A multimodal, label-free tool for gentle and efficient cell separation

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ABSTRACT

LevitasBio has developed the LeviCell™ platform, a powerful new solution for cell processing and characterization that utilizes magnetic fields to levitate cells. Unlike other single cell separation and cellular analysis methods, levitation does not require dyes, antibodies or specific markers, and the cells are not modified or perturbed in any fashion. In this novel cell separation platform, an inert, paramagnetic compound is added to the media in which the cells are suspended. The cells are added to a single-use cartridge, which is inserted into the LeviCell instrument where the cells equilibrate in the presence of an externally applied magnetic field. The cells levitate in solution to specific heights determined by the cells' intrinsic properties, including density and magnetic susceptibility. The cell suspensions are imaged in real-time, enabling complete analysis and characterization of the sample with real-time control over the cell separation. The simplicity of the system allows cells to be treated gently without use of high pressure or other perturbations that commonly lead to increased cellular stress responses, cell activation, or cell death. The simple, direct flow path leads to high yields of cells even with very low input cell numbers.

This novel new technology has been applied in a variety of applications, including the enrichment of viable cells from dead or dying cells and debris, separation of different cell types possessing distinct densities, and the separation of differentiated from undifferentiated cells. Here we demonstrate the performance of the LeviCell platform in the enrichment of viable cells from a variety of samples, including cell lines and primary samples such as peripheral blood mononuclear cells (PBMCs) and dissociated tumor samples. Dissociated tumor samples with a starting viability of 50-60% are readily enriched to viabilities of greater than 85%. Gene expression studies comparing cells pre- and post-separation highlight the gentleness of the enrichment approach. The ability to enrich live cells with high recovery is particularly significant for analytical applications such as single cell sequencing, as well as cellular expansion workflows such as cell line development, iPSC workflows, and CRISPR recovery. Here, we demonstrate significant improvements in the number of cells and transcripts identified in single cell sequencing following enrichment of viable cells on the LeviCell system.

BASIS OF LEVITATION

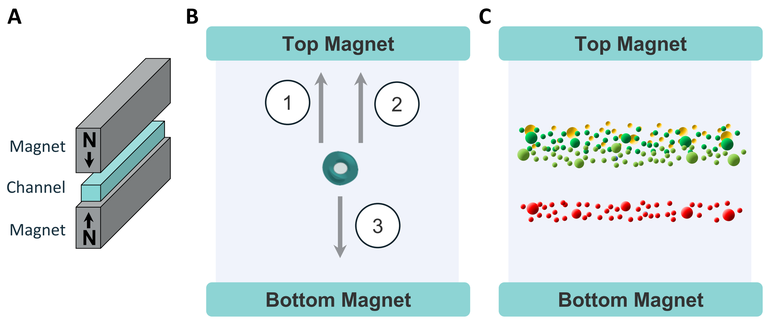

Figure 1. Illustration of the LeviCell system's magnetic field and resulting cell levitation
A) The flow channel containing cells in a paramagnetic medium is placed between two magnets.
B) Within the cross-section of the levitation chamber, cells reach their final levitation height determined by a combination of cell buoyancy (1), magnetic forces created within the paramagnetic fluid (2), and gravity (3), generalized in this simple illustration.
C) This view illustrates different cell populations at different levitation heights. Live cells (shown in yellow and green) will levitate high and according to their density or magnetic properties. Dead cells (red) will levitate lower in the channel since their membranes tend to be permeable, and they take up the paramagnetic media.

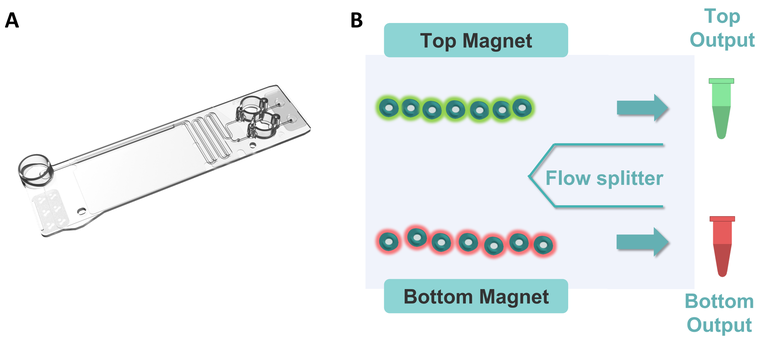

Figure 2. Cell separation in the LeviCell instrument
A) The design of the LeviCell cartridge includes an inlet port, a separation channel, and two output ports.
B) After levitation, the separated cells are pulled past a splitter in the levitation channel and into the output ports, where they can be collected for downstream use.

