TECHNICAL NOTE

Rescue Your Live Cells From Challenging Samples

Introduction

Preparation of single-cell suspensions from solid tissues can be complex and time-consuming. Furthermore, techniques used to obtain cell suspensions from tissue often damage the cells, resulting in membrane breakage, cell death, and debris. Additionally, multi-step workflows required to prepare samples can be lengthy and thus require cryopreservation prior to analysis: a step that can further compromise sample quality. The low-viability and low-quality single-cell suspensions obtained from these techniques greatly diminish the usefulness of their downstream investigation. For example, samples with high amounts of debris can cause inefficiencies in flow cytometry analyses by generating a slowdown of the flow rate and a higher incidence of instrument clogging. Samples with a high percentage of dead cells (≥30%) are not recommended for single-cell RNA sequencing (scRNA-seq) library preparation as a minimum viability of 70% is required for benchmark downstream scRNA-seq data quality when using the 10x Genomics platform ("Getting Started with Single Cell Gene Expression, Experiment Planning Guide", 10x Genomics).

The LeviCell™ system rescues poor-quality cell suspensions obtained from tissue dissociation, cryopreservation, or other methods that compromise cell viability, producing samples with high viability and live cell yield. The LeviCell workflow requires only three steps and 20 minutes of minimal hands-on time. To demonstrate the LeviCell system's performance, we tested 33 cryopreserved PBMC and DTC-derived cell suspensions with a starting viability ranging from 16 to 82% and cell input range from 18,500 to 7 million cells. All samples showed significant levels of enrichment of viable cells with a consistent live cell yield average of 64%. In parallel, we tested a subset of the DTC samples for viability enrichment with the Dead Cell Removal Kit (Miltenyi Biotec). The Dead Cell Removal Kit recovered 0.2% to 8% of the live cells when a total of 90,000 cells were loaded into the column, whereas the LeviCell system recovered 47% to 64% of the live cells loaded from similar samples.

Results

To evaluate the LeviCell system's performance in the enrichment and recovery of live cells from challenging samples, a total of 19 cryopreserved PBMC samples from different subjects were thawed and processed. Multiple operators processed the samples at different time points across several instruments. We used a cell input range of 18,500 to 7 million across all samples with a post-thaw viability of 16 to 82% (all cell concentration and viability measurements were performed using Propidium Iodide & Acridine Orange assay on the Cellometer K2, Nexcelom). After the independent LeviCell system runs for each of the 19 samples, cell suspension viability increased up to 5.7x for low viability samples and a viability range of 72 to 99% (Figure 1). Live cell recovery was accessed on all samples and all input ranges with the LeviCell system. The average live cell yield obtained was 64%, ranging from 31 to 84% (Figure 2).

We also used DTC samples to evaluate the performance of the system. This sample set is particularly interesting because it contains unwanted dead cells and debris resulting from tissue dissociation methods. A total of 14 DTC samples were tested; two were obtained from lung cancer, six from ovarian cancer, and six from breast cancer. This sample type has a limited number of cells for analysis, so the total number of cells loaded in the LeviCell system was lower than with the PBMC experiments. Between 52,000 and 900,000 cells were loaded into the LeviCell cells loaded in the LeviCell system was lower than with the PBMC experiments. Between 52,000 and

