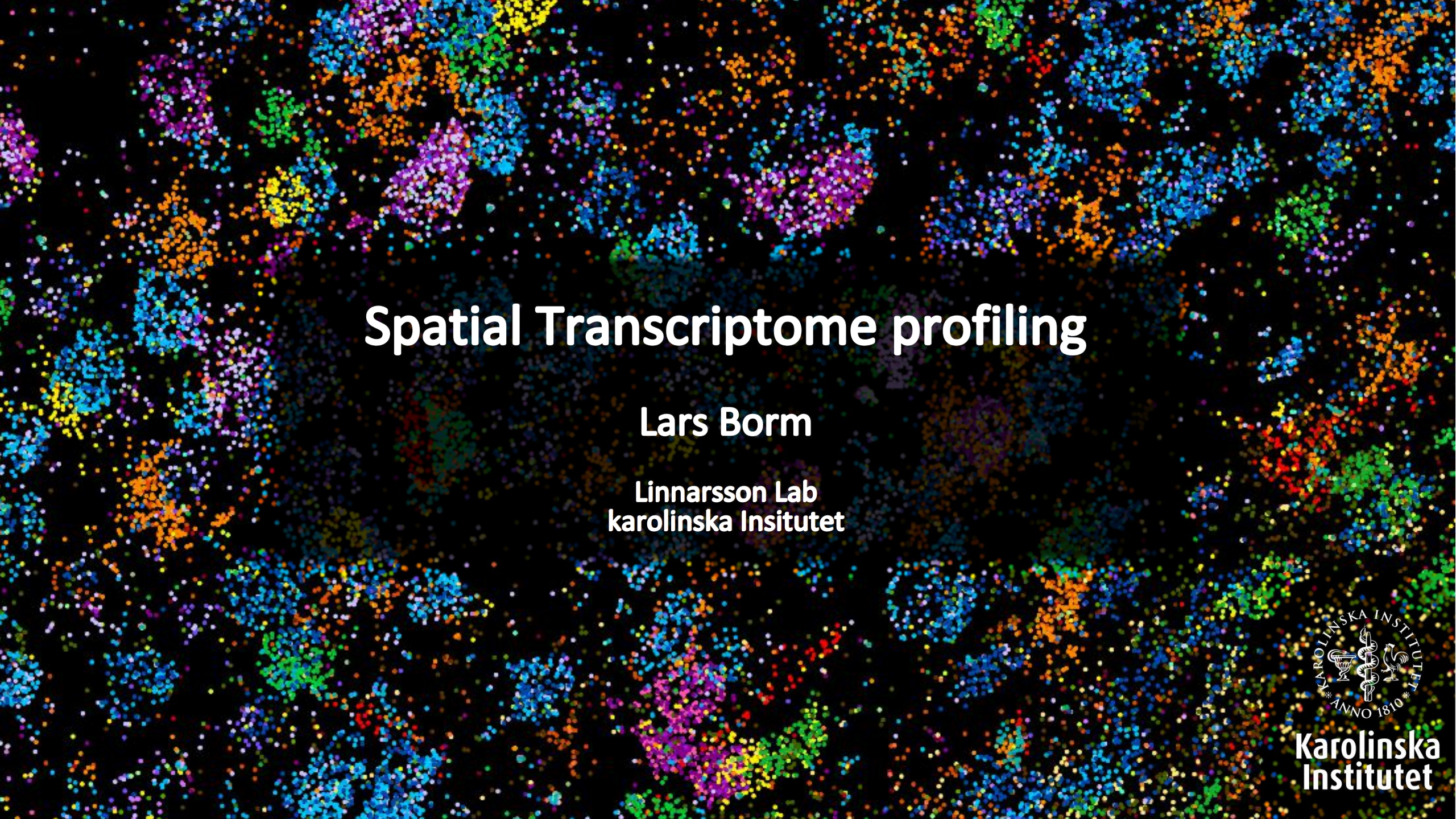


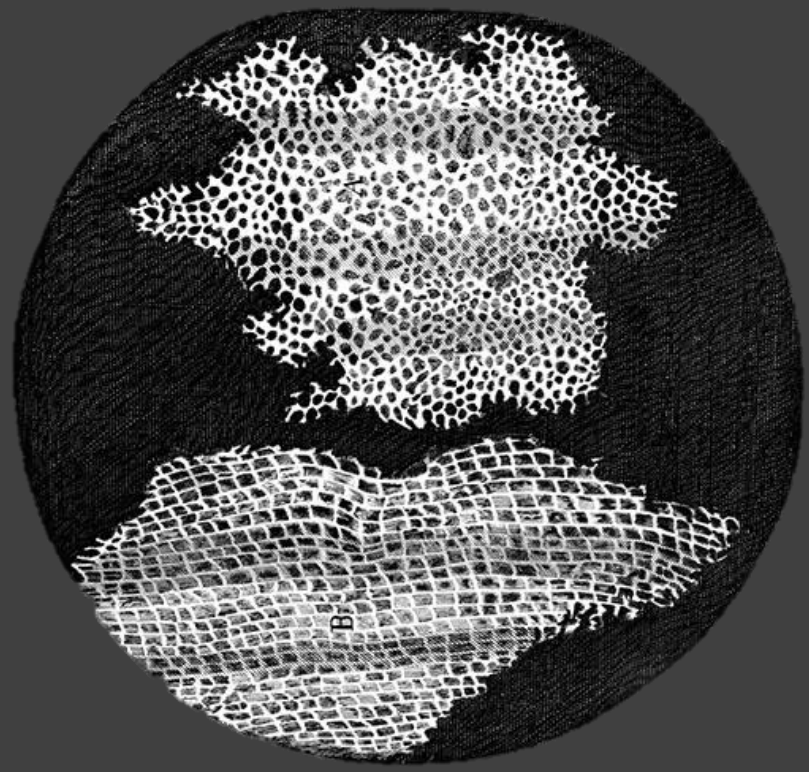
Spatial Transcriptome profiling

Lars Borm

Linnarsson Lab
karolinska Insitutet

Karolinska Institutet

Single cell biology



Robert Hooke 1665

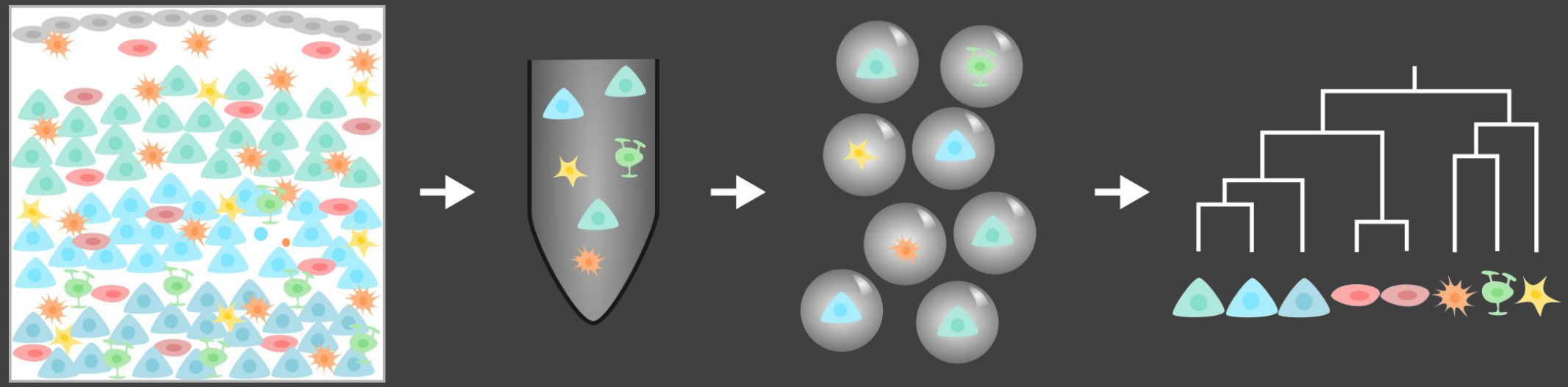
Single Cell RNA sequencing

Tissue

Dissociation

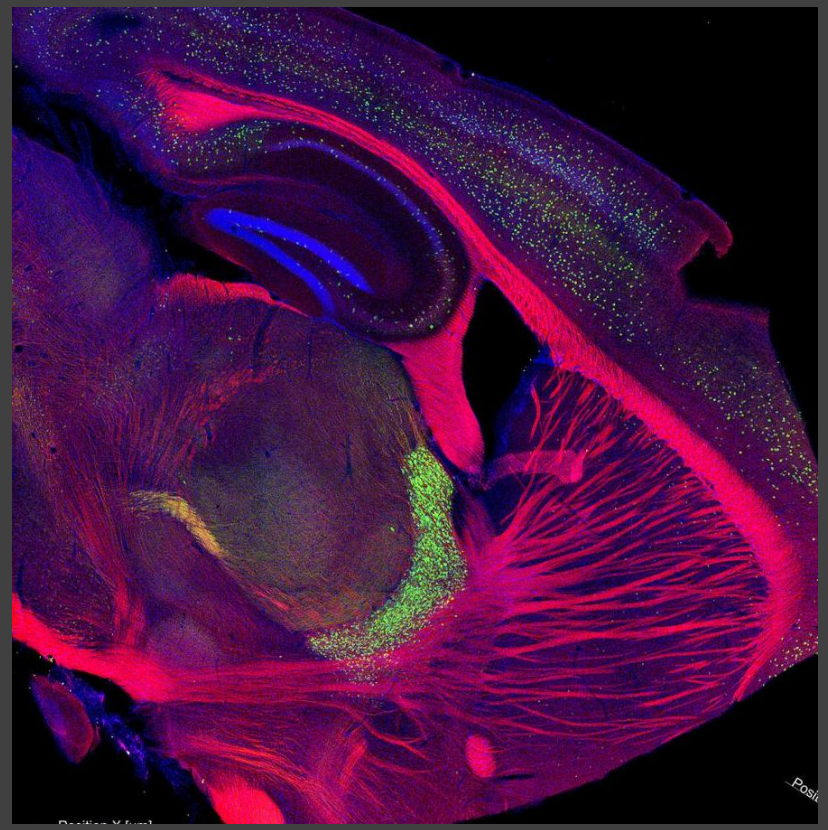
compartmentalize

Sequencing & Analysis



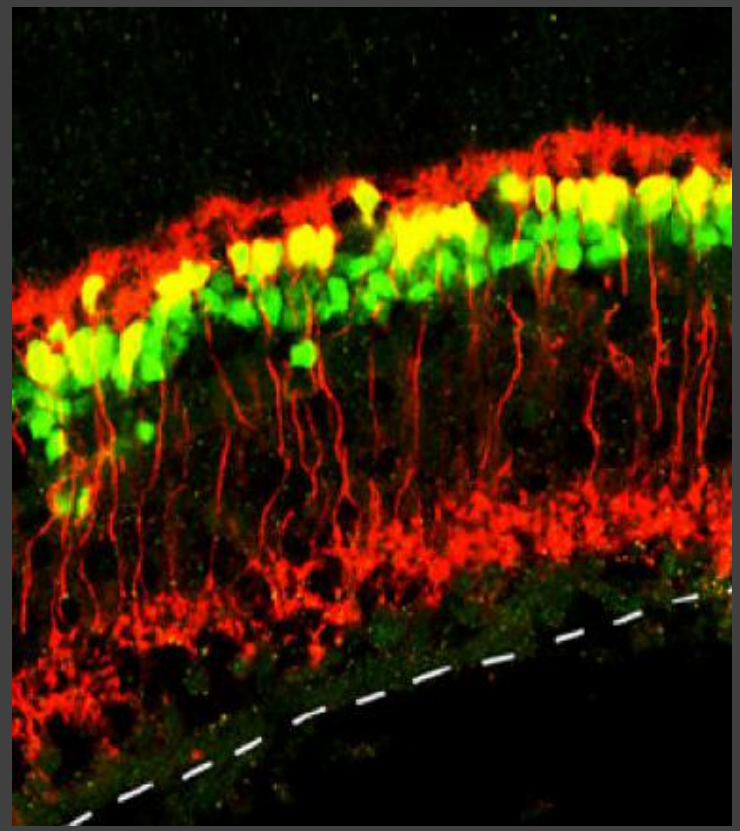
Tissue architecture

Brain



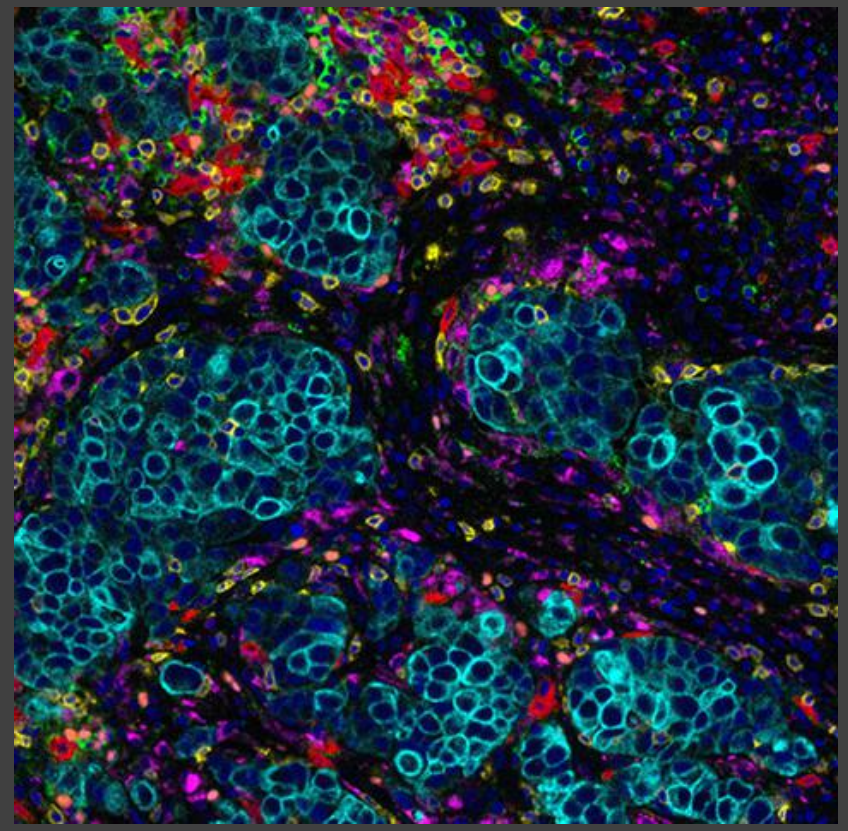
IGB Illinois

Retina



Morrow *et al.* 2008

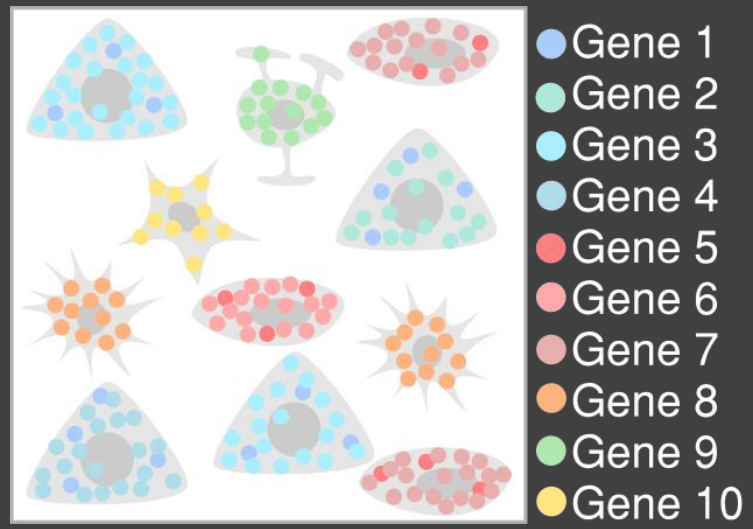
Cancer



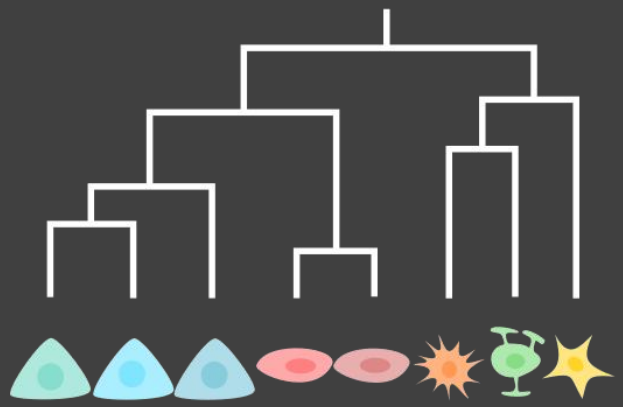
Perkin Elmer

Detecting RNA in tissue

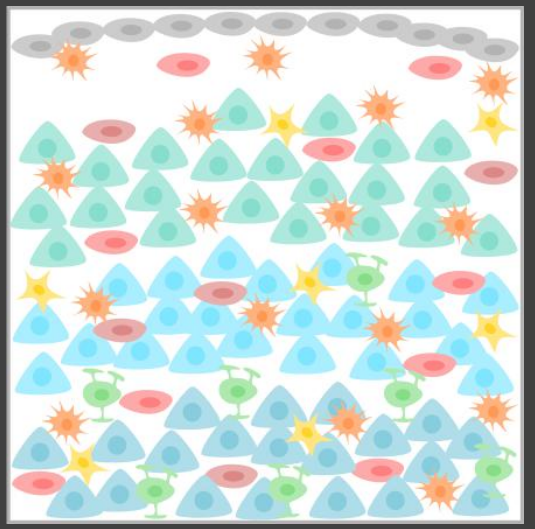
RNA detection *in situ*



Analysis

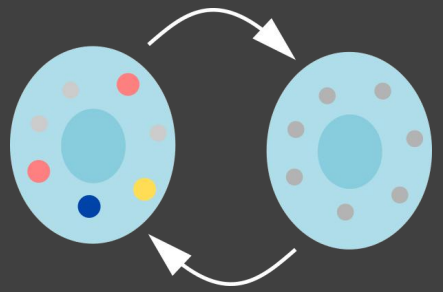


Spatial transcriptomics

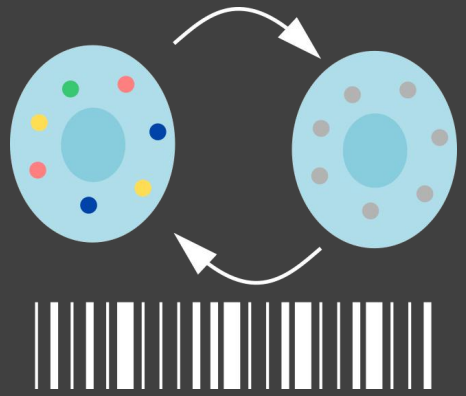


RNA spatial detection

Cyclic FISH

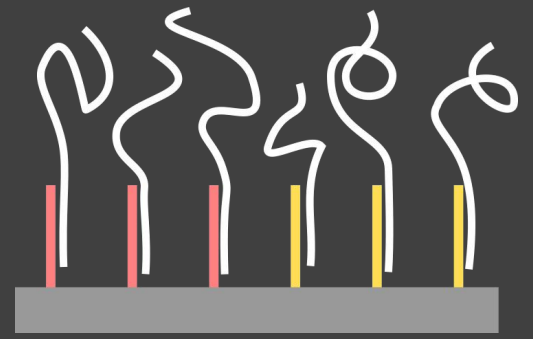


Barcoded FISH



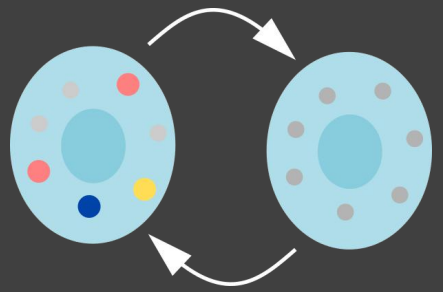
in situ Sequencing

Spatial Sequencing

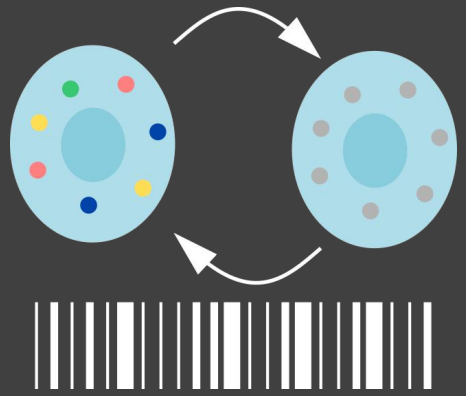


Microscopy methods

Cyclic FISH



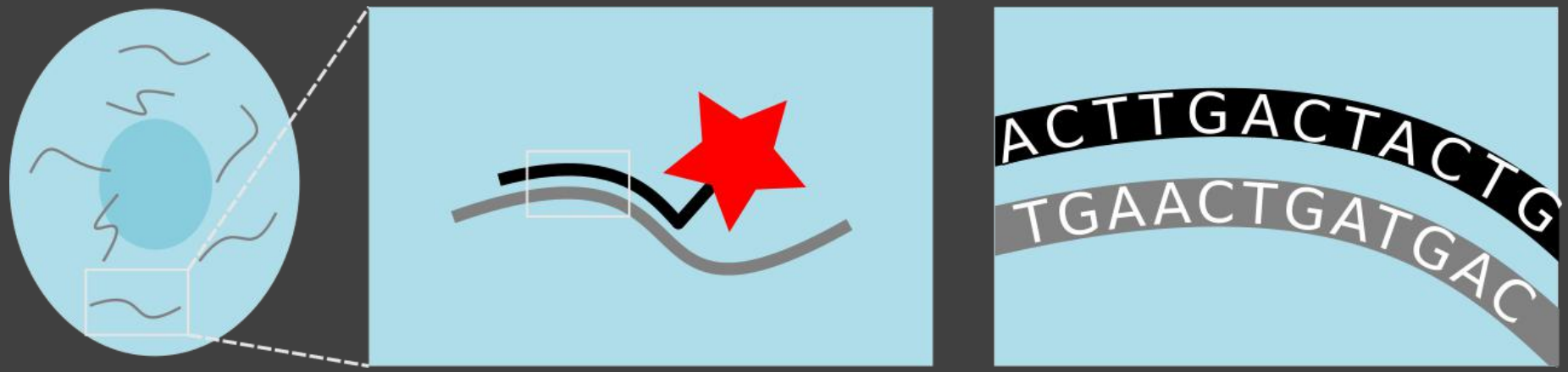
Barcoded FISH



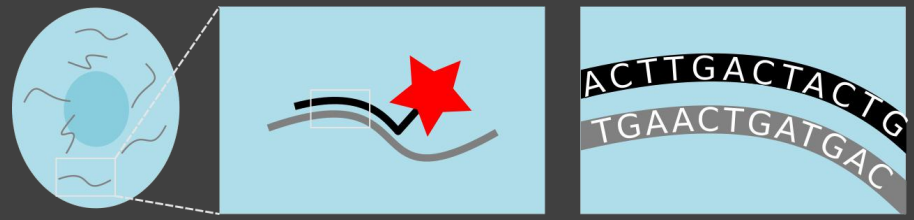
in situ Sequencing

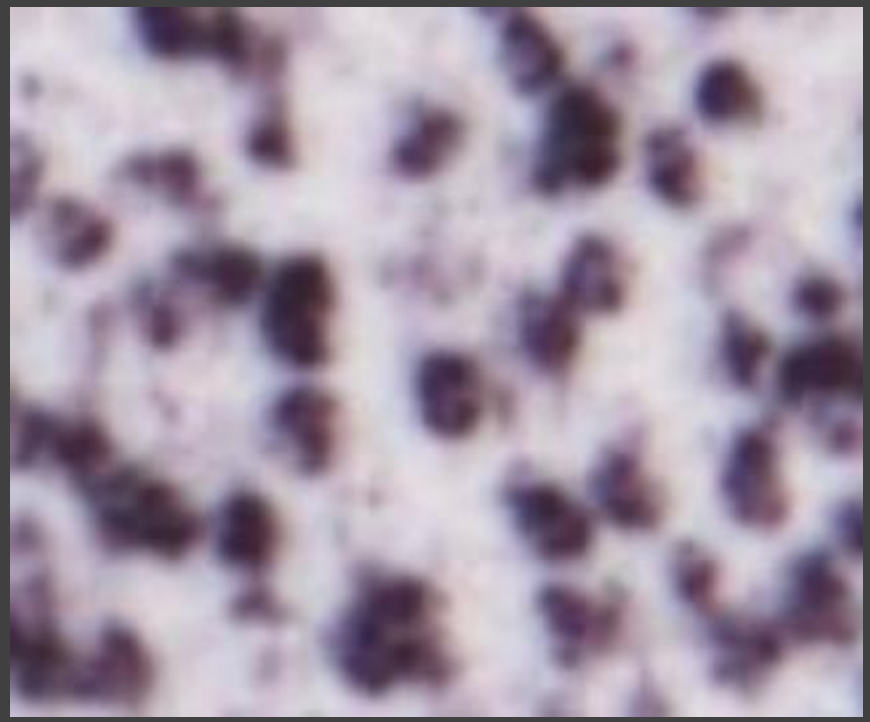
Spatial Sequencing

in situ Hybridization



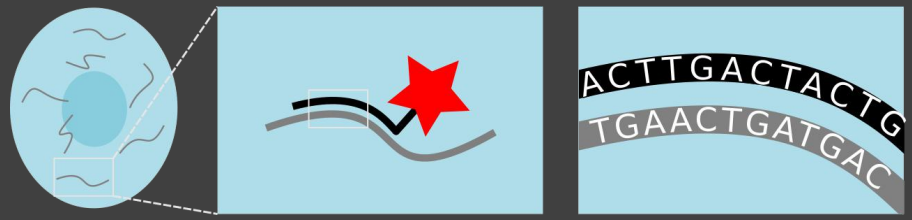
in situ Hybridization (ISH)

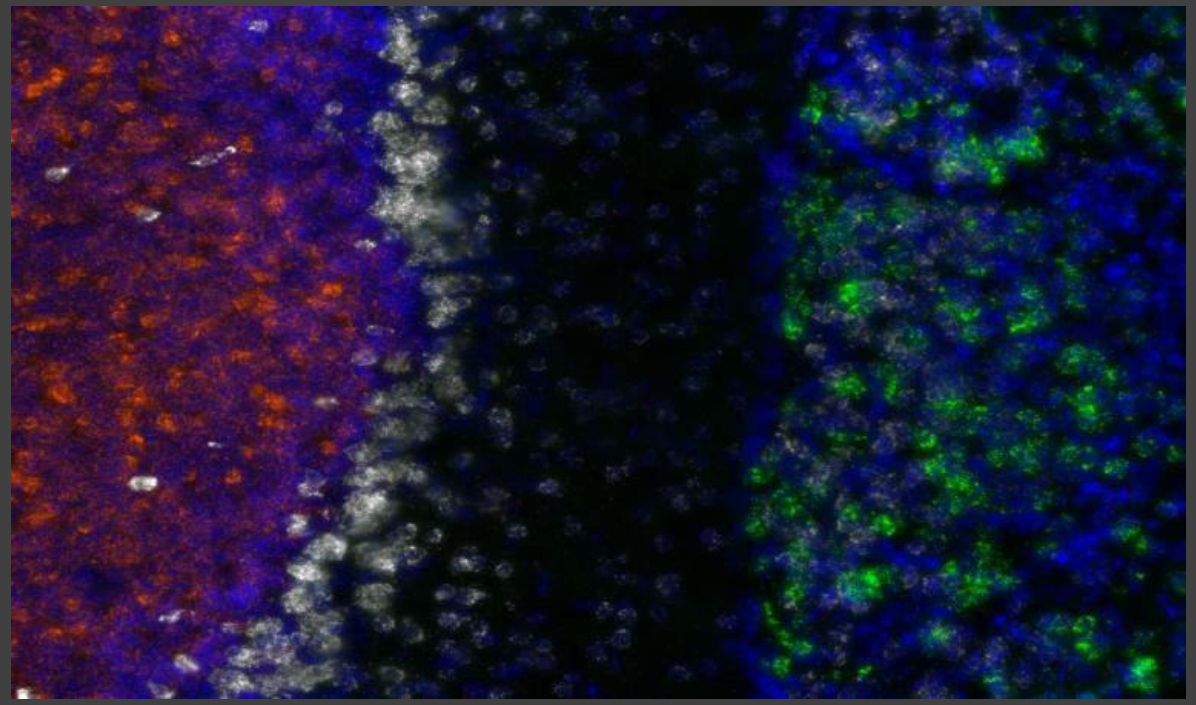




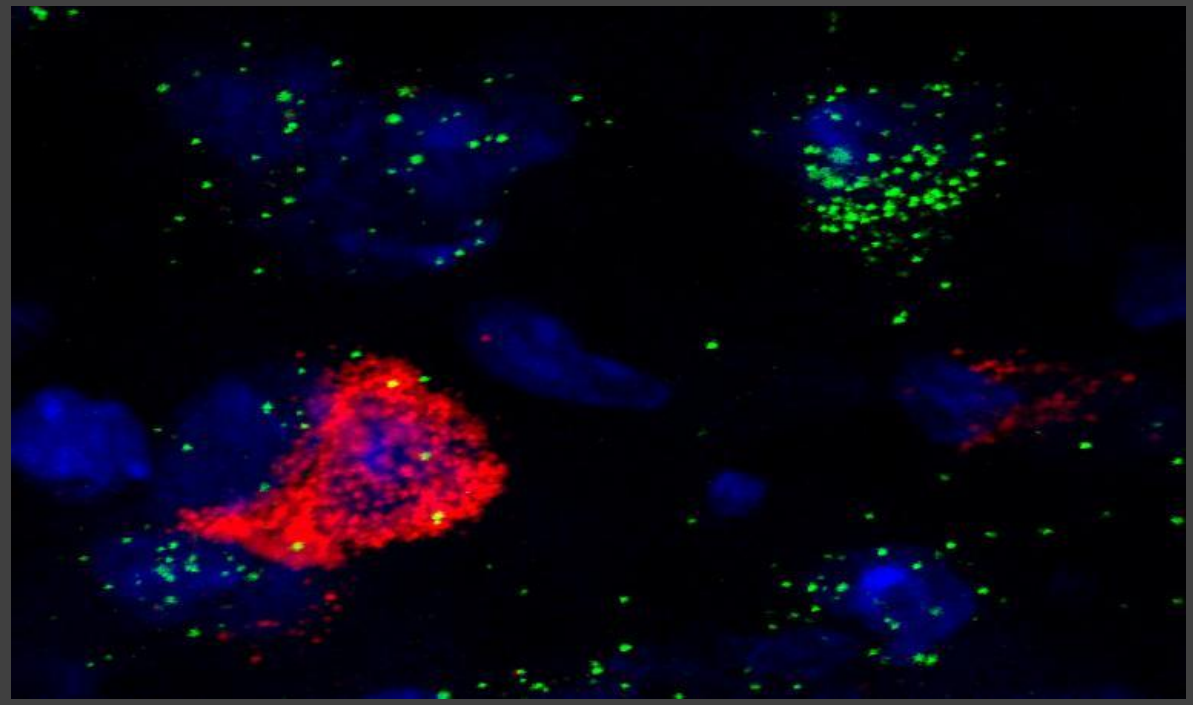
Lein *et al.* 2008

Fluorescent in situ Hybridization (FISH)



