TECHNICAL NOTE

ICP - Mass Spectrometry

Patented Asperon Single Cell Spray Chamber Delivering Intact Individual Cells to the ICP-MS Plasma

Introduction

Single Cell ICP-MS (SC-ICP-MS) is a technique that allows for the analysis and quantification of metal content in an individual cell. This technique can be used to quantify the uptake and bioaccumulation of metals and metal nanoparticles in to cells, as well as the intrinsic metals within the cells themselves, which can be a measure of metabolomic changes related to cell stress or lifecycle. SC-ICP-MS provides insights on:

- Metal content per cell
- Metal distribution in the cell population
- Number of cells containing metal or metal nanoparticles
- Number of nanoparticles per cell

PerkinElmer's award-winning NexION® ICP-MS Single Cell Analysis System offers a first-to-market complete solution to quantitatively measure the metal content in individual cells, unveiling new capabilities to study intrinsic metals content and the uptake of dissolved (ionic) and nanoparticulate metals into cells, providing new insights into drug delivery, mobilization/immobilization of metal content, bioavailability, and bioaccumulation mechanisms. The solution includes:

- Single Cell Micro DX Autosampler
- Single Cell Sample Introduction Kit with patented Aperon™ spray chamber (US Patent No. 10,147,592)
- Syngistix™ Single Cell Application Software Module

This technical note provides an overview of SC-ICP-MS system validation methods:

- Optimization of sample flow rate for optimal cell transport efficiency into the plasma
- Quantification of the mass of metal per cell using a standard reference material
- Comparison of total digested metal in a sample compared to manufactures data

For information related to sample preparation or workflow, please consult our white paper "Single Cell ICP-MS Analysis: Quantification of Metal Content at the Cellular Level".[1]

Methods

ICP-MS Instrumental Conditions

All analyses were carried out on a PerkinElmer NexION® ICP-MS series using two types of spray chambers: baffled cyclonic and Asperon. The conditions used for both spray chambers are shown in Table 1.

Table 1. ICP-MS Conditions for Acid-Digested Beads and SC-ICP-MS.

Parameter	ICP-MS	SC-ICP-MS
Spray Chamber	Baffled Cyclonic	Asperon
Nebulizer	Glass Concentric	High Efficiency Glass Concentric
Injector	2 mm Quartz	
RF Power (W)	1600 W	1600 W
Spray Chamber Path	Cyclonic	Linear Pass
Neb Gas Flow (mL min^{-1})	0.92	0.32
Make-Up Gas (mL min^{-1})	0	0.7
Sample Flow Rate (mL min^{-1})	0.283	0.015
Sample Size Required for Analysis (µL)	800-1000	100-200
Elements	^{140}Ce, ^{151}Eu, ^{153}Eu, ^{165}Ho, ^{175}Lu, ^{197}Au	

PerkinElmer®
For the Better

Polymer beads (Fluidigm, USA): Polymer beads laced with lanthanide metals (^{140}Ce, ^{151}Eu, ^{153}Eu, ^{165}Ho and ^{175}Lu) were used as a suitable reference material due to the similarity in size (2.5 µm) and density (1.05 g/cm^3) to cells. The presence of lanthanides in the beads enables them to be measured using SC-ICP-MS. Although the size cut-off for the baffled cyclonic spray chamber is in the 1-5 µm range,[2,3] the transport of these larger beads (2.5 µm) is low, as illustrated later in Figure 2. The beads were supplied at a concentration of 330,000 beads per mL and diluted 10 fold for analysis. The mass of metal per bead can be found in Table 2.

Table 2. Supplier information on lanthanide laced polystyrene beads.

