# Multi-element determination in populations of single cells by quadrupole ICP-MS

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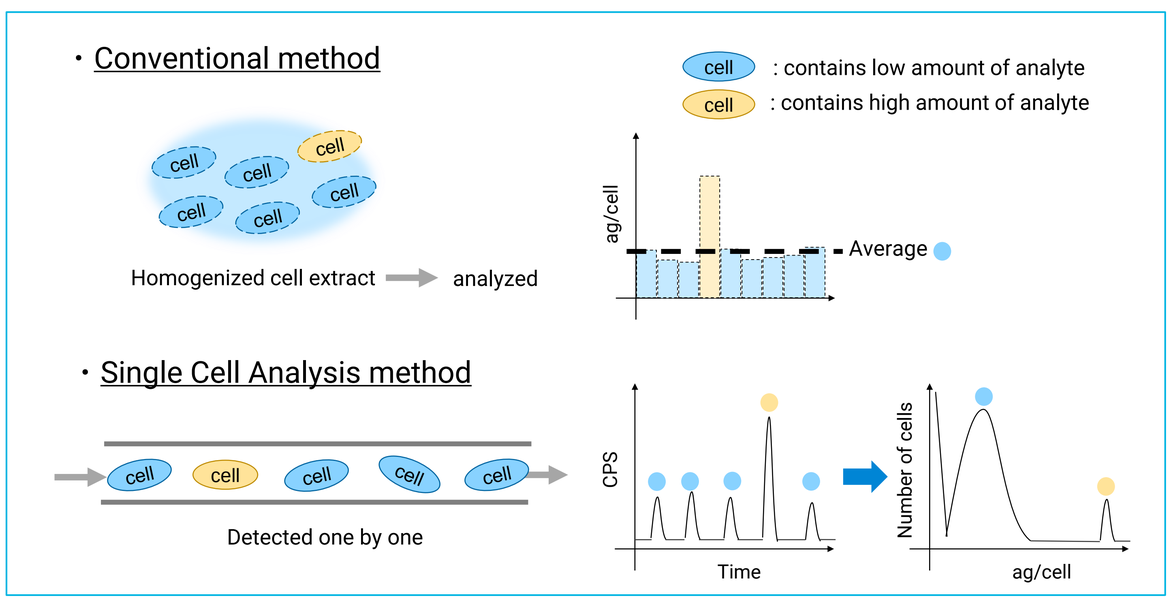
## Introduction

### What is “Single Cell Analysis” ?

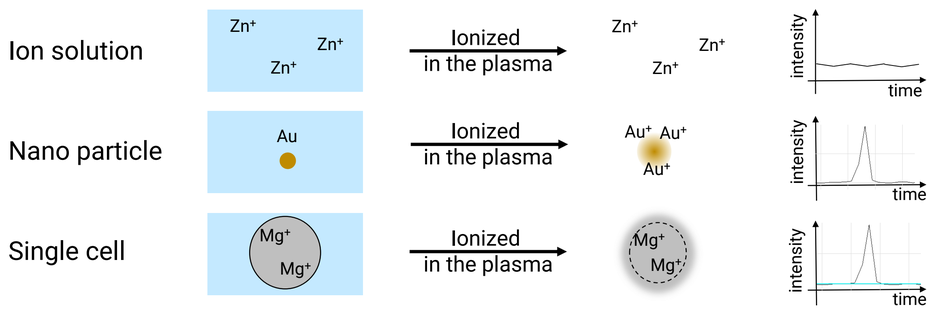
Elemental quantification of cells for life science research has been performed by measuring analyte concentration as an average value in cell extraction. However, this method assumes that the cell population is homogenous, and therefore it might miss minority cell clusters which exhibit significant differences from the normal major cells.

In contrast, single cell analysis allows cells to be injected individually, enabling us to understand the detailed elemental profiles of cell clusters. Furthermore, multi-element scanning in single cell analysis enables to acquire high-dimensional parameters of cell components tagged by antibodies which contain rare metals. [1]

In this study, we developed the new hardware and software for the single cell analysis by ICP-MS and measured the amount of elements in a single yeast cell.




Single cells are ionized in the ICP and detected as pulse signals such as nano particles signals.

