Genetics, Biomedical Computing, and Genomics Monthly Meeting –
New Technologies in the UCI Genomics Research and Technology Hub (GRT Hub)

Spatial Transcriptomics

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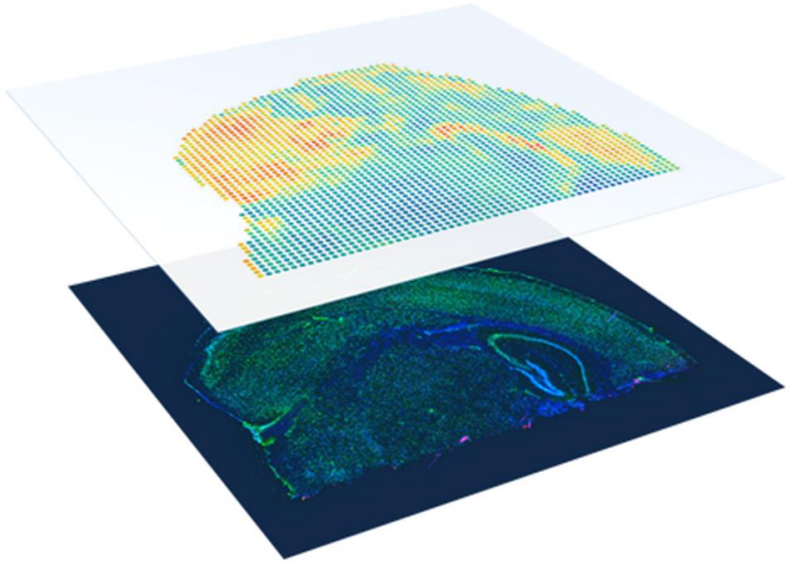
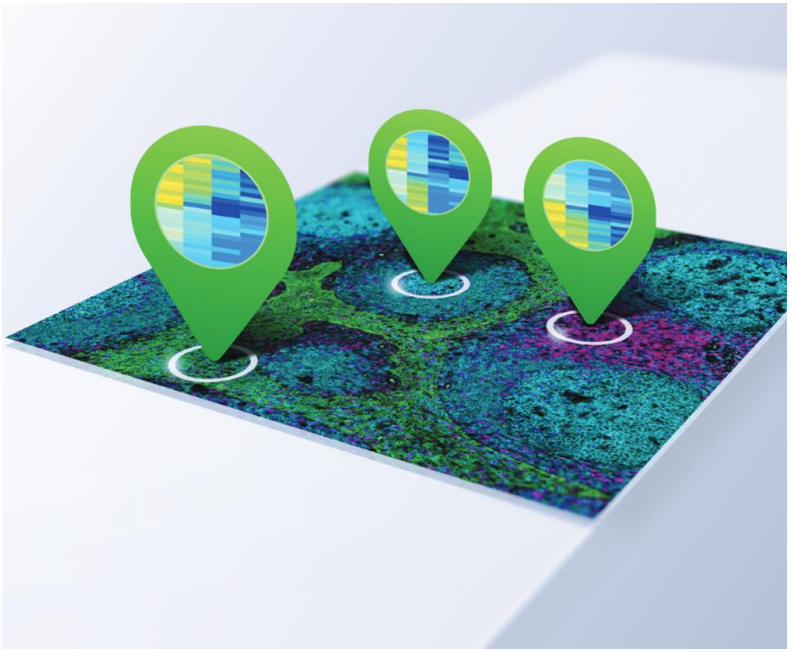
April 4, 2023

