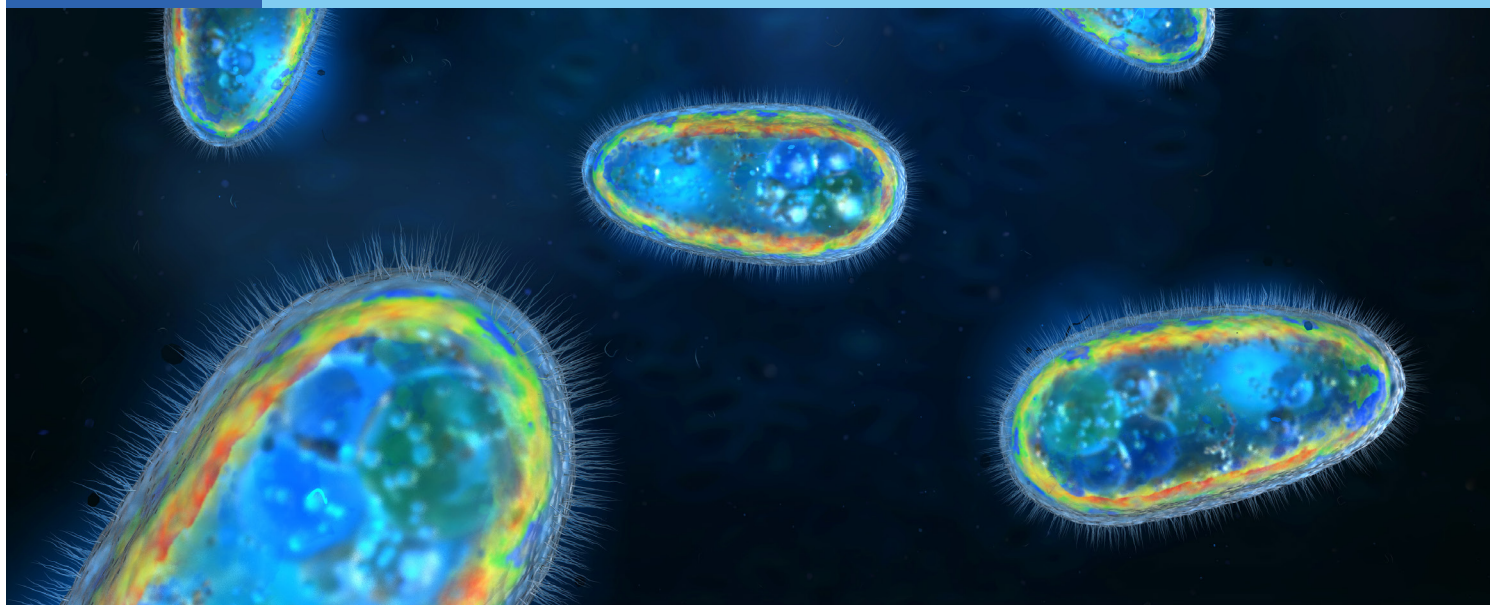


Single Cell ICP-MS Analysis: Quantification of Metal Content at the Cellular Level



Introduction

Accurately quantifying the metal content in an individual cell can offer an insight into understanding the uptake and elimination mechanisms of metals and/or metal-containing nanoparticles by individual cells, the mechanisms of interactions between metal-containing drug and cells, and the distribution of nutrients among a population of cells. Traditional methods of measuring the metal content of cells use nominal mass concentrations, which assume an equal distribution of metals among the cells, thereby overlooking vital information on the metal distribution and variation on a unicellular level. Here we introduce Single Cell ICP-MS (SC-ICP-MS), a technique capable of rapidly measuring the metal content of individual cells. Hardware for SC-ICP-MS includes both the NexION® family of ICP-MS instruments and a dedicated sample introduction system (Asperon™) capable of delivering individual, intact cells into the plasma of the ICP-MS. Data collection and processing is done through the Syngistix™ Single Cell Application Module; a dedicated software package which, when coupled with the fast data acquisition capabilities of the NexION ICP-MS, quantifies the metal content within individual cells down to the attogram-per-cell level and determines the metal mass distribution and the number of cells containing the metal. When considered in unison, this information is able to evaluate and quantify the degree of heterogeneity within a cell population.

How Does Single Cell ICP-MS Work?

