RESEARCH SNAPSHOT

Retain More Single Cell Data to Gain Deeper Insights

Introduction

Single-cell RNA sequencing (scRNA-seq) is used to reveal rare and complex cell populations, uncover the role of genes involved in numerous disorders, and shed light on developmental pathways of distinct cell lineages[1]. Such relevant biological findings depend on generating high-quality scRNA-seq data, which can only be obtained from intact and viable single cell suspensions. Generating high-quality single cell suspensions from biological material remains a challenge, particularly when the number of cells is limited and the sample has been cryopreserved for a long period.

The **LeviCell® system** rescues cell suspensions from different sample sources, and here, we show how it performs using cryopreserved human bone marrow biopsy samples. The system uses gentle levitation to separate viable from non-viable fractions, increasing cell viability from 57% to 77% prior to scRNA-seq library preparation. The result is higher quality data produced when using LeviCell compared to an alternative method. Additionally, after applying stringent filters to remove stressed, dying, or dead single cells, the LeviCell dataset retained 10% to 15% more high-quality single cells over other methods. This underscores the value of the LeviCell system to improve the viability of single-cell suspensions derived from challenging samples to generate the highest data quality while maximizing the number of single cells represented in the dataset.

KEY HIGHLIGHTS

- 60% more genes and 100% more UMIs detected when using LeviCell to generate scRNA-seq data
- 4x lower single-cell loss in quality filtering steps in the LeviCell workflow
- LeviCell shows superior performance in generating more data for single-cell experiments as compared to FACS

Methods

We evaluated the efficiency of the LeviCell system for the enrichment of high-quality viable human bone marrow cells (BMCs) from cryopreserved multiple myeloma biopsies. These samples were cryopreserved for approximately five years and stored in cryotubes containing FBS+10% DMSO. The samples were thawed and processed using three distinct workflows: (1) control (no viability enrichment), (2) viability enrichment using flow cytometry, and (3) viability enrichment using LeviCell. Each condition was then followed by scRNA-seq library preparation on 10x Genomics's Chromium Platform and sequencing on Illumina's NextSeq 550 System. After sequencing, all three scRNA-seq data sets were assessed for the following key quality metrics using 10x Genomics' Cell Ranger software:

1. Median genes per cell: Median number of genes detected per cell-associated barcode. Detection is defined as the presence of at least 1 Unique Molecular Identifier (UMI) count.

2. Reads mapped confidently to the transcriptome: The fraction of reads mapped to a unique gene in the transcriptome with a high mapping quality score reported by the aligner.

3. Fraction reads per cell: Fraction of confidently mapped reads with cell-associated barcodes.

4. Median UMI counts per cell: The median number of UMI counts per cell-associated barcode.

Additionally, we used threshold filters for the percentage of reads mapping to mitochondrial DNA to remove stressed, dying, or dead single cells from the final dataset. This is a commonly used metric in singlecell data quality analysis and implemented in a public R package that is a reference for quality control and analysis of scRNA-seq data[2] (Seurat v4.0).

Results

Cell suspensions enriched for viability using the LeviCell system before scRNA-seq library preparation show consistent improvement of scRNA-seq metrics compared to cell suspensions enriched using flow cytometry (Figure 1). Even though both sample sets are enriched, the LeviCell workflow produces more transcriptional information than the flow cytometry dataset. LeviCell enriched samples outperformed the control samples for reads mapping to the transcriptome while delivering comparable performance in three of the four metrics (Figure 1).

To evaluate the impact of LeviCell sample enrichment on single-cell data quality, we used two cutoff values to filter out single cells based on the percentage of reads mapping to mitochondrial DNA, a stringent cutoff at 10% and a lenient cutoff at 20% (Figure 2).

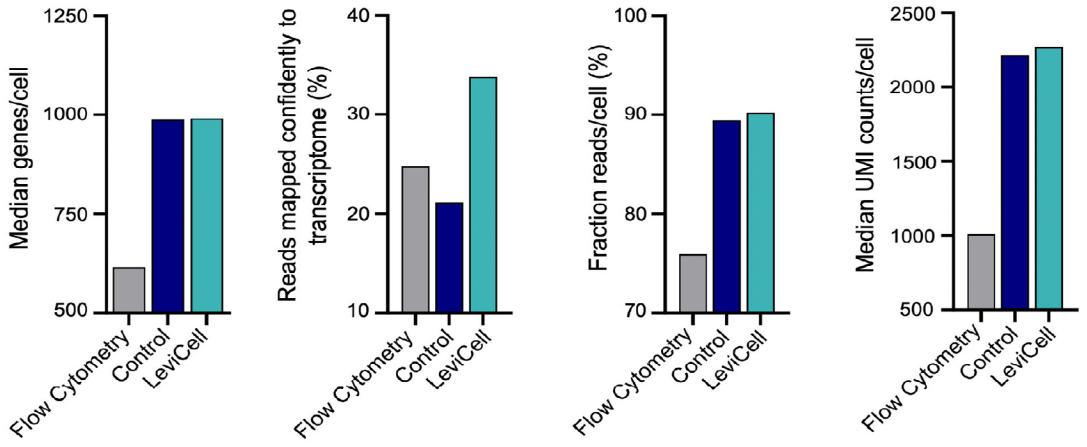

Figure 1. Analysis of key quality metrics from scRNA-seq data. *Data generated from LeviCell outperforms the flow cytometry dataset across all metrics evaluated, producing higher data quality.*

