PARAMETERS TO ADJUST WHEN USING LEVICELL FOR VIABLE CELL ENRICHMENT

OVERVIEW

LeviCell technology together with its simple workflow, offers a good viable cell enrichment for most cells using the standard recommendations for levitation agent concentration (150 mM, final) and 20 min of levitation time. This approach requires no cell staining, labeling, or use of cell surface antibody markers. The gentle process virtually eliminates undue stress on cells and allows users to ultimately analyze their cells as close to their in vivo state as possible.

This technical note, while applicable to all, will be of particular use to those who have unusual samples (e.g., cells with lower densities like adipocytes, smaller or larger than average cells, or complex samples) or who simply want a deeper understanding of the user-modifiable parameters of LeviCell technology. Understanding how these parameters work empowers our customers and users to have more control over their samples and explore new applications and uses.

INTRODUCTION TO LEVITATION TECHNOLOGY PARAMETERS

The underlying physics that determine how objects behave in the LeviCell™ system can be described by a few key equations and parameters which are fixed and inherent to the design of the technology.However, parameters like levitation agent concentration and cell concentration are user-controlled and interact directly on the inherent physical properties and innate density of the cells, and how those cells behave within the magnetic field of the LeviCell system. Similarly, the time a sample is allowed to levitate and how the split line is set are factors that can be modified and determine the success of levitation and collection of the sample. These user-controlled parameters are explained and summarized.

LEVITATION AGENT CONCENTRATION

One of the main parameters that users can, and often should, optimize is the concentration of Levitation Agent (LA) in the final Levitation Buffer containing the cells. Levitation Agent is supplied at a stock concentration of 1M and should be added directly to the Levitation Buffer containing cells at a final concentration between 50 mM to 150 mM (<50 and >150 in rare cases). In general, the concentration of LA is directly related to the levitation height of the cells within the magnetic field (Figure 1).

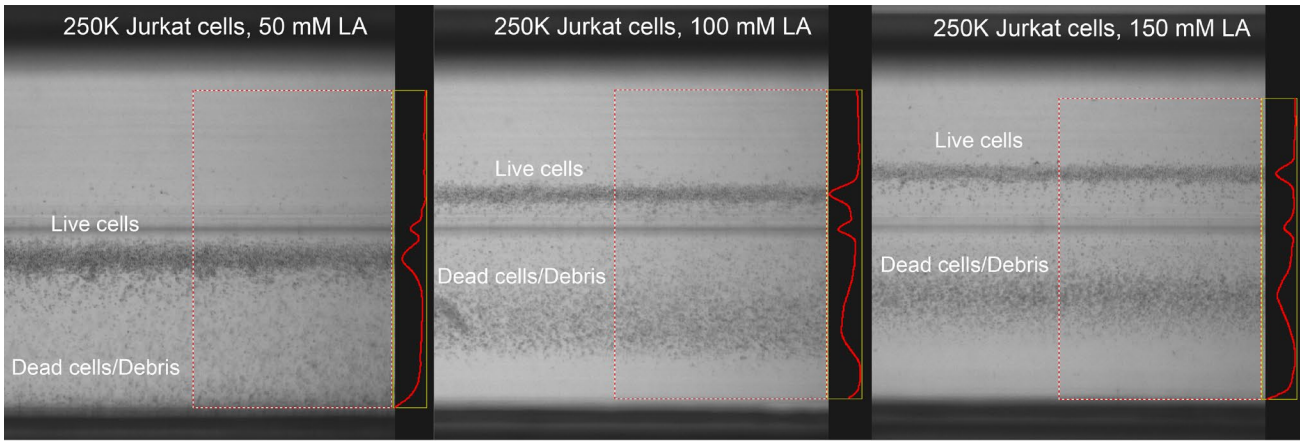

Figure 1. LA concentration affects the levitation height of aged Jurkat cells. *In all 3 cases, when levitation is complete, live cells levitate far from the dead cells and debris. The effect of LA concentration can be observed in the final height and "tightness" of the live cell band. Note: the cells in the 50 mM LA required 30 min of levitation (vs. 20 min) to reach equilibrium and the dead cell band is more spread in the bottom part of the chamber. Further, note the wider spread of the dead cell band in the 100 mM vs. the 150 mM samples (observed as a broader "peak" in the projection analysis histograms to the right of each image). LeviCell platform records the images perpendicular to the channel.*

In addition to affecting levitation height, LA concentration also influences the "tightness" of the cell band as well as the time it takes for a cell to reach its final levitation height. Higher LA concentration results in a tighter cell band, and generally requires less time for cells to reach final levitation height.

