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Time-resolved ICP-MS Analysis of Mineral Element Contents and Distribution Patterns in Single Cells

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Abstract

Novel single cell techniques are attracting increasing interest for the clinical application because they can elucidate the cellular diversity and heterogeneity instead of the average masked by bulk measurements. Herein, the time-resolved ICP-MS for the determination of essential mineral elements in single cells has been developed and is used to analyze the contents and distribution patterns of Fe, Cu, Zn, Mn, P and S in two types of cancer cells (Hela and A549) and one type of normal cells (16HBE). The results show that there are obvious differences in contents and distribution patterns of the elements among the three types of cells. The mass of Fe, Zn, Cu, Mn, P, and S in individual Hela cells is significantly higher and span a broader range of values than in single 16HBE and A549 cells. The contents of Fe, Zn, and Cu follow log-normal distributions, and Mn, P, and S follow Poisson distributions with high λ values in single Hela cells, indicating a large cell-to-cell variance. Comparatively, the contents of Cu, Zn, P, and S in 16HBE cells show the narrowest distribution-range among the three tested cells, presenting the homogenous distribution of the elements in the cells. The method of single cell ICP-MS (SC-ICP-MS) provides potential application for the monitoring of the variation of mineral elements at a single cell level.

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3 **Keywords:** Time-resolved ICP-MS; single cell analysis; mineral element distribution;
4 cells.
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10 **Introduction**

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14 Recent genome-wide assays have revealed that cellular heterogeneity is a
15 widespread event within an isogenic cell population, which is associated with
16 different cell type, physiological feature, bio-environment, and therapeutic response.¹
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18 ² Thus, single cell analysis (SCA) can provide the valuable information of cell-to-cell
19 variance and identify the rare cell type in a mixed population of cells, which are
20 important for the need of cancer research as well as the basic biomedical study and
21 the fundamental principle of cell biology.³ So far, multiple novel single-cell
22 techniques, such as genomics, transcriptomics, proteomics, and metabolomics at a
23 single cell level that directly analyze the changes from the chemical contents (genes,
24 proteins and metabolites) of individual cells, have emerged, which exhibit potential
25 applications in the identification and detection of circulating tumor cells,² embryo
26 development at early stage,⁴ and monitoring immune responses.⁵ However, single cell
27 analysis is still in its infancy. There is a great demand to develop new techniques for
28 achieving high temporal, spatial and molecular resolution at a single cell level that can
29 differ significantly from the average patterns over the cell populations.
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39 The endogenous trace metals (*e.g.*, Fe, Cu, Zn, Mn and Co) and non-metals (*e.g.*,
40 P and S) are essential and important constituents in cells, which are found in
41 metalloproteins, metalloenzymes, nuclei acid, adenosine triphosphate (ATP), etc., and
42 play critical roles in a myriad of biological processes, such as oxidative-reductive
43 balance, gene expression, cell cycle regulation and energy metabolism.⁶⁻⁸ The
44 contents of these essential elements in biological tissue or cells are associated with
45 various patho-physiological conditions.⁹ For instance, the cellular requirement for Fe
46 is directly correlated with the cell type, the rate of cell growth, and the stage of cell
47 differentiation. Neoplastic cells are found to have higher iron requirements than normal,
48 non-malignant cells. Fe levels have been found elevated in serum and tumor tissues of
49 many types of cancer patients, including breast cancer, leukaemia, cervical carcinoma,
50 bladder cancer, lung cancer and so forth.^{10,11} In addition, the elevated iron levels have
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3 been shown to correlate with tumor angiogenesis, tumor growth and progression.^{10,12}
4 Intracellular iron depletion leads to G1/S cell-cycle arrest, DNA damage, and
5 apoptosis.¹³ Likewise, a number of tissue abnormalities and disease states in humans
6 have been reported to associate with either reduced or elevated levels of copper.¹⁴
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8 Serum and tumor copper levels are elevated in both solid tumor and blood cancer, and
9 have been reported to directly associate with the severity and progression of cancer,
10 involving in modulating tumor angiogenesis and affecting oxidative stress and so
11 forth in cancer cells.¹⁵ However, the knowledge about to what extent does the metal
12 content heterogeneity and how much diversity of them between normal cells and
13 cancer cells is still limited to date. To explore these questions, we developed a SCA
14 technique based on inductively coupled plasma mass spectrometry (ICP-MS) to
15 determine endogenous elements (Fe, Cu, Zn, P and S) at a single cell level from
16 several batches of human cancer cells and normal cells.

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18 ICP-MS is one of the most useful methods for ultratrace element analysis due to
19 its extremely high sensitivity to a wide range of elements. Recently, time-resolved
20 ICP-MS (usually using a quadrupole analyzer) and ICP-MS applied mass cytometry
21 (usually using a time of flight analyzer) is receiving much attention for elemental and
22 multiparametric analysis of single cells.^{16,17} As for single cell ICP-MS (SC-ICP-MS)
23 analysis, the intensity of spike signal is proportional to the quantity of the analyte ions
24 in a cell and the distribution of spike intensity is correlated with the mass distribution
25 of analyte ions in cells.¹⁸ So far, SC-ICP-MS has been used to determine the
26 intracellular major elements such as calcium¹⁹ and magnesium,⁹ or exogenous
27 uranium,²⁰ bismuth-based drugs,²¹ and quantum dots¹⁸ in single cells. Through
28 combination with elemental tagged techniques, intracellular biomolecules, including
29 surface antigens, amino acids, peptides, proteins, and DNA, can be successfully
30 quantified in single cells by mass cytometry.¹⁷ In recent years, although the
31 application of time-resolved ICP-MS in SCA has made great progress in single cell
32 analysis,^{22,23} the accurate determination of essential mineral elements, *e.g.* Fe, Cu, Zn,
33 Mn, P, and S in mammalian cells, because of the insufficient detection limits,
34 overlapping of spike signals and high cellular background.

