Fibroblast enrichment from a mixed cell population

Efficient, gentle enrichment by magnetic levitation with the LeviCell System

Overview

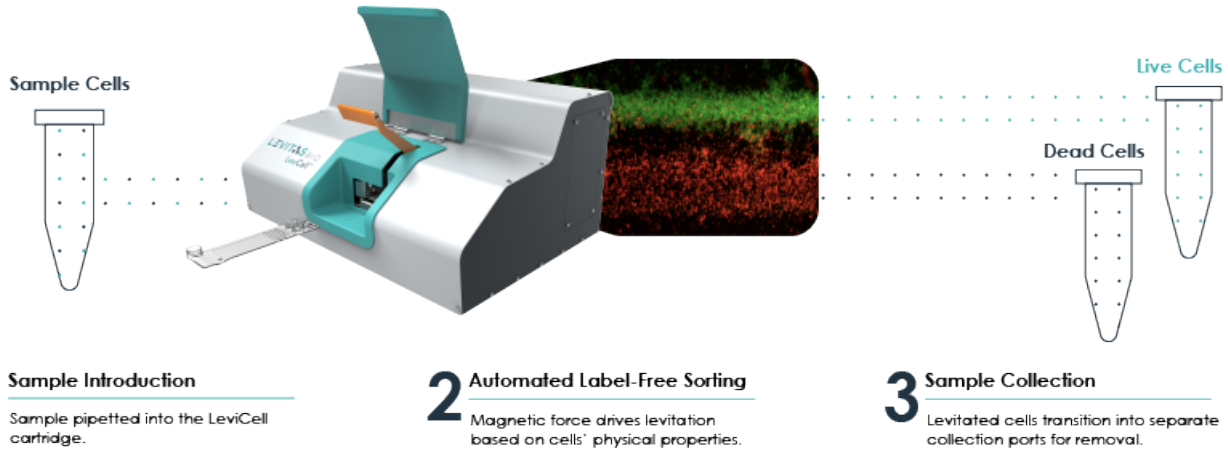
The tumor microenvironment (TME) regulates tumor initiation, progression, angiogenesis, metastasis, drug and therapy resistance and other aspects of cancer biology. Cancer associated fibroblasts (CAF), which often make up most of the TME, provide a scaffold to tumors and promote tumor cell growth, but these fibroblasts can be difficult to isolate due to the lack of CAF-specific markers. With the LeviCell system, fibroblasts can be efficiently and gently isolated and purified from all the other types of cells in the TME such as immune cells, pericytes, adipocytes, endothelial cells, as well as cellular debris.

Key Benefits of LeviCell System

- Gently enrich and purify mixed cell populations, as well as sensitive or fragile cell types
- Obtain significant enrichment and high recovery rates regardless of starting cell number
- Maintain cell physiology
- Reduce hands-on time and handling steps by 80%

The LeviCell System

The LeviCell instrument and protocol purifies and enriches cells via a simple, 3-step workflow that can be completed in less than 20 minutes: (1) pipette sample into a single-use levitation cartridge; (2) load cartridge into the LeviCell; and (3) collect target cells from output well(s) for downstream analysis.

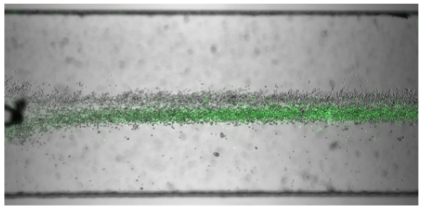


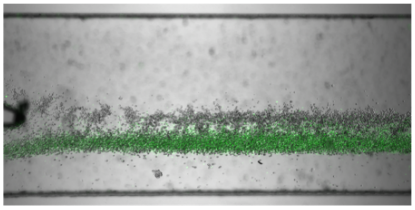
The LeviCell instrument separates cells based on their buoyancy as the cells flow through an inert solution. This flow medium, which can be calibrated to cell type, creates a density gradient due to an externally applied magnetic field. Cell types are easily separated, and distinguished from unwanted debris, without the use of dyes, beads, antibodies, or any other additives that could damage cells or alter gene expression. The LeviCell instrument operates at less than 1 PSI, orders of magnitude lower than most current cell sorting techniques where pressures start at 30 PSI. The gentle LeviCell process preserves the integrity of each cell population, maximizes yield and purity, and does not alter gene expression which is critical for single cell/single nucleus genomic research.

