

Biopharma

Single cell ICP-MS analysis using the scQuant Plug-in

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Keywords

ICP-MS, metals, single cell,
metallome, biological cells

Goal

To demonstrate how many individual entities, such as single cells collected from a biological system, can be screened for the content of trace metals using the Thermo Scientific™ scQuant Plug-in for the Thermo Scientific™ Qtegra Intelligent Scientific Data Solution™ (ISDS) Software

Introduction

Trace elements play a vital role in biological life, and minor differences in the contents of individual cells can provide insights into a variety of processes and mechanisms from metabolic pathways to drug uptake. It is therefore beneficial to be able to analyze the contents of individual cells.

The analysis of small entities such as individual cells or nanoparticles is based on a measurement principle developed by Degueldre et al.¹⁻⁴ in the mid-2000s. In short, individual packets of ions are analyzed using a time-resolved acquisition mode using short dwell times (less than 10 ms) so that one single measurement corresponds to a single nanoparticle or individual cell.

The scQuant Plug-in allows these types of measurements to be conducted in a guided and user-friendly manner. It contains a series of features to lead the user through every step of the workflow.

Methodology

When an individual cell enters the plasma of an ICP-MS Instrument, it is completely broken down and its contents atomized and ionized. This results in an ion burst (of typically around 0.5 ms in duration) reaching the detector of the ICP-MS instrument, which in turn yields discrete spikes of signal for any chosen analyte appearing in a time-resolved trace on the ICP-MS instrument. The intensity of an integrated signal spike is directly proportional to the mass of that analyte present within the cell processed by the plasma.

The scQuant Plug-in analyzes these integrated signal spikes to determine both the mass distribution of analyte within individual cells (using the integrated signal size) and the number concentration of cells containing that analyte within the sample (by counting the number of events detected in a volume of sample).

Integrated control over sample delivery

Conventional ICP-MS uses a peristaltic pump to deliver the sample to the nebulizer and on to the plasma. However, typical sample introduction systems capable of introducing individual cells intact and with high efficiency typically require low flow rates and create high back pressures, which are incompatible with peristaltic pumps.

The Qtegra ISDS Software comes with two options for sample delivery compatible with any nebulizer and spray chamber suitable for this purpose:

1. A dedicated plug-in is available to allow integrated control of a syringe pump, which permits the direct transfer of flow rates set in the Qtegra ISDS Software to the syringe pump.

Using the dashboard view, the syringe pump can also be used outside of a Qtegra ISDS Software LabBook, for example, to set up and tune the ICP-MS system (Figure 1).

The syringe pump plug-in also allows sample infusion to be stopped automatically after data acquisition is complete so that any sample not required for analysis is preserved for further use or storage (Figure 2).

Syringe metadata, such as a unique name for the syringe for traceability, applied flow rate, and uptake times, can be included in Qtegra ISDS Software Reports for additional traceability.

2. Support is provided for dedicated single cell autosamplers through either direct plug-in control or by using digital IO triggers to control data acquisition.

