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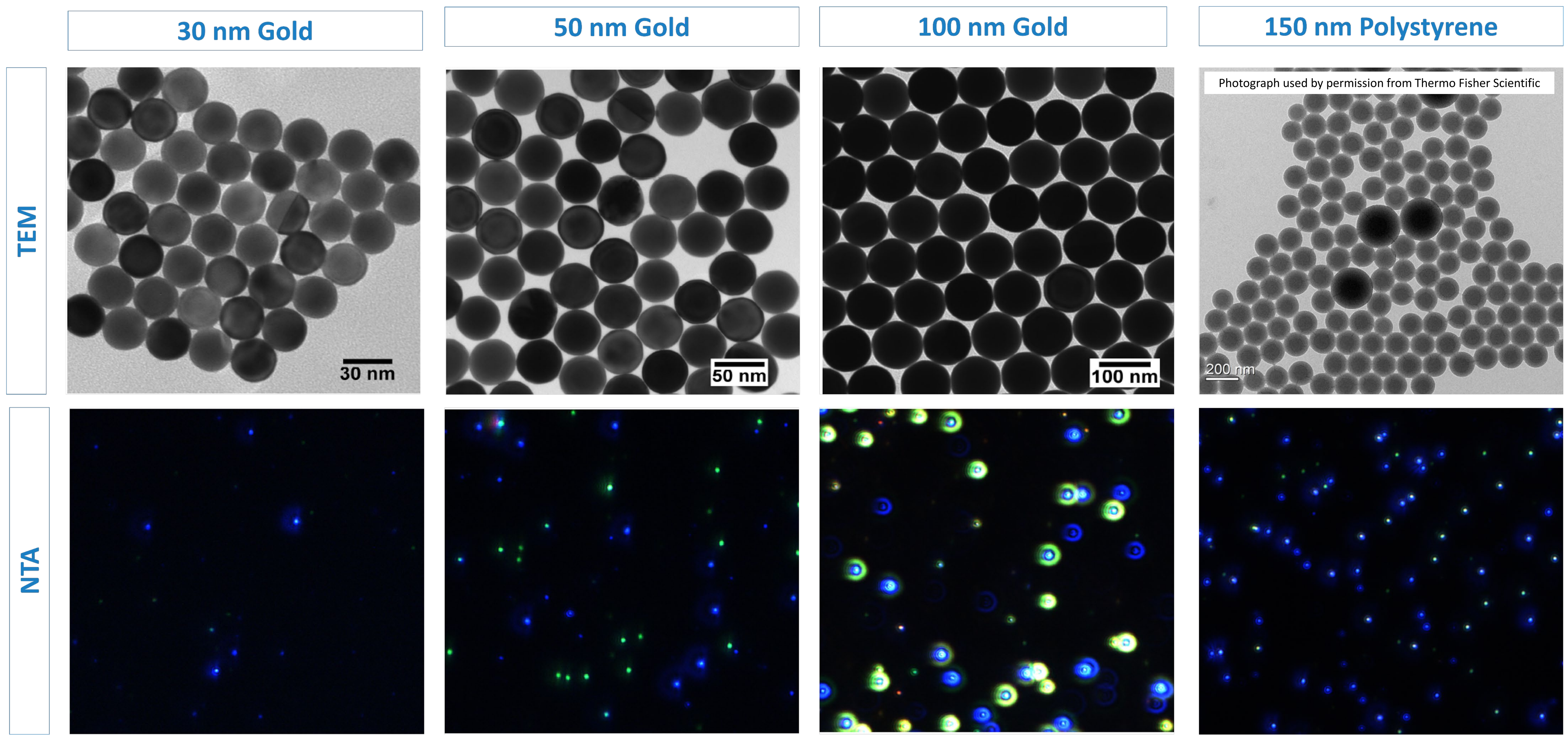
# Orthogonal Measurement of Number Concentration Standards for NTA Calibration

Jeffrey Bodycomb, PhD, Julie Y. Chen, HORIBA | Irvine, CA 92618, USA. **Contact Information**: Julie.Chen-EX@horiba.com

