Aura+: High Throughput, Low volume Product Stability and Purity Analysis for Gene and Cell Therapies

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Stability and Purity of Cell Therapies

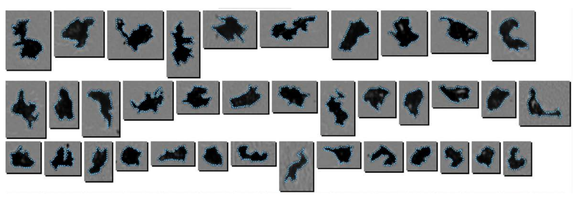
Cell, protein, and viral aggregates are critical quality attributes for all biological products. Subvisible biotherapeutic product aggregates indicate low product stability and low shelf life. In addition, these attributes are a crucial indicator of potential immunogenicity for a given biological drug. 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 eliminates the need to use unreliable morphological metrics and cumbersome spectroscopy by combining membrane microscopy with labeled fluorescence.

BMI Explained

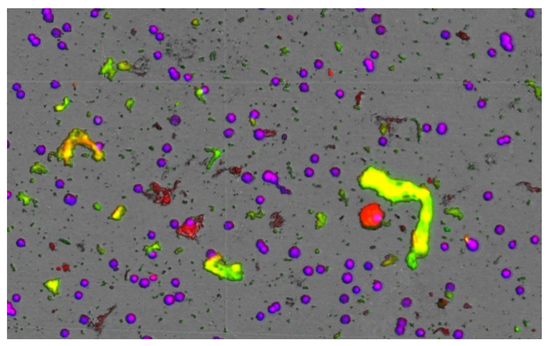
Backgrounded Membrane Imaging (BMI) is based on the USP <788> compendial method and has been modernized using automation, image processing, and innovative optics. First, a background image of a filter membrane is taken, then samples are vacuumed through the filter and reimaged. The background and sample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



BMI + Fluorescence Membrane Microscopy

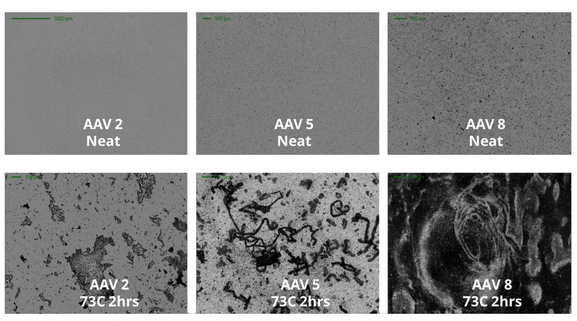
Combining BMI with Fluorescence Membrane Microscopy (FMM) allows rapid and specific identification of particles. After particles are counted with BMI, a fluorescent dye is added to the membrane to label particles of interest. Highly specific stains to identify cellular, proteinaceous, lipid and other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



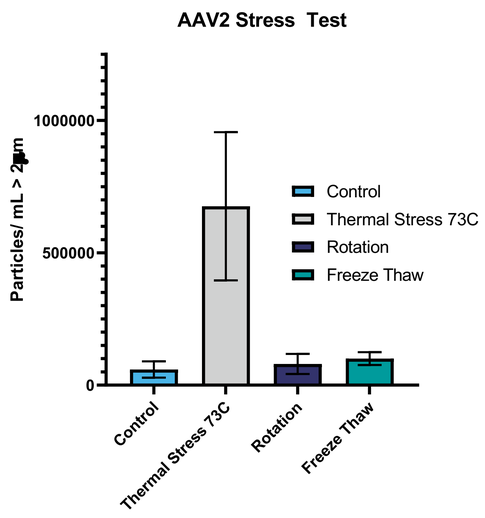
Protein aggregates: red particles
IgG fluorescently labeled protein: yellow particles
Stained cells: pink particles

Subvisible AAV Aggregate Characterization

Aura enables low volume, high throughput characterization of biological aggregates. This is of critical importance for characterizing the stability and manufacturability potential of AAV formulations, where sample volume is severely limited compared to traditional antibody manufacturing.

