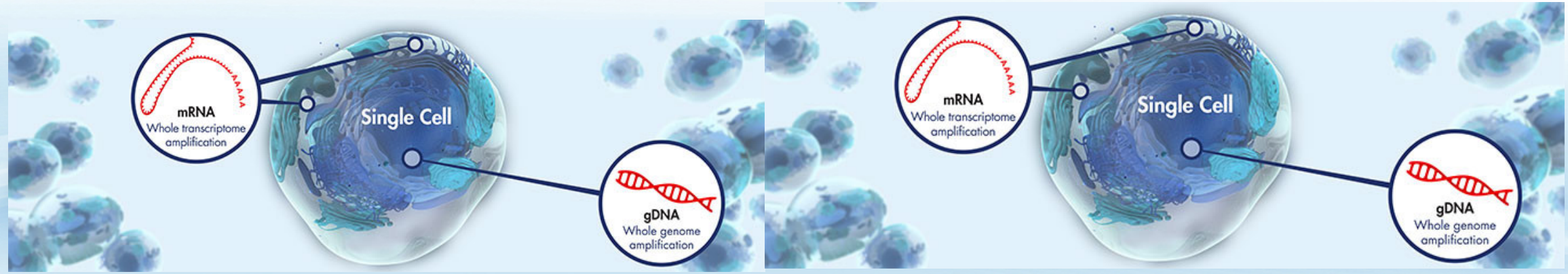
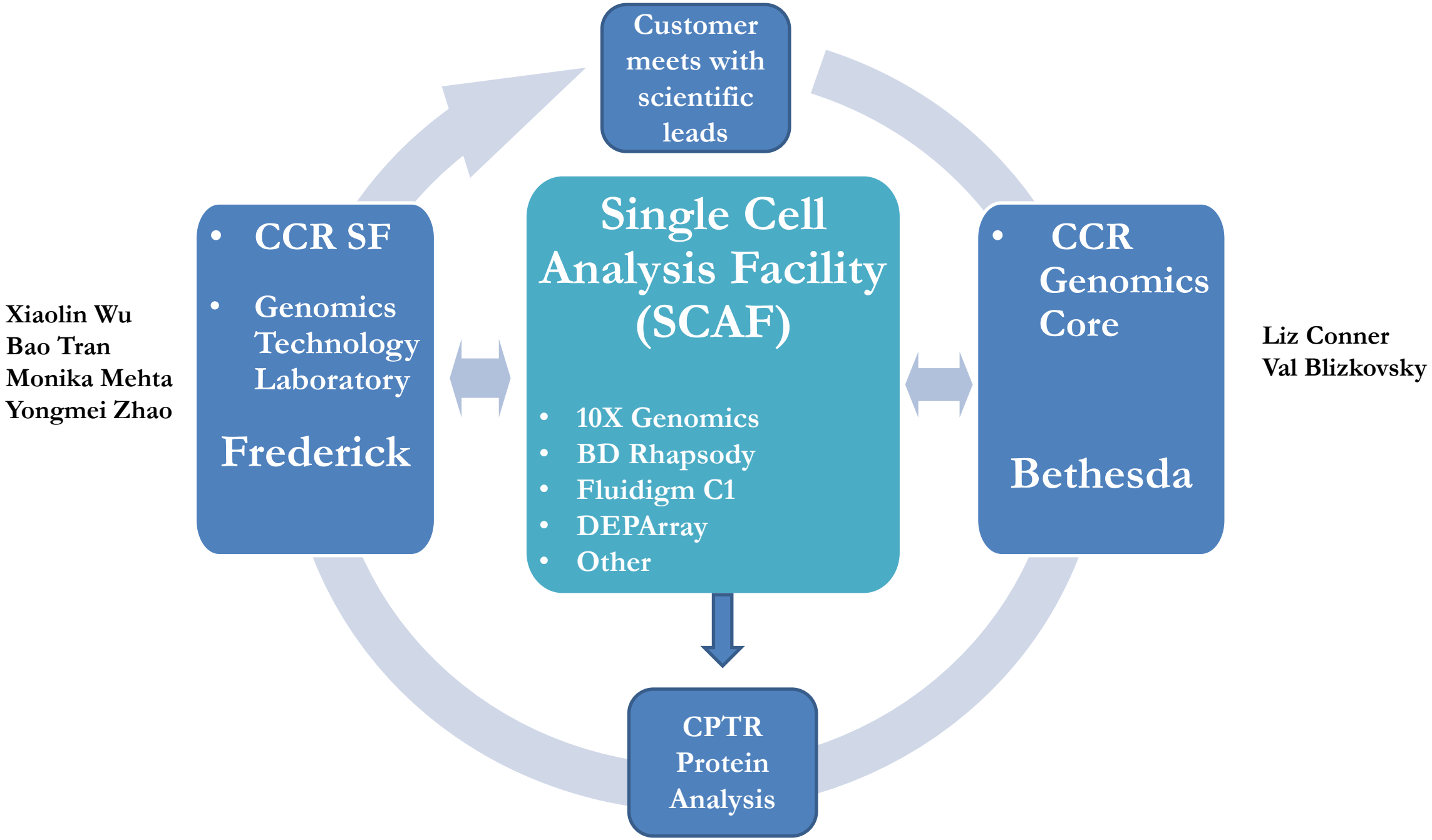


The New CCR Single Cell Analysis Facility (SCAF)

Xiaolin Wu, PhD

NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research


Customer meets with scientific leads
Single Cell Analysis Facility (SCAF)
10X Genomics
BD Rhapsody
Fluidigm C1
DEPArray
Other
CCR SF
Genomics Technology Laboratory
Frederick
CCR Genomics Core
Bethesda
CPTR Protein Analysis
Xiaolin Wu
Bao Tran
Monika Mehta
Yongmei Zhao
Liz Conner
Val Blizkovsky

Low throughput
Tens to hundreds cells

High throughput
Thousands to millions of cells

Fluidigm C1

DEPArray

Single Cell Analysis Facility
(SCAF)
Bldg37-1stFloor
(new lead scientist)

10xGenomics
Chromium

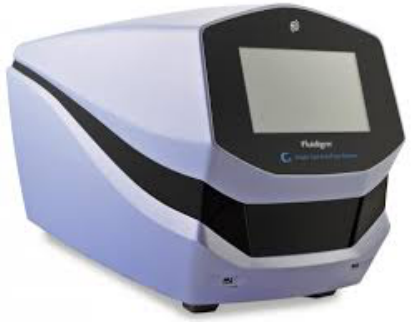
BD Genomics
Rhapsody

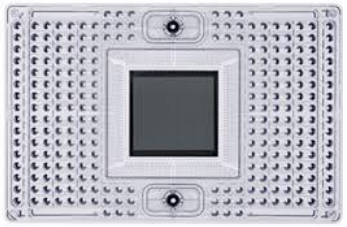
Other new
platforms

Chip (Microfluidics) Based Single Cell Technologies

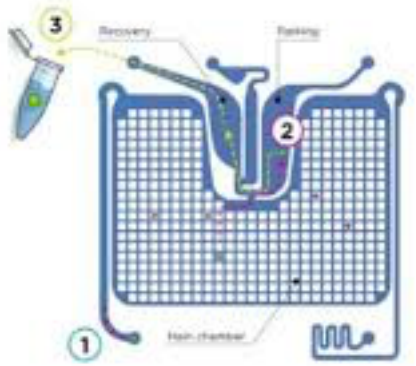
Frederick National Laboratory for Cancer Research