INTRODUCTION OF THE LEVICELL PLATFORM

Figure 3. LevitasBio has developed the LeviCell platform to perform cell separation
The instrument on the left is the original LeviCell-1.0 instrument. This instrument provides an easy-to-use workflow that allows the user to separate cells using magnetic separation in just 20 minutes. The instrument on the right is the LeviCell EOS, which provides the same simple workflow, but with four samples at a time. Multiple instrument modules can be run in parallel from the same computer, allowing for dramatic increase in sample throughput. Both systems provide imaging of the samples during levitation in brightfield and two fluorescence channels.

THE LEVICELL SYSTEM ENRICHES VIABLE CELLS AND REMOVES DEBRIS

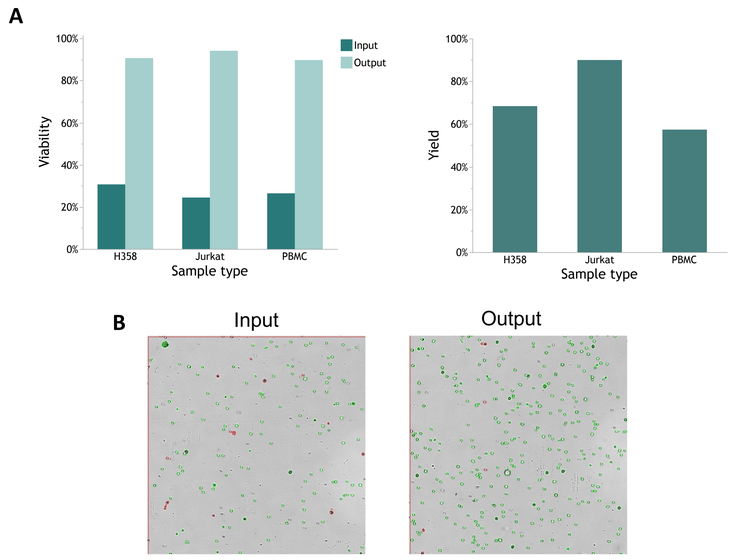

Figure 4. Live cell enrichment applied to different cell types
A) Populations of two different cell lines and PBMCs were killed using 70% ethanol and then mixed with live cells from the same starting stock to generate starting populations with a viability of ~25%. The resulting populations were enriched for viable cells using the LeviCell. The average final viability after enrichment ranged from 90% to 94%. The average yield of viable cells ranged from 57% to 90%.
B) A population of Jurkat cells was allowed to overgrow, resulting in reduced viability as well as debris (left image). Viable cells are indicated with green circles, dead cells in red circles, and debris with no color. Levitation resulted in a population with increased viability and reduced debris quantity (right image).

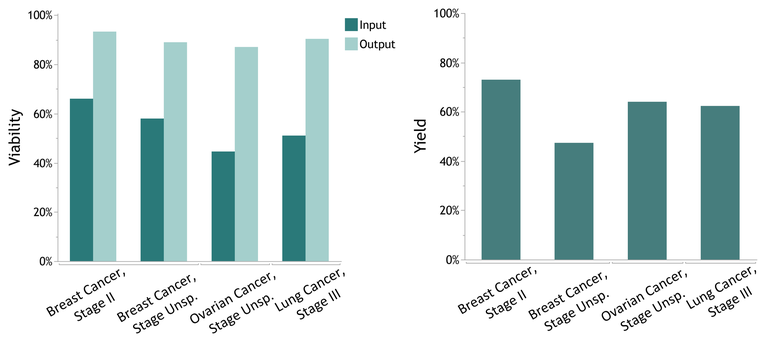

Figure 5. Live cell enrichment applied to a more complex primary sample
DTC samples representing a variety of different cancer types were purchased from Discovery Life Sciences. Each sample starts with a viability ranging from 45-66% based on the properties of that specific sample. After passing each sample through the LeviCell, the average viability of the output samples range from 87% to 93%, and the yield ranges from 47% to 73%. This improvement in viability increases the overall quality of downstream applications, such as single cell sequencing.

