Frederick National Laboratory
for Cancer Research
sponsored by the National Cancer Institute

CCR Single Cell Analysis Facility (SCAF): An Overview of Supported Single Cell Sequencing and Spatial Profiling Assays

Michael Kelly, PhD

Team Lead, CCR SCAF

BTEP Single Cell Seminar Series
April 3rd, 2024

Overview of SCAF Support Services

Note: CCR SCAF is dedicated to NCI CCR-affiliated laboratories

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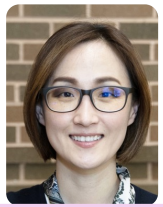
Single Cell Analysis Facility (SCAF) Staff


Kimia Dadkhah


Anna Lee Fong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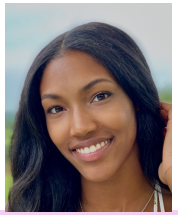
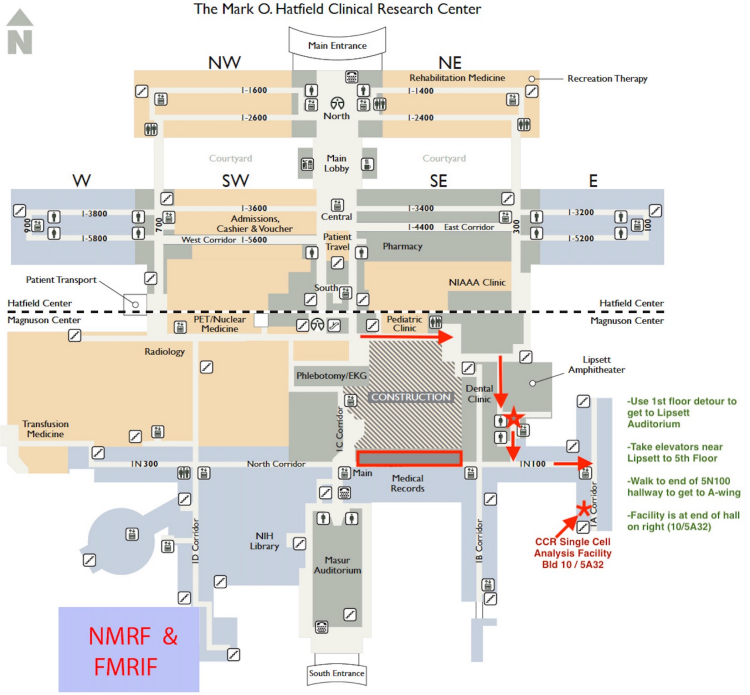

Farin Debose

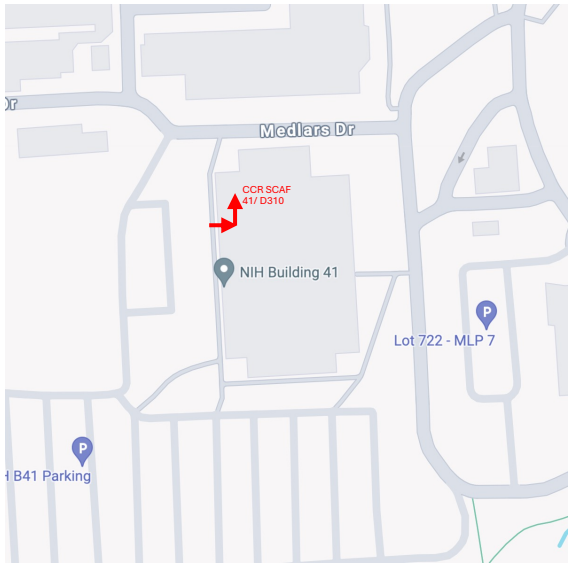
Childhood Cancer Data Initiative (CCDI)-Dedicated Satellite Facility Staff

- Neeraja Syed
- Steven Toms



Bld 10 Location (5A32):

- Droplet-based single cell sequencing support
- Walk-up equipment, including Miltenyi dissociator, single cell capture instruments



Bld 41 Location (D310):

- Spatial profiling support
- SCAF sequencing instrumentation
- Advanced method testing

Single Cell Project Support Workflow

Frederick National Laboratory for Cancer Research
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Project Consultations & Onboarding

- Discuss overview of project, sample prep, cost, timeline, and any custom processing needs for project support
- Investigator submits request via NCI Accessioning System (NAS), SCAF provides estimate for Investigator, subsidy and AO approval

Sample Capture Schedule Coordination

- Investigator chooses available date and time for capture support via SCAF iLab system
- Investigator provides additional project information to document important project details

Sample Intake & Project Commitment

- Investigator submits high quality sample, free of debris in compatible buffer and sample intake form
- SCAF performs cell count and sample quality check; communicate deviations to Investigator
- Investigator can choose on how to proceed with any less than ideal sample

Sample Capture to Results

- SCAF performs single cell capture, library generation, and sequencing
- SCAF processes data, checks basic metrics and attempts corrective actions for problematic samples in communication with Investigator

Data Sharing & Ongoing Support

- Access to generated project data provide and discussed with Investigator
- Investigator and collaborators curate and analyzes results, in consultation with SCAF, as needed

Project Cost Estimates

Project estimates provides and funding approved via NCI Accessioning System (NAS). Editable draft excel sheets can be shared ahead for project planning or grant purposes.

Part 1: Capture and Library Prep Costs

- Unit cost of the specific assay * number of samples
- Additional reagent and consumable costs are factored into assay estimate
- Failed samples (e.g. due to poor quality cell suspensions) are still invoiced
- CCR OSTR subsidy applied for on CCR Investigator's behalf at time of estimate

Part 2: Sequencing Costs

- Number of Samples * Number of Cells Per Sample * Target Read Depth = Total Reads Needed
- Accuracy of high quality cells captured defines accuracy of sequencing need for project
- Sequencing costs applicable / tracked for STARSseq subsidy mechanism

Other Notes:

- Pricing includes ~10% overhead charge of supported assays to CCR Investigators in order offset facility operational costs to CCR OD
- CCR SCAF prefers Investigators not purchase capture / library kits or sequencing kits

Sequencing cost savings coming soon with Illumina NovaSeq X

	Cost	Output (Paired Reads)	Unit Cost ($/M Reads)
NovaSeq 6000 S1 100-cycle	$4,400	$1.30x10^9$	$3.38
NovaSeq X 1.5B 100-cycle	$2,120	$1.50x10^9$	$1.41

~42% of NovaSeq 6000 S1 100-cycle

- NovaSeq X Plus installed March 2024.
- Validation and optimization testing in progress.
- SCAF will provide pricing on new projects and offer option to switch pending projects soon.

1.5B

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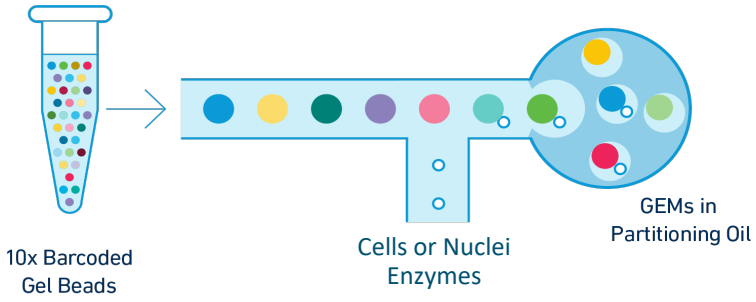
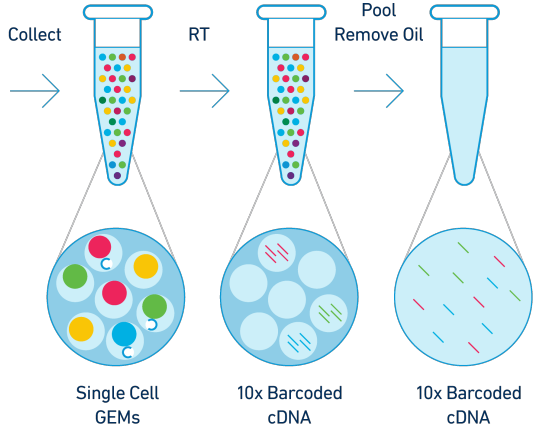
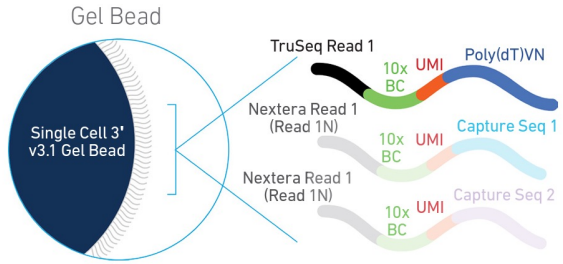
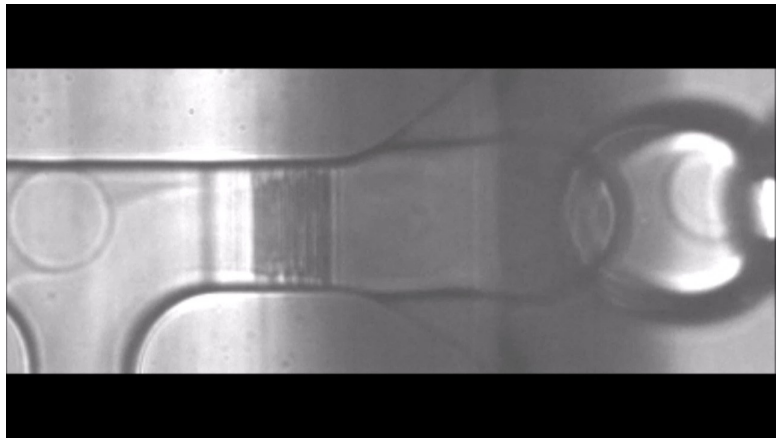
Single Cell (or Nuclei) RNA-Seq on 10x Genomics Chromium