Fluidigm C1



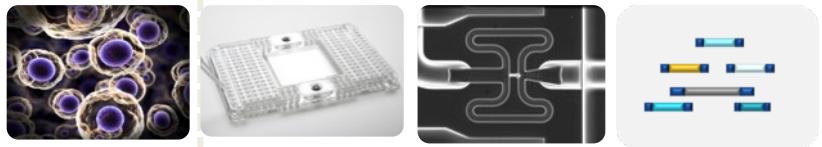


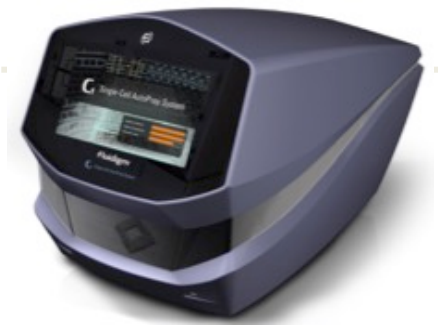
DEPArray



Fluidigm C_1 Single-Cell Auto Prep System

Enrich → Load & Capture → Wash, Stain & Image → Lyse, RT & Amplify





Fluidigm C_1 Single-Cell Auto Prep System

Chips optimized to isolate cells by cell size:
- 5-10 micron: stem cells and WBC
- 10-17 micron: iPS, progenitor cells, and others
- 17-25 micron: fibroblasts, keratinocytes, and others

Prepare Library → Sequence → Analyze

RNA-Seq on any Illumina System

Transfer → Load → Amplify & Detect

Gene Expression Profiling: BioMark System

- Self-service following training
- OSTR Subsidy available (50%)

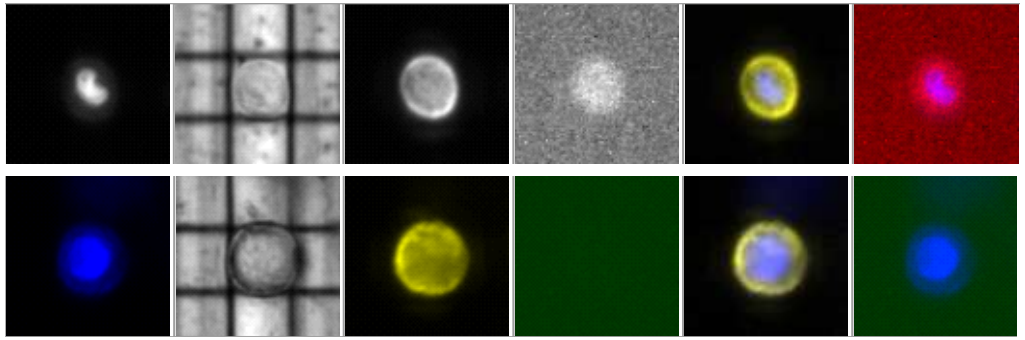
DEPArray Technology for Rare Cell Isolation

- Live cells
- Fixed cells
- FFPE
- Blood
- FNA
- Bone marrow
- Fresh Frozen

→ Enrichment

Stained single cell suspension

➢Imaged-based Cell Selection



➢Up to 5 fluorophores

Table 2: Configuration of DEPArray™ filter set-up with PerCP-Cy5.5.

Cube	Filter	Emission [nm]
1	DAPI	417 - 477
	PE	568.5 - 591.5
	BRIGHTFIELD	-
2	FITC	500 - 520
	APC	661.5 - 690.5
3	FITCsb	500 - 520
4	PerCP-Cy5.5	690-730

- Culturing cells
- Whole genome amplification
- Whole genome sequencing
- Expression analysis
- Mutation and CNV analysis

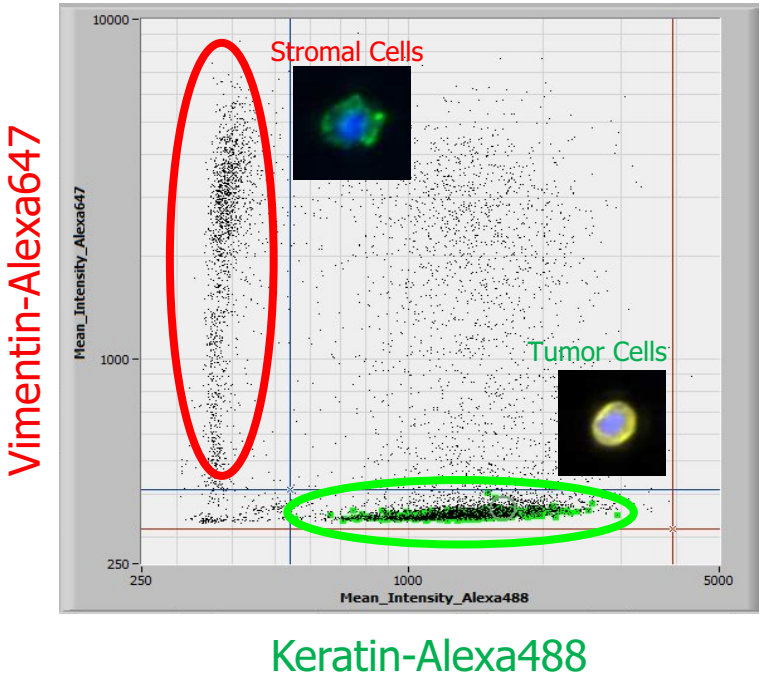
DEPArray Applications

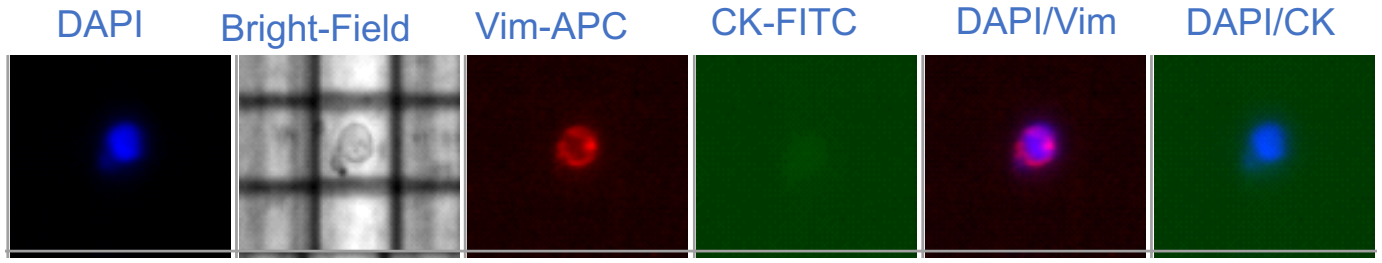
Liquid biopsy: CTCs enumeration and isolation from human and mouse models blood sample

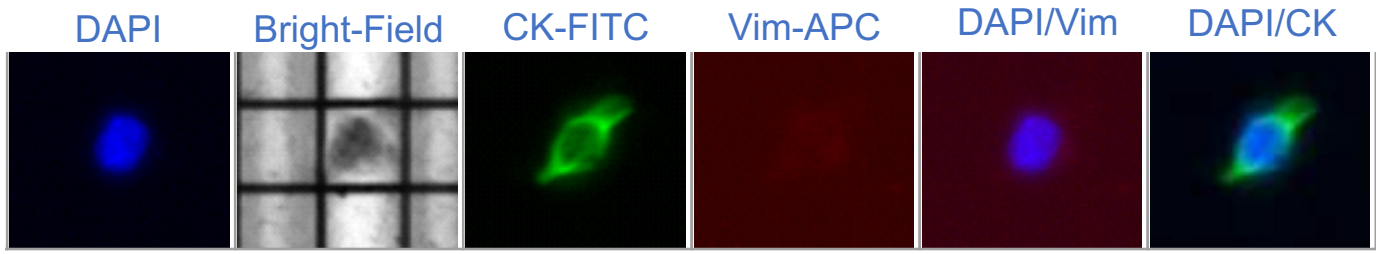
- Progression of disease-efficacy of therapy-predictive biomarker

