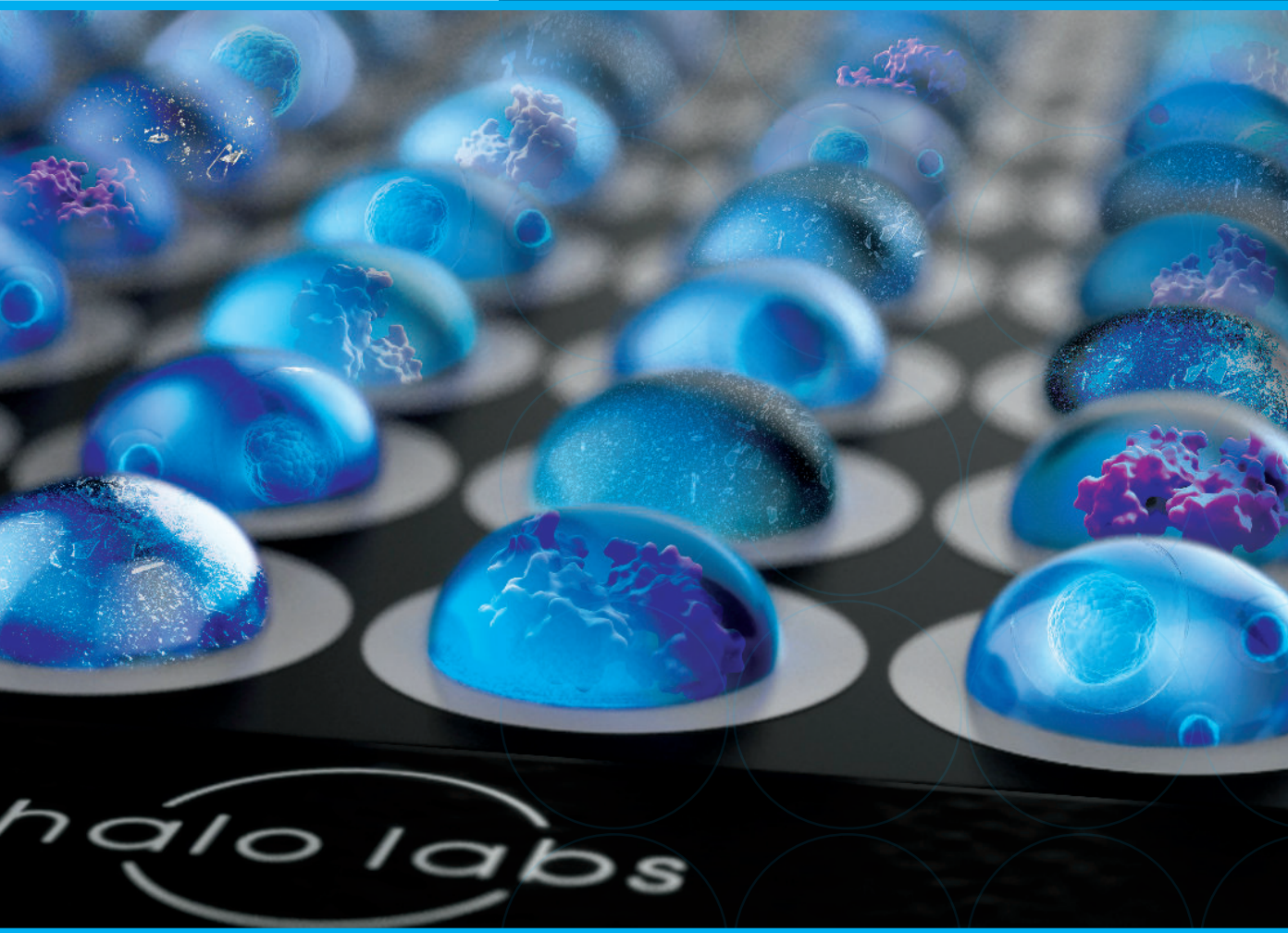
halo labs

# Get High-Throughput, Definitive Identification of Viable Cells and More in Your Cell Therapy




BioProcess International
SPECIAL REPORT

# Get High-Throughput, Definitive Identification of Viable Cells and More in Your Cell Therapy

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The percentage of viable cells present in a cell therapy is a key product-purity parameter. However, traditional methods used to track this parameter (e.g., flow cytometry and manual hemocytometry) are prone to cause clogging when used to analyze the high concentration of cells typically found in a cell therapy. Such methods also are user intensive, low throughput, and incredibly complicated, which can lead researchers to misidentify cellular and noncellular material and confuse cell viability results with product-purity issues. Providing rapid, reliable, and high-throughput viability and purity analyses for cell therapies has proven to be challenging.