Currently Available- Spatial Transcriptomic Platforms

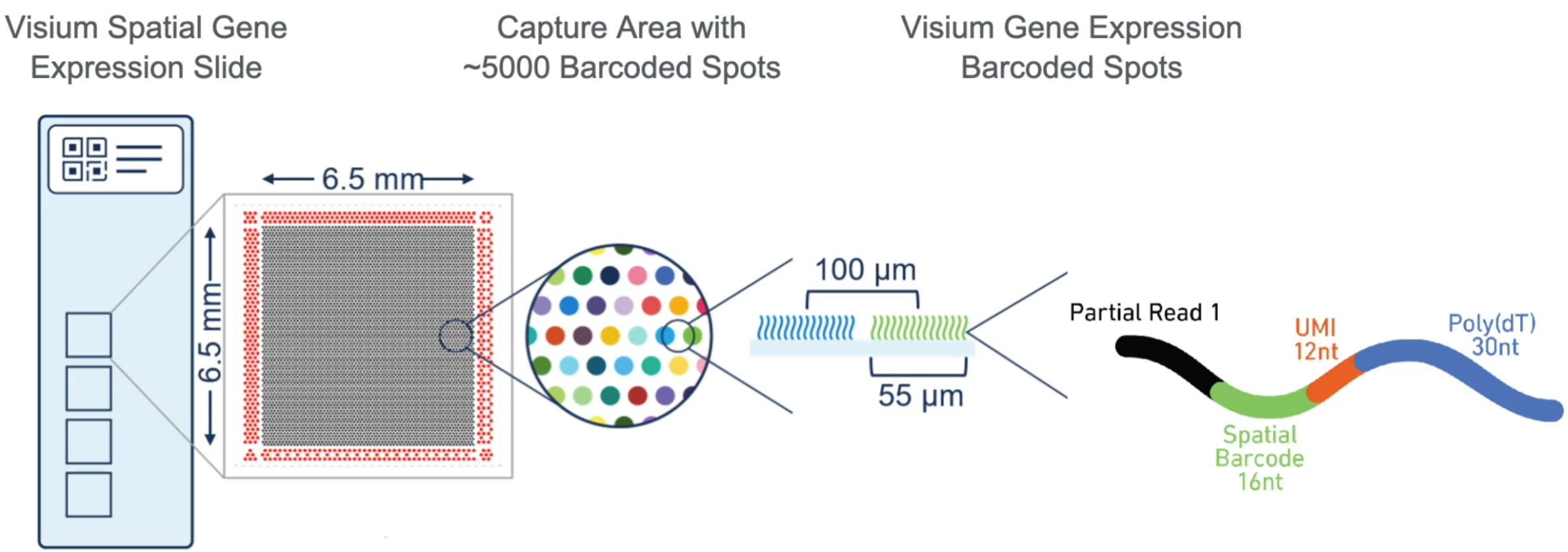
- 10X Genomics Visium



- NanoString GeoMx DSP



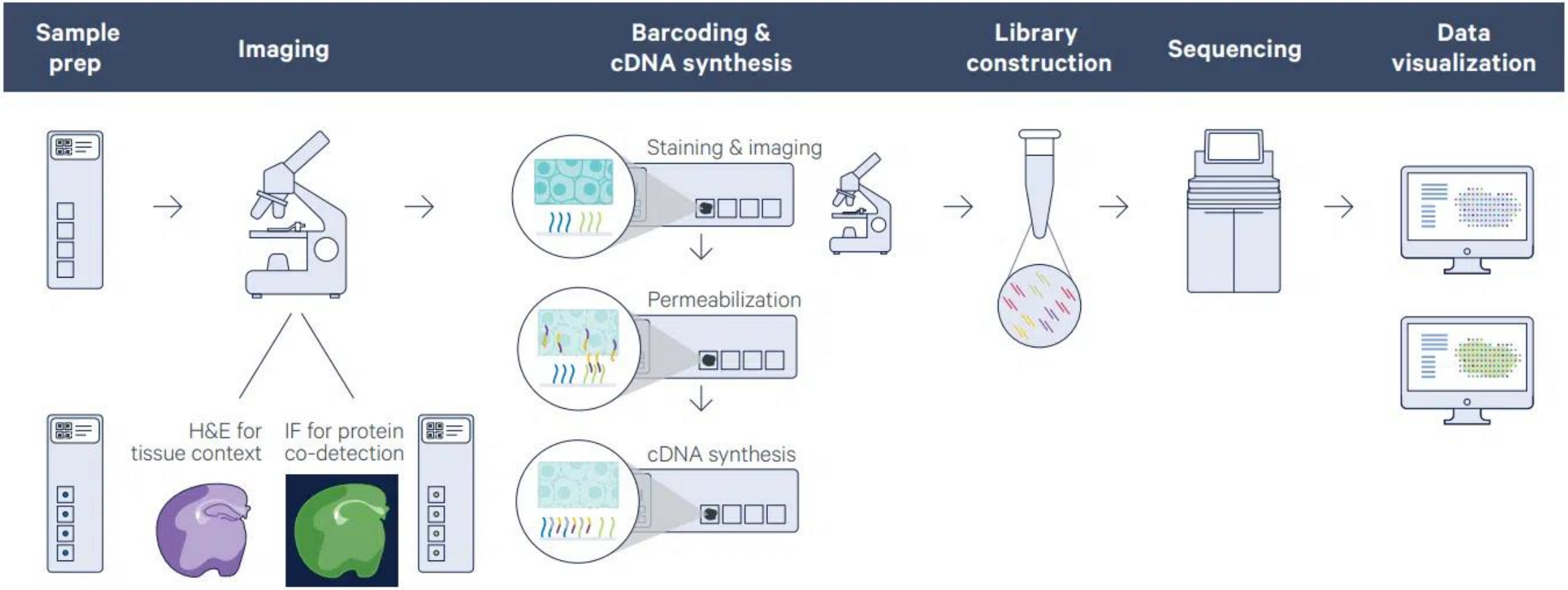
Visium for Fresh Frozen and FFPE



HE or IF image of tissues is overlaid with map of barcoded spots for spatial reconstruction of captured RNA.

10x GENOMICS

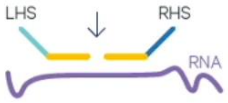
Visium for FF Workflow



Visium FFPE Chemistry

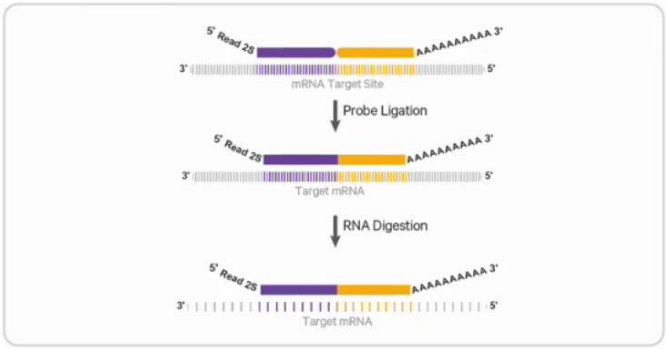
RNA Templated Ligation (RTL) for sensitive, specific RNA detection in FFPE samples

