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Automated Platform for Single Particle-ICPMS

Introduction

Single particle-inductively coupled plasma mass spectrometry (sp-ICPMS) has become an intriguing technique for understanding the elemental nature of nanoparticles (NPs). These nanoparticles have an impact in the food industry, environmental implications, and may pose toxic threats to biological life forms. The ability to detect these nanoparticles has improved over recent years, however, automation and the ability to analyze large sets of samples without compromising sensitivity, robustness, and transport efficiency has yet to be addressed. The micro*FAST* Single Cell system in combination with the single cell sample introduction kit addresses this need for both single cell and single particle analyses.

Elemental Scientific's sample introduction kit addresses the need for extremely high transport efficiencies, offers flexibility to adjust conditions to analyze cells or nanoparticles, and has low internal volume and dead volume. The micro*FAST* Single Cell autosampler addresses the need for automation, which includes in-vial syringe mixing of samples and robust analysis of large sample sets.

Figure 1. (Left) – micro*FAST* Single Cell Autosampler. (Right) – Single Cell sample introduction kit, includes CytoNeb, CytoSpray, and one-piece torch.

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2. University of Oviedo, Oviedo, Spain

Instrumentation

The micro*FAST* Single Cell autosampler (MF-SC-79) contains two high precision syringes for sample loading and carrier to deliver the sample to the sample introduction kit. Sample loops can easily be swapped out for a desired volume on the *FAST* valve, however, in this work a 100 µL sample loop (standard sample loop volume) was utilized. The sample loop may be changed to match a required sample volume or desired ICPMS measurement time (e.g. increasing from 100 µL to 200 µL loop, at 20 µL/min, will increase the max measurement time from 5 minutes to 10 minutes). The sample carrier is typically in the range of 10-50 µL/min (≤50 µL/min results in total consumption operation), for these experiments it was set to 10 µL/min.

The sample introduction kit (SC-SI-79) includes a CytoNeb, CytoSpray, one-piece torch with 2.0 mm injector, and all gas and sample line connections with quick release fittings. Two gas inlets are utilized in the CytoNeb and CytoSpray operation. The CytoNeb utilizes the nebulizer gas port from the ICPMS, whereas, the CytoSpray utilizes the make-up gas. This allows for optimization of gas flows to accommodate different sample types, such as cells or nanoparticles.

An Agilent 7900 ICPMS was employed for these experiments. RF power of 1.55 kW, RF matching of 1.8 V, sample depth of 8.0 mm. Isotopes of ^{107}Ag, ^{195}Pt, and ^{197}Au were monitored for 60 seconds each in single particle mode using a 100 µs dwell time (Fig. 2). The reference mass was set to ^{195}Pt which has a density of 21.45 g/cm and a reference size of 50 nm.

