Isolation of Unaltered Sub-Populations of Immune Cells Using Levitation Technology

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Abstract #255

ABSTRACT

The analysis and characterization of subsets of immune cells from heterogeneous samples, such as peripheral blood mononuclear cells (PBMCs), requires isolation and enrichment. Common methods for this enrichment step include fluorescence-activated cell sorting and positive or negative selection with magnetic beads. However, these methods alter native cell phenotypes during the enrichment process by the application of pressure, binding of antibodies, extensive manipulation and centrifugation, among other perturbations. This results in altered gene expression, generating molecular profiles that are not representative of true biological significance. These alterations are prevented by using magnetic levitation cell isolation and enrichment. The LeviCell™ platform performs hands-free cell enrichment by utilizing magnetic fields to levitate viable, healthy cells away from dead cells and debris. Unlike other single-cell separation methods, the enriched target cells are not subject to mechanical force, labeling, or staining to achieve viability enrichment. This results in a single-cell suspension that is ready for immediate downstream analysis, producing molecular profiles free of isolation-induced gene expression artifacts.

We have developed reagents to extend the benefits of this technology to the immuno-oncology research market. These new reagents target the enrichment of all T cells, the CD4+ or CD8+ subsets of T cells, or all B cells, with other target populations in development. In all cases, unwanted cells are captured within the levitation chamber, depleting them from the sample. These reagents leverage the magnetic field across the levitation chamber. Specifically tagged, unwanted cells are pulled to the edges of the levitation chamber while the target cells undergo viable cell enrichment. The end result is a cell population that has been enriched and at higher viability. In most cases, we have seen consistent depletion rates of >98%, resulting in a final targeted population with 90% or higher purity. Here we demonstrate the performance of the LeviCell platform in the enrichment of targeted cell populations relevant to the immuno-oncology research community.

SYSTEM OVERVIEW

Figure 1. LeviCell Systems for Cell Enrichment
The instrument on the left is the LeviCell-1.0, a single channel platform that enables gentle, viable cell enrichment of the most challenging sample types. The instrument on the right is the LeviCell EOS, which enables the same workflows with higher sample throughput, more powerful imaging capabilities, and more flexibility for working with different types of samples. Both instruments utilize the same simple, three step workflow.

BASIS OF MAGNETIC LEVITATION TECHNOLOGY

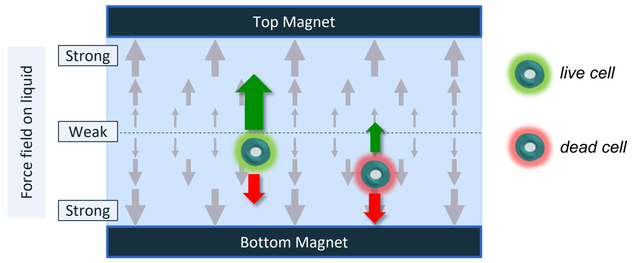


Figure 2. Basis of Levitation
Cells in suspension are placed in a channel held between two rare earth magnets. The solution containing the cells also contains a paramagnetic compound (Levitation Agent). As a result of the presence of this paramagnetic compound, the solution experiences a force pulling it towards the edges of the channel, shown in grey arrows. Live cells are impermeable to the Levitation Agent, and don't interact with it in any way. Because of this difference between live cells and the solution, the liquid displaces live cells towards the center of the channel (depicted in a green arrow). Gravity still acts on the suspended cells, pulling them down (depicted with a red arrow). As a result of these two forces acting on the live cells, they levitate at a height that corresponds to their density.

In contrast to live cells, dead cells tend to have permeable membranes that allow Levitation Agent into the cell. As a result, dead cells experience a greatly reduced buoyancy force and levitate lower in the channel than live cells. This levitation height difference allows magnetic levitation technology to separate live cells from dead.

APPLICATION TO TARGETED ENRICHMENT

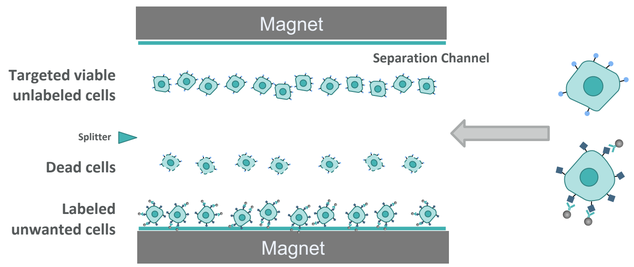


Figure 3. Enrichment of Specific, Targeted Cell Populations
Specific cell types can be targeted for enrichment by depleting other cell types. This is accomplished through the addition of antibody/magnetic nanosphere complexes that recognize specific markers expressed on the surface of cells to be depleted, as indicated in the cartoon images on the right. Once the cells are loaded into the LeviCell system, they are attracted to the magnets at the top and bottom of the separation channel. The remaining cells of interest continue to levitate as described in Figure 2, resulting in a separation of viable and dead cells. The depleted cells remain in the cartridge while the viable cells are removed. The output is therefore enriched in the viable targeted cells of interest.

