

# Separate Cell Types and States Based on Density Differences Down to 1%

## Overview

The **LeviCell™ system** purifies a heterogeneous population of cells via separation based on differences in magnetic susceptibilities between the cells, a suspending non-ionic paramagnetic reagent known as the Levitation Agent, and a balance between magnetic and corrected gravitational forces.

The instrument takes advantage of the fact that a wide variety of cellular processes, both physiological and pathological, are accompanied by transient or permanent changes in a cell's volumetric mass density or magnetic signature due to formation or quenching of intracellular paramagnetic reactive species, for example reactive oxygen species (ROS) or reactive nitrogen species (RNS). These events include cell-cycle stage, differentiation, cell-death (apoptosis/necrosis), malignancy, disease state, activation, phagocytosis, in vivo and ex vivo cell aging, viral infection, and specific as well as non-specific responses to drugs.

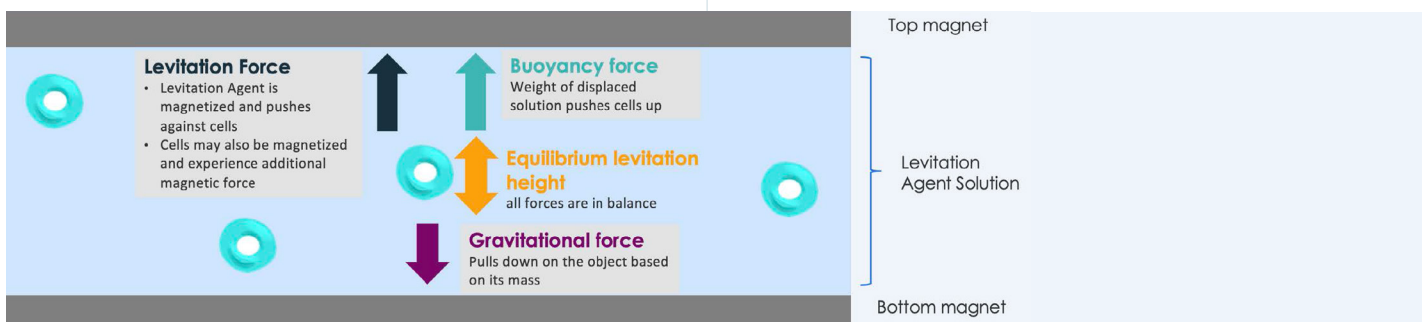
Magnetic levitation has previously been demonstrated in analyses of densities and magnetic susceptibilities of individual, macroscopic objects and as a means effective in separating foods, determining the fat content in milk, cheese, and peanut butter, comparing a

variety of grains on the basis of their intrinsic densities, guiding self-assembly of objects, and characterizing forensic-related evidence.

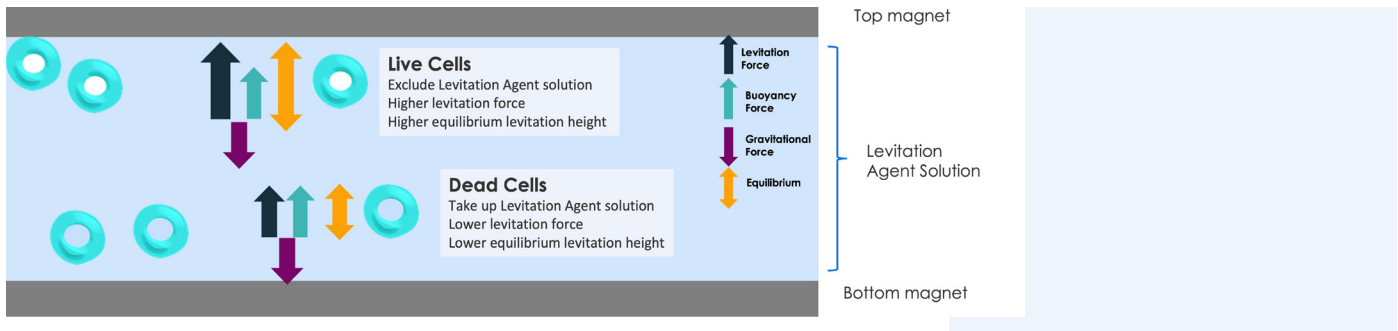
However, these earlier magnetic levitation-based experiments were performed using large setups that were not compatible with or geared towards microscopy, such as is needed in biological analyses.