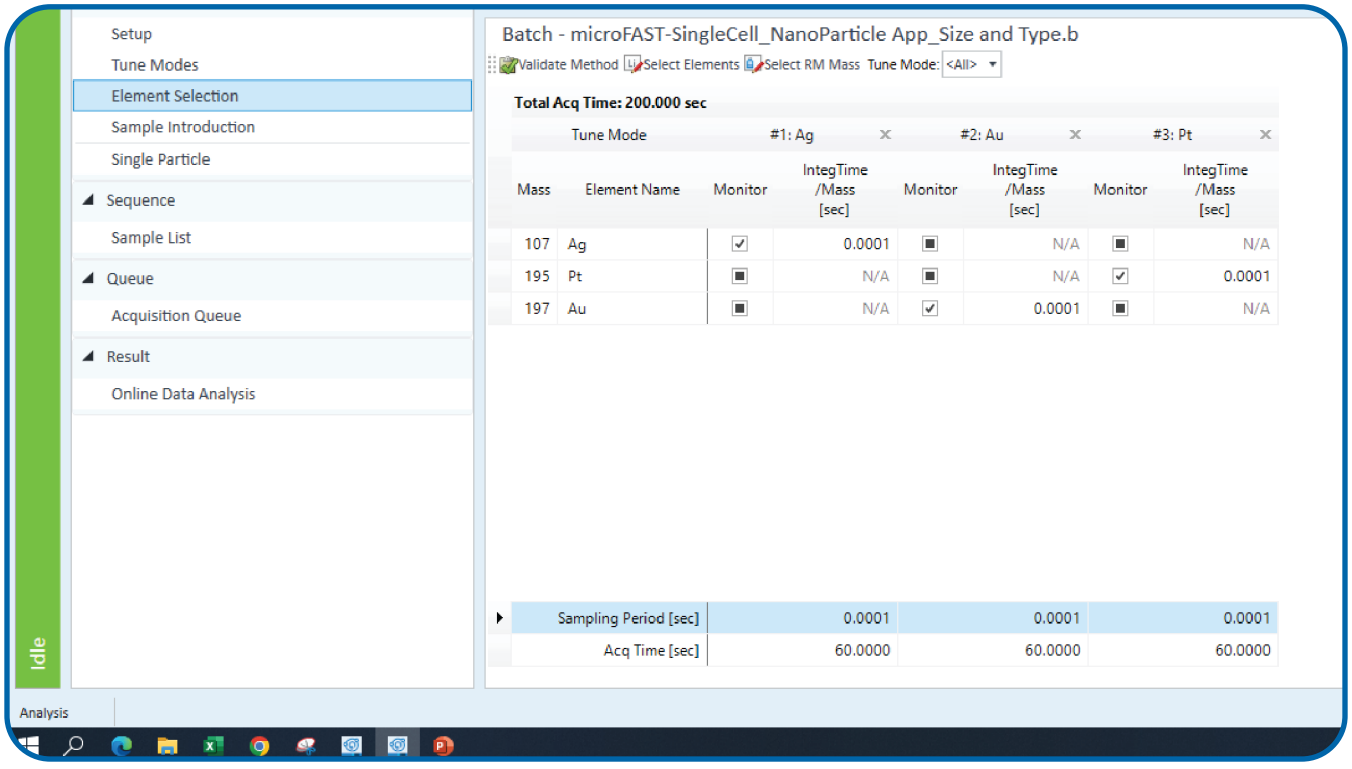


Figure 2. Screenshot of Mass Hunter "Element Selection" showing the selection of Ag, Au, and Pt for single particle mode analysis. The reference mass was selected as Pt, mass 195.

Results

For nanoparticles, higher nebulizer gas flows are utilized while cells require a much lower, gentle gas flow which can be aided by the make-up gas. Figure 3 displays the number of events detected (50 nm Pt NPs) vs nebulizer gas flow. The optimal nebulizer gas flow for nanoparticles is between 0.5 and 0.75 L/min Ar, with a fixed CytoSpray make-up gas of 0.5 L/min Ar. Figure 4 displays the number of events (20 µm human cells) vs nebulizer gas flow. Here, a gentler gas flow is required to not lyse the cells, thus 0.2 to 0.35 L/min Ar are found to be the optimal rate. This lower nebulizer gas flow requires a higher CytoSpray make-up gas (typically between 0.7 to 0.8 L/min Ar) to ensure good transport of the cells to the ICP torch/plasma. A unique aspect in Mass Hunter is the ability to detect mass concentration vs ionic concentration, which can be utilized to determine when the cells are breaking under higher nebulizer gas flows. Figure 5 displays the concentration (mass and ionic) vs the nebulizer flow rate, as the backpressure increases the cells break and the signal is detected as an ionic solution rather than a cell (mass concentration).

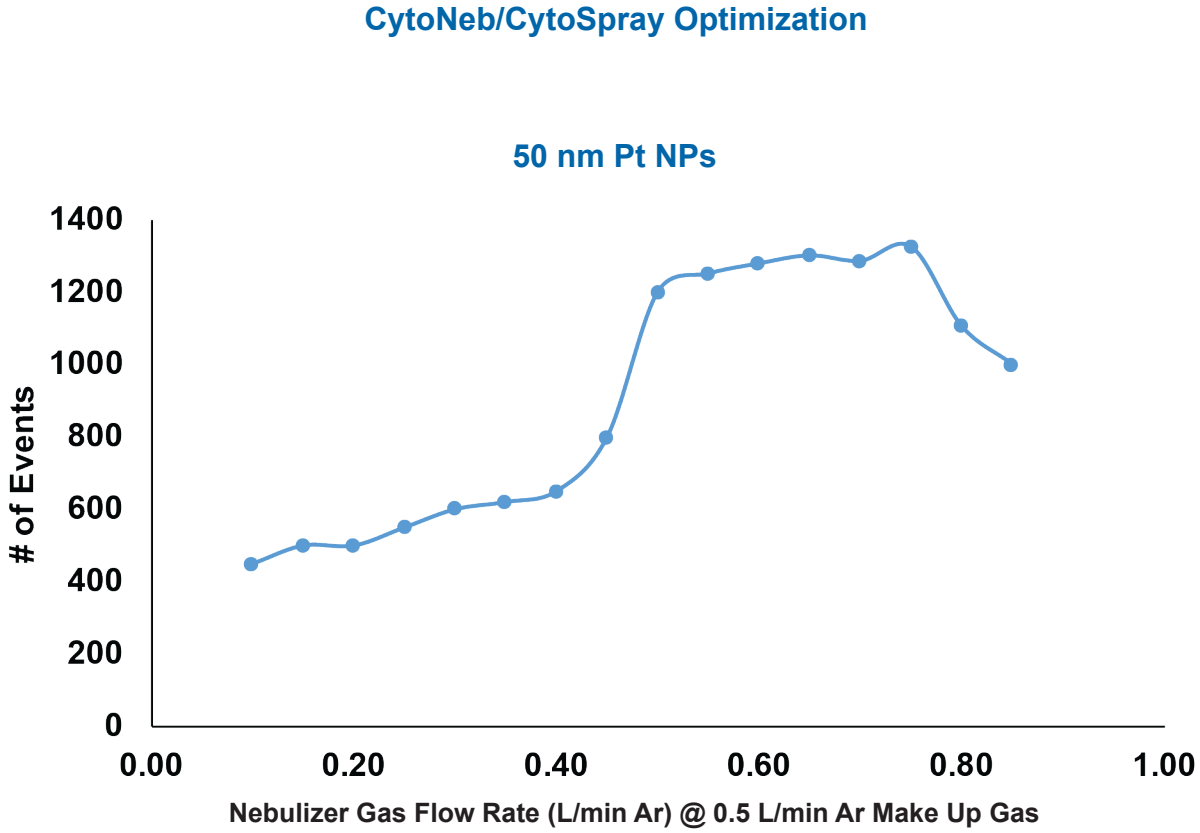


Figure 3. Number of Pt NP events detected as it relates to the nebulizer gas flow rate (L/min Ar) with a fixed make-up gas flow rate of 0.5 L/min Ar.

