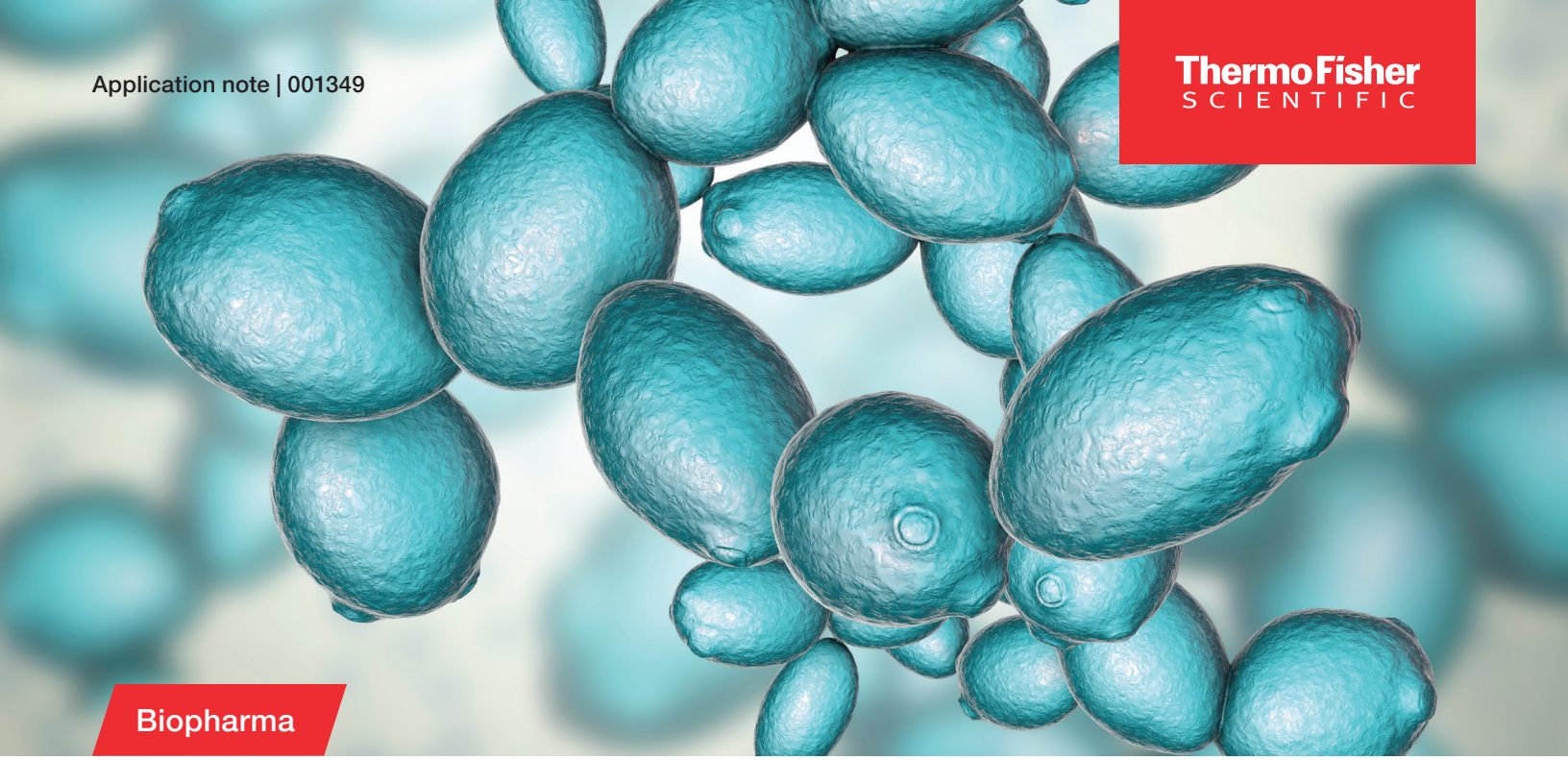
Application note | 001349



Biopharma

Assessing the level and distribution of selenium in selenized yeast cells using single cell ICP-MS analysis

Authors

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Keywords

Selenized yeast, single cell analysis, scQuant plug-in, ICP-MS, TQ-ICP-MS

Goal

This application note 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.

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.