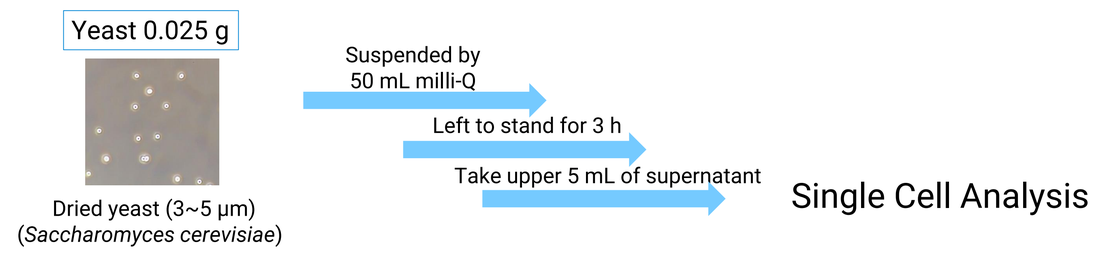



## Experimental

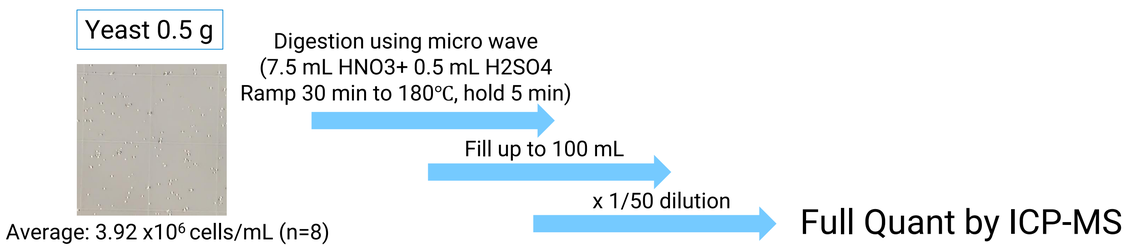
There are no reference materials of single cells, then the accuracy of measured amount of analytes was confirmed by cross checking using digested cell sample. [2]

### Method

#### ・Suspending and injecting the single cell sample




#### ・Cell digestion using microwave




(Concentration)/(number of cells)=(amount of analyte per cell)

### Instrumentation

- Agilent 7800/7900 ICP-MS (single Quadrupole ICP-MS)
- Software: Mass Hunter 4.4, Fast TRA Multi-Element Screening Mode
- 1.0 mm id quartz torch (G3280-80081)
- Total consumption nebulizer (G3280-80602) & single pass spray chamber (for CAP LC) (G3280-80603)
- Gas Mode

  No Gas mode : Au, Mg, P, Mn, Co, Cu,
  He mode : K
  H2 mode : Ca, Fe

Total consumption nebulizer (G3280-80602) & single pass spray chamber (G3280-80603)

Table 1. Instrument & Data analysis parameter

| parameter | value |
|---|---|
| RF power | 1600 W |
| Sample Depth | 7.0 mm |
| Nebulizer Gas | 0.77 L/min |
| Nebulizer Pump | 0.05 rps |
| Dilution Gas | 0.10 L/min |
| Cell Gas $H_2$ | 4.0 mL/min |
| Cell Gas He | 3.4 mL/min |
| dwell time | 0.1 msec |
| Acq time | 20 sec |

## Results and Discussion

### Time Scan Data

- Approximately 500 pulse signals were observed in 20 sec.
- The width of the signals was 0.4 msec.

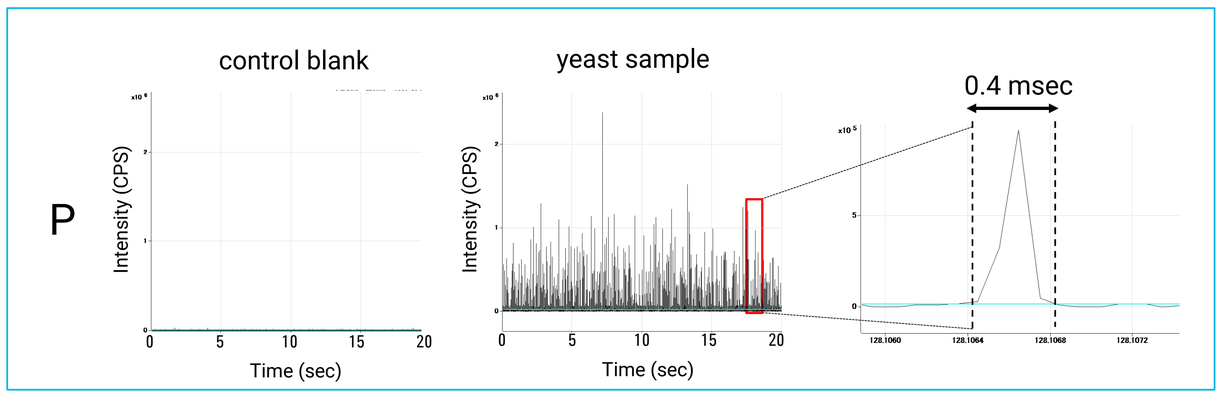



Figure 1. Time scan data and zoom-in

### Signal Distribution

- The result of this study indicates that the newly developed method can separate the analyte signals from background noise clearly.
- 16 elements were measured in one sequence, and in terms of Au, Mg, P, K, Mn, Fe, Co, Cu, and Zn, analyte signals were separated from background noise.
- The number of cells detected were the same level among the multiple elements, which suggested the density of cells in a sample was stable in one sequence.

