RESEARCH SNAPSHOT

# Study of Disease Enhanced by Modulation of Levitation Height

## Introduction to Levitation Technology

LevitasBio has developed the LeviCell™ platform, an exciting new technology that utilizes a magnetic field to separate cells based on the intrinsic properties of different cell types. In its simplest form, levitation occurs through the use of a magnetic field in the presence of an inert paramagnetic compound (Levitation Agent) which is added to the cell suspension media. The cell suspension is loaded into a separation channel within a single-use cartridge consumable, where it is then exposed to a magnetic field. The cells' intrinsic properties, including density and magnetic susceptibility, determine the height to which the cells levitate (or equilibrate) within the channel. Cell separation occurs when different cell types equilibrate to different levitation heights within the levitation media due to celltype density and/or magnetic susceptibility differences. The **LeviCell system** includes image capture to enable visualization during cell equilibration. This imaging capability allows for both real-time and postcollection sample analysis and characterization. It has the added benefit of real-time control over the cell separation during the post-equilibration separation flow.

The simplicity of **Levitation Technology** enables cells to be treated gently, since there is no cellular exposure to high pressures or other perturbations that commonly lead to increased cellular stress responses, specific cell type activation, or even cell death. The direct and gentle flow path leads to high yields of live cells even with low input cell numbers.

Levitation Technology does not require dyes, antibodies, specific markers, or magnetic beads, and the cells are not required to be modified or perturbed in any fashion to do live cell enrichment. Thus, cell separation can occur without any type of labeling that could affect cell physiology other than being in a single cell suspension. If more targeted methods for cell enrichment are required, however, levitation technology can be adapted to meet user needs. For example, if a mixed cell population in suspension contains cells of different types with similar levitation properties (i.e. that equilibrate to the same levitation height), being able to separate those cell types can be beneficial. In such a situation, various labeling technologies can be employed to augment the power of cell separation by levitation alone.

## Modulation of Levitation Height

Since levitation technology leverages the variations in the density and magnetic susceptibility properties of cells, any changes to these properties can alter levitation height. Examples of such cell alterations are: genetic disorders such as thalassemia[1]; cell differentiation (internal data); or binding of cell surface epitopes with an antibody conjugated to a low-density, high-density, or magnetic particle. Combining these factors can be used to add further dimensions to cell separation and sample processing.

To demonstrate how density difference affects levitation, we can levitate polymer microparticles composed of materials with different densities. After resuspending particles in a buffer containing the paramagnetic Levitation Agent and equilibrating on the LeviCell, we can clearly visualize the separation of two different density particles (Figure 1).

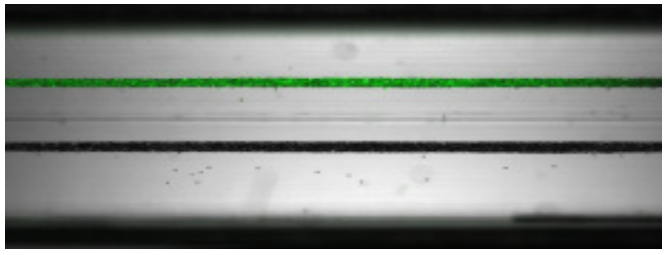

***Figure 1. Effect of low- and high-density particles on levitation height.*** *The particles shown in green are fluorescently labeled particles of a low-density polymer, and consequently levitate high in the separation channel. The non-labeled (black) particles consist of a different, higher density polymer and as a result levitate lower in the channel.*

The denser the particle, the lower it will levitate in the magnetic field compared to a less dense particle. Thus composition and magnetic susceptibility of particles can alter the levitation height.

Leveraging the availability of: 1) different material density micro/nano-particles, 2) magnetic micro/nano-particles, 3) libraries of antibodies to cell surface antigens, and 4) the cell surface antigens of different cell types, allows us to modulate cellular levitation height. Using this matrix of different particle and cell-attachment options can thus open a variety of target-specific magnetic levitation applications. Combined with viable cell enrichment inherent in the levitation technology, these additional features that can specifically target cells of interest for depletion or enrichment can be a powerful tool. Combining live-cell enrichment and levitation height manipulation can also reduce the number of steps required for sample processing.

To demonstrate the ability to alter cell levitation height away from the native phenotype, we first examined the use of small dense particles that are directly conjugated to a cell surface receptor specific antibody. Jurkat Clone E6.1 (T-cell) cells and H358 (lung epithelial) cells were mixed in this experiment. The Jurkat cells were labeled with Calcein-AM to visualize the populations more effectively and use the fluorescent imaging capabilities on the LeviCell system. In contrast, H358 cells were labeled with CellTracker™ Red CMTPX Dye (Thermo Fisher Scientific PN C34552). The cells were mixed at a 50:50 ratio prior to incubating both with and without anti-human CD45 antibody-labeled higher density microparticles.

The anti-human CD45 particles did not bind to the H358 cells, as demonstrated by the similarity in the levitation height in the presence of particles (Figure 2, right) compared with the levitation height in the absence of particles (Figure 2, left). The anti-human CD45 particles did bind to the Jurkat cells, as expected. This binding resulted in a lowering of the levitation height of the Jurkat cell population in the LeviCell System. Due to the number of particles bound per Jurkat cell, they equilibrated to a new height that is in between the levitation heights of unbound cells and particles alone. Particles alone, which are quite dense, levitated at a much lower height in the separation channel of the LeviCell cartridge, demonstrating that the particle density is higher than the complex formed between the Jurkat cells and particles.