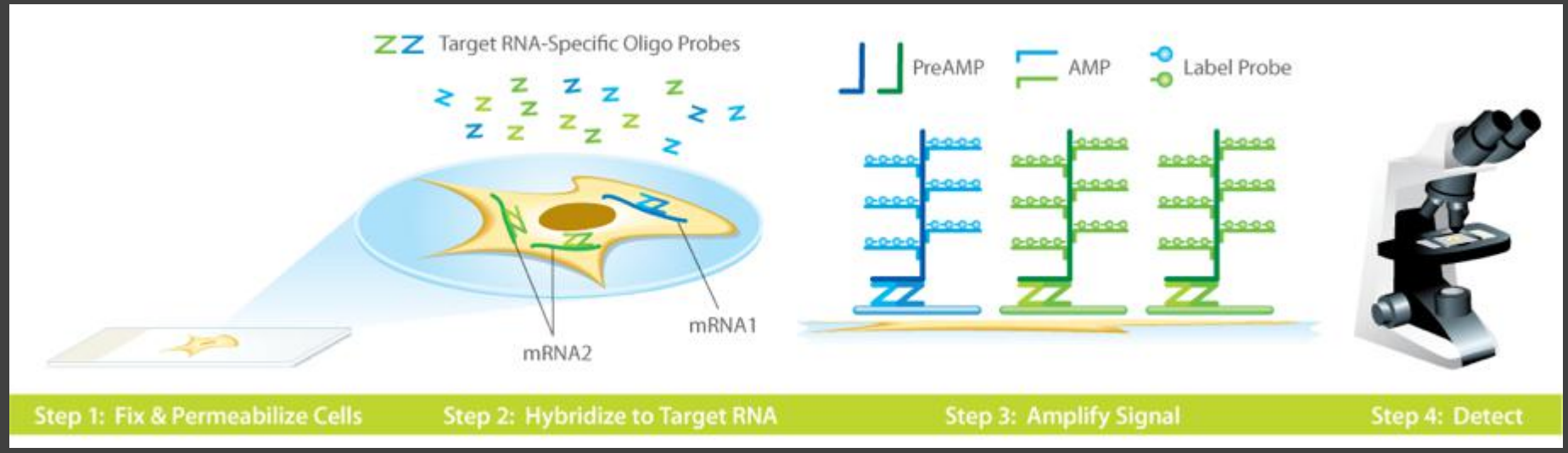


ACD Bio



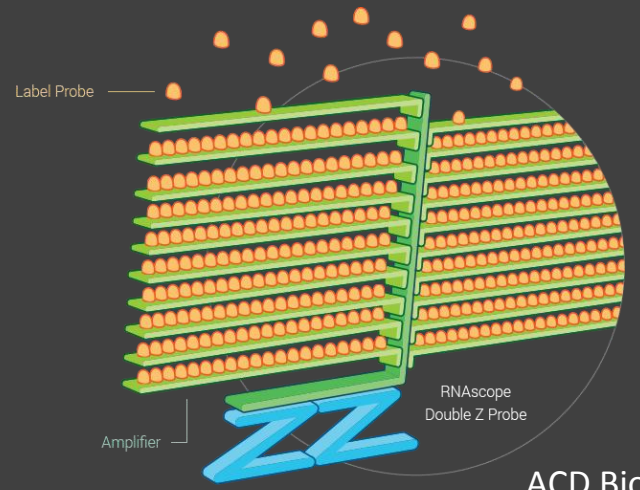
ACD Bio

RNAscope

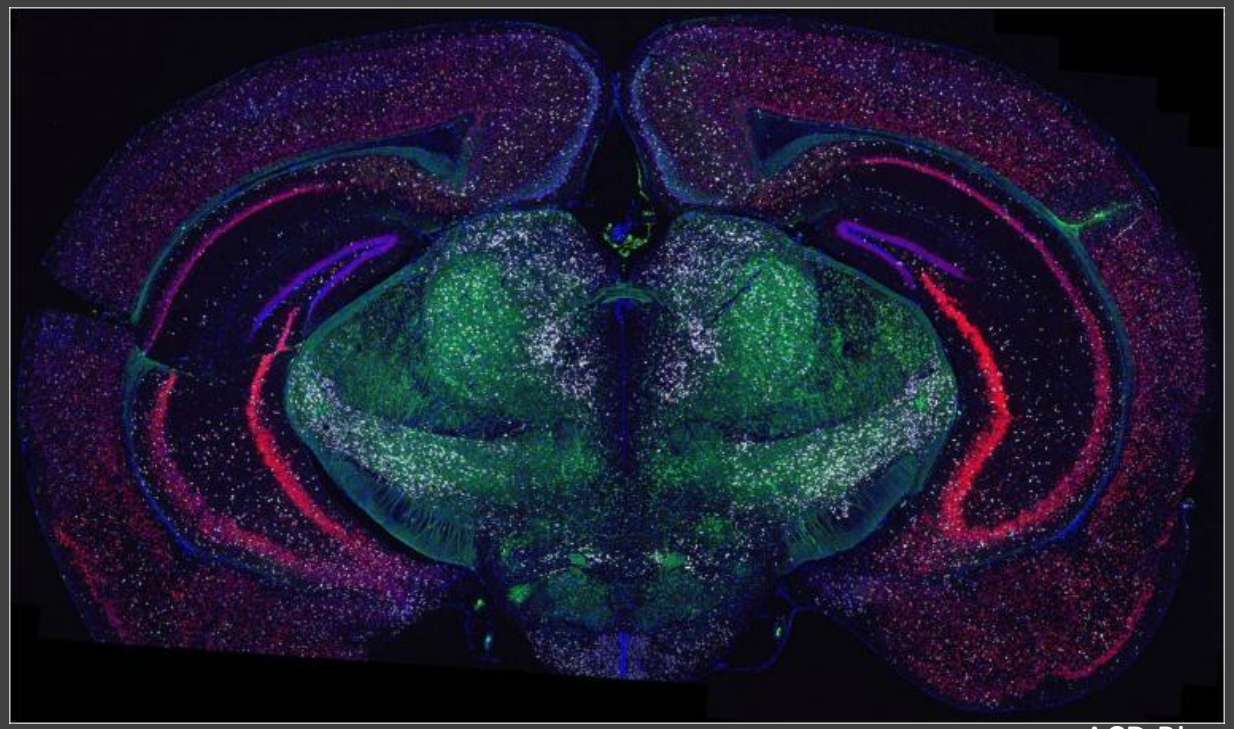


ACD Bio

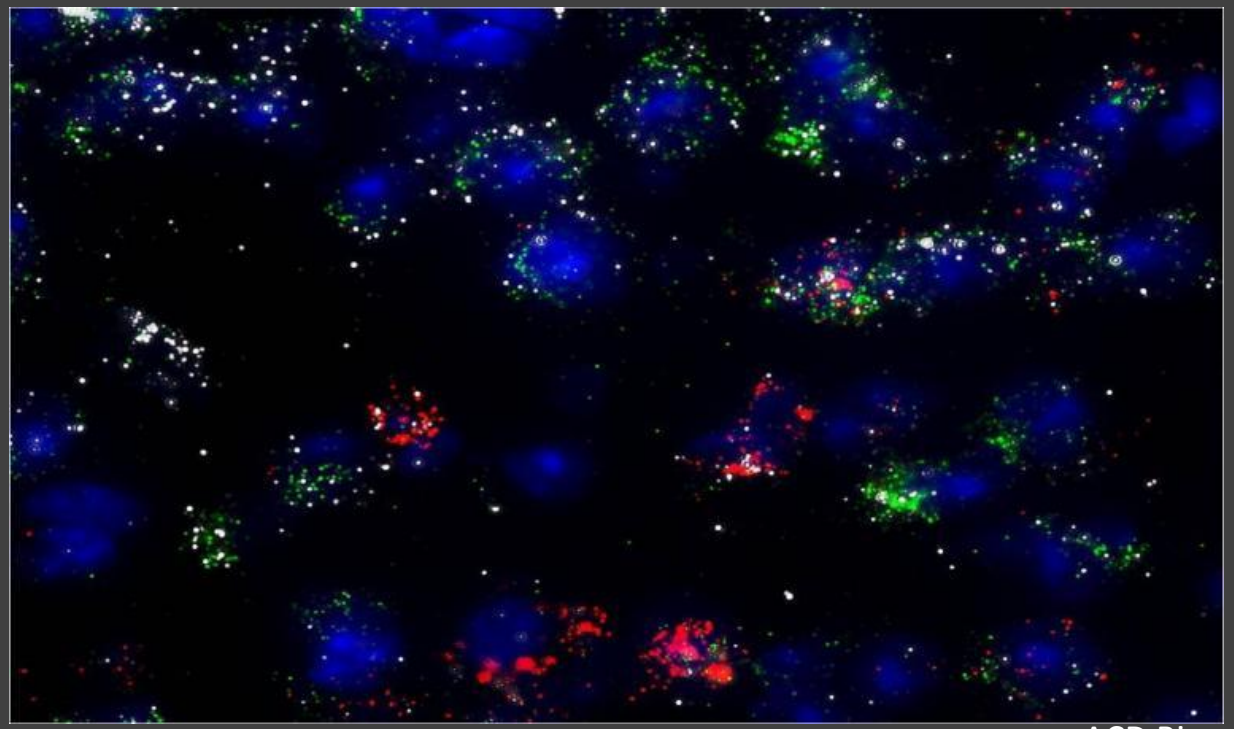
RNAscope



ACD Bio



ACD Bio



ACD Bio

single molecule FISH (smFISH)

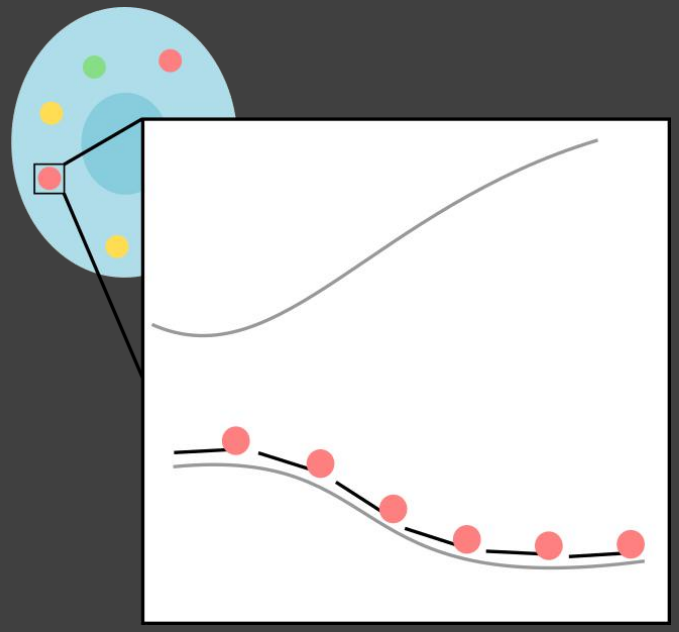
Probes

Gene 1 40X

Gene 2 40X

Gene 3 40X

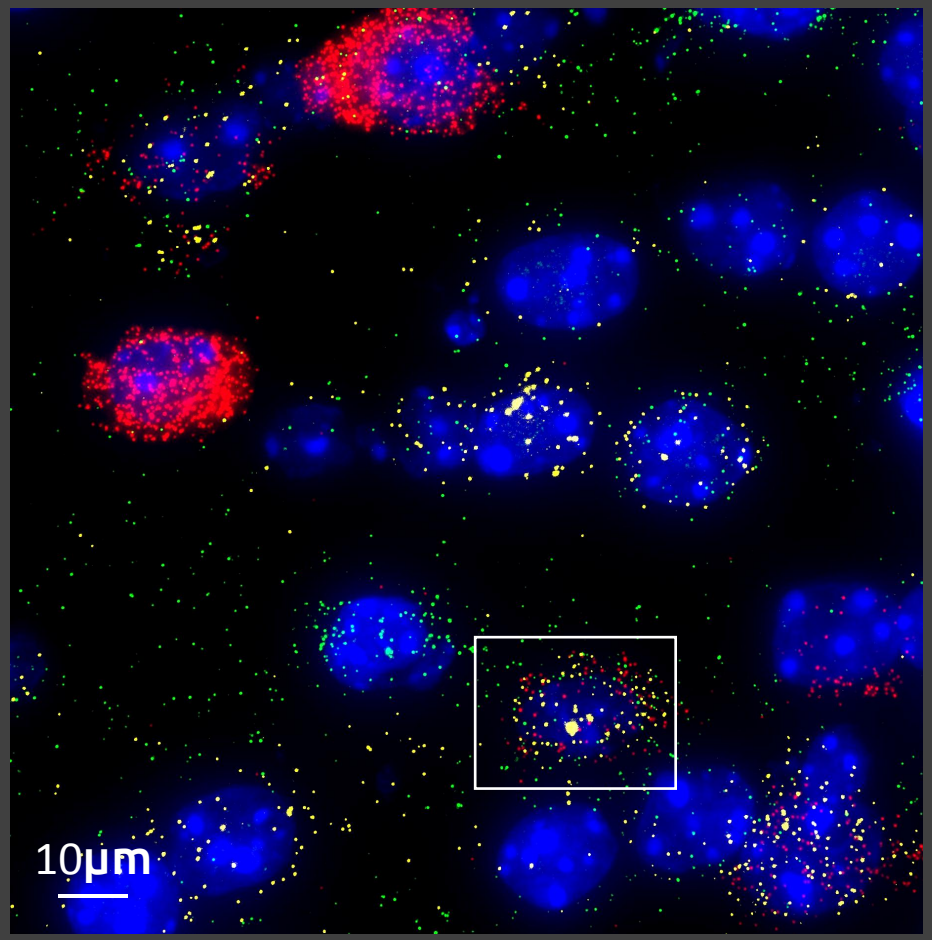
Gene 4 40X

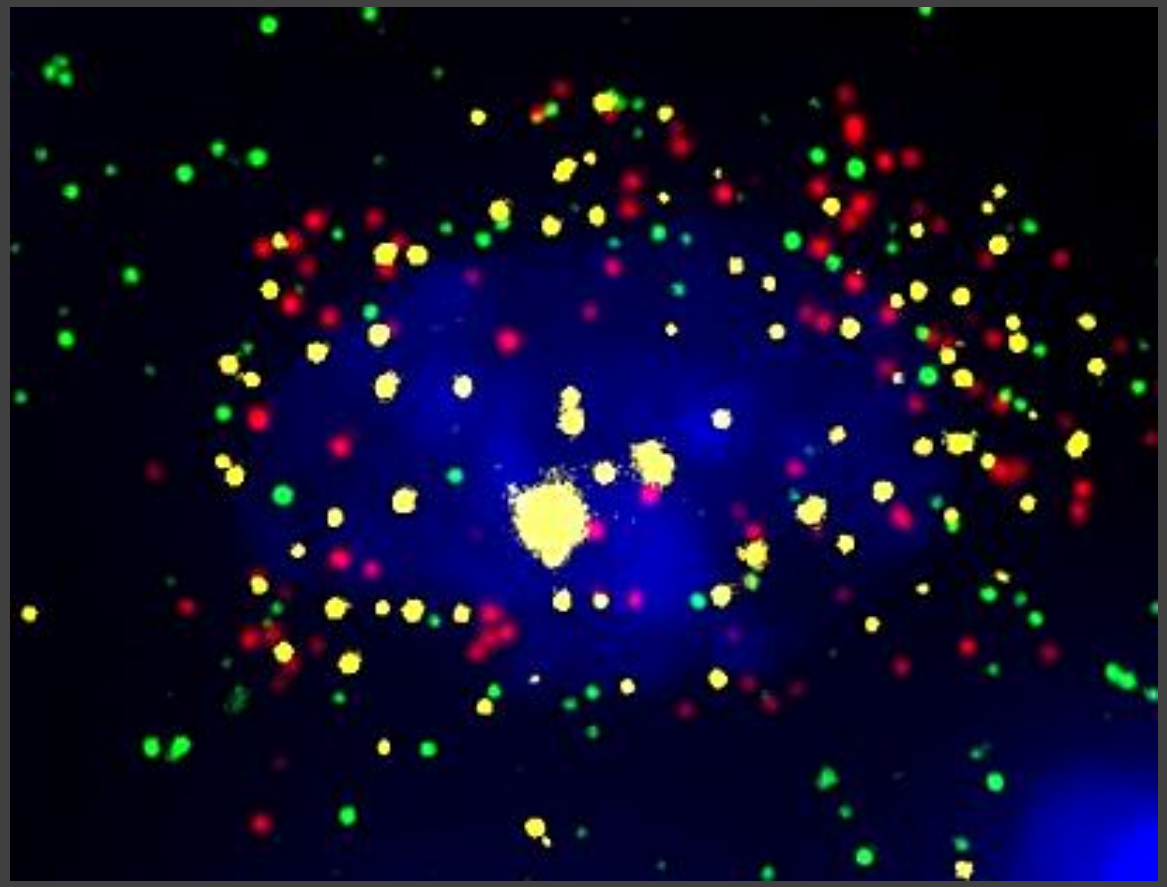


Raj *et al*. Nature Methods 2008

single molecule FISH (smFISH)

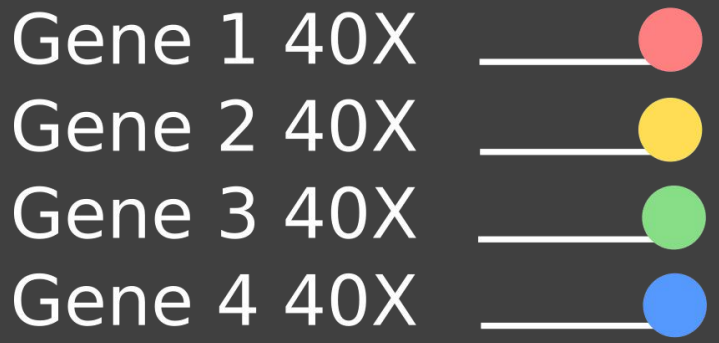
Gene 1 Gene 2 Gene 3 DNA

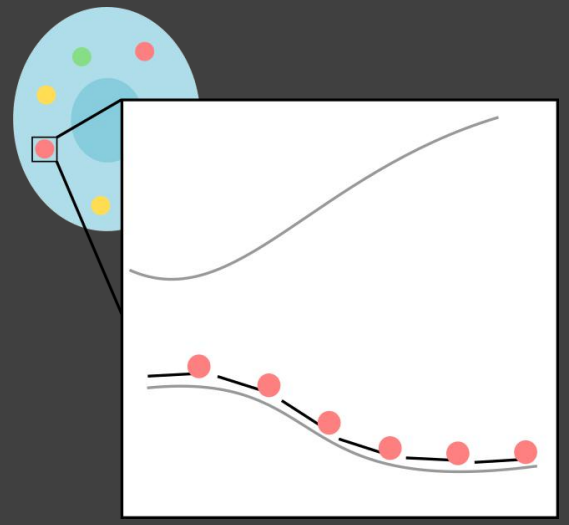




single molecule FISH (smFISH)

- High sensitivity
- Low false positives
- Low false negatives

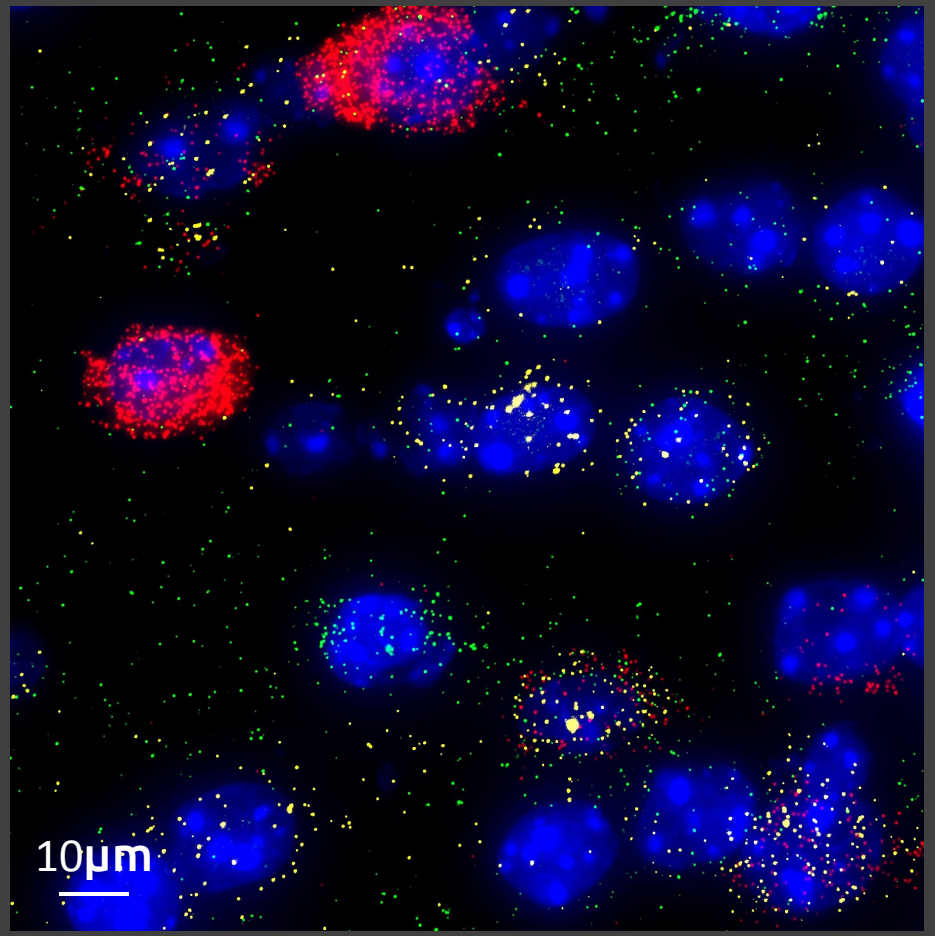




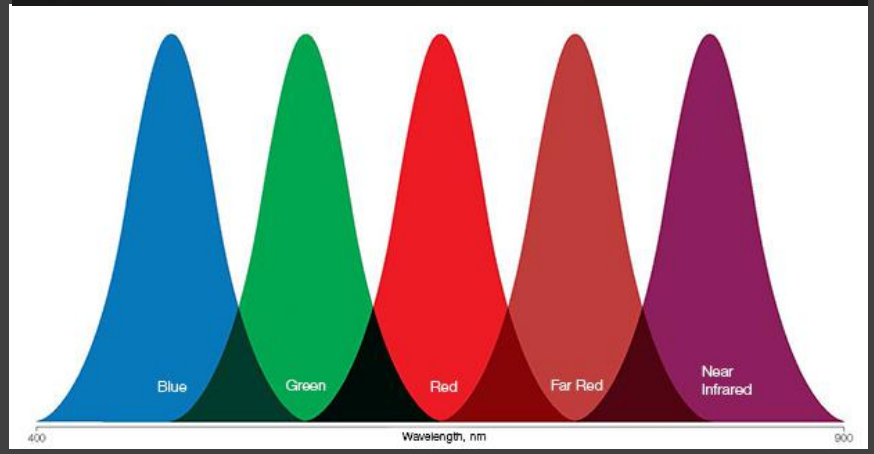
Raj *et al.* 2008

single molecule FISH (smFISH)

