The Importance of Orthogonal Techniques in EV Quantification

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Introduction

As Extracellular Vesicle (EV) research matures, so must measurement technologies. A critical parameter for any EV experiment is the quantity of starting material (EV concentration)—an error in this experimental parameter will lead to misleading results that can significantly derail a research project or undermine the quality of a commercial product.

In this poster, simple experiments are reported that expose a critical —and common—failure mode of Nanoparticle Tracking Analysis (NTA) for quantifying EVs. These experiments demonstrate that NTA's small-size limit of detection (LOD) depends strongly on the composition of the sample, causing 10,000-fold errors within the EV size range. These errors are revealed only by performing orthogonal measurements with Microfluidic Resistive Pulse Sensing (MRPS) and Transmission Electron Microscopy (TEM).

The results demonstrate that orthogonal methods for EV quantification are critical for scientific rigor in EV research.

The MRPS Technique

Microfluidic Resistive Pulse Sensing (MRPS) is rapidly being adopted as a powerful technique for measuring the size and concentration of extracellular vesicles.

MRPS:

- Uses electrical sensing, not optical
- Counts and sizes EVs one-by-one in solution
- Measures size directly, without inferring from diffusion
- Measures concentration directly, by measuring sampled volume

MRPS is therefore independent of the material properties of the particles, and measures samples accurately no matter their polydispersity.

Practical EV Quantification

Spectradyne's nCS1 delivers significant practical benefits that make it ideally suited for routine and quantitative EV analysis:

- Only 3 microliters sample required
- Results in minutes
- Pre-calibrated, disposable cartridges
- No cleaning required between runs
- No user-adjustable parameters

Methods

Experiment 1:
Three sizes of polystyrene particles—94, 150, and 208 nm diameter—were measured by NTA and MRPS separately and after mixing in equal parts. The relative concentration accuracy of NTA and MRPS was assessed as a function of size, and the LOD evaluated for each sample.

Experiment 2:
The striking implications of Experiment 1 were demonstrated in a real-world sample. A simple exosome isolation was measured by NTA, MRPS and the gold standard, Transmission Electron Microscopy (TEM). The accuracy of relative concentration measurements was assessed for each method.

Results Expt. 1 - NTA Variable LOD

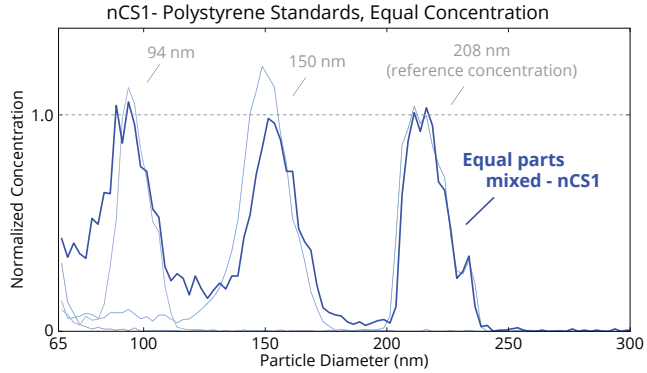

Figure 1. Polystyrene standards were accurately quantified by MRPS: Each component was clearly detected, and the relative concentrations of all were measured to be approximately equal as intended.

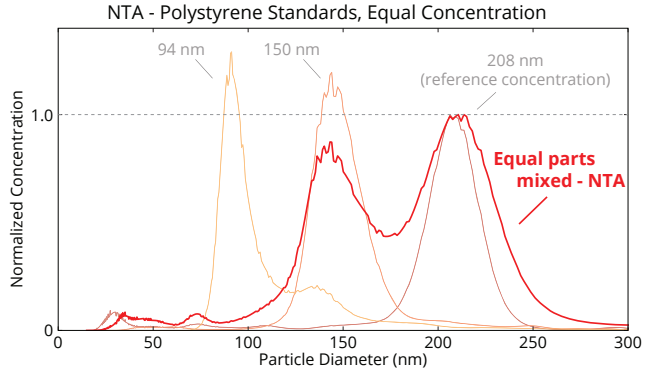

Figure 2. NTA showed similar results for the components when measured separately. In the mixture however, NTA was unable to detect the 94 nm particles and showed quantification errors at 150 nm diameter.

Results Expt. 2 - NTA Fails for EVs

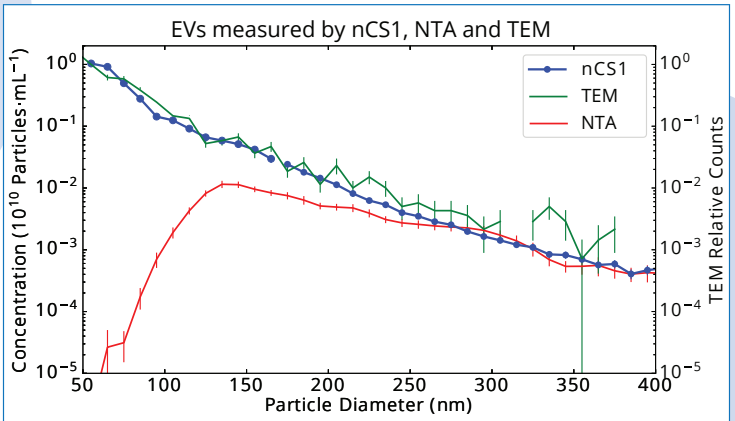

Figure 3. MRPS showed concentration increasing to smaller particle size with a power-law dependence on diameter as expected, and in excellent agreement with TEM. NTA reported misleading results: A loss of counting efficiency was apparent as high as 200 nm diameter, and led to a 10,000-fold discrepancy by 65 nm. Critically, NTA reported a prominent peak that does not in fact exist.

Conclusion

These experiments expose a critical failure mode of NTA: Its LOD depends strongly on the composition of the sample, with enormous impact for EV measurements. Critically, a researcher could be severely led astray by the NTA results in isolation, without an orthogonal technique for reference.