In some cases where 2 different cell types are distinguishable based on their levitation height, altering the LA concentration can result in even greater separation of the cell bands and the possibility of separating the two cell types directly from the LeviCell cartridge (Figure 2).

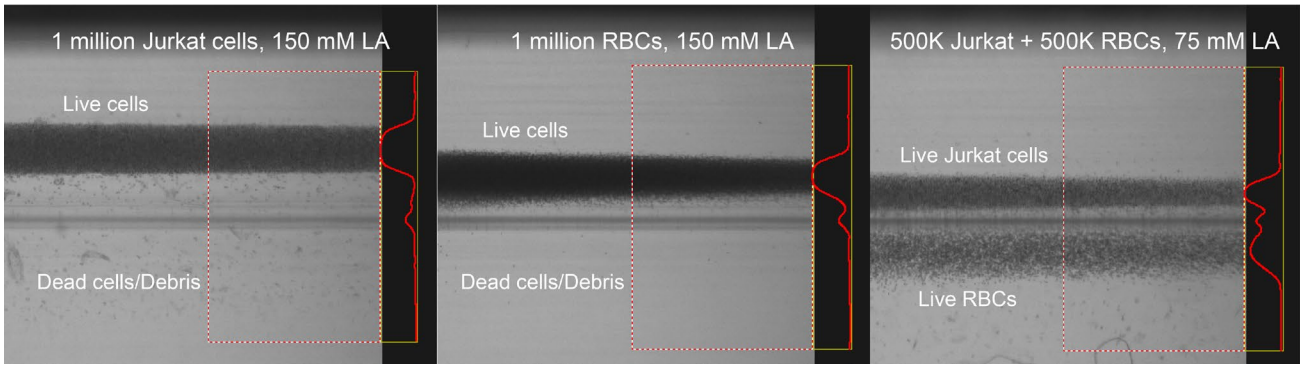

Figure 2. Example of how 2 different types of cells can be separated: Jurkat cells and red blood cells. *The images on the left and the middle show a band of Jurkat cells and a band of RBCs levitating in 150 mM LA, respectively. The projection analysis shows the levitation height and how this differs between the Jurkat and the red blood cells. The image on the right shows these same cells, mixed, at their final levitation height using 75 mMLA. In this case, both cell bands levitate lower in the chamber, but also separate from each other enough to harvest both cell types independently using a split line setting between the two bands of cells (explained further in the Setting the Split Line section of this technical note).*

CELL CONCENTRATION

The total number of cells loaded into the cartridge, for a given cell type, can determine the levitation behavior and ultimately how the bands appear at final levitation height (Figure 3). Generally, when testing a new cell type, it is best to start with a total cell input of 250,000 cells and avoid using more than 1 million cells. With this number of cells, a live cell and dead cell band can be distinguished. Clear bands can be seen as well when higher cell numbers are loaded, and the bands do appear broader as a greater number of live and dead cells are added.

