



Quality Assessment of Adenoviral Vectors

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Interferometric nanoparticle tracking analysis (iNTA) offers a unique capability: simultaneous measurement of particle size, optical contrast and number concentration. Get insights into the complexity of advanced therapeutic medicinal products (ATMP), allowing the assessment of critical quality attributes (CQAs) such as purity and quantity of adenoviral vectors (AdV).

About Adenoviral Vectors (AdV)

Adenoviral vectors (AdV) are widely used as delivery platforms for therapeutic payloads. However, conventional characterization methods like dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), or analytical ultracentrifugation (AUC) often lack the resolution or throughput required for routine quality control.

Interferometric NTA (iNTA) overcomes these limitations by enabling label-free, high-resolution analysis of biological particles (>50 nm) in solution, assessing size and quantity. It can distinguish intact AdV particles from other particles (e.g. contaminations) based on their contrast signal.

iNTA Provides Two Orthogonal Parameters in a Single Measurement

Like conventional methods, iNTA determines the hydrodynamic diameter of particles. Additionally, it assigns each particle another independent parameter (the contrast signal) which correlates with the particle mass.

The particle-wise representation of this 2D parameter space is shown in Figure 1 and displays the result of a measurement of several hundred AdV particles in a single sample.

By defining a region of interest (ROI) in the 2D plot, based on both size and contrast thresholds, users can define their target population. Guided by empirical experience, we define an oval ROI around 97nm (± 25 nm) and 0.69 (± 0.09 nm) to identify intact AdV particles.

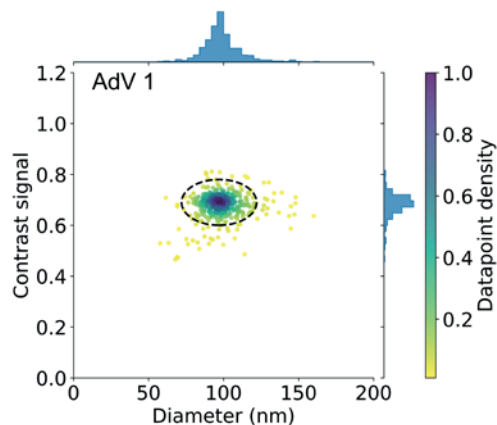


Fig. 1 Scattering plot displaying size and optical contrast of individual Adenoviruses of a single sample (AdV1). For that purpose, the sample was diluted 1:10³ in phosphate buffer saline (PBS) and measured for 5min. The colormap reflects how densely datapoints are clustered. Guided by empirical experience, a region of interest (ROI) within the 2D scattering plot was selected (dashed line) to define the target population (i.e. intact AdV).

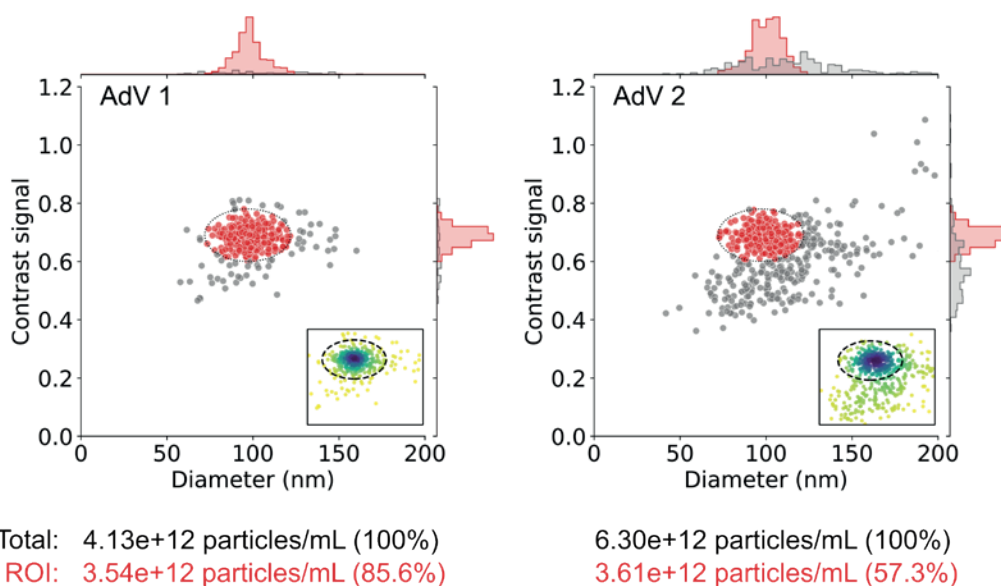


Fig. 2
2D scattering plots of two adenoviral vectors (AdV) samples measured via interferometric nanoparticle tracking analysis (iNTA). Viruses were diluted 1:10³ in PBS and recorded for 5min. Both samples show narrow size distributions (~97 nm and ~101 nm), but differ in contrast profiles. AdV 1 sample exhibits a uniform contrast around 70%, indicating high purity. AdV 2 sample reveals a broader contrast spread at similar sizes, suggesting a significant fraction of other particles. Gating over the ROI (particles highlighted in red) enables accurate determination of relevant particle concentration.

Accurate Quantification of AdV Samples

iNTA can be further used to determine the particle number concentration. Other methods that quantify samples in this way have the disadvantage that they can only determine the sample concentration in total. In contrast, iNTA makes it possible to determine the proportional concentration of different subpopulations without the use of labels, as shown in Figure 2.

If we determine the total concentration of both samples, AdV 2 contains >50% more particles than AdV 1. However, the scatter plot of AdV 2 (Fig. 2) shows that particles outside the ROI (grey) contribute significantly to the total concentration. It is important to note that this differentiation cannot be made on the basis of size distribution alone. For example, there are particles of the same size but with significantly lower contrast signal. It is conceivable that these are particulate impurities, e.g. empty AdV capsids.

Thanks to the possibility of label-free delimitation, this sample complexity can be considered in the concentration determination. The result shows that the concentration of intact AdVs (red) is almost identical (3.54e+12 particles/mL in AdV 1 and 3.61e+12 particles/mL in AdV 2).

Conclusion

Interferometric nanoparticle tracking analysis (iNTA) is a great tool for the quality control of adenoviral vectors. The high-resolution, label-free analysis of individual particles provides insights into size and optical contrast, enabling the differentiation of intact AdVs and particulate impurities.

The ability to resolve subpopulations label-free, makes iNTA very suitable for assessing sample quality and optimizing production workflows. With rapid measurements and minimal sample preparation iNTA is poised to become a standard method in viral vector analytics.

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