How to Choose an Optimal Spatial Technology from Six Available Options at TCGB

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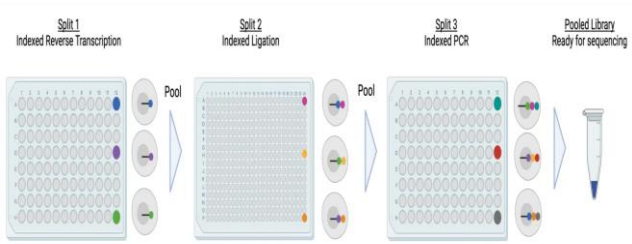
https://www.uclahealth.org/pathology/tcgb

Commercially Available Single Cell Sequencing Technologies

- **10X Genomics single cell system**
- BD Rhapsody single cell system
- Parse Biosciences single cell platform
- Scale Biosciences single cell platform
- Mission Bio single cell system

10X Chromium IX



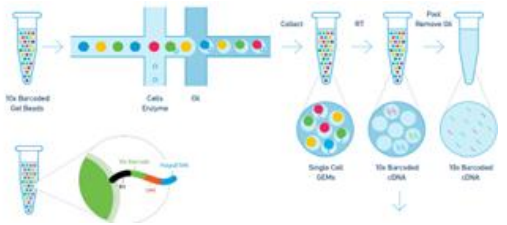
Parse/Scale Bioscience

BD Rhapsody™ System

Tapestri

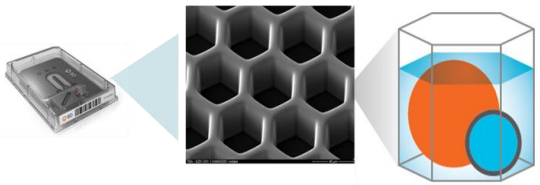
Three Major Single Cell Technologies Available at TCGB/JCCC GSR

10X Genomics



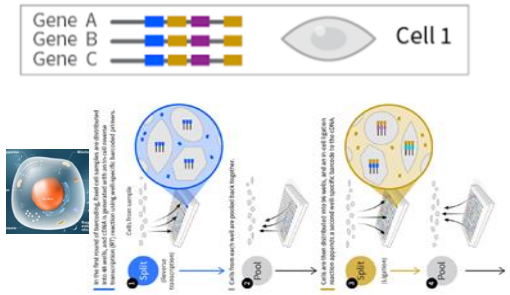
- Gold standard
- Comprehensive & Robust assays
- New product: GEM-X

BD Rhapsody



- known # of cells captured before seq
- Good for delicate cells
- Capturing >50K cells/sample

Scale Bio



- No instrument (cell itself as a barrier)
- High throughput
- Unique methy assay

Commercially Available Spatial Genomics Technologies

Two Types of Spatial Genomics Technologies

Imaging -based

- Molecular Cartography (Resolve Bios.)
- MERSCOPE (Vizgen)
- Xenium (10X genomics)
- CosMx SMI (Nanostring)

Sequencing-based

- 10X Visum (10X genomics)
- GeoMx DSP (Nanostring)
- Curio Seeker (Curio Bioscience)
- Visium HD (10X Genomics)
- Stereo-seq (STOmics)

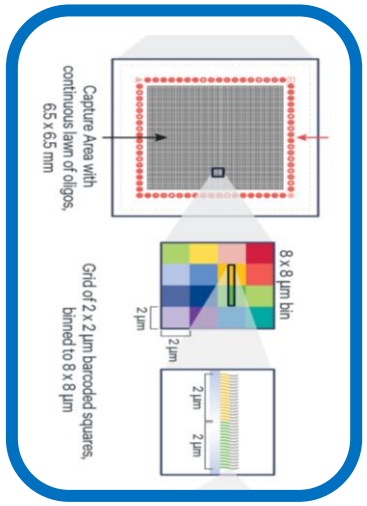
Six Spatial Technologies Available at TCGB