Element	Atoms Per Bead (± 15%)	Mass (ag) (± 15%)
Ce 140	19.9E6	4626.26
Eu 151	11.3E6	2833.38
Eu 153	12.0E6	3048.75
Ho 165	7.6E6	2082.32
Lu 175	9.8E6	2847.82

Standards for SP-ICP-MS and SC-ICP-MS: Standards of 1, 2 and 3 ppb ^{140}Ce, ^{151}Eu, ^{153}Eu, ^{165}Ho and ^{175}Lu were prepared for measuring the mass of metals in the polystyrene beads. All standards for SC-ICP-MS were prepared in ultra-pure water. The transport efficiency was determined with the 60 nm Au NPs (NIST 8013) at a concentration of 50,000 part. mL^{-1}.

Digestion of polystyrene beads: Digestion was accomplished by placing 5 mL of the bead suspension into a PTFE digestion vessel with 5 mL of hydrogen peroxide (Optima grade) and 10 mL of nitric acid (Optima grade). The mixture was allowed to sit for 10 minutes to allow gasses to be released from any initial reactions before the vessels were sealed. The beads were then digested in a Titan MPS™ Microwave (PerkinElmer), following the program in Table 3. After they had cooled, the samples were diluted to 2% acid for analysis. Standards of 10, 50, 100 and 200 ppb Lu, Eu, Ho and Ce were matrix-matched to the samples for ICP-MS analysis. Ge and In were spiked into the samples as internal standards.

Table 3. Conditions for microwave acid digestion of polystyrene beads.

Stage	Temperature (°C)	Pressure (bar)	Ramp (min)	Hold (min)	P (%)
1	150	70	2	5	60
2	190	75	2	10	80
3	210	80	2	15	90
4	50	80	1	10	0
5	50	0	0	0	0

Gas and Sample Flows Optimization

The make-up and nebulizer gas flows and sample flow rate had to be optimized to allow for equal transport of nano- and micron-sized objects through the introduction system. Figure 1 shows the transport efficiency for nano- and micron-sized objects through the introduction system for different sample flow rates. It can be seen that as the sample flow rate decreases, the transport efficiency for both nano- and micron-sized objects increases and the difference between their transport gets less pronounced with a transport efficiency of about 31% for both the 60 nm Au NIST standards and the lanthanide-doped micron beads (Fluidigm). The optimized conditions for this system can be seen in Table 1.

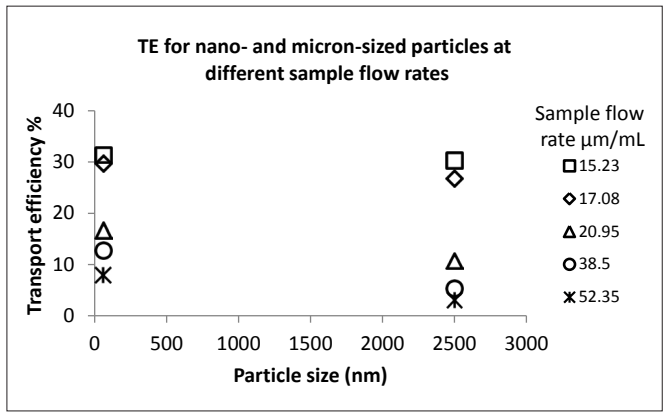

Figure 1. Optimization of sample flow rates through the introduction system to maximize the transport of nano- and micron-sized objects into the plasma.

Transport Efficiency Validation: Baffled Cyclonic vs. Asperon Spray Chamber

A comparison study of the transport efficiency of nano- and micron-sized particles between the baffled cyclonic and Asperon spray chambers is shown in Figure 2. The systems were both optimized for maximum intensities while keeping oxide and double-charge formation below 2.5% (Table 1). It can be seen in Figure 2 that both introduction systems transport a sufficient amount of NPs (NIST 8013 60 nm Au NPs) into the ICP-MS to provide a statistically significant measure of the mean and standard deviation of the NPs (either in diameter or mass per NP) and particle number concentration showing that both systems work equally well for nanoparticles. However, the number of micron-sized particles transported by the baffled cyclonic spray chamber is low, with only a few beads being analyzed compared to the Asperon spray chamber, where a significant number of beads were counted.

