

# Spatial Transcriptome profiling

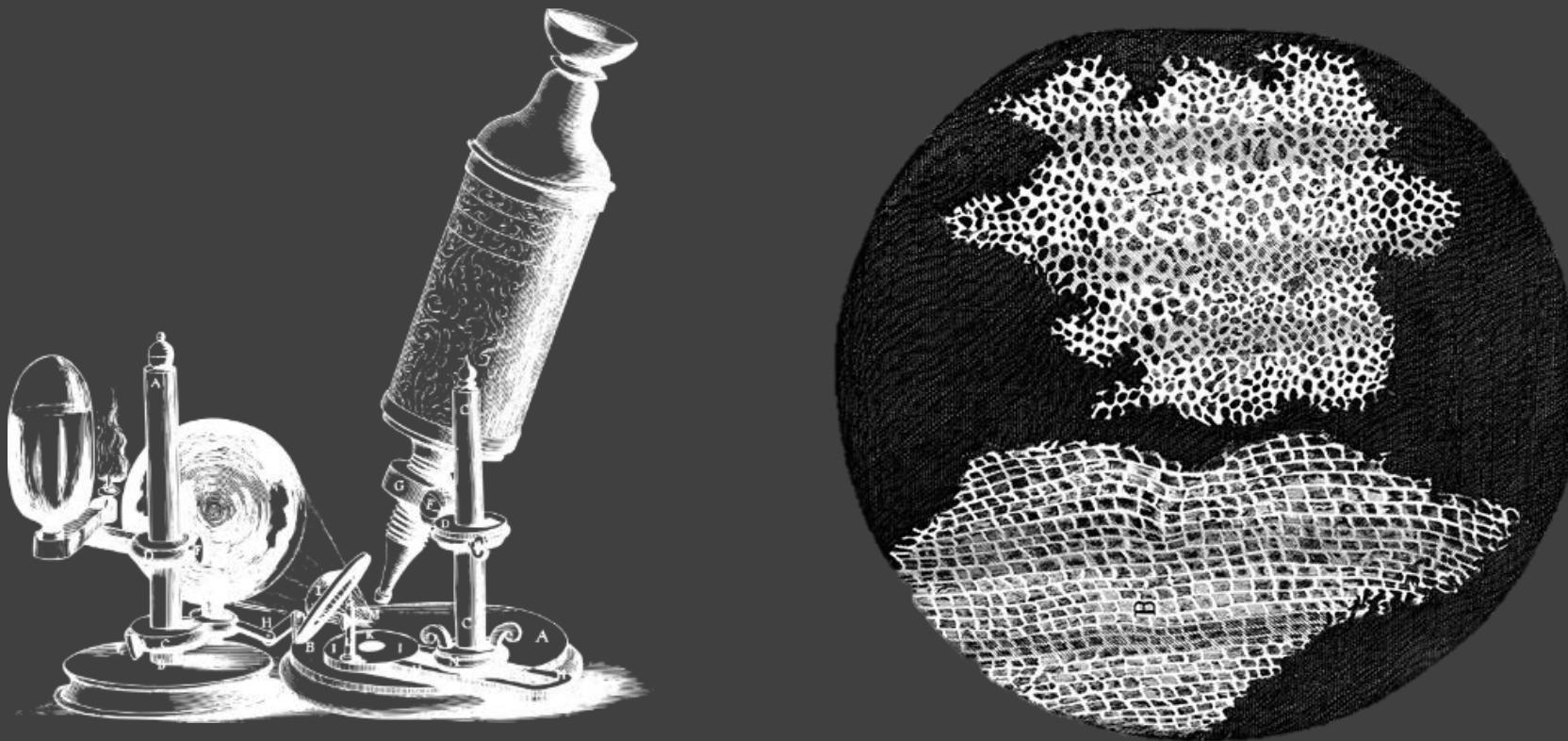
Lars Borm

Linnarsson Lab  
karolinska Institutet



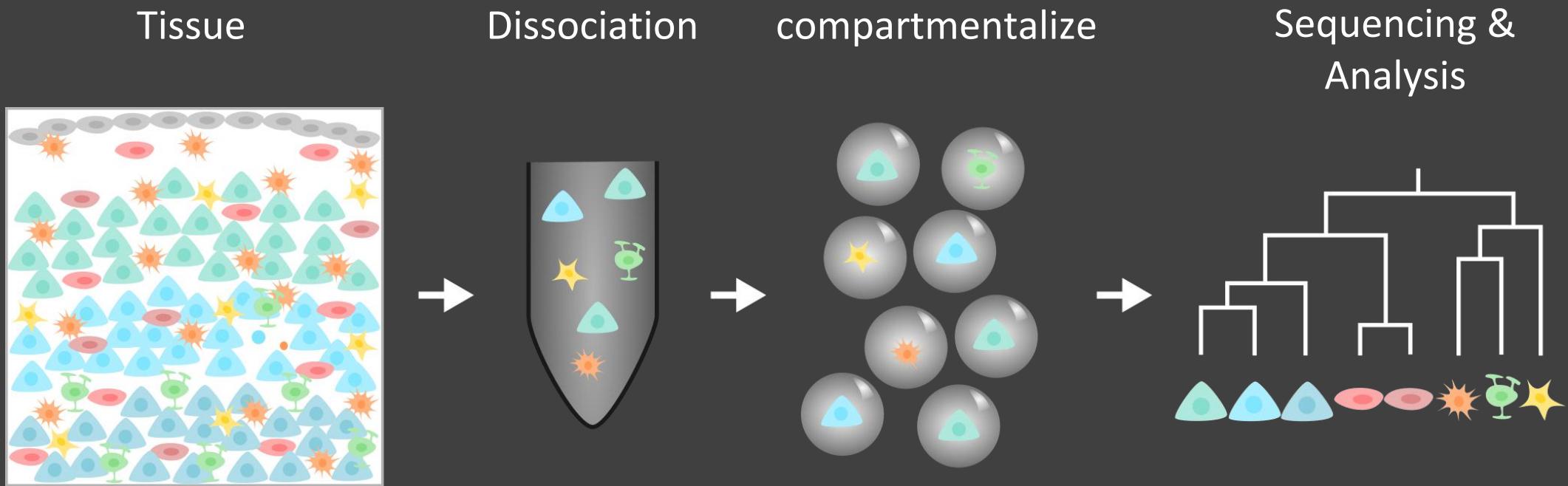
Karolinska  
Institutet

# Single cell biology



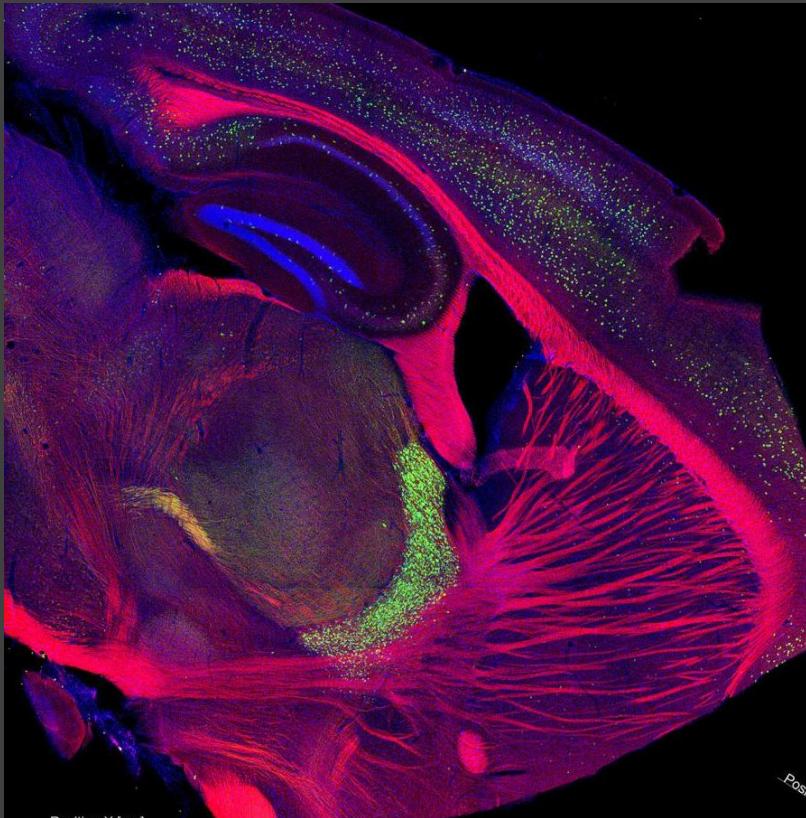
Robert Hooke 1665

# Single Cell RNA sequencing



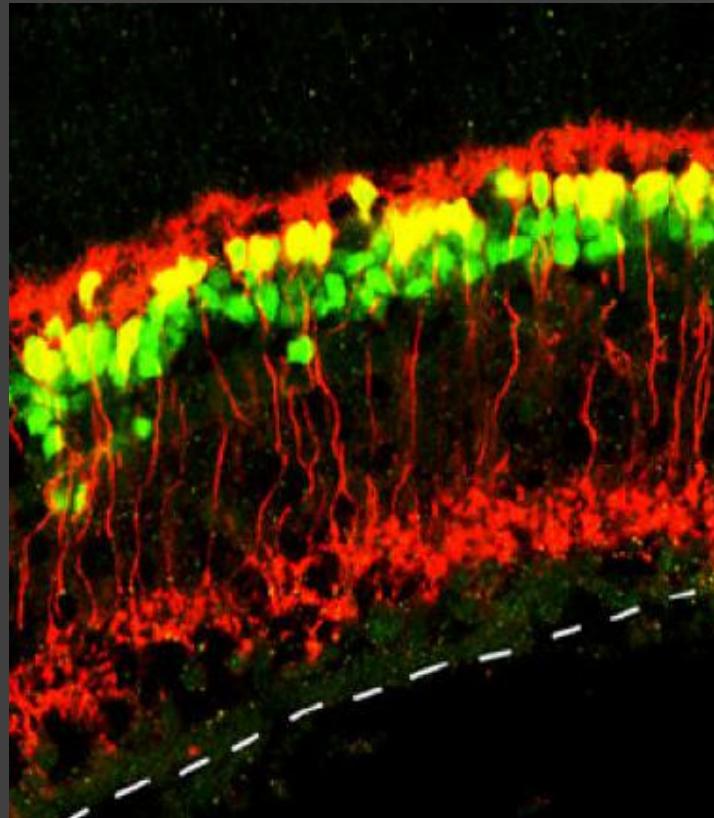
# Tissue architecture

Brain



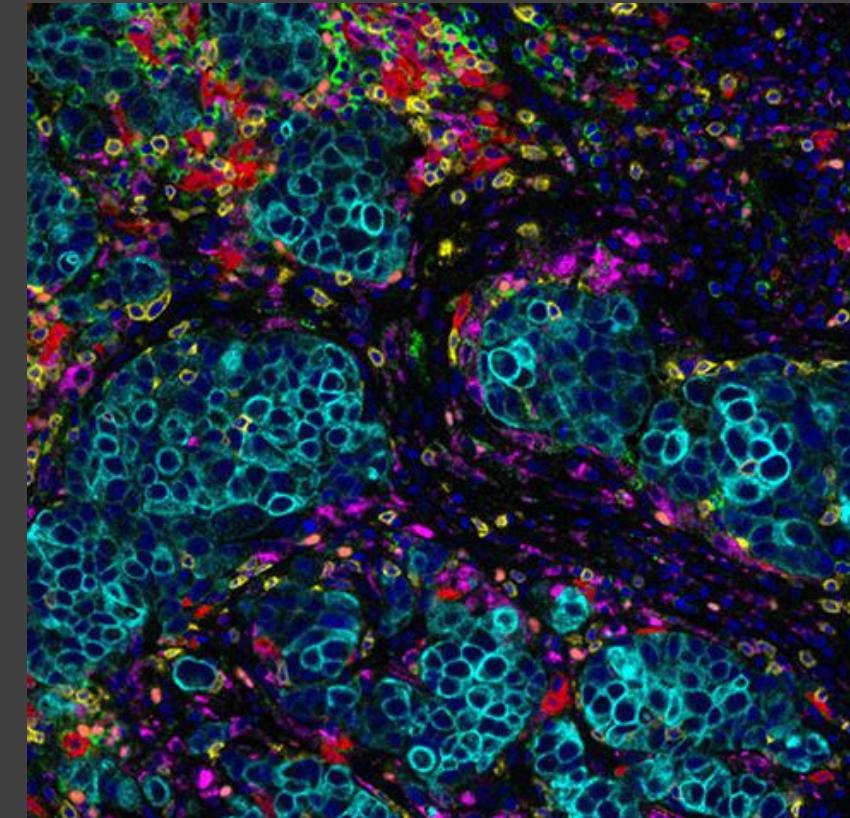
IGB Illinois

Retina



Morrow *et al.* 2008

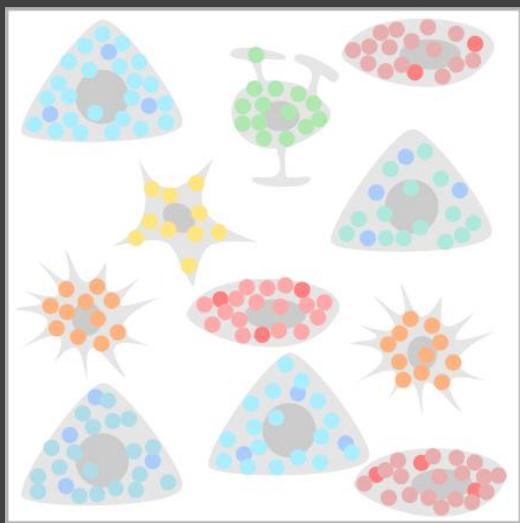
Cancer



Perkin Elmer

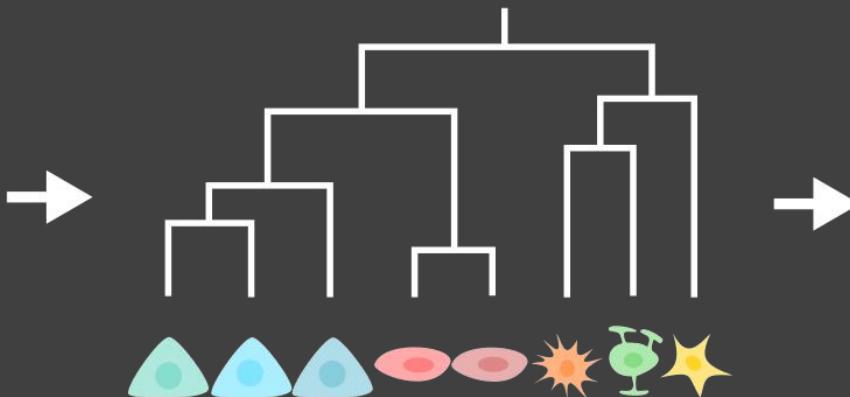
# Detecting RNA in tissue

RNA detection *in situ*

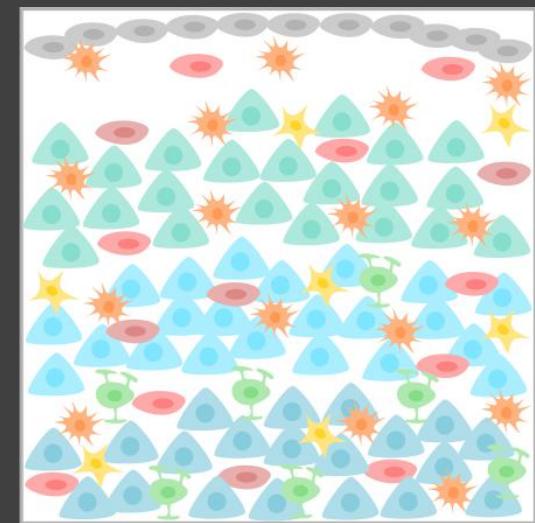


- Gene 1
- Gene 2
- Gene 3
- Gene 4
- Gene 5
- Gene 6
- Gene 7
- Gene 8
- Gene 9
- Gene 10