The Aura CL system is powered by backgrounded membrane imaging (BMI) and fluorescent membrane microscopy (FMM). The instrument performs cell viability assays with definitive cell identification for 96 40-µL samples in about an hour. The workflow is simple and reduces much of the variation found with more intricate and sometimes outdated methodologies. Now, a user can perform accurate viability assessment on induced pluripotent stem cell (iPSC) seed populations or expanded T cells while simultaneously understanding the purity state of the product.

BMI is a brightfield (BF) imaging technology that uses sophisticated image processing techniques to ensure that everything can be seen in a sample. First, a background image is taken of the membrane. After samples are filtered through and the particles are captured, the same membrane is reimaged, and the "background" image is aligned with the "measure" image. Pixels from the background image are subtracted to reveal only the particles on the membrane, which are counted, sized, and characterized automatically by Particle Vue software. Imaging particles on a membrane eliminates the optical limitations of flow-imaging methods, and increasing the reflective index by 10× reduces light obscuration.

Particle identification by FMM uses fluorescent reagents that bind to a particular particle type. Dyes used include thioflavin T (ThT), a commonly used fluorescent dye that stains protein aggregates, and boron-dipyrromethene (BODIPY) C16 (from Thermo Fisher), which stains degraded polysorbate products. Different dyes can be detected using different

**Figure 1:** In the Aura CL cell-viability assay, step 1 uses an in-solution staining step to identify populations of nonviable (dead) cells using 4′,6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.05 µg/mL. Step 2 stains the entire cellular population using a high concentration of DAPI (2 µg/mL). DAPI-positive cells are identified by their staining intensity in the fluorescence 2 (FL2) channel (FL2 intensity >30). BF is brightfield.

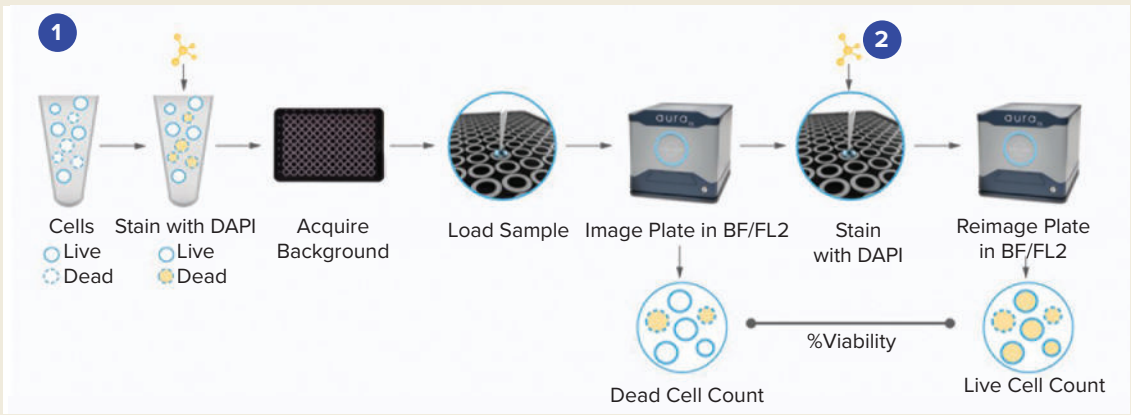
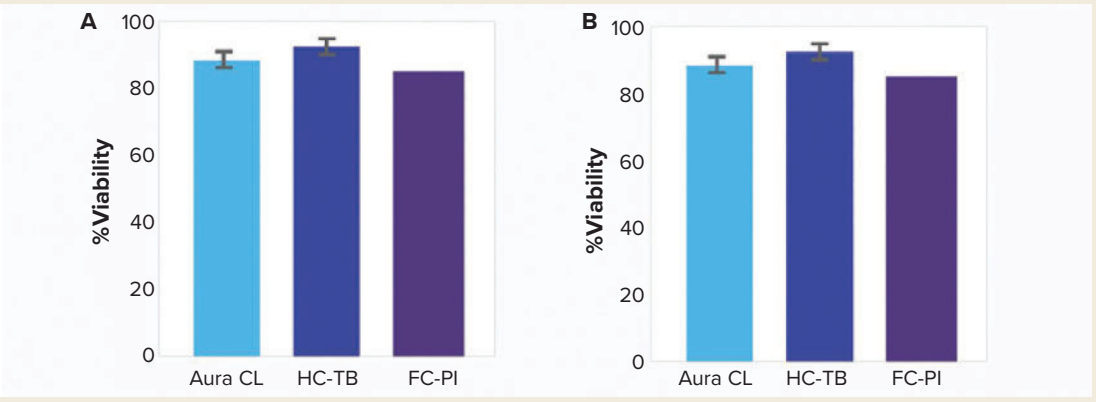


