Swiss Institute of Bioinformatics

INTRODUCTION TO SEQUENCING-BASED TRANSCRIPTOMICS DATA ANALYSIS

Introduction to spatial transcriptomics techniques

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December 9-10, 2025

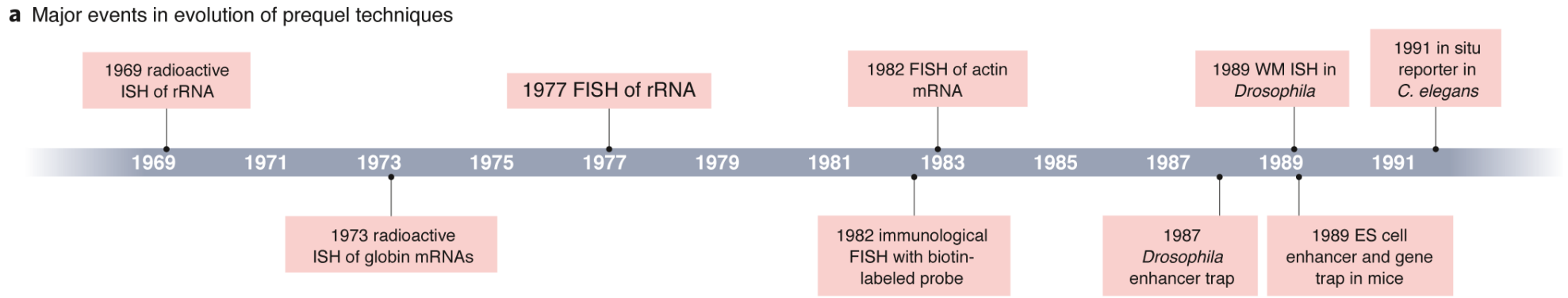
elixir SWITZERLAND

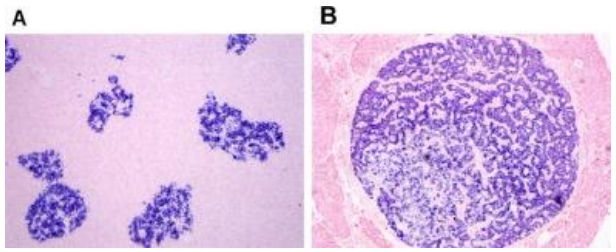
Learning objectives

- Describe the general differences between imaging-based and sequencing-based ST methods
- List different sequencing-based ST methods and their main particularities
- Identify important criteria to choose the adequate technology

Introduction to spatial transcriptomics

History of spatial transcriptomics





Chu Ying-Hsia et al. Sem in Diagn Pathology. 2019

Moses and Pachter, Nat Methods. 2022

Introduction to spatial transcriptomics

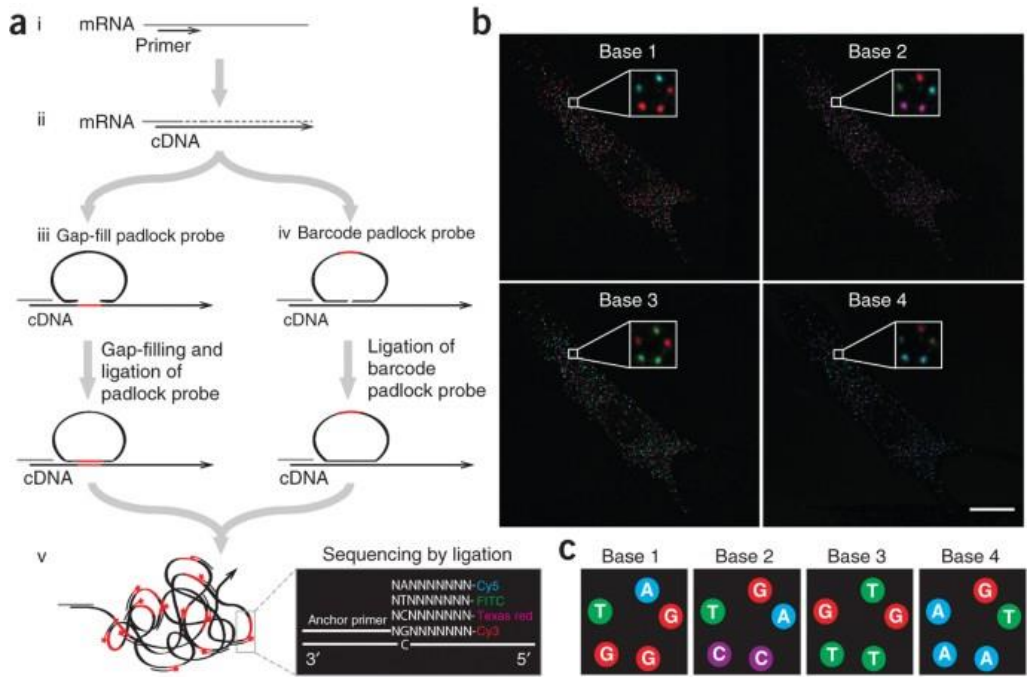
FISSEQ

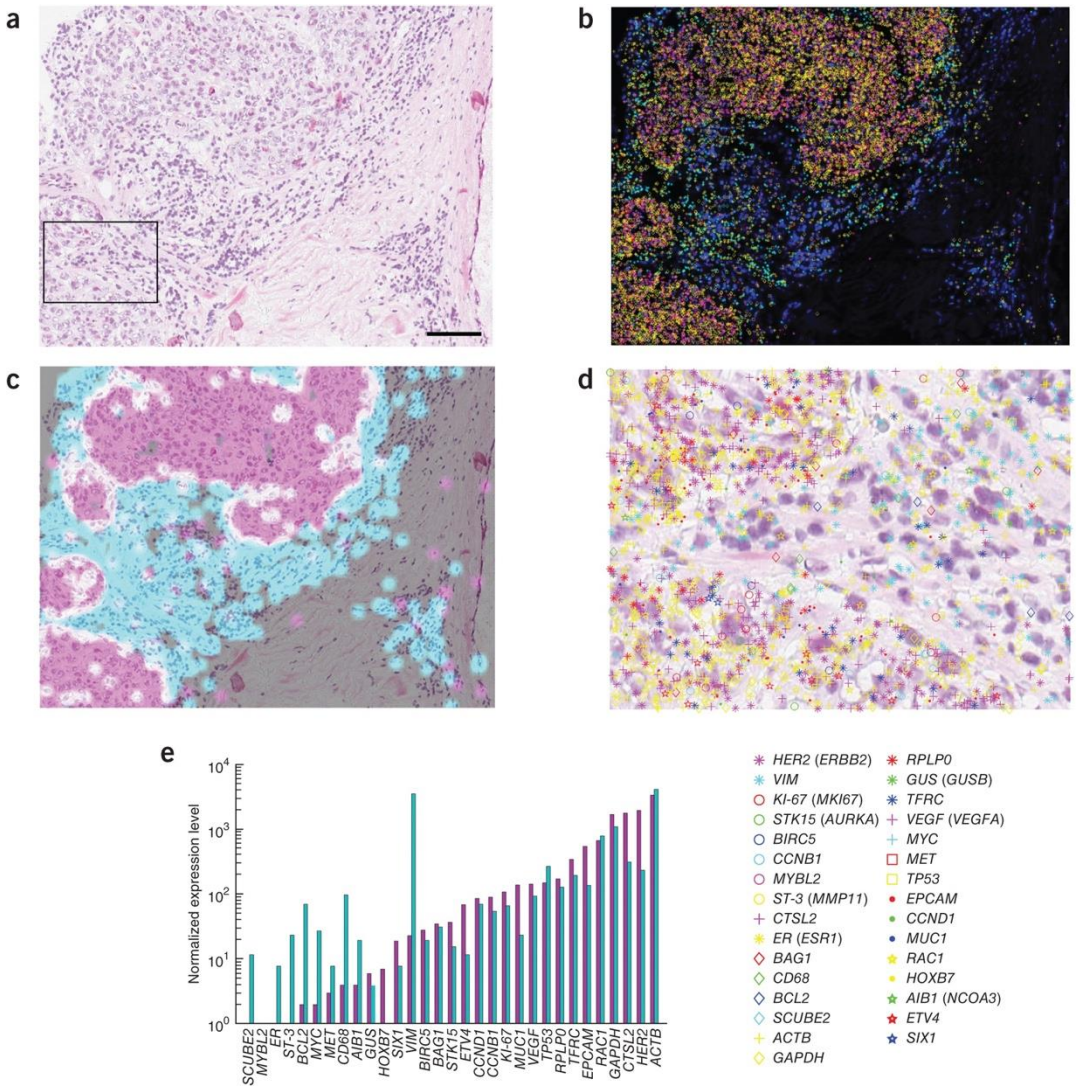
Brief Communication | Published: 14 July 2013

