM14
11	0	0	0	1	0	0	0	0	0	0	0	0	0	0	unidentified
12	0	0	1	0	0	0	0	0	0	0	0	0	0	0	unidentified

Chen *et al.* Science 2015

MERFISH

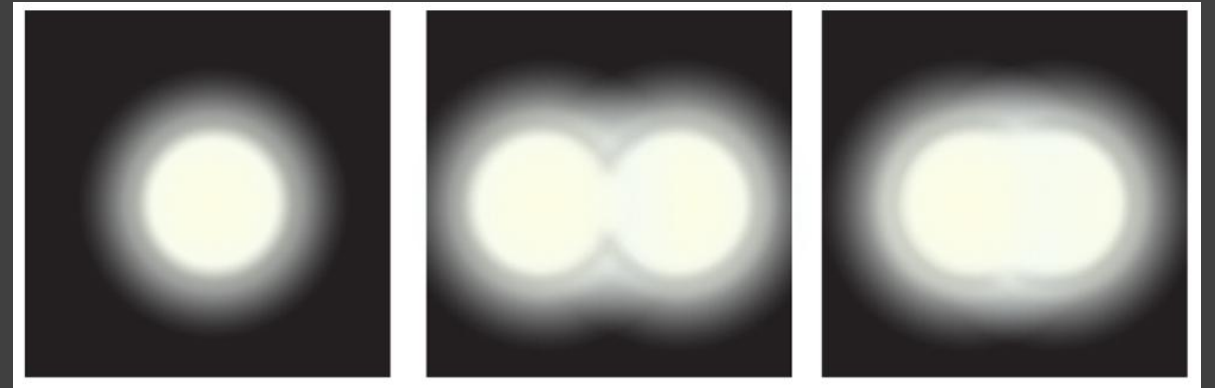
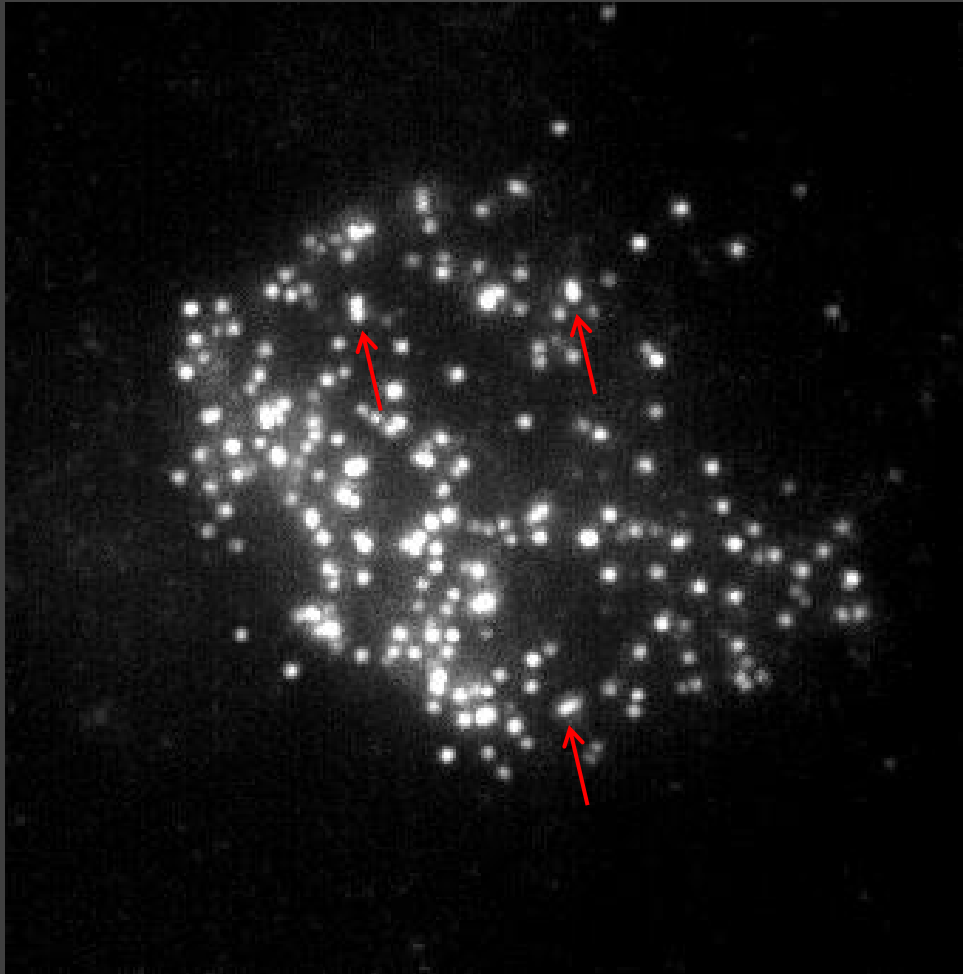
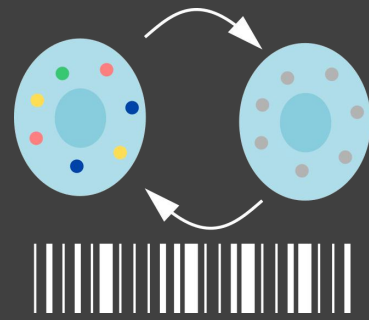


MERFISH

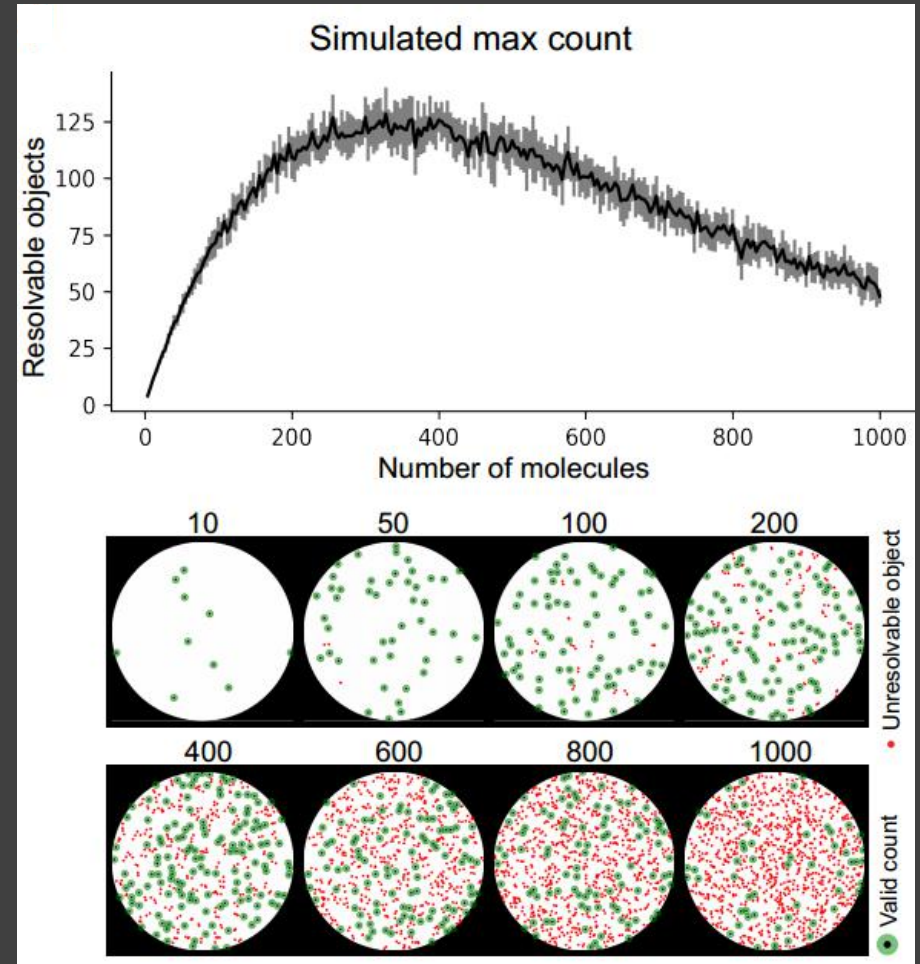
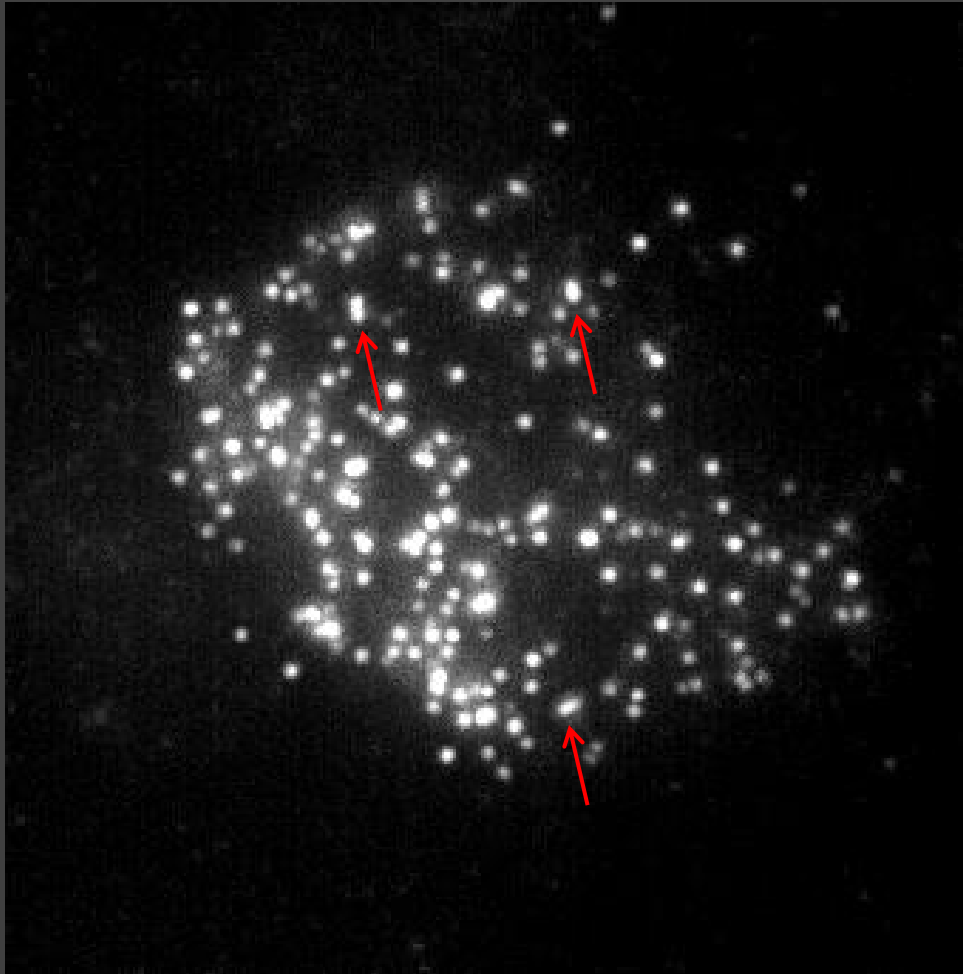
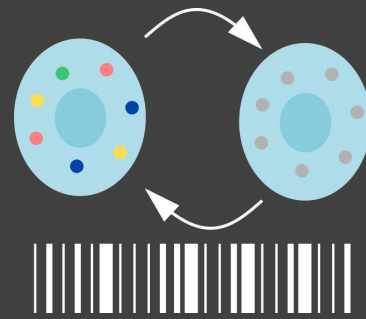


