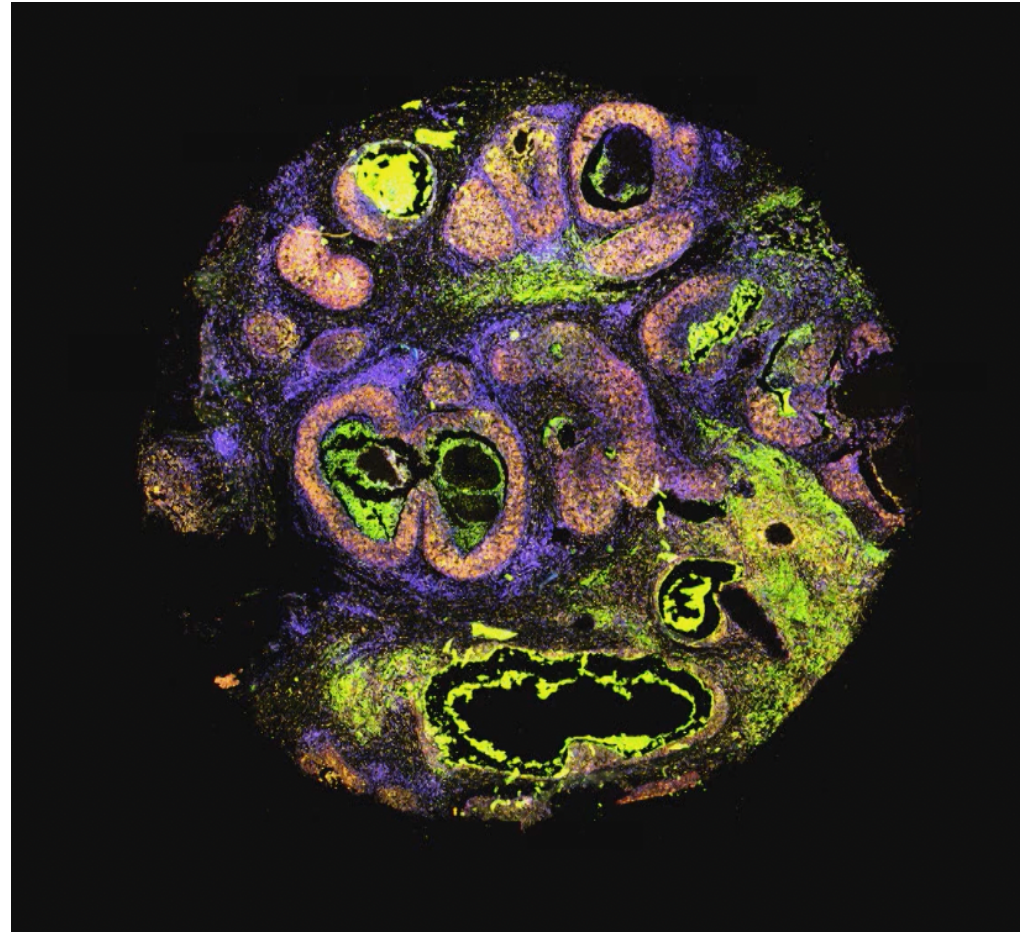
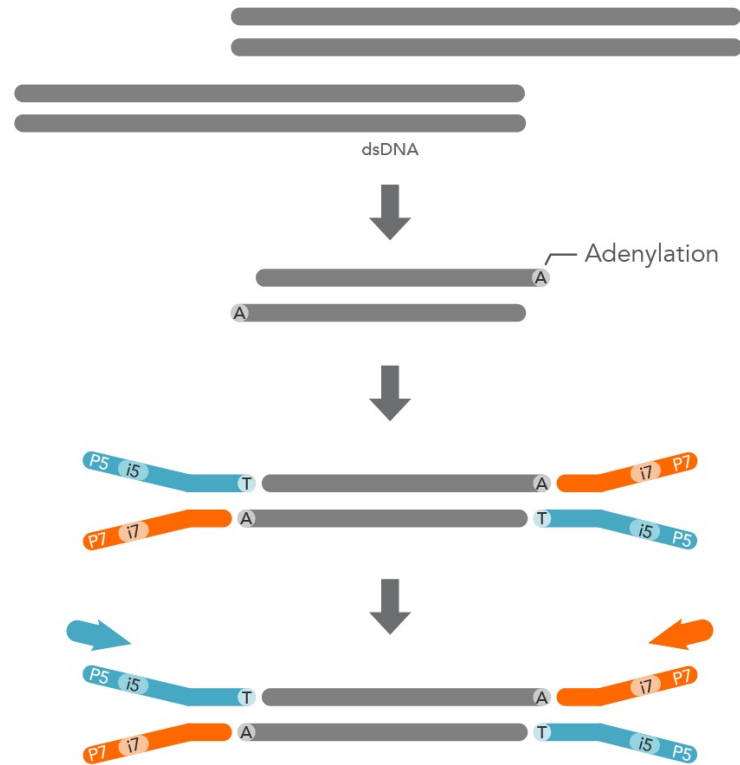
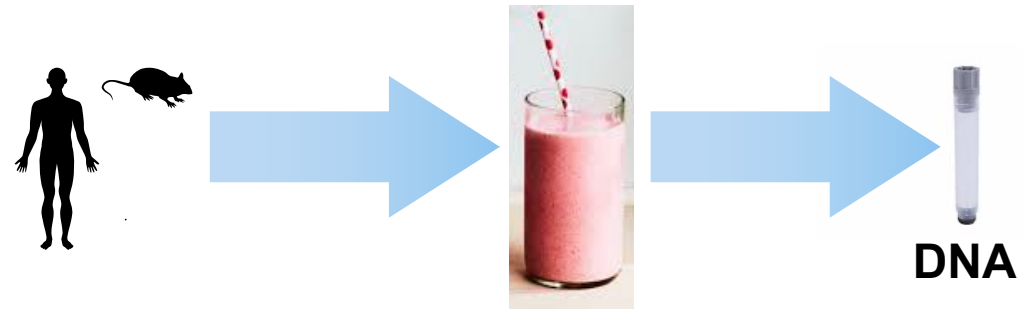


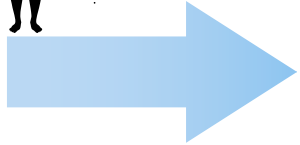
The next genomic frontiers



Neeman Mohibullah
Integrated Genomics Operation

Generic overview of Illumina library creation





Bulk



Genome



Epigenome



Transcriptome



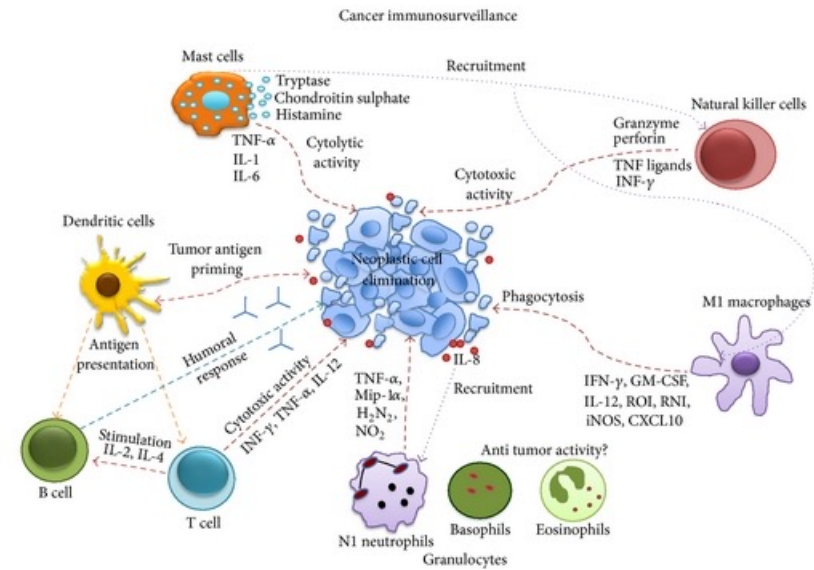
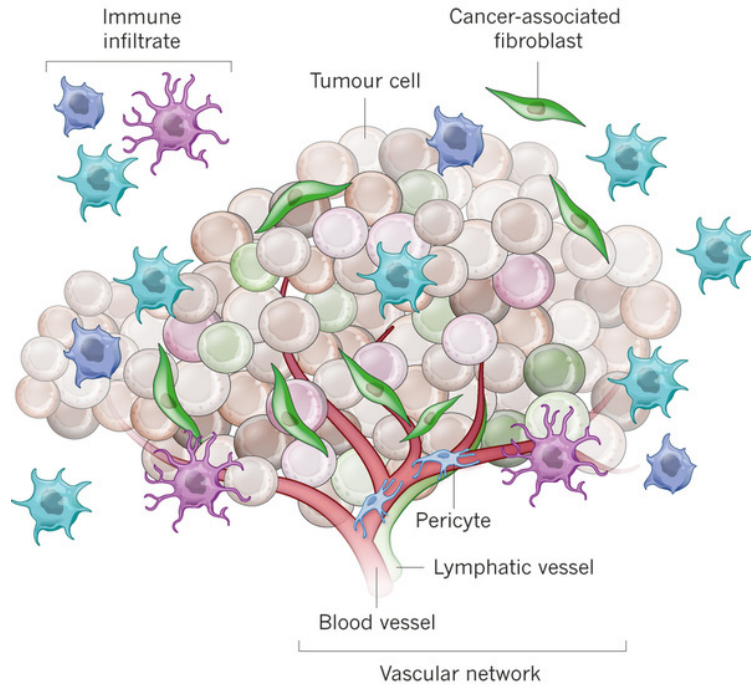
Proteome



Metabolome



Because tissues (and tumors) are very heterogeneous



Global RNAseq tells a very different story than single cell RNAseq

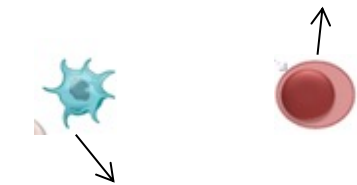


Gene	Expression level
Gene 1	10,000
Gene 2	1,000
Gene 3	5,000
Gene 4	200*
Gene 5	0

*:limit of detection

Gene	Expression level
Gene 1	0
Gene 2	2000
Gene 3	1000
Gene 4	800
Gene 5	0

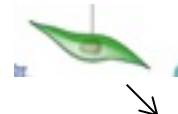
Gene	Expression level
Gene 1	0
Gene 2	2000
Gene 3	1000
Gene 4	0
Gene 5	0