G1

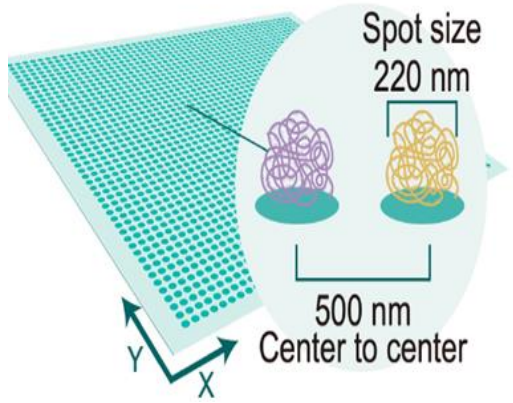
- 10X Visium
- GeoMx DSP

G2

Visium HD



Stereoseq



G3

- 10X Xenium
- CosMx SMI

Classified into Three Groups based on Resolution and Content

Comparisons within and across the Groups

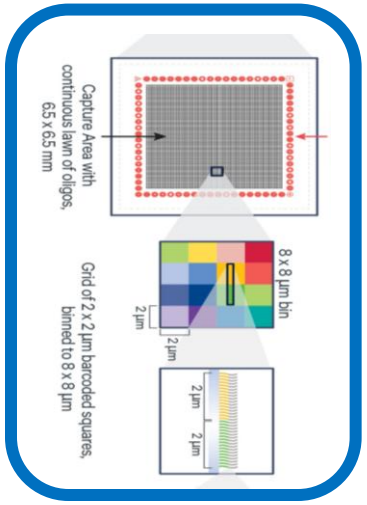
G1

10X Visium

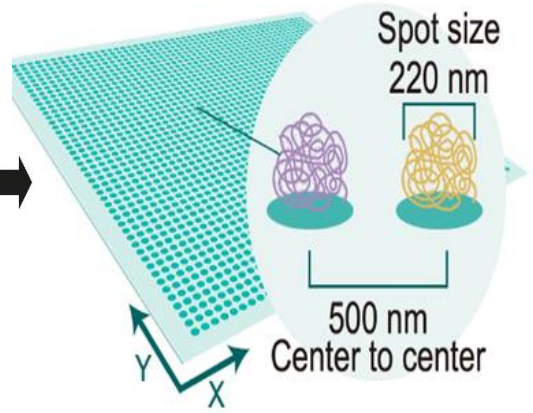
GeoMx DSP

G2

Visium HD



Stereoseq



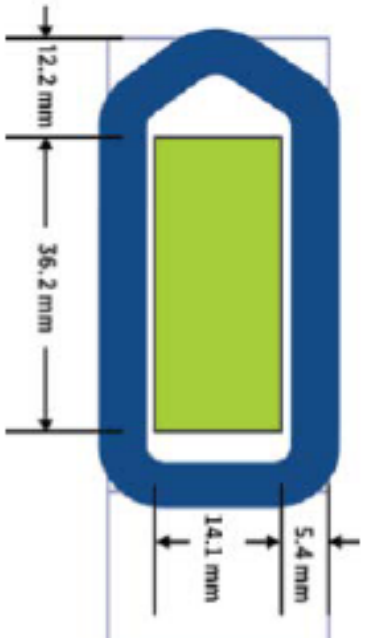
G3

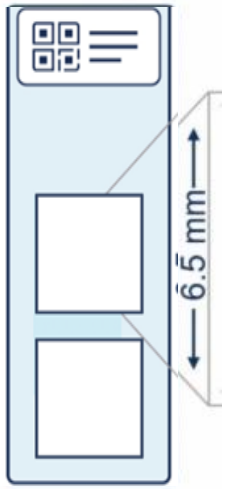
10X Xenium

CosMx SMI

G1: 10X Visium vs GeoMx DSP

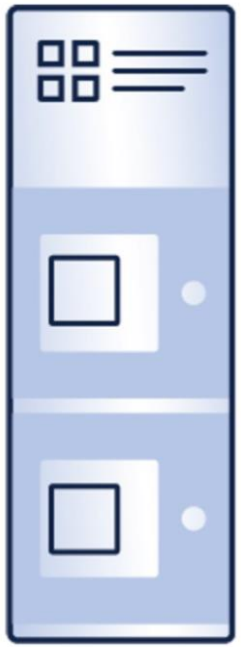
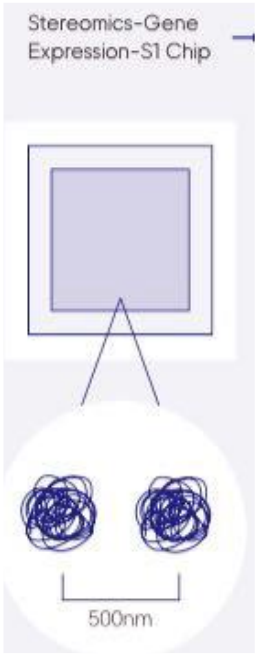
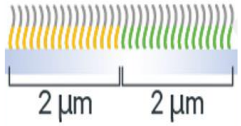
GeoMx	10X Visium
➢ Human & Mouse only	Any species (fresh tissue for V1 kit)
➢ Scan 36X14mm and sequencing selected ROI regions only	Scan & sequencing entire 6.5X6.5mm
➢ More samples or single large section with scattered cells of interest/slide	2 or 4 samples/slide
➢ Relatively low	Higher sensitivity & resolution
