Bioprocessing

Assessing the level and distribution of trace elements in individual cells using single cell ICP-MS analysis

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Abstract

Purpose: To demonstrates how ICP-MS can be used to assess the level of trace elements in individual yeast cells and display the mass distribution across a cohort of several hundred cells.

Methods: A triple quadrupole ICP-MS system, in conjunction with the scQuant plug-in for Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution software was used for all measurements.

Results: The levels of selenium and phosphorous were determined in selenized yeast successfully across several hundreds of individual cells.

Introduction

Trace elements play a key role in many processes involved in living beings. Some elements are found as constituent elements in biopolymers, such as phosphorous as part of the DNA backbone, or sulfur, as part of the key amino acids methionine and cysteine involved in the formation of proteins covering a wide variety of functions. Other elements (in particular, the transition elements copper, iron, and zinc) are involved in specific functions, such as acting as co-factors in enzymes. However, the distribution of trace elements cannot be assumed to be homogeneous across a cell cohort, even under ideal conditions. Ultimately, cell-to-cell variability of the metal content may be an important factor in understanding biological diversity. The analysis of specific biomarkers at the single cell level has therefore gained significant interest in recent years.

The analysis of the content of a given metal at the single cell level has remained a challenge. While inductively coupled plasma mass spectrometry (ICP-MS) is well known as a powerful and element-selective detection system, it is only recently that its capacity for analyzing individual cells has been recognized.

Materials and methods

Sample Preparation

Lyophilized yeast cells (SELM-1 certified reference material, National Research Council of Canada, Ottawa, Canada) were resuspended in water, washed twice by centrifugation, and diluted to a final concentration of around 50,000 yeast cells per mL in water (the exact cell count was determined by flow cytometry before the measurement). After calibration of the system using single element standards gravimetrically diluted to the appropriate concentration range, the yeast cells were analyzed for phosphorous (as a marker for the total number of cells) and selenium (to determine the number of cells that contain relevant amounts of selenium). For measurements of phosphorus and selenium, TQ-O_2 mode was selected to induce mass shift from $^{31}P^+$ to $^{31}P^{16}O^+$ and $^{80}Se^+$ to $^{80}Se^{16}O^+$, respectively, after reaction with oxygen in the reaction cell. This allowed the otherwise abundantly present polyatomic interferences on both elements to be removed from the respective analyte signals.

Test Method(s)

A Thermo Scientific™ iCAP™ TQ ICP-MS was used for all measurements. The instrument was equipped with a specialized nebulizer and spray chamber to allow the introduction of single cells with high transport efficiency (Glass Expansion, Port Melbourne, Australia). To achieve this, the sample flow rate had to be reduced significantly, such that sample delivery was accomplished using a syringe pump (Chemyx, Stafford, Texas, USA) instead of the conventional peristaltic pump commonly used.

Table 1. Typical instrument parameters used in this study.

Parameter	Value
Nebulizer	MicroMist HE U-Series Nebulizer
Spray chamber	Total consumption spray chamber
Sample delivery	Syringe pump
Sample flow	10 µL/min
Forward power	1550 W
Nebulizer gas flow	0.51 L·min^{-1}
Sheath gas flow	0.65 L·min^{-1}
Interface Configuration	High Sensitivity
Analysis time	60 s per element, total duration 240s incl. uptake and wash
CCT Settings	
CRC gas flow	0.35 mL·min^{-1}, 100% O_2

Data Analysis

The scQuant Plug-in was used for method creation and data evaluation. The Qtegra ISDS Software also contains a dedicated plug-in to integrate the operation of the syringe pump into the overall workflow, so that manual steps, for example rinsing, priming the system, and starting the sample delivery for data acquisition, can be accomplished in the main user interface of the Qtegra ISDS Software.

Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution Software

scQuant plug-in for Qtegra Software

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.