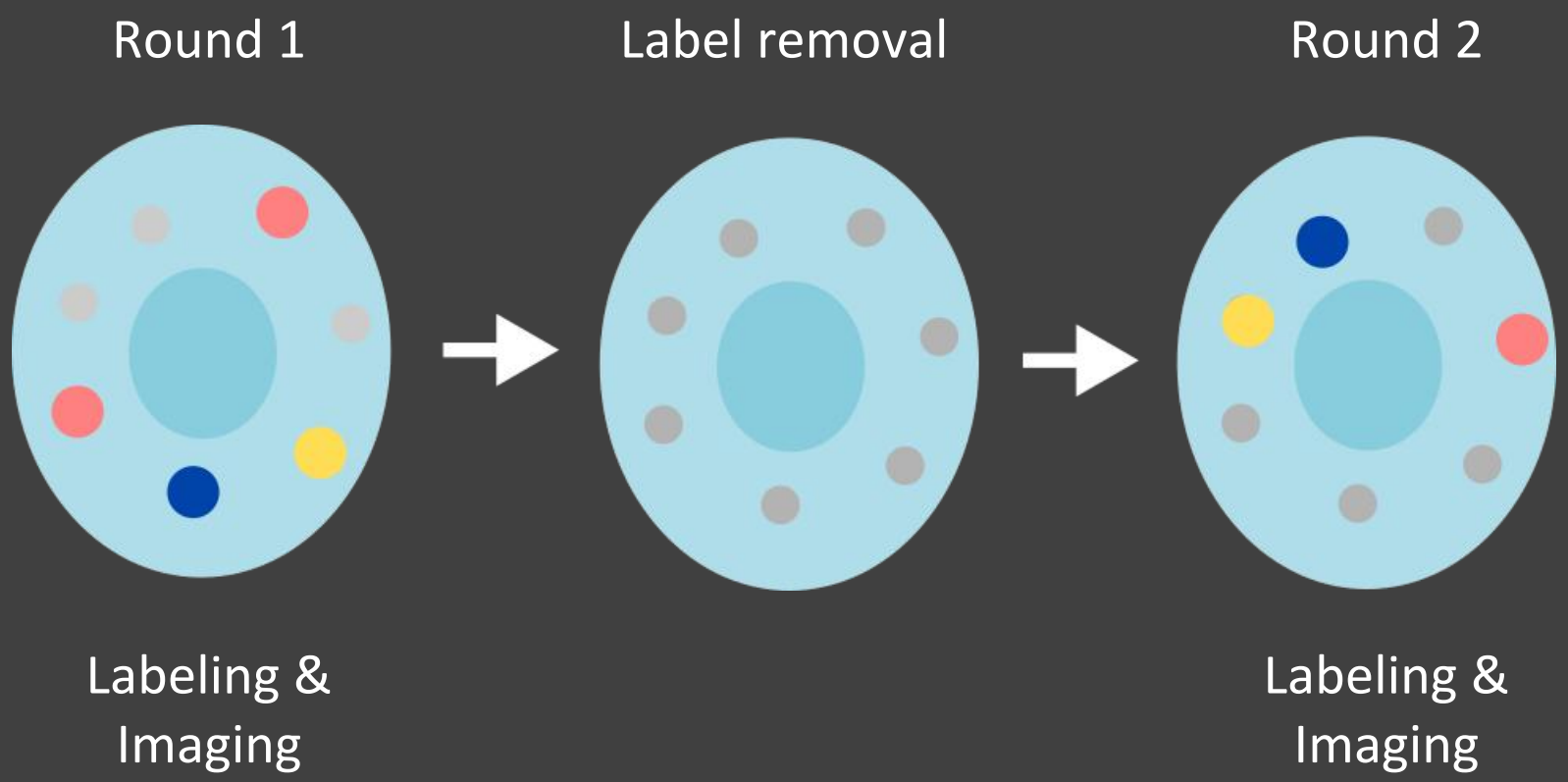
Gene 1 Gene 2 Gene 3 DNA



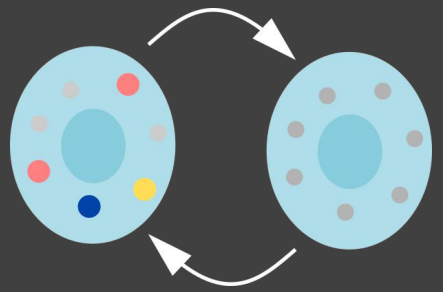
Limited fluorphores



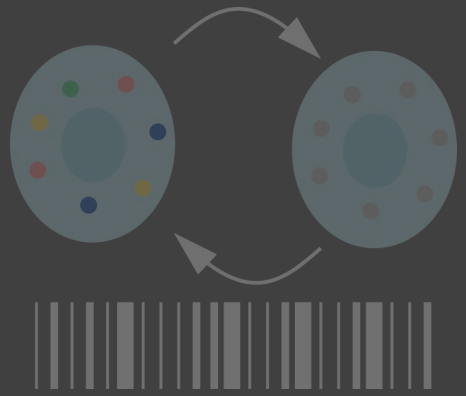
Cyclic labeling



Cyclic FISH



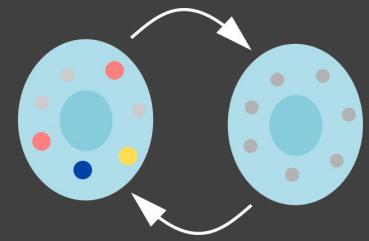
Barcoded FISH

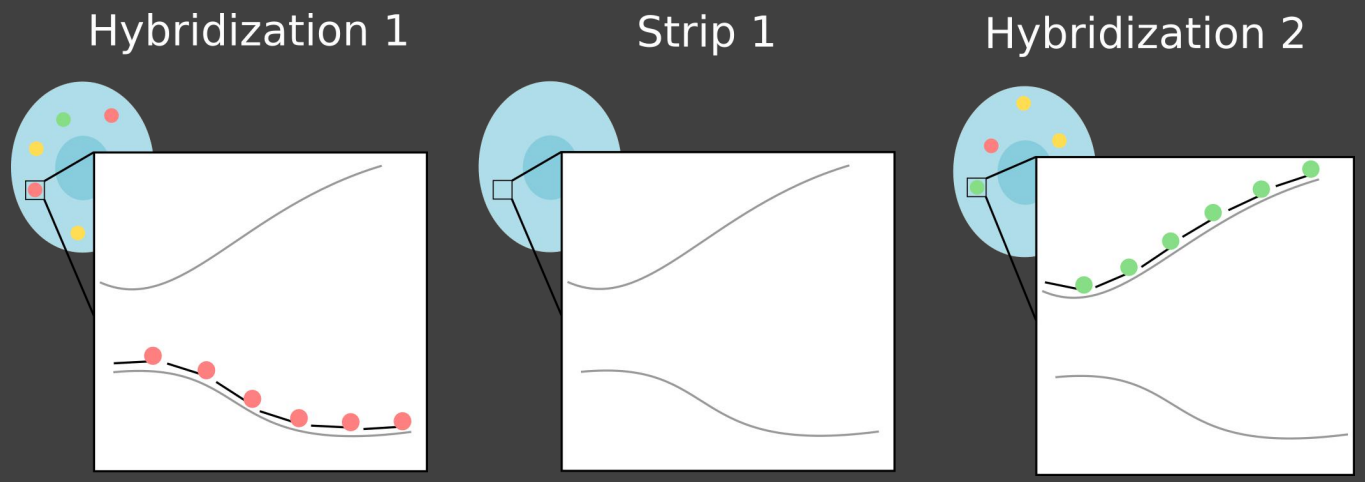


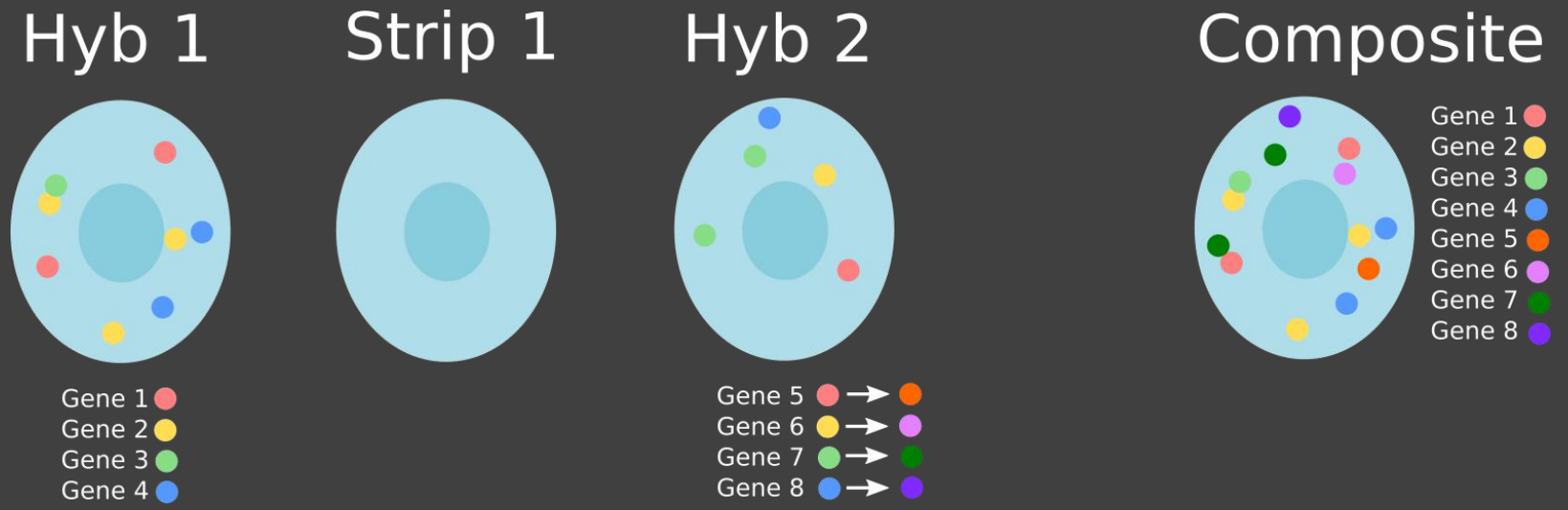
in situ Sequencing

Spatial Sequencing

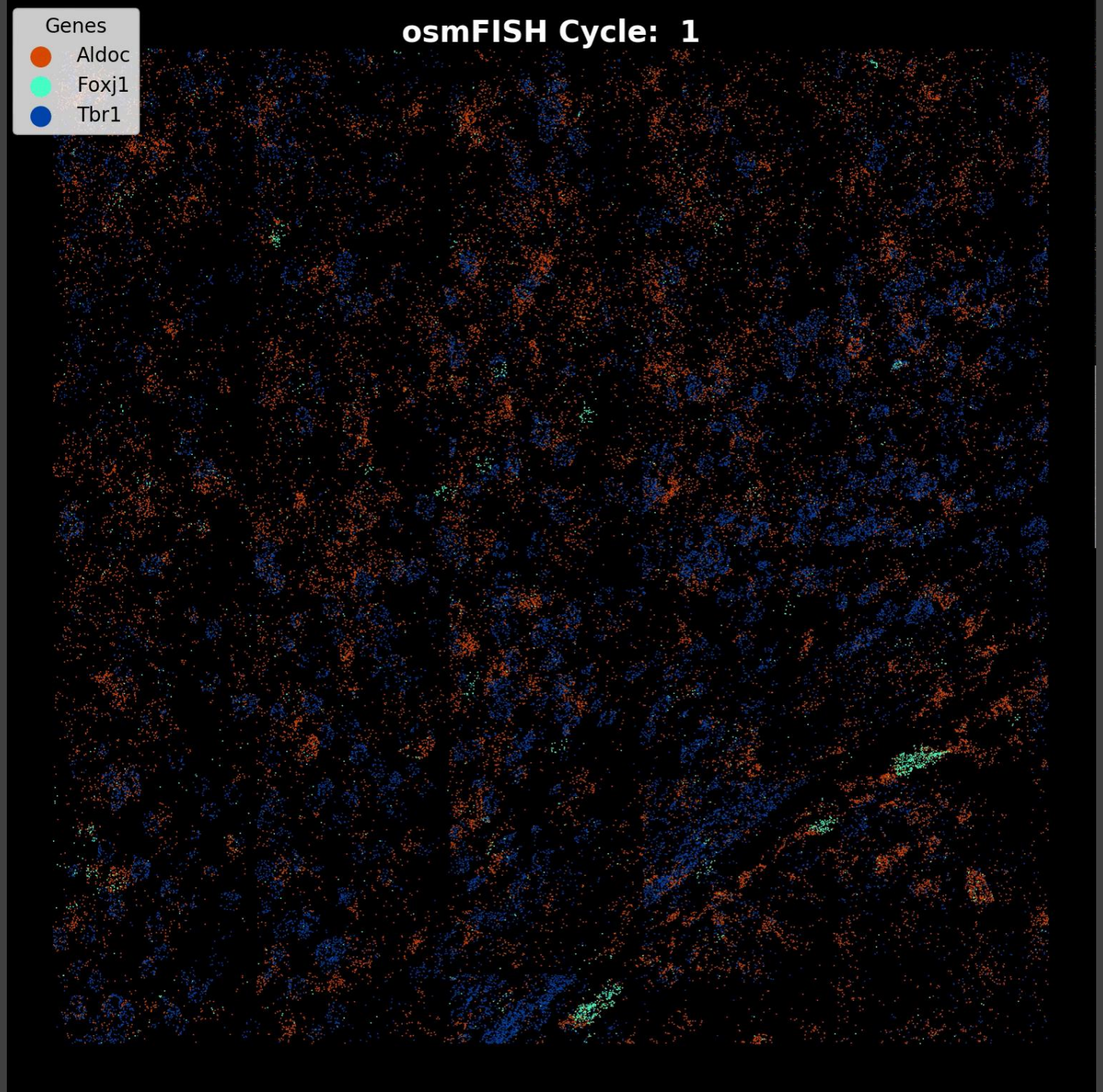
osmFISH

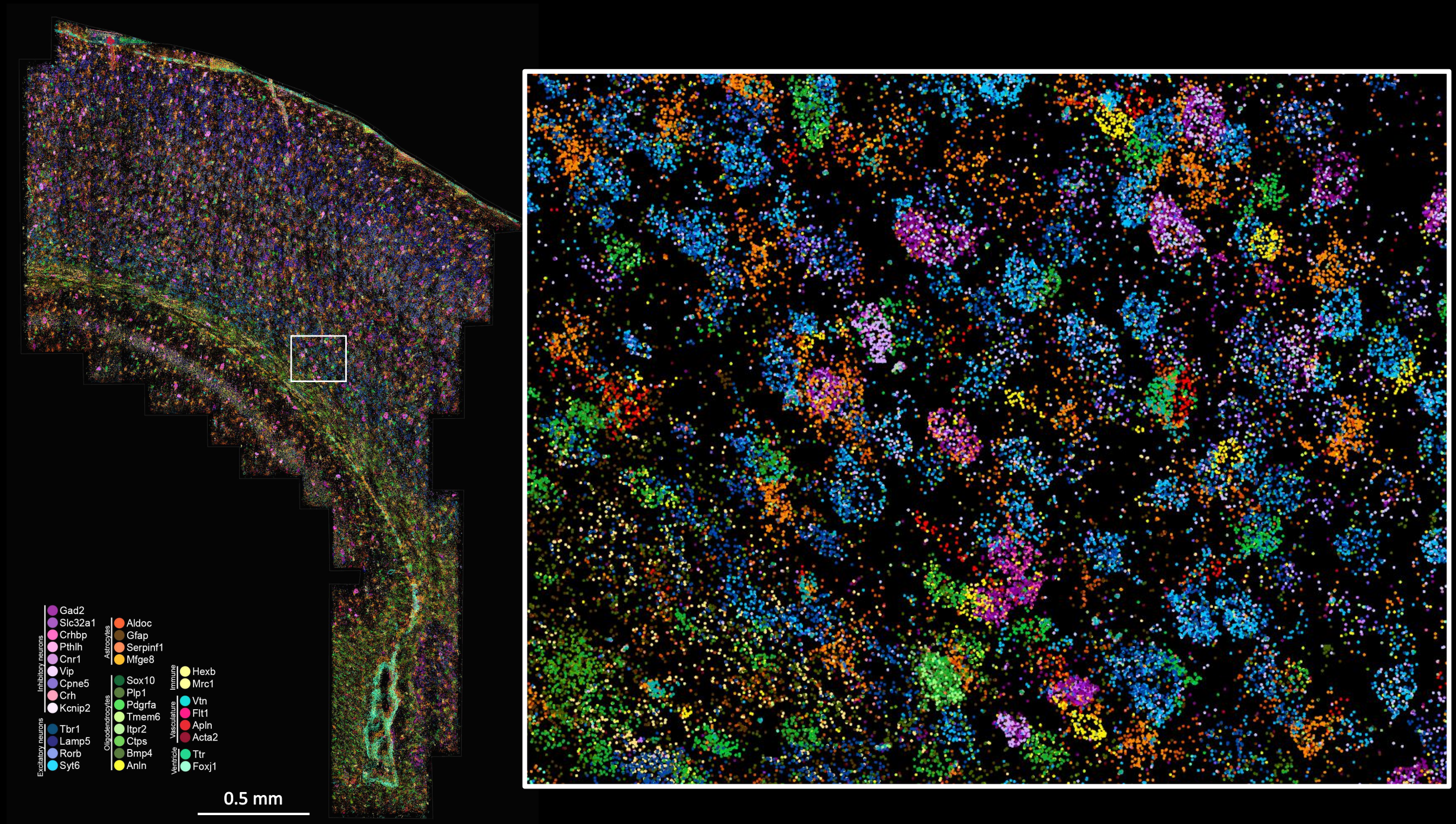




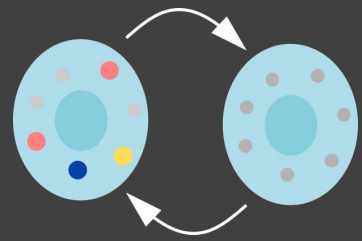


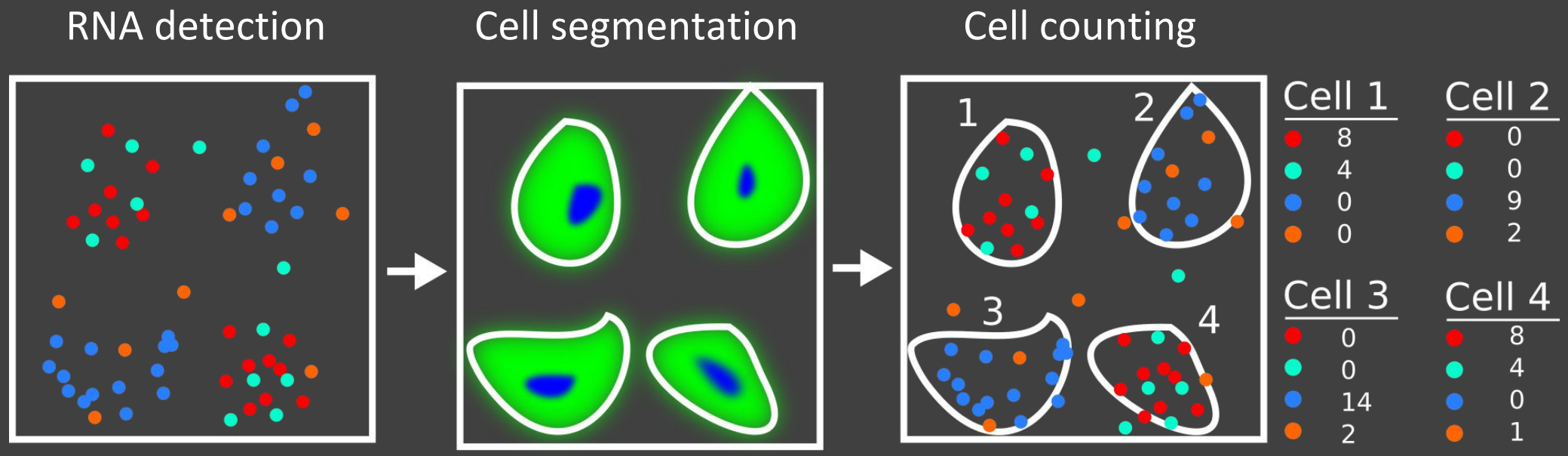
Codeluppi *et al.* Nature Methods 2019

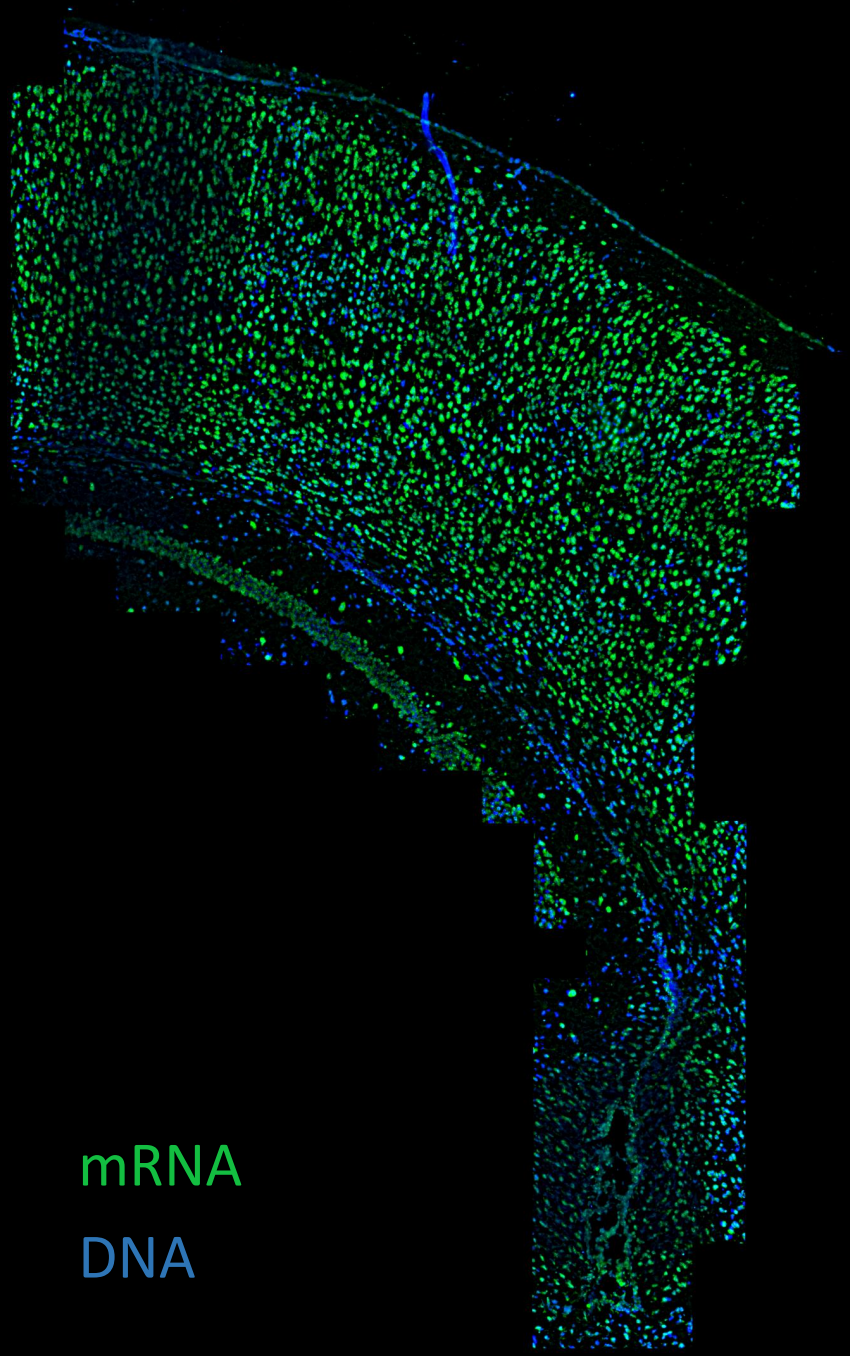

Genes
Aldoc
Foxj1
Tbr1
osmFISH Cycle: 1


Inhibitory neurons
Gad2
Slc32a1
Crhbp
Pthlh
Cnr1
Vip
Cpne5
Crh
Kcnip2
Excitatory neurons
Tbr1
Lamp5
Rorb
Syt6
Astrocytes
Aldoc
Gfap
Serpinf1
Mfge8
Oligodendrocytes
Sox10
Plp1
Pdgrfa
Tmem6
Itpr2
Ctps
Bmp4
Anln
Immune
Hexb
Mrc1
Vasculature
Vtn
Flt1
Apln
Acta2
Ventricle
Ttr
Foxj1
0.5 mm

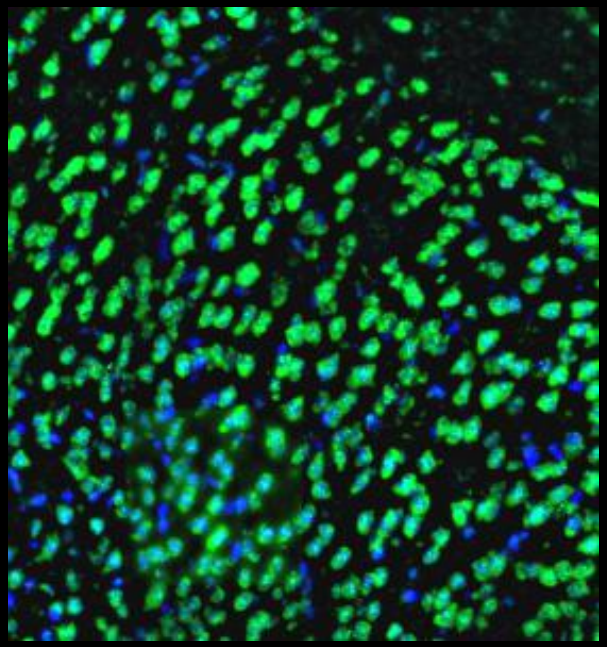
Pipeline



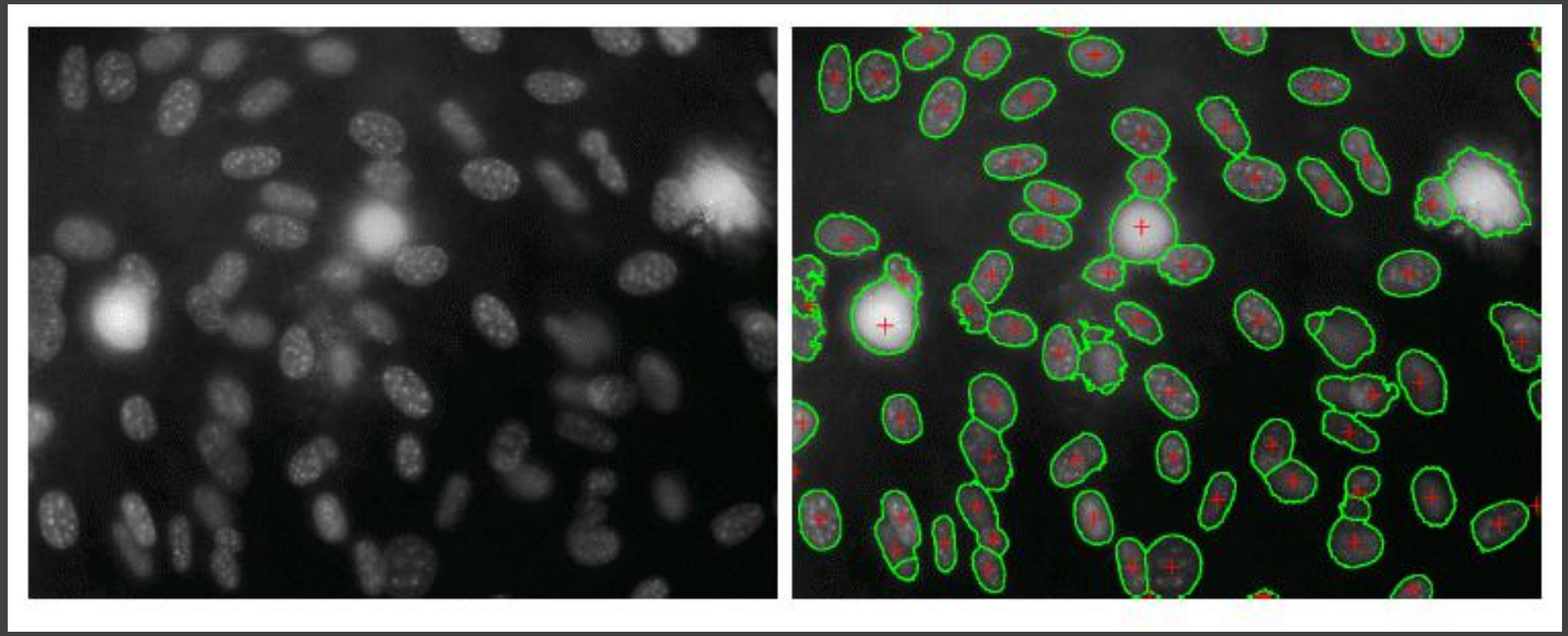

RNA detection
Cell segmentation
Cell counting
1
2
3
4
Cell 1
8
4
0
0
Cell 2
0
0
9
2
Cell 3
0
0
14
2
Cell 4
8
4
0
1