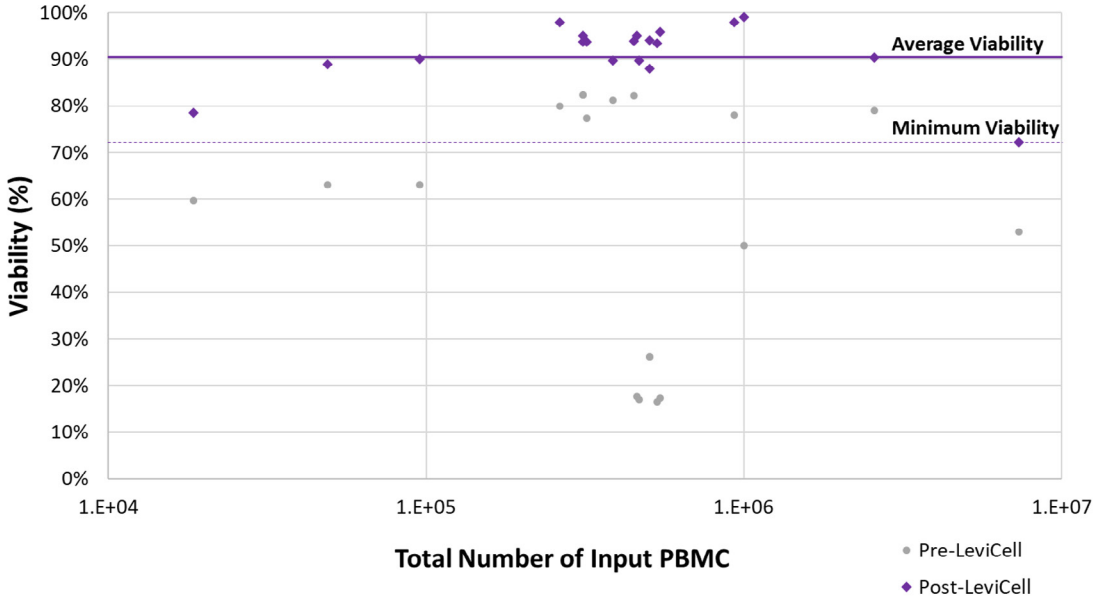


Figure 1: *Cell suspension viability of 19 cryopreserved PBMC samples before (gray) and after (purple) a LeviCell system run. The minimum increase in viability obtained was from 53% to 72% (dashed line), and on average, the viability reached was 91% (solid line).*

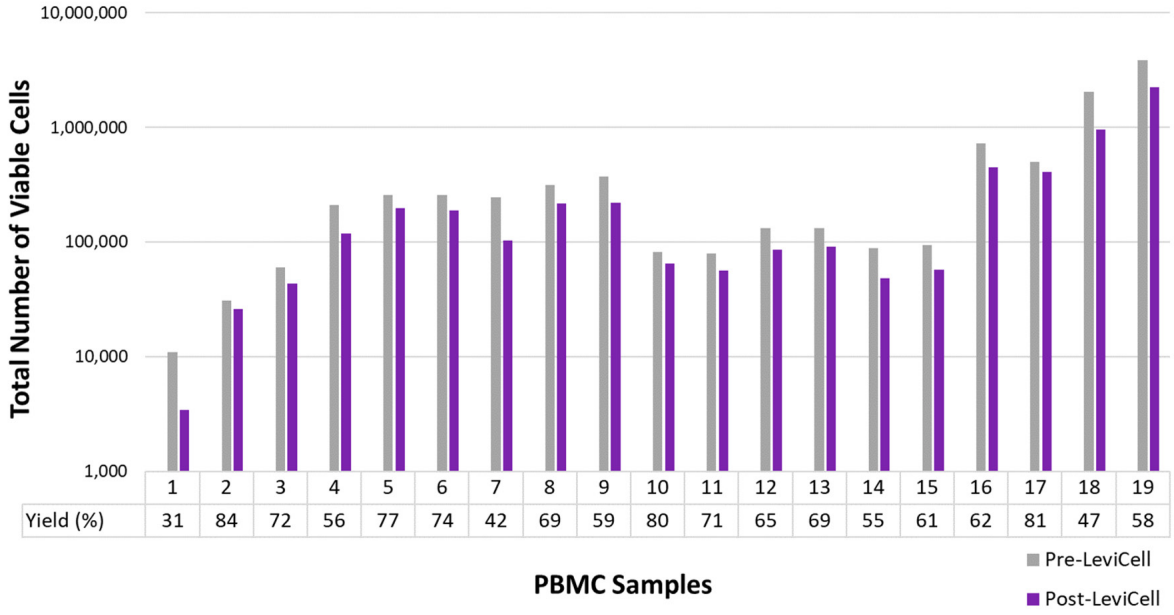


Figure 2: *Live cell yield obtained from 19 independent LeviCell system runs using varying PBMC input ranges. The gray bars show the viable cells input into the LeviCell system while the purple bars show the viable cells output from the system. On average, the live cell yield is 64%.*

900,000 cells were loaded into the LeviCell system with starting viability from 43% to 75%. The live cell output collected ranged from 23,000 to 257,000 cells, with an increased cell suspension viability of up to 2x for low viability samples and a recovered viability range of 84% to 96% (Figure 3).

The average live cell yield obtained was 64%, with a range from 34% to 88% (Figure 4). These results align with what was obtained using PBMC samples and demonstrate the robustness and reproducibility of the LeviCell system when enriching challenging samples.