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This application note demonstrates how single cell ICP-MS can help to determine the presence of individual trace elements using selenized yeast (*Saccharomyces cerevisiae*) as an example. Selenium supplementation with enriched yeast has been proposed as a preventive measure to reduce the incidence of cancer among the general public; however, a clearly identified compound or a conclusive mechanistic explanation has not yet been found.[1,2] Selenium can be present in a wide range of chemical forms in yeast, including organic (i.e., selenomethionine), and inorganic species (selenite and selenate), as well as selenium-containing nanoparticles.[3] As the identification of the species involved in a given cell culture requires the use of hyphenated techniques such as HPLC-ICP-MS, the use of a direct approach to scan the selenium content (and its variability under given conditions) can be viewed as an interesting option for characterization directly in fermentation processes. This can be accomplished using ICP-MS operated in a so-called single cell acquisition mode that allows direct analysis of a dilute cell suspension, the number concentration of which typically does not exceed 50,000 cells per mL of solution. In a time-resolved analysis of typical duration below 5 minutes, several hundred individual cells can be analyzed for trace elemental content of multiple analytes, and the results can be displayed using a mass distribution histogram or alternative visualization approaches, including, for example, box plots.

Experimental

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. The Thermo Scientific™ scQuant Plug-in available for the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software 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.

Sample preparation and analysis

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.

Data were acquired using time-resolved analysis mode at a dwell time of 5 ms. All typically applied parameters are summarized in Table 1.

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	Chemyx™ Fusion 100-X syringe pump.
Sample flow	10 µL/min
Forward power	1,550 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, 240 s total duration (including uptake and wash)
CCT settings	
CRC gas flow	0.35 mL·min^{-1}, 100% O_2

The detection sensitivity for all elements under study was determined using the scQuant Plug-in for the Qtegra ISDS Software. Typically, an instrument detection limit of 0.2 µg·L^{-1} was achieved for selenium, which corresponds to a minimum detectable amount of 0.17 fg per cell.

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.[3] These particles are chemically stable and well characterized not only in size (nominal diameter of 30 nm, particle modal diameter of 32.7 nm), but also with respect to the particle number concentration (1.47×10^{11}) and mass fraction of gold in solution (45.1 mg·kg^{-1}). The transport efficiency was usually found to be in the typical range of 50 to 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.

As the signal duration of a typical cell derived transient signal is only in the range of 0.5 to 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 measured for an identical period of time. The results were subsequently evaluated and displayed as one integral data set.

Results and discussion

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 was 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 to ensure that only a single cell was introduced to the plasma, ionized, and detected during one measurement duration.

The average signal intensity for phosphorous was found to be in the range of 165,000 cps, whereas signals obtained for selenium were found to show an average signal intensity of 46,000 cps. Additionally, the number of detected signals per unit time was also slightly lower for selenium in comparison to phosphorous. This is because 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 the determination of 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 Table 2.

