Introducing Aura CL™ for Gene Therapy Physical Stability, Aggregation, and Particle Identification

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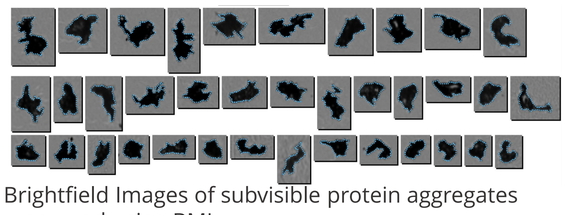
Physical Stability of Gene Therapies

Subvisible particle analysis is a key predictor of drug product stability and is a critical quality attribute for all biologics. Adeno-associated viruses (AAVs) have transformed gene therapies by enabling the targeted delivery of curative genetic payloads. However, the amount of AAV material available is consistently in short supply due to its inherently expensive and laborious production process. Until now, there has not been a simple, rapid, and easy way to both count and size AAV particles while at the same time, being able to ID their source. 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

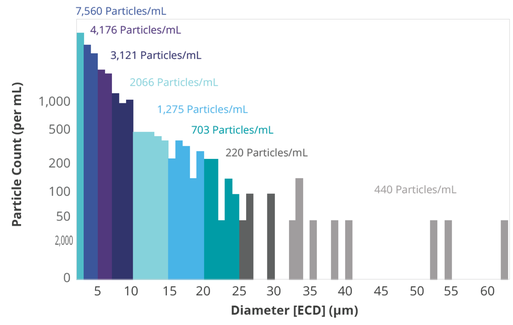
Backgrounded Membrane Imaging (BMI) is based on the USP <788> compendial method and has been modernized using automation, image processing, and innovative optics. First a background image of a filter membrane is taken, then samples are vacuumed through the filter and reimaged. The background and sample 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.



Brightfield Images of subvisible protein aggregates measured using BMI.

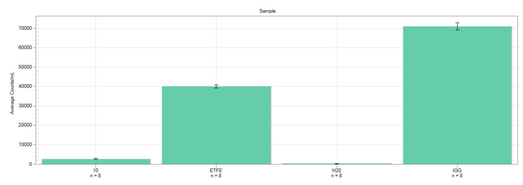
BMI's high refractive index imaging results in high contrast images essential for imaging subvisible particles and is sensitive compared to orthogonal techniques.[1,2]

Detailed to High Level Insights with BMI



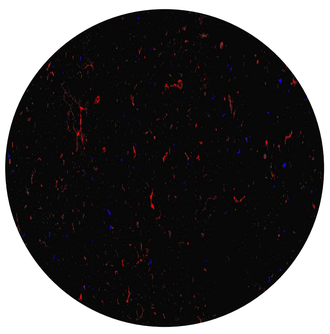
Counts by Condition (Counts/mL)

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 enables 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 and rapid stains such as Thioflavin T (ThT) can be used to stain and positively identify subvisible protein particles. The workflow can be adjusted to allow for solution or membrane-phase staining. Membrane-phase staining after the particles have already been counted using BMI does not result in false positive counts since only particles that were first counted with BMI are evaluated.

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.

Sample	Replicates	ECD > 5um (/ml)	ECD >5um & FL > Bck + 6σ (/ml)	% >5um above Threshold
ETFE	24	15857	244	**1.5%**
IgG	24	43494	43396	**99.8%**
IgG + ETFE	24	66343	58223	**87.8%**

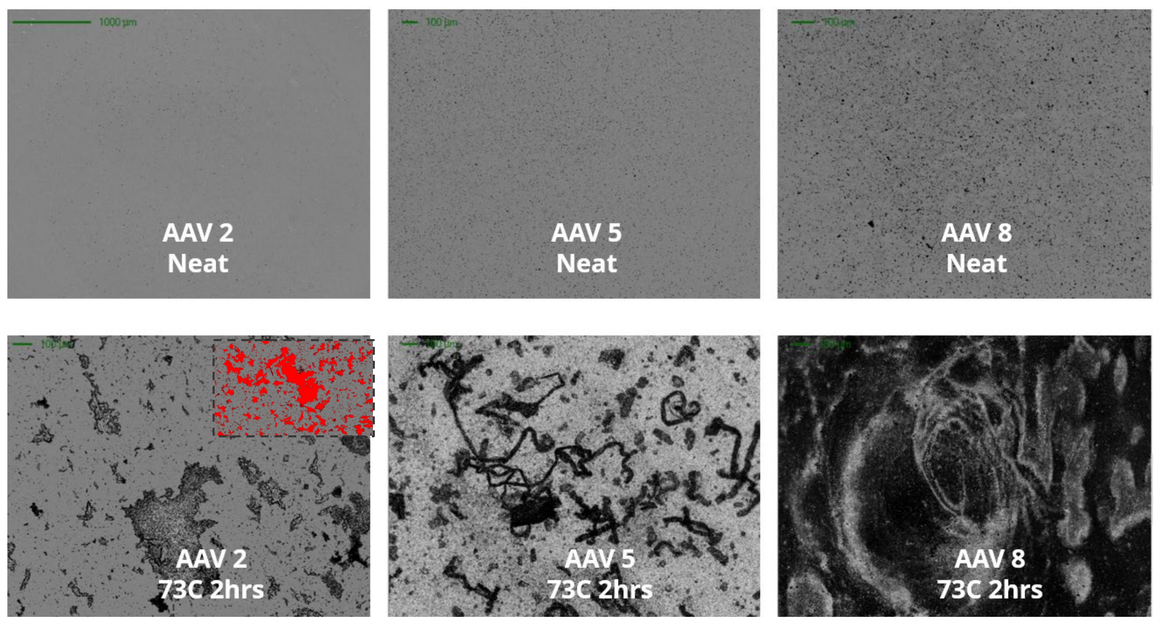
Summary table of protein vs. non-protein particles from a mixed sample.

