Introducing Aura CL™ for Cell Therapy Aggregation, Purity, Particle Analysis and ID

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Stability and Purity of Cell Therapies

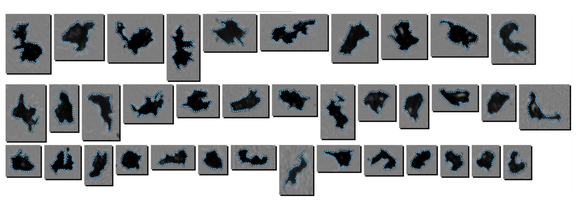
Cell, protein, and viral aggregates are critical quality attributes for all biological products. Subvisible biotherapeutic product aggregates indicate low product stability and low shelf life. In addition, these attributes are a crucial indicator of potential immunogenicity for a given biological drug. The FDA suggests that "strategies to minimize aggregate formation should be developed as early as feasible in product development." Cell therapies present a unique challenge in that cells themselves are subvisible in nature, and distinguishing cells vs. large cellular aggregates and other product impurities remains a challenge. Until now. Aura™ is the first system specifically designed to count, characterize, and ID particles in a rapid and low-volume assay.

Aura systems eliminate the need to use unreliable morphological metrics and cumbersome spectroscopy by combining membrane microscopy with labeled fluorescence.

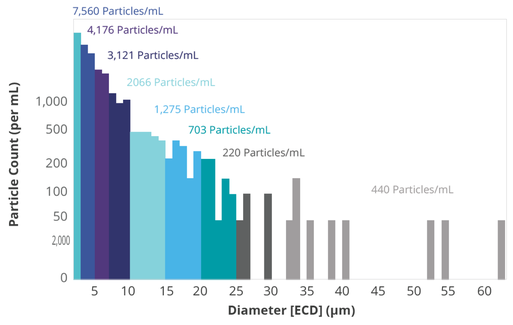
BMI Explained

Backgrounded Membrane Imaging (BMI) is based on the USP <788> compendial method and has been modernized using automation, image processing, and innovative optics. First a background image of a filter membrane is taken, then samples are vacuumed through the filter and reimaged. The background and sample images are processed together in order to remove the background texture and clearly identify the particles present in the sample. This step results in particle counts for particles 1 µm – 4 mm ECD, as well as shape and size distributions according to USP specifications and is easily validated for both size and counts.

BMI's high refractive index imaging results in high contrast images essential for imaging subvisible particles and is sensitive compared to orthogonal techniques.



Detailed to High Level Insights with BMI

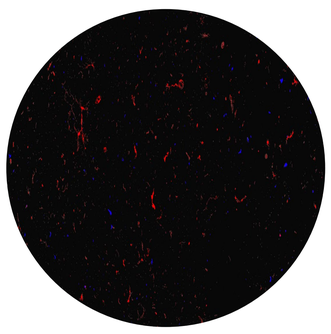


Counts by Condition (Counts/mL)

Sample	Replicates	%CV 2µm..50µm	2µm.. 50µm	Brightfield ≥2µm	≥5µm	≥10µm	≥25µm
10	8	11.85	2672	2675	1815	1445	38
ETFE	8	2.05	40121	40192	17012	6533	996
H20	8	40.32	215	218	22	8	2
IGG	8	2.57	70948	71235	27762	10585	1778

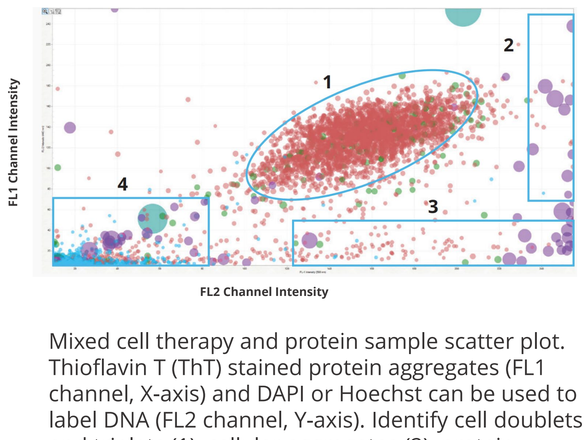
Aura systems enable the characterization of individual particle size distributions, and its high-throughput format also makes it possible to obtain high level insights at a whole 96-well experiment level. Running replicates and obtaining confidence in your subvisible particle results has never been easier.