Gene	Expression level
Gene 1	60,000
Gene 2	0
Gene 3	1000
Gene 4	200
Gene 5	0

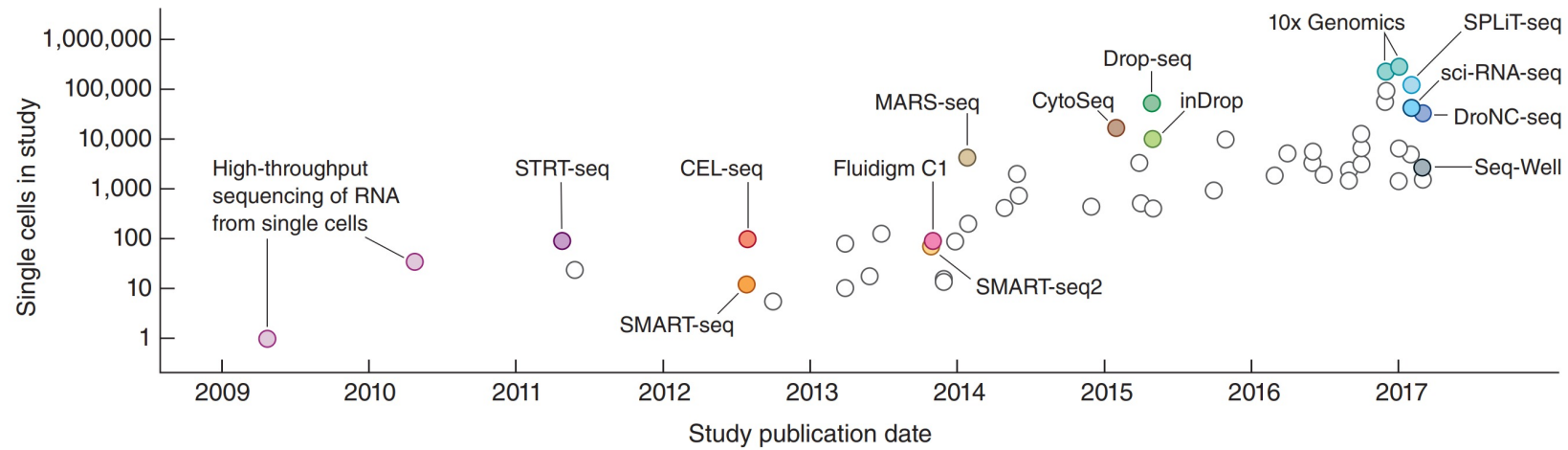


Gene	Expression level
Gene 1	0
Gene 2	1000
Gene 3	1000
Gene 4	0
Gene 5	250



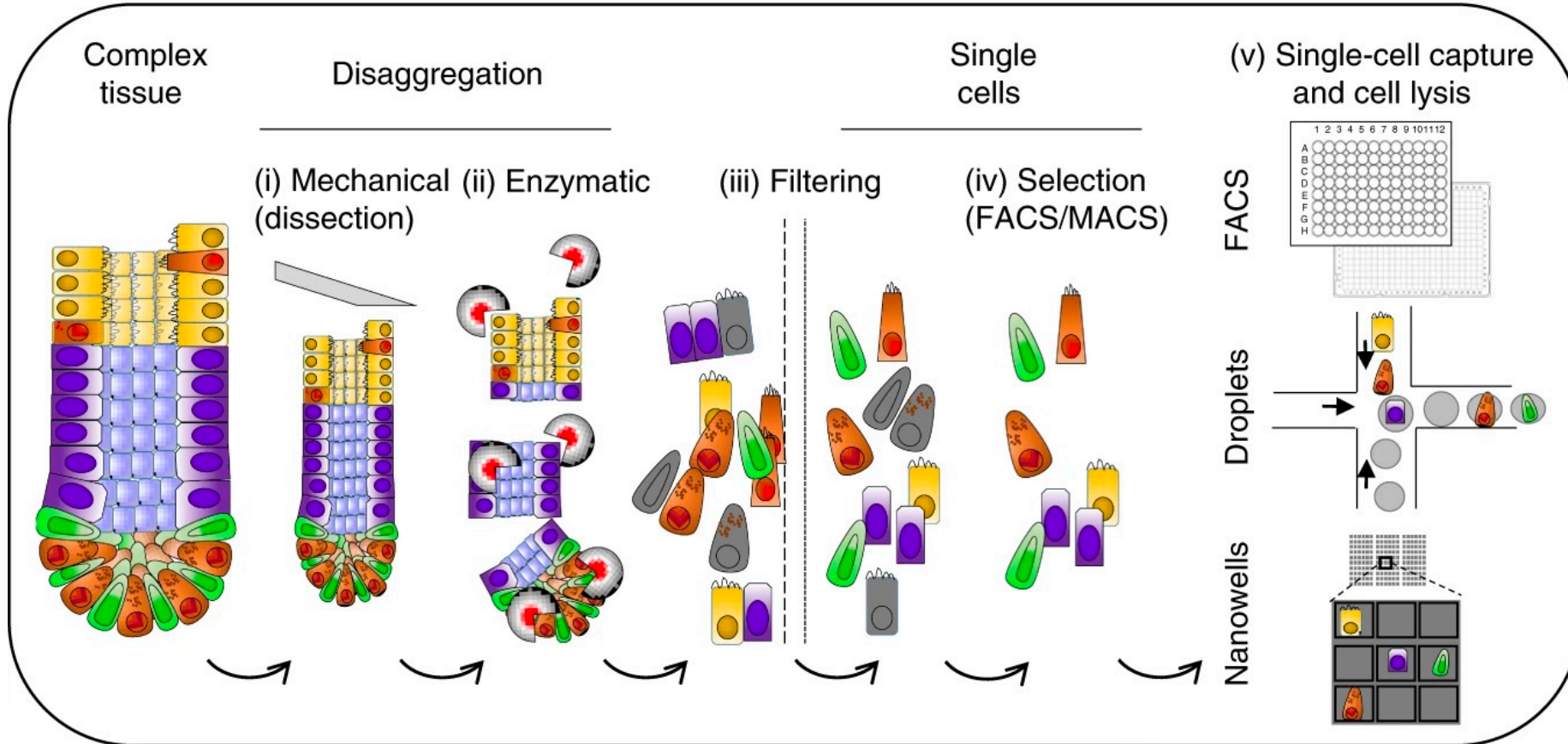
Gene	Expression level
Gene 1	0
Gene 2	0
Gene 3	21 000
Gene 4	0
Gene 5	0

Single cell RNA seq timeline



Single cell wet lab workflow

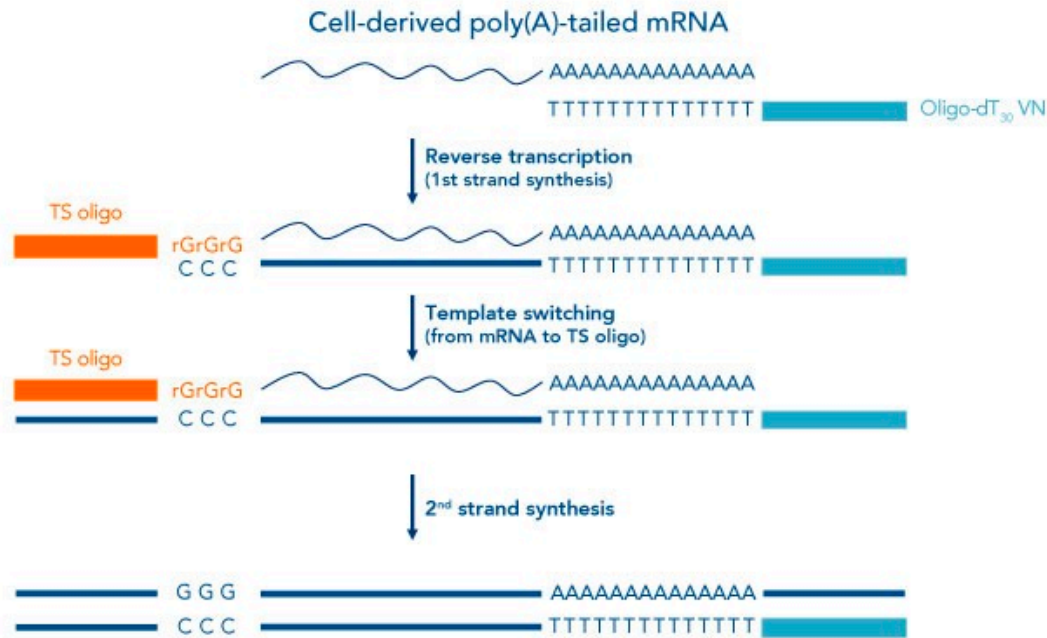
(1) Sample preparation



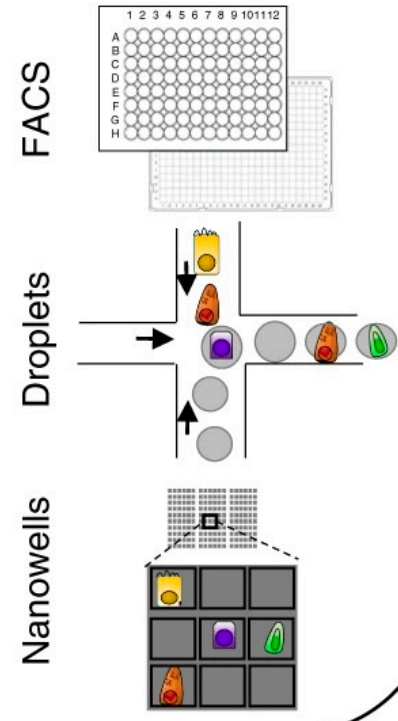
What could go wrong?