Moffit *et al.* PNAS 2016

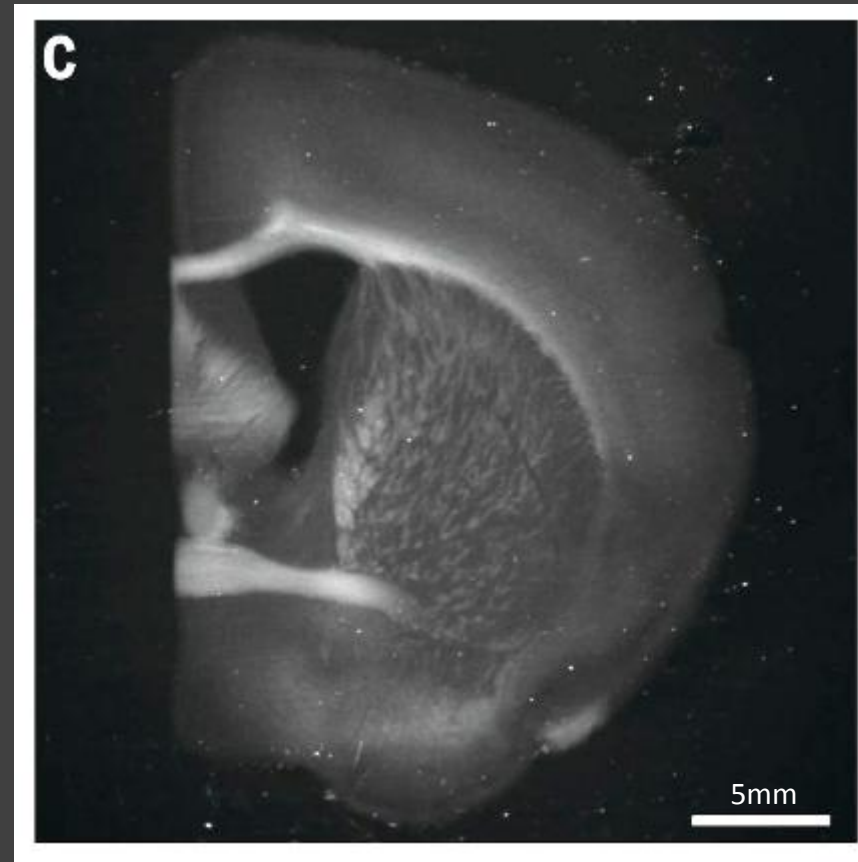
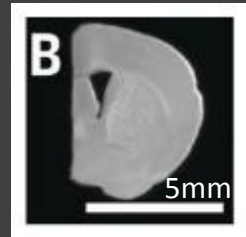
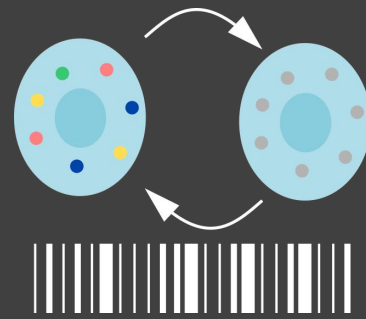
Barcoding Optical space



Barcoding Optical space

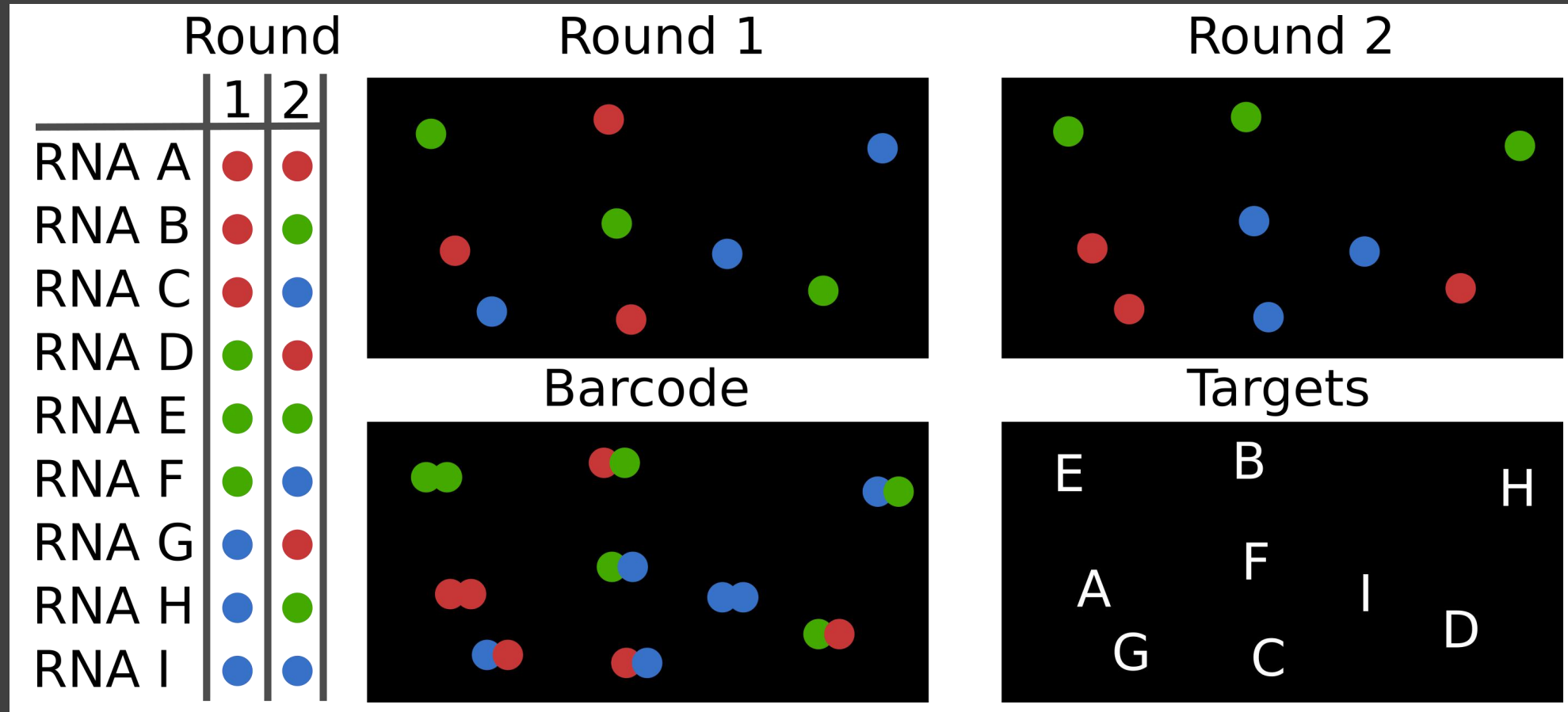
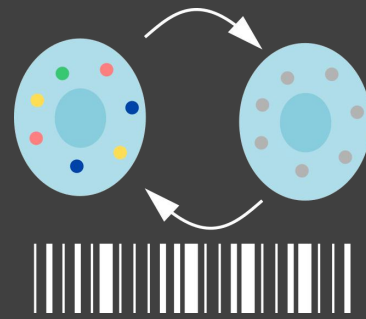


