BD Rhapsody™ Single-Cell Analysis System

A robust microwell-based single-cell partitioning system for high-dimensional biology research

BD

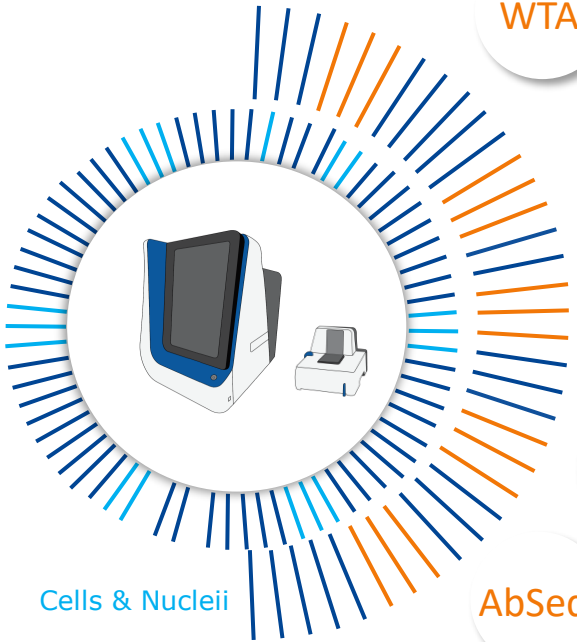
A complete single-cell multiomics offering

BD Rhapsody™ Single-Cell Analysis System

BD Rhapsody™ Assays & Reagents

BD Rhapsody™ Analysis Pipelines & SeqGeq™ Software

INSTRUMENT



Cells & Nucleii

WTA — Whole Transcriptome Amplification

Targeted — Targeted RNA-Seq

SMK — Sample multiplexing

VDJ — Immune repertoire

AbSeq — Protein Expression Analysis

ASSAYS & REAGENTS

BD Rhapsody™ WTA and Targeted Analysis Pipelines SevenBridges

SeqGeq™ Software

BIOINFORMATICS

BD Rhapsody™ Systems – two configurations

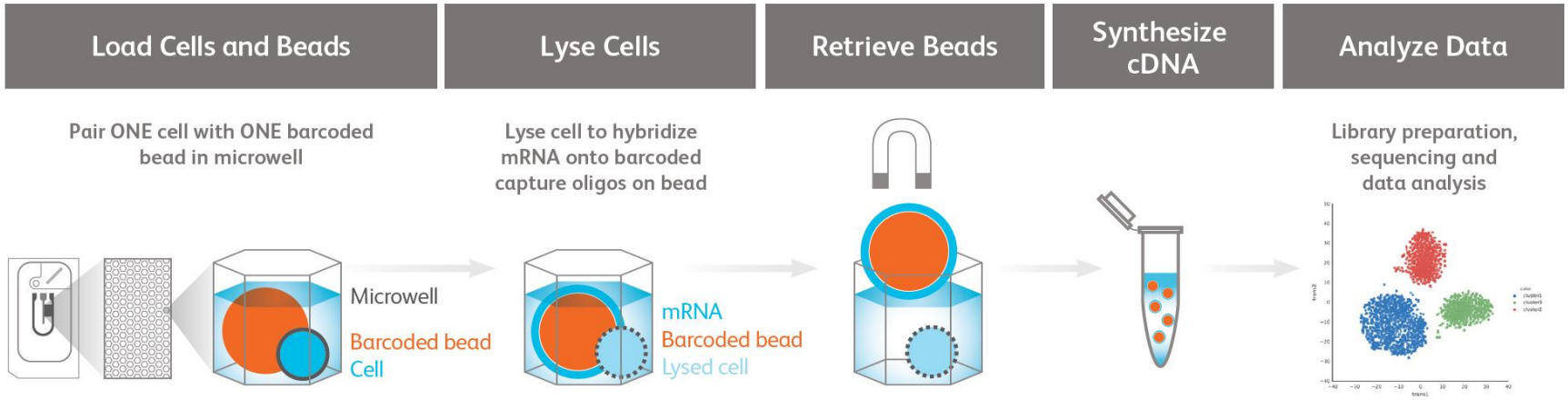
BD Rhapsody™ Single-Cell Analysis System

- BD Rhapsody™ Scanner and BD Rhapsody™ Express System
- Visual workflow QC
- Recommended for users new to single-cell workflows, developing protocols, working with novel cell types or with complex cell systems
- Allows control over every experiment and the ability to troubleshoot and optimize single-cell capture without sequencing

BD Rhapsody™ Express Single-Cell Analysis System

- BD Rhapsody™ Express System
- The same powerful single-cell partitioning system without visual workflow QC
- Recommended for experienced users working with well-established sample preparation methods and standard cell types

BD Rhapsody™ System workflow



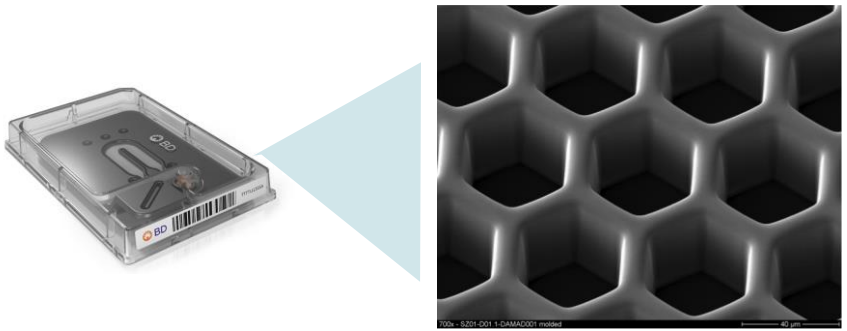
Does not use microfluidics

- Cells settle into microwells by gravity
- Volume of microwell array is ~600 µL, cell suspension loaded can be quite dilute
- Clogging of channels with cells is less of a concern

Requires minimal set of benchtop equipment

- Fluidic exchange in cartridge performed using automated pipettes on a mechanical station
- No fluidic pumps required

Unique benefits of the BD Rhapsody™ System



Microwell-based single-cell partitioning

Visual workflow QC

Stable bead-oligo complex

Microwell-based single-cell partitioning

Trust the BD Rhapsody™ System with your precious samples

- Superior Cell Capture Rates
- Low Multiplet Rates
- Highest Cell Throughput per Flow Cell
- Go Beyond PBMCs
- Reliable, Consistent Results

Superior cell capture rate

Across 75 runs, **~75%** of cells loaded onto cartridge are retrieved by sequencing

	Mean	Median	Overall variability (SD, all runs)	Intrasample variability (SD, replicate runs)
% of live cells loaded into cartridge recovered by sequencing	73.9%	75.2%	9.7%	3.4%

*number of cells seen in sequencing/live cells loaded to cartridge

- ~5000 Jurkat/Ramos cells (1:1 mix)
- Experiments performed on multiple days by multiple users
- Data taken from a total of 75 cartridge runs (~25 experiments, 2-5 replicate runs per experiment)
- Intrasample variability: ~ 3.4%
- Overall variability (including variability introduced by hemocytometer): ~ 9.7%

Cartridge capture rate for various cell types

Cell type	Tissue	Sample prep	Live cells loaded to cartridge*	Cartridge Capture rate**
CAR-T cells	Blood	Cell in-house manufacturing	25,000	72%
Mesenchymal stem cells (MSC)	Fatty tissue	Frozen, ON grown and trypsinised cells	25,000	73%
Tumor xenograft	Head and neck cancer	Dissociated tumor on Mice (PDX - paw)	25,000	67%
Total CD4+ T cells	Blood	Magnetic isolation (negative selection of total CD4 T cells)	25,000	74%
CD45+ immune cells	Duodenal biopsies	FACS sorted	25,000	68%
FACS sorted NK and T cell subsets	Blood	FACS sorted	15,000	66%
MSC (cyropreserved)	Cell lines	Cryopreserved	11,443	80%
iPSC, Adipocyte (primary fresh), GABA Neurons (cell line), Hepatocytes (primary)	Primary cells/Cell lines	Live/Cryopreserved	22,885	60%
Myeloma cell lines	Cell lines	Flask grown	11,000	73%

*Based on hemocytometer cell counts

**Capture rate = # of wells with viable cells and a bead/live cells loaded to cartridge
# of wells with viable cells and a bead is good estimation of the number of cells that could be recovered in sequencing

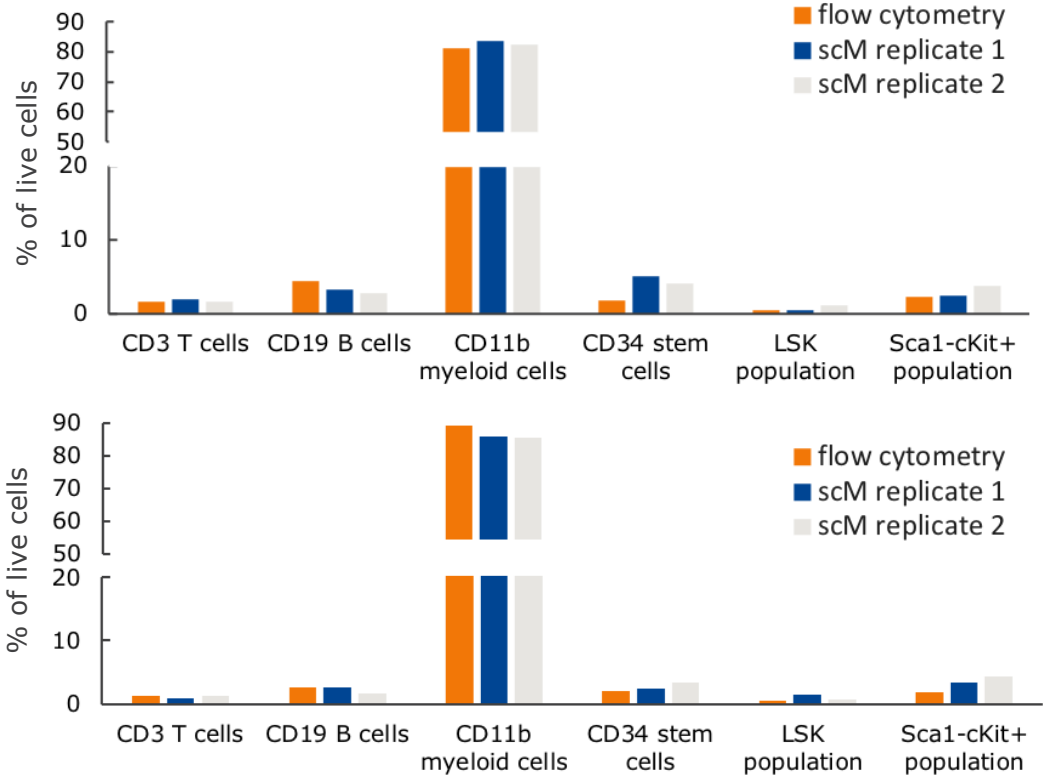
Examining capture of cell types in the BD Rhapsody™ System compared to flow cytometry

Bone marrow extracted

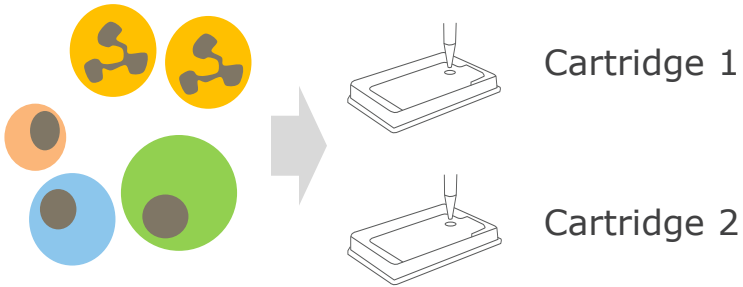
High correlation between cell types seen with the BD Rhapsody™ System and those seen using flow cytometry

Mouse 1

Mouse 2

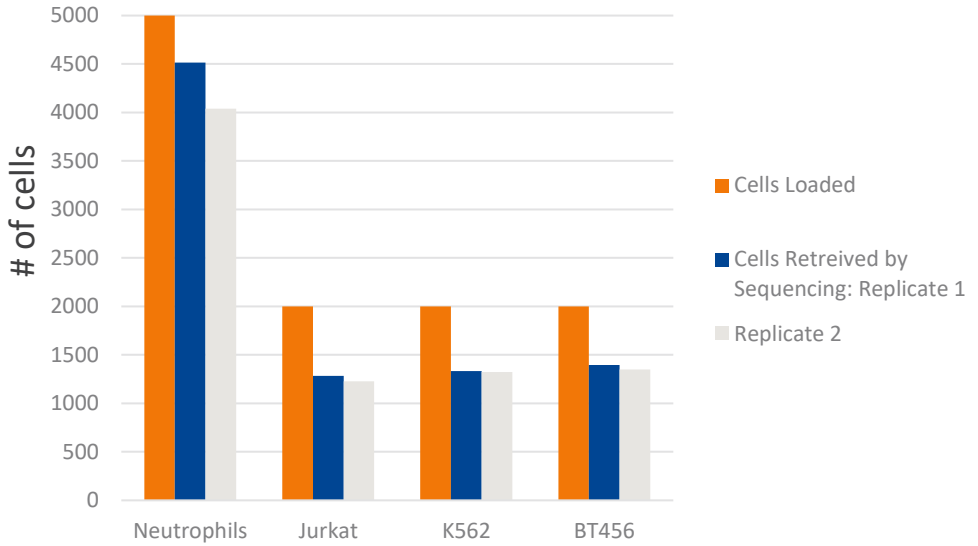


Faithful capture of cells with disparate size and morphology



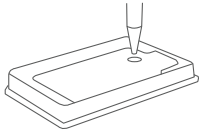
2.5:1:1:1 Neutrophils:Jurkat:K562:BT456

- Neutrophils* (10-14 um)
- Jurkat (11 um)
- K562 (16 um)
- BT546 (20 um)

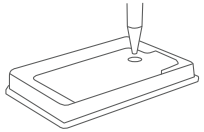


*Neutrophils (granulocytes) isolated using negative magnetic enrichment

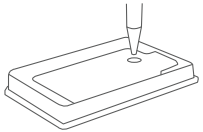
High cell capture and low multiplet rate across cell inputs



