RESEARCH SNAPSHOT

Improve Flow Cytometry Efficiency and Analysis with Superior DTC Cleanup

Introduction

The resection of tumor tissue for in-depth study is a staple of cancer research. Dissociation of these tissues into a single cell suspension via enzymatic and/or mechanical techniques yields a cellular milieu that provides the closest representation of the clinical scenario by retaining the characteristics and cell type fractions present in the tumor. Access to these samples is critical to understanding the innate biology of the tumor and the host response. The fields of personalized medicine, drug/biomarker discovery, and preclinical/translational research rely heavily on the ability to study dissociated tumor cells (DTCs) from a wide range of cancer types using several different techniques. Central to the ability to study DTCs using any of these technologies is sample quality post-cryopreservation, particularly cell viability and purity (absence of debris).

Common Challenges With DTCs

The freeze-thaw process that these samples undergo inherently damages some portion of the total sample, typically manifested through cell loss due to routine handling and removal of the cryopreservative via washing. The freeze-thaw process may negatively impact cell viability. Further, the dissociation process itself can vary widely depending on the tumor and the host organ. The quality of the DTC suspension plays a significant role in the ultimate quality and usability of the sample post-cryopreservation.

In addition to the adverse effects of tissue dissociation and cryopreservation on total cells and overall viability, these samples are often plagued by the presence of cellular debris. A common use of these samples is to characterize them using routine cell sorting or analysis, but the amount and characteristics of the cellular debris present can impede this.

Excessive debris and dead cells can confound the ability to efficiently analyze the sample via cell sorting or analysis. Small bits of debris and dead cells can cloud the analysis, while larger debris can clog the instrument, resulting in extra time spent cleaning the instrument while the cells sit idle, further degrading the quality of the sample.

During a typical flow cytometry run, if there is a lot of debris and dead cells, high nozzle pressure is often used to process enough events to ensure the viable cells of interest are observed. This higher pressure in turn, adds undue mechanical stress on the cells. Fragile cells may not tolerate these conditions, and if the cells of interest are particularly fragile, there is a risk of losing those cells altogether due to artificial cell death.

KEY HIGHLIGHTS

Levitation Enabling Gentler Cell Processing

- Lower nozzle pressure required for flow cytometry on sensitive cell types
- 3-fold increase in viable cells
- 5 -fold decrease in dead cells/debris

Viable Cell Enrichment and Debris Removal Using LeviCell System

Optimizing the condition of these precious samples by magnetic levitation on the **LeviCell system** enriches the most viable cells and removes both small and large debris, as well as dead cells from the suspension (Figure 1). There are two primary benefits of sample optimization by levitation upstream of the flow cytometry workflow. First, enriched viable samples generate more meaningful events in the final analysis. Fewer dead cells and small debris events are present, allowing more confidence in the analysis and gating strategy. Second, if the sample contains a higher relative percentage of viable cells, then lower nozzle

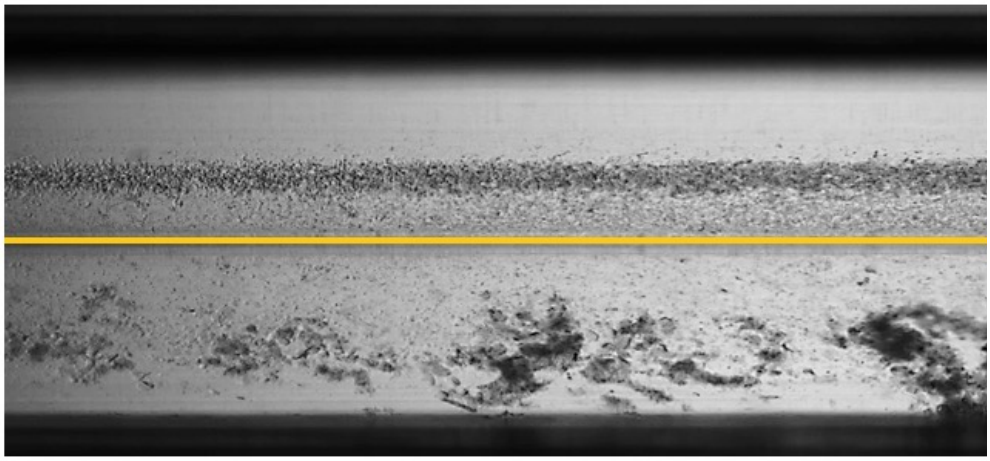

Figure 1. Viable cell enrichment with the LeviCell system. *Viable cells levitate above the split line (midpoint between the magnets), dead cells are immediately below the split line, and debris is observed as larger objects and clumps near the bottom of the levitation chamber.*

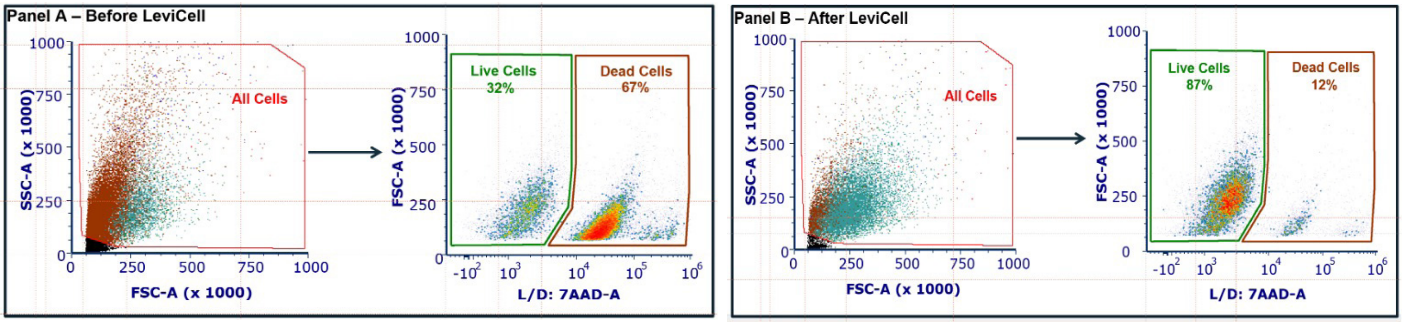

Figure 2. Flow cytometry analysis of a human lung DTC sample before (Panel A) and after (Panel B) viable cell enrichment using the LeviCell system. *After levitation, viability enrichment of the DTC cell suspension increased nearly 3-fold as shown by the increase in live cell events from 32% to 87% (Panel B). This was achieved by removing dead cells, which decreased over 5-fold, from 67% to 12%.*

pressure can be used which allows for more fragile cells to potentially withstand the mechanical stresses of flow cytometry.

As seen in Figure 2, this human lung DTC was characterized before and after magnetic levitation to enrich viable cells and remove dead cells and debris with the LeviCell system. 7-Aminoactinomycin D (7AAD) was used to distinguish between intact dead cells ($7AAD^{Pos}$) and viable cells ($7AAD^{Neg}$).

Conclusion

Fast and gentle magnetic levitation on the LeviCell system can significantly clean-up poor quality samples like DTCs, generating viable, high-quality cells prior to flow analysis. A higher viability, debris-free sample promotes more efficient flow cytometry analysis while enabling lower nozzle pressures, leading to better survival of particularly sensitive cell types.

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

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