Expansion microscopy

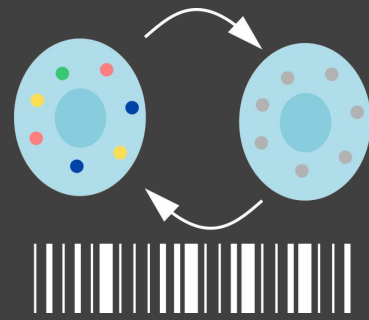


Chen *et al.* Science 2015

Dense barcode



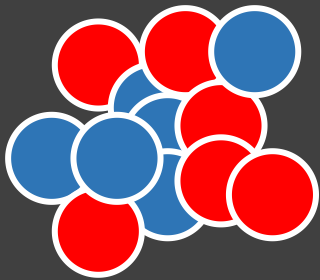
Dense vs Sparse barcode



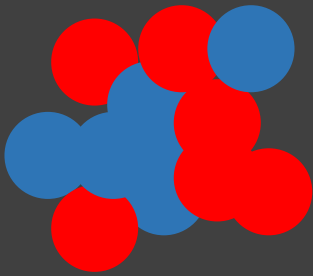
Dense

RRBRB

Molecules

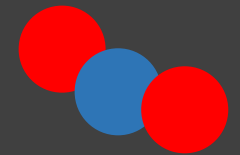
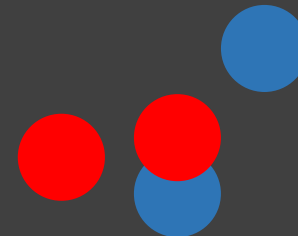
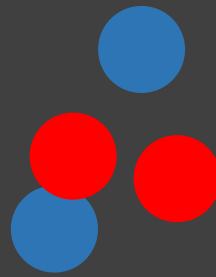
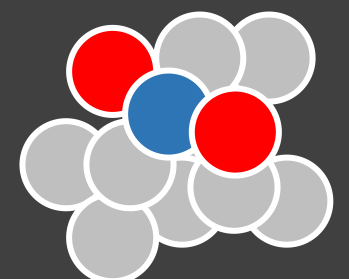
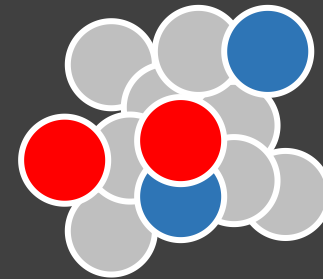
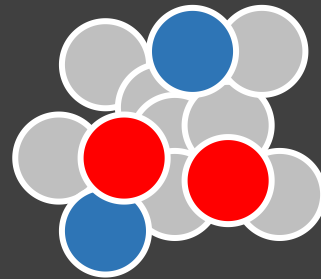


Image



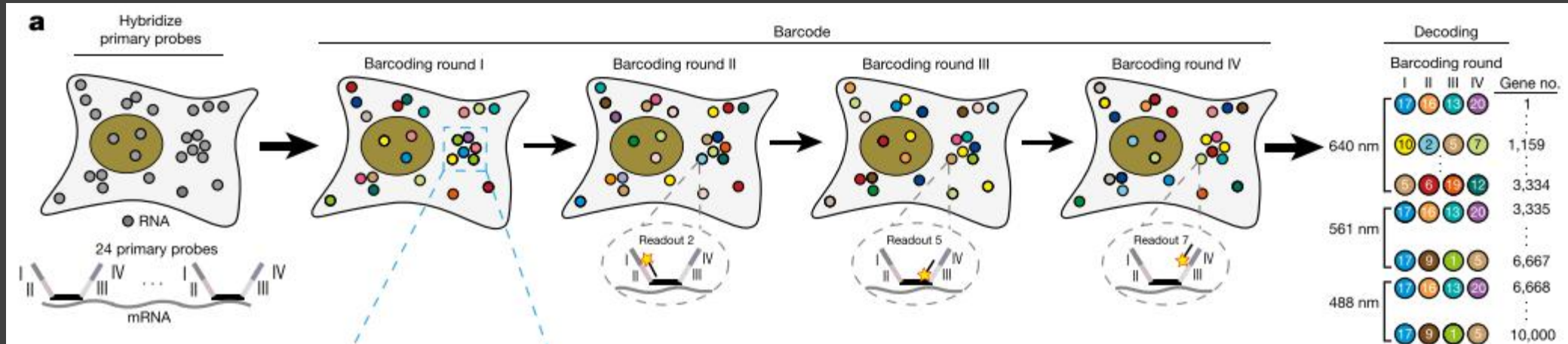
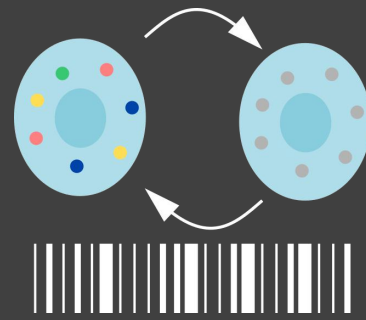
Sparse

R00B0B0



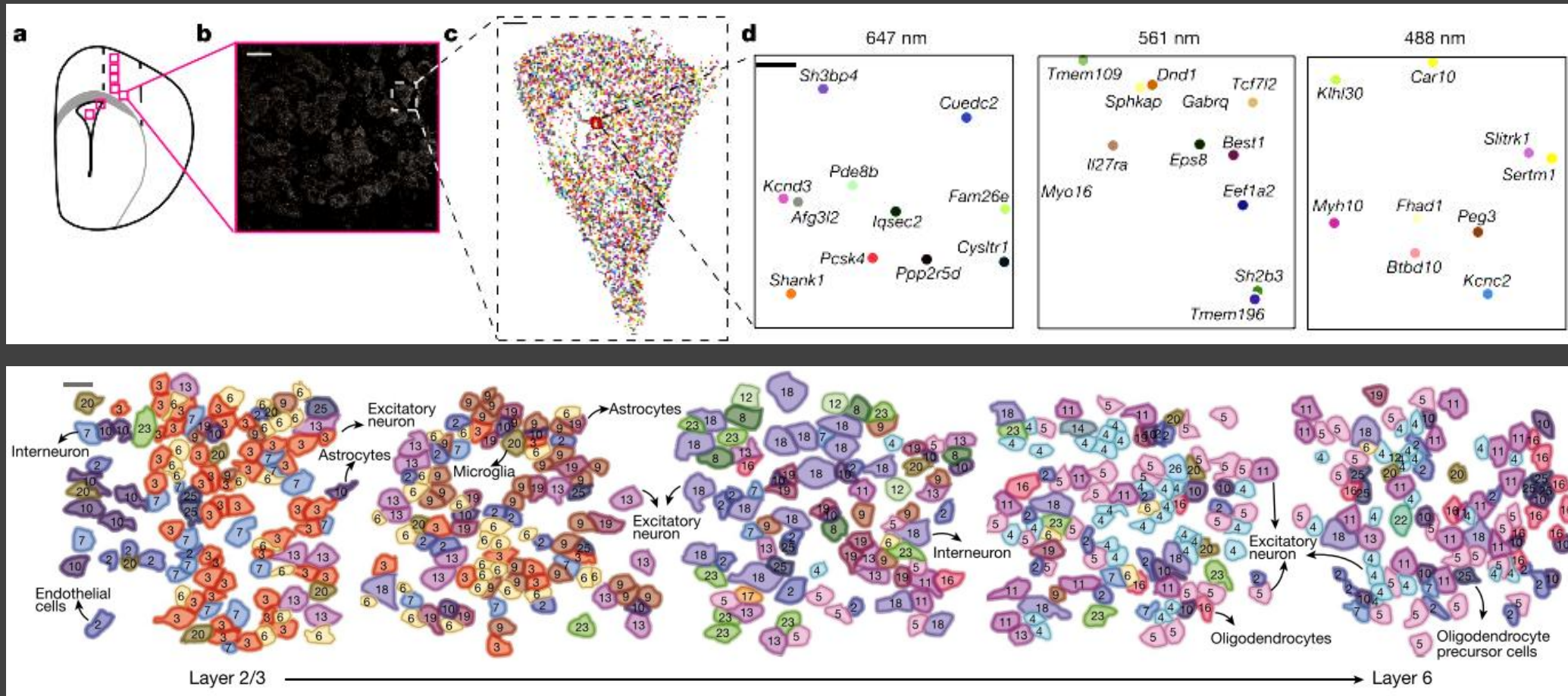
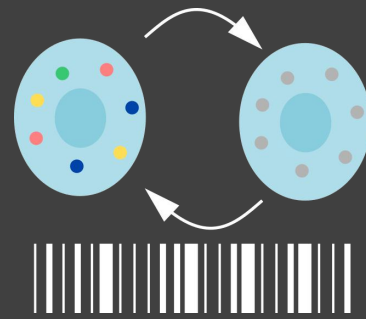
SeqFISH+

Sparse barcode

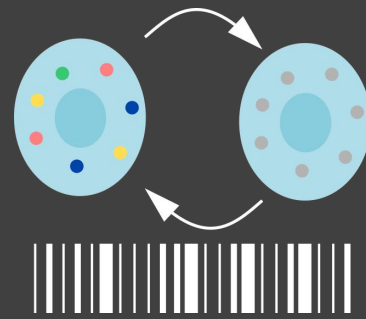


Eng *et al.* Nature 2019

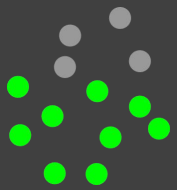
SeqFISH+



Barcoded smFISH



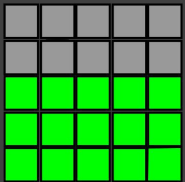
Resolution: Diffraction limited (150-300nm)