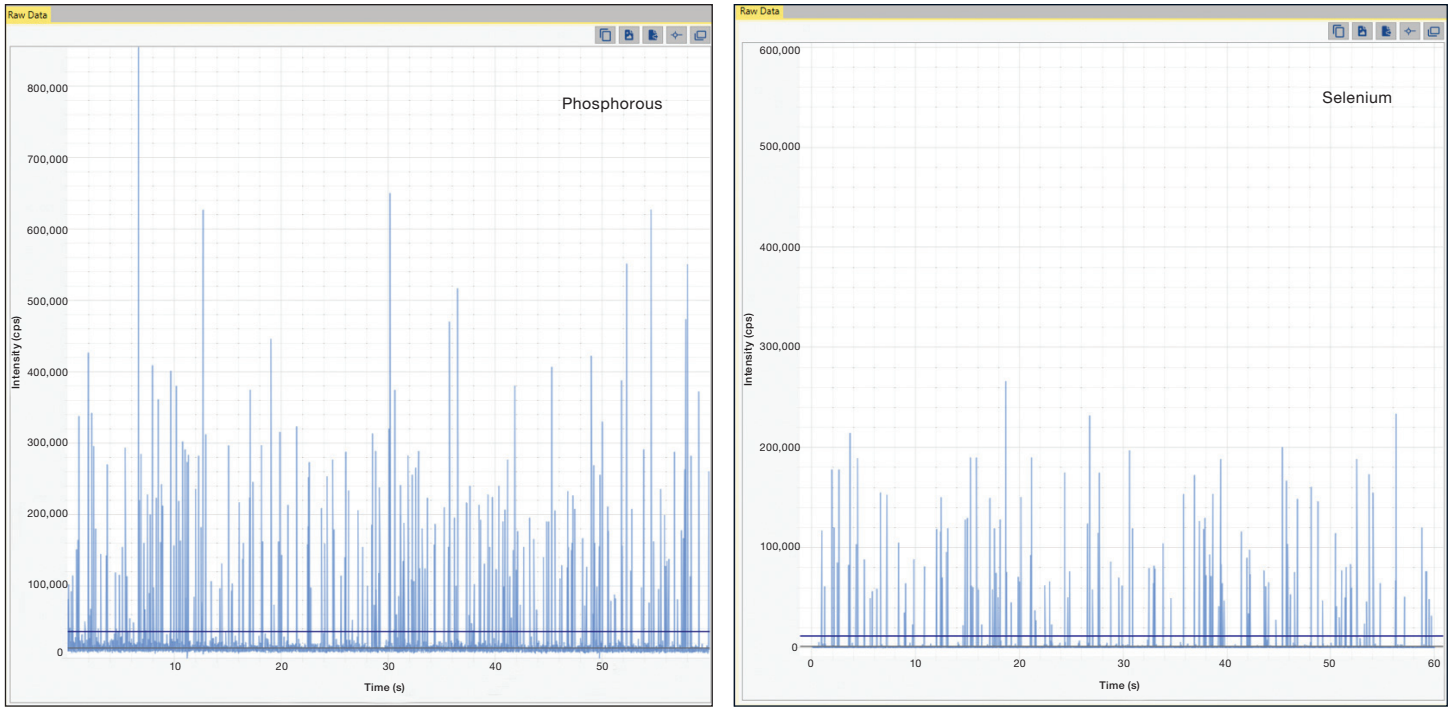


Figure 1. Raw data for the measurement of phosphorous and selenium in selenized yeast cells

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

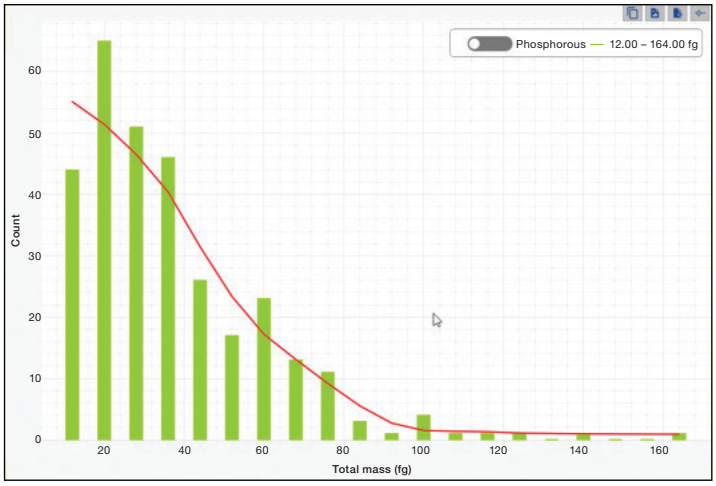
	Average content [fg/cell]	Number of cells measured		Number concentration [cells/mL^{-1}]		Fraction [vs. total # of cells, %]	Fraction of selenium containing cells [vs. measured cells, %]
		Run 1	Run 2	Run 1	Run 2		
Phosphorous	0.94 ± 0.56	309	152	47,617	23,423	68.3± 5.7	n.d.
Selenium	0.79 ± 0.33	285	175	43,923	26,970	n.d.	57.8 ± 15.6