Results *(Continued)*

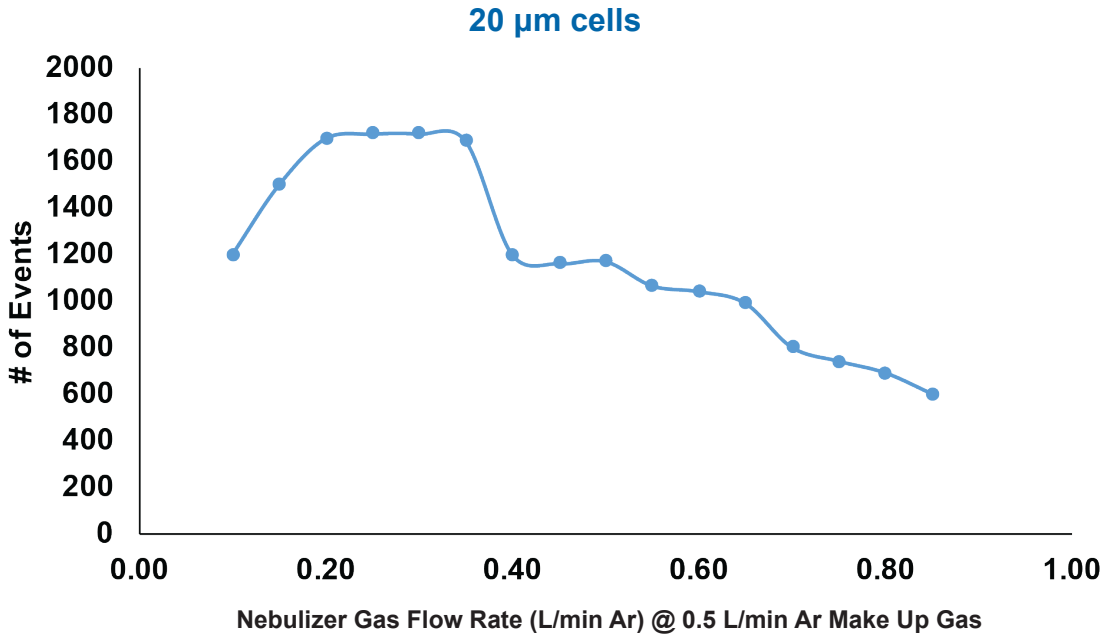


Figure 4. Number of cell events detected as it relates to the nebulizer gas flow rate (L/min Ar) with a fixed make-up gas flow rate of 0.5 L/min Ar.

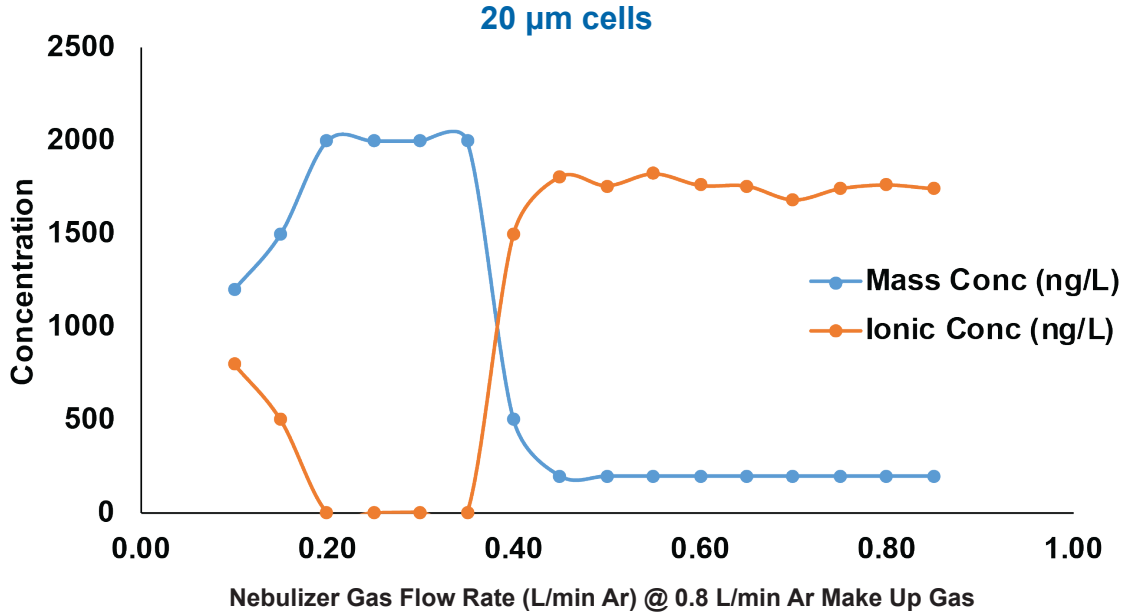


Figure 5. Mass and ionic concentration as it relates to the nebulizer gas flow rate (L/min Ar) with a fixed make-up gas flow rate of 0.8 L/min Ar.

Results *(Continued)*

Automated Sampling

Using the optimal conditions for nanoparticle introduction (CytoNeb – 0.7 L/min Ar, CytoSpray – 0.5 L/min Ar) 50 nm Pt NPs were analyzed over a 5 day period. Figure 6 displays the transport efficiency determined for each day using 50 nm Pt NPs. The overall transport efficiency ranged from 86-98% over the 5-day period. The analysis was repeated over a 12-hour time period to determine the with-in day variations. Figure 7 displays the transport efficiency within day two. The transport efficiency varied between 84-93%. In general, the transport efficiency for 50 nm Pt NPs is typically between 84-98% with slight variations in efficiency seen between CytoNebs.