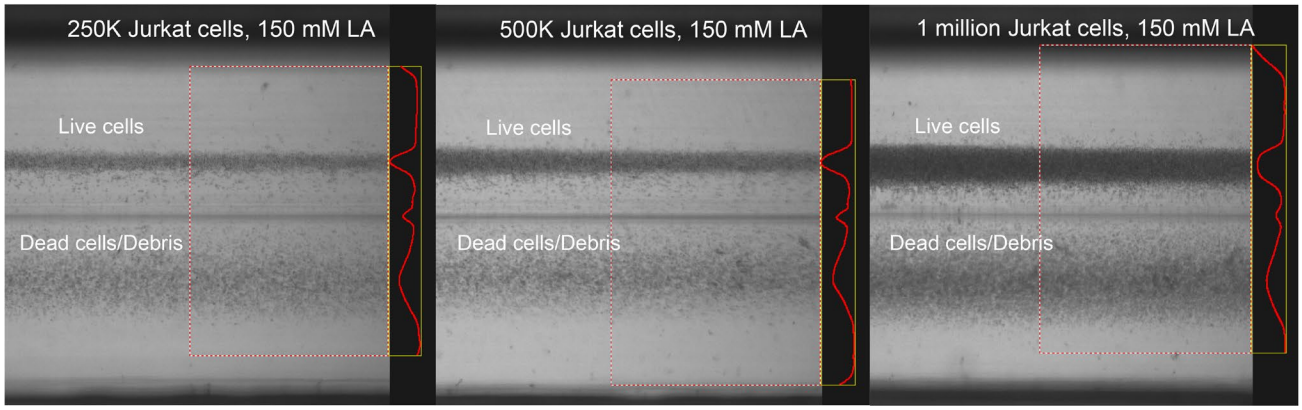

Figure 3. Live cell band visualization is related to the number of cells loaded into the LeviCell. *Different numbers of aged Jurkat cells were loaded (250K, 500K and 1 million) using 150 mM LA and their levitation height captured at 20 min post levitation. Note the broadening of the live cell band projection analysis peak in the histograms to the right of all images. The distance between the live and dead cell bands does not change, but the cell bands become broader as more cells are loaded.*

Additionally, cell size and cell viability should be considered as they can influence levitation behavior.

Cell size – given that the levitation chamber has a finite volume, larger cells will occupy more space than smaller cells, therefore the user should consider using fewer cells when cell size is > 20 µM.

Viability – determining the levitation behavior of viable cells requires that enough viable cells are introduced into the cartridge to visually confirm where they levitate relative to the accompanying dead cells and debris in the cell suspension. If viability is very low (e.g., < 30%), it may require more than 250,000 total cells input to visualize the live cell band adequately and to have a good recovery of your viable cells.

LEVITATION TIME

The amount of time required for cells to reach their final levitation height varies. Larger cells will reach equilibrium very quickly, often within 5-10 min, especially if cell size exceeds 20 µm. Most cells fall within the range of 5-20 µm and require up to 20 min of levitation time (see Figure 1-3 for examples). Cells smaller than 5 µm often need 30-40 min of levitation time (Figure 4). The LeviCell Experiment Manager software comes with 3 different protocols based on the size of the cells. The pre-programmed times are: 40 min for cells <5 µm; 20 min for “standard” cells 5-20 µm, and 5 min for cells > 20 µm.

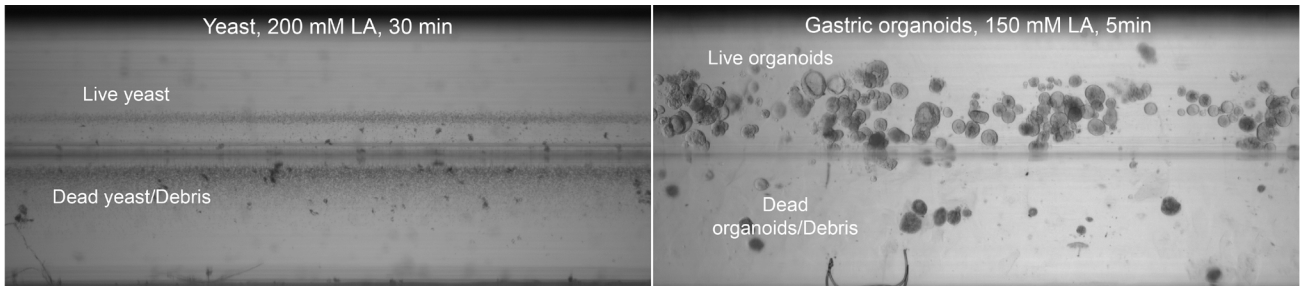

Figure 4. Different levitation time is needed depending on cell size. *Live yeast (3-4 µm diameter) reach levitation equilibrium after 30 min and form a tight band far away from dead yeast. Epithelial human gastric organoids (100-200 µm diameter) reach levitation equilibrium faster and in 5 min live organoids are far from debris and dead organoids.*