Detection efficiency: 70-90%

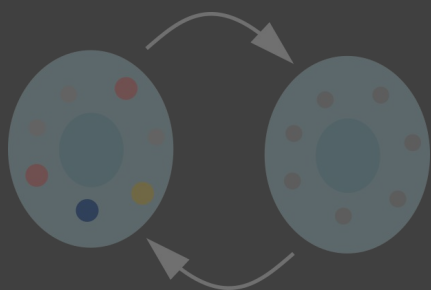


Gene throughput: 100 - 10,000



Spatial throughput: several mm²

Cyclic FISH



Barcoded FISH



in situ Sequencing



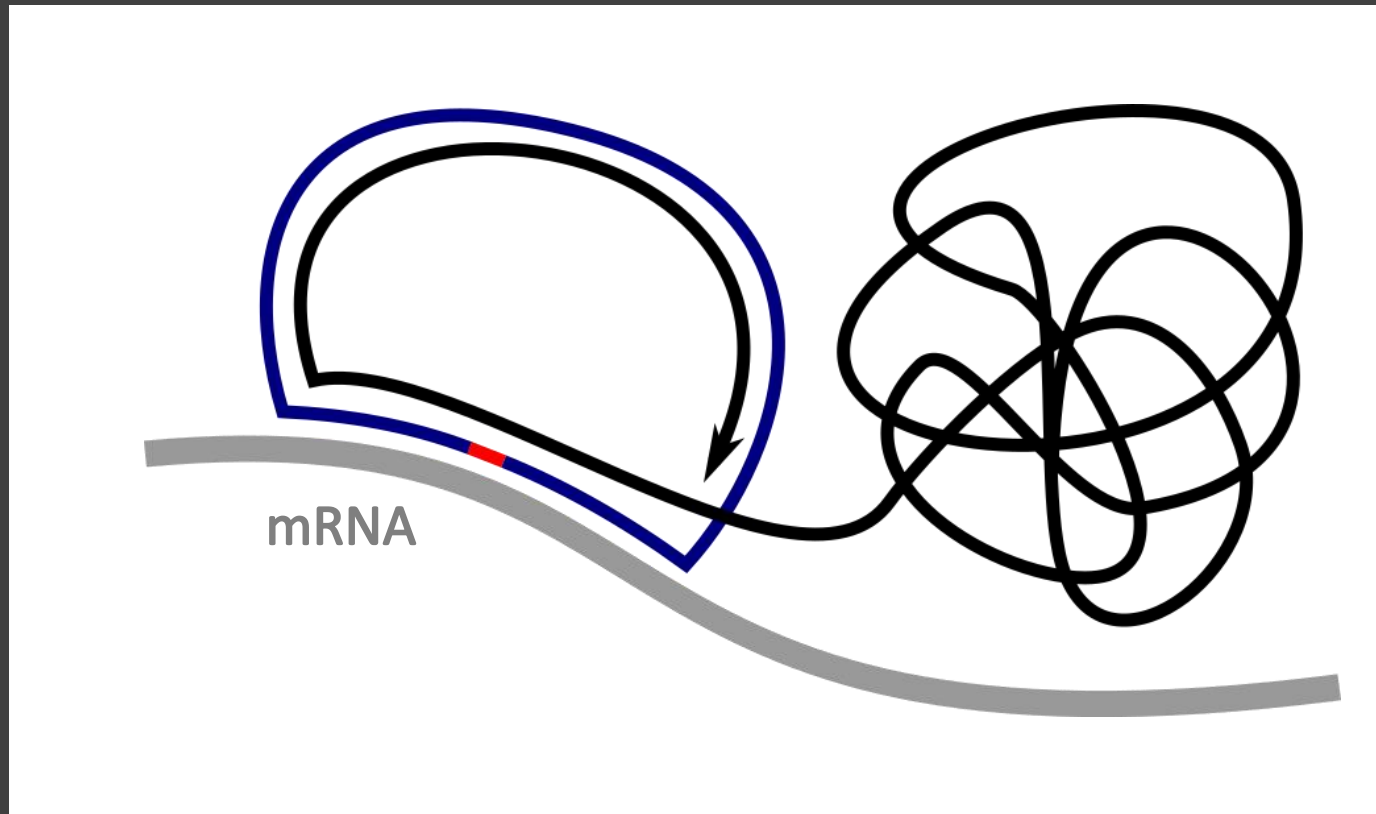
Spatial Sequencing



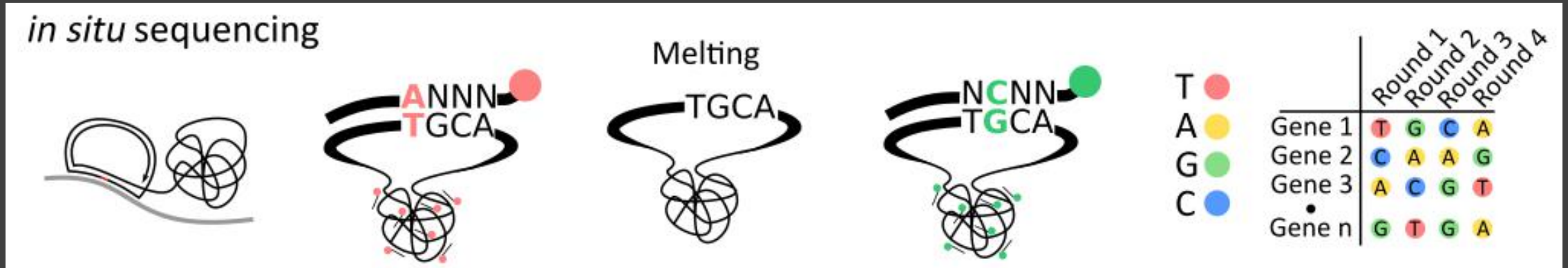
in situ sequencing



Rolling circle amplification

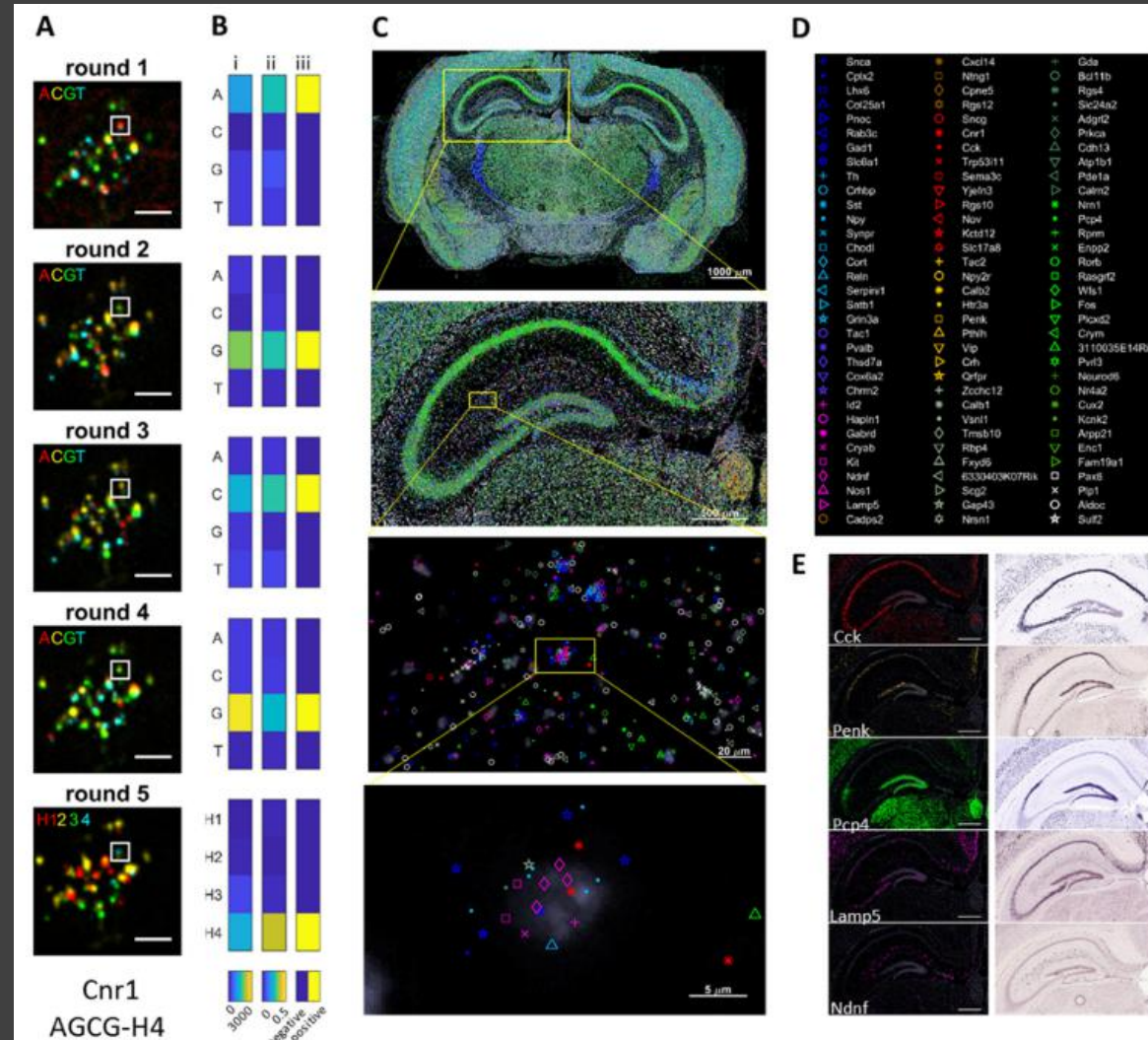


in situ sequencing



Sequencing by ligation

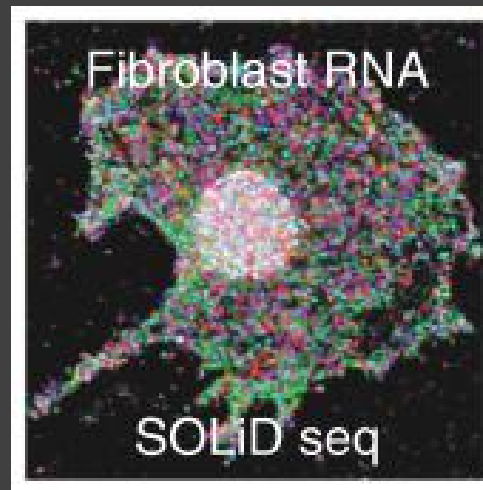
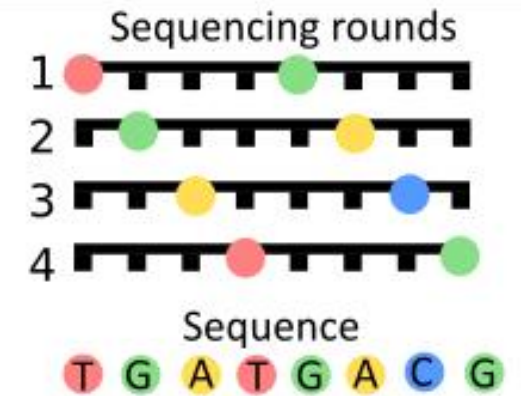
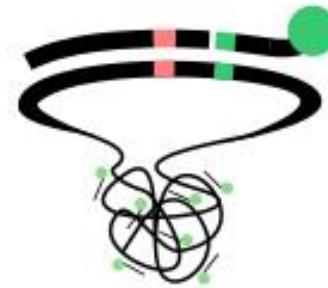
in situ sequencing