In situ sequencing for RNA analysis in preserved tissue and cells

Ronggin Ke, Marco Mignardi, Alexandra Pacureanu, Jessica Svedlund, Johan Botling, Carolina Wählby ✉ & Mats Nilsson ✉

Nature Methods **10**, 857–860 (2013) | Cite this article





Introduction to spatial transcriptomics

MERFISH

RESEARCH ARTICLE | SYSTEMS BIOLOGY |

High-throughput single-cell gene-expression profiling with multiplexed error-robust fluorescence in situ hybridization

Jeffrey R. Moffitt, Junjie Hao, Guiping Wang, +2, and Xiaowei Zhuang Authors Info & Affiliations

Fortner and Bucur. Frontiers in Cell and Development Biology, 2024

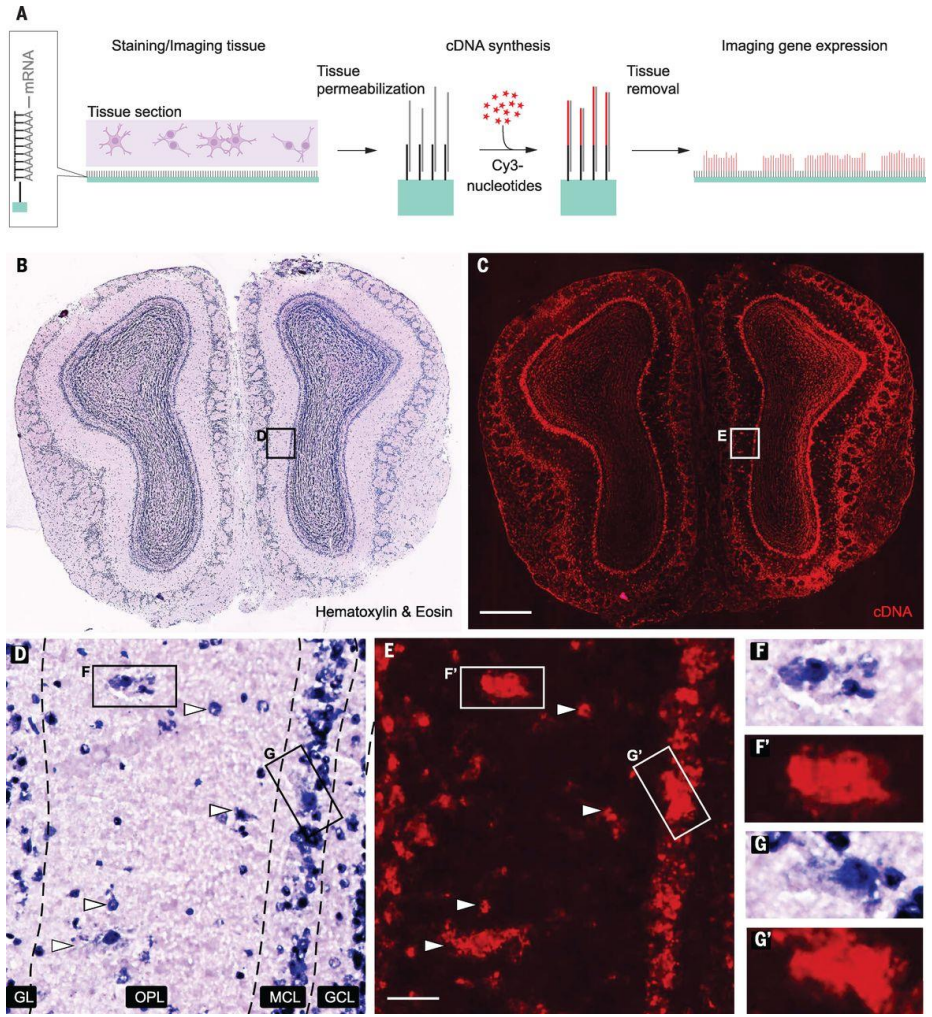
Introduction to spatial transcriptomics

Spatial transcriptomics (ST)

Visualization and analysis of gene expression in tissue sections by spatial transcriptomics

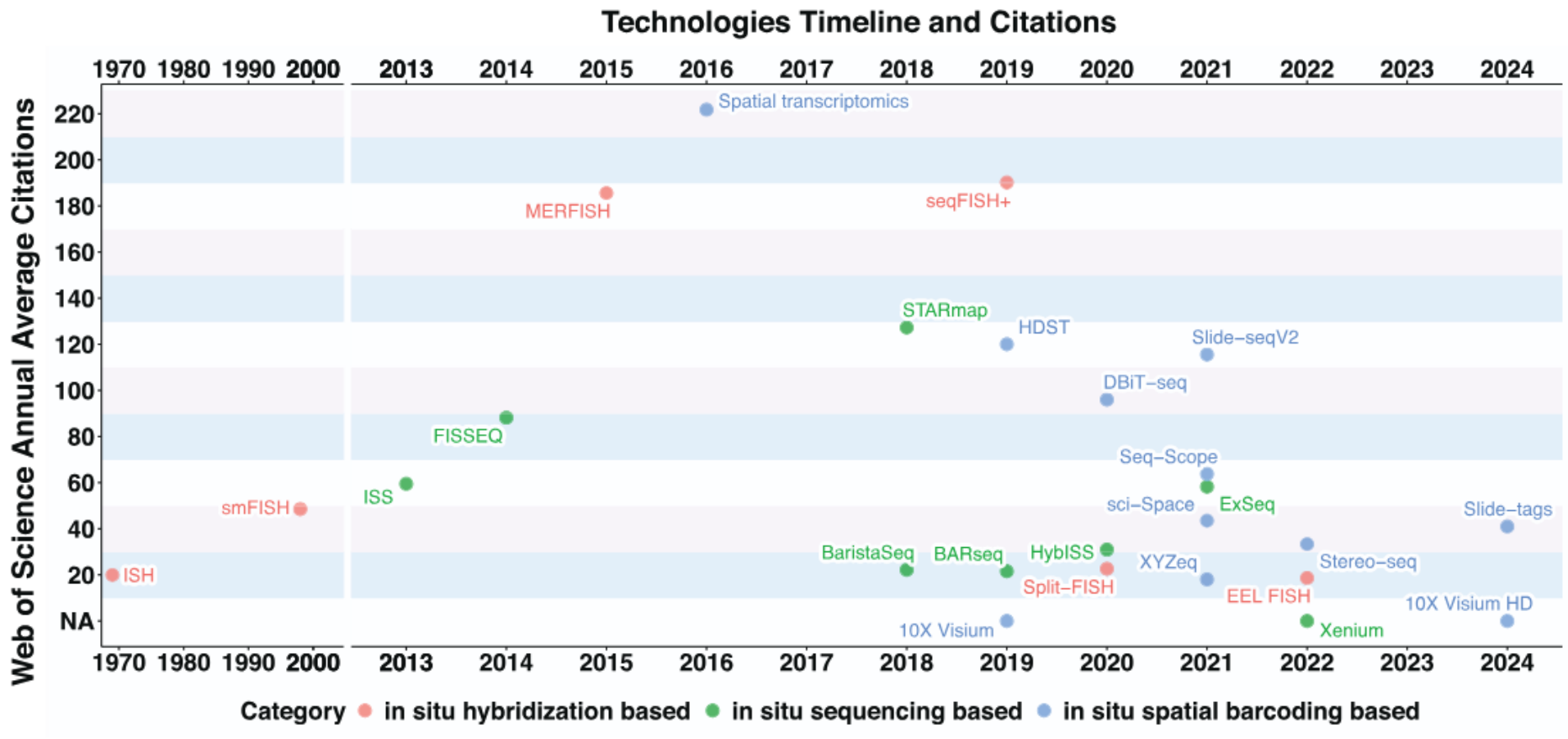
PATRIK L. STÅHL, FREDRIK SALMÉN, SANJA VICKOVIC, ANNA LUNDMARK, JOSÉ FERNÁNDEZ NAVARRO, JENS MAGNUSSON, STEFANIA GIACOMELLO, MICHAELA ASP, JAKUB O. WESTHOLM, [...], AND JONAS FRISÉN +11 authors Authors Info & Affiliations

