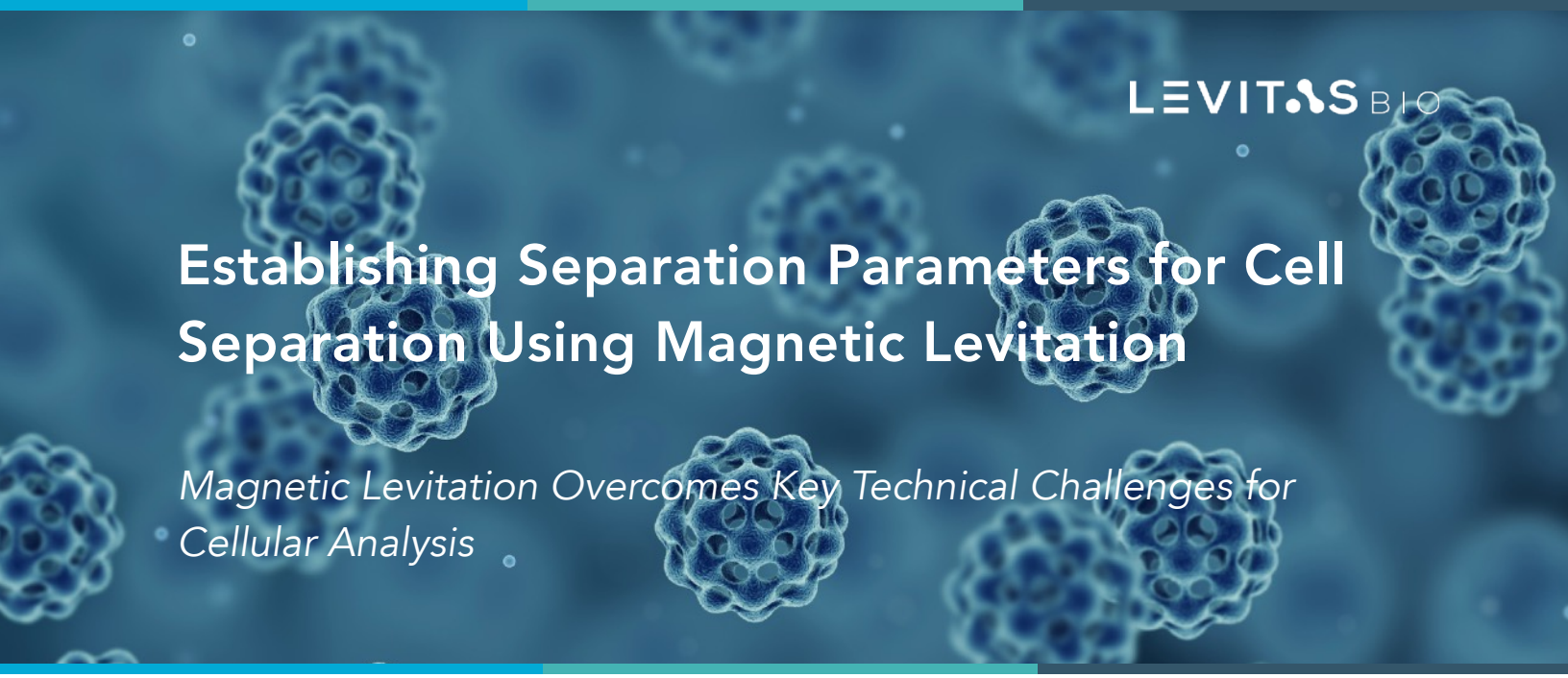

Establishing Separation Parameters for Cell Separation Using Magnetic Levitation

Magnetic Levitation Overcomes Key Technical Challenges for Cellular Analysis

Overview

Cell sorting and separation technologies are ubiquitous in molecular biology labs. Their popularity belies the serious limitations these tools have. For instance, flow cytometry requires antibody selection and therefore does not allow for a holistic look at a cellular population. Across cellular analysis technologies, challenges arise from the use of labels or stains — which fundamentally modify the cells — or from harsh processes that can damage or kill the cells of interest.

