TECHNICAL NOTE

Visualization Capabilities of the LeviCell Platform

Overview

Fluorescence visualization methods enhance understanding of the sample studied and help with comparisons and assessments. Often, the results of sample preparation are unknown, with the exception of cell counting. Sample visualization can be used to characterize sample quality prior to downstream analysis. The LeviCell™ platform enables real-time sample visualization through its imaging capabilities of brightfield and fluorescent images within common green (Ex 474/Em 524 nm) and red (Ex 560/Em 628 nm) channels.

The LeviCell 1.0 System is the first commercial product to use Levitation Technology, enabling the simultaneous enrichment of viable cells and removal of dead cells and debris. The instrument utilizes two permanent magnets to create a magnetic field that is used in levitation. A single-use cartridge is inserted between the two magnets and the sample is loaded into the cartridge. Brightfield microscopy is used as the primary visualization method for observing real time cell levitation and collection. Viable cells levitate to their equilibrium within the separation channel of the cartridge, while dying cells and debris sink toward the bottom. While Levitation Technology does not require any cell stains or surface markers to perform viable cell enrichment, use of the fluorescence capabilities of the LeviCell can be beneficial for real-time assessment of cell levitation behavior. When cells are stained prior to loading on the LeviCell, specific populations can be easily identified. Examples include differentiation of nucleated cells from non-nucleated cells such as red blood cells; identification of transfected cells by co-expression of a fluorescent protein to determine the success of the transfection protocol; and differentiation of cells in a mixed population through staining coupled with levitation height.

This technical note highlights the fluorescence detection and imaging capabilities of the LeviCell platform and the value of real-time insights gained with this method.

Visualization Capabilities of the LeviCell Platform

The LeviCell platform collects and displays a brightfield view of the levitating sample in real time, as well as optional green or red fluorescence channels. They are equipped with 3 light-emitting diodes (LEDs); 530 nm for brightfield transmission and two excitation LEDs at 474 nm and 560 nm for fluorescence. The brightfield channel is automatically displayed and images collected during a run, and the fluorescence channels can be independently activated depending on the stain(s) used. With an image pixel resolution of 2 µm, the system provides a detailed view of the sample that can be used to guide the collection or the sample. The system captures images every 3 seconds for each active channel, with default exposure times set at 100 µs for the brightfield channel, 1000 µs for the green channel, and 5000 µs for the red channel. Exposure times and image contrast can be adjusted during a run for better sample visualization. Once a run is complete, the collected images can be imported into Experiment Analyzer software to create a movie and/or panoramic view of levitation.

A LeviCell Installation and Calibration Kit is included during installation of the LeviCell system. This kit contains two bead mixtures that demonstrate how Levitation Technology separates objects of different densities as well as the basic fluorescence imaging capabilities of the instrument (Figure 1).

Leverage Sample Visualization Without Compromising Viable Cell Enrichment

Levitation Technology achieves label-free separation of viable and dead cells, but in some situations, the fluorescence channels may be beneficial for certain biological experiments. Table 1 (see page 5) provides a list of dyes, probes and fluorescent proteins that have been successfully tested on the LeviCell platform to visualize different cells. Most of the tested compounds are membrane-permeable fluorescent dyes which freely

cross the cell membrane and fluoresce green or red when bound to nucleic acid. Others, after entering the cell, become fluorescent due to the intracellular enzymatic reactivity. In general, the recommendations from the stain provider should be sufficient to provide good visualization of the cell on the LeviCell platform. In a few cases, the dye needs to be optimized for use with the LeviCell, and details are provided in Table 1.

Some dyes stain live and dead cells and are extensively used for cell counting. A common pairing is the dual staining of Acridine Orange (AO) and Propidium Iodide (PI). In Figure 2A, 1 million EL4 cells (mouse T-lymphoblast cell line) were dual stained with AO+PI, with each separate imaging channel shown from left to right. All nucleated cells (live and dead) absorb AO and can be visualized in the green channel. In contrast, only dead cells absorb PI due to their compromised membrane so they are only visible in the red channel. Due to the dual staining, some green fluorescence may appear in the final composite picture (Figure 2B).

Other dyes like Calcein AM only fluoresce by enzymatic activity of a cytoplasmic esterase from a live cell. Figure 3 shows a snapshot of 1 million Jurkat cells (human T-cell line) stained with Calcein AM + PI. In the composite image, the viable green fraction is observed in the top half of the channel while the dead cells and debris in red are observed in the bottom half.