Probe pairs designed against the protein-coding transcriptome, one pair per gene



Split two probe chemistry reduces nonspecific signal

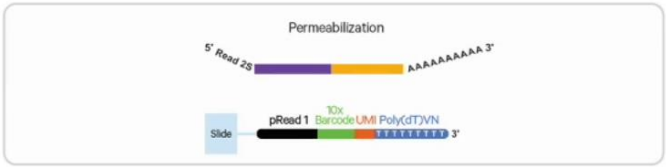
Probe hybridization and ligation



~18,000 genes targeted in human

Ligated probe pair released

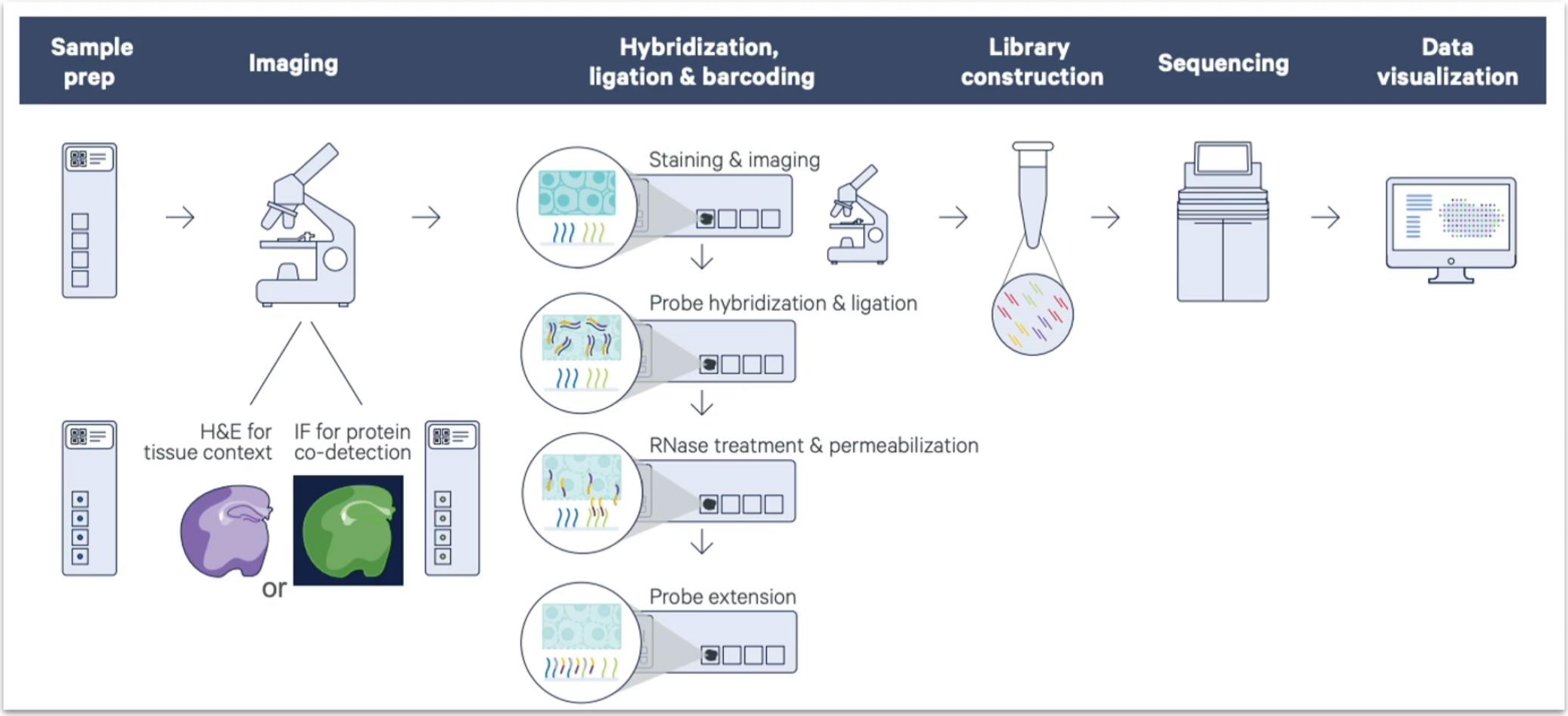
Tissue permeabilization and ligated probe pair capture



~20,000 genes targeted in mouse

10x GENOMICS

Visium for FFPE Workflow



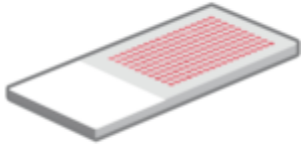
Visium Spatial Gene Expression for FFPE

10x GENOMICS

NanoString GeoMx

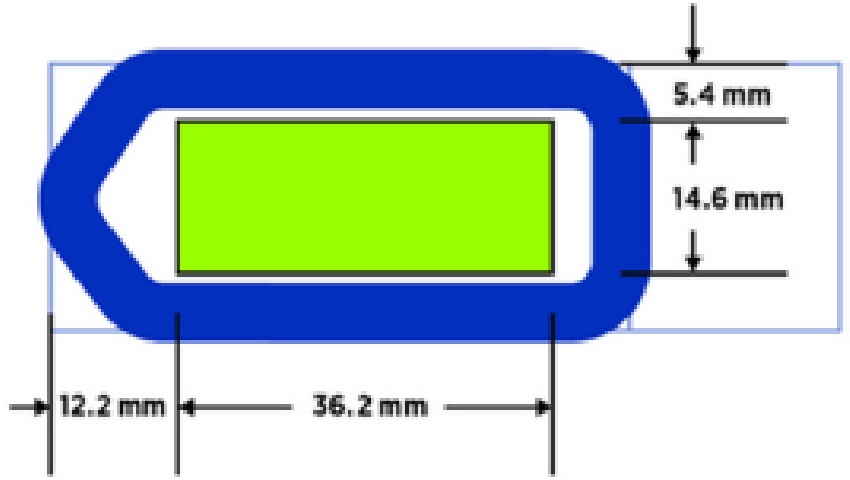
Versatile Sample Types, including Formalin-Fixed Paraffin-Embedded and Fresh Frozen Tissue