Lafzi et al Nature Prot 2018

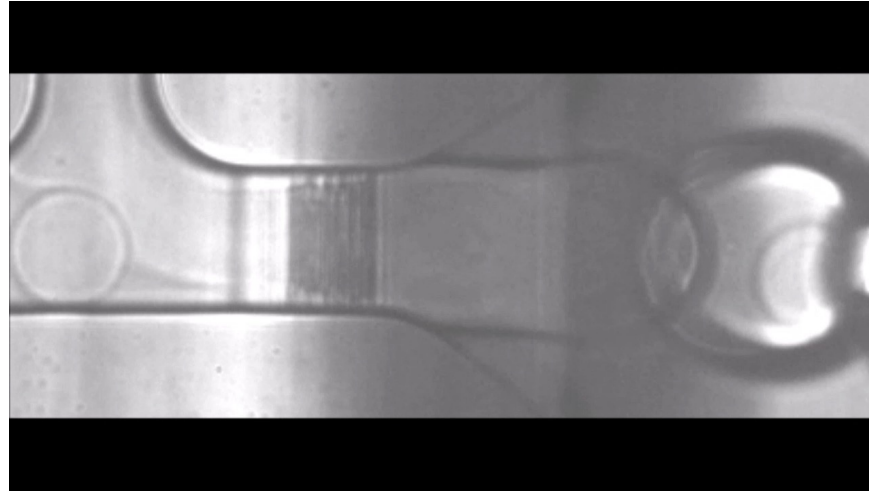
Template-switching



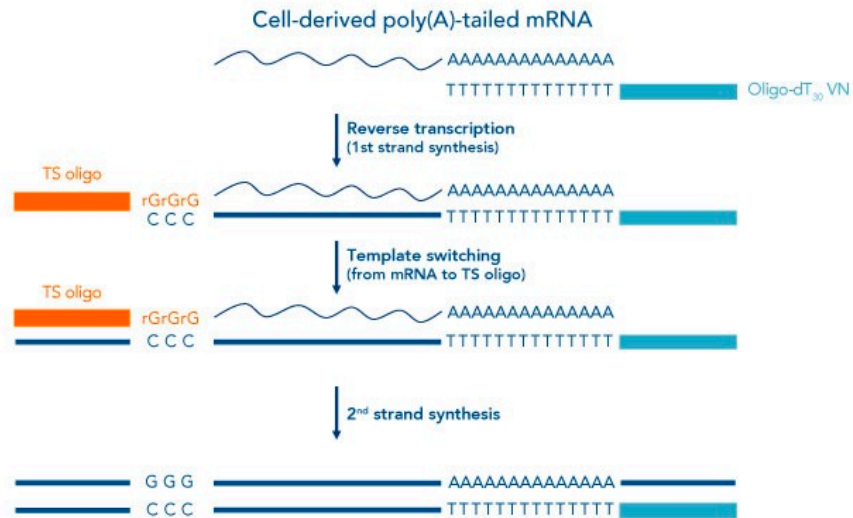
(v) Single-cell capture
and cell lysis



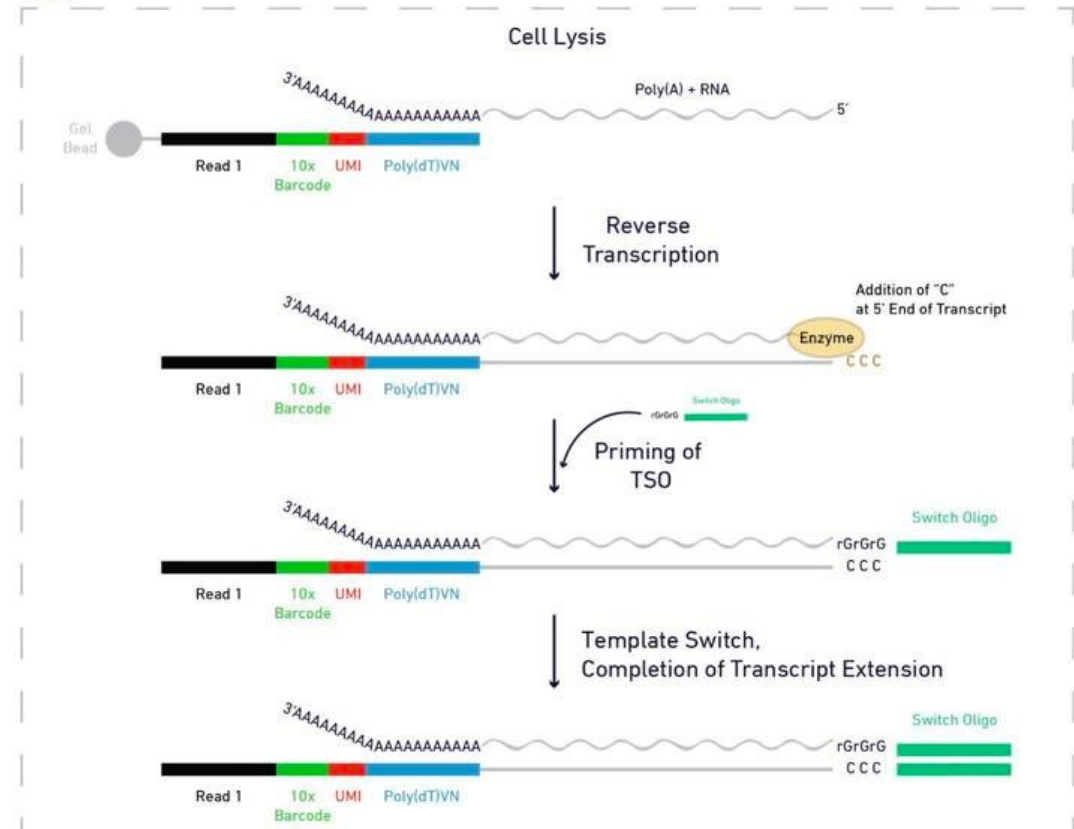
10X genomics



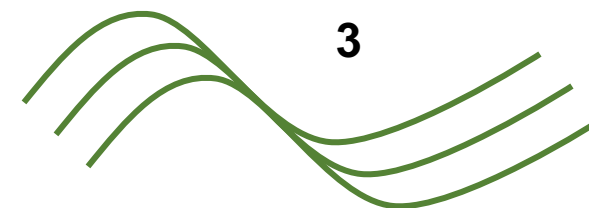
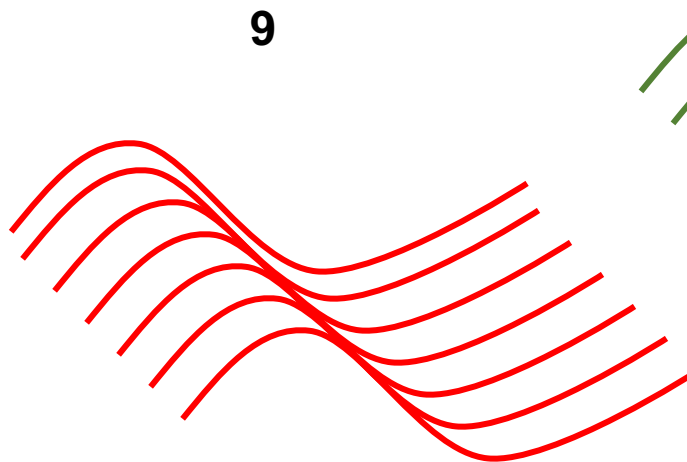
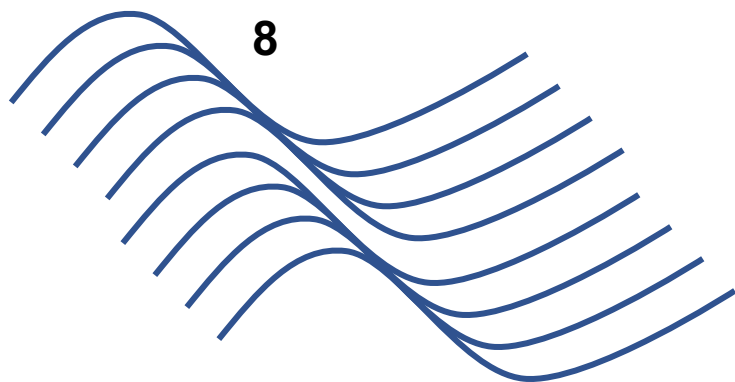
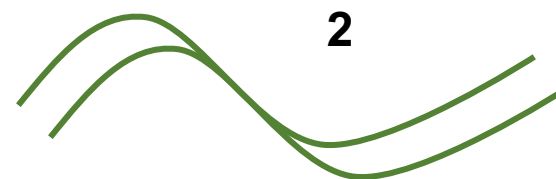
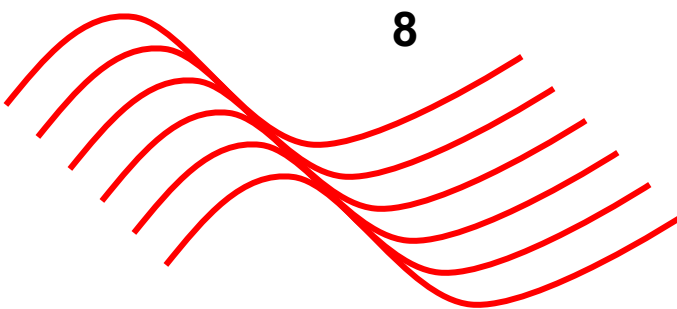
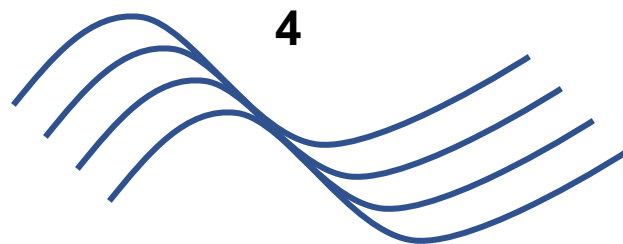
Template-switching with cell barcode



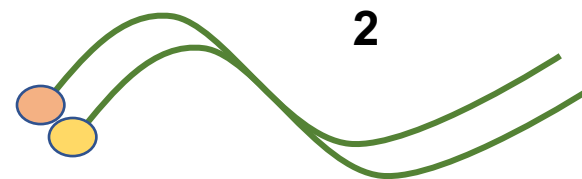
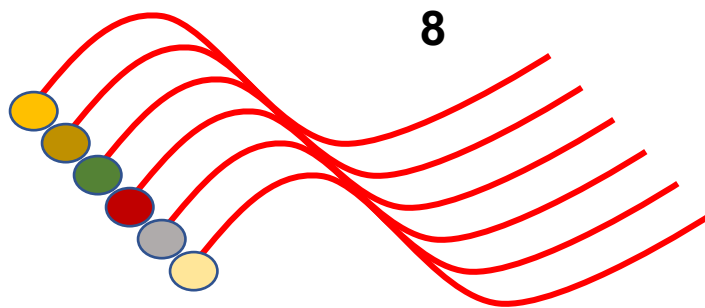
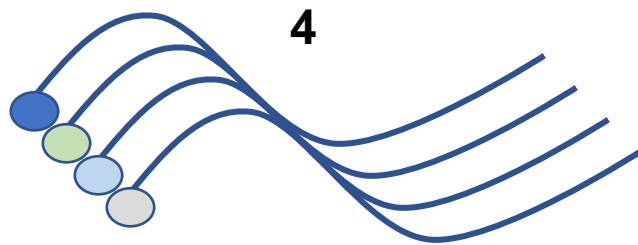
GEMs



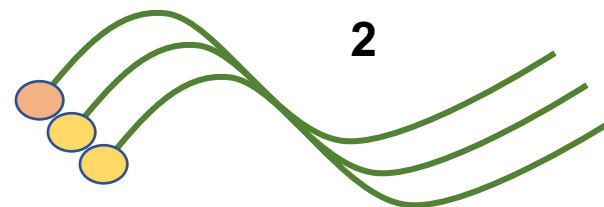
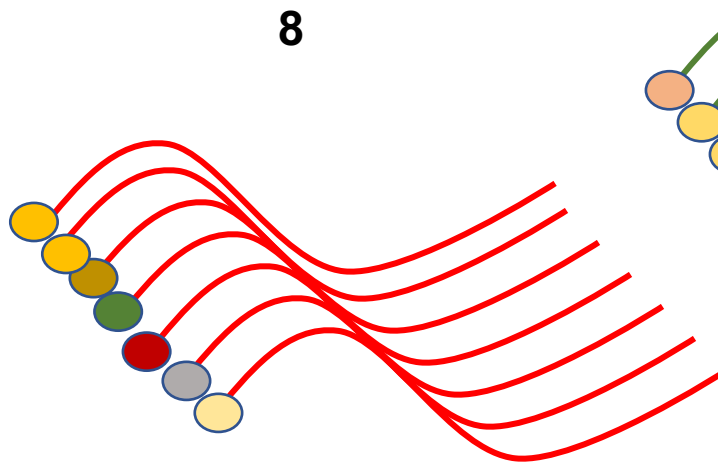
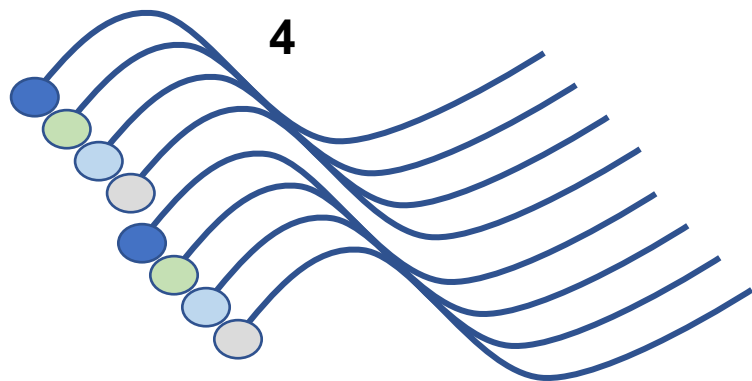
Unique Molecular Identifier