While fluorescent imaging is not required for viable cell enrichment to occur on the LeviCell system, it can be useful. The ability to simultaneously visualize different dyes associated with different cell populations help

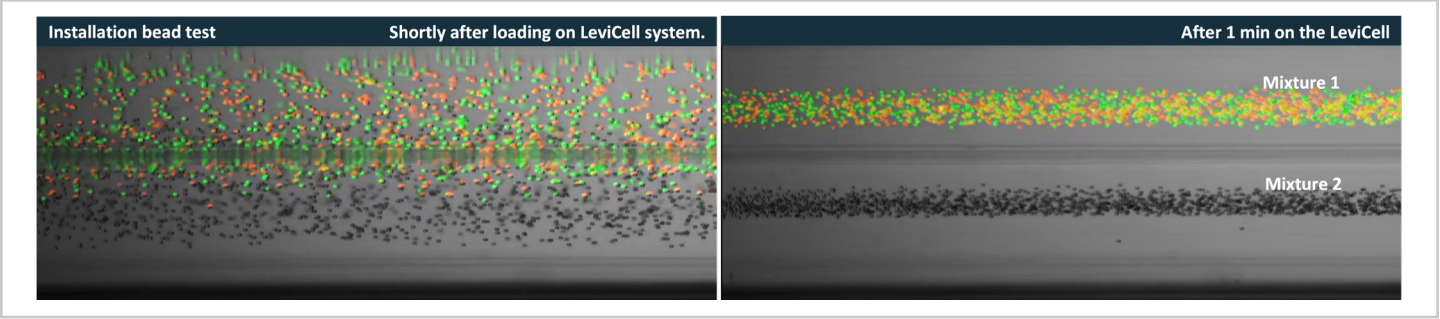

Figure 1. LeviCell installation bead test. *LeviCell Install Bead Mix 1 (fluorescent beads) and LeviCell Install Bead Mix 2 (non-fluorescent beads) are combined with levitation buffer and loaded on the LeviCell for a 3 minute run. When loaded, the mixture enters the separation channel randomly distributed. Quickly, the denser beads (non-fluorescent) form a band in the bottom fraction of the channel while the less dense beads (fluorescent) form a band in the top fraction. These images are snapshots of the composite movie created after the run is performed.*

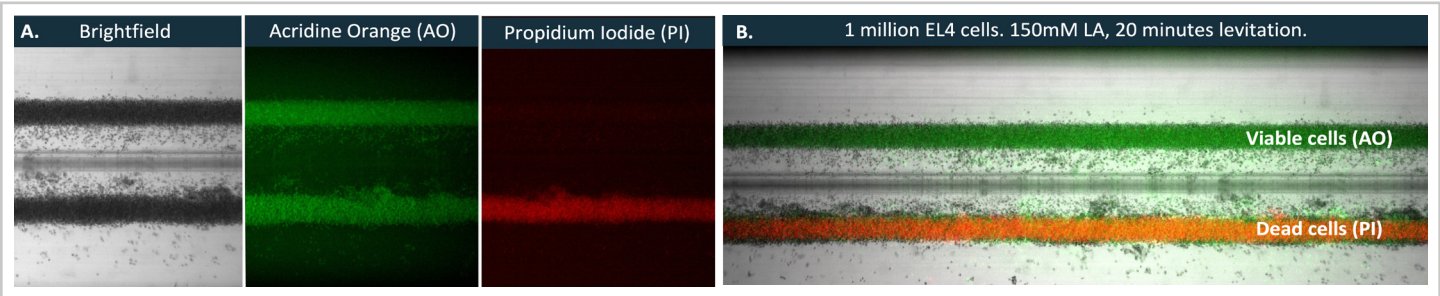

Figure 2. Acridine Orange + Propidium Iodide dual staining of EL4 cells on the LeviCell system. *EL4 cells with 50% initial viability were stained with AO + PI, combined with levitation buffer and loaded on the LeviCell instrument for a 20 minunte run. A) The left panel shows the brightfield image, middle panel shows all cells stained with AO, and the right panel shows only the dead cells stained with PI. B) Composite image of the run with all 3 channels overlaid. LA = Levitation Agent.*