With the soaring interest in single-cell analysis for genomics, oncology, bacteriology, and more, there is a pressing need for better tools to isolate, sort, and analyze cells. A new approach based on magnetic levitation technology overcomes many of the challenges seen in conventional tools.

Magnetic levitation is based on a simple and widely accepted observation: cells have unique density signatures that make it possible to sort and separate them. It's the same concept that makes the centrifuge an essential tool for molecular biology labs.

Levitas Bio has designed a technology platform that takes this idea one step further. Cells in a channel are subjected to a magnetic field, which makes them levitate. The unique magnetic and density profiles of different cell types allow for gentle sorting and collection. Robust experimental validation has shown that this approach can successfully sort cells by type (such as red blood cells, white blood cells, and circulating tumor cells) or state (such as live or dead, or by a response to a drug or stressor).[1-2]

This approach uses no stains, labels, or antibodies. The gentle process minimizes harm or stress, allowing users to analyze cells as close to their *in vivo* state as possible. The method is particularly useful with precious or difficult samples.

How it works

The LeviCell instrument from Levitas Bio is a cell densitometry and imaging platform that consists of two magnets with a channel between them. Cells in a non-ionic, paramagnetic medium flow into the channel, levitating to a final position where the magnetic force upward equals the gravitational force downward. The system monitors the equilibrium height of a cell to

determine how the sample will be separated. Cells reach an equilibrium position which is determined by their density and magnetic signature, and is independent of cell volume. Notably, live cells maintain their equilibrium within the chamber, while dying cells sink toward the bottom.

Polymer beads with a range of densities provide a clear demonstration of the LeviCell system's capabilities. A software model of magnetic levitation correlates well with experimentation using beads of known density (Figures 1-2).

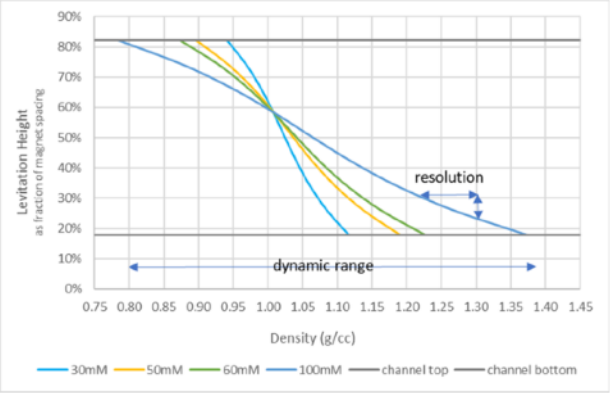

Image 1: Software Model of Levitation Heights vs. Density [3]

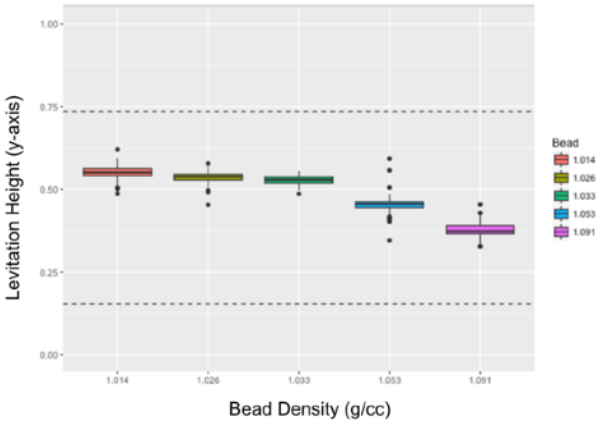

Image 2: Polymer bead height in levitation channel versus density specification from manufacturer (dotted lines reflect LeviCell flow cell top and bottom, Internal Levitas Data)

The LeviCell instrument's main enclosure houses the imaging system, flow controller, and flow cell. The flow cell passes between custom-designed rare earth magnets for sensitive and robust separation of different cell and particle types in continuous flow operation. The flow cell is reusable and resistant to bleach and some solvents. A camera images the cells and particles as they move through the flow cell. The instrument holds three tubes: one for sample input, and two to collect output products.