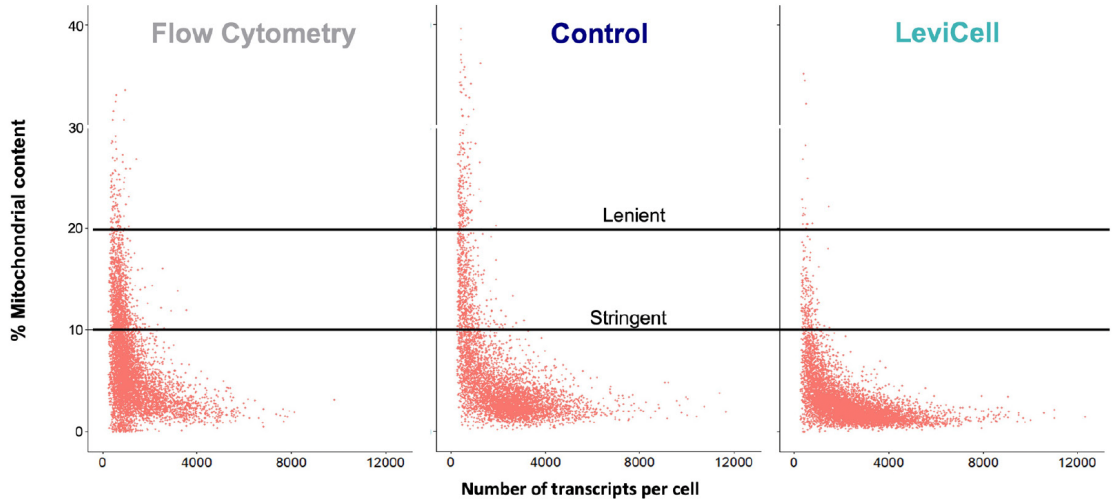

Figure 2. Filter thresholds applied to scRNA-seq data. *The LeviCell workflow retained more high-quality single cells in the final dataset as compared to the other workflows.*

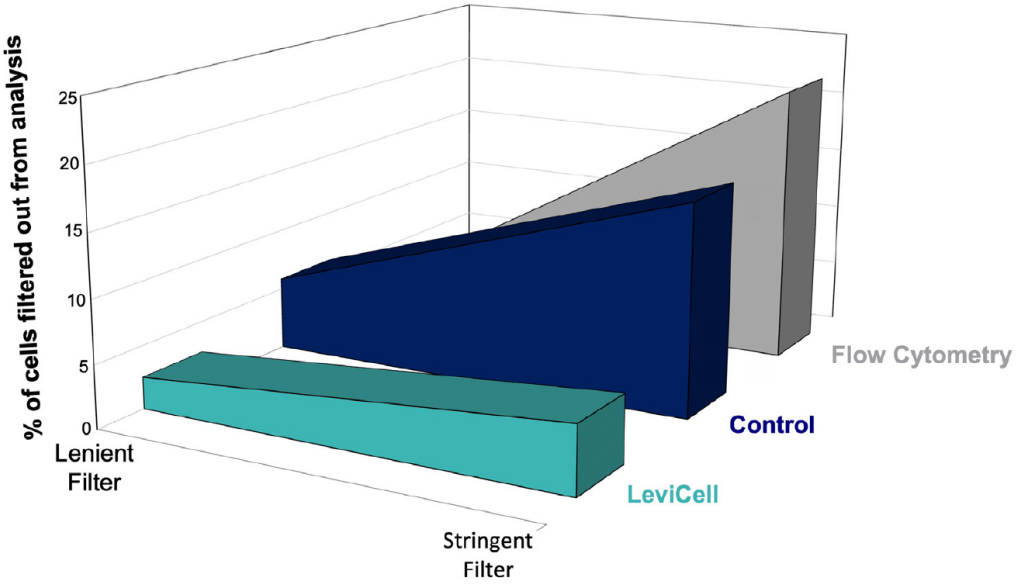

Figure 3: Cells filtered out from the analysis across all three workflows. *At the most stringent filtering criteria (10%), the LeviCell dataset retained 10 to 15% more high-quality single cells in the final dataset as compared to control and flow cytometry workflows, respectively.*

cells were filtered out from the LeviCell workflow compared to 22% from the flow cytometry and 16.7% from the unenriched, respectively (Figure 3). This means that 3-4x more cells were filtered out in the alternative workflows compared to the LeviCell workflow. At the 20% cutoff, the LeviCell workflow had comparable percentages of filtered cells as the flow cytometry workflow (2.5% and 2.2%, respectively) but a lower percentage compared to 5.8% from the unenriched (Figure 3). These results illustrate that LeviCell enriched samples deliver higher quality scRNA-seq data that tolerate the application of stringent quality filters without sacrificing the number of single cells retained in the final dataset.

Conclusions

The LeviCell system generates cell suspensions that deliver improved data quality compared to the control (no viability enrichment) and flow cytometry for scRNA-seq workflows. This is confirmed by the analysis of key scRNA-seq quality metrics as well as the use of a stringent quality filter using % mitochondrial DNA as a parameter to remove stressed, dying, and dead cells from the final dataset. When LeviCell was used prior to scRNA-seq there were no trade-offs required in quality versus the number of cells analyzed: 10% to 15% more single cells were retained in the final dataset for analysis as compared to the workflow without enrichment or that used flow cytometry, respectively.

References

1. Hwang, B., Lee, J.H. & Bang, D. ***Single-cell RNA sequencing technologies and bioinformatics pipelines.*** Experimental and Molecular Medicine, 2018.

2. Hao Y, Hao S, Andersen-Nissen E, et al. ***Integrated analysis of multimodal single-cell data.*** Cell, 2021.