FFPE TISSUE BLOCK



TISSUE MICROARRAY (TMA)

FRESH FROZEN TISSUE

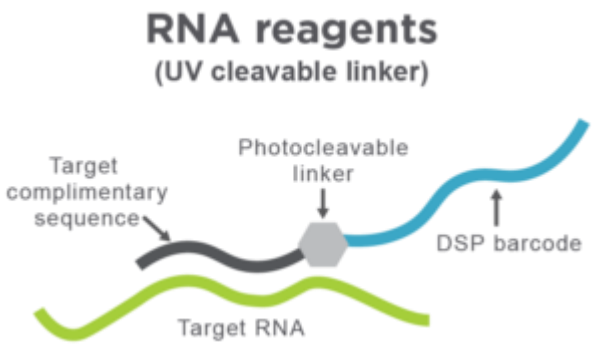


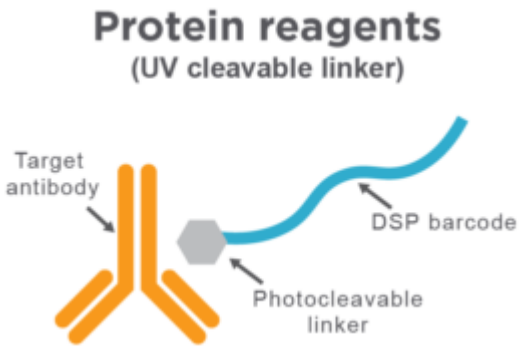
Tissue sections must be placed in the digital scan area (FOV, shown in green), measuring, at maximum, 36.2 mm long by 14.6 mm wide in the center of the slide.

www.nanostring.com

NanoString GeoMx

Whole Transcriptome Atlas, Cancer Panel and Validated Protein Assays



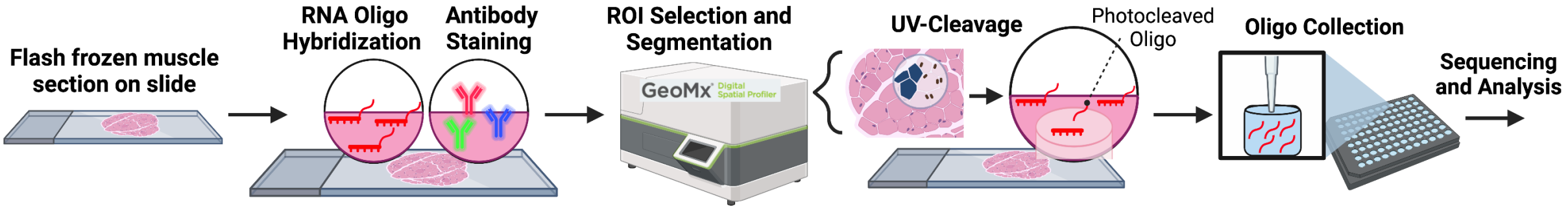


NanoString's digital spatial profiling technology enables analysis of both protein and RNA expression of FFPE samples.