mRNA
DNA


Segmented

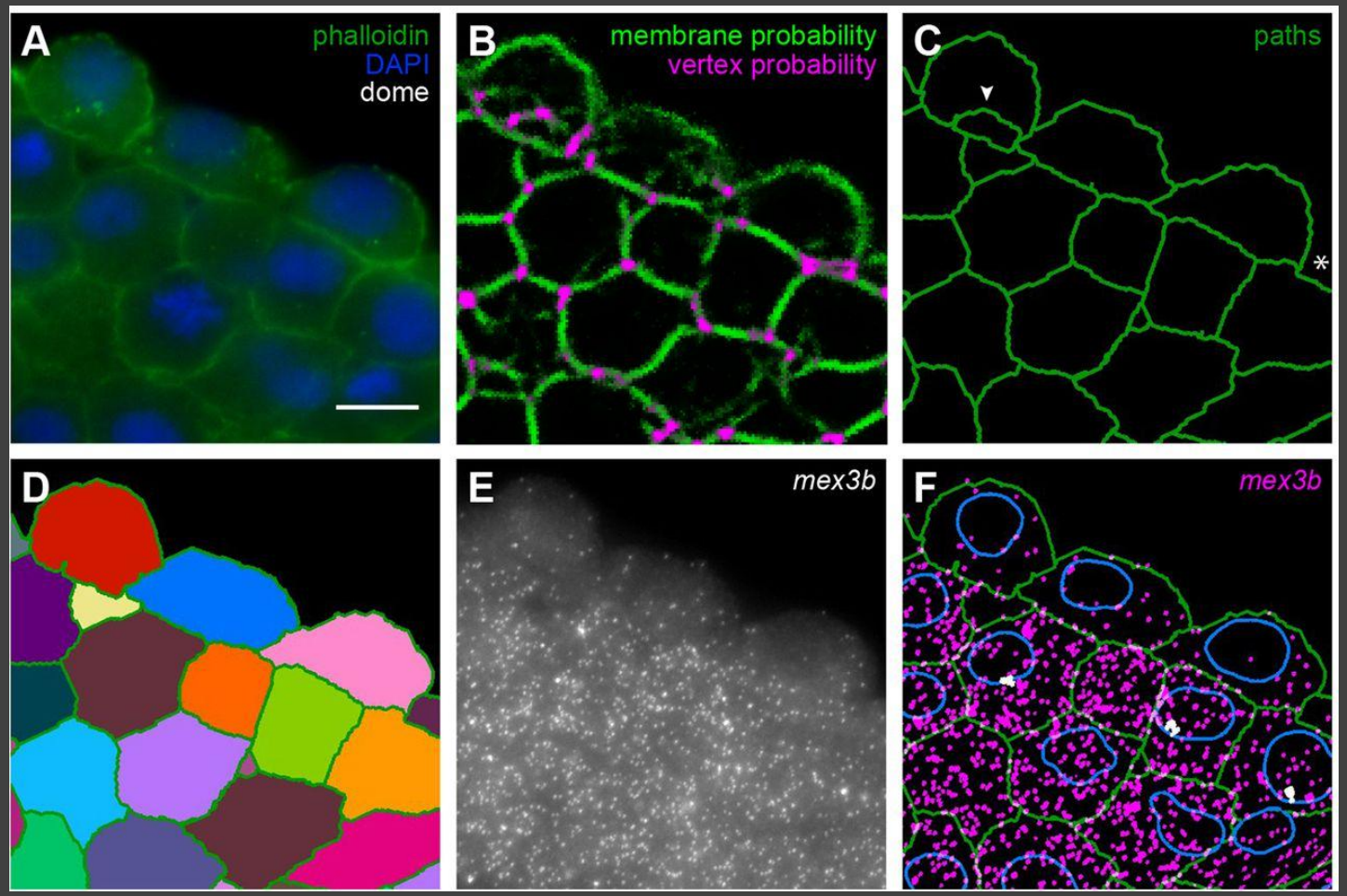


Cell segmentation



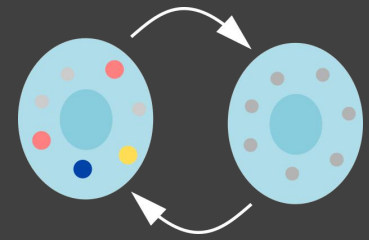
Panagiotakis *et al.* IEEE 2018

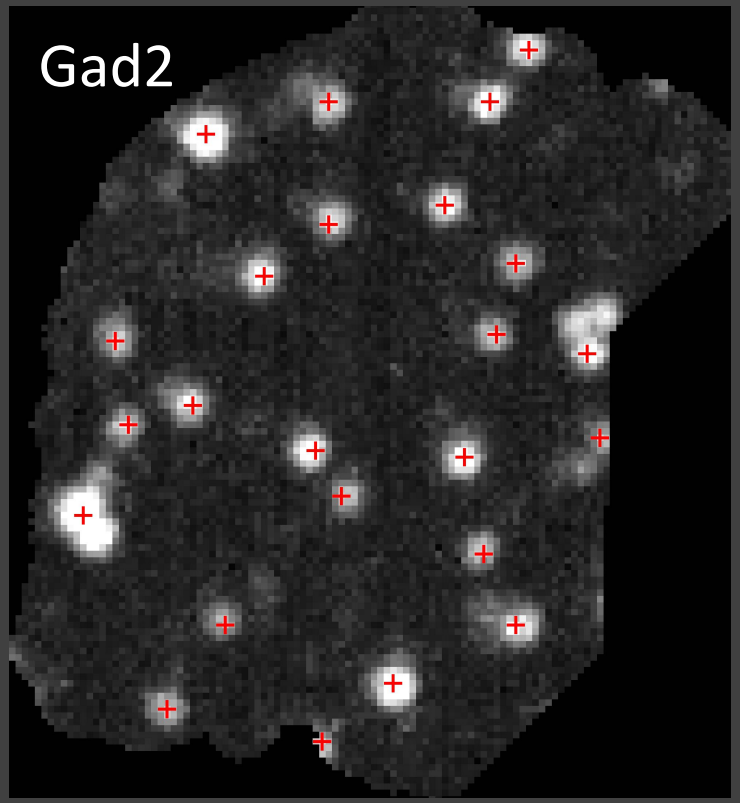
Cell segmentation



Stapel *et al.* Development 2016

Single cell expression





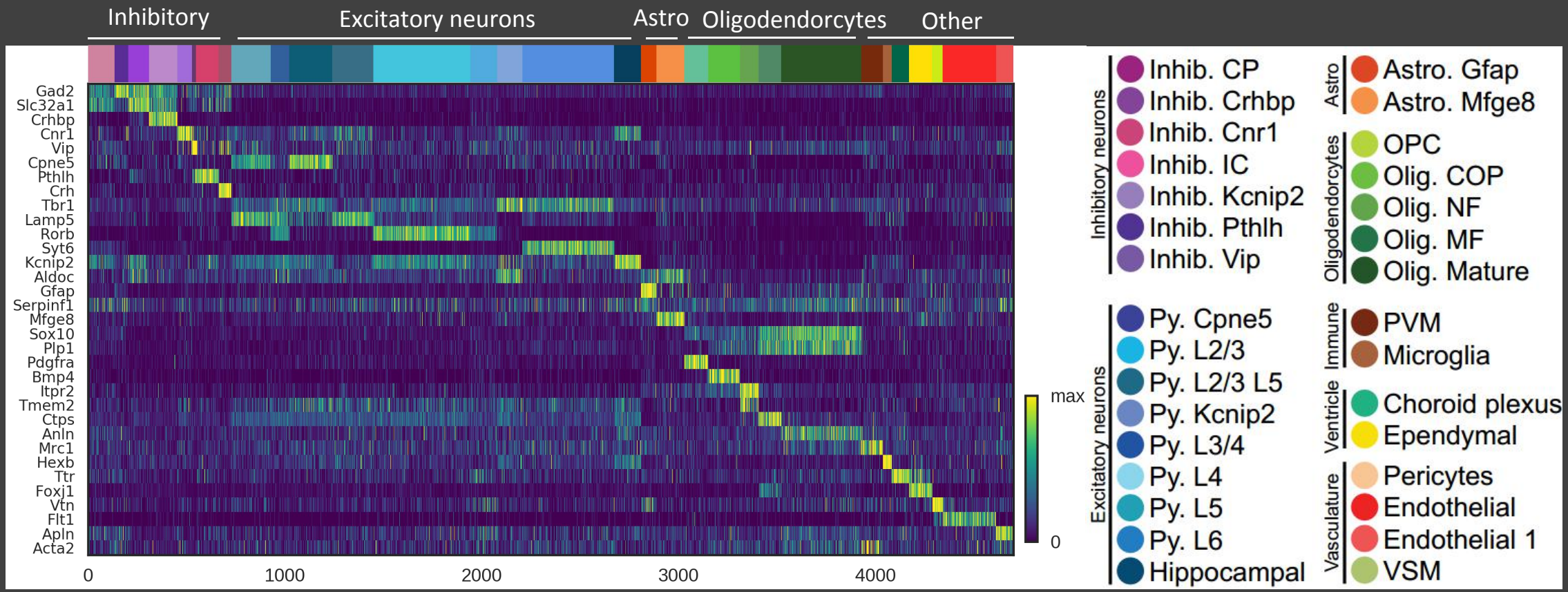
Genes

Cells

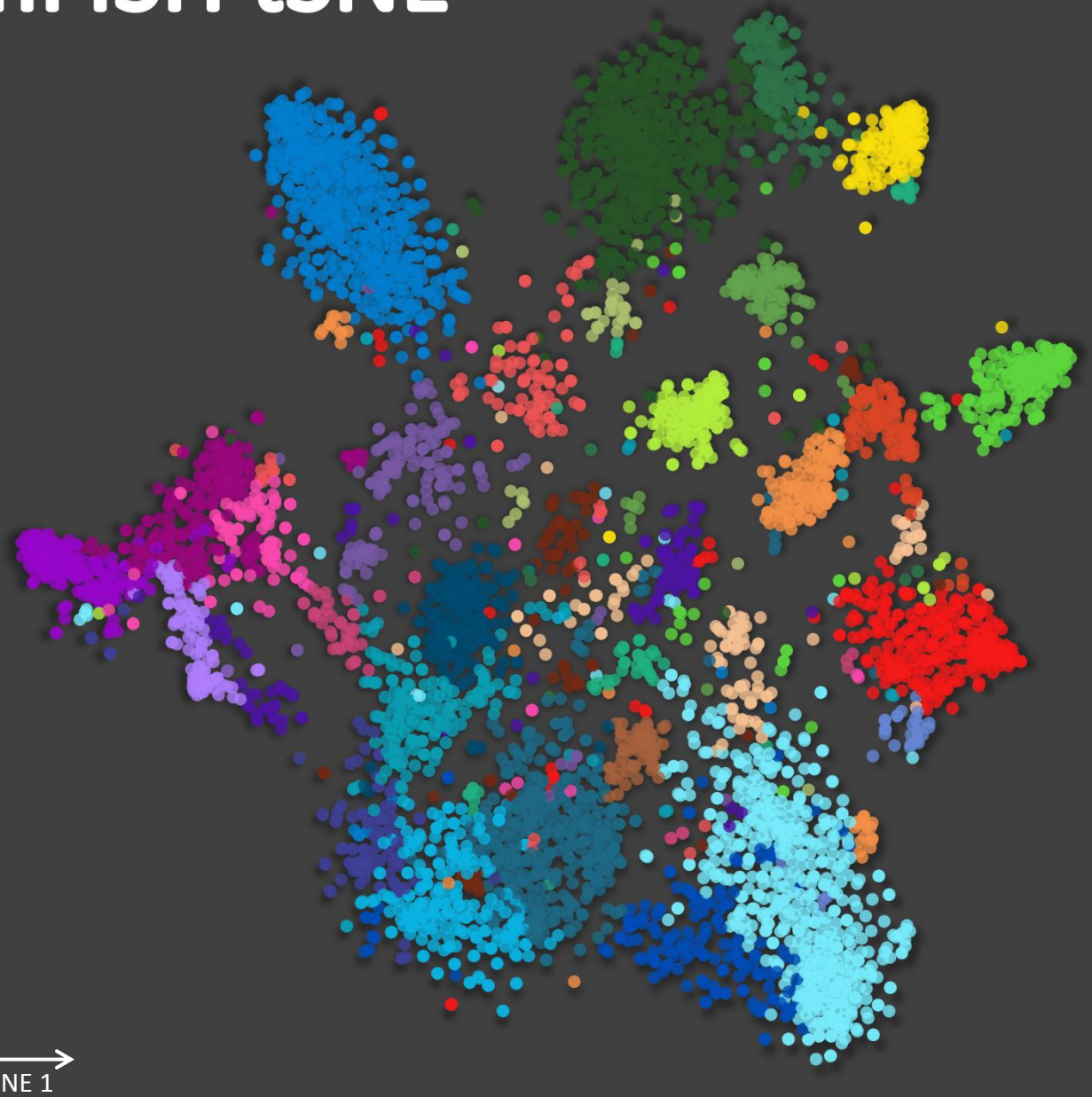
	1124	2325	2400	241	6248	5992	275	2573	330	1149	...	5162	532	3607	3251	7173	2757	1228	1234	7797	4653
Hybridization1_Tbr1	13	11	28	12	7	6	14	24	5	3	...	57	20	26	8	5	5	0	14	18	5
Hybridization1_Aldoc	38	0	9	5	38	2	4	3	7	10	...	11	10	5	4	10	2	0	2	2	9
Hybridization1_Foxj1	0	0	0	1	5	0	3	1	1	1	...	0	0	0	2	1	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_Itpr2	4	0	0	1	0	0	1	0	2	1	...	3	0	1	2	3	0	0	0	0	0
Hybridization6_Vip	13	1	2	4	30	1	3	2	1	4	...	0	11	2	5	1	7	2	3	6	1
Hybridization4_Cnr1	0	0	0	0	65	5	0	0	0	0	...	2	0	9	0	17	0	0	0	0	5
Hybridization4_Plp1	16	0	0	0	8	0	0	6	0	0	...	0	0	0	10	1	27	5	1	2	0
Hybridization4_Vtn	0	0	0	2	4	0	2	1	1	0	...	0	3	1	2	2	2	0	0	0	3
Hybridization7_Rorb	4	0	0	1	0	4	0	0	2	3	...	0	27	14	0	0	1	0	1	0	1
Hybridization7_Sox10	52	0	1	1	3	3	13	3	19	33	...	1	4	0	10	12	40	15	32	1	0
Hybridization7_Ctps	6	3	9	15	3	3	3	5	2	1	...	6	4	12	14	1	2	0	2	1	6
Hybridization11_Syt6	1	16	20	0	0	0	3	21	1	2	...	4	0	1	11	1	3	0	0	12	2
Hybridization11_Tbr1	4	13	36	6	2	5	9	12	6	15	...	30	19	30	0	3	2	0	0	10	4
Hybridization11_Tmem6	2	0	0	3	1	1	2	2	1	2	...	4	1	3	1	1	0	0	0	0	4
Hybridization8_Pdgfra	1	1	2	0	1	0	2	1	20	1	...	1	1	1	2	26	0	0	0	1	6
Hybridization8_Serpinf1	13	1	2	6	2	4	2	1	10	2	...	0	5	10	8	6	5	6	2	2	2
Hybridization8_Pthlh	2	0	0	0	8	0	1	1	0	0	...	0	0	1	1	0	0	0	0	1	0
Hybridization10_Crhbp	2	0	1	0	0	0	0	0	0	3	...	0	0	0	0	0	0	0	0	0	0
Hybridization10_Crh	2	0	2	0	3	0	6	1	0	2	...	1	1	1	1	0	0	0	3	1	0
Hybridization10_Apln	3	5	2	31	0	2	3	4	8	5	...	0	2	3	1	2	3	1	3	5	1
Hybridization9_Lamp5	6	38	51	126	0	1	52	44	51	0	...	4	1	168	5	5	0	0	1	3	90
Hybridization9_Lum	1	0	0	3	0	0	0	0	3	6	...	0	0	0	1	1	0	0	0	0	0
Hybridization9_Anln	19	1	1	1	2	2	2	6	3	23	...	0	1	10	3	8	14	8	11	3	0
Hybridization12_Kcnip	1	25	50	14	6	3	20	14	7	0	...	25	23	64	0	2	2	0	0	3	22
Hybridization12_Slc32a1	2	1	2	2	22	0	1	0	2	0	...	0	1	1	0	0	4	0	0	0	0
Hybridization12_Vtn	2	2	0	1	2	0	0	0	0	2	...	0	1	0	0	2	0	0	0	0	1
Hybridization5_Acta2	3	1	1	1	1	0	1	4	0	2	...	0	0	2	0	7	6	0	4	1	0
Hybridization5_Cpne5	0	4	1	1	1	0	2	9	2	0	...	3	0	10	0	3	0	0	0	3	16
Hybridization5_Klk6	0	0	0	0	0	0	0	0	0	0	...	0	0	0	0	0	2	0	1	0	0
Hybridization3_Mfge8	6	0	1	2	0	2	2	3	2	13	...	2	2	2	1	7	4	0	2	0	7
Hybridization3_Mrc1	14	2	2	3	2	0	0	6	1	19	...	2	2	26	4	9	0	1	6	2	5
Hybridization3_Hexb	10	0	3	6	1	0	3	4	1	3	...	9	2	4	3	6	2	0	0	1	2
Hybridization2_Gad2	7	4	3	5	65	1	7	1	2	12	...	2	6	9	2	2	2	1	9	3	11
Hybridization2_Flt1	0	0	0	0	0	0	0	0	0	0	...	0	3	0	0	0	0	0	0	0	0
Hybridization2_Gfap	57	0	1	0	1	0	3	3	0	32	...	3	1	4	5	0	0	1	2	0	1
Hybridization13_Cnr1	1	1	2	14	56	3	6	7	5	3	...	3	3	25	1	2	3	0	0	0	6
Hybridization13_Ttr	2	0	0	1	1	0	1	0	1	13	...	0	2	0	3	3	1	1	1	3	0
Hybridization13_Plp1	10	5	3	0	7	0	0	8	5	33	...	3	1	2	35	0	4	7	33	4	1

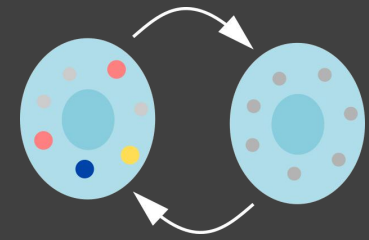
39 rows × 6036 columns

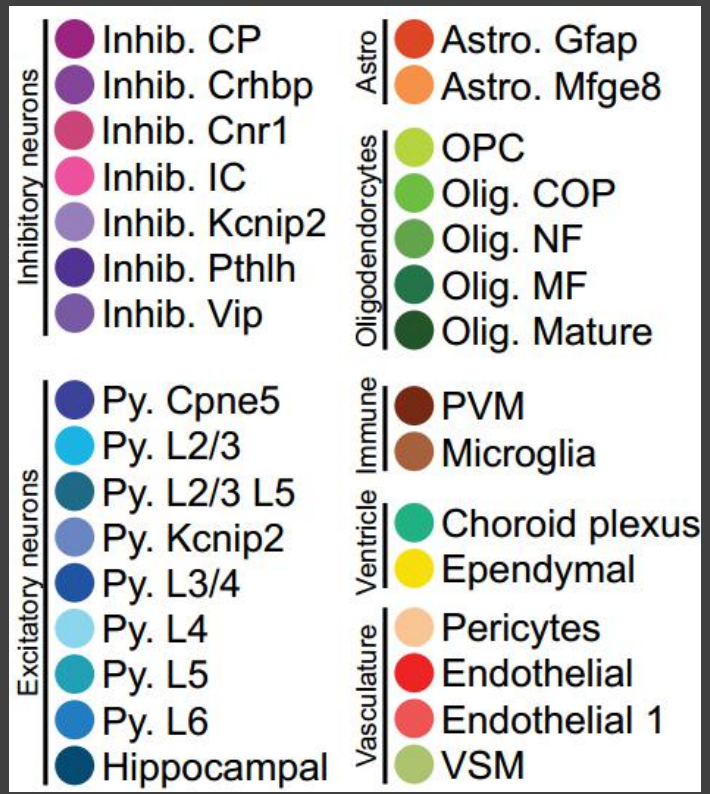
osmFISH clustering

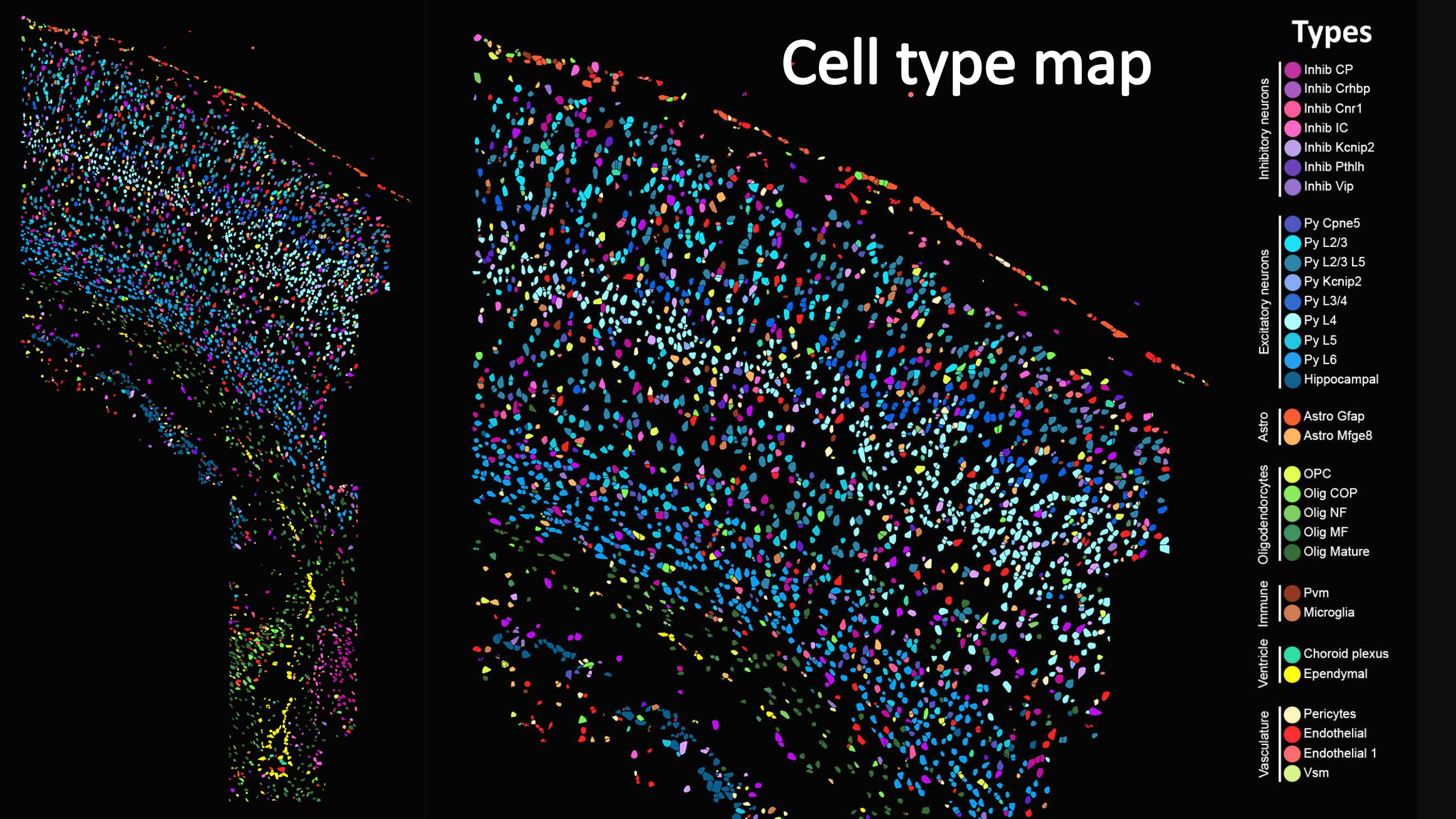


osmFISH tSNE

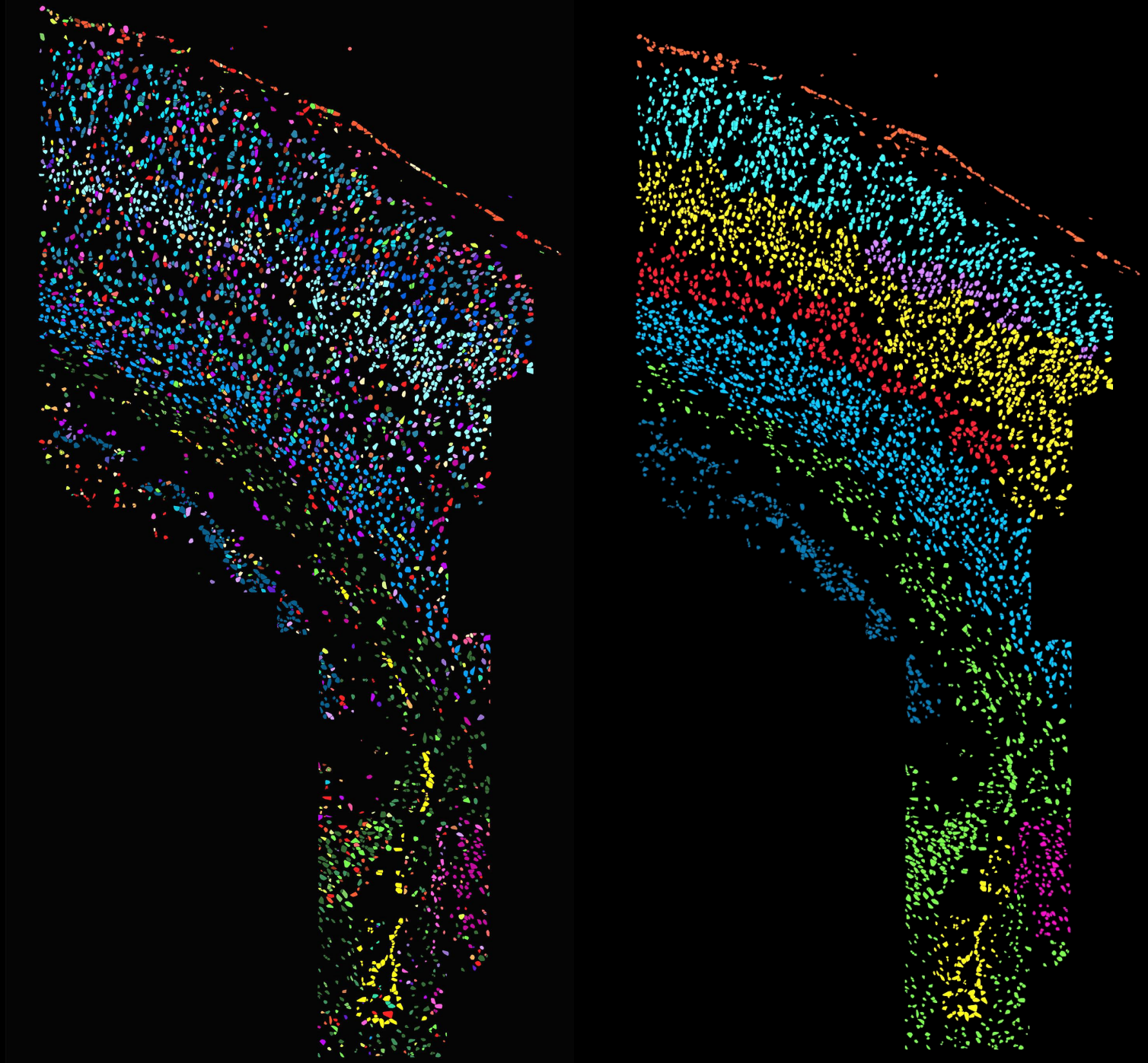


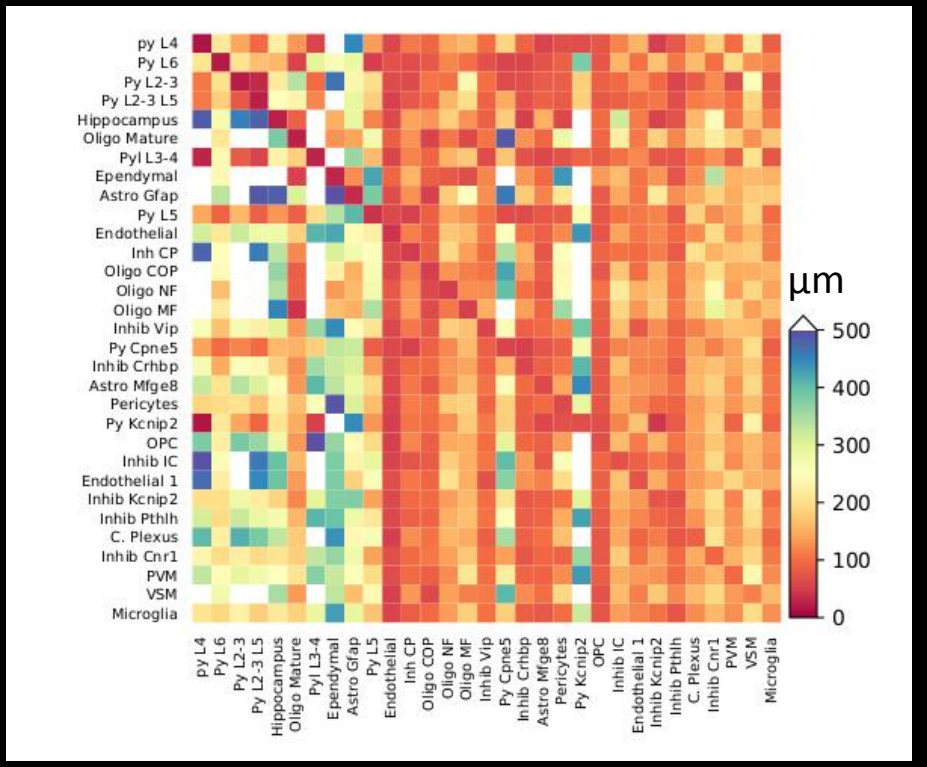




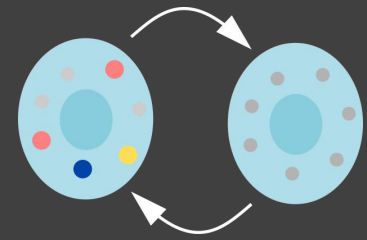

Cell type map
Types
Inhibitory neurons
Inhib CP
Inhib Crhbp
Inhib Cnr1
Inhib IC
Inhib Kcnip2
Inhib Pthlh
Inhib Vip
Excitatory neurons
Py Cpne5
Py L2/3
Py L2/3 L5
Py Kcnip2
Py L3/4
Py L4
Py L5
Py L6
Hippocampal
Astro
Astro Gfap
Astro Mfge8
Oligodendorcytes
OPC
Olig COP
Olig NF
Olig MF
Olig Mature
Immune
Pvm
Microglia
Ventricle
Choroid plexus
Ependymal
Vasculature
Pericytes
Endothelial
Endothelial 1
Vsm

Spatial analysis

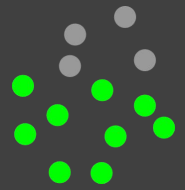




Cyclic smFISH

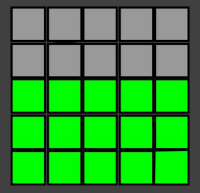


Resolution: Diffraction limited (150-300nm)



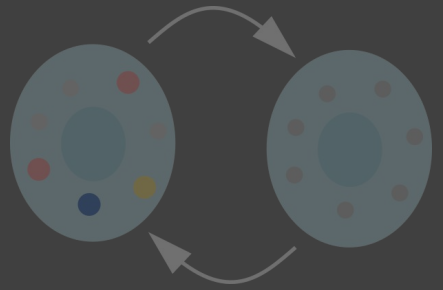
Detection efficiency: ~100%

Gene throughput: tens of genes (F x R)

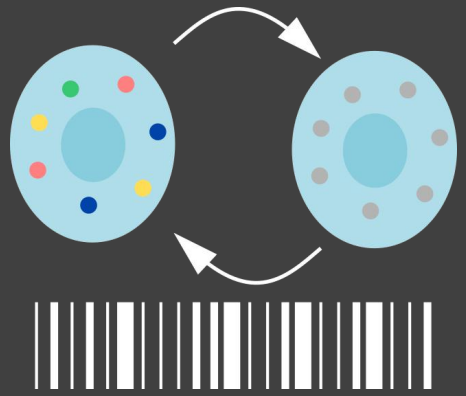


Spatial throughput: several mm^2

Cyclic FISH



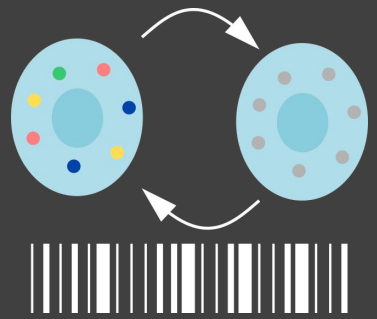
Barcoded FISH



in situ Sequencing

Spatial Sequencing

Reprobing same molecule

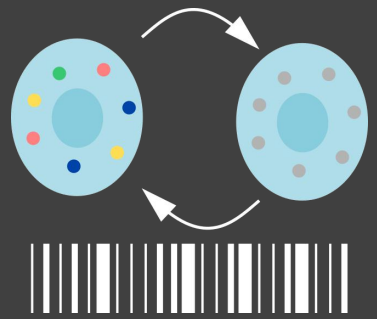


Round 1

Stripping

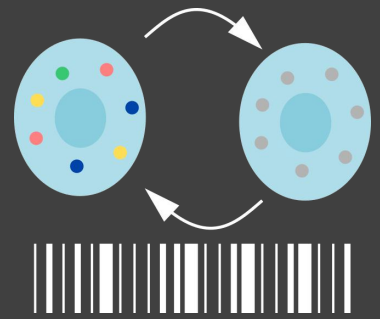
Round 2

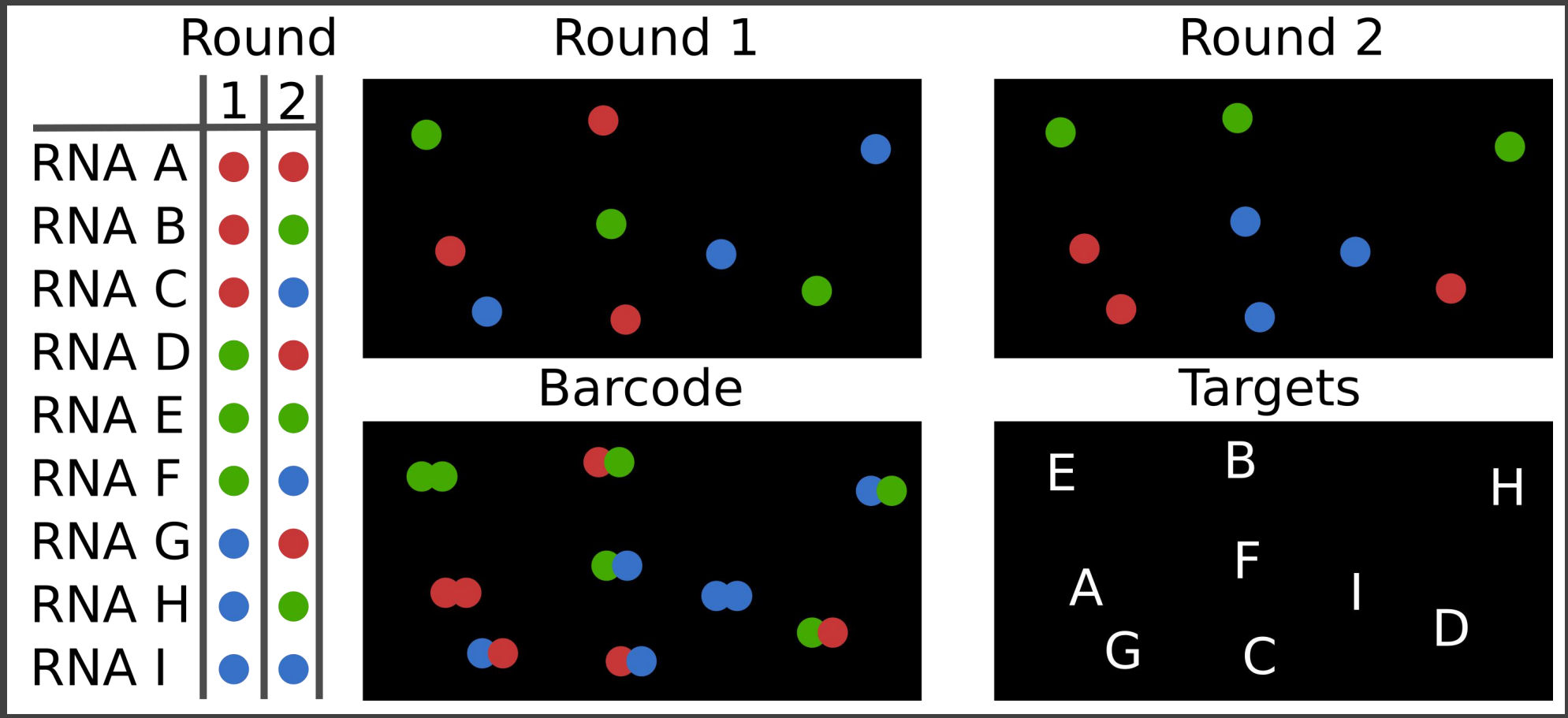
Barcoding



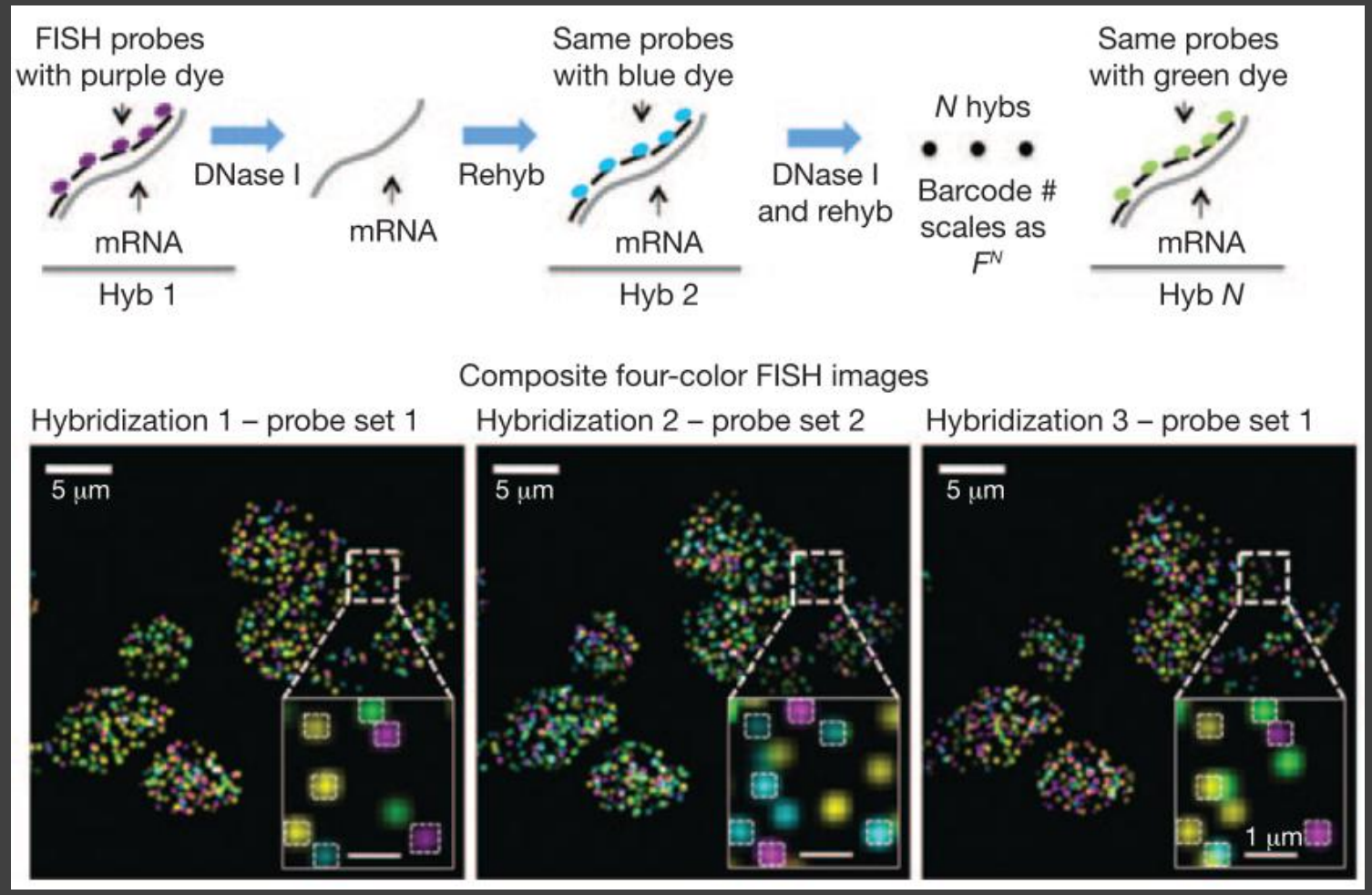
	Round 1	2
RNA A	●	●
RNA B	●	●
RNA C	●	●
RNA D	●	●
RNA E	●	●
RNA F	●	●
RNA G	●	●
RNA H	●	●
RNA I	●	●

