Single-Cell Genomics

Exploring new applications in microfluidics

Mark Lynch
April 10, 2019
Agenda

1. Microfluidics solutions for single-cell analysis
2. Single-cell total RNA sequencing
3. Single-cell high-throughput (HT) RNA expression and protein sequencing (REAP-seq)
4. Creating single-cell microenvironments
Microfluidic solutions for single-cell analysis
Cellular analysis cell by cell
The single-cell revolution is only beginning
Driving those advances are techniques for isolating thousands of intact cells from living organisms, efficiently sequencing expressed genetic material in each cell and using computers, or labeling the cells, to reconstruct their relationships in space and time. That technical trifecta ‘will transform the next decade of research,’ says Nikolaus Rajewsky, a systems biologist at the Max Delbrück Center for Molecular Medicine in Berlin.

—Elizabeth Pennisi, writing in ‘Science’ about the 2018 Breakthrough of the Year
Creating a cell atlas

The only way to identify and understand all cells in a tissue

Three cell-based approaches:

1. Classification
2. Characterization
3. Context
The benefit

Individual cells behave differently from the average of many cells

Modified from Dominguez et al. *Journal of Immunological Methods* (2014)
Cell characterization
Deep profiling of cells to study multiple modes

Microfluidics
Integrated fluidic circuit (IFC) technology to capture, verify, process and perturb individual cells

Microfluidics for single-cell genomics
C1 system and C1 IFCs
C1 IFC flexibility
To capture, verify, process and perturb individual cells

Processing cells using solid-state valve-formatted microfluidics enables a platform that is fully customized for flexibility.
### C1 IFC

An IFC range to offer full flexibility

<table>
<thead>
<tr>
<th>Capture and process up to 96 single cells</th>
<th>Input: 200–2,000 cells/µL</th>
<th>mRNA Sequencing Applications: • Full length • Total • End counting</th>
<th>Cell Size Formats: • Small • Medium • Large</th>
<th>Compatible with C1 Script Builder™</th>
<th>Compatible with C1 Script Hub™ Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture and process up to 800 single cells</td>
<td>Input: 2,000–5,000 cells/µL</td>
<td>mRNA Sequencing Applications: • End counting • C1 REAP-seq</td>
<td>Cell Size Formats: • Small • Medium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Formats:**
- Small captures cells from 5 to 10 µm.
- Medium captures cells from 10 to 17 µm.
- Large captures cells from 17 to 25 µm.
Visualizing cells using C1

Neuronal cells

Single cells

Debris
Single-cell total RNA sequencing
Benefits
Workflow, cost and deeper transcriptome characterization

<table>
<thead>
<tr>
<th>On-IFC amplification</th>
<th>Total RNA Seq</th>
<th>SMART-Seq v4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Back-loading indexing PCR</td>
<td>• Universal amplification</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post C1 workflow</th>
<th>Total RNA Seq</th>
<th>SMART-Seq v4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• No extra kit needed</td>
<td>• Nextera® XT, index kits required</td>
</tr>
<tr>
<td></td>
<td>• Samples pooled after harvest</td>
<td>• Samples pooled after final PCR</td>
</tr>
<tr>
<td></td>
<td>• rRNA depletion and final PCR performed in a single tube</td>
<td>• Tagmentation through indexing PCR performed in 96-well plate</td>
</tr>
</tbody>
</table>

With enhanced transcription data, C1 Total RNA Seq provides an easier and much cheaper workflow than SMART-Seq® v4.
ncRNA biotypes

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>lincRNA</td>
<td>Long intergenic noncoding RNA</td>
<td>Gene regulation, splicing, translation</td>
</tr>
<tr>
<td>eRNA</td>
<td>Enhancer RNA</td>
<td>Gene regulation</td>
</tr>
<tr>
<td>snRNA</td>
<td>Small nuclear RNA</td>
<td>Splicing</td>
</tr>
<tr>
<td>snoRNA</td>
<td>Small nucleolar RNA</td>
<td>Splicing, translation</td>
</tr>
<tr>
<td>miRNA</td>
<td>MicroRNA</td>
<td>Translation</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
<td>Translation</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer RNA</td>
<td>Translation</td>
</tr>
<tr>
<td>tmRNA</td>
<td>Transfer-messenger RNA</td>
<td>Translation</td>
</tr>
</tbody>
</table>