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36 In the study, the contents and distribution patterns of essential mineral elements
37 Fe, Cu, Zn, Mn, P, and S in human cancer cells, including human cervical carcinoma
38 (Hela), human lung carcinoma epithelial cell line (A549), and human bronchial
39 epithelial cells (16HBE) at single cell level were analyzed by time-resolved ICP-MS,
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3 which will provide the multiparametric analysis of cellular heterogeneity and may
4 help to understand the behavior of mineral elements in tumor cells and further
5 distinguish the tumor cells from the normal ones. The unique analytical capability of
6 SC-ICP-MS for the essential mineral elements in single cells is expected to provide
7 crucial information for cancer research and clinical applications.
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11 **Materials and methods**

12 **Chemicals**

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3 counted by hemacytometer. For the measurement of total cells, the cells were digested
4 with 1 mL of concentrated nitric acid (MOS grade) at 90 °C for 2 h. Then the digested
5 solution was diluted with 2% HNO₃ and determined by ICP-MS.
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9 **Time-resolved ICP-MS measurement**

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11 The quadrupole-based PerkinElmer NexION 300D ICP-MS equipped with a
12 concentric PFA-ST nebulizer and a baffled quartz cyclonic spray chamber was used
13 throughout the experiment. The ICP-MS instrumental and operating parameters for
14 SCA and bulk analysis are listed in Table 1. Before analysis, the ICP-MS was tuned
15 using a 10 µg/L multi-element standard solution (containing Li, Be, Mg, Fe, In, Ce,
16 Pb, U in 2% v/v nitric acid). In order to eliminate polyatomic interferences and obtain
17 high signal-to-background ratio (SBR), ⁵⁶Fe⁺, ⁶⁸Zn⁺, ⁶³Cu⁺ and ⁵⁵Mn⁺ in single cells
18 was measured separately in dynamic reaction cell (DRC) mode using NH₃ as a
19 reaction gas. Phosphorus (P) and sulfur (S) was measured in DRC mode by using O₂
20 as a reaction gas, and monitored *via* the oxide reaction products PO⁺ at m/z 47 and
21 SO⁺ at m/z 48 as the analytical species. The reaction gas rate, from 0.3 mL/min to 1.2
22 mL/min with a step of 0.1 mL/min, and the quadrupole rejection parameter q (RPq),
23 from 0.4 to 0.8 with a step of 0.05, were optimized based on obtaining the lowest
24 background equivalent concentrations (BEC) by using the 10 µg/mL standard solution.
25 The dwell time (t_{dwell}) was optimized from 0.1 ~ 20 ms and the cell density for test
26 was optimized from 5×10⁴/mL to 1×10⁶/mL.
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40 **Data processing**

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42 For SC-ICP-MS, an iterative algorithm based on a three times standard deviation
43 (3σ) was used to distinguish single cell events from the background signal.²⁴ Briefly,
44 the mean and standard deviation (σ) of the entire dataset are firstly calculated and the
45 data that are 3σ above the mean were collected. The reduced dataset is recalculated
46 with the same method until no data points are higher than 3σ the final mean. The
47 mean value of the entire data set plus 3σ is considered as the threshold value of the
48 cellular contents, at which equal or below this value are excluded. The collected data
49 represent the SC signals and the reduced data represent the cellular background. The
50 detection limit of Fe, Cu, Zn, Mn, P and S in tested cells was calculated from 3σ
51 cellular background. The frequency of the signals was directly related to the number
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3 of cells and the intensity of single-cell events was related to the mass of analyzed ion
4 in one individual cell. To quantitatively analyze the mass of the elements in a single
5 cell, the multi-element standards containing Fe, Cu, Zn, Mn, P and S were measured
6 under the conditions of SC as well. Box plots and Gauss/Poisson statistics was used to
7 visualize the distribution patterns of these elements in individual cells.
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14 **Results and discussion**

15 **Single cell analysis by time-resolved ICP-MS**

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18 For SC-ICP-MS analysis, the tested cells keeping intact cellular morphology and
19 staying in monodispersed state is important, so that each ICP-MS spike signal
20 corresponds to one individual cell event.⁹ The light microscope observation showed
21 that the cells remained morphological integrity and were well monodispersed in
22 aqueous solution (Fig. 1).
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29 The difficulty for SC-ICP-MS measurement is that the matrix of cells are
30 composed primarily of light elements C, H, O, and N, which easily generate
31 polyatomic interferences (such as the polyatomic interference of $^{40}\text{Ar}^{16}\text{O}^+$ and
32 $^{40}\text{Ca}^{16}\text{O}^+$ on $^{56}\text{Fe}^+$) that regularly masks the single cell events, particularly for the
33 masses between 40 and 82 amu. The DRC technique can help to remove these
34 interferences by using gas-phase chemical reactions. In the present study, NH_3 was
35 used as the highly reactive gas to react with the interference ion for analysis of ^{56}Fe ,
36 ^{63}Cu , ^{68}Zn , and ^{55}Mn . P and S were detected *via* $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$ as analyte ions using
37 O_2 as a reaction gas in DRC to oxidize S^+ and P^+ . Fig. 2 presents the effects of the
38 parameters of reaction gas flow rate and RPq on the BEC measurements. The
39 optimized parameters for the analysis of $^{56}\text{Fe}^+$, $^{63}\text{Cu}^+$, $^{68}\text{Zn}^+$, $^{55}\text{Mn}^+$, $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$
40 are listed in Table 1. Under the optimized condition, the reaction gas flow rate of 0.7
41 mL/min and RPq as 0.65 resulted in the lowest BEC (0.08 $\mu\text{g/L}$) for ^{56}Fe
42 measurement (Fig. 2).
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55 In order to obtain sensitive and accurate signals of single cells by ICP-MS, the
56 number density of cells must be controlled properly and only one cell should be
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3 transported to the plasma at any given time (t_{dwell}). Fig. 3 shows the effects of cell
4 density on signal profile. The cell events increased with the increase of cell density
5 from $5 \times 10^4/\text{mL}$ to $2 \times 10^5/\text{mL}$, while they decreased when the concentration was
6 higher than $5 \times 10^5/\text{mL}$, which might be caused by the overlapping of cell signals. As a
7 result, the cell density of $2 \times 10^5/\text{mL}$ was chosen.
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11 Fig. 4 presents the temporal profile of ^{56}Fe in single HeLa cells (2×10^5 cells/mL)
12 by ICP-MS at various t_{dwell} . The data show the number of single cell events per min
13 gradually increased with the t_{dwell} from 0.1 to 5 ms, and reach a maximum at the t_{dwell}
14 of 5 and 10 ms, and then decreased with the t_{dwell} to 20 ms (Fig. 5). Therefore, the
15 t_{dwell} of 5 ms is proved to ensure the best SBR. It is known that the dwell time and
16 settling time determines the transient data acquisition speed of time-resolved ICP-MS,
17 which is important for SCA.^{23,25} Generally, a minimum dwell time and no settling
18 time is a desirable situation for SCA. It has been reported that a cell event in the
19 plasma lasts 0.2-0.4 ms.²⁶ When the settling time of the data acquisition is shorter
20 than the t_{dwell} , the longer t_{dwell} than the duration of ion plume generation will result in
21 low spike signals that correspond to cell events, which is due to the signal average
22 with the background in this time window. Thus, a short t_{dwell} is generally desirable to
23 obtain high SBR. However, when the settling time of the data acquisition is longer
24 than the t_{dwell} , an inappropriate short t_{dwell} will decrease effective reading frequency
25 and waste the detection time. In the study, when operation in DRC mode, an
26 appreciable settling time (~ 4 ms) is required for the processes, including the charge
27 distribution, ion flow from cell entrance to exit, and ion signal settling down to stable
28 values. The obtained optimal t_{dwell} under cellular condition is consistent to this settling
29 time, which can guarantee the maximum reading efficiency.
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33 The feasibility for individual cell monitoring within the 5-ms integration window
34 are further demonstrated by calculating the probability of two cells existence in one
35 aerosol droplet and simultaneously entering the plasma during a t_{dwell} . The diameter of
36 the transmitted droplets in ICP is commonly considered as $10 \mu\text{m}$.⁹ Under the
37 conditions of 0.32 mL/min for the sample uptake rate, the rate of generating droplets
38 aerosol is about $6.1 \times 10^8/\text{min}$, thus, for the cell number density of $2 \times 10^5/\text{mL}$, the
39 probability of a cell presents in one aerosol droplet is calculated as about 1×10^{-4} ,
40 indicating the chance of two cells in one droplet can be ignored. In addition, the
41 probability of two cells in ICP within the 5 ms t_{dwell} is calculated as 0.005% and the
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3 cell transport efficiency is about 0.2% based on the average spike frequency of ^{56}Fe
4 (approximately 2/s) and Poisson statistics,²⁷ indicating the spike overlapping from two
5 cells is insignificantly under the experimental conditions.
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8 9 10 **Quantitative analysis and distribution patterns of mineral elements in single cells**