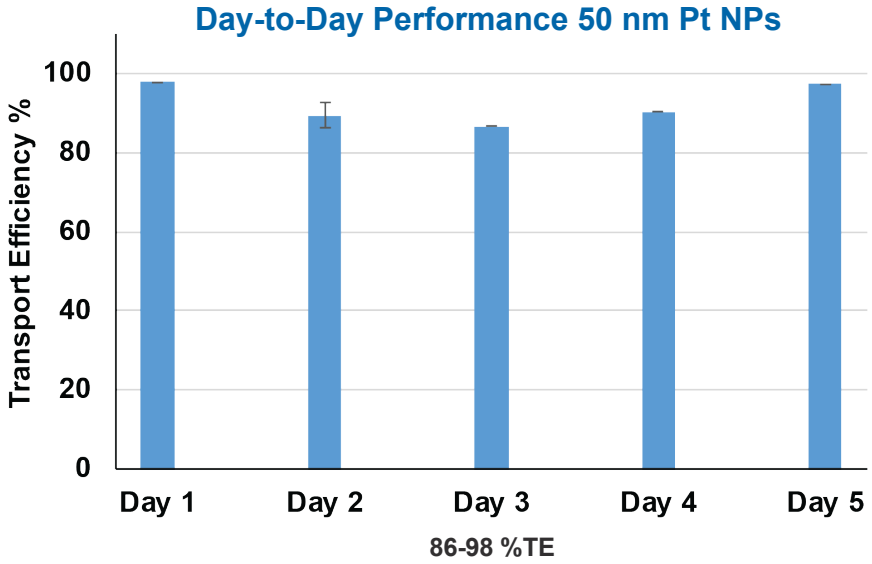


Figure 6. Displays the day-to-day performance of the micro*FAST* Single Cell autosampler and sample introduction kit. 50 nm Pt NPs were analyzed at the beginning and end of each analysis for 5 days.

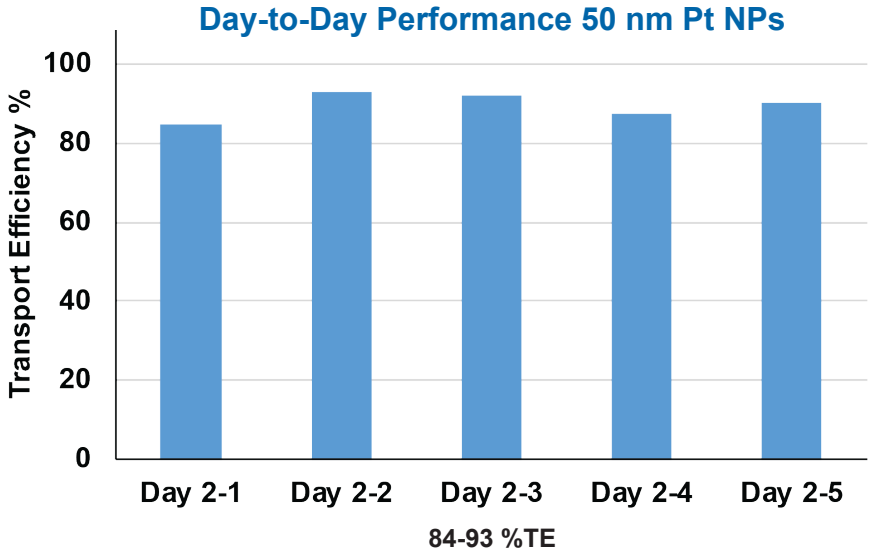
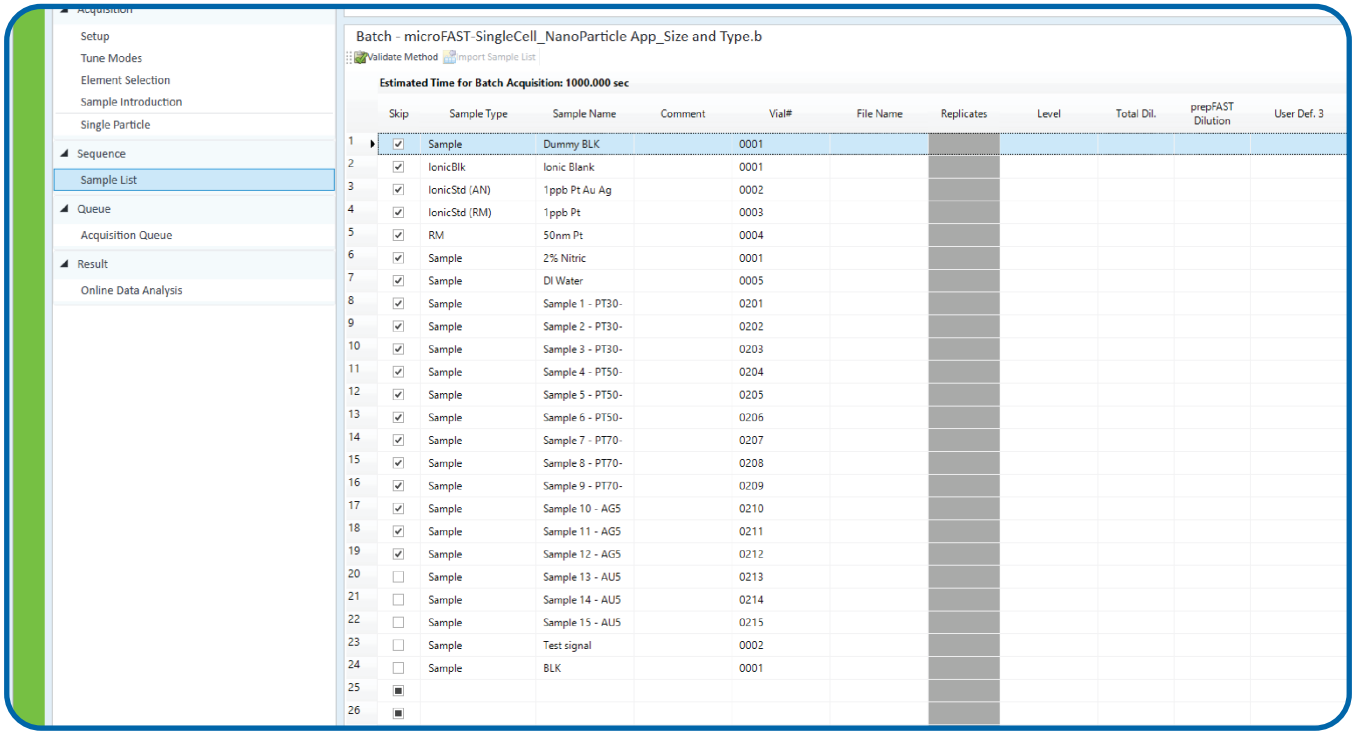