Analysis

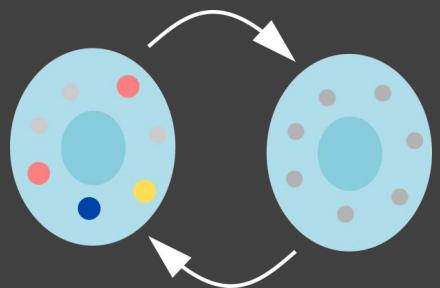


Spatial transcriptomics

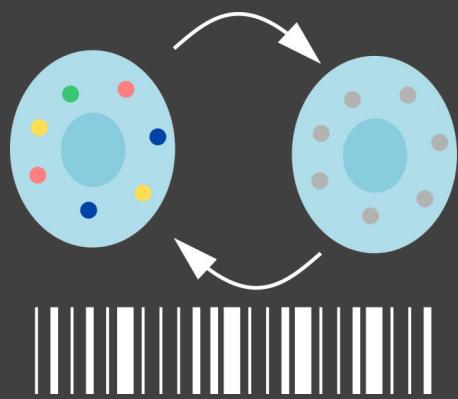


# RNA spatial detection

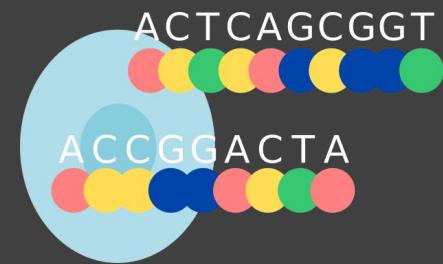
Cyclic FISH



Barcoded FISH



*in situ* Sequencing

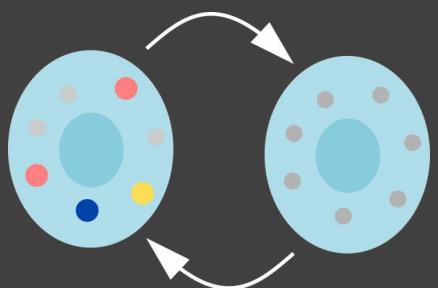


Spatial Sequencing

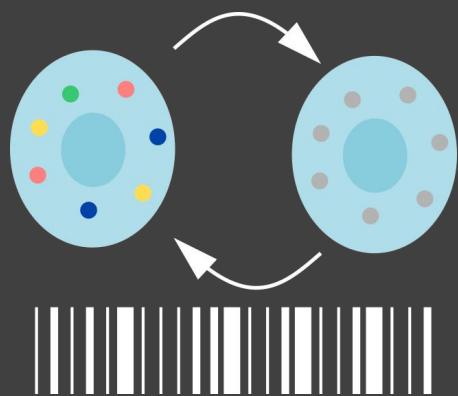


# Microscopy methods

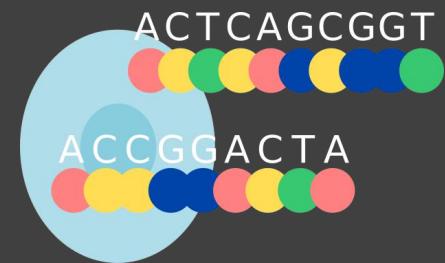
Cyclic FISH



Barcoded FISH



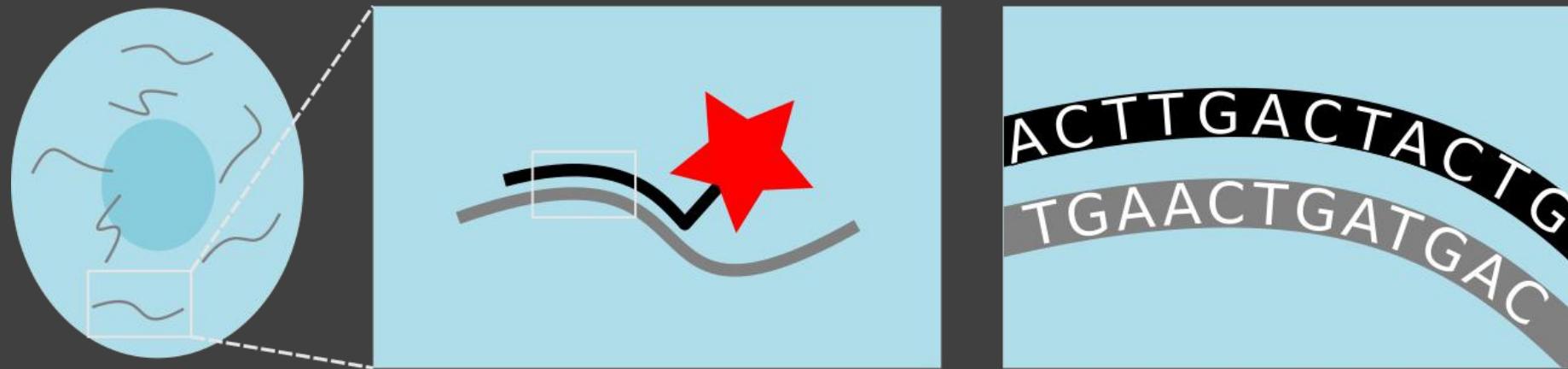
*in situ* Sequencing



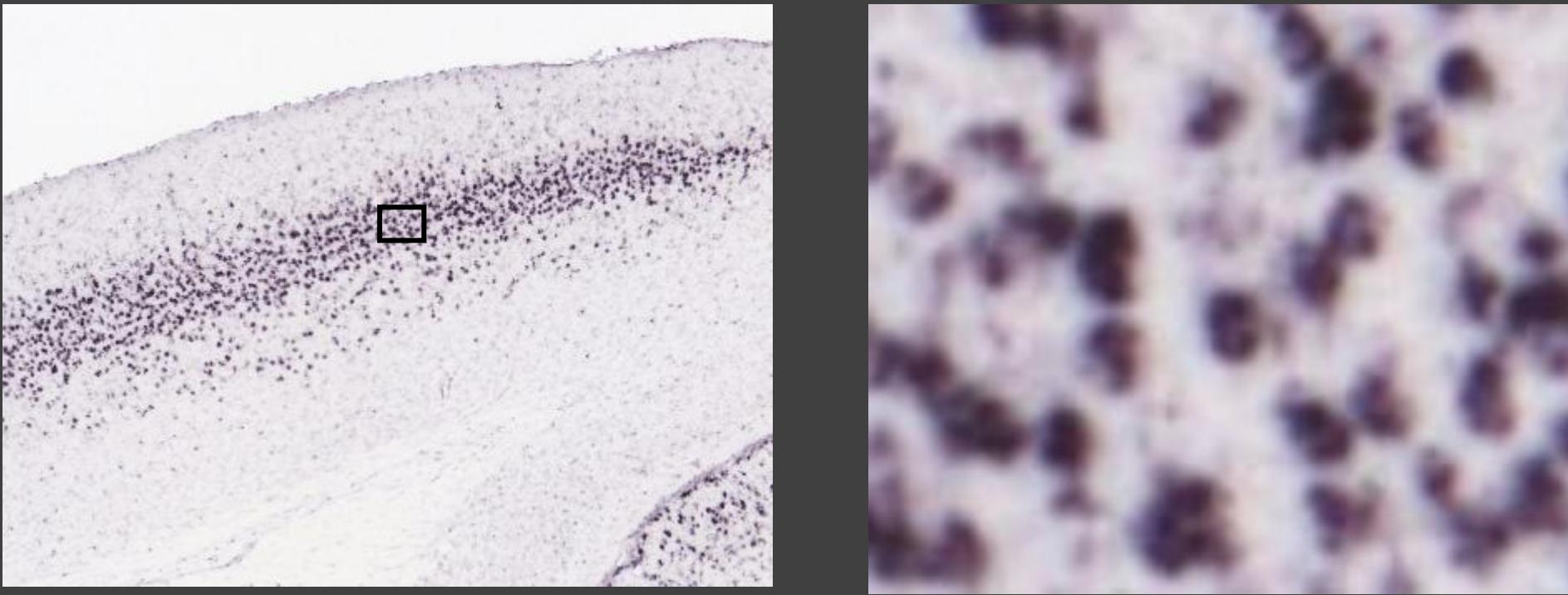
Spatial Sequencing



# *in situ* Hybridization

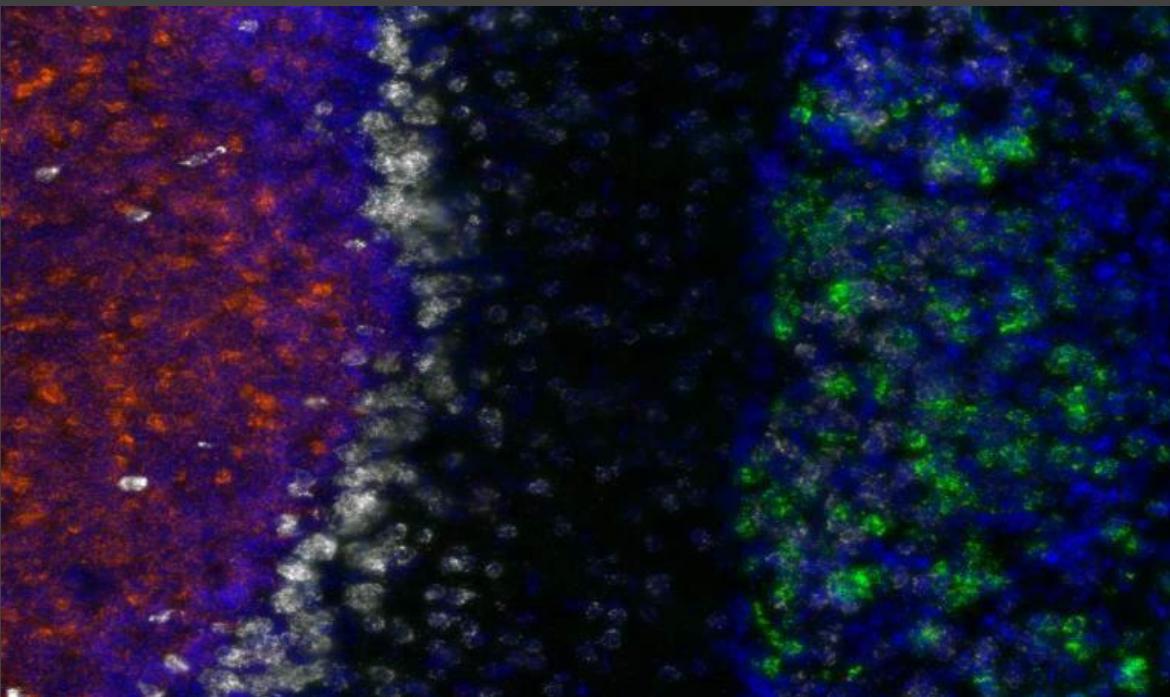


# *in situ* Hybridization (ISH)

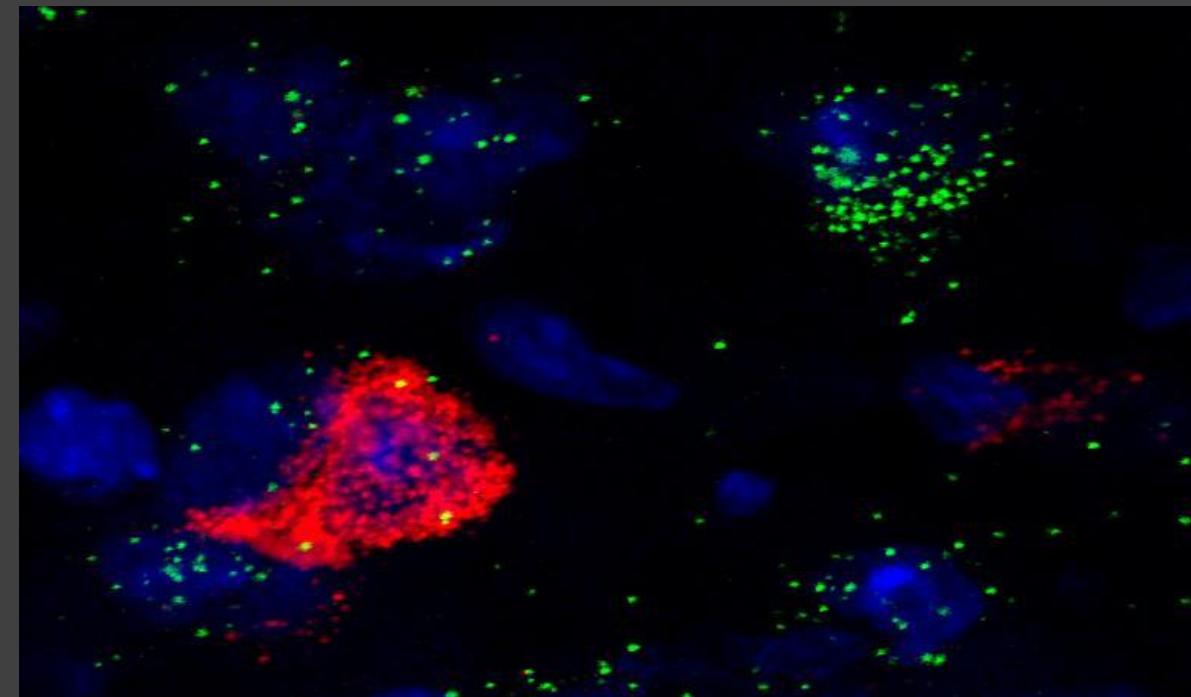


Lein *et al.* 2008

# *Fluorescent in situ Hybridization (FISH)*

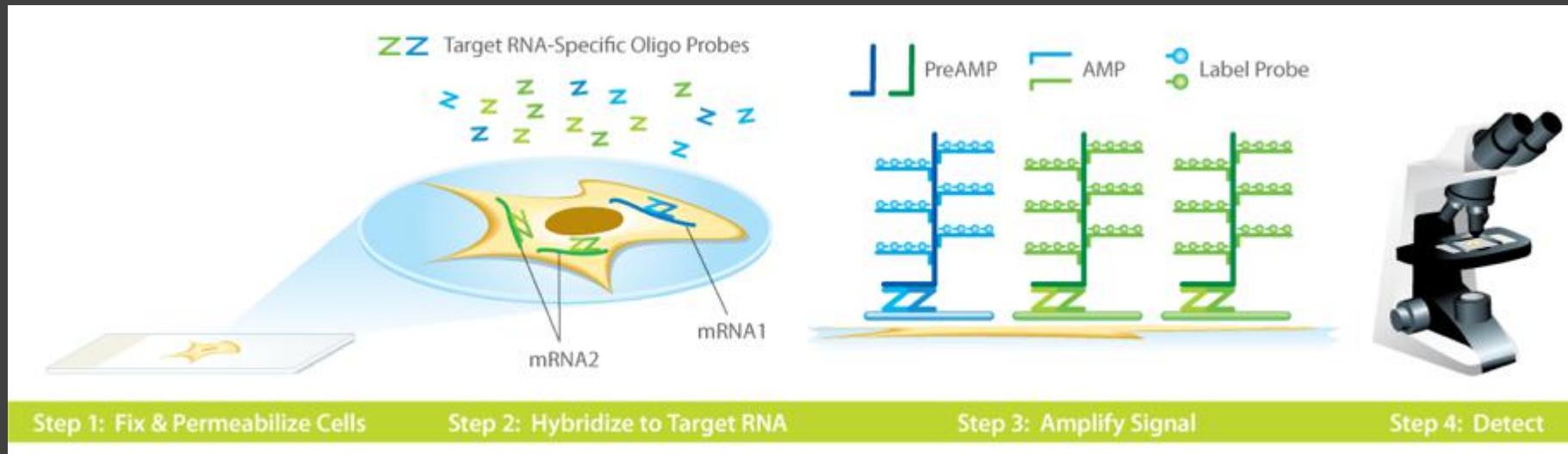


ACD Bio



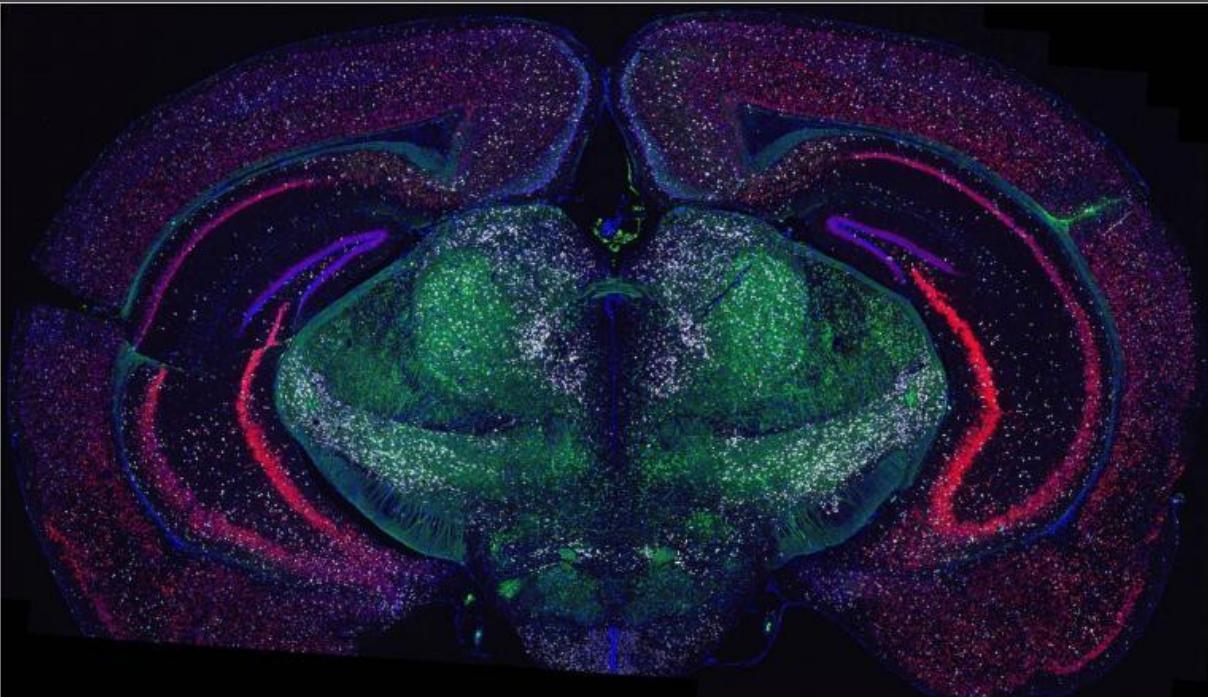
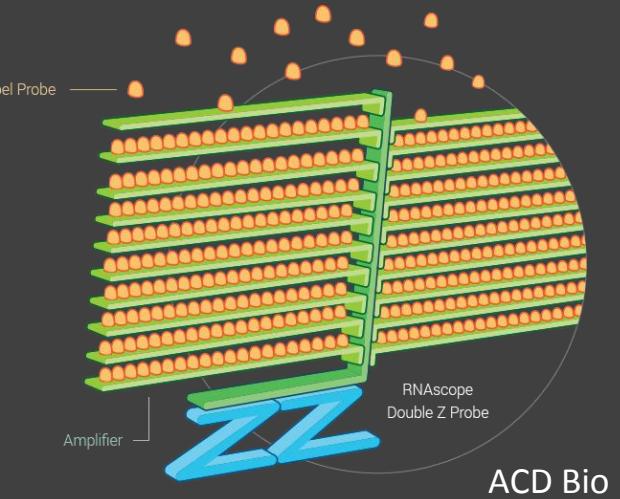
ACD Bio

# RNAscope

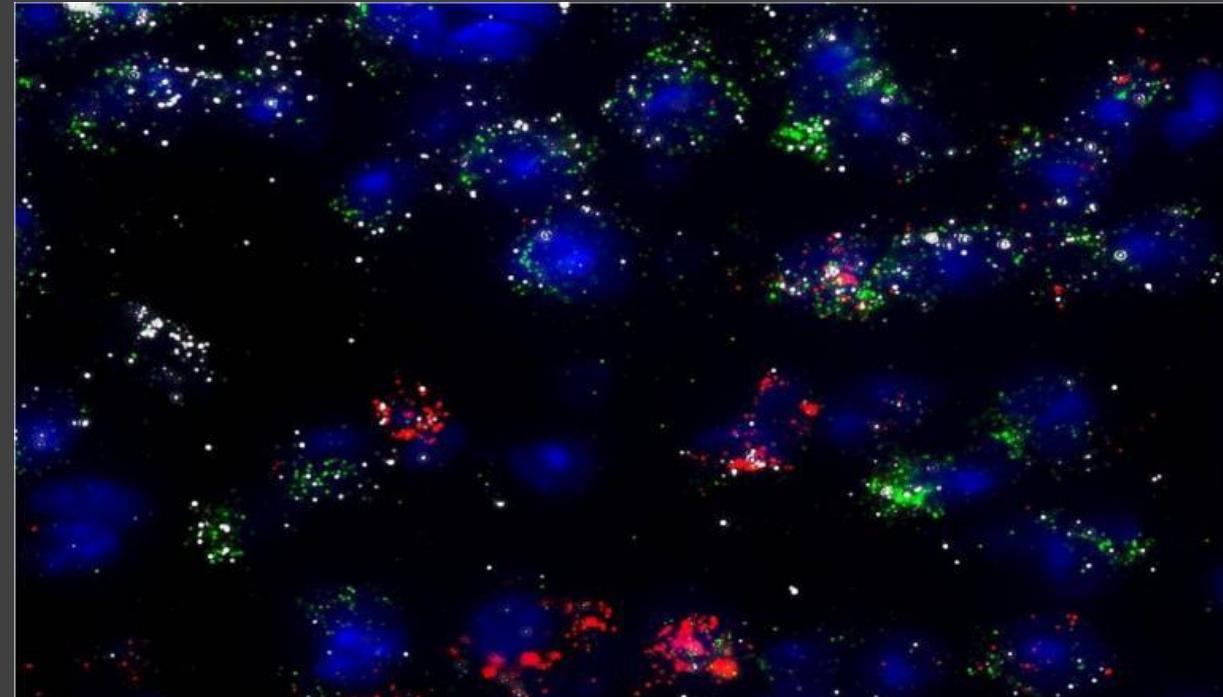


ACD Bio

# RNAscope



ACD Bio

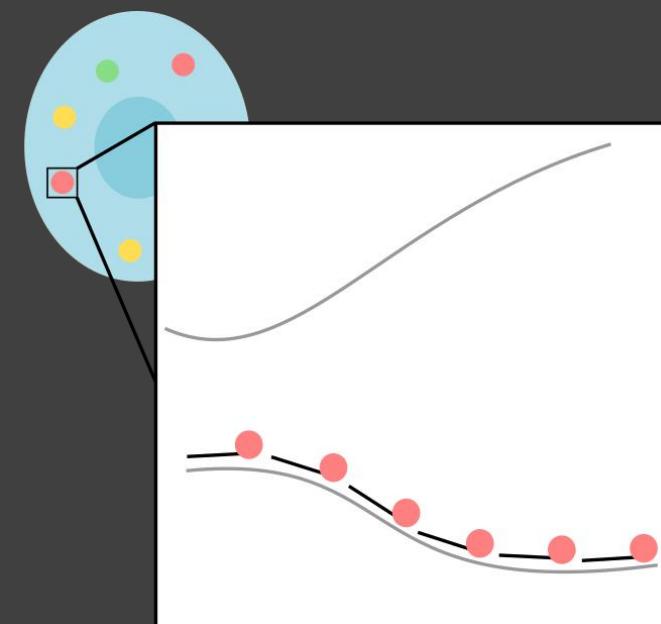


ACD Bio

# single molecule FISH (smFISH)

Probes

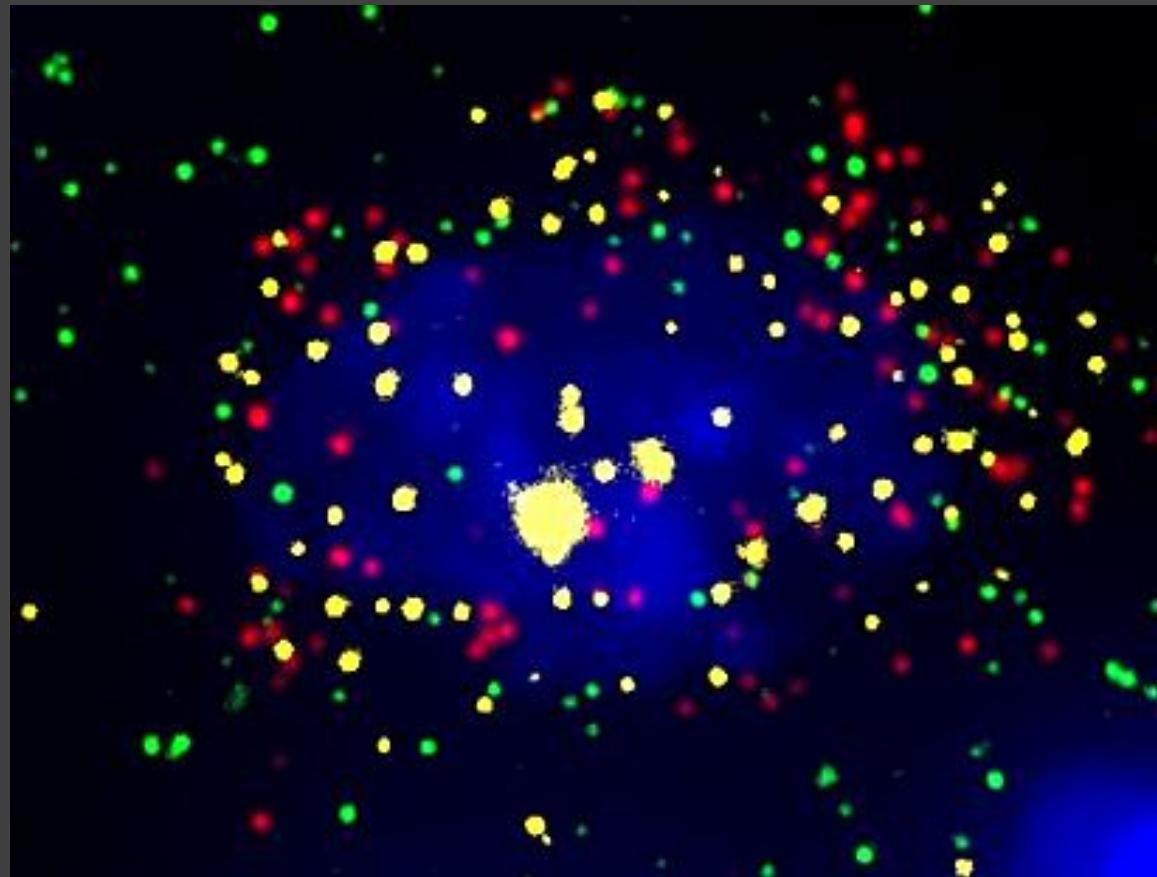
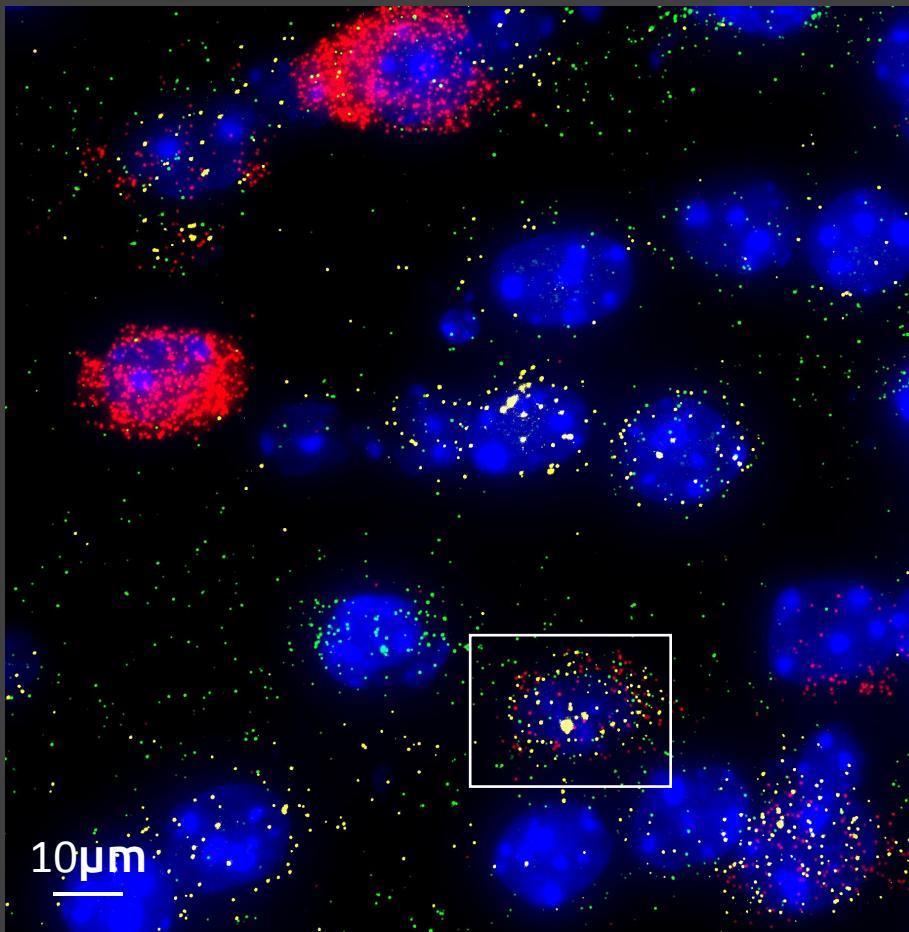
Gene 1 40X	
Gene 2 40X	
Gene 3 40X	
Gene 4 40X	



Raj *et al.* Nature Methods 2008

# single molecule FISH (smFISH)

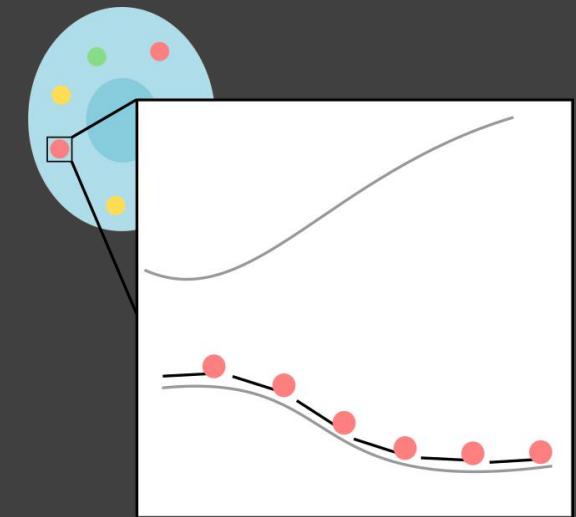
Gene 1 Gene 2 Gene 3 DNA



# single molecule FISH (smFISH)

- High sensitivity
- Low false positives
- Low false negatives

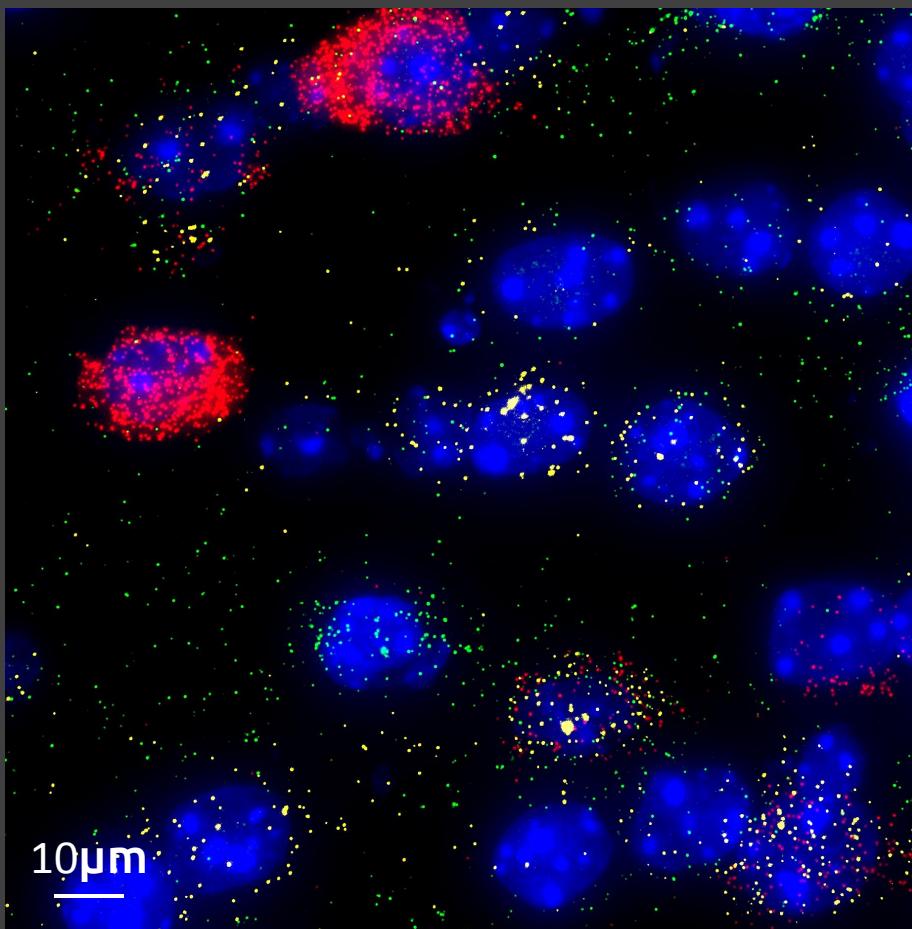
Gene 1 40X        
Gene 2 40X        
Gene 3 40X        
Gene 4 40X      



Raj *et al.* 2008

# single molecule FISH (smFISH)

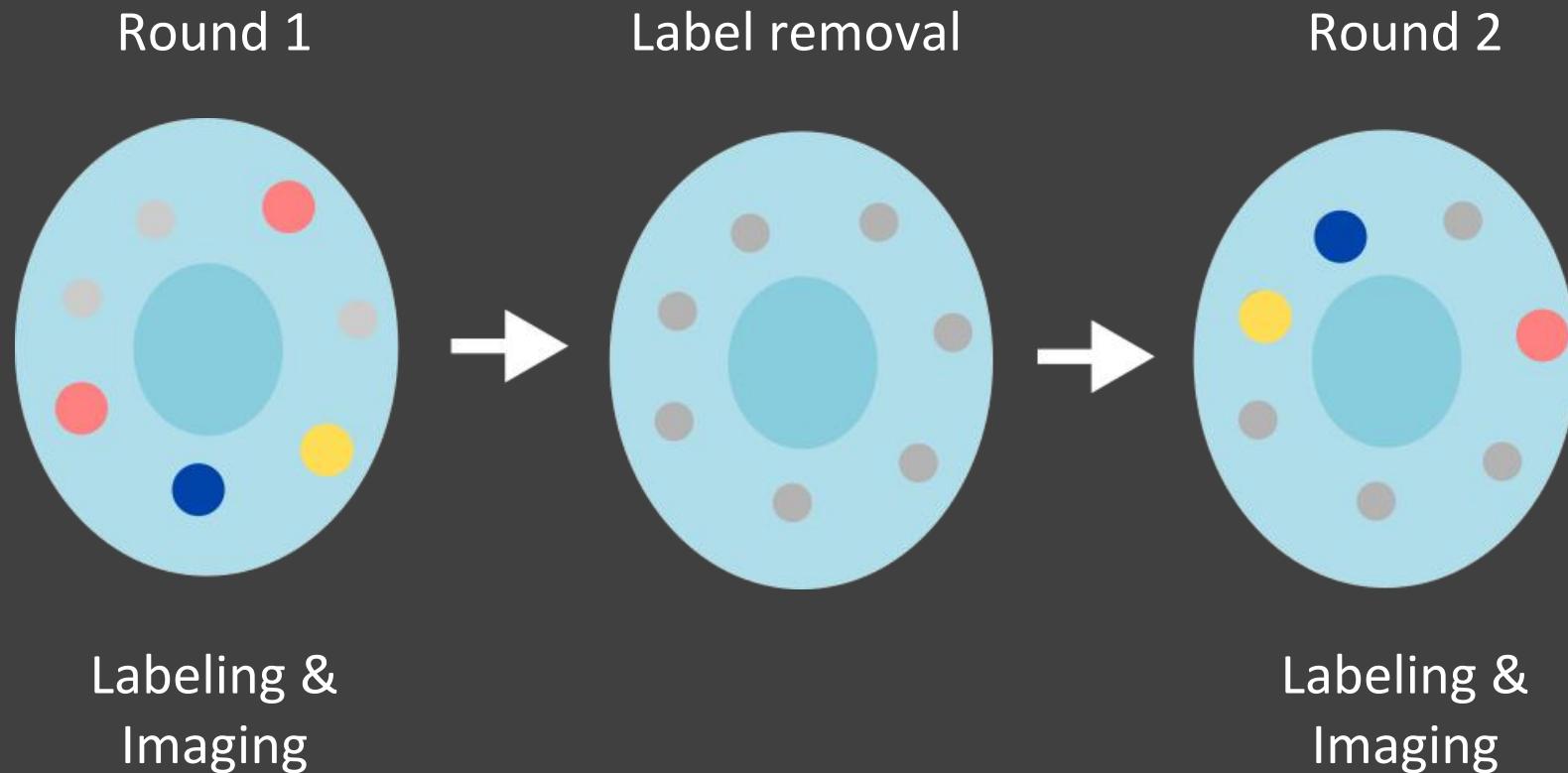
Gene 1 Gene 2 Gene 3 DNA



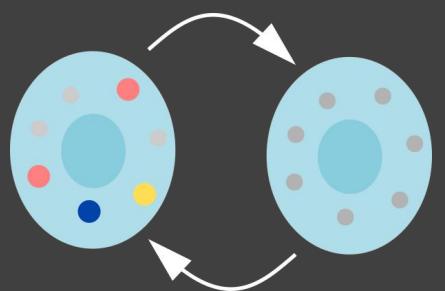
Limited fluorophores



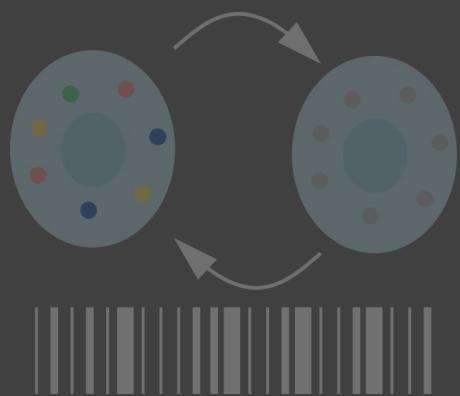
# Cyclic labeling



Cyclic FISH



Barcoded FISH



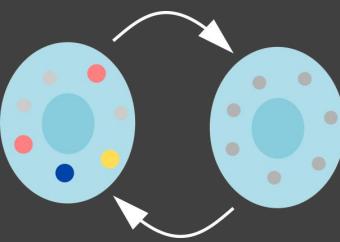
*in situ* Sequencing



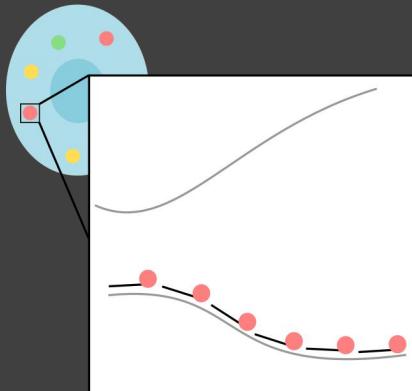
Spatial Sequencing



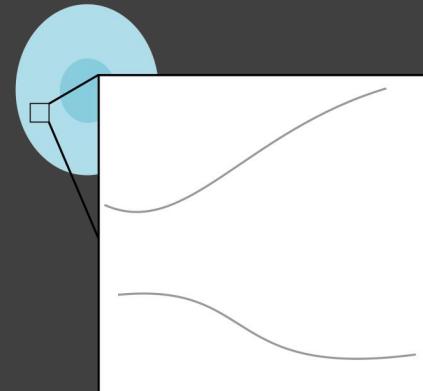
# osmFISH



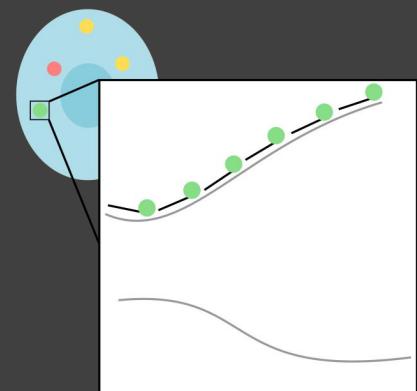
Hybridization 1



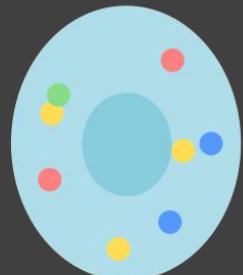
Strip 1



Hybridization 2

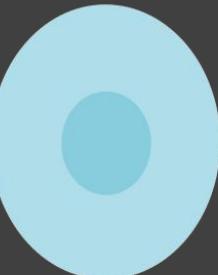


Hyb 1

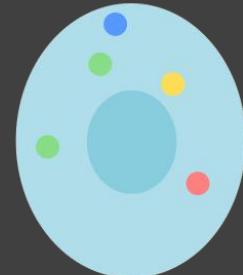


Gene 1 ●  
Gene 2 ●  
Gene 3 ●  
Gene 4 ●

Strip 1

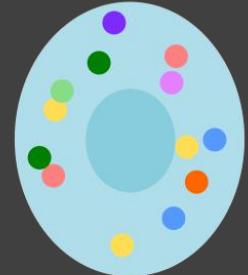


Hyb 2



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

Composite

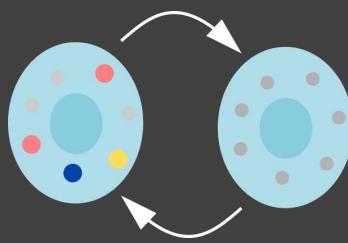
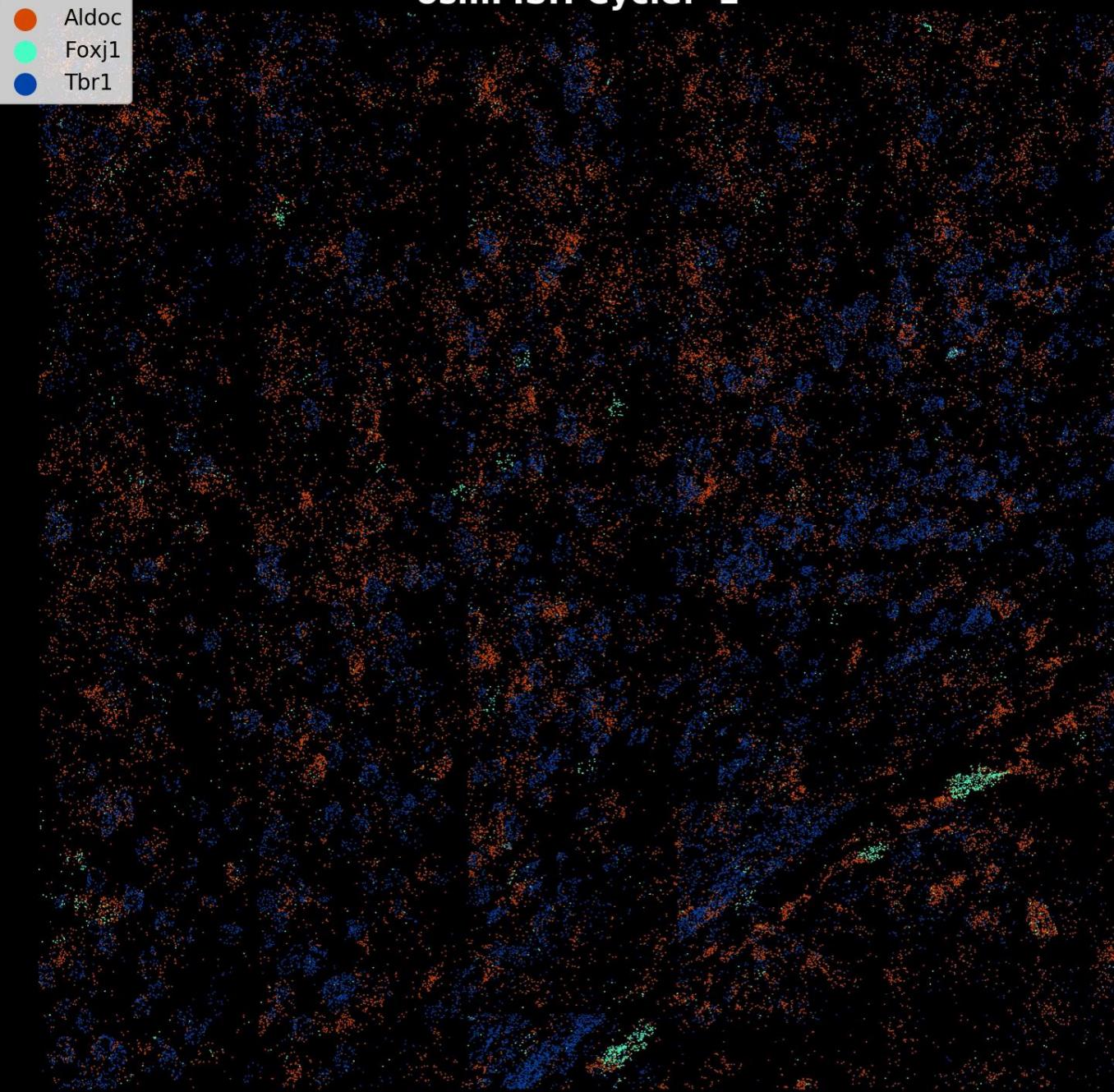


