

## **Time-resolved ICP-MS measurement for single-cell analysis and on-line cytometry**

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### **Method development**

The criteria for the selection of the model cell for the development of the time-resolved ICP-MS method of single-cell analysis are: (1) the cell poses no biohazard, (2) the cells exist individually in nature, (3) the cells are relatively tough and can survive the process of nebulization, and (4) each cell contains sufficient quantity of metal(s) for ICP-MS measurement. Green algae appear to meet all the criteria. Natural water generally contains multiple species of algae and the distribution of size and metal contents of the mixture is ill-defined. In this study, a single species, *Chlorella vulgaris*, was selected. The abundant quantity of chlorophyll in the cells provides sufficient quantity of Mg and the detection of the cells was successful in the first trial.

Typical experimental set up and operating parameters of the ICP-MS for solution samples were used with only a few changes. The sampling depth was 0.5 mm larger to increase the signal-to-background ratio of Mg measurement. Time resolved analysis (TRA) mode of operation was needed for single cell analysis. Micro-syringe pump was used to give precise sample uptake rate for cell counting. The other experimental

set up and operating parameters of the ICP-MS are specifically selected to be similar to those used in solution analysis so that the single-cell analysis method can be used in any laboratories equipped with an ICP-MS.

An important criterion of successful ICP-MS measurements of individual cells is that the cells do not overlap during the integration duration of each data point, *i.e.*, one ICP-MS intensity spike corresponds to one single cell. The integration duration of the ICP-MS used in this study is 10 ms, the sample introduction rate into the ICP must be  $<100$  /s. However, for proper sampling of the cells by the ICP with statistically insignificant degree of spike overlap, the sample introduction rate was further reduced to 4 /s in this study. The sample uptake rate and the number density of the algae suspension were carefully selected to meet the requirement (Section 3.2 of the manuscript).

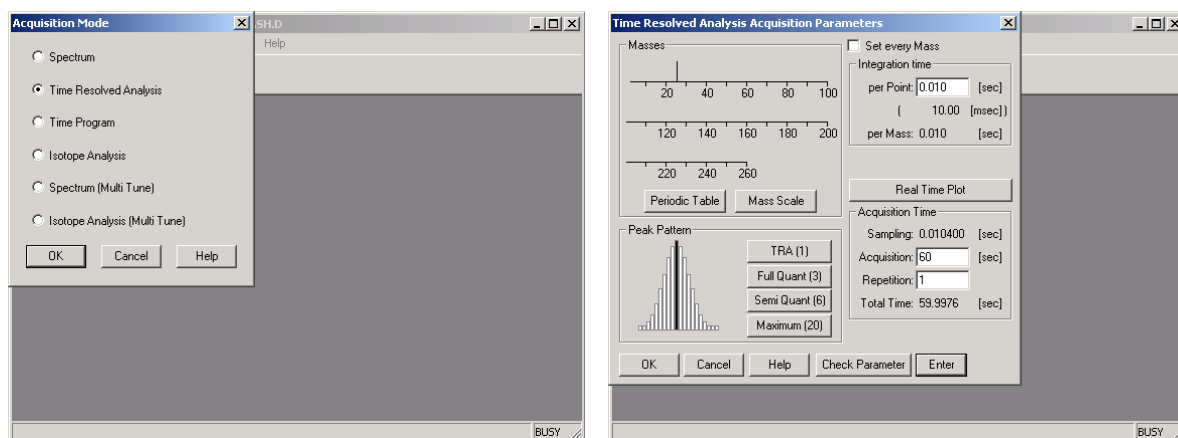
The number density and size distribution of the algal cells in a suspension were determined using a hemacytometer under an optical microscope. Microscopic examination of the cells is also necessary to ensure that the cells are in unicellular, not colonial, form and that the cells are intact. A low-cost digital eyepiece equipped with a CMOS array sensor of  $1280 \times 1024$  pixels was used for viewing and photographing the algae cells at  $1200 \times$  magnification. Under this magnification, 1 pixel corresponds to  $0.1 \mu\text{m}$ . Typical algal cell of diameter of  $3.4 \mu\text{m}$  takes up 34 pixels. The error in the size measurement of the algal cells is, therefore,  $\pm 1.5\%$ . The digital eyepiece enables rapid determination of the size distribution of a relatively large number of cells from the digital photographs of the cells. Measurement of the cell size directly under the microscope is tedious and inaccurate.