Figure 1: The scQuant plug-in for Qtegra software allows sequential multi-element analysis in a single run

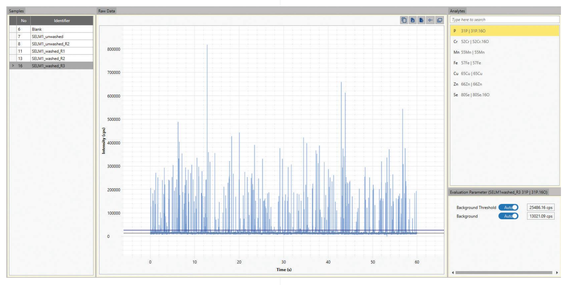
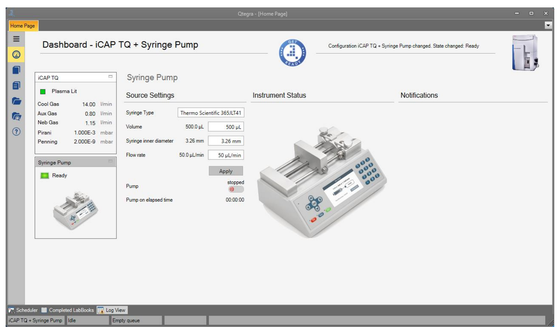


Figure 2: For operators working with a syringe pump for sample delivery, a dedicated plug-in is available to fully integrate the syringe controls in a single workflow



Workflow

As the signal duration of a typical cell derived transient signal is only in the range of 0.5-1 ms, it is not possible to scan two elements (or isotopes) on a single signal, as the mass jump of the quadrupole would be too slow. The scQuant plug-in therefore enables the sequential scanning of a sample for two (or more) elements in a single aspiration. This functionality was applied here, and all elements under investigation were screened for an identical period of time. The results are subsequently evaluated and displayed as one integral data set.

Determination of detection sensitivity

The detection sensitivity for all elements under study was determined using the scQuant module for Qtegra software. An instrumental detection limit of 0.2 µg·L^{-1} was achieved for selenium, which corresponds to a minimum detectable of 0.17 fg per cell.

Determination of transport efficiency

The transport efficiency of the liquid elemental standards for mass determination was calculated using 30 nm gold nanoparticles (LGCQC5050 Colloidal Gold Nanoparticles, LGC, Teddington, UK), as previously reported (4). The transport efficiency was usually found to be in the typical range between 50-70% using the proposed setup. On the day when the measurements described in the following were performed, the transport efficiency was determined to be 65%. This value was used for all subsequent calculations using the scQuant plug-in.

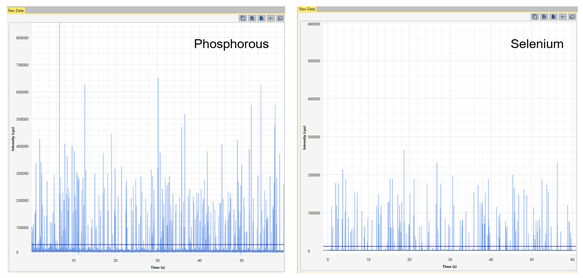
Sample analysis

Results

Analysis of selenized yeast

Figure 1 displays the untreated signals resulting from the measurement of selenized yeast cells using the proposed method. As can be seen, a series of signals is recorded for both traces, $^{31}P^{16}O^+$ and $^{80}Se^{16}O^+$, corresponding to the presence of the elements in single cells. The cell concentration in the measured solution was low enough so that only a single cell is entering the plasma at a time (and is subsequently becoming ionized and detected).

Figure 3: Raw data for the measurement of phosphorous and selenium in selenized yeast cells



The average signal intensity for phosphorous was found to be in the range of 165000 cps, whereas signals obtained for selenium were found to show an average signal intensity of 46000 cps. The number of detected signals per unit time was also slightly lower for selenium in comparison to phosphorous, which can be explained by the fact that each cell contains a significant amount of phosphorous as part of the DNA stored in the cell core, whereas only a fraction of all cells contain detectable amounts of selenium.

A quantitative assessment of the signals allows to determine the number of cells containing a detectable amount of each of the elements, as well as the average mass and its distribution across the full cell cohort. This data is summarized in Tables 2 and 3.