10,000 Cells



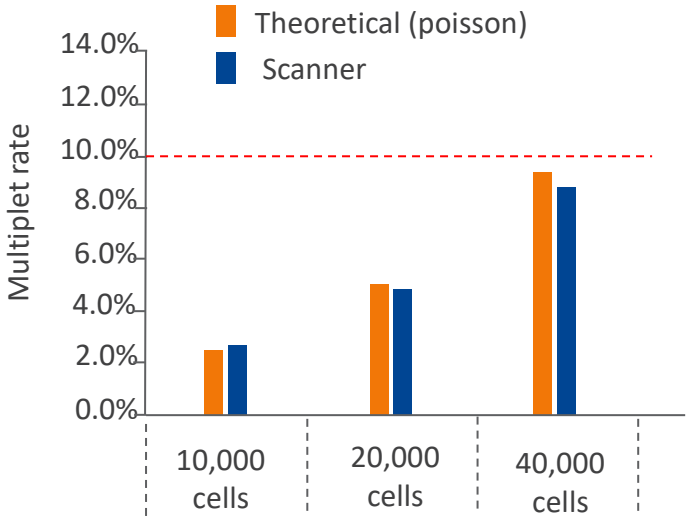
20,000 Cells



40,000 Cells

Target cell input	Live cells loaded (hemocytometer count)	Viable cells captured in wells with a bead	Capture rate
*40,000	52352	43432	0.83
*20,000	26176	22949	0.88
*10,000	13088	11789	0.90

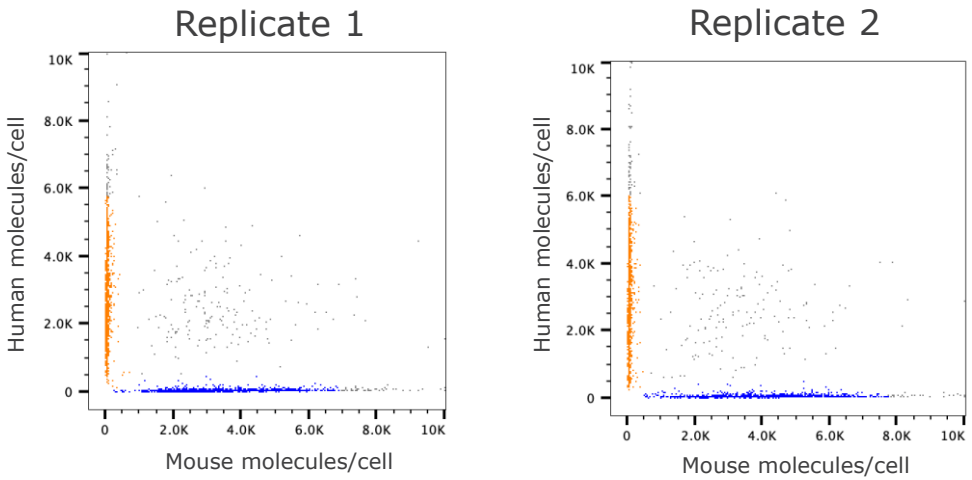
*Mix of PBMC, Jurkat, Ramos and THP1 cells

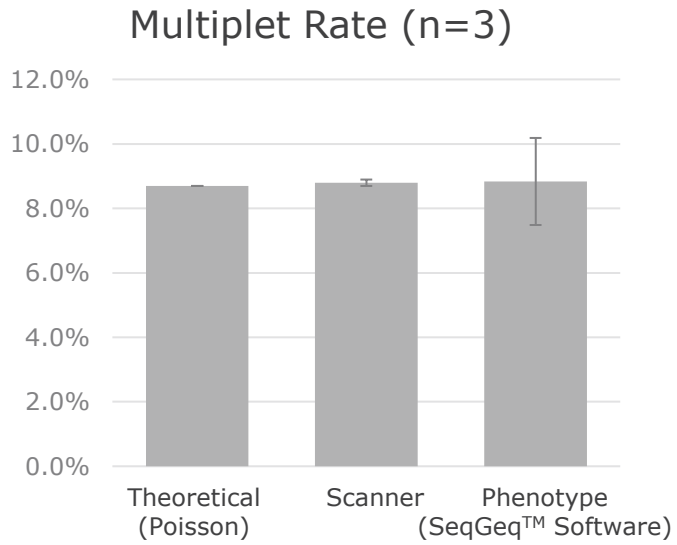


Very good correlation of theoretical multiplet rate (poisson distribution) and scanner values
~8-10% multiplets @40,000 cells/cartridge

Reproducible data with minimal cell-cell crosstalk from high cell inputs

Displaying 3,000 cells from a 40,000 cell load (1:1 mixture of human and mouse cell lines; shallow sequencing)





Superloading enables significant cost savings

Load 40,000 cells

~8% multiplet rate @ 40,000 cell load
>220,000 partitions per flow cell

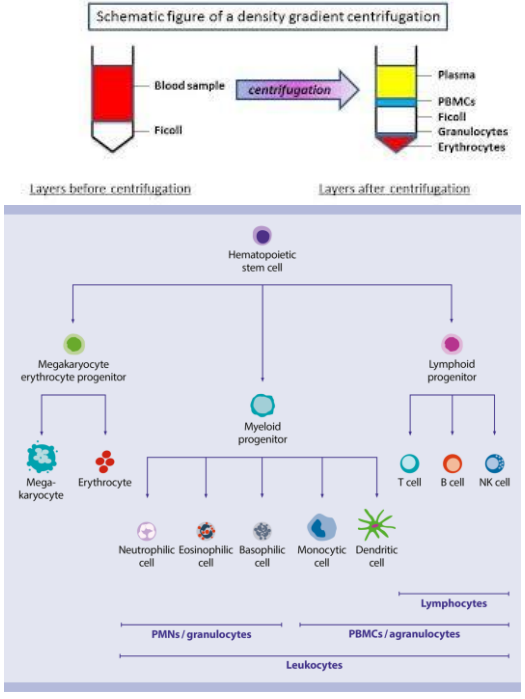
Process 40,000 cells
as one sample

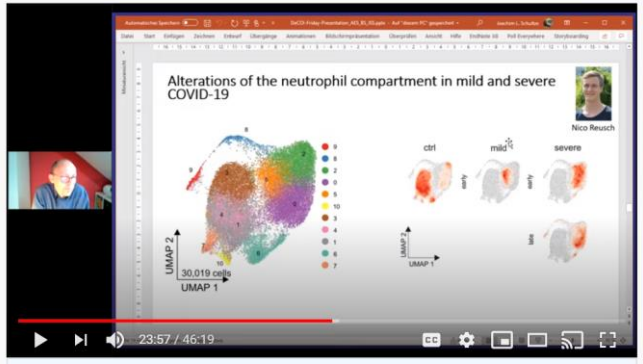
1 Sample

Platform	Assay	Sample Input	BD Rhapsody™ Cartridge + Reagents Cost	
BD Rhapsody™ System	Targeted	40,000 cells per cartridge	w/ SMK: $1,624 ($0.04/cell)	w/o SMK: $1,520 ($0.04/cell)

Go beyond PBMCs with the BD Rhapsody™ System

- Increasing recognition in the Immunology field about the importance of granulocytes in immune response



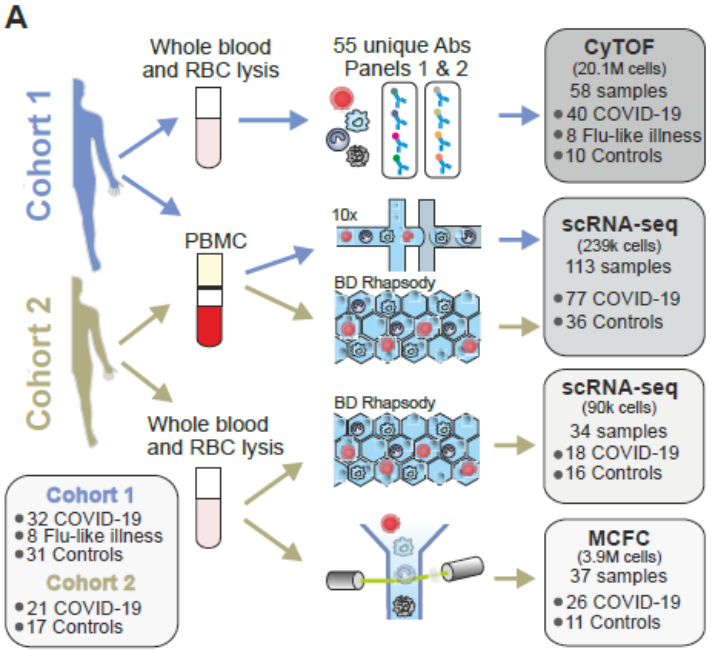


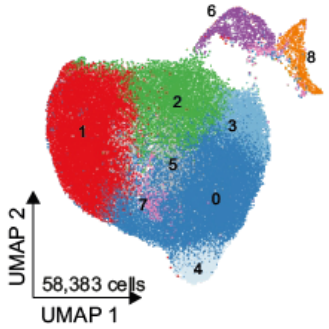
https://youtu.be/i7UIYdyFJvQ

- Microwell-based systems
  - Gold standard for fragile cells such as granulocytes
  - Reliable
  - Much less batch effects allowing easier integration of datasets

Severe COVID-19 is marked by a dysregulated myeloid cell compartment

https://doi.org/10.1016/j.cell.2020.08.001

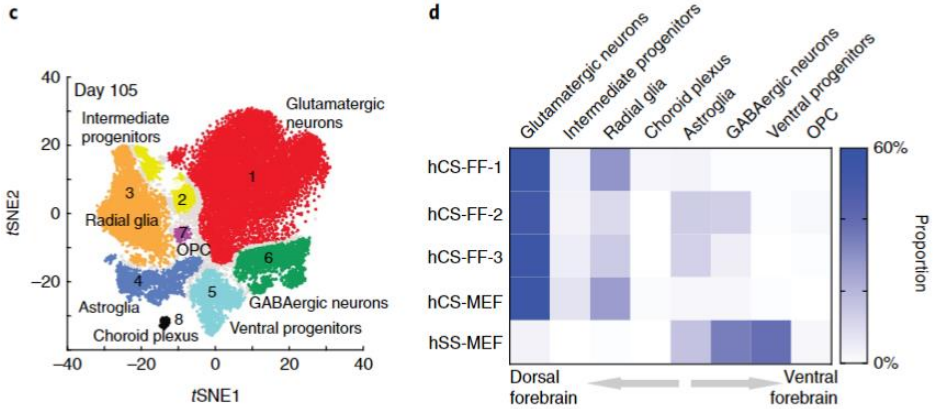




Cluster	Marker	Function
8	*FUT4*(CD15)+ CD63+ *CEACAM8*(CD66b)+	pro-Neutrophils
6	*ITGAM*(CD11b)+ CD101+	pre-Neutrophils
1	*CD274*(PD-L1)+ ISG15+ *ZC3H12A+MME*(CD10)-	mature (severe specific)
2	CD46+	mature (covid specific)
3	*MME*(CD10)+ S100A4+	mature (control)
4	*MME*(CD10)+ NFAT5+	mature (control)
0	*MME*(CD10)+ CXCR4+	mature (control)
5	HBB +	mature
7	ribosomal RNA +	mature

Whole blood CyTOF analysis (cohort 1) clearly indicated very distinct phenotypic alterations of the neutrophil compartment in mild and severe forms of COVID-19. To further delineate the underlying transcriptional programs, we performed scRNA-seq analysis on fresh whole blood samples of 23 individuals (34 samples, cohort 2, **Table S1**). Integrated visualization of all samples of cohort 2 (fresh/frozen PBMC, fresh whole blood, 229,731 cells, **Fig. S6A**) revealed the expected blood leukocyte distribution, including granulocytes (**Fig. 7A, S6A, Table S4**). Cell type distribution identified by scRNA-seq profiles (**Fig. S6B**) strongly correlated with MCFC characterization of the same samples (**Fig. S6C**). For further analysis of the granulocyte compartment, we first combined the whole blood samples with the fresh PBMC to guide the clustering of all major immune cells resulting in a total of 122,954 cells (**Fig 7A**). From these samples, we identified all neutrophil clusters and extracted the cells derived from whole blood for subsampling, which revealed a structure of 9 clusters (n=58,383 cells, **Fig. 7B+C**).

Single-cell characterization of human cortical spheroids



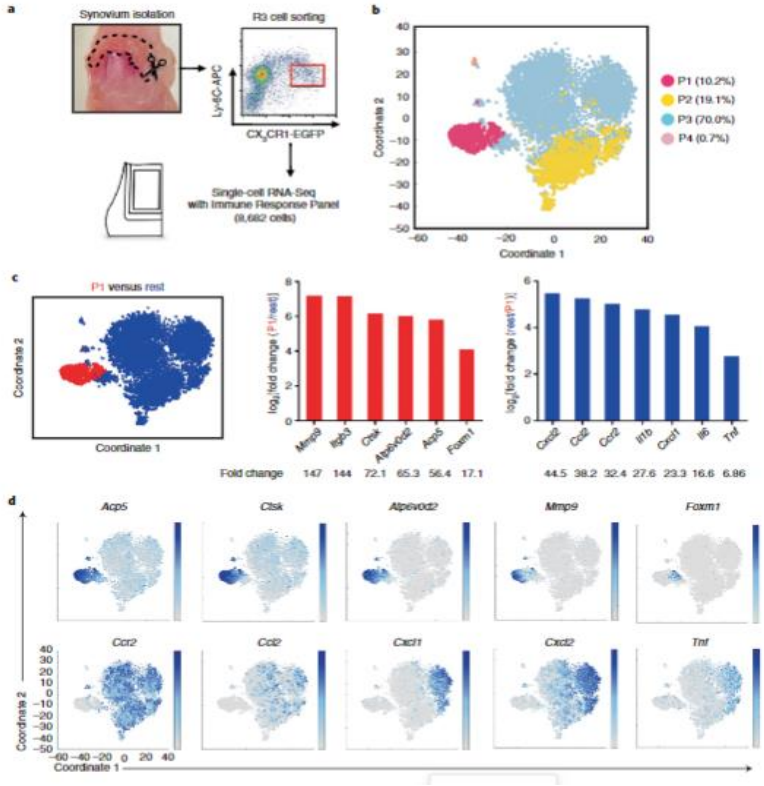
nature methods
BRIEF COMMUNICATION
https://doi.org/10.1038/s41592-018-0255-0