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12 The contents of Fe, Cu, Zn, Mn, P, and S in single cells were quantitatively
13 analyzed *via* time-resolved ICP-MS and cell digestion methods, respectively. Fig. 6
14 shows the SC-ICP-MS temporal profiles of $^{56}\text{Fe}^+$, $^{68}\text{Zn}^+$, $^{63}\text{Cu}^+$, $^{55}\text{Mn}^+$, $^{47}\text{PO}^+$, and
15 $^{48}\text{SO}^+$ spike signals in HeLa cells, indicating the single cell events were successfully
16 detected by the time-resolved ICP-MS. The obtained detection limits are: Fe (4 fg),
17 Cu (0.6 fg), Zn (8 fg), Mn (0.3 fg), P (2.8 pg), S (0.8 pg), which may satisfy the
18 requirement of SC measurement. The quantitative analysis of these elements in single
19 cells was achieved *via* measuring the standard solutions at the same experimental
20 conditions. Linear calibration curves for $^{56}\text{Fe}^+$, $^{68}\text{Zn}^+$, $^{63}\text{Cu}^+$, $^{55}\text{Mn}^+$, $^{47}\text{PO}^+$, and $^{48}\text{SO}^+$
21 (correlation coefficient $R^2 > 0.999$) were obtained and the mass of these elements in
22 SC sample was calculated using the the following equation:
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$$30 \quad m_c = \frac{\eta_n \cdot Q_{sam} \cdot t_{dwell} \cdot (I_c - I_{Bgd})}{m}$$

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32 where m_c is the mass of elements in a single cell; η_n is the transport efficiency of
33 standard solution (about 1% in the experiment); Q_{sam} is the sample uptake rate, t_{dwell} is
34 the dwell time of standard solution; m is the slope of the calibration curve; I_c and I_{Bgd}
35 are the signal intensity of a single cell and the cellular background, respectively. The
36 average masses of Fe, Cu, Zn, Mn, P, and S in HeLa, A549 and 16HBE single cells
37 determined by SC-ICP-MS are listed in Table 2, which shows good agreement with
38 the data by the cell digestion method (Table 2), demonstrating the SC-ICP-MS is a
39 reliable method for analysis of mineral element content in single cells.
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49 Importantly, the data by SC-ICP-MS can provide the distribution patterns of
50 elements in individual cells which reflect the cell variability. The relative frequency of
51 high-intensity elemental signals in single cells follows a Gaussian distribution,
52 whereas, at low intensity, the frequency can be described with a Poisson distribution,
53 assuming perfectly random arrival of ions to the detector.²⁸ The statistical analysis
54 shows that the distribution of ^{56}Fe , ^{68}Zn , ^{63}Cu , ^{55}Mn , ^{47}PO and ^{48}SO contents in the
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three cell lines presents different patterns (Fig. 7 and Fig. 8). The spike intensity of ^{56}Fe in single HeLa, A549, and 16HBE cells shows nearly log-normal distributions with a long right-hand tail, indicating a small number of cells possess high Fe contents. The full width at half maximum (FWHM) of the distribution plots of ^{56}Fe in HeLa, A549, and 16HBE cells are 0.07, 0.20, and 0.17, respectively, exhibiting that the Fe contents in HeLa cells are more homogeneous than in A549 and 16HBE ones. Similarly, the Zn content in single HeLa, A549, and 16HBE cells shows log-normal distribution patterns as well but with a small FWHM value of 0.03, indicating the homogeneity of intracellular Zn contents in the three types of cells. The intensity distribution of Cu in single HeLa cells approximately fits log-normal and has a FWHM of 0.17, whereas, in single A549 and 16HBE cells, the Cu content presents a Poisson distribution. The spike intensity of ^{55}Mn , ^{47}PO , and ^{48}SO in single-cell level of the three cells follows a Poisson distribution as well. In Poisson distribution, the parameter λ refers to the average value and the variance of distribution, which means the average intensity of events in a time interval and the width of distribution. Interestingly, the λ values of P and S distribution in HeLa cells are 41 and 35, respectively, which are obviously higher than those in A549 (4 and 12, respectively) and 16HBE cells (5 and 11, respectively), indicating the single HeLa cells have relatively high level and wide distribution of P and S contents comparing with A549 and 16HBE cells. Moreover, the box plots that display distribution profiles of the elemental masses in individual cells are shown in Fig. 9. It is noteworthy that the masses of Fe, Cu, Zn, Mn, P, and S in single HeLa cells span a broader range of values than those in A549 and 16HBE cells, demonstrating larger biological variations in a population of HeLa cells than in the other two cells. Comparatively, the masses of Cu, Zn, P, and S in 16HBE cells show the narrowest distribution among the three tested cells, indicating the homogeneity of the element contents in the single 16HBE cells.