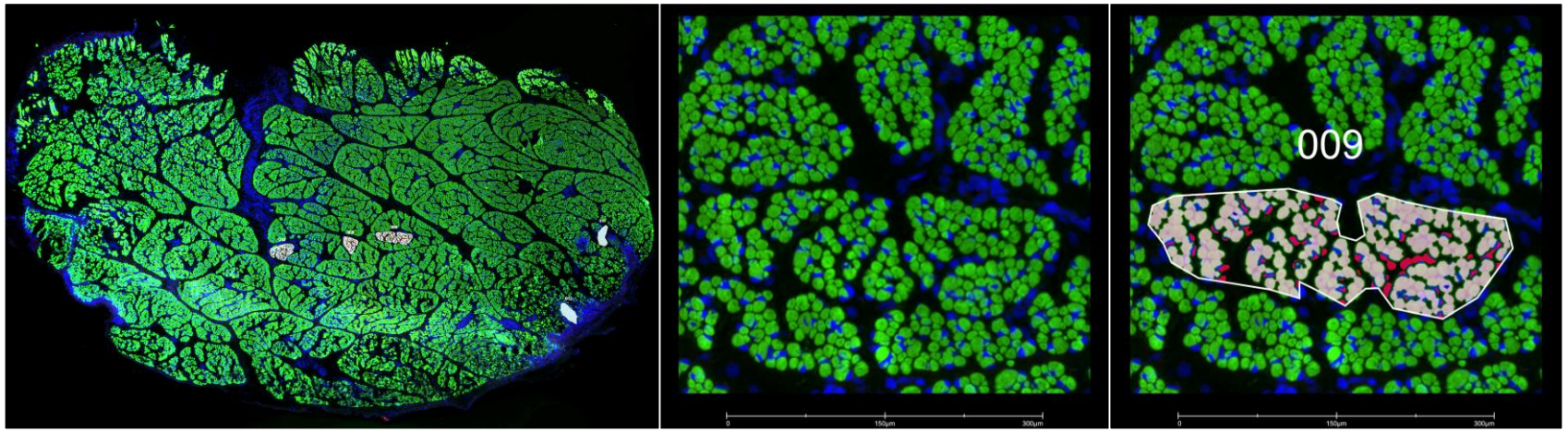
www.nanostring.com

GeoMx Workflow

High-plex analysis of precious and limited samples as it is non-destructive (the sample may be reanalyzed with alternative approaches such as whole exome sequencing (WES), RNAscope®, immunohistochemistry, etc.)



1. Image entire tissue
2. Zoom into area of interest
3. Apply a segmentation



Utilization of Nanostring GeoMX for segmentation and Spatial RNA-seq of human cell engrafted into mouse skeletal muscle

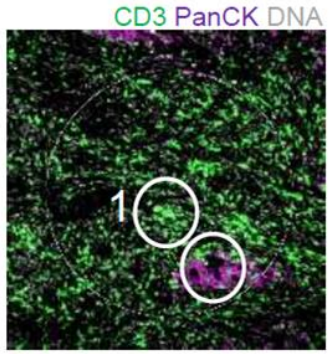
Michael Hicks Lab

ROI Selection

- The minimum ROI size tested at NanoString is a 32µm diameter circle with 93% of the Human IO and 81% of the Mouse IO panels being detected above background.
- Maximum ROI size is 660 µm x 785 µm

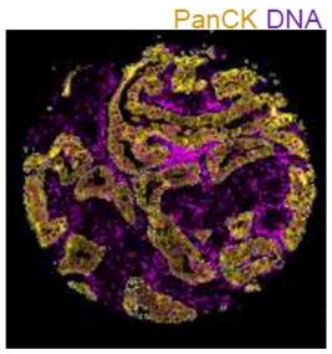
GeoMx: ROI Selection

Geometric



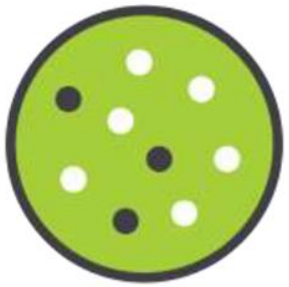
What is the heterogeneity of expression in different regions of my tissue?

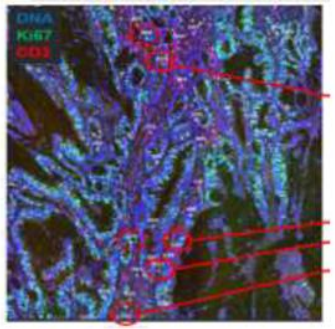
Segmentation



What is the expression profile of distinct biological compartments (e.g., Tumor-TME)?