The strategy of the calibration method using the peak maximum of the ICP-MS spike intensity distribution of MgO particles is unique. As discussed in the manuscript, polydisperse particles can be used as calibration standard. The sample uptake rate of the MgO suspension into the ICP-MS is not important as long as the ICP-MS spikes do not overlap and sufficient number of intensity spikes is collected for the construction of the spike intensity distribution. The nebulization and transport efficiency of the calibration standard (MgO particles) and the sample (algae) can also be different.

The requirements of the calibration standard are: (1) the particles are not soluble or react in water, (2) the particle size is small enough to be completely atomized in the ICP, and (3) the amount of metal in the standard particles and the sample particles are of similar order of magnitude. The last requirement reduces errors in the one-point calibration method.

### Operation of the ICP-MS in time-resolved analysis data acquisition mode

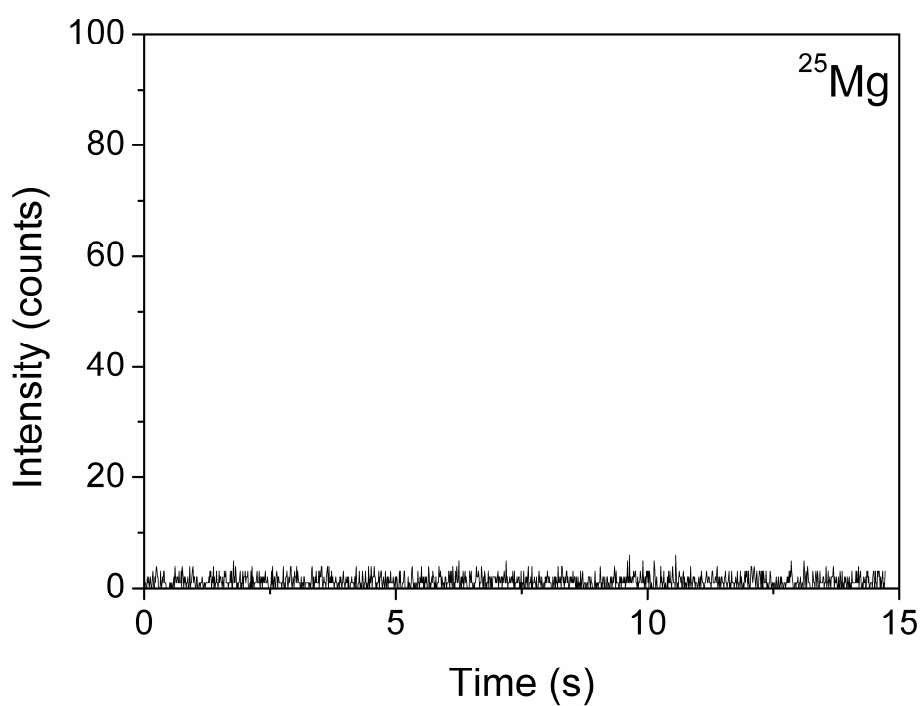
In this study, the quadrupole-based ICP-MS spectrometer (Agilent 7500a, Agilent Technologies, CA, USA) was set to Time-Resolved Analysis (TRA) mode of analysis. The minimum integration time of 10 ms was used and only one isotope was monitored in each experiment to maximize the data sampling rate of the isotope. The duration of each scan was 60 s (Figure S1). Each temporal profile contains 5769 data points. The acquired temporal profiles were converted into ASCII format using the “tabchart” function of the Chemstation software of the ICP-MS. The ASCII data were processed offline using custom software or third party software such as Origin or Excel. The maximum number of data points of each scan is limited to 65500 by the “tabchart” function.