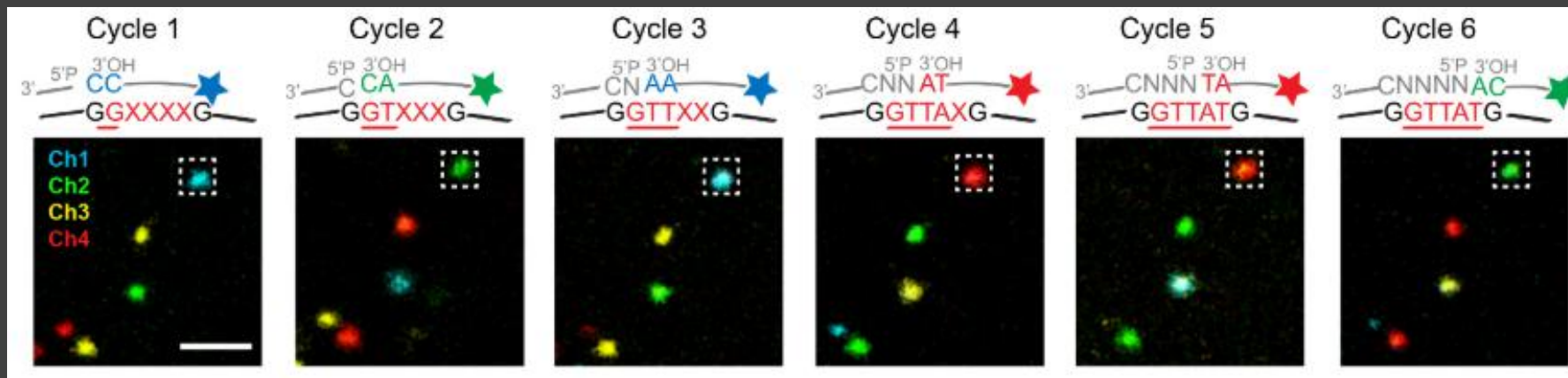
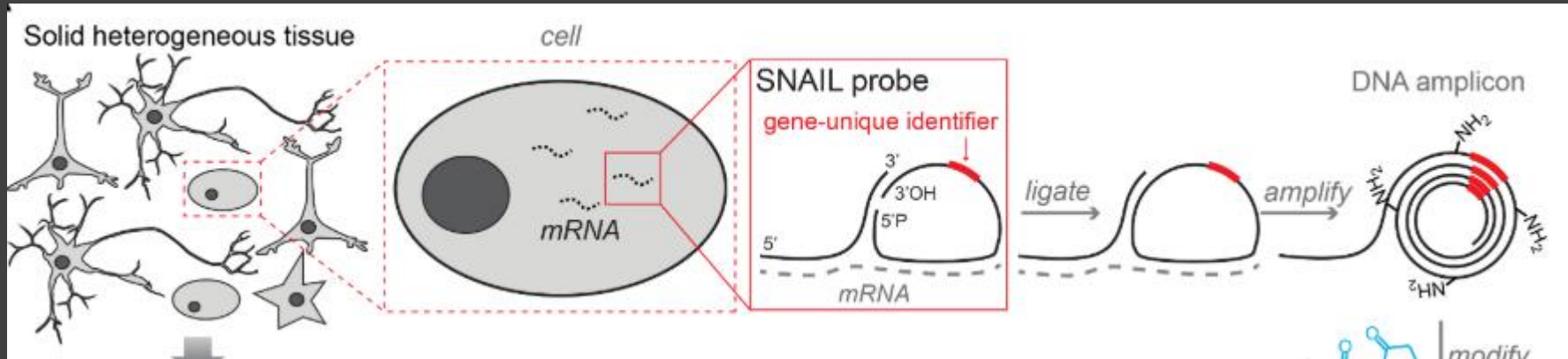
FISSEQ



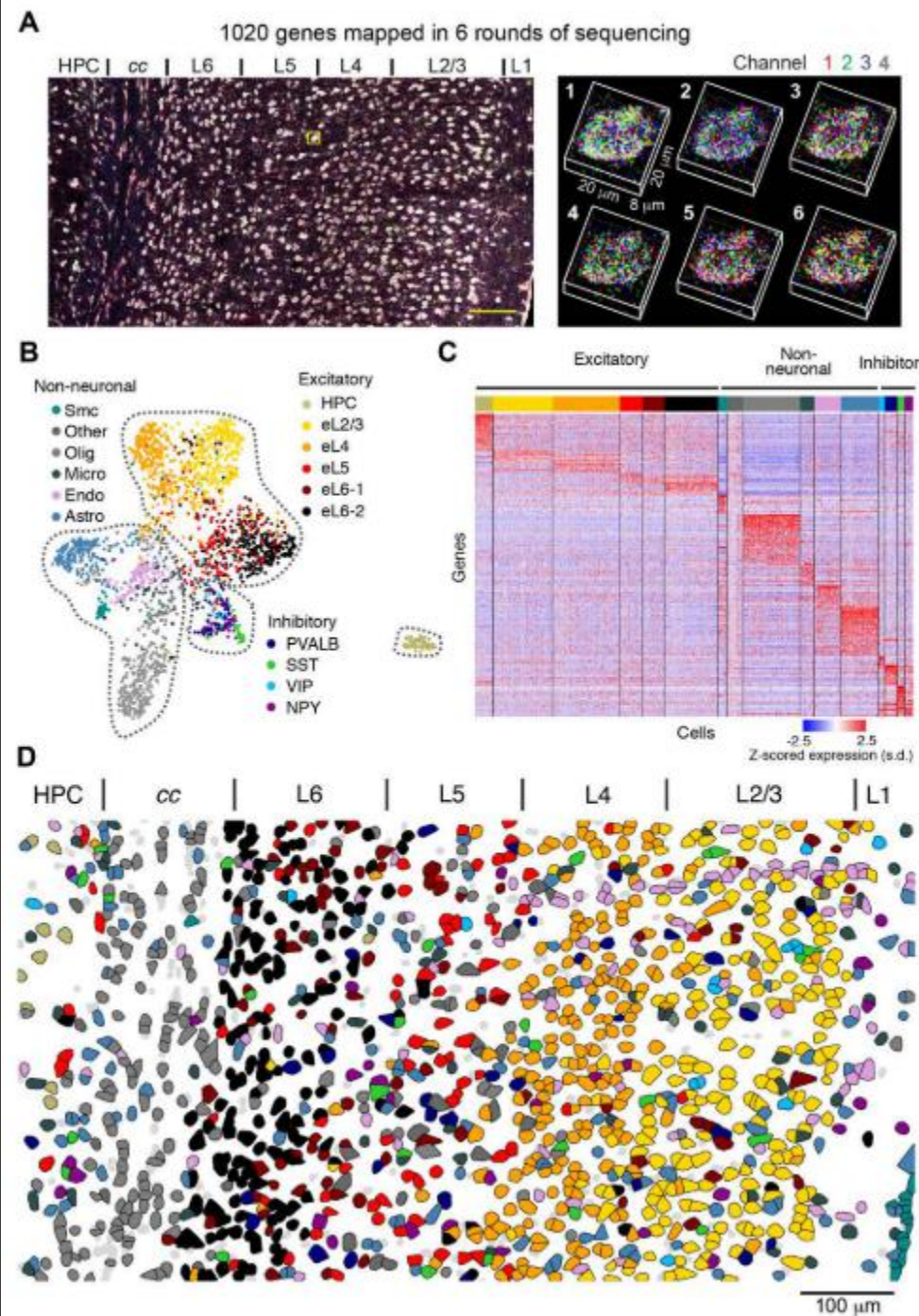
FISSEQ



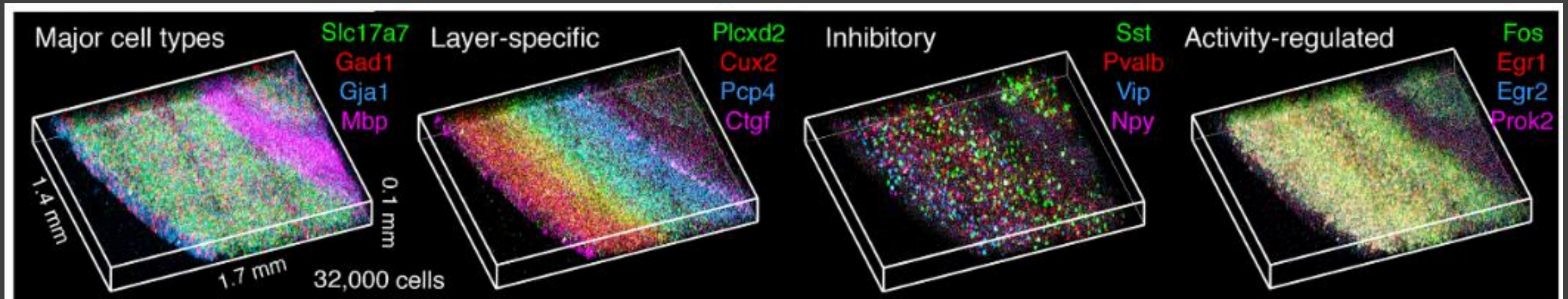
STAR MAP



STAR MAP



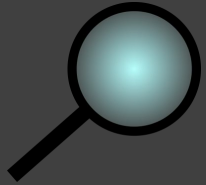
STAR MAP



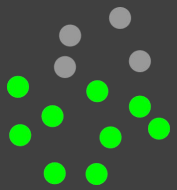
Wang *et al.* Science 2018



Sequencing *in situ*



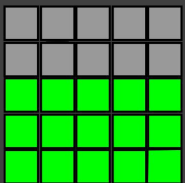
Resolution: Amplicon size (0.5 - 1um)