Figure 7. Displays the within day performance of the micro*FAST* Single Cell autosampler and sample introduction kit. 50 nm Pt NPs were analyzed at the beginning and end of each analysis over a 12-hour time period.

Results *(Continued)*

Automated Sampling

Using these optimized conditions, a set of NPs (30 nm Pt, 50 nm Pt, 70 nm Pt, 50 nm Ag, and 50 nm Au) were analyzed for size accuracy. Utilizing Mass Hunter's single particle module and ESI's micro*FAST* plugin, blanks, calibration standards, and samples were easily setup for analysis. The ionic standard was a 1 ppb Pt, Au, and Ag solution (if more elements were being measured, they would be included in the ionic standard). The ionic standard (RM) was a 1 ppb Pt solution to match the reference mass. The reference mass was set to Pt, mass 195, with a density of 21.4 g/cm3. Figure 8 displays the sequence that was setup, which includes ionic blank, ionic standard (AN), ionic standard (RM), RM, and samples



	Skip	Sample Type	Sample Name	Comment	Vial#	File Name	Replicates	Level	Total Dil.	prepFAST Dilution	User Def. 3
1	☑	Sample	Dummy BLK		0001						
2	☑	IonicBlk	Ionic Blank		0001						
3	☑	IonicStd (AN)	1ppb Pt Au Ag		0002						
4	☑	IonicStd (RM)	1ppb Pt		0003						
5	☑	RM	50nm Pt		0004						
6	☑	Sample	2% Nitric		0001						
7	☑	Sample	DI Water		0005						
8	☑	Sample	Sample 1 - PT30-		0201						
9	☑	Sample	Sample 2 - PT30-		0202						
10	☑	Sample	Sample 3 - PT30-		0203						
11	☑	Sample	Sample 4 - PT50-		0204						
12	☑	Sample	Sample 5 - PT50-		0205						
13	☑	Sample	Sample 6 - PT50-		0206						
14	☑	Sample	Sample 7 - PT70-		0207						
15	☑	Sample	Sample 8 - PT70-		0208						
16	☑	Sample	Sample 9 - PT70-		0209						
17	☑	Sample	Sample 10 - AG5		0210						
18	☑	Sample	Sample 11 - AG5		0211						
19	☑	Sample	Sample 12 - AG5		0212						
20	☐	Sample	Sample 13 - AU5		0213						
21	☐	Sample	Sample 14 - AU5		0214						
22	☐	Sample	Sample 15 - AU5		0215						
23	☐	Sample	Test signal		0002						
24	☐	Sample	BLK		0001						
25	▣										
26	▣										

Figure 8. Screenshot of Mass Hunter sample list for the analysis presented in this work.

Results *(Continued)*

Size Accuracy

Figure 9 displays the histogram for 30, 50, and 70 nm Pt NPs determined using the above Mass Hunter calibration strategy. The particle sizes were determined to be within specifications of the certificate of analysis, which can be seen in Table 1. Figure 10 displays the histograms for the 50 nm Pt, Au, and Ag NPs. The sizes are all within the specified ranges, with extremely accurate results for the Pt NPs. The Ag size value was measured to be slightly lower than the certified value, however, statistically they are the same based on the uncertainties. The Au size was measured to be slightly higher than the certified value, but again statistically the same.