It has been reported that tumor cells generally require larger amount of Fe, Cu, Zn, P and S than normal ones to sustain their growth and proliferation.^{8,29} For instance, cancer cells (*e.g.*, HeLa, breast cancer, pancreatic cancer and hepatocellular cancer) may reprogram the iron metabolism, including iron acquisition, efflux, storage and

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3 regulation, to satisfy their iron requirement.¹⁰ Similarly, high level of Cu is usually
4 necessary in cancer cells, which may act as a tumor promotor to stimulate cancer-cell
5 proliferation and regulate oxidative phosphorylation.¹⁴ The measurement of P in
6 single cells may be particularly useful in the identification of malignant tumor cells
7 because cancerous cells (lung cancer, breast cancer, etc.) have a tendency to
8 accumulate more phosphate in DNA than normal cells.^{30,31} Sulfur is shown to
9 significantly increase homocysteine levels in rapid proliferating tumor cell lines due
10 to increase of protein synthesis and transmethylation reactions.³² Therefore, the
11 content discrepancies and different distribution patterns of the essential mineral
12 elements (Fe, Zn, Cu, Mn, P, S, etc.) in single cells can be used to differentiate normal
13 and tumor cells in a mixed population of cells.
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23 Conclusions

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26 Cellular heterogeneity that arises from stochastic changes of genes, proteins,
27 metabolites and so forth is a fundamental principle of cell biology. Herein, the
28 time-resolved ICP-MS for the determination of essential mineral elements: Fe, Cu, Zn,
29 Mn, P and S, in single cells (SC-ICP-MS) have been developed. By using DRC mode
30 to eliminate polyatomic interferences and optimizing the sample uptake rate, cell
31 number density and effective dwell time for SC-ICP-MS analysis, the element mass
32 can be sensitively detected and quantified at the single cell level.
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38 The quantitative analysis by SC-ICP-MS shows obviously cellular variation of the
39 mineral elements in HeLa, A549, and 16HBE cells among individual cells. The mass
40 of Fe, Cu, Zn, Mn, P and S in single HeLa cells is significantly higher than in A549
41 and 16HBE cells, and they span an obviously broader range of values in HeLa than in
42 the other two types of cells, demonstrating greatly biological variations in HeLa cell
43 populations. Furthermore, the masses of Fe, Cu, Zn, Mn, P and S in the three types of
44 cells follow different distribution patterns. In single HeLa cells, Fe, Zn and Cu masses
45 follow log-normal distributions, and Mn, P and S masses follow Poisson distributions
46 with high λ values, indicating the large variation of the masses of elements among
47 single HeLa cells. The study demonstrates the method of SC-ICP-MS provides
48 potential application for monitoring the variation of distribution patterns of mineral
49 elements at the single cell level.
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Table 1 NexION 300D ICP-MS instrumental and data acquisition parameters for cell analysis

Instrumental Parameters	SCA	Cell digestion
<i>ICP parameters</i>		
Nebulizer	Concentric PFA-ST	
Spray Chamber	Baffled Quartz Cyclonic	
Torch	Glass Torch	
ICP RF Power	1600 W	
Plasma Gas	18 L/min	
Auxiliary Gas	0.9 L/min	
Nebulizer Gas	1.0 L/min	0.92 L/min
<i>Measurement parameters</i>		
Acquisition mode	Peak Hopping	
Sweeps	1	20
Dwell time	5ms	50ms
<i>DRC parameters</i>		
RPa	0	
RPq	0.40 (for $^{56}\text{Fe}^+$), 0.45 for ($^{63}\text{Cu}^+$), 0.55 (for $^{68}\text{Zn}^+$, $^{55}\text{Mn}^+$, $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$)	
DRC gas (NH_3)	0.7 mL/min (for $^{56}\text{Fe}^+$ and $^{55}\text{Mn}^+$), 0.5 mL/min (for $^{68}\text{Zn}^+$), 0.9 mL/min (for $^{63}\text{Cu}^+$)	
DRC gas (O_2)	2.2 mL/ min (for $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$)	

Table 2 Quantitative analysis of elements in single cells by SC-ICP-MS and cellular digestion ICP-MS

Elements	Analytes	Hela		A549		16HBE	
		SC	Digestion	SC	Digestion	SC	Digestion
Fe (fg)	$^{56}\text{Fe}^+$	26.6±2.6	22.3±2.7	17.2±1.6	16.2±1.3	15.1±1.0	11.3±1.0
Zn (fg)	$^{68}\text{Zn}^+$	72.9±5.7	68.4±4.0	42.9±4.9	31.5±2.4	25.7±1.4	27.4±0.7
Cu (fg)	$^{63}\text{Cu}^+$	3.7±0.8	3.4±0.3	1.6±0.2	1.4±0.1	1.4±0.1	2.1±0.1
Mn (fg)	$^{55}\text{Mn}^+$	0.5±0.1	0.6±0.1	0.4±0.0	0.4±0.0	0.2±0.0	0.2±0.0
P (pg)	$^{47}\text{PO}^+$	8.9±0.1	14.6±1.3	4.7±0.1	6.7±0.5	5.2±0.5	5.1±0.4
S (pg)	$^{48}\text{SO}^+$	6.1±0.1	5.2±0.5	2.7±0.1	3.7±0.3	1.3±0.1	1.8±0.1

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Figure captions

Fig. 1 Microscope images of HeLa (a), A549 (b) and 16HBE (c) cells in ultrapure water after immobilization with 70% v/v methanol.