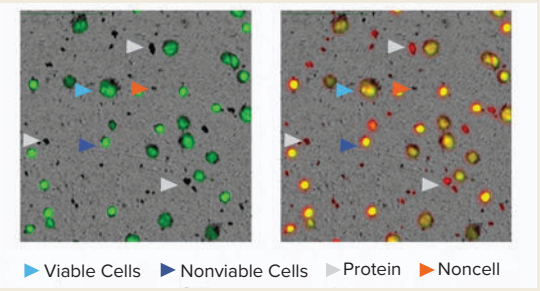
**Figure 2:** Comparative analysis of the Aura CL viability assay with manual hemocytometry (HC) and flow cytometry (FC) shows that the Aura CL analysis compared favorably with both methods. Results for both (A) Jurkat T cells and (B) THP-1 monocytes were comparable, with all percent differences <4.7%. Each cultured cell population was tested for baseline viability in each assay. TB is trypan blue, and PI is propidium iodide.




fluorescent channels, so multiple particles can be identified in one sample. The Aura CL system is designed for cell therapy analysis and labels cells with Hoechst or 4′,6-diamidino-2-phenylindole (DAPI) DNA stains.

Below, we introduce a simple, two-step cell labeling method that distinguishes between viable (live) and nonviable (dead) cells with a high degree of specificity and accuracy. The Aura CL results were validated against flow cytometry and manual hemocytometry. We also analyzed the same samples for other particles (e.g., proteinaceous particles and nonprotein/noncellular contaminants) to assess product purity. The Aura CL instrument generates cell-viability and product-purity data for the same sample.

**Figure 3:** Cell and protein analysis on Aura CL. (LEFT) DAPI is effective at staining nuclei on the membrane, enabling the positive identification of both viable (blue arrow) and nonviable (dark blue arrow) cells and allowing for their differentiation from other particles resident on the filtered membrane. (RIGHT) Staining with ThT allows for additional evaluation through staining both cells and protein aggregates (grey arrow), thus enabling distinction among cells, protein aggregates, and other noncellular material (orange arrow).




## Easy Determination of Cell Therapy Product Viability

A cell viability assay is built on the fact that DAPI is an impermeable dye when used at a low concentration (<0.05 µg/mL) but can stain the DNA of viable (live) cells when used at higher concentrations (>1 µg/mL) (**1**). Cell membranes, however, lose integrity when undergoing cell death. Thus, DAPI can stain the DNA of nonviable (dead) cells even when low concentrations are used.

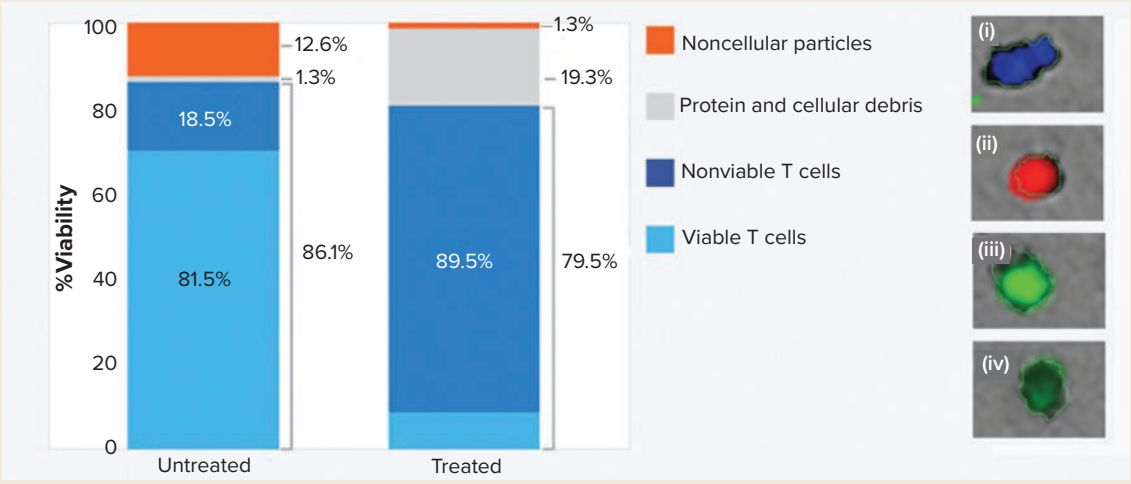
To perform an Aura CL cell viability assay, a cell sample is stained in solution with a low concentration of DAPI so that only the dead cells are labeled (Figure 1). Next, the sample is imaged in the BF channel and fluorescence channel 2 (FL2), capturing information about the number of dead cells in the sample. The same sample is stained on membrane with a high concentration of DAPI to label all cells before the plate is reimaged. The percent viability then can be determined by comparing the number of nonviable to total cells in the sample.