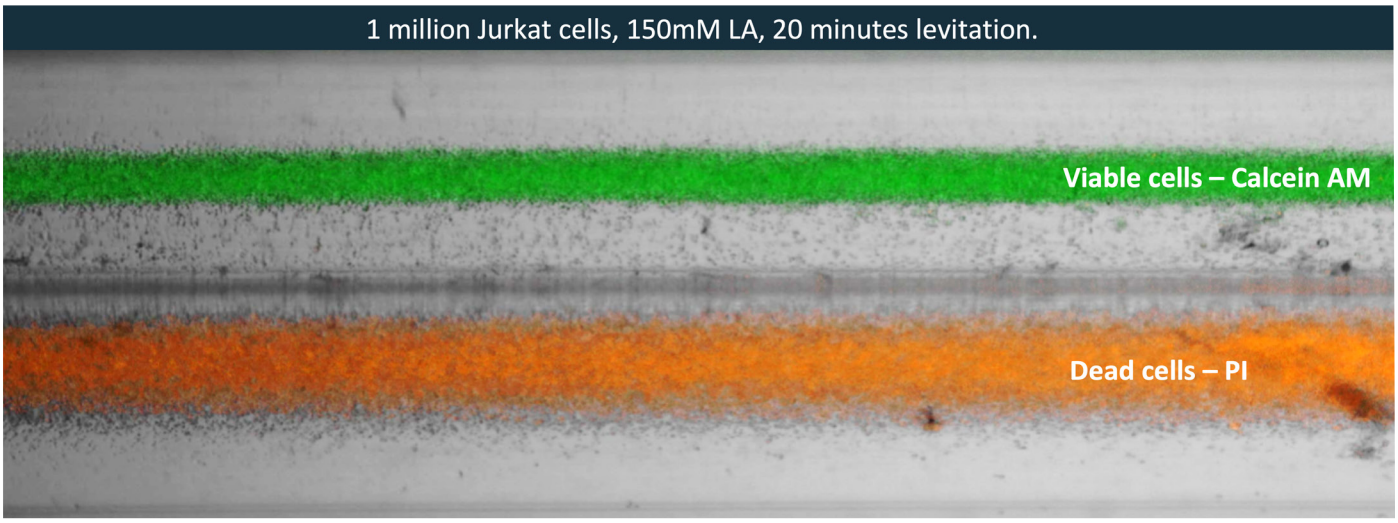

Figure 3. Calcein AM + PI stained Jurkat cells on the LeviCell. *Aged Jurkat cells (50% live) were stained with Calcein AM + PI, combined with levitation buffer and loaded on the LeviCell for a 20 min run. This image is a snapshot of the composite movie created after the run is performed and all 3 channels can be observed. LA=Levitation Agent.*