## Levitation Agent - the required paramagnetic medium

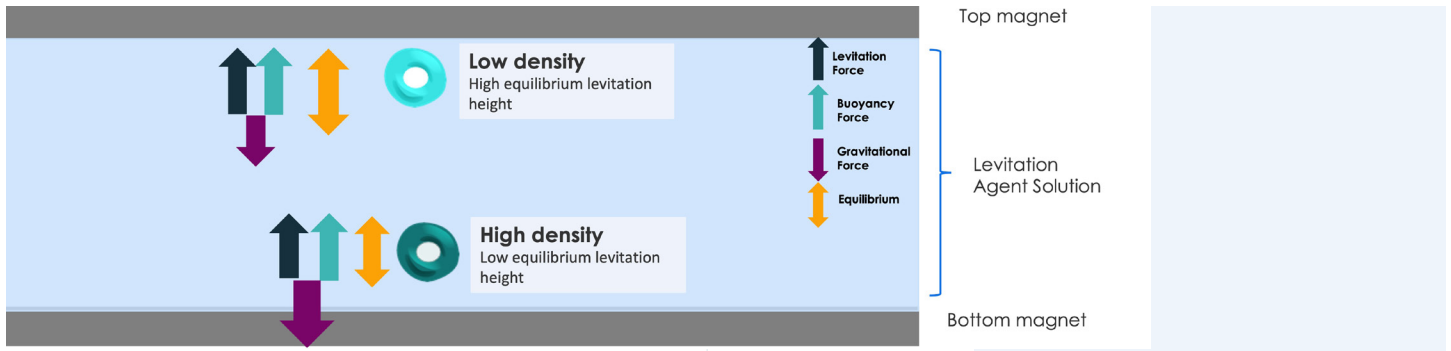
The paramagnetic medium, or reagent, employed in the LeviCell system, is called the Levitation Agent. The Levitation Agent contains purified, sterile water and gadobutrol with the final concentration customized to your experiment or lab protocol. Figure 1 shows conceptually how the Levitation Agent interacts with physical forces - magnetism, buoyancy and gravity - to yield an equilibrium levitation height. Insofar as live cell enrichment, Figure 2 refers to a unique property of the LeviCell: the reagent is preferentially taken up by non-viable cells. This creates a clear demarcation in levitation height, viable versus non-viable cells, making enrichment easy. Apart from viable cell enrichment, almost any cell type, or state, can be distinguished within the LeviCell instrument based on minute (1%) differences in cell density: the concept is depicted in Figure 3.



**Figure 1. The Levitation Agent is attracted to the magnets more strongly than the cells, creating a similar effect to a gradient. This illustration demonstrates the different forces that needs to reach equilibrium before a cell reaches its final levitation height.**



**Figure 2. The Levitation Agent only enters a cell if the membrane is compromised or altered, resulting in membrane permeability. Live, viable cells have intact, impermeable membranes that exclude the Levitation Agent, result in higher levitation force and equilibrium levitation height. Dead or cells with compromised membranes, such as DMSO-treated samples, will take up the Levitation Agent, resulting in lower levitation force and equilibrium levitation height.**