Enabling study of these ncRNA biotypes at the single-cell level will allow for a more comprehensive understanding of cellular mechanisms.
Stranded Total RNA Seq workflow

Automated steps on C1

1. RNA fragmentation

2. First-strand synthesis and tailing by RT

3. Template switching and extension by RT

4. PCR 1: Addition of Illumina® adapters with barcodes

Single-tube library prep after C1

1. Cleavage of ribosomal cDNA

2. PCR 2: Enrichment of uncleaved fragments

Adaptation of SMART-Seq Stranded Kit (Takara Bio)
Building the application

<table>
<thead>
<tr>
<th></th>
<th>K562 Cells</th>
<th>HL-60 Cells</th>
<th>Activated T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell description</strong></td>
<td>Myelogenous leukemia cell line, robust cell type</td>
<td>Leukemia cell line, fragile cell type</td>
<td>Primary cell, stimulated with anti CD3/CD28 beads</td>
</tr>
<tr>
<td><strong>IFC size</strong></td>
<td>Medium</td>
<td>Small</td>
<td>Medium</td>
</tr>
<tr>
<td><strong>Total RNA Seq</strong></td>
<td>3 IFCs*</td>
<td>3 IFCs*</td>
<td>2 IFCs</td>
</tr>
<tr>
<td><strong>SMART-Seq v4</strong></td>
<td>2 IFCs</td>
<td>2 IFCs</td>
<td>2 IFCs</td>
</tr>
</tbody>
</table>

* Initial Total RNA Seq experiments with K562 and HL-60 cells were run with the SMARTer® Stranded Total RNA Seq Kit v2 —Pico Input Mammalian. The remaining Total RNA Seq experiments were performed using the updated SMART-Seq Stranded Kit after its launch in May 2018.
Bioinformatic pipeline

Denovo assembling (kallisto)
Generate gene-level expression tables (tximport)

Ensembl hg38 CDNA reference (release 92) (FASTA file)
Ensembl hg38 ncRNA reference (release 92) (FASTA file)

Raw sequencing data from MiSeq® v3 150-cycle kit (FASTQ files)

(kallisto index)

Kallisto index (T-DBG)

abundance.h5
abundance.tsv
run_info.json

Generate metrics (custom Python® and R scripts)
Batch correction of expression table (Seurat linear regression)

Sequencing metric visualizations
Hypothesized gene expression
Biotype proportions
Dimensional reduction (custom Python and R scripts, Seurat)

FASTQ files were also processed to visualize transcript coverage (Bowtie, Picard).
Processing live cells
Only cells with >20,000 mapped reads received secondary analysis

![Single live cell](image1.png)

One IFC is shown as an example. Dashed line indicates 20,000 mapped reads.

<table>
<thead>
<tr>
<th></th>
<th>K562</th>
<th>HL-60</th>
<th>Activated T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMART-Seq v4</td>
<td>57 cells</td>
<td>40 cells</td>
<td>38 cells</td>
</tr>
<tr>
<td></td>
<td>90 cells</td>
<td>42 cells</td>
<td>24 cells</td>
</tr>
<tr>
<td>Total RNA Seq</td>
<td>58 cells</td>
<td>69 cells</td>
<td>38 cells</td>
</tr>
<tr>
<td></td>
<td>83 cells</td>
<td>45 cells</td>
<td>40 cells</td>
</tr>
<tr>
<td></td>
<td>76 cells</td>
<td>65 cells</td>
<td></td>
</tr>
</tbody>
</table>

A total of 765 cells passed filters for further analysis.