One of the main challenges of SC-ICP-MS is the process of getting cells to enter into the plasma. Since cells are typically 1- 100 μm in size, they will be filtered out by traditional cyclonic spray chambers, which only allow droplets smaller than 3-5 $\mu\text{m}^{1,2}$ to enter the plasma, where the majority of droplets are smaller than 1 μm . To overcome this obstacle, the new Asperon™ introduction system, developed by scientists at PerkinElmer, allows larger, micron sized entities to enter the ICP. This was accomplished by modifying the spray chamber design and incorporating new flow patterns within the spray chamber. Specifically, a dual make-up gas inlet was positioned to create a tangential flow to the spray chamber walls, thereby preventing cells from colliding with and sticking to the walls. In addition, an inner tube was equipped with microchannels, to which some of the make-up gas was diverted. The purpose of these channels is to prevent liquid deposition within the flow path. Finally, a laminar flow was incorporated to ensure the maximum transport of the cells.

What Can Single Cell ICP-MS Measure?

With the Single Cell Application Software Module, it is possible to measure:

- Mass of metal per cell
- Mass distribution within a cell population
- Concentration of cells containing metal or nanoparticles
- Number of nanoparticles per cell

What are the Important Parameters to Consider?

There are six main parameters to consider or optimize for SC-ICP-MS. Figure 1 shows a schematic diagram of the set up with the six important parameters highlighted.