Characterizing Stability of Different AAVs

In this poster, we demonstrate how Aura characterized the physical stability for AAV2, AAV5, and AAV8, at titer concentrations 1E13 GC/mL. The virus was suspended in PBS + 0.01% Pluronic F68 surfactant. Viral samples were thawed at room temperature (RT) and then split into separate tubes and subjected to the following stress conditions :

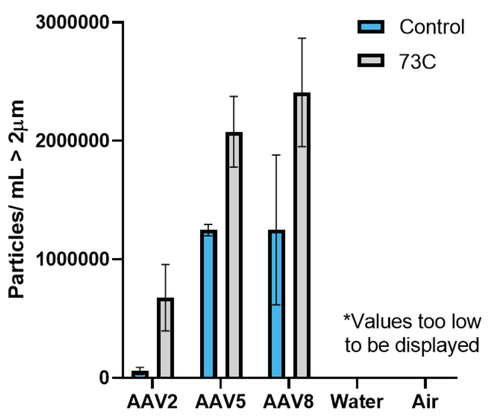
(1) no stress
(2) Heat stress 73 °C for 2 hours
(3) Rotation tumbling stress
(4) 2 Rounds of Freezer Thawing

AAV and Subvisible Particle Formation

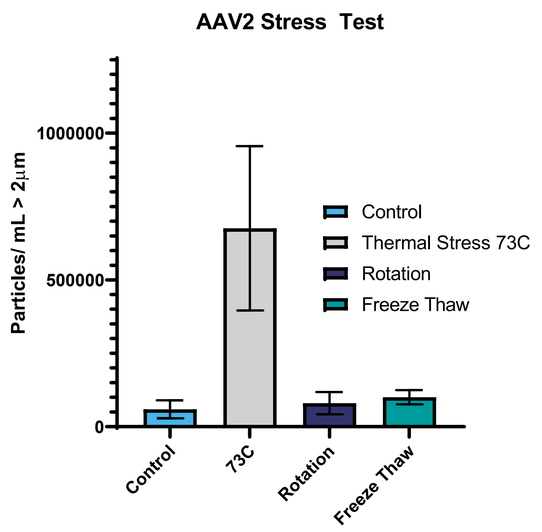


Brightfield membrane images for different AAV serotypes. Unstressed AAV2, AAV5, and AAV8 samples (top). Thermally-stressed (73 °C, 2 hours) AAV2, AAV5, and AAV8 samples (bottom). All images were magnified to 100 µm.

Subvisible particle aggregation was observed as a function of serotype and stress conditions with as little as 10 µL per sample as shown in the figure above. Of the 3 serotypes, only the AAV2 sample did not form significant subvisible particles when no stress was applied. When heat stress was applied, the particle formation varied drastically between the three serotypes, with AAV2 forming large, discrete particles, AAV5 forming large fibrils and AAV8 showing large amounts of small particles. The inset in the AAV2 heated figure shows the FMM image of AAV2 when dyed with a protein stain, confirming that the particles observed are AAV2 aggregates. The particulate content was numerically obtained for every serotype under every stress condition. Particle formation did not correlate with Tm values from the literature, indicating they need to be measured independently.



Particle formation for AAV2,AAV5, and AAV8 samples under no stress and thermal stress.



Subvisible particle formation in AAV2 subjected to thermal, rotation and freeze thaw stresses.

	AAV2	AAV5	AAV8
nanoDSF (T_m)	Low	High	Medium
BMI (Diameter)	High	Medium	Low

Melting temperature and subvisible particle formation do not necessarily correlate.

References

[1] Helbig *et al. J Pharm Sci.* 109 (1) Jan 1, 2020

[2] Vargas *et al. Intl. J Pharm*. 2020 Mar 30;578:119072.