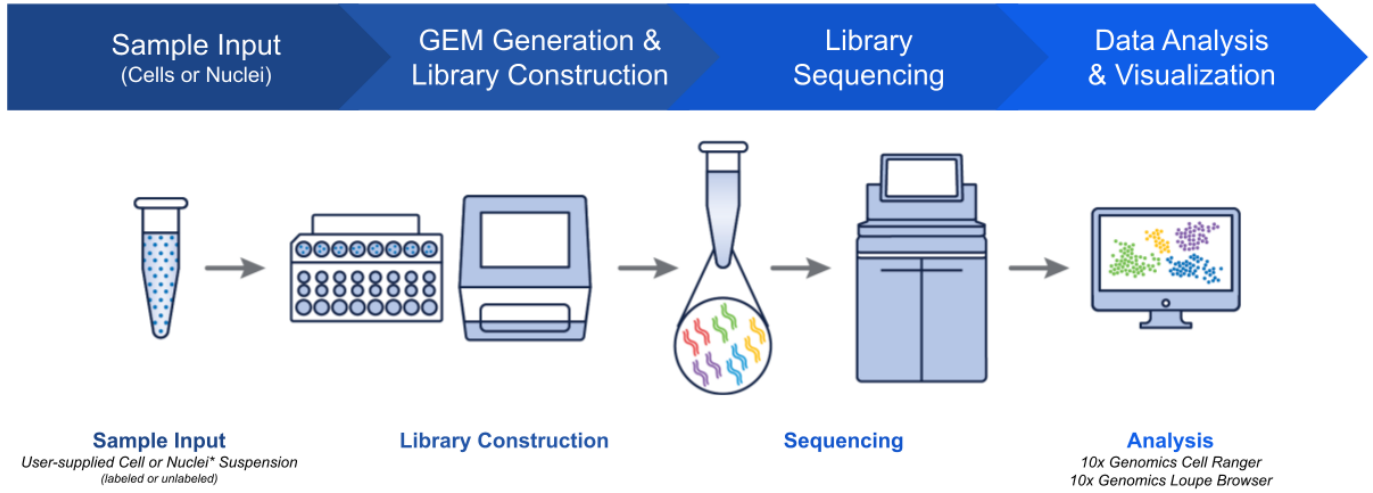
10x Genomics Chromium provides robust high-throughput single cell / nuclei RNA-Seq







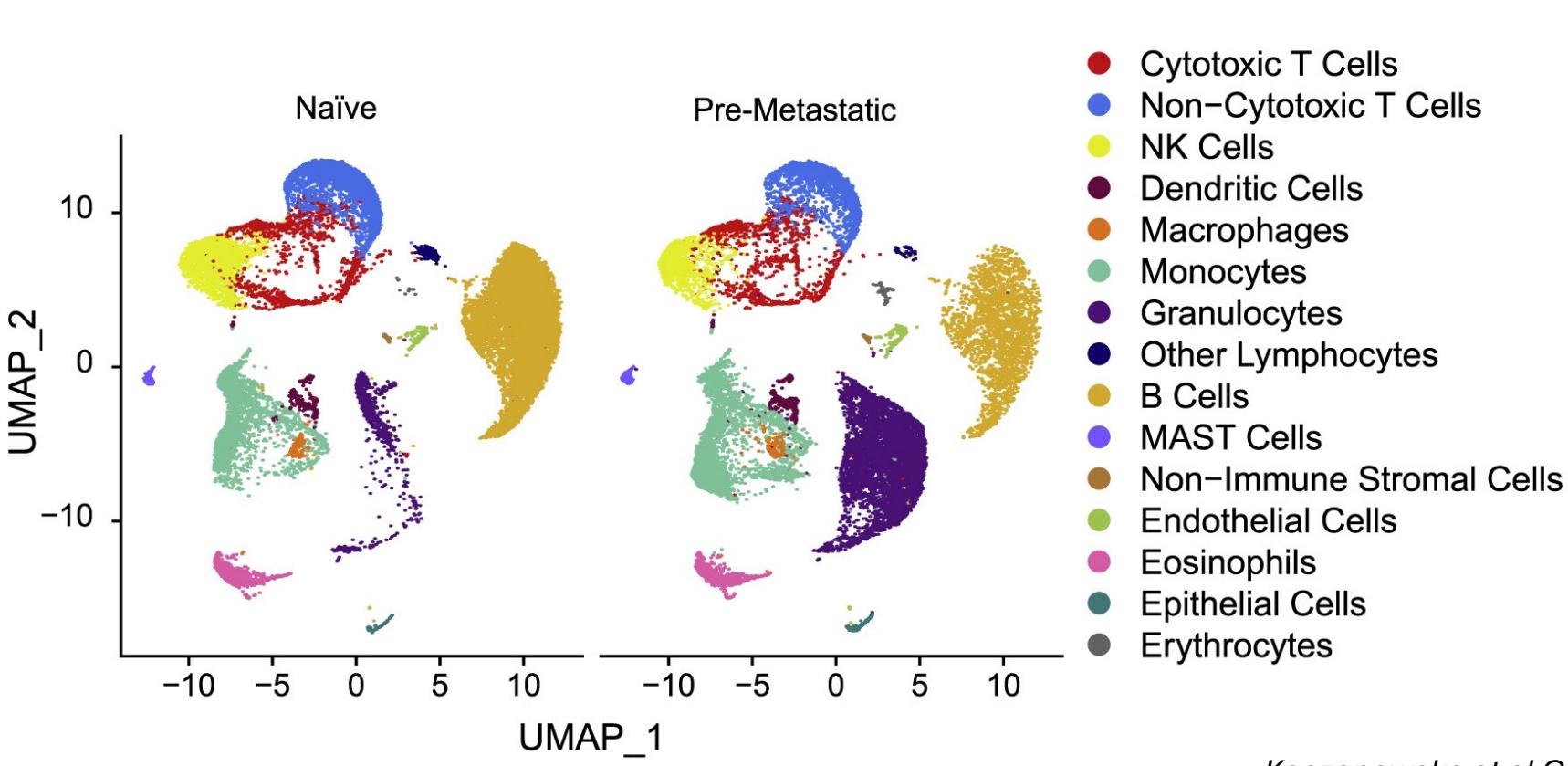


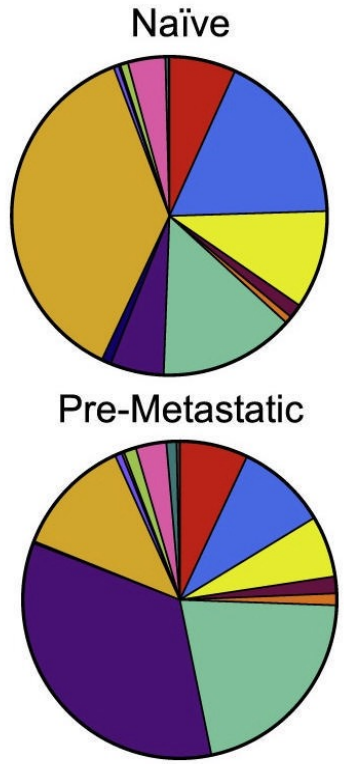


Cost per sample capture lane =
$1950 (typically targeting 6000 cells)
*Assay costs before sequencing

Diagrams from 10x Genomics

High-Throughput scRNA-Seq allows survey of complex biological systems across various cell types and conditions