Formalin-Fixed Paraffin Embedded FFPE samples: Isolation of pure tumor and stromal cell populations for genomic analysis

- Analysis of sample with low tumor cellularity and resolve tumor heterogeneity

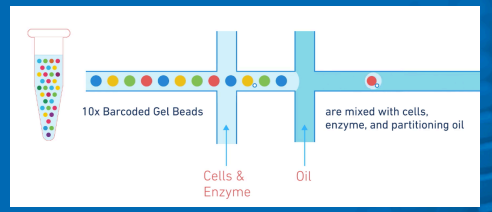




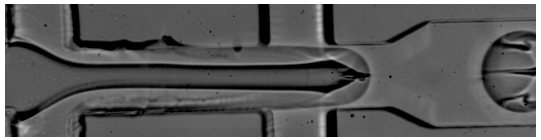


						class	unsrt		V+K-		K+V-									
						DNA index	-		DIx		DIx					1.7				
						n. cells	478	956	85	300	85			300		600				
						uniformity	98.3	99.6	96.8	97.9	95.9	96.5	96.9	97.0	97.8	98.1				
	gene	chrom	pos	ref	alt	L22_06	L24_06	L16_05	L17_05	L19_05	L20_06	L12_04	L15_05	L21_06	L13_04	GVC	event	eff	ann
1	APC	chr5	112175770	G	A	100	100	100	100	100	100	100	100	100	100		grm HOM		C/rs
2	FGFR3	chr4	1807894	G	A	100	100	100	100	100	100	100	100	100	100		grm HOM		rs
3	PDGFRA	chr4	55141055	A	G	100	100	100	98.3	100	100	100	100	100	100		grm HOM		C/rs
4	CSF1R	chr5	149433596	TG	GA	98.3	98.7	98.6	98.9	98.8	100	100	98.6	100	98.8		grm HOM		rs
5	TP53	chr17	7579472	G	C	80	88.1	59.1	50.1	98.6	94.8	98.3	95.3	94.6	96.8		LoH	M	C/rs
6	KDR	chr4	55980239	C	T	76.7	80.1	43.1	33.4	100	100	98.8	100	99.9	95.1		LoH		rs
7	MET	chr7	116339642	G	T	67.8	67.3	40.5	54.6	64.4	60.1	70	71.7	70.4	65.7		grm HET CNVg	M	C/rs
8	EGFR	chr7	55249063	G	A	63.3	64.4	41.7	56.5	73.4	58.2	66.4	58.3	70.4	72.3		grm HET CNVg		C/rs
9	TP53	chr17	7578461	C	A	61.2	60.6	0	0	96.6	100	100	98.3	94.5	91.2		som HOM	M	C/rs
10	FLT3	chr13	28610183	A	C	50.5	57.5	56.1	44.4	59.7	46.4	49.9	56.6	60.5	45.2		grm HET	S	C/rs
14	KDR	chr4	55962546	-	G	9.1	9.75	48.3	50.5	0	0	0	0	0	0		LoH		rs
15	VHL	chr3	10183852	C	A	2.9	4.6	6.5	3.3	2.7	5.9	6.9	5.1	5.3	5		noise		
16	KDR	chr4	55972974	T	A	2.1	2.1	11.1	18	0	0	0	0	0	0		LoH	M	C/rs
17	PTEN	chr10	89711834	-	T	0	0	4.95	6.2	1.32	0	0	0.2	0	0		noise		

Droplet Based Single Cell Systems



Frederick National Laboratory for Cancer Research

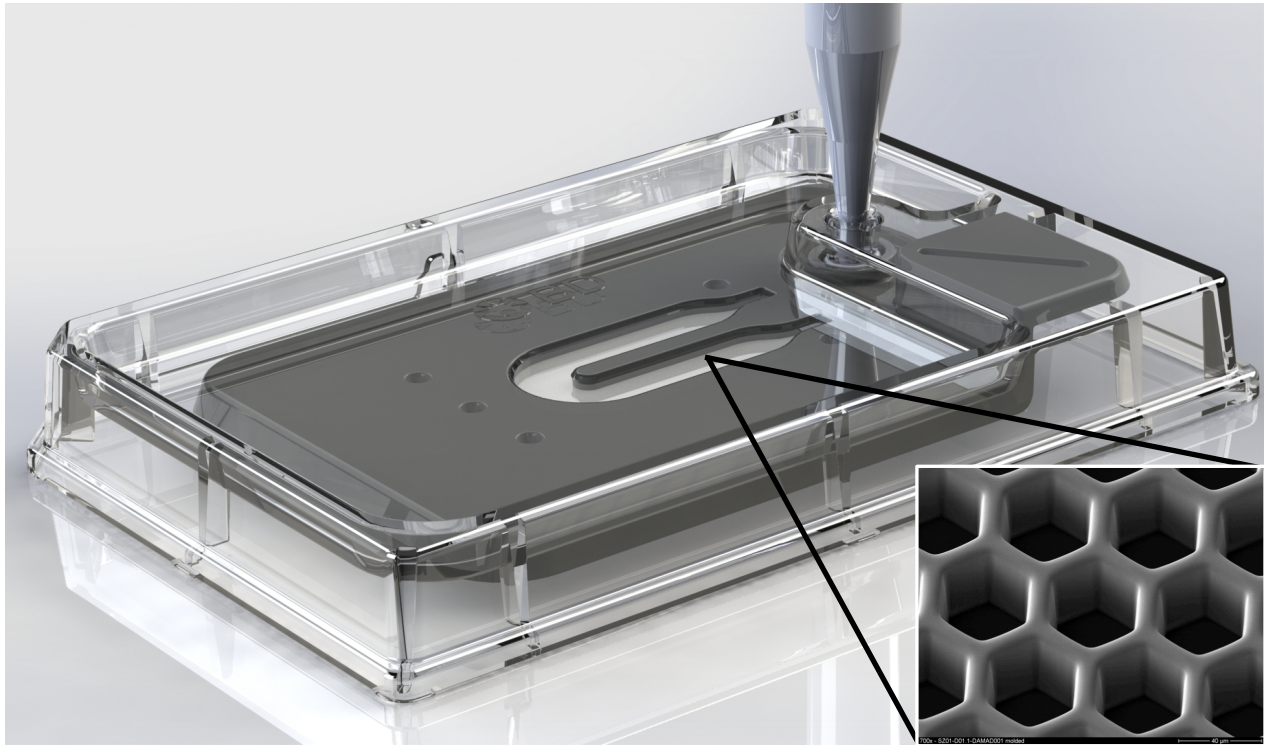


- Custom-Built DropSeq
- Droplet based
- Process 10,000 or more cells
- Not commercial system, needs lots of optimization
- Initially offered as core service
- No longer offered

- Illumina ddSEQ
- Droplet based
- Process 300-1,200 cells
- Tested, will wait for next update to see if it's worth to offer as service

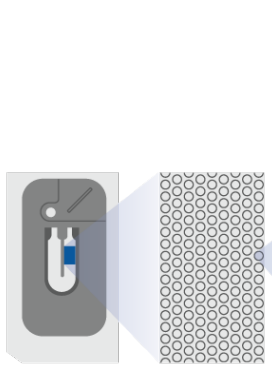
- 10xGenomics Chromium
- Droplet based
- Process 500-10,000 cells per well
- 8 wells per cartridge
- Purchased, will be offered at the SCGF when open