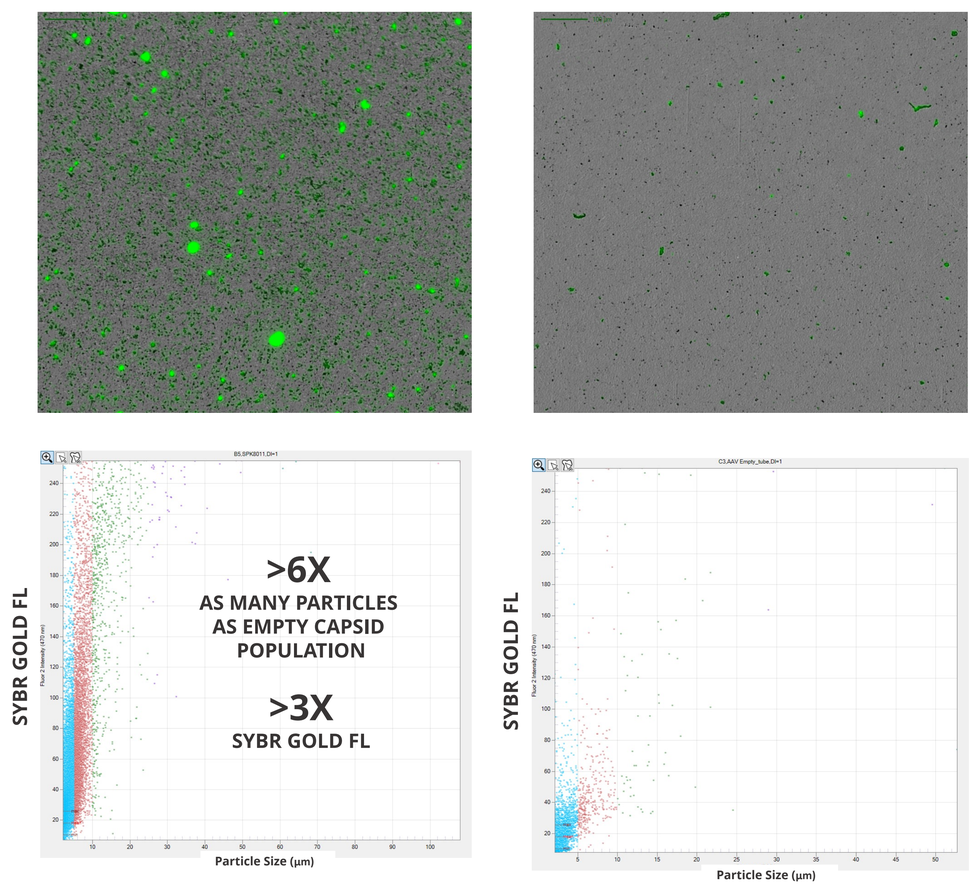


Images of subvisible particle formation in three different AAV serotypes under no stress and heat stress conditions. Only 10 µL of sample were required to quickly assess subvisible stability