Fig. 2 BEC concentration histogram with the reaction gas flow rate and RPq.

Fig. 3 Effects of cell density on signal profile of single cells.

Fig. 4 ICP-MS temporal profile of ^{56}Fe in HeLa cells with a cell number density of 2×10^5 cells/mL at different t_{dwell} ranging from 0.1 to 20 ms.

Fig. 5 Single cell event rate at different t_{dwell} .

Fig. 6 ICP-MS signal profile of $^{56}\text{Fe}^+$, $^{68}\text{Zn}^+$, $^{63}\text{Cu}^+$, ^{55}Mn , and $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$ in HeLa cells at a cell number density of 2×10^5 cells/mL.

Fig. 7 Intensity distribution patterns of Fe, Zn, Cu, and Mn in HeLa, A549 and 16HBE cells.

Fig. 8 Intensity distribution patterns of P and S in HeLa, A549 and 16HBE cells.

Fig. 9 Box plot for the mass of Fe, Zn, Cu, Mn, P and S in HeLa, A549 and 16HBE cells. The boxes mark the intervals between the 25th and 75th percentiles; the lines inside the boxes denote medians; whiskers denote the intervals between the 5th and 95th percentiles and filled circles indicate data points outside the 5th and 95th percentiles.

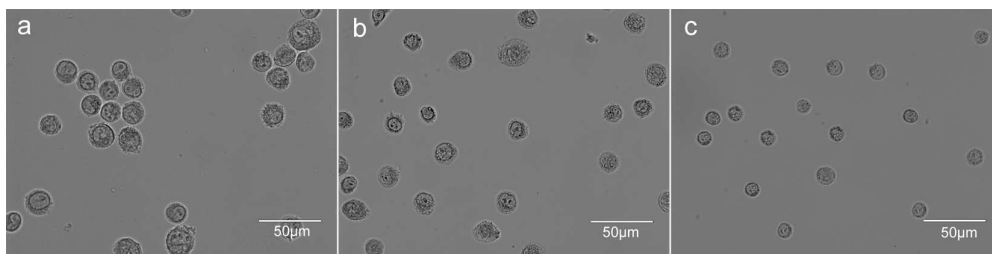


Fig. 1

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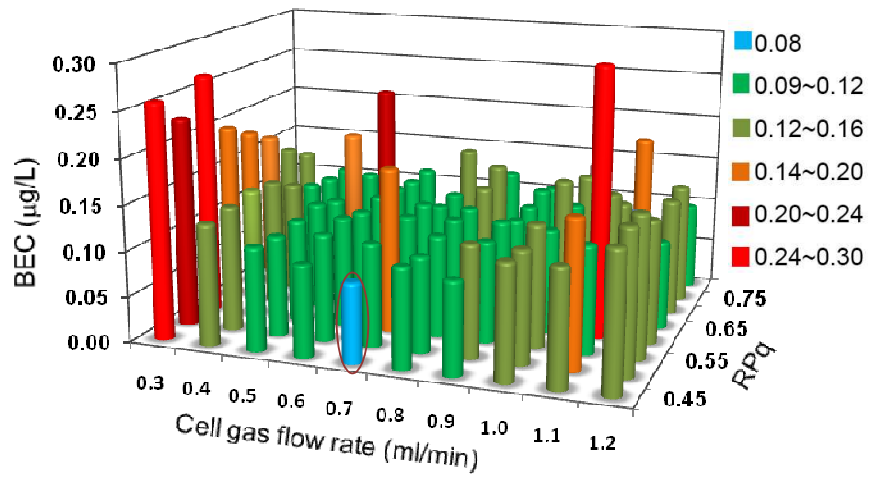


Fig. 2

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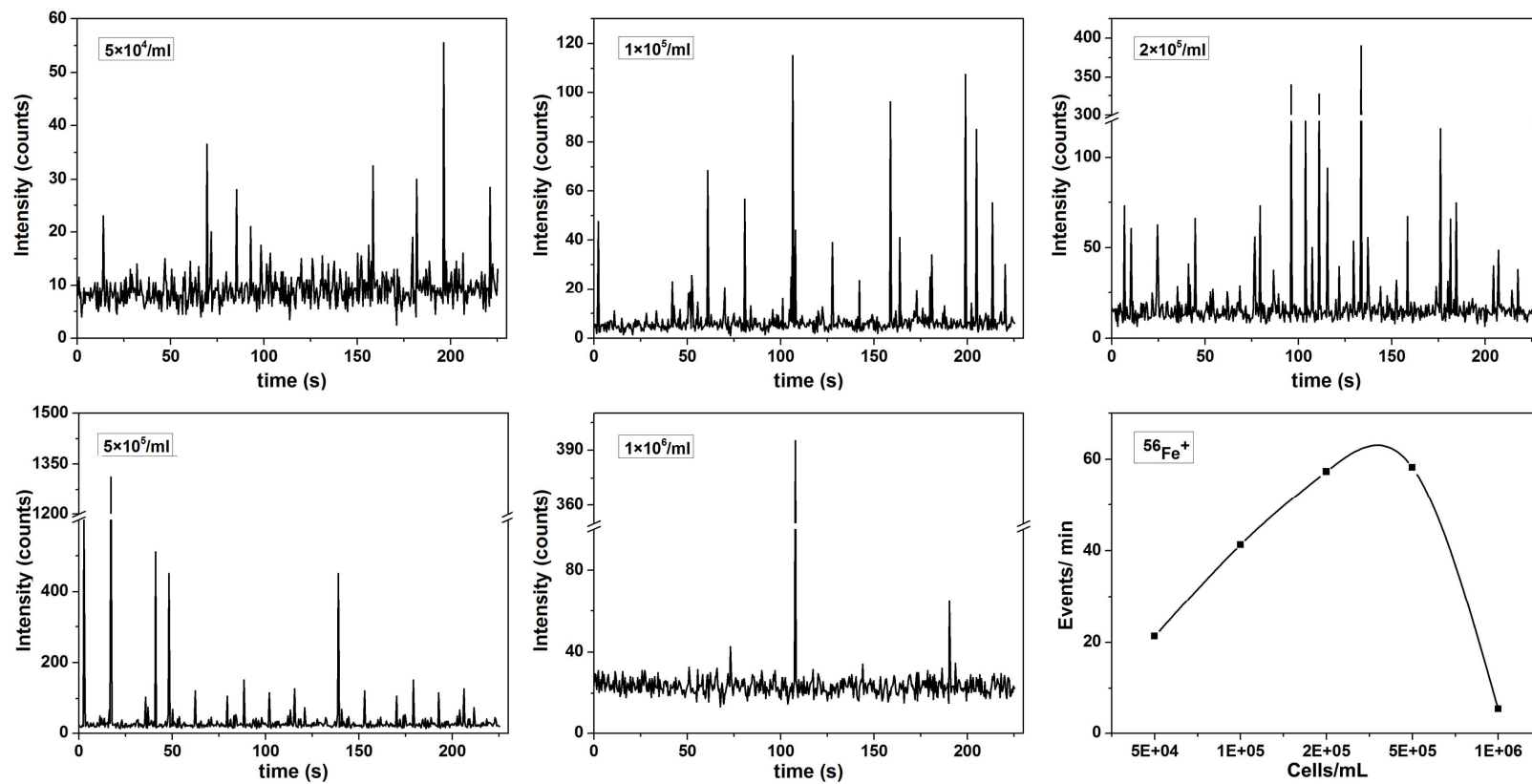


