Uncertainty in exosome concentration measurement by nanoparticle tracking analysis (NTA)

Jeff Bodycomb, Ph.D. and Julie Chen Nguyen
HORIBA Scientific, Irvine, CA

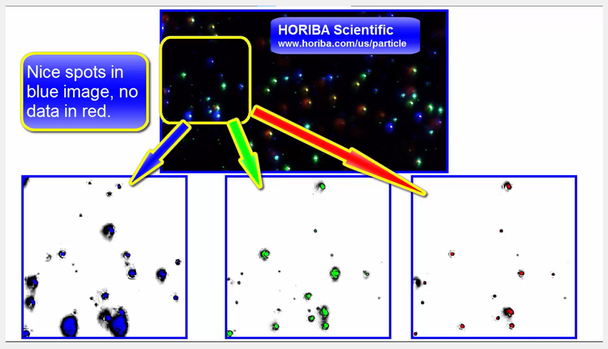
ABSTRACT

Repeated measurements can be used to estimate concentration measurement uncertainty for stable dilutions of EV's. The observed coefficient of variation, 4.3% is close to but somewhat higher than the expected from the number of particles counted and Poisson statistics. The predicted value is 1/sqrt(1640) or 2.5%. Repeatability for the concentration in each size class roughly followed Poisson statistics. This indicates that repeatability improves in proportion to the square root of measurement time.

INTRODUCTION

EV concentration measurement results are routinely reported. Thus, measurement uncertainty and its control is important. Experimental replicates will show variation. This variation has multiple components such as variation due to differences between animals and uncertainty of the final measurement (e.g., NTA). Understanding final measurement uncertainty improves interpretation of experimental data. In addition, understanding how to control uncertainty can guide experimental design by clarifying the trade-off between effort and uncertainty.

METHODS

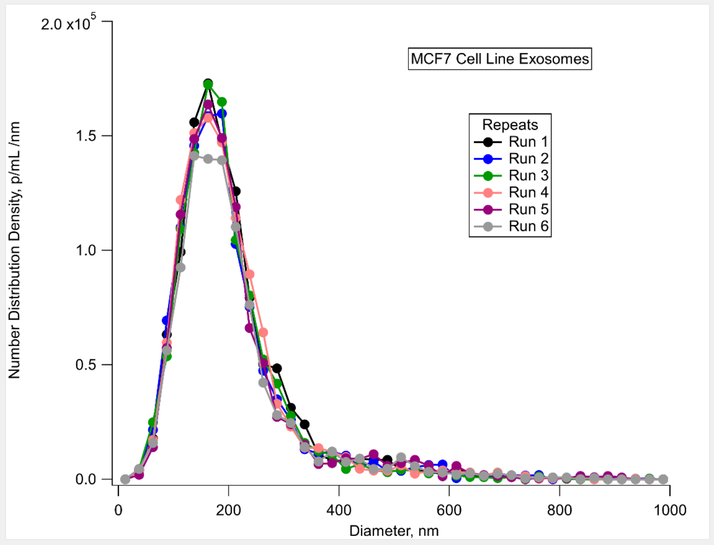


Lyophilized exosomes were obtained from Abcam (Lyophilized MCF7 Exosome Standards, lot GR3401014-1). These are derived from MCF7, a human cancer cell line. Exosomes were reconstituted following manufacturer instructions and diluted in Dulbeccos phosphate buffered saline (dPBS).

Nanoparticle tracking analysis, NTA, was performed with a HORIBA ViewSizer 3000 multi-laser NTA instrument. Multiple lasers were used to ensure analysis of all EV sizes in the sample. Laser power settings were 210 mW blue, 12 mW green, 8 mW red and camera gain was 30 dB.

RESULTS

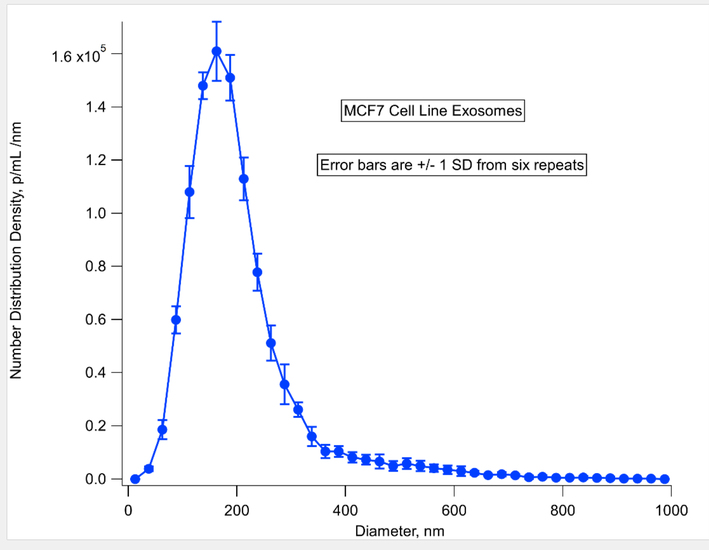
A single aliquot of diluted exosomes was measured six times in succession. The obtained size distributions (concentration as a function of size) are shown below.