Unique Molecular Identifier

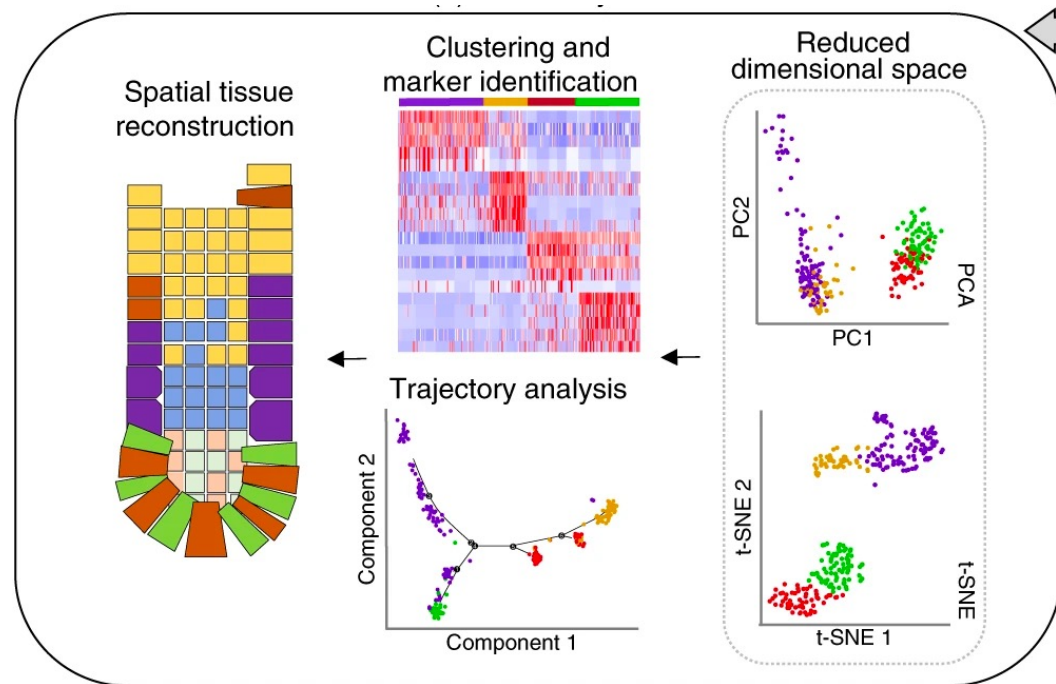
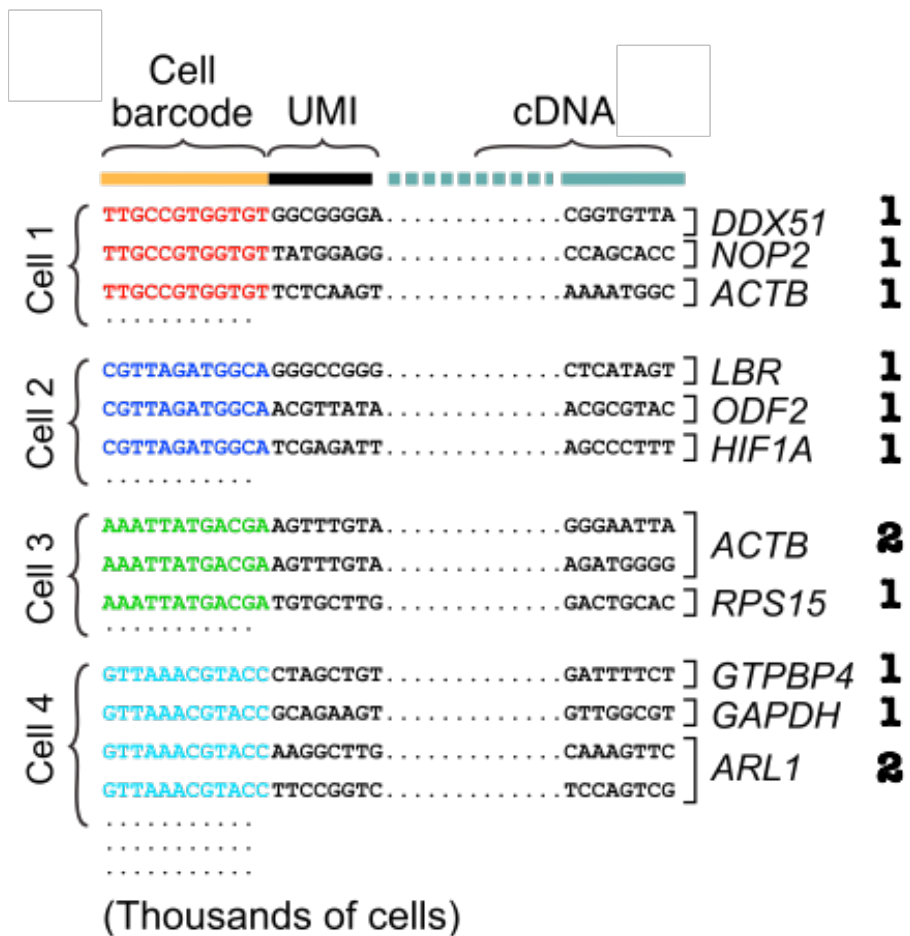


PCR

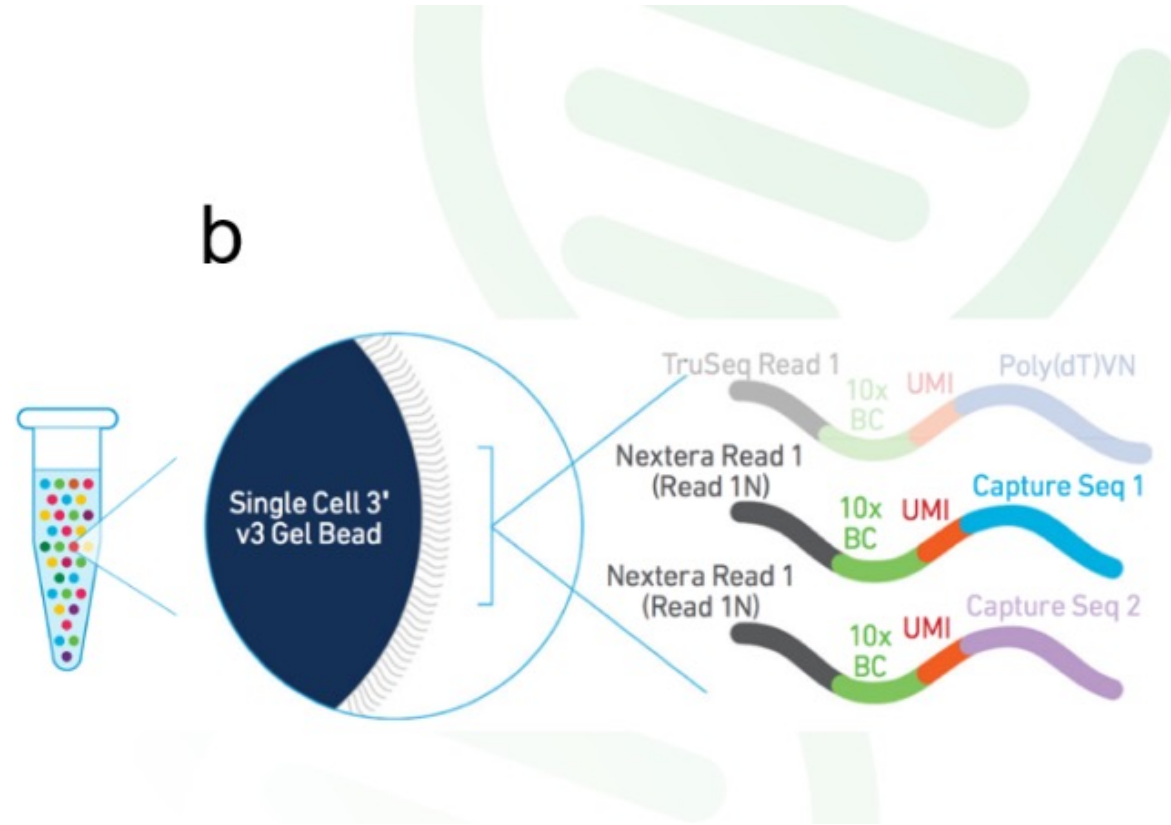


counting UMIs instead of transcripts

Pipeline to analyze data



Using the same chemistry to perform proteogenomics





SPATIAL GENE EXPRESSION

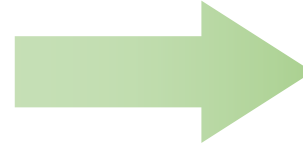
Visualize Gene Expression within Tissue Organization