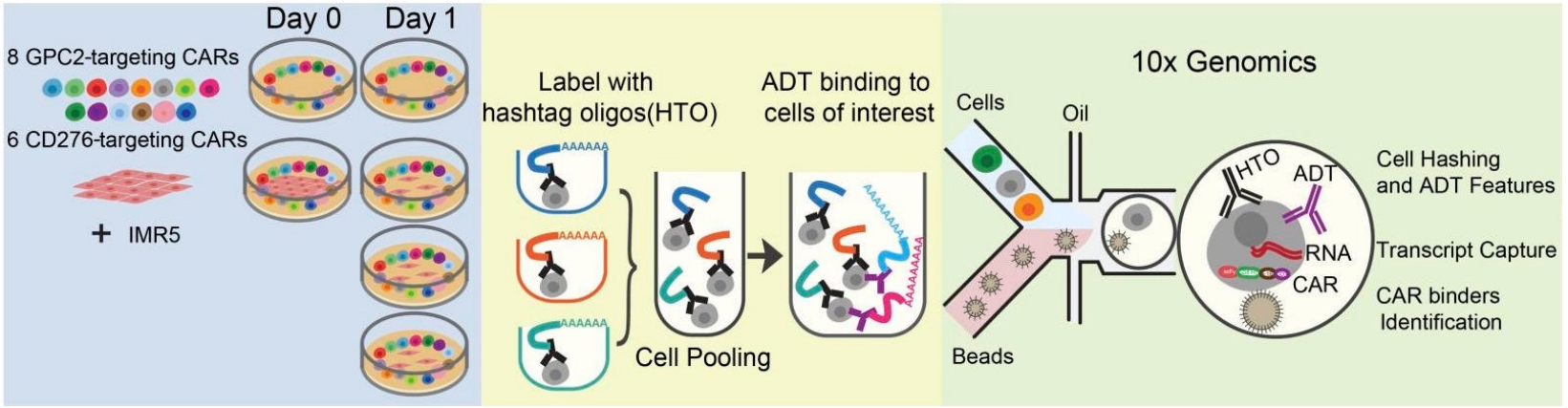


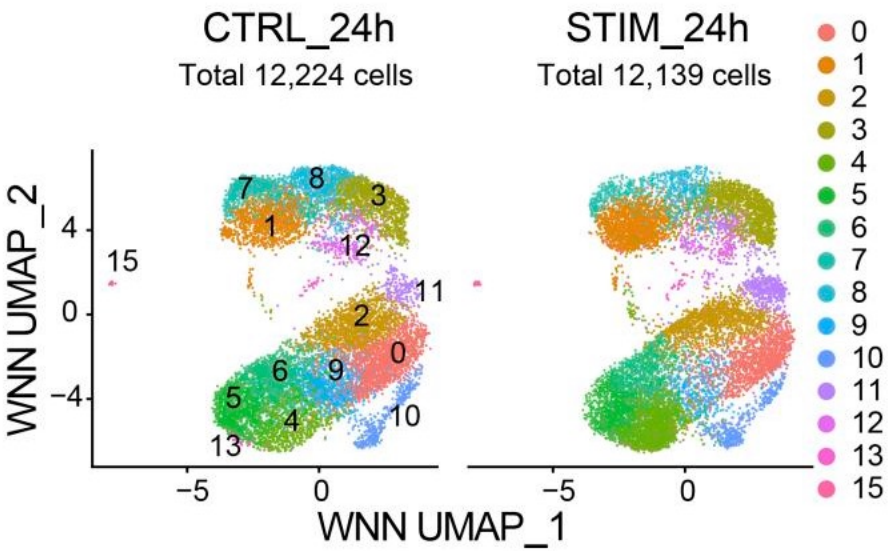


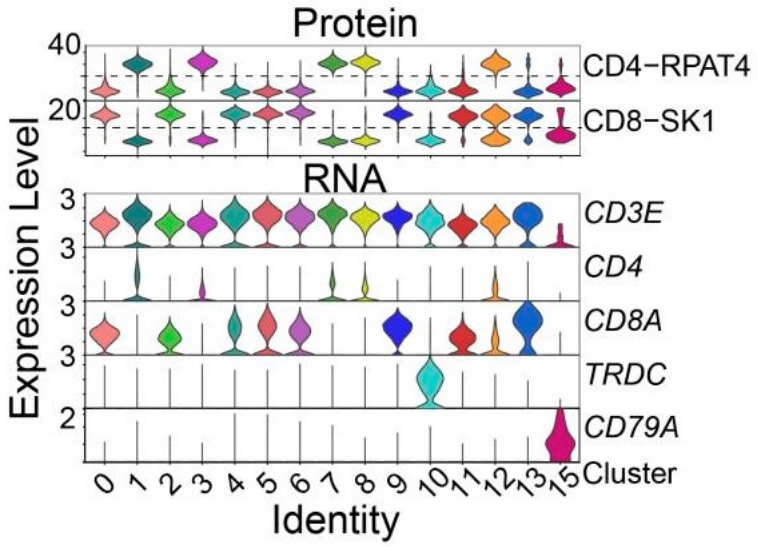
Kaczanowska et al Cell, 2021

- Cell types identified by their gene expression profiles clustered together
- Difference in observed number of cells and changes in expression profile across conditions can be made

Additional features and modalities can be assayed in parallel to standard scRNA-Seq gene expression profiles – CITE-Seq / Cell Hashing



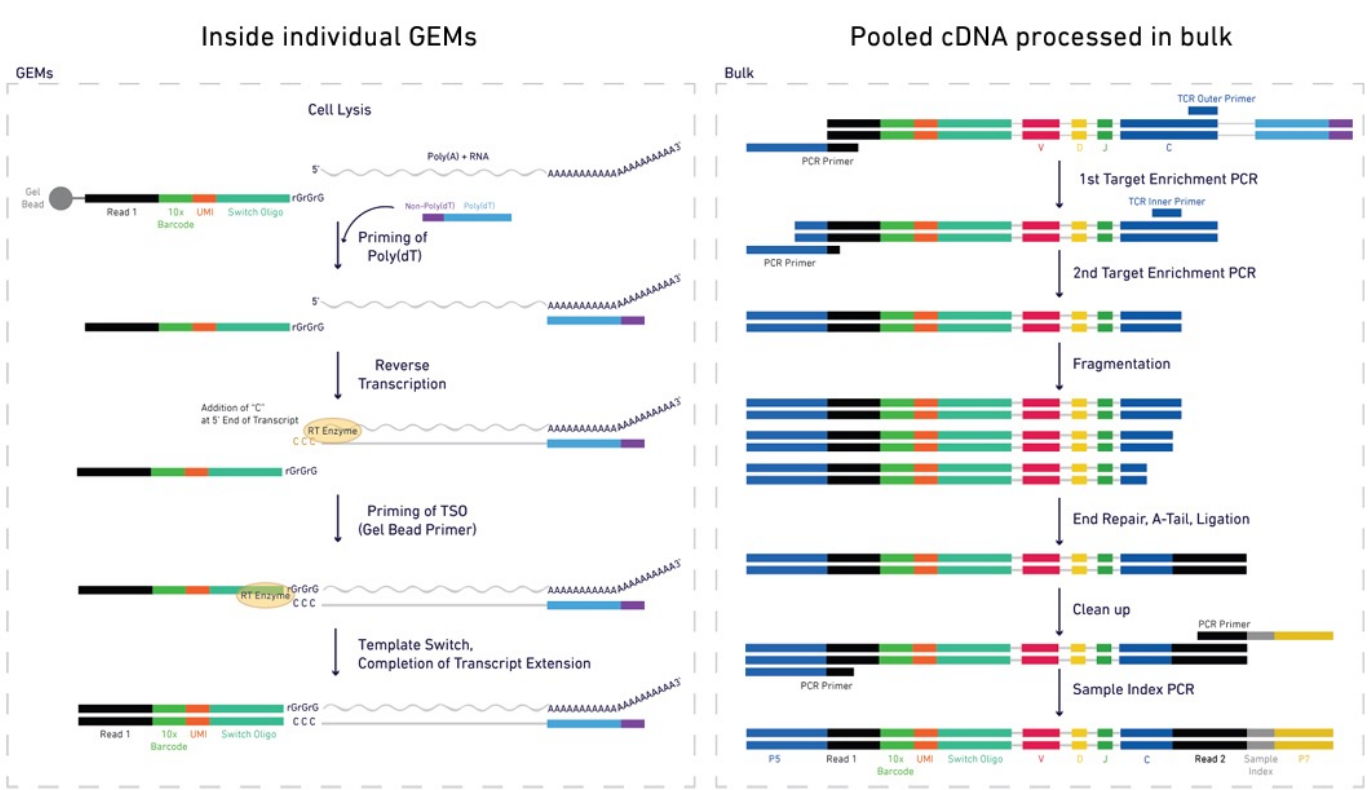


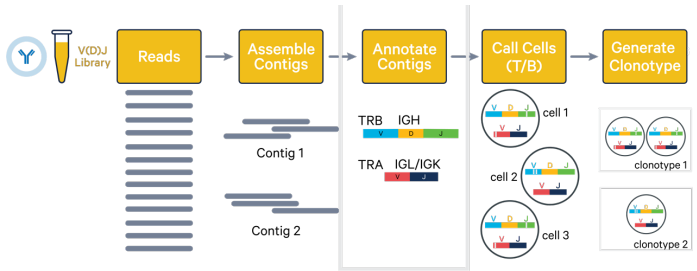


- Antibodies against cell surface proteins can conjugated with oligo tag and picked up at same time as expressed RNA
- Can be used to measure cell surface protein expression and/or identify component multiplexed samples

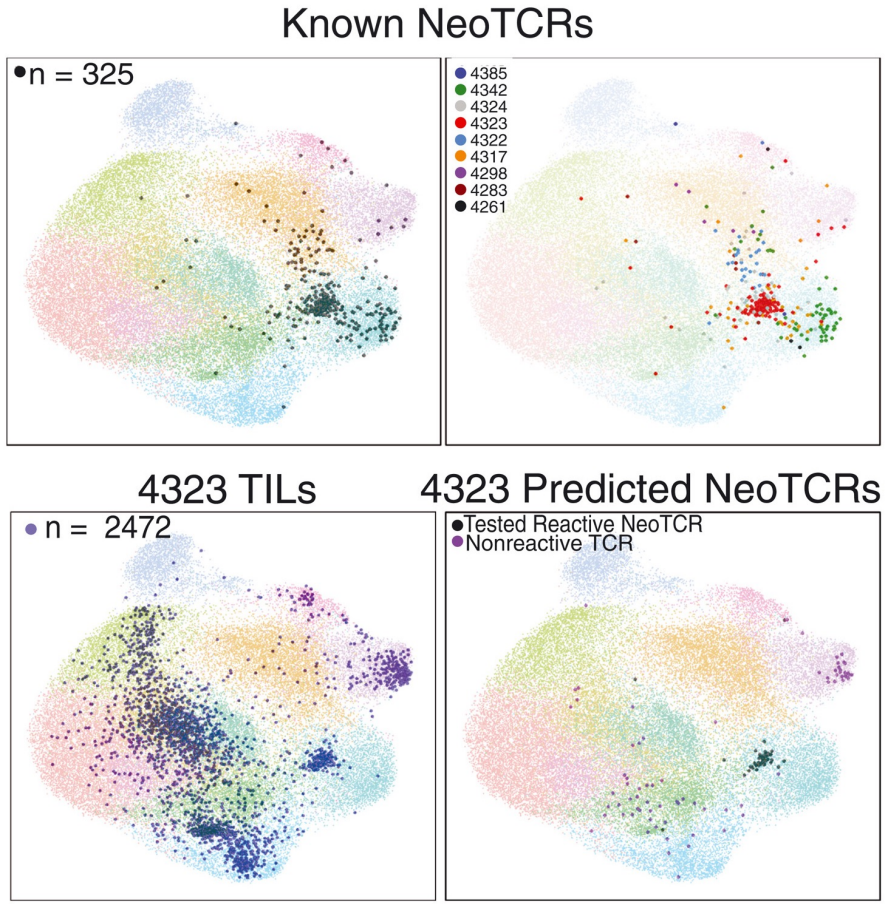
Tian et al JCI, 2022

Specific expressed transcripts can be enriched for improved detection – example T-Cell receptor sequences