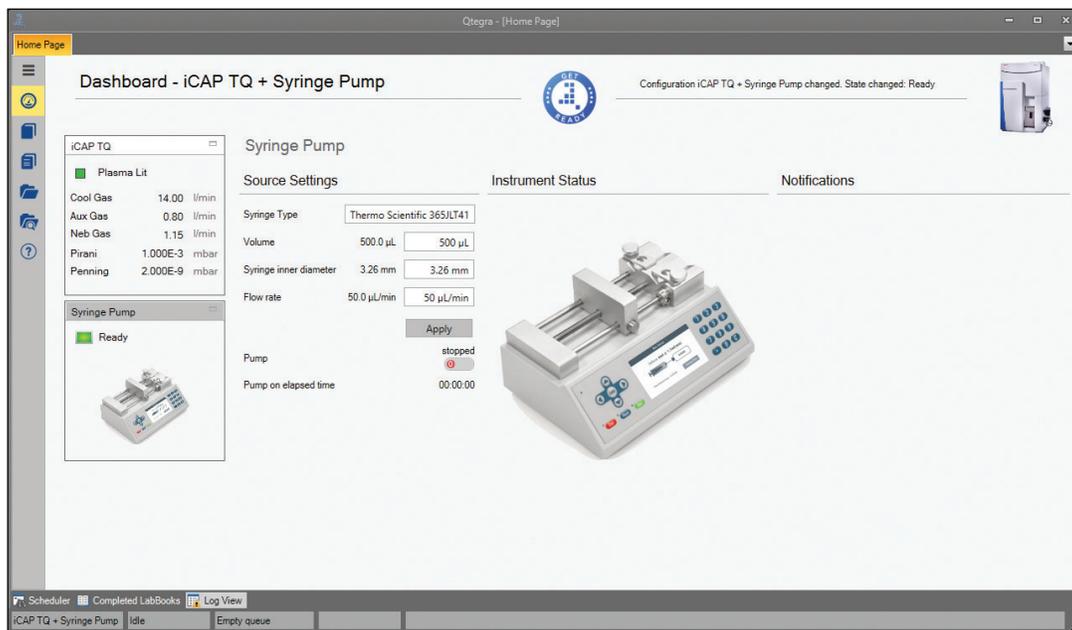


Figure 1. Dashboard controls for the Chemyx Syringe Pump Plug-in for the Qtegra ISDS Software

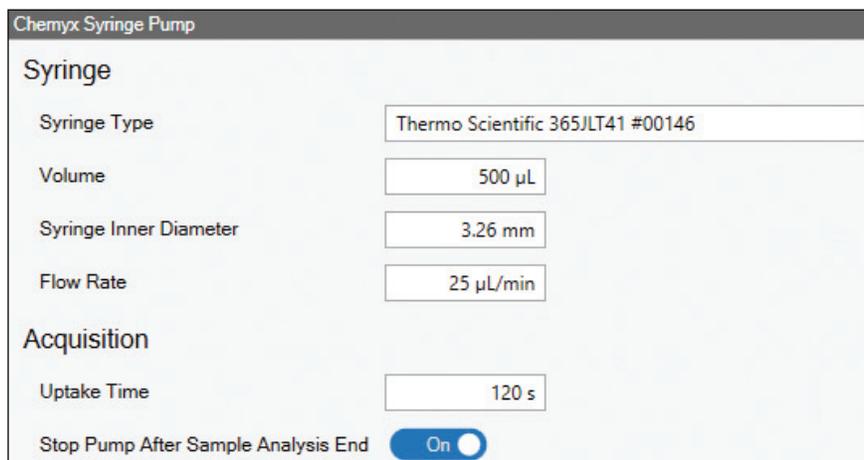


Figure 2. LabBook view for the Chemyx Syringe Pump – here, the user can define whether the pump is stopped between samples and enter metadata for syringe traceability.

Analysis of multiple elements in a single run

In contrast to the analysis of nanoparticles, where the chemical composition of the particles is often known and well defined, the analysis of individual cells may often require an untargeted approach, meaning that a series of elements need to be screened for in a single run.

As an implicit limitation for sequentially scanning instruments, such as quadrupole-based ICP-MS systems, only one element (or, more specifically, one isotope) can be scanned at the time resolution required to resolve signals from individual entities. The scQuant Plug-in allows multiple elements (or isotopes) to be analyzed in a single LabBook, which are then sequentially measured during the analysis without the need to restart the analysis and within a single aspiration of a sample.

This feature allows, for example, measurement of a constituent element to count the cell concentration in the sample suspension, such as phosphorous, while probing for the presence of other elements in the cell population, ultimately allowing the amount present to be accurately estimated and also the proportion of

cells with the target analyte element present to be determined (Figure 3).

For Thermo Scientific™ iCAP™ TQ Series ICP-MS instrument users, the Reaction Finder Method Development Assistant aids with the selection of the most appropriate reaction product ion for the chosen reaction gas in the method.

Automatic determination of key evaluation parameters

Signal discrimination and automatic background determination are achieved using an n -times standard deviation ($n\sigma$) criterion.⁵ The average intensity (\bar{I}) and standard deviation (σ) of the dataset is calculated, and any data greater than $(n\sigma + \bar{I})$ are recorded as cell derived events and removed from the working dataset. This process is repeated iteratively until no more cell-derived events are rejected. The remaining data now represents the background signal, which is the instrument background and dissolved ionic background. The value for n is set to 5 by default but can be changed by the user depending on the analytical requirements.