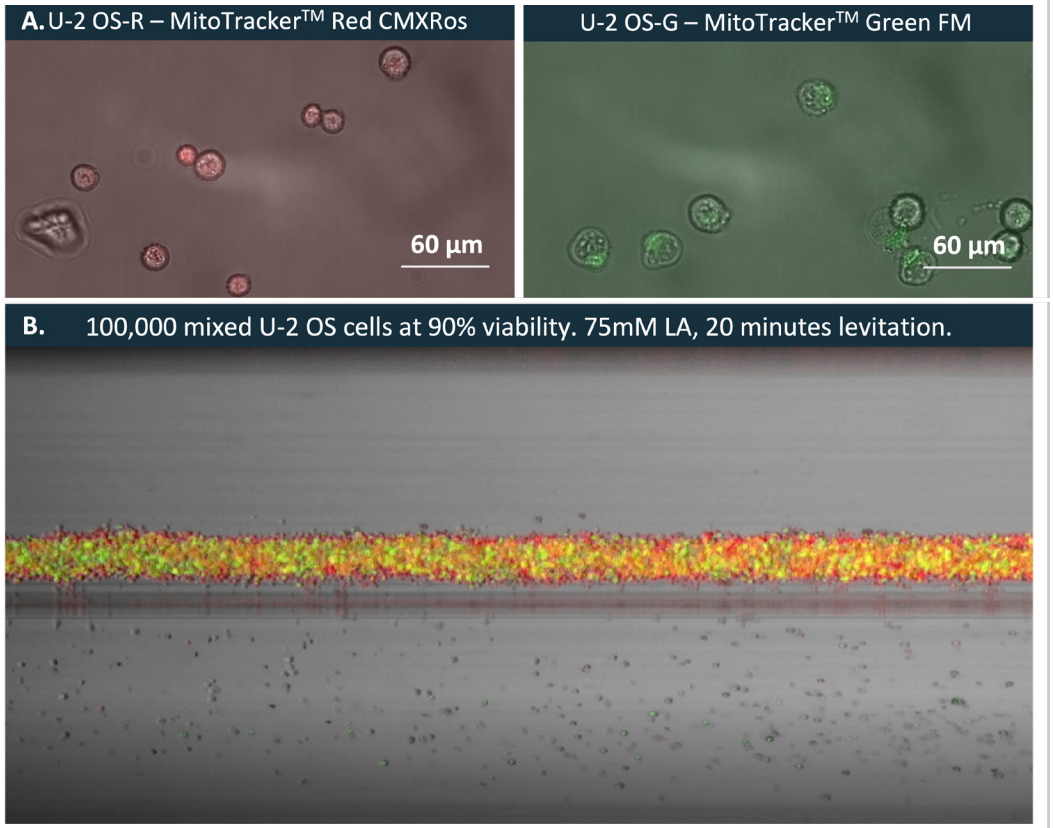

Figure 4. Visualization of MitoTracker Green and Red stained U-2 OS cells on the LeviCell system.
A) Two populations of U-2 OS cells were stained with either MitoTracker Red or Green dye, combined with levitation buffer and enriched through a 20 minute LeviCell run. Images shown were generated by overlaying the brightfield with each fluorescent image. B) Here, a composite image with all 3 chanels overlaid was captured , demonstrating the choice of stain does not affect levitation.

LA=Levitation Agent.

differentitate them during the run and makes it easier to troubleshoot based on their levitation heights.

In Figure 4, the mitochondria of U-2 OS cells (human bone osteosarcoma epithelial cells) were stained either with MitoTracker™ Green or Red (Figure 4A). In this case, the differential staining of U-2 OS cells demonstrated that levitation was not affected by the stain used (Figure 4B). Thus, the clear visualization of these dyes on the LeviCell can be used for the staining of different populations before mixing them with no concern that the stain used might contribute to any levitation differences.

Many readily available cell lines have innate fluorescence and can be identified on the LeviCell instrument. During the transfection process, the gene of interest is often associated with a fluorescent protein, like green fluorescent protein (GFP), to enable visualization of the transfection and whether it was successful. In Figure 5, GFP-expressing NIH/3T3 cells (a mouse fibroblast cell line) can be easily visualized. This visualization confirms the majority of these cells were alive and viable when loaded on the LeviCell instrument, and they remained viable post-enrichment.

Fluorescently labeled antibodies, such as FITC-conjugated anti-CD45 antibody, are commonly used for identifying different cell types that express specific markers. They can also be used for real time sample visualization during a LeviCell run. In Figure 6, anti-CD45 FITC positive Jurkat cells are easily identified and visualized by the LeviCell system due to their green fluorescence.

Conclusion

With this ability to view and overlay dyes or fluorescent probes on top of brightfield imaging, the LeviCell platform allows real-time sample visualization providing valuable insights for sample assessment and optimization. For the first time, desired and undesired populations can be visualized as part of the sample preparation process and the information used to guide the collection of the population of interest.

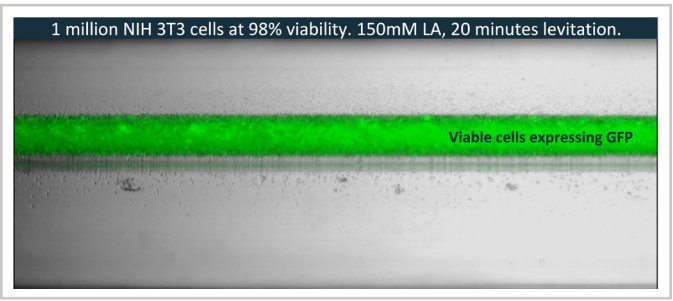

Figure 5. GFP expressing NIH/3T3 cells on the LeviCell. *NIH3T3 cells expressing GFP (98% live) were combined with levitation buffer and loaded on the LeviCell for a 20 min run. This image is a snapshot of the composite created after the run is performed and the GFP expression can be observed for most of the cells. LA=Levitation Agent.*