LeviCell Separation of Fibroblasts from Lung Cancer Cells

A recent experiment demonstrates the efficiency of the LeviCell system in separating and purifying fibroblasts from lung cancer cells. Lab assay requirements vary but, in this demonstration, the mixed cells and debris were processed through the LeviCell to first remove dead cells and debris via the bottom outlet. In a second round, utilizing a new cartridge, the clean, live fibroblast and lung cancer cells, that were collected from the top output well, were processed to enrich for the two cell populations.

The LeviCell instrument separates and enriches each cell type due to the inherent difference in buoyancy of cell types while traversing a density gradient created by the action of a magnetic field on the flow medium (aka, the "Levitation Agent"). The concentration of the levitation agent can be calibrated to create the desired visual separation between cell types. For example, Figure 1 (a, b, c) depicts fibroblasts (no dye) versus lung cancer cells (labeled green) that were prepared with levitation agent concentrations of 50 mM, 35mM and 25 mM. Note that the labeling is solely to illustrate the effectiveness of the separation and thus is not at all required in the assay.

1a. 

1b. 

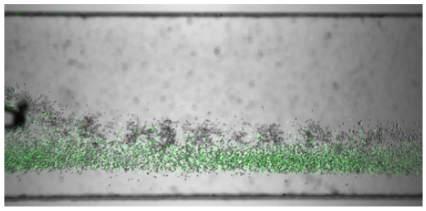
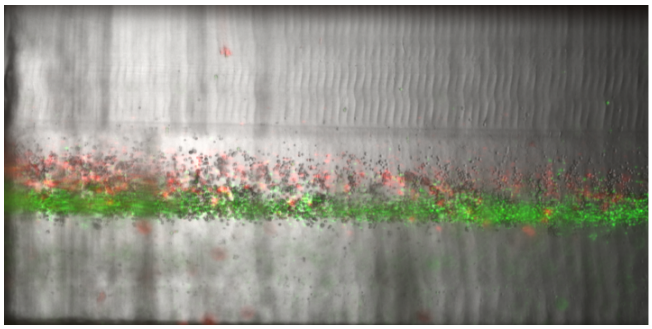
1c. 

Figure 1. Different levels of cellular separation can be achieved by adjusting the final concentration of the Levitation Agent. HFL1 fibroblasts (no dye) and H358 lung cancer cells (green) were pooled and prepared using three different concentrations of Levitating Agent - 50mM (1a), 35 mM (1b), and 25 mM (1c). All three were then processed per the standard enrichment protocol with the LeviCell instrument. The H358 lung cancer cells were stained with Vybrant™ DyeCycle™ Green Stain (Thermo Fisher Scientific) for visualization purposes only.

Based on the level of separation observed, 35 mM was chosen as the final Levitation Agent concentration, and the LeviCell enriched for the two fractions of cells, resulting in final yields of 55% for the fibroblasts and 85% for the lung cancer cells.



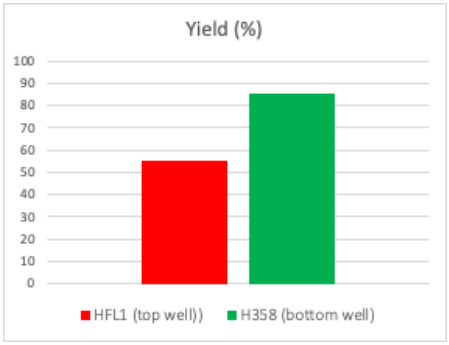


Figure 2. Fibroblasts were successfully separated from lung cancer cells and confirmed by flow cytometry. **Left:** Fibroblasts (red) and lung cancer cells (green) were separated with the LeviCell system using a final concentration of 35mM Levitation Agent. **Right:** The cells collected in the top and bottom output wells were independently analyzed by flow cytometry using a Sony SH800S cytometer. HFL1 fibroblast cells (labeled red with CellTracker™ Red CMTPX dye from Thermo Fisher Scientific) were harvested from the top output well and had a final yield of 55%. H358 cancer cells (labeled green with Vybrant™ DyeCycle™ Green Stain from Thermo Fisher Scientific) were harvested from the bottom output well and had a final yield of 85%.

The LeviCell system, with its innovative cell enrichment and characterization technology, delivers robust yields, excellent enrichment, and ultimately, a significant reduction in processing time. This ability to rapidly enrich for targeted cells of interest without altering their expression profile enables researchers to save time, gain more relevant insights into the tumor microenvironment, and accelerate their cancer research.

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