Application Brief

Clinical Research

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Single Cell Analysis using Agilent 7900 ICP-MS in scICP-MS Mode

Measurement of four elements in single cells using only 100 µL of sample

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Determine the elemental content of intact cells

Expanding the understanding of the role metals play in cell biology is an emerging field of study. Many elements are essential for cell health, and an imbalance, deficiency, or excess may disrupt natural cell processes. Traditional bulk analysis methods for measuring metals in cells rely on sample solubilization, extraction, or digestion, followed by analysis by atomic spectroscopy. The sample preparation step destroys the individual cell structure, so the reported metal concentration results are derived from the mean values measured from thousands of cells.

In single cell-ICP-MS (scICP-MS), intact cells contained in the sample liquid are nebulized so each cell is suspended in an aerosol droplet. Individual cells are introduced into the plasma using an approach similar to the well-established method used for single nanoparticle analysis by ICP-MS (spICP-MS).

High sensitivity ICP-MS with short dwell times

A yeast cell sample was prepared in an aqueous solution. The Agilent 7900 ICP-MS was fitted with a quartz torch with small (1.0 mm) i.d. injector and standard nickel cones. An AIF-3 triple tube nebulizer and spray chamber (both from S.T. Japan) were specially designed for the introduction of intact cells to the ICP-MS. A syringe pump (AS ONE Corporation, Japan) was fitted to the ISIS 3 to control the low sample flow rate to the ICP-MS. The 7900 ICP-MS provides optimum conditions for acquisition of the short-duration signals that are characteristic of single cell and single particle applications. It combines very high sensitivity with short (0.1 ms) dwell times, enabling fast time resolved analysis (TRA) mode. Fast TRA allows single element acquisition at a sampling rate of 10,000 measurements per second with no settling time between measurements. Very high sensitivity is essential to allow detection of attogram (ag, 1.0×10^{-18} g) levels of analytes in single cells.

Multi-element acquisition

Method setup, acquisition, and data processing were performed using the Rapid Multi-Element Nanoparticle Analysis mode of the Single Nanoparticle Application Module of Agilent ICP-MS MassHunter software. Using the software module, multi-element data is collected and combined into a table in ICP-MS MassHunter's Data Analysis pane, shown in Figure 1.

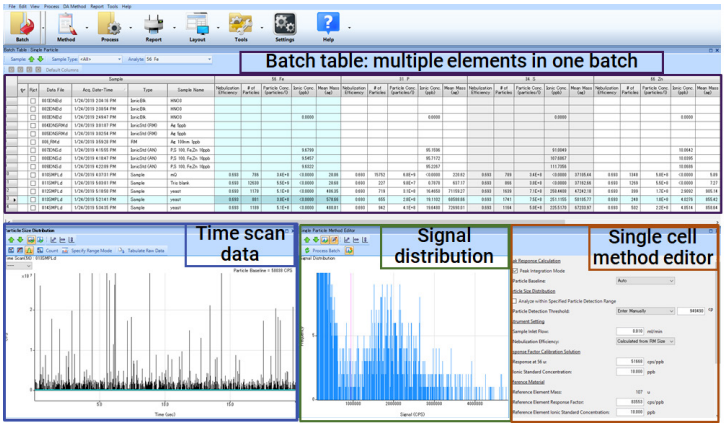

Figure 1. ICP-MS MassHunter data analysis overview. Final single-cell analysis results are reported in tabular and graphical formats.

Cell nebulization and transport efficiency

To establish the cell transport efficiency, the number of cells calculated by ICP-MS was divided by the number of cells counted by microscope. The cell transport efficiency was found to be 25%. Ensuring a high number of cells are nebulized and analyzed improves the accuracy of the data.

Signal distribution

Single yeast cells were analyzed using scICP-MS in multi-element mode. The signal distributions for $^{31}P^{+}$, $^{34}S^{+}$, $^{56}Fe^{+}$, and $^{66}Zn^{+}$ are shown in Figure 2. Each element in single cells can be clearly distinguished from the background. Being able to measure multiple elements in a single acquisition saves time and reduces the sample volume required for each analysis.

The total acquisition time was 240 s from a sample volume of approximately 100 µL. If each analyte had been determined individually, the acquisition time would have been 640 s, requiring a sample volume of 400 µL.

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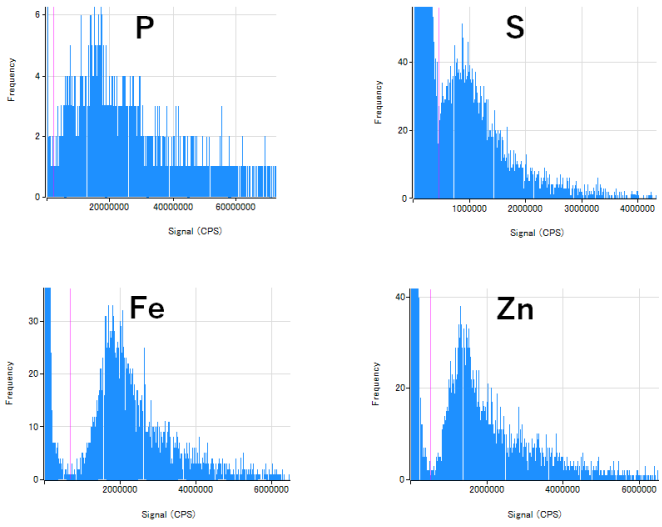

Figure 2. Signal distribution of four analytes in single cells.

Mean mass

The mean mass data for P, S, Fe, and Zn shown in Table 1 was automatically calculated by the ICP-MS MassHunter software. In addition to P and S, which are major components of nucleic acid compounds and proteins, Fe and Zn were measured at sub-femtogram (fg, 1.0 x 10^{-15} g) levels per cell.

Table 1. Mean mass (attogram) and precision of each analyte in single cells (n=3).

Analyte	Mean mass (ag)	RSD (%)
P	70,800	2.4
S	54,900	16.1
Fe	485	0.7
Zn	873	2.6

New research possibilities

The Agilent multi-element scICP-MS method enables the role of multiple metals in cell biology to be measured and explored in detail. The technique provides valuable information on the intrinsic metal content and metal associations in individual cells. scICP-MS can also be expanded to research the uptake, accumulation, and release of metals and metal-containing nanoparticles from cells.

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