Barcoding



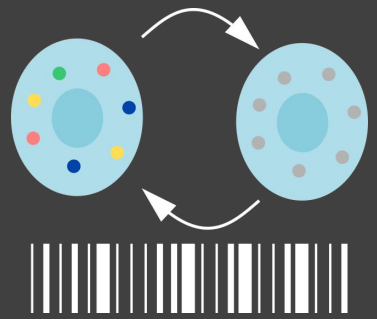

Round
1
2
RNA A
RNA B
RNA C
RNA D
RNA E
RNA F
RNA G
RNA H
RNA I
Round 1
Round 2
Barcode
Targets
E
B
H
F
A
I
D
G
C

SeqFISH



Lubeck & Cai Nature Methods2014

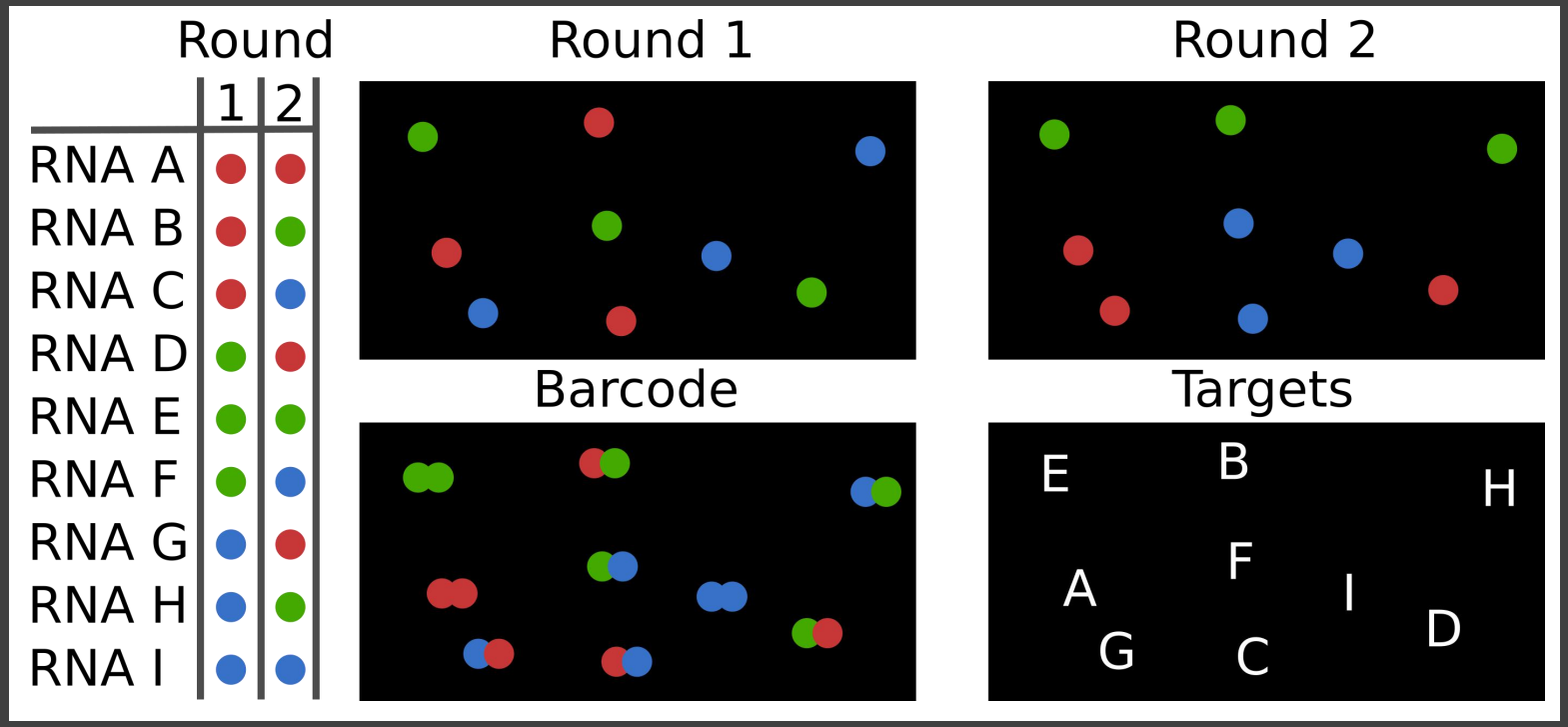
Barcoding



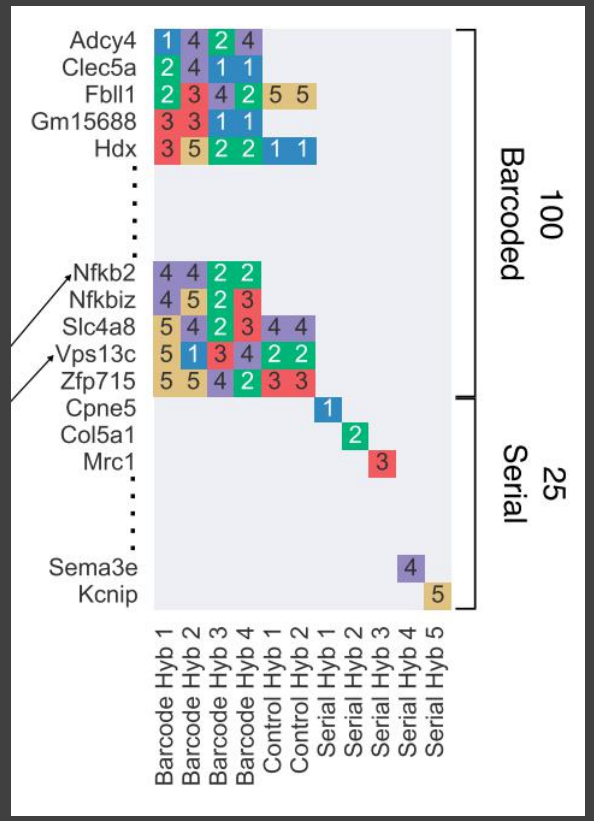
Scaling: $Fluo^{Rounds}$

4^7 = 163,84 genes

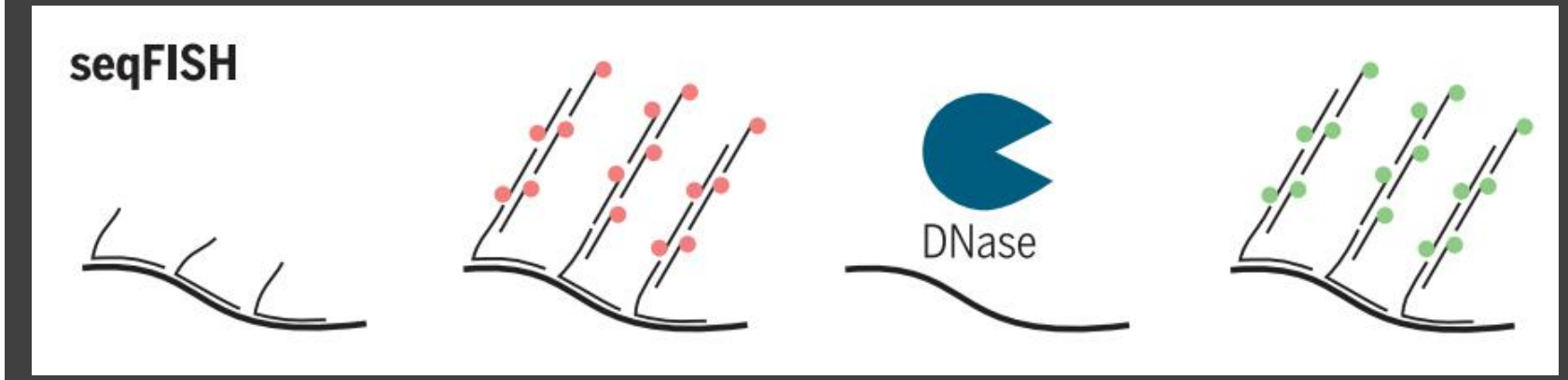
4^8 = 65,536 genes



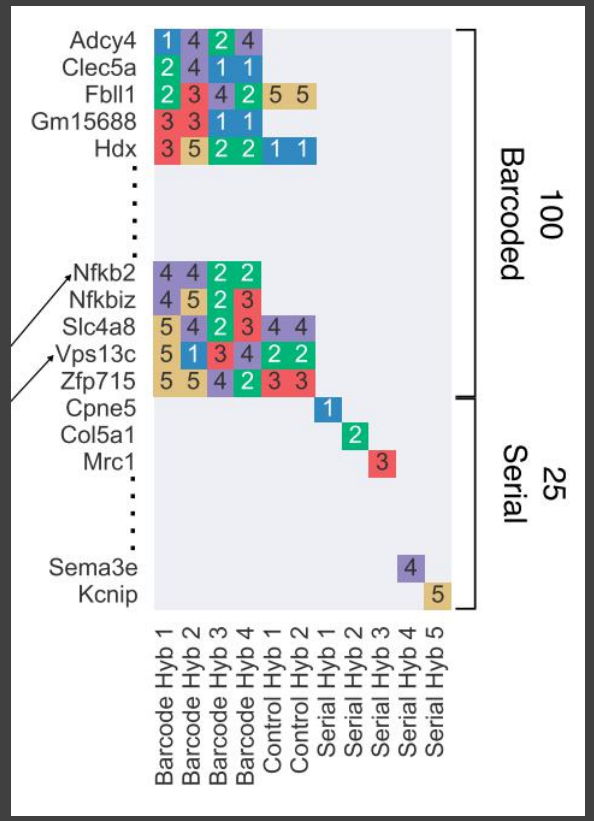
SeqFISH

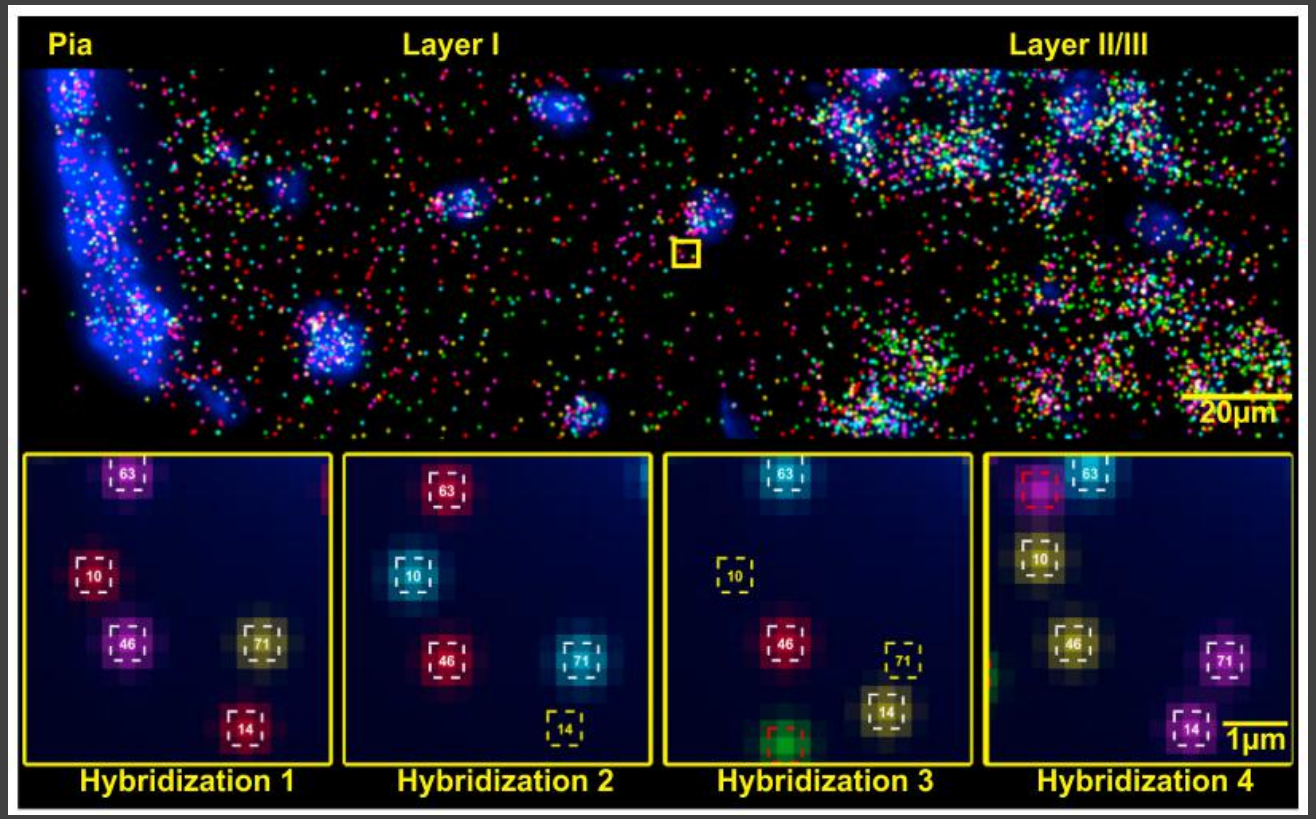


Shah *et al.* Neuron 2016



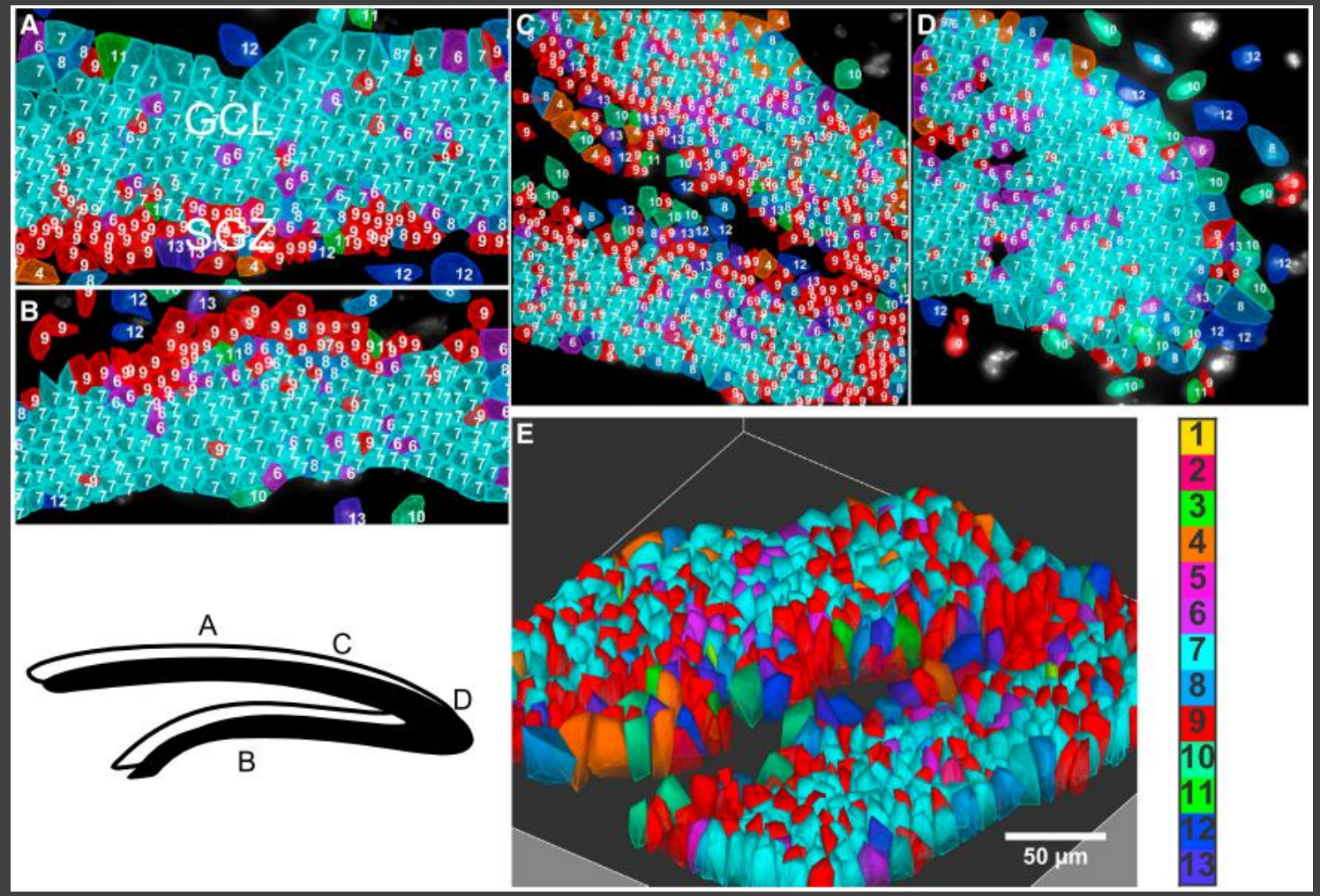
SeqFISH





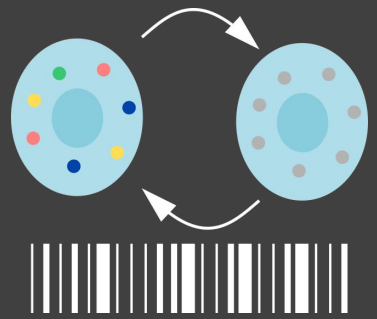
Shah *et al.* Neuron 2016

SeqFISH



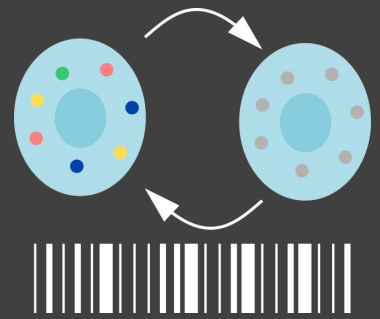
Shah *et al.* Neuron 2016

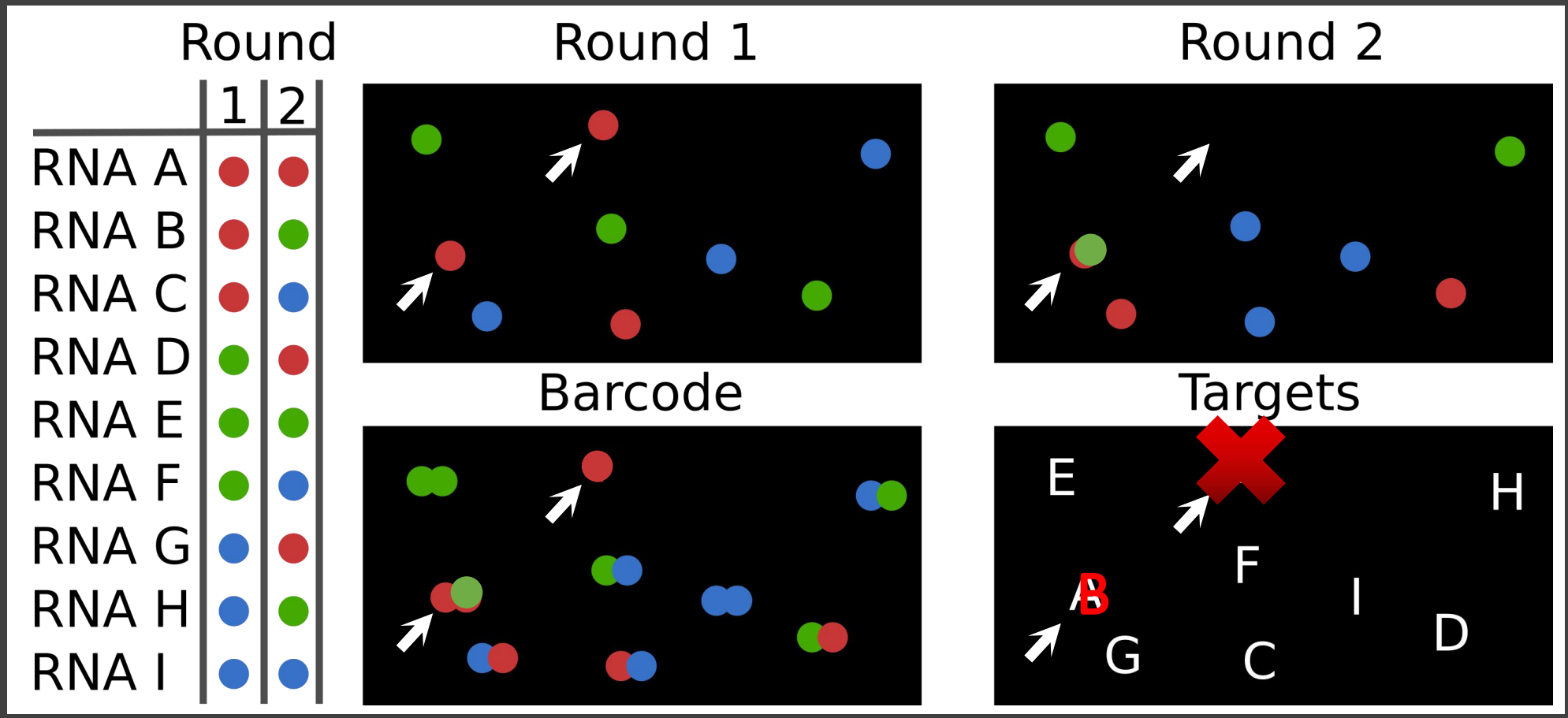
Challenges



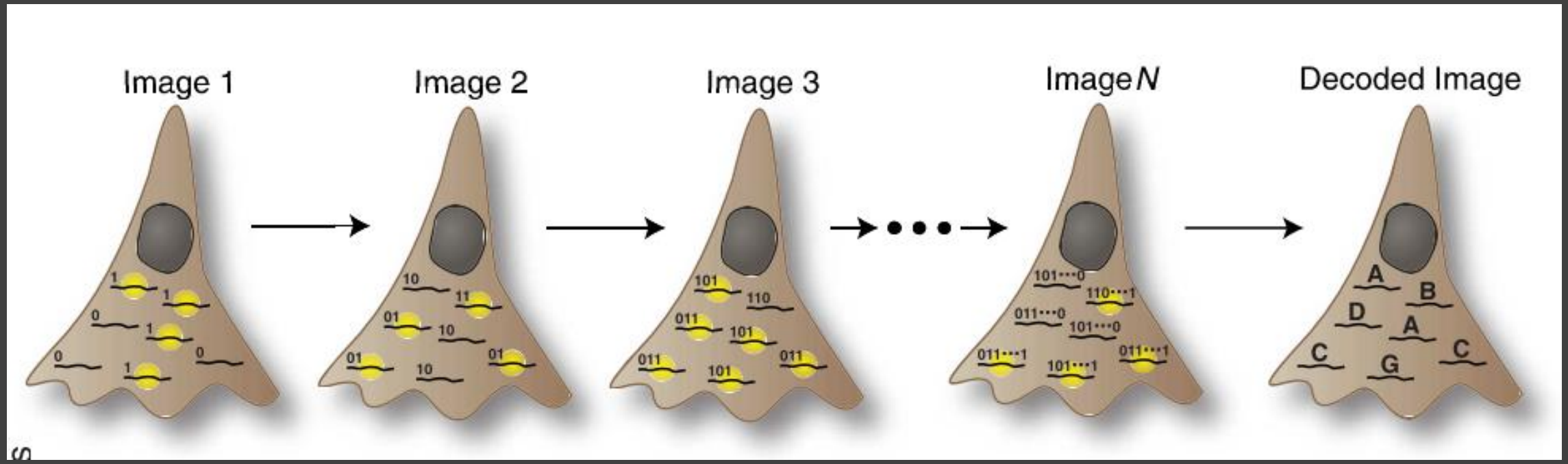
- Errors in barcodes
- Optical density

Errors



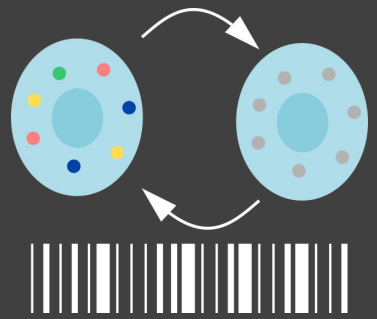

Round
1
2
RNA A
RNA B
RNA C
RNA D
RNA E
RNA F
RNA G
RNA H
RNA I
Round 1
Round 2
Barcode
Targets
E
H
F
I
D
G
C