BMI + Fluorescence Membrane Microscopy



Protein Aggregates: Red Particles
ETFE: Blue Particles. Images acquired with FMM.

Combining BMI with Fluorescence Membrane Microscopy (FMM) allows rapid and specific identification of particles. After particles are counted with BMI, a fluorescent dye is added to the membrane to tag particles of interest. Highly specific stains that identify cellular, proteinaceous, lipid, and other particles can be used. Fluorescence is not used for sizing, but rather to the chemical and biological nature of the particles in question.

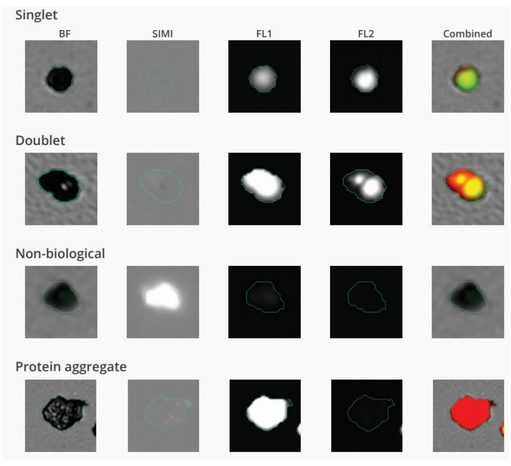


Mixed cell therapy and protein sample scatter plot. Thioflavin T (ThT) stained protein aggregates (FL1 channel, X-axis) and DAPI or Hoechst can be used to label DNA (FL2 channel, Y-axis). Identify cell doublets and triplets (1), cellular aggregates (2), protein aggregates (3), and plastic contaminants (4) in a cell therapeutic sample.

Aura software automatically detects fluorescence from stained particles and displays them in relation to unstained particles for easy individual particle and whole population ID and differentiation in just 1 minute per sample.

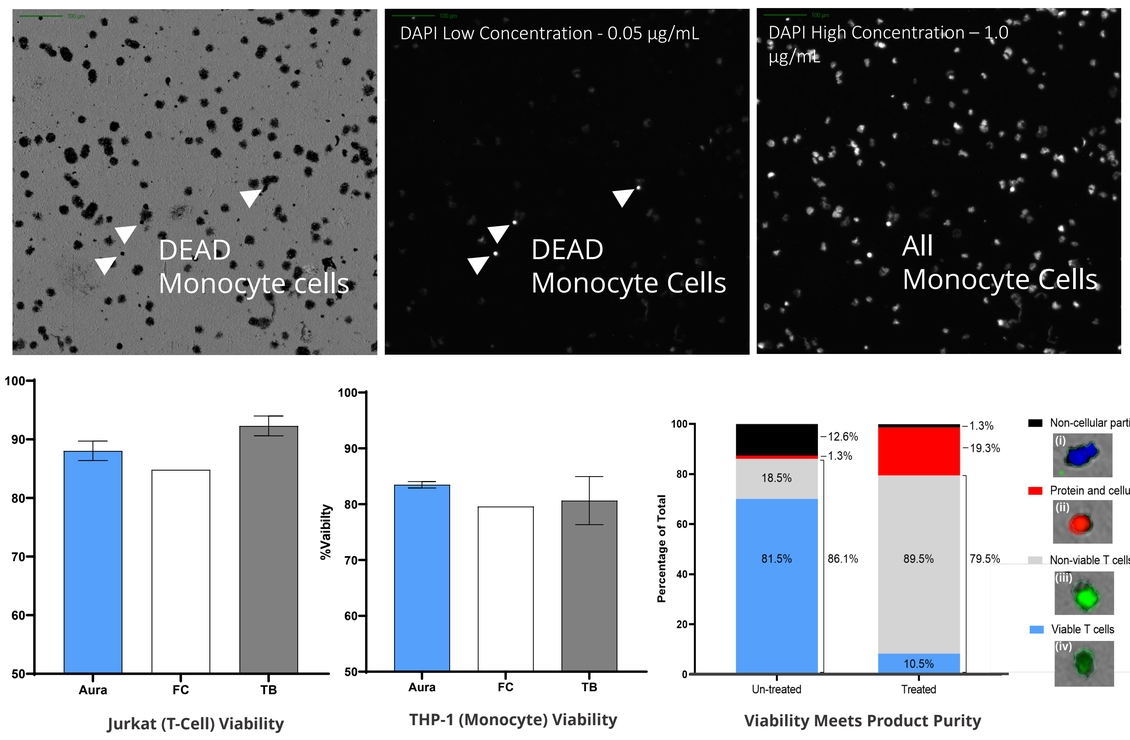
Characterizing CAR-T Cell Products with Aura CL™