Bulk



Spatial



Genome



Epigenome



Transcriptome



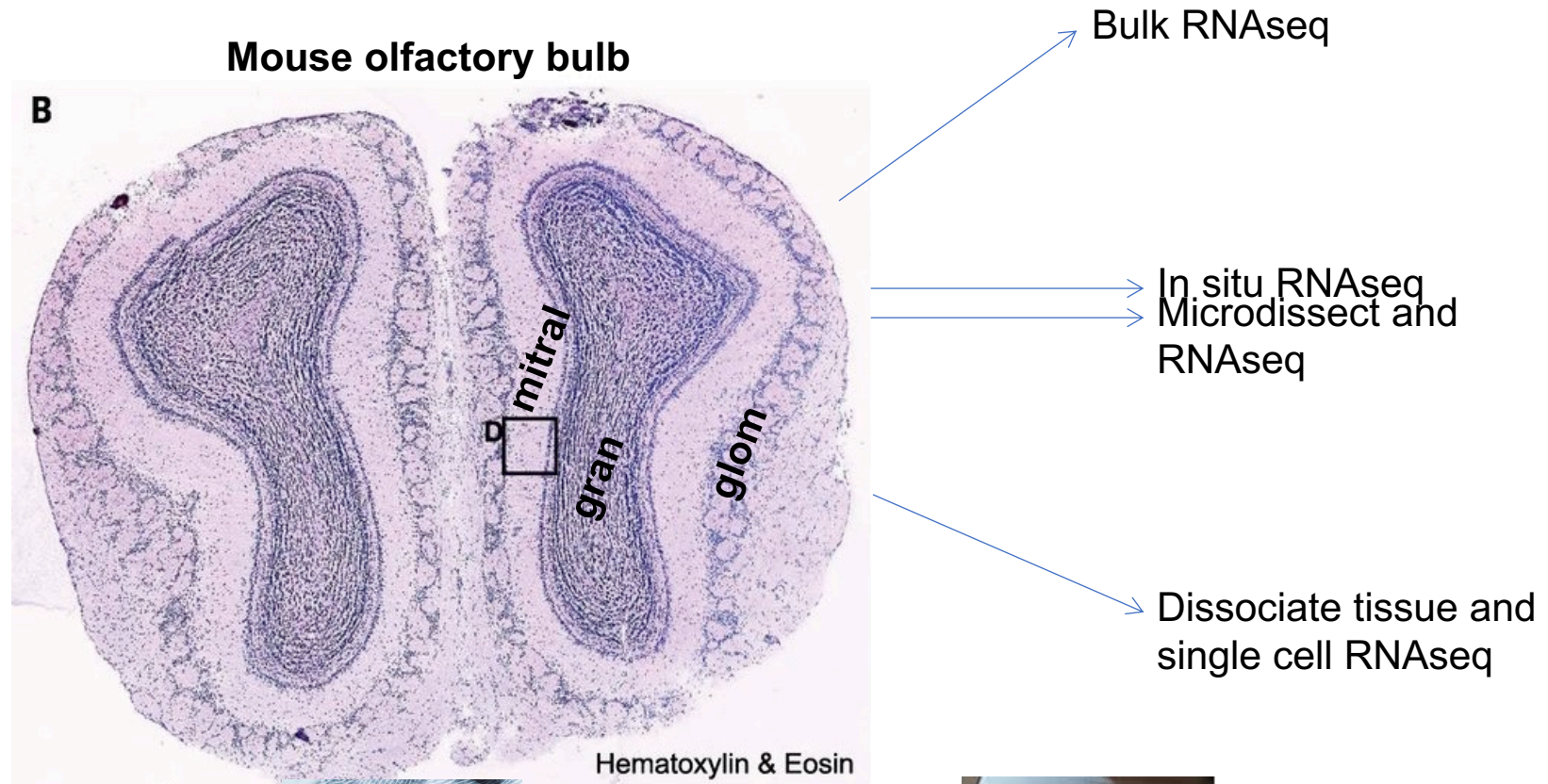
Proteome



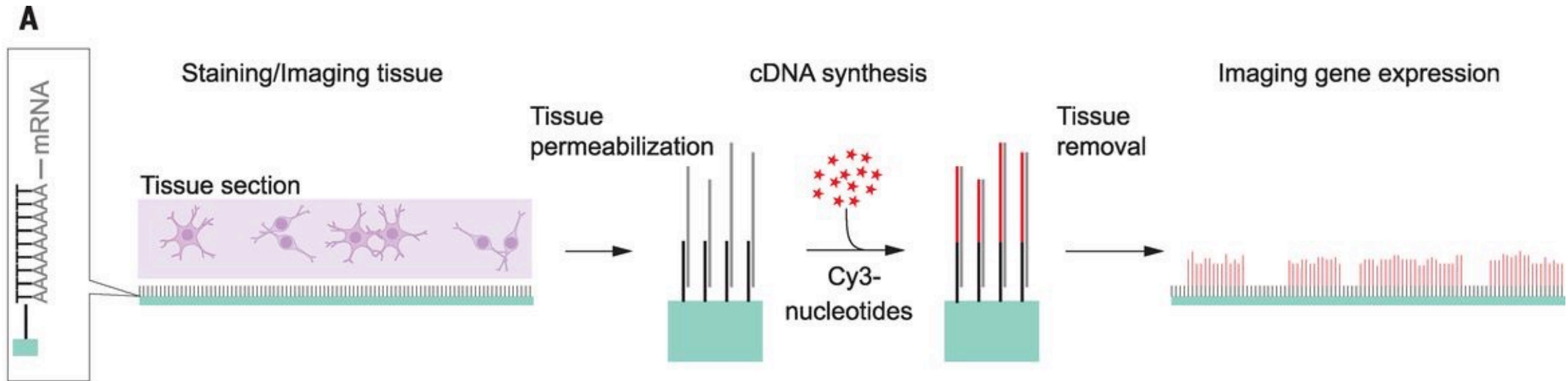
Metabolome

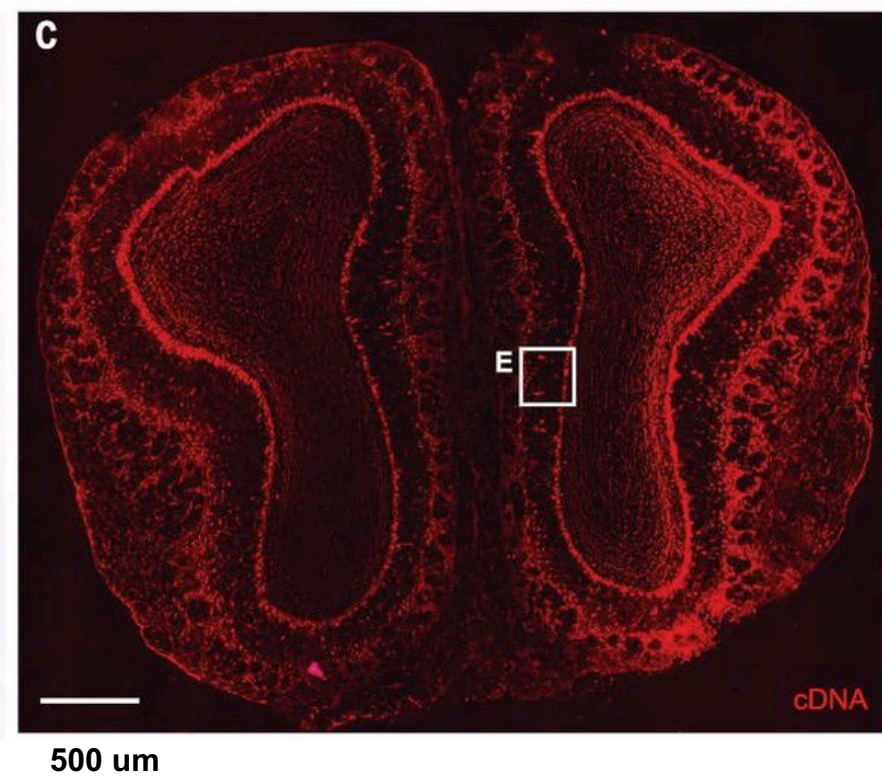


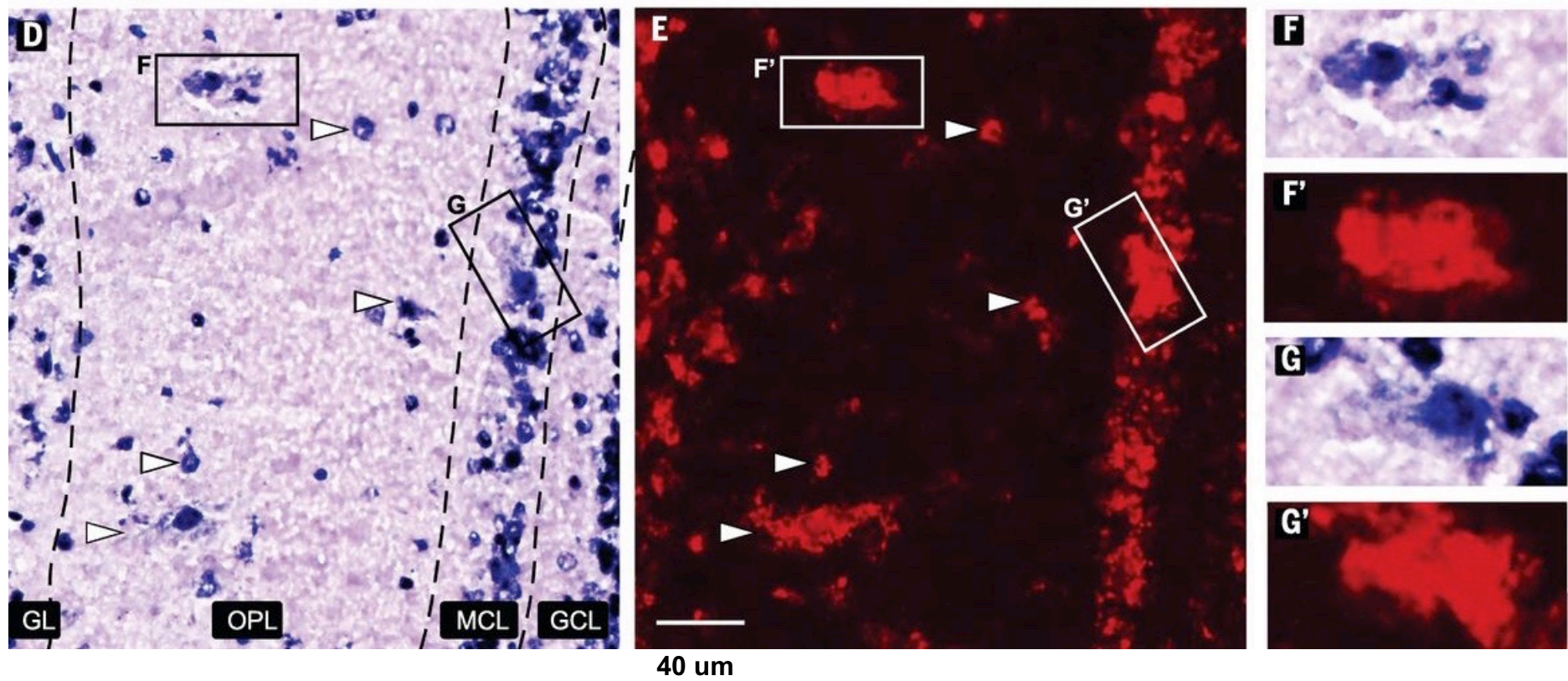
Spatial transcriptomics

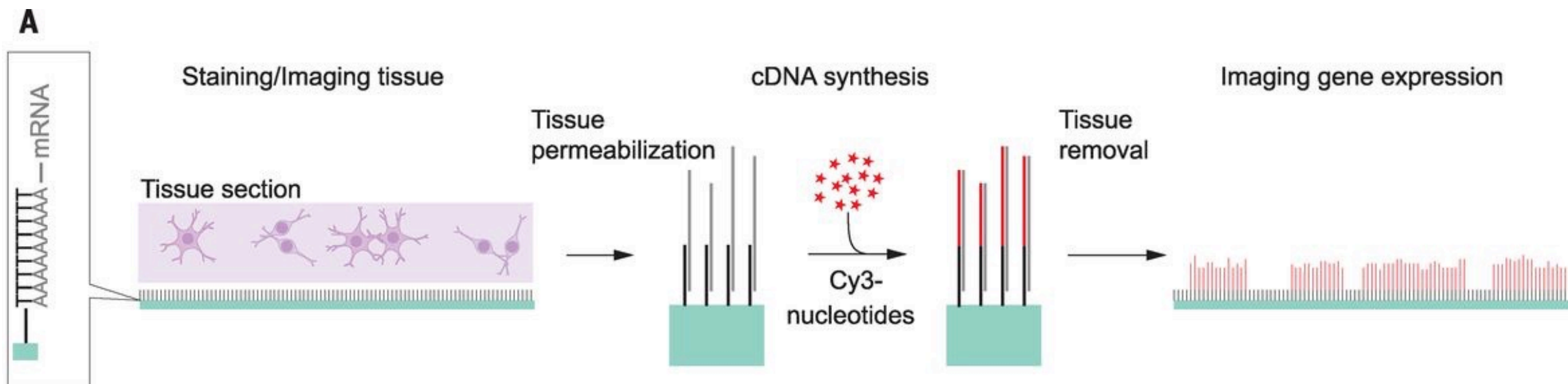


Spatial transcriptomics workflow

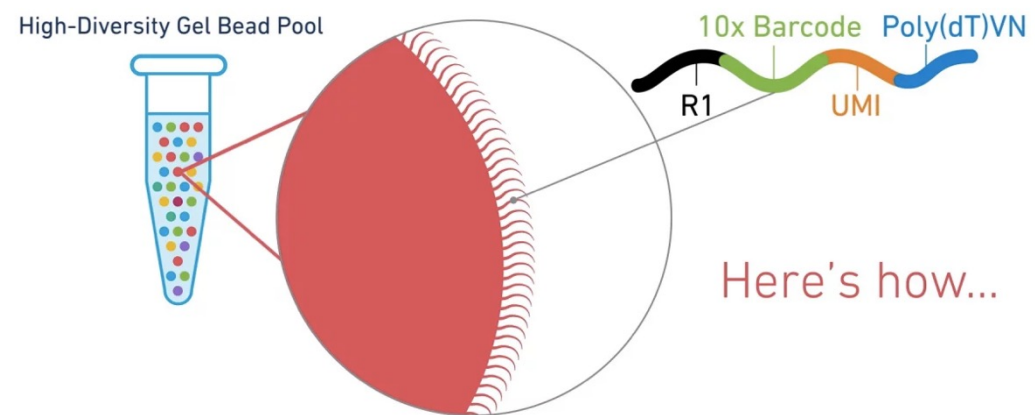
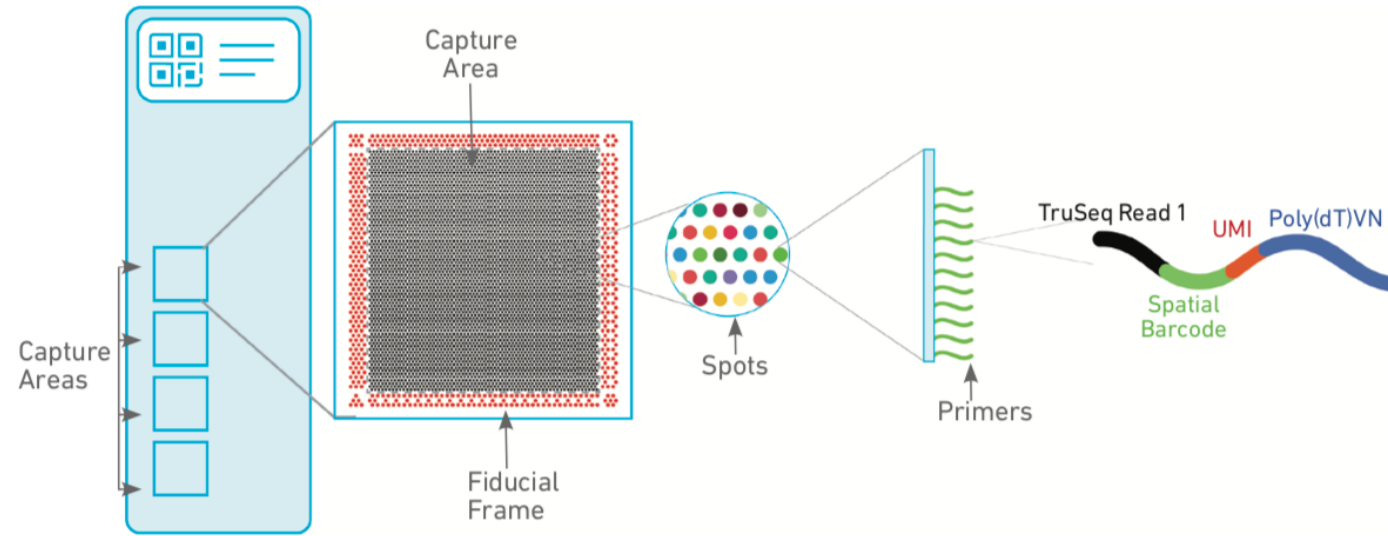