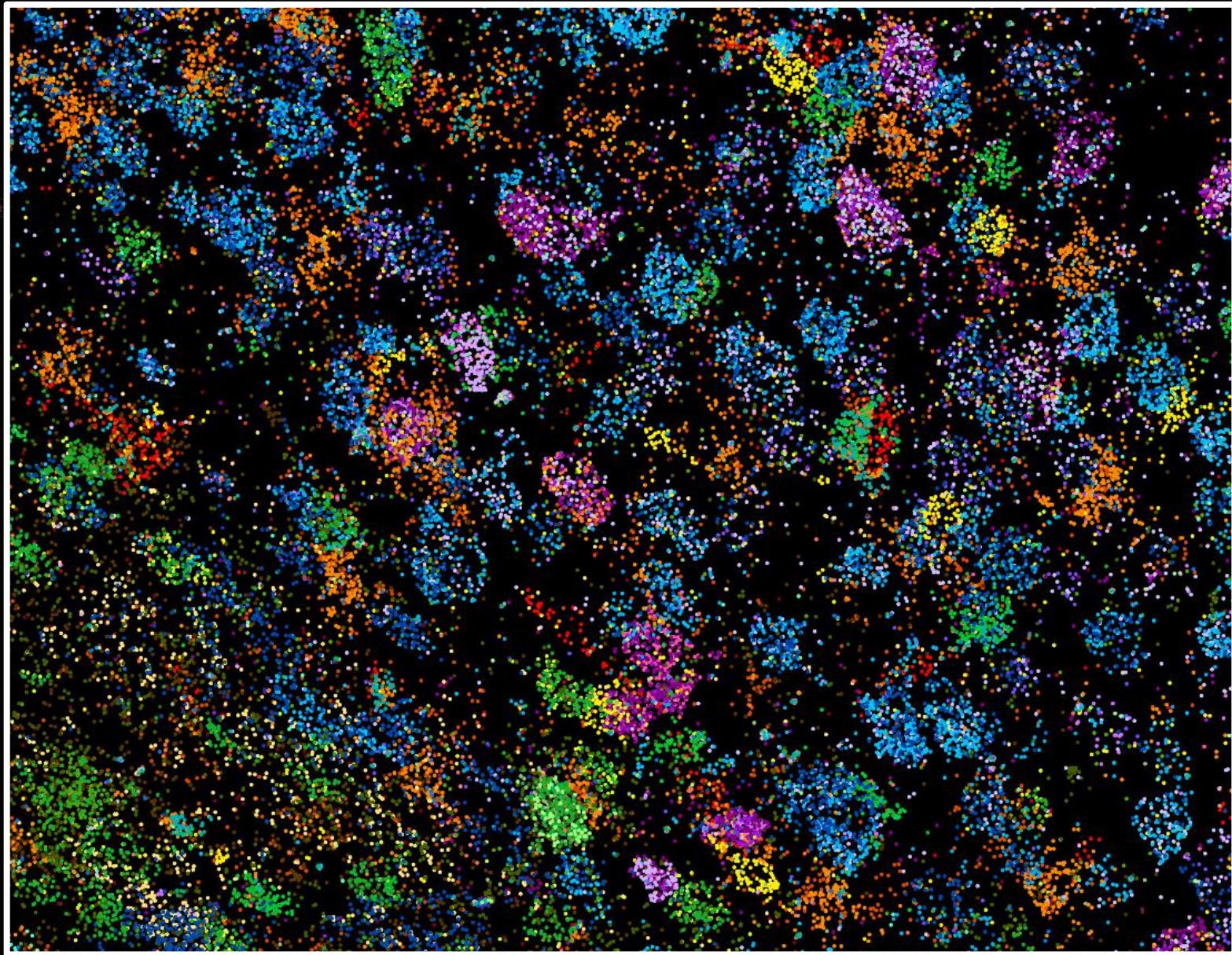
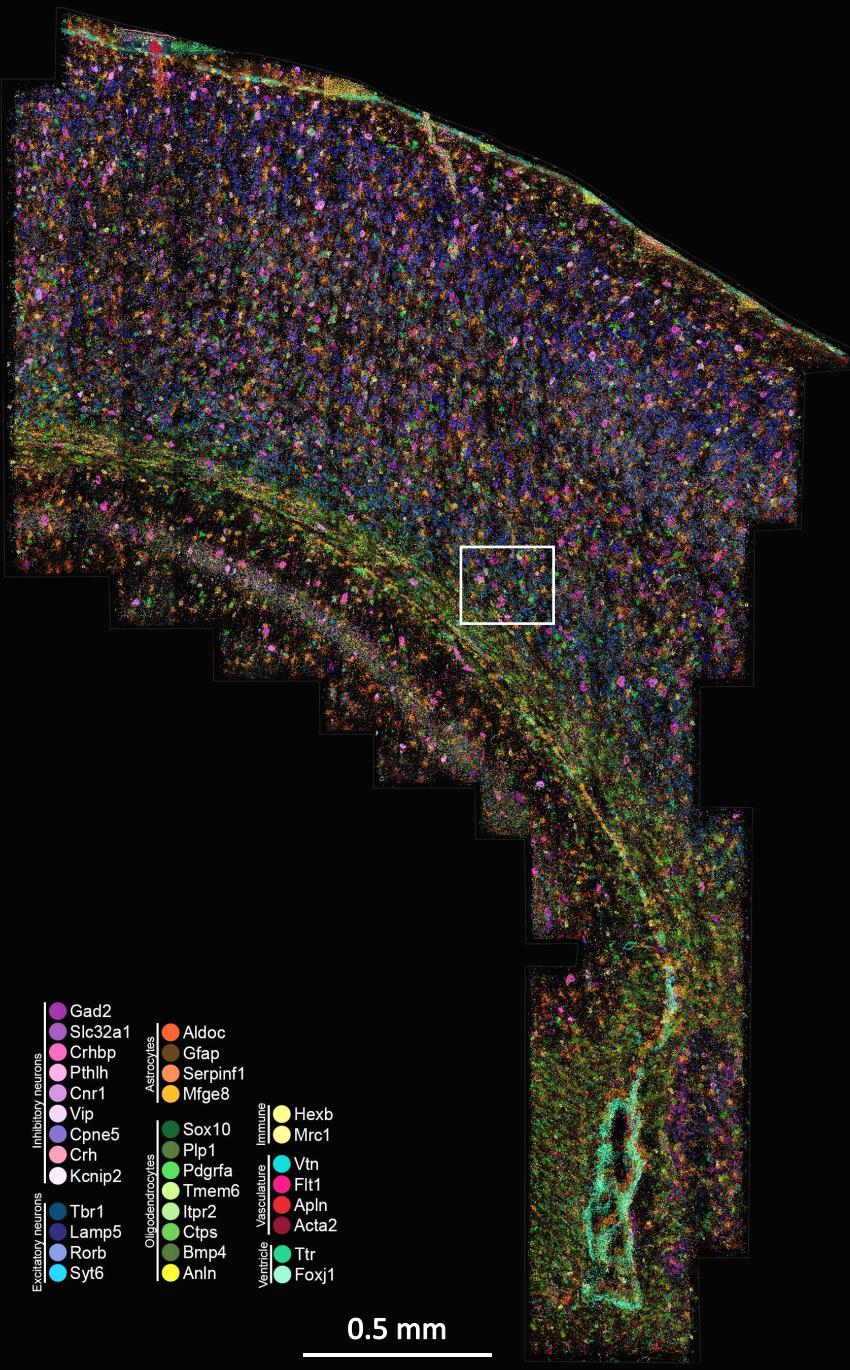
Gene 1 ●  
Gene 2 ●  
Gene 3 ●  
Gene 4 ●  
Gene 5 ●  
Gene 6 ●  
Gene 7 ●  
Gene 8 ●

Genes

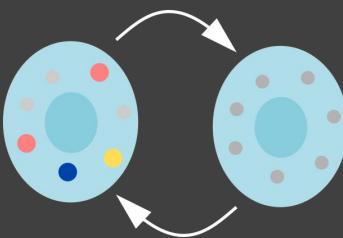
- Aldoc
- Foxj1
- Tbr1

osmFISH Cycle: 1

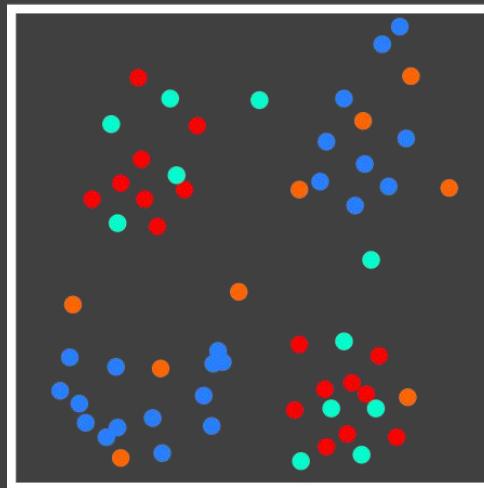




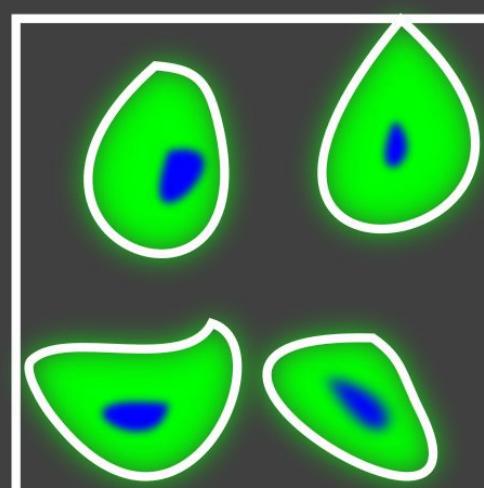
# Pipeline



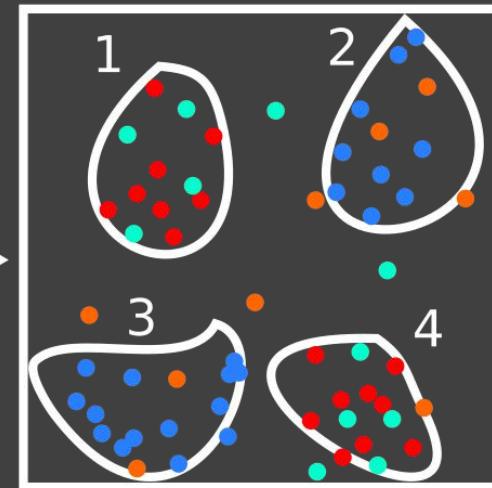
RNA detection



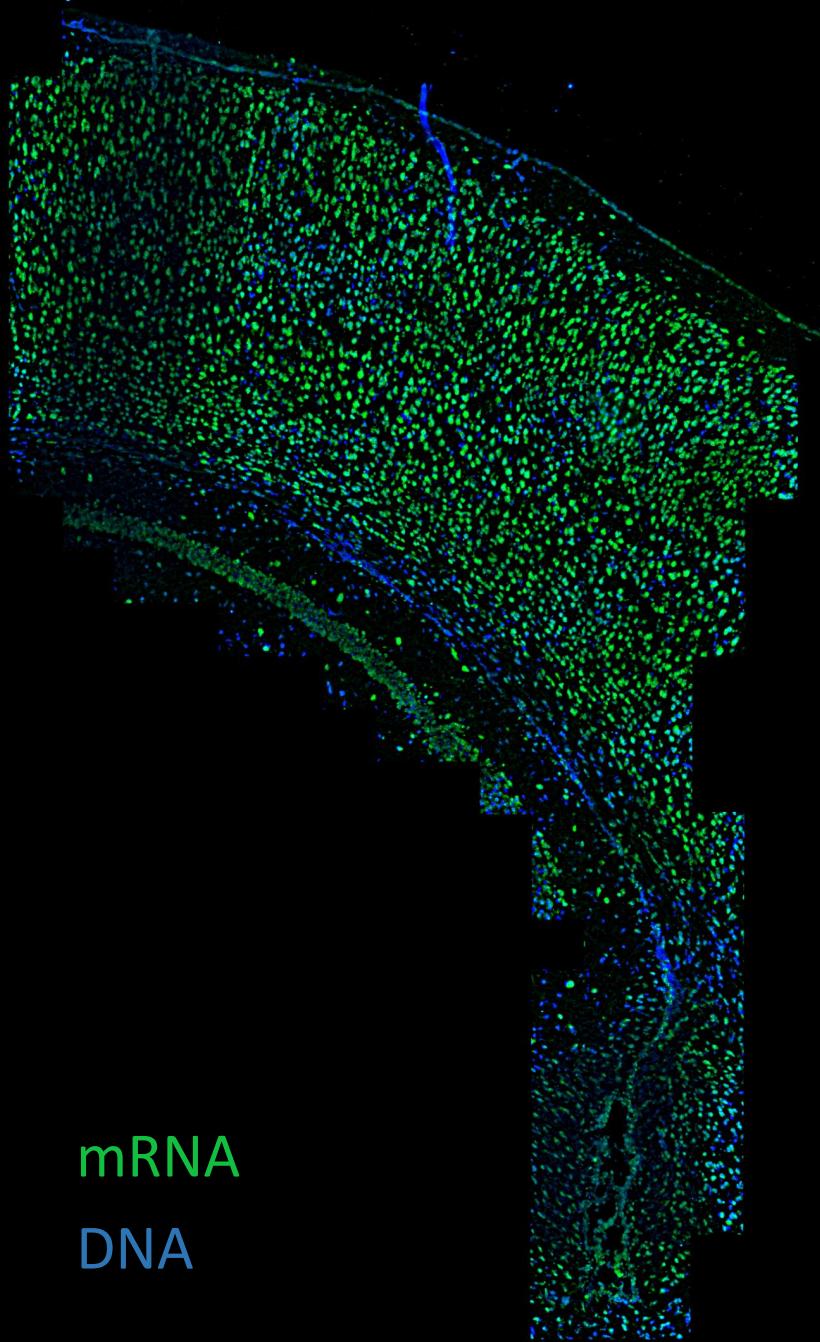
Cell segmentation



Cell counting



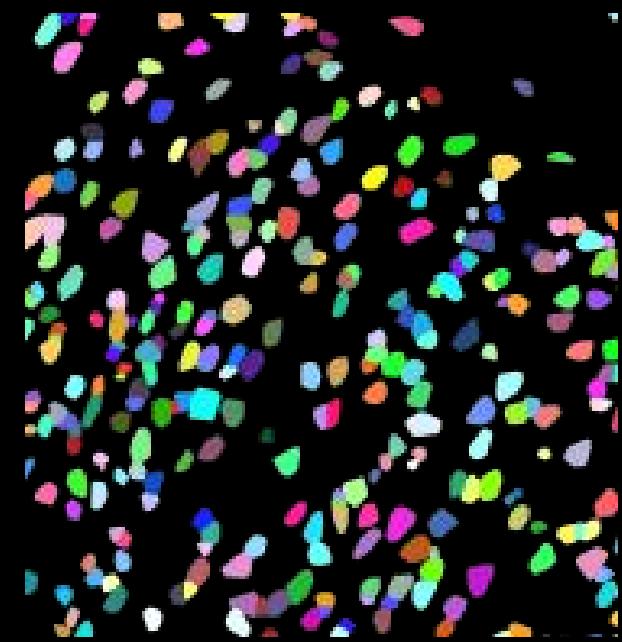
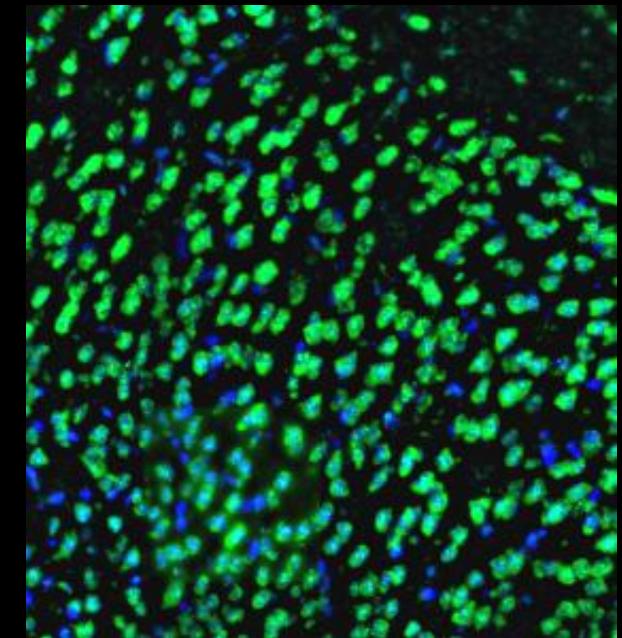
Cell 1	Cell 2
● 8	● 0
● 4	● 0
● 0	● 9
● 0	● 2
Cell 3	Cell 4
● 0	● 8
● 0	● 4
● 14	● 0
● 2	● 1



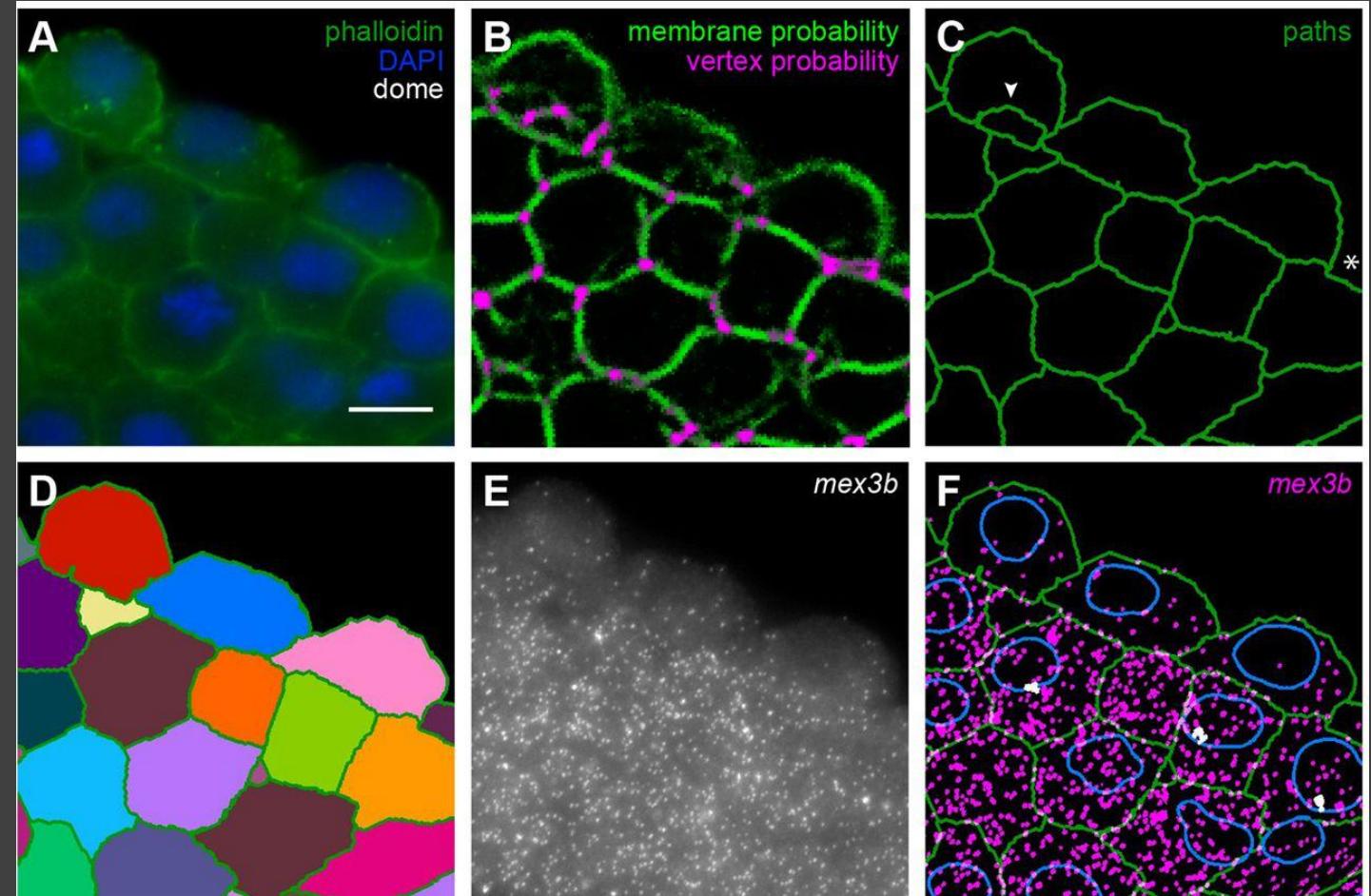
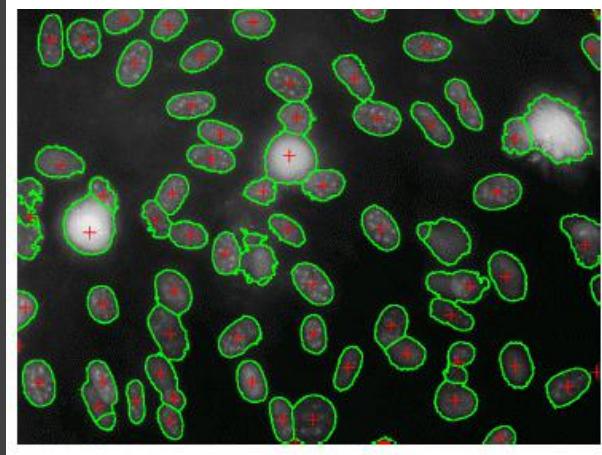
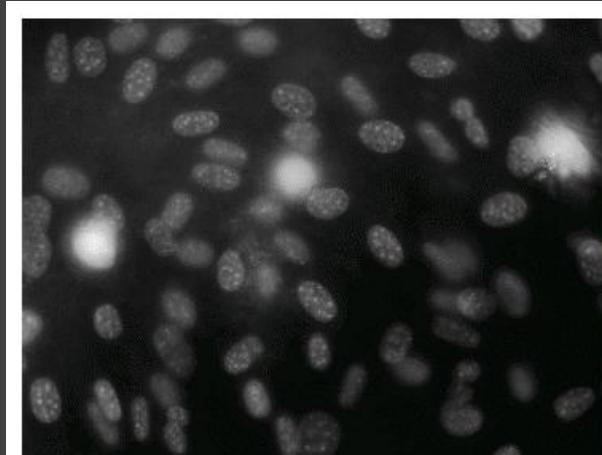
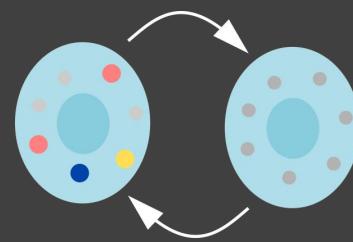
mRNA

DNA

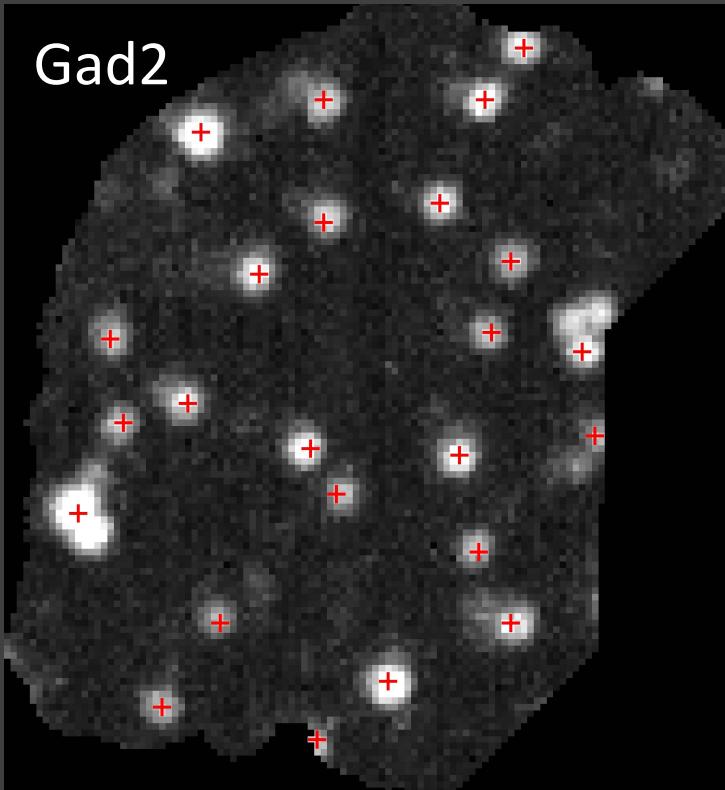
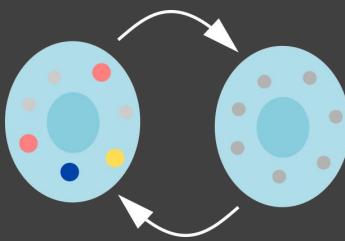
Segmented



# Cell segmentation



# Single cell expression



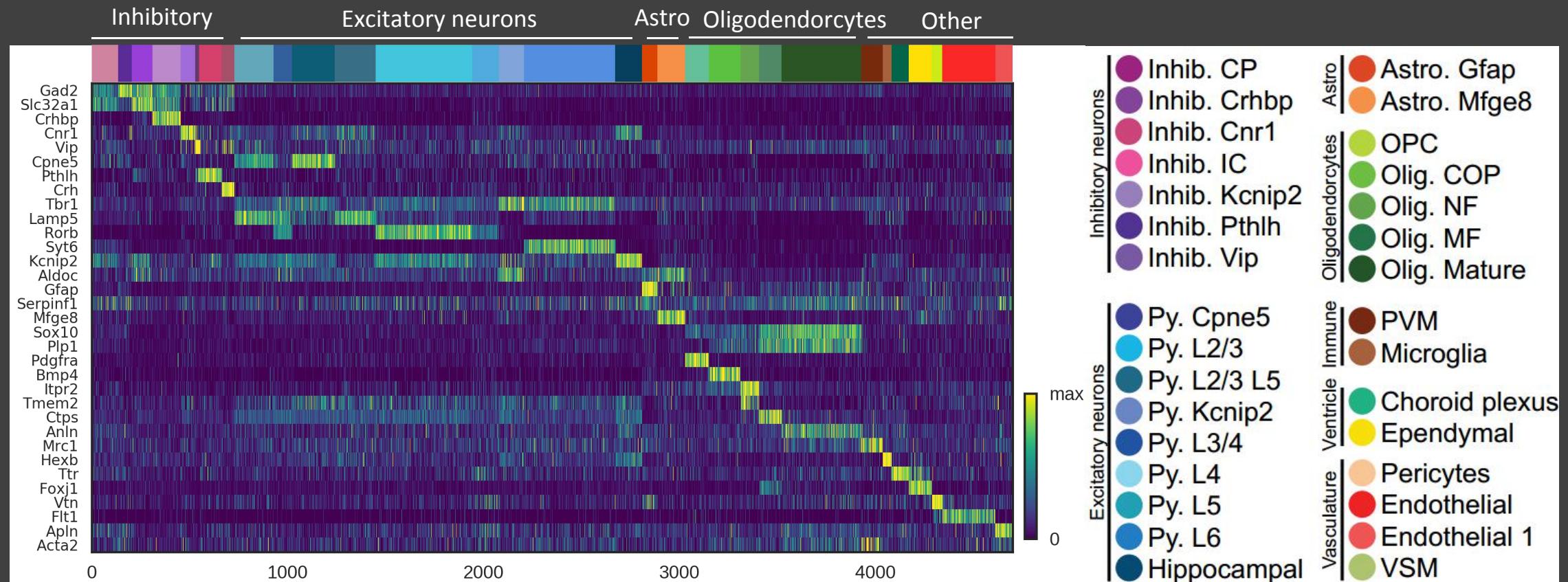
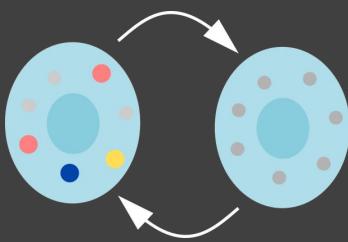
Genes