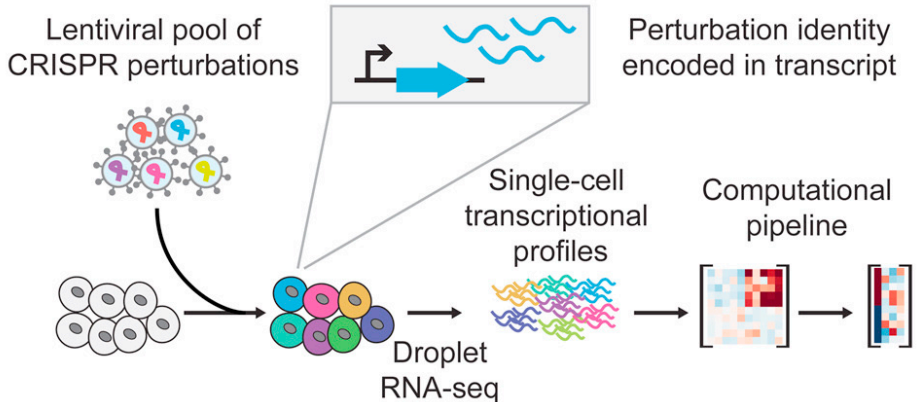


Diagrams from 10x Genomics

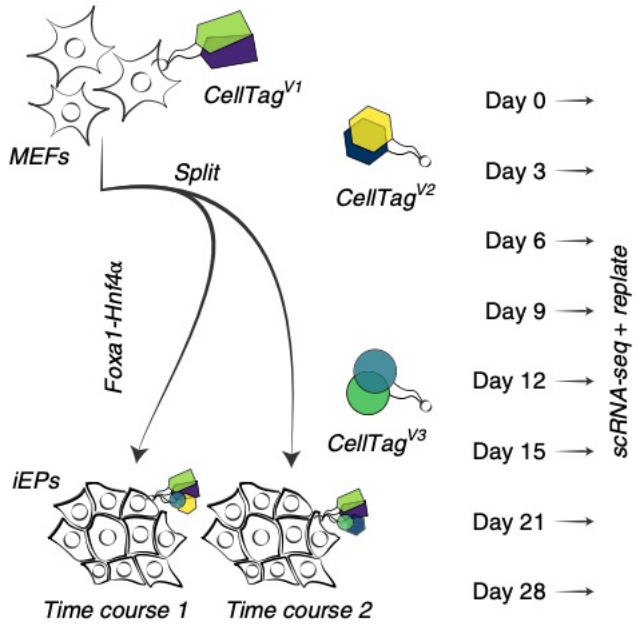


Lowery, Krishna et al Science, 2022

Single cell molecular barcoding can enable functional genomic screens and lineage barcoding



Adamson et al Cell 2016



Kong et al Nature Protocols 2020

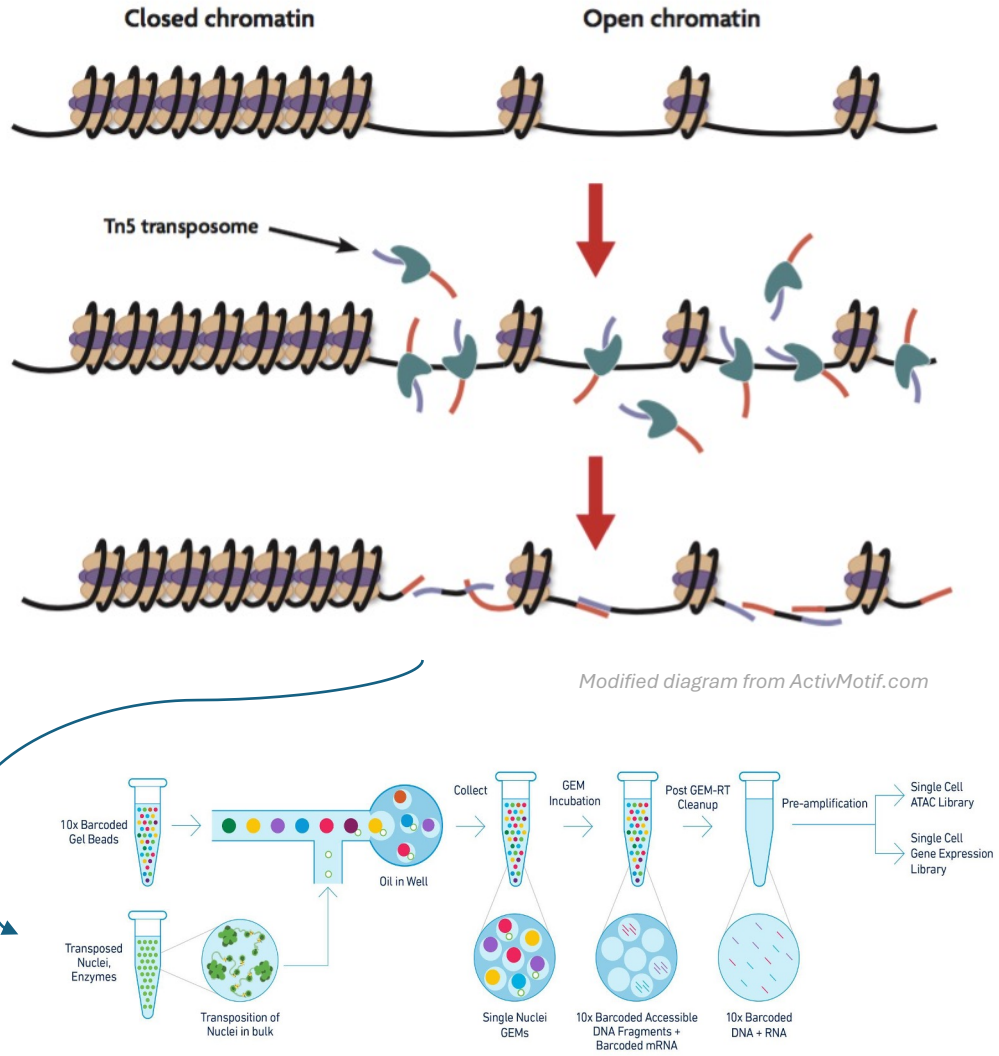
Single cell libraries containing both the transcriptome, as well as the barcode of the CRISPR guide RNA are able to be sequenced from the same cell

CRISPR knockdown and CRISPR activation are compatible, allowing a dissection of gene regulation and phenotyping

Other applications of using an expressed barcodes can also be employed, such as lineage tracing clone

Other Modalities on 10x Genomics Chromium

Combined single nucleus RNA-Seq with chromatin accessibility – expression phenotype with epigenetic state



Modified diagram from ActivMotif.com

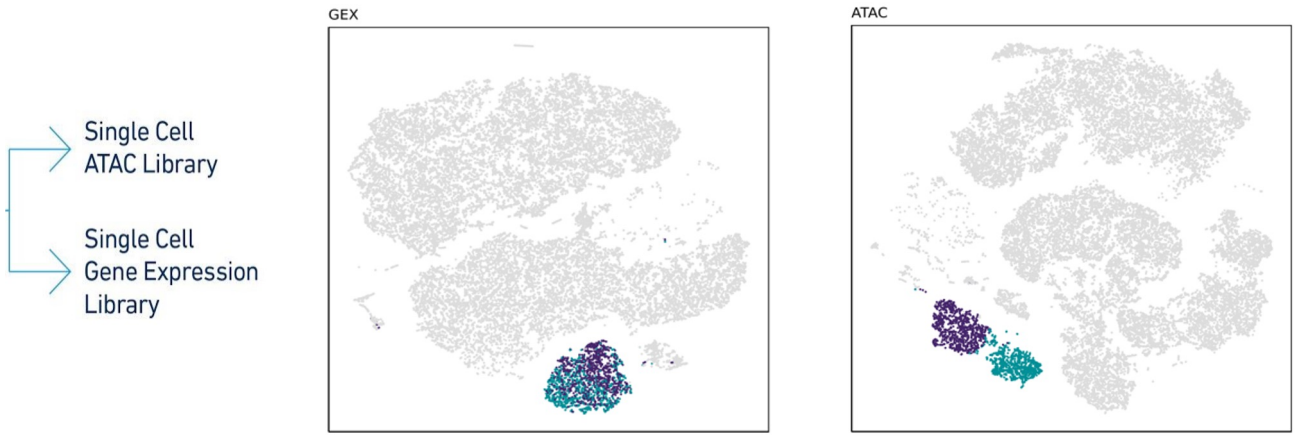
From 10x Genomics Multiome User Guide

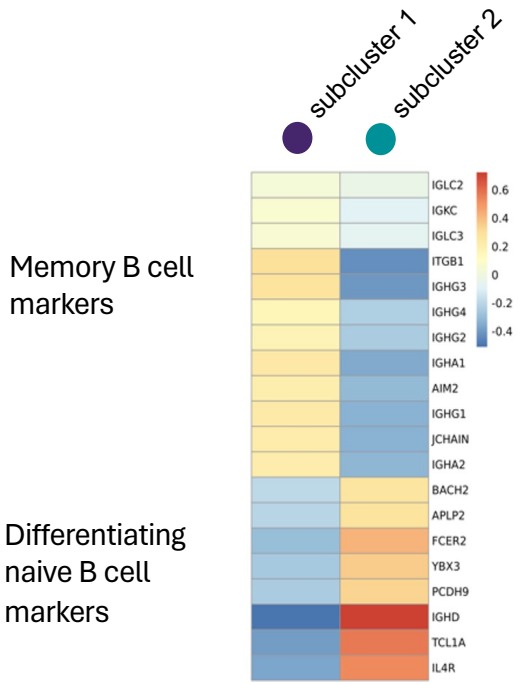
→ Gene Expression → Cell Phenotype

- Chromatin state can be efficiently profiled by Assay for Transposase-Accessible Chromatin with Sequencing (ATAC-Seq)
- Both RNA-Seq and ATAC-Seq can be performed at single cell resolution, and a combined assays on same cells can refine subtypes and confirm ATAC <-> GEX relationship
- Single cell RNA-Seq+ATAC-Seq can improve understanding of transcriptional control during developmental, disease, treatment, etc.

