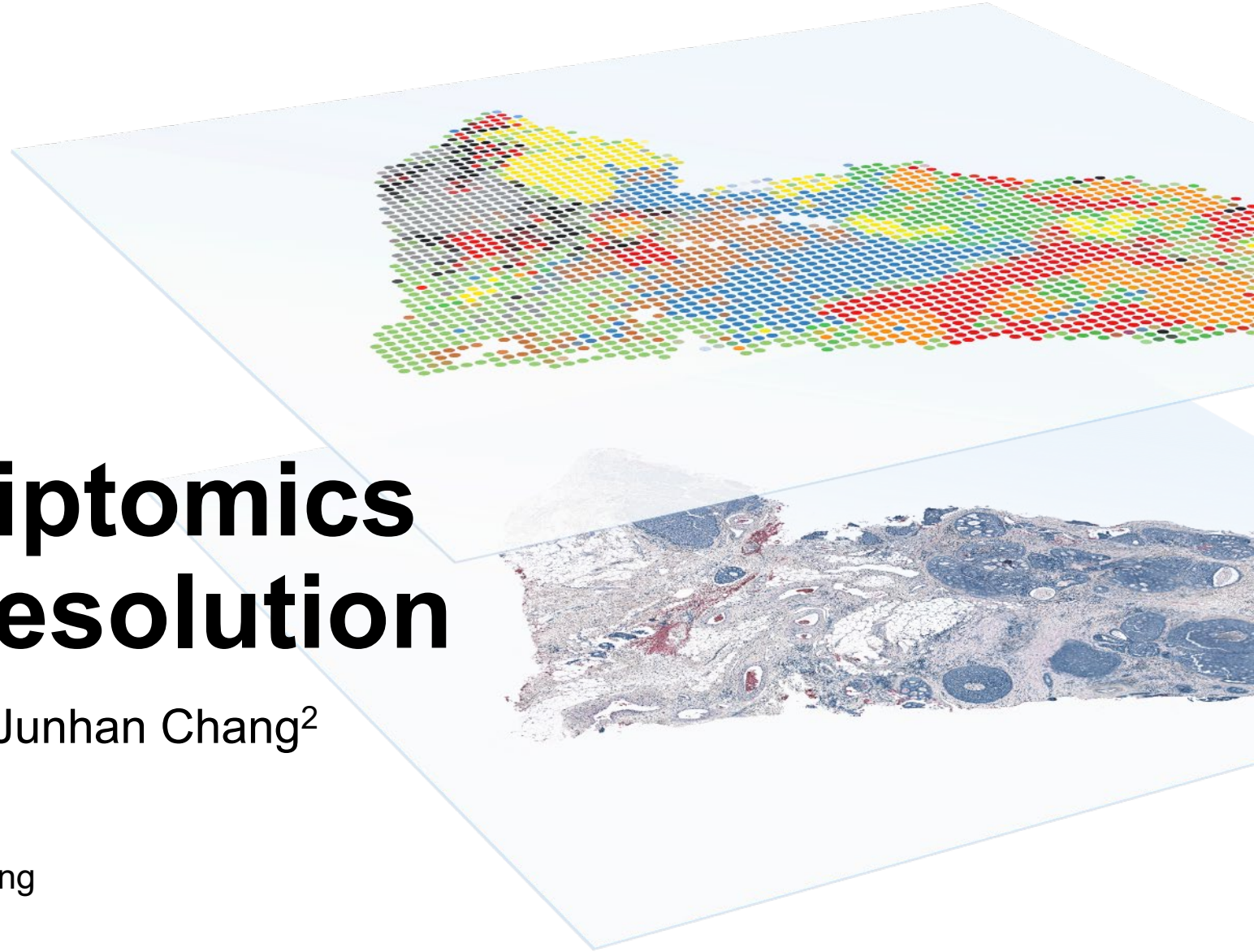
2021.05.15 UHPB Journal Club

Spatial Transcriptomics at Single-cell Resolution

Yongcheng Jiang[1], Wuji Han[1] and Junhan Chang[2]

[1] Integrated Science Program, Yuanpei College

[2] College of Chemistry and Molecular Engineering



Honored guest of today

Prof. Letian Tao
School of Life Sciences, PKU

Prof. Guoqiang Li
BIOPIC, PKU

Outline of the Journal Club

PART 1. Introduction

Asp, M., Bergenstråhle, J., & Lundeberg, J. (2020). Spatially resolved transcriptomes—next generation tools for tissue exploration. *BioEssays*, 42(10), 1900221.

PART 2. seqFISH+

Eng, C. H. L., Lawson, M., Zhu, Q., Dries, R., Koulena, N., Takei, Y., ... & Cai, L. (2019). Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+. *Nature*, 568(7751), 235-239.

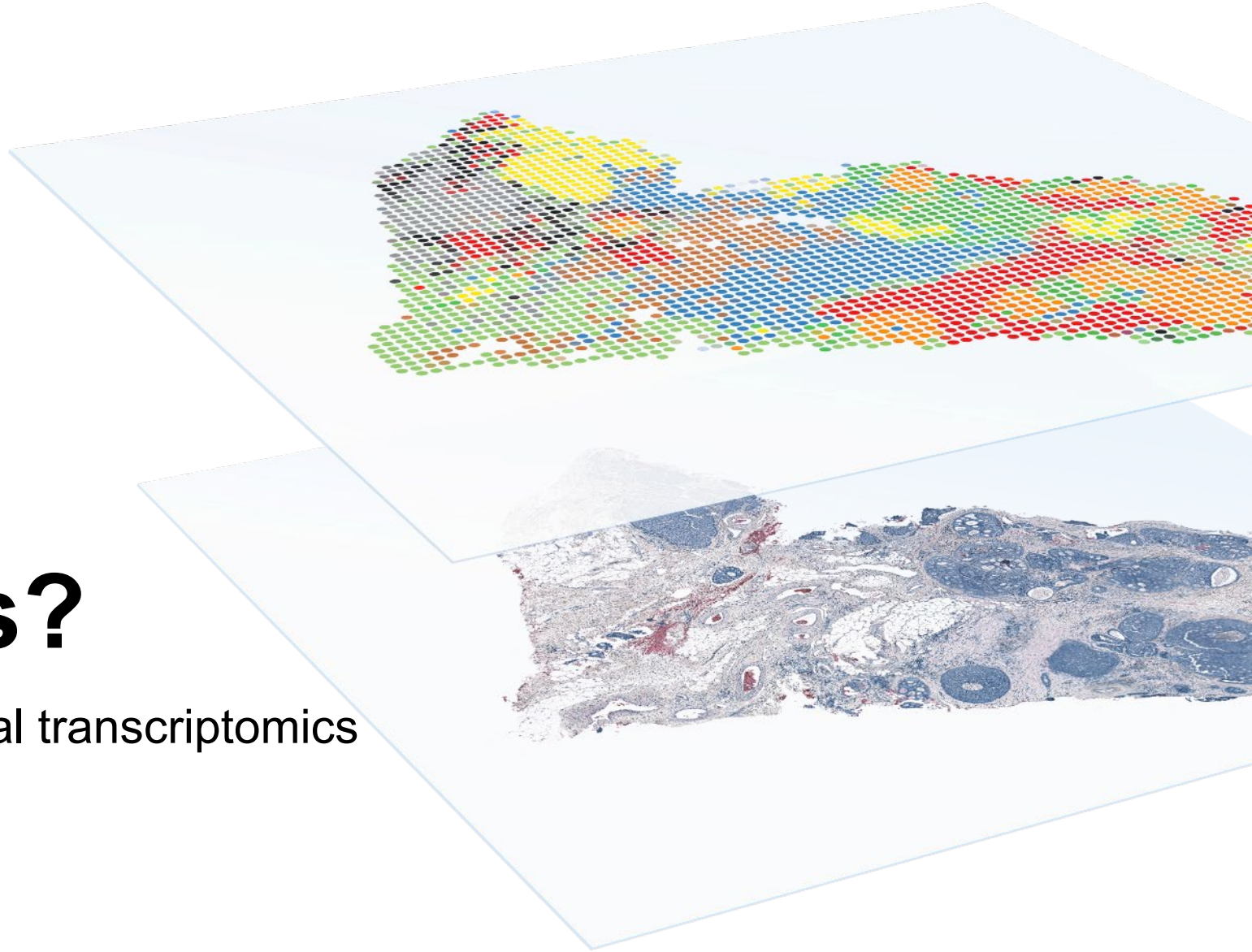
PART 3. CSOmap

Ren, X., Zhong, G., Zhang, Q., Zhang, L., Sun, Y., & Zhang, Z. (2020). Reconstruction of cell spatial organization from single-cell RNA sequencing data based on ligand-receptor mediated self-assembly. *Cell research*, 30(9), 763-778.

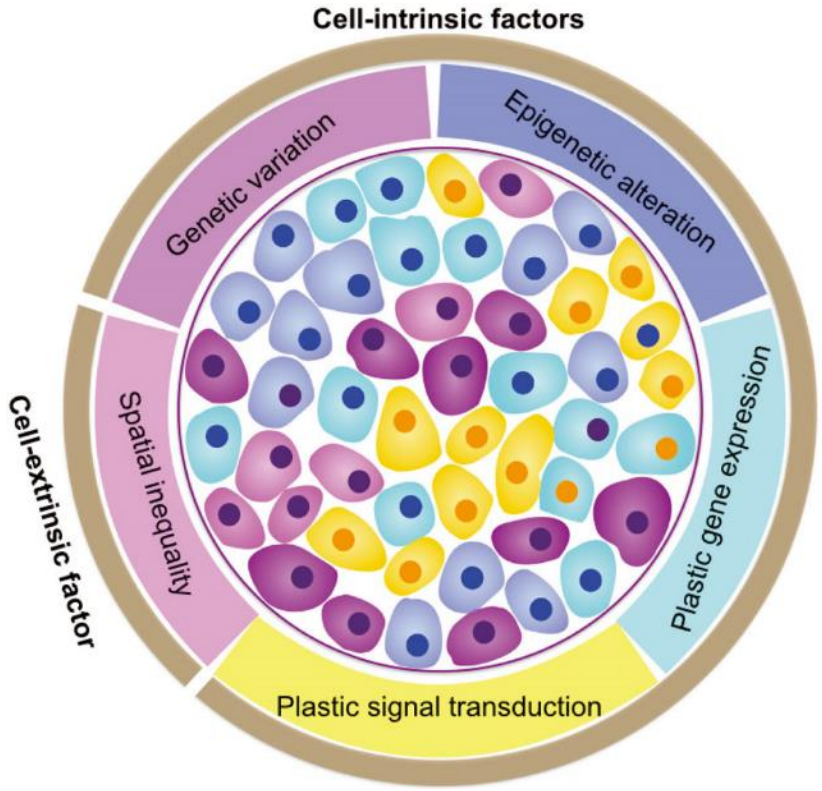
PART 4. Discussion

Why spatial transcriptomics?

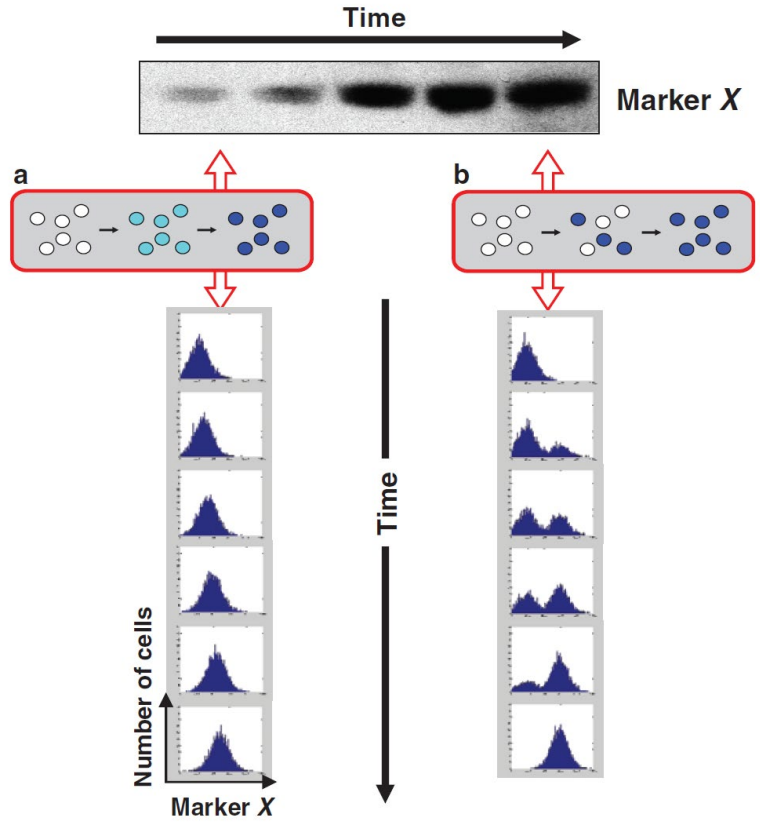
From single cell techniques to spatial transcriptomics



Cell population shows heterogeneity



Extrinsic and intrinsic heterogeneity

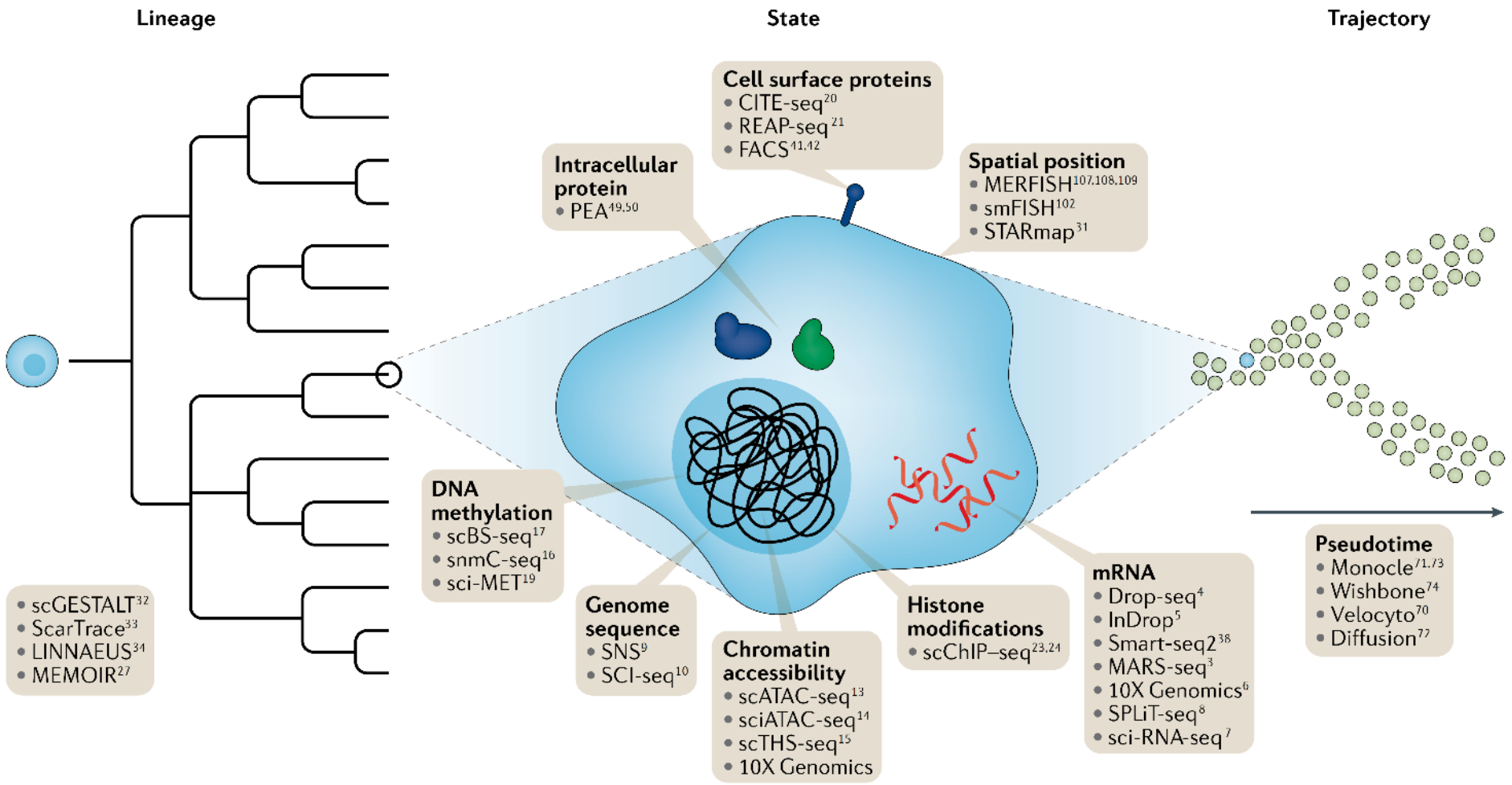


Bulk vs. single cell level data

Sun, X. X., & Yu, Q. *Acta Pharmacologica Sinica* (2015)

