Recovery of limited viable cells from low-quality precious samples for scRNA-seq

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ABSTRACT

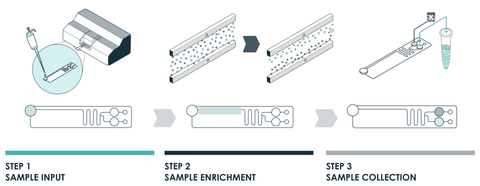
Biomarker discovery is a critical milestone in pursuing personalized and targeted cancer treatments, yet it faces formidable challenges hindering success rates. Limited access to high-quality samples restricts in-depth biological investigation, with scarce and compromised viable cells acting as barriers to progress. Levitation Technology™ addresses these challenges by significantly improving sample quality and preserving the maximum number of viable cells for single-cell RNA sequencing (scRNA-seq). This innovative approach to sample preparation overcomes limitations in sample quality and availability and heralds a new era of possibilities, propelling significant advancements in biomarker discovery for personalized cancer treatment.

To assess the performance of Levitation Technology using the LeviCell® 1.0 system, 105 cryopreserved samples derived from diseased human tissues were processed by Discovery Life Sciences™. This diverse sample set covered 20 disease indications, including 17 types of cancer. A staggering 70% of the samples (n=74) exhibited cell viability ranging between 14-69%, underscoring the need for enhanced viability for effective scRNA-seq analysis. Notably, 45 of these samples presented fewer than 500,000 cells, a scenario marked by low quality and limited viable cells. Traditional methods fail to recover viable cells from such samples without considerable or complete viable cell loss. Enrichment using the LeviCell 1.0 system improved the quality of all samples, with an impressive 90% of the most difficult samples (n=40) presenting a viability increase to 70-94%, meeting the minimum quality criteria for scRNA-seq. A notable success story was a particular breast cancer sample with only 31,000 cells and 14% viability. Enrichment with the LeviCell 1.0 system yielded 13,300 viable cells and a substantial 4.5-fold increase in viability to 64%. Such enhancements in sample quality translate into significant improvements in scRNA-seq data quality, enabling more single-cell data extraction from each invaluable sample.

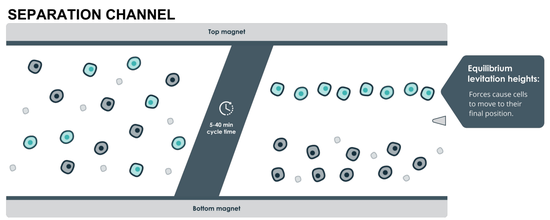
Levitation Technology represents a paradigm shift in sample preparation, rescuing even the most challenging samples. This groundbreaking technology maximizes the retention of viable cells while efficiently eliminating dead cells and debris, factors that have traditionally compromised scRNA-seq data quality. By revolutionizing this aspect of sample preparation, Levitation Technology opens unparalleled opportunities to explore disease biology deeper, providing a leap forward in biomarker discovery.

SYSTEM OVERVIEW

LeviCell Systems for Cell Enrichment
The LeviCell-1.0 is a single-channel system that enables gentle, label-free viable cell enrichment of the most challenging sample types. The LeviCell EOS enables the same workflows and has the added benefits of 4x higher sample throughput, more powerful imaging capabilities, and more flexibility for working with different types of samples.



Workflow
The LeviCell systems utilize the same simple, three step workflow. 1) Sample is loaded into the inlet well of a single-use cartridge. 2) The sample is drawn into the separation channel, where a balance of buoyancy, magnetic, and gravitational forces lead to the separation of viable cells from dead cells and debris. 3) Separated fractions are collected into two outlet wells and the enriched samples are removed.



Gentle Forces Act on Cells
Cells levitate at a height determined by the balance of buoyancy, magnetic, and gravitational forces. Dead cells and debris aren't affected as strongly by the magnetic forces and levitate lower than live cells.

IMPROVING THE QUALITY OF DISSOCIATED TUMOR SAMPLES

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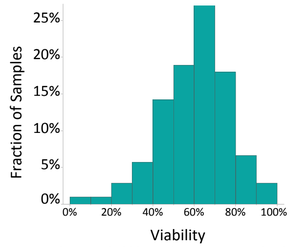
Many dissociated tumor and bone marrow samples have a low viability, making them unsuitable for single-cell sequencing. In this population of 105 samples, 72% of the samples have a viability <70%. The number of samples from each cancer type is shown in the table on the right.



Indication	N
Adenoma	1
AML	12
Benign Endometrial	1
Bladder Cancer	11
Brain Cancer	2
Breast Cancer	14
Carcinoid	1
Cervical Cancer	2
Colorectal Cancer	11
Crohn's Disease	1
Endometrial Cancer	9
Gastric Cancer	2
Head & Neck Cancer	2
Kidney Cancer	1
Liver Cancer	1
Lung Cancer	8
Multiple Myeloma	19
Ovarian Cancer	4
Polyp, Endometrial	1
Thyroid Cancer	2

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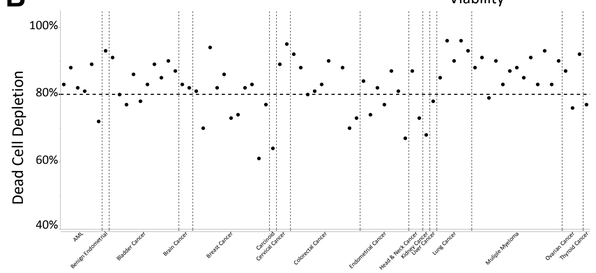

Examining only those samples with an initial viability less than 70% (n=76), on average 80% of the dead cells are removed from the sample using the LeviCell platform's viable cell enrichment workflow (indicated with the dashed line).