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_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	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_Serpint1	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
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_Vtr	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	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
Hybridization13_Plip1	10	5	3	0	7	0	0	8	5	33	...	3	1	2	35	0	4	7	33	4	1

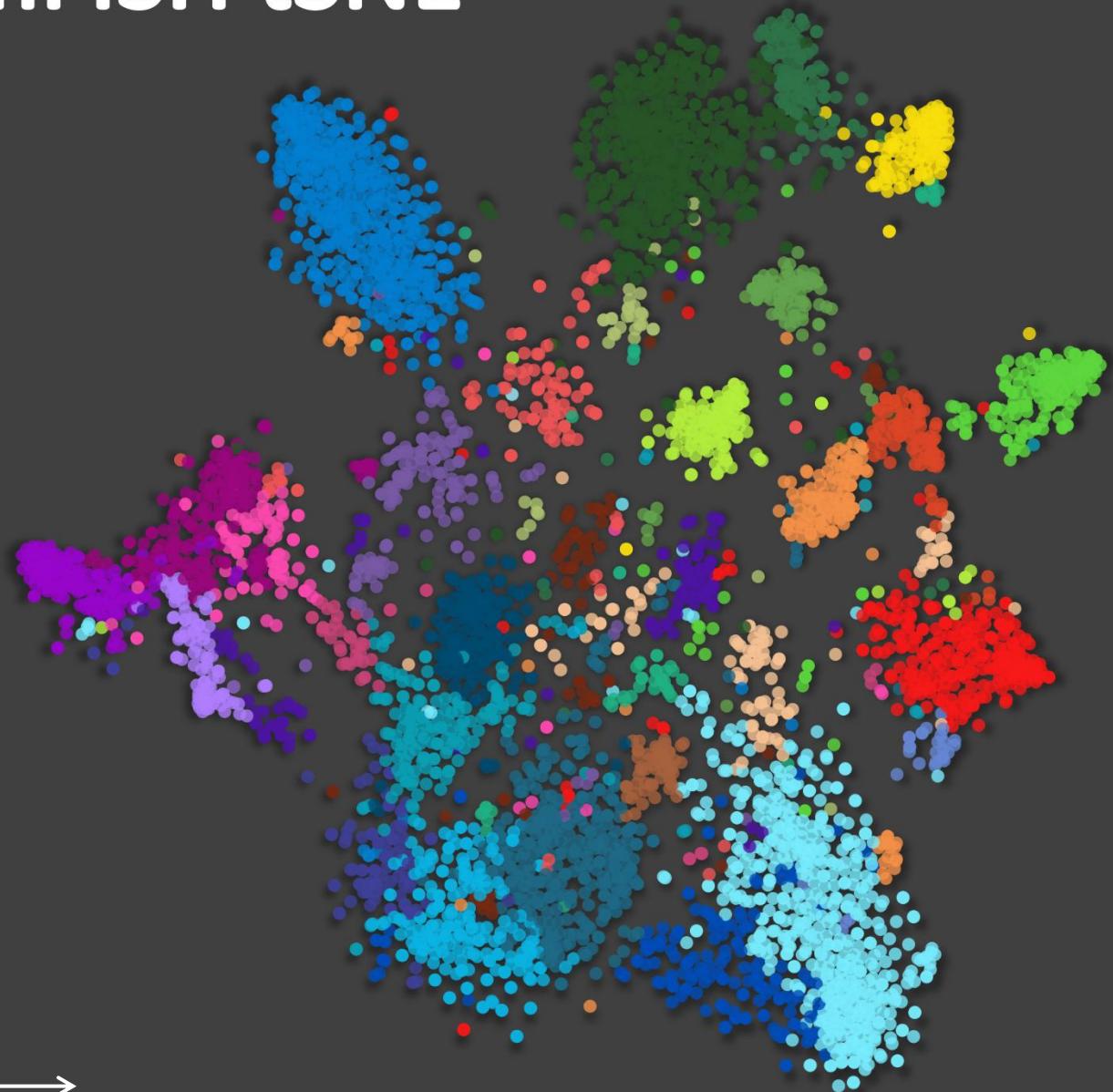
39 rows × 6036 columns

# osmFISH clustering

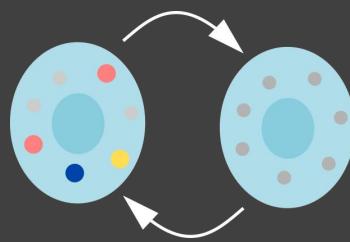


# osmFISH tSNE

tSNE 2  
tSNE 1



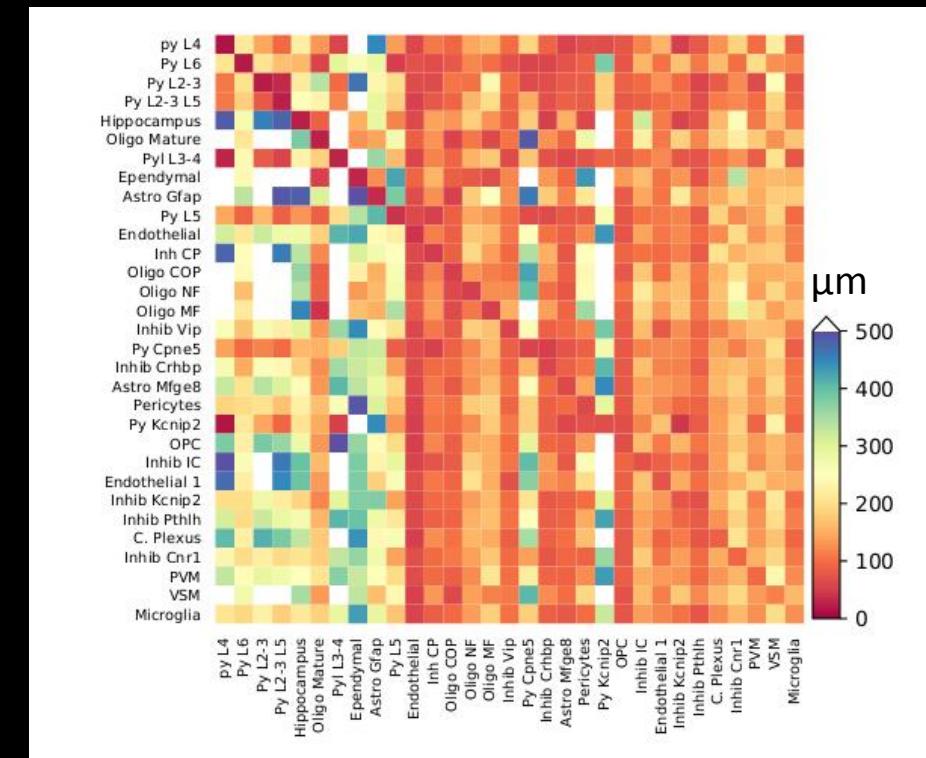
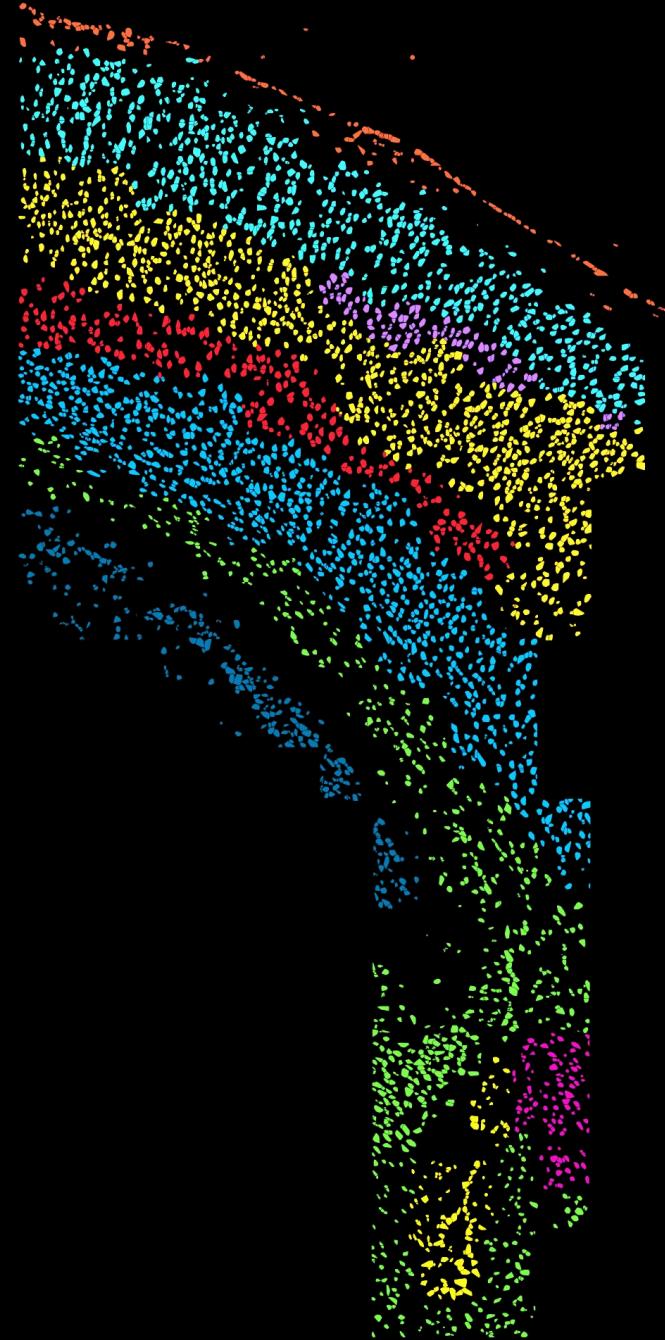
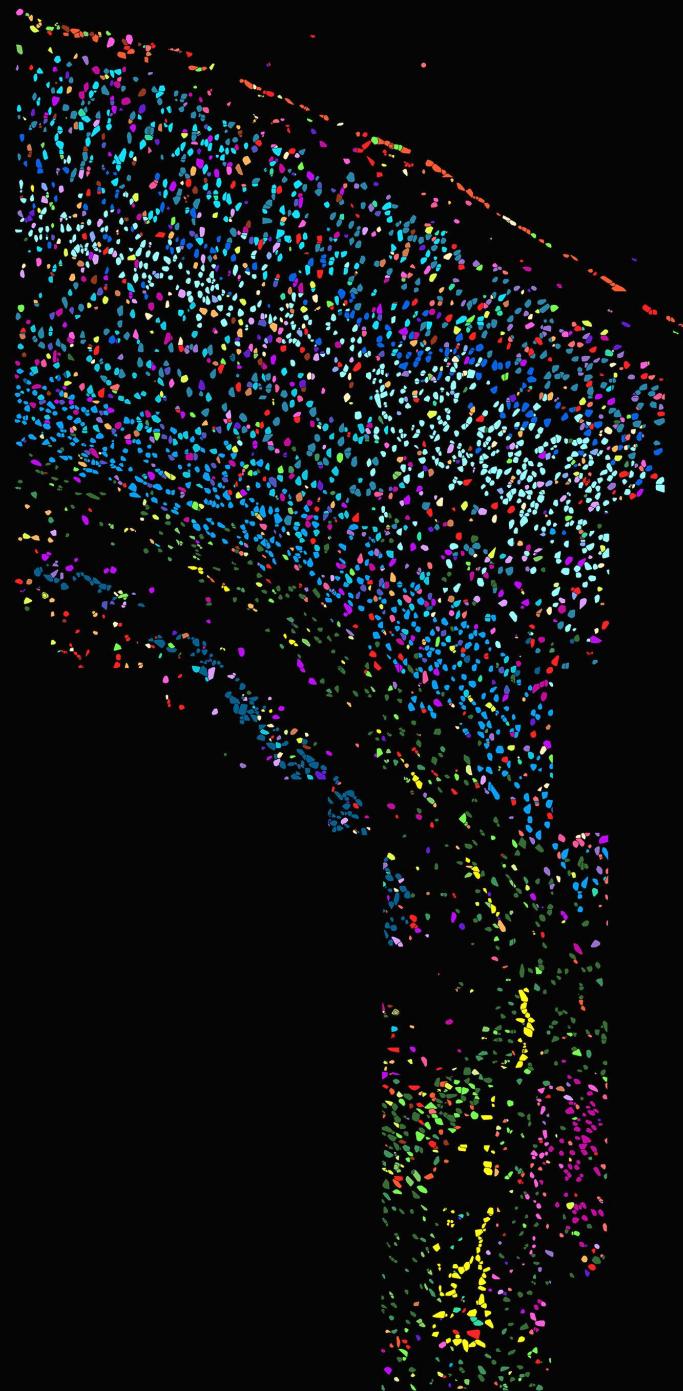
Inhibitory neurons	Inhib. CP	Astro	Astro. Gfap
	Inhib. Crhbp		Astro. Mfge8
	Inhib. Cnr1	Oligodendrocytes	OPC
	Inhib. IC		Olig. COP
	Inhib. Kcnip2		Olig. NF
	Inhib. Pthlh		Olig. MF
	Inhib. Vip		Olig. Mature
Excitatory neurons	Py. Cpne5	Immune	PVM
	Py. L2/3		Microglia
	Py. L2/3 L5	Venticle	Choroid plexus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



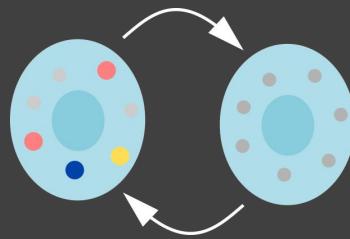
# Cell type map



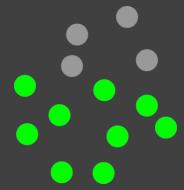
# Spatial analysis



# Cyclic smFISH



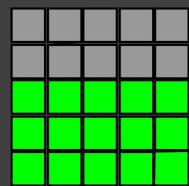
Resolution: Diffraction limited (150-300nm)



Detection efficiency: ~100%

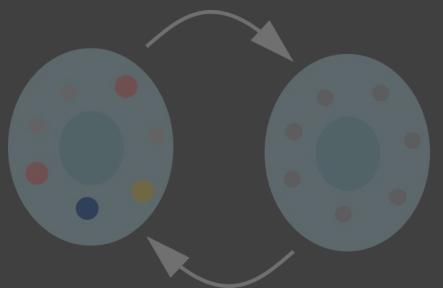


Gene throughput: tens of genes (F x R)

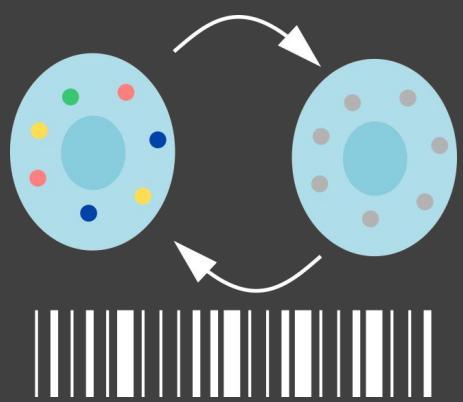


Spatial throughput: several mm<sup>2</sup>

Cyclic FISH



Barcoded FISH



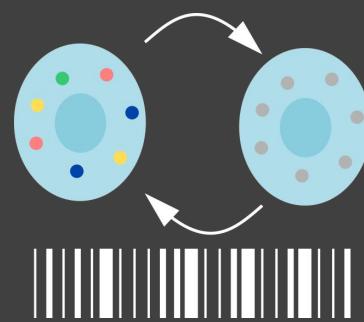
*in situ* Sequencing



Spatial Sequencing



# Reprobing same molecule



Round 1



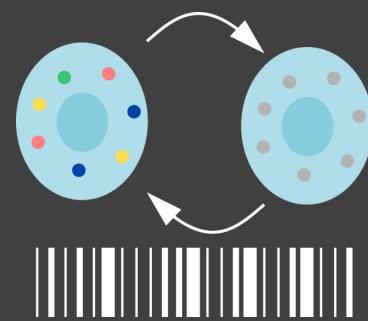
Stripping



Round 2

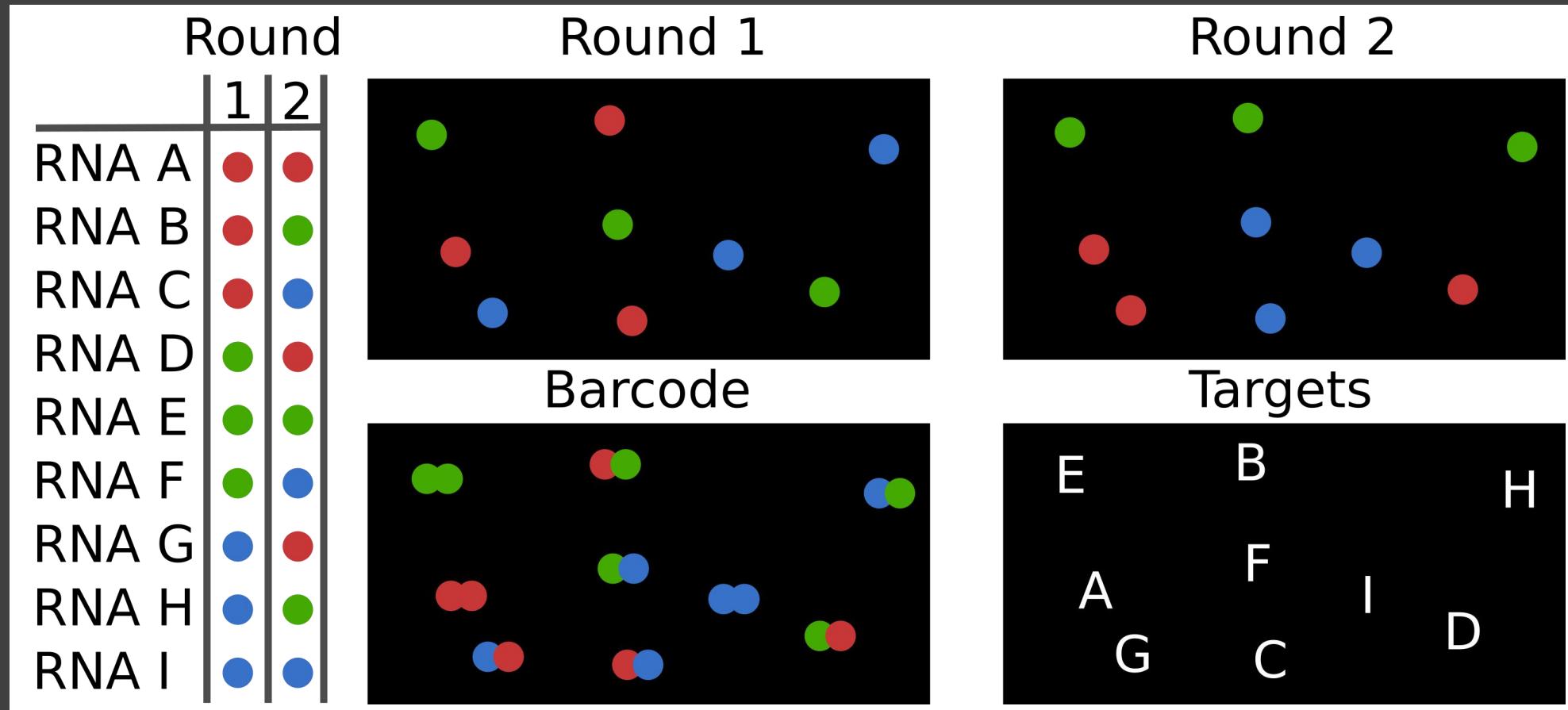
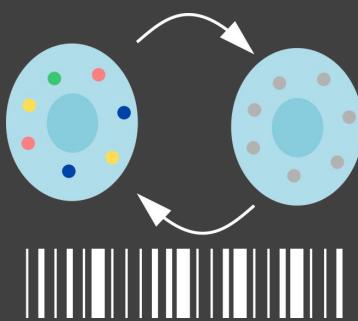


# Barcoding

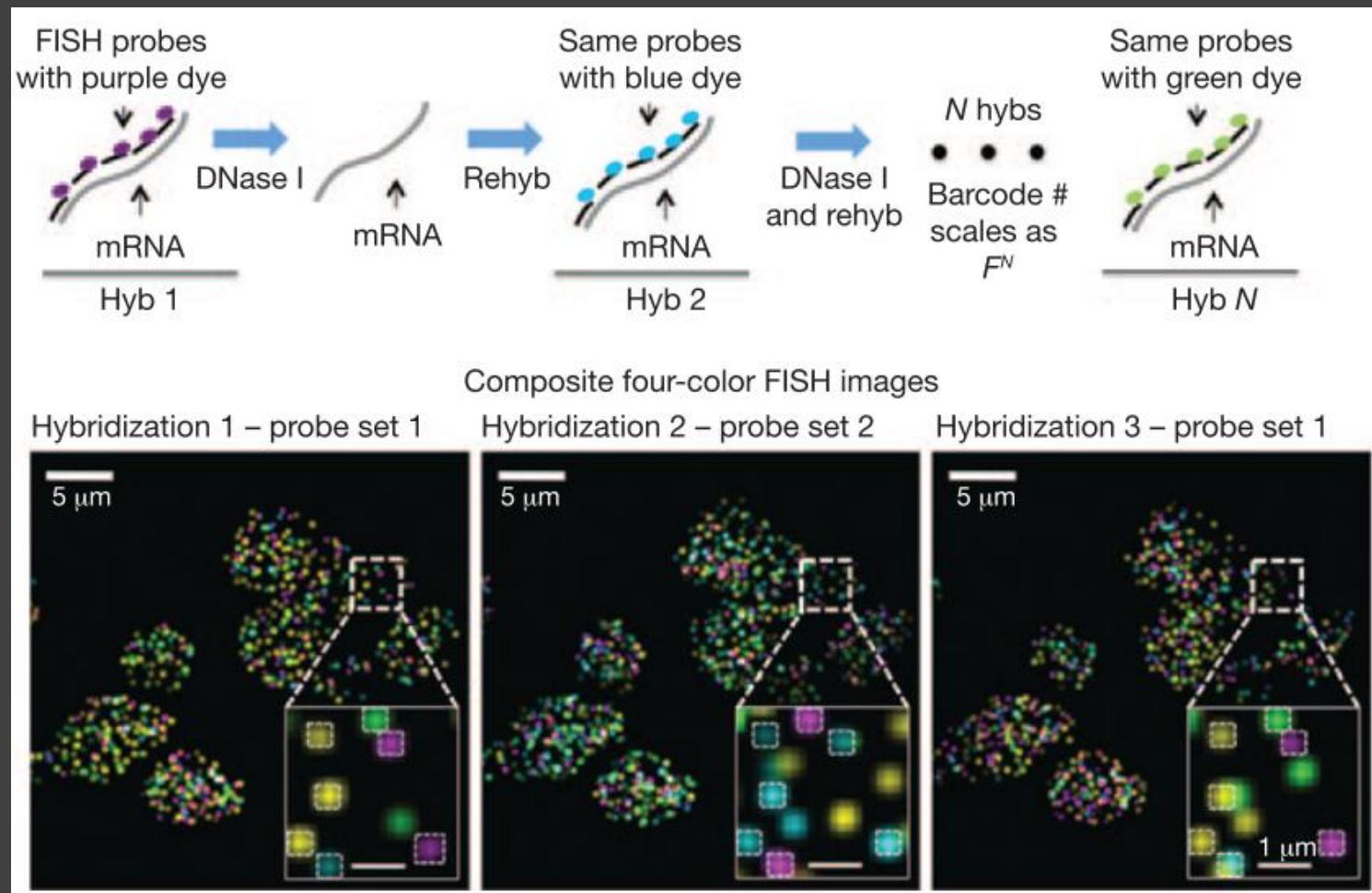
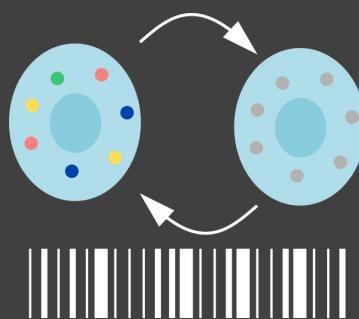


	Round	
	1	2
RNA A	●	●
RNA B	●	●
RNA C	●	●
RNA D	●	●
RNA E	●	●
RNA F	●	●
RNA G	●	●
RNA H	●	●
RNA I	●	●

# Barcoding

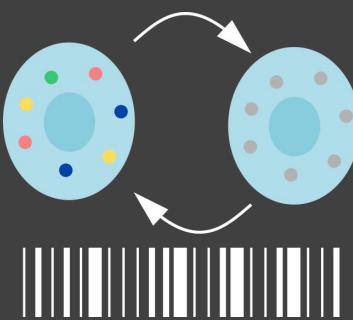


# SeqFISH



Lubeck & Cai Nature Methods 2014

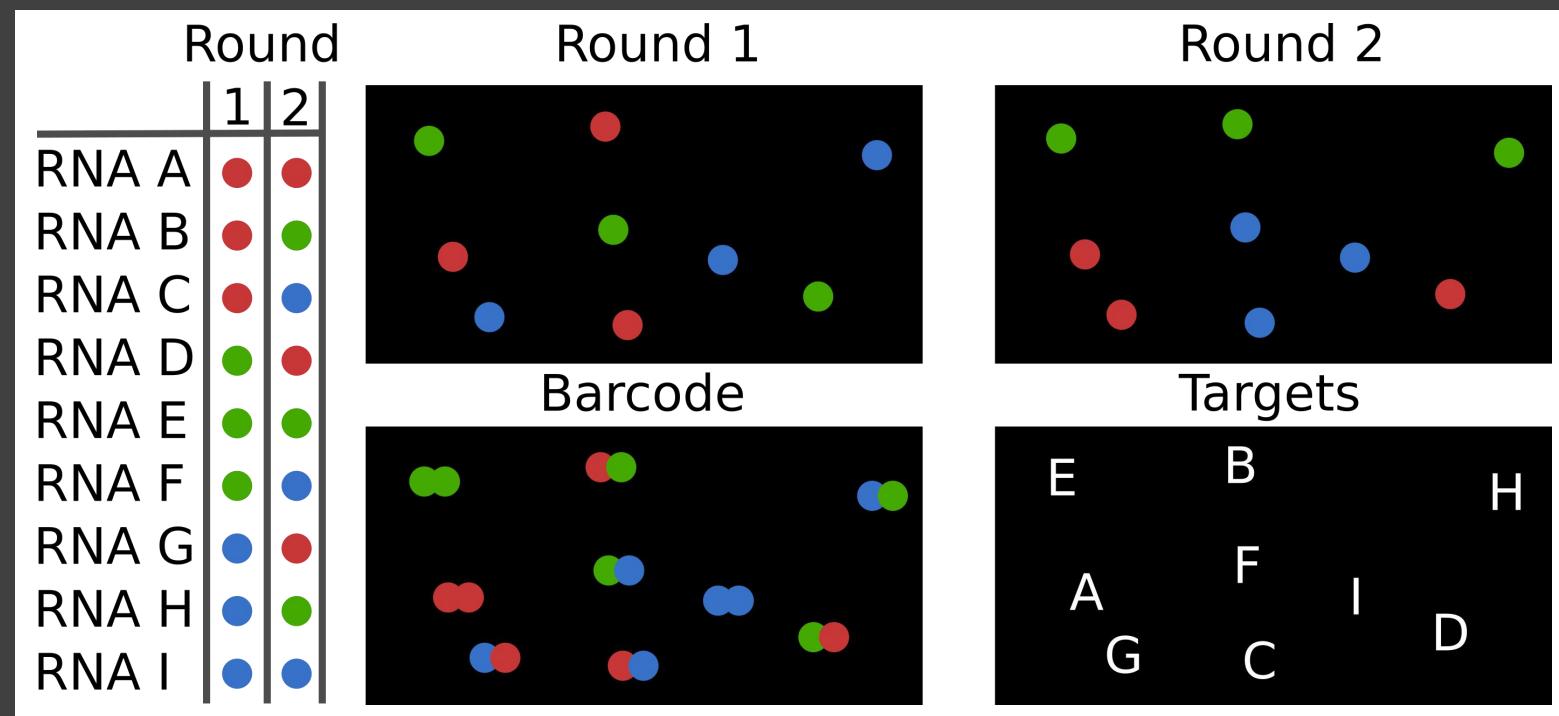
# Barcoding



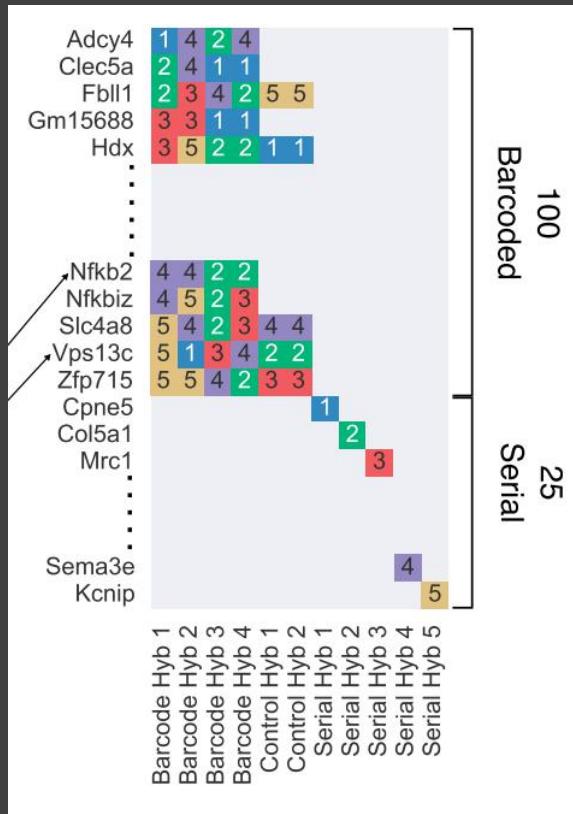
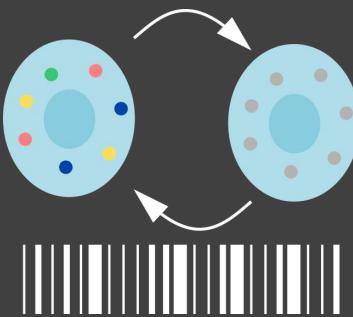
Scaling: Fluo<sup>Rounds</sup>