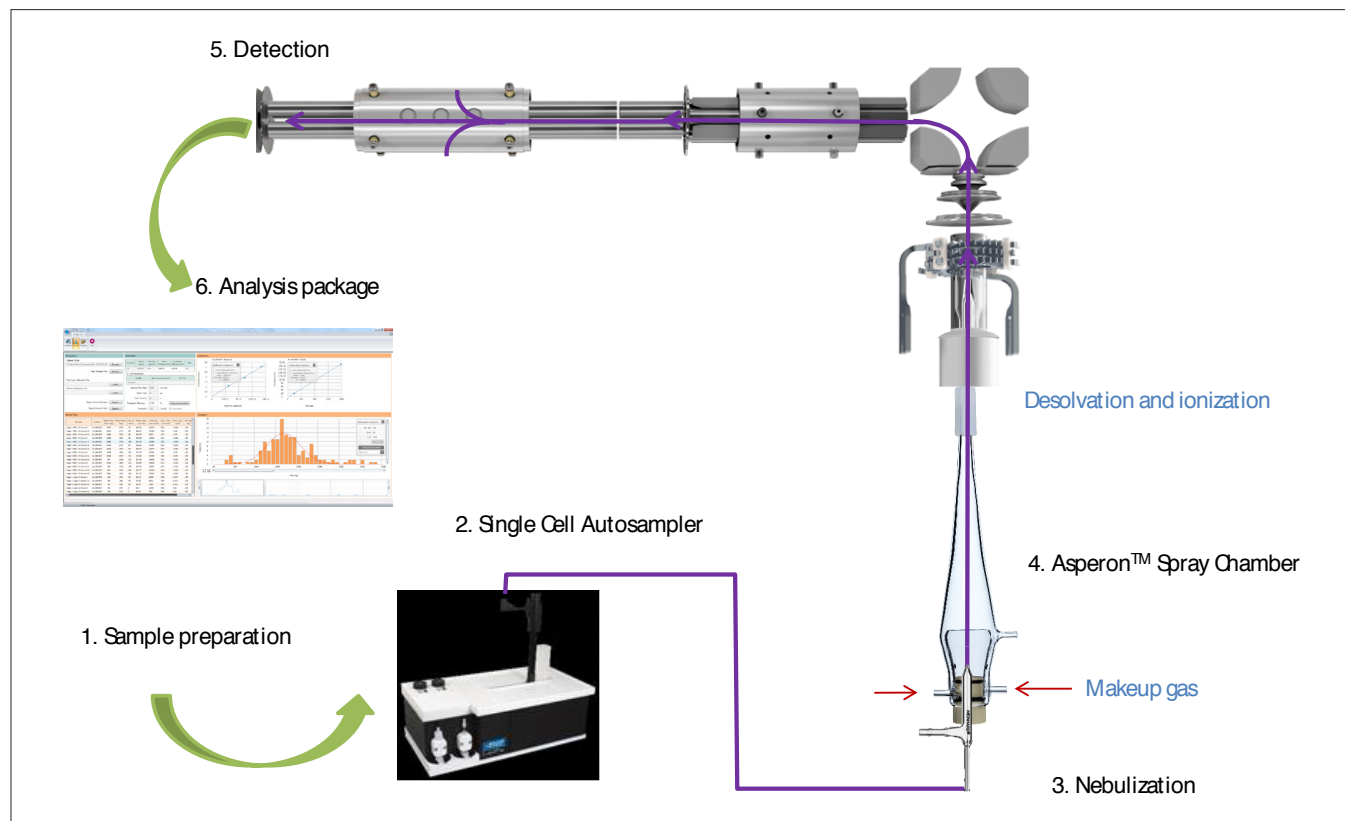


Figure 1. Schematic diagram of SC-ICP-MS set up.

1. Sample Preparation

Single celled organisms come in many different shapes and sizes. Some are more fragile or adverse to changes in osmotic pressures, while others are harder and can tolerate such change before their membrane is disrupted and cell lysis occurs. As such, it is important to understand how to prepare particular cell line being analyzed. Some requirements for sample preparation include:

- Forming a solution of single cells (removing any cell aggregations)
- Washing the cells from the original exposure media without causing aggregation or membrane disruption
- Resuspending cells in a media not containing high levels of the analyte metal (more necessary for measuring some intrinsic metals)
- Quantifying the number of cells present prior to analysis via ancillary counting methods

Care needs to be taken during washing procedures as cells can be easily damaged in this step. Figure 2 shows a typical procedure used for cells in suspension. The g-force required for cell separation must be low as to not rupture cell membranes; however this can lead to a loss in cell number over the wash cycles with typically a 30-50% loss over three cycles.

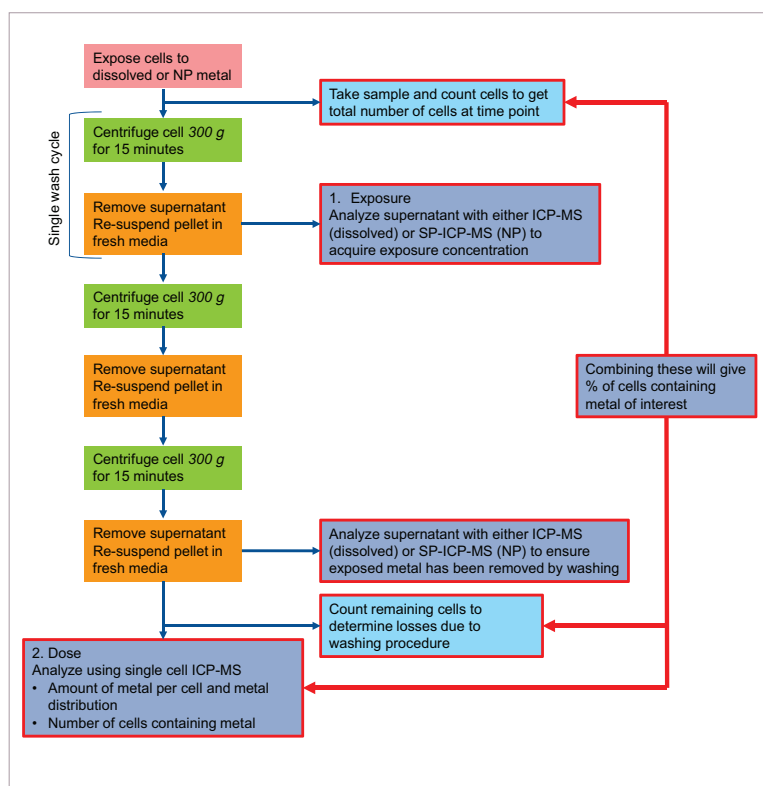


Figure 2. Wash steps required for removing algal cells from exposure media.

2. Automated Sample Introduction

Automated sampling for SC-ICP-MS needs to be able to cope with certain cell-sampling issues:

- Typically cells do not remain in suspension and rapidly settle over time
- Some cells may require fixed temperatures for survival or to reduce cellular lysis
- Sample volumes or cell numbers may be limited
- The introduction system requires low sample flow rates.