Instrument control and analysis are both performed by Levitas Bio's Experiment Manager software application. Raw results are available for further processing on other platforms.

Establishing Run Conditions

In order to optimize the LeviCell system, users first establish the density range associated with their cells of interest. For some cases, this information is accessible in the literature or from density centrifugation techniques. That data can be combined with Levitas Bio software models to help determine the resolution that can be achieved under specified conditions. When this information is not already available, it can be generated through the use of polymer density calibration beads or through model cell populations such as cell lines or purified populations.

To illustrate this, Levitas Bio scientists compared the image of levitating cells from a mouse fat pad sample with an image of levitated polymer beads of density 1.033 and 1.091 g/cc (Figure 3). Literature suggests a density range of 0.93-0.97 g/cc for adipocyte cells, which, based on the bead positions, correlates well with these levitating cells.[4] Given this information, separation of mouse fat pad adipocytes from dead cells, debris, and blood cells, was performed with the LeviCell system, using a total flow rate of 1.2 ml/hour using a 1:1 top:bottom flow rate ratio.

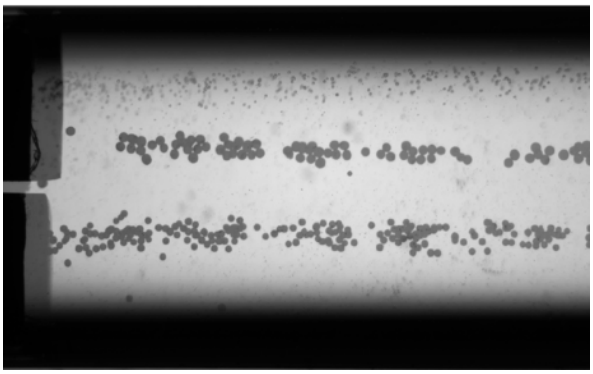

Image 3: Image of adipocytes (top, estimated density of <1.0 g/cc) overlaid with 1.033 g/cc and 1.091 g/cc density polymer beads, as an example of literature use to estimate cell levitation heights and separation.

Model populations such as cell lines or purified populations of interest can also be mixed with a representative background population to provide a sample for separation on the LeviCell system. In an example of this, scientists spiked the HEPA 1-6 mouse liver cancer cell line into red blood cell-depleted mouse blood at a 5:1 cell ratio (Image 4). The LeviCell run conditions (1:1 top/bottom flow rate ratio, 2.4 ml/hour total flow rate) were determined and employed to separate cancer cells from real patient samples.

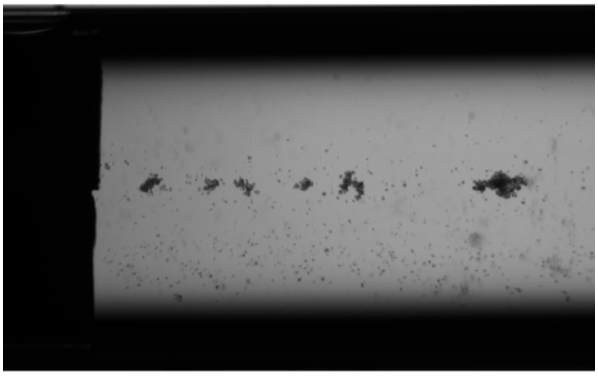

Image 4: HEPA 1-6 (Mouse Liver Cancer Cell Line), spiked into RBC depleted mouse blood to determine flow conditions for patient sample separation (Levitas Internal Data).

The LeviCell system was also designed for use with cell tracker dyes when needed to allow for fluorescent identification of the population of interest. The device contains two fluorescence channels, as well as bright-field imaging, specifically for this purpose.

Conclusion

The LeviCell system enables real-time imaging of density-based cell separation, along with multiple approaches to calibrating or optimizing the separation profile for each experiment. Because it does not use labels or dyes and minimizes harm to cells, it is ideally suited to workflows with low-volume samples, rare or precious samples, and heterogeneous samples that can be challenging for fluorescence-activated cell sorting. For more information, please visit www.levitasbio.com or contact support@levitasbio.com.

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