TECHNICAL NOTE

Redefining Efficiency: A Comparative Analysis of LeviCell EOS and LeviCell 1.0 Performance

Overview

Biomedical research often involves analyzing isolated cell populations, necessitating efficient cell processing technologies. The LeviCell® 1.0 system uses unique levitation technology for gentle and unbiased live cell enrichment. The LeviCell EOS system expands this capacity, offering a four-fold increase in throughput and up to 16x total scalability by interconnecting EOS modules. This study compares both systems' performance across a total of 89 runs with three sample types. Both systems exhibited a comparably high performance, achieving an overall average viability enrichment of 94% and an average live cell yield of 84%. The EOS system enables parallel processing in four lanes, supporting simultaneous diverse sample processing or single-sample maximization. Over 43 runs, live cell yield, and viability were consistent across lanes, demonstrating robust reproducibility. EOS also includes a comprehensive imaging system for real-time sample analysis across all four lanes during levitation, facilitating immediate quality control decisions for downstream workflows. Thus, LeviCell EOS mirrors LeviCell 1.0's reliability and presents enhancements for efficiency and scalability, further supporting its utility for cell processing and analysis.

KEY HIGHLIGHTS

LeviCell EOS enhances cell enrichment throughput while maintaining the same live cell yield and viability performance as LeviCell 1.0

- Same consistent and robust viability enrichment across systems, tested on samples with starting viability as low as 20% and reaching final viability of up to 96%
- Over 85% of dead cell removal, on average
- Four-fold throughput increase facilitates single-step enrichment, enhancing efficiency in sample processing workflows
- The EOS optical system allows visualization of all samples during the run allowing on-the-spot comparative analysis and decision-making for next step of the workflow

Enhanced Cell Processing Efficiency With Levitation: From LeviCell 1.0 to LeviCell EOS

Using magnetic levitation technology, the LeviCell 1.0 system efficiently delivers gentle, unbiased, and consistent enrichment of viable cells. Researchers' desire for greater throughput, scalability, and flexibility motivates the pursuit of even more innovative solutions. Building on the strengths of its predecessor, LeviCell EOS delivers an upgraded user experience for parallel sample processing and significantly greater throughputs (Figure 1).

With LeviCell EOS, researchers can maximize single-sample enrichment by loading up to four lanes in parallel to obtain more cells for the same sample or process up to four different samples simultaneously. Both scenarios minimize sample processing time for improved cell viability while achieving the required quantity of cells for downstream steps. The EOS Manager software allows universal control of up to four interconnected EOS Modules for even further scalability, enabling the processing of up to sixteen sample lanes at once.

The LeviCell EOS system's upgraded optics allows researchers to visualize the entire levitation process across samples in parallel. The new design of the LeviCell EOS cartridge supports optical scanning of the separation channel across all four samples during a single run. This enhanced feature permits the user to

perform a qualitative analysis to evaluate distinct sample characteristics, such as the presence of cell clumps, dead cells, debris, and persistent contaminants such as myelin, and observe the presence of heterogeneous cell subtypes. This visualization feature empowers researchers to make informed decisions on utilizing their enriched samples in downstream studies. The LeviMetrics software also allows for easy review and visualization of samples post-run and video generation of levitation and sample collection.

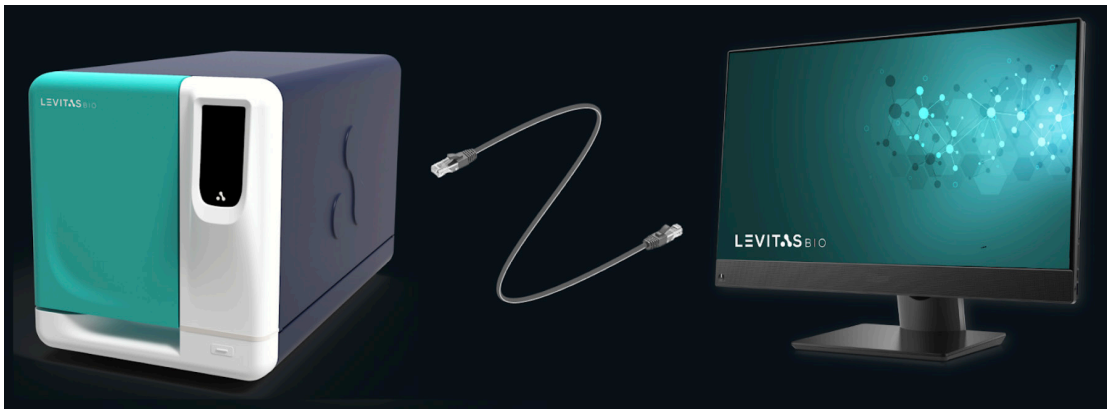

Figure 1. LeviCell® EOS System. *The system includes the EOS Module, computer, and EOS Manager software for instrument run control.*