MERFISH



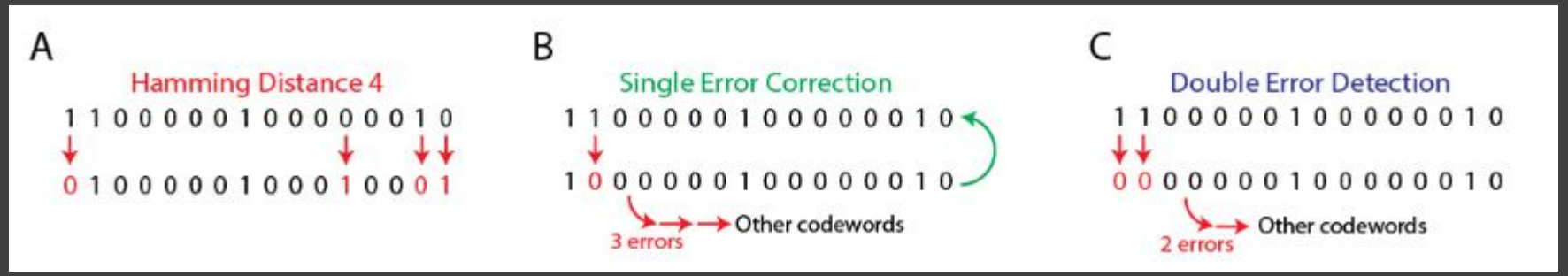
Chen *et al.* Science 2015

MERFISH



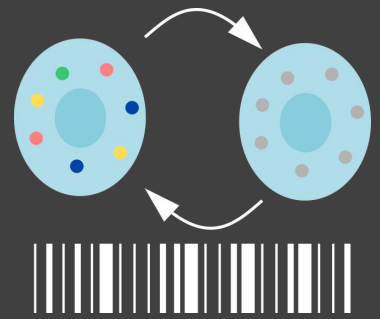
Barcode 1: 1 1 0 0 0 1 0 0 1 0

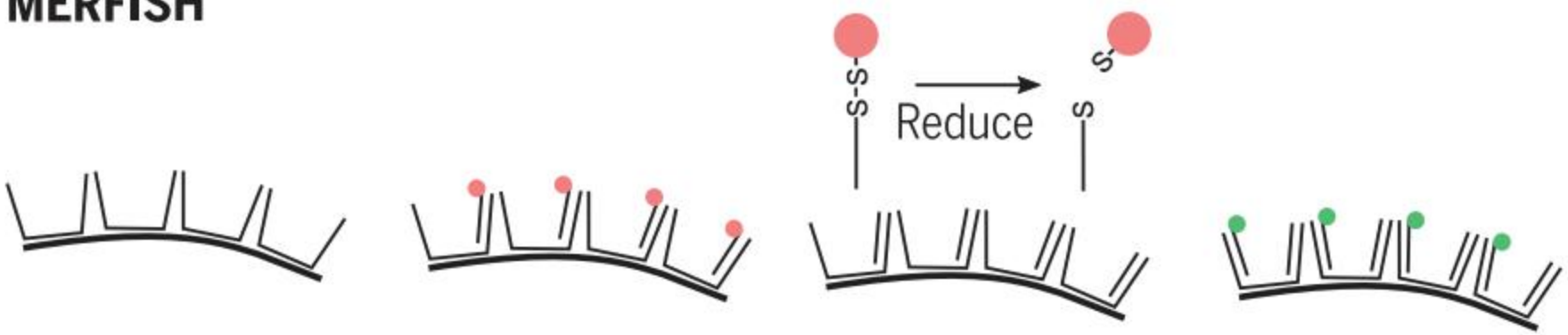
Barcode 2: 0 1 0 0 0 1 0 0 1 0



Chen *et al.* Science 2015

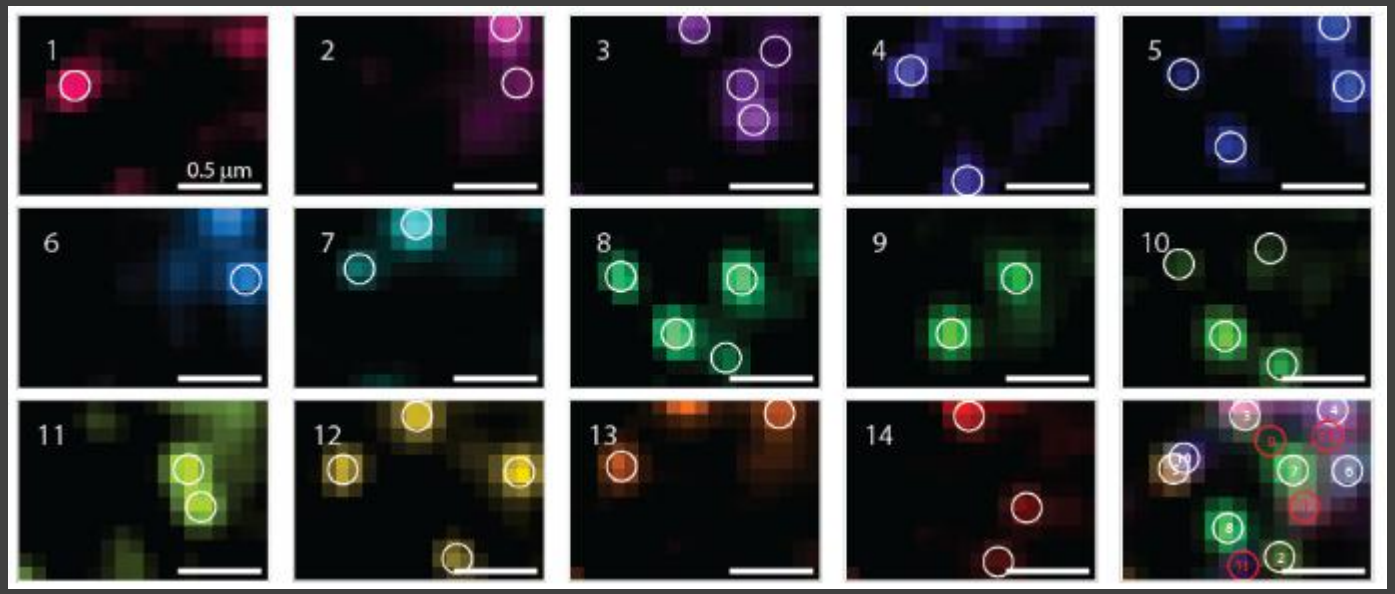
MERFISH





	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

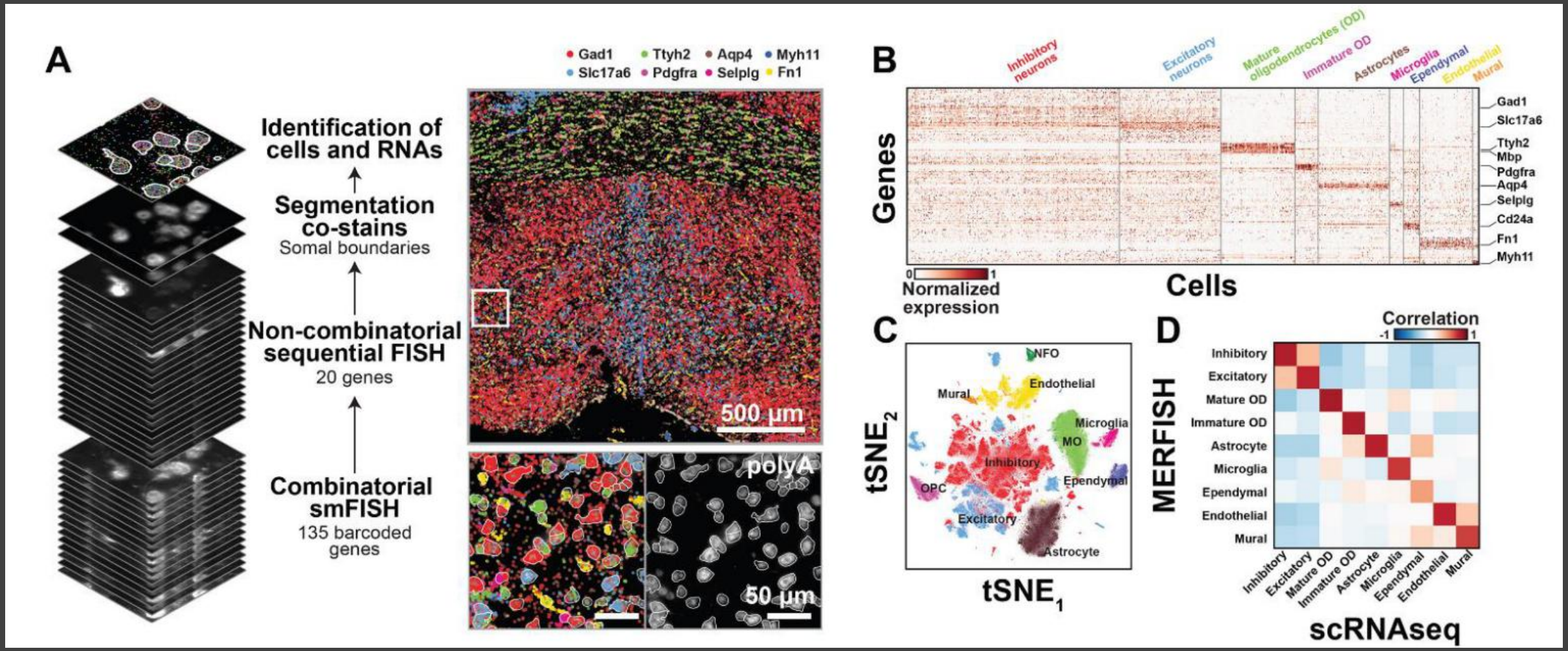


Hybridization round

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

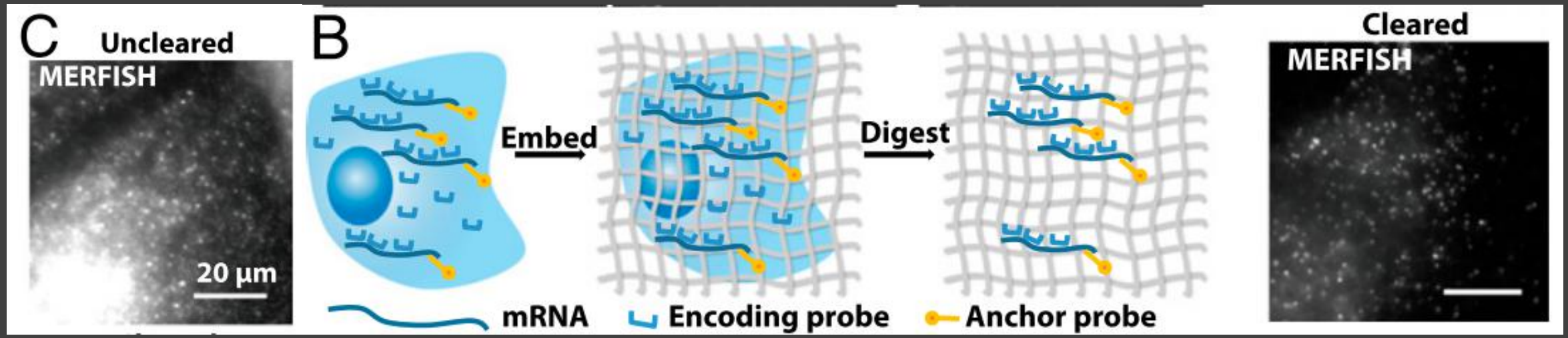
Chen *et al.* Science 2015

MERFISH



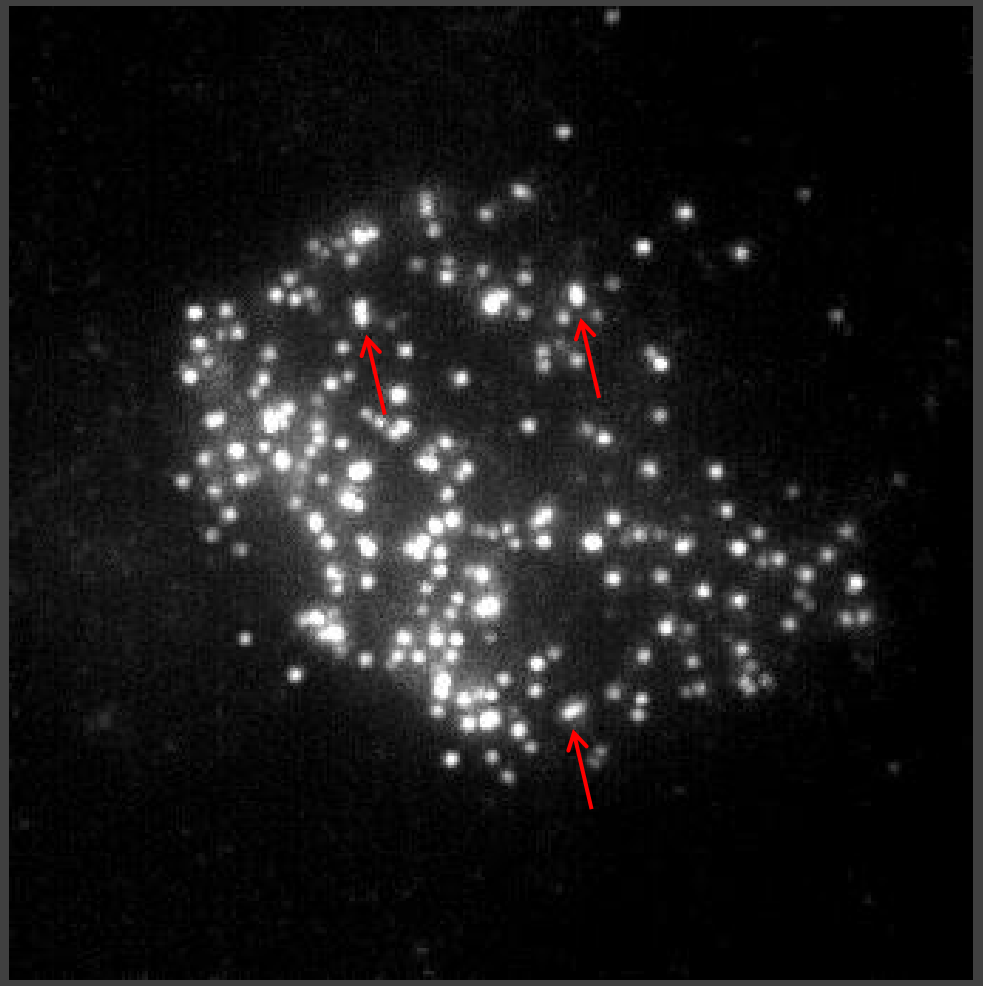
Moffit *et al.* Science 2018

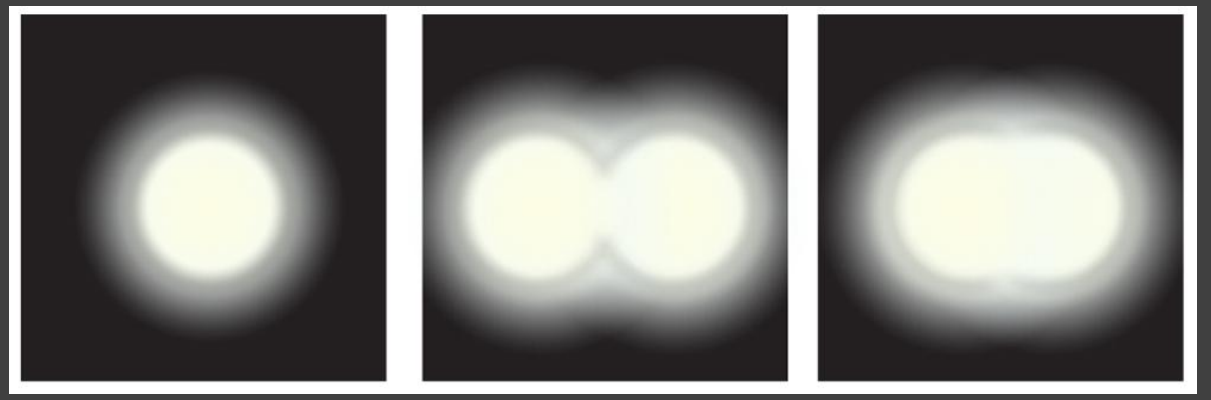
MERFISH



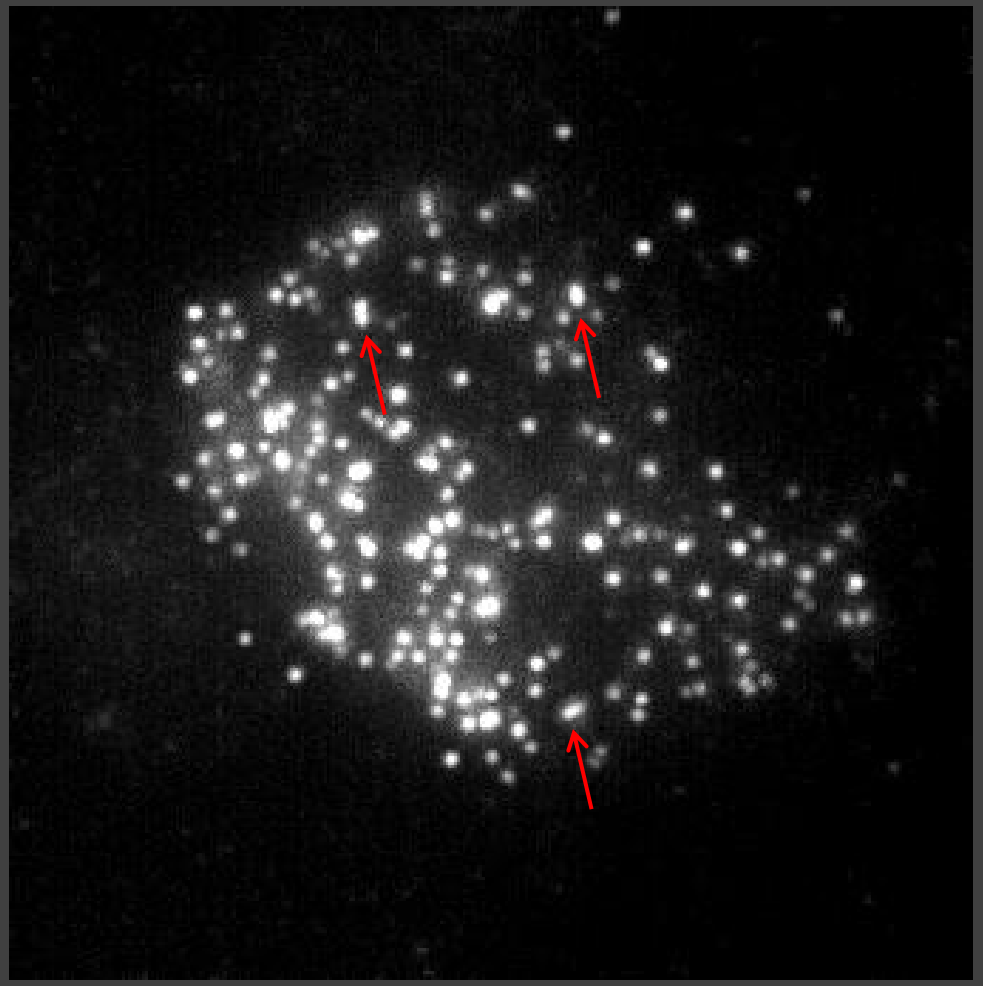
Moffit *et al.* PNAS 2016

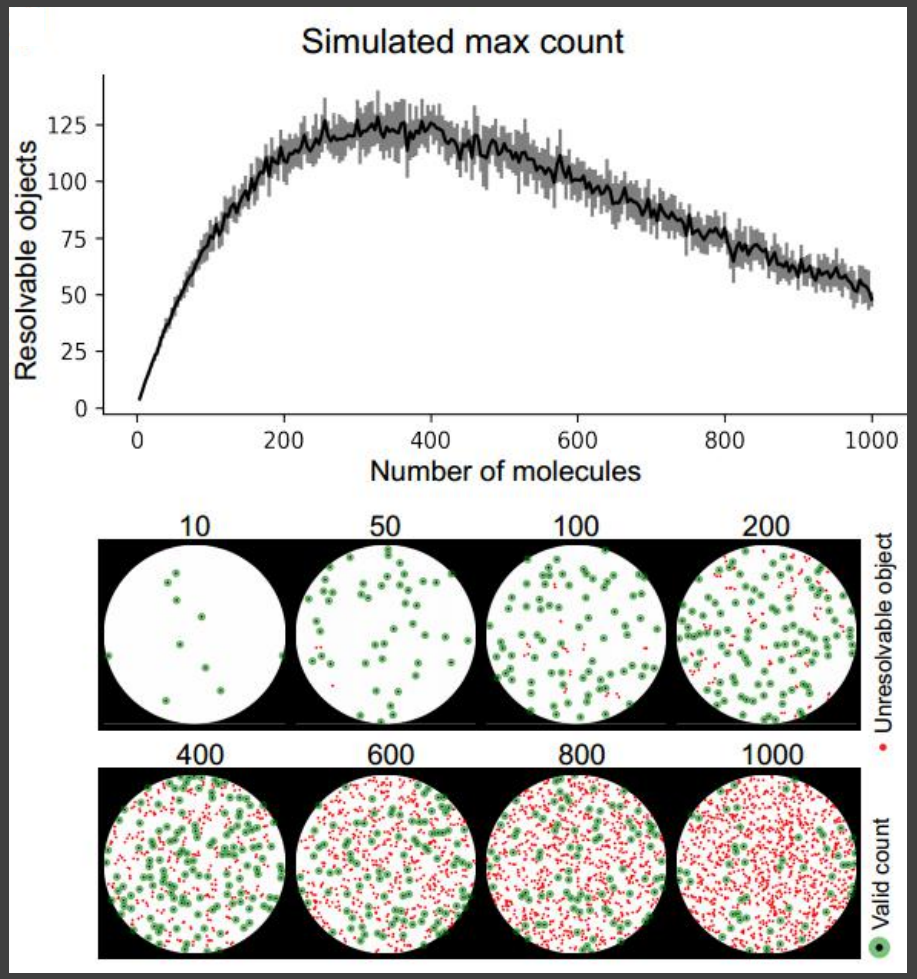
Barcoding Optical space





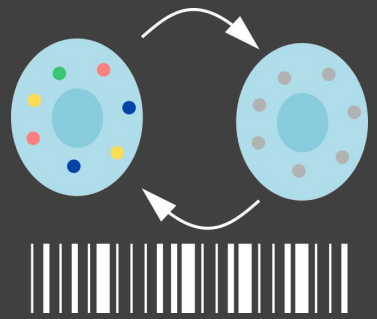
Barcoding Optical space

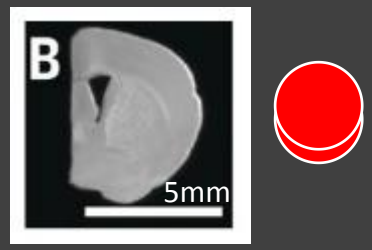


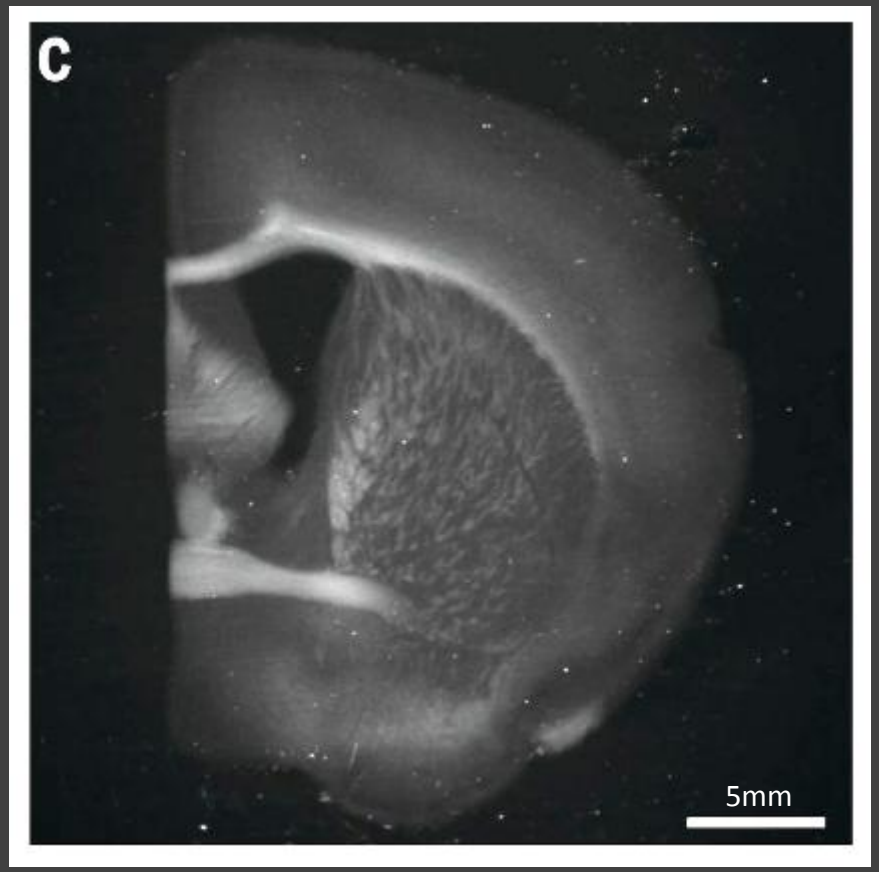


Codeluppi *et al.* Nature Methods 2019

Expansion microscopy

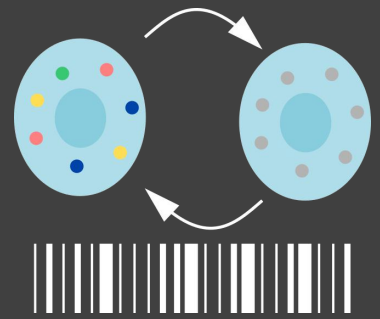


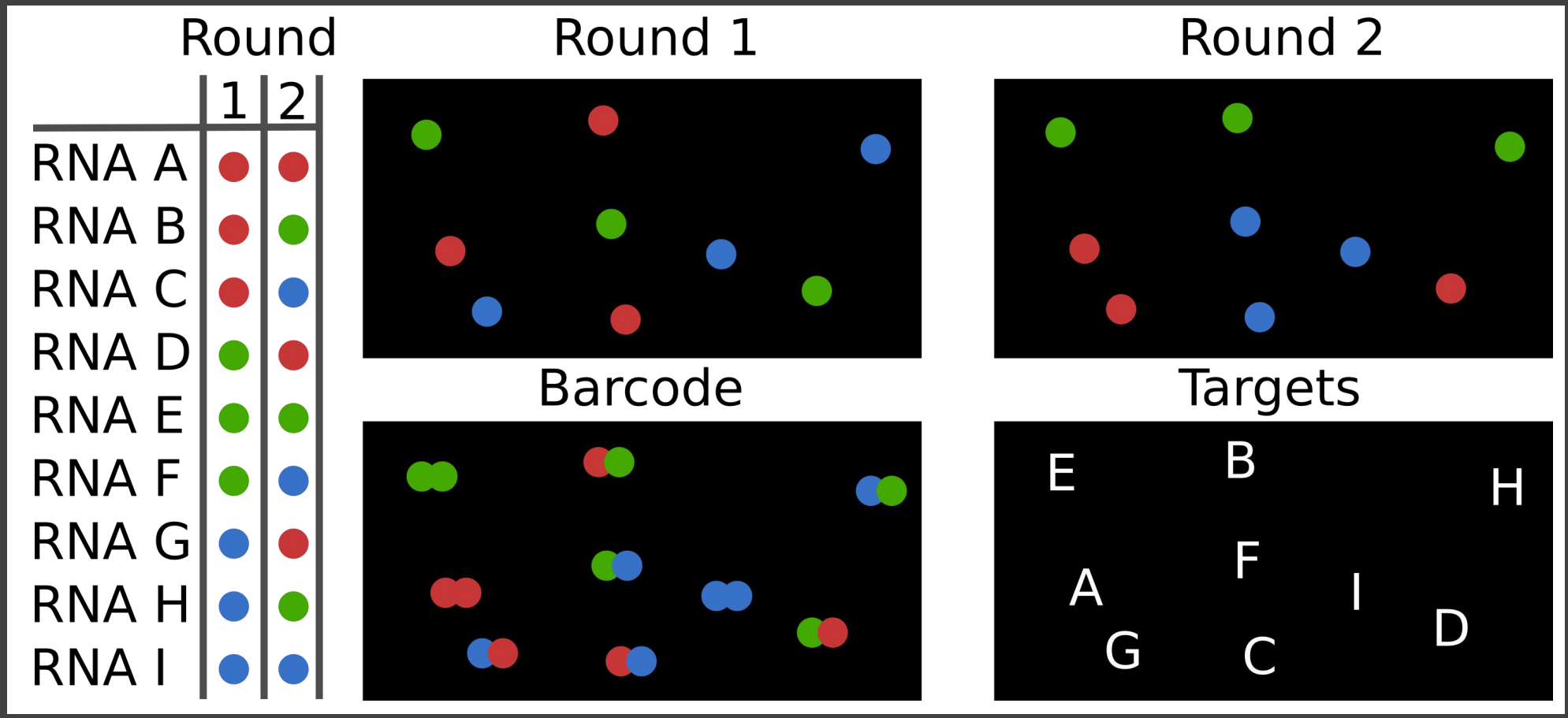




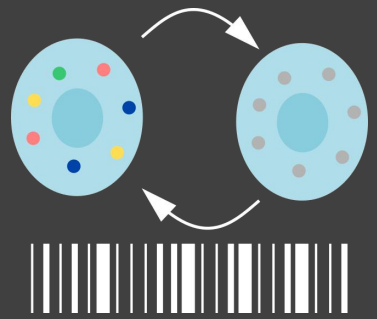
Chen *et al.* Science 2015

Dense barcode





Dense vs Sparce barcode



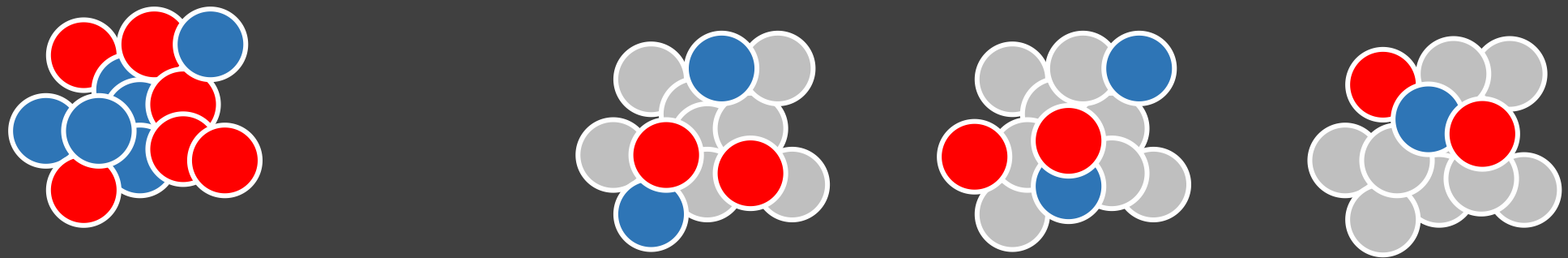
Dense

RRBRB

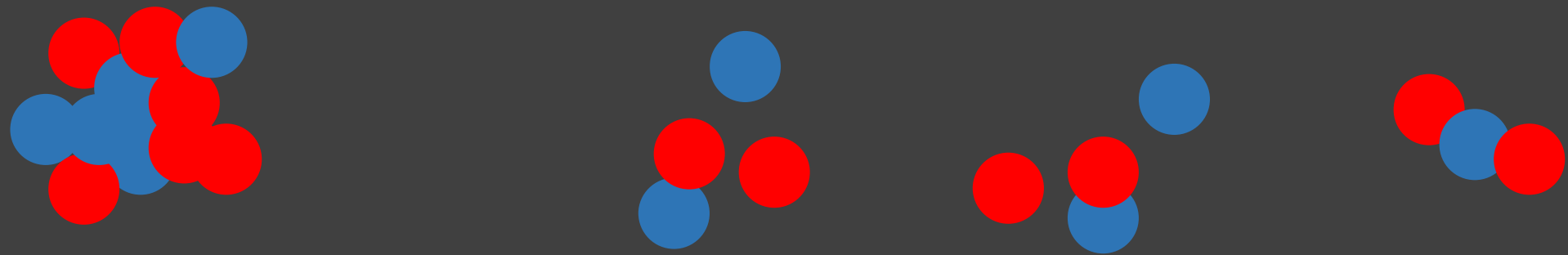
Sparce

R00B0B0

Molecules