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

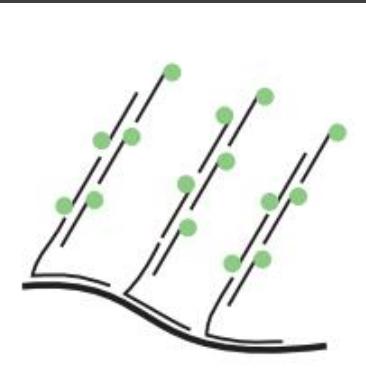
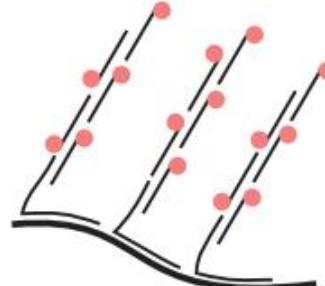
$$4^8 = 65,536 \text{ genes}$$



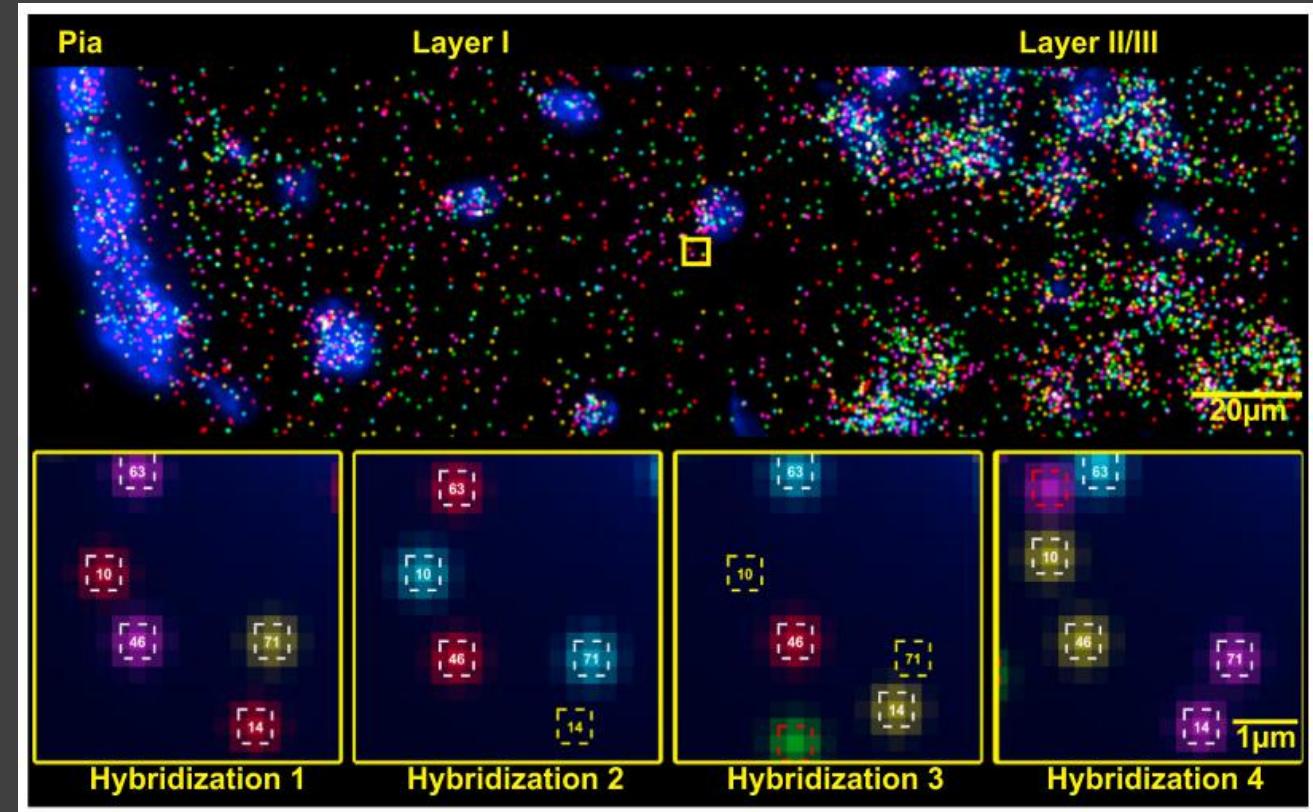
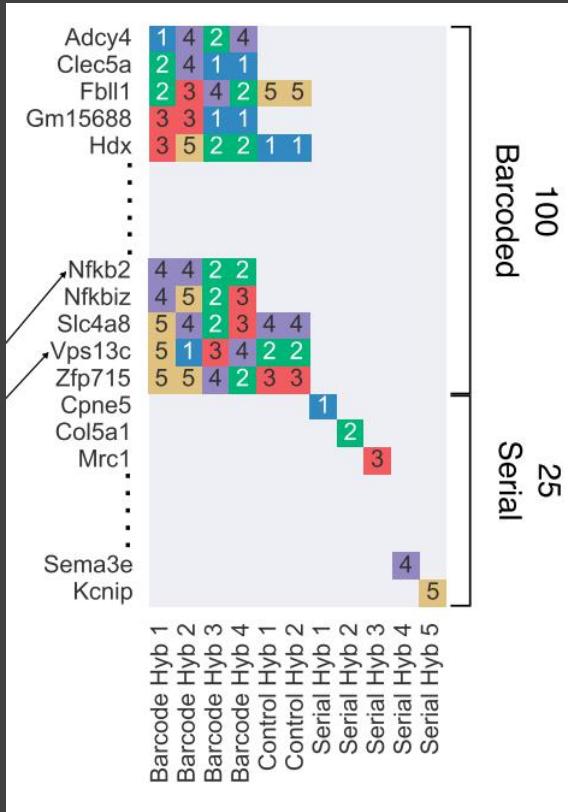
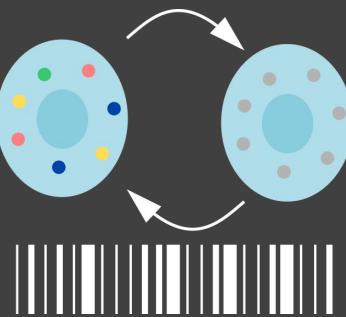
# SeqFISH



seqFISH

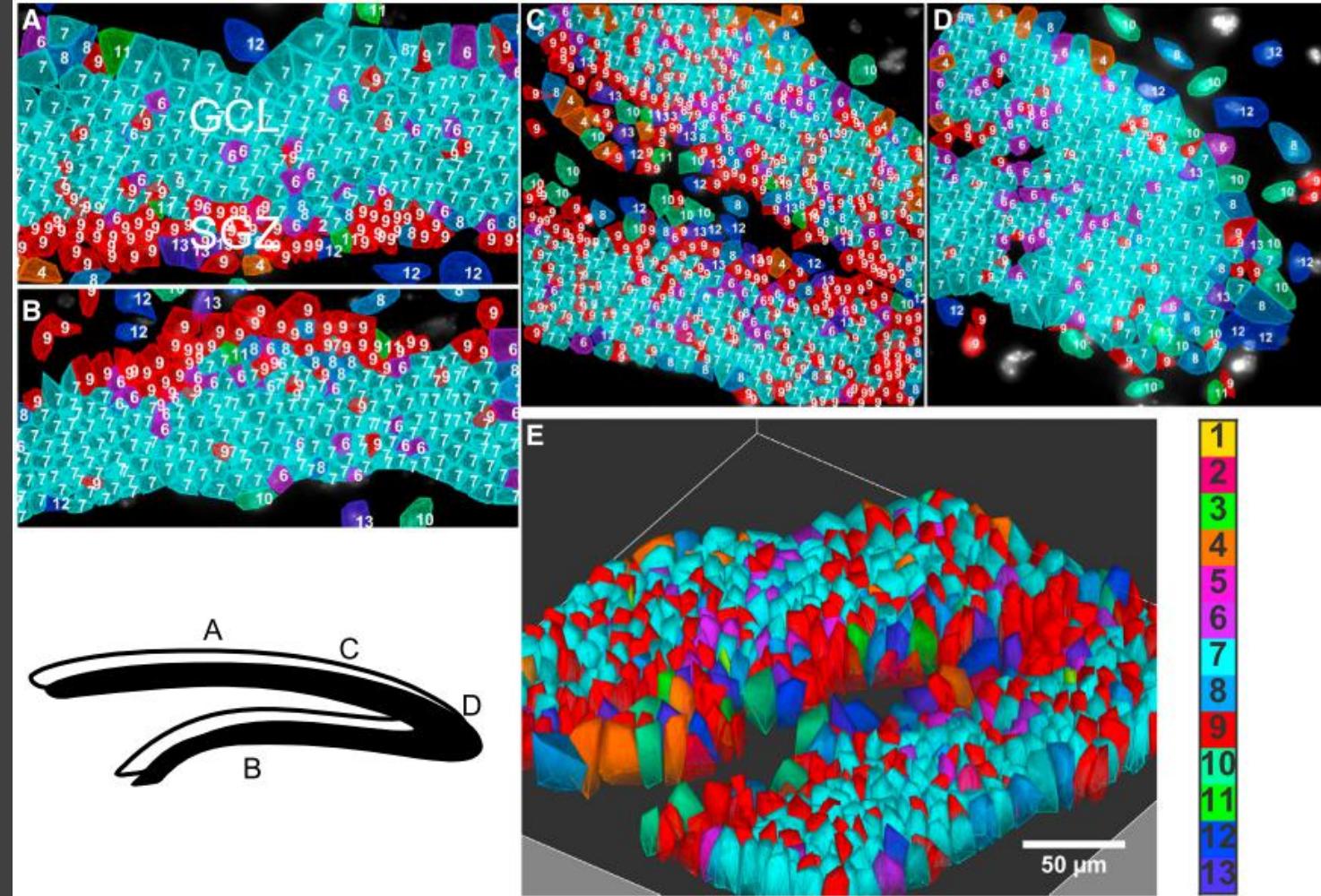
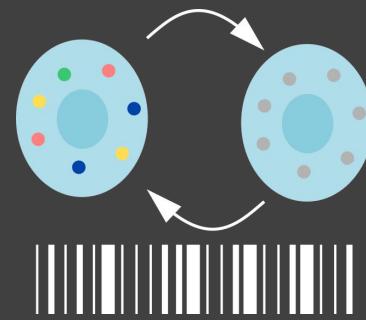


# SeqFISH

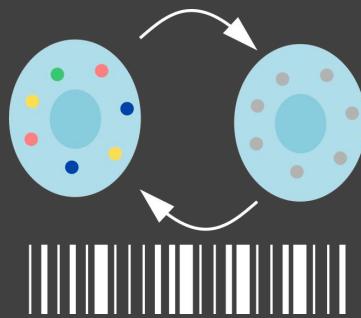


Shah *et al.* Neuron 2016

# SeqFISH

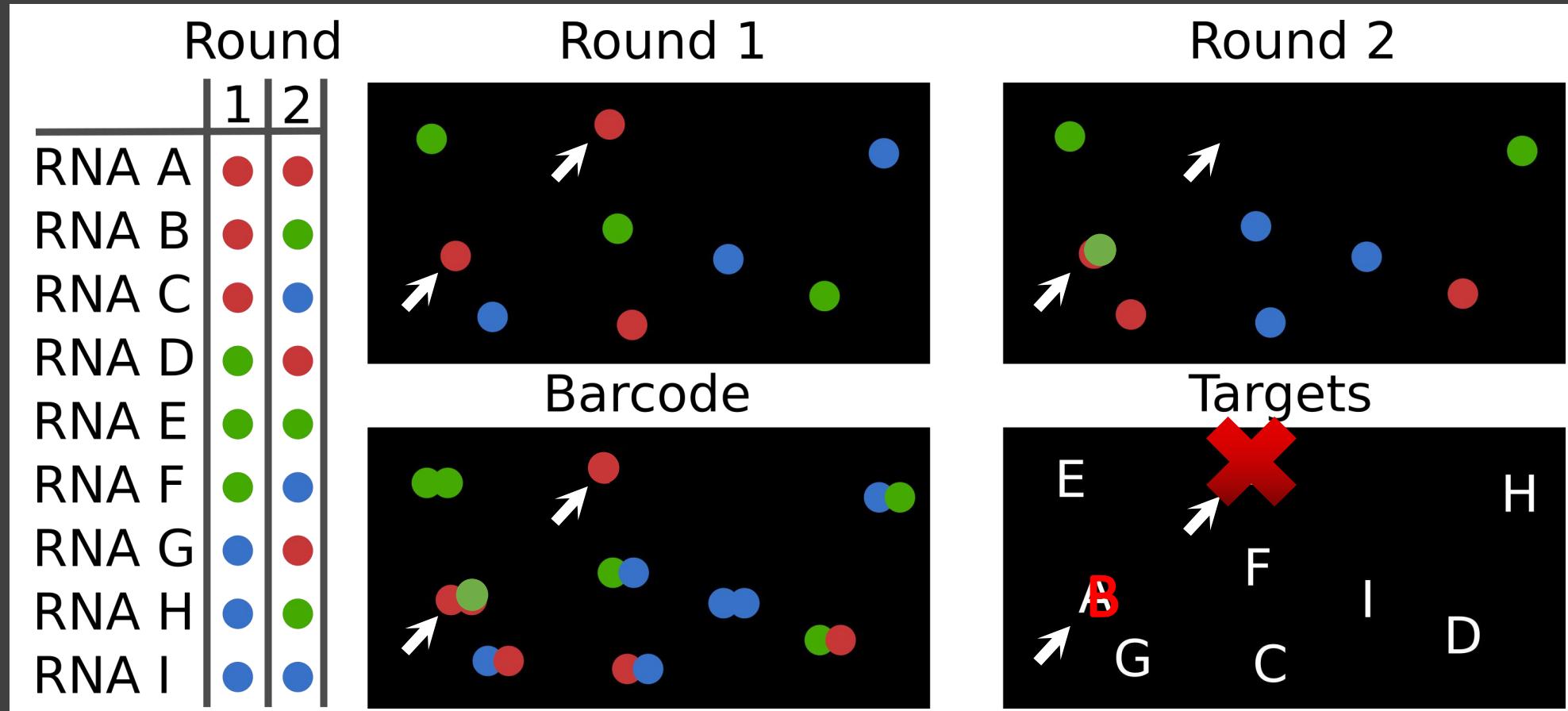
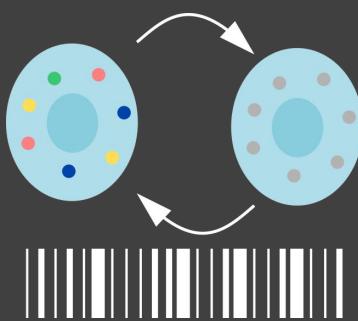


# Challenges

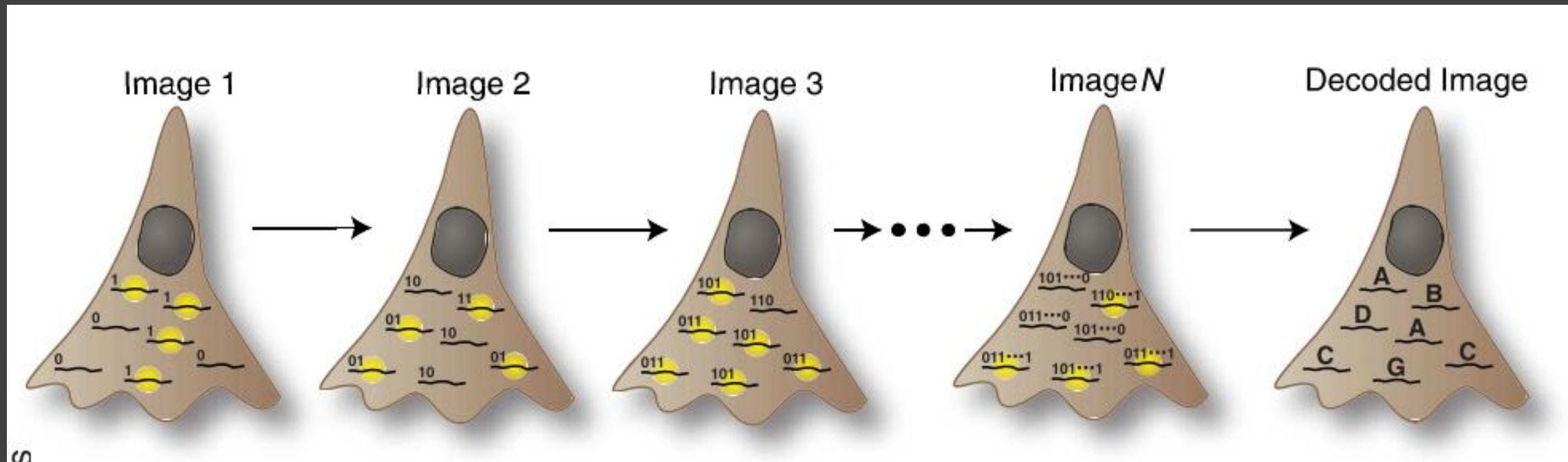
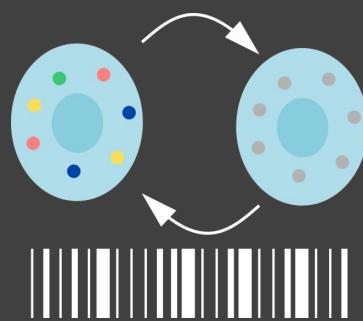


- Errors in barcodes
- Optical density

# Errors

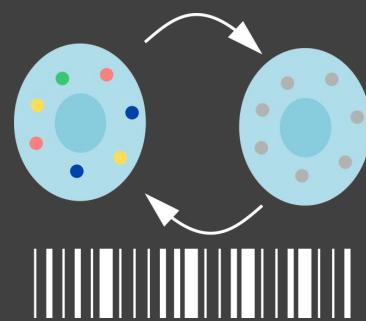


# MERFISH



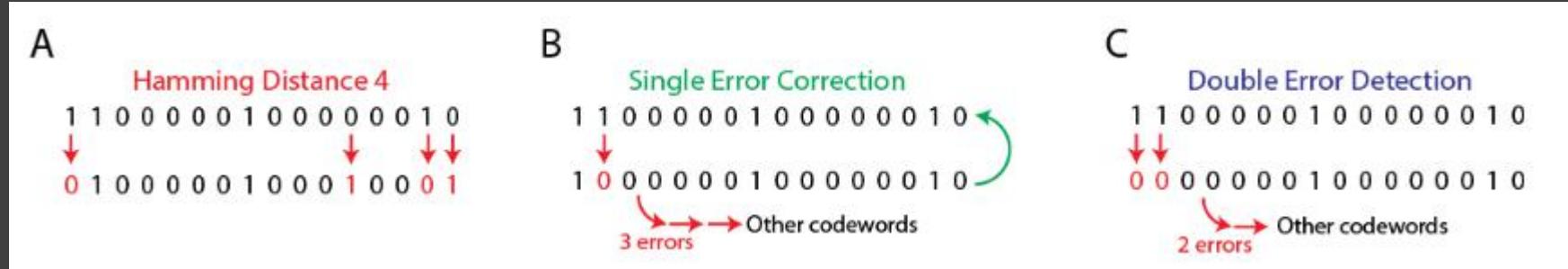
Chen *et al.* Science 2015

# MERFISH



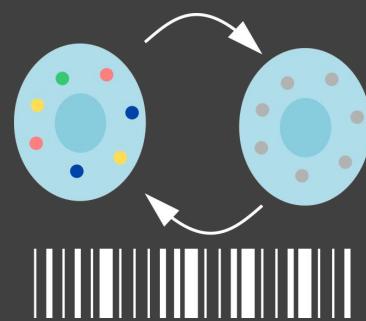
Barcode 1: 1 1 0 0 0 1 0 0 1 0

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

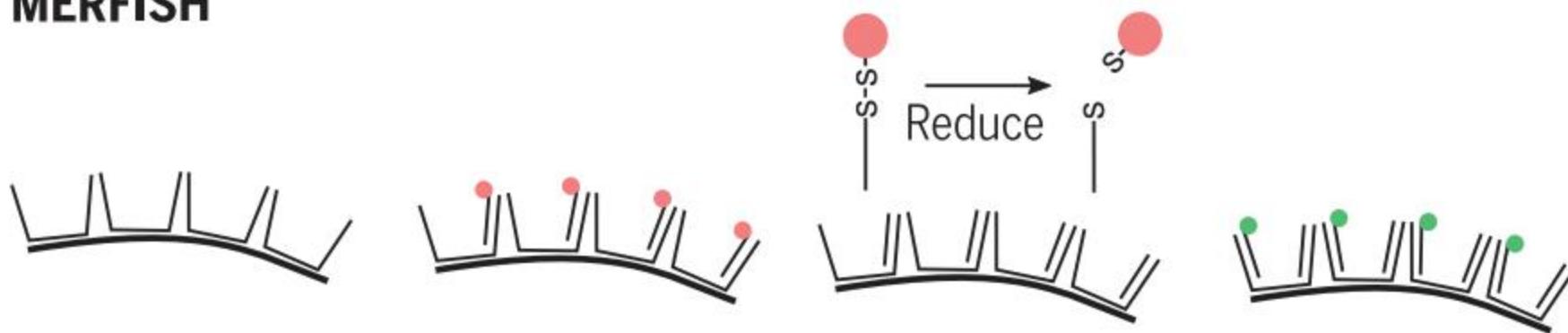


Chen *et al.* Science 2015

# MERFISH

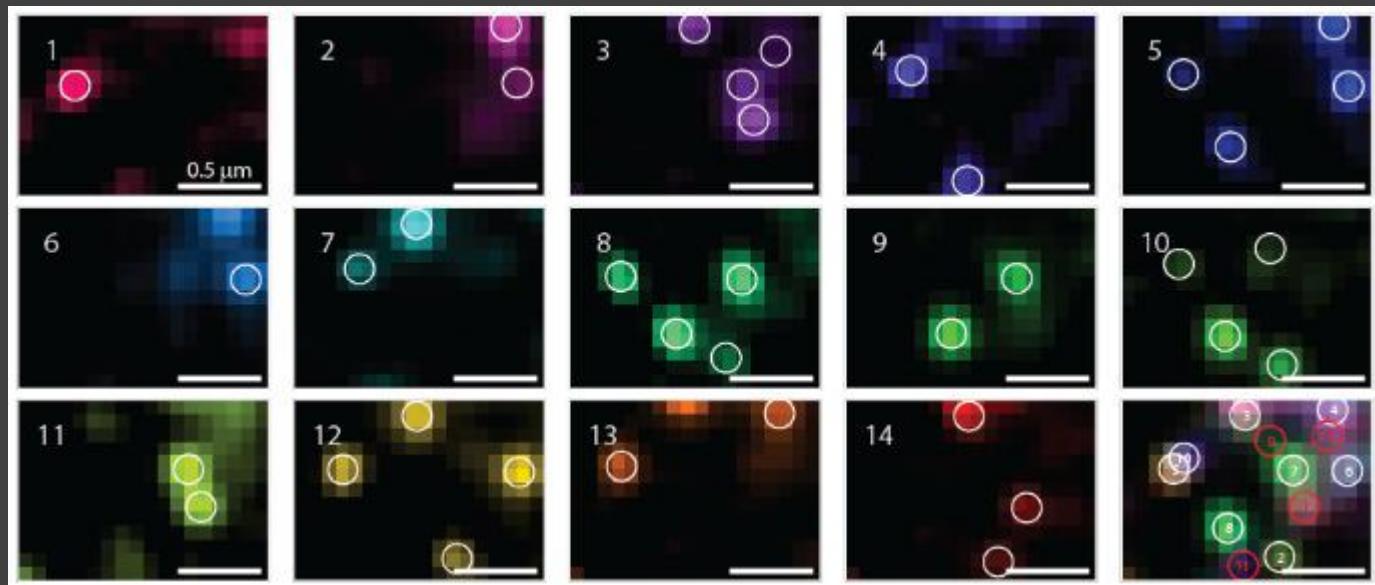
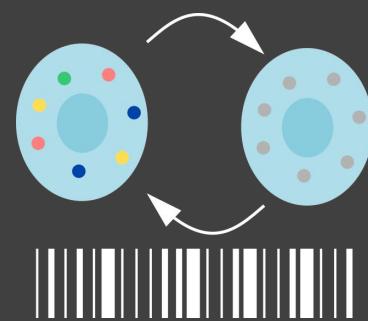


## MERFISH



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

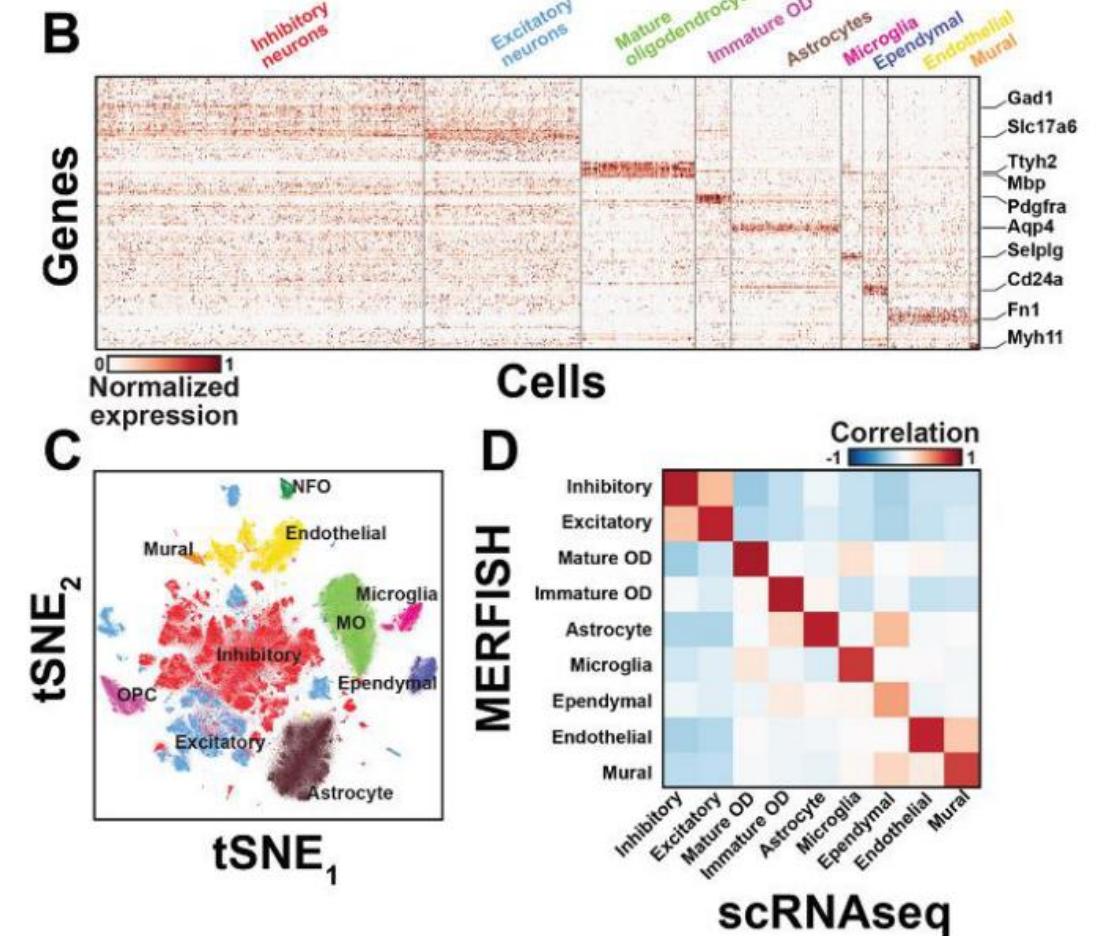
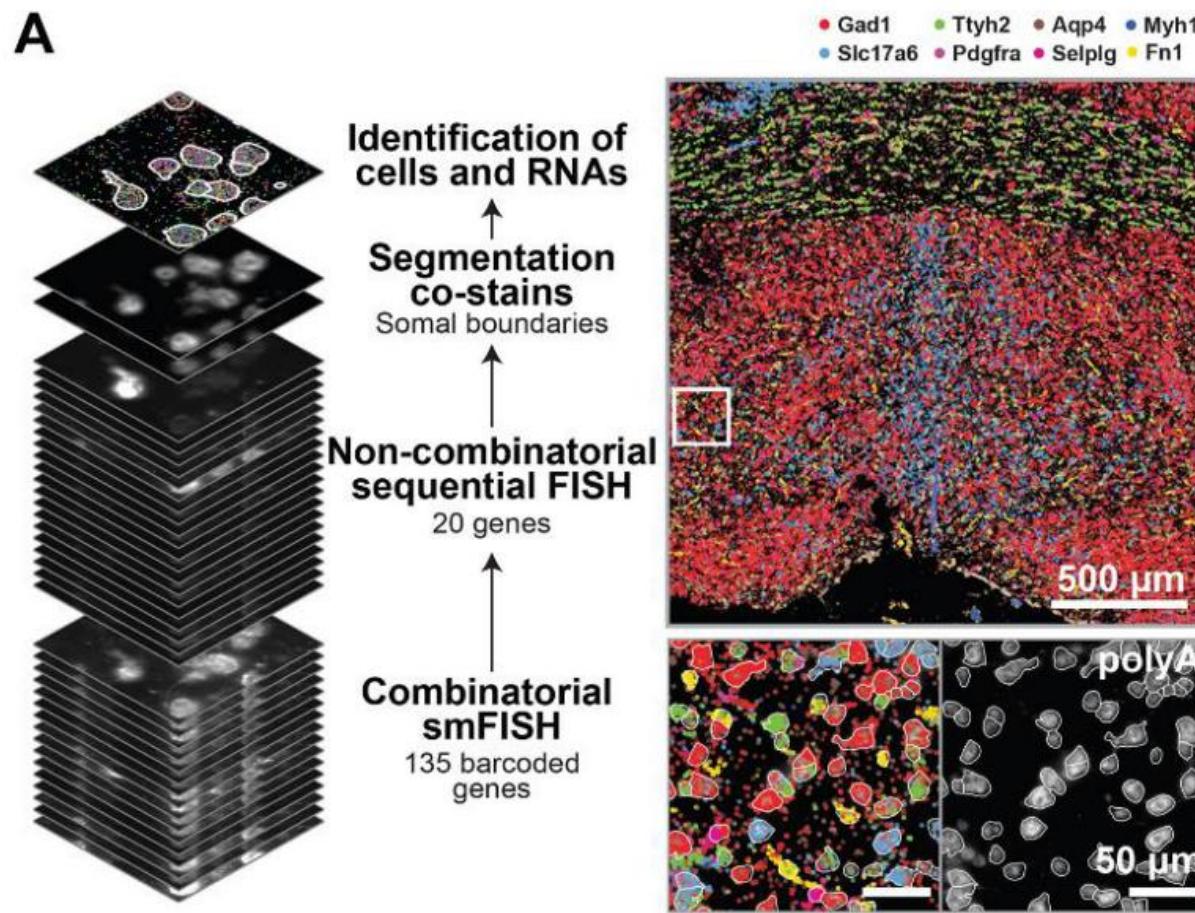
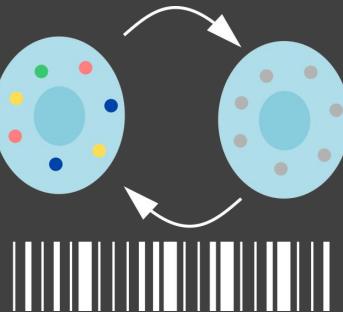
# MERFISH