Fig.3

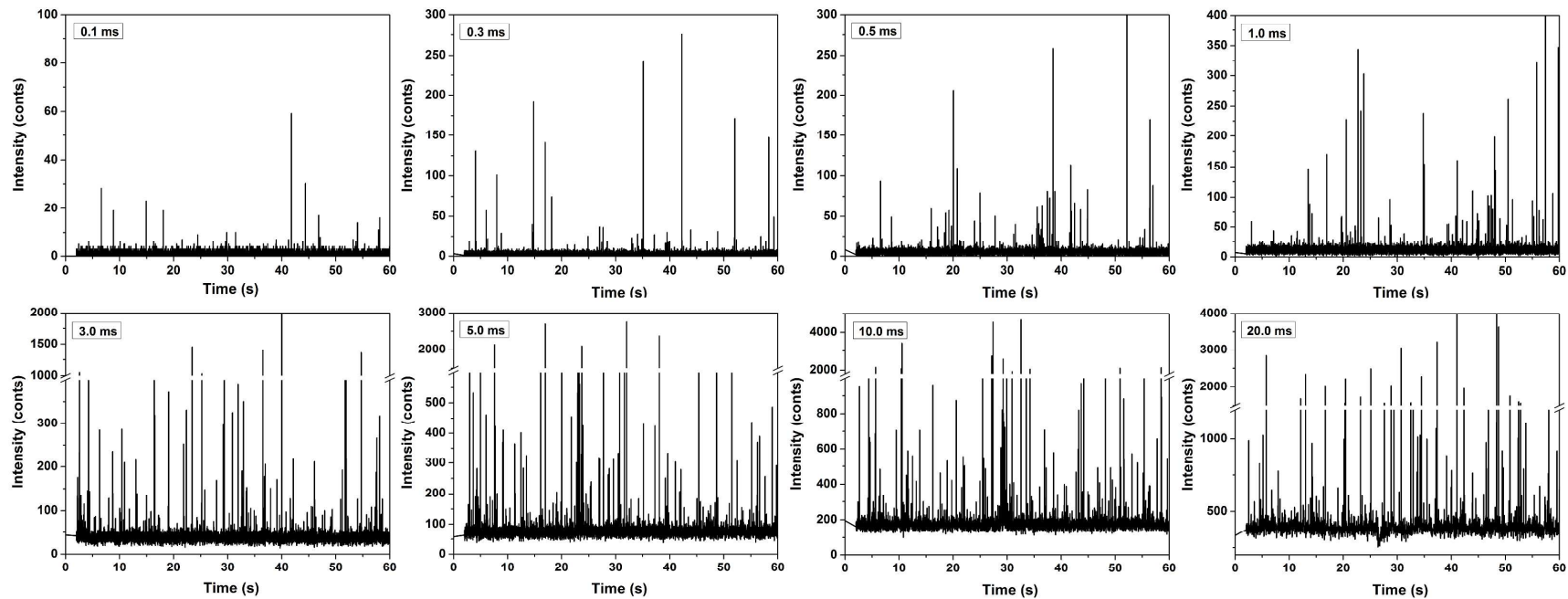


Fig. 4

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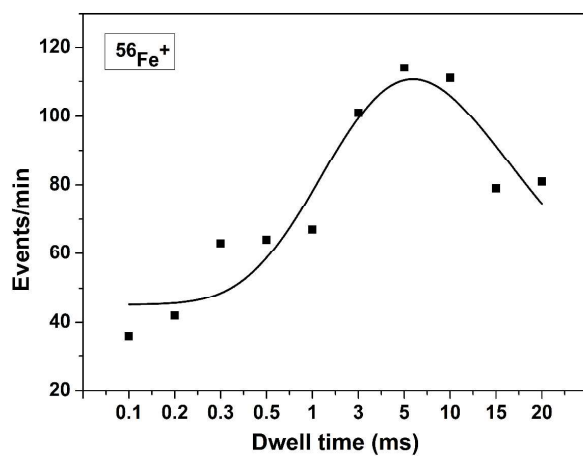