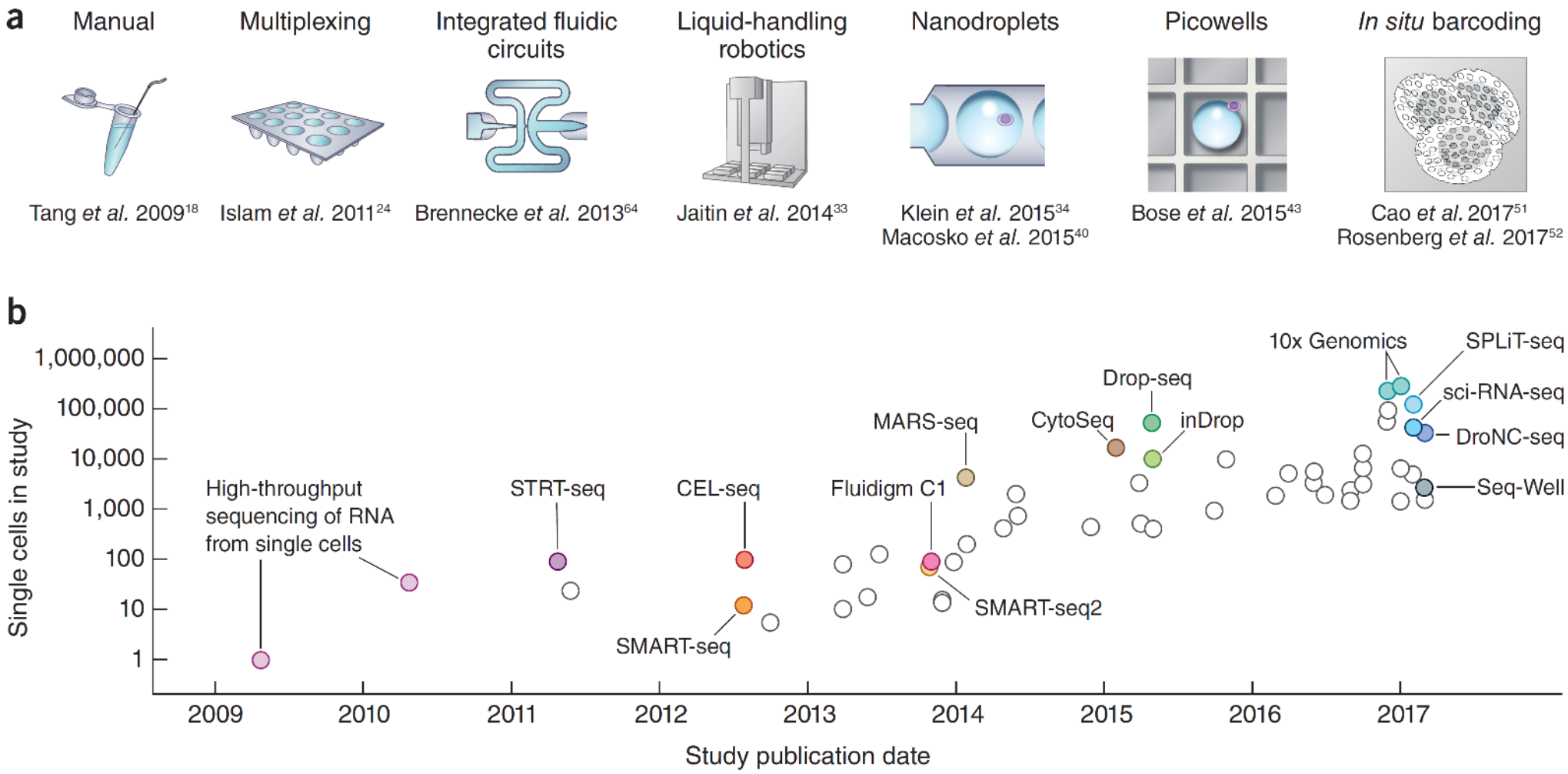
Huang, S. *Developments* (2009)

Single-cell analysis helps reveal cellular heterogeneity



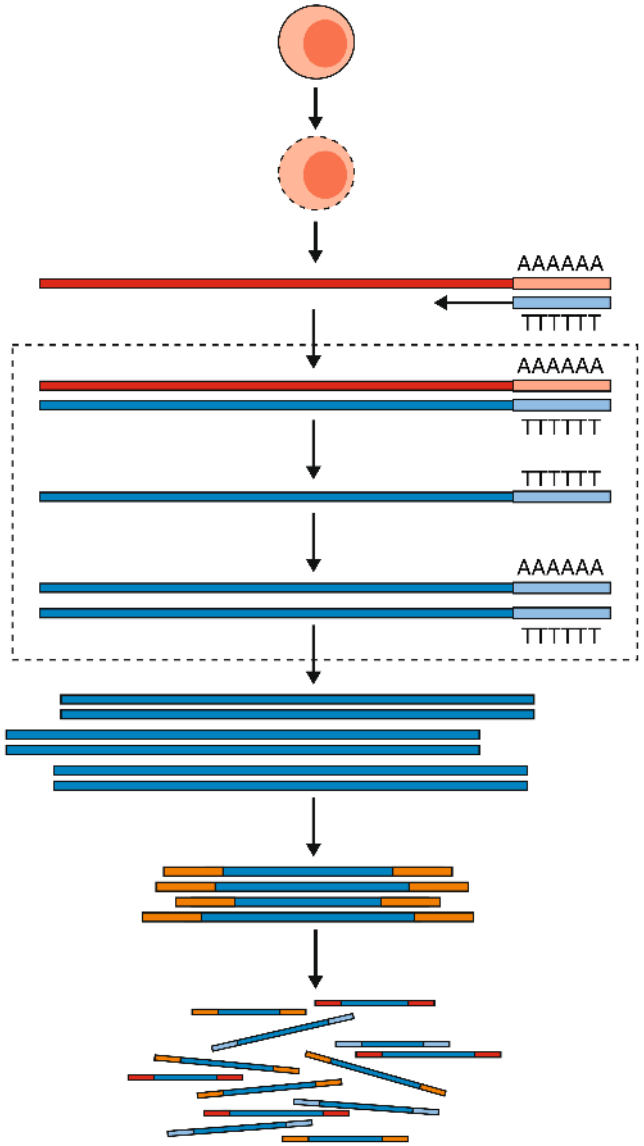
Stuart, T., & Satija, R. *Nature Reviews Genetics* (2019)

Scaling up single-cell RNA sequencing (scRNA-seq)



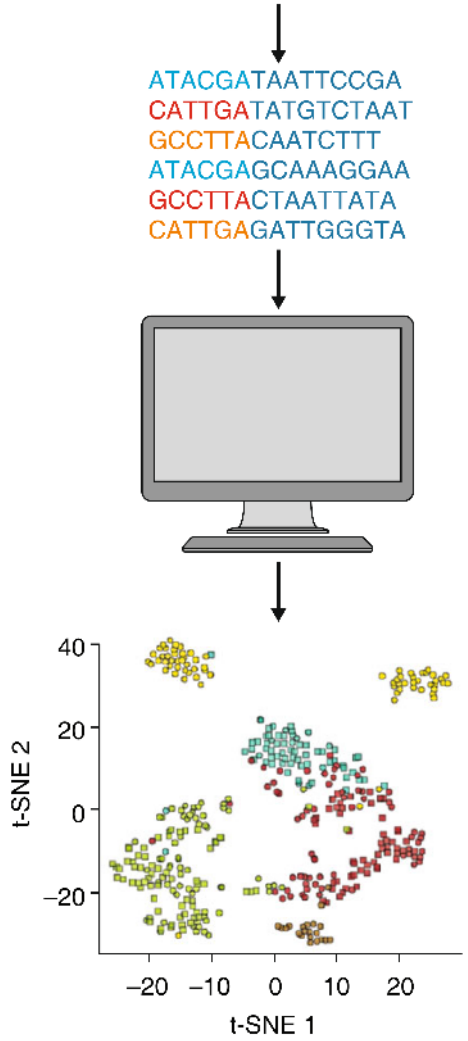
Svensson, V. *et al. Nature Protocols* (2018)

Workflow of scRNA-seq



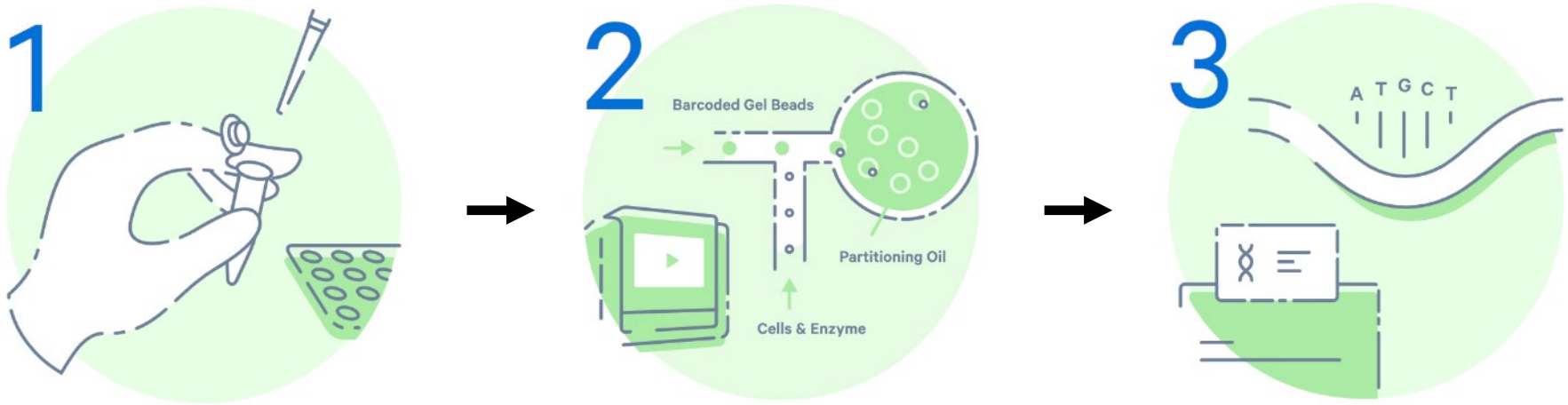
1. Isolate single cells from a tissue sample (including micro-dissection and manipulation, flow cytometric cell-sorting, microfluidic platforms, and droplet-based methods)
2. Single cell lysis in a way that preserves cellular mRNA
3. mRNA molecule capture using poly[T] sequence primers that bind to mRNA poly[A] tails
4. Convert poly[T]-primed mRNA into cDNA using reverse transcription
5. cDNA amplification (usually by PCR or by *in vitro* transcription)
6. cDNA sequencing library preparation (insert 'index' nucleotide barcodes to identify each library)
7. Pool cDNA sequencing libraries

Sequence libraries (via Next Generation Sequencing)



Haque, A. *et al. Genome Medicine* (2017)

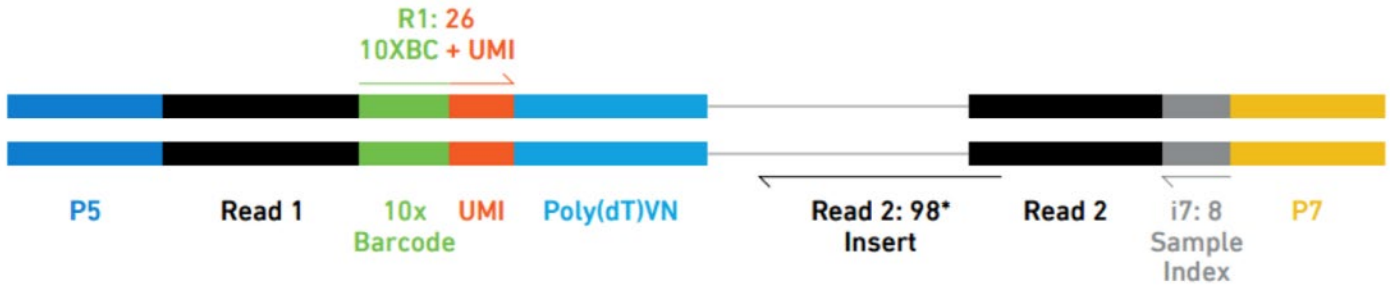
Workflow of scRNA-seq



Sample preparation

Reverse transcription and library construction

Sequencing



Poly(dT): cDNA synthesis

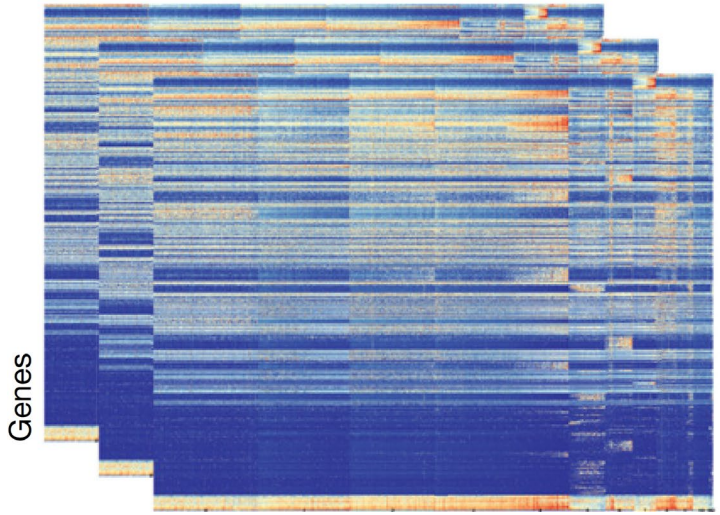
BC: Cellular barcode

UMI: Unique molecule identifier

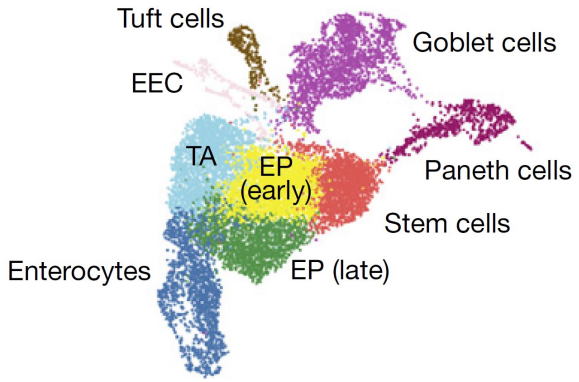
P5, P7: Adaptor → Sequencing

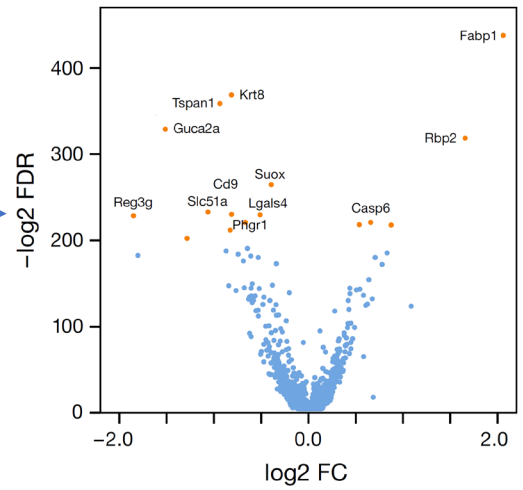
https://www.10xgenomics.com/products/single-cell-gene-expression
https://hbctraining.github.io/scRNA-seq/lessons/02_SC_generation_of_count_matrix.html