For K562 and HL-60, two IFCs each were run with an older version of Total RNA Seq chemistry before the current version was launched.
Total RNA Seq shows transcript coverage similar to or better than SMART-Seq v4

Total RNA Seq provides complete full-length mRNA sequencing with better coverage across transcripts in cell types that show high 3’ bias, which is important for characterizing the complete transcriptome.
Total RNA detects significantly more noncoding genes

SSv4: SMART-Seq v4
TRv1: Total RNA Seq

Total RNA applications enable characterization of the full transcriptome including regulatory elements that control transcription.
Total RNA Seq
Shows gene expression similar to or more consistent than SMART-Seq v4

Within a cell type, Total RNA Seq detects genes with greater consistency across the full sample than SMART-Seq v4.
Total RNA Seq
Shows greater resolution when visualized by PCA

Total RNA Seq samples
SMART-Seq v4 samples

Total RNA Seq chemistry provides better resolution because more genes are detected, enabling complete transcriptome characterization.

PCA generated with Seurat
Total RNA Seq
Exhibits a greater detection of noncoding RNA

Total RNA Seq detects more ncRNA and a greater diversity of noncoding RNA biotypes.
Total RNA Seq methods can detect full-length nonpoly(A) isoforms

<table>
<thead>
<tr>
<th>Method</th>
<th>Read Depth</th>
<th>Transcript Coverage</th>
<th>Poly(A) Transcript Isoforms</th>
<th>Nonpoly(A) Transcript Isoforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet-based methods</td>
<td>Low</td>
<td>3’ only</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C1 high-throughput</td>
<td>Medium</td>
<td>3’ only</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C1 96 (SMART-Seq v4)</td>
<td>High</td>
<td>Full-length</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C1 96 Total RNA Seq</td>
<td>High</td>
<td>Full-length</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Total RNA Seq

Summary

• Provides one of very few methods to sequence both poly(A) and nonpoly(A) RNAs in single cells

• Simpler workflow than most protocols, enabling on-IFC cell-indexing PCR and single-tube post-C1 prep (primarily ribosomal RNA depletion)

• Maintains full-length transcriptome coverage with little 5′–3′ bias in cell types where SMART-Seq v4 shows a strong 3′ bias

• Provides a method for researchers to perform deeper single-cell characterization by enabling analysis of novel noncoding RNA features

Download the C1 Total RNA Seq poster today.
Single-cell high-throughput RNA expression and protein sequencing
What is REAP-seq?

- RNA expression and protein from the same isolated single cell
- With the enablement of REAP-seq on C1 Single-Cell mRNA Seq HT IFCs, researchers can simultaneously detect protein and gene expression from the same cell.
- Detecting both RNA and protein from the same cell eliminates the bioinformatic challenges of stitching datasets together.
- REAP-seq labels cells with antibodies conjugated to unique DNA sequences, circumventing the limitations of fluorescence and stable isotope-conjugated antibodies. Our method allows for more than 80 proteins to be detected per single cell.
Building the application
Antibody oligo conjugates are specific to the intended protein targets for transcriptome and protein marker detection
C1 REAP-seq workflow

1. Obtain barcoded antibodies.
2. Stain cells.

Preparation

3. Isolate antibody stained cells.
4. Image.
5. Perform REAP-seq chemistry followed by harvest.

C1 processing

7. Sequence and analyze.

Data collection and analysis

>500 bp  ~193 bp
REAP-seq chemistry

1. Reverse transcription

2. Preamplification

Antibody barcode library prep

cDNA library prep
REAP-seq chemistry

3. Harvest and clean up to separate and process cDNA and antibody (Ab) barcode (BC) library in parallel.

**Antibody barcode library prep**

Antibody library barcoding and adapter addition by PCR

**cDNA library prep**

Library tagmentation

3′-end enrichment

Final Ab BC library structure

Final cDNA library structure
Building the application

Antibody barcode
- 22 human Ab BCs targeting various protein markers are pooled before incubation.
- Well-characterized proteins of particular interest are CD10, CD19, CD3 and CD45RA.