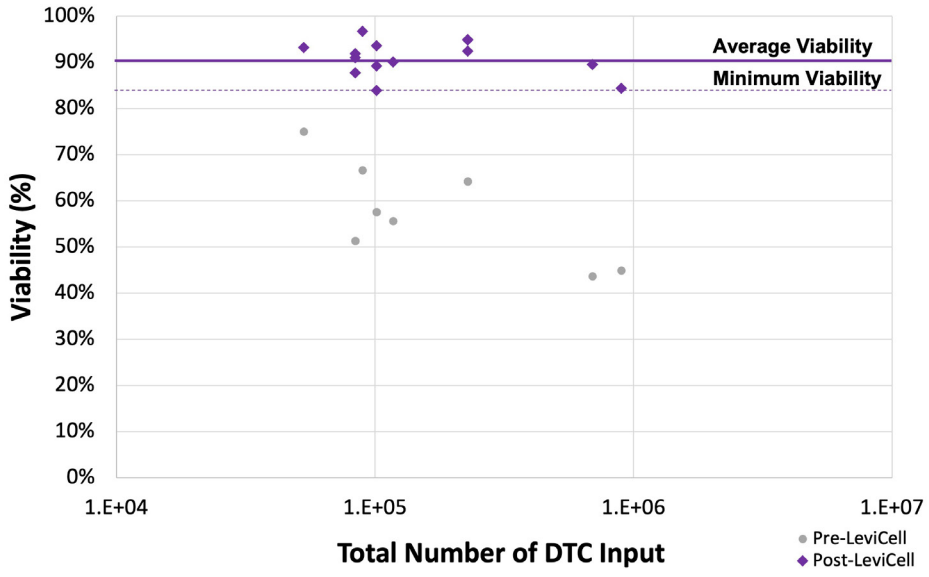


Figure 3: *Cell suspension viability of 14 DTC samples before (gray) and after (purple) the LeviCell system. The minimum increase in viability was from 53% to 72% (dashed line), and on average, the viability reached was 91% (solid line).*

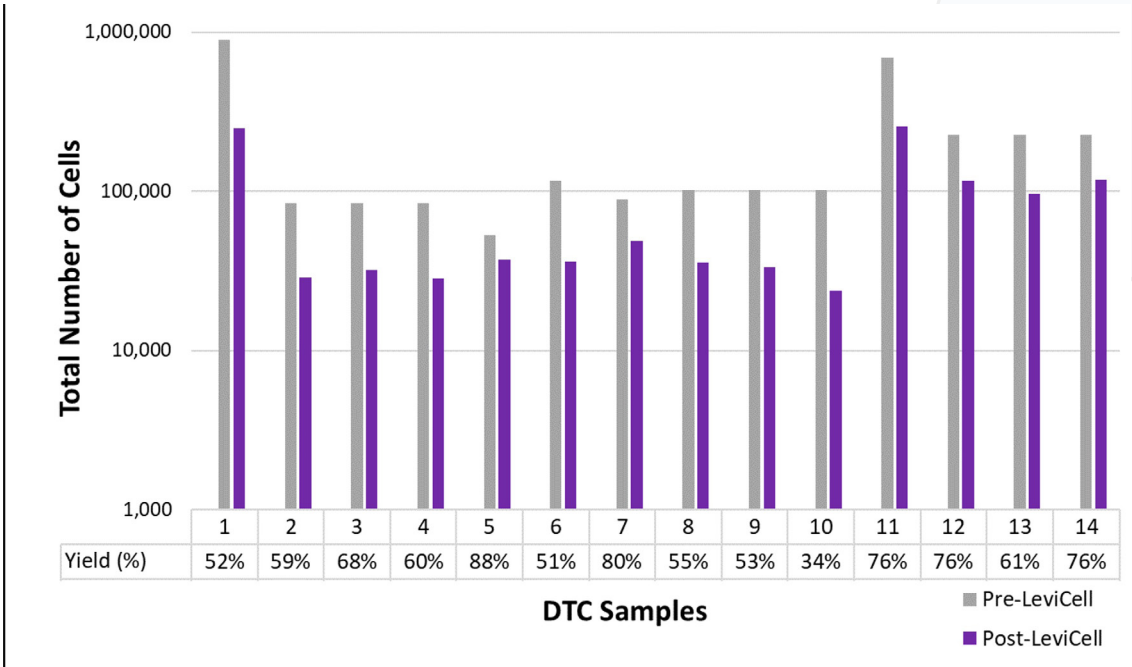
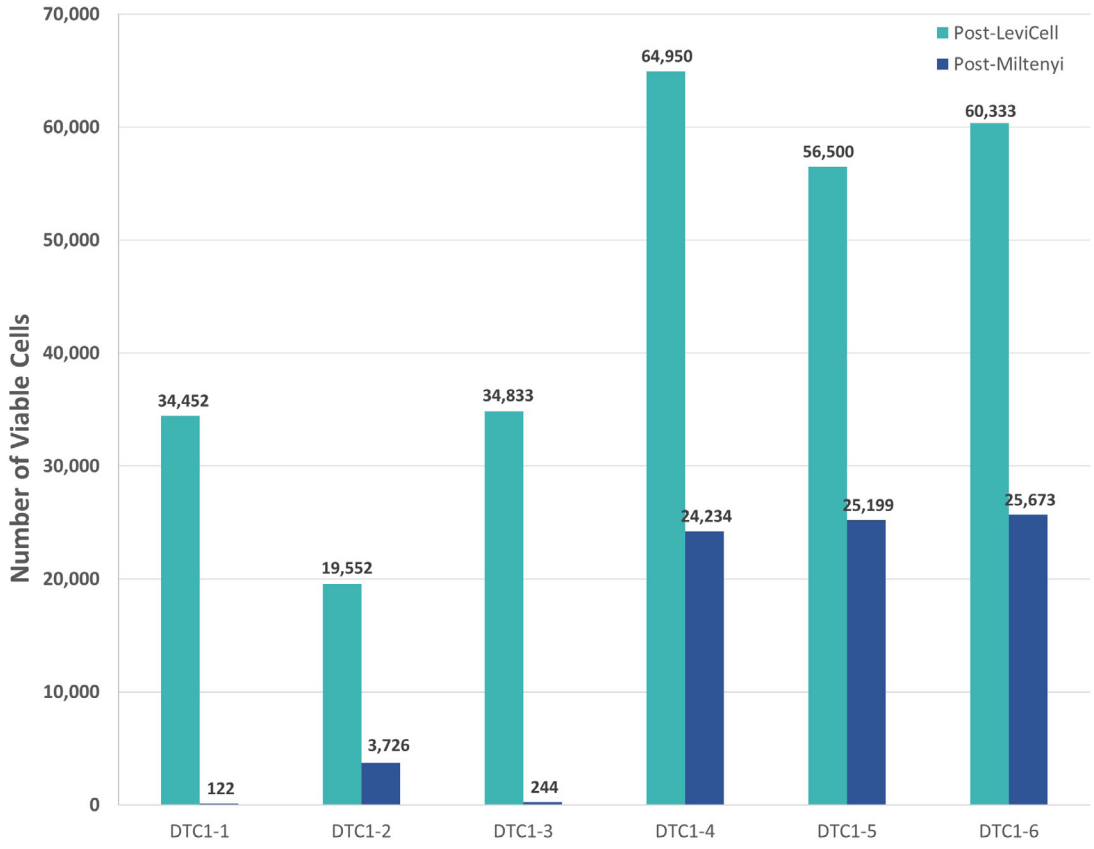