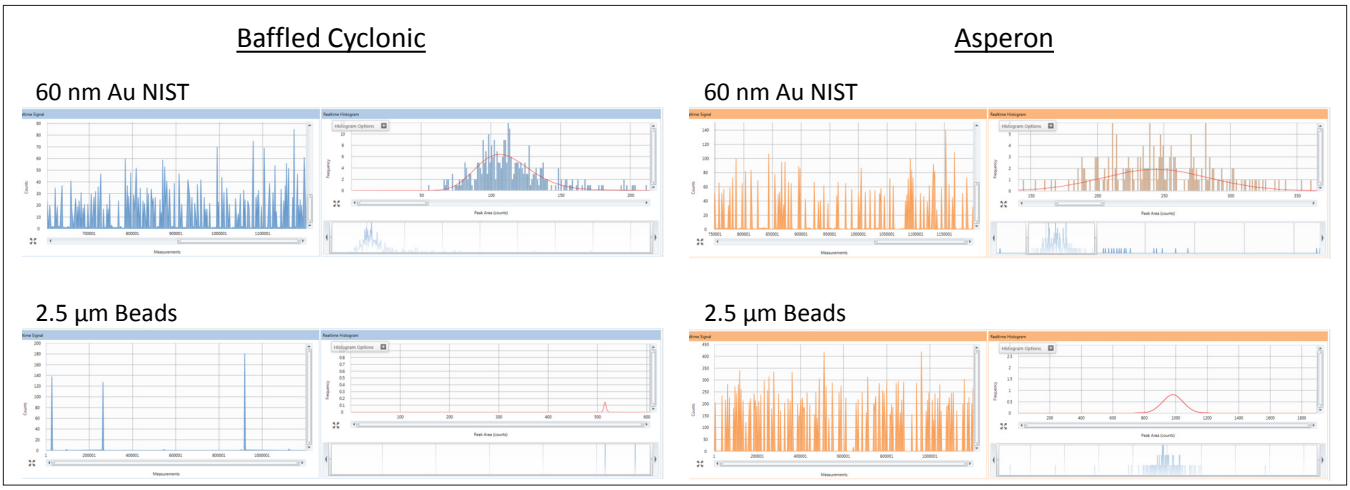

Figure 2. Real-time screen shots comparing the number of Au NIST 60 nm NPs and 2.5 µm polystyrene beads making it to the plasma for the baffled cyclonic and Asperon spray chambers.

As shown in Table 4, the transport efficiencies for the 2.5 µm polystyrene beads and 60 nm Au NPs in the baffled cyclonic spray chamber are 0.04 ± 0.02 % and 2.42 ± 0.06 %, respectively, which is significantly different (calculated probability (p) < 0.05, Student's t-distribution). This translates to approximately 3.5 ± 1.9 beads per minute reaching the plasma in a suspension containing 33,000 beads mL^{-1} and 342.3 ± 8.7 NPs per minute for a suspension containing 50,000 NPs mL^{-1}. Clearly, the cyclonic spray chamber is not suitable for single cell analysis. In contrast, the Asperon spray chamber has a transport efficiency which is not significantly different for the beads or the NPs: 31.33 ± 2.54 % and 30.31 ± 1.85 %, respectively. This corresponds to 229.5 ± 19.5 NPs and 208.3 (± 12) micron-sized beads for suspensions containing 50,000 particles mL^{-1} and 33,000 beads mL^{-1} respectively.