## INTRODUCTION

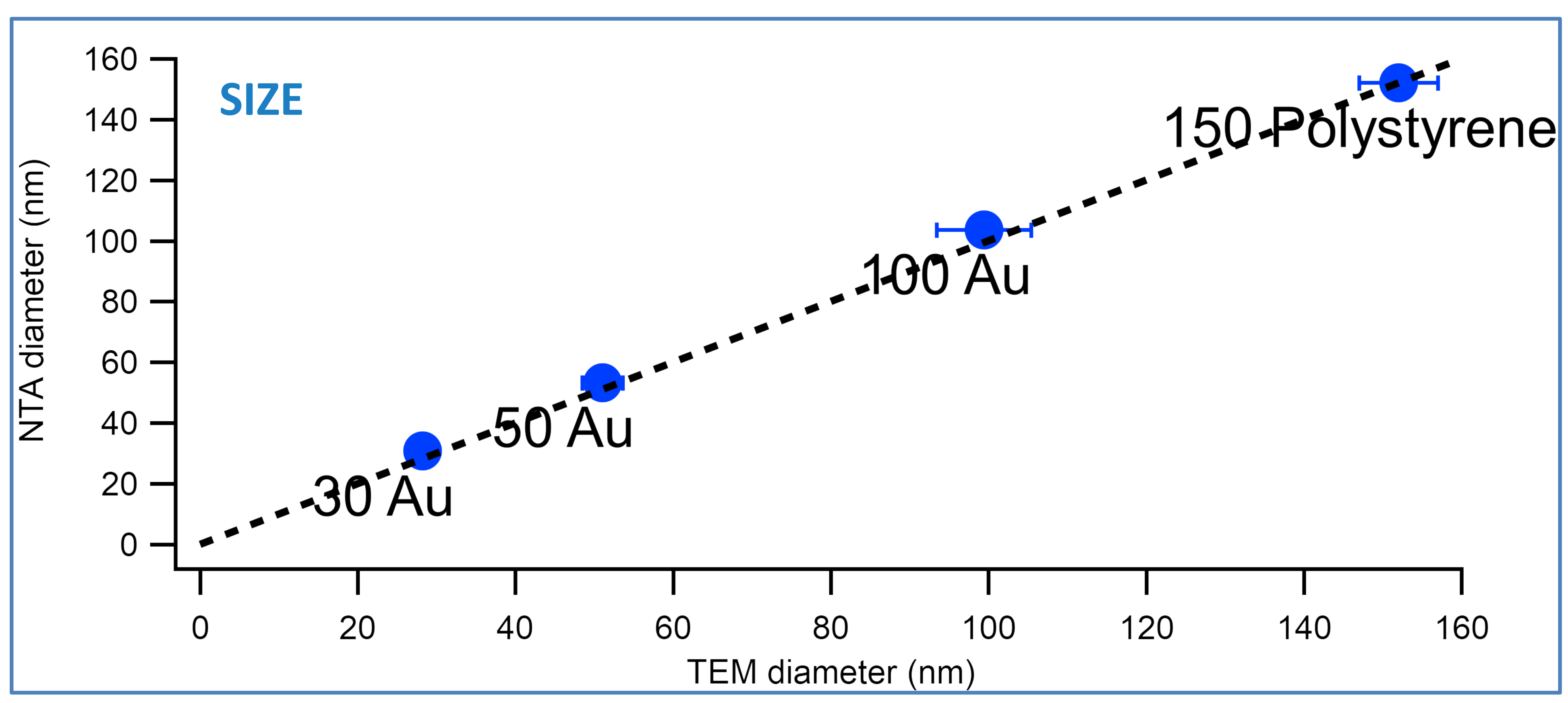
Instrument calibration is one of the standardization requirements in EV (biomarker) research. The process involves comparing the measurement values of an instrument with those expected from a known standard to determine measurement accuracy. Latex standards are commonly used for instrument calibration and have number particle concentrations derived from the stated fraction (1%) solids. This value is not reported as carefully as, for example, particle size. This implies a rather large uncertainty in the calibration standard which is propagated into NTA measurements. In this study, size and number concentration standards are analyzed using orthogonal techniques and subsequently incorporated for NTA calibration. The use of orthogonal techniques will significantly reduce concentration measurement uncertainties.