➢ Focused study with known cells of interest	Discovery purpose without pre-knowledge of cells of interest





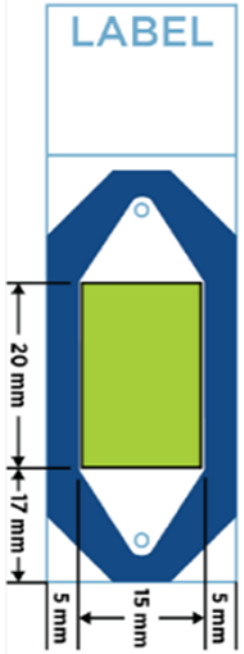
Recommend a minimum of 50-100 cells per ROI. The largest ROI area that you can image is 660um x 785um

G2: 10X Visium HD vs Stereoseq

10X Visium HD	Stereoseq
	
➢ Human & Mouse only	Any species
➢ FFPE tissue	Fresh frozen tissue
➢ Capturing size: 6.5X6.5mm	10X10mm, 20X30mm, 130X130mm
➢ Feature size: 2um 	0.2um
➢ Data output: 8X8um	Bins as needed
➢ Single cell scale	Single cell resolution

Note: Trade off between resolution and # of gene detected

G3: 10X Xenium vs CosMx SMI

10X Xenium	CosMx SMI
	
➢ RNA only	RNA and 64 proteins
➢ 247-379 genes, 480 transcripts (1-100 custom genes)	1000 and 6000 genes (7- to 200 custom genes)
➢ Scanning time: 2-3 days	3-7 days
➢ Hands-on time: over 3 days	over 2 days
➢ High specificity and sensitivity	Low specificity and sensitivity