Figure 4: *Live cell yield obtained from 14 independent LeviCell system runs using DTC samples with varying cell input ranges. The gray bars show the viable cells input into the LeviCell system, while the purple bars show the viable cells output from the system. On average, the live cell yield is 64%.*



Yield (%)	DTC1-1	DTC1-2	DTC1-3	DTC1-4	DTC1-5	DTC1-6
LeviCell	64.2	46.8	63.3	81.8	73.6	80.7
Miltenyi	0.2	8.3	0.5	32.3	33.6	34.2

Figure 5: *Comparison of live cell recovery (yield) obtained from the LeviCell system and the Miltenyi Dead Cell Removal Kit when testing DTC samples at total cell inputs of 90,000 (DTC1-1 to DTC1-3) and 140,000 (DTC1-4 to DTC1-6) using a DTC sample at 50% viability.*

To benchmark the viability enrichment performance of DTC samples, we tested the LeviCell system alongside the Miltenyi Dead Cell Removal Kit. We used approximately 90,000 and 140,000 cells in both methods, with technical triplicates and a starting viability of approximately 50%. When 90,000 total cells were loaded into the Miltenyi Dead Cell Removal Kit column, only 8% of the viable cells were recovered in one technical replicate while the other two replicates recovered less than 1% of the viable cells loaded. In the same experimental conditions, the LeviCell system yield ranged from 46% and 64% across all of the three technical replicates (Figure 5). At 140,000 total cells loaded, the LeviCell system consistently recovered 73% to 81% of the viable cells present in the suspension compared to 32% to 34% with Miltenyi (Figure 5). For samples at higher input, which yield live cells from the enrichment methods, an equivalent viability % was obtained (Figure 6). The LeviCell system consistently enriched for viability over a range of low cell concentrations.