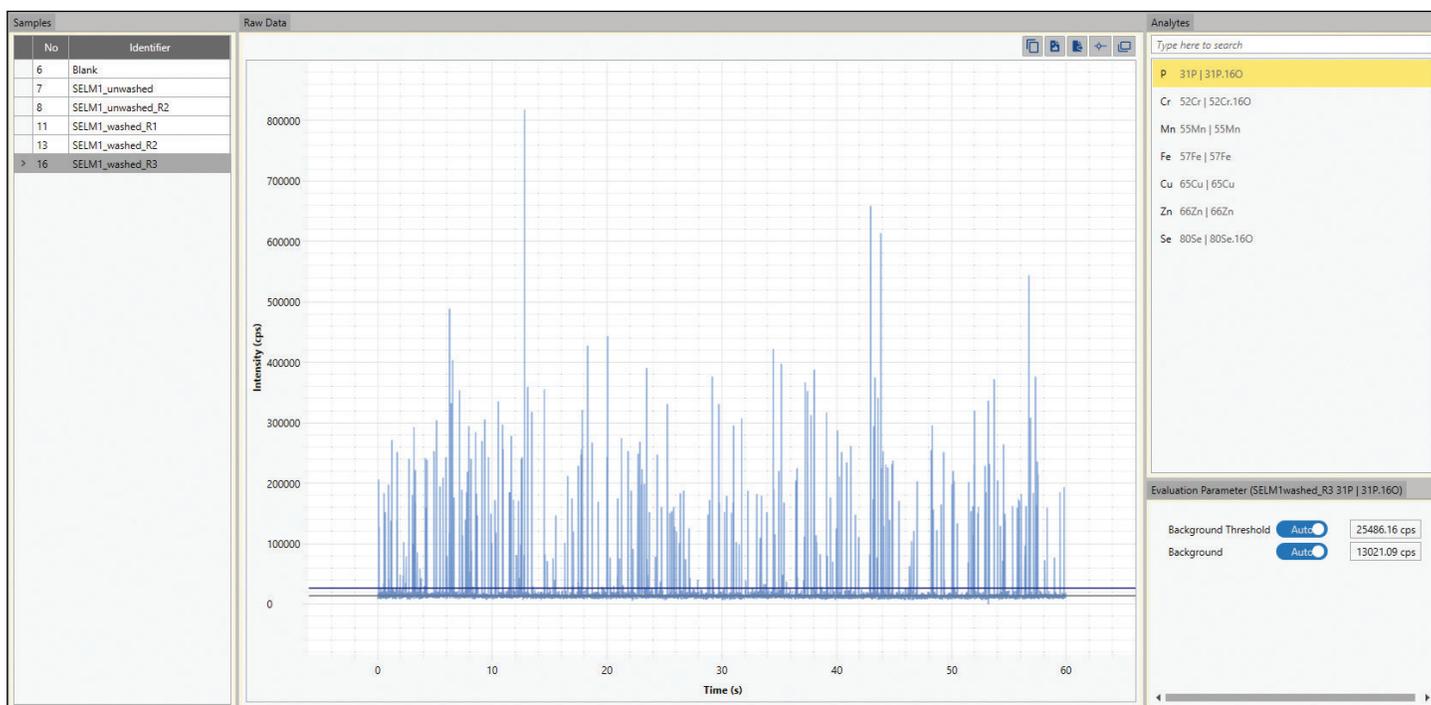


Figure 3. Raw data view in the scQuant Plug-in highlighting the ability for the plug-in to analyze multiple analytes in the same sample infusion

The transport efficiency of the system is a crucial parameter to consider in determining both the mass of analyte contained within each cell and the number of cells in the sample suspension.⁶ There are two ways to estimate transport efficiency—by mass and by counting:

- Transport efficiency by mass accounts for the fact that ionic solutions can partition continuously in the spray chamber between reaching the plasma and being rejected to waste, whereas individual cells (or nanoparticles) cannot. They either wholly reach the plasma, or they do not.
- Transport efficiency by counting not only accounts for the above, but also for physical effects that reduce the number of particles reaching the spray chamber intact, for example static effects or cell lysis during transport.

The scQuant Plug-in is capable of automatically determining both transport efficiencies separately for greater accuracy in each determination, as illustrated in Figure 4. By determining these two transport efficiencies independently, the mass distribution of the analyte within the cells can be determined accurately without being affected by physical phenomena that only affect the transportation (i.e., count) of the individual cells from the sample vial to the nebulizer.

The transport efficiency by mass is therefore only used for determining analyte mass within subsequent unknown sample analyses, and the transport efficiency by counting is used for determining the cell concentration per volume in those samples.

Data visualization

Visualization of results is key to understanding phenotypical differences between different samples, for example, cells exposed to different conditions during an experiment. The scQuant Plug-in offers the ability to visualize the results of an analysis as either mass distribution plots, showing the number of individual events versus the calculated mass as a histogram, or as a box plot, allowing visualization of the locality, spread, and skewness of the data set. Box plots are either presented in a tabbed window in the Total Mass Distribution pane that can be switched to easily or moved and docked in the Qtegra ISDS Software for easy viewing side-by-side with the mass distribution histogram.

