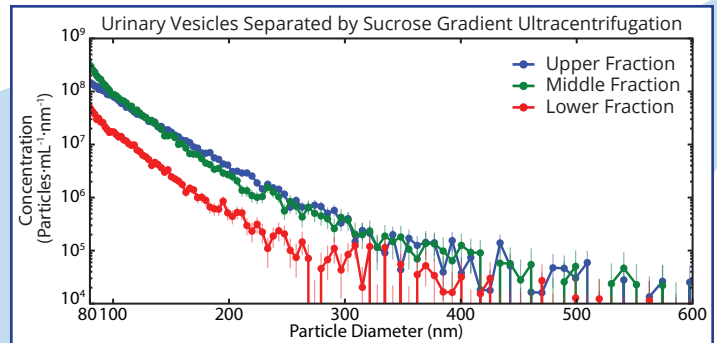![Spectradyne® Particle Analysis]

Analysis of Extracellular Vesicles Using the nCS1™

Adapted from Fraikin et al., "Size and concentration determination of extracellular vesicles as small as 50 nm in diameter at a rate beyond 10,000 EV/s", *Proceedings of ISEV 2017*

Extracellular vesicles (EVs) hold great promise as biomarkers, as novel therapeutics and, generally, as a source for new understanding of biological processes. Their physical characterization, however, has until now presented two significant challenges: Both the size and concentration of EVs span multiple orders of magnitude, and they scatter light weakly, making accurate characterization by optical techniques difficult. Spectradyne's nCS1 instrument, based on microfluidic resistive pulse sensing (MRPS)[1], delivers practical solutions to both challenges in a fast and robust analysis platform.

Fraction Analysis. Urinary exosomes were separated using sucrose gradient ultracentrifugation[2], and three fractions were analyzed with the nCS1. Results are shown in the figure at right. The nCS1 measurements revealed subtle differences in the size distribution of EVs in the fractions, and importantly, show that each fraction comprises EVs spanning more than 400 nm in diameter, and 4 orders of magnitude in concentration. Characterization of these samples in such fine detail would not be possible using optical techniques due to the low refractive index contrast of the samples.
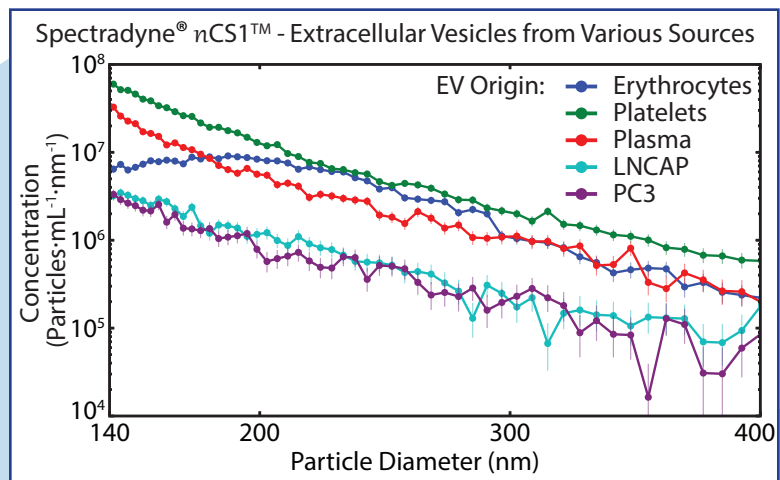

Robustness & Repeatability. Particle characterization techniques based on light scattering lack consistency when used for EV measurements such as those above. In contrast, the nCS1 instrument demonstrates very high fidelity: When triplicate measurements were performed in separate cartridges of the EV size distribution in cell culture supernatant, variability was < 3% in concentration in the size range of 250-2000 nm (see table at right). Importantly, no sample preparation of any kind was required before measurement.

Run	Measured Concentration
1	5.44×10^7 particles/mL
2	5.25×10^7 particles/mL
3	5.09×10^7 particles/mL

Distinguishing EVs of various origin. The high resolution of the nCS1 enables quantitative differentiation of EVs from different sources. The figure on the right demonstrates this application for 5 different EV samples, with the concentration values shown below over the range of 140-400nm in diameter[3].

Sample	Measured Concentration
#4 Erythrocyte	$(9.7 \pm 0.1) \times 10^9$ particles/mL
#5 Platelet	$(2.44 \pm 0.02) \times 10^{10}$ particles/mL
#6 Plasma	$(1.09 \pm 0.01) \times 10^{10}$ particles/mL
#7 LNCAP	$(1.98 \pm 0.05) \times 10^9$ particles/mL
#8 PC3	$(1.58 \pm 0.05) \times 10^9$ particles/mL

The Spectradyne nCS1 is a practical tool for high-fidelity EV characterization: Typical measurement time is ~3 min/sample, and the system delivers resolution and accuracy that are superior to other metrologies. Disposable microfluidic cartridges require only 3μL of sample per measurement, eliminate cleaning between samples, and provide engineered features to reduce or eliminate blockages of the sensing constriction. For more information or to arrange a demonstration of the technology, please visit our website, http://nanoparticleanalyzer.com, or contact us by email.

1.) Fraikin et al., Nature Nanotechnology 6, 308-313 (2011)
2.) Chen et al., Methods Enzymol. 524, 225-241 (2013)
3.) Van der Pol et al., J. Thromb. Haemost. 12 1182-1192 (2014)