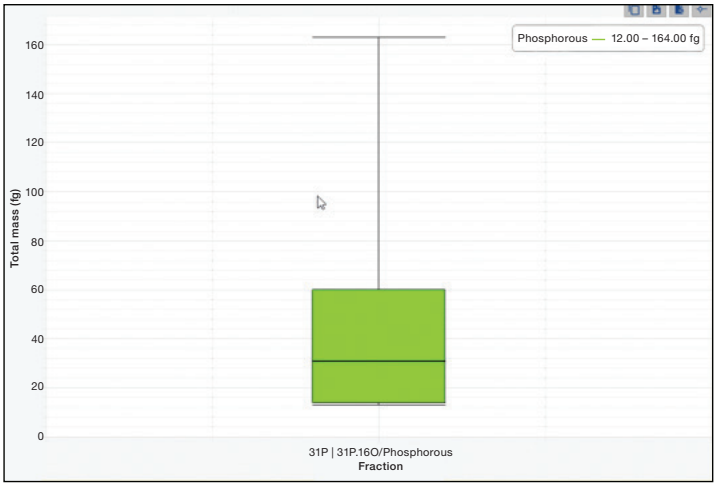
As can be seen from the data, the measurement of phosphorous allows the number of cells entering the plasma in a given measurement to be counted. In comparison to the total number of cells, previously determined using flow cytometry, the number of cells counted using the ICP-MS measurement based on the 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. However, the data delivers a clear indication that selenium, if present in the cell, occurs in amounts beyond a clear threshold enabling its detection. This is true for approximately 57% of all cells, where the detectable amount of selenium was found in the range between 2.50 fg to approximately 72.50 fg There is a broad distribution of the selenium content within the cell population, which is demonstrated by the difference in the mean and median value, corresponding to 18.6 fg (mean) and 16.8 fg (median) per cell (standard deviation ± 12.5 fg). 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. When extrapolating the number of cells in solution, the inhomogeneity would become much more obvious.

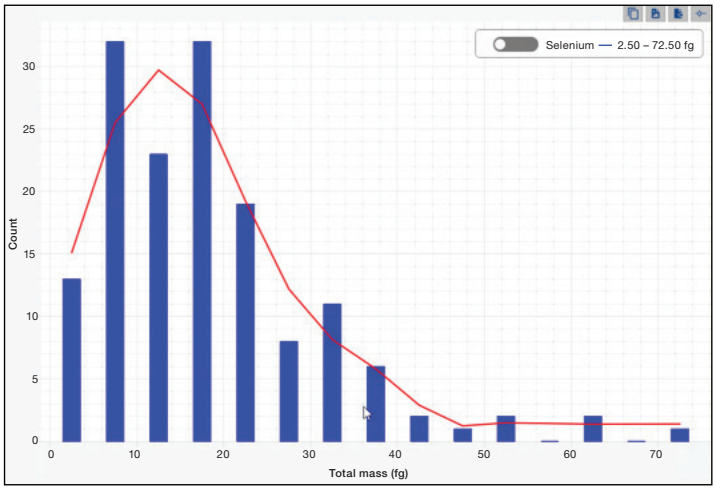
Figure 2 displays the distribution of the detected content in all measured cells. For both elements, the distribution is displayed in a histogram (left column) as well as a box plot (right column of the figure). For the histogram view, the bin size can be freely applied by the user.

Phosphorous





Selenium



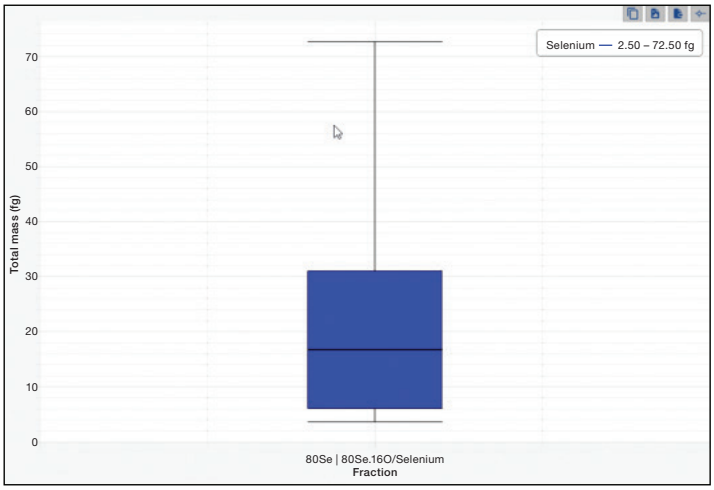


Figure 2. Mass distribution for phosphorous and selenium in selenized yeast, left column shown as a histogram (bin size 8 fg for phosphorous and 5 fg for selenium)

Conclusion

This application note 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. This is made possible by the scQuant Plug-in for the Qtegra ISDS Software. The core features of this software tool provide the following benefits to the user:

- 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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