Atomic Spectroscopy

Review

ICP-MS-based Methodology in Metallomics: Towards Single Particle Analysis, Single Cell Analysis, and Spatial Metallomics

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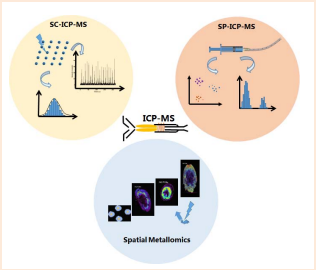
Received: *June 08, 2022;* ***Revised:*** *June 25, 2022;* ***Accepted:*** *June 25, 2022;* ***Available online:*** *June 27, 2022.*

DOI: 10.46770/AS.2022.108

ABSTRACT: As an emerging interdisciplinary science, metallomics aims to integrate research fields related to metals and metalloids in biological systems from a systematic perspective. Inductively coupled plasma mass spectrometry (ICP-MS) is considered one of the most versatile tools for metallomics research. This review presents a brief overview of ICP-MS and describes recent advances in ICP-MS instrumentation. Then, ICP-MS-based methods and applications are discussed, focusing on single particle analysis, single cell analysis, and spatial metallomics. With the rapid developments in instrumentation and methodology, ICP-MS-based methodologies will evolve further and play a dominant role in metallomics research.



METALLOMICS: CONCEPT AND HISTORY

Metals play important roles in cells and organisms by participating in fundamental processes such as cell signaling, gene expression, and enzyme catalysis.[1] Accordingly, a cell (or an organism) must be characterized not only by its nucleic acids (genome), proteins (proteome), and metabolites (metabolome), but also by its metals, *i.e.* metallome.[2–5] The term "metallome" was first introduced by R.J.P. Williams to describe the content and distribution of metal ions in a cell or an organism.[6] Later, the meaning of metallome was extended to the entirety of metal and metalloid species. Hiroki Haraguchi coined the term "metallomics" to denote the research activities related to metallome.[2] In 2010, the International Union of Pure and Applied Chemistry (IUPAC) proposed the definitions of "metallome" and "metallomics."[4] In this paper, metallome is defined as "the entirety of metal and metalloid species present in a biological system", including their concentration, distribution, speciation, identification, and metabolism; metallomics is defined as "study of the metallome, interactions, and functional connections of metal ions and other metal species with genes, proteins, metabolites, and other biomolecules in biological systems."[4]

Various research topics related to metals and metalloids, such as essential physiological processes, toxicological effects, and complex interactions with other biomolecules, have been studied independently. As an emerging interdisciplinary science, metallomics aims to integrate the research fields related to metals and metalloids in biological systems from a systematic perspective.[3,7] In this context, metallomics is extremely complicated. On the other hand, the metallome is highly dynamic because many metal complexes and intermediates are thermodynamically unstable and easily change.[8] This complexity and variation pose huge challenges to analytical methods because many techniques that are successfully used in other omics are not suitable for metallomics.

The analytical techniques for metallomics must have specific responses to metals and metalloids, a high-throughput capability, and sufficient sensitivity with a limited sample amount, such as single cells.[3,9] The techniques should allow not only the quantitative analysis of multiple elements but also the determination of their species in the samples. To completely characterize the metallome, it is also necessary to understand the

localization, speciation, and metabolism of the metals and metalloids at a subcellular level and study their connections and interactions with the genes, proteins, or metabolites in the spatial dimension, which is a new topic in metallomics and is referred to as *spatial metallomics*.

New analytical tools and strategies are necessary to address these challenges. Inductively coupled plasma–mass spectrometry (ICP-MS) is considered one of the most versatile tools for metallomics research because of its outstanding characteristics, including high analytical throughput, excellent detection limits for most elements, minimal matrix effects, wide linear dynamic range, and simple coupling to other analytical methods (*e.g.*, laser ablation or high-performance liquid chromatography (HPLC)).[10,11] However, ICP-MS-based methods can only provide qualitative and quantitative information on elements and must be integrated with other analytical tools for metallomics research. For example, in parallel with the detection of metal species, metal ligands or metal-binding molecules have been identified using biological mass spectrometric methods coupled with a separation method such as two-dimensional gel electrophoresis or HPLC.[12]

This review provides a brief overview of ICP-MS and describes recent advances in ICP-MS instrumentation. Then, ICP-MS-based methods and applications are discussed, focusing on single particle analysis, single cell analysis, and spatial metallomics. Lastly, the conclusion and the prospect of ICP-MS-based methodology in metallomics are presented.