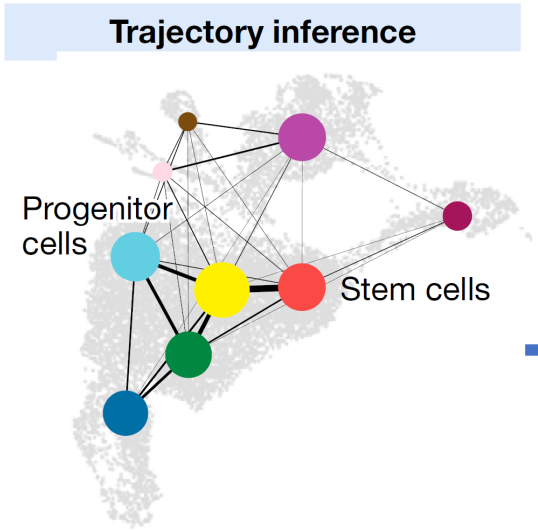
Workflow of scRNA-seq

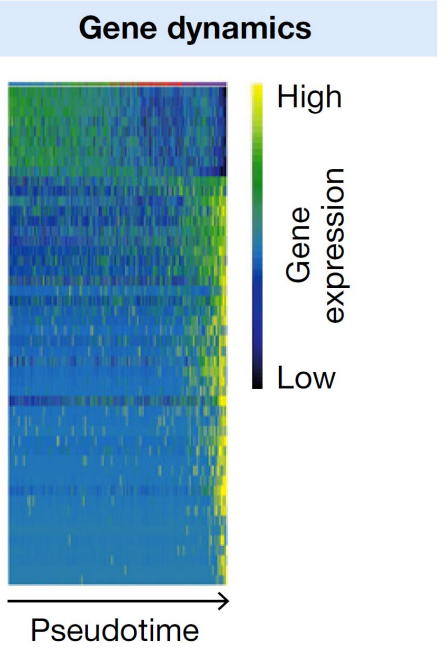


Count matrices





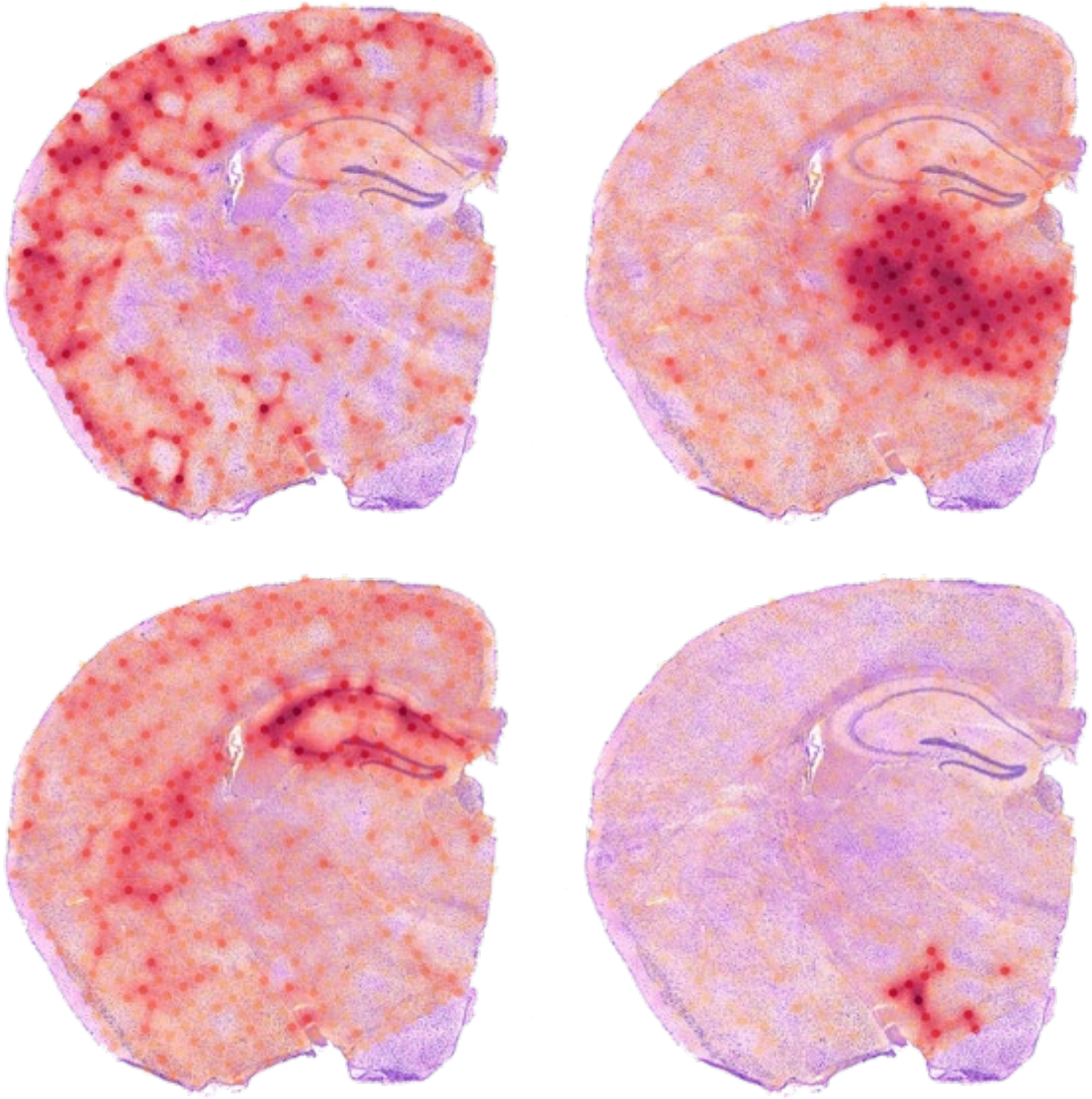


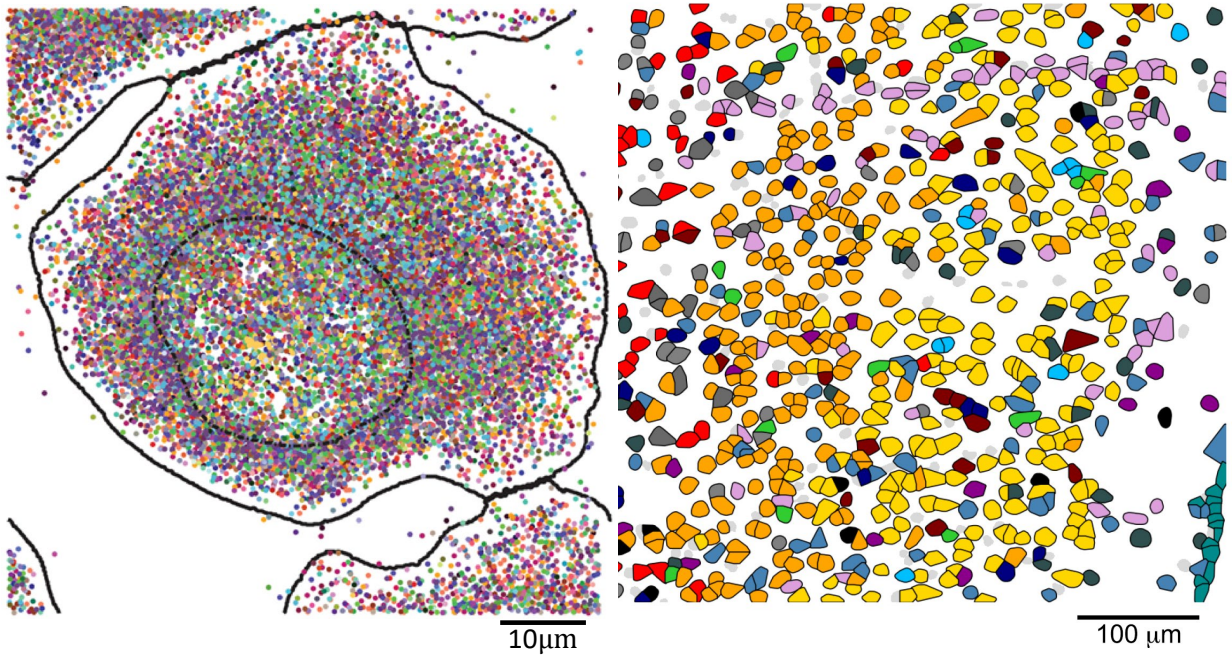


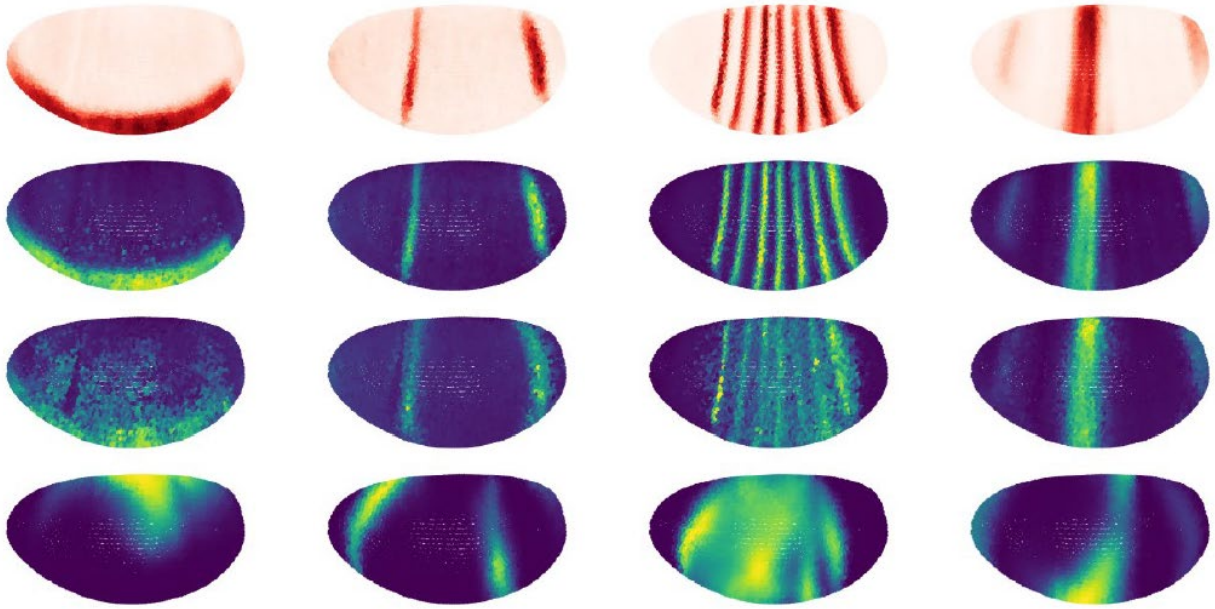
Spatial information is lost!

Luecken, M. D., & Theis, F. J. *Molecular Systems Biology* (2019)

Spatial transcriptomics



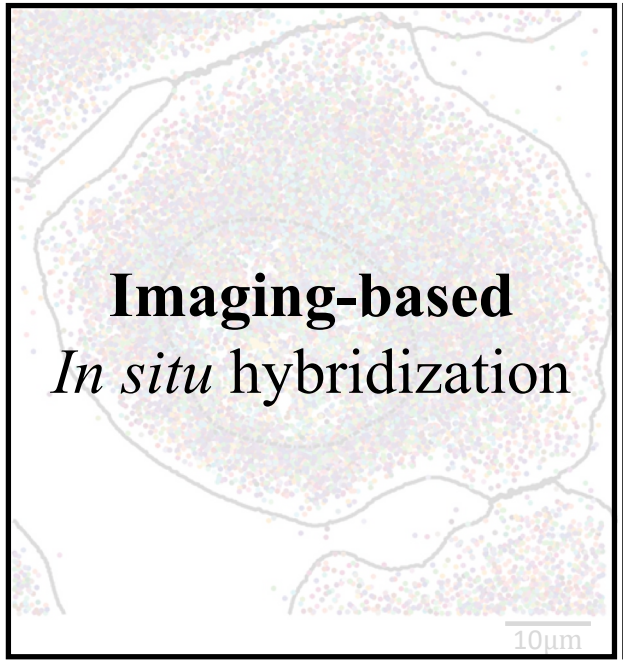




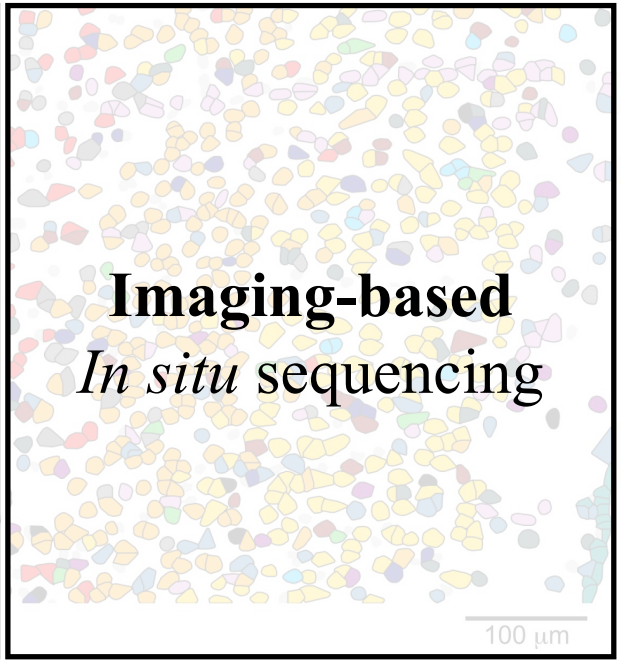
https://spatialtranscriptomics.com/showcase/ Wang, X. *et al. Science* (2018) Wang, G. *et al. Scientific Reports* (2018) Nitzan, M. *et al. Nature* (2019)