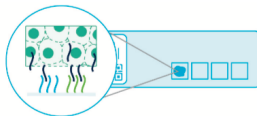




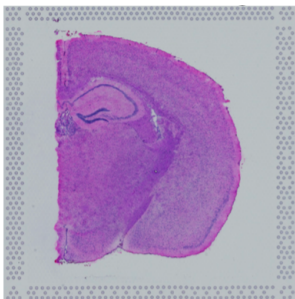
10x genomics Visium



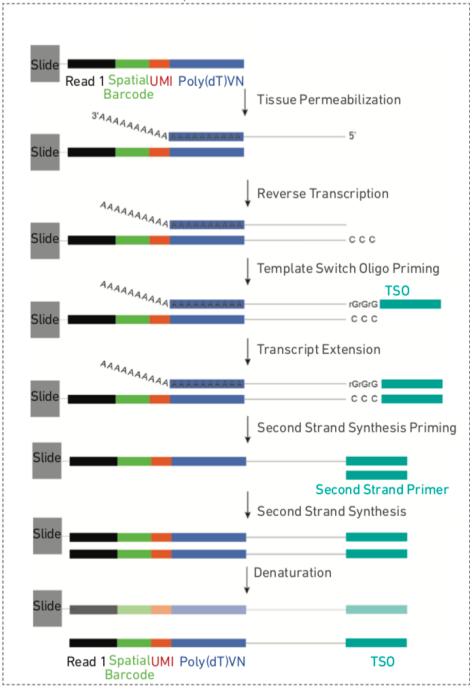
Permeabilize and capture H&E stain



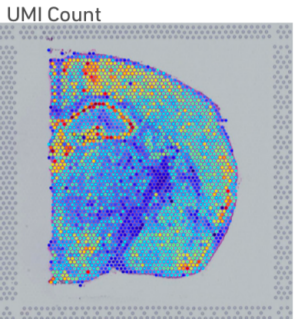
Place tissue on slide H&E stain



Reactions on slide Capture Areas



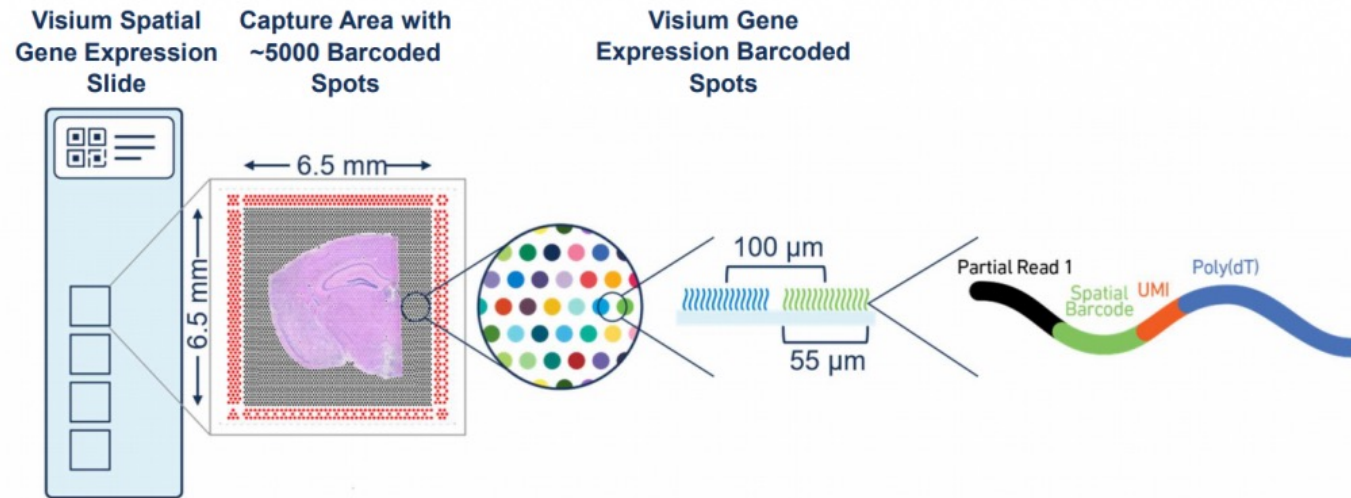
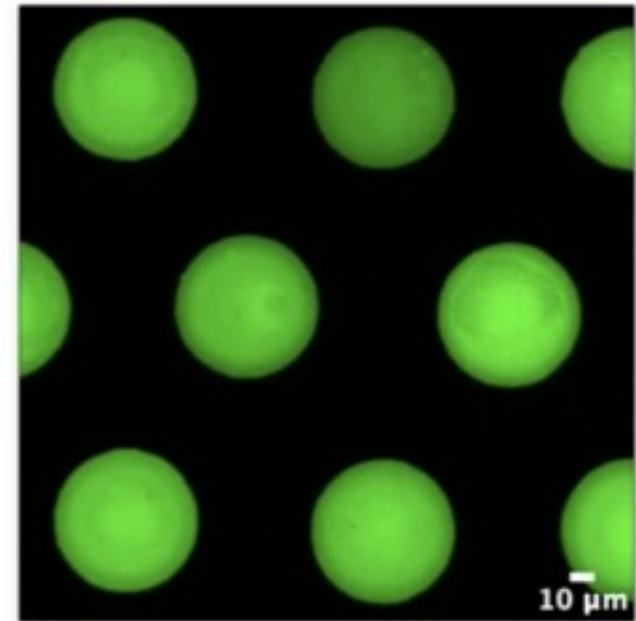
Prepare libraries Sequence



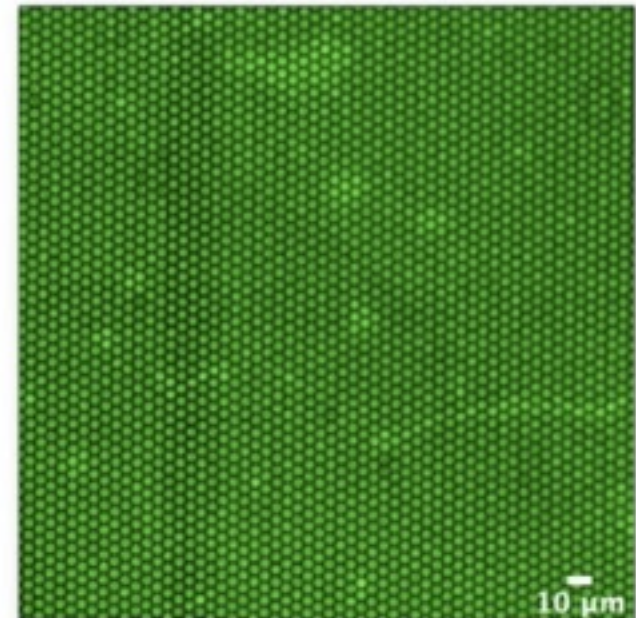
Cell barcode	UMI	RNA
ACAGTATAAAGACT	GGGCCCCG
TGACAAATAAAGACT	TCTAGCTG
CGTTAGGTTACGTC	GATTATAG
TGACAAATTACGTC	ACAATGCT
GTTAGCTGATGCCG	CTTTGCAT
GTTAGCTGATGCCG	TCTCGACT
CGTTAGTGTGCGG	CCTCGAGC
ACAGTAGTTACGTC	GTCACATC
TGACAAATGATGCCG	GTCACATC
ACAGTATGATGCCG	TCGACGAT
GTTAGCTAAAGACT	ACATGCTG
CGTTAGGTTACGTC	TAGCCAGT

Visium HD | Resolution

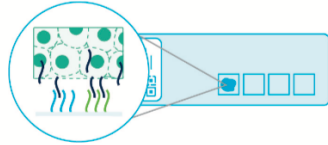
Visium



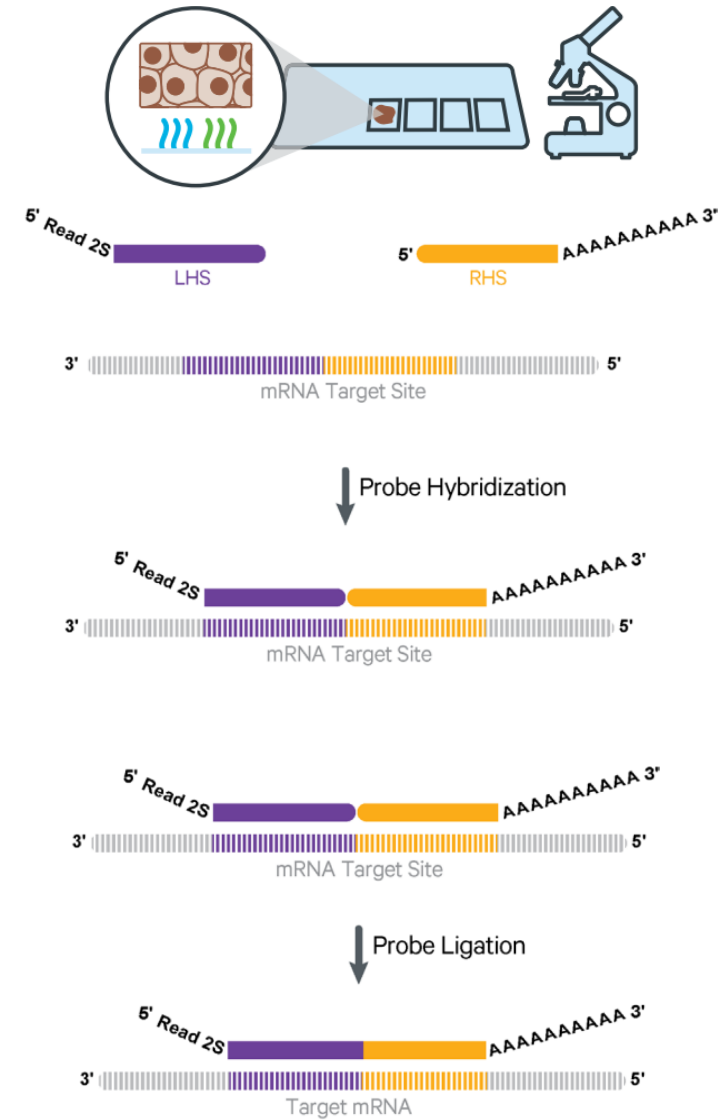
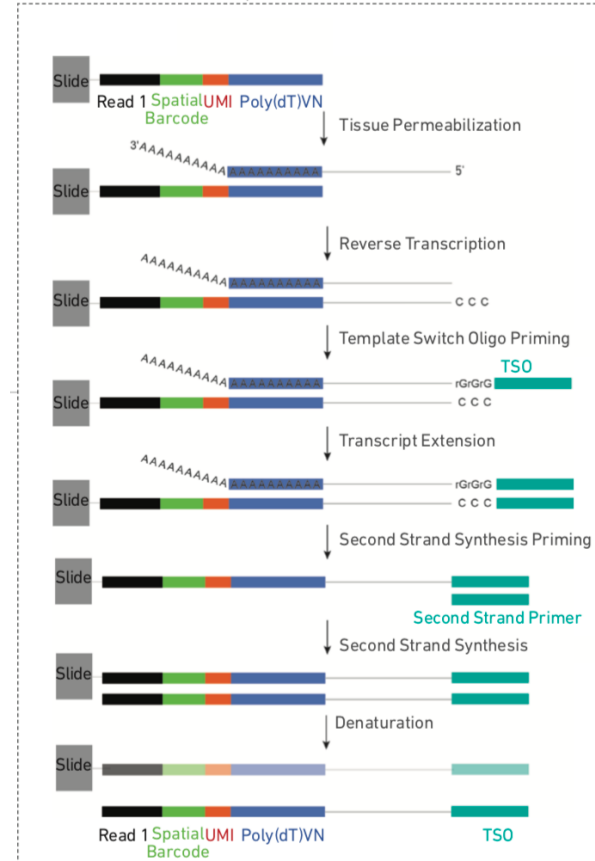
Visium HD



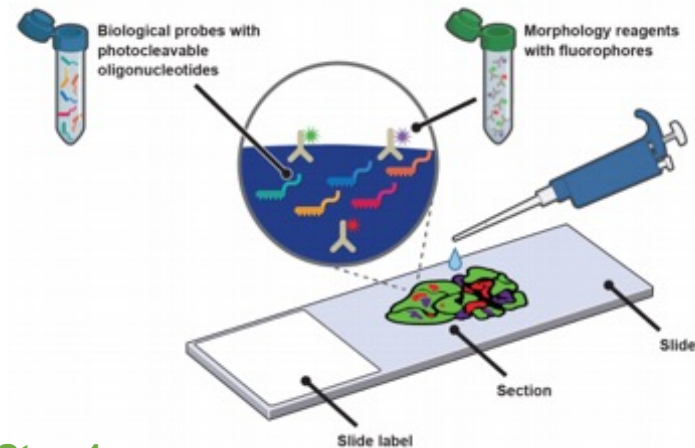
How to make this compatible for FFPE?