The PerkinElmer Single Cell Autosampler is designed to address these concerns by mimicking pipette-driven agitation prior to sample injection so as to resuspend the cells. Interchangeable temperature controlled sample racks provide optimal conditions to promote cell survival before analysis. After resuspension, the sample is transported into a sample loop before it is introduced into the ICP-MS via a syringe driven mechanism capable of delivering flow rates between 2-100 $\mu\text{L min}^{-1}$.

3. Nebulization

The amount of pressure placed on the cells while being aspirated into the spray chamber can be significant causing cell lysis, which in turn will lead to anomalies in the results. The integrity of the cell during aspiration will depend on the sample flow rate, the nebulizer gas flow rate and the size of the cells. Generally, in SC-ICP-MS the sample flow rate is between 15-20 $\mu\text{L min}^{-1}$ and

the nebulizer gas flow rate is between 0.3 and 0.4 mL min^{-1} , with the highest pressures being exerted at the higher sample and gas flow rates.

Figure 3 shows three types of algae cell: *Chroomonas sp.*, *Cryptomonas ovata*, *Gonyostomum semen* with size ranges of 5-7 μm , 20-30 μm and 50-70 μm , respectively, along with graphs of intact cell numbers against nebulizer gas flow rate (Figure 2). It can be seen that at these flow rates the smaller two algae species are intact up to nebulizer flow rates of 0.5 mL min^{-1} and sample flow rates of 0.015 mL min^{-1} . In contrast, the largest cell species can be seen to become unviable at nebulizer gas flows as low as 0.3 mL min^{-1} , showing the need for a cell viability tests to be performed prior to analysis.

4. Transport of Cells Into the Plasma

Traditionally, spray chambers (such as the baffled cyclonic or parallel path, Figure 3) are designed to prevent the majority of large water droplets ($\approx 4 \mu\text{m}$ and larger) from entering the plasma, with the transport of the larger droplets being significantly lower than the smaller ones.^{1,2} In these cases, limited transport of single-celled organisms to the plasma will occur both due to the size limitation and the lysis of biological cells from impacts with the chamber walls. The new proprietary spray chamber (Asperon™ by PerkinElmer, Figure 4) has overcome this limitation and has increased cell transport to the plasma. This is accomplished by modifying the spray chamber design and incorporating new flow patterns within the spray chamber.

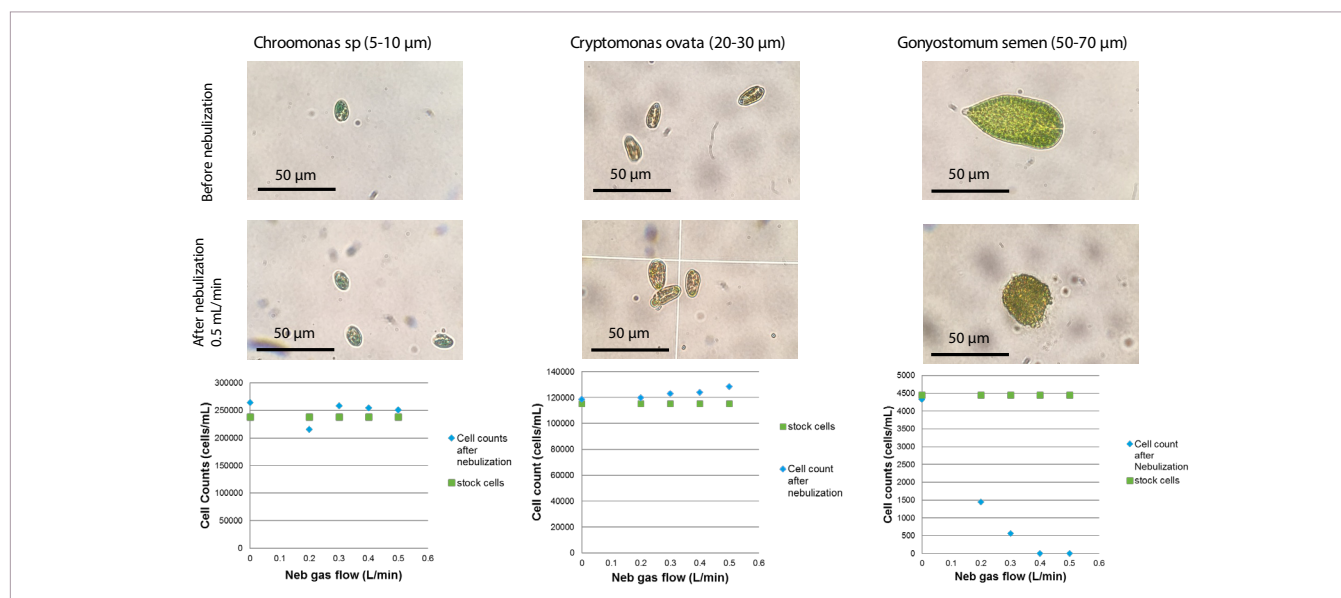


Figure 3. The relationship between nebulizer gas flow and cell viability during cell nebulization for various sizes of cells.