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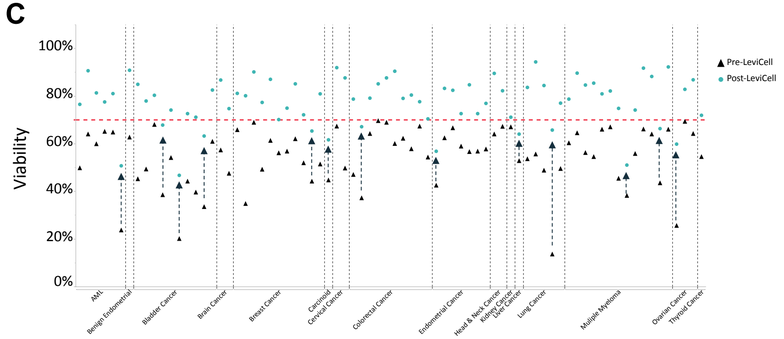
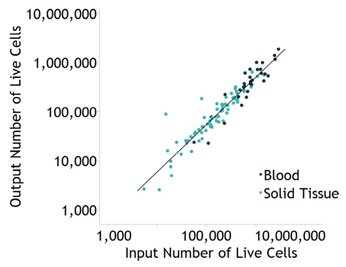


As a result of removing these dead cells, the viability of the output sample is increased. In most cases (83%) the final viability is greater than 70%, recovering these samples for downstream single-cell sequencing workflows. In all cases in which the final viability is less than 70%, the samples were still significantly improved from their initial viability (indicated with up-pointing arrows), rescuing them for single-cell sequencing.

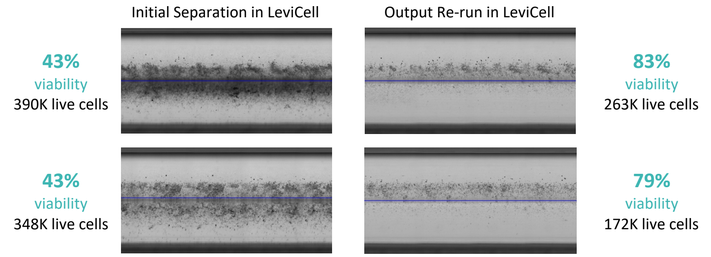
CONSISTENT PERFORMANCE ACROSS ALL INPUT AMOUNTS



The output number of cells remains proportional to the number of input cells across a broad range of input viable cell numbers, from ~5,000 cells to ~3 million cells. As a result, Levitation Technology and the LeviCell platform address the need for successful preparation of low input cell numbers.

VISUAL QC OF VIABLY-ENRICHED SAMPLES

BLADDER CANCER DTCs



Two different bladder cancer DTC samples were run through the LeviCell 1.0 system. Using the imaging capabilities built into the instrument, we see a significant amount of non-viable cells and debris, visible as the dark mass of material levitating lower in each image on the left. Both samples are significantly improved in viability after levitation. The images on the right were obtained by collecting the output of the first LeviCell runs and re-loading them on a second cartridge. The dead cells and debris have clearly been removed and are no longer present in the bottom half of the separation channel.

LEVITATION TECHNOLOGY APPLIED TO scRNA-SEQ

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Workflow	Viability	No. of Cells Sequenced	N. of Unique Transcripts/ Cell (median)	N. of Genes Detected/ Cell (mean)
No Enrichment	39%	1,150	3,411	1,370
Levitation Technology	87%	3,392	8,328	2,585

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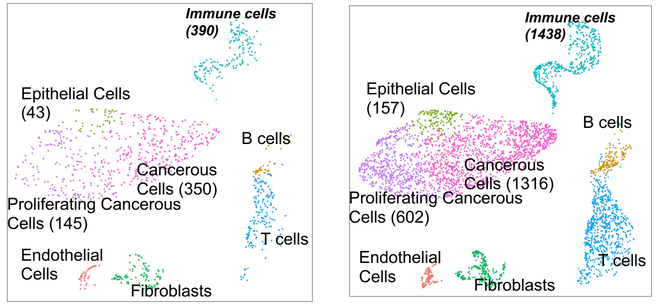

A dissociated sarcomatoid carcinoma sample with a starting viability of 39% was enriched using the LeviCell 1.0 system to a final viability of 87%. The unenriched sample and the viability-enriched sample were both put through a single sequencing workflow.
A) A comparison of several high-level metrics with and without using the LeviCell 1.0 for viable cell enrichment illustrates rescue of a low-quality sample using Levitation Technology.
B) t-SNE plots comparing the clusters of cells observed from a single-cell sequencing analysis of the sample with and without viable cell enrichment. After enrichment, more overall cells are observed with the same number of input cells.

CONCLUSIONS

- Levitation Technology allows for efficient viable cell enrichment across a wide variety of low-quality, dissociated tumor samples.
- The cell separation performance of Levitation Technology is consistent across a wide range of cell numbers, enabling viable cell enrichment with less than 10,000 cells.
- Levitation Technology provides a gentle, stress-free method for sample improvement and cell enrichment, removing both dead cells and debris contaminants.
- Viable cell enrichment enabled by levitation results in greater cell recovery and greater resolution of cells of interest in single-cell sequencing workflows.