- Single nuclei needed as input and nuclei prep needs to be optimized; balance permeabilization for ATAC but keep RNA in nuclei
- Data (particularly for single nucleus ATAC) is very sparse for each single cell sample

Co-Measured modalities at single cell resolution are powerful – co-leveraging via integrated analysis can extend resolution





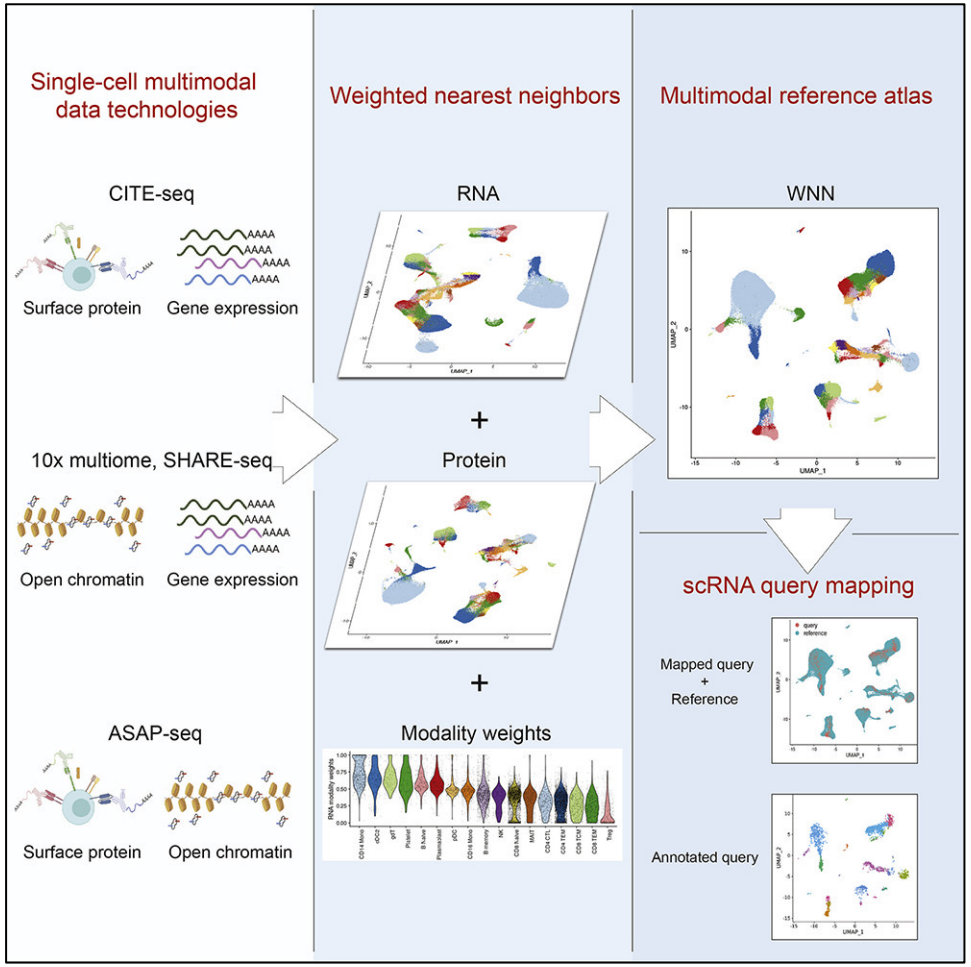
Example data from 10x Genomics

Pre-Integration:

- Results from multiple modalities are handled separately, but linked via cell ID
- Annotation from one analysis can be used to query other data

Post-Integration:

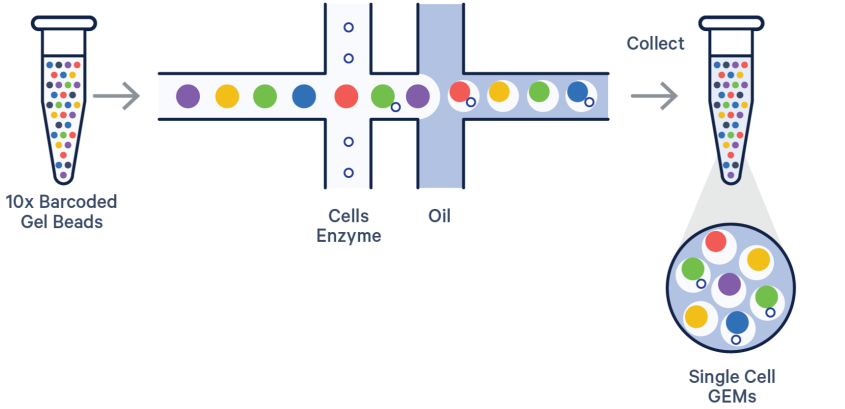
- Data from multiple modalities used in the analysis for increased sensitivity, resolution or depth of information

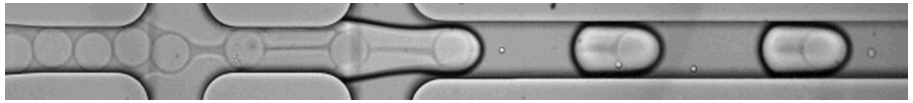


Hao et al 2021

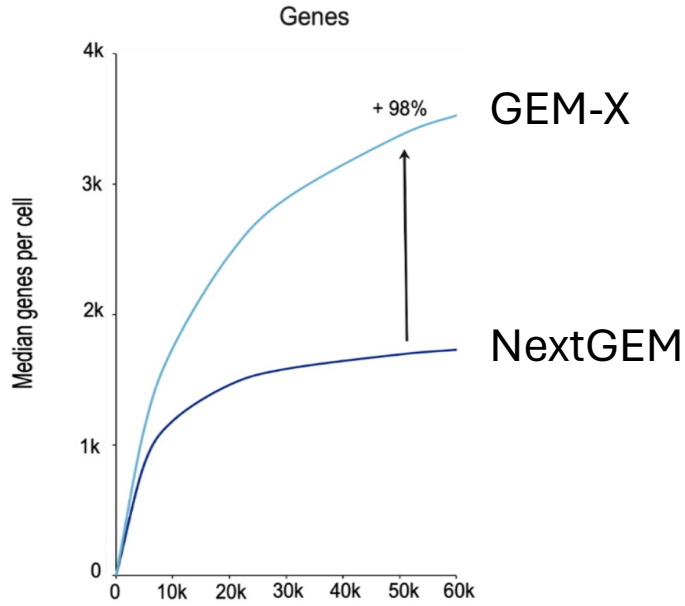
GEM-X: Updated 5’ and 3’ scRNA-Seq for 10x Chromium

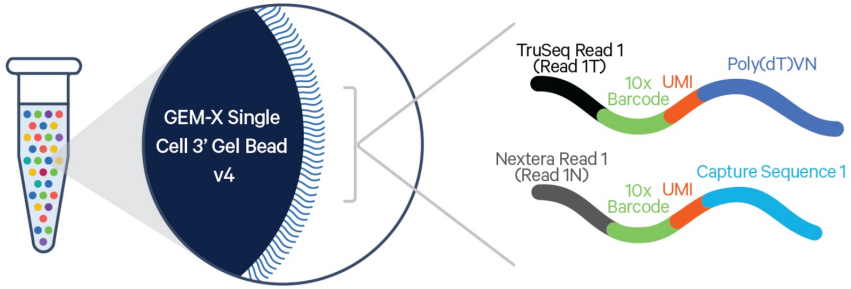
10x Genomics GEM-X now available – improved capture rates and sensitivity





Multiplet Rate	# of Cells Recovered	
	Next GEM	GEM-X
0.8%	1,000	2,000
1.6%	2,000	4,000
2.4%	3,000	6,000
3.2%	4,000	8,000
4.0%	5,000	10,000
4.8%	6,000	12,000
5.6%	7,000	14,000
6.4%	8,000	16,000
7.2%	9,000	18,000
8.0%	10,000	20,000



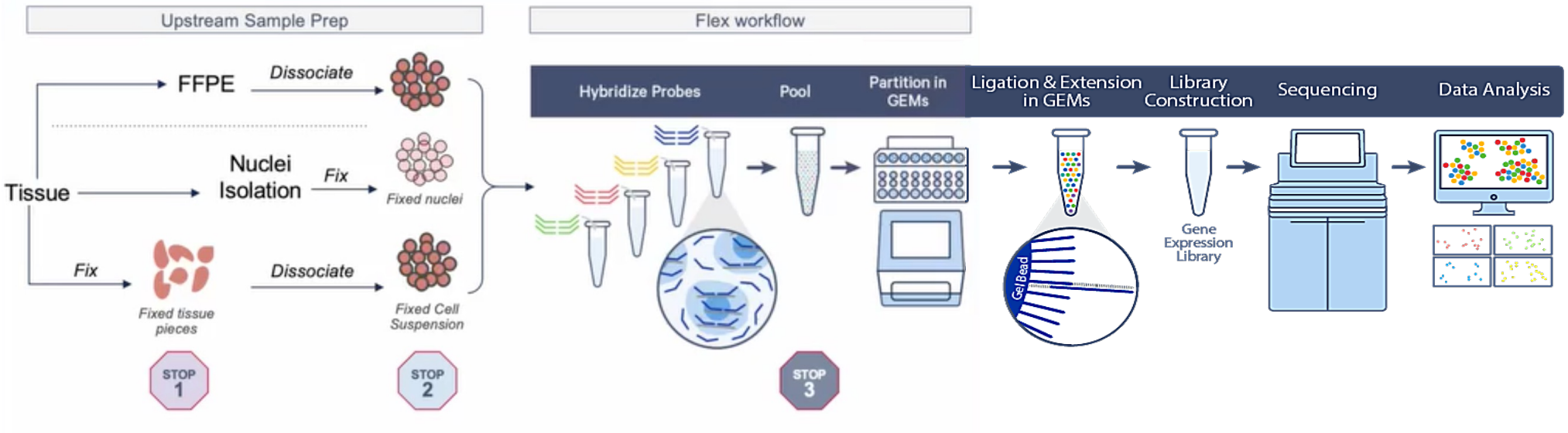


Cost per sample capture lane = $1800 (typically targeting 12,000 cells)
*Assay costs before sequencing

- New microfluidics and chemistry increases sensitivity and doubles available target cell capture
- Must be run on Chromium X and does not include Capture Sequence 2
- Not yet available for ATAC or Multiome Assays
- SCAF now providing estimates with new GEM-X chemistry – consider requesting previous version if combining with existing data

Fixed Cell Profiling with 10x Genomics FLEX

10x Genomics scFLEX is a whole transcriptome probe-based assay that enable multiple sample sources / designs