Figure 4. The design of the Cyclonic, double path and Asperon™ SC spray chambers.

5. Detection

The detector is capable of fast acquisition, with dwell times down to 10 μ s and zero settling time allowing for an accurate measurement of the total metal content of each individual cell.

6. Analysis Package

Finally, the data analysis is achieved with a dedicated software package that allows for real-time visualization of the individual cell events and the peak area distribution of the cell population during data acquisition (Figure 5). The peak areas are then converted into mass of metal per cell (ag per cell) and displayed in a histogram of mass per cell against frequency of that mass range.

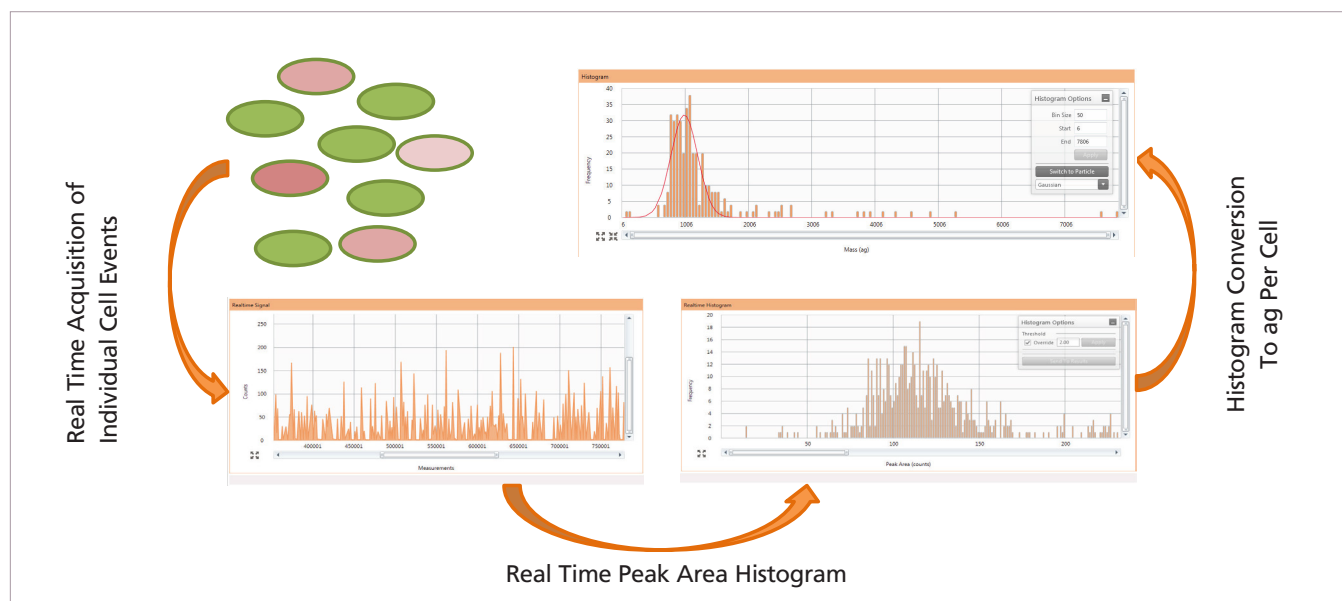


Figure 5. Conversion of metals within individual cells to mass per cell histogram using the Syngistix Single Cell Application Software Module.

Summary

The PerkinElmer Single Cell application module consists of a dedicated introduction system and software module. Together they are capable of:

- Delivering intact individual cells to the plasma
- An increased transport of larger micron sized objects into the plasma
- Mass of metal per cell (attograms / cell)
- Mass distribution per cell population
- Concentration of cells containing metal or nanoparticles
- Number of nanoparticles per cell

References

1. 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.
2. 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 Analytyczna* **2009**, 54, (6), 1219-1244.