Reliability of human cortical organoid generation

Se-Jin Yoon[1], Lubayna S. Elahi[1], Anca M. Pașca[2], Rebecca M. Marton[1], Aaron Gordon[3], Omer Revah[1], Yuki Miura[1], Elisabeth M. Walczak[4], Gwendolyn M. Holdgate[4], H. Christina Fan[4], John R. Huguenard[5], Daniel H. Geschwind[3,6] and Sergiu P. Pașca[1,7]*

hSS-MEF is indicated. **c**,**d**, Clustering (**c**) and proportions (**d**) of all single cells across conditions (glutamatergic neuron cluster 1, $n = 11{,}367$ cells; intermediate progenitor cluster 2, $n = 1{,}018$ cells; radial glia cluster 3, $n = 4{,}217$ cells; astroglia cluster 4, $n = 2{,}036$ cells; ventral progenitor cluster 5, $n = 1{,}915$ cells; GABAergic neuron cluster 6, $n = 2{,}520$ cells; OPC cluster 7, $n = 194$ cells; choroid plexus cluster 8, $n = 170$ cells; 800 cells not assigned to a cluster). **e**, Representative hCS cryosection at day 150 of differentiation stained for deep (CTIP2) and superficial (SATB2) neuronal markers and the glial

Identification of a distinct arthritis associated macrophage population, AtoM



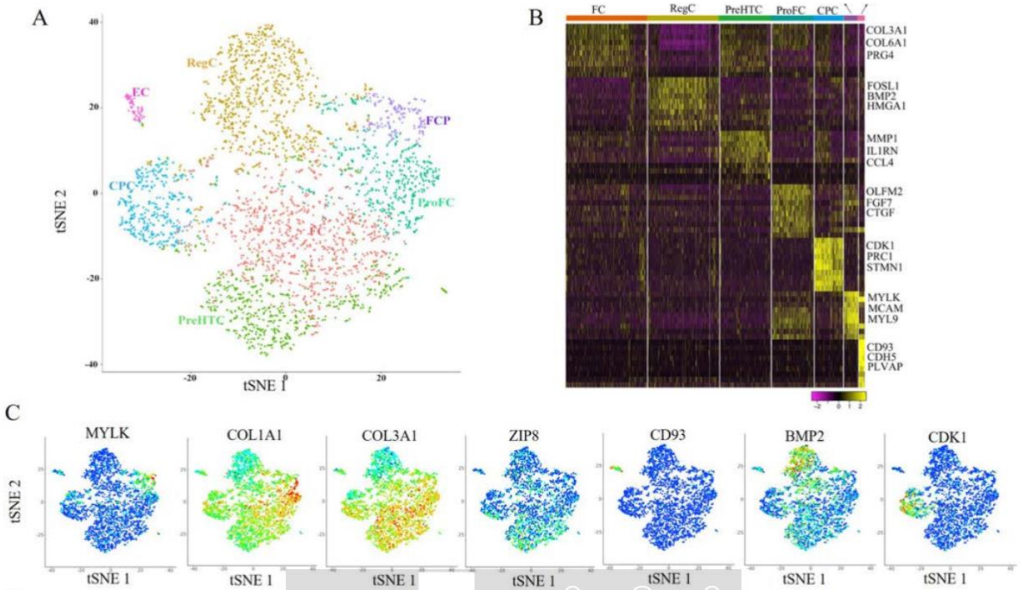
nature immunology
ARTICLES
https://doi.org/10.1038/s41590-019-0526-7

Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1

Tetsuo Hasegawa[1,2], Junichi Kikuta[1,3], Takao Sudo[1], Yoshinobu Matsuura[1], Takahiro Matsui[1], Szandor Simmons[1], Kosuke Ebina[4], Makoto Hirao[4], Daisuke Okuzaki[5], Yuichi Yoshida[6], Atsushi Hirao[7], Vladimir V. Kalinichenko[8], Kunihiro Yamaoka[2], Tsutomu Takeuchi[2] and Masaru Ishii[1,3]*

Fig. 5 | Single-cell RNA-Seq analysis of synovial R3 cells. a, Schematic outlining the single-cell RNA-Seq analysis. R3 cells were isolated from inflamed knee synovium 1 week after CIA onset. **b**, t-SNE plot of the single-cell RNA-Seq data of 8,682 R3 cells from mice with CIA. Hierarchical clustering based on gene expression profiles was performed using BD DataView software. **c**, Differential expression analysis of cluster P1 and the other cell clusters. Fold changes were calculated for differentially expressed genes. The cell numbers of the 'P1' and 'rest' groups analyzed were 784 and 1,461 (*Mmp9*), 652 and 255 (*Itgb3*), 885 and 6,011 (*Ctsk*), 885 and 1,098 (*Atp6v0d2*), 886 and 6,093 (*Acp5*), 258 and 241 (*Foxm1*), 239 and 5,685 (*Cxcl2*), 77 and 3,709 (*Ccl2*), 138 and 6,883 (*Ccr2*), 14 and 1,395 (*Il1b*), 107 and 2,662 (*Cxcl1*), 4 and 98 (*Il6*), and 180 and 3,791 (*Tnf*), respectively. **d**, Single-gene expression plots on the t-SNE plot of the single-cell RNA-Seq data.

Comprehensive census of human meniscus cells using single-cell RNA-Seq



Osteoarthritis

TRANSLATIONAL SCIENCE

OPEN ACCESS

Single-cell RNA-seq analysis identifies meniscus progenitors and reveals the progression of meniscus degeneration

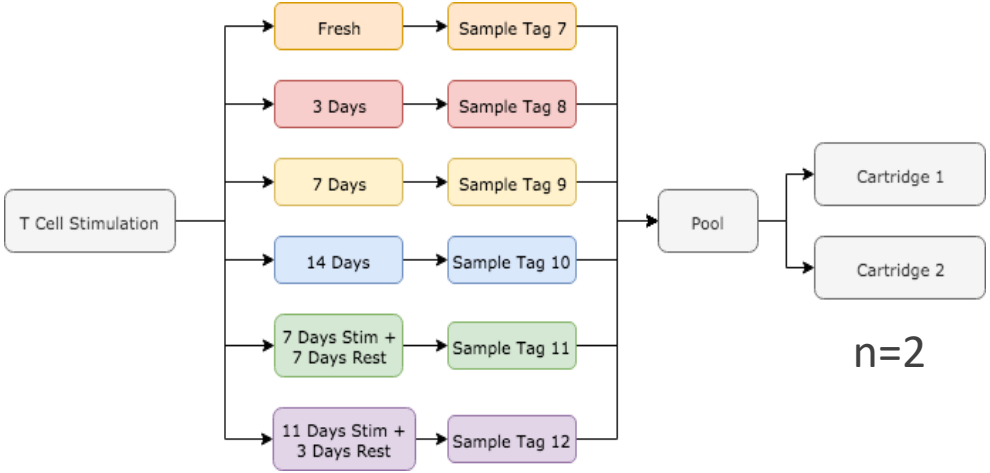
Hao Sun [1,2] Xingzhao Wen,[2] Hongyi Li,[2] Peihui Wu,[2] Minghui Gu,[2] Xiaoyi Zhao,[2] Ziji Zhang,[2] Shu Hu,[2] Guping Mao,[2] Ruofan Ma,[1] Weiming Liao,[2] Zhiqi Zhang [2]

Figure 1 A single-cell atlas of healthy human meniscus. (A) Seven healthy human meniscus cell clusters. t-Distributed stochastic neighbour embedding (t-SNE) of 3639 cells (mixed with cell fractions, n=3), annotated post-hoc and coloured by clustering. (B) Heatmap revealing the scaled expression of differentially expressed genes for each cluster. (C) Dot plots showing the expression of the indicated markers for each cell cluster on the t-SNE map. (D) Representative immunohistochemistry staining of MYLK, COL1A1, COL3A1, ZIP8, CD93, BMP2 and CDK1 in white and red zones

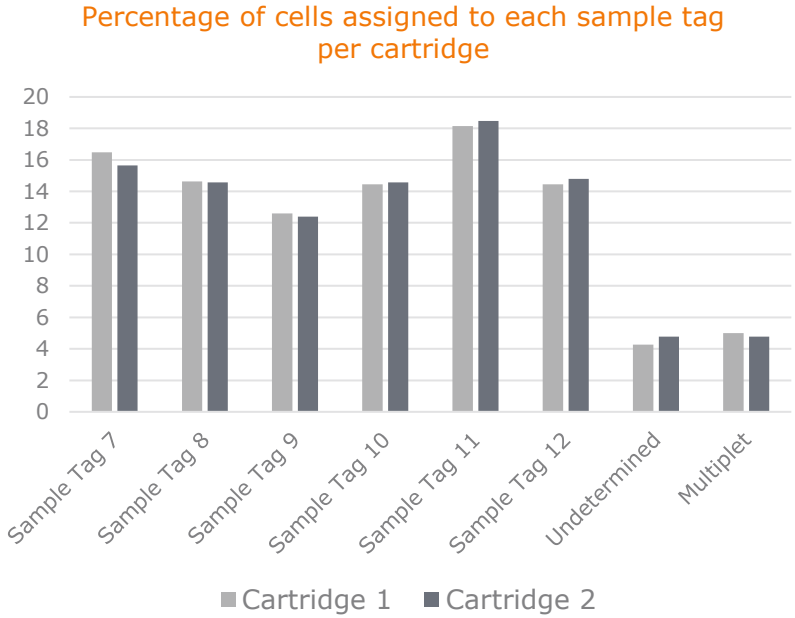
Reliable, consistent results

- Minimal batch effects
  - Technical replicates
  - Biological replicates
  - Site to site
  - User to user
- Minimum sample bias
- Ability to integrate large data sets without correction for batch effects
- System of choice for translational, multi-center, multiple cohort studies

T cell activation time-course examined across technical replicates

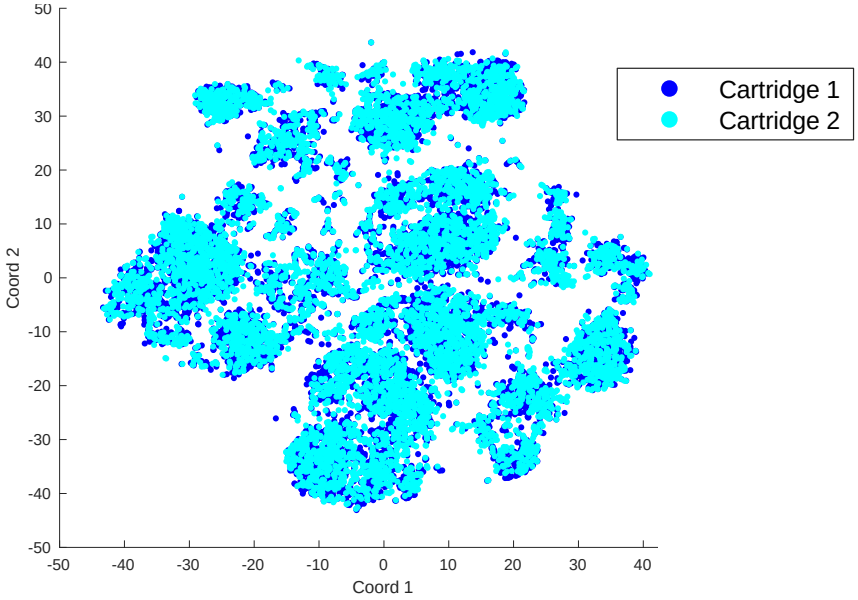


n=2

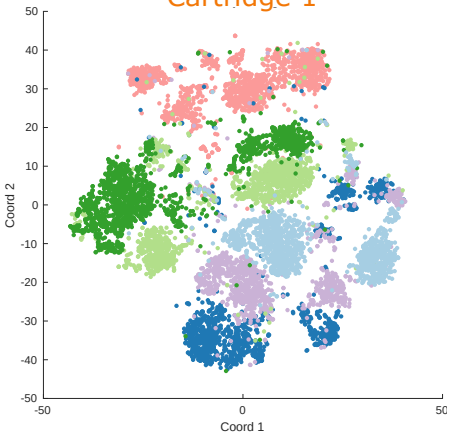


No batch effect from technical replicates

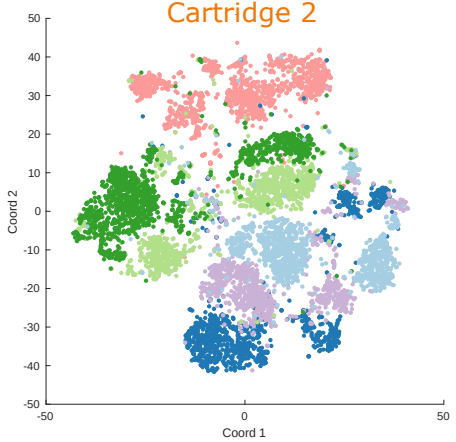
tSNE projection: Cartridge 1 vs Cartridge 2

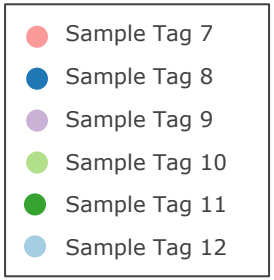


Cartridge 1



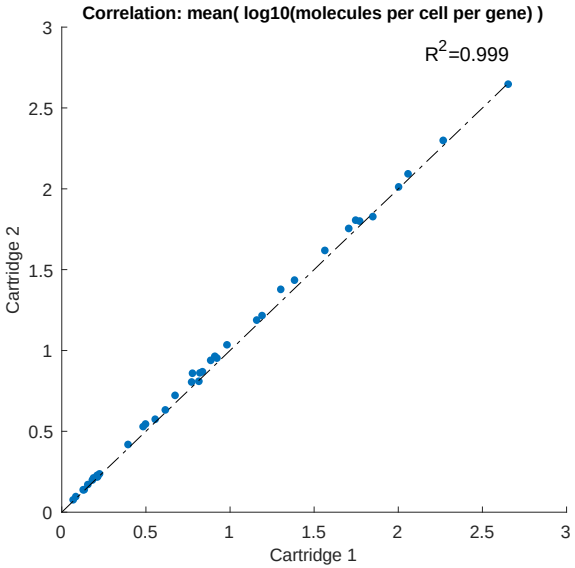
Cartridge 2





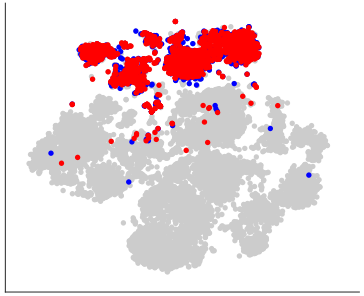
Strong correlation of mRNA and protein expression in resting and activated T cells between cartridges