Spatial transcriptomics

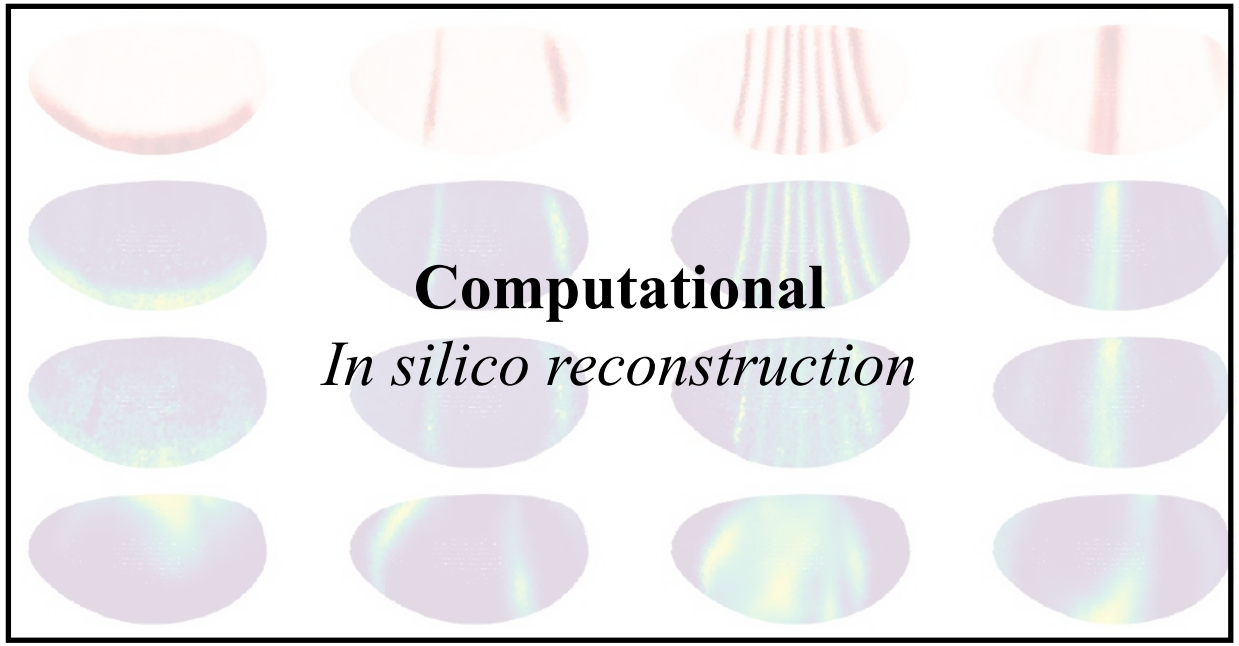
Spatial mapping
In situ capturing



Imaging-based
In situ hybridization



Imaging-based
In situ sequencing

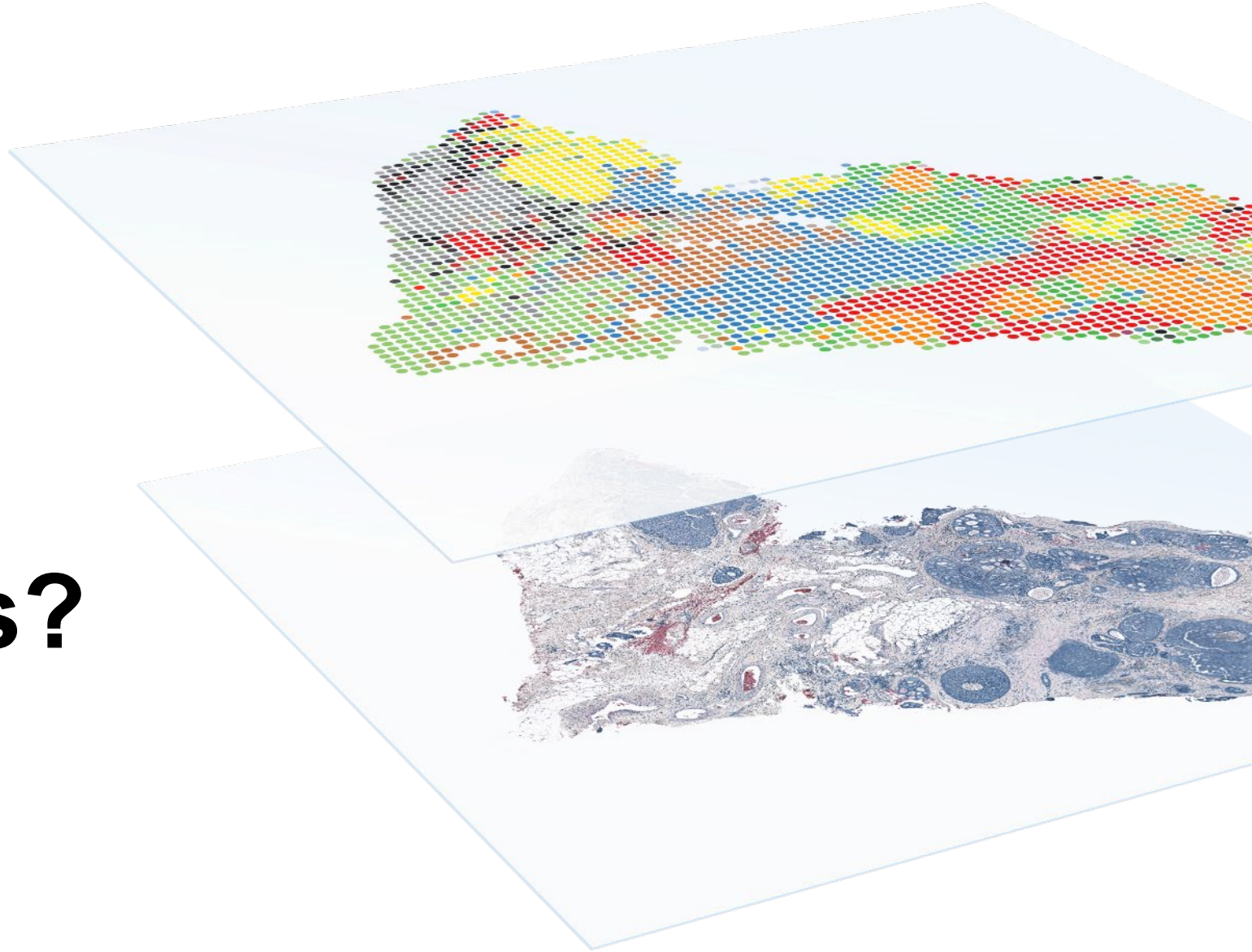


Computational
In silico reconstruction

https://spatialtranscriptomics.com/showcase/ Wang, X. *et al. Science* (2018) Wang, G. *et al. Scientific Reports* (2018) Nitzan, M. *et al. Nature* (2019)

What is spatial transcriptomics?

Look into four distinctive methods

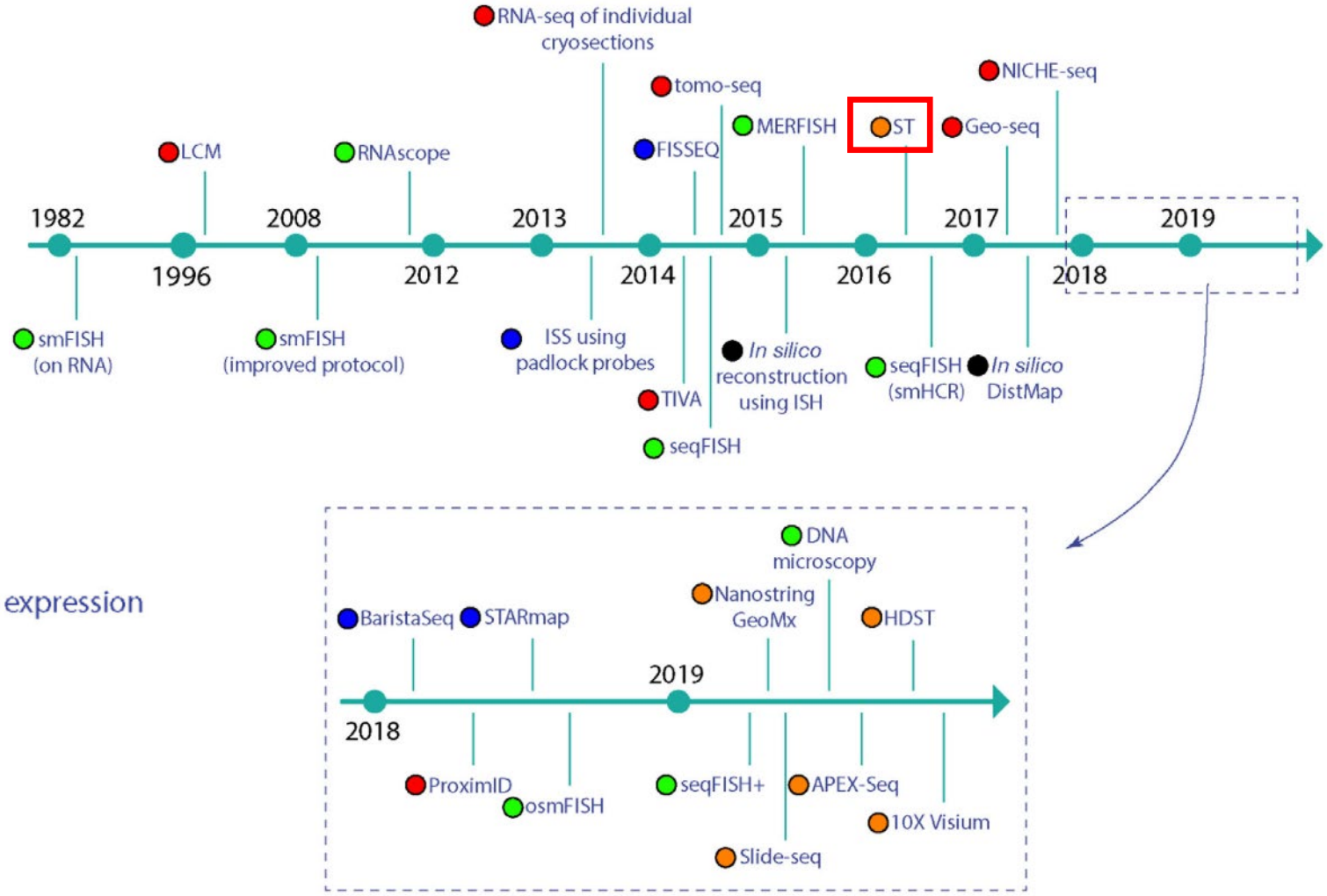


History timeline

FOCUS | 06 JANUARY 2021

Method of the Year 2020: spatially resolved transcriptomics

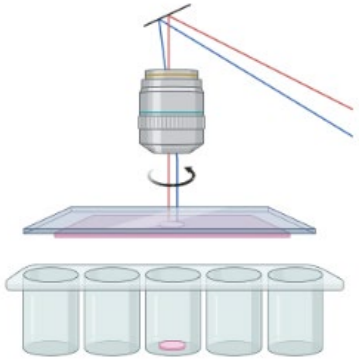
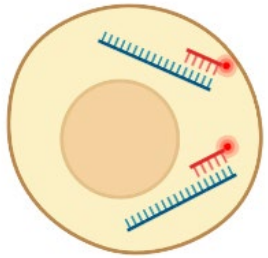
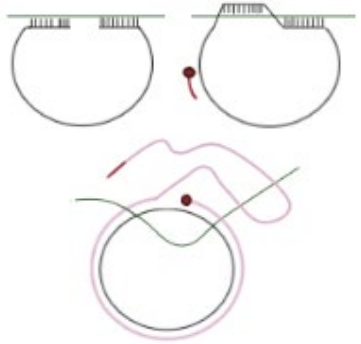
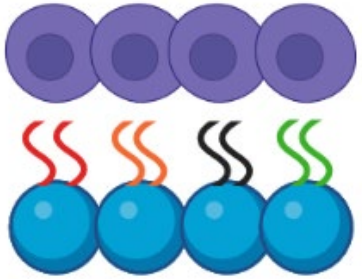
Spatially resolved transcriptomics is our Method of the Year 2020, for its ability to provide valuable insights into the biology of cells and tissues while retaining information about spatial context.



- Section 1. Technologies based on microdissected gene expression
- Section 2. *In situ* hybridization technologies
- Section 3. *In situ* sequencing technologies
- Section 4. *In situ* capturing technologies
- Section 5. *In silico* reconstruction of spatial data

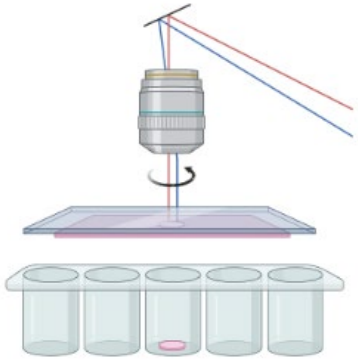
Asp, M. *et al. BioEssays* (2020)

Four paradigms of spatial transcriptomics

Spatial info. source	Microdissection	Imaging-based methods		Spatial mapping	Computation
					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

Four paradigms of spatial transcriptomics

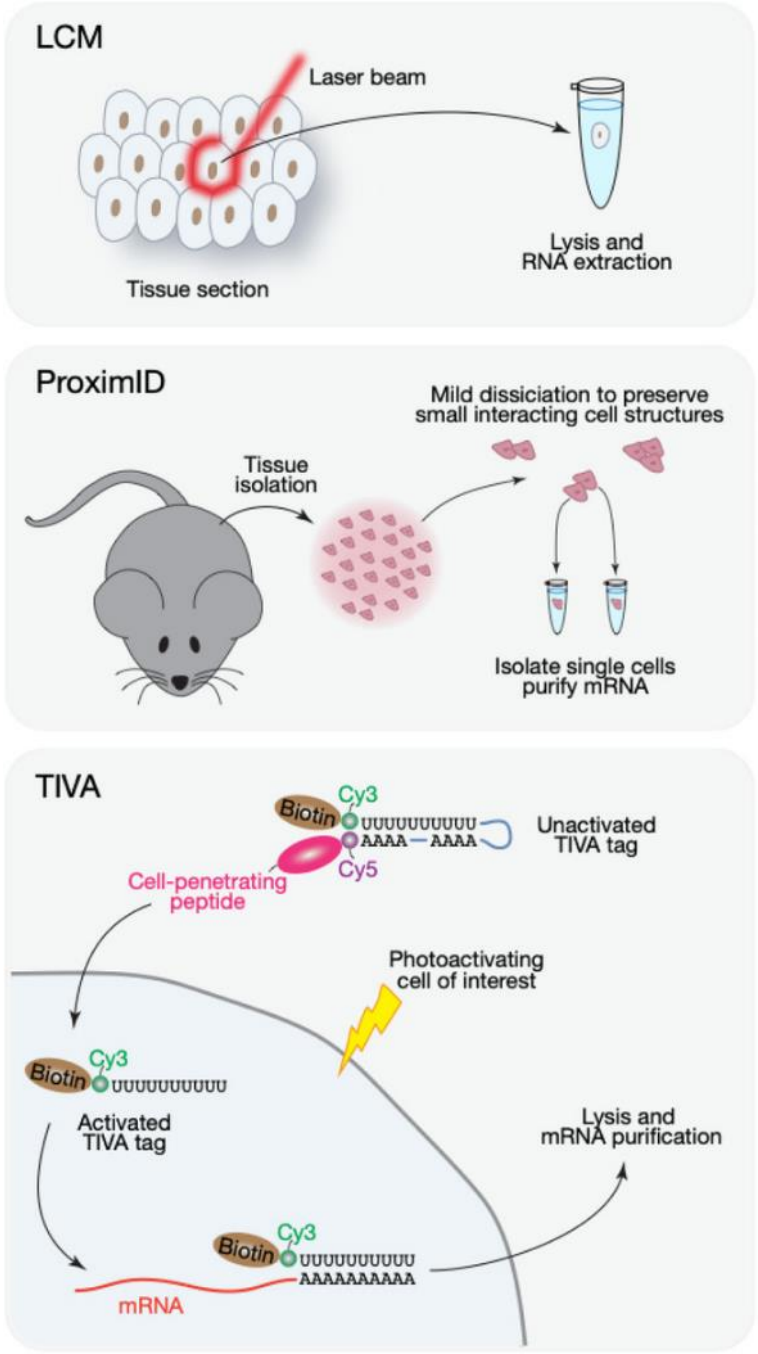
Spatial info. source	**Microdissection**	Imaging-based methods	Spatial mapping	Computation
				
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+** DNA microscopy	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST APEX-seq DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

Microdissection methods isolate individual areas

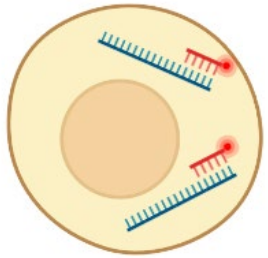
+ Cellular resolution
– Low cell throughput
– Can't be applied to limited samples

LCM: laser capture microdissection
TIVA: transcriptome *in vivo* analysis



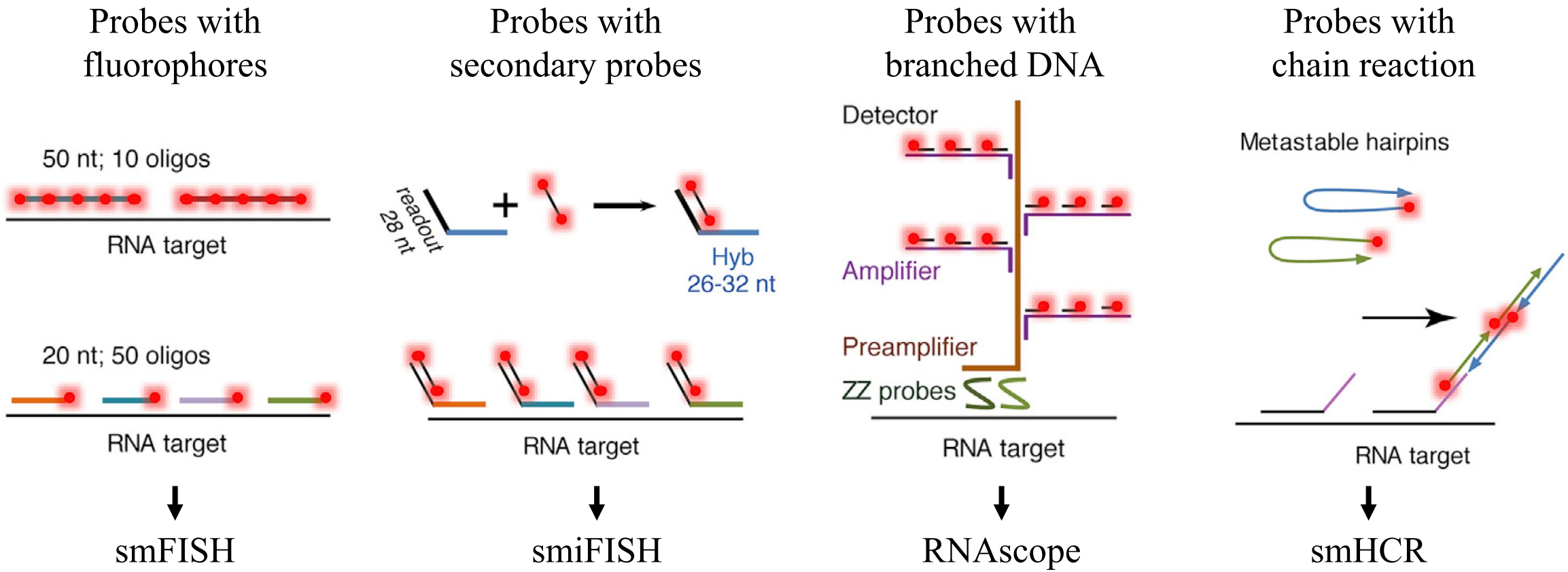
Asp, M. *et al. BioEssays* (2020)

Four paradigms of spatial transcriptomics

Spatial info. source	Microdissection	**Imaging-based methods**		Spatial mapping	Computation
					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

DNA probes hybridization localizes cellular transcripts



sm: single molecule
smi: single molecule inexpensive
HCR: hybridization chain reaction