System Performance Comparison Between LeviCell EOS and LeviCell 1.0

Live Cell Yield

We analyzed the outputs from 46 independent runs on LeviCell 1.0 and 43 runs on LeviCell EOS. The test samples comprised three types: a Leukemic T cell line (Jurkat), a non-small cell lung cancer cell line (H358), and peripheral blood mononuclear cells (PBMCs). Initial sample viabilities were between 20% and 60%. All samples were loaded at a total cell input between 100,000 and 600,000 cells.

The average live cell yield was consistently high across both systems. LeviCell 1.0 demonstrated a yield of 81%, while LeviCell EOS achieved an average yield of 86% (Figure 2).

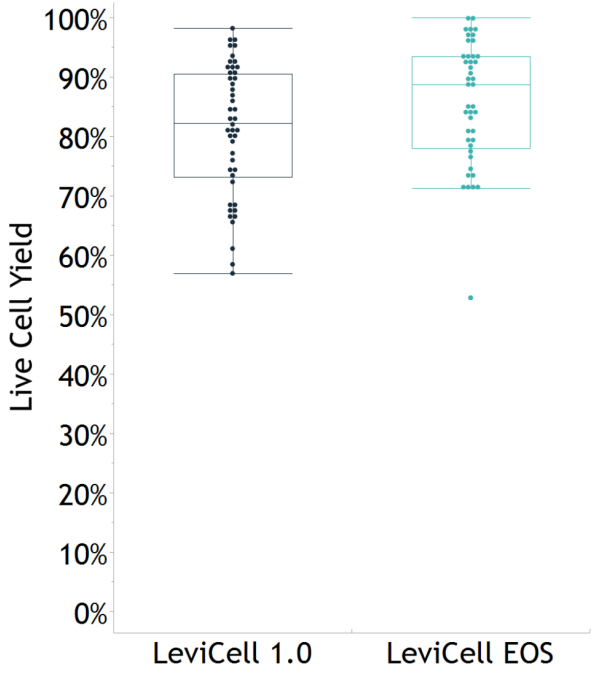

Figure 2: Live cell yield comparison between LeviCell® 1.0 and EOS systems. *Samples from various cell types (Jurkat, H358, and PBMCs) with initial viabilities between 20% and 60% were put through multiple runs, n=46 for LeviCell 1.0 and n=43 for LeviCell EOS. LeviCell 1.0 live cell yield was 81% and LeviCell EOS 86%, on average.*

Output Cell Viability

We used the same dataset shown in Figure 2 to evaluate final cell suspension output viability post-LeviCell(R) 1.0 and EOS system's sample processing. Both systems exhibited high average output viability, with an average of 94% for the LeviCell 1.0 system, closely mirrored by the LeviCell EOS system at 93% (Figure 3A).

Across both technologies and all 89 runs, the minimum number of viable, high quality and native-state cells obtained was 80,000, which meets the minimum number of cells required for downstream analysis using single-cell RNA sequencing (scRNA-seq), in addition to other applications. The average number of cells obtained across all 89 runs was 240,000.

Dead Cell Removal Performance

Beyond the preservation of live cells, a critical aspect of cell enrichment involves the removal of dead cells and debris from samples, as these can significantly impact the quality of downstream applications such as scRNA-seq and flow sorting. We defined dead-cell removal performance using the formula:

$$\text{dead cell removal} = \frac{\text{(dead cells input) - (dead cells output)}}{\text{(dead cells input)}}$$