ICP-MS: A POWERFUL METHOD IN METALLOMICS

ICP-MS instrumentation was introduced to the commercial market in the early 1980s[13] and has since become the most powerful tool for ultratrace element analysis. In ICP-MS, the high-temperature plasma (6000–10000 K) is used as an ion source; the energy is sufficient for desolvation, atomization, and ionization of the analyte.[14] Different mass analyzers are utilized in ICP-MS, such as the quadrupole, magnetic sector analyzer, and ion trap.[15] Besides solution analysis, *in situ* solid sampling can be achieved when laser ablation (LA) is used as the sample introduction system. In LA-ICP-MS analysis, solid samples are ablated with high-power laser shots, and the resulting aerosol is transported and analyzed by ICP-MS.[16] LA-ICP-MS has the unique advantages of high spatial resolution (down to ~1 μm), minimal sample preparation, and high sensitivity (<1 fg for many elements). Recent advances in ICP-MS instrumentation are discussed in the following section.

Solution introduction system and plasma source. Currently, the main application of ICP-MS involves the analysis of liquid samples. The standard sample introduction systems in ICP-MS suffer from low transport efficiencies. For example, the typical transport efficiency of single cells using ICP-MS is reported to be less than 1%.[17] In addition, polydisperse aerosols from standard systems induce a sampling bias and adversely affect the accuracy of the results of single particle analysis.[18] New introduction systems have been developed to improve the transport efficiency and reduce the sampling bias. Miyashita *et al.* achieved approximately 100% transport efficiency of single cells using a microflow concentric nebulizer coupled to a total consumption spray chamber.[19] A heated single-pass spray chamber with a sheath gas was reported to improve the transport efficiency and reduce the sampling bias in single cell analysis.[20] Zhou *et al.* developed a novel oil-free passive microfluidic system coupled to ICP-MS with a direct infusion micronebuliser and achieved >70% transport efficiency of single cells.[21]

Monodisperse droplets generated by either a commercial piezoelectric dispenser[22] or a microfluidics-based droplet dispenser[23] have proven to be ideal calibrations for single cell/particle analysis. Monodisperse droplets exhibit almost the same behavior in the plasma (*e.g.*, trajectory, ionization efficiency, and transport efficiency) so that the sampling bias in standard sample introduction systems is reduced.[24] Using the sample introduction system with the monodisperse droplets resulted in more accurate distributions in single cells or single particles.[25]

Better detection limits are continuously pursued in metallomic studies. The detection efficiency of current ICP-MS instruments ranges from 10^{-4} to 10^{-6} counts per atom.[26] Achieving the better detection limit is hindered by several conventional ICP-MS designs. A new conical torch with a reduction of argon consumption and power density was developed to realize a stable plasma with a 1000–1700 K higher excitation temperature and a 5-fold increase in the electron number, compared with conventional torches.[27] The ICP-MS equipped with the conical torch has a higher sensitivity, better signal-to-background ratios, and better trajectories, thus achieving a better performance in single particle analysis.[28]

Almost all commercial ICP-MS systems have a horizontal orientation of the plasma in which the trajectories of sample droplets in different sizes are affected by gravity and transport efficiencies of the droplets become mass-dependent, particularly for droplets tens of micrometers in diameter. Vonderach *et al.* designed a vertical downward orientation of the plasma, allowing sample introduction from the top.[29] This gravity-assisted sampling approach is beneficial for transporting large-sized droplets that are difficult to transport in a horizontally oriented plasma. In addition, the transport efficiency of the droplets was independent of the droplet size.[29] This new plasma source design has the potential to be widely applied in single cell/particle analysis.

Table 1. Comparison of three modern ICP-TOF-MS instruments

	icpTOF 2R	Vitesse	CyTOF Helios
Mass range (m/z)	6–280	6–280	75–209
Mass resolution (FWHM)	6000	4500	900
Minimum integration time (μs)	46	25.5	13
Sensitivity (kcps/ppb)	30 (^{238}U)	40 (^{238}U)	300 (^{159}Tb)
Abundance sensitivity (ppm)	30	30	3000
Linear dynamic range	10^6	10^7	$10^{4.5}$
Time resolution (continuous acquisition, μs)	3000	77	13
Ion blanking	Notch filter	Bradbury–Nielsen gate	Quadrupole high-pass filter