Choosing the appropriate protocol based on cell size is important as levitation cannot be aborted until the timer for the chosen protocol reaches 0. That said, levitation time can be extended beyond any of the set protocol times by simply not starting separation flow (which requires a manual start if a predetermined split line setting has not been defined). Therefore, users should be conscious of the time in the instrument for experimental documentation and reproducibility. In addition, when working with samples containing a mixed population and different particle sizes, smaller particles will need more time to reach their final levitation height.

There can be consequences to ending levitation too early. Even though a viable cell band may become obvious (Figure 5), and it seems appropriate to begin separation flow, there may still be cells both above and below the visible band that have not reached their final levitation height. This may be especially true when higher numbers of cells are used.

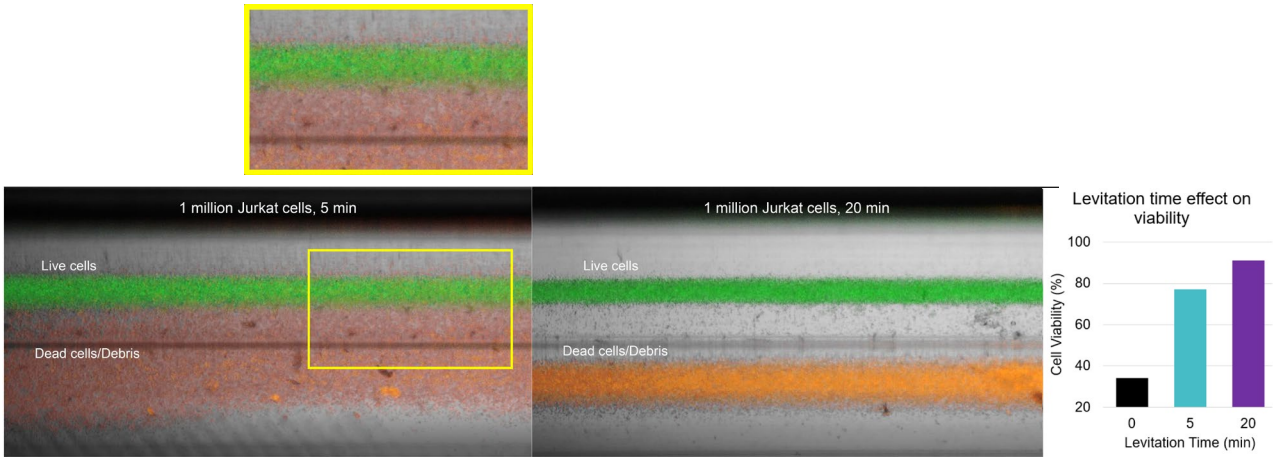

Figure 5. Levitation time influences the final viability of the collected sample. ***Left panel:*** *After 5 minutes of levitation a live cell band is clearly observed using Calcein AM stain (green) while the dead cells stained with PI are visualized in red. Note the blown up image from the left panel; dead cells and small debris are visible above the live cell band indicating additional levitation time is needed for those object to fall below the live cell band to their final levitation height.* ***Right panel:*** *Waiting another 15 minutes, clearly increases the separation of the live and dead cell bands and significantly increases sample viability as shown in the graph. LA concentration of 150 mM and a split line of +13 were used in both cases. Cell staining with Calcein-PI, while not required, is used here for visualization purposes.*

Individual cells are destined for a specific levitation height within the vertical column of the separation channel, but cells enter the channel *randomly distributed*. Therefore, some cells will reach their destination faster than others based solely on where they started within the channel. Cells that, by chance, start at or very near their *destined levitation height* start to build a "ribbon" of cells that expands both above and below their final levitation height. Cells that, by chance, start very near the top or bottom of the chamber have the greatest distance to travel. During the time it takes for these cells to reach their desired levitation height, a great many other cells will have already arrived at their position. Therefore, the later-arriving cells are forced to "pack" into the ever-developing ribbon of cells creating the outermost edges of the final cell band.