Fig. 5

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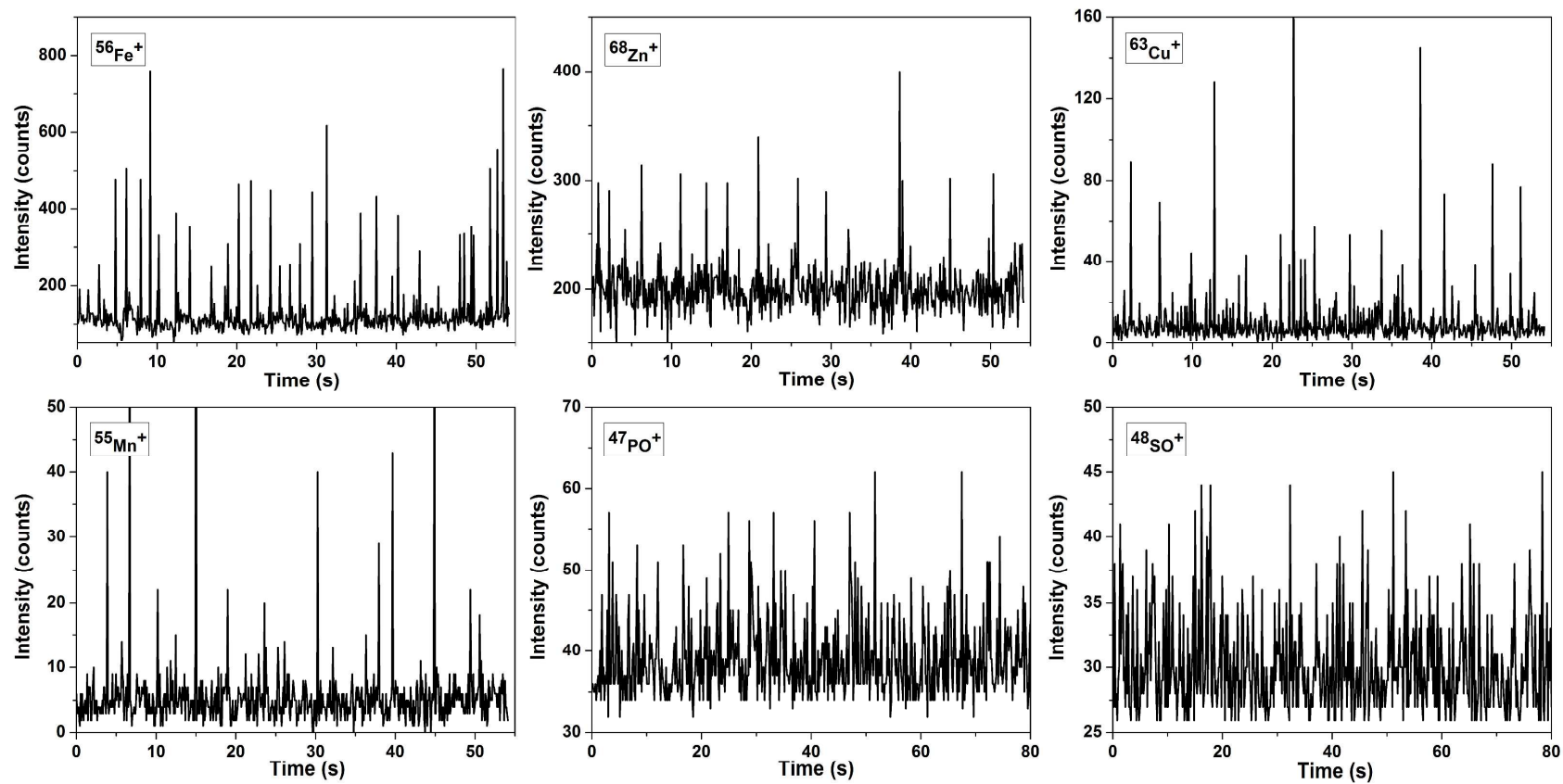