A summary of the numerical results is displayed per fraction for each sample underneath the mass distribution or box plots, which enables a full overview of the mass content of the cells in the sample, as illustrated in Figure 5.

Analyte	Calculate Sensitivity	Sensitivity
31P 31P.16O	On	625.26 cps/(µg/L)
80Se 80Se.16O	On	950.13 cps/(µg/L)
197Au 197Au	On	42,735.12 cps/(µg/L)

Figure 4. scQuant Plug-in Evaluation Parameters page showing the transport efficiency calculation by mass and by counting options

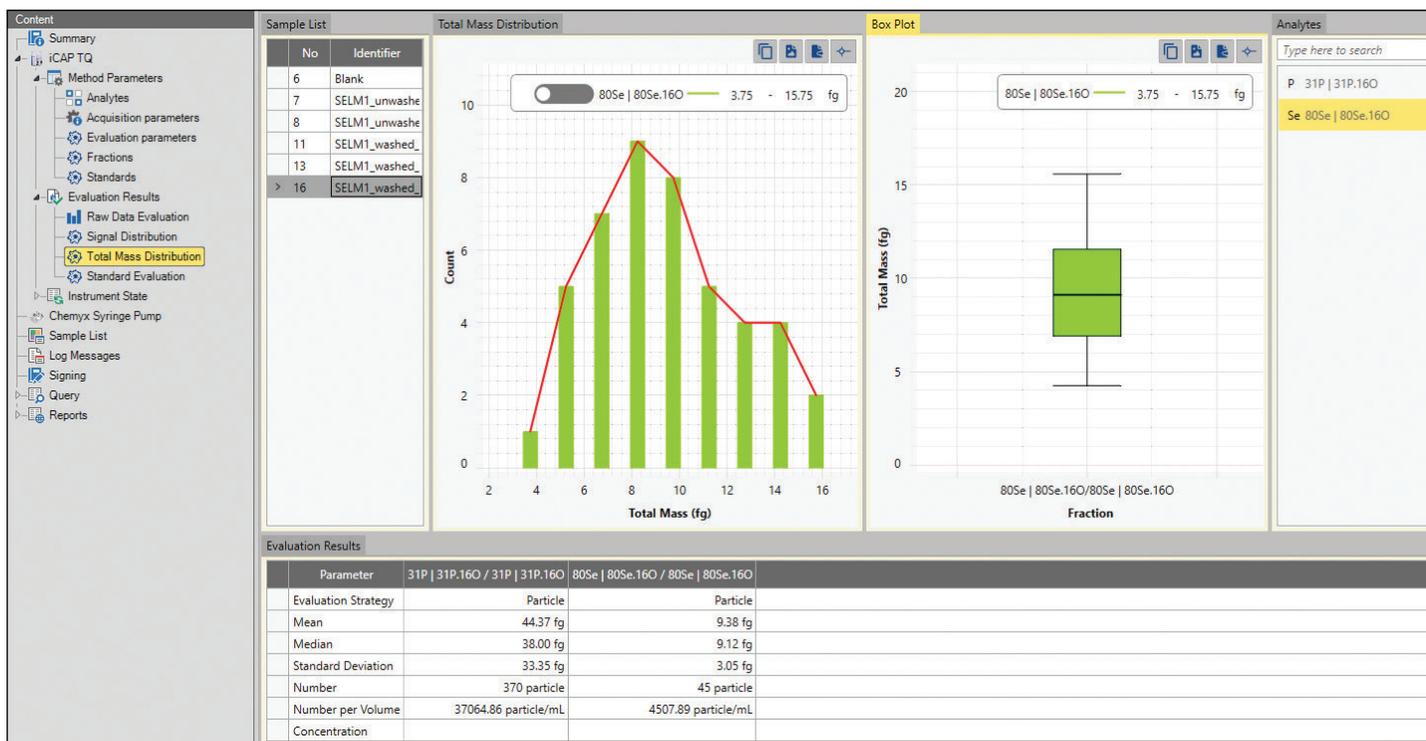


Figure 5. Cell mass distribution histogram and box plots available in the scQuant Plug-in

Conclusion

The scQuant Plug-in for the Qtegra ISDS Software enables the analysis of single cells, as well as other small entities such as microplastic particles, containing ICP-MS detectable elements in an integrated manner. The plug-in is capable of automatically determining key evaluation parameters, calculating accurate mass content of analyte in each cell, and displaying mass distribution, box plots, and summary data for each sample analysis in one place.

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