Now consider the population of cells that are destined for low in the chamber, primarily the dead cells and debris (which tend to be smaller objects than viable cells). If those cells start their journey from very near the top of the chamber, they have the greatest distance to travel and they must work their way through the developing viable cell band, which takes time (particularly when higher numbers of cells are present). These cells may not be visible to the naked eye during levitation, and even though it may appear that nothing is moving (vertically) any longer, there may still be non-viable cells working through the viable cell band and they should be given adequate time to reach their final levitation. If levitation is terminated to soon, these dead cells will artificially decrease the output cell viability measurement. Therefore, it is recommended that when evaluating the levitation behavior of a new cell or sample type, the minimum levitation time should be 20 minutes.

SETTING THE SPLIT LINE

The LeviCell platform provides a real-time visualization of your sample throughout the entire levitation process. Once final levitation height is reached, a decision about where to place the split line is necessary. For instance, PBMC's contain multiple cell types and these cell types do levitate at slightly different heights (Figure 6). This results in a viable cell band that may appear "broader" than a more homogeneous population of cells (such as a cultured cell line), and care should be taken when setting the split line too close to the bottom edge of viable cell band so as not to inadvertently exclude a specific PBMC cell type from collection. Another example would be when working with dissociated tissue or tumor samples.

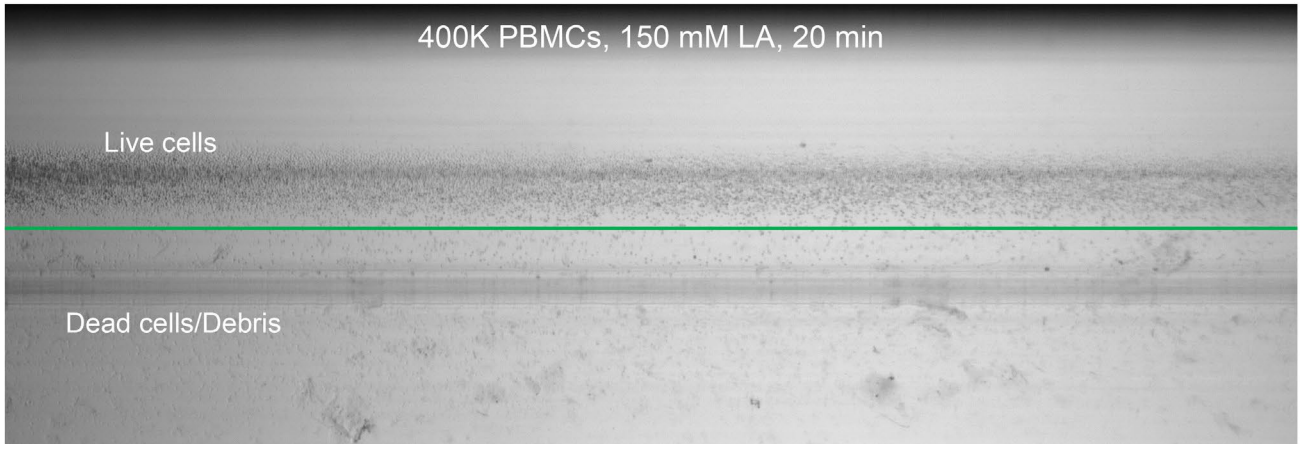

Figure 6. PBMC sample, 80% input viability. *The green split line has been set more "conservatively" to ensure collection of all possible cell types within this heterogenous sample type.*

When working with a *homogenous population* of cells, for example a Jurkat cell line, there are two factors related to the intended use of the viable cells being collected that need to be considered when choosing the appropriate split line setting: Purity or Yield of viable cells (Purity = Viable cells out/Total cells out; Yield = Viable cells out/Viable cells input).

If a high purity of viable cells is most desired, the split line may be set very close to the bottom of the viable cell band (Figure 7 see orange split line). Setting the split line very close to the viable cell band will ensure capture of predominantly viable cells, and because the cells are homogenous, there is no risk of inadvertently excluding a specific cell type from capture.