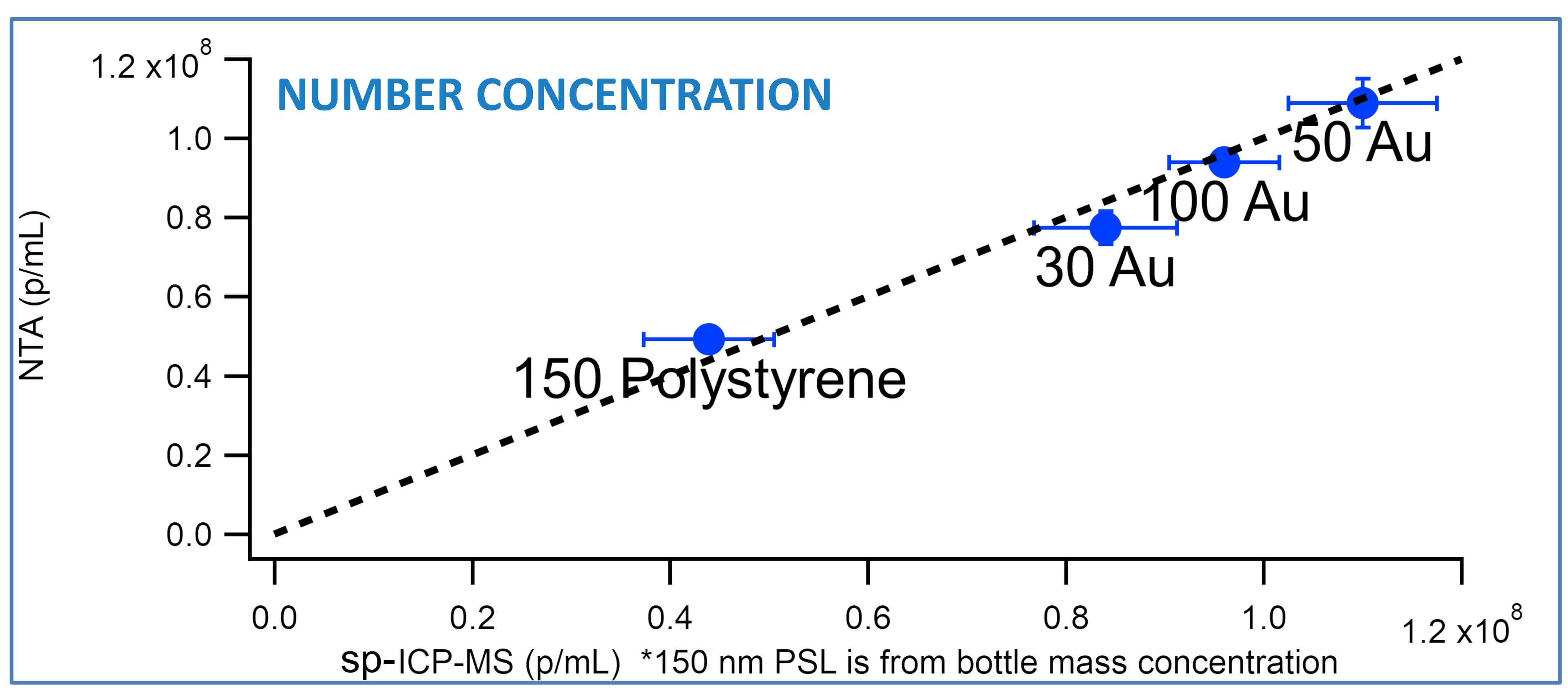



## METHODS

150 nm NIST traceable polystyrene latex size standard (Thermo Fisher Catalog Number 3150A) and 30 nm, 50 nm, and 100 nm diameter Ultra Uniform™ Gold Nanospheres Number Standards (nanoComposix) were obtained for this study. These standards were analyzed in triplicates using the **ViewSizer® 3000 simultaneous multi-laser NTA** from **HORIBA**. Multiple lasers with individually adjusted laser power settings and camera gains were carefully selected for each sample. Gold nanoparticle NTA size results were verified using JEOL 1010 Transmission Electron Microscopy (**TEM**); concentration (number standards only) were verified using Agilent 7800 single particle Inductively Coupled Plasma Mass Spectroscopy (**sp-ICP-MS**).

To facilitate comparison with other orthogonal techniques, the average of the median diameters reported from each NTA measurement is used. Diameter distributions will be reported separately.




## RESULTS

NTA size and concentration from four standards were obtained and plotted as a direct comparison to TEM and sp-ICP-MS reporting. Percent Coefficient of Variation (CoV %) was used to assess differences and the results of each technique were compared. NTA results after calibration by latex standards were similar to NTA concentration measurement of gold nanoparticles.

| | 30 nm Gold | | 50 nm Gold | | 100 nm Gold | | 150 nm Polystyrene | |
|---|---|---|---|---|---|---|---|---|
| | Median Diameter (nm) | Conc. (p/mL) | Median Diameter (nm) | Conc. (p/mL) | Median Diameter (nm) | Conc. (p/mL) | Median Diameter (nm) | Conc. (p/mL) |
| NTA | 30.80 ± 0.71 | 7.74E+7 ± 4.16E+6 | 53.41 ± 0.70 | 1.09E+8 ± 6.24E+6 | 103.76 ± 0.18 | 9.40E+7 ± 2.77E+6 | 152.14 ± 0.61 | 5.81E+12 ± 1.52E+11 |
| TEM | 28.2 ± 1.41 | NA | 51.0 ± 2.55 | NA | 99.4 ± 5.96 | NA | 152 ± 5.02 | NA |
| sp-ICP-MS | NA | 8.40E+7 ± 7.22E+6 | NA | 1.10E+8 ± 7.48E+6 | NA | 9.60E+7 ± 5.57E+6 | NA | 5.20E+12 (calculated) ± 7.80E+11 |