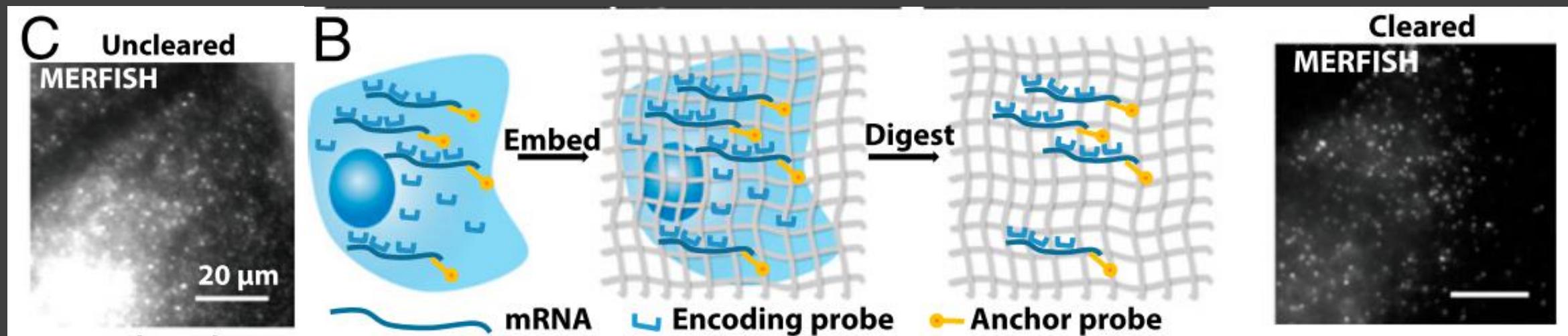
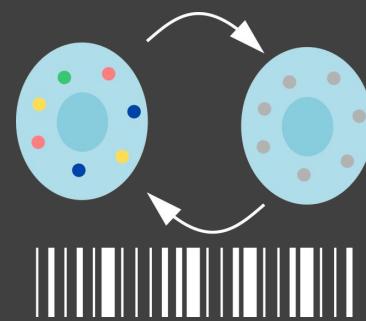
Spot number	Hybridization round													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0	1	0	0	0	0	0	0	0	1	0	0	1
2	0	0	0	0	0	0	0	1	0	1	0	1	0	1
3	0	0	1	0	0	0	1	0	0	0	0	1	0	1
4	0	1	0	0	1	1	0	0	0	0	0	0	1	0
5	1	0	0	0	0	0	0	1	0	0	0	1	1	0
6	0	1	0	0	1	1	0	0	0	0	0	1	0	0
7	0	0	1	0	0	0	0	1	1	0	1	0	0	0
8	0	0	1	0	0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

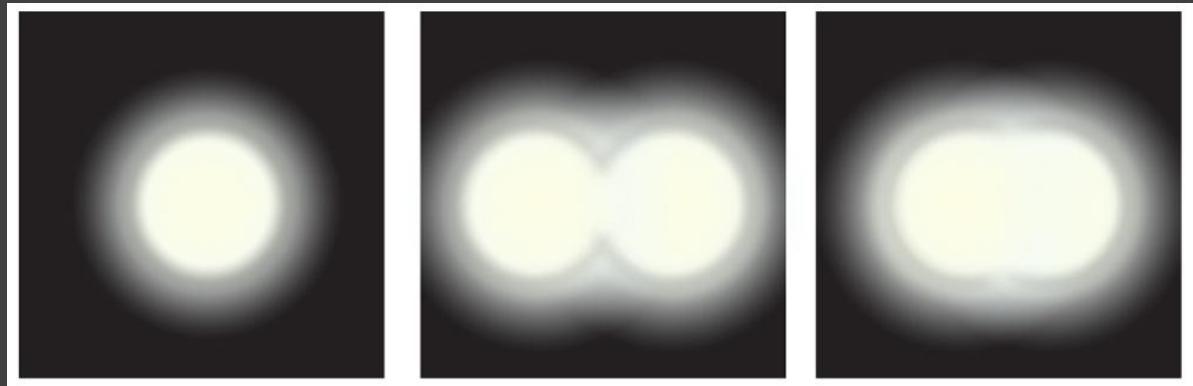
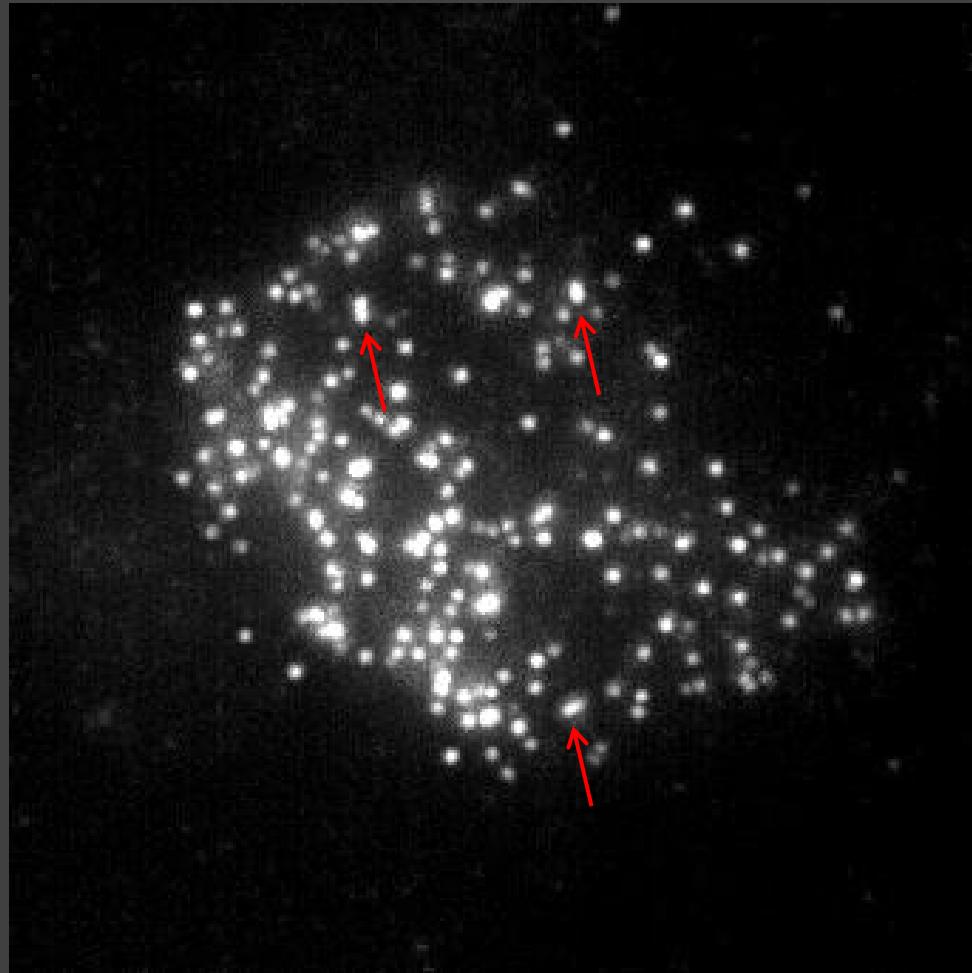
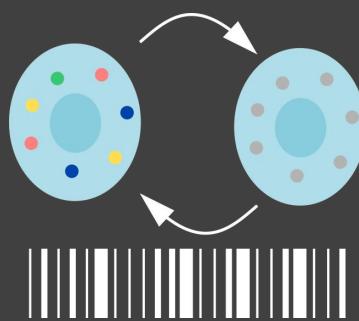


# MERFISH

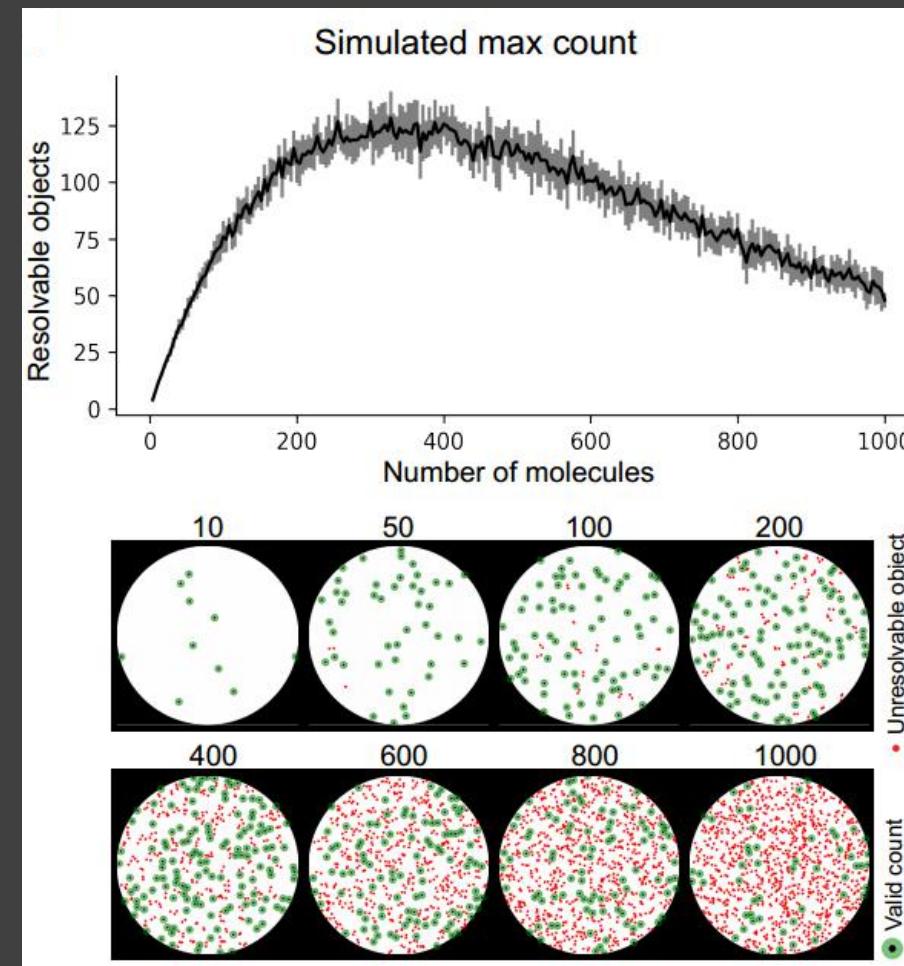
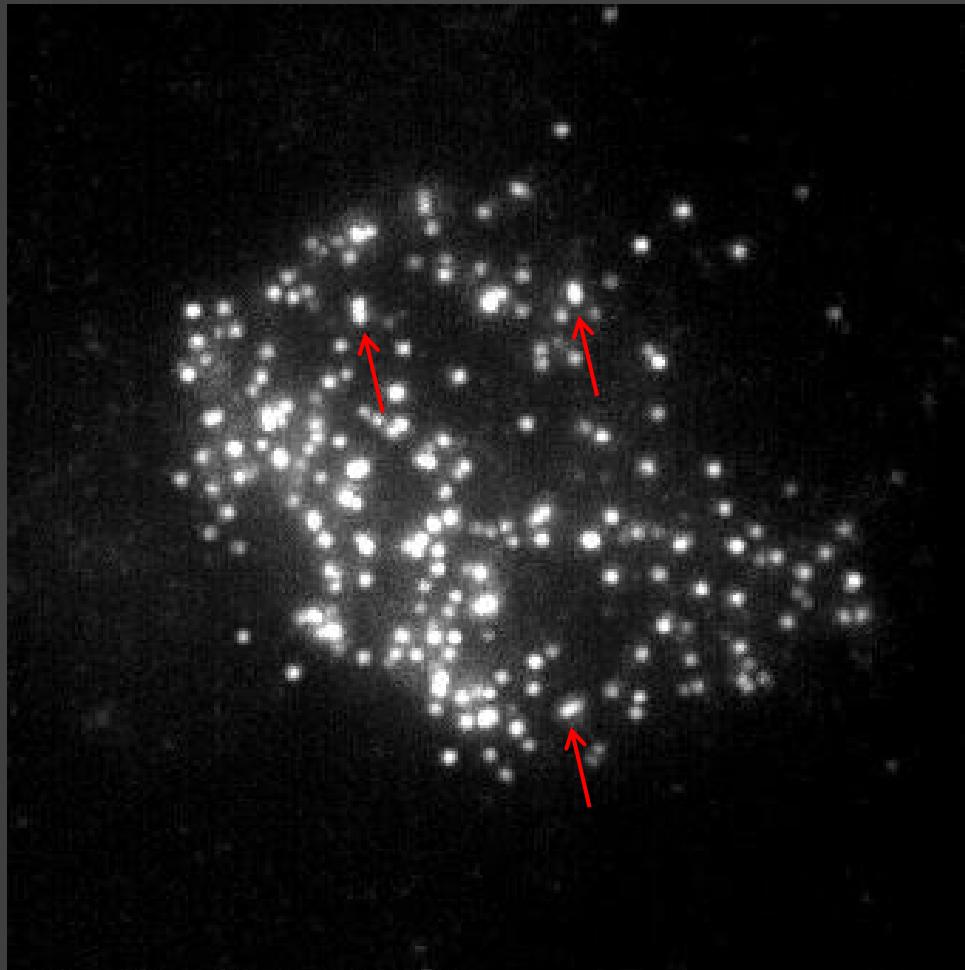
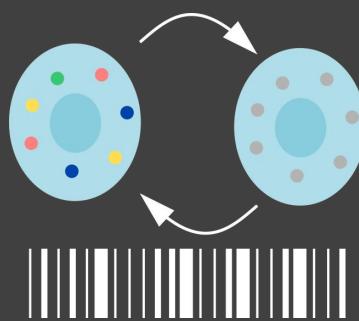


Moffit *et al.* PNAS 2016

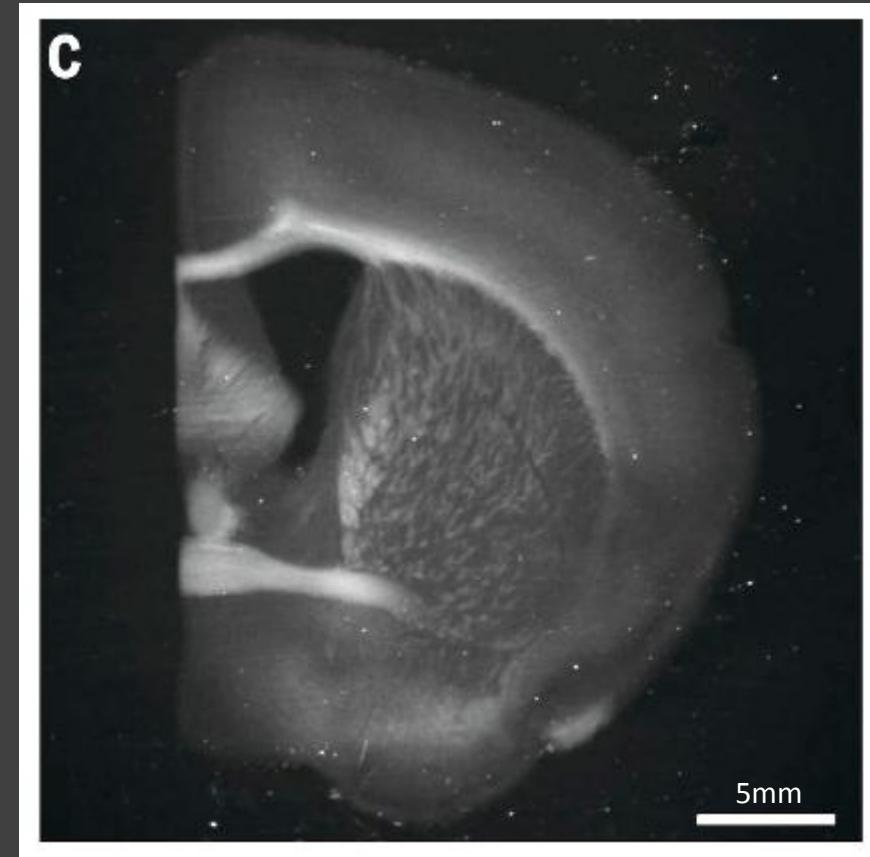
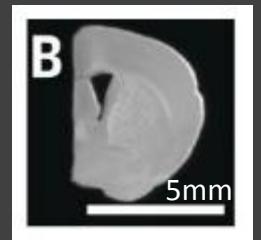
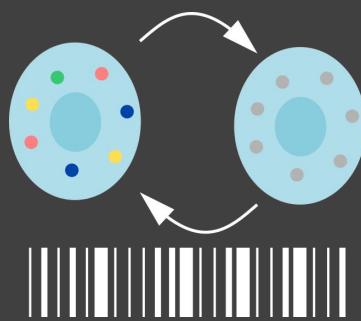
# Barcoding Optical space



# Barcode Optical space

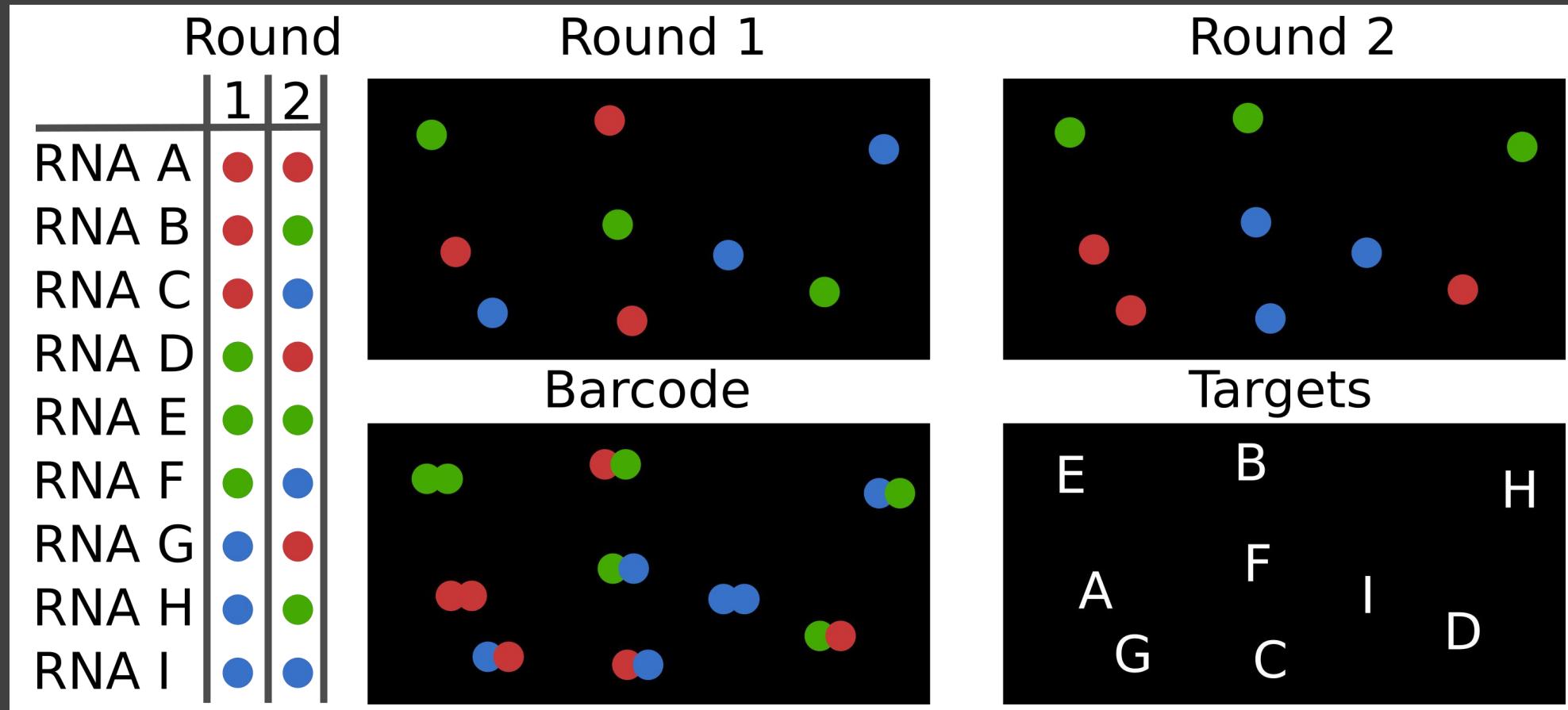
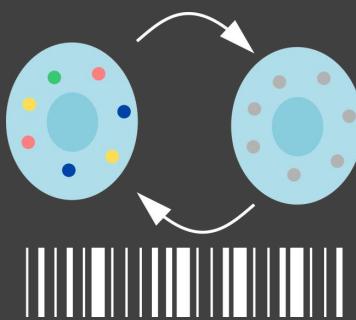


# Expansion microscopy

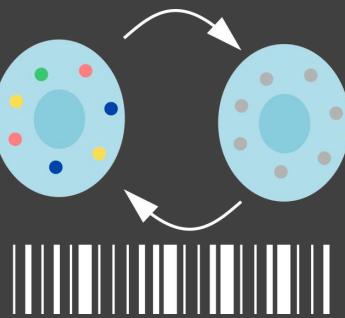


Chen *et al.* Science 2015

# Dense barcode

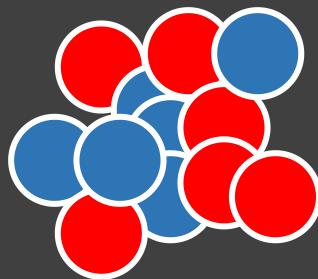


# Dense vs Sparse barcode

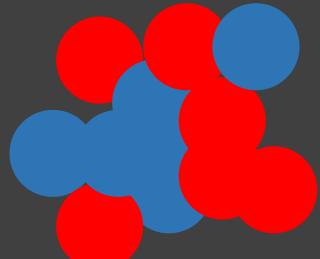


Dense

**RRBRB**

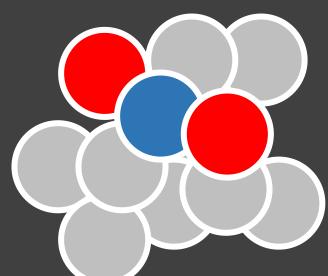
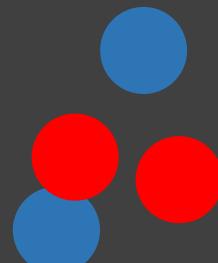
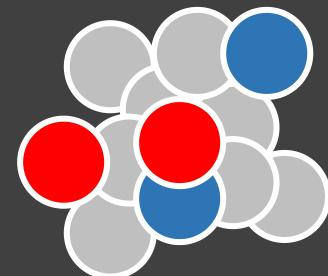
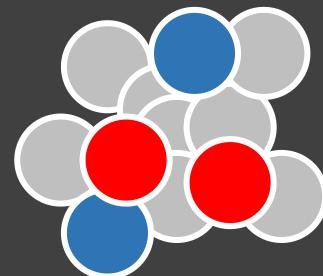


Image

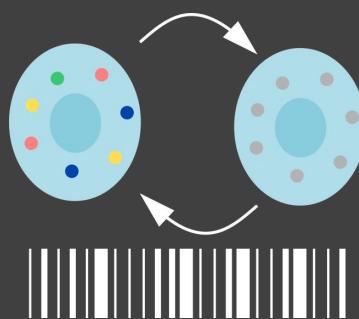


Sparce

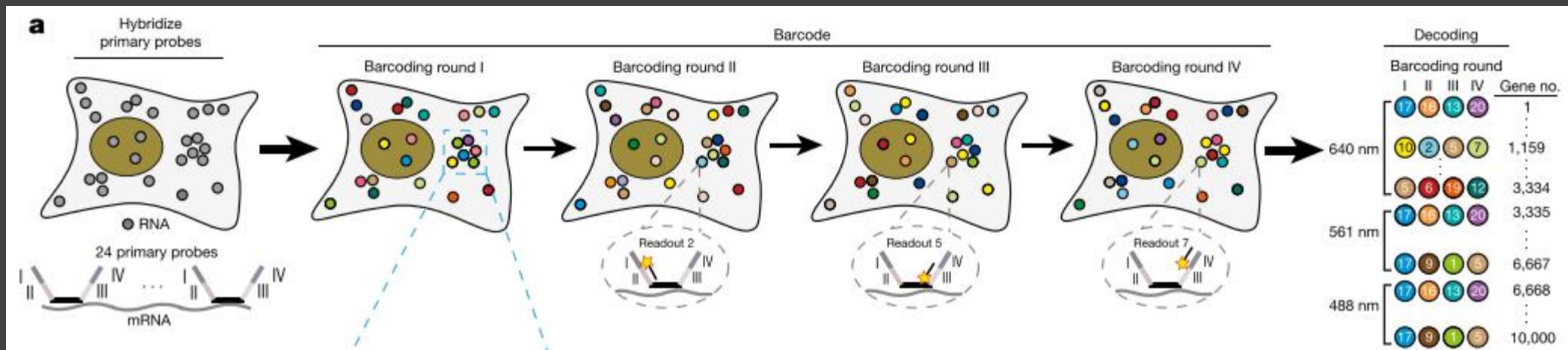
**R00B0B0**



# SeqFISH+

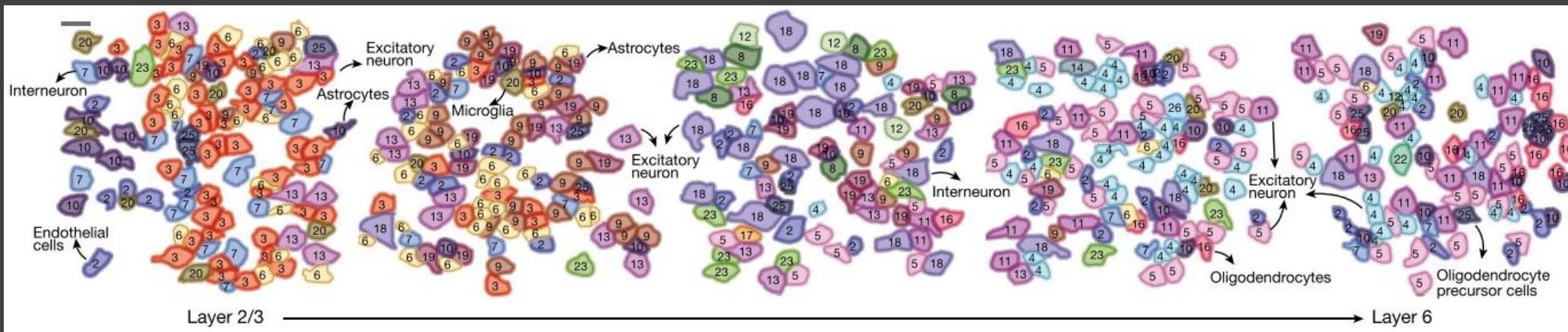
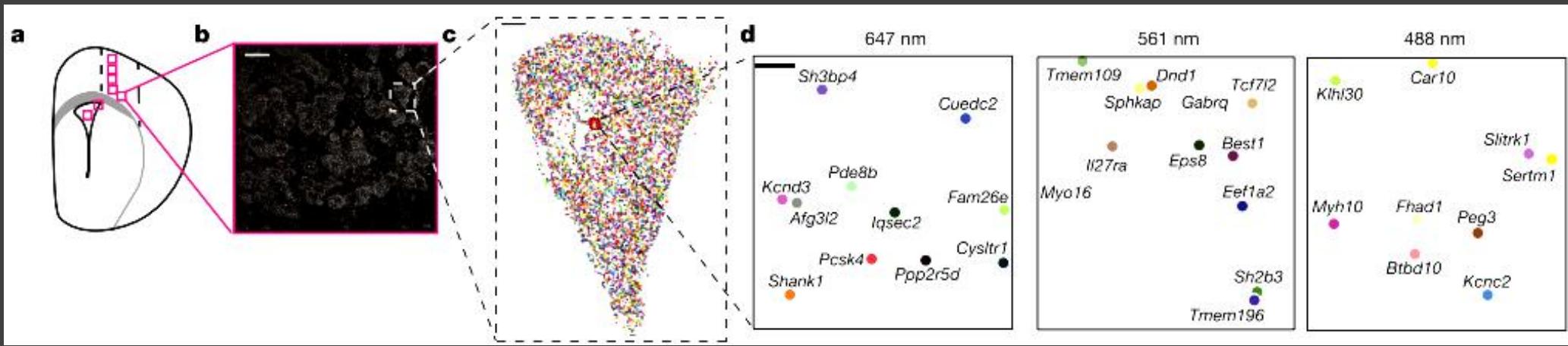
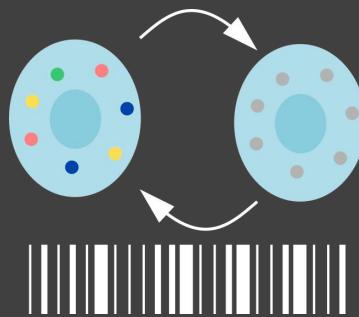