SCIENCE · 1 Jul 2016 · Vol 353, Issue 6294 · pp. 78-82 · DOI: 10.1126/science.aaf2403



Introduction to spatial transcriptomics

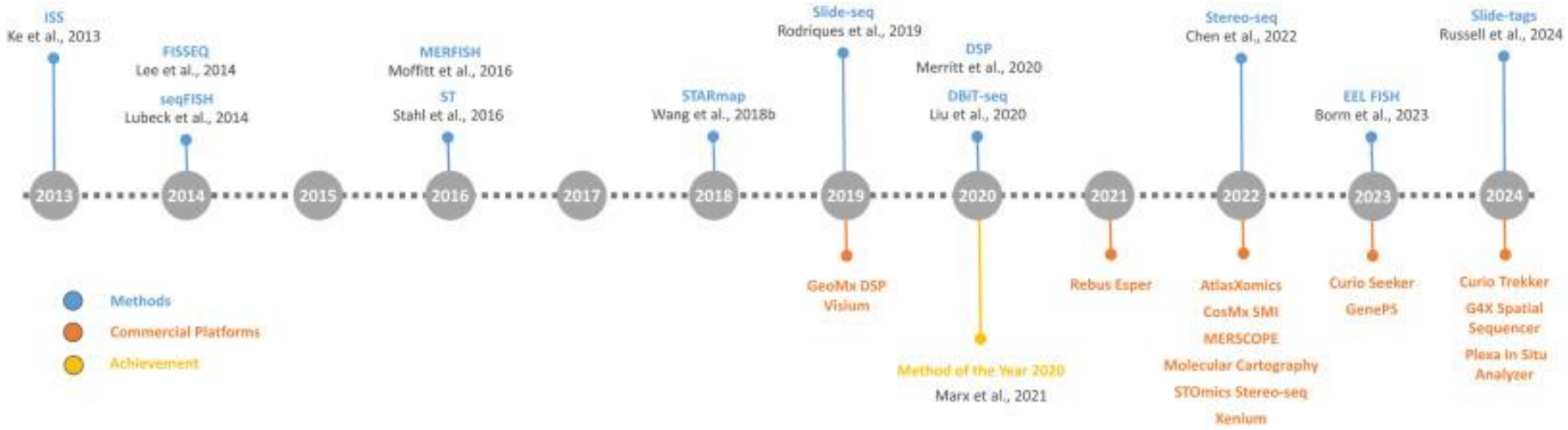
History of spatial transcriptomics



Wang et al. Small methods. 2025

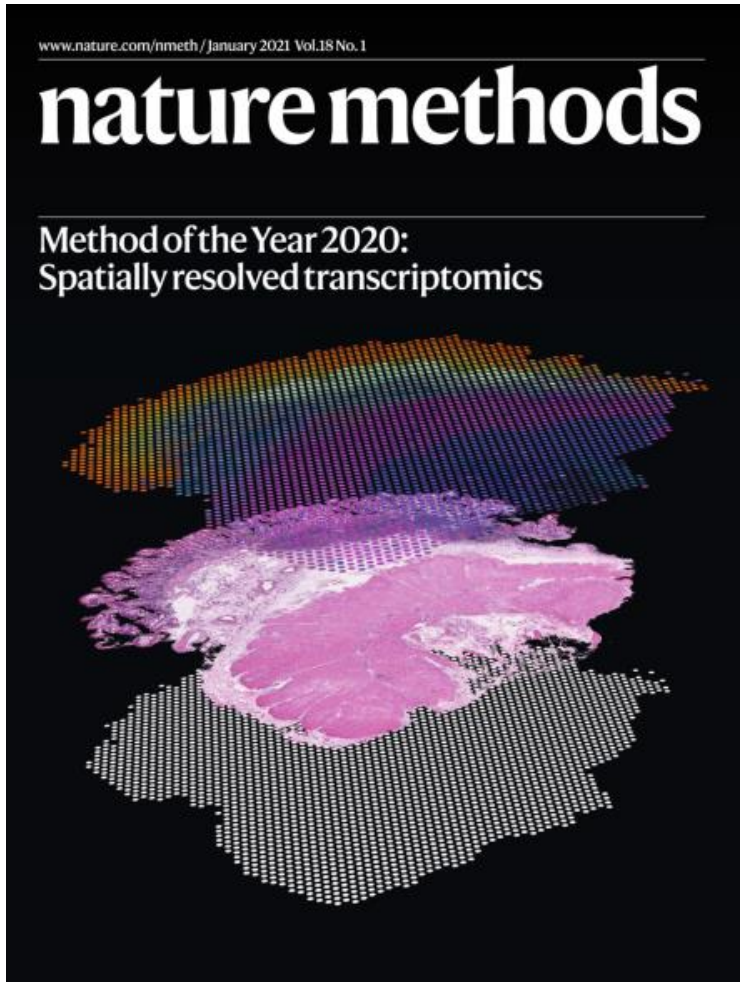
Introduction to spatial transcriptomics

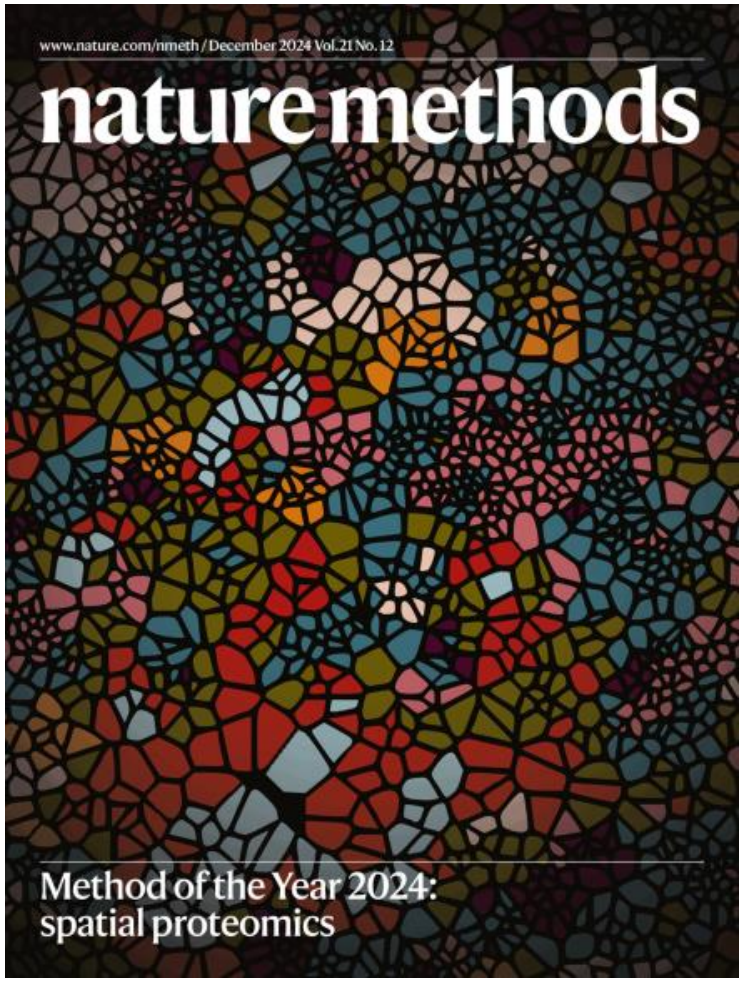
Methods and commercialisation over the last years



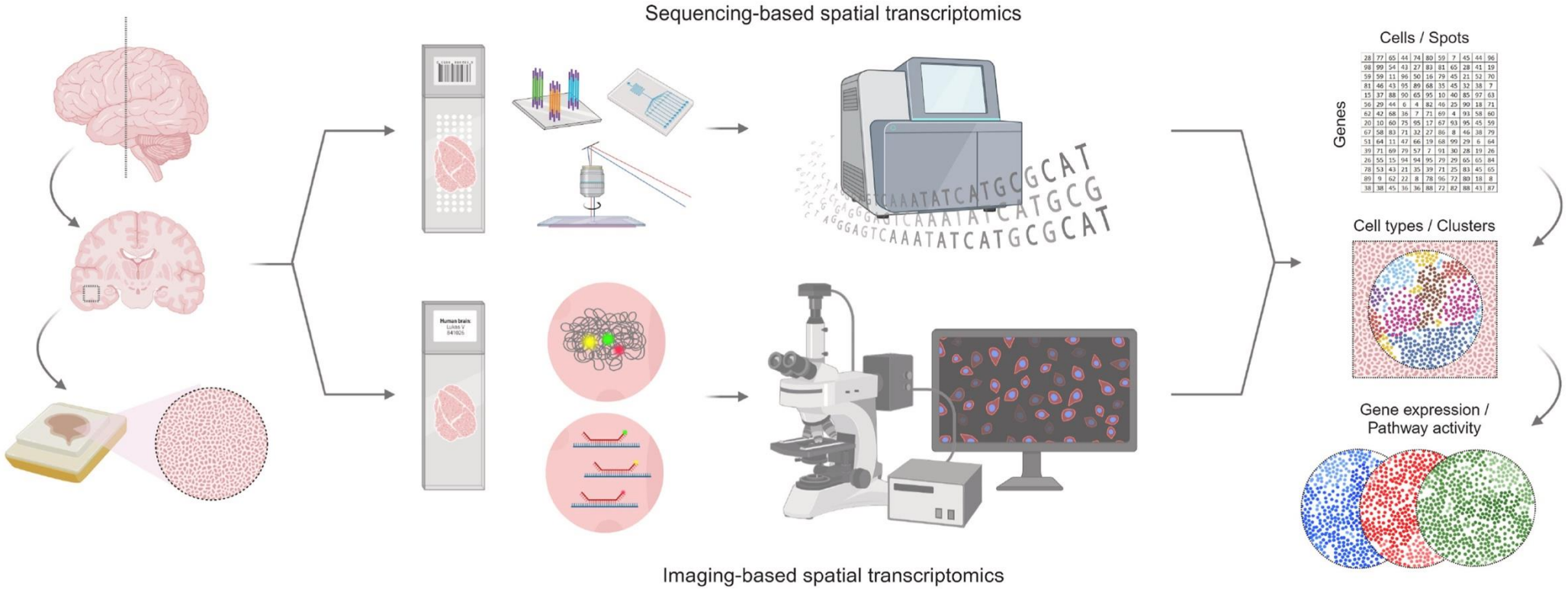
Valihrach et al. Molecular Aspects of Medicine, 2024

Spatial Omics





Modalities and technological platforms



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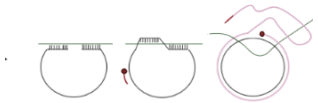
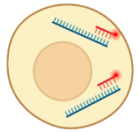
Imaging-based spatial transcriptomics

	In-situ sequencing methods (ISS)		In-situ hybridization methods (ISH)	
				
Examples:	ISS, STARmap, Barcode padlock probes		osmFISH, MERFISH, seqFISH(+), 10X Xenium	
Advantages:	• Allowes 3D localisation of transcripts • Independent of gene selection • Better signal-to-noise ratio		• Targeted measurement • Highly sensitive	
Limitations:	• Lower sensitivity		• Targeted measurement • Labor expensive and expertise/equipment required	

Sequencing-based spatial transciptomics

In-situ capturing (ISC)

Examples: 10X Visium (HD), slide-seq (vs), seq-Scope, Stereo-seq

Major point of innovation is the **capture array** design.

Caputre array: clusters of oligonucleotides carrying specific sequence that encodes the spatial position. mRNA difuses from slice to the capture array. Ex-situ sequencing of the library.

Spatial measurement locations often referred to as “spots”, “beads”, or “bins”

Visium technology

https://www.youtube.com/watch?v=VwNk4d-oRJc

10X spatial transcriptomics is similar to 10X scRNAseq technology, adding a spatially resolved barcode to the library