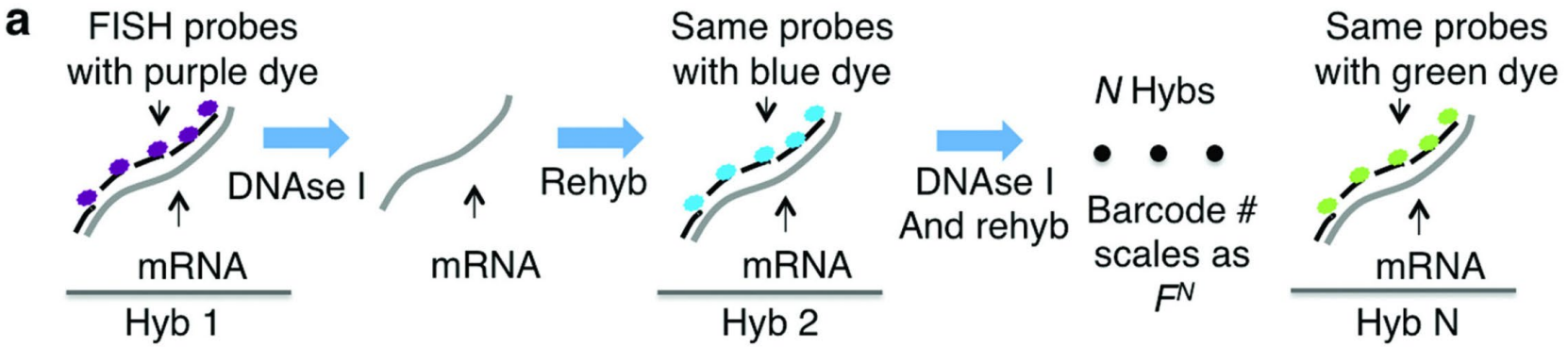
\+ Subcellular resolution – Low transcript throughput

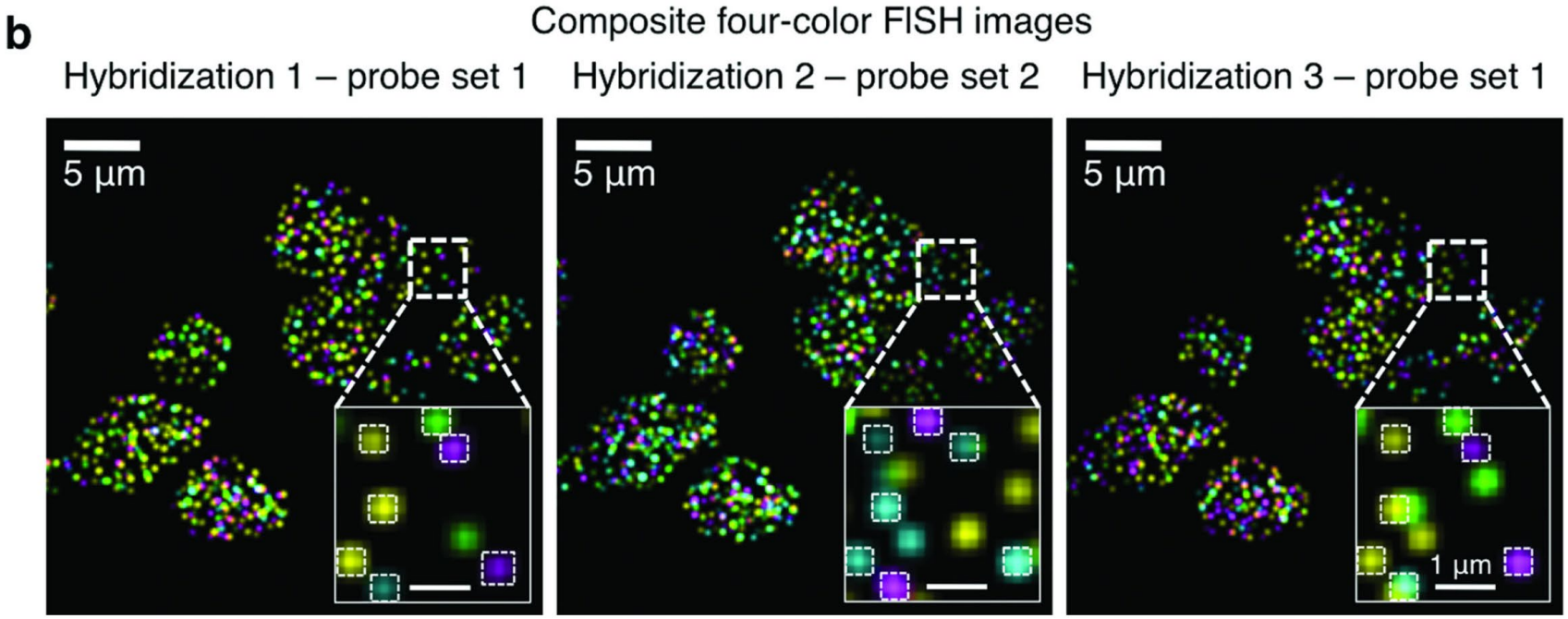
Pichon, X. *et al. Molecular Cell* (2018)

seqFISH realized multiplexing by multiple hybridization rounds

Prof. Long Cai
Caltech, USA

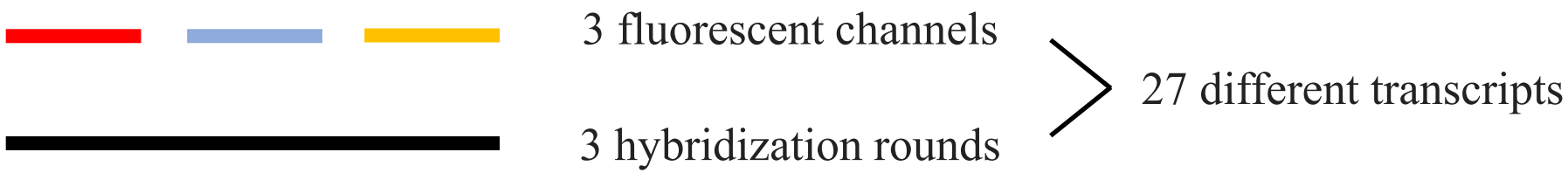
+ Subcellular resolution
+ Highly multiplexed
– Optical crowding



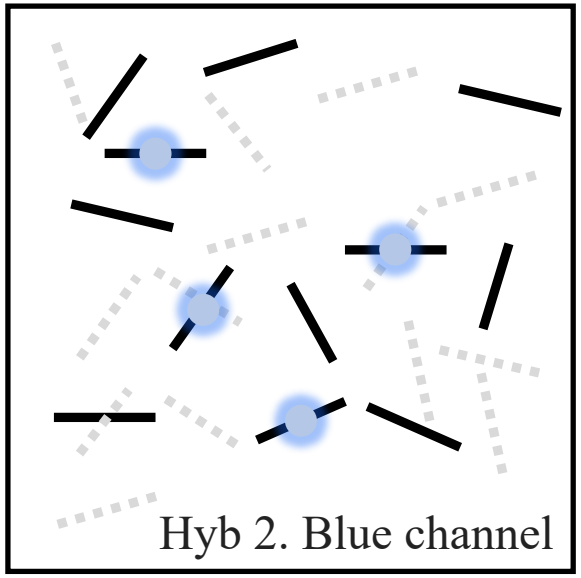


Lubeck, E. *et al. Nature Methods* (2014)

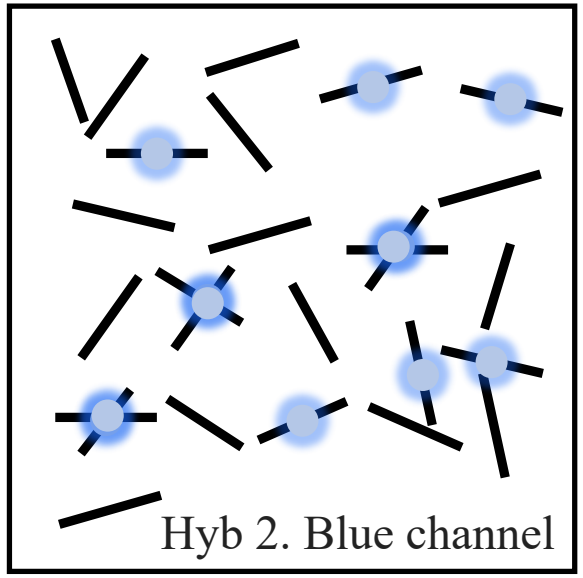
Optical crowding problem presents a challenge to multiplexing



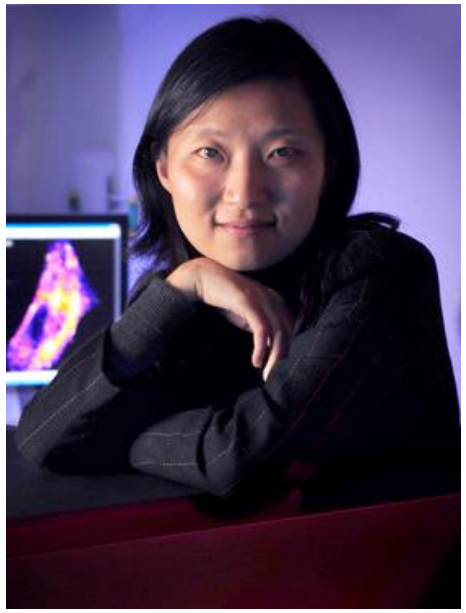
12 targets (no crowding)



27 targets (crowding)



MERFISH and seqFISH+ resolved optical crowding by an encoding-readout labeling strategy



Prof. Xiaowei Zhuang
Harvard University

RESEARCH ARTICLE SUMMARY

RNA IMAGING

Spatially resolved, highly multiplexed RNA profiling in single cells

Kok Hao Chen,[1*] Alistair N. Boettiger,[1*] Jeffrey R. Moffitt,[1*]
Siyuan Wang,[1] Xiaowei Zhuang[1,2†]

LETTER

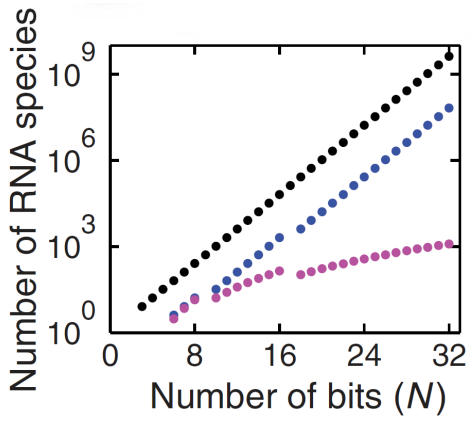
https://doi.org/10.1038/s41586-019-1049-y

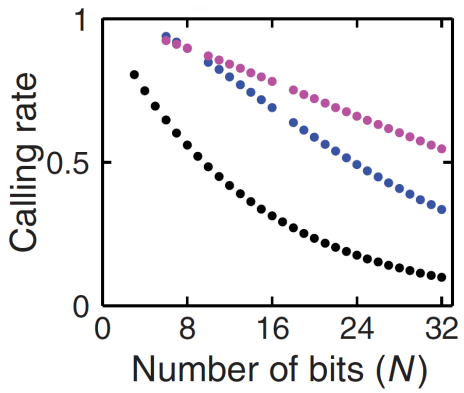
Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+

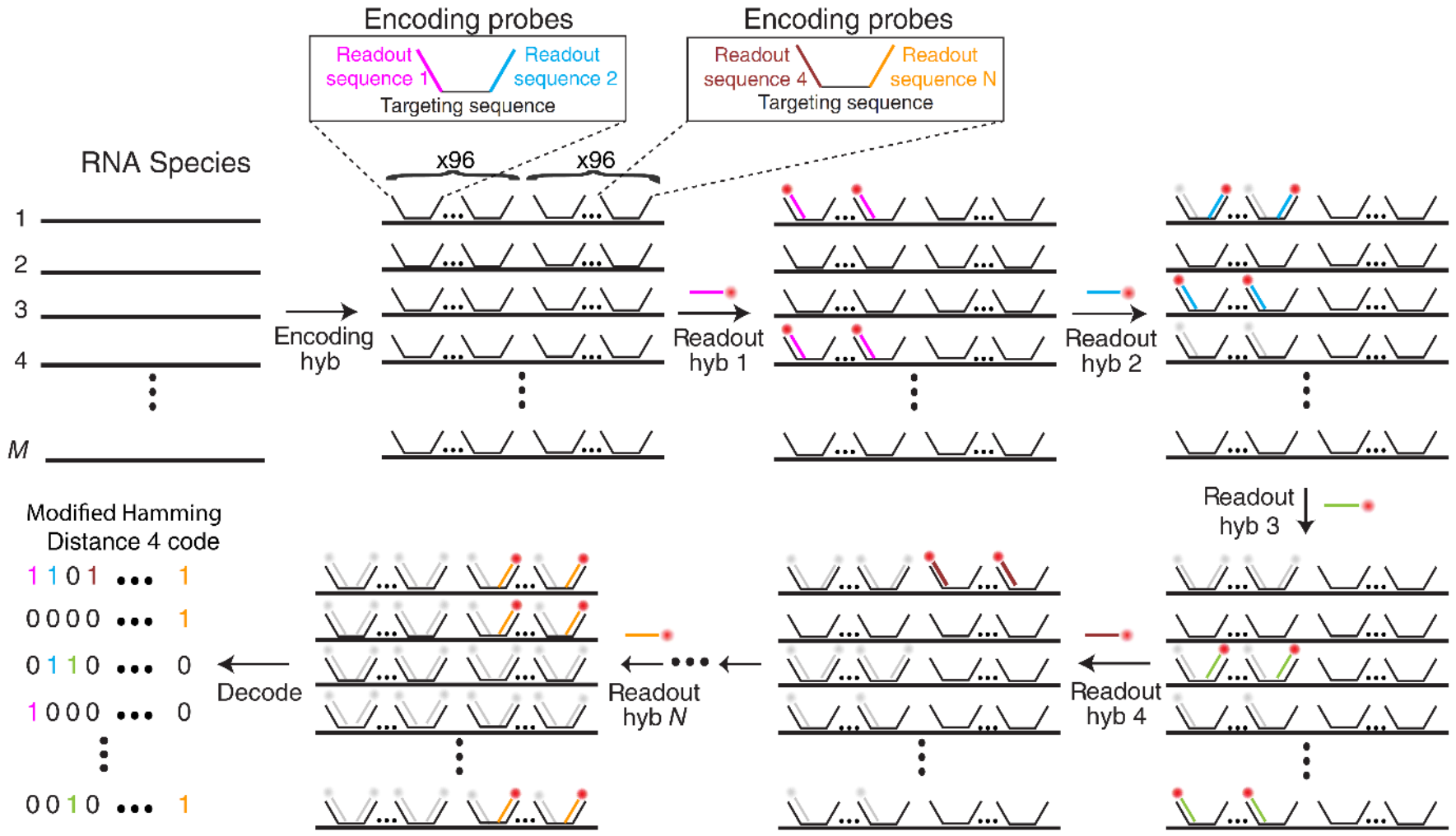
Chee-Huat Linus Eng[1], Michael Lawson[2], Qian Zhu[3], Ruben Dries[3], Noushin Koulena[2], Yodai Takei[2], Jina Yun[2], Christopher Cronin[2], Christoph Karp[2], Guo-Cheng Yuan[3] & Long Cai[2*]