## Sparse barcode

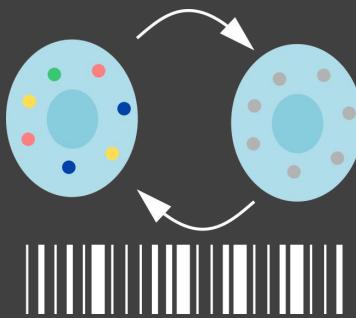


Eng *et al.* Nature 2019

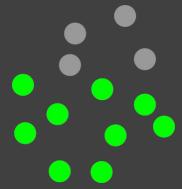
# SeqFISH+



# Barcoded smFISH



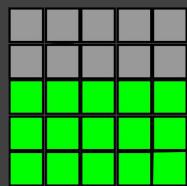
Resolution: Diffraction limited (150-300nm)



Detection efficiency: 70-90%

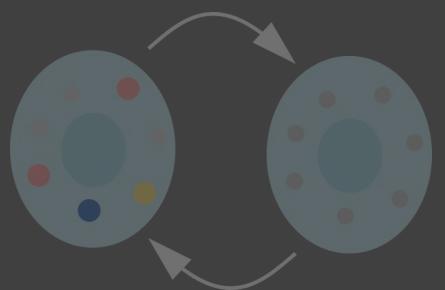


Gene throughput: 100 - 10,000

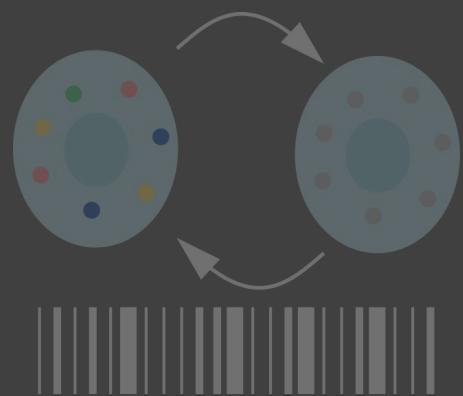


Spatial throughput: several mm<sup>2</sup>

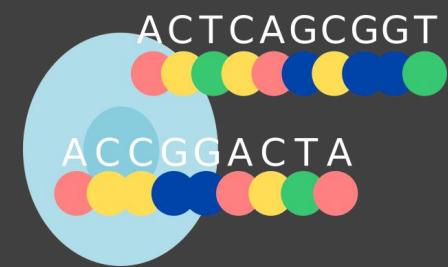
Cyclic FISH



Barcoded FISH



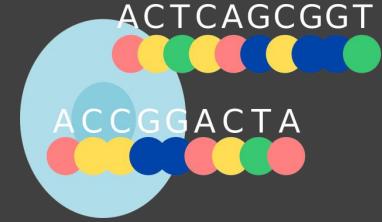
*in situ* Sequencing



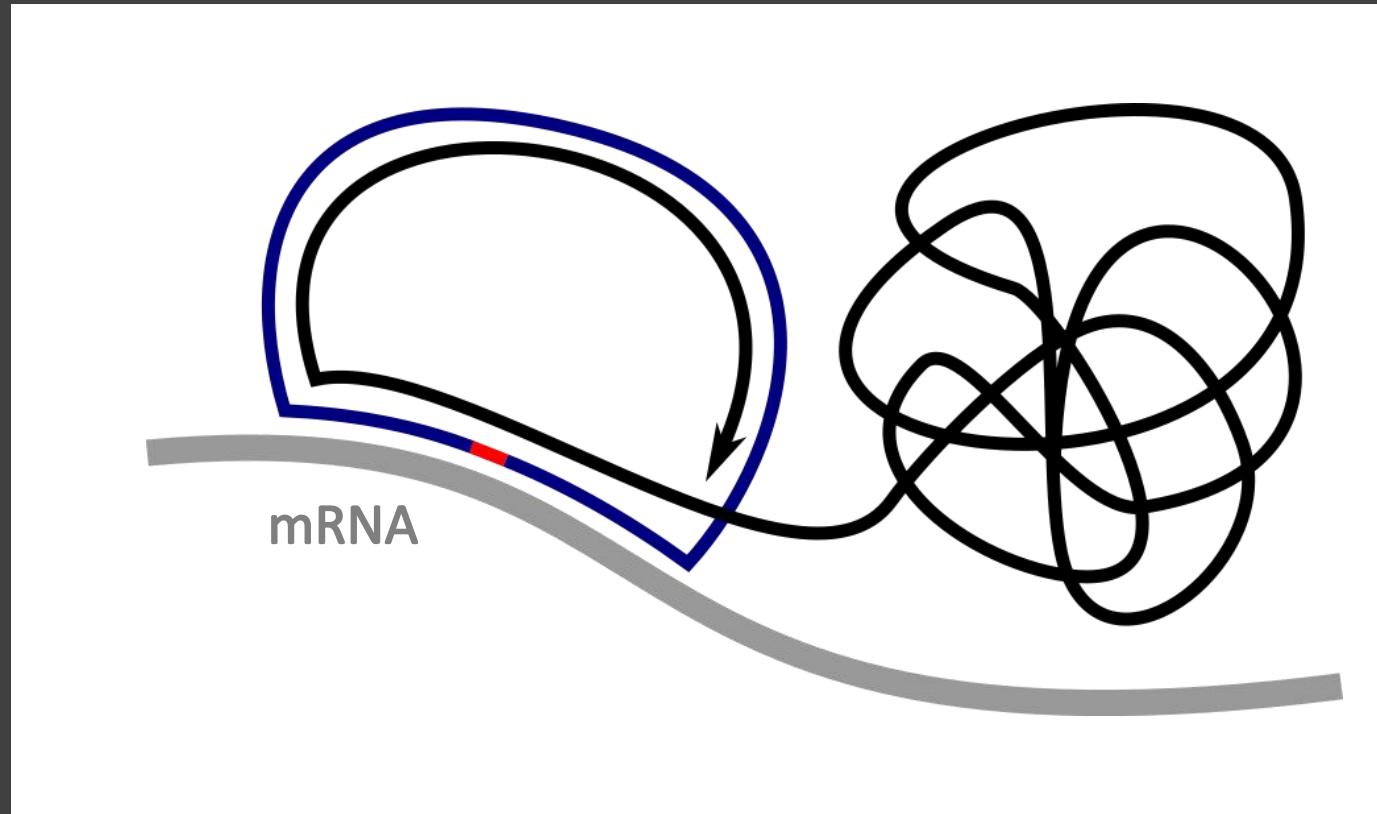
Spatial Sequencing



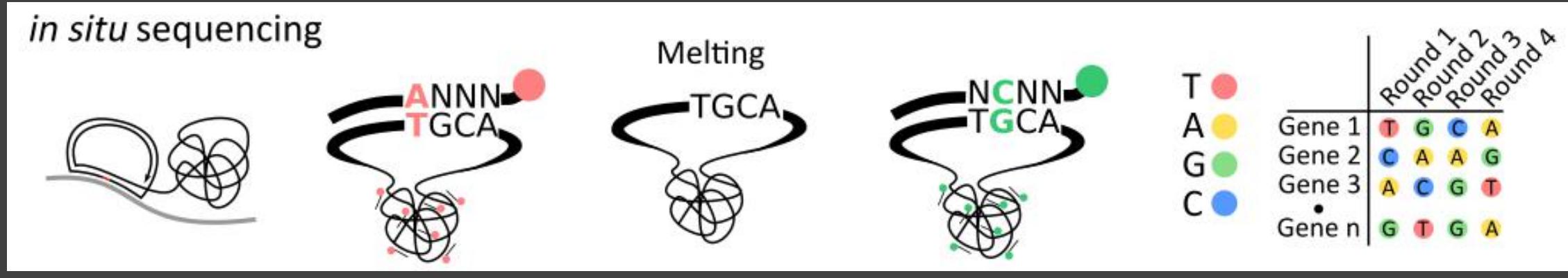
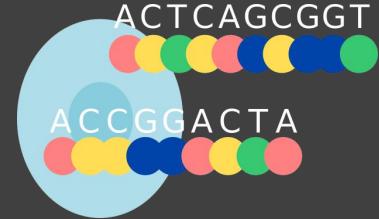
# *in situ* sequencing



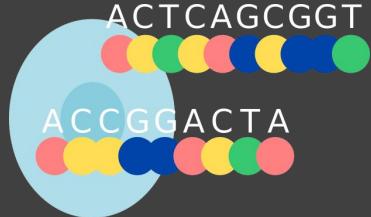
Rolling circle amplification



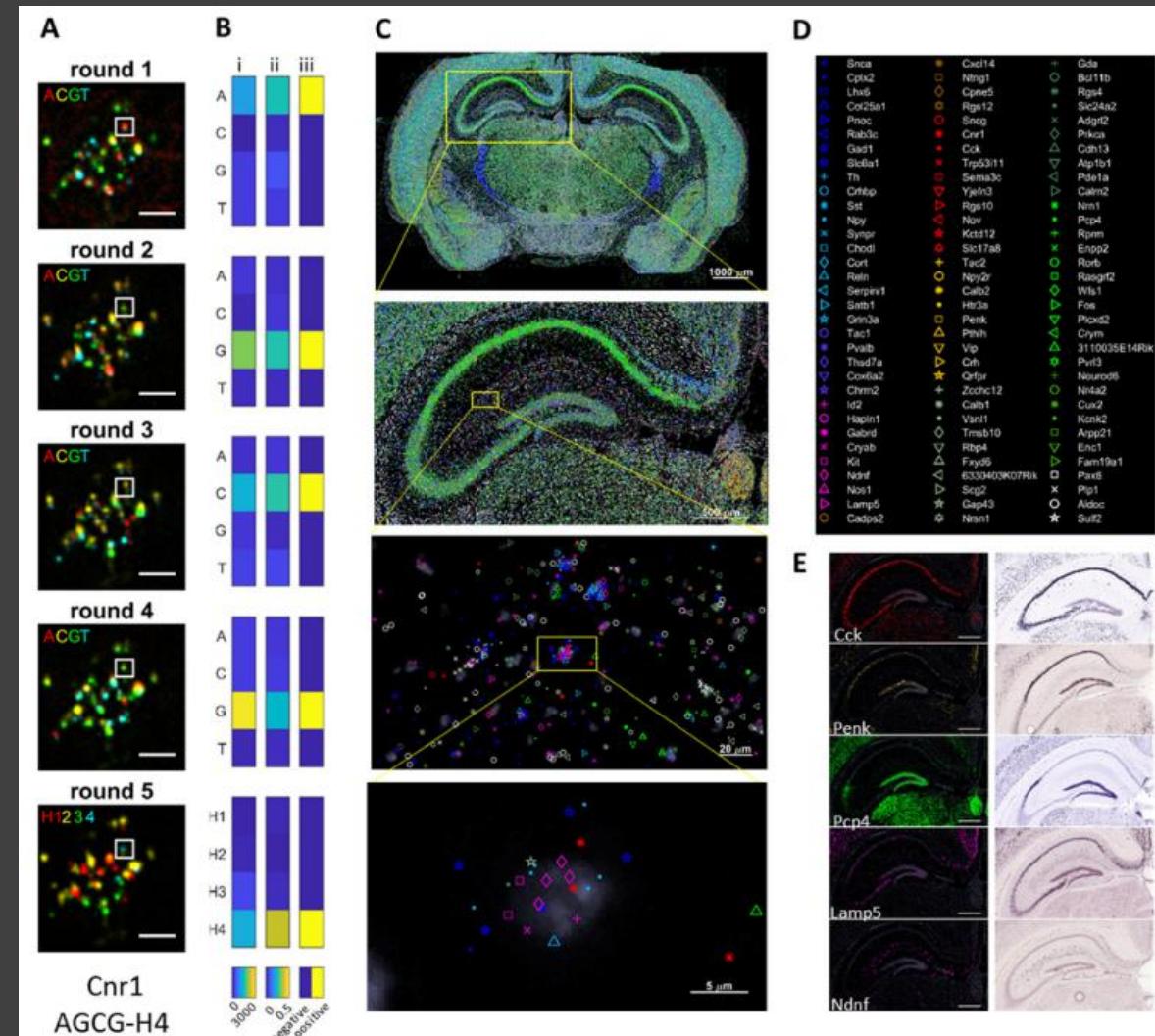
# *in situ* sequencing



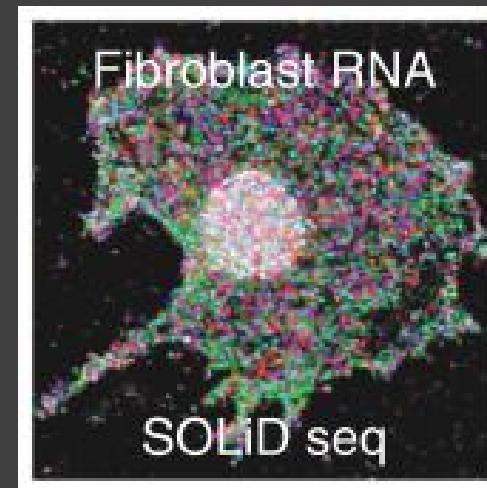
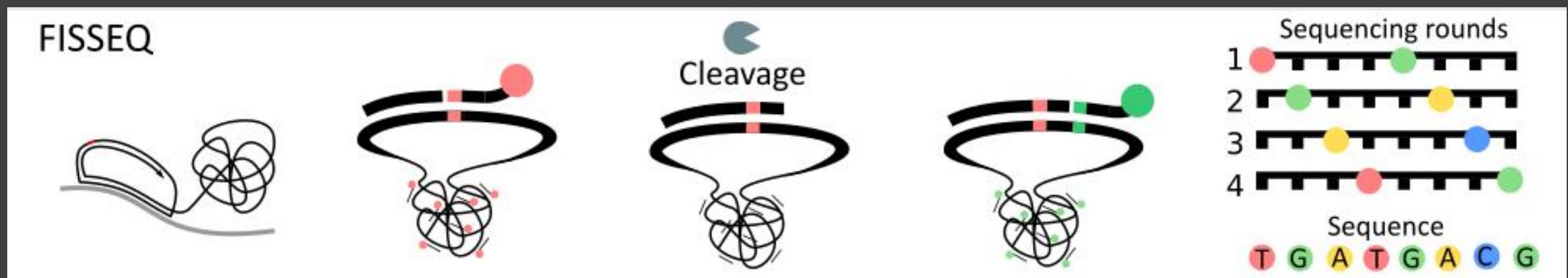
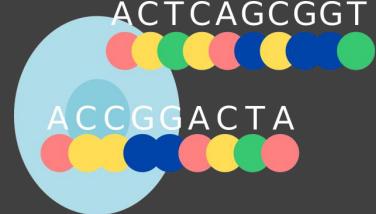
Sequencing by ligation



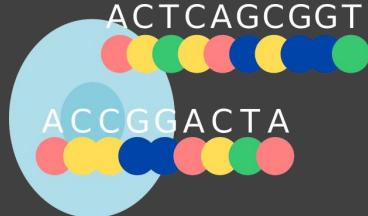
# *in situ* sequencing



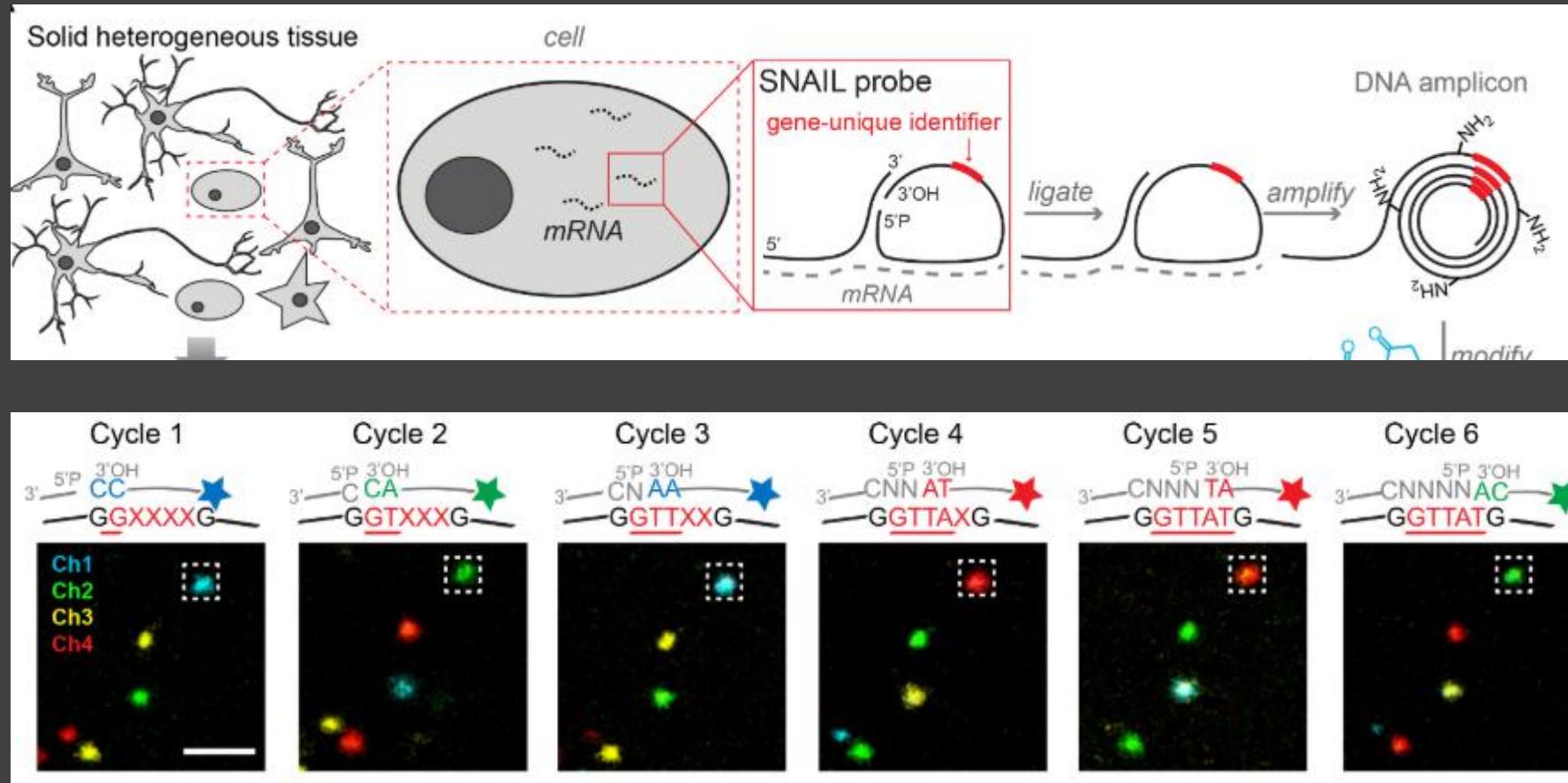
# FISSEQ



Lee *et al.* Science 2014

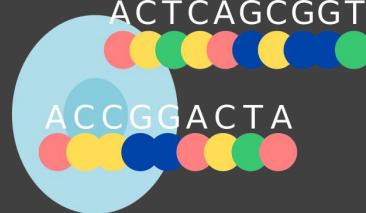
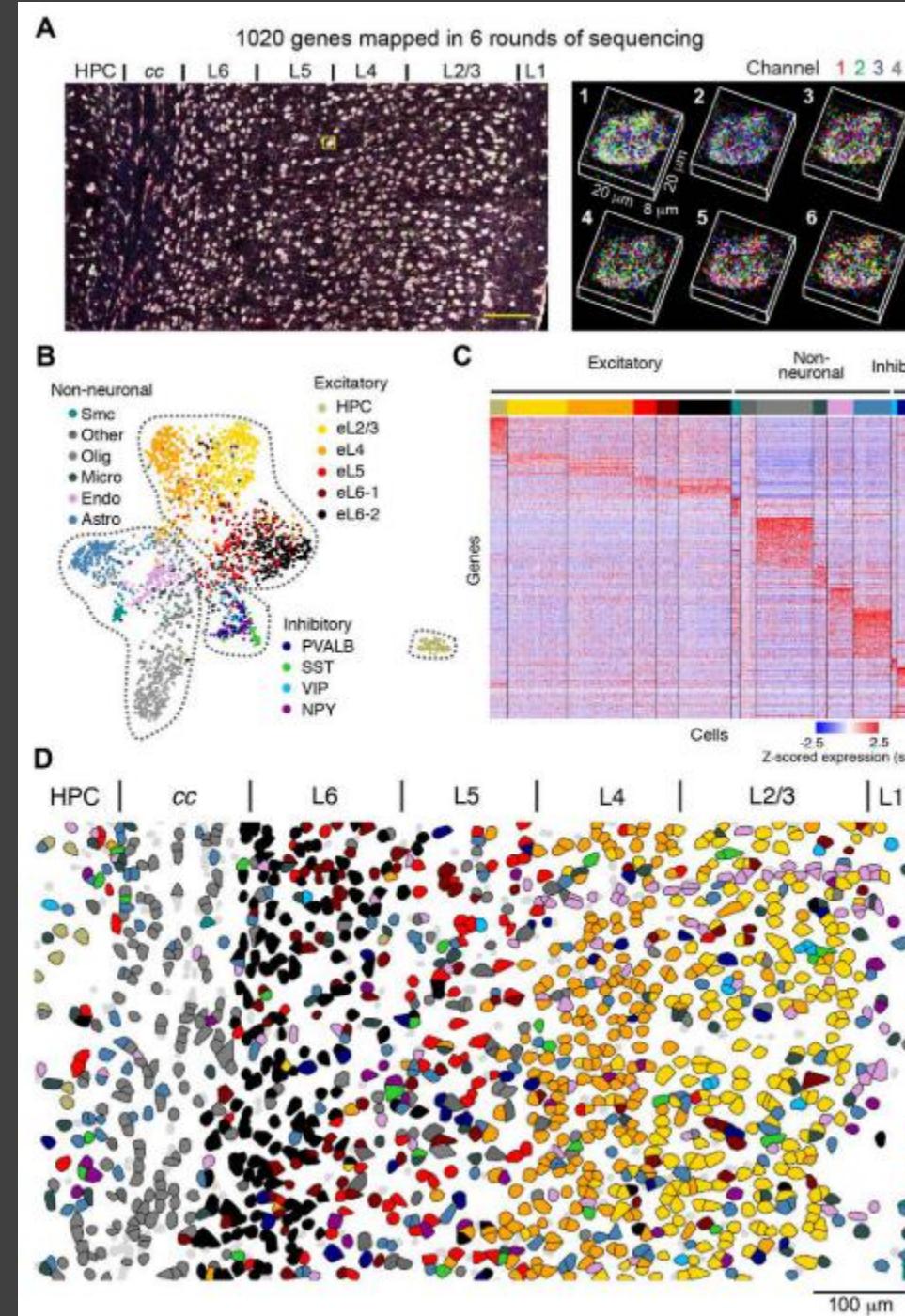


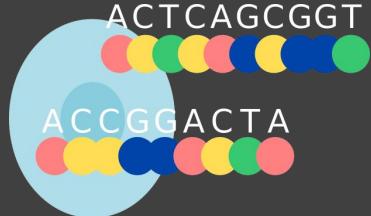
# STAR MAP



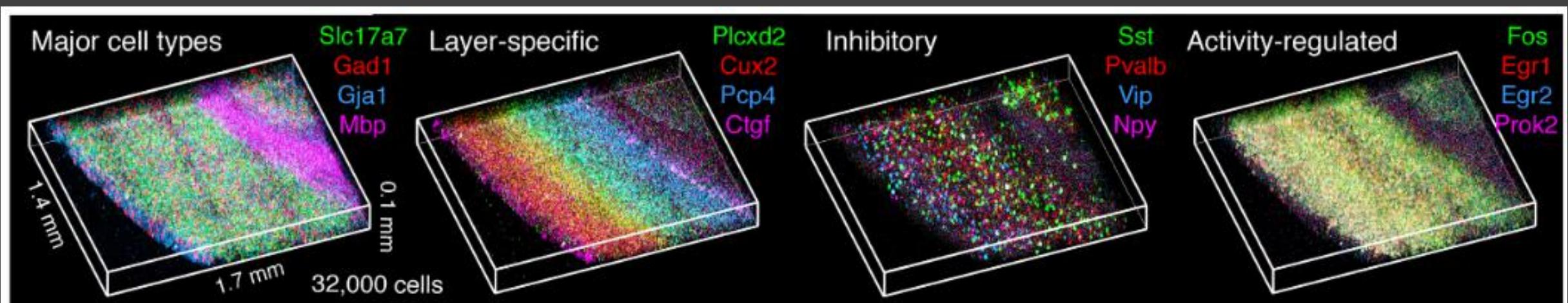
Wang *et al.* Science 2018

# STAR MAP

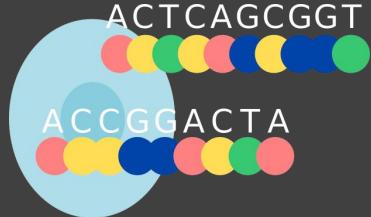




# STAR MAP



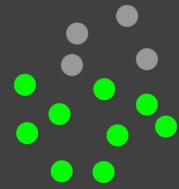
Wang *et al.* Science 2018



# Sequencing *in situ*



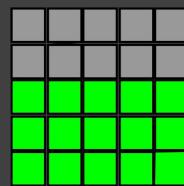
Resolution: Amplicon size (0.5 - 1 $\mu$ m)