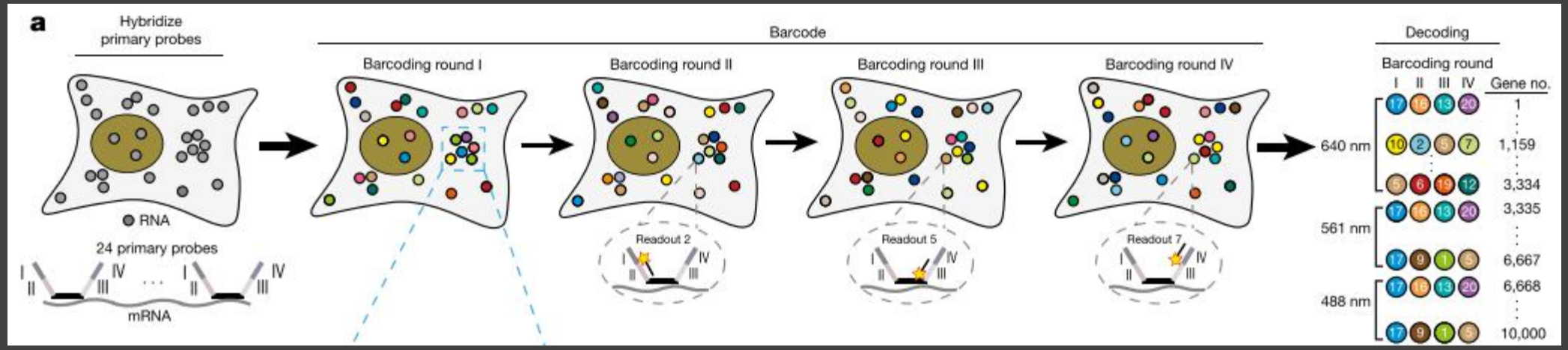


Image



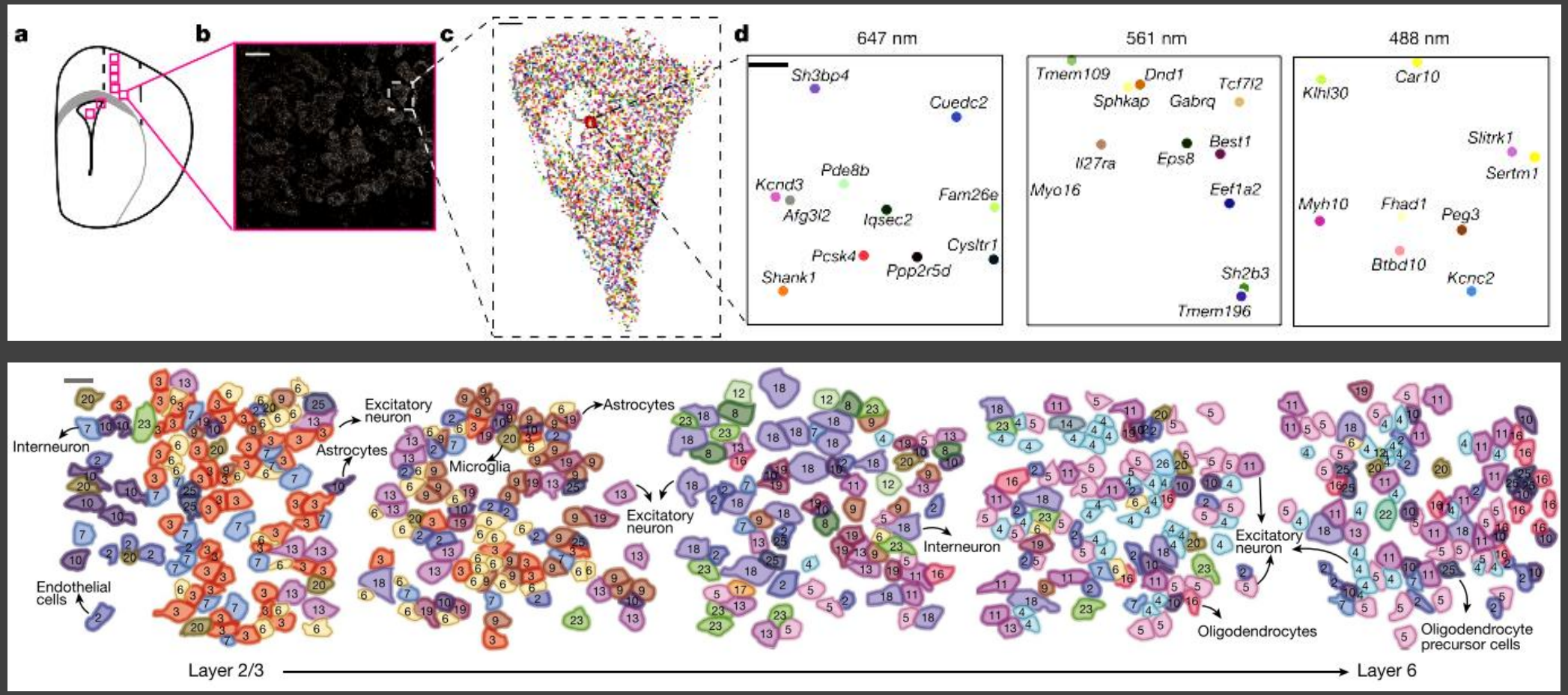
SeqFISH+

Sparce barcode



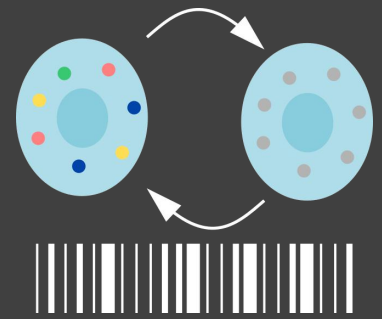
Eng *et al.* Nature 2019

SeqFISH+

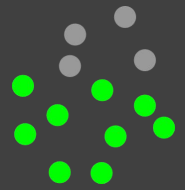


Eng *et al.* Nature 2019

Barcoded smFISH

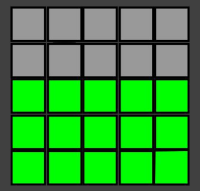


Resolution: Diffraction limited (150-300nm)



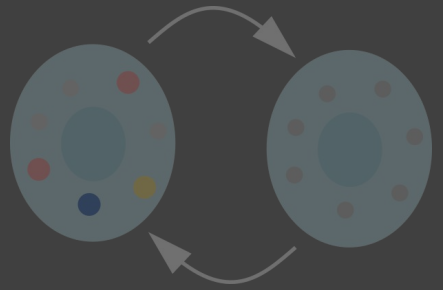
Detection efficiency: 70-90%

Gene throughput: 100 - 10,000

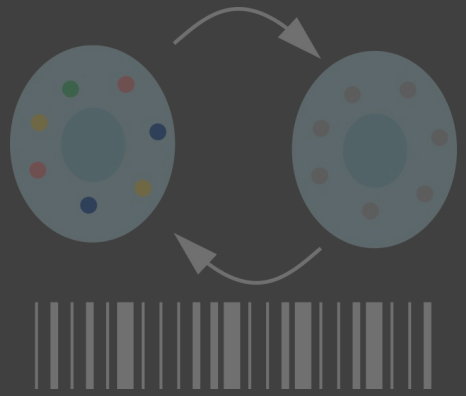


Spatial throughput: several mm^2

Cyclic FISH



Barcoded FISH

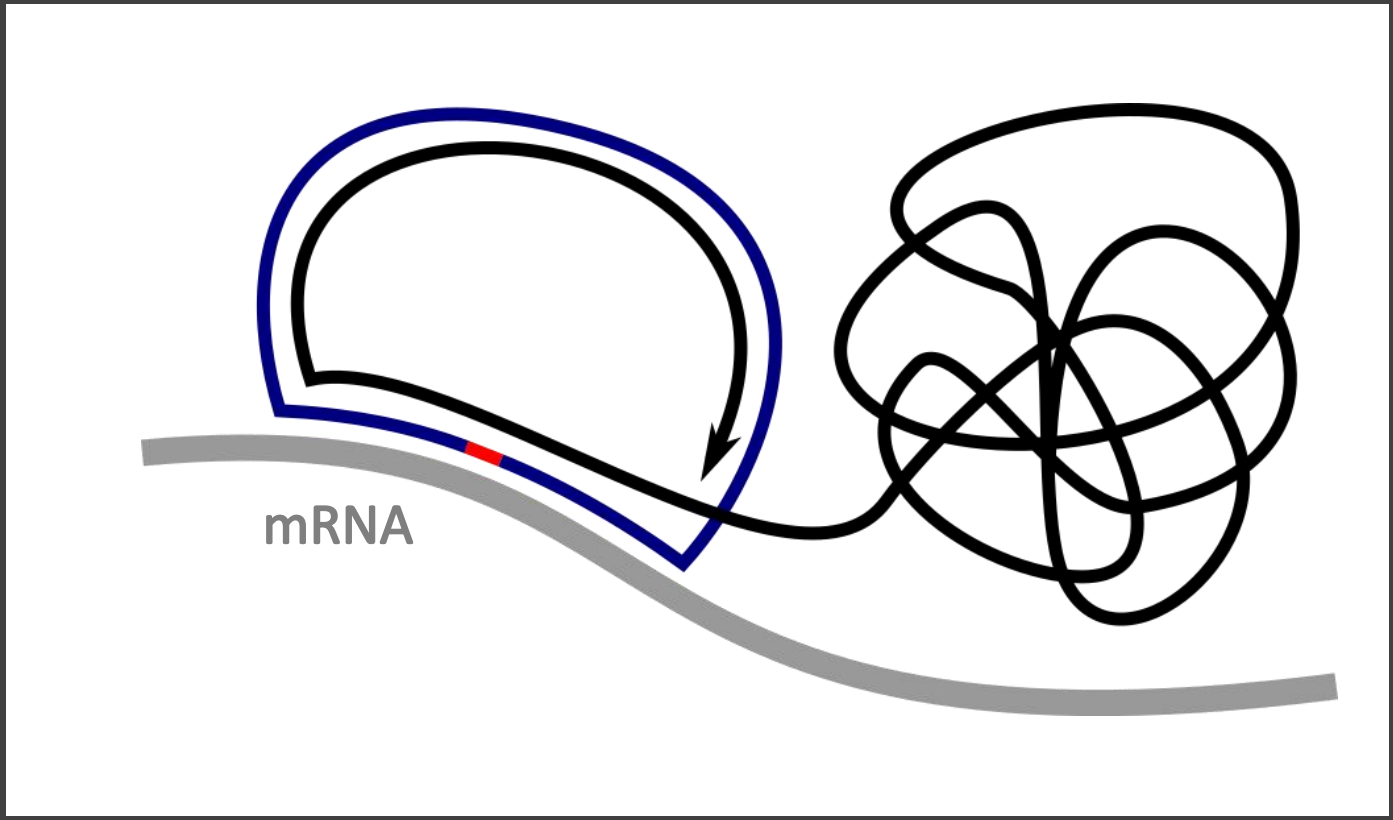


in situ Sequencing

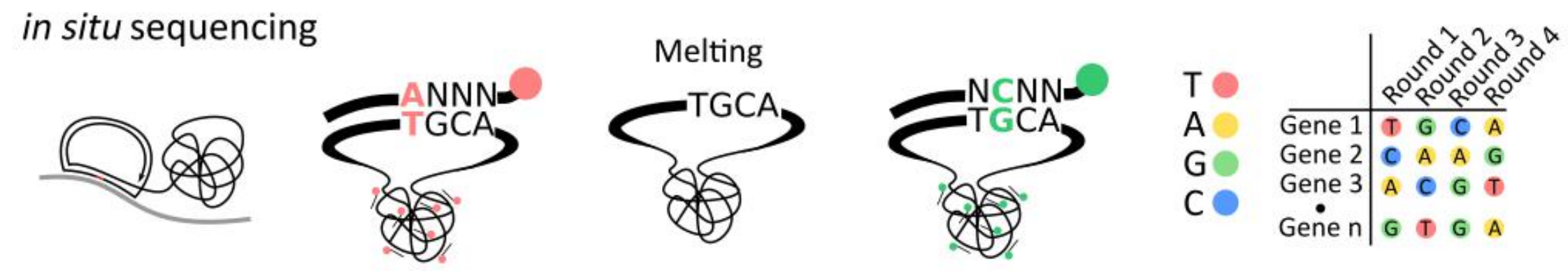
Spatial Sequencing

in situ sequencing

Rolling circle amplification

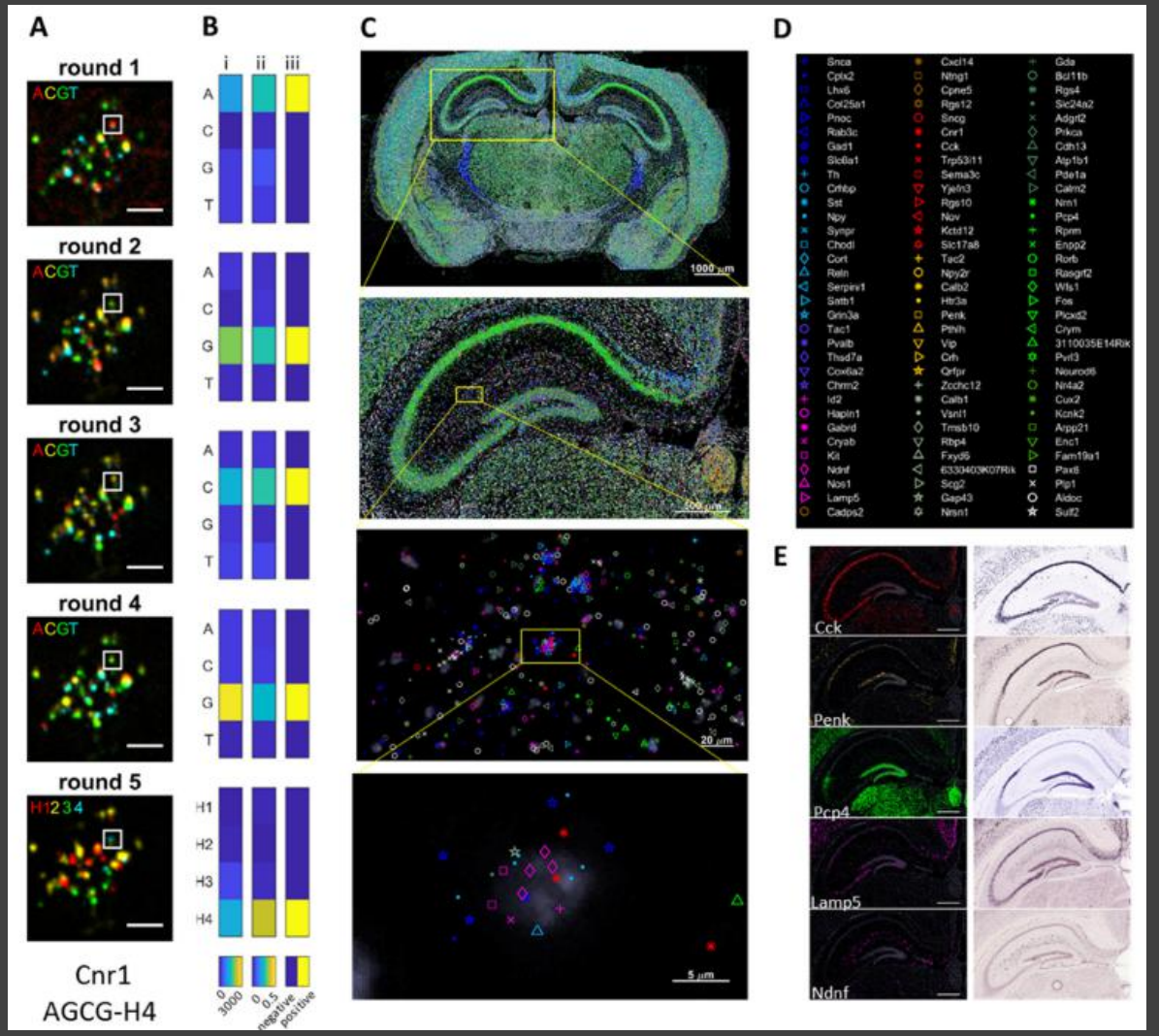


in situ sequencing



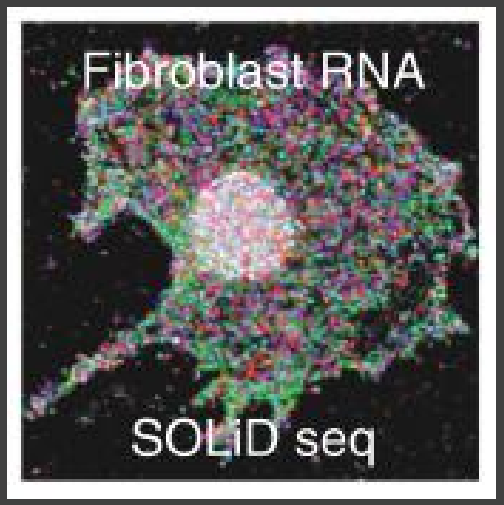
Sequencing by ligation

in situ sequencing



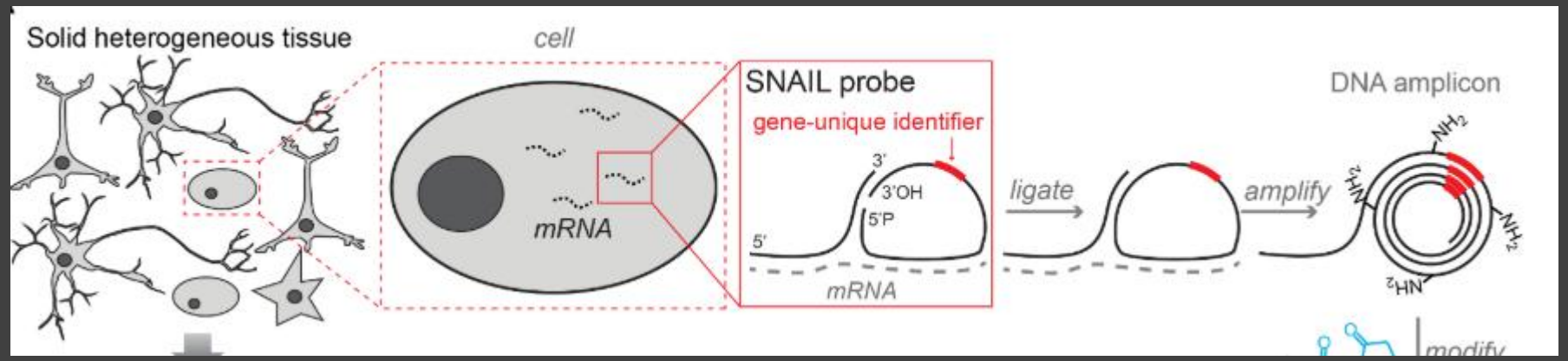
Qian *et al.* BioRxiv 2018

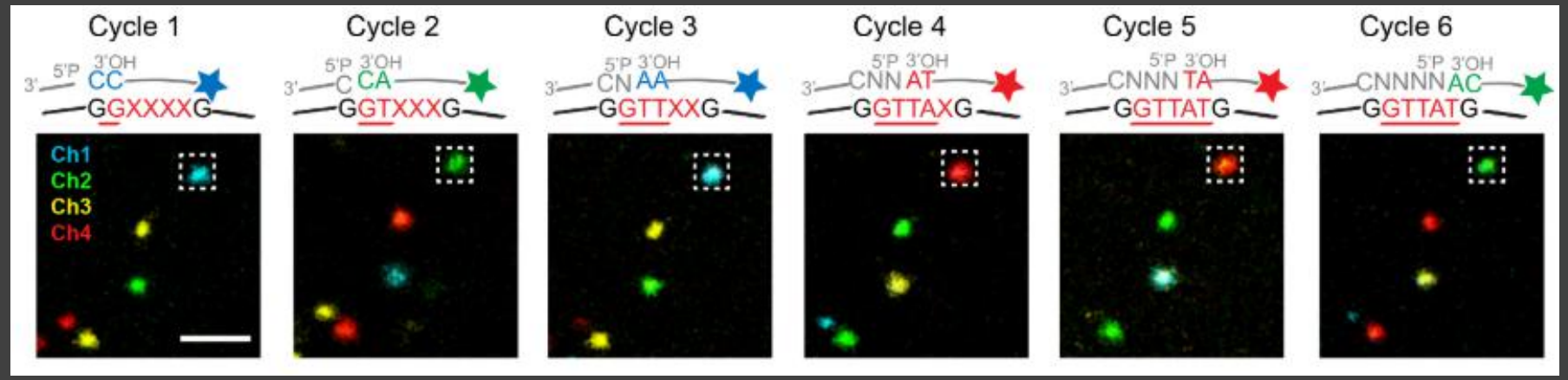
FISSEQ



Lee *et al. Science* 2014

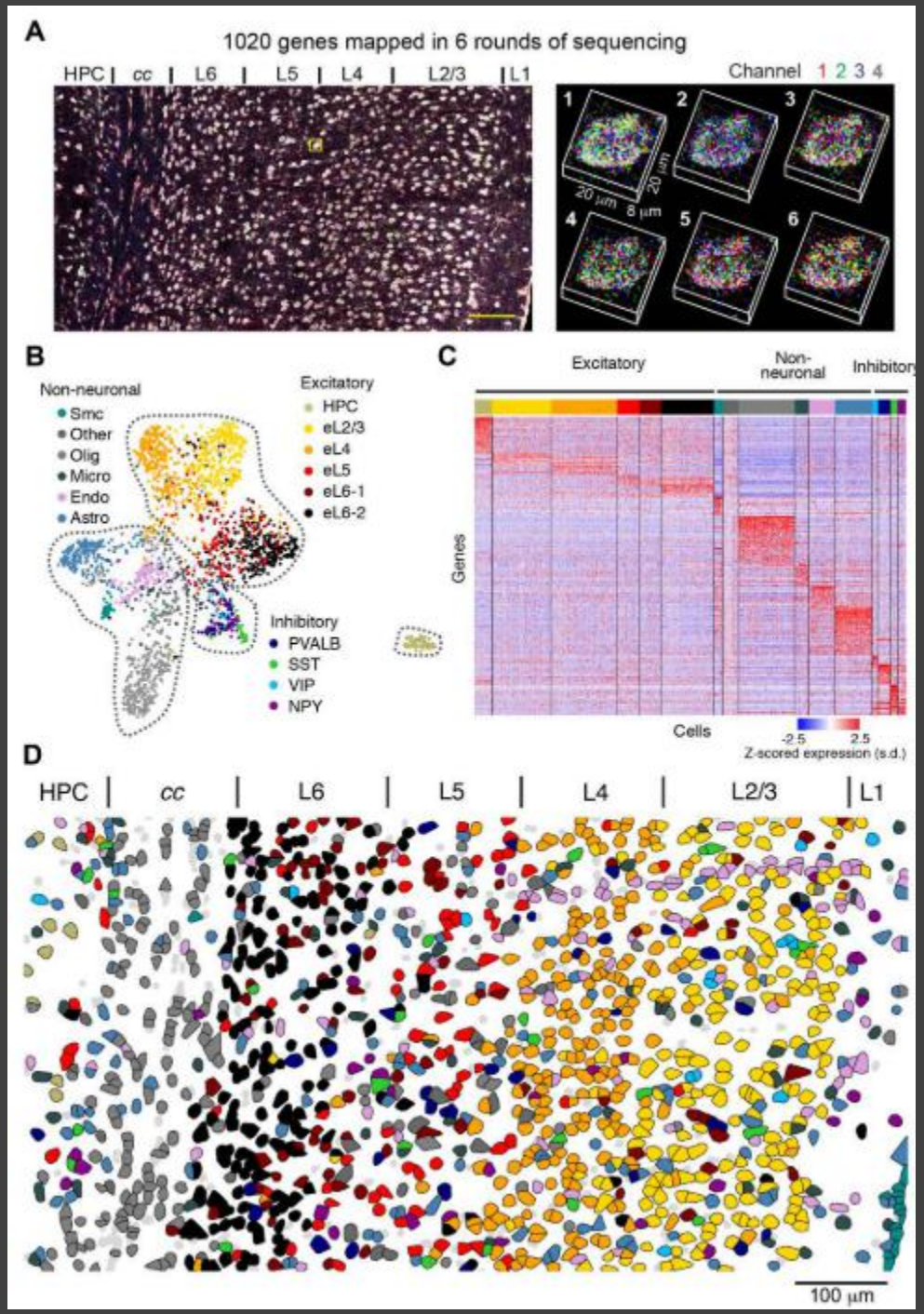
STAR MAP





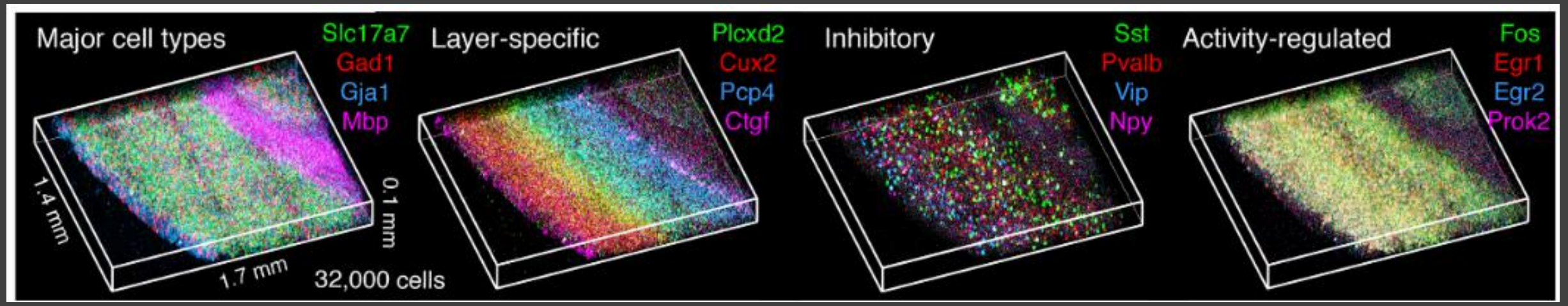
Wang *et al.* Science 2018

STAR MAP



Wang *et al.* Science 2018

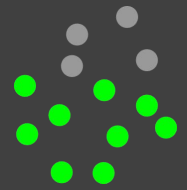
STAR MAP



Wang *et al.* Science 2018

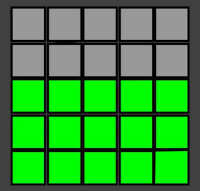
Sequencing *in situ*

Resolution: Amplicon size (0.5 - 1um)



