Spectradyne®
Particle Analysis

Rapid Viral Titer in Complex Media with Spectradyne's ARC™ Particle Analyzer

DNA-loaded virus and extracellular vesicles are used to demonstrate:

1. Fluorescent labeling of internal virus cargo and quantification with the ARC particle analyzer
2. Labeling and detection of subpopulations in complex, heterogenous samples

Experimental Design:
A heterogenous mixture comprising human adenovirus (HAdV) and extracellular vesicles (EVs) in tissue culture media was prepared. The mixture, and each of the components separately, were labeled with the fluorescent nucleic acid stain SYBR Gold (Thermo Fisher Scientific) according to manufacturer's instructions. Particle size, concentration and fluorescence were measured for all three samples using Spectradyne's ARC particle analyzer.

Results:
Figure 1 shows the total particle size distribution for each sample as measured by Microfluidic Resistive Pulse Sensing (MRPS) on the ARC. The particle size distribution for the mixture (red) is equal to the sum of the component distributions (HAdV in green, EVs in blue). For the mixture, an important experimental question is: "What is the concentration of just the adenovirus?" The ARC answers this question directly using fluorescence.

Figure 2 shows fluorescence brightness versus MRPS diameter for each particle detected in the HAdV + EV mixture sample (red) and the stained EV-only control (blue). Adenovirus particles are clearly identified by their diameter (~90 nm) and strong fluorescence, which is caused by intercalation of the SYBR Gold stain with the DNA present in the virus. The background EVs in the mixture do not show any detectable fluorescence because they do not contain sufficient DNA—as confirmed by measurements of the stained EV-only sample. Note by default, fluorescence brightness is reported in pre-calibrated, absolute, traceable units (ERF or MESF) in the ARC software, making results immediately comparable across instruments and between researchers.

Fun fact! HAdV dimers are readily identified in the sample by their two-fold higher brightness and two-fold larger particle volume (~110 nm effective spherical diameter)!

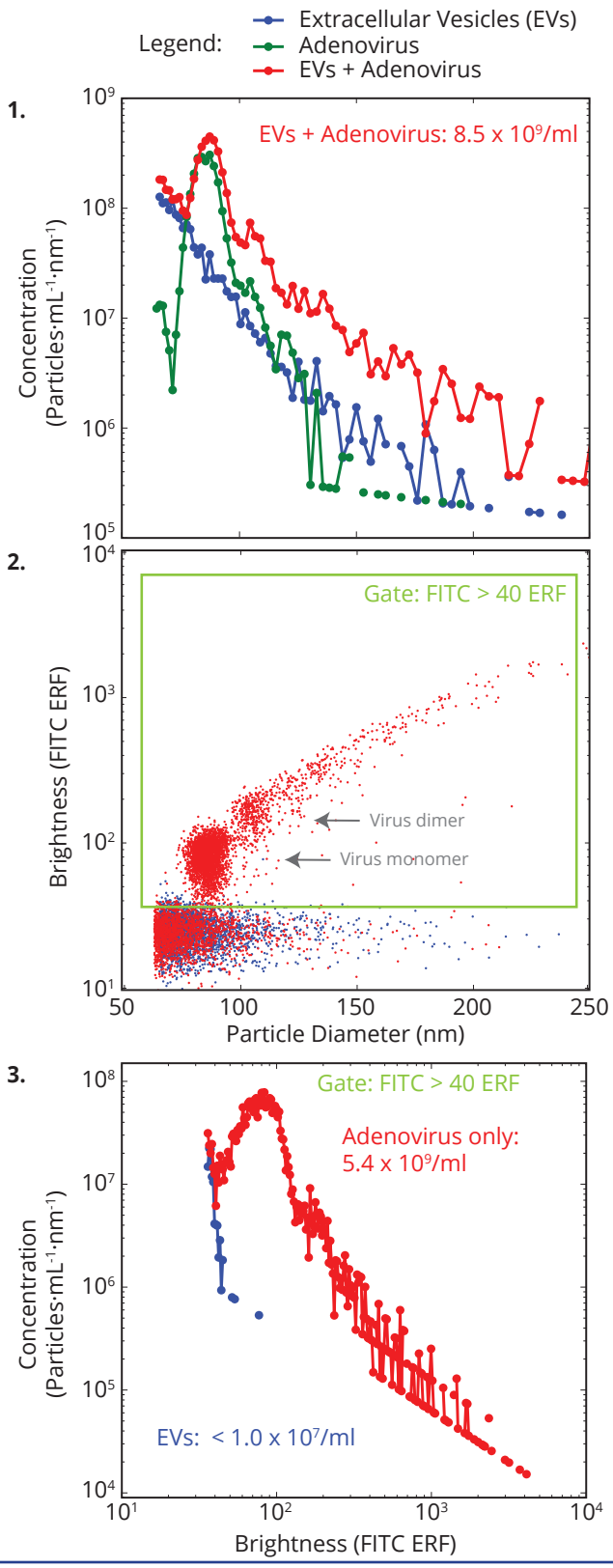
Figure 3 shows the distribution of particle concentration versus brightness for the mixture and EV-only control sample after gating to include only particles brighter than the fluorescence limit of detection (LOD ≈ 40 FITC (ERF)). The HAdV monomers are tightly distributed with mean brightness of approximately 82 FITC (ERF). Essentially no fluorescent particles are detected in the EV-only control.

Discussion:
In the mixed sample, over the size range of 65 nm – 400 nm diameter, adenovirus with DNA cargo and brightness > 40 FITC (ERF) is present at 5.4 x 10^9/mL, representing 64% of all particles within the measurement range. Mean brightness of the adenovirus was found to be ~82 FITC (ERF) with this staining protocol.

Conclusions:

1. The ARC quantitatively measures nanoparticle size, concentration, and internal payload fluorescence for viral applications.
2. The ARC accurately analyzes fluorescent virus subpopulations even in complex, heterogeneous samples.

Results



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