Where's My Peak? Separating Truth from Fiction in Measurements of Nanoparticles

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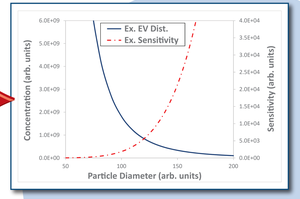
Spectradyne® Particle Analysis

Problem Statement

Regardless of an instrument's underlying principle of operation, all nanoparticle analysis instruments (and, indeed, instruments generally) are eventually limited by their intrinsic sensitivity and by instrument noise. This poster attempts to clarify relevant considerations in the detection of submicron particles near an instrument's limit of detection, providing a guide for researchers facing this kind of small-signal challenge.

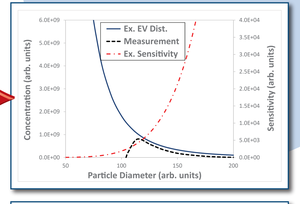
The figure at right shows a common situation in nanoparticle analysis:

- As particle diameter, *d*, increases, concentration (blue) decreases rapidly. In this case, ~ $d^{-4.2}$, as found in EV samples.
- As particle diameter increases, sensitivity (red) increases rapidly, ~ d^6, as in light scattering.



At some point as the particle size decreases, the instrument no longer reliably detects particles.

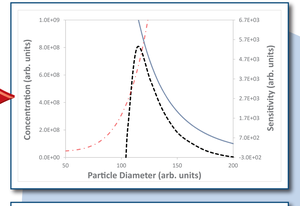
The black curve in the figure at right shows a hypothetical measurement result. Note that all axis scales are arbitrary. The detection threshold for state of the art instrumentation typically lies somewhere below 200 nm.



Zooming in on the measurement result, the potential for drawing a misleading conclusion is clear.

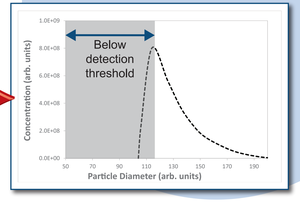
While the measurement indicates an absence of particles below 100 nm, in reality there is an increasing concentration of particles, but the instrument cannot detect them.

The peak is false information!



A more accurate way of representing the data is to exclude information below the detection threshold, represented at right by the grey shaded region.

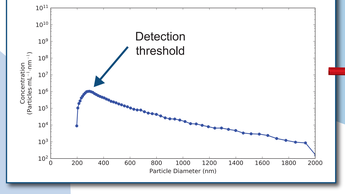
In this hypothetical example, we can only conclude that there is a rapidly increasing concentration of particles going down to 110 nm. We don't really know what happens below that limit.



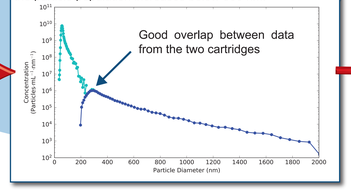
Example 1: Extracellular Vesicle (EV) Measurement with the nCS1

The data shown below was collected on Spectradyne's nCS1 instrument using two different cartridges, with two different measurement ranges. The data emipirically demonstrates the impact of sensitivity limitations on detection threshold.

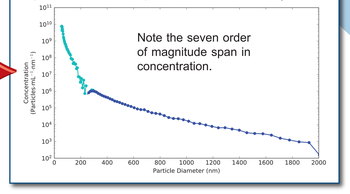
Measurement with a TS-2000 cartridge, with a max size of 2 microns and a min detection limit of 250 to 300 nm. We can't be confident in the data shown below the detection limit.



Data from the TS-400 cartridge (light blue), with a max size of 400 nm and a min detection limit of 65 nm, shows that indeed the particle population increases below 300 nm.



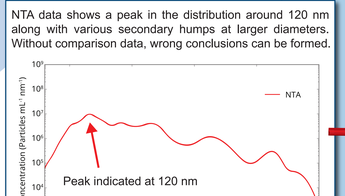
The figure below shows the measurement after excluding data below the detection limit of each cartridge. This avoids the presentation of false peaks, which is misleading.



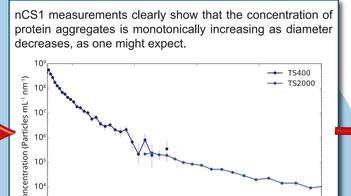
Example 2: Protein Aggregation Measurement with the nCS1 and NTA

Sensitivity differences between instruments result in different detection thresholds, as is clear in these protein aggregation measurements.