With Aura CL, different particle types can be identified based upon various morphological, light scattering, and fluorescent properties. Depending on their responses to specific chemical and biological stains, as well as their morphology, millions of particles can be identified simultaneously in high-throughput. Example particles from each quadrant are included here. Singlet and doublets present dual positive identification for DNA and protein (high FL1 and FL2 intensity), non-biological aggregate shows particles negative for DNA and protein (low FL1 and FL2 Intensity), and protein aggregate shows particles positive for protein only (high FL1 and low FL2 intensity).



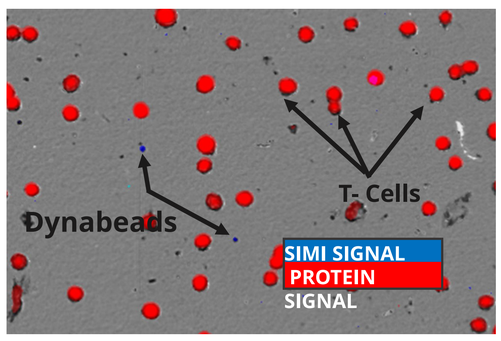
Cell Viability Meets Product Purity

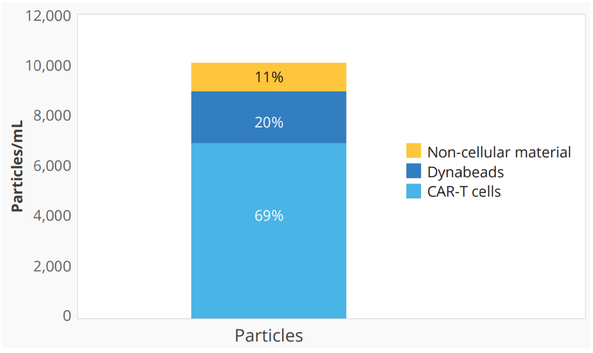
Aura CL enables high-throughput cell viability assays that are comparable with the most well-established and validated flow cytometry and hemocytometry assays. In addition to outperforming them in low volume requirements, ability to work with concentrated samples without clogging the system, and having visual confirmation for every cell and particle measured, Aura CL enables the characterization of cell viability in tandem with product purity measurements.

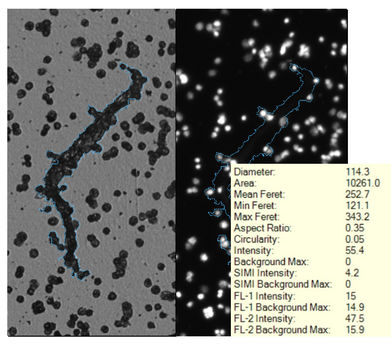


Cell viability and product purity with Aura CL. Using a low concentration of nucleic acid stains will only permeate through dead cells, a while high concentration will stain all cellular material. We use this differential property to conduct viability analysis that matches the most established cytometry and hemocytometry techniques. In addition to viability, Aura CL enables a simultaneous measurement of product purity, identifying protein aggregates and other contaminant impurities that may be present. Contaminant impurities tend to confound viability measurements, since non fluorescent materials will either be missed or identified as live cells in flow cytometry.

Finding Trace Particulate Impurities in Cell Products







Aura CL can be used to find trace amounts of subvisible and visible particle contaminants in cell therapy materials. Some examples included find trace amounts of Dynabeads® in CAR-T cell products, or identifying the presence of fibers and distinguishing them from cellular material. Aura enables the ability to characterize, size, and identify every particle in across every cell therapy experiment.