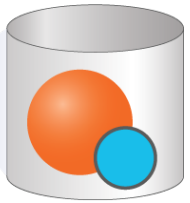
MicroWell cartridge-Based, BD Rhapsody



- Single use
- Easy to load
- 200k+ MicroWells for capture of cells and beads

BD Rhapsody workflow

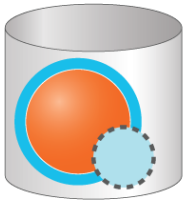




Microwell

Barcoded bead

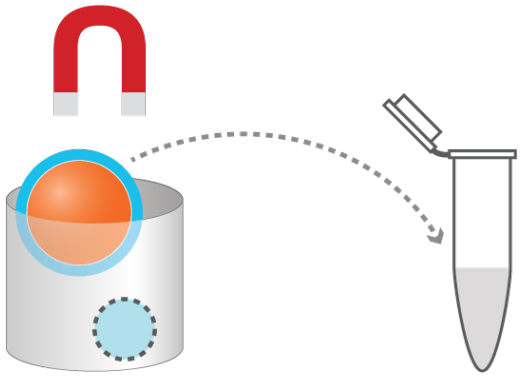
Cell

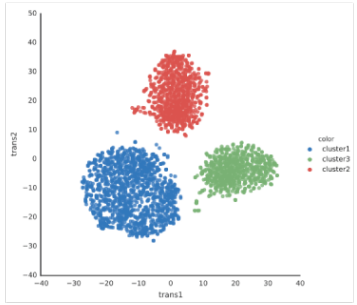


mRNA

Barcoded bead

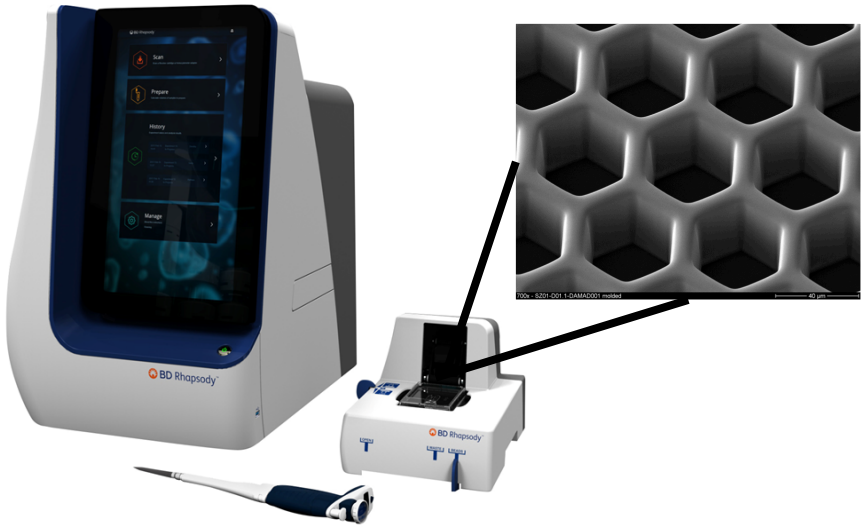
Lysed cell



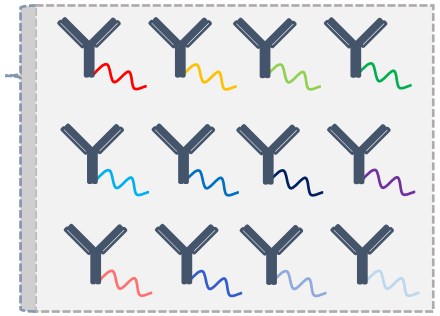


Pair ONE cell with ONE barcoded bead in microwell → Lyse cell to hybridize mRNA onto barcoded capture oligos on bead → Retrieve beads → Synthesize cDNA → Sequence and construct single cell gene expression profiles

BD Rhapsody System



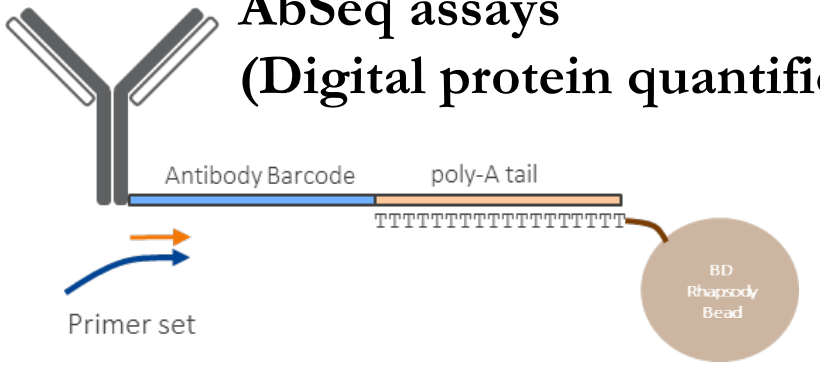
Targeted gene expression assays



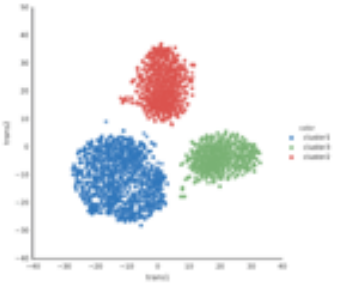
Sample multiplexing kit

Pre-designed and custom base panels available

- Flexibility to customize panels
- New BD tools and workflows for RNA and protein analyses
- Simple workflow; no oils
- Re-amplification and archiving
- Greater transcript detection sensitivity with targeted panels



AbSeq assays
(Digital protein quantification)



Data analysis suite

The Benefits of a Targeted Approach

Higher Sampling Sensitivity

Lower detection threshold of rare transcripts compared to WTA or 3’ mRNA Profiling

Better Resolution

With higher sensitivity, resolve more rare and interesting cell populations & subpopulations

Economy of Sequencing Budget

Save on experimental cost by using sequencing reads for genes that matter
100,000 reads/cell x 10,000 cells=1,000,000,000 reads, a few HiSeq lanes

Simplify Analysis

Smaller datasets mean easier alignment and fewer issues with rRNA and highly expressed species

Custom & Supplemental Panels

Discovery
- Small sample numbers
- High cost per experiment

Whole transcriptome data
- Bulk RNA-seq
- Gene expression microarray
- Low-throughput single-cell 3′ RNA-seq
- High-throughput single-cell 3′ RNA-seq

OPTION 1
Fixed panel

OPTION 2
Fixed panel + Supplemental (up to 100 targets)

OPTION 3
Full Custom Panel (up to 500 targets)

BD Rhapsody

Routine analysis
- Low cost per sample
- High sample numbers
- Improved sensitivity