Controls
- α-mouse and isotype controls to gauge nonspecific binding of antibody barcodes
- Performance monitoring in the K562/HEK293 quality control
# Antibody barcode pool

<table>
<thead>
<tr>
<th>Antibody barcode pool</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isotype Controls</strong></td>
</tr>
<tr>
<td>Mouse IgG1, κ isotype Ctrl</td>
</tr>
<tr>
<td>Mouse IgG2a, κ isotype Ctrl</td>
</tr>
<tr>
<td>Mouse IgG2b, κ isotype Ctrl</td>
</tr>
<tr>
<td>Rat IgG1, κ isotype Ctrl</td>
</tr>
<tr>
<td>Rat IgG2a, κ isotype Ctrl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mouse Antibodies</strong></th>
<th>Specificity</th>
<th>Clone</th>
<th>Reactivity</th>
<th>Barcode Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-mouse/rat CD29</td>
<td>CD29</td>
<td>HMβ1-1</td>
<td>Mouse, Rat</td>
<td>ACGCATTCTTGTTG</td>
</tr>
<tr>
<td>anti-mouse CD24</td>
<td>CD24</td>
<td>M1/69</td>
<td>Mouse</td>
<td>TATATGCTTGCGCCA</td>
</tr>
<tr>
<td>anti-mouse CD106</td>
<td>CD106</td>
<td>429 (MVCAM.A)</td>
<td>Mouse</td>
<td>CTCCCTACTACCT</td>
</tr>
<tr>
<td>anti-mouse CD63</td>
<td>CD63</td>
<td>NVG-2</td>
<td>Mouse</td>
<td>ATCCGACAGCTA</td>
</tr>
<tr>
<td>anti-mouse CD140a</td>
<td>CD140a</td>
<td>APA5</td>
<td>Mouse</td>
<td>GTCATTGCGTGCTCA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Human Antibodies</strong></th>
<th>Specificity</th>
<th>Clone</th>
<th>Reactivity</th>
<th>Barcode Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-human CD10</td>
<td>CD10</td>
<td>H110a</td>
<td>Human</td>
<td>CAGCCATTCATTAGG</td>
</tr>
<tr>
<td>anti-human CD11c</td>
<td>CD11c</td>
<td>S-HCL-3</td>
<td>Human</td>
<td>TACGCTTACTCTTACT</td>
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<tr>
<td>anti-human CD14</td>
<td>CD14</td>
<td>M5E2</td>
<td>Human</td>
<td>TCTCAGACCTCGTA</td>
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<tr>
<td>anti-human CD16</td>
<td>CD16</td>
<td>3G8</td>
<td>Human</td>
<td>AAGTCACTCTTGCA</td>
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<tr>
<td>anti-human CD19</td>
<td>CD19</td>
<td>H1100</td>
<td>Human</td>
<td>CAGCCATTCATTAGG</td>
</tr>
<tr>
<td>anti-human CD195 (CCR5)</td>
<td>CD195 (CCR5)</td>
<td>J418F1</td>
<td>Human</td>
<td>CCCAGTAAGAGCCA</td>
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<tr>
<td>anti-human CD197 (CCR7)</td>
<td>CD197 (CCR7)</td>
<td>G043H7</td>
<td>Human</td>
<td>AGTCAGTCAACCA</td>
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<tr>
<td>anti-human CD29</td>
<td>CD29</td>
<td>TS2/16</td>
<td>Human</td>
<td>GTCATTCCCTAGTA</td>
</tr>
<tr>
<td>anti-human CD3</td>
<td>CD3</td>
<td>UCHT1</td>
<td>Human</td>
<td>CTCACTGTAACCT</td>
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<tr>
<td>anti-human CD34</td>
<td>CD34</td>
<td>581</td>
<td>Human</td>
<td>GCAGAAATCTCCCTC</td>
</tr>
<tr>
<td>anti-human CD4</td>
<td>CD4</td>
<td>RPA-T4</td>
<td>Human</td>
<td>TCTCCCGCTCAACT</td>
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<tr>
<td>anti-human CD43</td>
<td>CD43</td>
<td>CD43-10G7</td>
<td>Human</td>
<td>GATACACTCTGCA</td>
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<tr>
<td>anti-human CD45RA</td>
<td>CD45RA</td>
<td>H1100</td>
<td>Human</td>
<td>TCACTGTCGCTTT</td>
</tr>
<tr>
<td>anti-human CD45RO</td>
<td>CD45RO</td>
<td>UCHL1</td>
<td>Human</td>
<td>CTCGAACATCGTTG</td>
</tr>
<tr>
<td>anti-human CD55</td>
<td>CD55</td>
<td>JS11</td>
<td>Human</td>
<td>GCTCATTACCCATTA</td>
</tr>
<tr>
<td>anti-human CD56 (NCAM) Recombinant</td>
<td>CD56 (NCAM) Recombinant</td>
<td>Q17A16</td>
<td>Human</td>
<td>TTCGCAGCATTGAGT</td>
</tr>
<tr>
<td>anti-human CD63</td>
<td>CD63</td>
<td>H5C6</td>
<td>Human</td>
<td>GAGATGCTCACAAT</td>
</tr>
<tr>
<td>anti-human CD8A</td>
<td>CD8A</td>
<td>RPA-T8</td>
<td>Human</td>
<td>GCTTGCTTTCAT</td>
</tr>
<tr>
<td>anti-human CD9</td>
<td>CD9</td>
<td>H19a</td>
<td>Human</td>
<td>GAGTCACACACCTG</td>
</tr>
<tr>
<td>anti-human CD95</td>
<td>CD95 (Fas)</td>
<td>DX2</td>
<td>Human</td>
<td>CCAGCTTACGAAGGC</td>
</tr>
<tr>
<td>anti-human CD98</td>
<td>CD98</td>
<td>MEM-108</td>
<td>Human</td>
<td>GCACCAACACGCTA</td>
</tr>
<tr>
<td>anti-Human Podoplanin</td>
<td>Podoplanin</td>
<td>NC-08</td>
<td>Human</td>
<td>GTTACTCGTGTTG</td>
</tr>
</tbody>
</table>
Bioanalyzer quality control
cDNA and antibody barcode