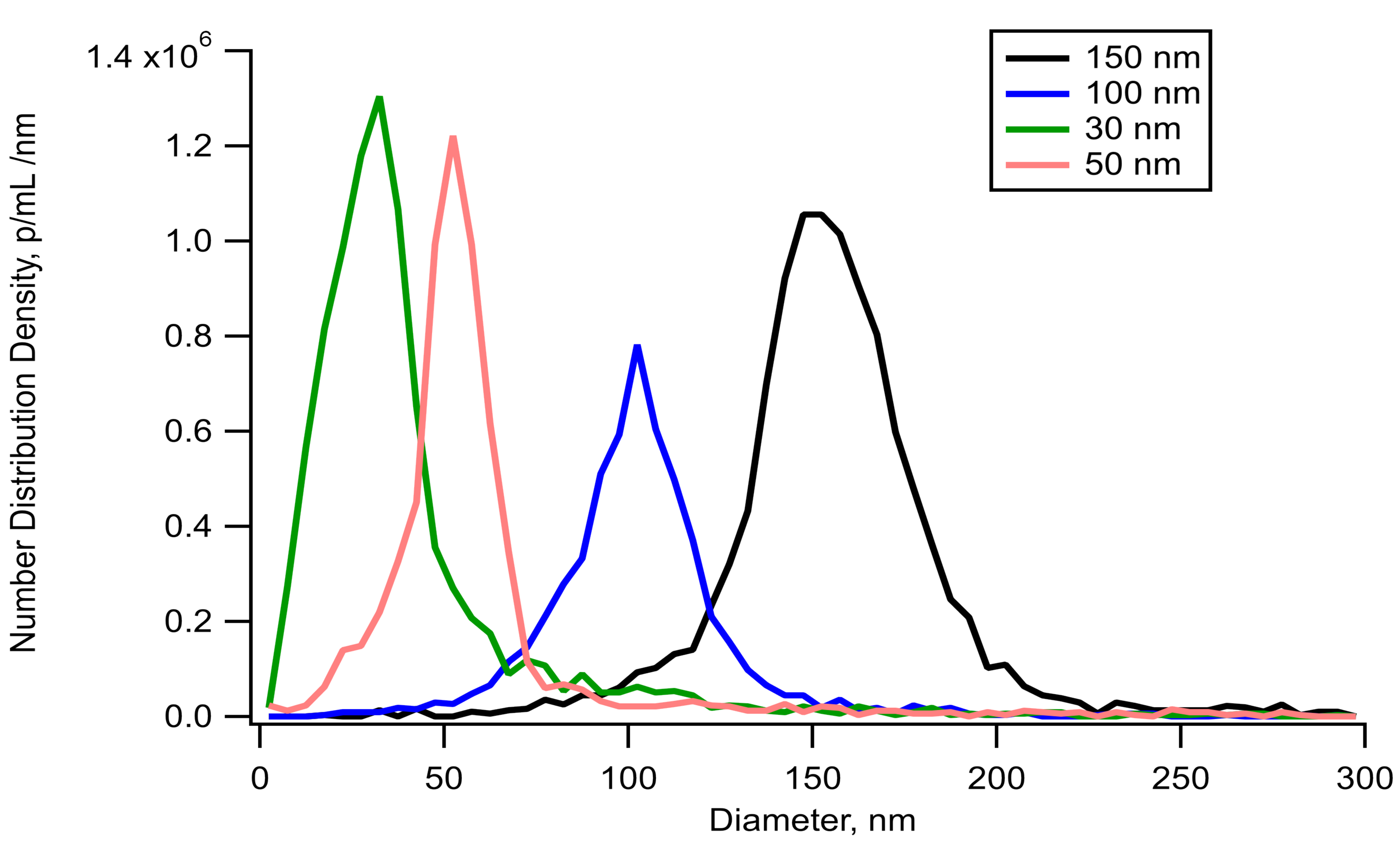



*Figure 1 (left to right): 30 nm, 50 nm, and 100 nm Ultra Uniform Gold Nanospheres number standards and 150 nm (Cat# 3150A) Polystyrene Latex beads were used for this study.*

## CONCLUSION

Orthogonal techniques serve as independent confirmation to evaluate and qualify NTA's ability to accurately size and count number standards as small as 30 nm in diameter, thereby improving accuracy of EV (biomarker) characterization.

**ACKNOWLEDGEMENTS**

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