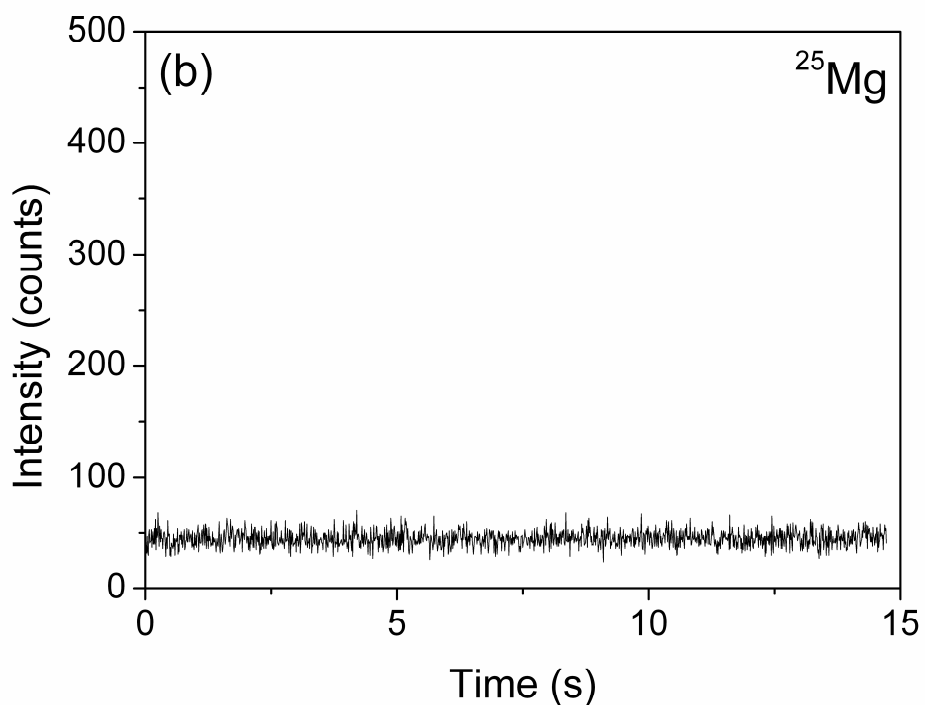
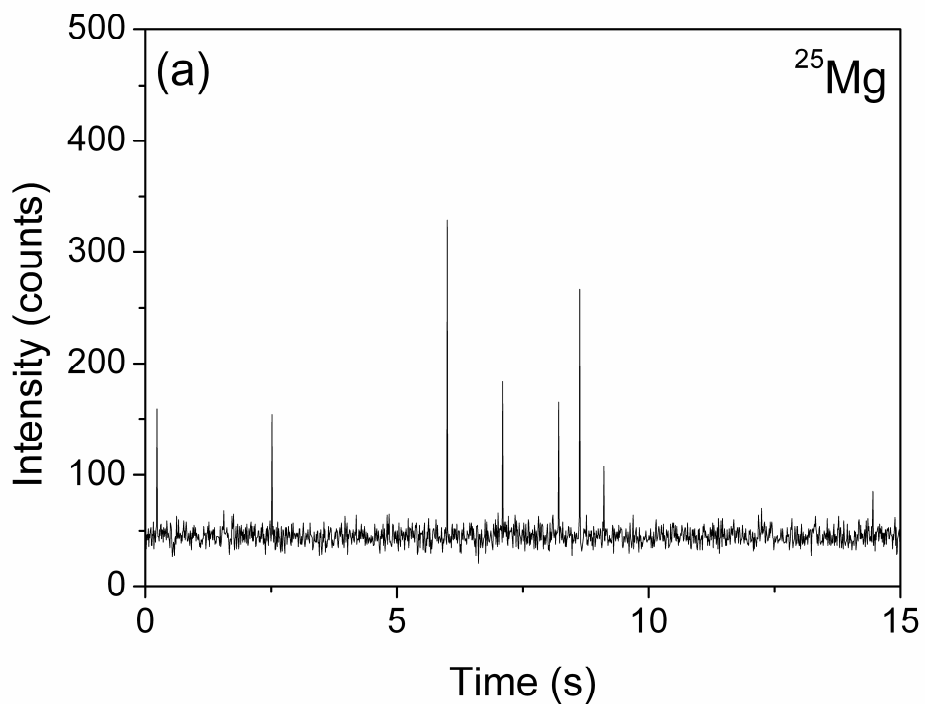
**Figure S1.** Setting the acquisition mode and time-resolved analysis acquisition parameters in the Chemstation software of the ICP-MS.

**Additional data on time-resolved ICP-MS measurement of the blank and diluted culturing solution with and without algae**

The ICP-MS temporal profile of water (Figure S2). No ICP-MS intensity spike of Mg is observed. The average count of the blank is 1.28.



**Figure S2.** ICP-MS temporal profile of water. The ICP-MS intensity corresponds to ICP-MS ion counts in 10 ms.



**Figure S3.** ICP-MS temporal profile of (a) diluted stock algae suspension and (b) diluted stock algae suspension with algal cells removed by centrifugation. The