Comparisons across the Groups

G1

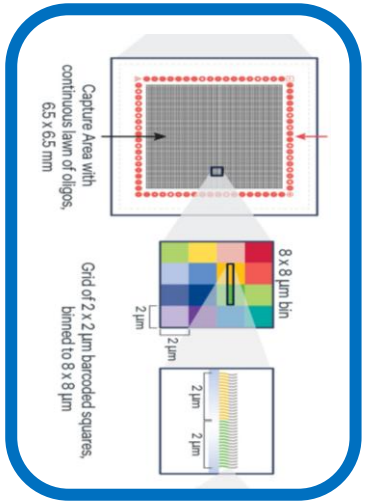
10X Visium

GeoMx DSP

WTA + Protein panel at around 50um resolution

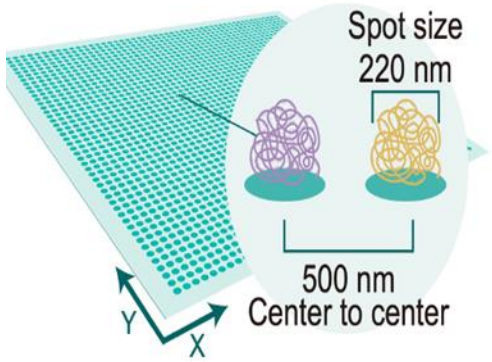
G2

Visium HD



WTA at single cell scale, 2x2um feature size, 8x8um Bin

Stereoseq



WTA at subcellular Resolution, 0.2um feature size, 0.2x0.2um Bin

G3

10X Xenium

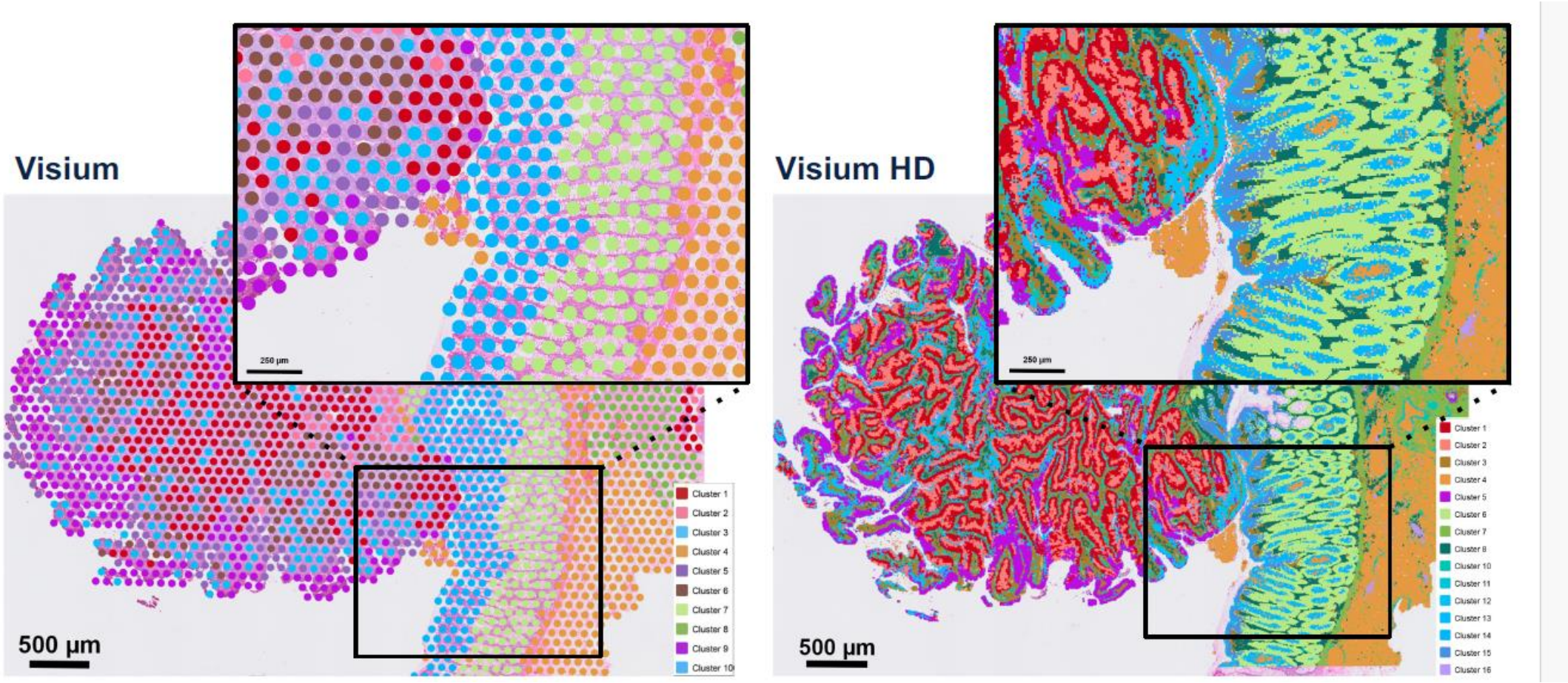
CosMx SMI

Gene panels+ protein at single cell resolution
CosMx: 1000 and 6000 genes
Xenium: 247-379 genes

Resolution

Resolution Makes a Difference (55um vs 2um)