Detection efficiency: 0.01 - ~50%

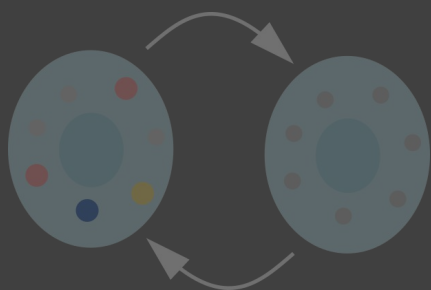


Gene throughput: 10 - 1,000

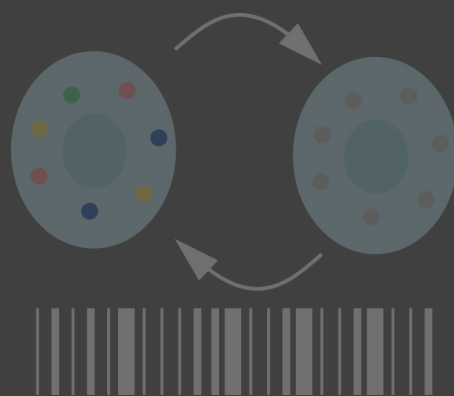


Spatial throughput: several mm² - several cm²

Cyclic FISH



Barcoded FISH



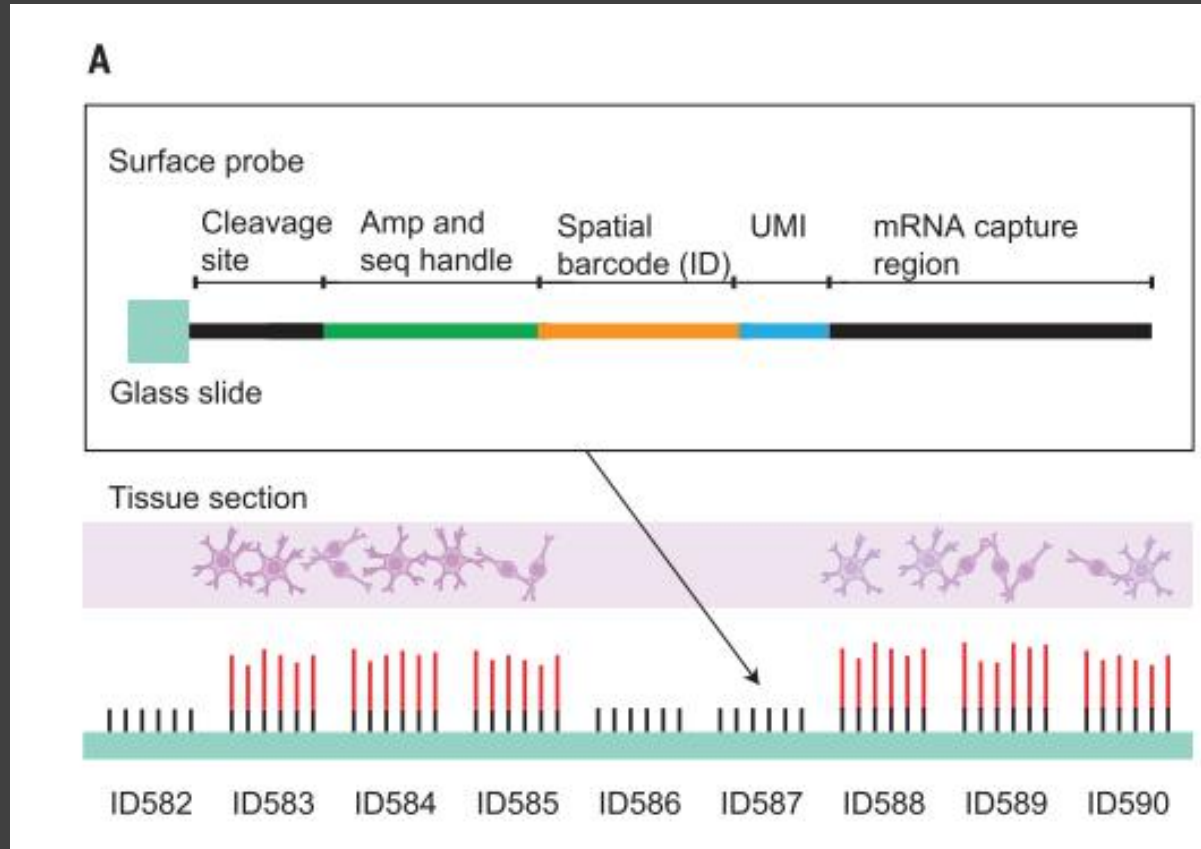
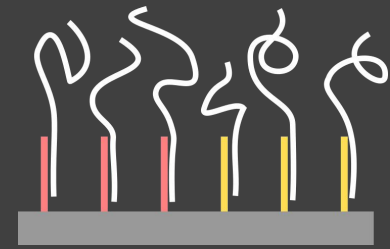
in situ Sequencing



Spatial Sequencing

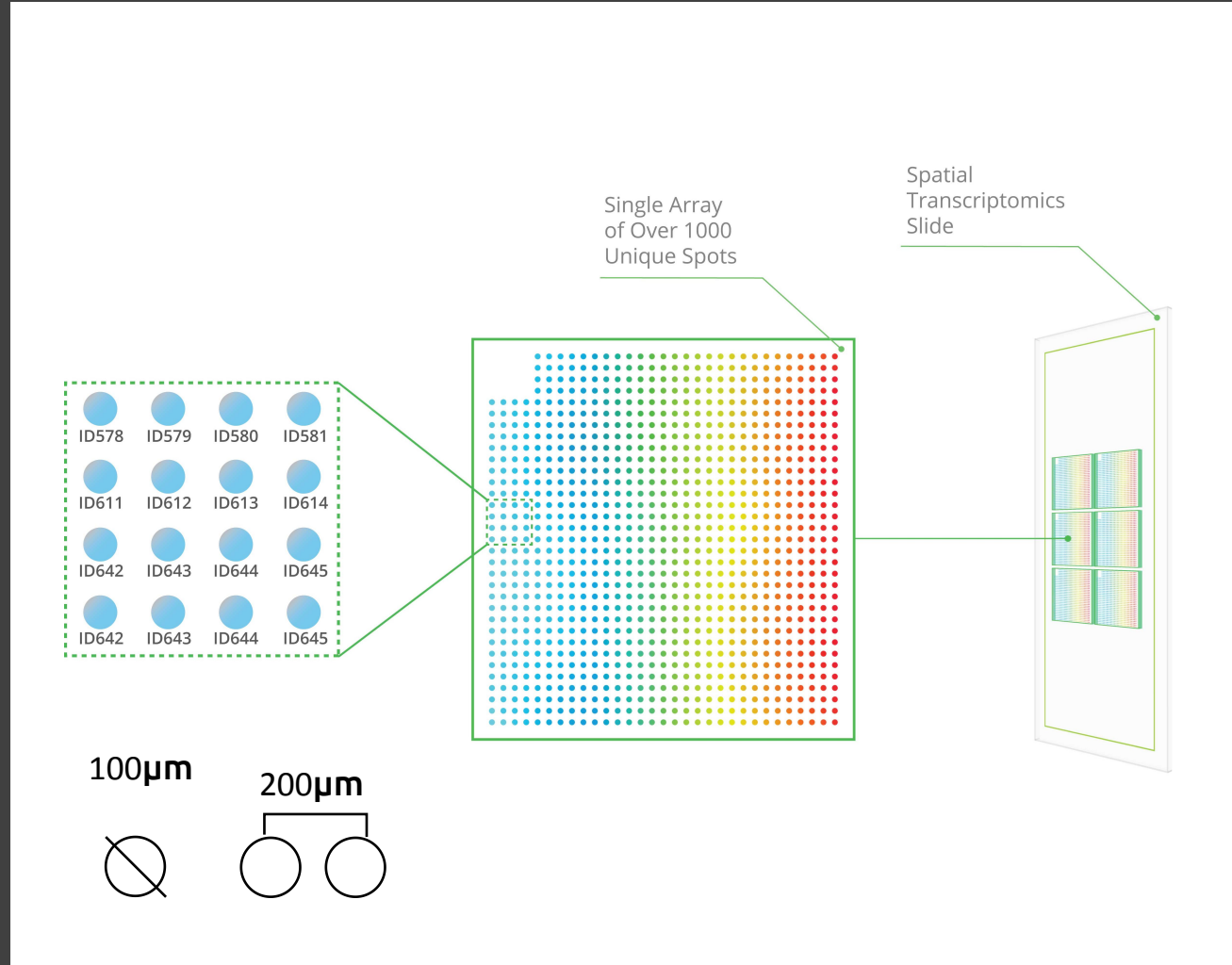
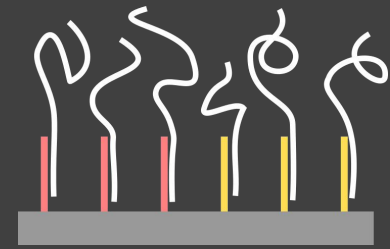