Since Levitation Technology relies on a magnetic field created by magnets, the ability to tune magnetic strength or use magnetic particles to alter cell levitation height can also be used as another parameter to enhance cell separation. The number of magnets or the magnet positions relative to the

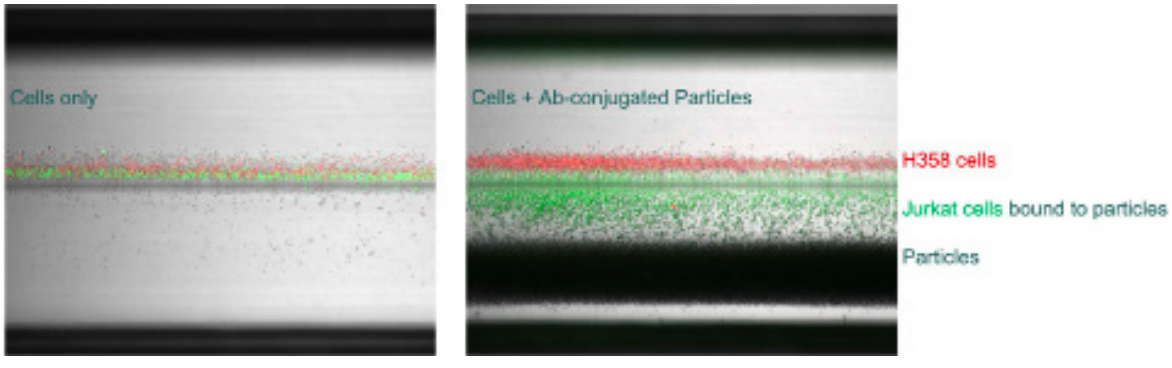


***Figure 2. Separation of cell types with density particles.*** *In the first two images on the left, the fluorescently labeled Jurkat cells (green) are located at the bottom of the channel due to the highest concentrations of magnetic particles. As the concentration is decreased, the Jurkat cells can be observed levitating at increased heights. In the final image on the right, there are no magnetic particles present, resulting in no differences in levitation height between the two cell types.*

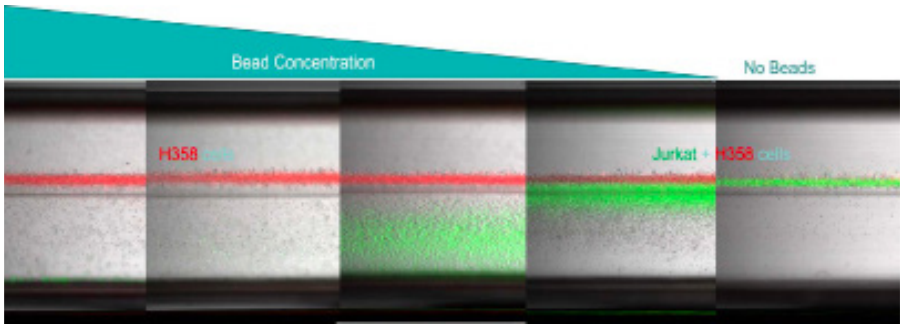


***Figure 3. Inverse correlation between magnetic particle concentration and levitation height.*** *(Left) In their natural state, Jurkat (green) and H358 (red) cells levitate at similar heights. (Right) Separation of the two cells types was achieved with the addition of antibody-conjugated CD45 high-density polymer particles, which bound to the Jurkat cells and lowered their levitation height. CD45 high-density polymer particles, which bound to the Jurkat cells and lowered their levitation height.*

separation channel can create differential magnetic fields thereby changing the way cells ultimately levitate, with and without the introduction of magnetic particles.

In another example experiment, we looked at anti-human CD45 antibody-labeled magnetic particles and their ability to alter levitation height. By varying the concentration of cellbound magnetic particles, we found measurable differences in the levitation height of CD45+ Jurkat cells. A cell mixture in a ratio of 85:15 of Calcein-AM-labeled Jurkat to CellTracker™ Red CMTPX Dye (Thermo Fisher Scientific PN C34552)-labeled H358 cells, respectively, was added to varying amounts of antibody- conjugated magnetic particles. The levitation height of the H358 cells was unchanged as compared to the no magnetic bead control sample (Figure 3, next page). At intermediate ratios of magnetic particles to input cells, the Jurkat cells levitated at different heights compared to the no bead sample. The balance of magnetic forces, the Jurkat cells own buoyancy and magnetic susceptibility within the paramagnetic medium, and the number of magnetic particles added to the cell suspension (i.e. the number of particles bound per cell) enabled the separation of the Jurkat cells from the H358 cells. This experiment demonstrated that when the concentration of magnetic particles on the cell surface was high, the Jurkat cells were pulled to the bottom of the separation channel. In contrast, when the concentration of magnetic particles on the cell surface became more limited the cells remained in view but at varying lower levitation heights. The concentration of paramagnetic Levitation Agent can also influence the spread of the Jurkat cell band in the separation channel (data not shown).

## Conclusion

Identifying and enriching cells of interest from a mixture of different cell types is fundamental to the study of disease models and basic cell biology. While magnetic levitation alone can enrich live cells from dead or dying cells and can separate cells based on their inherent differences, the ability to modulate levitation height will further enhance the power of this technology. We have demonstrated the use of a surface antigen targeted approach combined with a particle that can alter the effective density or magnetic susceptibility of cells. Together, the successful enrichment of highly purified, viable cells can be enabled and accelerated in a gentle and effective manner.

### References

1. Knowlton et al., Science Reports. 2015;5:15022.

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