From 10X

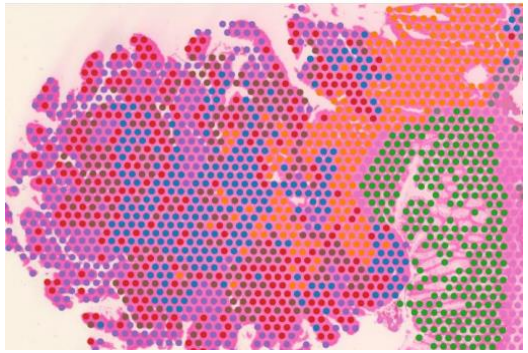


Comparison between Sequencing-based and Imaging –based Technologies

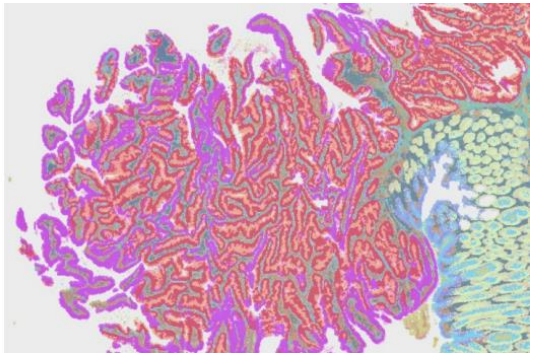
Unbiased Discovery

Precision Insights

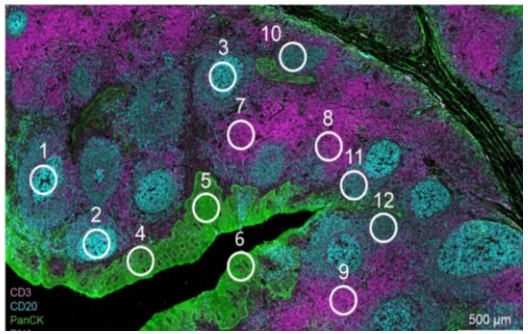
Visium



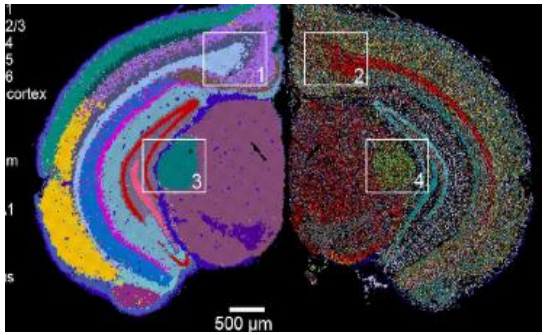
Visium HD



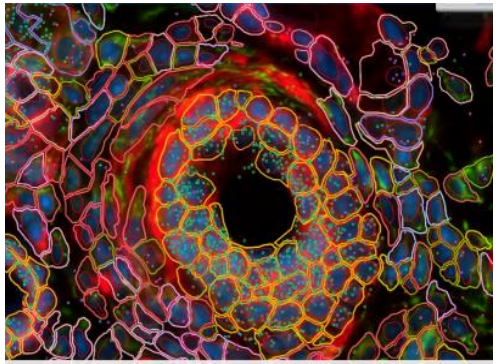
GeoMx



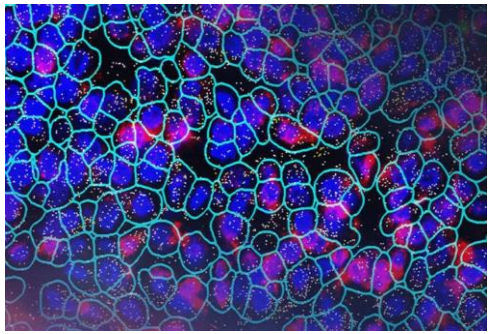
Stereoseq



Xenium



CosMx



Important Considerations - What You Have

- Biological question – spatial info is critical to answer your biological question
- Species – Visium (many species) vs GeoMx DSP (human and mouse only, morphology markers)
- Sample number & size (GeoMx – many small tissues or 1 large tissue vs Visium – 2 or 4 tissues), 13.2X13.2cm with Stereo-seq, 6.5X6.5mm with 10X visium
- Tissue type – Fresh frozen vs FFPE (optimization?)
- RNA quality - DV200 of ≥ 50% or RIN>5 or 7
- Budget

Additional Technical Considerations – What You Need

Having considered species, sample number, tissue type, tissue size, RNA integrity and your budget, following technical parameters should be considered:

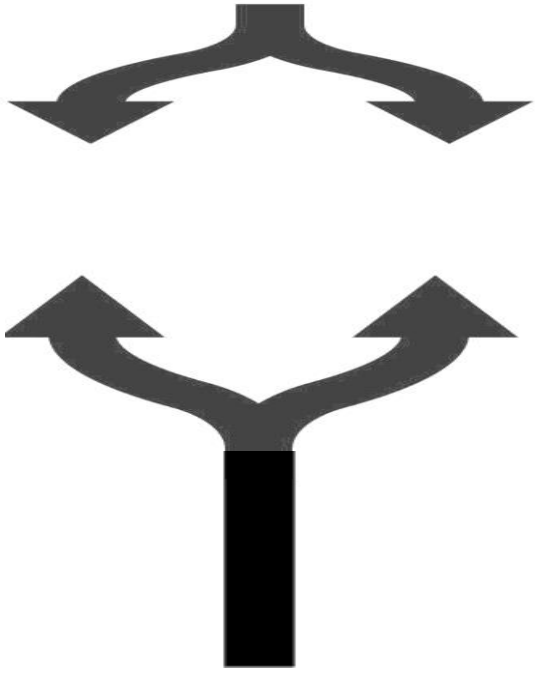
- mRNA capture efficiency (Imaging based vs sequencing based
- Spatial resolution
- Number of genes profiled (WTA vs Panels, need custom genes?)
- Protein panels (Available vs no available, # of proteins)
- Imaging area (0.65cm vs 13cm)
- Sensitivity & specificity

Based on What You Have and What You Need, Check the Key Parameters of Different Spatial Platforms

Platform	Species	Tissue Type	# of genes profiled	# of Proteins profiled	Spatial Resolution	Capture efficiency	Imaging Area	Rate/slide
10X Visium	Any with V1 kit	Fresh & FFPE	WTA	35	55um	Low	0.65X0.65cm, 1.1X1.1cm	~$3293+seq
GeoMx DSP	Human & mouse	Fresh & FFPE	WTA	570	50um (claimed)	Low	3.6X1.4cm (select ROI)	$3837+seq
Visium HD	Human & mouse	FFPE	WTA	N/A	Single cell scale	Low	0.65X0.65cm	$6645+seq
Stereo-Seq	Any	Fresh	WTA	N/A	Single cell	Low	1X1cm,2X3nm 13.2X13.2cm	$3054+seq
Xenium	Human & mouse	Fresh & FFPE	247-379,	N/A	Single cell	High	1X2 cm (select FOV)	$3878
CosMX SMI	Human & mouse	Fresh & FFPE	1000, 6000	64	Single cell	High	2X1.5cm (select FOV)	$6325