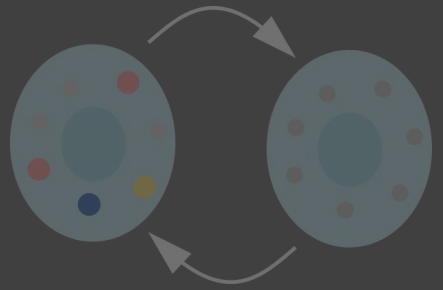
Detection efficiency: 0.01 - ~50%

Gene throughput: 10 - 1,000

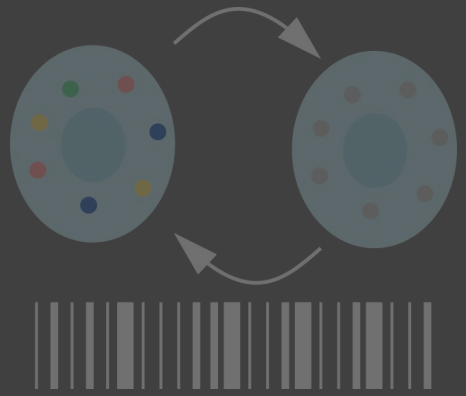


Spatial throughput: several mm^2 - several cm^2

Cyclic FISH

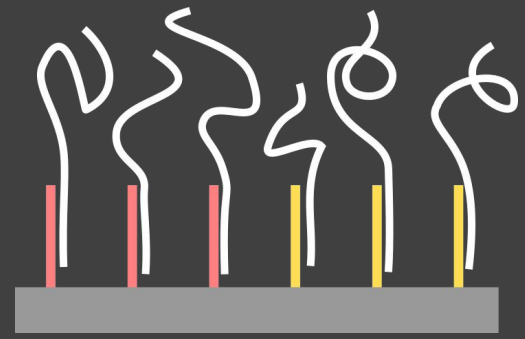


Barcoded FISH

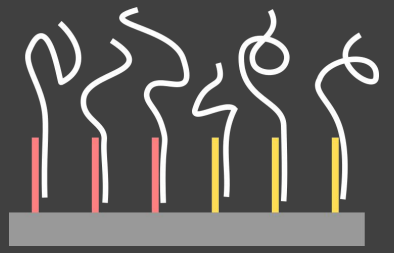


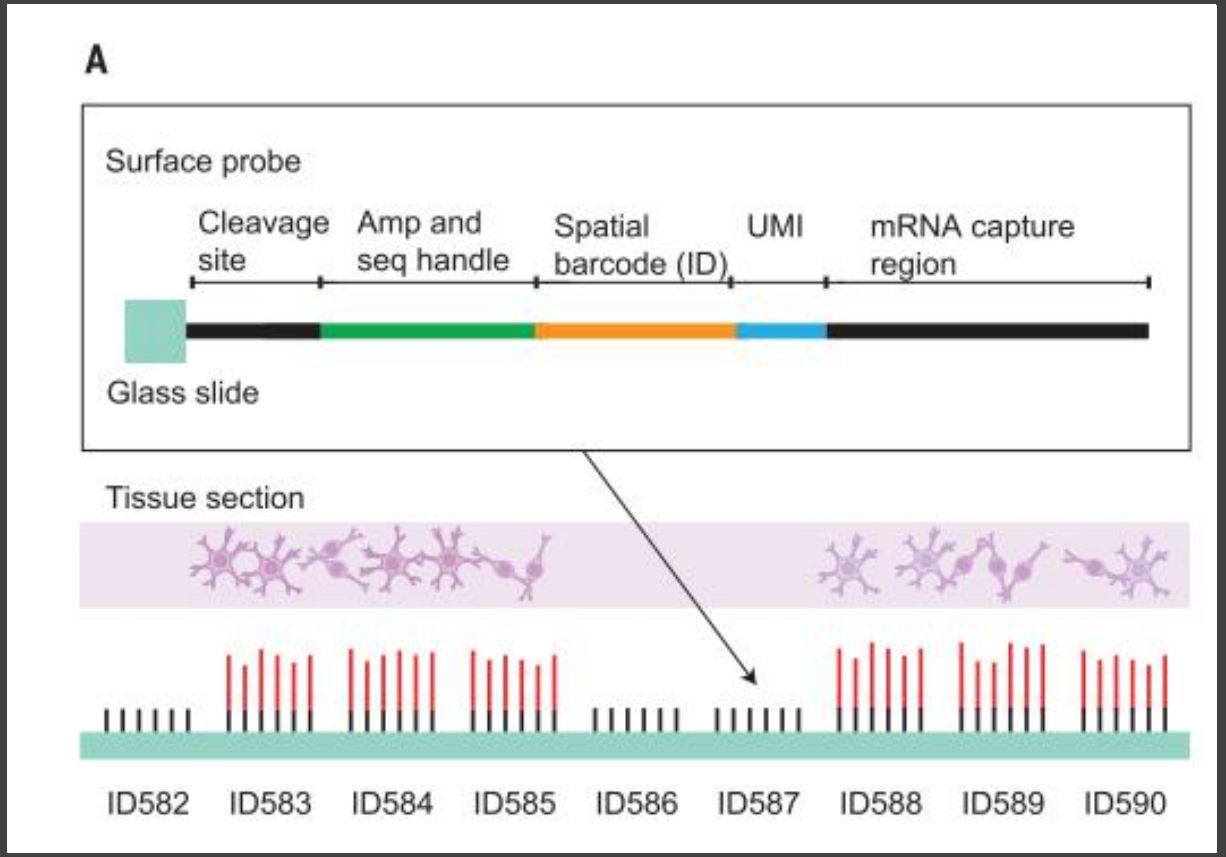
in situ Sequencing

Spatial Sequencing



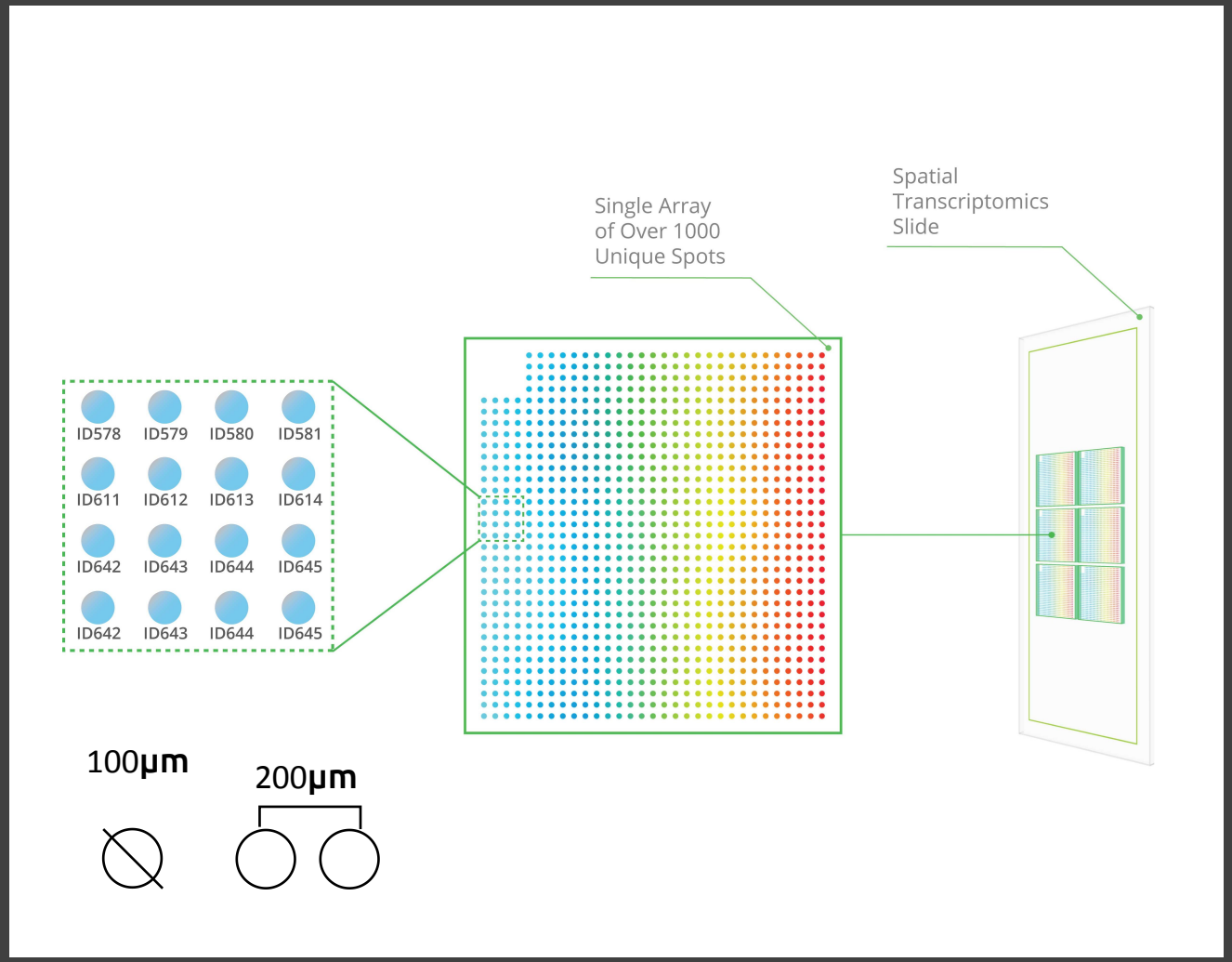
Spatial transcriptomics





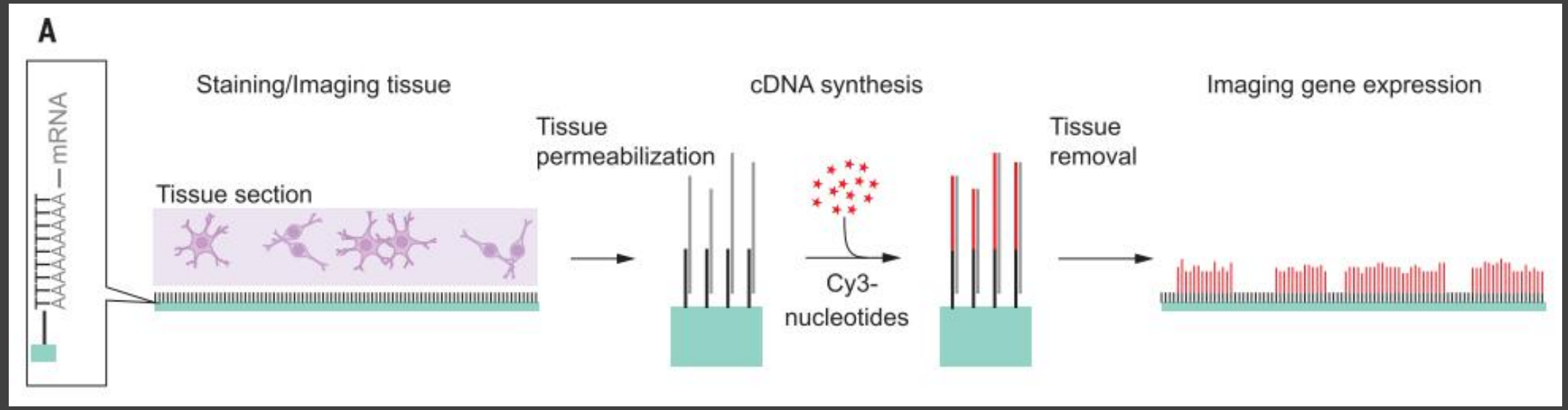
Stahl *et al.* Science 2016

Spatial transcriptomics



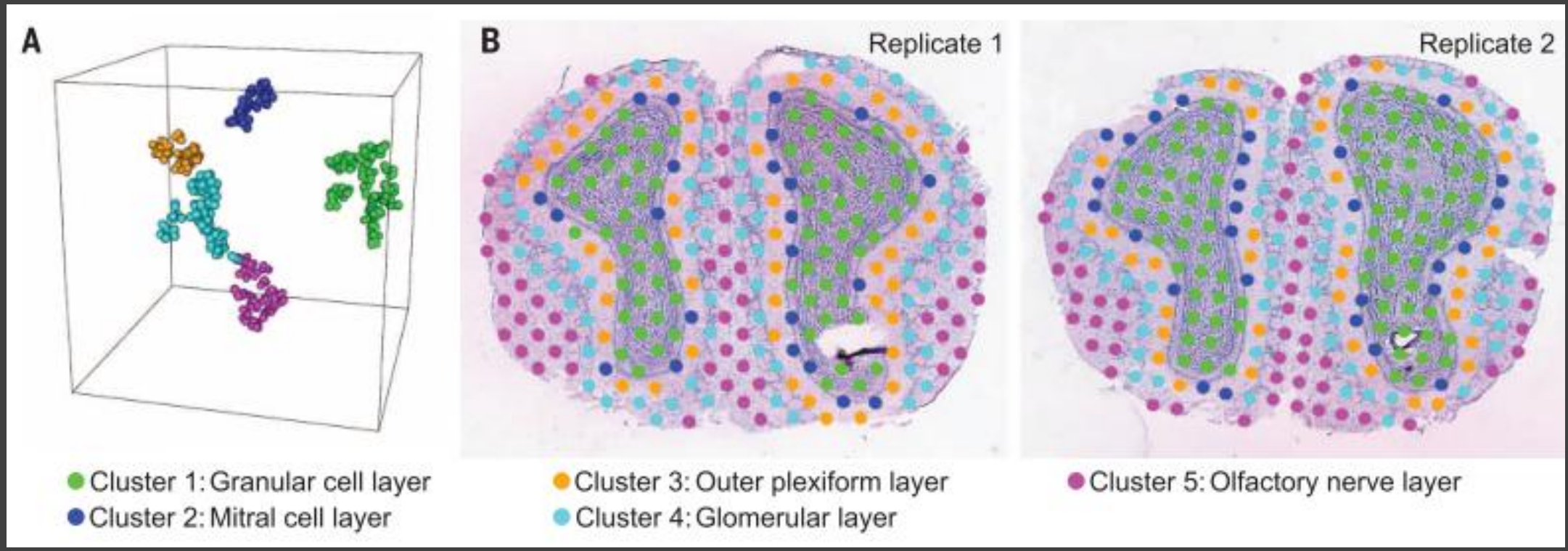
Spatial Transcriptomics

Spatial transcriptomics



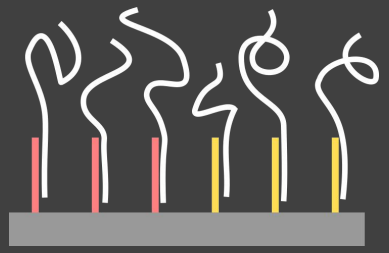
Stahl *et al.* Science 2016

Spatial transcriptomics



Stahl *et al.* Science 2016

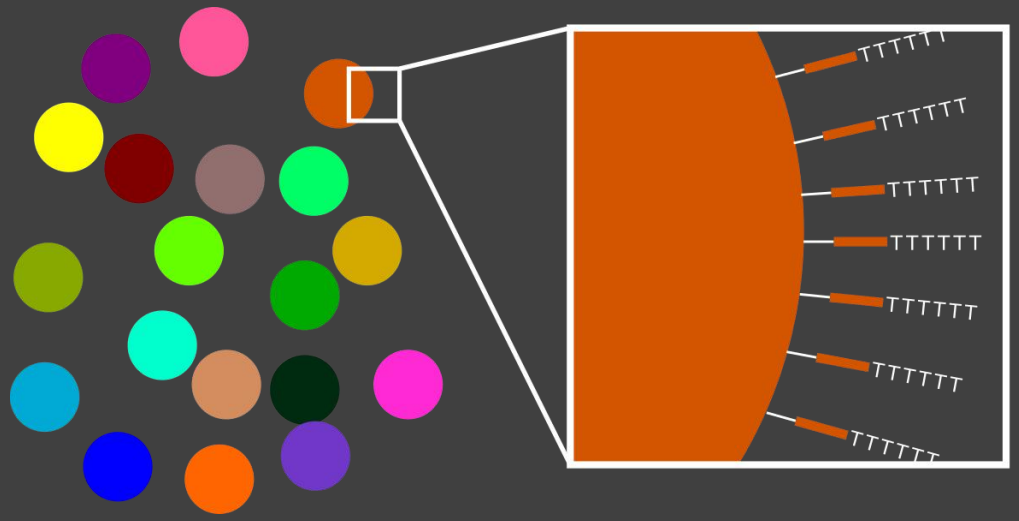
Array of barcoded beads



Random barcoded beads

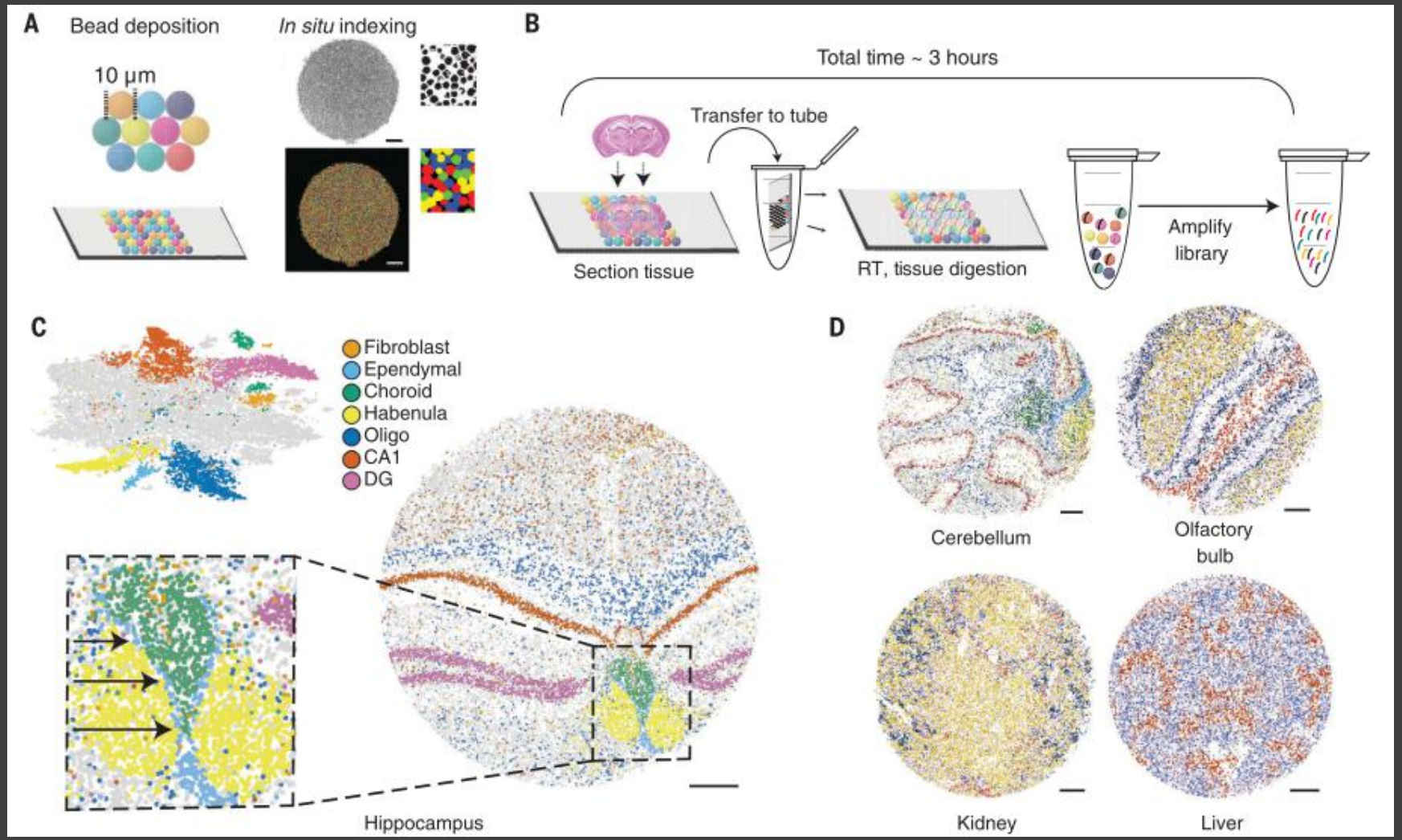
Array

Decoded array



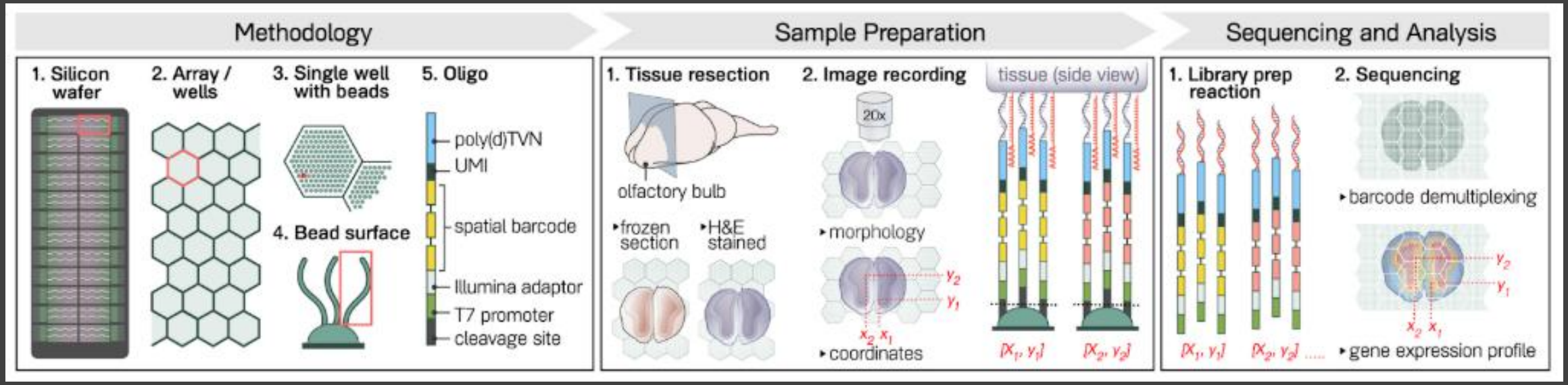
Barcode	X	Y
AACGTC	1	2
CGTTCA	2	5
TCGATC	1	6
GGTACT	5	3
TACCGA	2	3

Slide-Seq



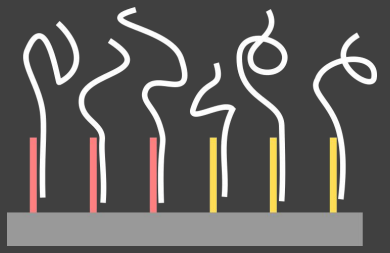
Rodriques *et al.* Science 2016

High density Spatial transcriptomics

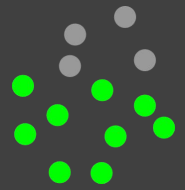


Vickovic *et al*. BioRxiv 2019

Spatial Sequencing

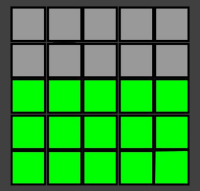


Resolution: Spot size 2um - 100um



Detection efficiency: 0.1 - 1%

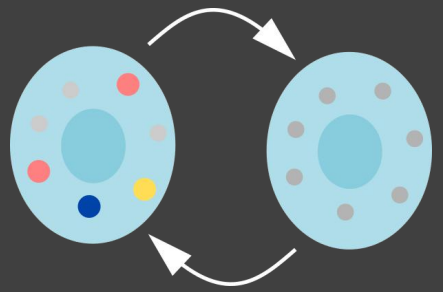
Gene throughput: Full transcriptome



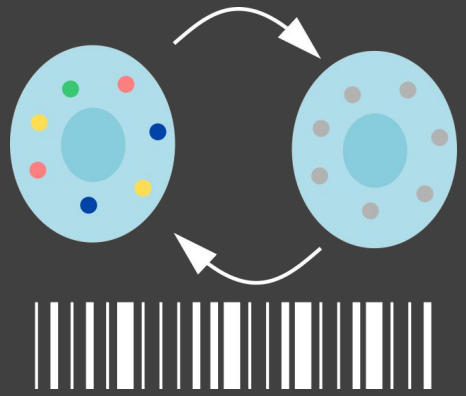
Spatial throughput: several cm^2

RNA spatial detection

Cyclic FISH

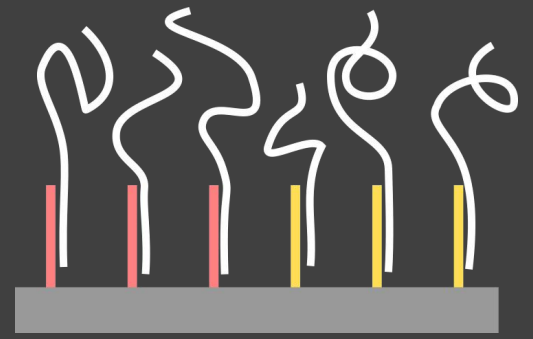


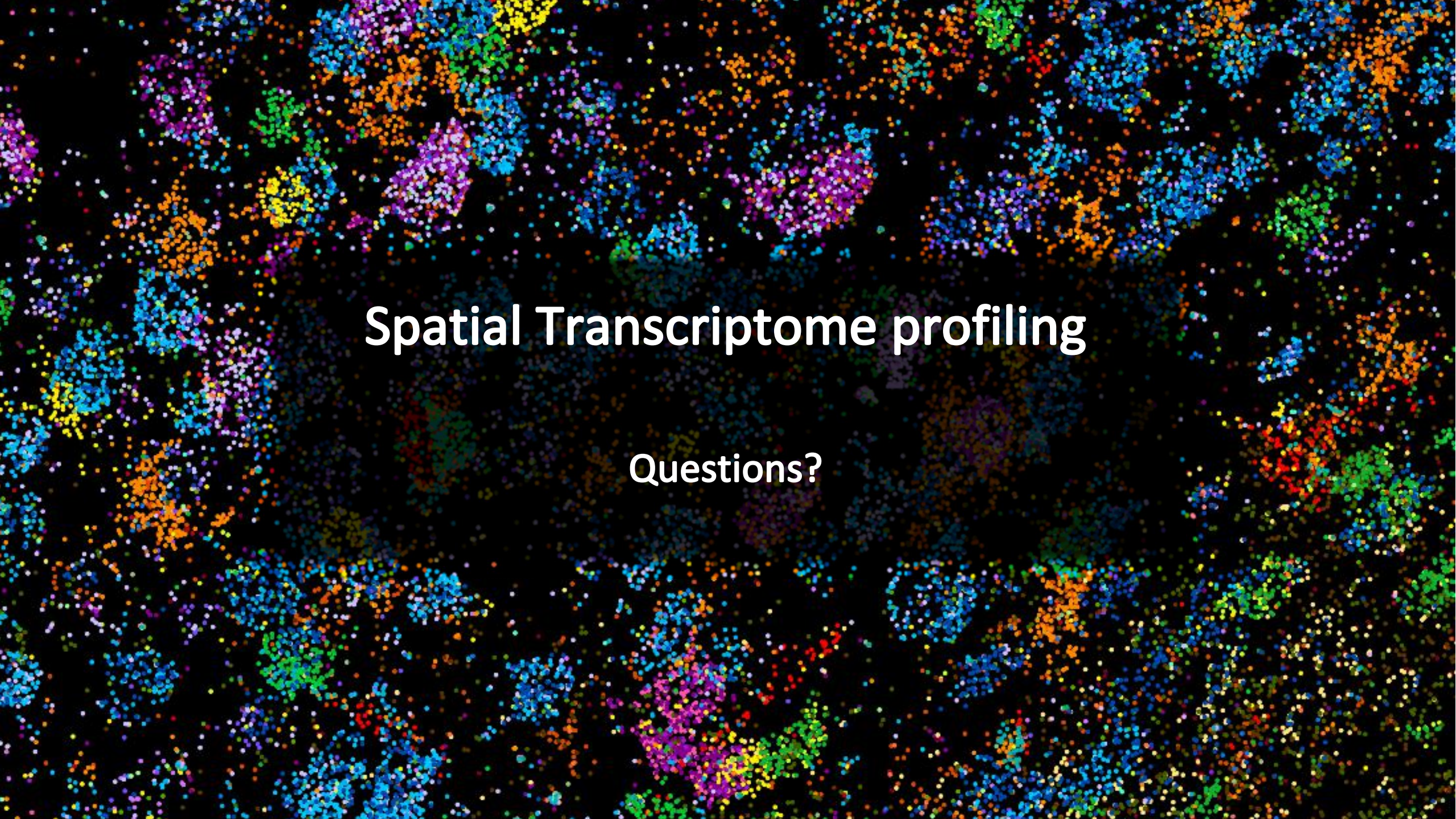
Barcoded FISH

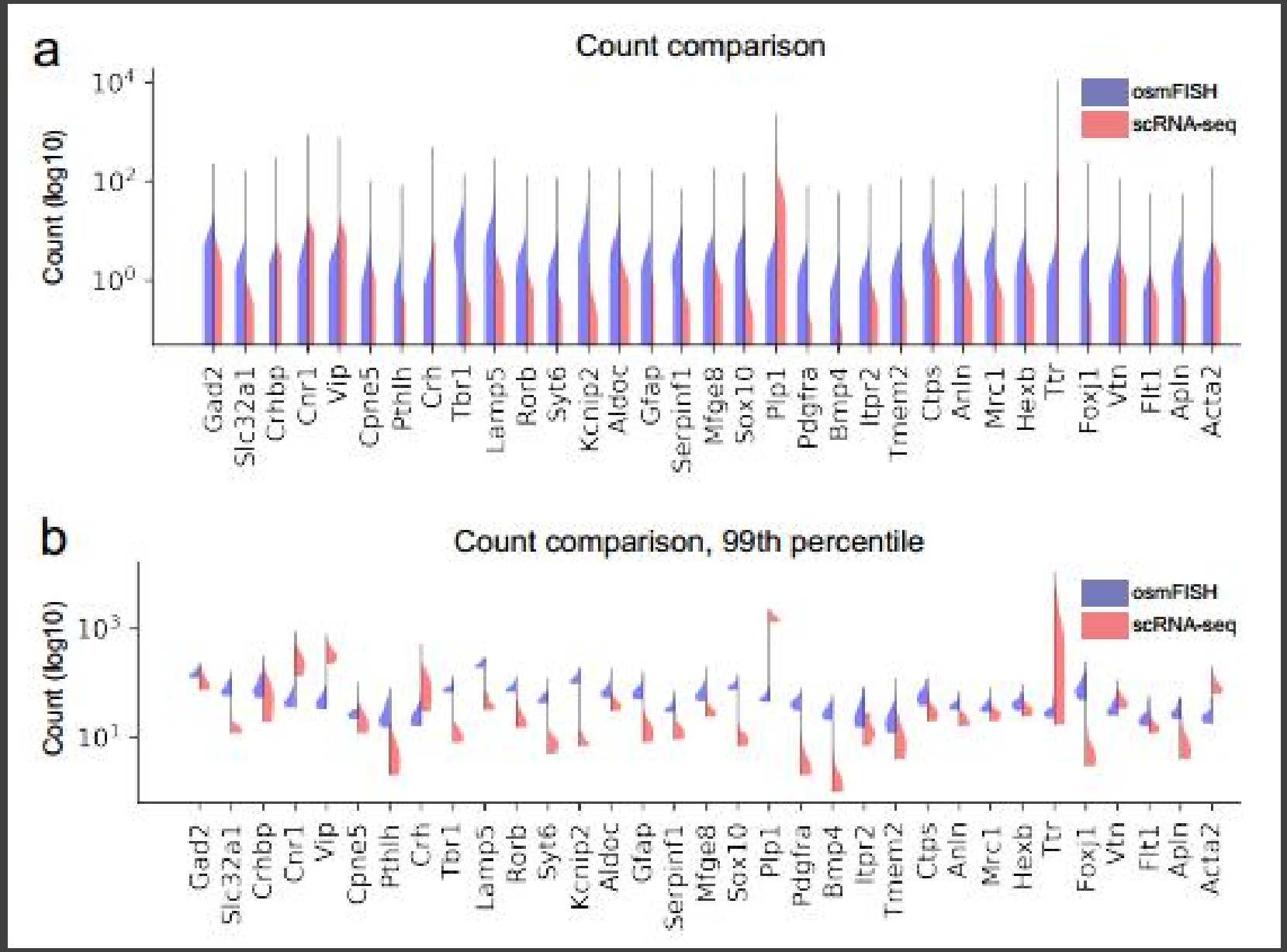


in situ Sequencing

Spatial Sequencing




Spatial Transcriptome profiling
Questions?


a
Count comparison
Count (log10)
10^4
10^2
10^0
osmFISH
scRNA-seq
Gad2
Slc32a1
Crhbp
Cnr1
Vip
Cpne5
Pthlh
Crh
Tbr1
Lamp5
Rorb
Syt6
Kcnip2
Aldoc
Gfap
Serpinf1
Mfge8
Sox10
Plp1
Pdgfra
Bmp4
Itpr2
Tmem2
Ctps
Anln
Mrc1
Hexb
Ttr
Foxj1
Vtn
Flt1
Apln
Acta2
b
Count comparison, 99th percentile
Count (log10)
10^3
10^1
osmFISH
scRNA-seq
Gad2
Slc32a1
Crhbp
Cnr1
Vip
Cpne5
Pthlh
Crh
Tbr1
Lamp5
Rorb
Syt6
Kcnip2
Aldoc
Gfap
Serpinf1
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Foxj1
Vtn
Flt1
Apln
Acta2