Complete panel flexibility

Pre-designed panels

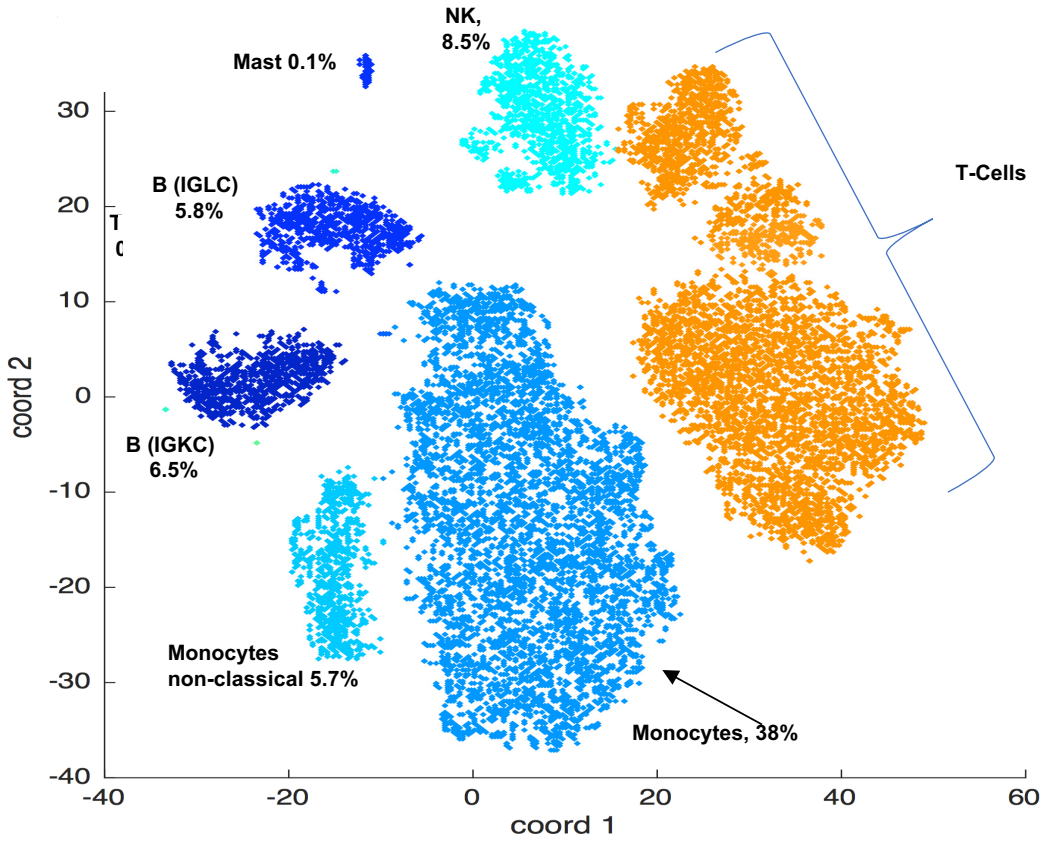
~400 targets selected and wet lab validated

- Breast cancer – Oncology
- T-cell Expression
- Immune response panels

In Development

Immune-Oncology and Oncology
Developmental Biology/IPS, Neurobiology
Panels of thousands of targets

Power of single cell analysis



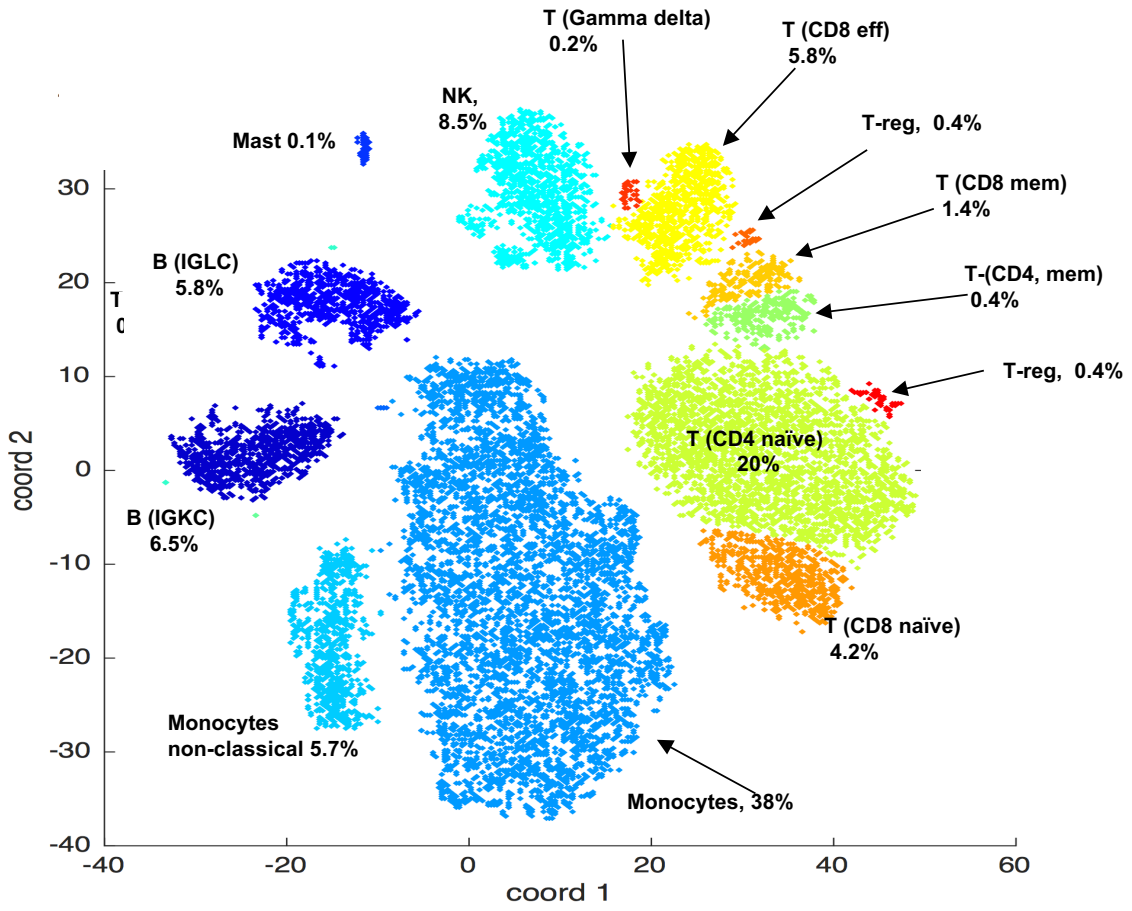
10,000 PBMCs –Healthy Donor

Immune Response Panel (399)

CD marker	52
Cell type-specific marker	44
Immunoglobulin	33
Interleukin	33
Chemokine/chemokine receptor cytokine/cytokine receptor	60
Other*	196