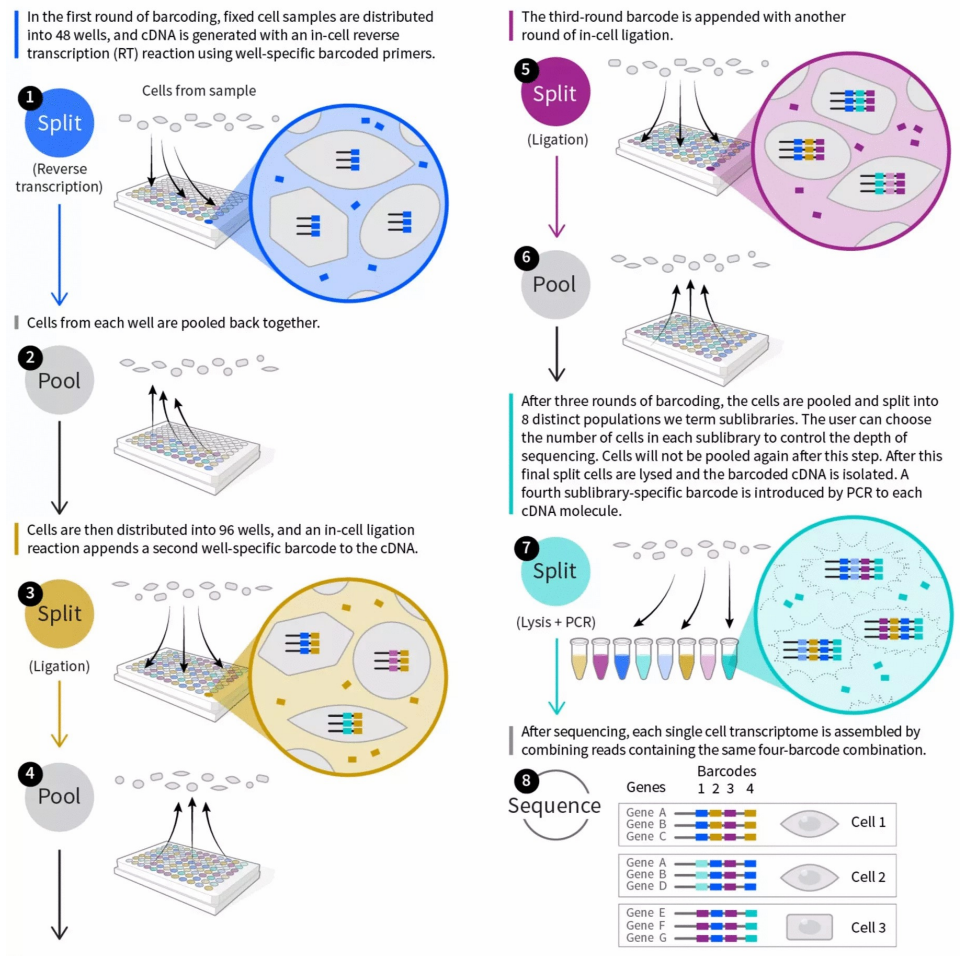
- Allows access to various input sample sources, including cells dissociated from FFPE tissue blocks
- Allows cells to be fixed at different timepoints and stored safely until ready to run
- Samples can be efficiently multiplexed using pre-indexed barcodes on probes
- Multiplexed captures can be cheaper per sample ($1060 with 6k cells each, before sequencing)

- ”Whole Transcriptome” by diverse probe set to measure expression of transcripts
- Limited to Mouse or Human (no multi-species)
- Must be run on the Chromium X instrument

- Quality control checks and optimizing scFLEX can be more challenging than traditional scRNA-Seq

Single Cell RNA-Seq Alternatives to 10x Genomics Chromium

Combinatorial indexing (e.g. Parse Evercode v3) allows massive-scale experiments lower cost



Pools of **fixed** cells / nuclei are deposited into PCR wells and undergo a first-stage tagging of the molecules – cells are the microreaction vessels

Multiple rounds of re-pooling and splitting to add combinatorial layers of index tags

Single cell index tags can be read by sequencing to determine the cell origin of each molecule sequenced

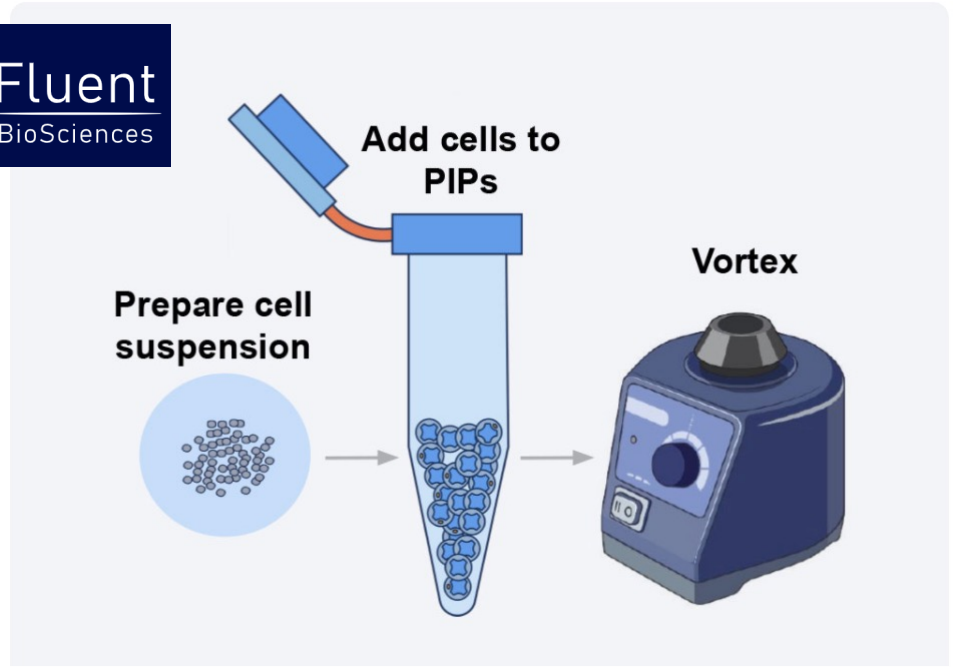
Cost and design advantages for large scale experiments where all cells and samples of similar type are to be analyzed at same time

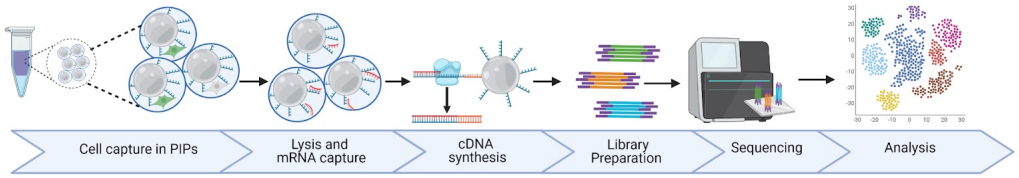
100k cell experiment with 16 samples = ~$800 per sample (6k cells each)
1M cell experiment with 96 samples = ~$300 per sample (10k cells each)
*Assay costs before sequencing

Combinatorial indexing experiments require more upfront planning and coordination

Diagram from Parse Evercode User Guide

PIP-Seq is a low-cost alternative droplet-based standard scRNA-Seq assay for smaller scalability





T2 (~2k cells) = ~$350 per sample
T20 (~20k cells) = ~$1,020 per sample
**Assay costs before sequencing*

Droplet generation by vortexing single cell suspension with 'PIPs'. Barcoded beads with lysis solution allows cell mRNA to be captured and barcoded

Similar library preparation to other droplet-based single cell assays

Some advantages includes:

- Less expensive capital equipment to run assay
- All cells partitioned at same time during vortexing
- Sample can be stored after partitioning
- Has scalability because of how the PIPs are aliquoted in tubes (increments of 2k or 20k cells per capture tube)

Some things to consider:

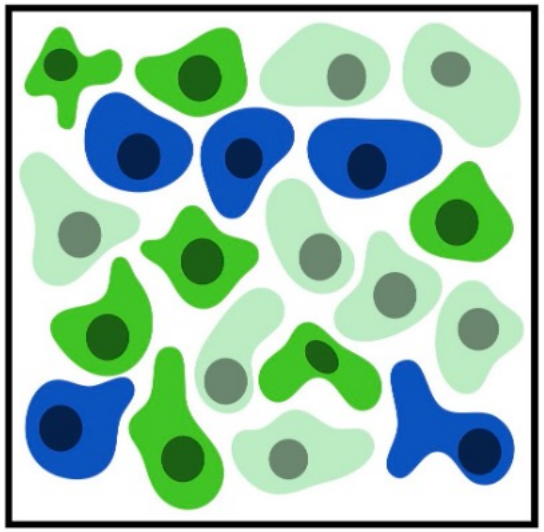
- Sensitivity of gene detection not on par with 10x assays
- Allows cell surface protein (CITE-Seq) measurement, but not the full array of add-on modalities

Diagrams from Fluent Biosciences

Spatial Profiling as a ‘Single Cell’ Approach to Understand Biology

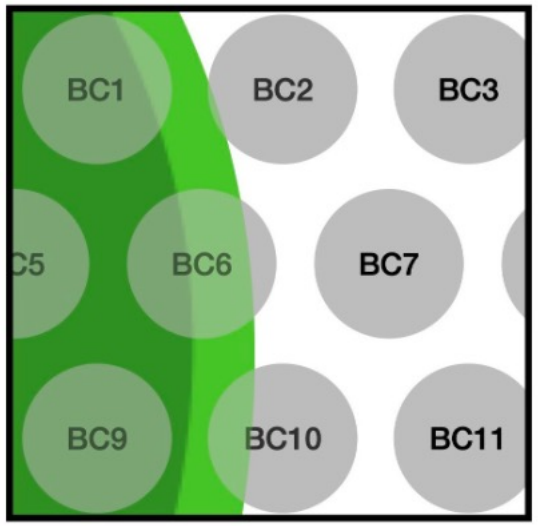
True single cell resolution with spatial profiling approaches is approximated with different strategies

Single-cell resolution



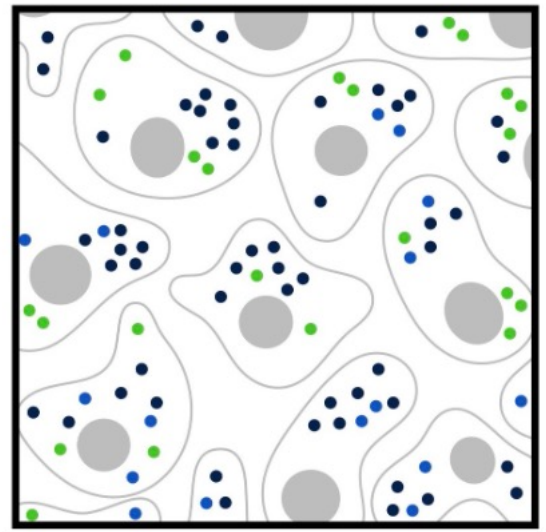
Ideal: Whole transcriptome profiling of each clearly distinct cell with spatial location represented accurately