Overall concentration results are tabulated below. The overall average measured particle concentration was 2.63e7 p/mL with a standard deviation of 1.13e6 p/mL. This reflects a coefficient of variation, COV, of 4.31%. In these measurements, an average of 1640 particles were observed in each measurement. Note that the COV is reduced to 2.03% if only the first five measurements are analyzed.

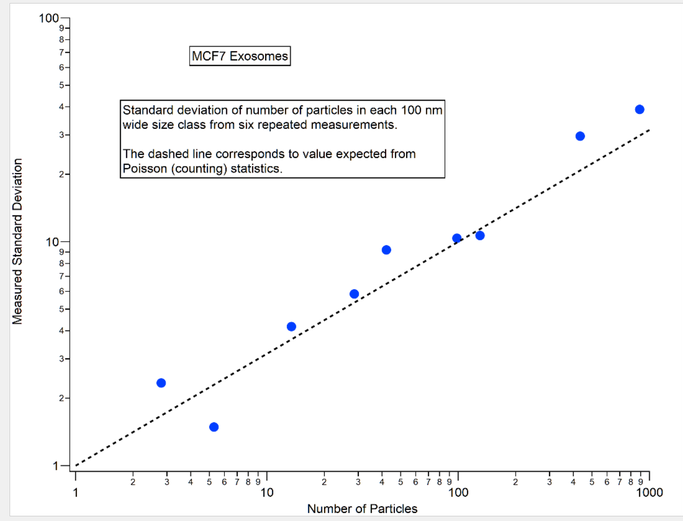
Run	Dn50, nm	DnAvg, nm	DnSD, nm	Measured Concentration, p/mL	Particles
1	179.8	162.1	45.1	2.75E+07	1718
2	178.3	159.5	46.0	2.64E+07	1652
3	178.1	161.6	44.9	2.64E+07	1653
4	178.9	161.2	46.3	2.70E+07	1685
5	178.3	160.7	43.7	2.61E+07	1632
6	179.4	162.2	45.8	2.42E+07	1511
Avg.	178.8	161.2	45.3	2.63E+07	1642
Std. Dev.	0.68	1.03	0.94	1.13E+06	71
CoV	0.38%	0.64%	2.08%	4.32%	4.32%

Since there were repeated runs, the observed standard deviation for each size class can be calculated and used to add error bars to the overall average for each size class. This is shown in the plot below.



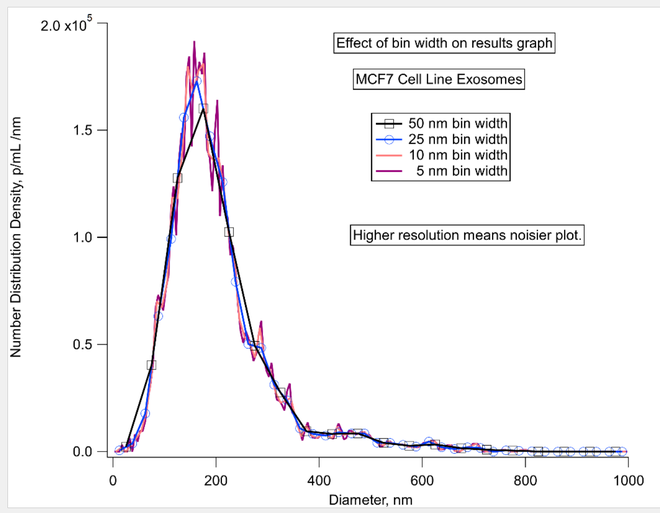
RESULTS (cont.)

Uncertainty as a function of the number of particles in each size class can be plotted and compared to the theoretical value for Poisson (counting) statistics.



As seen in the plot, as the number of particles in a size class increases, the uncertainty increases more slowly. That is, the relative uncertainty decreases.

The effect of number of particles on uncertainty is also illustrated by plotting the same data set with different bin widths. As the bins become narrower, the plot becomes noisier.



CONCLUSIONS

Repeated measurements can be used to estimate measurement uncertainty for stable dilutions of EV's. The observed coefficient of variation, 4.3% is close to but somewhat higher than the expected from the number of particles counted and Poisson statistics. The predicted value is 1/sqrt(1640) or 2.5%. Repeatability for the concentration in each size class roughly followed Poisson statistics. Since the number of particles increases with measurement time, this indicates that repeatability improves in proportion to the square root of measurement time.