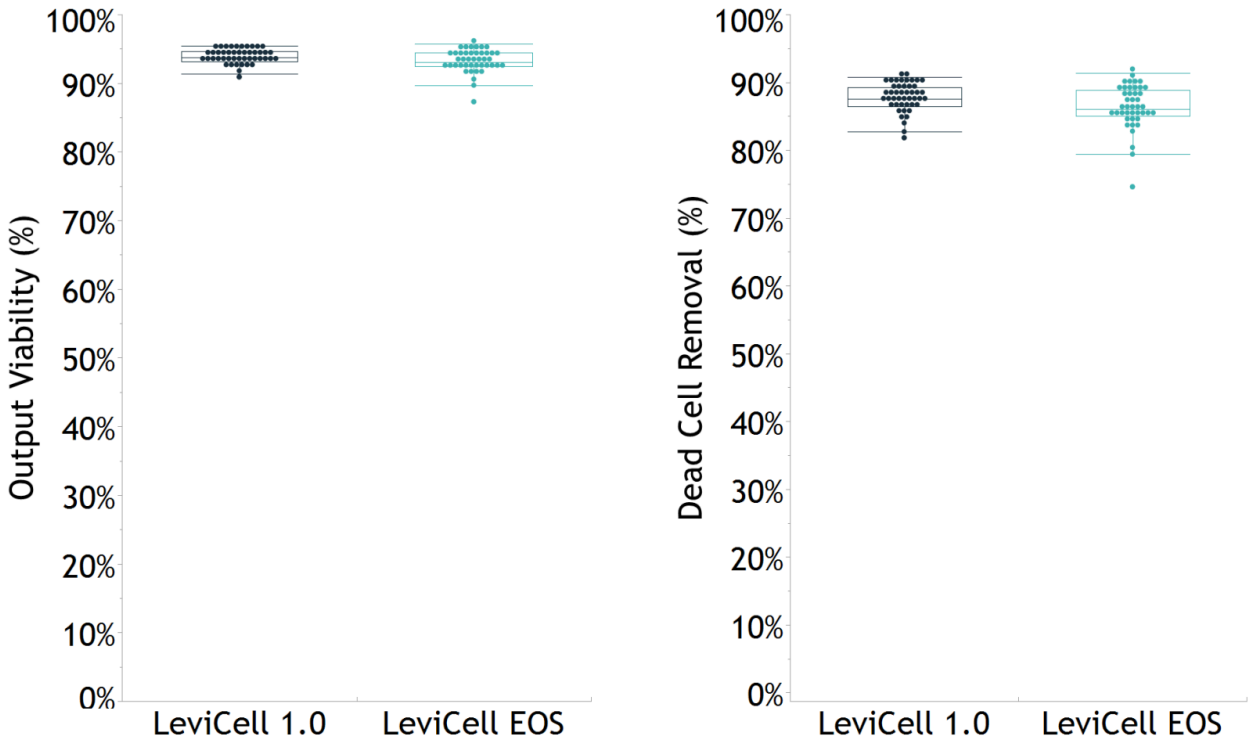

Figure 3: A. Output viability comparison (%) between LeviCell® 1.0 and EOS from the same dataset used in Figure 2. *Both systems delivered high cell suspension output viability: 94% for LeviCell 1.0 and 93% for LeviCell EOS.* ***B. Dead cell removal comparison (%) between LeviCell 1.0 and EOS from the same dataset used in Figure 2.*** *Dead cell removal % was estimated using the formula: (dead cells input)-(dead cells output)/(dead cells input). Both systems showed high dead cell removal results: 88% for LeviCell 1.0 and 86% for LeviCell EOS. This metric does not account for debris removal, a simultaneous outcome of LeviCell processing.*

High-Consistency Parallel Processing With Real-Time Visualization Through LeviCell EOS

In addition to achieving comparable and robust results to LeviCell® 1.0, the LeviCell EOS system has been designed to ensure higher throughput and simultaneous targeted selection of viable cells in parallel. For experiments requiring a high number of cells processed from a single sample, four aliquots of the same sample can be loaded across all four lanes of the LeviCell EOS system. Alternatively, researchers may need to process up to four different samples at a time. Both scenarios require high reproducibility, as researchers must have confidence that the system does not add variability to their experiments.

We conducted multiple runs with LeviCell EOS over each of the four lanes to validate the lane-to-lane reproducibility. The performance was consistent across the lanes, with comparably high live cell yields (Figure 4) and output viabilities.