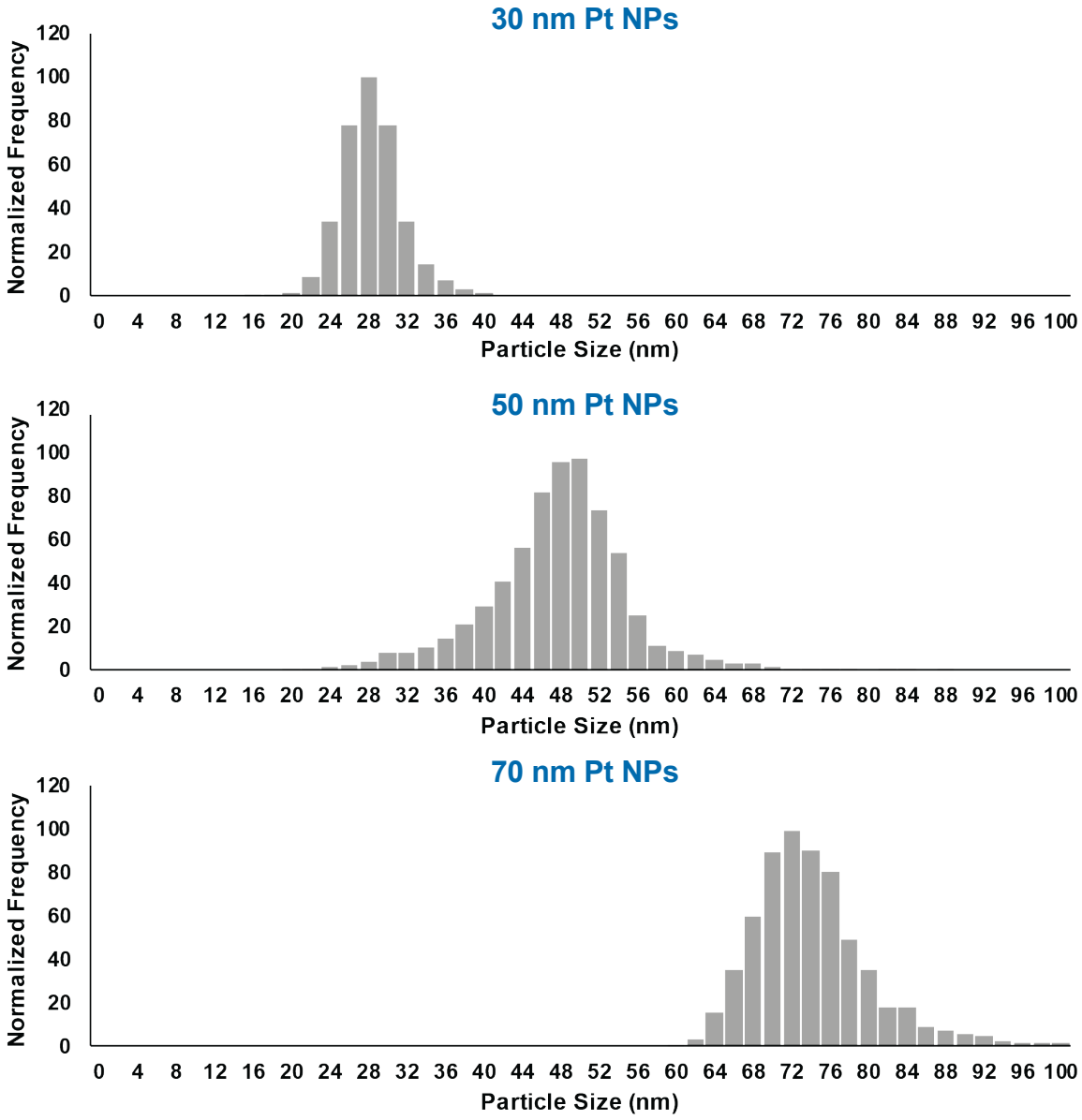


Figure 9. Histograms from the Pt NP data displaying the particle size (nm) vs normalized frequency.