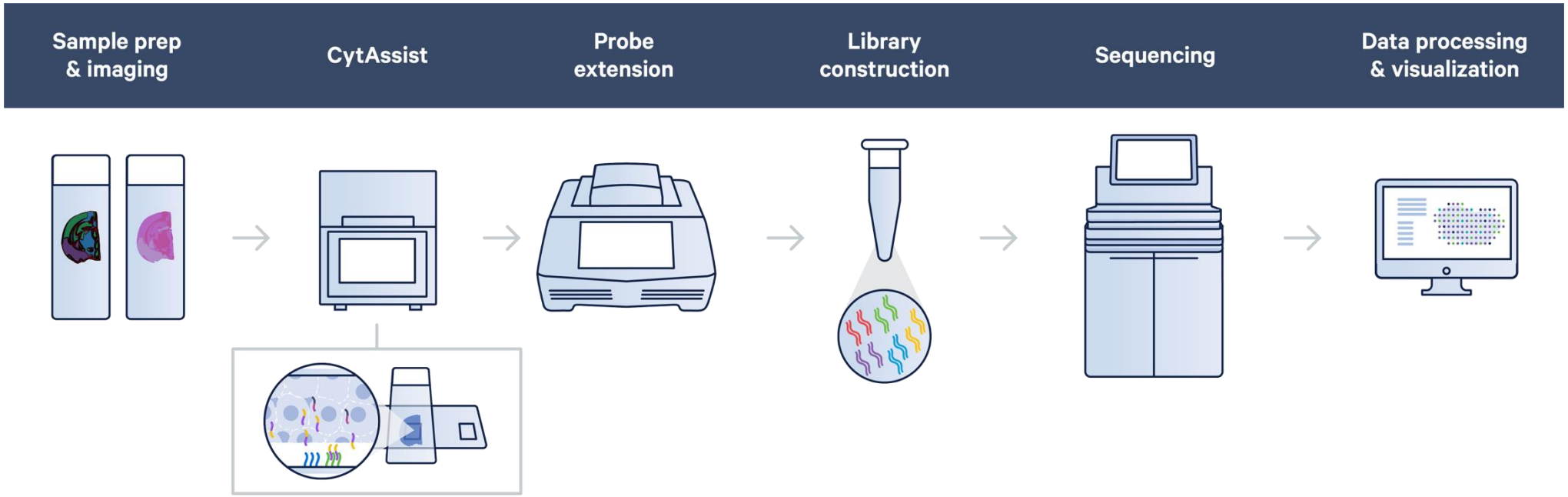
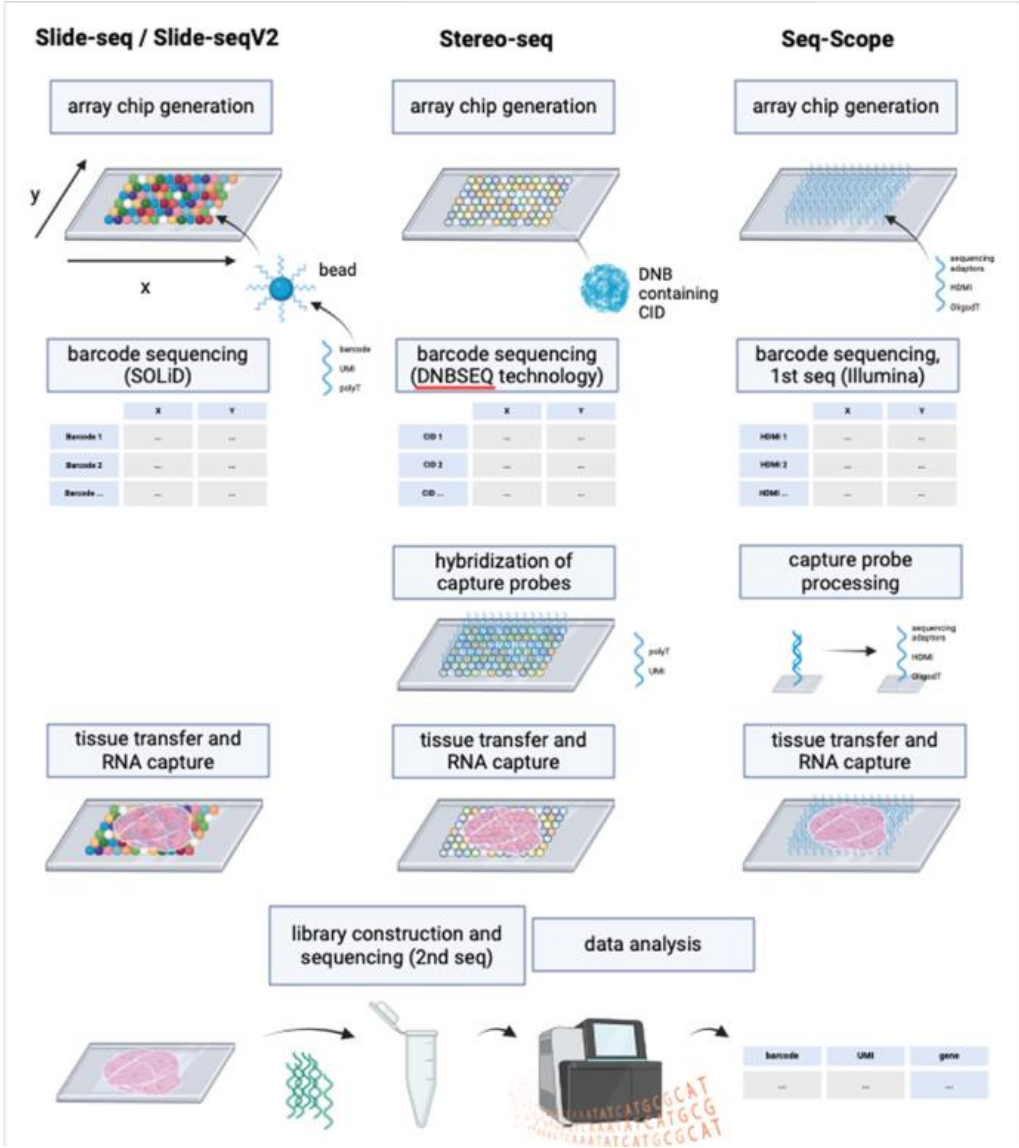


Image from https://www.10xgenomics.com

Sequencing-based technologies

- Spatial transcriptomics (ST): capture oligos printed in glass slide
- Nanobeads: Oligos anchored to nano-beads (lower diameter)
  Nanobeads are organised in a monolayer on the array
- Spatiotemporal enhanced resolution omics:
  DNA nanoballs localy amplified
  Different chip sizes (including custom ones)
- Homogeneous oligonucleotide colonies