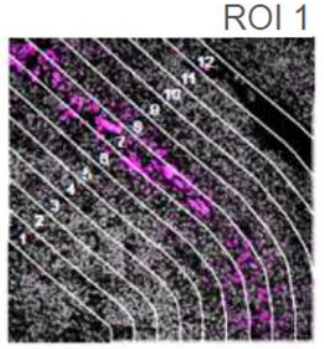
Cell Type Specific





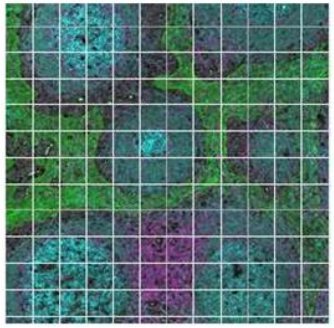
What is the expression profile of a specific cell population in my tissue?

Contour



How does the immune environment change on either side of an infiltrate boundary?

Gridded



What novel targets are uncovered with deep mapping of a specific tissue region?

Shared access

Vizgen MERFISH (Multiplexed Error-Robust Fluorescence in situ Hybridization)

Localize RNA transcripts at a subcellular level with ≤100 nm resolution.

High multiplexing with custom gene panel design. Current chemistry up to 500 genes.

MERSCOPE workflow: the sample is sliced, loaded onto a MERSCOPE slide, stained with the custom gene panel, embedded into a polyacrylamide to digest away the proteins to reduce the auto fluorescent background, and imaged.

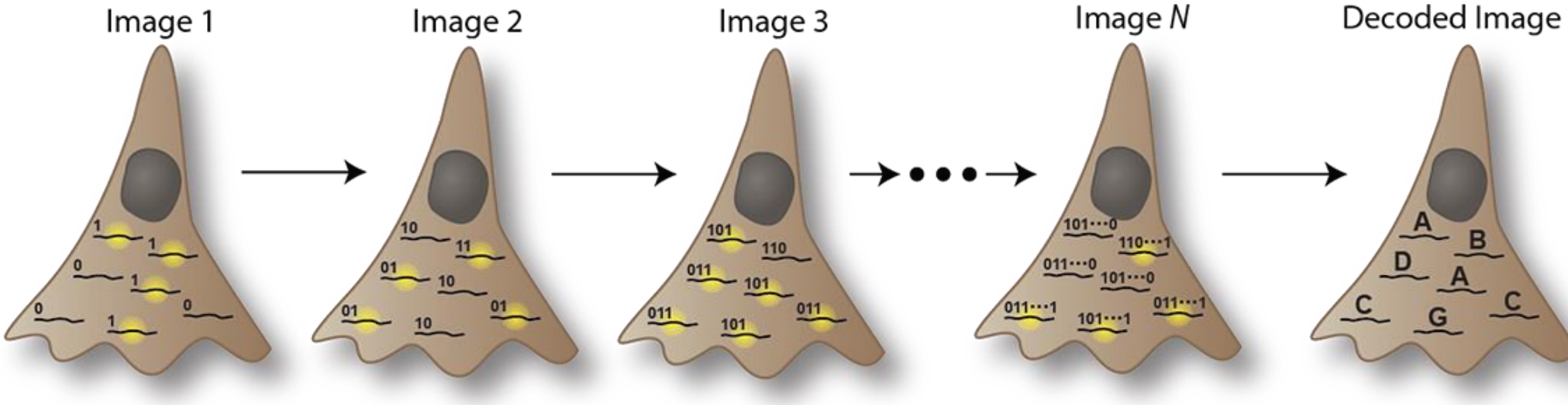
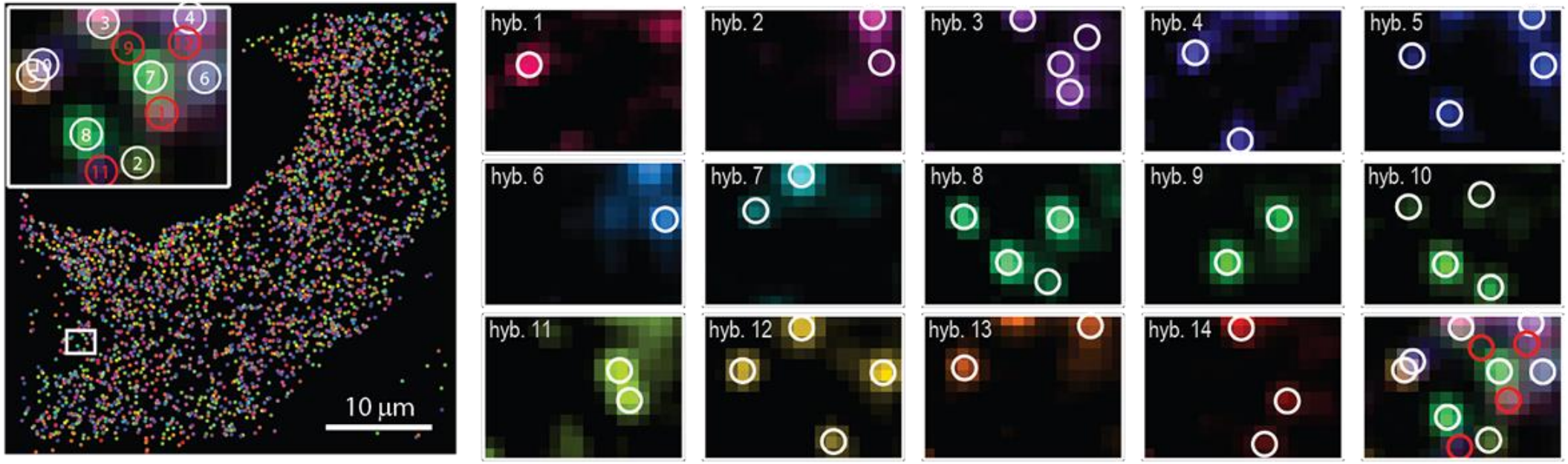