Prof. Long Cai
Caltech

Multiplexed error-robust FISH



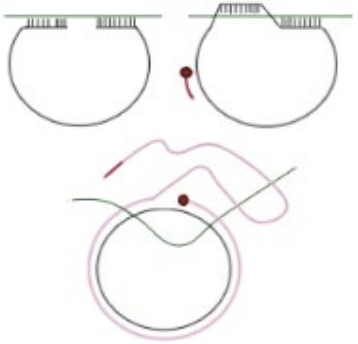




Modified HD4 code

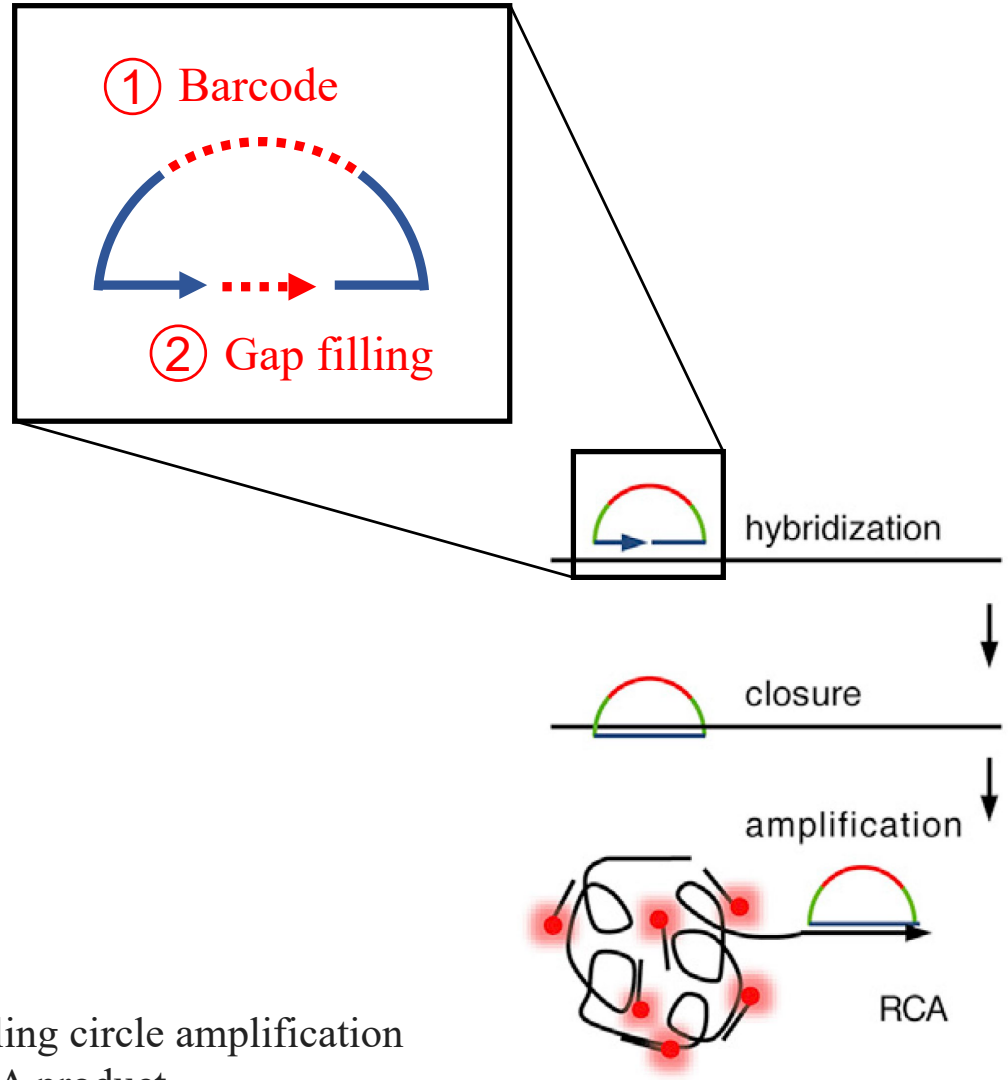
Chen, K. H. *et al. Science* (2015)

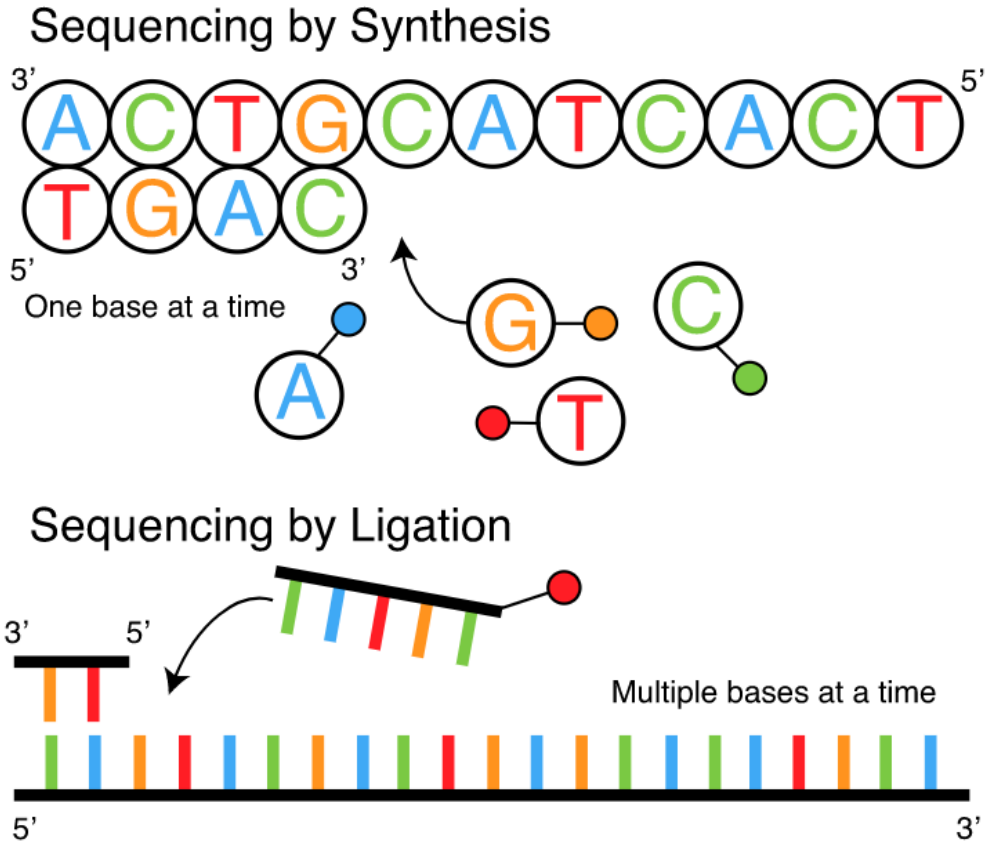
Four paradigms of spatial transcriptomics

Spatial info. source	Microdissection	**Imaging-based methods**		Spatial mapping	Computation
					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

Padlock probes hybridization localizes cellular transcripts



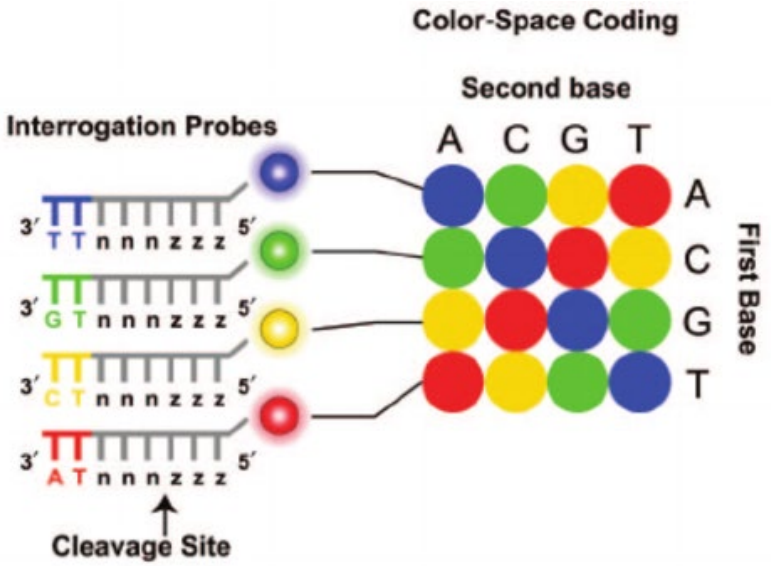


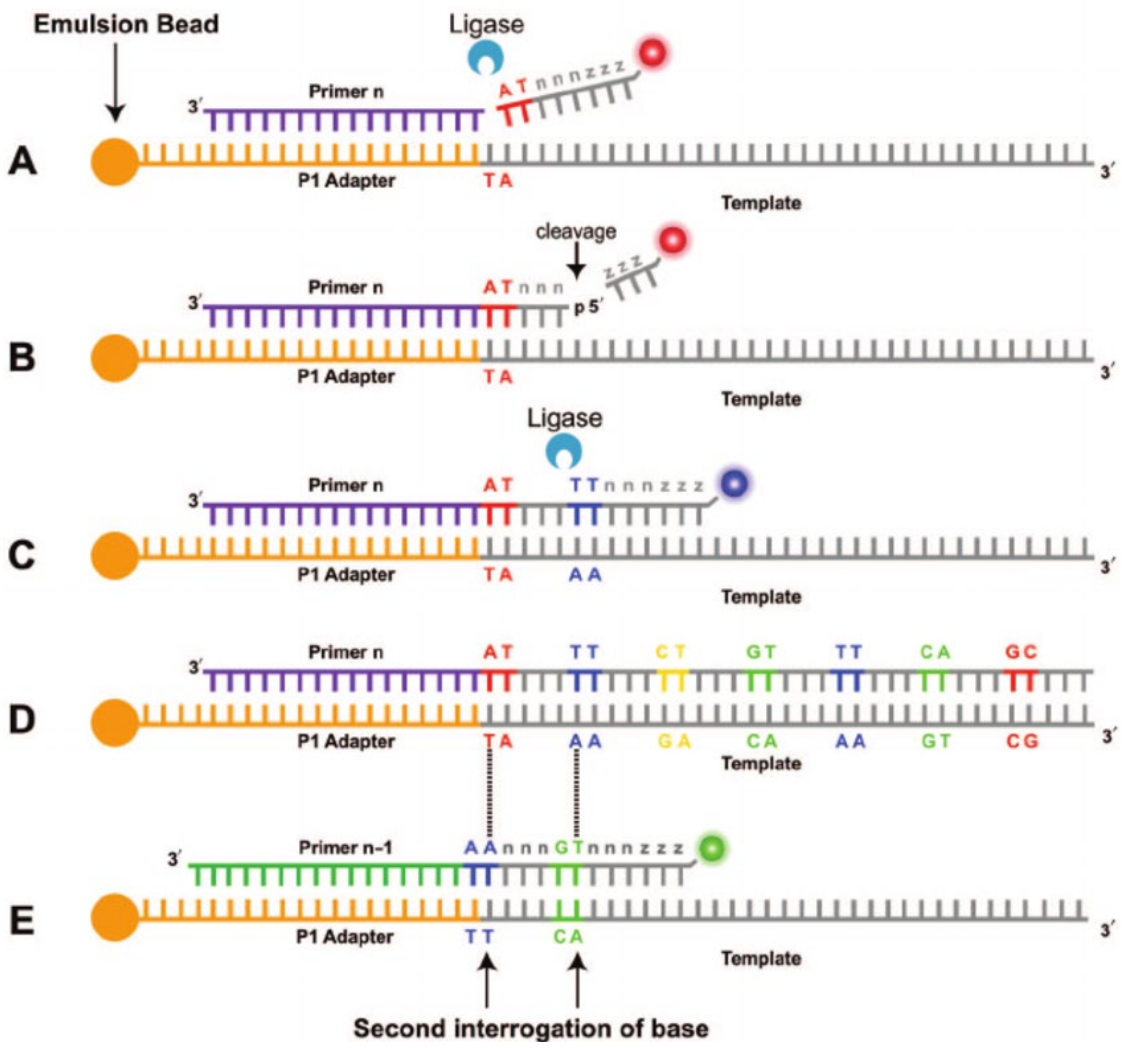
RCA: rolling circle amplification
RCP: RCA product

Pichon, X. *et al. Molecular Cell* (2018)
https://snipcademy.com/ngs-techniques

Sequencing by oligonucleotide ligation and detection

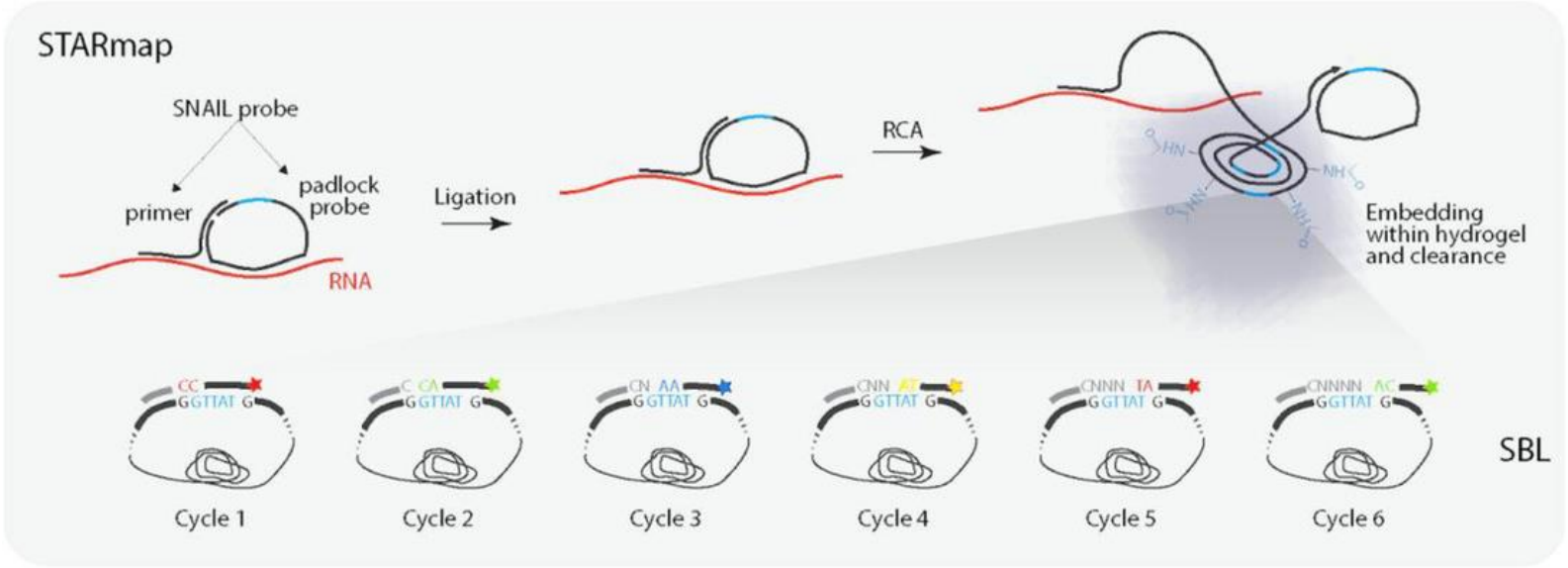
SOLiD sequencing

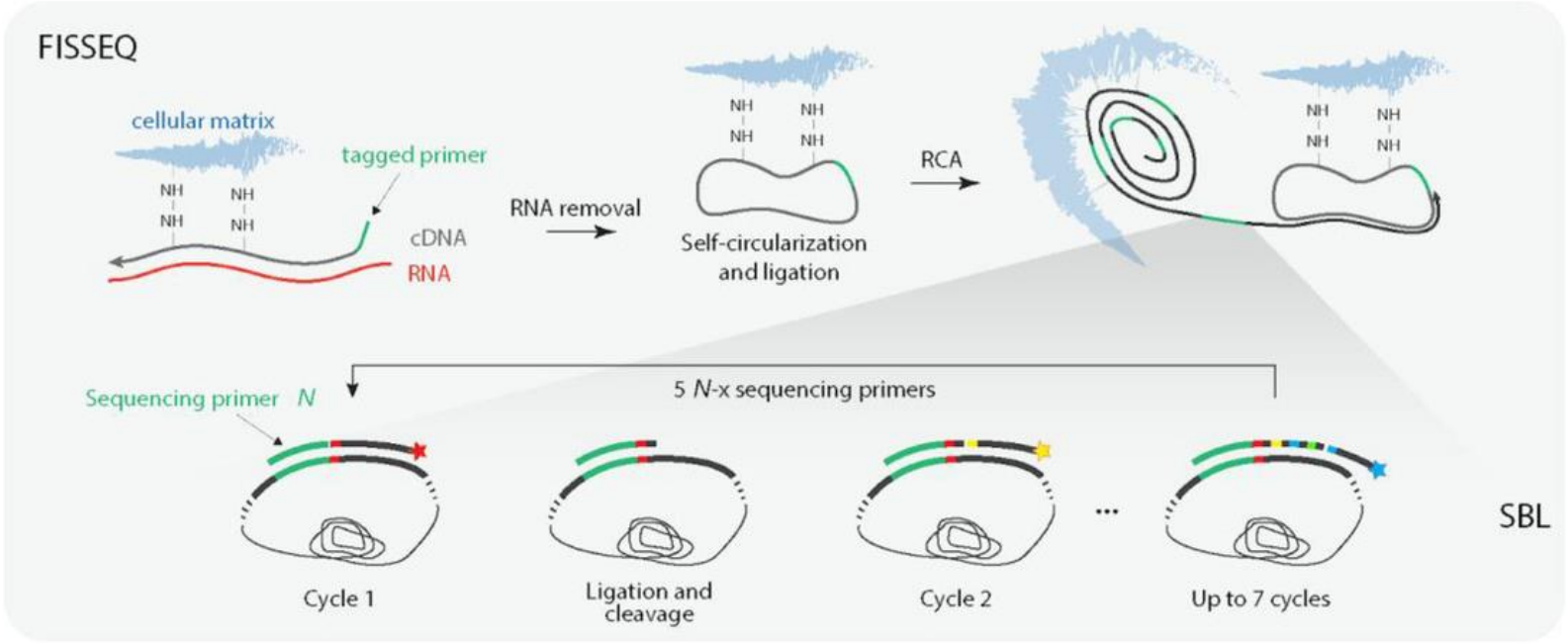




https://zannanzahattusili.blogspot.com/2017/12/dna4solid.html

Targeted or untargeted *in situ* sequencing



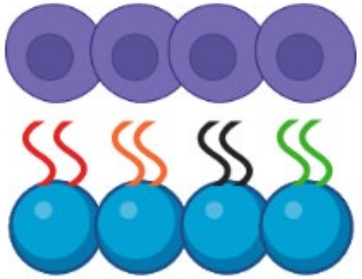


\+ Subcellular resolution
– Limit quantity
– Some only applied in cultured cell

FISSEQ:fluorescent in situ RNA sequencing
STARmap: spatially-resolved transcript amplicon readout mapping