Multi-cell resolution



Spatial Transcriptomics (e.g. Visium): Whole transcriptome profiling survey of entire tissue, but barcoding of molecules not defined by cell borders

Sub-cellular resolution

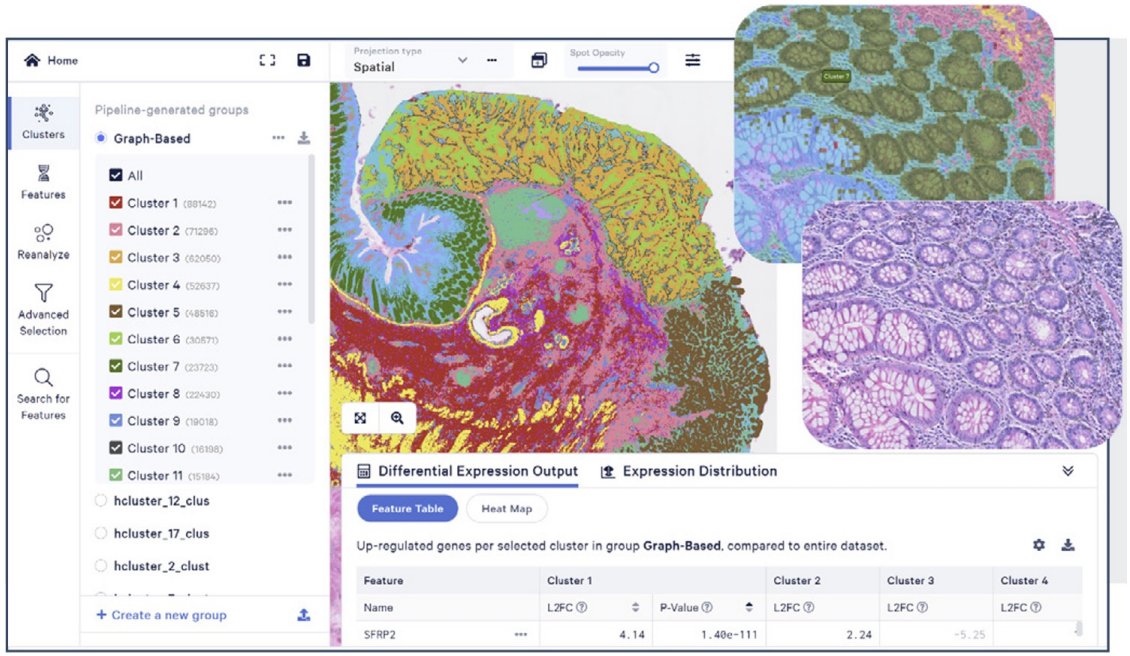


In Situ Sequencing (e.g. Xenium): Target transcript localization with subcellular localization, but assignment of transcripts to cells depends on accuracy of image segmentation

Diagrams from https://www.sc-best-practices.org/spatial/introduction.html

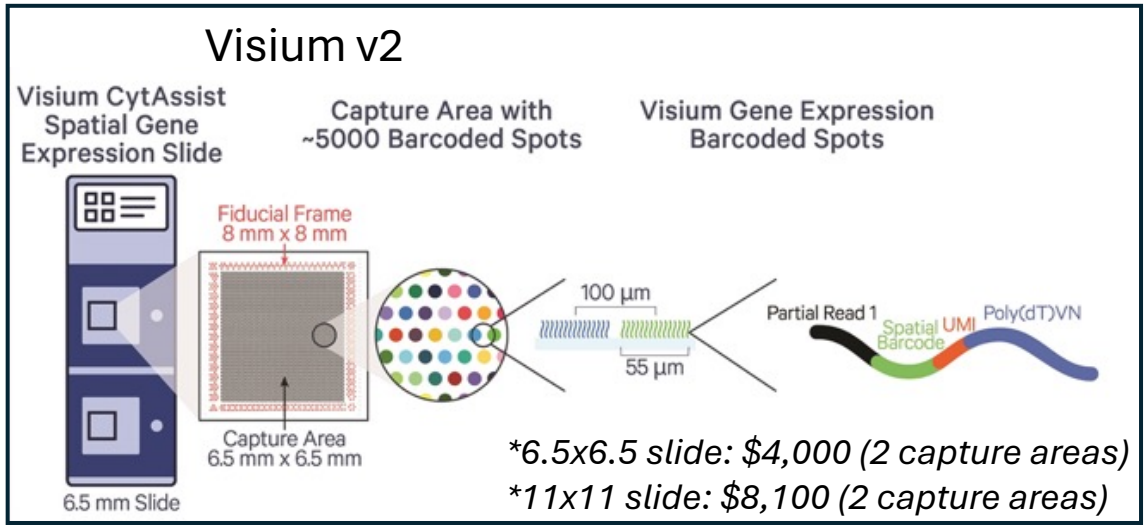
Spatial Profiling: Spatial Transcriptomics with Visium

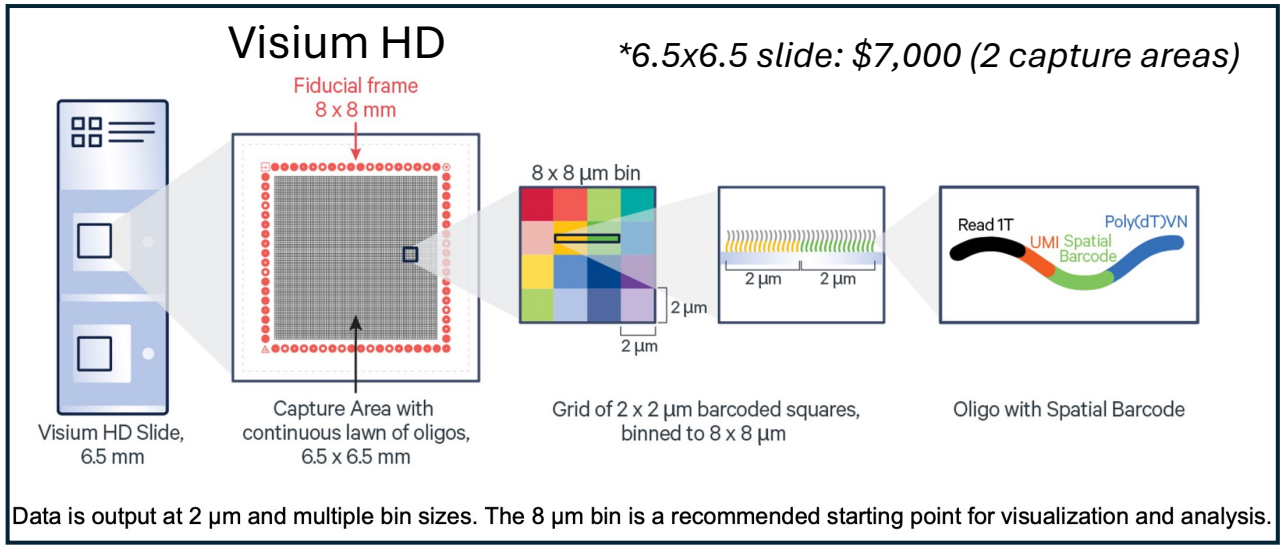
Visium provides a probe-based whole-transcriptome survey of a FFPE tissue section – now with 2-micron resolution in Visium HD



[Visium HD Demo Video]

- Whole-transcriptome probe set (mouse or human only, no multi-species)
- Visium v2 (100-micron resolution) has 6.5x6.5mm or 11x11mm capture window
- Visum HD (2-micron resolution) in 6.5x6.5mm format currently



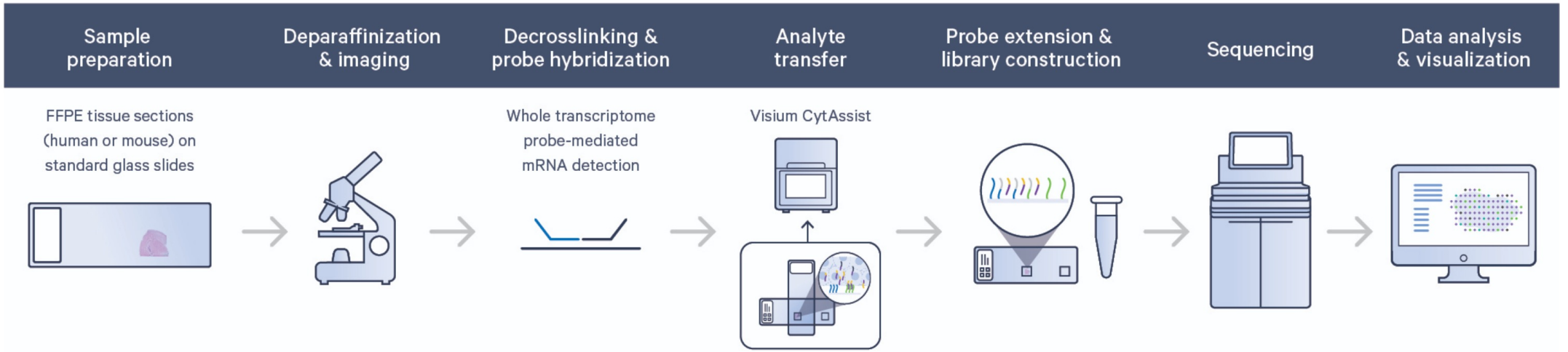


Figures from 10x Genomics

*Assay costs before sequencing

Visium workflow allows leveraging of expert histology support and flexible selection of input sample section along with SCAF support

Reminder: if you are interested in SCAF project support, please start with a project consultation



- Investigator prepares sample, has sections cut onto normal glass slides
- Sample are H&E stained and imaged
- Coordination between Investigator and SCAF to select target capture regions

