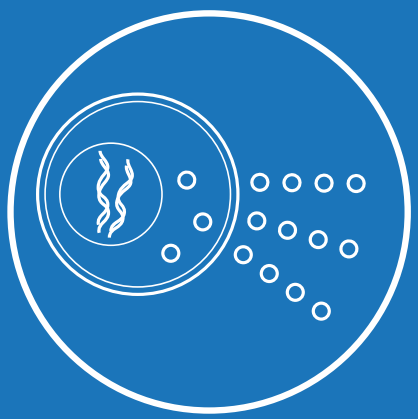
Spectradyne®
PARTICLE ANALYSIS

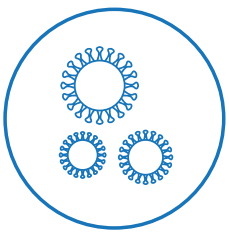
Extracellular Vesicles

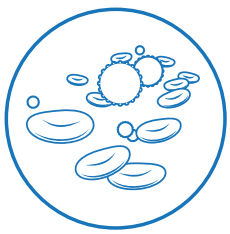


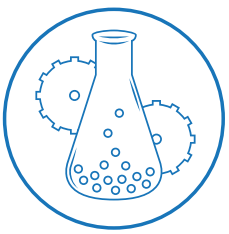
ARC

Application Note

Extracellular Vesicle and Cargo Quantification with Spectradyne's ARC™ Particle Analyzer







Application Note: Extracellular Vesicles

Extracellular Vesicle and Cargo Quantification with Spectradyne's ARC™ Particle Analyzer

Extracellular vesicles (EVs) expressing GFP and human tetraspanin CD63 were measured before and after staining with PEanti-CD63 to demonstrate:

1. Immunolabeling and co-localization analysis with the Spectradyne's ARC particle analyzer
2. Accurate size and concentration measurements of fluorescent subpopulations

Experimental Design:

Vesi-Ref CD63 GFP EVs (Vesiculab, UK) were measured first without modification and subsequently labeled with PE-conjugated antibodies: A monoclonal antibody targeting CD63 and an IgG isotype control. A dye-only control was prepared in the same manner without Vesi-Ref EVs using the PE-conjugated CD63 antibody. Particle size, concentration and fluorescence were measured for each staining condition using Spectradyne's ARC particle analyzer.

Results:

Figure 1 shows particle concentration vs. size for all particles in the unstained sample (purple), all particles in the stained sample (blue), and subpopulations of the stained sample after gating for GFP+ (green), CD63+ (yellow), and double-positive GFP+/CD63+ (red) populations.

The overall particle size distribution for both the unstained and PE-stained samples exhibits an approximate power-law dependence of concentration on size that extends to 65nm, the lower size limit of detection for this cartridge. The presence of a peak near 75nm diameter is visible in each of the fluorescent subpopulations, revealing the population of EVs that express the target biomarkers.

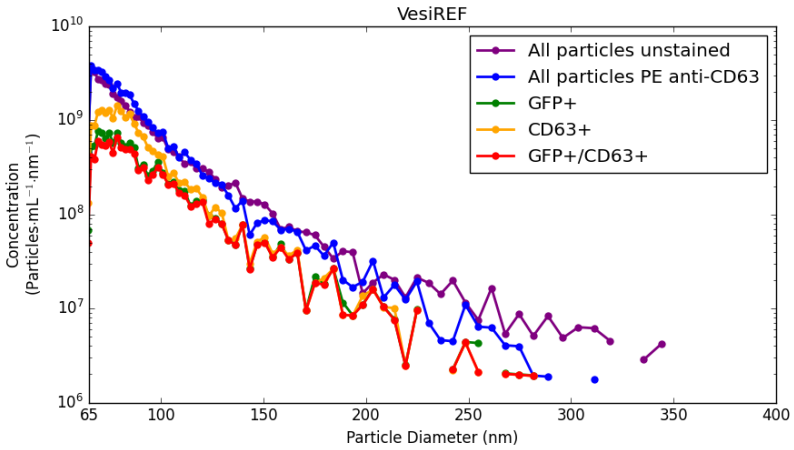


Figure 1

CSD (Concentration Spectral Density) plot of total particle concentration and subpopulations after staining of GFP+, CD63+, and GFP+/CD63+ expressing EVs.

Results (cont.):

Figure 2 The 2D scatter plot shows the brightness of each particle in FITC and PE channels for the PE-anti-CD63 stained data. Absolute concentration and relative abundance of particles are reported for each quadrant, the bounds of which are defined by the fluorescence limits of detection for each channel: 30 FITC ERF and 2.5 PE MESF. By default, Spectradyne's ARC reports fluorescence intensity in transferrable, NIST-traceable units of either ERF (Equivalent Reference Fluorophore) or MESF (Molecular Equivalent Soluble Fluorophores).
Quadrant 2 (Q2) shows that 25.4% fraction of the total particles are double-positive for both GFP and CD63 markers, while 23.8% are CD63+ but not GFP+ (Q4). Only 4% of EVs are GFP+ but not CD63+ (Q1). Almost half of all particles, 46.8%, are negative for both (Q3). Note that the purple events in the scatter plot are GFP+ signals from the EVs in the unstained sample.

