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High Resolution Size And Concentration Analysis of Polydisperse Nanoparticle Mixtures

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Spectradyne®
Particle Analysis

Introduction

The proliferation of nanomaterials has outpaced industry's ability to measure their properties accurately and efficiently. The unprecedented capabilities of **Spectradyne's nCS1** close this gap:

- ***Individual particle measurements: no averaging.***
- ***Sizing range: 30 nm - 2 μm diameter.***
- ***Fast count rate: 10,000 particles/second.***
- ***Absolute concentration measurements.***
- ***All particle compositions:***
  e.g., conducting/insulating, transparent/opaque.
- ***Any level of polydispersity.***

The nCS1's novel implementation of resistive pulse sensing was patented by two of Spectradyne's founders and published in *Nature Nanotechnology* (cover article, May 2011).

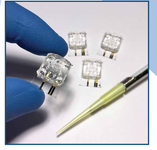
Fraikin, J-L., Teesalu, T., McKenney, C. M., Ruoslahti, E., Cleland, A. N. "A high-throughput label-free nanoparticle analyser," *Nature Nanotechnology* **6**, 308-313 (2011).

Purpose

Spectradyne's nCS1 is a new platform for characterizing submicron particles in liquid that meets a critical need felt by a broad set of industries for improved nanoparticle metrology. The instrument provides high resolution size and concentration analysis of particles in the 30 nm – 2.0 μm size range without the drawbacks of existing light scattering techniques. In this poster Spectradyne demonstrates the unprecedented capabilities of the nCS1 by fully characterizing a mixture of synthetic nanoparticles having high polydispersity, and by measuring the size distribution of biological nanoparticles in serum, a highly complex biological matrix.

Methods

Measurements were obtained with a proprietary nanofluidic cartridge employing resistive pulse sensing to measure individual nanoparticles. Typical concentration ranges were in the range of 10^8 to 10^{12} analyte particles per mL, mixed with standard polystyrene beads in saline, and measurement rates were in the range of ~3,000 particles per second. The nanofluidic cartridge is disposable and interfaces to Spectradyne's nCS1 benchtop instrument, which employs automated fluidics and electronics. The presence of the beads allows for direct calibration of nanoparticle concentration and size.



The Spectradyne nCS1 occupies a small bench top footprint approximately 1.5 sq ft (far left). Custom software running on a laptop computer automates the operation of the instrument. Each sample is analyzed using a disposable microfluidic cartridge (right), which prevents contamination between measurements and eliminates cleaning requirements. The cartridge includes a highly engineered microfluidic network that ensures repeatable, calibration-free measurements. On-board filtration in the cartridge eliminates the need for pre-filtering of the sample. The raw sample, of which only 3 uL is required, is loaded into the cartridge by pipette and the cartridge is loaded into the instrument. Subsequent sample analysis in the cartridge is automated, carried out by the click of a button in the control software. Measurement results are obtained using Spectradyne's data analysis software.

Results

Polydisperse Particle Mixtures.
The Spectradyne nCS1 delivers high-resolution analysis of complex particle mixtures. Analysis of a four-component mixture is shown in **Figure 1.** Polystyrene nanoparticles having NIST-certified mean diameters **63 nm, 94 nm, 122 nm and 150 nm** were mixed to a final concentration of $5x10^{10}$ particles/mL each and the mixture was analyzed in the nCS1. The nCS1 accurately measures each component of the mixture to within 1-2 nm of the NIST-certified values. The peak widths in the histogram reflect the true distribution of particles in each sub-population: CV measurements agree with manufacturer estimates.

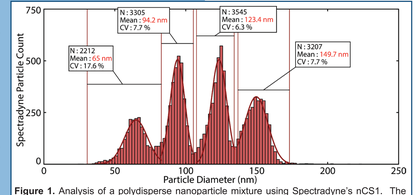


Figure 1. Analysis of a polydisperse nanoparticle mixture using Spectradyne's nCS1. The technology's high resolution enables the four components of the mixture to be fully characterized: measured mean diameters **63 nm, 94 nm, 122 nm and 150 nm** agree with the NIST certified values to within 1-2 nm. Histrogram data was collected over **20 s**, and required only **3 uL of sample**. No other commercially available technology is capable of such a measurement.