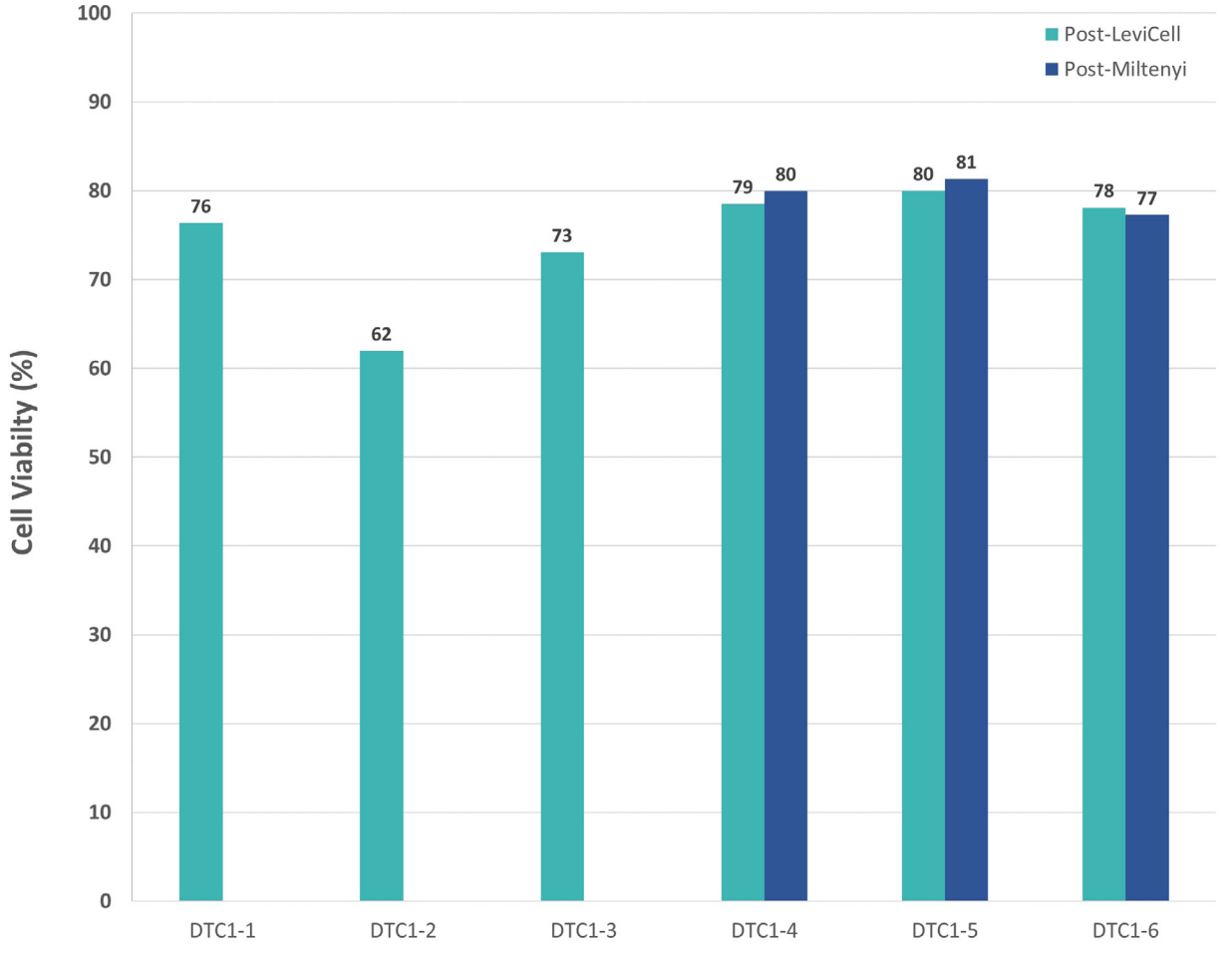


Figure 6: *Comparison of cell suspension viability obtained from the LeviCell system and the Miltenyi Dead Cell Removal Kit when testing DTC samples at two total cell inputs of 90,000 (DTC1-1 to DTC1-3) and 140,000 (DTC1-4 to DTC1-6) using a DTC sample at 50% viability. Comparable cell viability was obtained from both methods at higher sample loading albeit at lower yield (Figure 5) with the Dead Cell Recovery Kit.*

Conclusions

The LeviCell system consistently rescued viable cells from low-quality suspensions containing dead cells and debris providing usable samples in instances where the Miltenyi Dead Cell Removal Kit failed. The system delivered high performance across a range of cell inputs of 18,500 to 7 million cryopreserved cells with starting viability from 16% to 82%. The routine use of the LeviCell system ensures successful analysis of viable cells across a wide range of starting samples of varying quality quantity and sample types, such as PBMC and DTC-derived single-cell suspensions.