~10,000 reads per cell

Power of single cell analysis



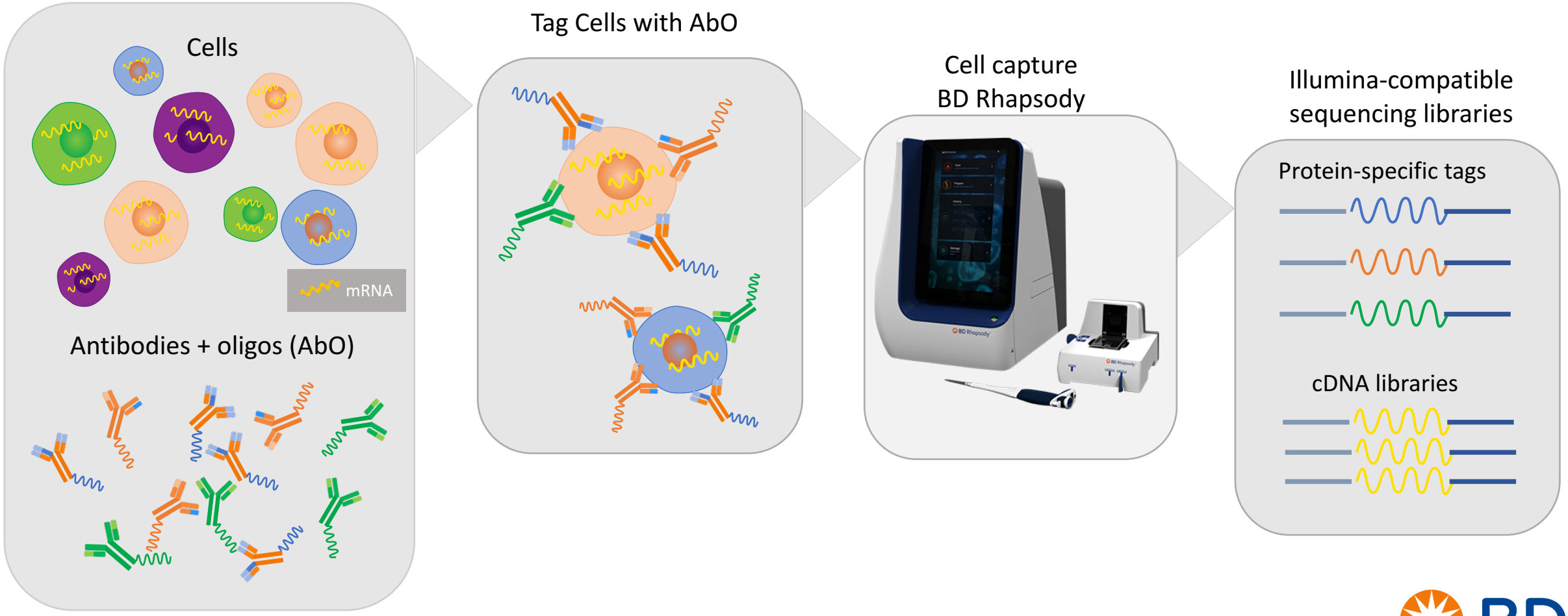
10,000 PBMCs –Healthy Donor

Immune Response Panel (399)

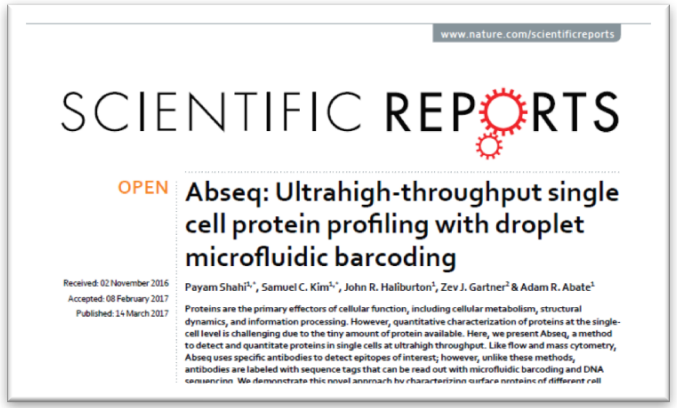
CD marker	52
Cell type-specific marker	44
Immunoglobulin	14
Interleukin	33
Chemokine/chemokine receptor cytokine/cytokine receptor	
Other*	196

~10,000 reads per cell

AbSeq: Simultaneous RNA and Protein



Antibody-Oligo Construct


www.nature.com/scientificreports

SCIENTIFIC REPORTS

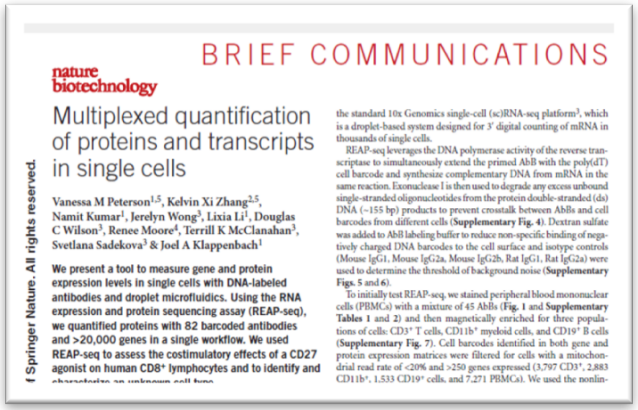
OPEN

Abseq: Ultrahigh-throughput single cell protein profiling with droplet microfluidic barcoding

Received: 02 November 2016
Accepted: 08 February 2017
Published: 14 March 2017

Payam Shahi[1,*], Samuel C. Kim[1,*], John R. Haliburton[1], Zev J. Gartner[2] & Adam R. Abate[1]

Proteins are the primary effectors of cellular function, including cellular metabolism, structural dynamics, and information processing. However, quantitative characterization of proteins at the single-cell level is challenging due to the tiny amount of protein available. Here, we present Abseq, a method to detect and quantitate proteins in single cells at ultrahigh throughput. Like flow and mass cytometry, Abseq uses specific antibodies to detect epitopes of interest; however, unlike these methods, antibodies are labeled with sequence tags that can be read out with microfluidic barcoding and DNA


BRIEF COMMUNICATIONS

nature biotechnology

Multiplexed quantification of proteins and transcripts in single cells

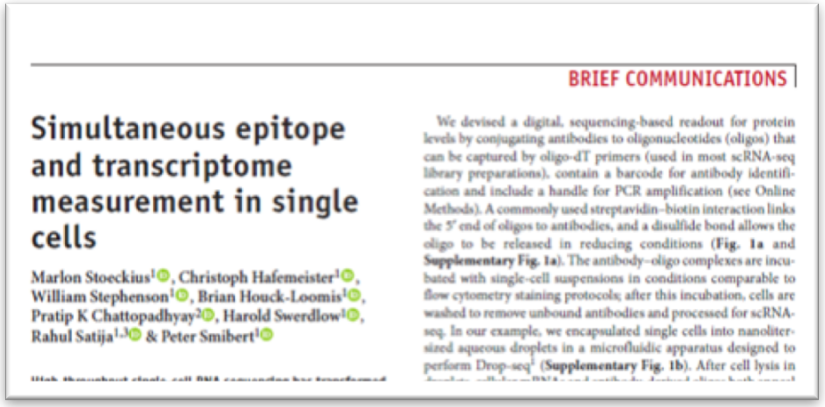
Vanessa M Peterson[1,5], Kelvin Xi Zhang[2,5], Namit Kumar[1], Jerelyn Wong[3], Lixia Li[1], Douglas C Wilson[3], Renee Moore[4], Terrill K McClanahan[3], Svetlana Sadekova[3] & Joel A Klappenbach[1]

We present a tool to measure gene and protein expression levels in single cells with DNA-labeled antibodies and droplet microfluidics. Using the RNA expression and protein sequencing assay (REAP-seq), we quantified proteins with 82 barcoded antibodies and >20,000 genes in a single workflow. We used REAP-seq to assess the costimulatory effects of a CD27 agonist on human CD8⁺ lymphocytes and to identify and

the standard 10x Genomics single-cell (sc)RNA-seq platform[3], which is a droplet-based system designed for 3′ digital counting of mRNA in thousands of single cells.

REAP-seq leverages the DNA polymerase activity of the reverse transcriptase to simultaneously extend the primed AbB with the poly(dT) cell barcode and synthesize complementary DNA from mRNA in the same reaction. Exonuclease I is then used to degrade any excess unbound single-stranded oligonucleotides from the protein double-stranded (ds) DNA (~155 bp) products to prevent crosstalk between AbBs and cell barcodes from different cells (**Supplementary Fig. 4**). Dextran sulfate was added to AbB labeling buffer to reduce non-specific binding of negatively charged DNA barcodes to the cell surface and isotype controls (Mouse IgG1, Mouse IgG2a, Mouse IgG2b, Rat IgG1, Rat IgG2a) were used to determine the threshold of background noise (**Supplementary Figs. 5** and **6**).