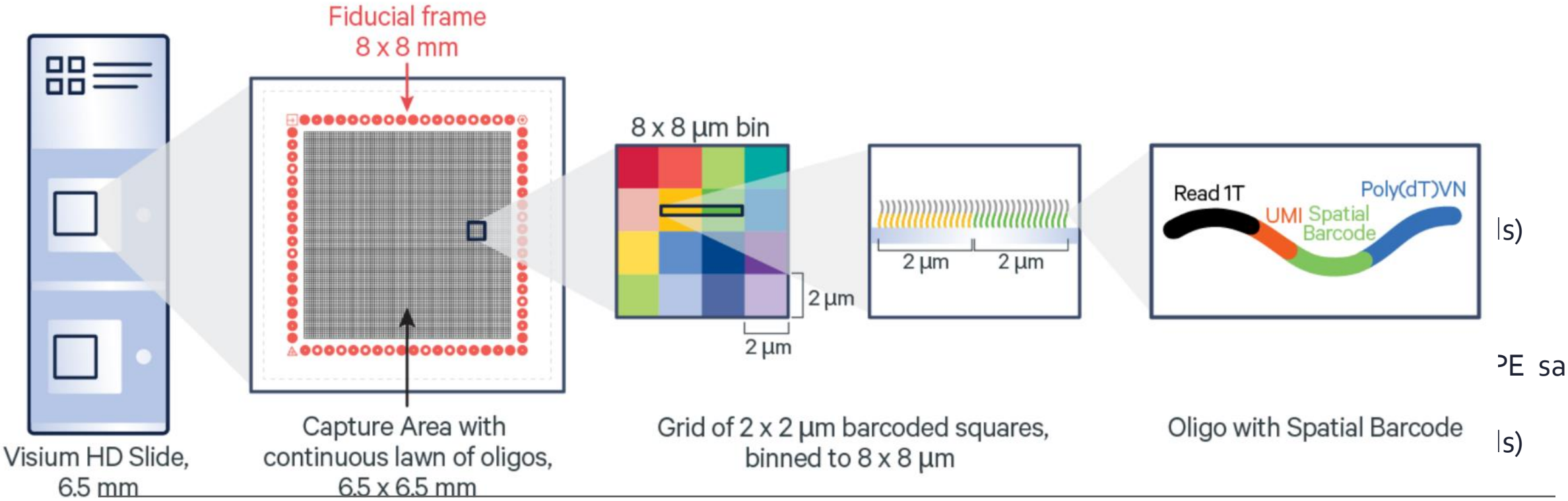


Fortner and Bucur. Frontiers in Cell and Development Biology, 2024

Sequencing-based technologies

methods	resolution (μm)	sensitivity/UMIs per 100 μm² (tissue)[a]	capture area (mm²)	detected target	accessibility[b]	ref
ST	100	31.88 (MOB)[c]	~40	polyA-tailed mRNAs	medium cost; commercialization as 10x Visium	(39)
10x Visium	55	508 (MOB)	~40	polyA-tailed mRNAs/target genes	high cost; commercially available	(77)
Ex-ST	~20	~496 (MOB)	~40	polyA-tailed mRNAs	high cost; tissue expansion technique and Visium slides	(40)
DBiT-Seq	10, 25, 50	~1320–4910 for mRNAs; 121.52 for proteins (ME)[c,d]	25[h]	polyA-tailed mRNAs and ADTs[i]	medium cost; homemade microfluidic chip	(64)
Decoder-seq	15, 25, 50	4010 (MOB)[e]	25[h]	polyA-tailed mRNAs	low cost; homemade microfluidic chip	(63)
xDBiT	50	200–800 (mouse organs)	116.64	polyA-tailed mRNAs	medium cost; homemade microfluidic chip	(62)
RRST	55	173.39 (MB)	~40	target genes	high cost; Visium slides and probe panel design	(44)
STRS	55	9–36 (mouse heart)[f]	~40	total RNAs	high cost; Visium slides	(45)
SHM-seq	100	~52 (mouse colon)	~40	host polyA-tailed mRNAs and microbiome rRNAs	high cost; Visium slides	(46)
SM-Omics	100	13.75 for mRNAs and 1.42 for proteins (MS)[c]	~40	polyA-tailed mRNAs and ADTs[i]	low cost; automated operating systems	(41)
spatial CITE-seq	25	78.88 for mRNAs; 35.4 for proteins (MS)	6.25	polyA-tailed mRNAs and ADTs[i]	high cost; ADTs and homemade microfluidic chip	(53)
spatial ATAC–RNA-seq	20, 50[g]	189.36 (ME)	25[h]	open chromatin and polyA-tailed mRNAs	NA; homemade microfluidic chip	(49)
spatial CUT&Tag–RNA-seq	20, 50[g]	144.12 (P22 MB)	25[h]	polyA-tailed mRNAs and histone modification	NA; homemade microfluidic chip	(49)
MISAR-seq	50	~450 (ME)	6.25	open chromatin and polyA-tailed mRNAs	NA; homemade microfluidic chip	(52)
Slide-seq	10	59 (E12.5 ME)	~7	polyA-tailed mRNAs	high cost; spatial decoding	(65)
Slide-seqV2	10	550 (E12.5 ME)	~7	polyA-tailed mRNAs	high cost; spatial decoding	(66)
HDST	2	12 (MOB)	13.68	polyA-tailed mRNAs	high cost; spatial decoding	(72)
Seq-Scope	<1	~1000 (ML)	0.8	polyA-tailed mRNAs	high cost; illumina flow cells	(68)
Pixel-seq	~1	977 (MOB)	315	polyA-tailed mRNAs	low cost; homemade polony gel	(69)
Stereo-seq	0.22	1450 (MOB)	up to 132 × 132	polyA-tailed mRNAs	high cost; has been commercialized	(67)
XYZeq	single cell	1.40 (ML[c]/tumor tissue)	~500	polyA-tailed mRNAs	NA; special facilities for tissue separation	(74)
sci-Space	single cell	41.23 (E14 ME)	324	polyA-tailed mRNAs	medium cost; sci-RNA-seq	(75)
Slide-tags	single cell	342.02 (E14 ME)	~7	polyA-tailed mRNAs, open chromatin, and T cell receptors	high cost; droplet-based snRNA-seq	(50)

Shi et al. 2024

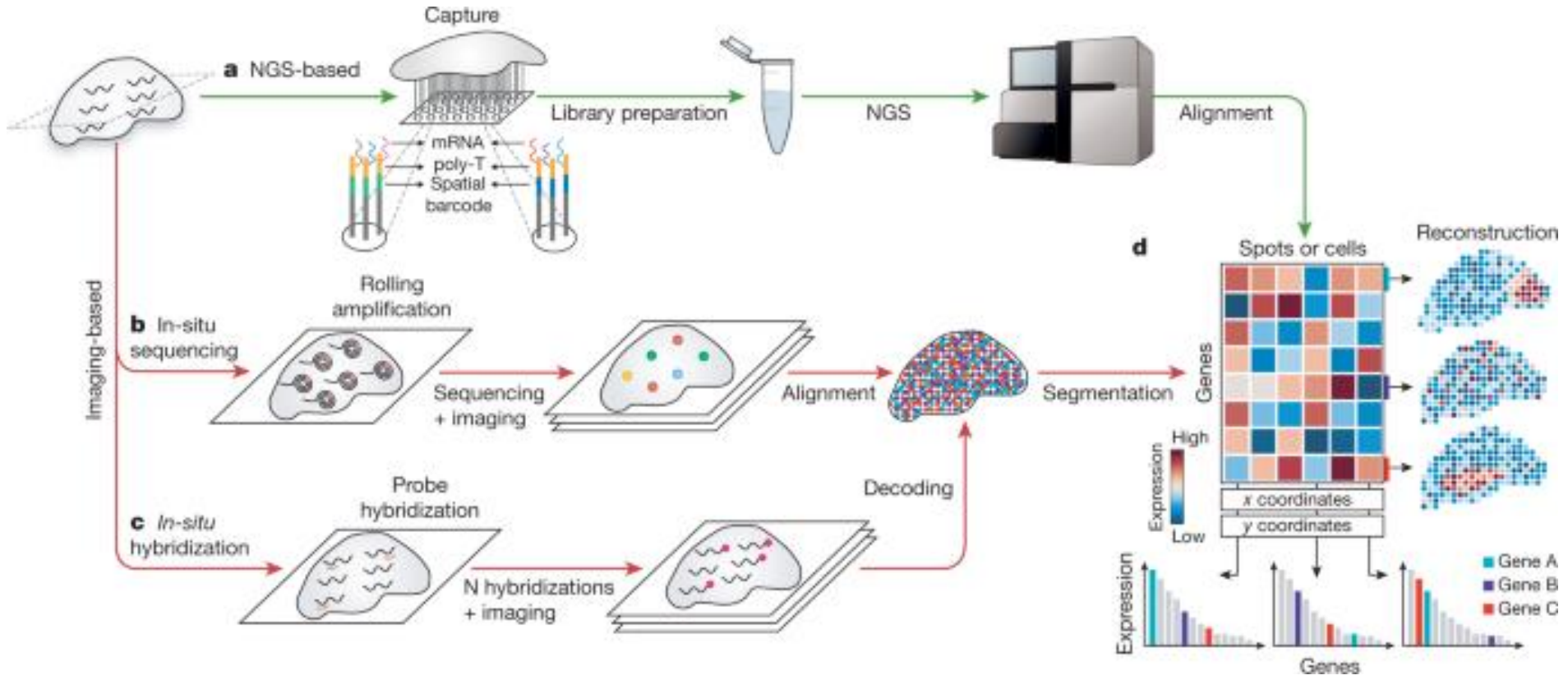


10x Visium HD

- **Visium HD**
  - Probe based chemestry
  - Fresh frozen, fixed frozen, FFPE samples
  - Human and Mouse
  - 2µm barcoded spot
- **Visium HD 3'**
  - 3' poly(A) captured based
  - Fresh frozen samples
  - Diverse species
  - 2µm barcoded spot