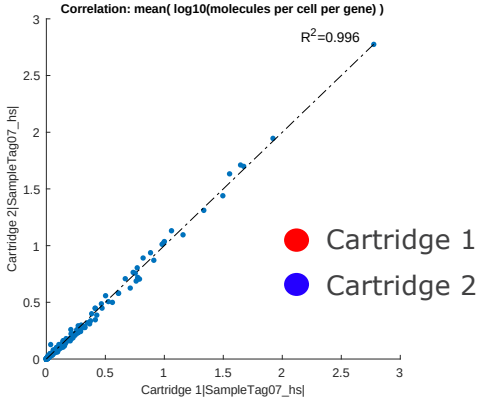
40-plex BD® AbSeq Reagents



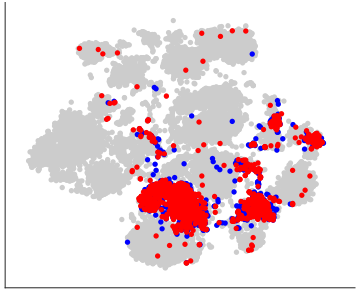
All mRNA and protein markers

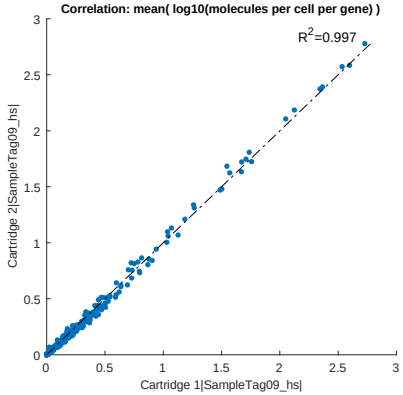
Resting T cells





Activated T cells

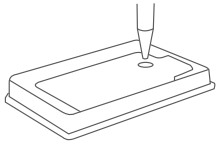




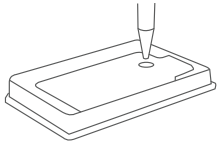
Bone-marrow derived cells examined from biological replicates

n=2

Bone marrow extracted

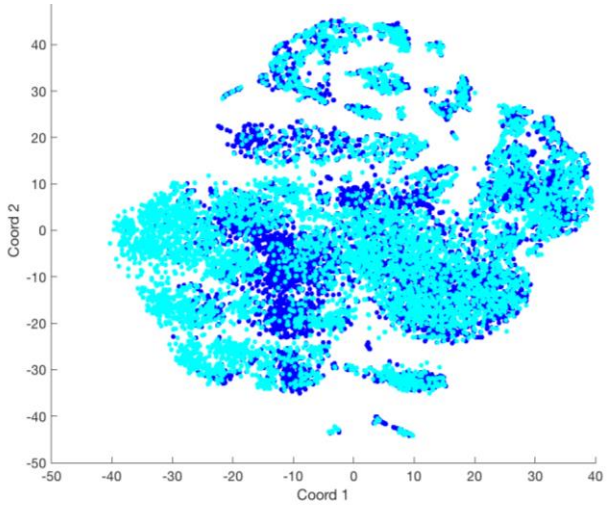


Cartridge 1 (Mouse 1)



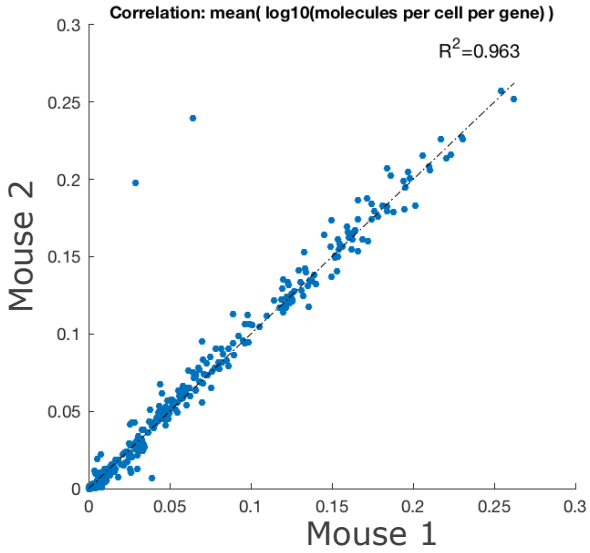
Cartridge 2 (Mouse 2)

Similar proportions of each cell type recovered from biological replicates

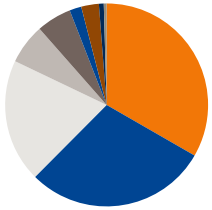


Mouse 1

Mouse 2



Mouse #1



myeloid derived supressor cells

neutrophils

immature neutrophils

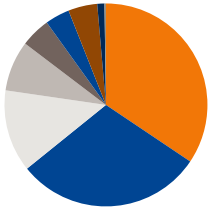
stem cells

monocyte

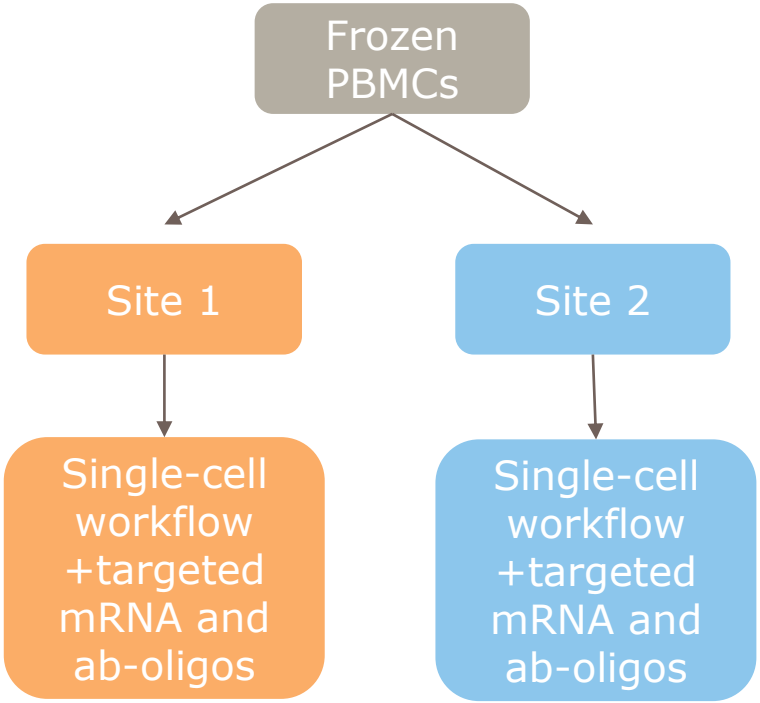
B cells

CD8 T cells

Mouse #2

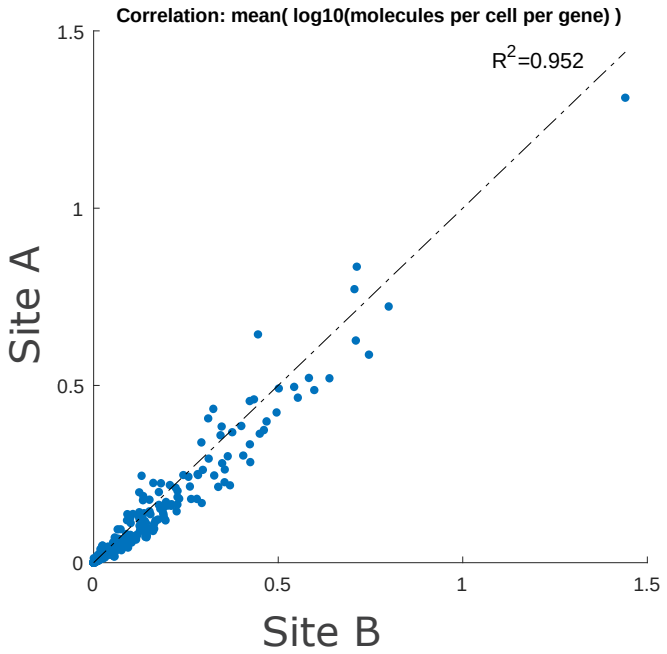


Site-to-site reproducibility examined by targeted gene expression and protein expression analysis when comparing two sites

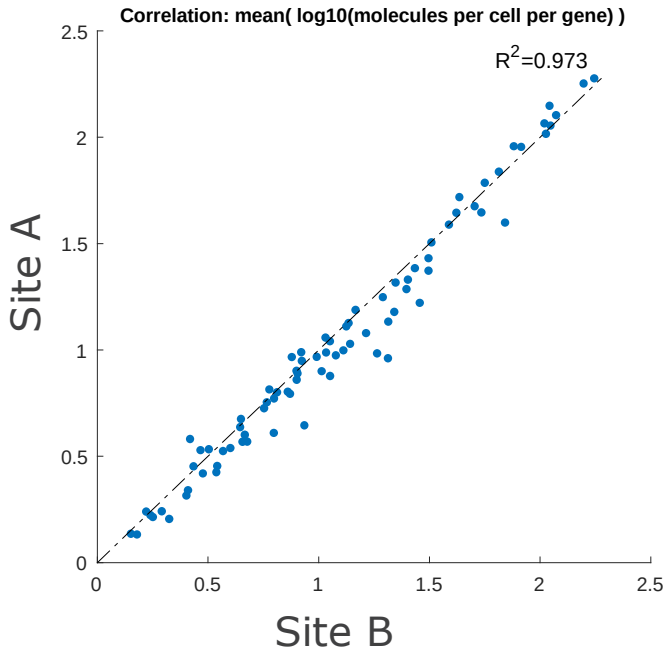


Good correlation between mRNA and protein markers between sites

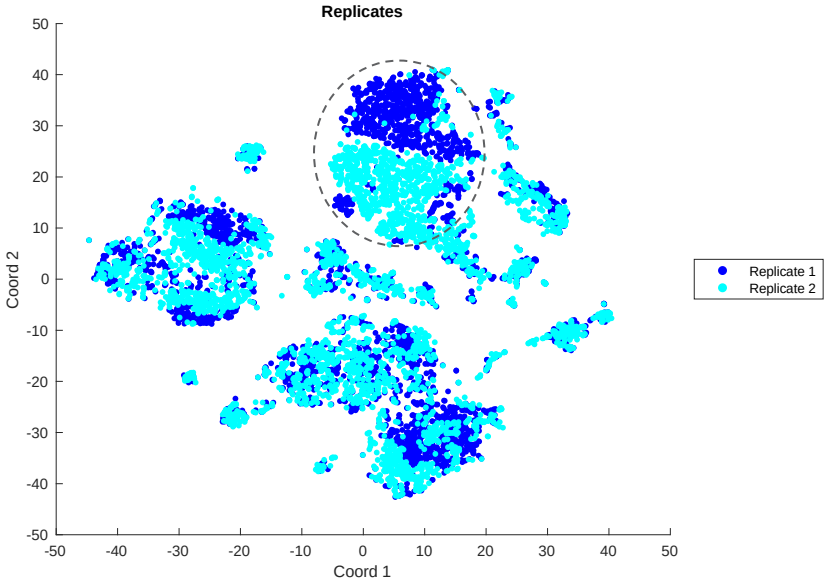
mRNA

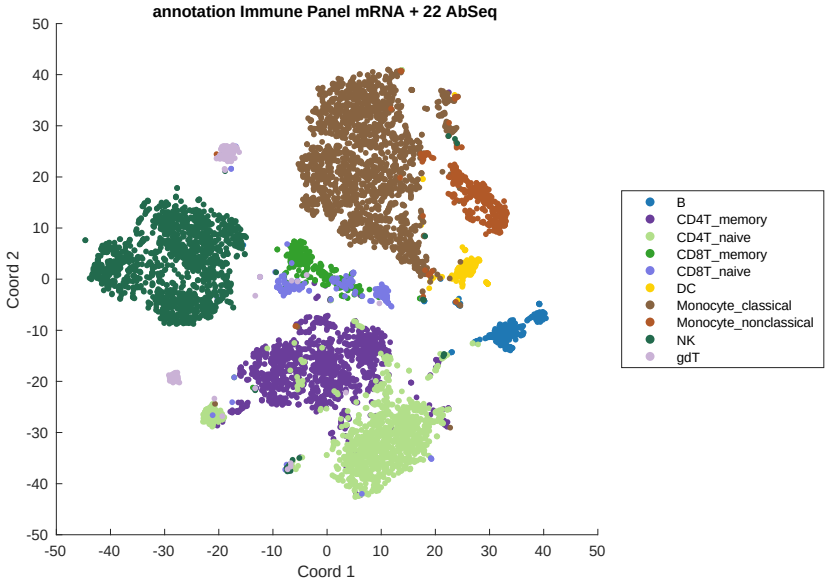


Protein (BD AbSeq™ Antibodies)

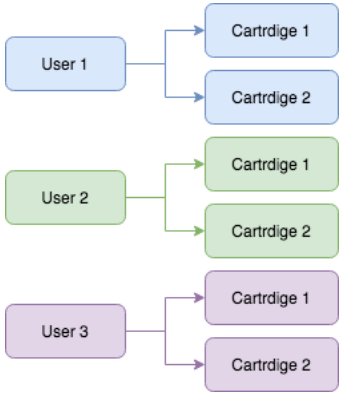


Little batch effect observed even with no batch correction applied when comparing two sites