TARGETED ENRICHMENT OF IMMUNE CELLS

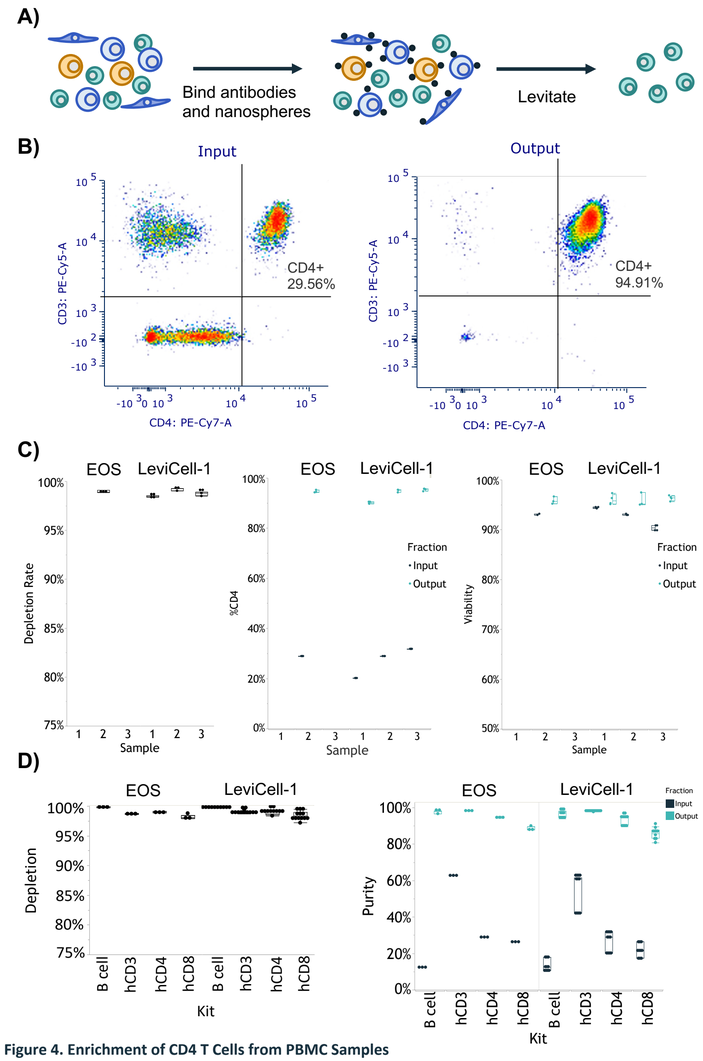


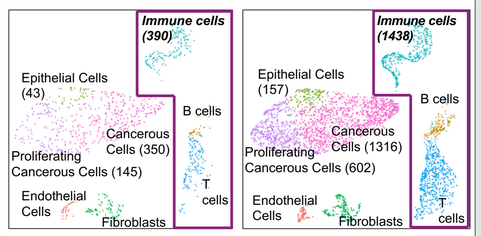
Figure 4. Enrichment of CD4 T Cells from PBMC Samples
In this case, the LeviSelect™ Human CD4 T Cell Kit was used to enrich CD4 T cells from a peripheral blood mononuclear cell (PBMC) sample. **A)** An illustration of the strategy for targeting the enrichment of a population of CD4 T cells. The desired cells are depicted as green cells. All other cells express cell surface markers that allow them to bind to specific antibody/magnetic nanosphere complexes (shown as small black circles). In the LeviCell system, these tagged cells will bind to the walls of the separation chamber, depleting them from the suspension. **B)** Flow plots illustrate the frequency of CD4 T cells in the input to and the output from the LeviCell. These plots are shown after gating for live cells, based on propidium iodide staining, and after gating for CD45 binding. After depletion, the CD4 T cells represent >90% of the final population. **C)** Comparison of multiple enrichment runs across multiple runs with different samples. Depletion (left; defined as the percentage of the non-target cells that are removed during the enrichment) is consistently >98%. This high depletion rate results in a significant increase in purity after levitation (middle). In addition, the viability of the samples is consistently increased due to enrichment using the LeviCell system (right). **D)** Depletion rates and output purity are shown for multiple LeviSelect kits, each targeting enrichment of a different population of immune cells.

LEVITATION TECHNOLOGY APPLIED TO scRNA-SEQ

A)

Workflow	Viability	N. 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

B)



C)

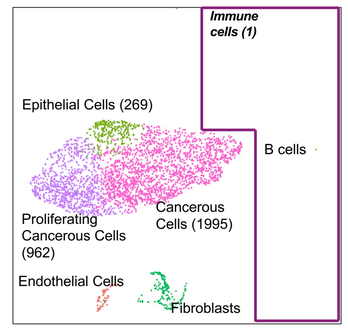


Figure 5. Enrichment of Tumor Cells in a Tumor Sample
A dissociated sarcomatoid carcinoma sample with a starting viability of 39% was enriched using the LeviCell system to a final viability of 87%. An additional aliquot of the sample was simultaneously depleted of CD45+ cells during viable cell enrichment. All three aliquots were put through a single sequencing workflow. **A)** A comparison of several high-level metrics with and without LeviCell viable cell enrichment illustrates the ability of the LeviCell system to rescue a low-quality sample. **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. **C)** After CD45-depletion, only one immune cell remains in the sample, greatly increasing the number of tumor cells that are sequenced.

CONCLUSION

- Levitation technology provides a gentle, stress-free method for cell separation.
- Levitation technology enables excellent viable cell enrichment due to the removal of dead and necrotic cells based on their permeability to the levitation agent.
- LeviSelect reagents can be used to enrich specific target populations, with >90% depletion of antibody-targeted cells.