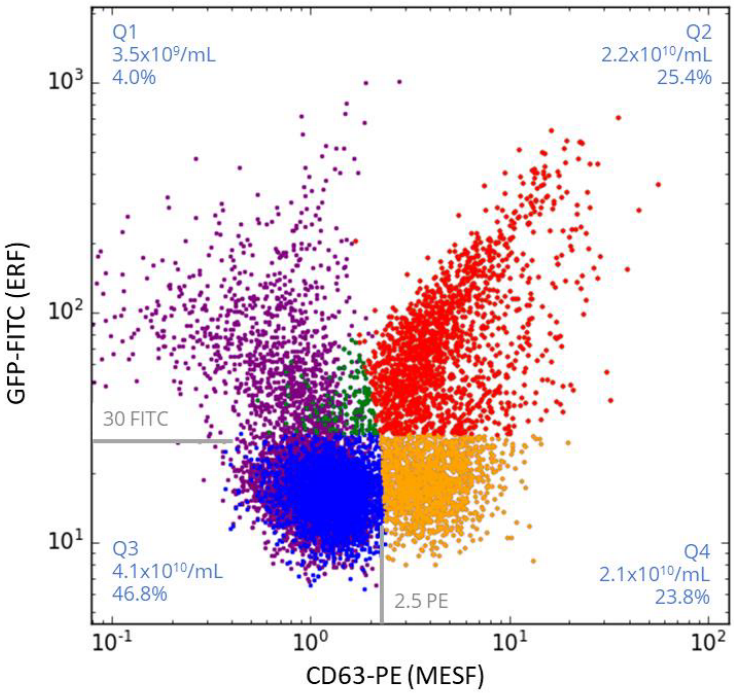


Figure 2

Scatter plot of the brightness of each particle in FITC and PE channels for the PE-anti-CD63 stained EVs.

Figure 3 The scatter plot of the PE fluorescence intensity vs MRPS diameter (right) for the particle detection events in the Vesi-Ref CD63 GFP EVs labeled with PE-anti-CD63 compared to PE-isotype labeled control and dye-only control. The limit of detection in the PE channel is shown as the horizonal line (grey) on the scatter plot, at 2.5 PE MESF. The bottom plot displays the particle concentration as a function of brightness in each sample after gating for the PE+ subpopulation. The isotype control stain shows few fluorescent particles, as expected, confirming the immunostaining is specific to the target, CD63. False positives or background fluorescence arising from the dye itself is negligible, as evident by the lack of fluorescent detection events in the dye-only control.

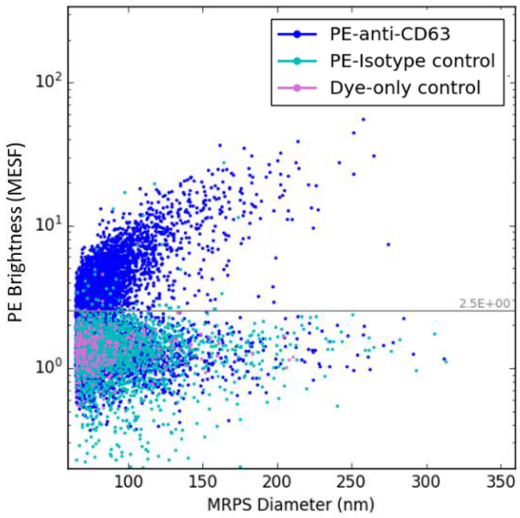


Figure 3

Scatter plot of the PE fluorescence intensity vs MRPS diameter for the particle detection events in the Vesi-Ref CD63 GFP EVs labeled with PE-anti-CD63 compared to PE-isotype labeled control and dye-only control.

Results (cont.):

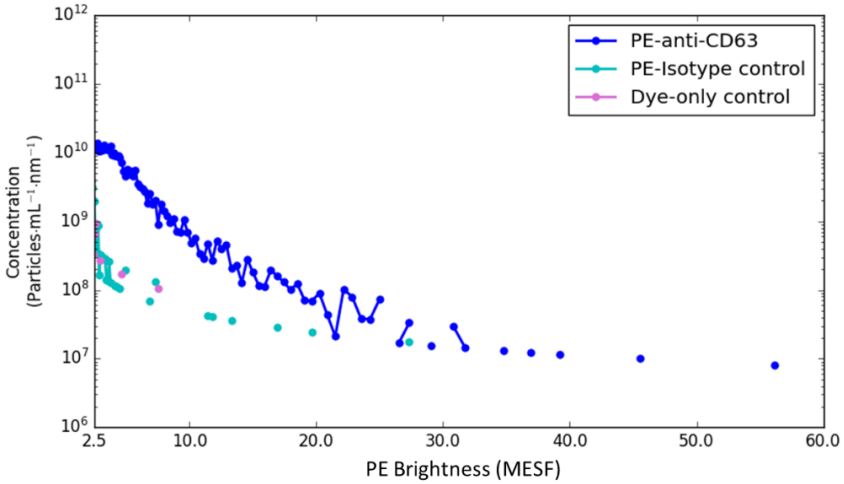


Figure 4

CSD (Concentration Spectral Density) plot of the Concentration vs PE Brightness (in MESF units) for the particle detection events in the Vesi-Ref CD63 GFP EVs labeled with PE-anti-CD63 compared to PE-isotype labeled control and dye-only control.

Discussion:

The ARC delivers accurate size and concentration of EVs and simultaneous quantitative subpopulation analysis with single-particle fluorescence. With up to three optical detection bands, the ARC is a powerful tool that enables the direct evaluation of the phenotype of EVs and their cargos. Common techniques such as immunostaining and co-localization of target biomarkers are easily transferrable from flow cytometry. Standard units are reported for each measurement (size in nanometers, concentration in particles/mL, fluorescence intensity in MESF/ERF units), facilitating data comparison across analytical systems and between researchers.