Reactions on slide Capture Areas



Nanostring GeoMx



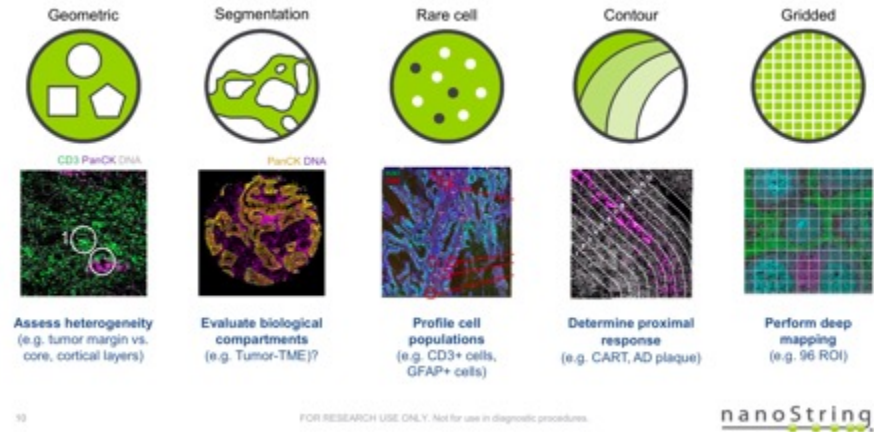
Step 1

IHC+ISH on the same slide

- Panel of oligos (for RNA detection)
- Fluorescent Antibodies

PROS:

Easy selection of ROI
Cheaper Sequencing



Step 2

Regions of Interest (ROI) selection

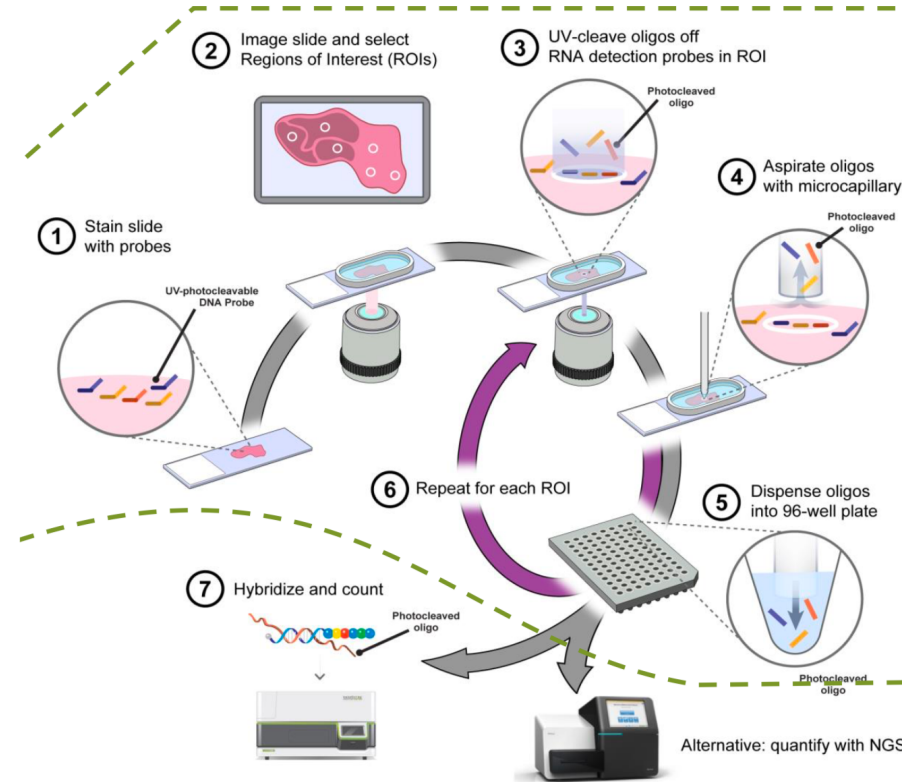
CONS:

Capital equipment purchase
Expensive (\$140/ROI); ROI 10-700um
Targeted Panels (Whole txome)
Software in early phases

Nanostring GeoMx

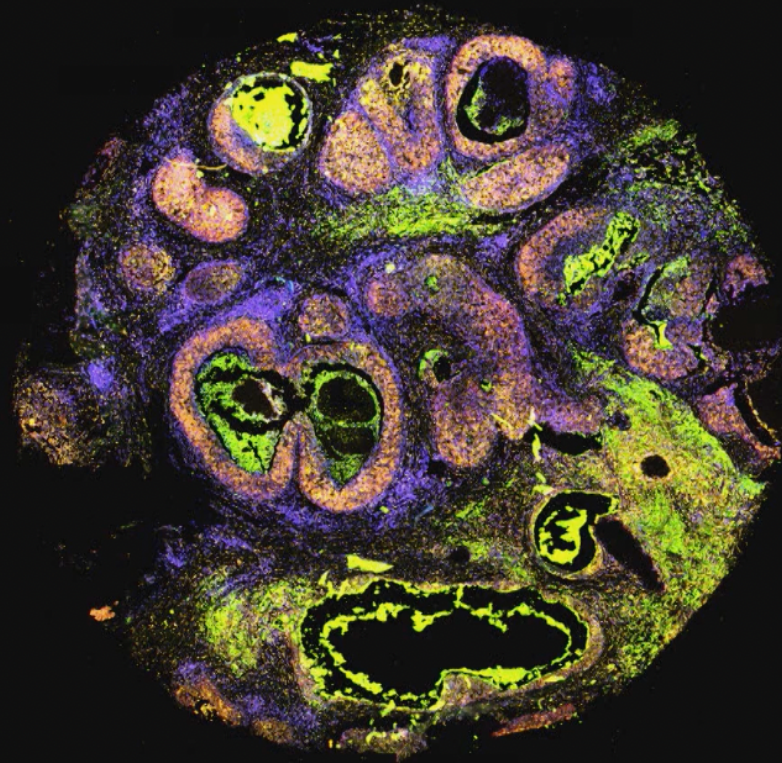


GeoMx Digital Spatial Profiler
Your GPS for Immuno-Oncology



Where do we go from here?

**Xenium
In Situ**



Where do we go from here?

From Discovery to Focused with Complementary Workflows

Discovery

Focused

Chromium
Single Cell

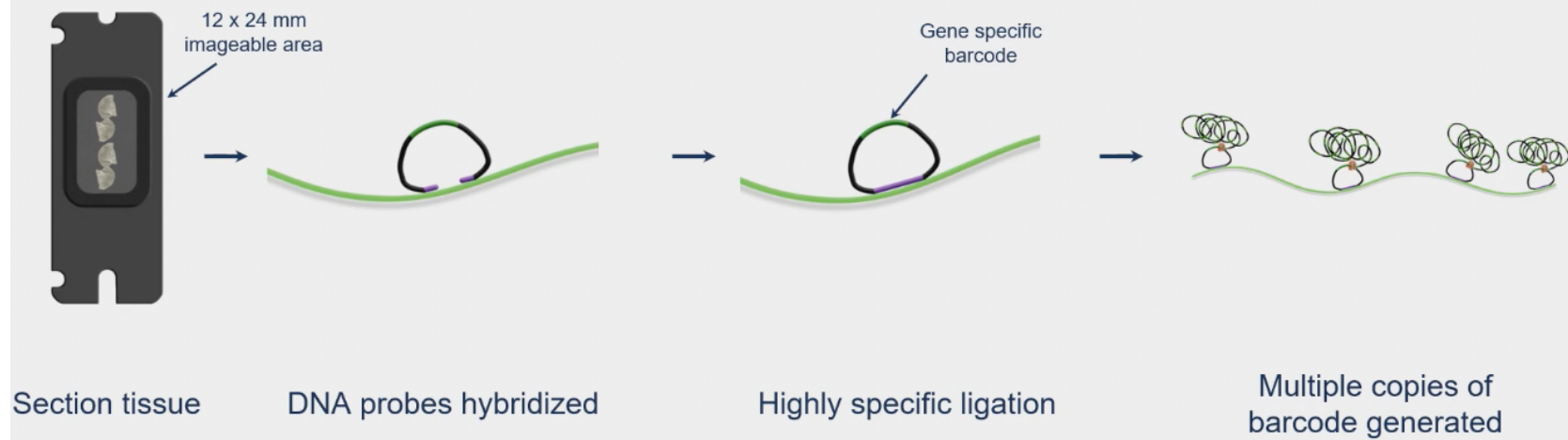
- Entire transcriptome measurement

Visium Spatial

Xenium
In Situ

- Targeted measurement
- Subcellular resolution
- High sensitivity
- High throughput

Xenium Workflow

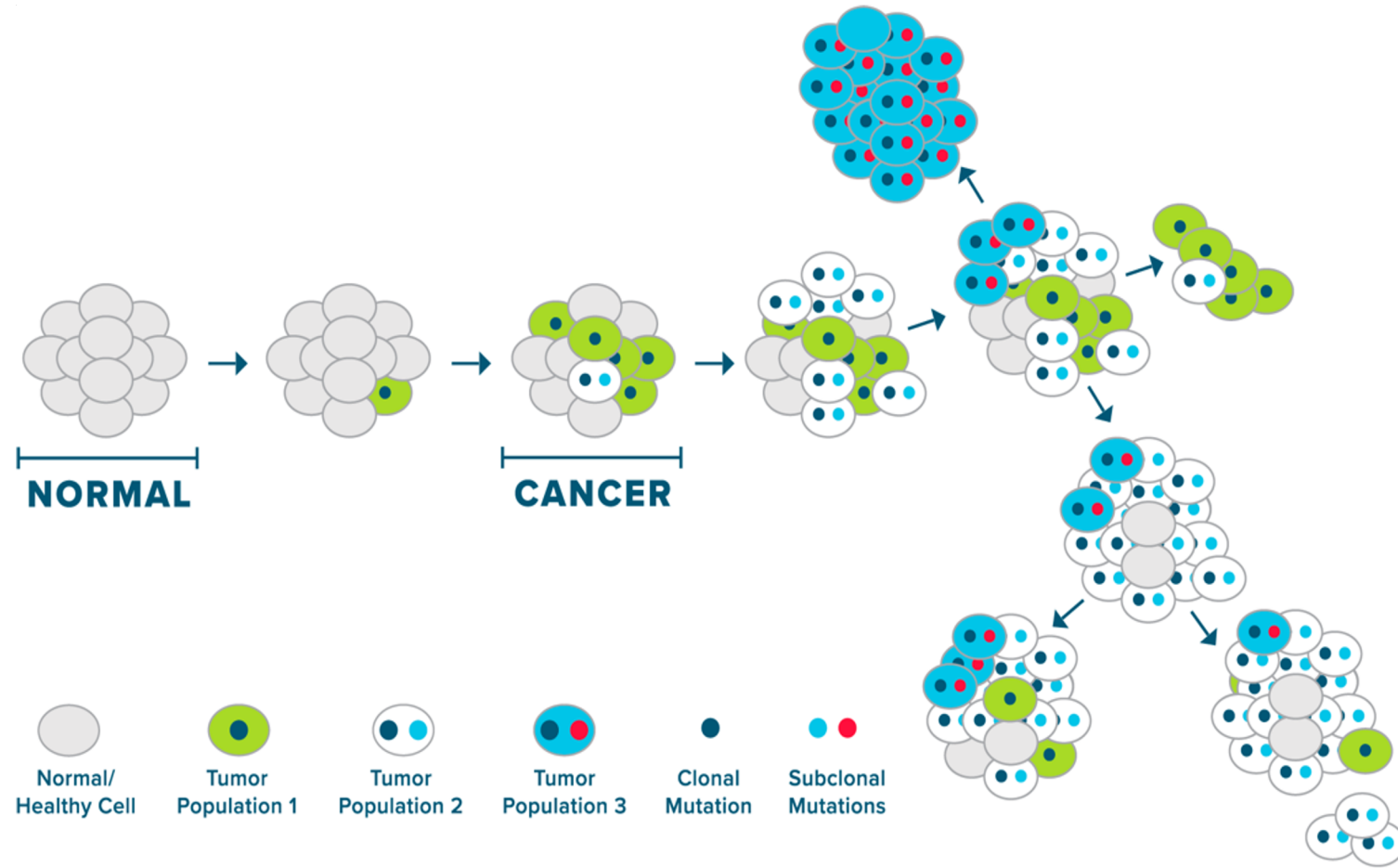




Flexible and Robust Platform to Build Future Capabilities Faster

	Accessible content	More analytes	Throughput & plexy	Software
NOW	Curated panels for specific tissues Custom for flexibility	RNA	Up to ~400 genes Up to 6 slides of 12 x 24 mm each/week	On-board analysis Nuclei-based segmentation Xenium Explorer
ROADMAP	Expanded menu of tissue and application panels	Simultaneous protein on same section/slide Expressed SNPs, isoforms, other	Flexible plexy to suit application needs Higher throughput/week	Cell segmentation Analysis features Simplified storage & collaboration with 10x cloud