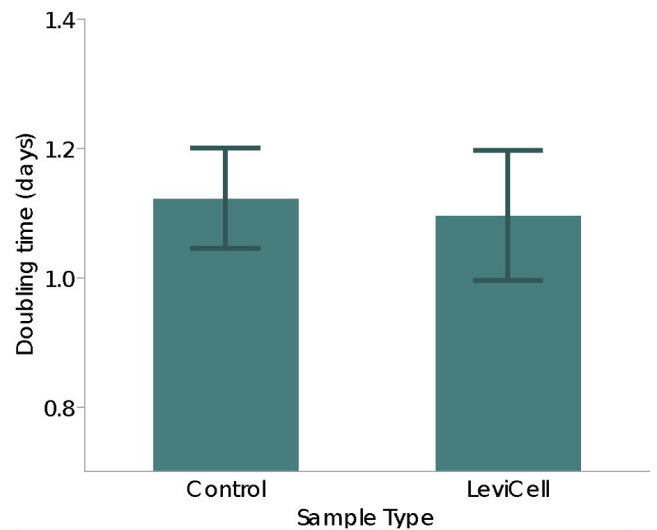


**Figure 3. Cell density inversely influences levitation height.**

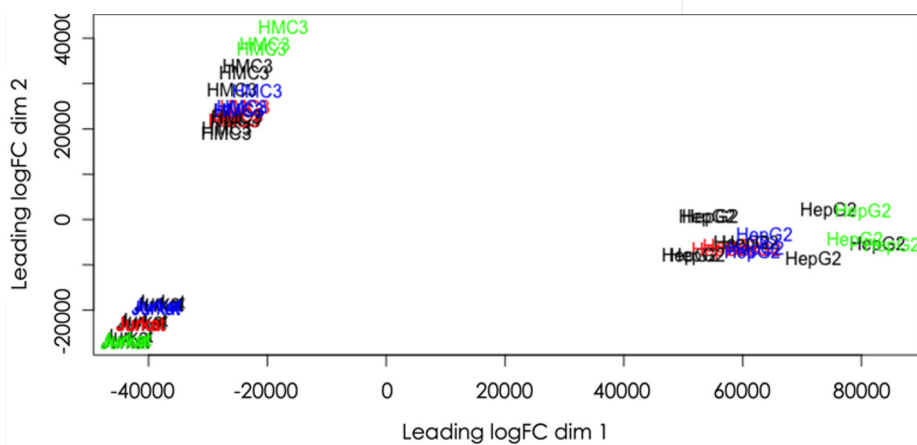
### Minimal Impact on Downstream Processes

Experiments prove that cell viability is not affected by limited exposure to the Levitation Agent. In Figure 4, the doubling time was compared for exposed cells versus controls: cells exposed to the Levitation Agent during LeviCell processing have the same doubling time as control cells in culture.

Experiments have also evaluated the potential for altered gene expression in comparison with conventional enrichment methods such as FACS and MACS. Four cell lines were tested: HEPG2, Jurkat, HMC3, RAW264.7. As shown below in Figures 5-6, alterations in gene expression are both small and comparable across all three enrichment methods.



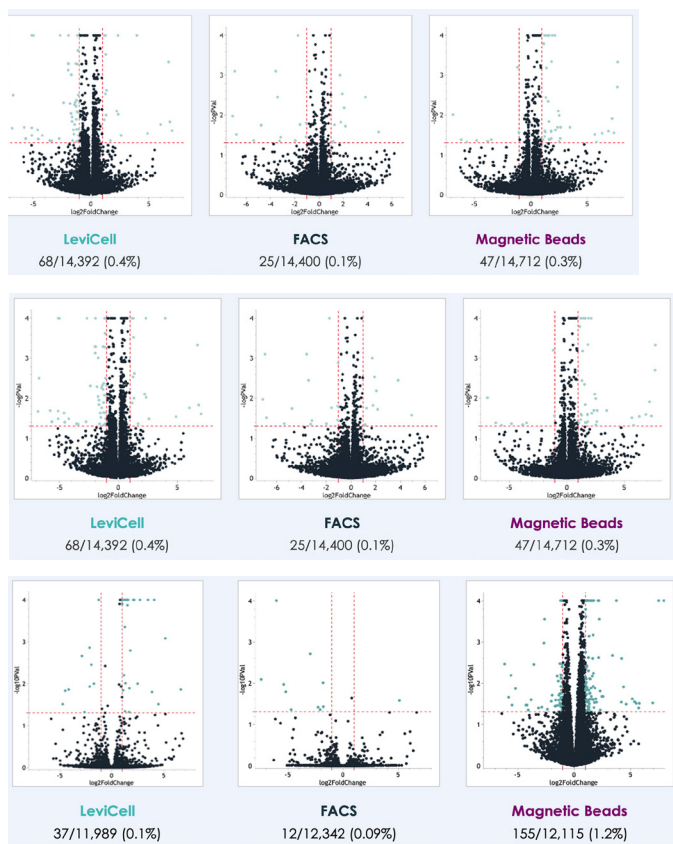
**Figure 4. Cell growth is not affected by Levitation Agent.**



**Legend**

- Control
- Beads
- FACS
- LeviCell

**Figure 5.** In this multidimensional scaling (MDS) plot, also known as a clustering graph, each human cell line clusters together, independent of enrichment method. This confirms gene expression alterations across different methodologies are comparable.



**Figure 6.** Comparable gene expression changes were observed across different cell lines from different separation methodologies. (A) - These volcano plots depict the actual observed changes in gene expression from (top to bottom) Jurkat, HMC3 and RAW264.7 cell lines.

## Conclusion

Levitation Agent is a vehicle that enables the LeviCell system to distinguish cell type and/ or cell state based on density differences down to 1%. Through analysis of gene expression profiles and viabilities, Levitation Agent is proven to have no adverse effect on cells.

## References

1. "Optimizing Magnetic Levitation: How to Tune Parameters for Best Results" at LevitasBio website: <https://levitasbio.com/wp-content/uploads/2020/05/Optimizing-Magnetic-Levitation-How-to-Tune-Parameters.pdf>

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