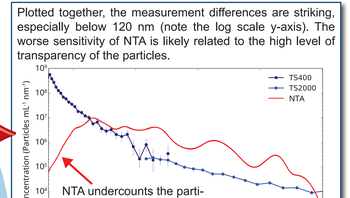
NTA data shows a peak in the distribution around 120 nm along with various secondary humps at larger diameters. Without comparison data, wrong conclusions can be formed.



nCS1 measurements clearly show that the concentration of protein aggregates is monotonically increasing as diameter decreases, as one might expect.



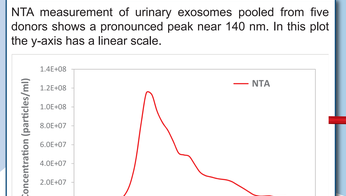
Plotted together, the measurement differences are striking, especially below 120 nm (note the log scale y-axis). The worse sensitivity of NTA is likely related to the high level of transparency of the particles.



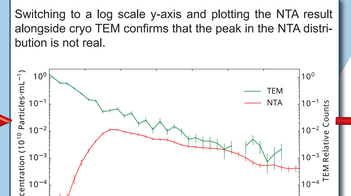
Example 3: Exosome Quantification with the nCS1, NTA, and Cryo TEM

A three-way comparison of an exosome measurement illustrates the NTA false peak issue clearly. Cryo TEM and nCS1 agree closely.

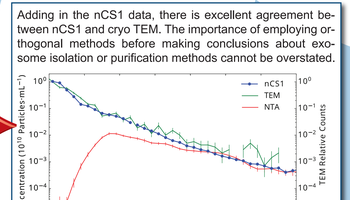
NTA measurement of urinary exosomes pooled from five donors shows a pronounced peak near 140 nm. In this plot the y-axis has a linear scale.



Switching to a log scale y-axis and plotting the NTA result alongside cryo TEM confirms that the peak in the NTA distribution is not real.

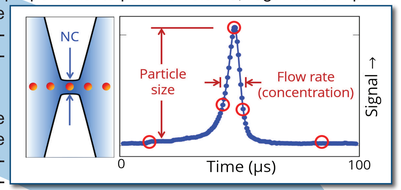


Adding in the nCS1 data, there is excellent agreement between nCS1 and cryo TEM. The importance of employing orthogonal methods before making conclusions about exosome isolation or purification methods cannot be overstated.



Overview of Spectradyne's Technology

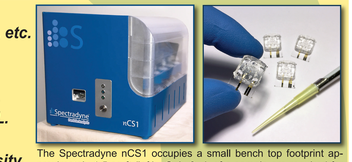
MRPS is a state-of-the-art microfluidic implementation of Resistive Pulse Sensing, aka the Coulter Principle. In RPS, the electrical resistance of a conducting fluid is monitored as particles flow through a constriction (see illustration below), thereby blocking the flow of ions and temporarily increasing the resistance. Maximum resistance modulation is obtained when the particle is midway through the constriction. The size of the resistance spike is proportional to particle volume, regardless of particle material, and the transit time gives the fluid flow rate, which enables absolute concentration measurements.



There is no dependence on the index of refraction of the particle and, because particles are detected and sized individually, high resolution measurements are obtained. Spectradyne's proprietary MRPS technology utilizes disposable cartridges to greatly improve ease-of-use and reduce measurement time compared to other nanoparticle implementations of RPS.

Key Features of the nCS1 and MRPS:

- ***A truly orthogonal method to DLS, NTA, etc.***
- ***Sizing range: 50 nm - 10 µm diameter.***
- ***Peak sizing resolution of 3% or better.***
- ***Absolute concentration measurements.***
- ***Concentration range: 10^5 to 10^{12} NPs/mL.***
- ***All particle materials.***
- ***Measurements unbiased by polydispersity.***
- ***Total sample analysis in minutes.***



The Spectradyne nCS1 occupies a small bench top footprint approximately 1.5 sq ft (left). Only 3 µL of a sample are required for analysis using a disposable microfluidic cartridge (right), which prevents contamination between measurements and eliminates cleaning requirements.

Recommendations

As with any measurement, researchers must understand instrumentation limitations prior to drawing conclusions about nanoparticle distributions. Detection thresholds, which may vary by particle type (for light scattering-based instruments, at least) need to be understood and any peaks in the distribution near the threshold should be greeted with skepticism until orthogonal methods can be employed. Failing that, dilution series can be run to confirm that detection events scale with anticipated concentration changes. In addition, checks in data analysis procedures should be in place to screen out false positives. Although taking care to confirm that peaks are real requires time and money, this investment is far less expensive than the consequences of drawing erroneous conclusions about one's formulations, which can lead to poor therapeutic and diagnostic outcomes.

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