To initially test REAP-seq, we stained peripheral blood mononuclear cells (PBMCs) with a mixture of 45 AbBs (**Fig. 1** and **Supplementary Tables 1** and **2**) and then magnetically enriched for three populations of cells: CD3⁺ T cells, CD11b⁺ myeloid cells, and CD19⁺ B cells (**Supplementary Fig. 7**). Cell barcodes identified in both gene and protein expression matrices were filtered for cells with a mitochondrial read rate of <20% and >250 genes expressed (3,797 CD3⁺, 2,883 CD11b⁺, 1,533 CD19⁺ cells, and 7,271 PBMCs). We used the nonlin-


BRIEF COMMUNICATIONS

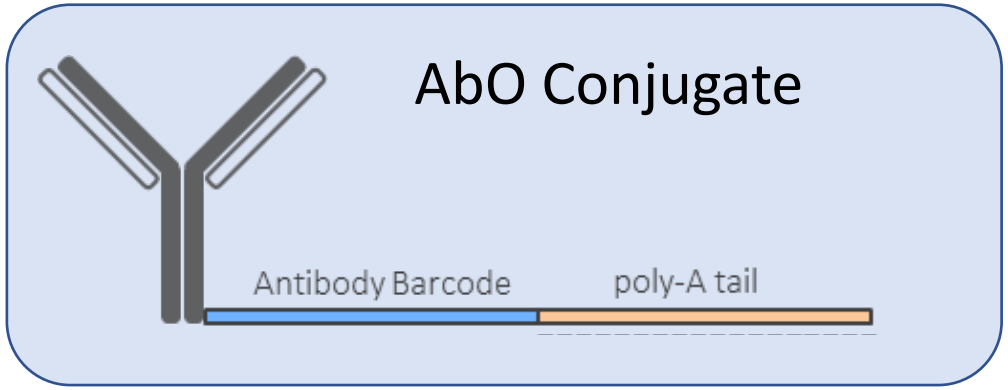
Simultaneous epitope and transcriptome measurement in single cells

Marlon Stoeckius[1], Christoph Hafemeister[1], William Stephenson[1], Brian Houck-Loomis[1], Pratip K Chattopadhyay[2], Harold Swerdlow[1], Rahul Satija[1,3] & Peter Smibert[1]

We devised a digital, sequencing-based readout for protein levels by conjugating antibodies to oligonucleotides (oligos) that can be captured by oligo-dT primers (used in most scRNA-seq library preparations), contain a barcode for antibody identification and include a handle for PCR amplification (see Online Methods). A commonly used streptavidin–biotin interaction links the 5′ end of oligos to antibodies, and a disulfide bond allows the oligo to be released in reducing conditions (**Fig. 1a** and **Supplementary Fig. 1a**). The antibody–oligo complexes are incubated with single-cell suspensions in conditions comparable to flow cytometry staining protocols; after this incubation, cells are washed to remove unbound antibodies and processed for scRNA-seq. In our example, we encapsulated single cells into nanoliter-sized aqueous droplets in a microfluidic apparatus designed to perform Drop-seq[1] (**Supplementary Fig. 1b**). After cell lysis in

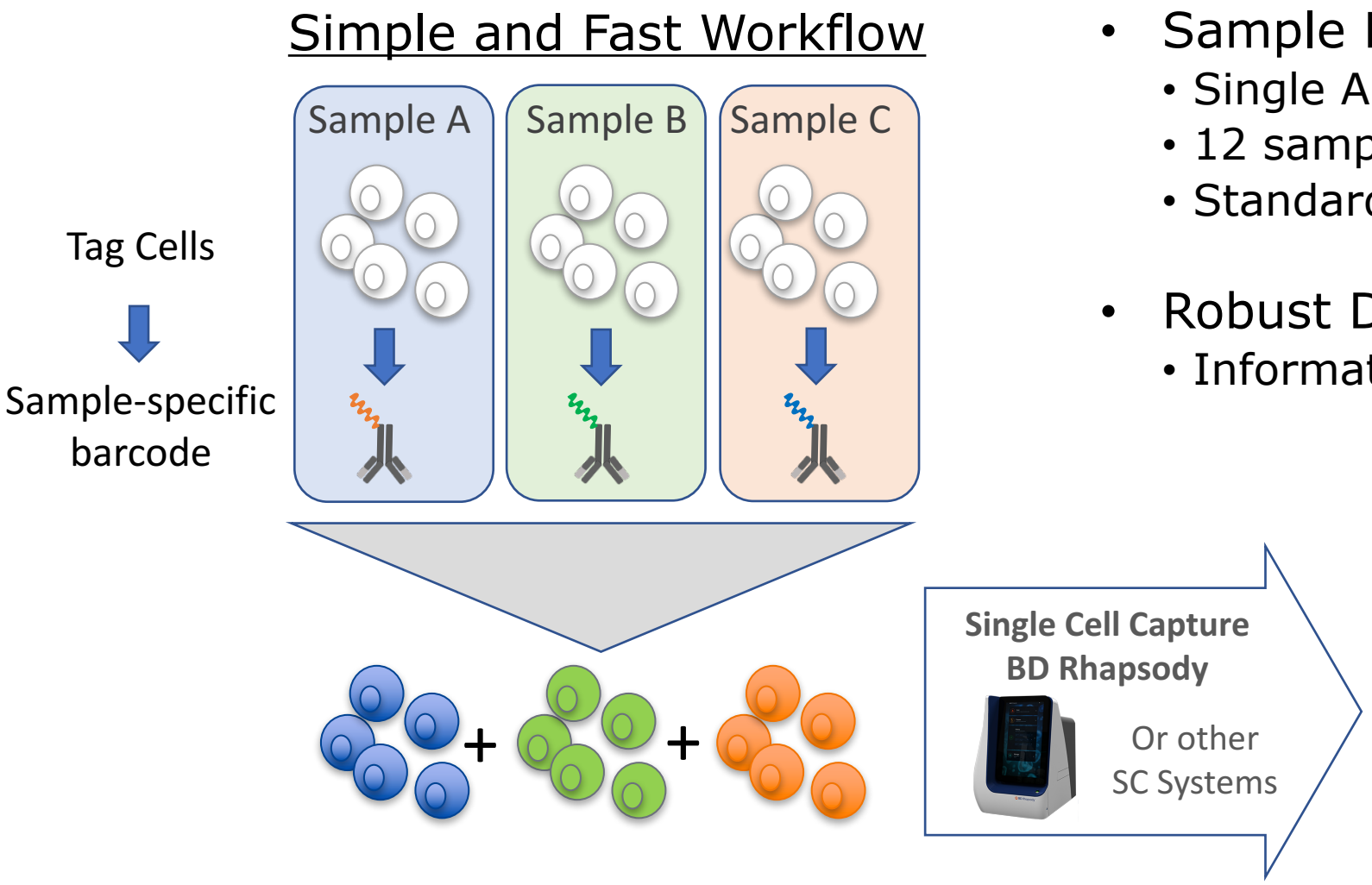
BD Developed Applications

- Sample Multiplexing
- Simultaneous RNA + Protein
  - High parameter proteomics
  - Combined with mRNA profiling
  - Simple workflow