Quantitative Validation: Measurement of Metal Mass per Bead

The polymer beads are doped with lanthanide metals (^{140}Ce, ^{151}Eu, ^{153}Eu, ^{165}Ho and ^{175}Lu) at the concentrations shown in Table 5, as supplied by the manufacturer. The mean mass of metal per bead as measured by SC-ICP-MS can also be seen in Table 5. Comparing the experimental data with the supplied concentrations, there is no statistical difference (all p values >> 0.05) between the mean mass provided by the supplier and those quantified using SC-ICP-MS. The number of beads per mL was measured to be 34,821 (± 661.7) having no statistical difference to the manufacturer's value of 33,000 (± 1650).

Table 4. A comparison of the number of nano- and micron-sized particles entering the ICP-MS and subsequent transport efficiencies.

Spray Chamber	Baffled Cyclonic	Asperon
TE 60 nm NIST (%)	2.42 (±1.85)	31.33 (± 2.54)
Number of 60 nm NIST NPs Measured in a One-Minute Scan	342.3 (± 8.7)	229.5 (±19.1)
TE 2.5 µm beads (%)	0.04 (± 0.02)	30.31 (±1.85)
Number of 2.5 µm Beads Measured in a One-Minute Scan	3.5 (± 1.9)	208.3 (±12)

Table 5. Metal Content per Bead and Bead Concentration: A Comparison of SC-ICP-MS vs. Certificate Values.

Element	Data Provided by Supplier			Measured by SC-ICP-MS (Ag Per Bead)					
	Atoms Per Bead (±15%)	Mass (ag)	Size (nm)	1	2	3	4	Average	STDEV
Ce 140	19.9E6	4626	109.7	4250	4561	4750	4699	4632	95
Eu 151	11.3E6	2833	101.1	2961	3012	3123	3075	3043	61.6
Eu 153	12.0E6	3049	103.6	2904	33240	3114	3061	3080	120.4
Ho 165	7.6E6	2082	76.8	2157	2228	2576	2354	2329	159.3
Lu 175	9.8E6	2848	82.1	2921	2228	2965	2982	2889	68.8
Number of Particles				34323	33145	34696	34821	34246	661.7

The total metal content of the bead suspension was established after acid digestion and was found not to be statistically different from those calculated from the mass per bead and bead number measured by SC-ICP-MS (Table 6).

Table 6. Total amount of metal in bead suspension after acid digestion.

Element	Values From Supplier (ppb)	Measured Values (ppb)
Ce 140	152.67	160.3 (7.4)
Eu 151	93.50	71.6 (7.6)
Eu 153	100.61	80.6 (8.0)
Ho 165	68.72	67.8 (8.4)
Lu 175	93.98	83.9 (8.1)

Conclusion

This work has shown that the patented Asperon spray chamber provides increased transport of micron-sized objects into the ICP-MS compared to traditional introduction systems. Coupled with the fast data acquisition capabilities of the NexION ICP-MS, the Asperon spray chamber has allowed for the quantification of masses from micron-sized objects down to the attogram per-bead level as well as providing accurate measurements of the number concentration of the beads per milliliter.

References

1. Single Cell ICP-MS Analysis: Quantification of Metal Content at the Cellular Level, PerkinElmer Inc., 2017.
2. Schaldach, G.; Berger, L.; Razilov, I.; Berndt, H., Characterization of a cyclone spray chamber for ICP spectrometry by computer simulation. *Journal of Analytical Atomic Spectrometry* 2002, 17, (4), 334-344.
3. Matusiewicz, H.; Slachcinski, M.; Almagro, B.; Canals, A., Evaluation of Various Types of Micronebulizers and Spray Chamber Configurations for Microsamples Analysis by Microwave Induced Plasma Optical Emission Spectrometry. *Chemia Analityczna* 2009, 54, (6), 1219-1244.

SC-ICP-MS Components

Component	Part Number NexION 1000/2000	Part Number NexION 300/350
Single Cell Micro DX Autosampler	N8150039	N8140039
Single Cell Sample Introduction Kit with Asperon Spray Chamber	N8150032	N8140032
Syngistix Single Cell Application Software Module	N8150321	N8150321

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013655A_01 PKI