Detection efficiency: 0.01 - ~50%

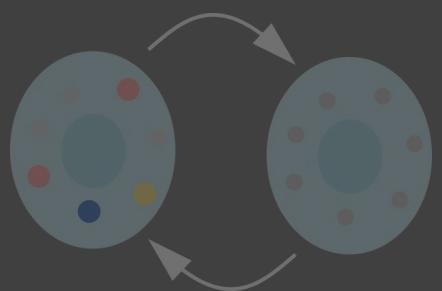


Gene throughput: 10 - 1,000

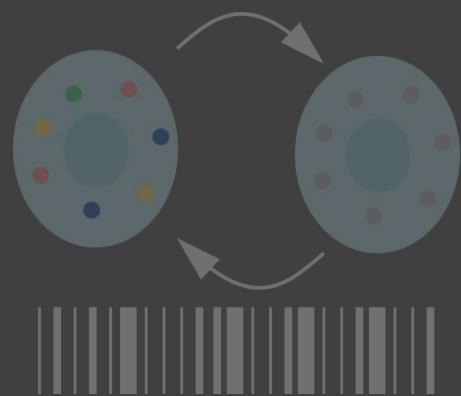


Spatial throughput: several mm $^2$  - several cm $^2$

Cyclic FISH



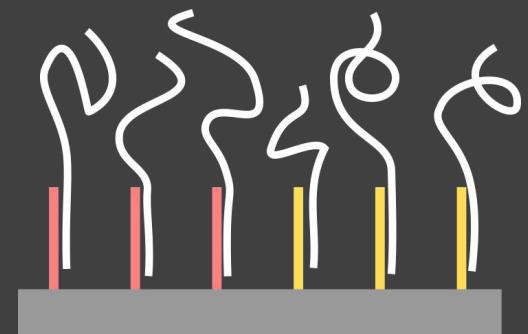
Barcoded FISH



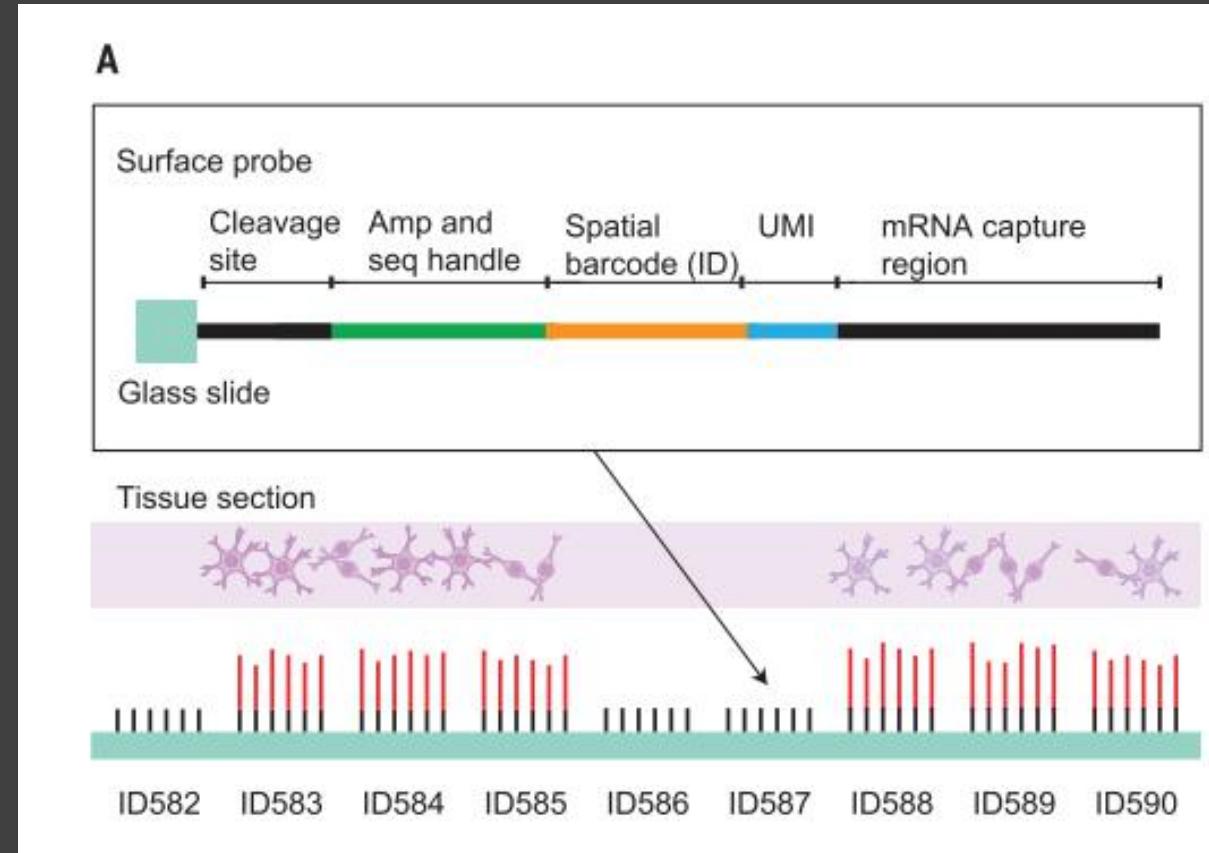
*in situ* Sequencing



Spatial Sequencing

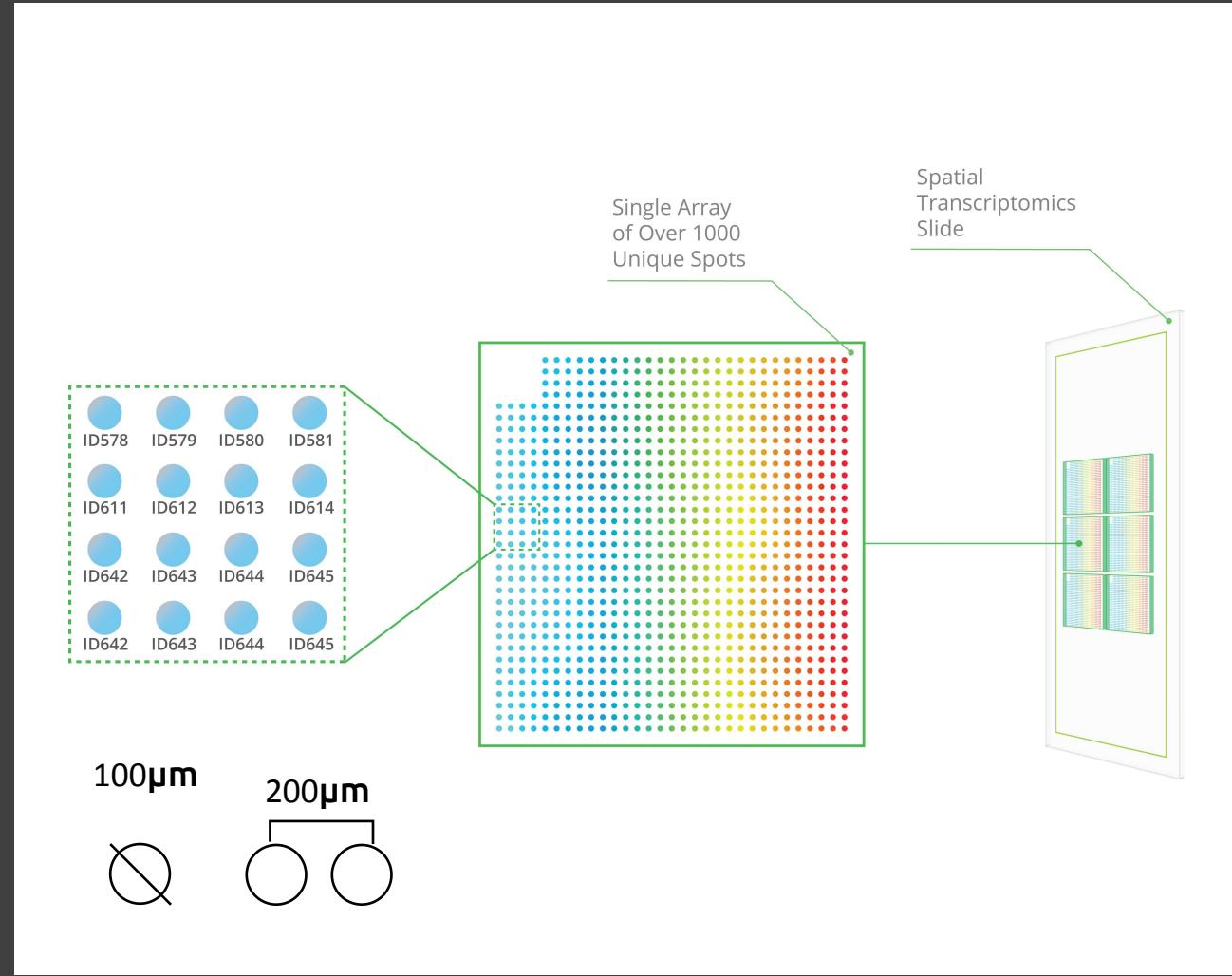
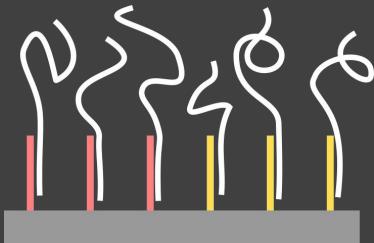


# Spatial transcriptomics

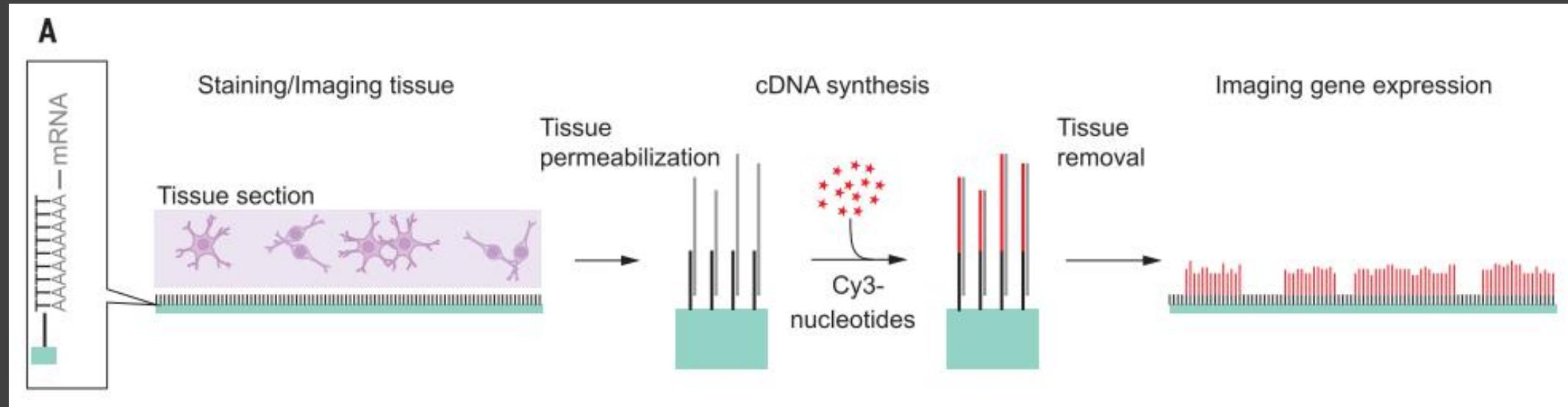


Stahl *et al.* Science 2016

# Spatial transcriptomics

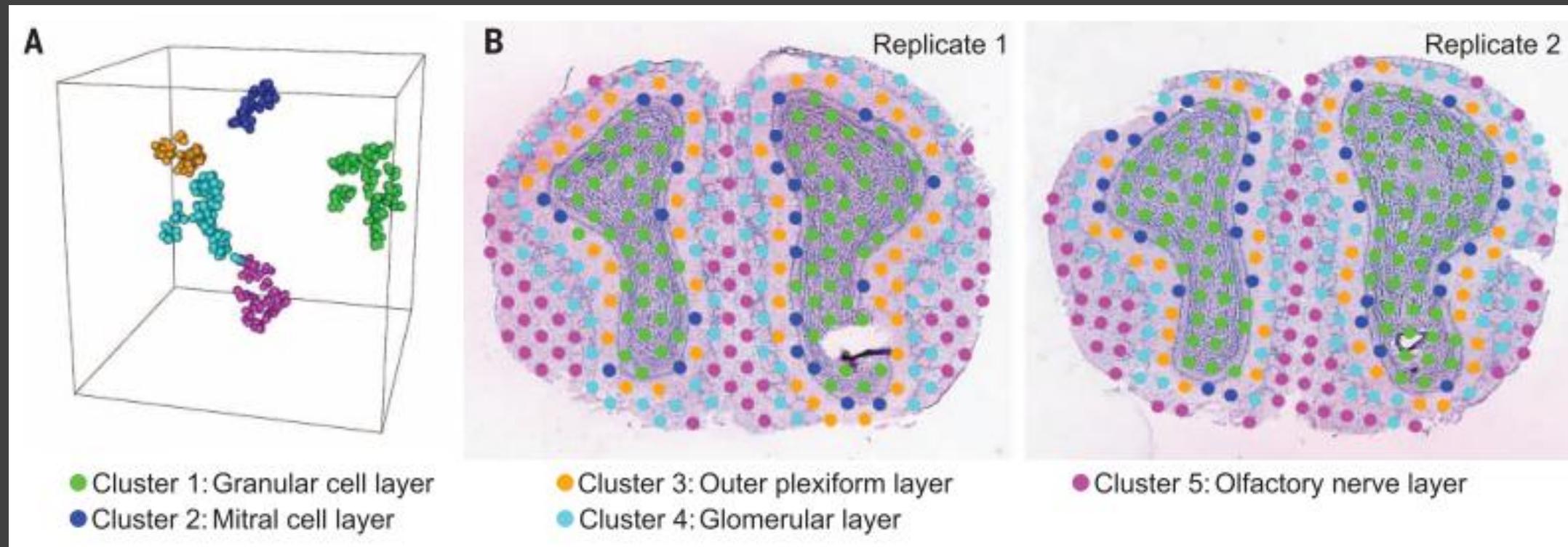
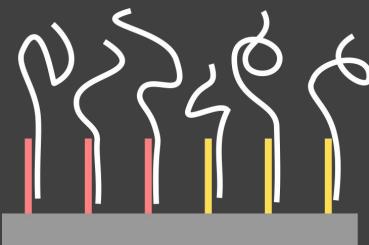


# Spatial transcriptomics



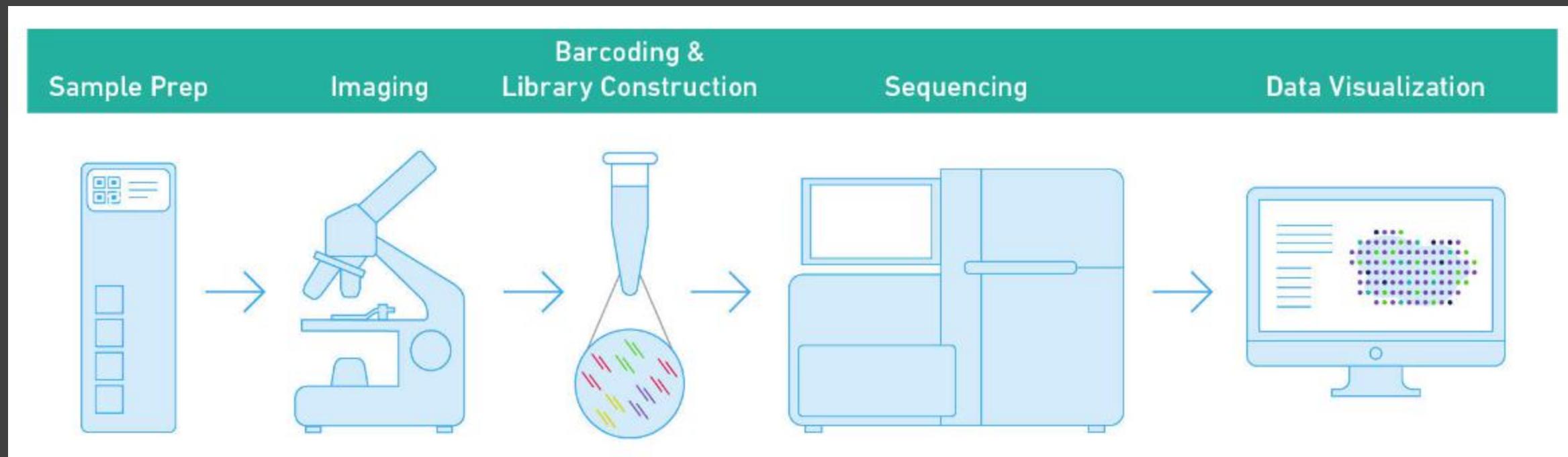
Stahl *et al.* Science 2016

# Spatial transcriptomics



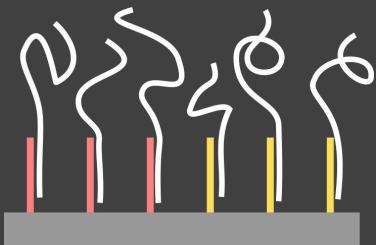
Stahl *et al.* Science 2016

# 10X Visium

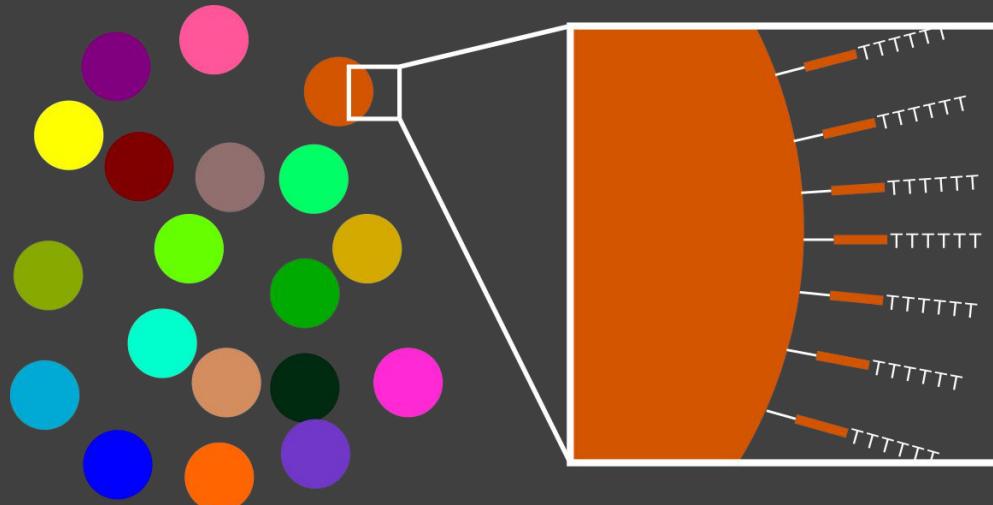


10X Visium

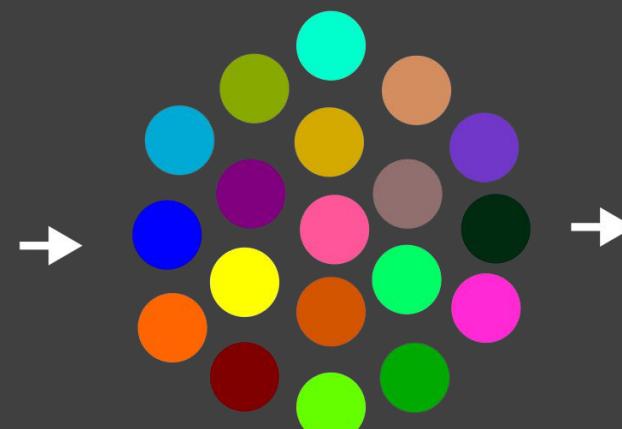
# Array of barcoded beads



Random barcoded beads



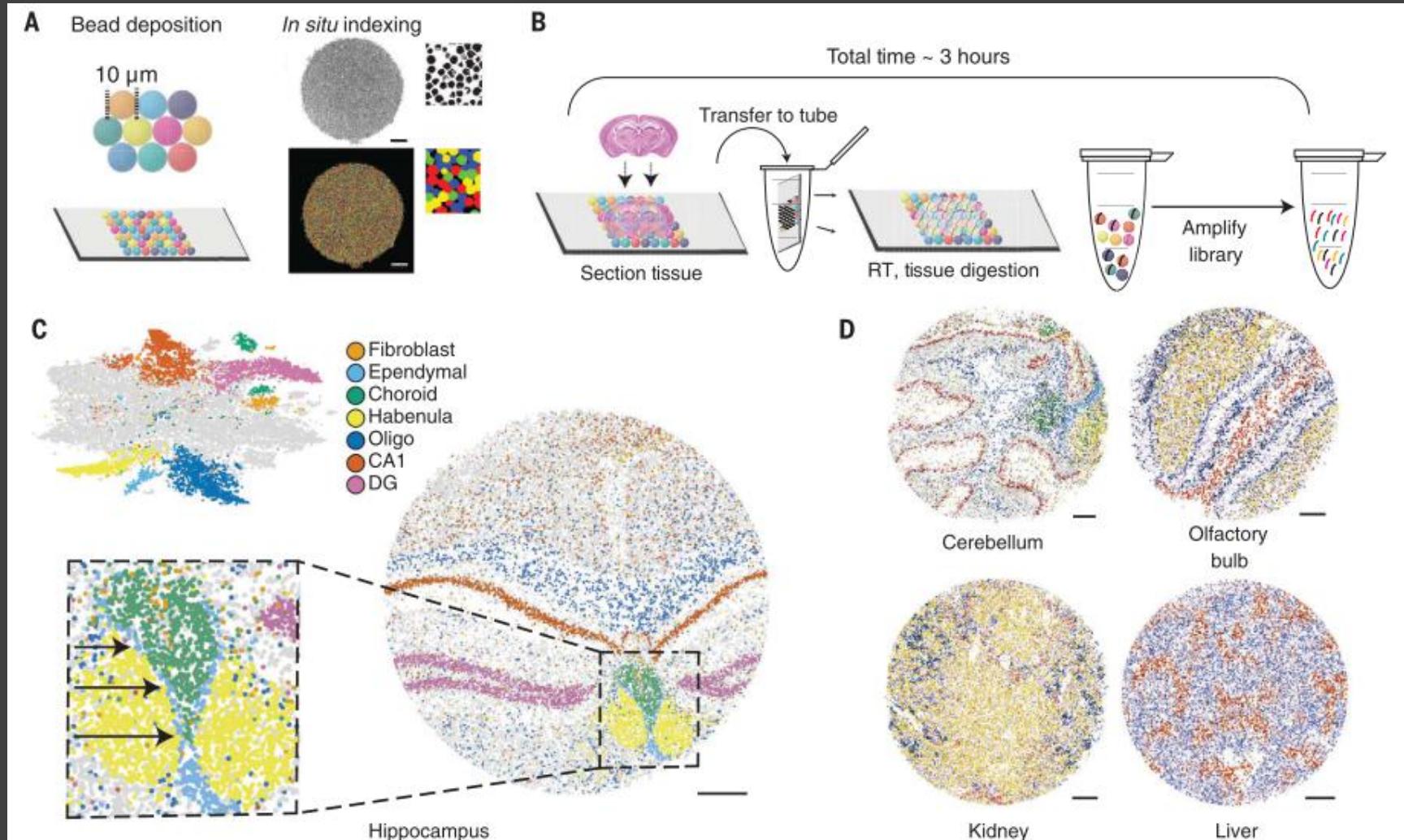
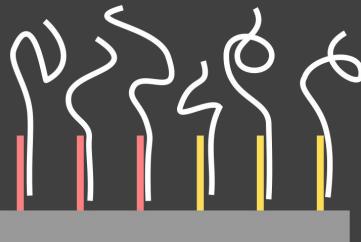
Array



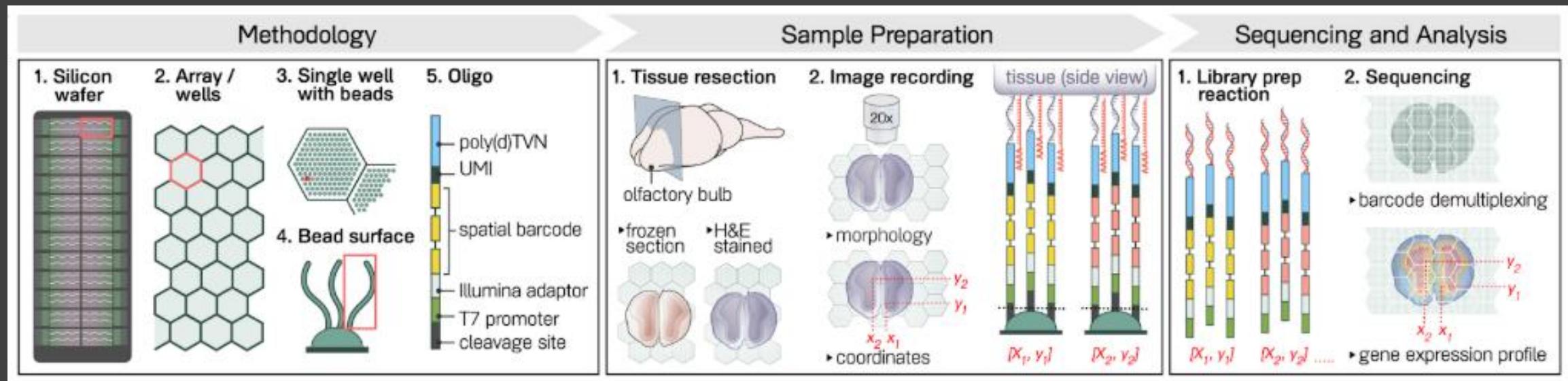
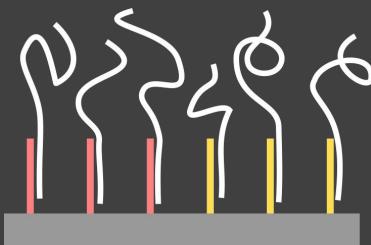
Decoded array

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

# Slide-Seq



# High density Spatial transcriptomics

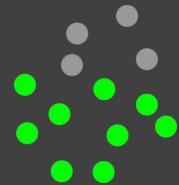


Vickovic *et al.* BioRxiv 2019

# Spatial Sequencing



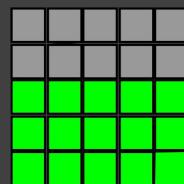
Resolution: Spot size 2um - 100um



Detection efficiency: 0.1 - 1%



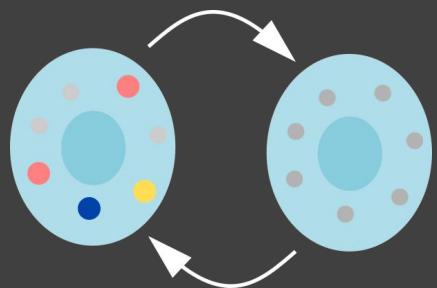
Gene throughput: Full transcriptome



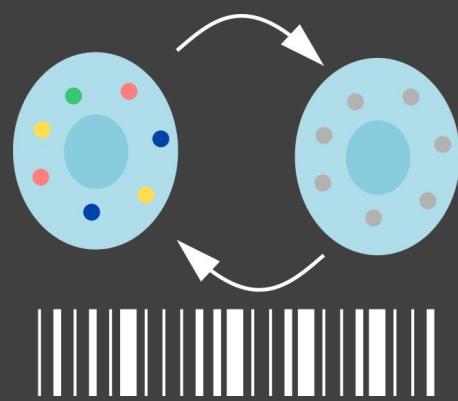
Spatial throughput: several cm<sup>2</sup>

# RNA spatial detection

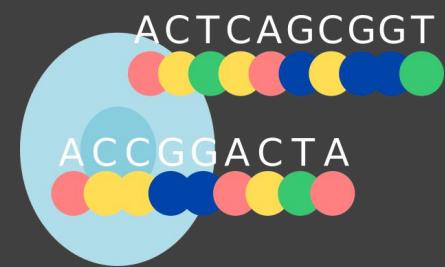
Cyclic FISH



Barcoded FISH



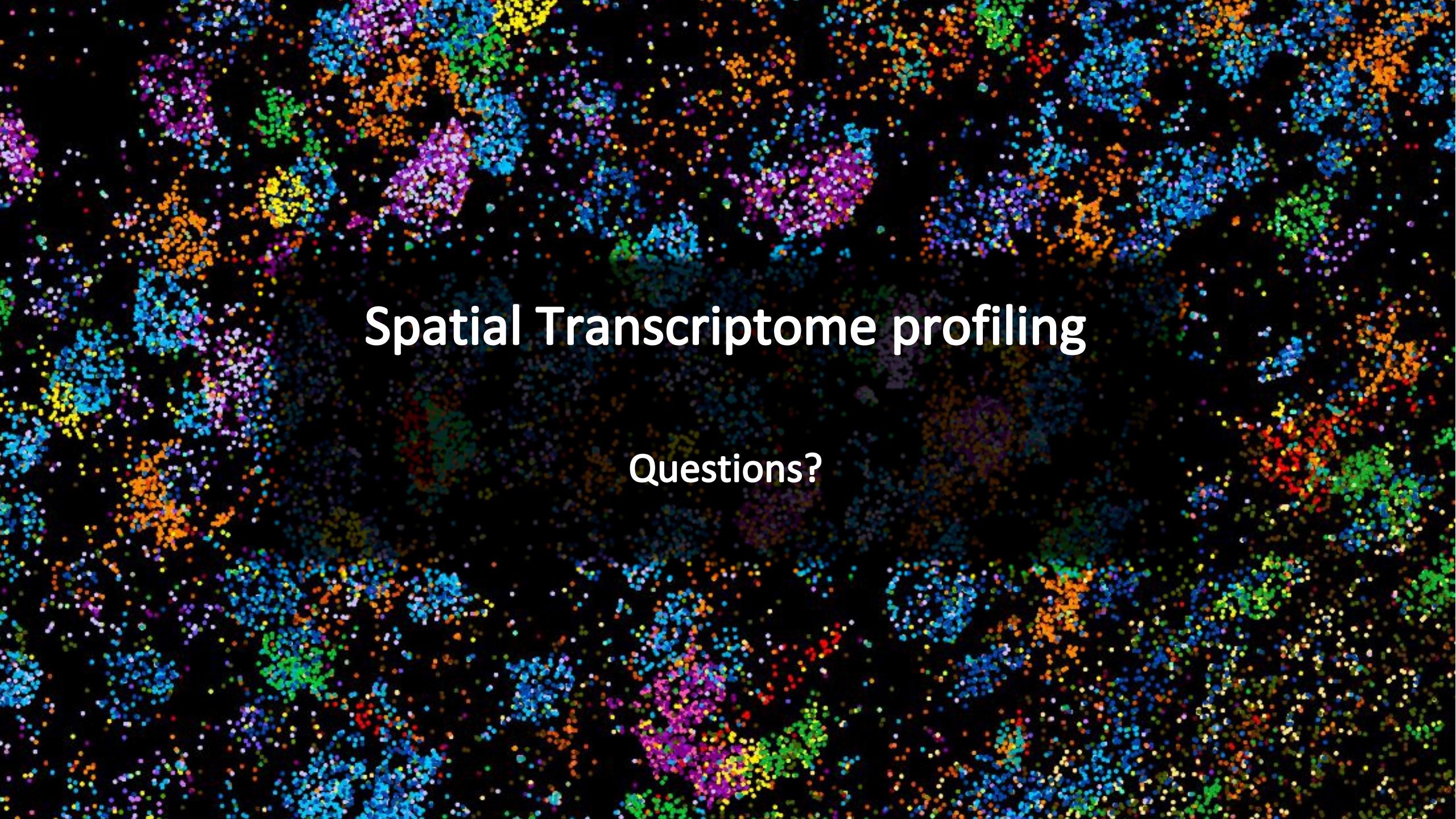
*in situ* Sequencing



Spatial Sequencing



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



# Spatial Transcriptome profiling

Questions?