- Determined quality and average base pair size of the cDNA and antibody barcode DNA-pooled libraries
- Pooled less than 20 columns from each IFC to increase reads per cell
- cDNA pool and antibody barcode curves look standard with average bp in the expected range.

The average size of the antibody barcode pool was 186.8 bp and the average size of the cDNA pool was 602 bp.

1 Antibody peak
2 cDNA peak
Samples cluster in concordance with cell sorting and cell type analysis, again illustrating good on-IFC performance for mRNA sequencing.
Protein sequencing cluster analysis

Samples cluster in concordance with cell sorting and cell type analysis, again illustrating good on-IFC performance for protein sequencing.
Protein sequencing cluster analysis

Samples cluster in concordance with cell sorting and cell type analysis, again illustrating good on-IFC performance for protein sequencing.
Qualifying protein expression specificity

CD10 and CD19 on Nalm6

CD3 and CD45RA on Jurkat
CD10 antibody barcode is expected to be specific to Nalm6. Comparing Jurkat, K562 and HEK293, we demonstrate that CD10 reads occur in Nalm6.
CD3 protein expression and specificity

CD3 antibody barcode is expected to be specific to Jurkat. Comparing Jurkat, K562 and HEK293, we demonstrate that CD3 reads occur in Jurkat.
Protein expression results
CD29 co-expression total reads

CD29 was expressed in all cell lines as predicted. Expression of CD29 between all four cell lines was higher than the number of reads from α-mouse and IgG Ab BC controls.
REAP-seq
Summary

• Antibody barcode specificity used with C1 REAP-seq is shown to be very specific to the intended targets.

• Highly characterized proteins such as CD10, CD19, CD3 and CD45RA were differentially expressed by the appropriate cells as shown by clustering, flow cytometry and box plots.