Time-of-flight mass analyzer. Most commercial ICP-MS instruments use a scanning analyzer (*e.g.*, a quadrupole filter); thus, only ions of a certain mass-to-charge ratio (m/z) can be determined at every moment. However, in many metallomics applications, multiple elements must be determined in a short period. For example, a typical temporal duration of ion clouds from a single cell is between 200 and 500 μs.[30] Scanning analyzers fail to determine more than one isotope at such a short pulse. Time-of-flight (TOF) technology offers unique advantages over scanning analyzers.[31] The ions are sampled simultaneously, and a full mass spectrum can be obtained in tens of microseconds. ICP-TOF-MS also offers a higher mass resolving power, minimizing polyatomic interferences in the mass spectrum.[32] In addition, ICP-TOF-MS offers improved precision in isotope ratio analysis owing to its quasi-simultaneous characteristics.[32]

TOF was first introduced in ICP-MS in the 1990s.[33] However, the last generation of ICP-TOF-MS suffered from a limited sensitivity and dynamic range compared with other types of ICP-MS instruments, such as the quadrupole ICP-MS. With breakthroughs in high-speed digitization electronics and related techniques,[34] ICP-TOF-MS has evolved into a powerful tool for metallomics studies. New generation of ICP-TOF-MS instruments are available in the market, including the icpTOF series from TOFWERK, Vitesse from Nu Instruments, and CyTOF from Fluidigm. These instruments use the same orthogonal design and single-pass reflectron TOF design. The CyTOF was designed to analyze samples labeled by lanthanide stable isotopes and it has a mass range of 75 to 209 m/z.[35] The other two ICP-TOF-MS instruments cover a wider mass spectrum and thus are more versatile in metallomics studies,[32,36] particularly for the study of important trace elements in biological systems (*e.g.*, iron, copper, and zinc). A comparison and the specifications of the ICP-TOF-MS instruments are presented in Table 1.

As an emerging technique, ICP-TOF-MS is expected to be the instrument of choice in metallomics studies that require high-throughput and high-resolution analyses, such as single cell/particle analysis[37] and high-resolution elemental bio-imaging.[38]

Laser ablation systems. Laser ablation coupled with ICP-MS (LA-ICP-MS) enables *in situ* quantitative analysis and imaging of metals and metalloids in sample sections. However, the slow analytical speed and limited spatial resolution of traditional instruments hinder the wider application of LA-ICP-MS.

Many commercial ablation cells produce a signal response to a single laser shot, which is also called a single-pulse response (SPR), in the range of a second. In the past several decades, numerous efforts have been made to optimize ablation cell geometries to improve the aerosol transport efficiency, reduce the signal duration, and decrease the aerosol dispersion.[39] Tanner *et al.* designed an in-torch laser ablation cell that greatly reduced the cell volume and achieved an SPR of 4 ms.[34] The signal-to-noise ratios were also significantly improved because the ablated aerosols were less diluted during the transport.[34] However, bio-imaging analysis was not possible because the cell was not able to sample a normal specimen. Liu *et al.* developed a two-volume cell by placing a movable inner cell inside an external cell, which was sufficiently large to accommodate large samples. The cell also greatly reduced the memory effects and the SPR.[40] Different designs of fast and low-dispersion ablation cells have since been developed and commercialized.[41,42] These cells all achieved a single pulse response of less than 10 ms at FW0.01M (*e.g.*, a 1% height of the maximum peak) and a higher sensitivity compared to the traditional ablation cells.[41] The stable distance between the cell inlet and sample surface in these low-dispersion cells is reported to be key for a shorter SPR and improved data precision,[43] thus, some of the latest laser ablation systems contain a z-axis drive to control the distance.[44]

In addition to the ablation cell, the laser performance must also be considered. Selective removal of biological tissues from glass substrates can be achieved after careful control of the laser fluence using an energy meter.[45] Compared to lasers with a low repetition rate (*e.g.*, ~20 Hz), an excimer laser can operate at 200–1000 Hz, enabling fast analysis and imaging. Van Malderen *et al.* achieved submicrometer LA-ICP-MS imaging at a pixel acquisition rate above 250 Hz with an ArF excimer laser.[44] A laser with a short wavelength also produces smaller thermal effects during the ablation, a more controlled ablation process, and a better distribution of ablated particles, which are beneficial to a successful LA-ICP-MS analysis.[46] The sizes of the laser spots in most modern laser systems are down to a few microns, making subcellular imaging possible. However, the lateral resolutions of

commercial systems are limited to ~1 μm, owing to the diffraction limit.