Asp, M. *et al. BioEssays* (2020)

Four paradigms of spatial transcriptomics

	Microdissection	Imaging-based methods		Spatial mapping	Computation
Spatial info. source					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

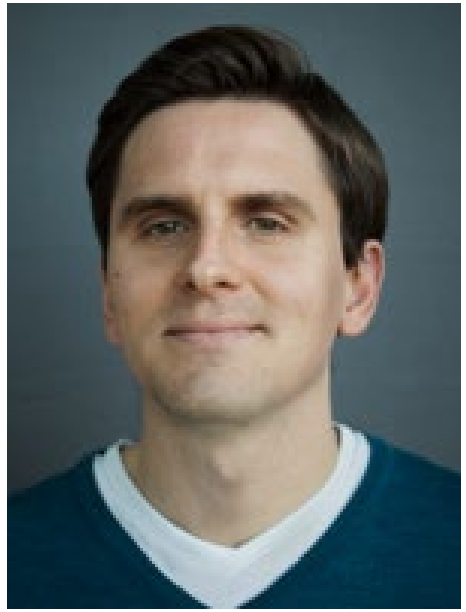
Spatial mapping methods

Pioneers of the field

Spatial transcriptomics (ST)
Slide-seq

High-definition spatial transcriptomics (HDST)

Prof. Joakim Lundeberg
KTH, Sweden



Prof. Patrik Ståhl
KTH, Sweden

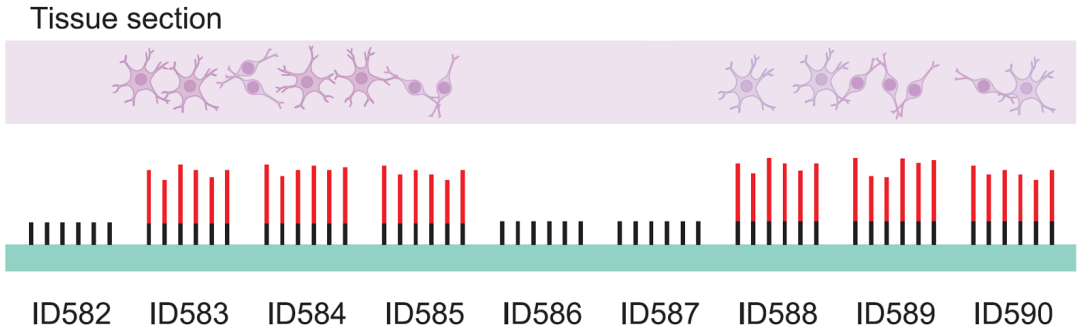
Prof. Evan Macosko
Broad Institute, USA

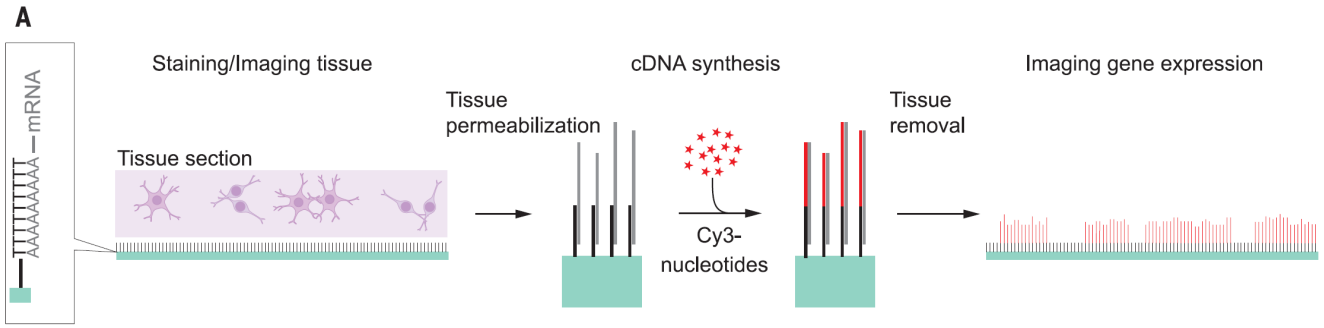
Prof. Fei Chen
Broad Institute, USA

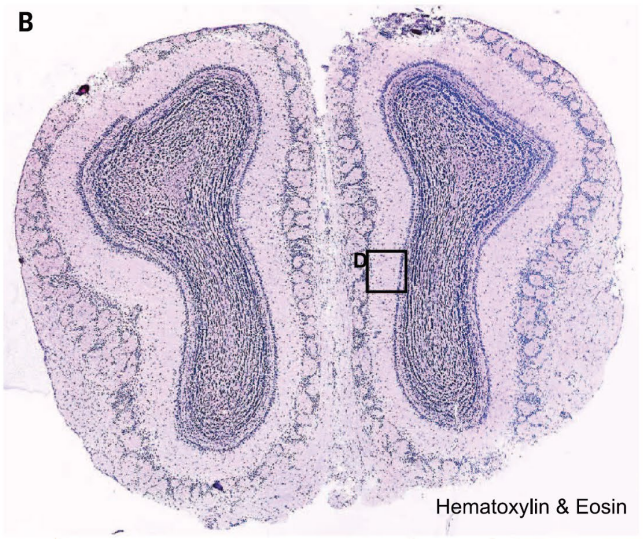
Spatial mapping methods

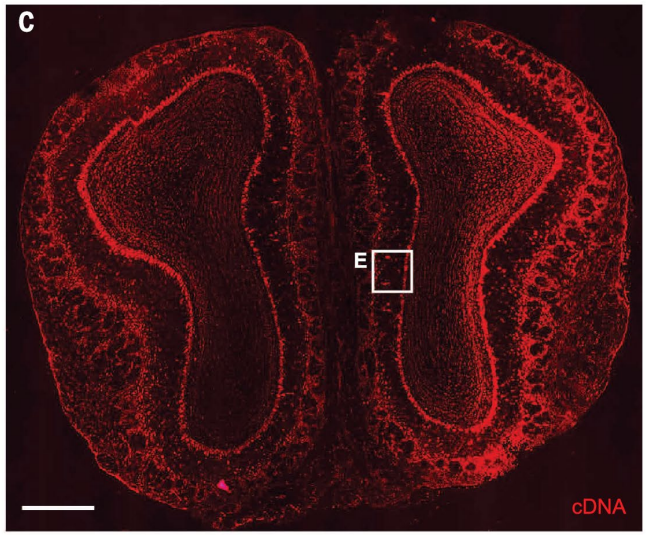
Spatial transcriptomics (ST)

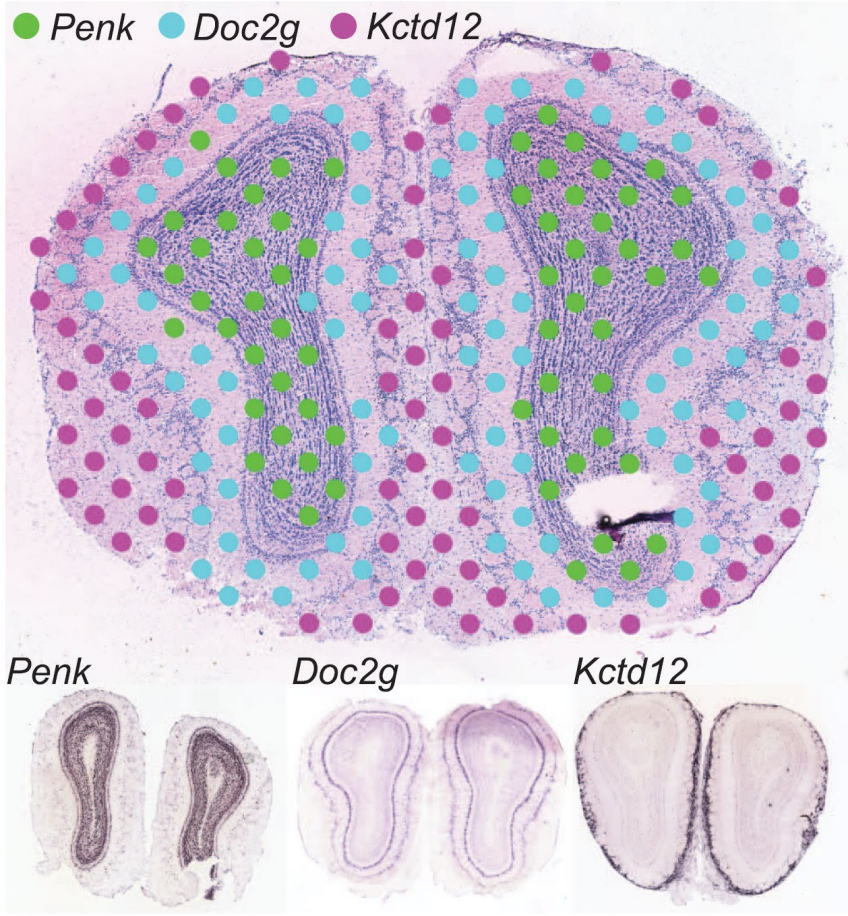
+ Whole mRNA – 100 µm resolution





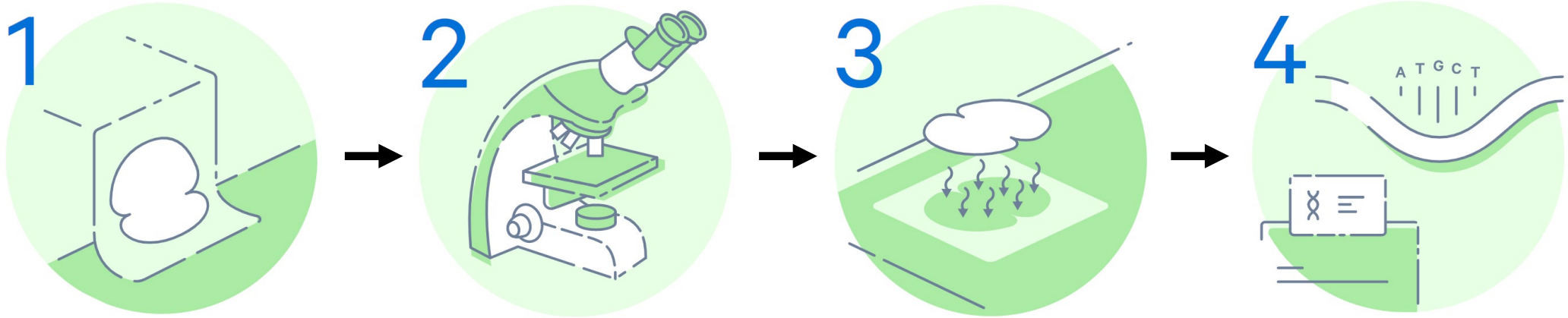




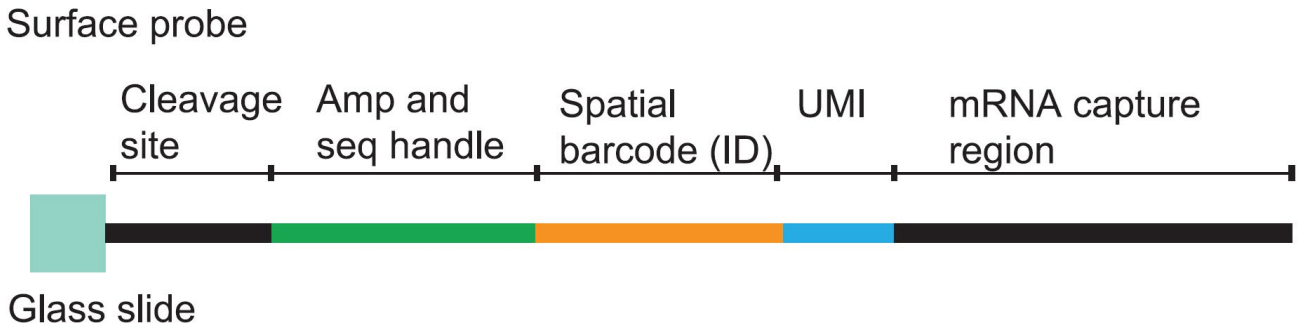


Patrik L. Ståhl *et al. Science* (2016)

Workflow of spatial mapping methods



Sample preparation → Stain and imaging → Permeabilize and Library construction → Sequencing



Spatial barcode: spatial ID

UMI: Unique molecule identifier

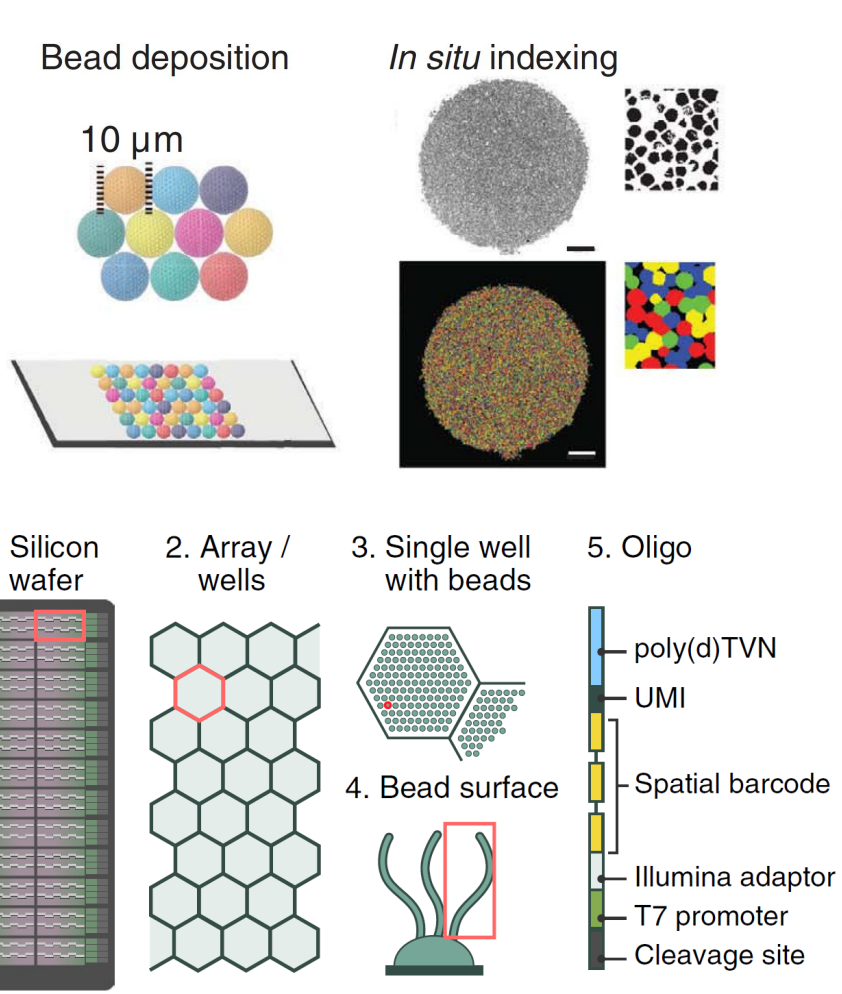
AmpSeq: PCR + Sequencing

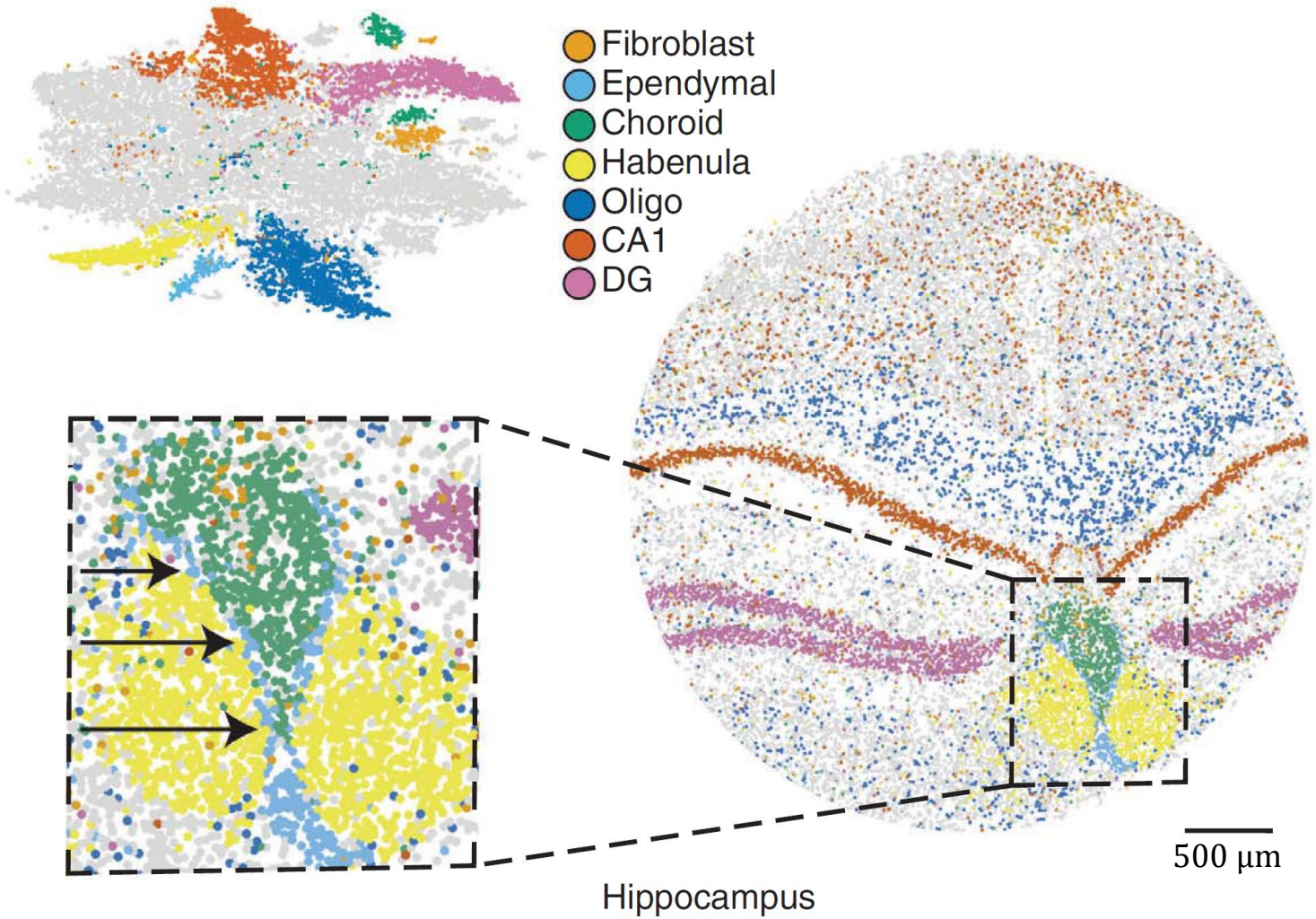
Patrik L. Ståhl *et al. Science* (2016)