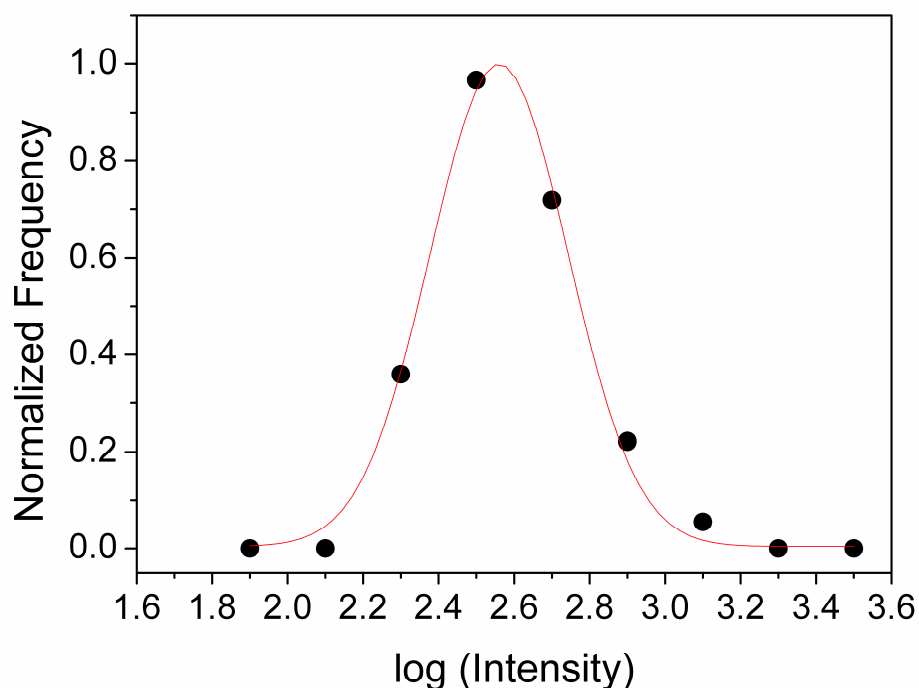
ICP-MS intensity corresponds to ICP-MS ion counts in 10 ms.

The ICP-MS temporal profiles of diluted stock algae suspension and the diluted stock algae suspension with algal cells removed by centrifugation are shown in Figure S3.

The average counts of the steady “background” intensity of the suspension with or without algae are both 45 counts. The steady intensity is due to  $\text{Mg}^{2+}$  ions in the culturing solution. (Please also refer to Figure 2 in the manuscript).

Please note that the sample preparation procedure of the suspension is different from that of Figure 2 in the manuscript. The algae suspension was not cleaned by the filtration procedure described in manuscript. Instead, the stock algae suspension in the culturing solution was diluted by 2000 times and was measured directly. The culturing solution was not removed. The steady intensity in Figure 2 of the manuscript is, therefore, different from the value in the figures above.

**Additional data on distribution of ICP-MS spike intensity using ICP torch with smaller injector tube diameter**



**Figure S4.** Distribution of ICP-MS spike intensity of  $^{25}\text{Mg}$  of a cleaned algae suspension. The diameter of the ICP torch injector was 1.5 mm. MCN nebulizer / single-pass spray chamber was used for aerosol generation. Sample uptake rate was 0.02 mL/min.

The distribution ( $n = 148$ ) is approximately log-normal with FWHM of 0.43. The FWHM of the size distribution of the algae is 0.15 (Figure 1b of the manuscript). The ratio of the FWHM is 2.9, consistent with the expected value of 3. Compared to the FWHM ratio of 4.2 in the manuscript (injector tube diameter = 2.5 mm), the smaller injector tube appears to produce a smaller ICP central channel and reduces the broadening of the ICP-MS spike intensity distribution.



### Diffusion of Mg ions in the ICP

Diffusion of Mg ions in the ICP can be estimated using the Chapman-Enskog Theory.<sup>1</sup> The diffusion coefficient of Mg is calculated to be 19.2 cm<sup>2</sup>/s in the ICP, assuming ICP temperature of 6000K. Particles pass through the ICP central channel at velocity of 20 m/s.<sup>2</sup> The diffusion length is, therefore, 0.223 cm at sampling depth of 6.0 mm. The Mg ion plume of the micron-size algae expands quickly in the ICP to a diameter of 0.446 cm at the sampling depth used in this study. The plume is much larger than the diameter of the sampler cone (0.1 cm) and loss of ions is expected to be significant. In contrast, in the case of solution nebulization, the concentration gradient of the Mg ions in the central region of the central channel is relatively small. Diffusion loss of Mg ions by solution nebulization is expected to be less severe.

### References

1. E. L. Cussler, *Diffusion, Mass Transfer in Fluid Systems*, Cambridge University Press, New York, 1997.
2. K. Jorabchi, K. Kahen, C. Gray and A. Montaser, *Anal. Chem.*, 2005, **77**, 1253-1260.