• Total CD10, CD19, CD3 and CD45A reads were above the number of reads produced by α-mouse and IgG control antibody barcodes, as shown by the dot plots.

Download the C1 REAP-seq technical note today.
Creating a single-cell microenvironment
Single-cell omics and multi-omics

Single-cell processing

- Classify and identify new cell types but with loss of cellular context.

Single-cell functional analysis

- Create and control a single-cell environment to understand single-cell function.

Modified from Wang et al., *Cell Biology and Toxicology* (2018)
Single-cell functional analysis
Microenvironment and single-cell interaction

Enable

• Single-cell culture with time course dosing, imaging and molecular readout
• Single-cell contextual analysis

Discover

• Molecular mechanisms
• Drug efficacy and toxicity
• The correlation of single-cell mRNA sequencing with cell heterogeneity, response, interaction and phenotype using drug combinations

Cell suspension
Polaris system
cDNA
Polaris IFC
Active single-cell selection and treatment correlating phenotype to molecular readout

Micrograph (4X magnification) of the cell selection, capture and culture/dose sections of the Polaris IFC.

| 64 single-cell partitions | Selection and dosing path | Cell culture chambers | Chemistry |

Micrograph (4X magnification) of the cell selection, capture and culture/dose sections of the Polaris IFC.
### Polaris workflow

#### Prime

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prime</td>
<td>Hemo-cytometer</td>
<td>Cell Selection</td>
<td>Dose and Feed or Time Course</td>
<td>Post Stain/Wash</td>
<td>mRNA Seq</td>
<td>Results</td>
</tr>
</tbody>
</table>

- Prepares the control lines for microfluidic control
- User prepares chambers for culturing conditions:
  - **Suspension**: No extracellular matrix (ECM) deposited on culture chambers
  - **Adherent**: Deposit ECM on culture chambers (for example, fibronectin, 25 ng/μL)
Prime
Extracellular matrices tested on Polaris IFCs

- Fibronectin
- Laminin
- Vitronectin
- Collagen
- Untreated PDMS
Polaris workflow

Cell Selection

- Select 48 target single cells (3% to 100% of total cell population) across 3 fluorescence channels.
- Two-population selection feature allows for study of two samples: control vs. treatment study design, etc.
- Low cell input numbers needed. Start from an input of 300 to 8,000 cells per inlet (5 inlets/25 µL total).
- Size-independent selection of desired cells based on fluorescent markers
- Gentle path to capture chamber suits delicate cells
## Example of cell types tested

### Cell Selection

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Suspension/Adherent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431</td>
<td>Adherent</td>
</tr>
<tr>
<td>A549</td>
<td>Adherent</td>
</tr>
<tr>
<td>BJs</td>
<td>Adherent</td>
</tr>
<tr>
<td>Neurons</td>
<td>Adherent</td>
</tr>
<tr>
<td>PC3</td>
<td>Adherent</td>
</tr>
<tr>
<td>Basophils</td>
<td>Suspension</td>
</tr>
<tr>
<td>CTCs</td>
<td>Suspension</td>
</tr>
<tr>
<td>HL-60</td>
<td>Suspension</td>
</tr>
<tr>
<td>K562</td>
<td>Suspension</td>
</tr>
<tr>
<td>PBMC</td>
<td>Suspension</td>
</tr>
<tr>
<td>T cells</td>
<td>Suspension</td>
</tr>
<tr>
<td>CD34+</td>
<td>Suspension</td>
</tr>
</tbody>
</table>
Single-cell selection

Cells in partition during flow

Cells in partition when flow stops

Qualified single cells redirected to capture site

Cells flowed through multiplexer to capture site.