If yield is most important for the downstream workflow, the split line may be set further below the bottom of the viable cell band to ensure collection of the maximum number of viable cells (Figure 7 see purple split line).

It is important to recognize that selecting for *purity* or *yield* can be a trade-off. Choosing purity over yield will result in some fraction of viable cells separated into the waste outlet of the cartridge. On the other hand, choosing yield over purity will result in maximum capture of viable cells in the sample but may also capture a few cells that, while “live/not dead”, are not fully viable (e.g. apoptotic) and are levitating just below the live cell band.

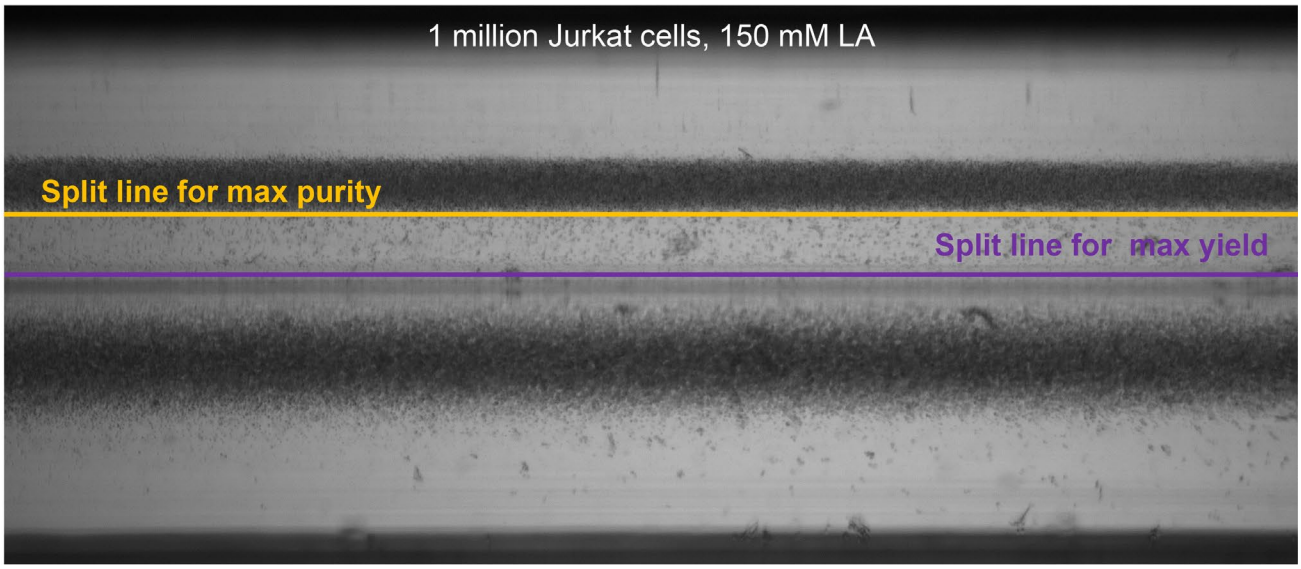

Figure 7. Purity vs yield. *Examples of split line settings determined by the goal of downstream cell analysis needs.*

CONCLUSION

Levitation technology uses the intrinsic properties of cells (density, volume, buoyancy) to separate and *enrich viable cells away from dead cells and debris* in a paramagnetic buffer, created by the addition of Levitation Agent, in the presence of a magnetic field. The LeviCell platform can process a broad spectrum of samples, from tiny microbes to bigger and more complex structures like organoids. Most cells reach their final levitation height within 20 minutes and the viable cells can be harvested for further downstream workflows (e.g., sequencing, culture, transfection, etc.) without the requirement for cell surface marker labelling, staining, or high-pressures/mechanical forces. However, by understanding the impact of modifying some key parameters, as described in this technical note, the user can effectively develop the best experimental design and optimize their protocol for low-volume samples, rare or precious samples, and heterogeneous complex samples that can be challenging for other cell enrichment/isolation methods.