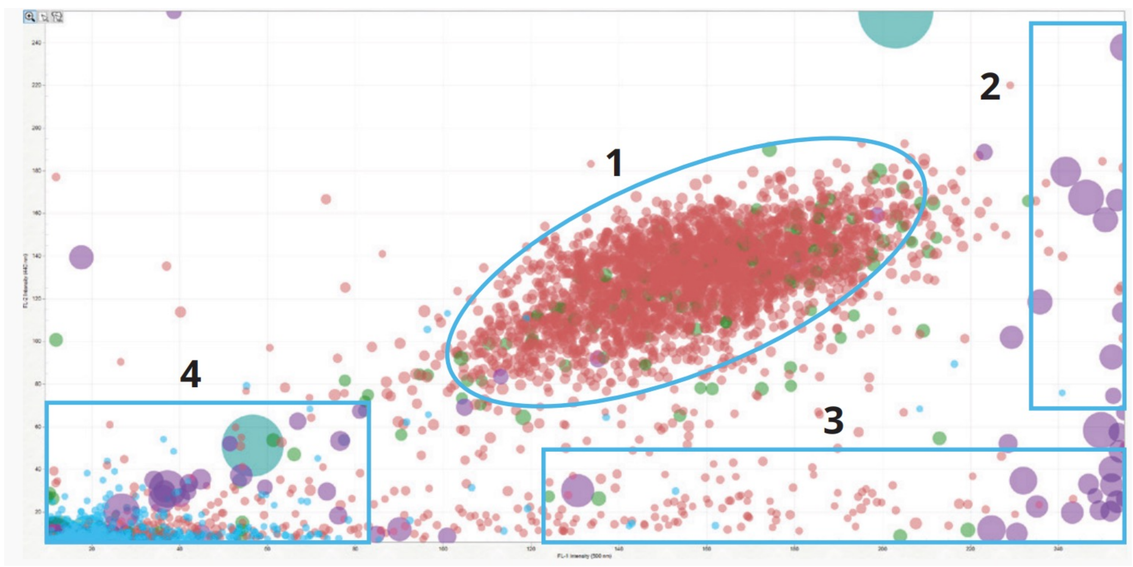
In depth particle analysis for AAV2 samples subjected to thermal, transportation and freeze thaw stresses. Thermal stress produced the most particles, while rotation and freeze thaw produced slightly more particles than the control condition.

Correlating AAV Stability and Role of DNA Leakage



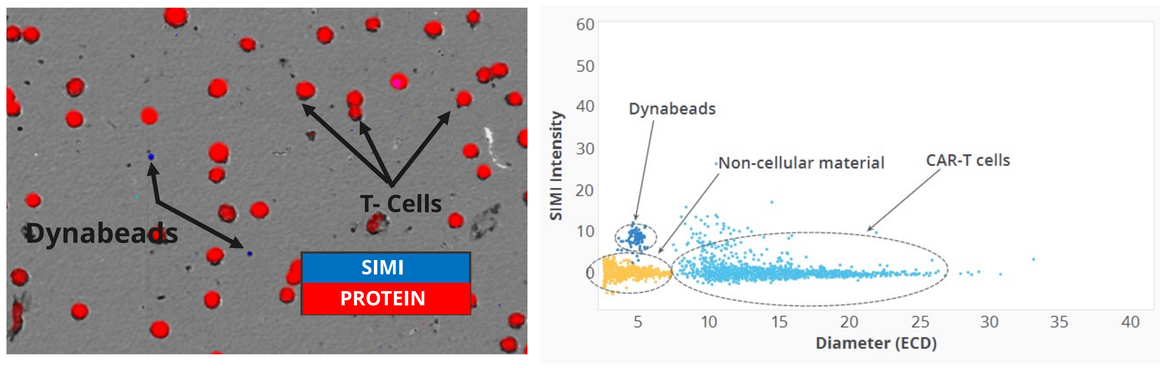
AAV samples separated between full and empty capsid fractions chromatographically.
Top two Images - *left*: full fraction - subvisible AAV particles stained with SYBR gold. *Right:* empty fraction shows less subvisible particle formation and less DNA leakage.
Bottom two graphs – *left*: full fraction - subvisible AAV particles stained with SYBR gold. *Right*: empty fraction 6-fold shows less subvisible particle formation and 3 times less DNA leakage.

Characterizing CAR-T Cell Products with Aura+



Mixed T cell therapy and protein sample scatter plot: ThT for protein aggregates (FL1 channel, X-axis) and DNA (FL2 channel, Y-axis). Identified were cell doublets and triplets (1), cellular aggregates (2), protein aggregates (3), and plastic contaminants (4) in a cell therapeutic sample.

Finding Trace Particulate Impurities in Cell Products



Aura can be used to find trace amounts of subvisible and visible particle contaminants in cell therapy materials. Some examples included finding 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 ID every particle in across all cell therapy experiment.

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