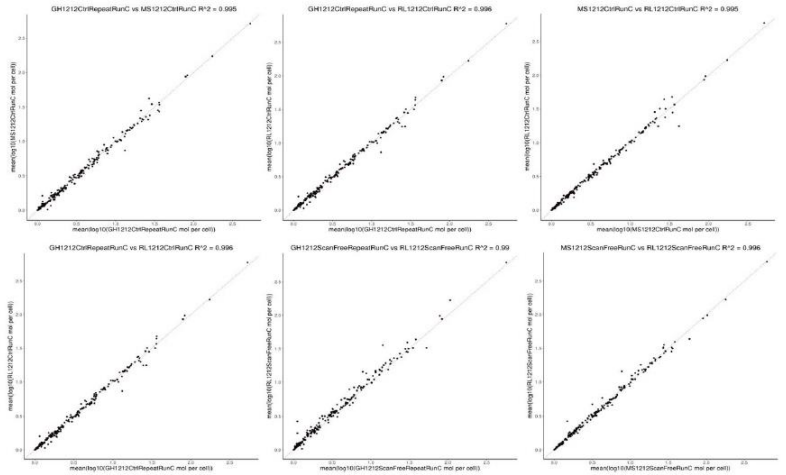




High correlation between data generated by three different operators

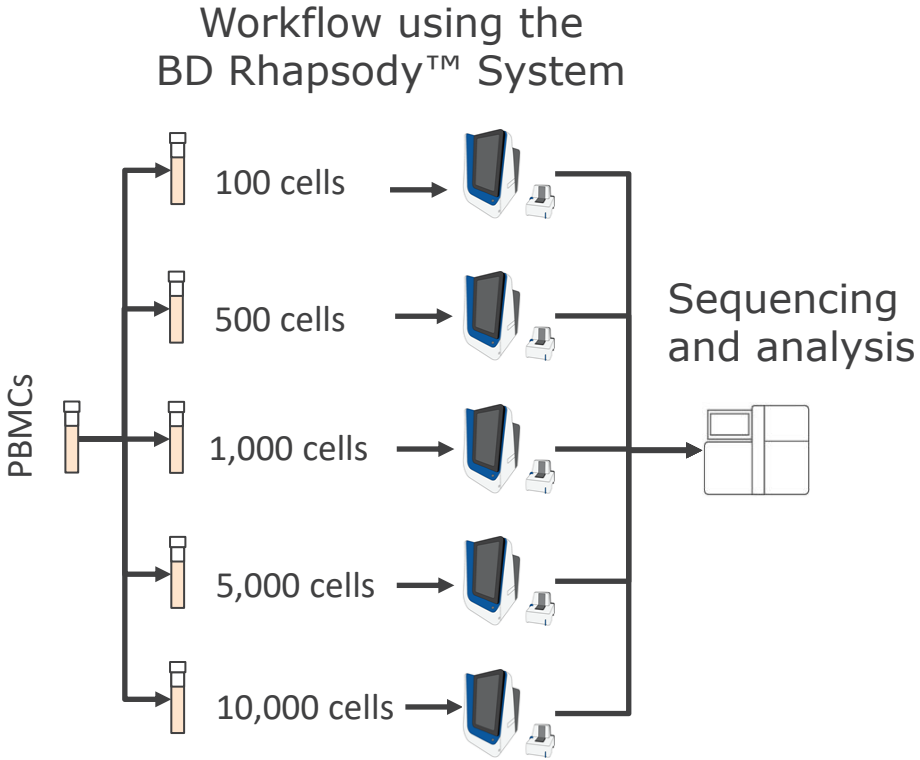


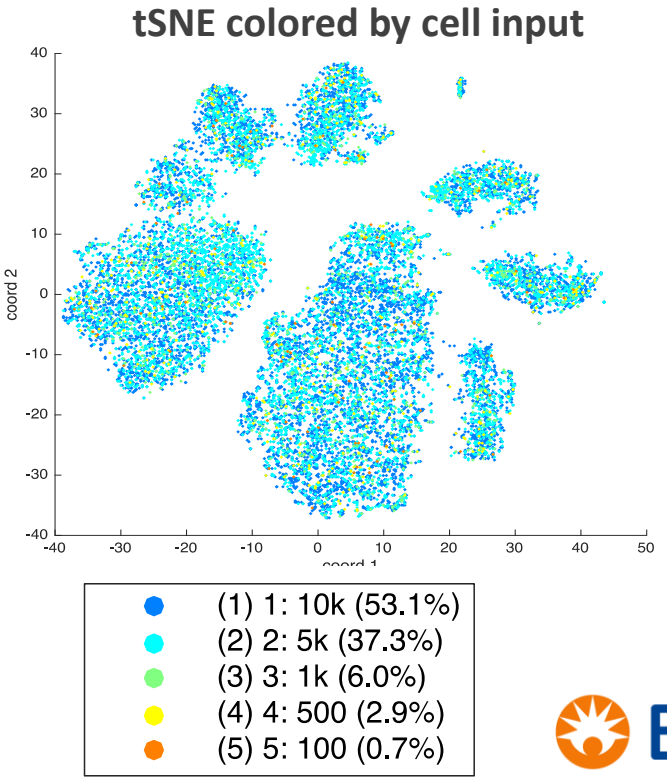
- BD Rhapsody™ Human Immune Response Panel
- 1:1 mix of 5,000 Jurkat and Ramos cells were loaded onto each cartridge



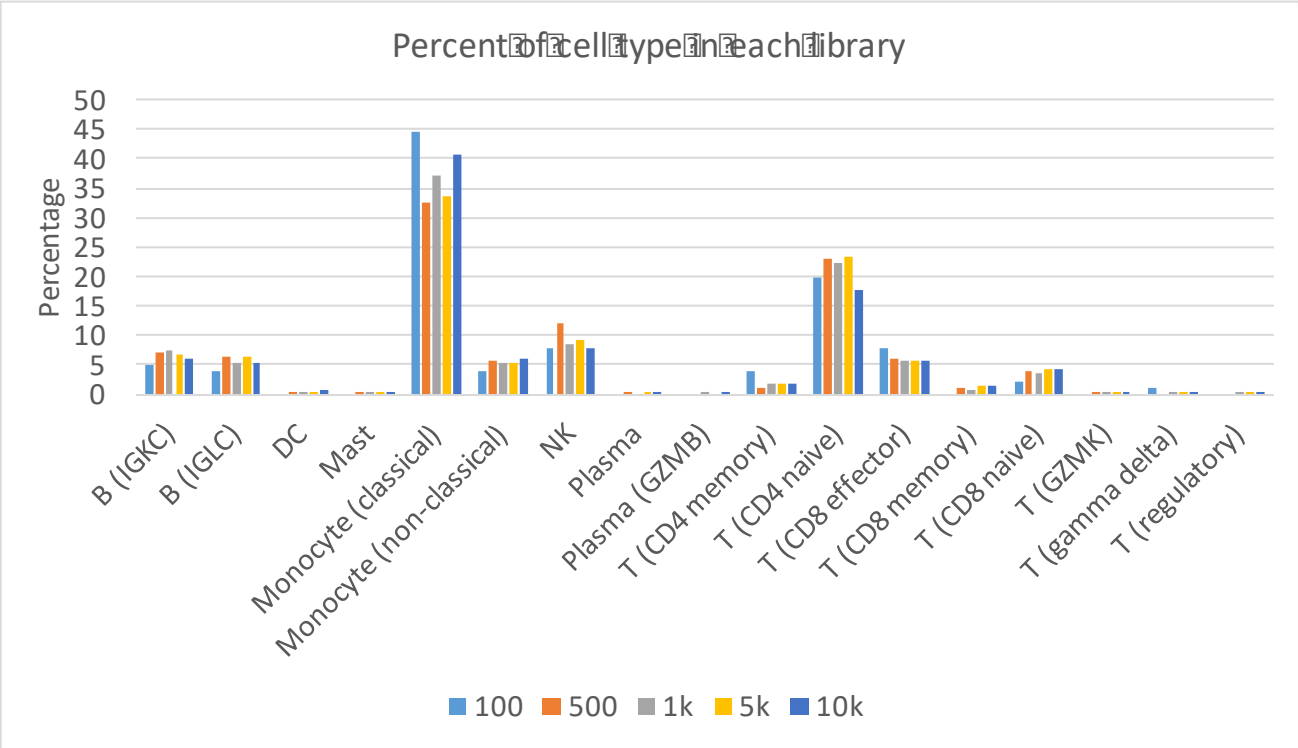
Gene-wise correlation (R^2)	User 1 vs. User 2	User 1 vs. User 3	User 2 vs. User 3
with scanner condition	99.5%	99.6%	99.5%
scanner free condition	99.6%	99.0%	99.6%

Consistent results with minimum sample bias across different cell inputs

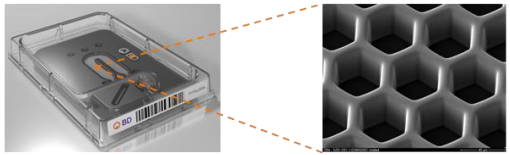




Similar proportions of each cell type recovered from different cell inputs



Consistent performance with pre-formed microwell partitions

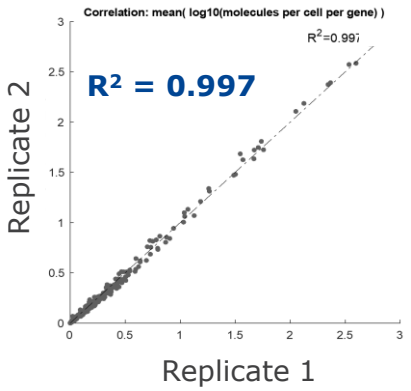


Flow Profile and Leak Testing

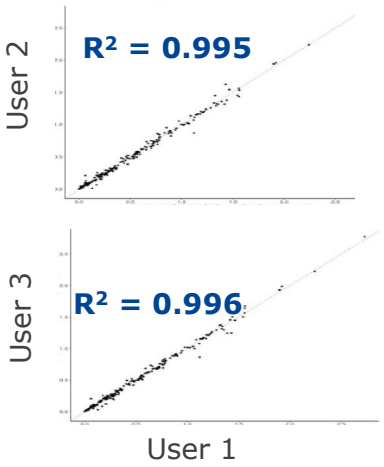
Functional Testing

Physical Attribute Testing

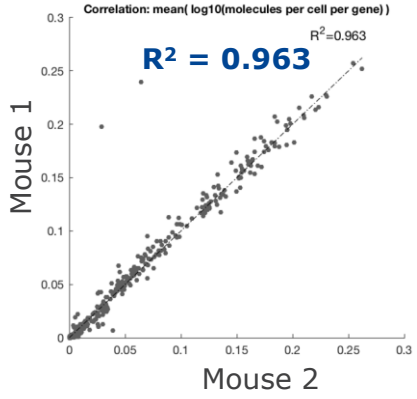
Technical replicates



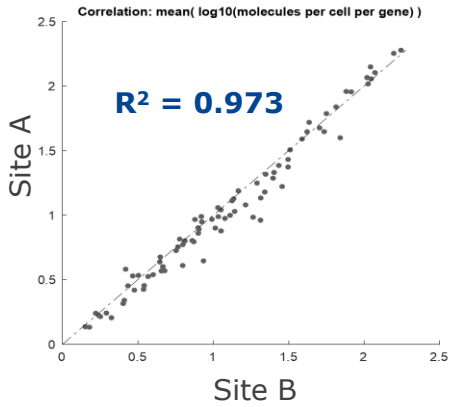
User-to-user



Biological replicates



Site-to-site

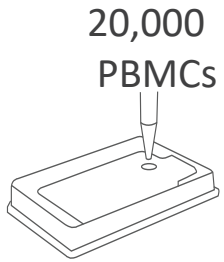


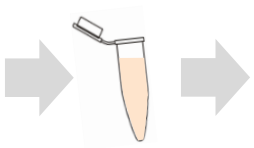
Stable bead-oligo complex

Peace of mind and efficiency

- Similar Data from Subsampled Beads
- Similar Data from Archived Beads
- Iterate on Experiment Design
- Tool to Standardize Performance

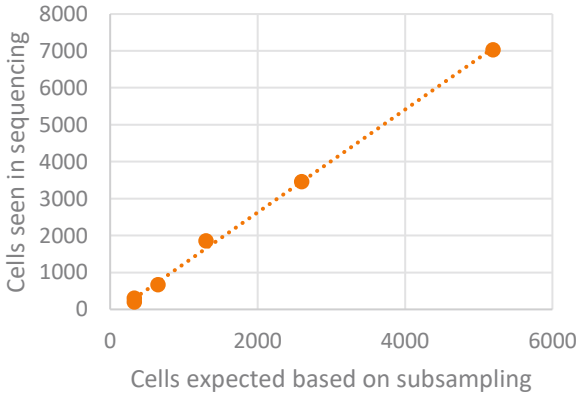
Expected number of cells seen after bead subsampling

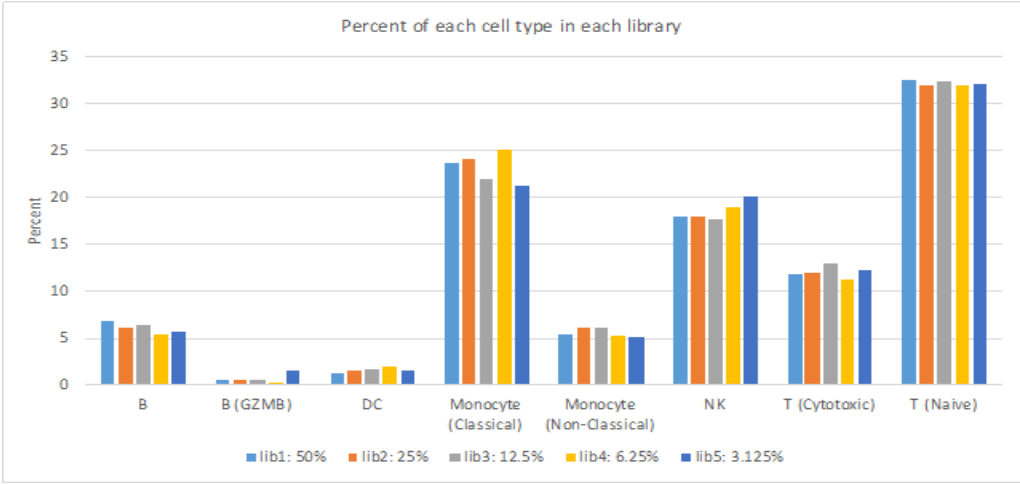




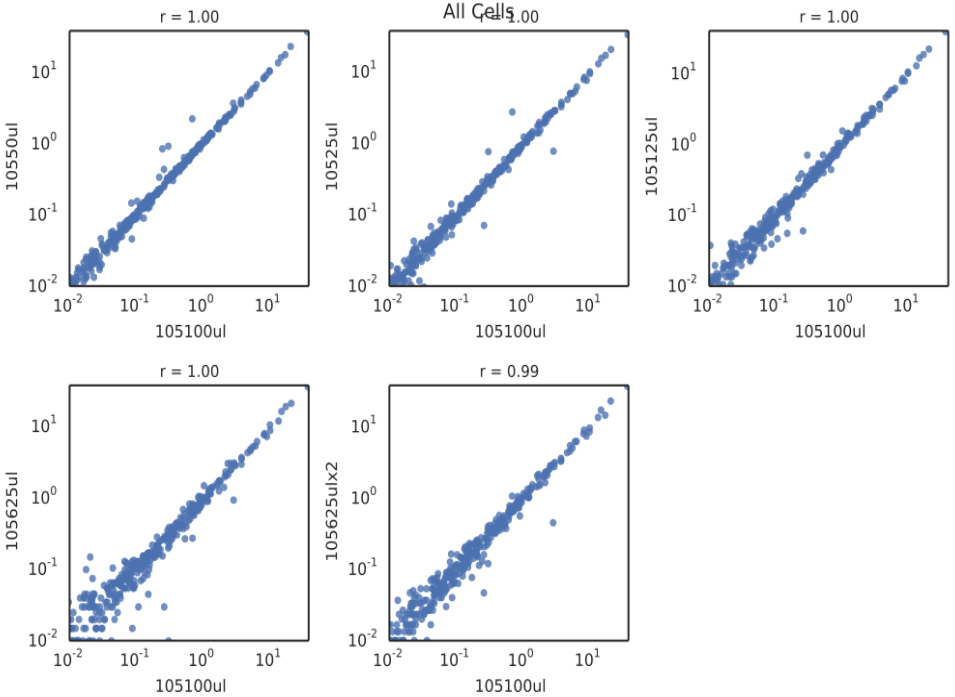
Split beads:

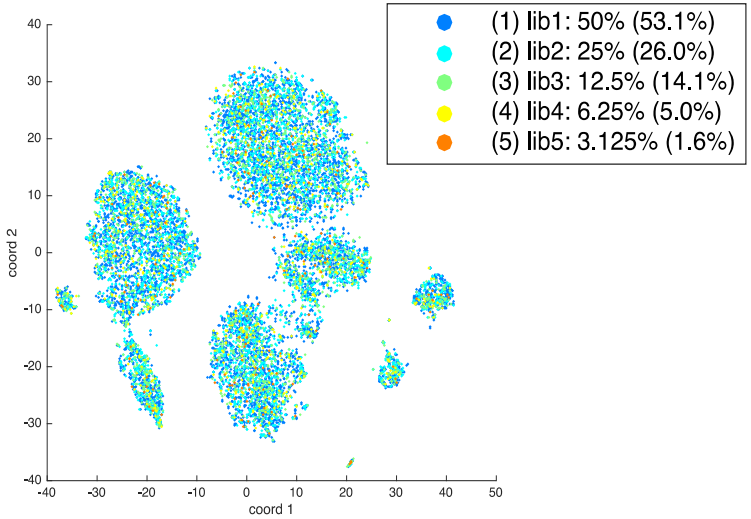
- 100 µL
- 50 µL
- 25 µL
- 12.5 µL
- 2x6.25 µL



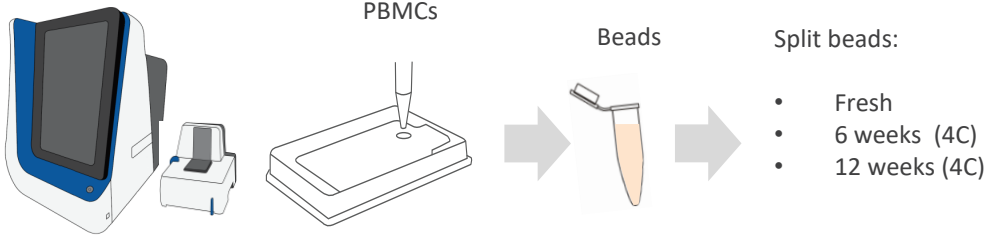


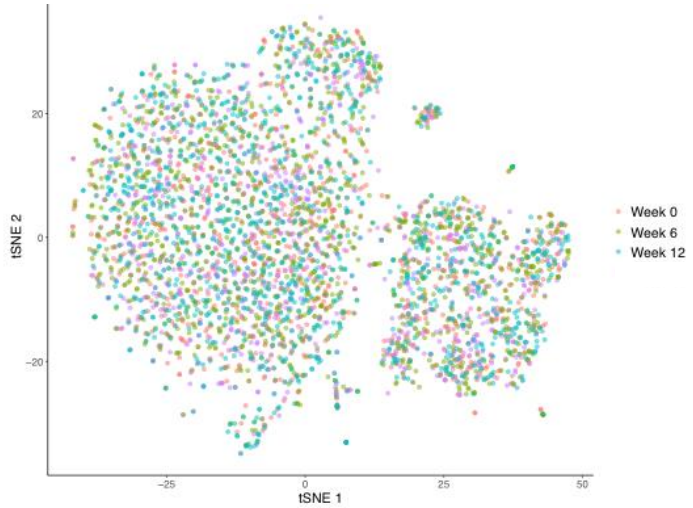
High correlation and no batch effect observed with bead subsampling



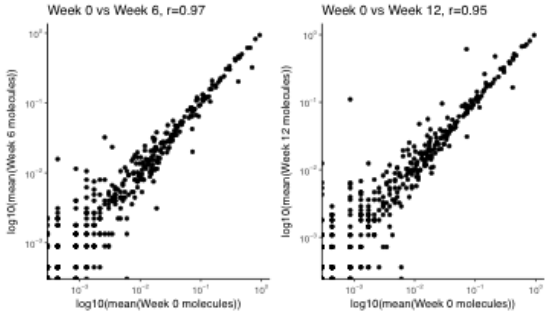


Equivalent data obtained from stored beads



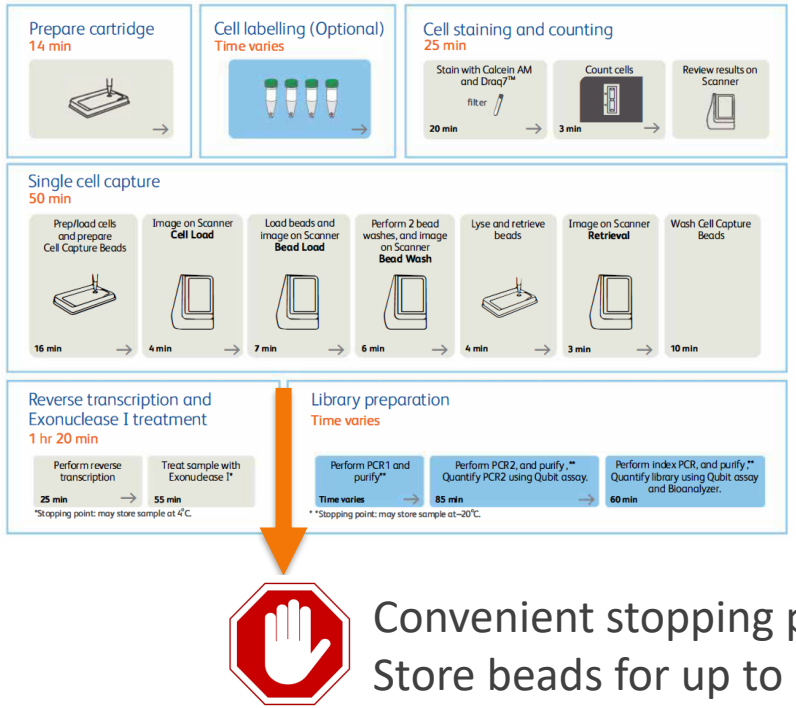


- No batch effect between beads stored for 12 weeks
- Good correlation of gene expression between data from fresh beads to beads stored for 12 weeks



Focus on strengths, leverage partners

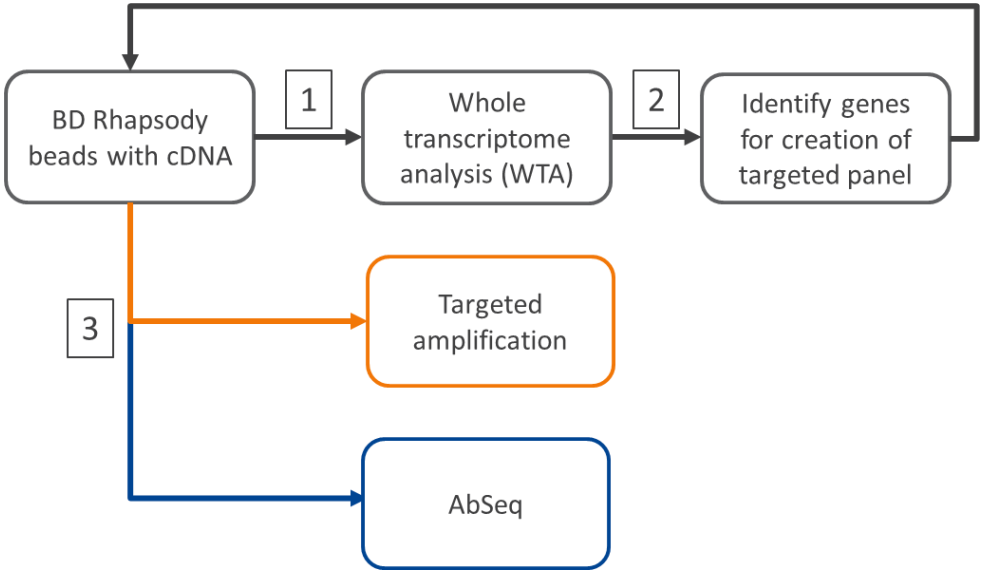
- Generate beads with stable cDNA that can be stored for up to 3 months
- Perform single-cell capture to RT and Exo treatment steps and store beads. Collect all clinical or research samples to process further, so all libraries can be processed on same day to reduce batch effects that may arise from library prep on different days
- Pool libraries to run on one flow cell for optimized cost efficiency in sequencing
- Store and send beads to CROs, collaborators and genomics core facilities



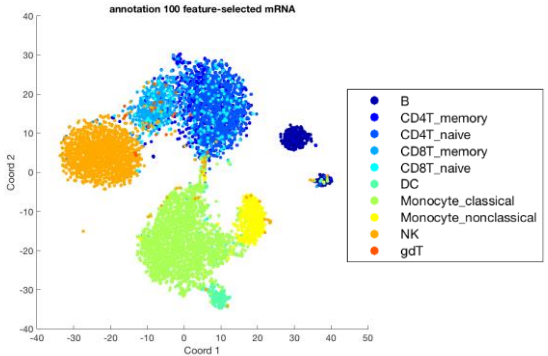
Convenient stopping point
Store beads for up to 3 months

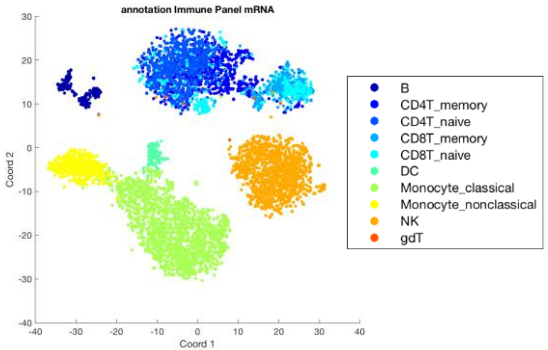
Iterate on experiment design

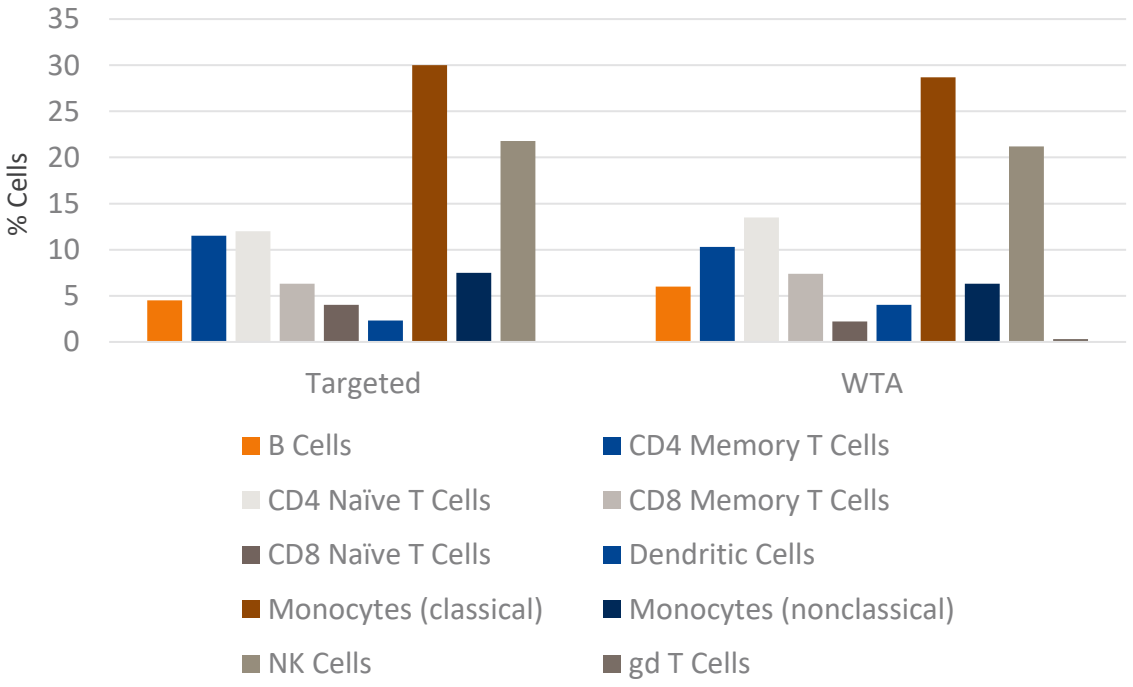
- Perform multiple assays over time on the beads prepared from the same set of single cells
- Targeted mRNA, AbSeq, and whole transcriptome libraries can be generated from the same stock of oligo-bead complex
- Allows for recovery from experimental failures
- Gain confidence in your data by running technical replicates



Similar proportions of each cell type are recovered from WTA and Targeted assays performed on subsampled beads

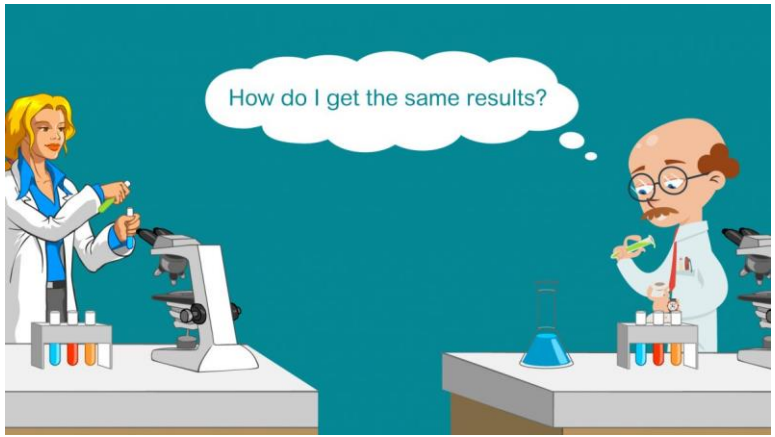






Tool to standardize performance

- Stored, subsampled beads as a tool to:
  - Test technical skills of users
    - New user vs Experienced user
    - Users across different laboratories
    - CROs and collaborators
  - Develop SOPs
  - To control for day-to-day variability in downstream library prep



