Understanding NTA and NanoFACS Reports

A Technical Guide for EV Analysis

1. NTA (Nanoparticle Tracking Analysis)

What is NTA?

NTA uses a ZetaView instrument that tracks individual particles moving in solution via Brownian motion. A 488nm laser illuminates the particles, and a high-speed camera records their movement. Particle size is calculated based on how fast they move - smaller particles move faster.

Size Statistics (X Values)

Metric	Example	Interpretation
D10	82.5 nm	10% of particles are smaller than this
D50 (Median)	127.5 nm	Half are smaller, half are larger
D90	217.5 nm	90% of particles are smaller than this
Span	1.1	(D90-D10)/D50 - polydispersity measure
Mean	143.8 nm	Average size (affected by outliers)
Mode	97.5 nm	Most frequently occurring size
StdDev	62 nm	Spread of sizes around the mean

Size Distribution Charts

Left Chart (Differential): Shows number of particles at each size bin. Peak indicates most abundant size. Peak width indicates heterogeneity.

Right Chart (Cumulative): Running total of particles up to each size. D50 is where curve reaches 50%. Steeper curve = more uniform population.

2. NanoFACS (Flow Cytometry)

What is NanoFACS?

A specialized high-resolution flow cytometer for nanoparticle detection. Each particle passes through laser beams, generating scatter (size/complexity) and fluorescence (marker) signals. Can detect particles as small as 50nm.

Channel Naming Convention

Channel	Full Name	Measures
VFSC-H	Violet Forward Scatter Height	Particle SIZE
VSSC1-H	Violet Side Scatter 1 Height	Internal COMPLEXITY
BSSC-H	Blue Side Scatter Height	Granularity
V447-H	Violet 447nm detector	Fluorescence marker
B531-H	Blue 531nm detector	FITC/GFP markers

Scatter Plot Interpretation

FSC vs SSC Plot: Forward Scatter (X-axis) indicates SIZE. Side Scatter (Y-axis) indicates COMPLEXITY. Tight cluster = homogeneous population. Multiple clusters = different subtypes.

Sample Types in Experiment

Sample	Purpose
PC3 EXO1.fcs	Main exosome preparation from PC3 cancer cells
Exo+CD81.fcs	EVs stained with anti-CD81 antibody (exosome marker)
Exo+CD9.fcs	EVs stained with anti-CD9 antibody (exosome marker)
+ISOTYPE.fcs	Negative control for non-specific binding

3. NTA vs NanoFACS: Complementary Techniques

Aspect	NTA (ZetaView)	NanoFACS
Principle	Brownian motion tracking	Light scattering + fluorescence
Size Range	10-1000nm	50-1000nm
Throughput	~1,000 particles	~1,000,000 particles
Markers	No (size only)	Yes (antibody staining)
Concentration	Absolute (particles/mL)	Relative (events)
Best For	Size distribution, concentration	Phenotyping, marker expression

4. Key Results for PC3 Exosome Samples

Metric	Result	Status
NTA D50	127.5 nm	✓ Typical exosome size
Span	1.1	✓ Uniform population
Traced particles	630	✓ Sufficient for statistics
FCS events	914,326	✓ High-quality acquisition
CD81/CD9 markers	Positive	✓ Confirmed exosome identity

Conclusions:

- **Size:** Particles are in expected exosome range (30-150nm)
- **Purity:** Low span indicates minimal contamination
- **Identity:** CD81/CD9 markers confirm exosome nature
- **Yield:** High particle counts from PC3 cell culture