Image 1
Image 2
Image 3
Image N
Decoded Image
A
B
D
A
C
G
C


10 µm
hyb. 1
hyb. 2
hyb. 3
hyb. 4
hyb. 5
hyb. 6
hyb. 7
hyb. 8
hyb. 9
hyb. 10
hyb. 11
hyb. 12
hyb. 13
hyb. 14

	Sample Type & Area	Spatial Resolution	Capture Technology	Protocol	Reagent Cost	IHC/Protein Detection
GeoMx	FFPE, FF 32.2mm x 14.6mm 5um section	Custom- lowest 10um recommended 50um-400um	Targeted or Probe based whole transcriptome	Easiest; 12 slides/day	$1.5k-$1.8k CTA (1.8k genes) $2.6K WTA (18k genes) per slide	IF – High plex protein detection by barcode; separate run from RNA
Visium FFPE	FFPE 6.5mm x 6.5mm 5um section	55um; 50um space between	Probe based whole transcriptome	Check RNA QC, Tissue Adhesion	~$1.6k per tissue slice 5k spots	H&E or IF
Visium FF	FF 6.5mm x 6.5mm 10um-30um section	55um, 50um space between	Poly(A) capture whole transcriptome	Requires Tissue Optimization	~$1.2k per tissue slice 5k spots	H&E or IF
Merfish	Cultured Cells, F, FF, FFPE (10um section) 20cm gel coverslip	<100nm Single molecule; up to 10 ROI for total of 100mm^2	Combinatorial labeling	Up to 500 genes; 3days sample prep	$3.75k per coverslip	
CosMx	FFPE; Fresh Frozen FOV 0.9 x 0.7 mm	50nm smallest spot size; 3 D resolution	Probe based; 5 probes per transcript	1cm^2 4 slides/week 16mm^2 4 slides/day	100 plex - $1500 1cm^2 1000 plex - $3000 1cm^2	IF – 100 plex protein detection by barcode; separate run from RNA

Challenges for Spatial Transcriptomics

The need for both subcellular detail and tissue-wide data presents a major challenge for the field.

Spatial transcriptomic technologies must maximize on a few key capabilities:

- the size of the tissue area that can be analyzed per experiment
- the number of genes that can be assessed per experiment
- the detection efficiency
- the spatial resolution of the image.

So far, however, most spatial transcriptomics solutions have been forced to compromise the quality of one or more of these capabilities for the sake of the others.