Spatial transcriptomics

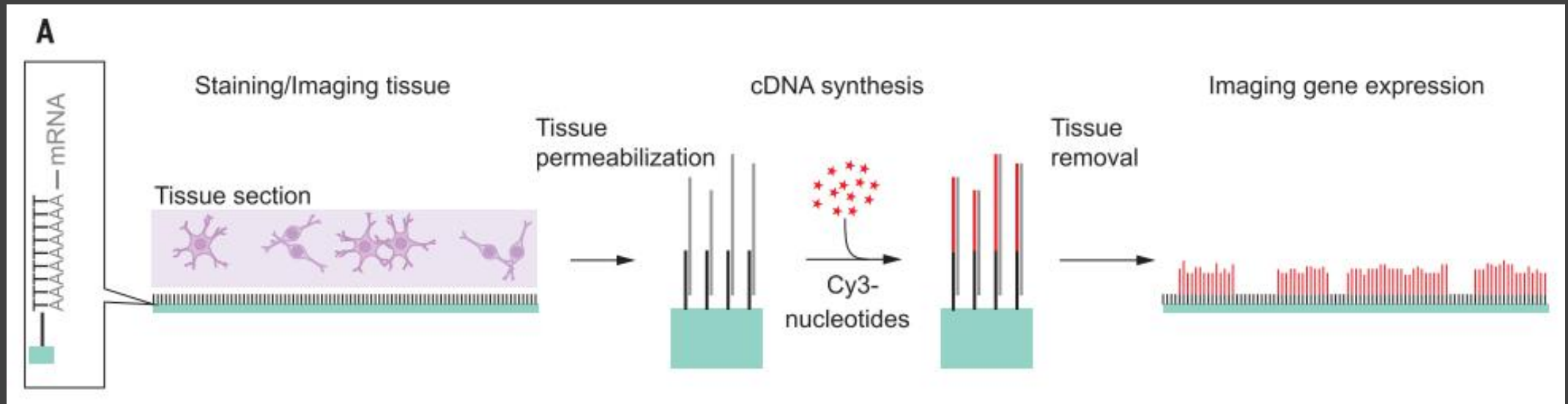
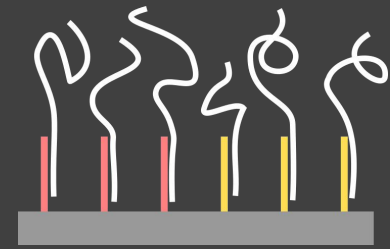


Stahl *et al.* Science 2016

Spatial transcriptomics

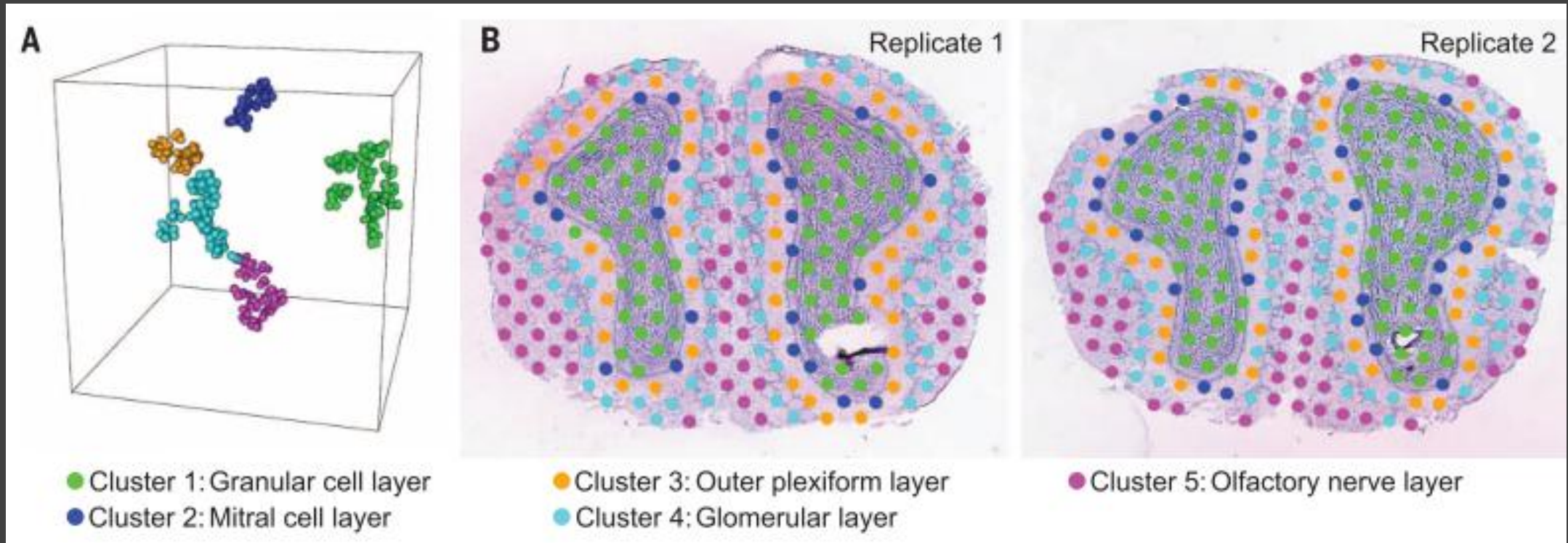
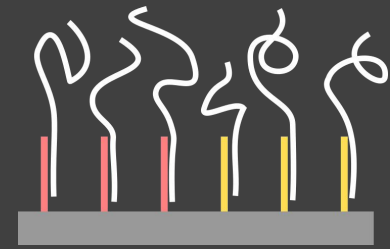


Spatial transcriptomics

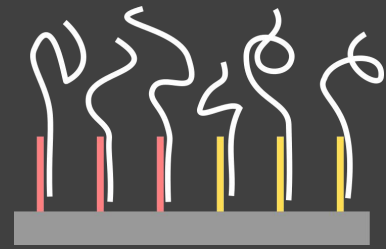


Stahl *et al.* Science 2016

Spatial transcriptomics



10X Visium



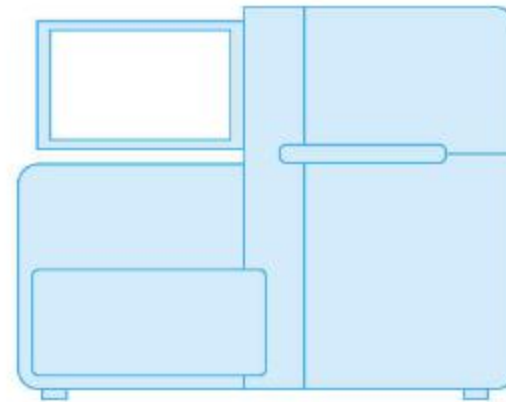
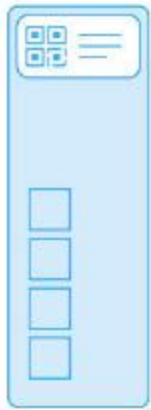
Sample Prep