Table 2: quantitative assessment of the intracellular amounts of phosphorous, selenium in selenized yeast cells (SELM-1 CRM).

	Average content [fg/cell]	Cells measured		Number concentration [1/mL^{-1}]	
		Run 1	Run 2	Run 1	Run 2
Phosphorous	0.94 ± 0.56	309	152	47617	23423
Selenium	0.79 ± 0.33	285	175	43923	26970

Table 3: % Fraction of detected cells after cell count and fraction of selenium containing cells vs. measured cells

	Fraction [vs. Total # of cells, %]	Fraction of Selenium containing cells [vs. measured cells, %]
Phosphorous	68.3± 5.7	n.d.
Selenium	n.d.	57.8 ± 15.6

The measurement of phosphorous allows the number of cells entering the plasma in a given measurement to be counted. In comparison to the total number of cells, previously determined using flow cytometry, the number of cells counted using the ICP-MS measurement based on the phosphorous signals returns only 68%. This number corresponds to the transport efficiency of these cells into the system. The cells that are lost during the transport, however, can be accounted for in the single cell ICP-MS measurement.
The distribution of selenium across the cell population is less homogenous. Comparing the cell number determined by the signals obtained via selenium versus the number determined previously using phosphorous, it becomes clear that not every cell contains selenium at levels above the determination level.

- ✓ If present in the cell, occurs in amounts beyond a clear threshold enabling its detection. This is true for approximately 57% of all cells.
- ✓ The detectable amount of selenium was found to be from 2.50 fg to 72.50 fg with a broad distribution of the selenium content within the cell population.
- ✓ For phosphorous, the amount found in the population under investigation here was 37.0 fg (mean) and 30.9 fg (median) (standard deviation ± 23.1 fg).
- ✓ Although the measurement reveals a lower standard deviation for selenium (in comparison to phosphorous), this includes only the cells that have been detected in the measurement.

Figure 4 displays the distribution of the detected content in all measured cells. For both elements, the distribution is displayed in a histogram (left column) as well as a box plot (right column of the figure). For the histogram view, the bin size can be freely applied by the user.

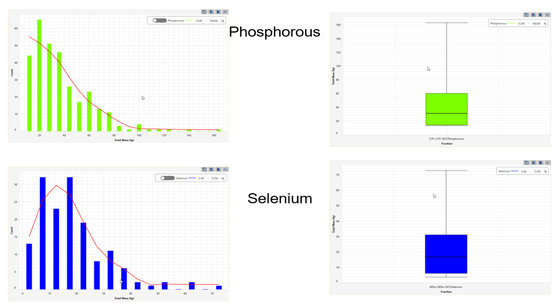


Figure 4: Mass distribution for phosphorous and selenium in selenized yeast, left column shown as a histogram (bin size 8 fg for phosphorous and 5 fg for selenium).

Conclusions

This poster highlights how ICP-MS operated in the single cell mode can determine the amount of different trace elements in individual cells and can therefore allow not only assessment of the average element mass per cell, but also the element distribution across the cohort.

- Scanning for multiple elements is possible using a sequential approach, in which all the elements of interest are measured for an identical period in a single aspiration of a sample, and the results are summarized in a single data set.
- The scQuant plug-in allows integrated control over sample delivery devices, in this case, a syringe pump, to facilitate a stable sample flow at the required low flow rates.
- Key method parameters, such as detection sensitivity and transport efficiency can be determined as part of the same Qtegra ISDS Software LabBook or can be independently determined (if no suitable standards are available) and applied to a data set.
- The data visualization features of the scQuant plug-in allow raw data and intermediate data (signal distribution) to be comprehensively evaluated, as well as enabling representation of results in either a histogram or a box plot. All results can be exported in a spreadsheet compatible format if required.

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References

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3. Álvarez-Fernández García, R.; Corte-Rodríguez, M.; Macke, M.; LeBlanc, K.L.; Mester, Z.; Montes-Bayón, M.; Bettmer, J. Addressing the presence of biologic selenium nanoparticles in yeast cells: analytical strategies based on ICP-TQ-MS; Analyst 2020, 145, 1457–1465.

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