When we compared the Aura CL assay method with other standard methodologies, our analysis proved that Aura CL performs comparably with manual hemocytometry using trypan blue (TB) staining and flow imaging using propidium iodide (PI) (Figure 2). The difference in viability percentages calculated across all three techniques was <4.7%, well within error. However, the Aura CL method is significantly less laborious, intricate, and dependent on individual user skill than are manual hemocytometry and flow cytometry.

## Determine Cell Viability and Product Purity in the Same Sample

A key regulatory requirement for all injectable drug products is the accurate determination of subvisible

**Figure 4:** The expression engine tool can be applied to count an entire data set. The relative contribution of particle subpopulations can be identified using the expression engine tool available with Particle Vue software. Untreated T cells exhibit a different population profile than do T cells treated with repeated freeze–thaw cycles. Representative fluorescent images of individual particles are shown where noncellular particles show a high SIMI intensity (i, blue), protein particles are positive for ThT (ii, red), and nonviable and viable cells are positive for DAPI (iii and iv, green).




particles (SVPs) and aggregates that can form during a manufacturing process, providing essential information about product quality and safety (**2**). The inherent capability of the Aura CL system to differentiate between cellular and noncellular particles is a powerful tool for such an assessment.

In one study, imaging cells were stained on a membrane with a high concentration (2 µg/mL) of DAPI. Cells were subsequently stained on the membrane with 5 mM of ThT to label aggregated protein in the sample (Figure 3) and imaged in fluorescence channel 1 (FL1). The Aura CL system also imaged the membrane using side illumination membrane imaging (SIMI), which images the membrane from the side to identify noncellular, nonprotein particles such as glass and plastic. Using that technique, scientists can examine the contribution of each particle type to the total number of particles within a given cell therapy product. In this case study, viable cells exhibited strong fluorescence in the FL1 (ThT) channel and in the FL2 channel after the measuring image was taken and the sample was stained with low concentrations of DAPI dye. Nonviable cells exhibited strong dual fluorescence in the FL1 (ThT) and FL2 channels, protein particles stained positively for only ThT, and nonbiologic materials were negative for both ThT and DAPI but were detected using SIMI.

Particle Vue software can simplify analyses. The expression engine identifies particles based on user-defined parameters such as fluorescence intensity and particle size to automate the quantitation of specific particle populations (Figure 4). That helps users define particles in a complex mixture (e.g., for a cell therapy) using a combination of BF, SIMI, and two-channel fluorescence. It also enables determination of the relative contribution of each particle subpopulation.

The Aura CL instrument empowers users who need to characterize SVPs in cell therapies. It can be used to perform cell viability assays, adding to its arsenal the ability to distinguish between viable and nonviable cell populations. Thus, users can obtain protein and nonprotein, cell and noncell, viable and nonviable cell information. They also can establish accurate counts for different subpopulations in a sample to determine the viability and purity of any cell therapy with 96 samples within a few hours (for two imaging steps) using only 40 µL per sample.

## References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**2** Wen Y, Jawa V. The Impact of Product and Process Related Critical Quality Attributes on Immunogenicity and Adverse Immunological Effects of Biotherapeutics. *J. Pharm. Sci.* 110(3) 2021: 1025–1041; https://doi.org/10.1016/j.xphs.2020.12.003.



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