https://www.10xgenomics.com/products/spatial-gene-expression

Slide-seq and High-definition spatial transcriptomics (HDST)

+ Cellular resolution – Low efficiency – Require scRNA-seq data





Sanja Vickovic *et al. Nature Methods* (2019)

Rodriques, S. G. *et al. Science* (2019)

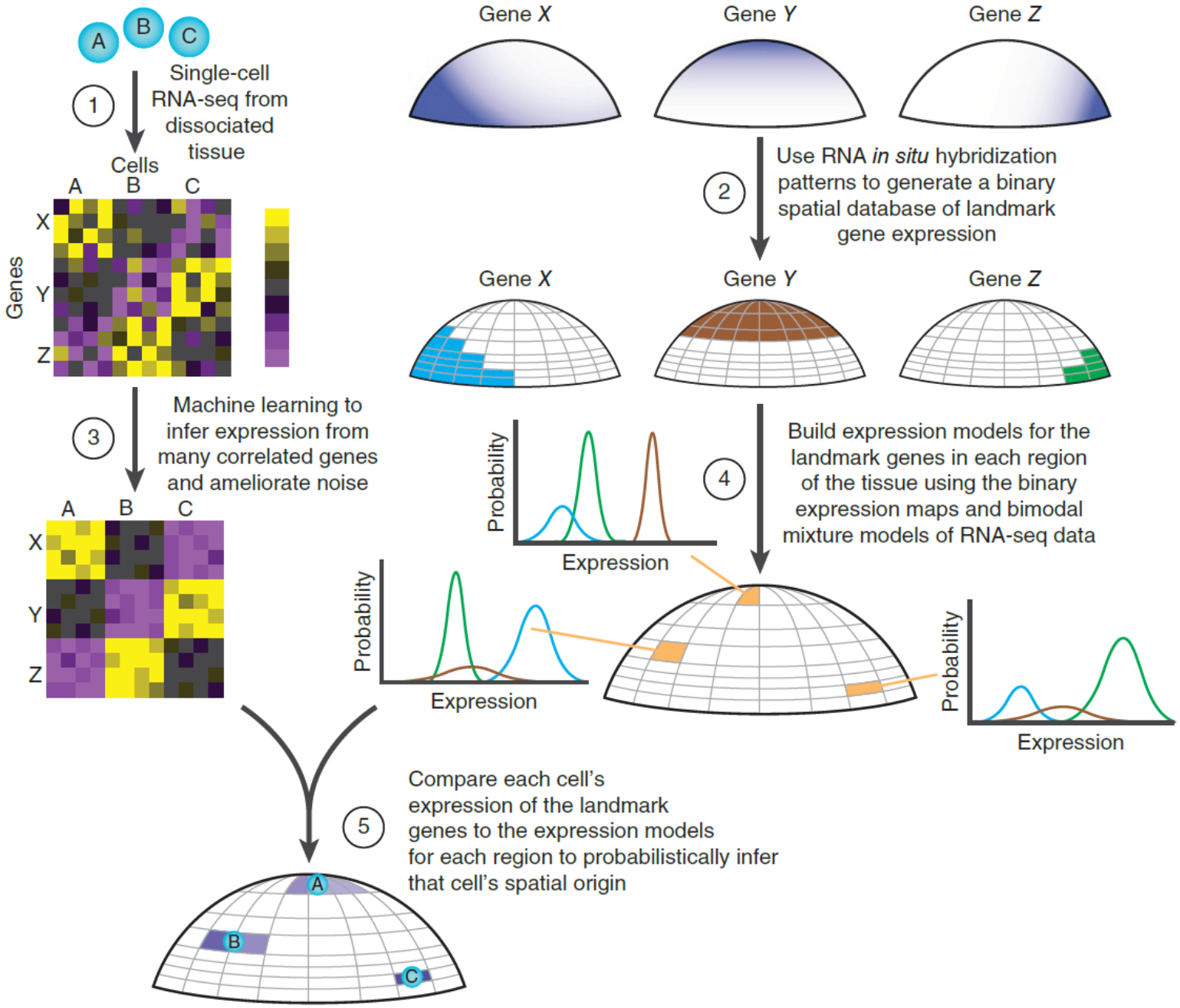
Four paradigms of spatial transcriptomics

Spatial info. source	Microdissection	Imaging-based methods		Spatial mapping	Computation
					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

Computational methods

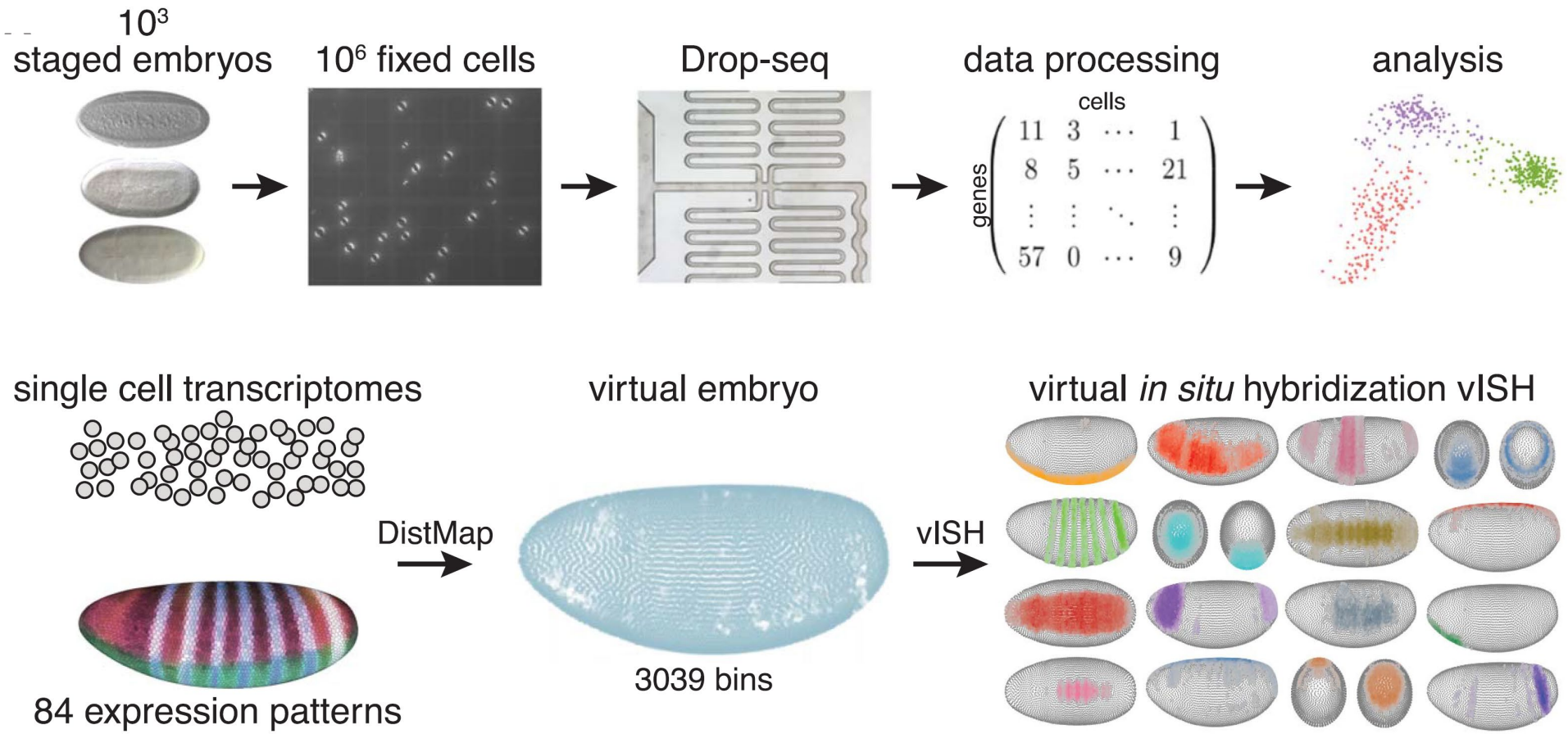
Reference-based or *de novo* reconstruction



Satija, R. *et al. Nature Biotechnology* (2015)

DistMap reconstructed *Drosophila* embryo based on ISH references

+ Cellular resolution – Require ISH database – Can't be applied to limited samples

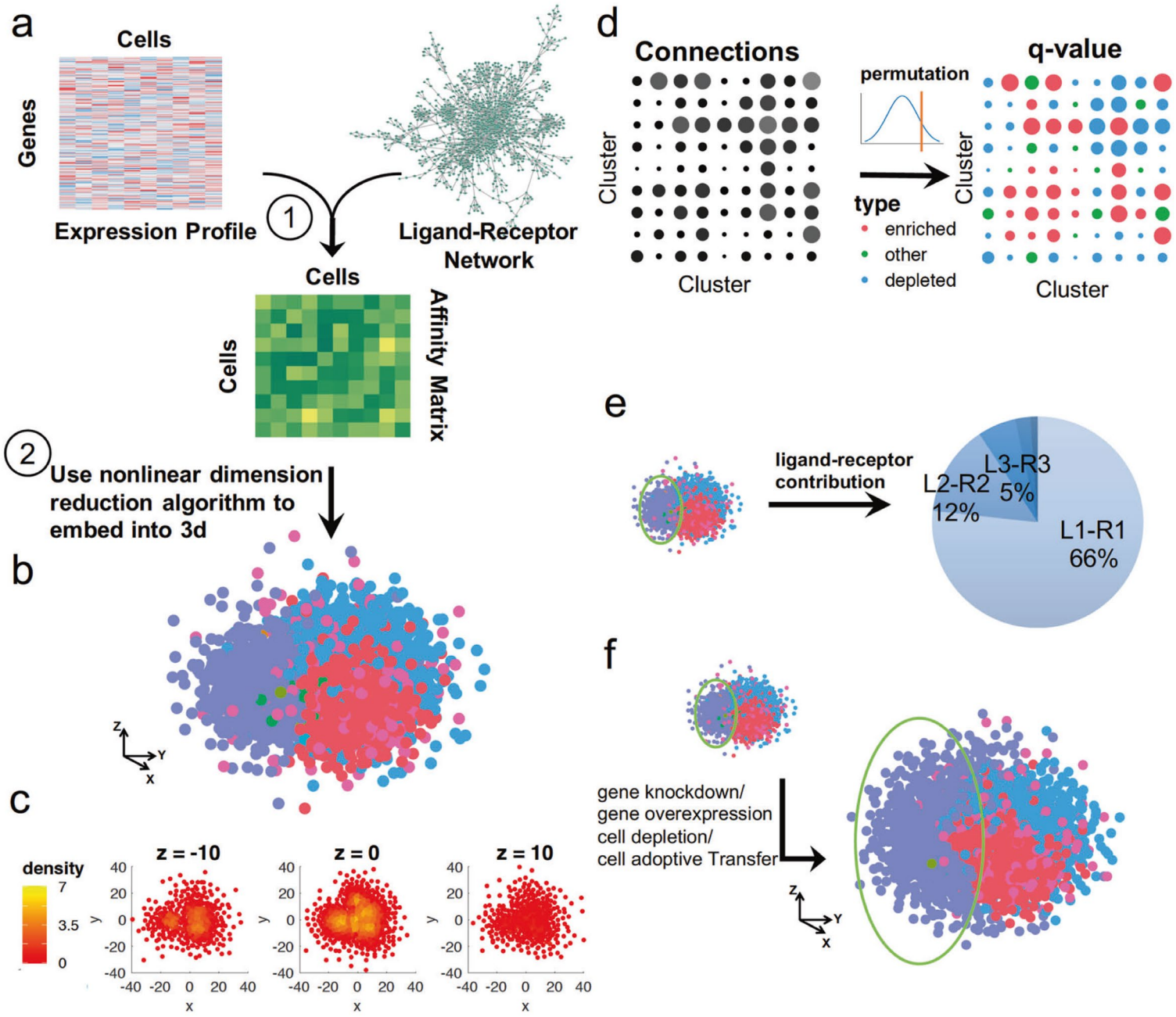


vISH: virtual *in situ* hybridization

Karaiskos, N. *et al. Science* (2017)

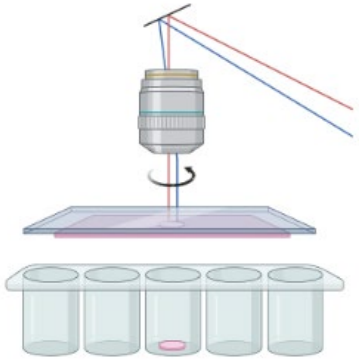
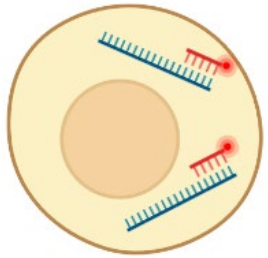
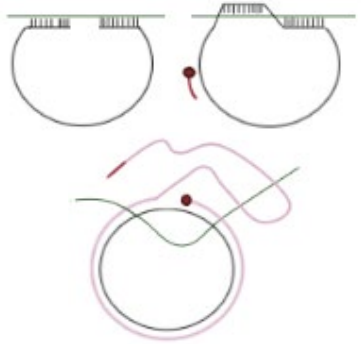
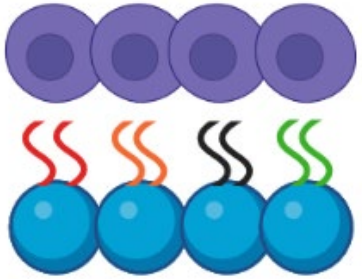
Computational methods

CSOmap reconstructed cell organization based on ligand-receptor interactions



Ren, X. *et al. Cell Research* (2020)

Four paradigms of spatial transcriptomics

Spatial info. source	Microdissection	Imaging-based methods		Spatial mapping	Computation
					
Key feature	Dissociation	*In situ* hybridization	*In situ* sequencing	*In vivo* capturing	*In silico* reconstruction
Available methods	Geo-seq (LCM) tomo-seq TIVA NICH-seq ProxmID	smFISH seqFISH MERFISH **seqFISH+**	Padlock probes BaristaSeq STARmap FISSEQ	ST Slide-seq HDST DBiT-seq	DistMap novoSpaRc **CSOmap**

https://en.wikipedia.org/wiki/Spatial_transcriptomics#cite_note-:22-8

The end of introduction

Any question?