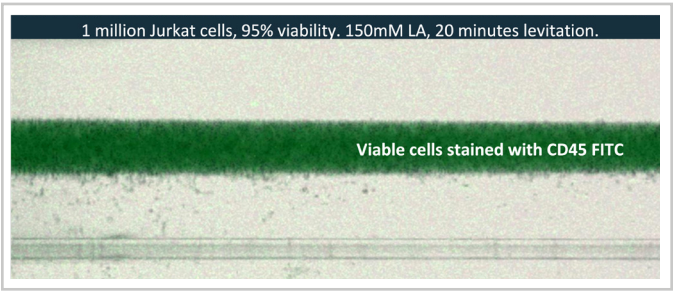

Figure 6. Jurkat cells stained with anti-CD45 FITC antibody. *Jurkat cells express CD45, a transmembrane protein on the cell surface. These cells were labeled (95% live) with anti-CD45 FITC antibody (30 minutes at RT) and later combined with levitation buffer and loaded on the LeviCell for a 20 min run. This image is a snapshot of the composite created after the run is performed and the FITC expression can be observed for most of the cells. LA=Levitation Agent.*

Dyes/Probes/ Fluorescent Proteins	Ex/Em Channel	Protocol	Use Cases	Mechanism
Propidium Iodide (PI)	Red (Ex 493/Em 636)	[Working solution (WS): 1 mg/mL] Use 1:300 into the Levitation Buffer + cells	Detection of dead cells and nuclei	DNA intercalating dye which is permeable to cells with compromised membrane only
Acridine Orange (AO)	Green (Ex 500/ Em 526)	[WS: 0.2 mg/mL] Use 1:100 into the Levitation Buffer + cells	Detection of all nucleated cells	Cell nucleic acid binding dye that emits green when bound to dsDNA
Calcein AM	Green (Ex 495/ Em 515)	[WS: 5 µM] Resuspend 1 million cells into 100 µL of WS. Incubate 15 min, spin and resuspend with the levitation buffer.	Detection of viable cells	Passive diffusion of dye. Inside live cells calcein AM is hydrolysed to green-fluorescent calcein by intracellular esterases.
Fluorescein Diacetate (FDA)	Green (Ex 498/Em 518)	Follow vendor's staining recommendations.	Visualization of live protoplasts and pollen	Passive diffusion of dye. Inside live cells it is deacetylated to green-fluorescent fluorescein by intracellular esterases.
FITC Annexin V	Green (Ex 488/Em 517)	Follow vendor's staining recommendations.	Detection of apoptotic cells	During early apoptosis Annexin V is exposed to the extracellular membrane and the probe recognizes it.
FITC-conjugated antibody, eg. anti-CD45 FITC	Green (Ex 488/Em 517)	Incubate with anti-CD45 FITC (1:10 dil) for 30 min at RT	Detection of cell surface protein CD45	CD45 is a glyoprotein expressed on the cell surface of all nucleated hematopoietic cells
Mitotracker™ green FM	Green (Ex 490/ Em 516)	[WS: 200 nM] Use phenol red free media to incubate cells during 45 min with the dye, spin the cells and resuspend with levitation buffer.	Detection of live cells	Passive diffusion of dye. It accumulates in active mitochondria in a potential-dependent manner.
Mitotracker™ red CMX-ROS	Red (Ex 554/Em 576)			
Cell Tracker™ Red CMTPX	Red (Ex 577/Em 602)	[WS: 10 mM] Resuspend 1 million cells into 200 µL of WS. Incubate 15 min at 37 C, spin and resuspend with levitation buffer.	Detection of stained cells in a mixture	Passive diffusion of dye. It is retained in living cells through several generations.
FluoroMyelin™ Green	Green (Ex 479/Em 598)	Follow vendor's staining recommendations.	Visualization of myelin	It binds to myelin protein of brain digestions
mCherry	Red (Em 585/Ex 611)		Detection of cells that have been successfully transfected using fluorescent protein expression marker	Expression in a cell
Green Fluorescent Protein (GFP)	Green (Em 498/Ex 509)		Detection of cells that have been successfully transfected using fluorescent protein expression marker	Expression in a cell

Table 1. Dyes, probes and fluorescent proteins with optimal concentrations as tested on the LeviCell platform.

Ex=Excitation, Em=Emission, WS=Working Solution, [WS]=Working Solution Concentration.

Levitation buffer is composed of the media or buffer plus the levitation agent. Levitation Buffer volume = 300 µL.

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