General Take-home Message

Experimental aim



Hypothesis Generation for Unbiased Discovery

Visium, GeoMx DSP

Hypothesis Testing for Precision Insights

Xenium, CosMx SMI,

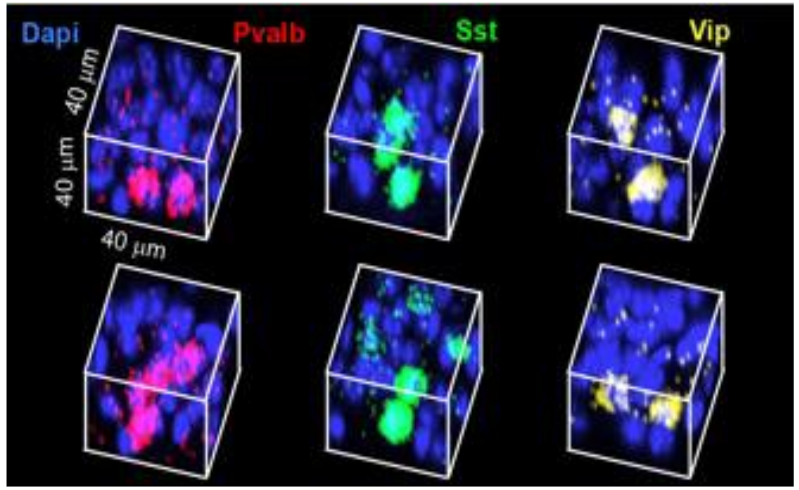
Visium HD, Stereoseq

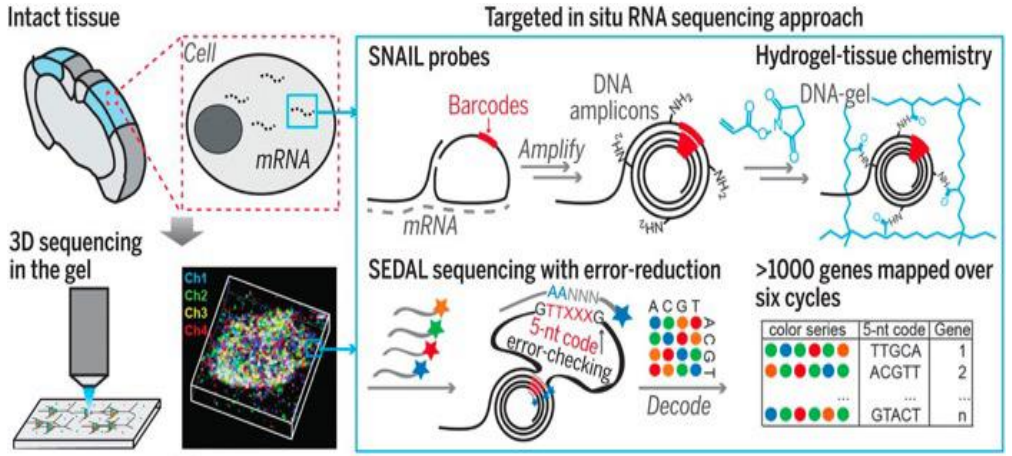
Upcoming Spatial Technologies

- 3-D Spatial Multiomics (StellarOmics) - Beta test stage
- In-situ Sequencing (Singular Genomics) - Early access
- Single-Cell Spatial Mapping (Curio Bioscience) – Early access

StellarOmics: 3D spatial Multiomocs

- Imaging-based in situ sequencing
- single cell resolution
- 15mm X15mm X 0.2mm capturing area
- Multi-cell-layer profiling
- 250 gene panel with up to 250 custom genes



