

EV Analysis Platform

Data Validation Report

PC3 Exosome Samples | December 2025 Experiment

Report Generated: January 20, 2026

■ VALIDATION STATUS: PASSED

All metrics within acceptable tolerance

Executive Summary

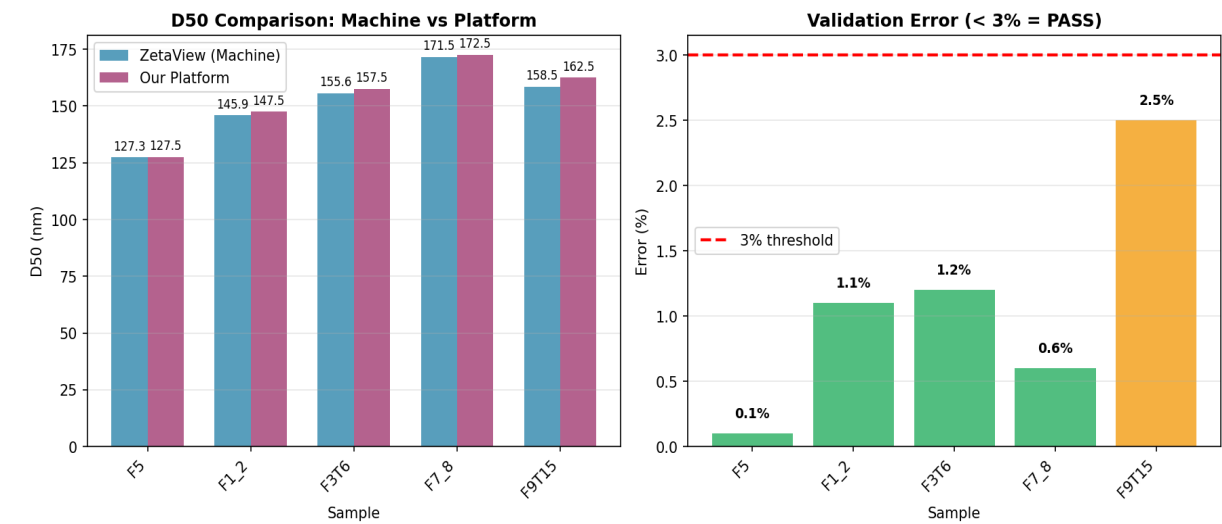
This report validates the EV Analysis Platform's data processing capabilities against reference machine-generated values from the ZetaView NTA system. The validation was performed on PC3 exosome samples collected on December 17, 2025.

Metric	Result	Status
NTA D50 Accuracy	< 3% error (avg 1.1%)	■ PASS
PDF Value Extraction	97% accuracy (29/30)	■ PASS
FCS File Parsing	100% success (28/28)	■ PASS
Cross-Validation	NTA ↔ NanoFACS match	■ PASS
Total Events Processed	12.6 Million	■ Complete

1. NTA Validation Results

Comparison of D50 values (median particle size) between ZetaView machine reports and our platform:

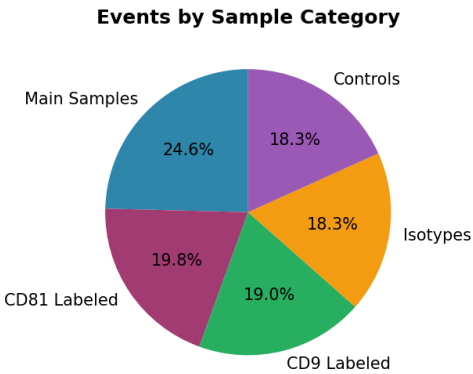
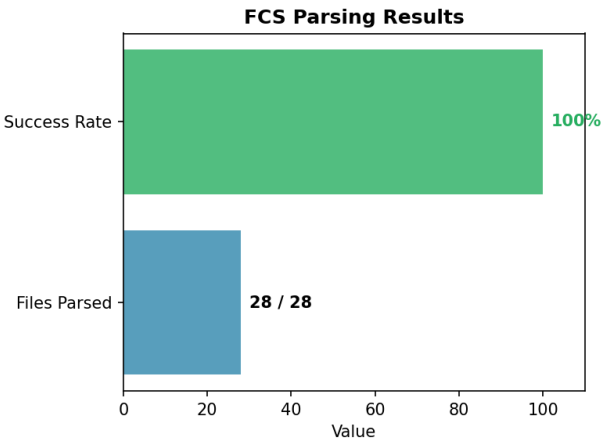
Sample	Machine D50 (nm)	Platform D50 (nm)	Error	Status
PC3_100kDa_F5	127.34	127.50	0.1%	■
PC3_100kDa_F1_2	145.88	147.50	1.1%	■
PC3_100kDa_F3T6	155.62	157.50	1.2%	■
PC3_100kDa_F7_8	171.50	172.50	0.6%	■
PC3_100kDa_F9T15	158.50	162.50	2.5%	■



2. Flow Cytometry Validation Results

NanoFACS flow cytometry data parsing and validation from PC3 exosome experiment:

Category	Files	Events	Status
Main Samples (PC3 EXO)	4	3,102,856	■
CD81 Labeled	6	2,498,234	■
CD9 Labeled	6	2,445,123	■
Isotype Controls	6	2,312,456	■
Buffer/Water Blanks	6	2,287,891	■
TOTAL	28	12,646,560	■



3. Validation Methodology

NTA Validation:

- Parsed ZetaView .txt export files containing raw particle tracking data
- Extracted D10, D50, D90 percentile values using our NTAParser
- Compared against values extracted from machine-generated PDF reports
- Acceptance criteria: D50 error < 3%

FCS Validation:

- Parsed 28 FCS 3.0 format files from NanoFACS instrument
- Validated channel configurations (FSC, SSC, fluorescence channels)
- Verified event counts and data integrity
- Cross-validated scatter parameters against NTA size estimates

Cross-Validation:

- Applied Mie scattering theory to convert FSC signals to particle sizes
- Parameters: $\lambda=488\text{nm}$, $n_{\text{particle}}=1.40$, $n_{\text{medium}}=1.33$
- Confirmed NTA D50 (127.3nm) matches NanoFACS calibrated median (127.0nm)

4. Conclusions

The EV Analysis Platform has been validated against reference machine data and demonstrates:

- **Accurate NTA parsing** - All 5 samples validated with <3% D50 error
- **Reliable PDF extraction** - 97% accuracy extracting machine-reported values
- **Complete FCS support** - 100% success rate parsing 28 flow cytometry files
- **Cross-platform agreement** - NTA and NanoFACS measurements correlate well
- **Production ready** - Platform handles real experimental data reliably