Images from https://www.10xgenomics.com

Imaging vs sequencing based technologies



Rao et al. Nature, 2021

Imaging vs sequencing based technologies

Imaging-based

- Higher spatial resolution
- Higher sensitivity

ISS
Xenium

- Lower genome coverage (targeted RNAs)
- Complex instrumentation nedded
- Large amount of data generation

Sequencing-based

Visium HD

- Lower resolution
- Lower sensitivity (capturing efficiency)
- Less flexibility in sample preparation

- Higher genome coverage (untargeted)
- Simpler instrumentation and more scalable
- NGS (widely available)

Technologies keep evolving to overcome the limitations…

Choosing the right technology

Put your scientific question first, don't choose over methodology

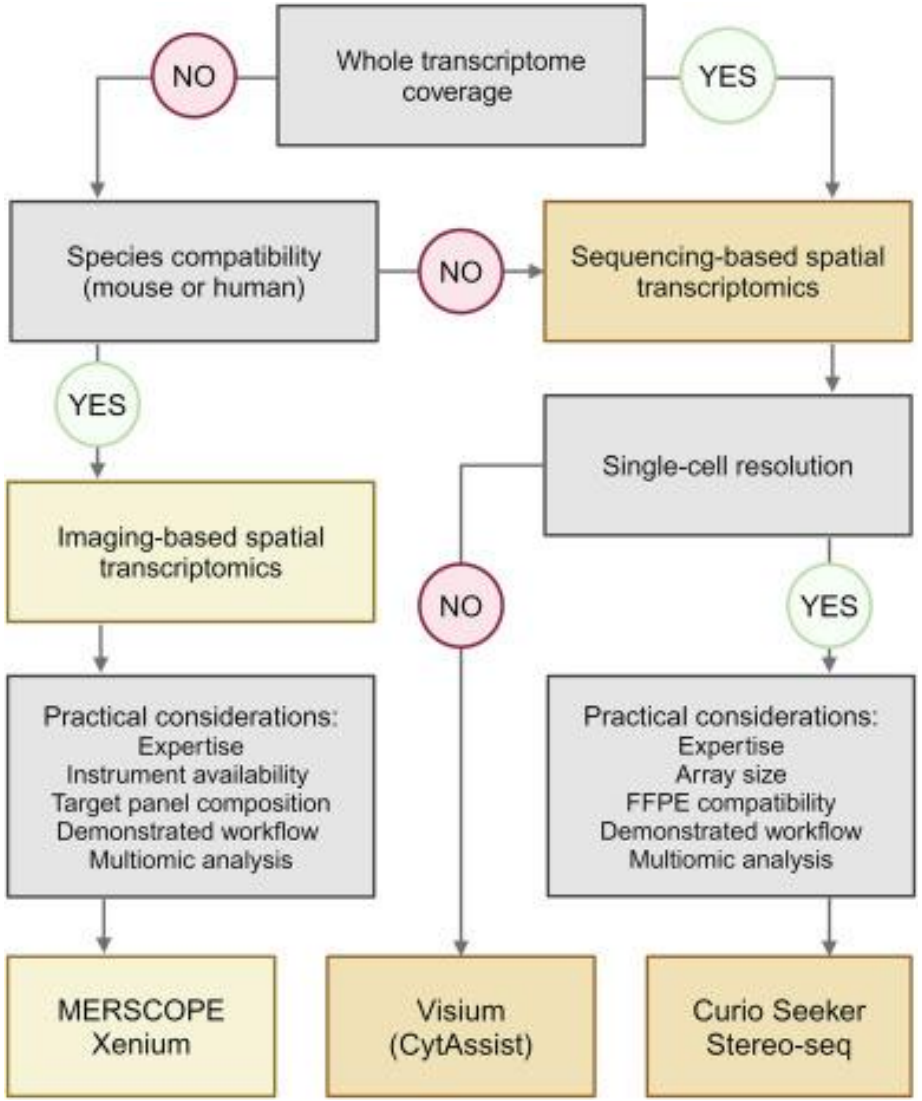
- Gene throughput: unbiased or targeted?
- Sequence information: do you need actual sequence information?
- Sensitivity: interested in all genes? Known marker genes available? Highly expressed?
- Resolution: do you need single cell information?
- Feasibility: which access to tissues can you have? Are there reference datasets? Instrument/data analysis access? Cost?

Very generalised!!!!

Imaging-based: precise localisation of transcripts, annotate cell types within subregions, spatial interactions between cell types…

Sequencing-based: transcriptome-wide studies, new markers discovery, sequencing-read downstream analysis like velocity…

Choosing the right technology



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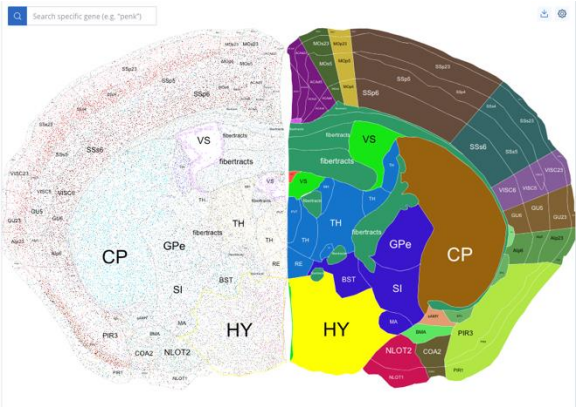
Applications



Mouse Spatial Transcriptomics Atlas

- Indetification of cell types and tissue organisation: cell atlas, detection cell proportions in tissue structures
- Study of microenvironments and cell-cell interactions: exploration of many-to-many ligand receptor interactions, characterisation of tumour microenvironment
- Elucidate molecular and cellular gradients/patterns in complex tissues, providing insights into physiology, molecular biology and anatomy of organs
- Time resolved analysis: lineage tracing and cell fate inference