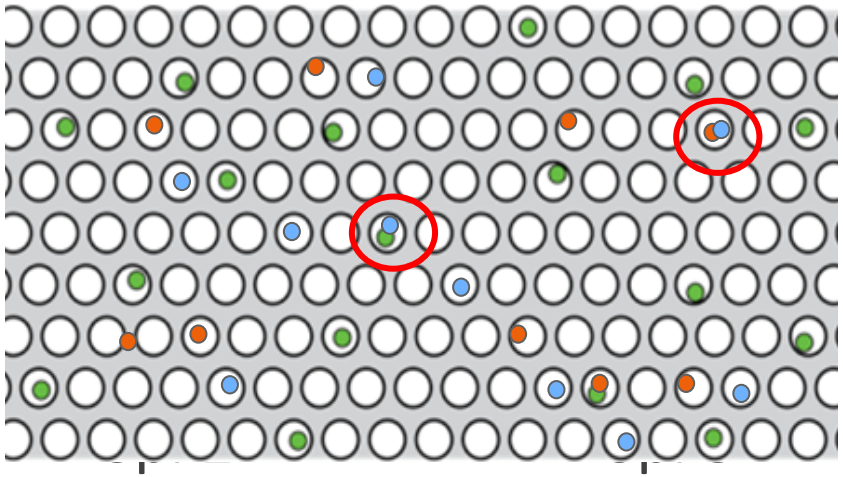


BD

Single Cell Sample Multiplexing



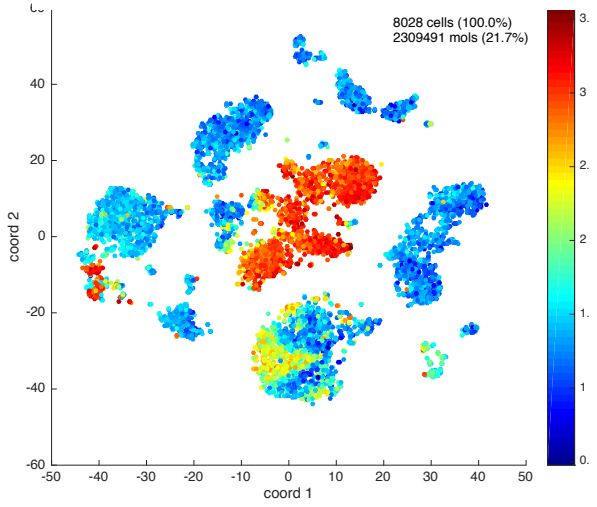
- Sample Multiplexing
  - Single Ab reagent
  - 12 sample-specific oligos
  - Standardized results and supply
- Robust Doublet detection
  - Informatics tool parse seq. data



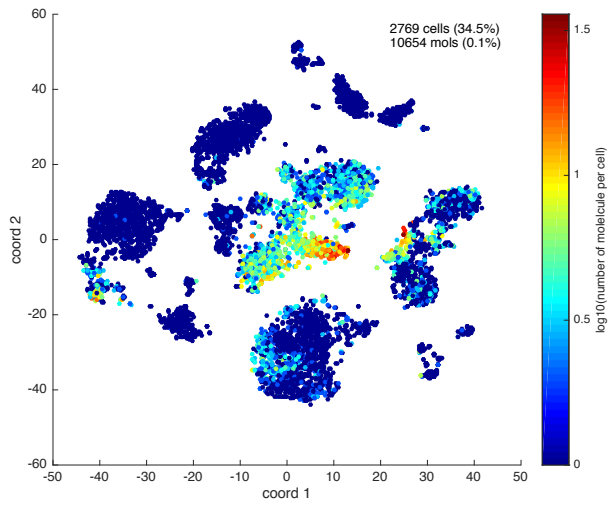
Simultaneous RNA and Protein

- BD's validated antibody portfolio
  - Broad range of specificities
  - Currently validating larger panels
- High quality reagent supply
  - Clean, efficient data
- Optimized library prep assay
- Standardized barcode design
  - Simplified bioinformatics and analysis

CD8 Cell Surface Protein



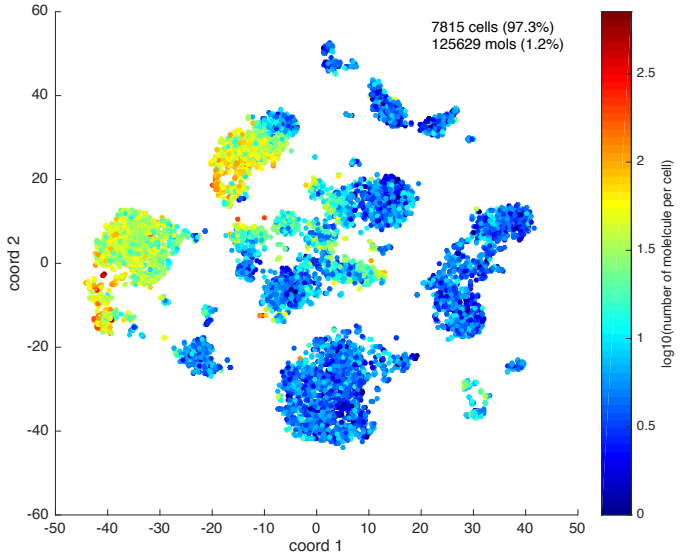
CD8 mRNA



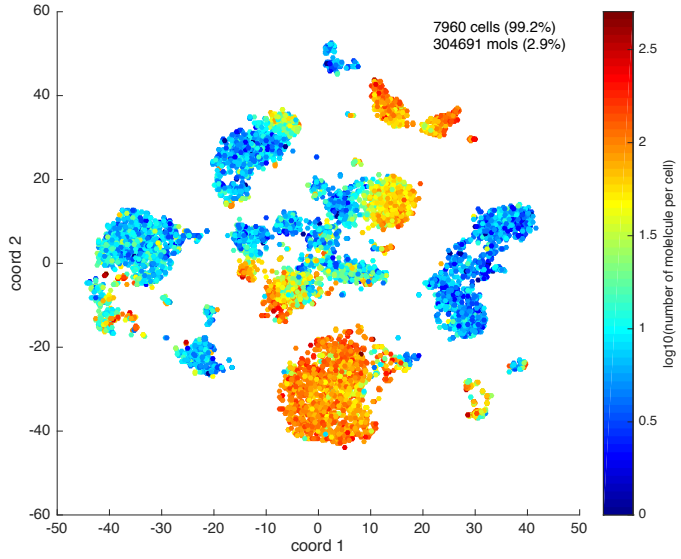
AbSeq allows detection of genes difficult to detect by 3’RNA seq assays

BD AbSeq allows detection of targets that are not in Rhapsody panel due to:
1. Low mRNA expression
2. Splice variants with splice sites away from the 3’ end

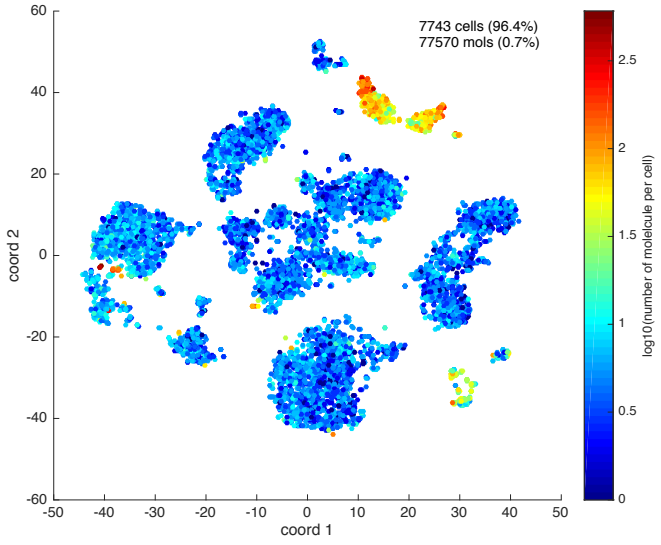
CD45RO (Memory T-cell marker)



CD45RA (Naïve T-cell marker)



CD19 (Pan B-cell marker)



SCAF is here to help you with your single cell experiments!

- Contact:
  - OSTR: Dr. David Goldstein, Dr. Mariam Malik
  - GTL: Dr. Xiaolin Wu
  - SF: Dr. Monika Mehta, Bao Tran
  - GC: Dr. Liz Conner
  - Informatics: Vishal Korpade (CCBR), Dr. Maggie Cam (CCBR), Dr. Yongmei Zhao (SF),
- In the near future, when the facility is up and running with additional staff, single contact (to be announced):

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BD