Scanner for workflow QC

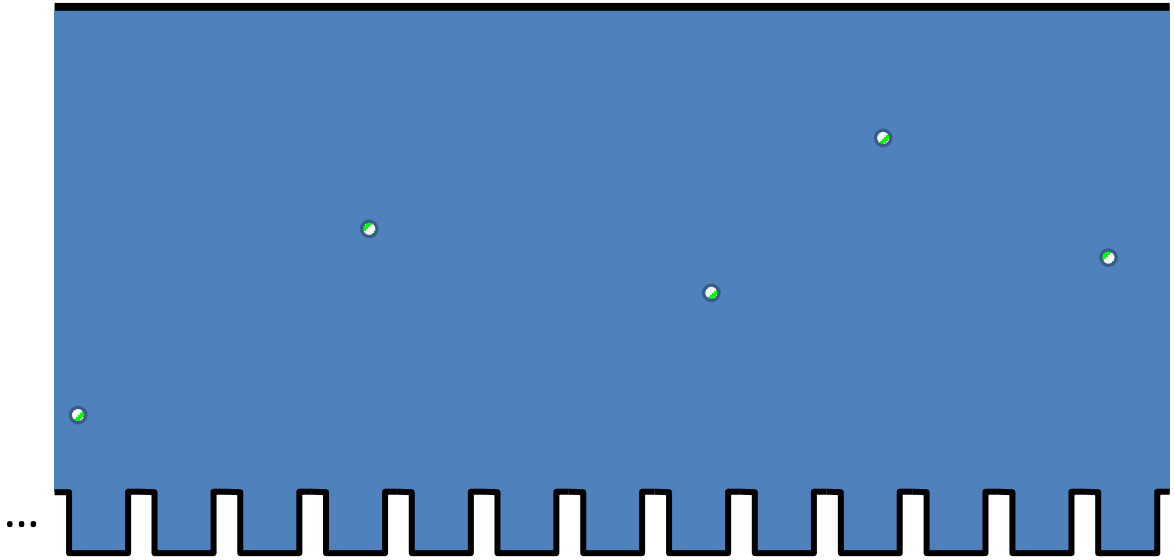
Not flying blind, confidence with every experiment

- Visual Workflow QC with Analysis Metrics at Every Step
- Estimate Cells Expected from Sequencing
- See Before You Pay
- Troubleshooting and Optimization of Single-Cell Capture without Sequencing

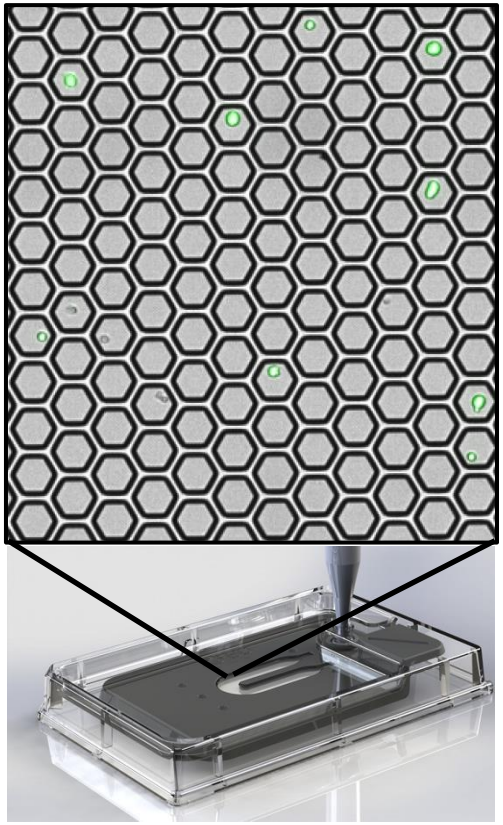
Visual workflow QC

Pairing cells with beads in microwells:

Load cells



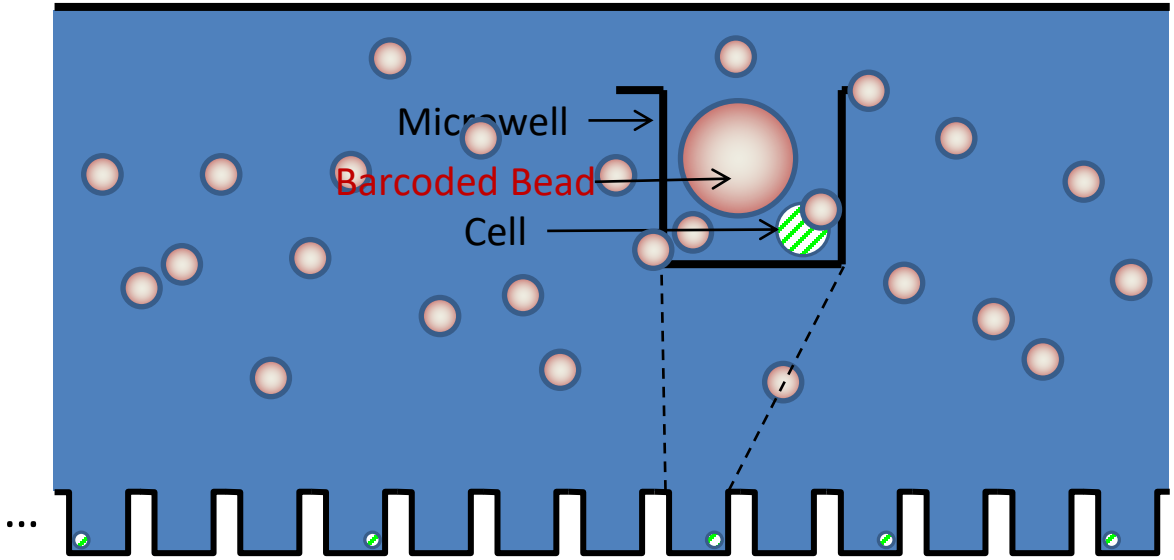
Array with > 200,000 microwells

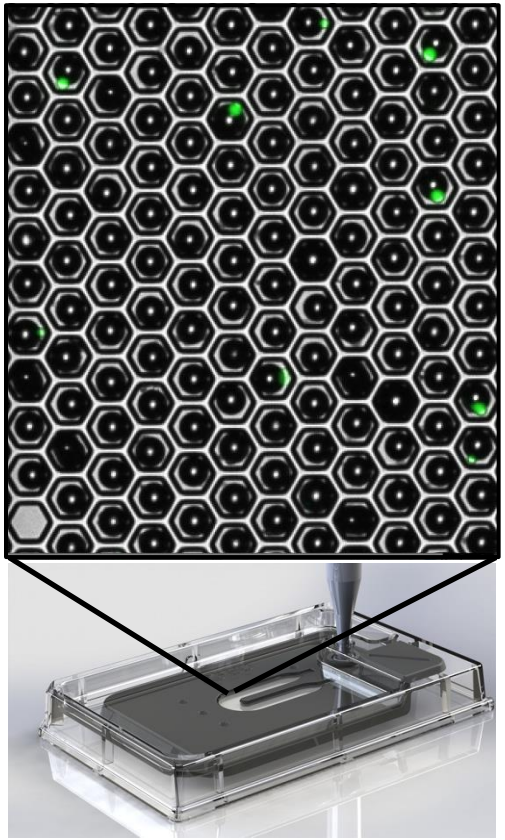


Bead loading

Pairing cells with beads in microwells:

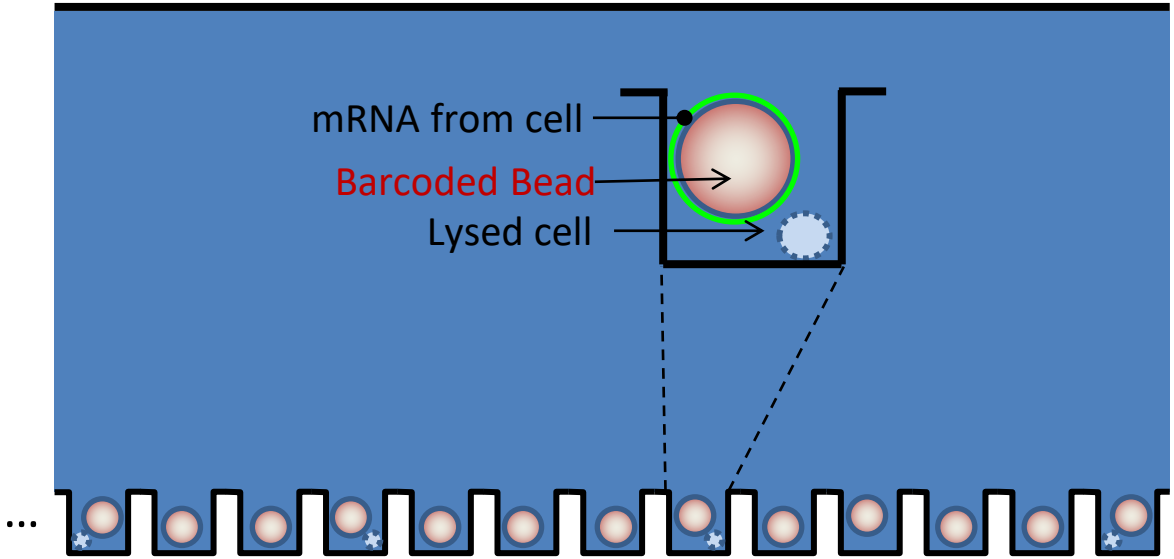
Load beads



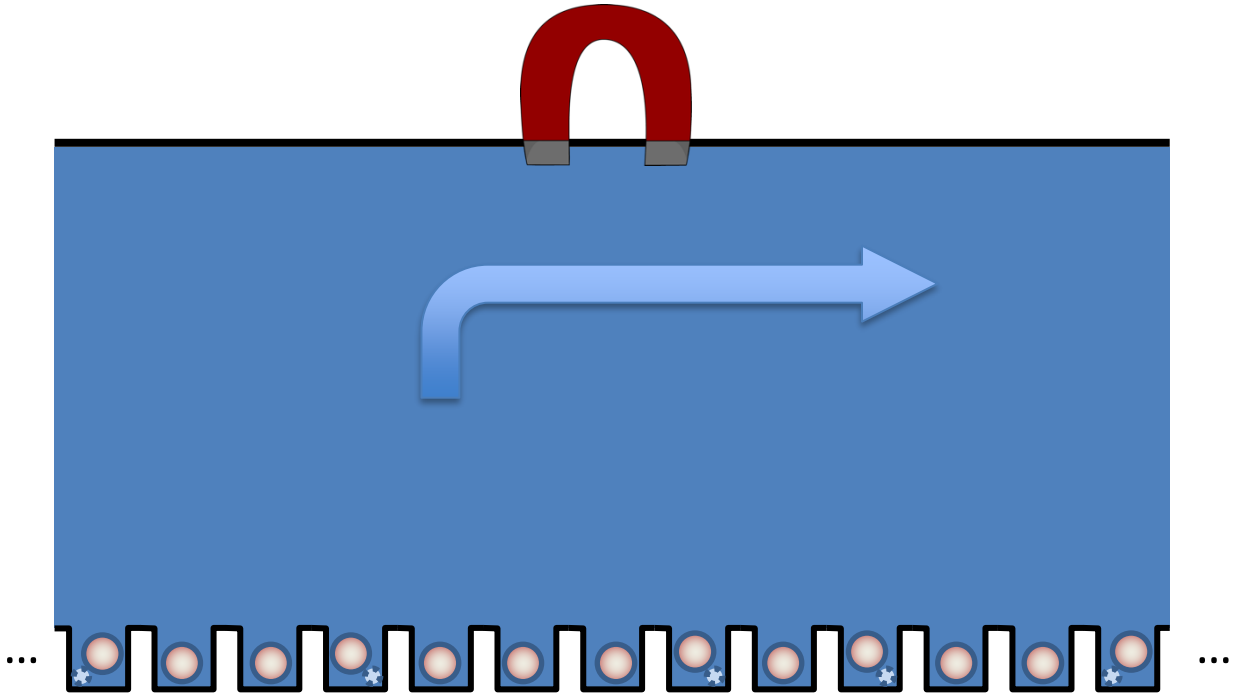


Cell lysis

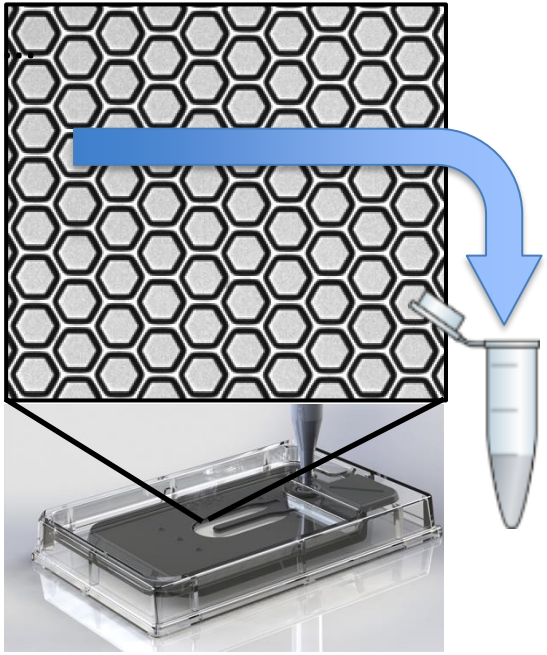
Lyse cells to release mRNA, which hybridizes to label molecules on bead