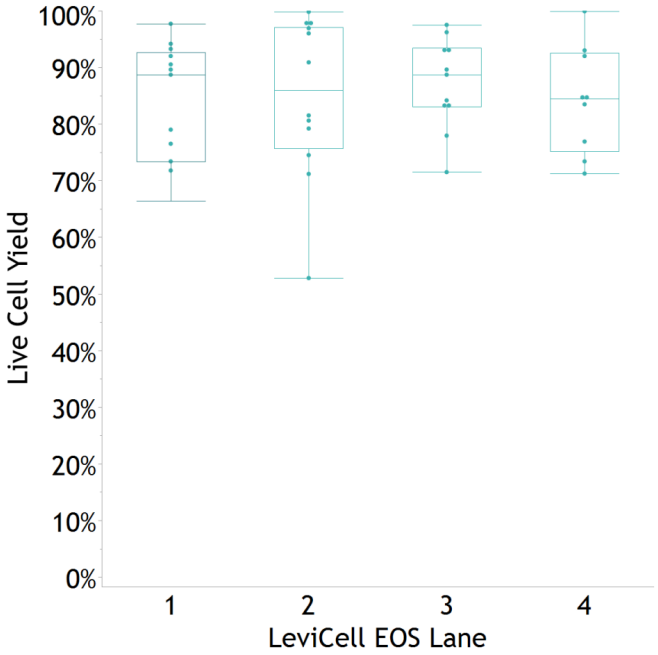

Figure 4: Live cell yield per lane in the LeviCell® EOS system across 43 runs. *The yield across the lanes is consistent, with an average yield of 83%, 85%, 87%, and 84% for lanes 1-4, respectively. No statistically significant differences exist between lanes for yield and viability metrics, proving the system's reproducibility.*

Another key advantage of LeviCell EOS is that the built-in optical system allows real-time visualization of all four samples during levitation. This powerful feature, carried over from LeviCell 1.0, has been upgraded to enable researchers to perform qualitative analysis and comparison across all four samples loaded in a cartridge during runs (Figure 5). This step allows for prompt decision-making, to proceed with downstream applications. For example, visual qualitative analysis can be performed during the enrichment step to determine the best samples to be used for expensive and time-consuming downstream analyses such as scRNA-seq. Additionally, live cell band thickness (top band of each lane in Figure 5) is correlated with number of live cells present in the sample. When a very thin band is observed, the user might decide to skip additional steps for cell quantification and move directly to the next application when working with rare samples.

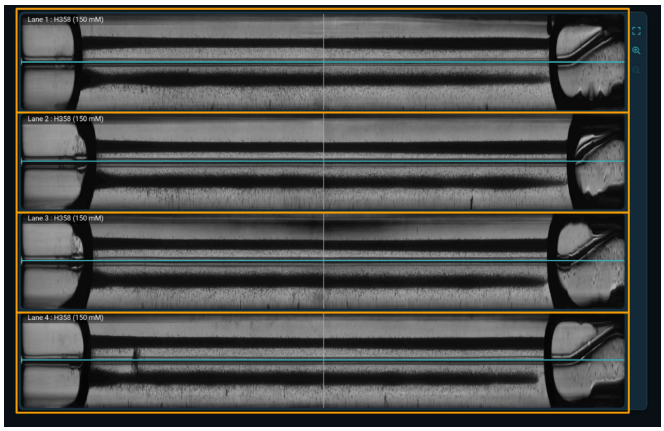

Figure 5: User interface for the LeviCell® EOS system's built-in optical system. *The fully integrated imaging system allows for parallel visualization of all four samples loaded for viability enrichment. The system scans the entire separation channel to obtain images of each sample during levitation. Viable cells levitate above the split line (top band above cyan line shown in each of the four separation channels); dead cells and debris are immediately below the split line. The user interface allows for a qualitative assessment of sample quality and comparison across the four samples loaded in the cartridge, allowing for prompt decision-making for downstream steps of the workflow.*

Conclusion

The LeviCell® EOS system mirrors the robust performance of the indispensable LeviCell 1.0 system for improved sample quality. In 89 runs involving three sample types and initial viabilities between 20%-60%, the systems exhibited an average viability enrichment of 94% and a live cell yield of 84%. Statistical analysis of output viability and dead cell removal showed no performance difference across systems. This consistency allows researchers to switch between the platforms seamlessly in the same study without experiment repetition or modification.

What distinguishes LeviCell EOS is its capacity for increased efficiency and scalability, with its ability to process and visualize four samples simultaneously. Researchers can use four parallel lanes to enhance single-sample enrichment or process four different experimental samples concurrently. The EOS Manager software facilitates control of up to four EOS Modules for further scalability, enabling the processing of up to 16 sample lanes simultaneously. This increased throughput is crucial for research facilities aiming to enhance their sample processing workflows by reducing processing time across multiple samples while improving cell viability to obtain quality downstream data.

Furthermore, the LeviCell EOS system includes an upgraded optical system that allows for full scanning and real-time qualitative assessment of all four samples during the levitation process. This on-the-spot visualization feature allows researchers to compare quality across samples for timely decision on downstream steps.

The transition from LeviCell 1.0 to LeviCell EOS marks a significant step in cell processing efficiency, offering increased scalability and flexibility while retaining the system's core strengths. Researchers can confidently utilize LeviCell EOS in their sample processing workflow with the assurance that they will benefit from the same superior performance in sample quality enhancement as achieved with the LeviCell 1.0 system.

For more information, visit **levitasbio.com,** or contact **sales@levitasbio.com**.