Results *(Continued)*

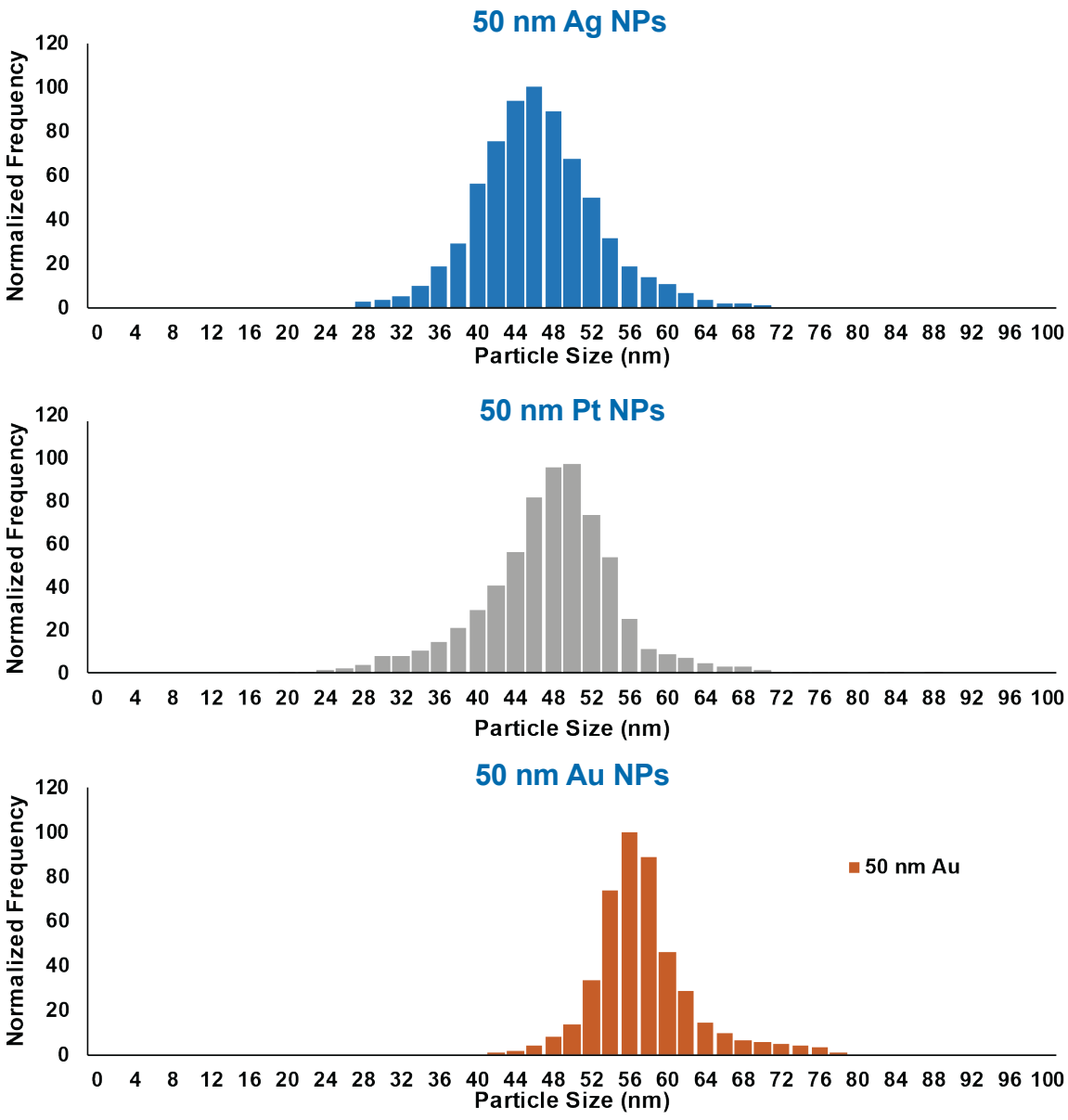


Figure 10. Histograms from the 50 nm Ag, Pt, and Au NP data displaying the particle size (nm) vs normalized frequency.

Results *(Continued)*

The ability to automate large data sets will only be valuable when you can guarantee reproducible data over hours of analysis time. To demonstrate the capabilities, 20 samples were prepared with 50 nm Pt NPs and placed into the 1 mL vials on the autosampler rack. The ICPMS measurement time was set such that the 20 samples took ~2 h to complete, to ensure the analysis covered a larger time period. Two different experiments were carried out, with and without mixing of the samples prior to syringe loading into a 100 µL sample loop. Figure 11 displays the no mixing method for the 50 nm Pt NPs. The average particle count without mixing was 879±109 particles (12.4% RSD). Figure 12 displays the mixing method for the 50 nm Pt NPs. The average particle count with mixing was 907 ± 47 particles (5.2% RSD). The mixing method provided an ~2.5x improvement in robustness when comparing identical samples being analyzed over a 2 hour analysis time.

Table 1. Certificate of analysis and measured particle size distributions for each NP used in this study. Measured values were determined based on the median distribution.

	Particle Size Distribution (nm)	
	Certificate of Analysis	microFAST Single Cell + Agilent 7900 ICPMS
30 nm Pt	30 ± 3	30 ± 2
50 nm Pt	51 ± 7	50 ± 5
70 nm Pt	72 ± 4	72 ± 5
50 nm Ag	51 ± 6	46 ± 6
50 nm Au	50 ± 2	56 ± 5