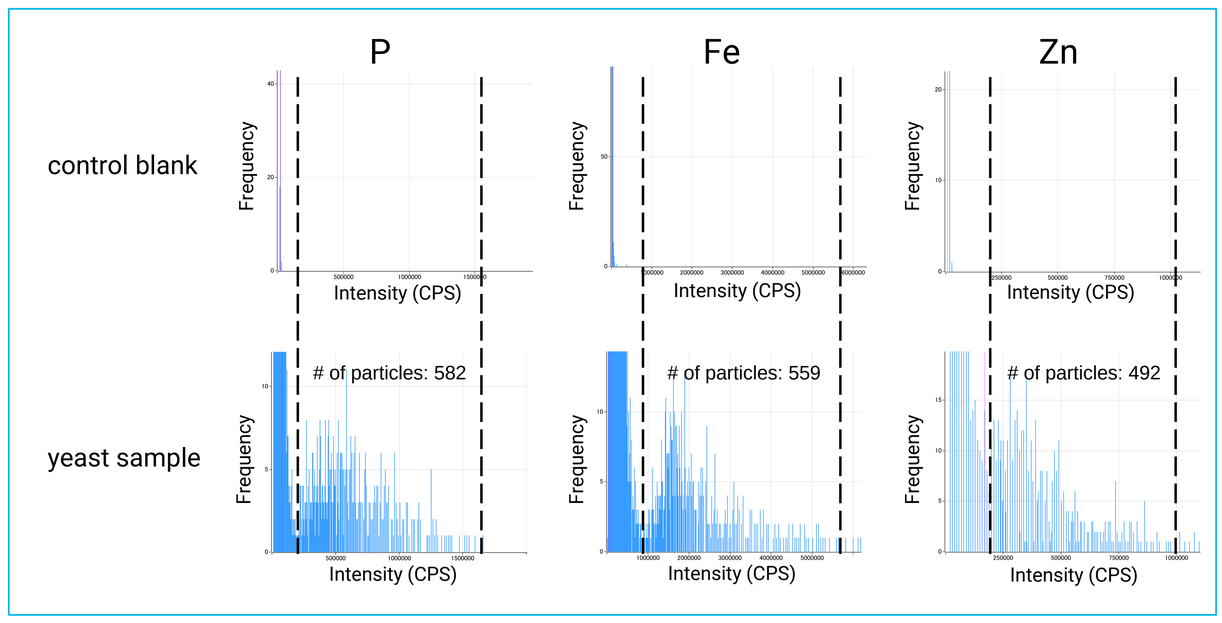



Figure 2. Signal distribution

### Cross Checking of the measured amount of analytes

- Thresholds between noise and signal were set manually. Threshold values mean the lowest limits of ag/cell detected as cells in this method.
- The amount of elements in single cell sample was about half as much as that of digested yeast sample. In terms of the signal distribution, it is not always clear to separate signals from noises clearly. Also cell counting results are not always stable, which might affect the result of digested cell. These factors need to be improved.

Table 2. The value of minimum , the amount of analytes in single cell, and that of digested cell

| Mass & Element | Single Cell | | Digested Cell |
|---|---|---|---|
| | Lowest limit (ag/cell) | Mean mass (ag/cell) | Mean mass (ag/cell) |
| 24 Mg | $4.25 \times 10^{2}$ | $4.37 \times 10^{3}$ | $1.23 \times 10^{4}$ |
| 31 P | $2.26 \times 10^{3}$ | $8.04 \times 10^{4}$ | $1.39 \times 10^{5}$ |
| 39 K | $3.56 \times 10^{4}$ | $1.21 \times 10^{5}$ | $3.08 \times 10^{5}$ |
| 55 Mn | 31.9 | 63.1 | $1.11 \times 10^{2}$ |
| 56 Fe | $2.42 \times 10^{2}$ | $3.82 \times 10^{2}$ | $6.06 \times 10^{2}$ |
| 59 Co | 6.00 | 19.7 | 51.7 |
| 63 Cu | 16.4 | 44.0 | 96.0 |
| 66 Zn | $2.24 \times 10^{2}$ | $8.39 \times 10^{2}$ | $1.37 \times 10^{3}$ |

(1.0 ag = $1.0 \times 10^{-18}$ g)

## Conclusions

- Agilent 7800/7900 ICP-MS using newly developed hardware and software enables to perform the Single Cell Analysis.
- It is possible to measure multiple-elements within the same batch, and calculate the amount of analytes in the cells automatically by the new software.
- The result of single cell analysis showed a similar order of magnitude to that of digested cell sample.

## References

[1] C. Giesen, H. A O Wang, D. Schapiro, et al., Nature Methods 11, 417-422 (2014)
[2] A. S. Groombridge, S. Miyashita, S. Fujii, et al., Anal Sci. 2013;29(6):597-603.