Bead retrieval

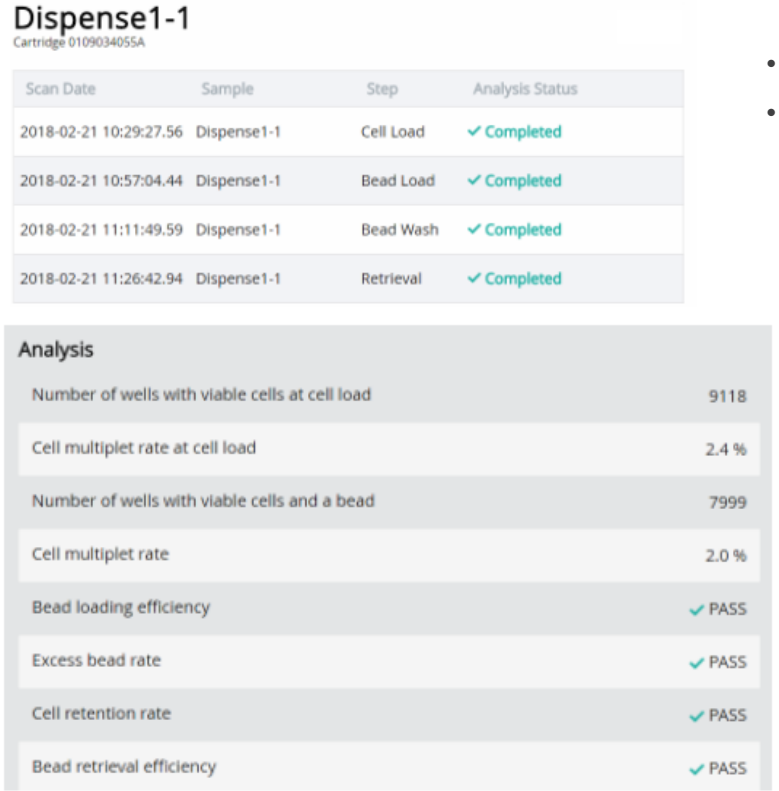


Retrieve beads and pool to a single tube



Analysis metrics

- Compare cell multiplet rates by imaging vs calculated by Poisson distribution
- Make Go/No-Go decisions
- Estimate cells retrieved by sequencing



Dispense1-1

Cartridge 0109034055A

Scan Date	Sample	Step	Analysis Status
2018-02-21 10:29:27.56	Dispense1-1	Cell Load	✓ Completed
2018-02-21 10:57:04.44	Dispense1-1	Bead Load	✓ Completed
2018-02-21 11:11:49.59	Dispense1-1	Bead Wash	✓ Completed
2018-02-21 11:26:42.94	Dispense1-1	Retrieval	✓ Completed

Analysis

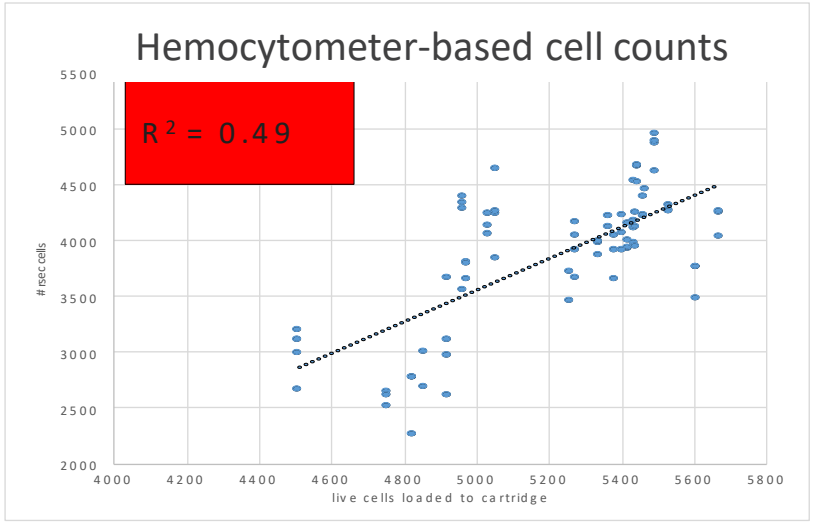
Number of wells with viable cells at cell load	9118
Cell multiplet rate at cell load	2.4 %
Number of wells with viable cells and a bead	7999
Cell multiplet rate	2.0 %
Bead loading efficiency	✓ PASS
Excess bead rate	✓ PASS
Cell retention rate	✓ PASS
Bead retrieval efficiency	✓ PASS

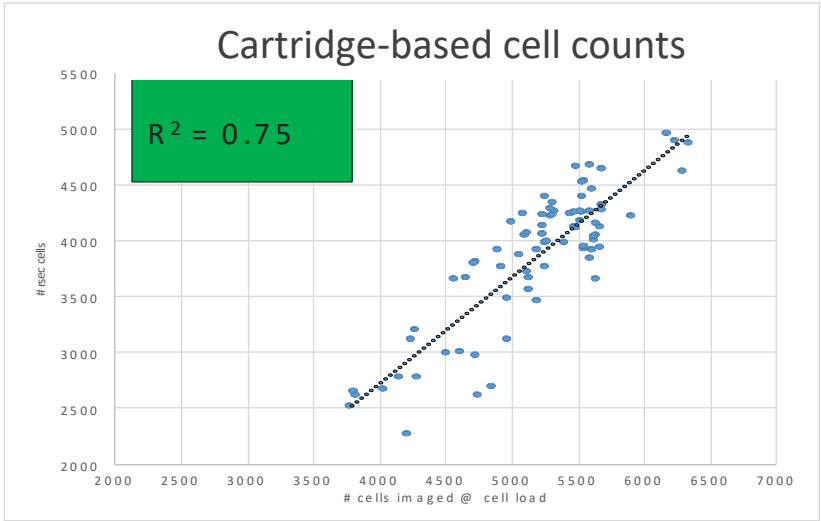
Scan start date and time	6/5/2019 10:20
Operator	PC
Analysis Software Version	1.3.0.12
Cell load analysis last updated	6/5/2019 11:10
Total number of tiles	26
Cell load number of tiles processed	26
Number of wells with viable cells at cell load	4001
Number of viable cells captured in wells at cell load	4052
Cell multiplet rate at cell load	1.30%
Bead wash analysis last updated	6/5/2019 11:37
Bead wash number of tiles processed	26
Number of wells with viable cells and a bead	3665
Number of viable cells captured in wells with a bead	3710
Cell multiplet rate	1.20%
Bead loading efficiency	94.70%
Bead loading acceptance	PASS
Excess bead rate	0.00%
Excess bead acceptance	PASS
Cell retention rate	96.90%
Cell retention acceptance	PASS
Bead retrieval analysis last updated	6/5/2019 11:47
Bead retrieval number of tiles processed	26

Analysis metrics at every step

Scanner metric	Use
Number of wells with viable cells at cell load	Provides a preliminary estimate of the number of wells with viable cells captured
Number of viable cells captured in wells at cell load	Provides a preliminary estimate of the total number of viable cells captured in the cartridge
Cell multiplet rate at cell load	Provides a measure of cell clumping
Number of wells with viable cells and a bead	Provides an estimate of the number of wells with viable cells captured with beads
Number of viable cells captured in wells with a bead	Estimates the total number of viable cells captured on a BD® Cell Capture Bead at cell lysis
Cell multiplet rate	Provides an estimate of the occurrence of multiple cells captured by the same bead in a well
Bead loading efficiency	Indicates if the cartridge is significantly underloaded with beads
Cell retention rate	Indicates if a significant number of cells initially loaded into wells are lost or died during the workflow prior to cell lysis
Bead retrieval efficiency	Indicates if the number of beads retrieved is significantly lower than expected

Scanner metrics are highly correlated with recovery by sequencing, helping users better plan for sequencing cost





Across 75 runs, **~75%** of cells loaded onto cartridge are retrieved by sequencing

See before you pay!

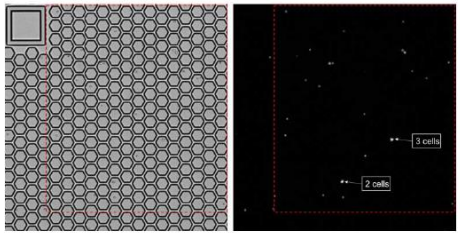
Live cells loaded	# wells with viable cells and a bead	Total number of reads needed (assuming targeted + 10-plex BD® AbSeq Reagents)	Sequencing cost
20,000 cells	20,000 cells	233M	NextSeq® System High Output ($2,600, ~400M reads)
20,000 cells	9,000 cells	105M	NextSeq® System Mid Output ($999, ~130M reads)

~$1,600
~130M
wasted reads

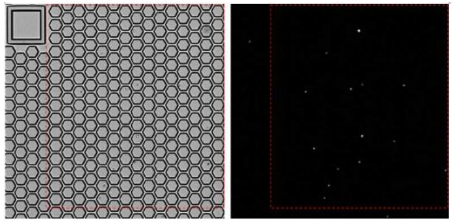
Troubleshooting without scanner is tedious

- Troubleshoot cartridge loading
  - Multiplet rate higher than expected - may indicate cell clumping
  - Cell viability and total number of cells captured in cartridge, after cell load vs in cell prep – indicates if a significant number of cells initially loaded into wells are lost or died during the workflow prior to lysis
  - Bead loading
  - Bead retrieval

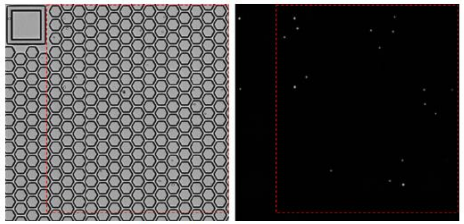
Region 1: 26 cells



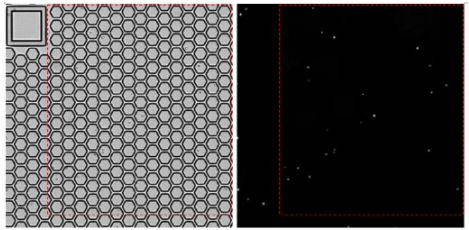
Region 2: 14 cells



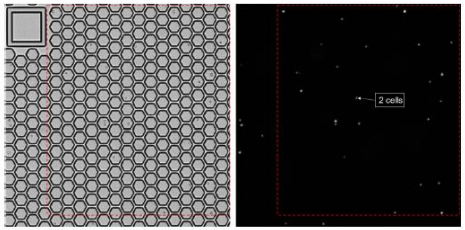
Region 3: 17 cells



Region 4: 22 cells



Region 5: 26 cells



Region	Cell count	No. of wells
1	26	225
2	14	225
3	17	225
4	22	225
5	26	225
Total	105	1,125

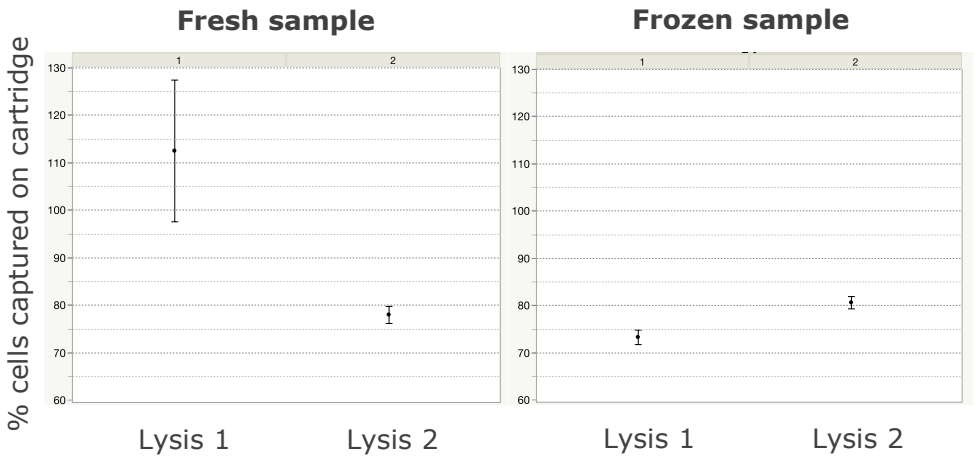
Cells/well = $105/1,125 = 0.0933$

Total no. cells captured in the cartridge = $0.0933 \times 221,891$

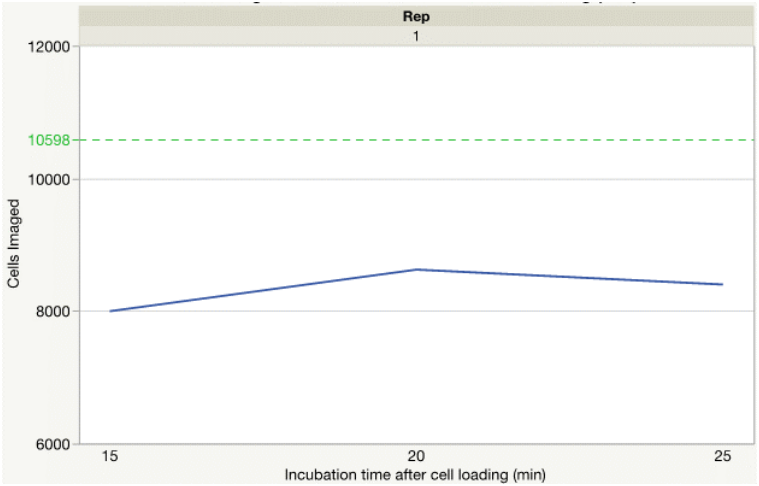
Total no. cells captured in the cartridge = $20,702$

Use scanner to optimize experimental workflow to maximize cell recovery without paying for sequencing

Upstream sample prep that may affect cell capture

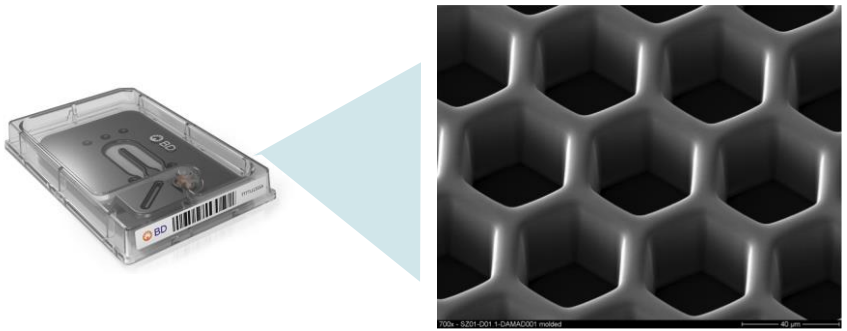


BD Rhapsody™ System workflow optimization



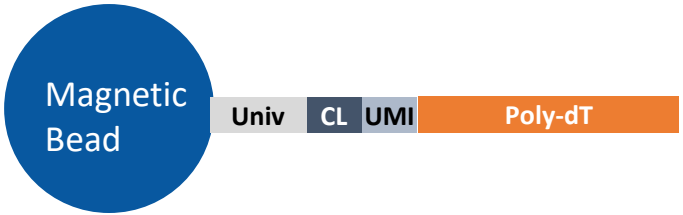
Settling time

BD Rhapsody™ System - A robust single-cell partitioning system with unique features



Microwell-based single-cell partitioning

Visual workflow QC



Stable bead-oligo complex

Thank you!

Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.
23-23087-00

BD Life Sciences, San Jose, CA 05131, USA

bdbiosciences.com