Fig. 6

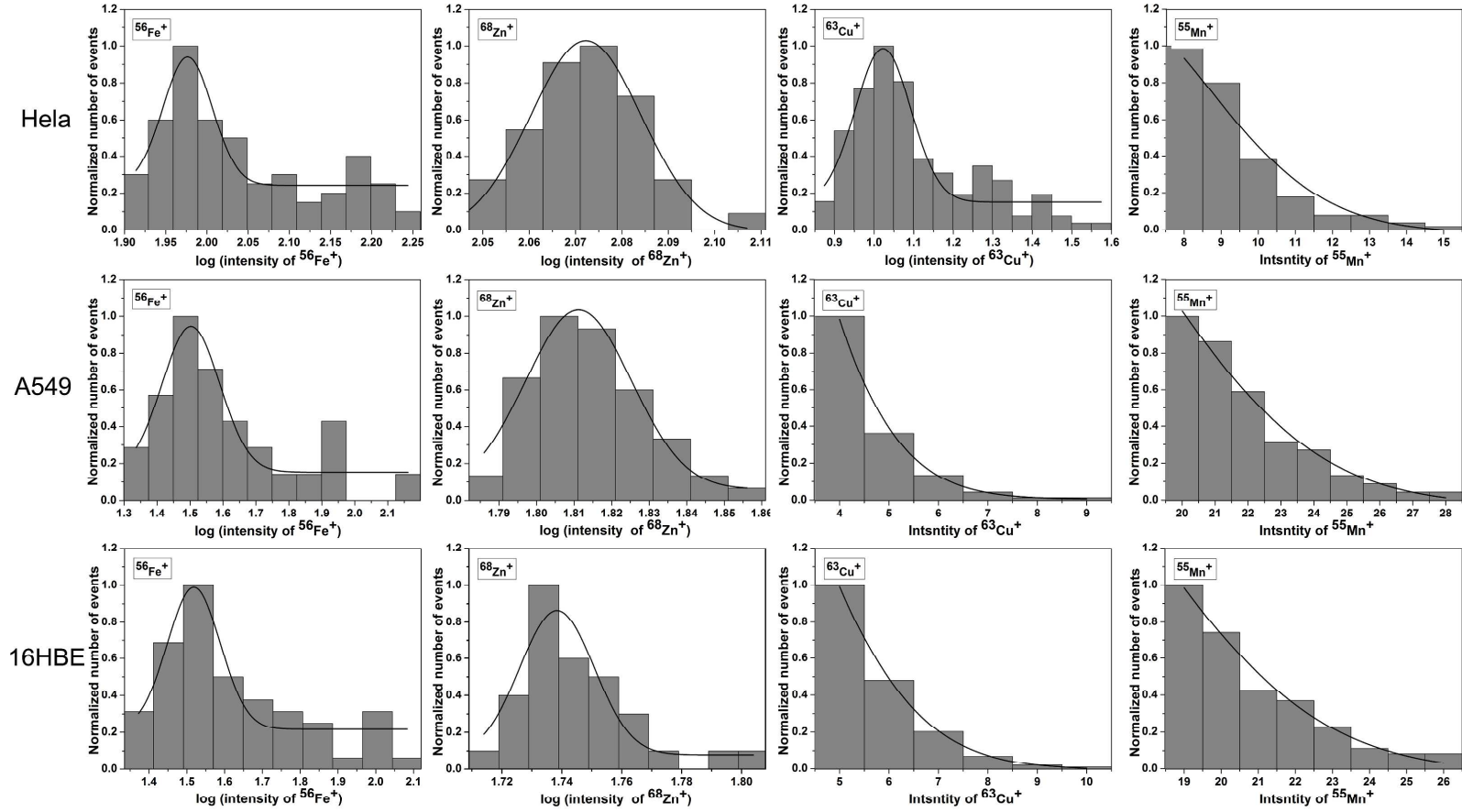


Fig. 7

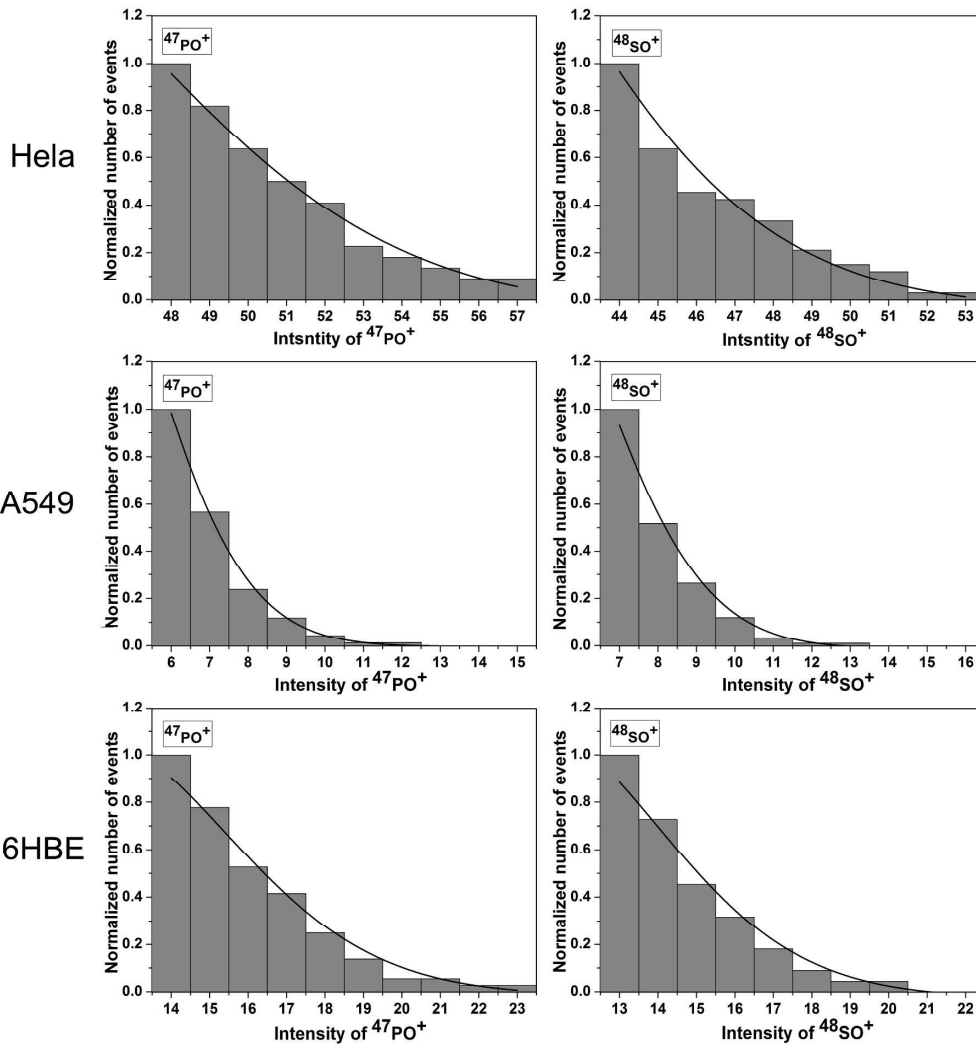


Fig. 8

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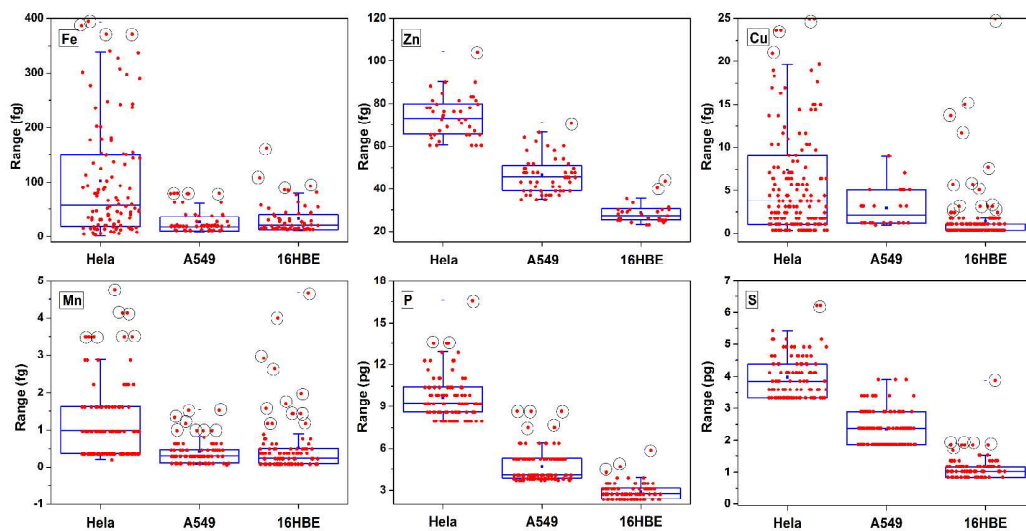


Fig.9