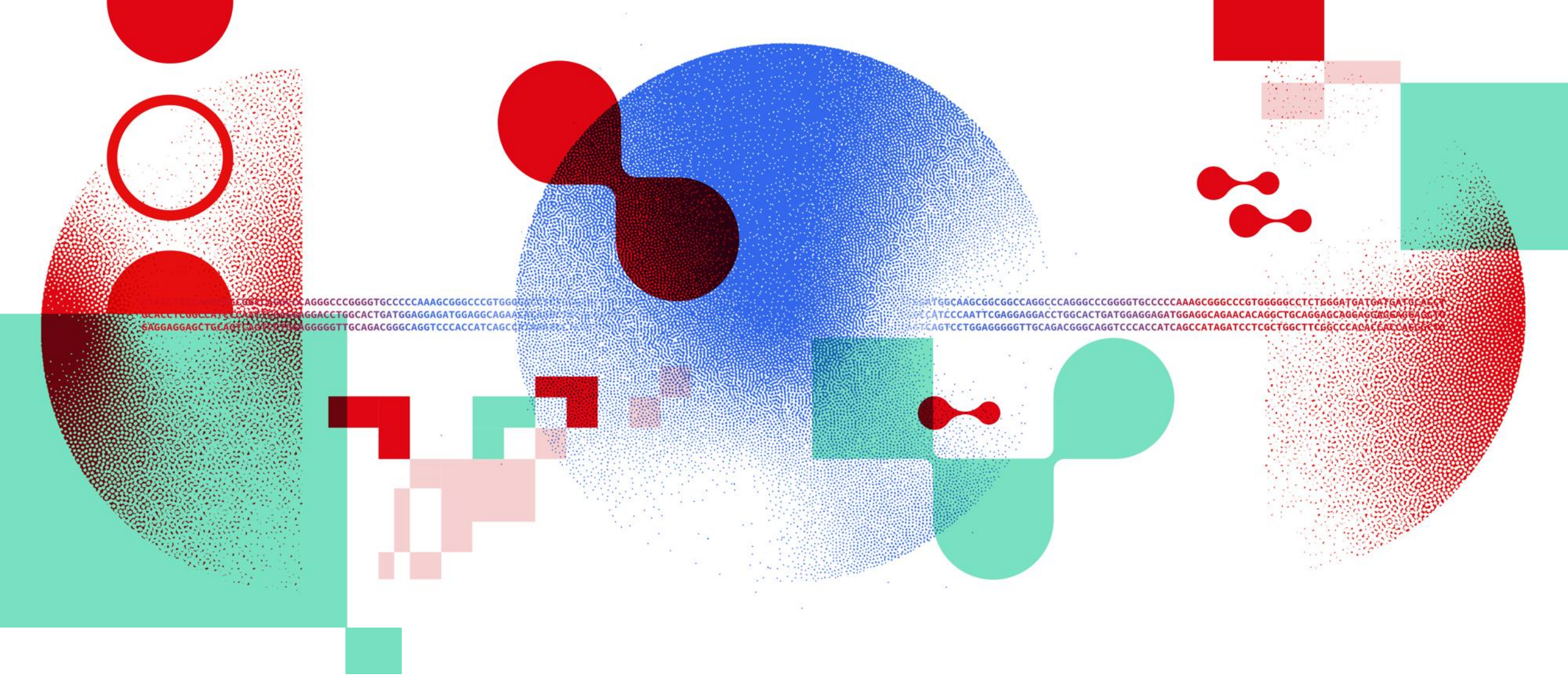
Challenges and future directions

Limitations:

- Lacks multidimentional profiling
- Low capture sensitivity and RNA difusion
- Compatibility with sample preparations
- Large amounts of data, problem to store, computational time and costs
- Scalability: Technology keeps scaling up to more resolution, more spots, etc. Challenge for methods to scale up

Future directions:

- Holistic view with application of spatial multi-omics (epigenetics, proteomics, metabolomics…)
- Couple ST with long-read sequencing technologies
- 3D reconstruction of ST slices



Thank you

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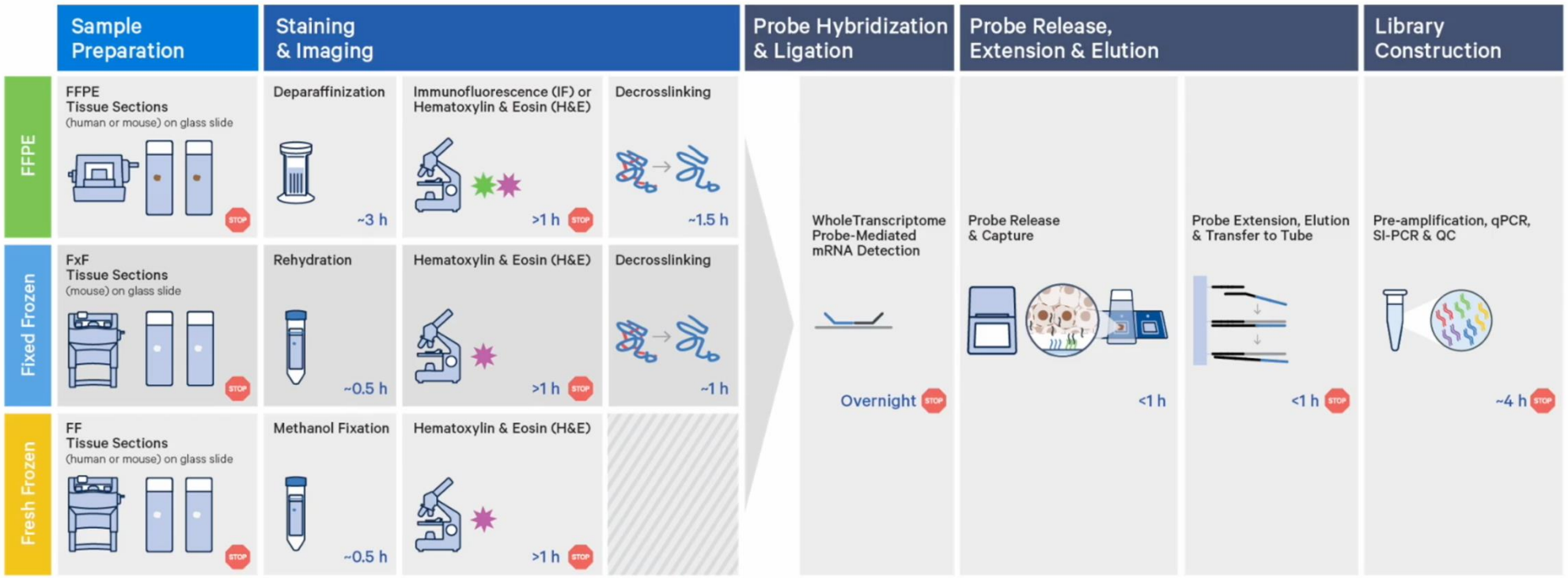
Table 1. Overview of commonly used ST platforms

Platform (type)	Resolution and panel type	Sample types	RNA quality	Best use cases
Visium (FF, sequencing)	~55 μm; whole transcriptome	Human, mouse, all species (polyA+)	RIN ≥ 7 (≥4 w/ CytAssist)	Broad discovery in fresh tissue
Visium (FFPE, sequencing)	~55 μm; whole transcriptome	Human, mouse FFPE	DV200 ≥50% (≥30% w/ CytAssist)	Archived samples; full profiling
Visium HD (sequencing)	~2 μm; whole transcriptome	Human, mouse FFPE or OCT	RIN ≥4; DV200 ≥30%	High-res + whole transcriptome
Xenium (imaging)	Subcellular; targeted (up to 5000 genes; customizable)	Human, mouse FFPE or fresh-frozen; non-model (custom)	DV200 ≥10%	Cell typing; high-res profiling; cross-species (custom panels)
CosMx (imaging)	Subcellular; targeted panels or whole-transcriptome	Human, mouse FFPE or fresh-frozen	DV200 ≥10%	Multiplexed profiling; spatial cell state mapping
MERFISH/ seqFISH (imaging)	Subcellular; highly multiplexed (customizable)	Fresh-frozen; FFPE (with optimization)	Protocol-dependent	Deep profiling in microscopy-capable labs
Stereo-seq (sequencing)	500 nm; whole transcriptome (species-specific probes)	Human, mouse, non-model (custom probes)	RIN ≥7 recommended	Nanoscale mapping; large area profiling
Non-model species	Varies by platform and probe design	Visium (polyA+), Xenium, Stereo-seq	Variable	Cross-species studies (requires custom panels or transcriptomes)


Sample Preparation
Staining & Imaging
Probe Hybridization & Ligation
Probe Release, Extension & Elution
Library Construction
FFPE
FFPE Tissue Sections (human or mouse) on glass slide
Deparaffinization
~3 h
Immunofluorescence (IF) or Hematoxylin & Eosin (H&E)
>1 h
Decrosslinking
~1.5 h
Fixed Frozen
FxF Tissue Sections (mouse) on glass slide
Rehydration
~0.5 h
Hematoxylin & Eosin (H&E)
>1 h
Decrosslinking
~1 h
Fresh Frozen
FF Tissue Sections (human or mouse) on glass slide
Methanol Fixation
~0.5 h
Hematoxylin & Eosin (H&E)
>1 h
WholeTranscriptome Probe-Mediated mRNA Detection
Overnight
Probe Release & Capture
<1 h
Probe Extension, Elution & Transfer to Tube
<1 h
Pre-amplification, qPCR, SI-PCR & QC
~4 h
STOP