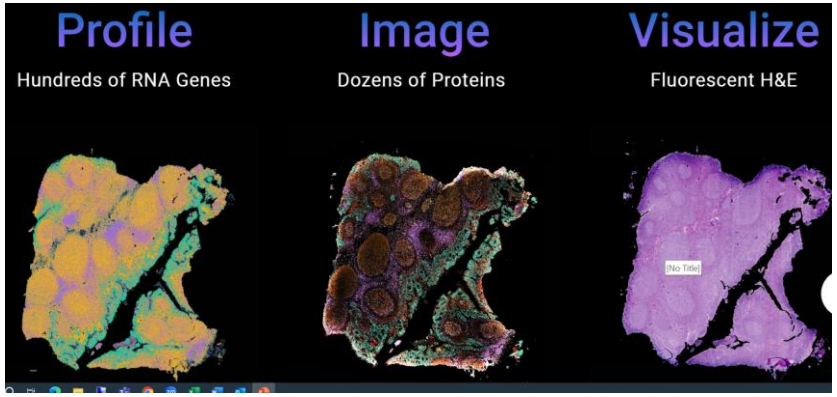


Singular Genomics: G4X Spatial Sequencing

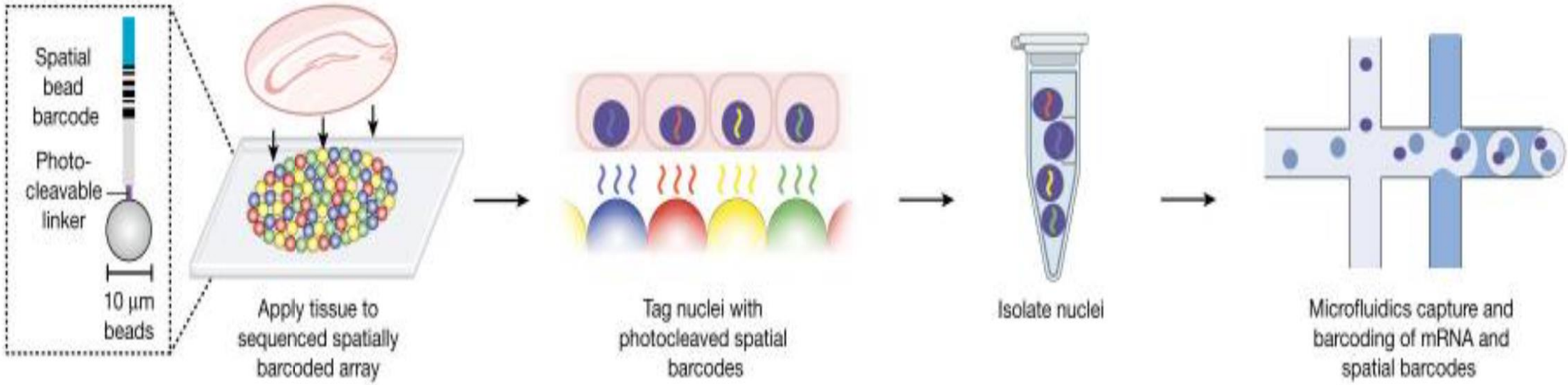
The G4X Sequencer combines two technologies into one singular platform: high-throughput in-situ spatial multiomics and next-generation sequencing

- Direct sequencing of RNA at subcellular resolution
- Profiling 300 genes at single cell resolution
- Imaging 10-15 proteins
- Visualizing tissue morphology (Fluorescent H&E)

G4X Spatial Sequencer



Curio Bioscience:
Curio Trekker – Single Cell Spatial Mapping



Schematic of Slide-tags. A 20-µm fresh-frozen tissue section is applied to a monolayer of randomly deposited, DNA-barcoded beads that have been spatially indexed. These DNA spatial barcodes are photocleaved and diffused into 20 µm fresh frozen tissue sections to associate them with nuclei (2um resolution) associate with nuclei. Spatially barcoded nuclei are then profiled using established droplet-based single-nucleus sequencing technologies. (Russell et al. Nature 2024)

Q&A