Single cell DNA studies



Direct Library Prep

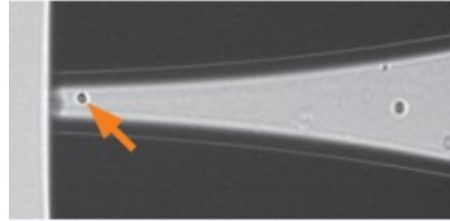


Contactless piezo
electric dispenser

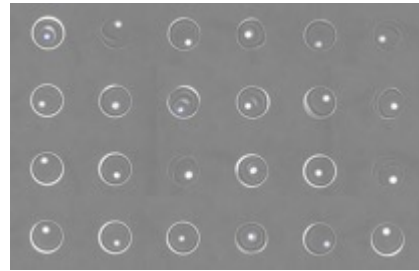
high throughput
dispensing of
individual cells from
cell suspensions
50 pl to 1 ul



Direct Library Prep

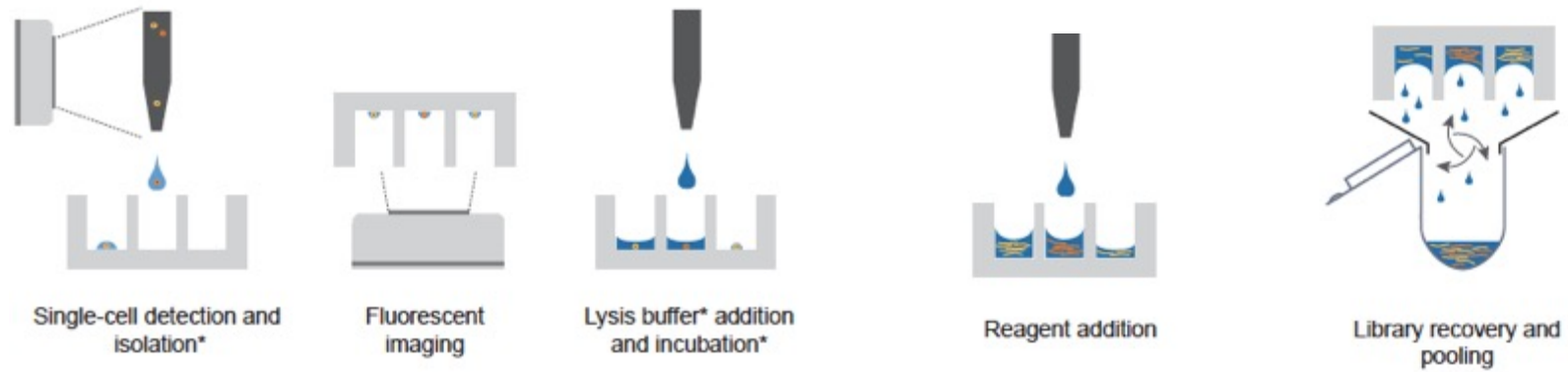


Real-time object recognition during dispensation
On-board fluorescent module



Imaging of cells on chip
Link cell state to sequencing data

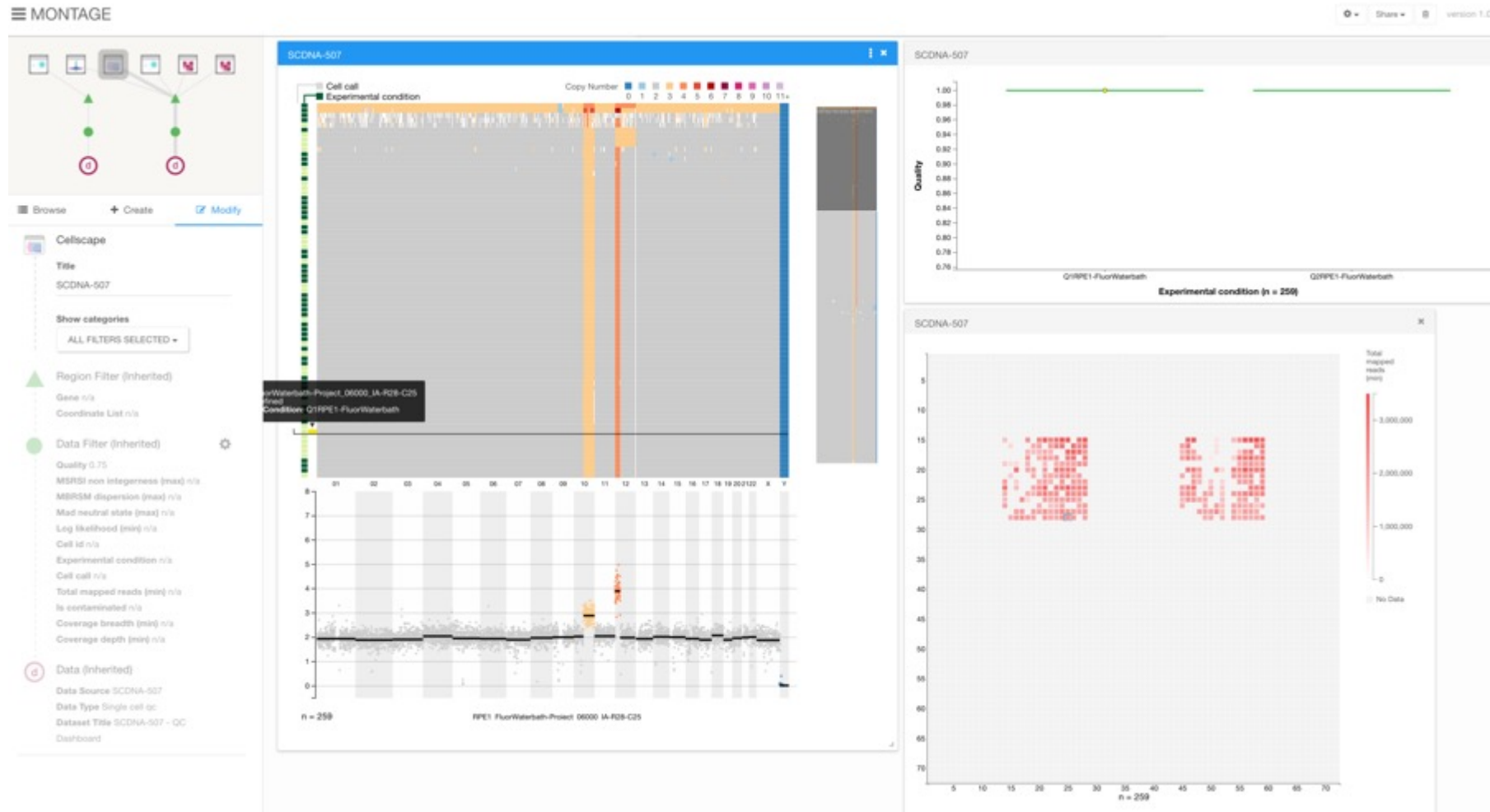
Single cell DNA libraries



Sohrab Shah, Sam Aparicio et al

Scalable whole genome sequencing of 40,000 single cells identifies stochastic aneuploidies, genome replication states and clonal repertoires Cell 2019

Data analysis to look at copy number aberrations



Questions?

This afternoon:

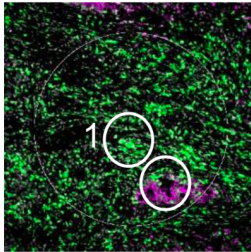
Quantitation of DNA

Meet at ZRC 3rd floor by elevator bank at 2 pm

Geometric



CD3 PanCK DNA

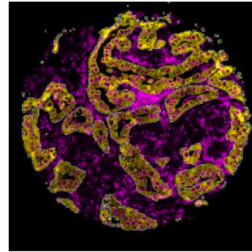


Assess heterogeneity
(e.g. tumor margin vs. core, cortical layers)

Segmentation

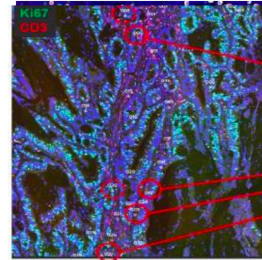
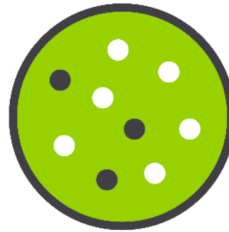


PanCK DNA



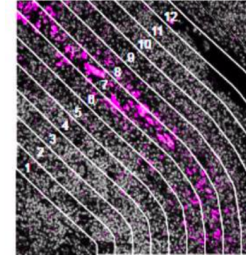
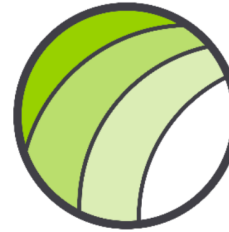
Evaluate biological compartments
(e.g. Tumor-TME)?

Rare cell



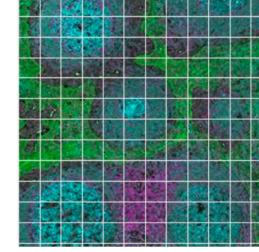
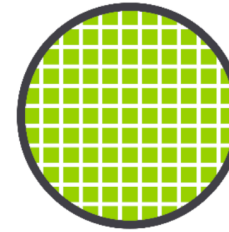
Profile cell populations
(e.g. CD3+ cells, GFAP+ cells)

Contour



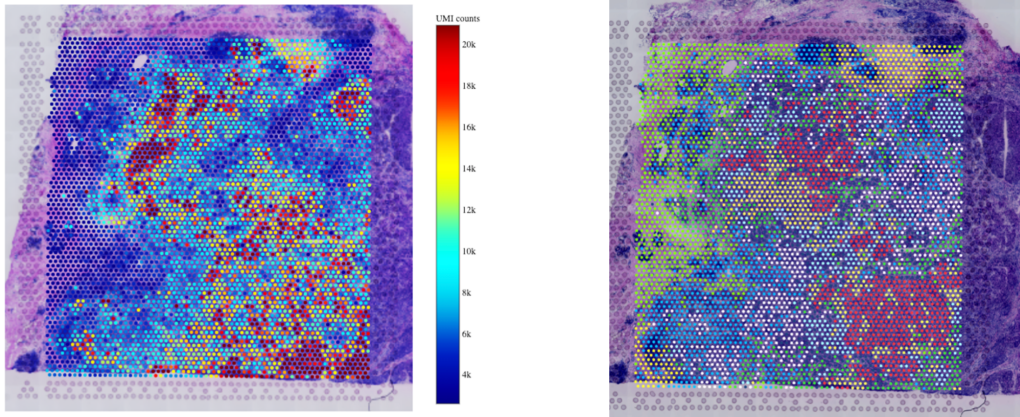
Determine proximal response
(e.g. CART, AD plaque)

Gridded



Perform deep mapping
(e.g. 96 ROI)

Breast tumor sample



Considerations

Frozen tissue only
55 um resolution (not single cell)
No capital equipment purchase