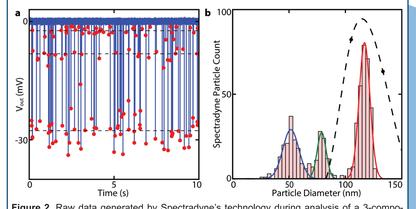


Figure 2. Raw data generated by Spectradyne's technology during analysis of a 3-component, polydisperse mixture is shown in (a). Each voltage peak is highlighted with a red dot and indicates the passage of a single nanoparticle through the sensing constriction. The size of each peak corresponds to the size of the particle. The distribution of particle sizes corresponding to the raw data in panel (a) is shown in panel (b). Three clear peaks are resolved by Spectradyne's technology, corresponding to the three particle sizes in the mixture: 51 nm, 75 nm, and 117 nm diameter. Results of a dynamic light scattering (DLS) analysis of the mixture are shown by the dashed black line, which misrepresents the true compoosition of the sample.

Comparison to Dynamic Light Scattering.
Figure 2 shows example raw data output (left) and a comparison between the Spectradyne instrument and DLS measurements (right). The three-component population, with beads of diameter **51 nm, 75 nm, and 117 nm** is clearly represented by the trimodal histogram generated by the Spectradyne nCS1. Data from a DLS measurement on the same three-component mixture is given by the dashed black line. DLS is unable to detect the smaller particles.

Biological Nanoparticles in Plasma.
Figure 3 shows data taken on mouse blood plasma without (blue) and with (red) the addition of T7 bacteriophage. The signal centered at 117 nm is from calibration polystyrene beads. Spectradyne's technology clearly detects **bacteriophage, approximately 55 nm diameter** in the spiked sample, and characterizes the distribution of naturally occuring nanoparticles in the plasma. The plasma was measured directly in the instrument, without modification to the samples.

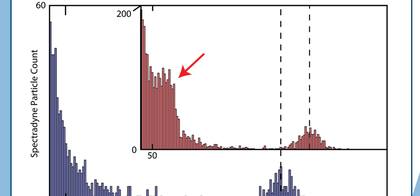


Figure 3. Analysis of the nanoparticle content in pure mouse plasma (blue histogram) and in mouse plasma spiked with bacteriophage (red histogram). The unlabeled bacteriophage are clearly detected, as well as a wealth of plasma nanoparticles that may serve as useful indicators of health and disease. Particles 117 nm in diameter were added to each sample to serve as positive controls for the measurement.

Application Areas

1. Pharma/Biopharma. Unwanted aggregation during drug formulation is a critical problem because it reduces a drug's bioavailability and can lead to severe immunogenic reactions. Detection of aggregate particles is therefore a critical part of the drug development process but has been limited by a lack of practical, high-resolution metrology for sizing particles in the sub-micron size range.

2. Nanomedicine and Drug Delivery. Nanoparticles that target specific tissues are able to deliver drug payloads more effectively in the body and produce better patient outcomes than conventional drugs. The physical characteristics of these new particles, in particular their size, are critical to their performance as delivery vehicles. Spectradyne's NPA technology will enable new characterization abilities for nanoparticles that do not currently exist in the market.

3. Cosmetics and Food. Certain cosmetics and food products contain nanoparticles as additives (e.g. TiO2 particles in sunscreen), and the quality control of these particles is critical to the safety and performance of the end products. Spectradyne's nCS1 platform will provide an important method for characterizing these materials additives.

4. Health Research. Particles in the sub-micron size range play natural and important roles in various physiological processes including intercellular signaling and regulation of mineralization processes. As a result, these particles have the potential to serve as significant biomarkers of human health and disease. However, the lack of characterization tools for particles in this size range—especially for polydisperse samples—has significantly limited research in these areas. The Spectradyne nCS1 has demonstrated measurements of nanoparticles in blood plasma with minimal sample prep—an important step in delivering a useful tool for medical research.

5. Safety and Regulation. Ensuring the safety of nanomaterial-containing products requires appropriate standards and regulations. As a result of the current lack of effective particle sizing technology, regulation of nanoparticles lags far behind their deployment. Spectradyne's innovative product design will enable better controlled, and therefore safer, implementation of nanotechnology.

Conclusions

Spectradyne's nCS1 has demonstrated the ability to resolve arbitrary levels of polydispersity in deep sub-micron particle populations. Estimated size resolution is ±3% and ultimate size sensitivity is in the range of 30 nm. Applications include improved detection of small shifts in aggregation levels, process control for synthetic nanoparticle production, and rapid detection of nanoparticle biomarkers in blood.

NSF & NIH SBIR Phase I Awardee

NIH National Institute of Biomedical Imaging and Bioengineering

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TechConnect National Innovation Award