IMPROVEMENTS IN SINGLE CELL SEQUENCING

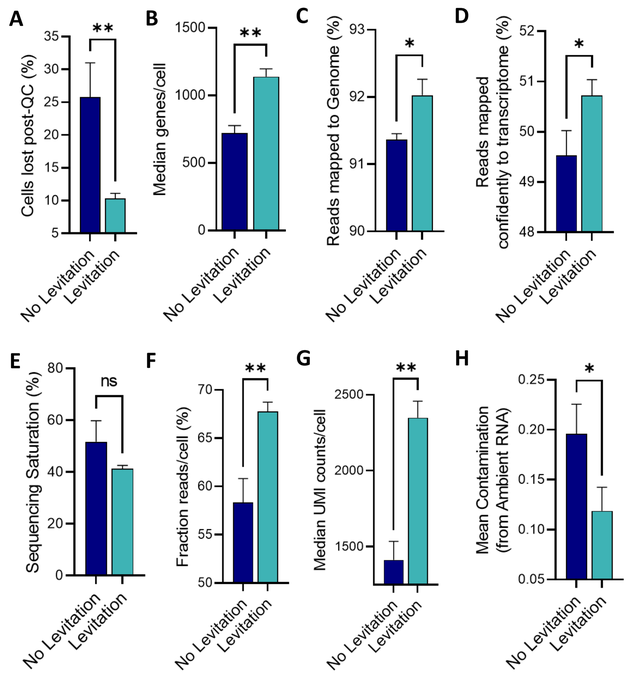

Figure 6. LeviCell cleanup improves single cell sequencing performance
An adult murine brain from a perfused animal was obtained from The Jackson Laboratory. After enzymatic dissociation, myelin was removed using a Percoll® centrifugation protocol, and the resulting single cell suspension was enriched for viable cells using the LeviCell system. Enriched samples were compared to non-enriched after processing using 10x's Chromium Next GEM Single Cell 3' Kit v3.1 chemistry. These plots compare a variety of QC metrics. In most cases, the levitated sample significantly out-performed the non-enriched.

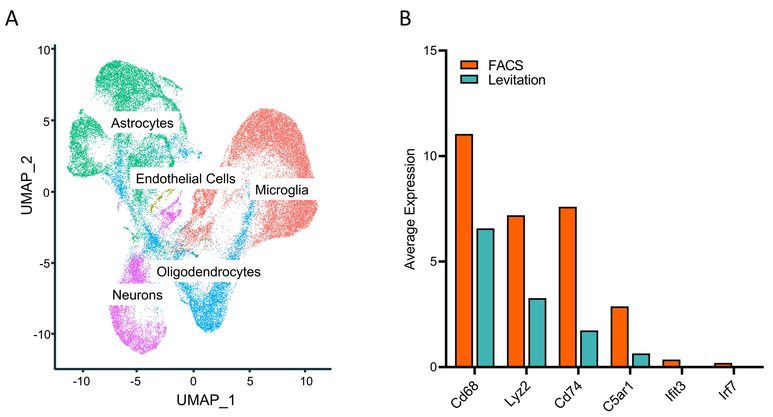

Figure 7. Analysis of scRNA-Seq data following LeviCell cleanup
A) The single cell RNA-Seq data from the experiment described in Figure 6 are plotted as a UMAP. This visualization of the data indicates that all major cell types from the brain are represented in the output cells.
B) To illustrate the gentle handling of cells, we focused on the microglia and compared the expression levels of a series of stress markers between cells enriched using the LeviCell to cells enriched using FACS. The LeviCell prevents the activation of microglial cells.

CONCLUSIONS

- The LeviCell platform's 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. Levitation can be applied to separate cell types based on their intrinsic differences in density and magnetic susceptibility. Enrichment of viable samples improves the quality of data that can be obtained from single cell sequencing experiments.

REFERENCES

Durmus, N.G., et al., "Magnetic levitation of single cells", Proceedings of the National Academy of Sciences, 2015, 112(28):E3661-8