Note: Value determined using histogram median

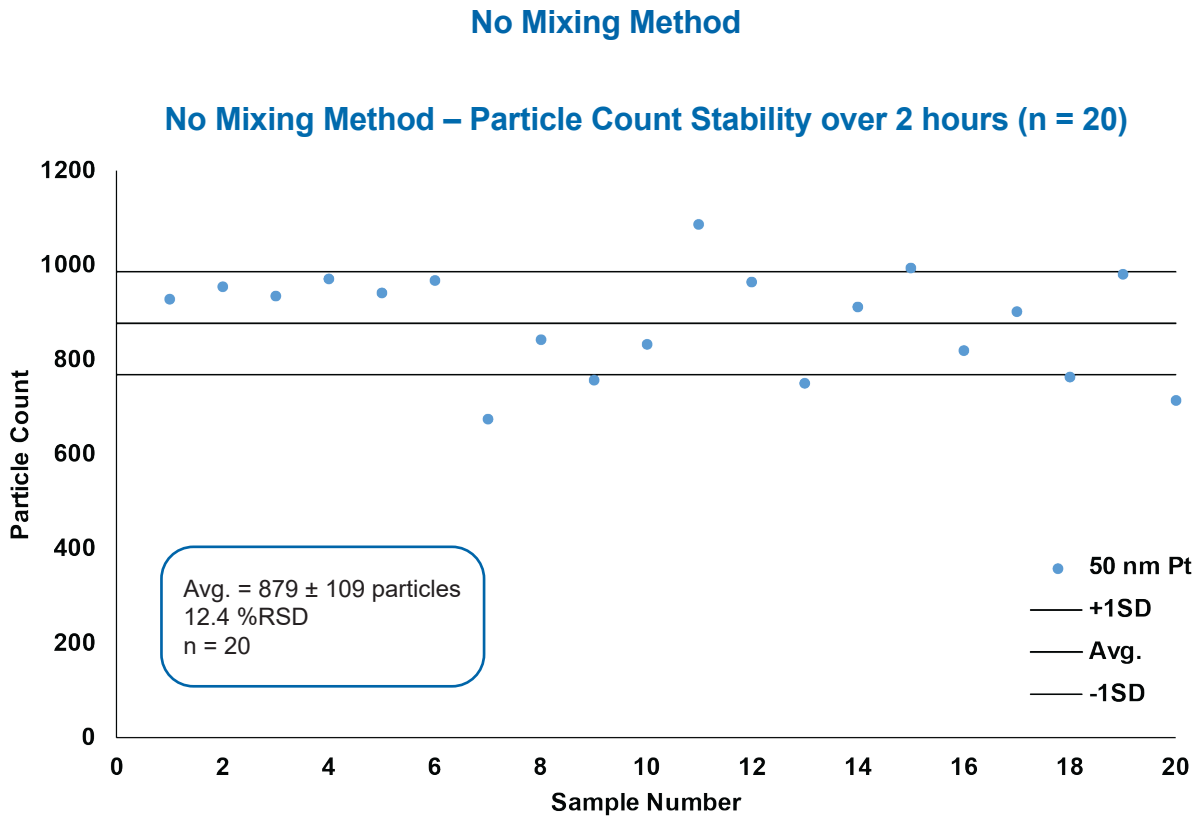


Figure 11. Particle count for 50 nm Pt NPs analyzed over a 2 h time period from 20 identically prepared samples in separate vials using the no mixing method. The analysis time was set to ensure the 20 samples took 2 h to complete.

Results *(Continued)*

Mixing Method

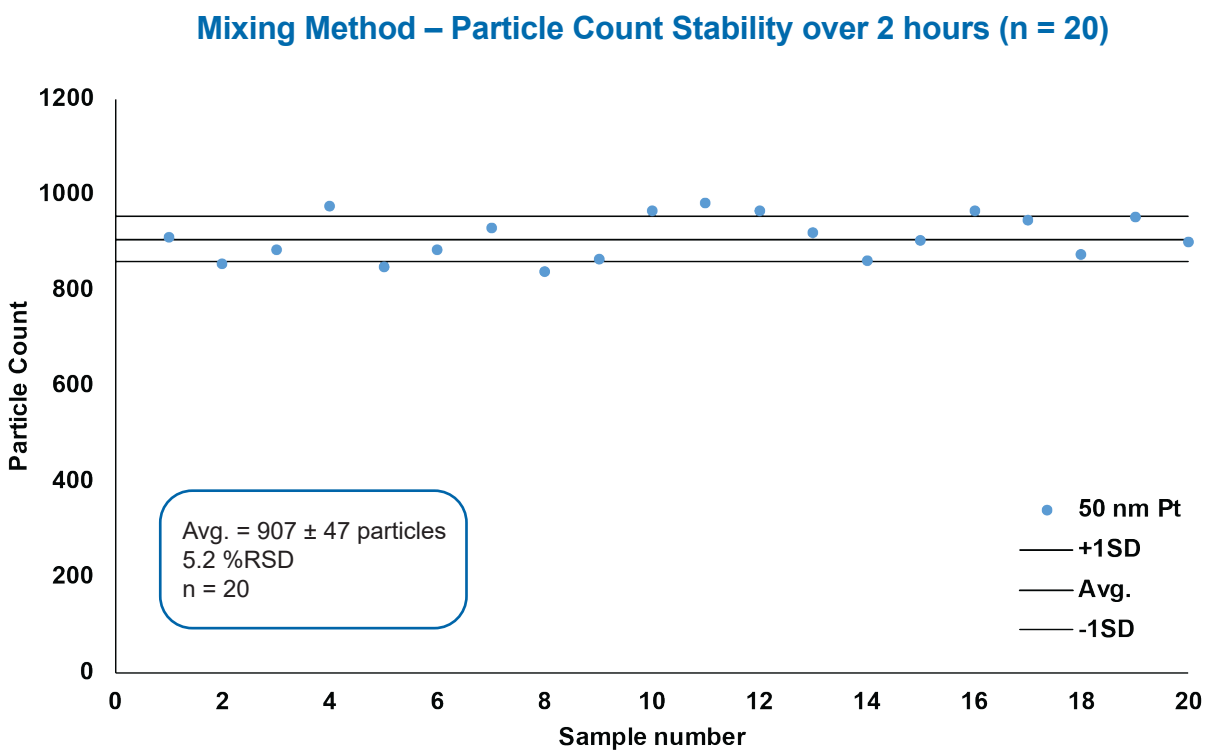


Figure 12. Particle count for 50 nm Pt NPs analyzed over a 2 h time period from 20 identically prepared samples in separate vials using the mixing method. The analysis time was set to ensure the 20 samples took 2 h to complete.

Conclusion

With the micro*FAST* Single Cell autosampler and ESI's sample introduction kit, sp-ICPMS now has a truly automated option that does not compromise transport efficiency or robustness. With the in-vial mixing method large sample sets can be analyzed with confidence. Day-to-day performance shows excellent transport efficiency ensuring the highest data quality. Utilizing Mass Hunter, calibrations for size and concentration are easily determined for the isotopes of interest.

Notes