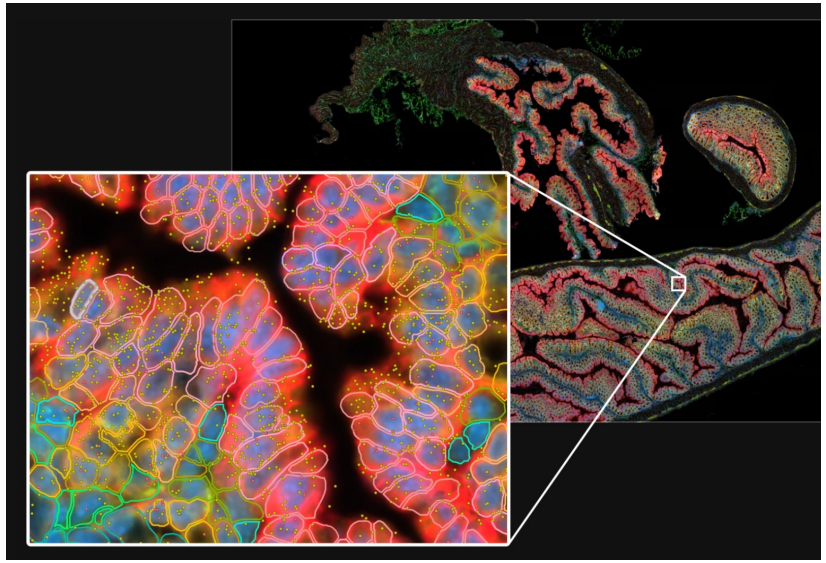
- SCAF performs decrosslinking and subsequent steps
- Bound and ligated probes (active) transferred to Visium barcoded slide to spatially barcode expressed transcript location

- SCAF generates libraries, sequences and processes data
- Resulting data is shared with Investigator, which includes both raw data and data processed with 10x pipeline (*spaceranger*)

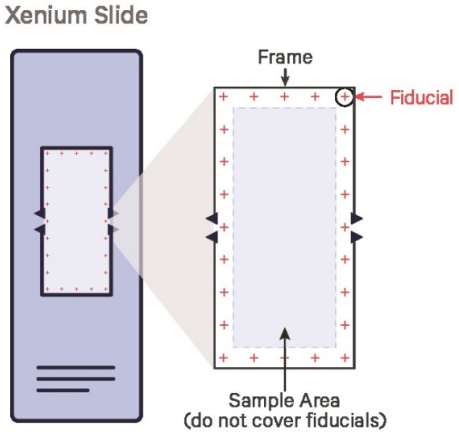
Figures from 10x Genomics

Spatial Profiling: In Situ Sequencing with Xenium

Xenium provides in situ localization of 100's (now) to 1000's (soon) of target transcripts in a robust assay and platform

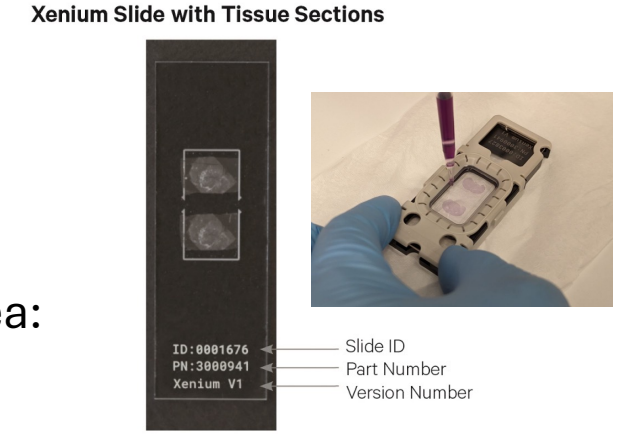


[Xenium Demo Video]



Sample Area:
10.45 x 22.45 mm

Imageable Area:
12 x 24 mm



(2) Xenium Slides Per Run

- Target probe sets selected by user – off-the-shelf panels with option for custom add-on or fully custom designs
- Advanced designs can include non-model sample types, viral or other targets, and expressed variant detection
- A multimodal stain can now be included to improve image segmentation
- Each Xenium run can accommodate 2 slides, each with a 10.45 x 22.45mm sample area

- Xenium Explorer app makes easier to query data quickly; Data output is easily imported into downstream analysis workflows

Xenium workflow is flexible for targeted probe panels and robust in generating high quality data with decent sensitivity

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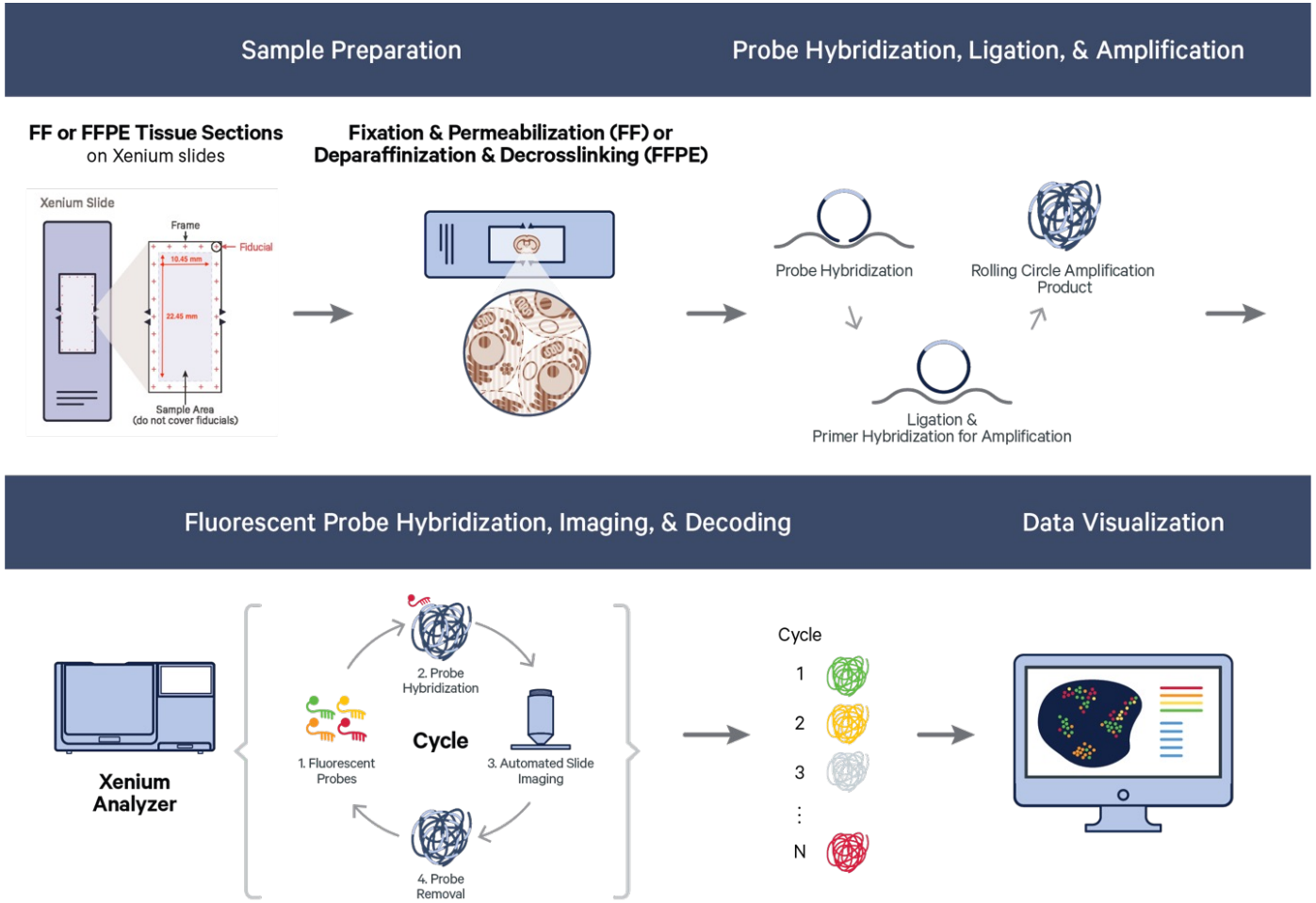


- Investigator chooses and/or designs probe panel; coordinate with SCAF about ordering
- Investigator prepares sample, obtains Xenium slide from SCAF, and has sections cut onto imageable area of slide
- Optional: Decide if multimodal segmentation stain is to be included
- SCAF processes slide for Xenium run, sequences slide, and shares project data back with Investigator and collaborators
- Optional: Post Xenium H&E or other downstream processing (IF by Investigator or Visium)

Diagrams from 10x Genomics

Primary Data Processing and Data Sharing

SCAF provides raw and primary processed for further curation and downstream data analysis

NextSeq 2000

NovaSeq 6000 / X Plus

Sequencing
Project-tailored sequencing with sample optimized coverage
Data Processing
Project-specific sequencing data processing with custom references
Expert troubleshooting based on knowledge of sample input to resulting data
Downstream Analysis
Downstream analysis based on project goals
(**limited capacity*)

BCL files to Fastqs

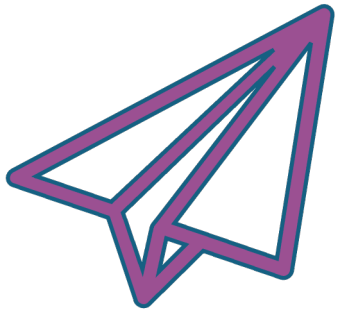
Aligned to Target Reference

SCAF Checks basic Metrics and Seq Performance

Data Shared & Ready For Investigator Curation

Continued Project Support (Consultations or Analysis*)

- Additional info requested by SCAF inform how data should be processed, checked and who the data will be shared with at time of delivery
- SCAF is working to utilize a permanent shared archive of all prospectively generated data in NCI Data Management Environment (DME). In meantime, Investigators should safely store copy of data when shared at project delivery
- Data curation and public sharing of data is reserved for the Investigator



Contact us at:

CCR-SCAF@nih.gov

Project support is dedicated to CCR-affiliated labs, but we are always happy to share experience and info with the greater NIH single cell and spatial community

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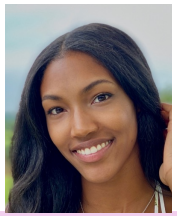
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CCR Investigator labs and Others we've worked with

Greater NIH Single Cell and Spatial Sequencing Community

FNLCR Colleagues
CRTP Program Office
Other CCR Dedicated Cores (SF, GTL, +Others)
Molecular Histopathology Lab (MHL)
LASP Genome Modification Unit

Other CCR Core Facilities
Genomics Core
CPTR
LGI Flow Core, Vaccine Branch Flow, +Others
LGCP Microscope Core and HiTiF
BTEP and CCBR

NIH HPC and NCI CBIIT Resources

And thanks to the developers of new methods, open source computational techniques, and vendors!