Imaging

Barcoding &
Library Construction

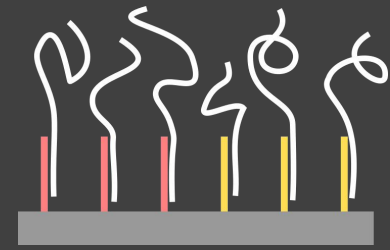
Sequencing

Data Visualization

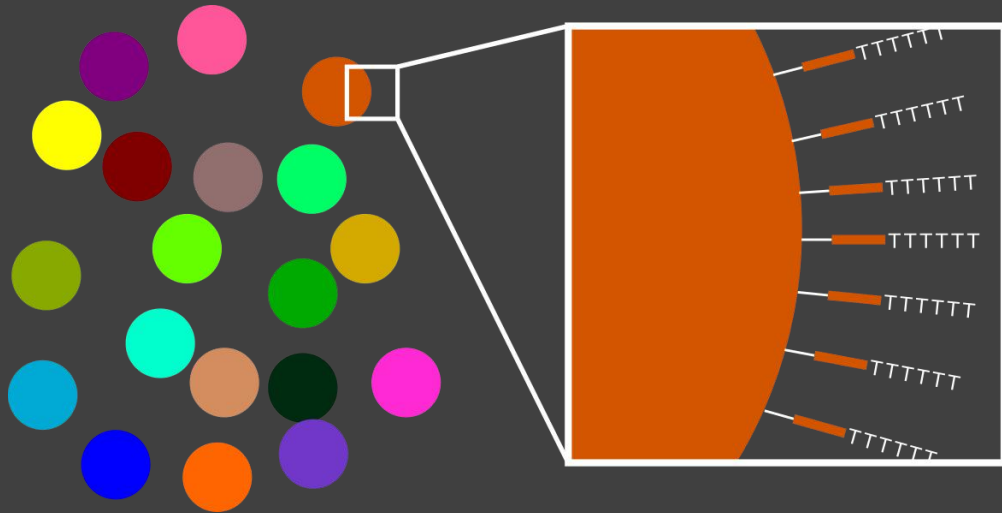


10X Visium

Array of barcoded beads



Random barcoded beads



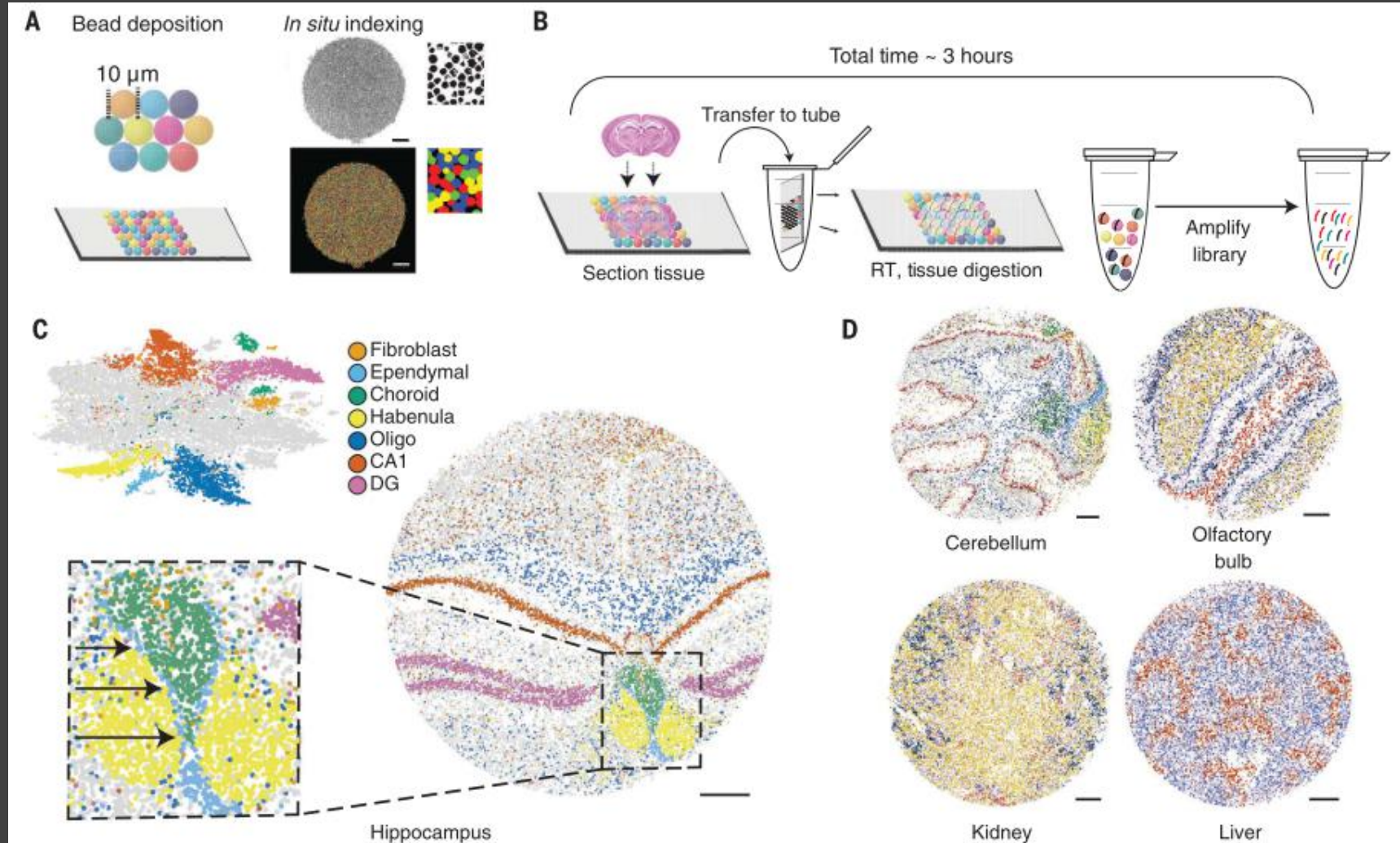
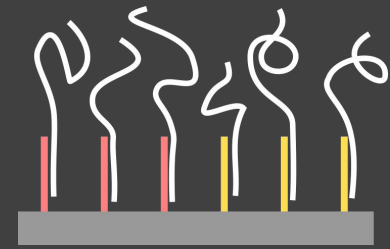
Array



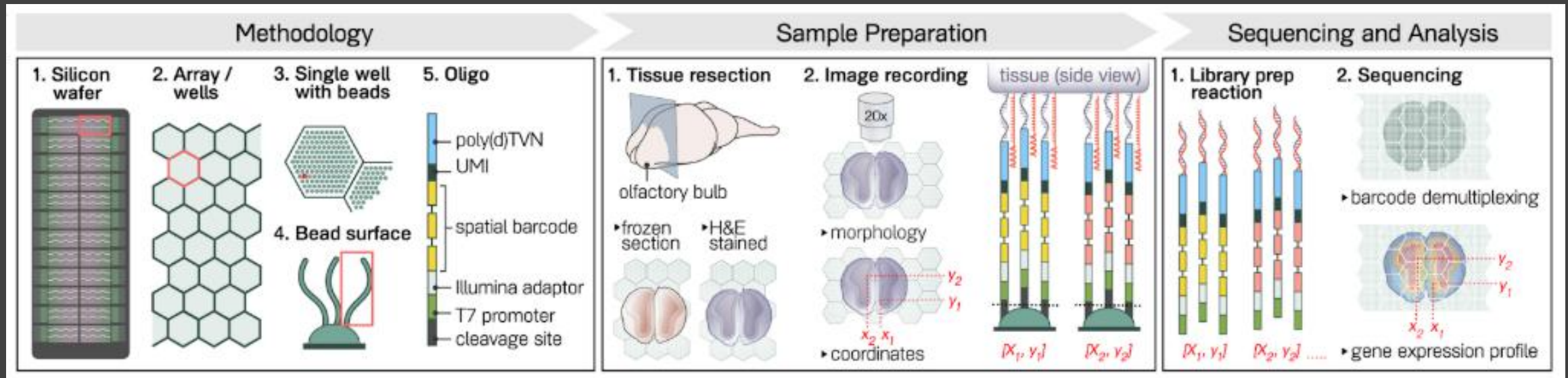
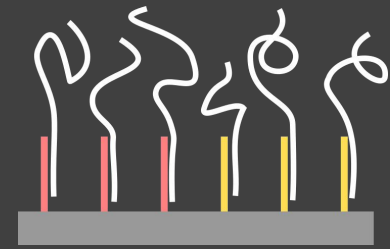
Decoded array

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

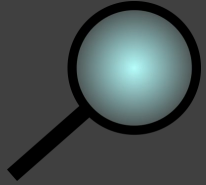
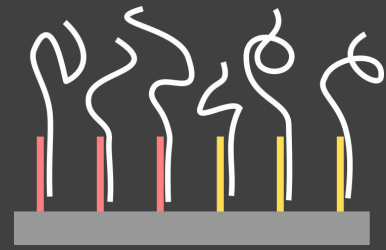
Slide-Seq



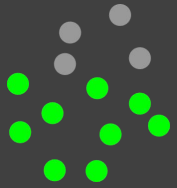
High density Spatial transcriptomics



Spatial Sequencing



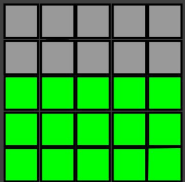
Resolution: Spot size 2 μ m - 100 μ m



Detection efficiency: 0.1 - 1%



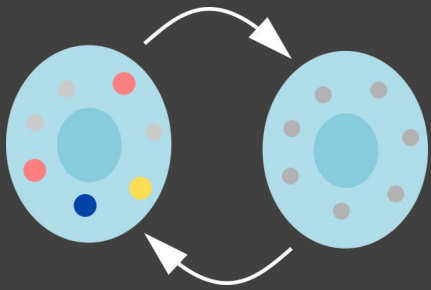
Gene throughput: Full transcriptome



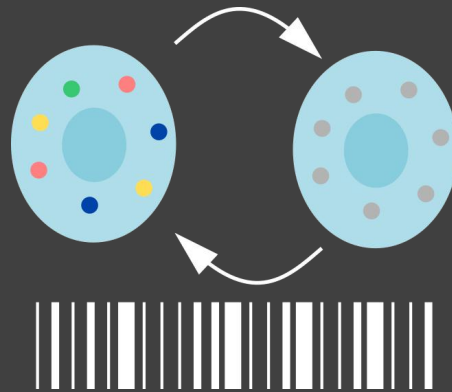
Spatial throughput: several cm²

RNA spatial detection

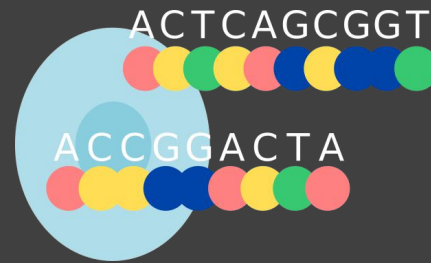
Cyclic FISH



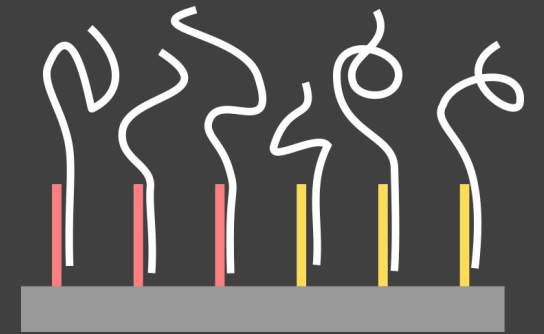
Barcoded FISH



in situ Sequencing



Spatial Sequencing



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



Spatial Transcriptome profiling

Questions?