Imaging confirms arrival and viability of single cell.
# Single-cell selection

## Supported wavelengths

<table>
<thead>
<tr>
<th>Channel Name</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>Suggested Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zombie</td>
<td>438/28</td>
<td>570/30</td>
<td>Viability assessment (used in Post Stain only)</td>
</tr>
<tr>
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The following options are presented at this step:

- **Dose and Feed**: Different reagent groups treated at the same time points
- **Time Course**: Same reagent treated for all cells at different time points
- **No Treatment**: Skip treatment of single cells and proceed straight to chemistry.
Single-cell culture
Dose and Feed

- Flexible dosing routines to expose cells to a variety of extracellular compounds
- Up to 8 treatment groups of 6 single cells in each group
- All cells dosed at same times with a maximum duration of 24 hours
- Fluorescence imaging every hour
Single-cell culture
Dose and Feed

K562 cells transfected with nGFP mRNA (>15 hr)
Single-cell culture
Time Course

• Same reagent for all cells, provided at different time points
• Fixed at 6 groups of 8 cells per time point with a maximum duration of 24 hours
• Fluorescence imaging every hour

19 hr of feeding (K562)
Single-cell culture
Cell adheres, spreads and migrates with time

Selected BJ fibroblast

Capture site imaged on Polaris to confirm placement

After 20 hours in culture

20X high-resolution image on inverted microscope
**Polaris workflow**

**Post Stain/Wash**

- Provides the option to assess viability with staining (for example, Zombie stain) before initiating chemistry
- Default incubation time is 10 minutes and maximum incubation time is 1 hour.
- Images recorded before and after the incubation period.
Workflow options

1. Prime
2. Hemo-cytometer
3. Cell Selection
4. Dose and Feed or Time Course
5. Post Stain/Wash
6. mRNA Seq
7. Results

- Different reagent groups treated at the same time points
- No Treatment
- Same reagent treated for all cells at different time points

Interested in different treatment groups or different treatment time points?
Polaris enables study of extracellular matrix effect on hematopoietic stem cells (HSCs)

- Granulocyte-macrophage progenitors (GMP) are a subset of CD34+ hematopoietic stem/progenitor cells (HSPCs).

- Polaris single-cell culture allows evaluation of ECM’s effect on CD34+ cells, without intercellular signaling interference.

- Polaris features enable study of migratory phenotype and differential gene expression following mRNA-seq chemistry.
Polaris imaging revealed that laminin increases motility of CD34+ HSCs

Relative to untreated culture chambers, motility increased ~2-fold on laminin (24-hour single-cell culture on Polaris with imaging).
Polaris mRNA Seq chemistry reveals that motility was integrin-driven
Gene expression outlines motility triggered by integrin via small Rho GTPases

Differentially expressed between Ln and PDMS
Not observed in this dataset
Events related to motility
Polaris enables study of activity responses to agonist application on neural stem cells

‘Multimodal Single-Cell Analysis Reveals Physiological Maturation in the Developing Human Neocortex’


Neuron 102 (2019): 143–158.e7
Polaris enables study of activity responses to agonist application on neural stem cells

- Embryonic neural stem cells give rise to functioning neurons.

- Development of neural stem cells depends in part on secreted molecules.

- Polaris single-cell isolation allows evaluation of agonist-specific neural responses, without confounding extracellular signals.

- Polaris enables study of neural activity and differential gene expression following mRNA Seq chemistry.
Polaris dosing applied short-acting agonist on primary neural cells

A. Stain cells (Ca reporter + CTO)
B. Select Ca(+) + CTO(+)
C. Dose with agonist
D. Rapid imaging
E. Gene expression analysis

Primary neuron

Nonresponder

Responder
Use gene expression data to improve selection of responders based on novel biomarkers.
Summary
Single-cell functional analysis with Polaris

Cell selection:
• Low cell input numbers
• Size-independent selection of desired cells based on fluorescent markers
• Gentle path to capture chamber (great for delicate cells)

Short-term culture (24 hr):
• Suspension or adherent culture on extracellular matrix of choice
• Control of microenvironment

Dosing: Use addressable fluidics to deliver dosing reagents.

In situ assays: Determine the functional response of single cells.

Single-cell genomics: Generate mRNA-seq libraries.
Thank you.