RECENT ADVANCES IN ICP-MS-BASED METHODOLOGY

Single particle analysis. Single particle ICP-MS (spICP-MS) is a new technique that utilizes the excellent detection capabilities of ICP-MS. spICP-MS can provide the elemental composition, number concentration, and size distribution of nanoparticles. In addition, ions dissolved from nanoparticles can be determined under the optimal conditions. This section discusses the basic theory and the application of spICP-MS in metallomics.

The spICP-MS method was developed by C. Degueldre and P.Y. Favarger.[47] The theory of spICP-MS can be found in many excellent reviews,[30,48] therefore, only a brief overview is given here. spICP-MS requires a sufficiently diluted nanoparticle solution at a constant flow rate. Under these conditions, only a single particle is statistically introduced into the ICP-MS instrument at a time. After being atomized and ionized, the nanoparticle is detected as a signal pulse in ICP-MS. The frequency of the nanoparticle pulses is directly proportional to the number concentration of the nanoparticle solution, and the intensity of the pulse is a function of the element mass of the nanoparticle. After the correction of transport efficiencies, standard solutions can be used for calibrations of single particles. spICP-MS can provide valuable information about the nanoparticles and reach extremely low number detection limits between 10^3 and 10^5 mL^{-1}.[49]

Despite the remarkable progress made to date, spICP-MS is still a developing technique that faces many challenges. The current size detection limits for metal nanoparticles, most of which are in the range of 10–80 nm in diameter,[50] often fail to meet the requirements of metallomics studies. Accordingly, more sensitive ICP-MS instruments and improved sample introduction systems are required. Hadioui *et al.* used a high-sensitivity sector field ICP-MS and a dry aerosol sample introduction system to successfully lower the size detection limits of Ag and TiO_2 nanoparticles to 3.5 and 12.1 nm, respectively, by spICP-MS.[51]

Multi-isotope analysis of a single particle is another challenge for spICP-MS, particularly for instruments equipped with a scanning analyzer, such as a quadrupole. ICP-MS instruments with a simultaneous analyzer can circumvent this challenge. Multi-collector (MC)-ICP-MS has been applied to multi-isotope analysis in single particles,[52] but the determined isotopes must be close to each other due to the instrument limitations. As mentioned in Section 2.2, TOF is considered a better choice for multi-isotope analysis of single particles. Praetorius *et al.* analyzed multi-isotopes in single particles with ICP-TOF-MS.[53] In this way, the engineered CeO_2 nanoparticles were distinguished from the natural Ce-containing particles in soils by multi-isotope fingerprints that were classified using a machine learning method.[53]

Sample preparation is crucial for spICP-MS analyses. The unique physical and chemical characteristics of nanoparticles should be considered to avoid changes in the form, size distribution, or aggregation state. The dilution of samples is always necessary in spICP-MS, and for real samples, further extraction and separation are also necessary to remove the matrix and minimize interference.[54] The nanoparticles from biological matrices have been successfully extracted from biological matrices using acids, alkalis, or enzymes, as reported in the literature.[55] However, standard protocols cannot be applied to nanoparticles in different biological matrices. Reliable protocols for spICP-MS sample preparation are urgently needed, for example, the development of certified reference materials for nanoparticles in biological matrices for method validation.[56]

In combination with nanoparticle labeling, spICP-MS offers a new opportunity for highly sensitive bioassays. Hu *et al.* developed a competitive heterogeneous immunoassay for the determination of α-fetoprotein in serum with an AuNP-tagged antibody and spICP-MS analysis. The quantification limit was 0.016 μg/L with a relative standard deviation of 4.2% for α-fetoprotein.[57] Later, Han *et al.* reported a novel method of a one-step homogeneous DNA assay using spICP-MS.[58] The hybridization of DNA targets with probes immobilized on the AuNP surfaces resulted in the formation of dimers, trimers, or even large aggregates of AuNPs. These changes were detected and the DNA concentration was obtained with spICP-MS. After the above pioneer works, spICP-MS based methods were successfully applied for the analysis of DNAs,[59] rRNAs,[60] and carcinoembryonic antigens.[61] Table 2 shows the selected applications of spICP-MS.

Single cell analysis. Cell heterogeneity is always present in all cell populations. Therefore, single cell analysis can provide valuable insights that are often covered in cell populations.[62] For the analysis of metals and metalloids in single cells, ICP-MS is considered to be the first choice.[63] Two main ICP-MS-based methods have been applied for single cell analysis: single cell ICP-MS (scICP-MS) for the analysis of cells in a suspension and LA-ICP-MS for the analysis of cells on a substrate.

The scICP-MS shares a similar theory and technique with spICP-MS. The single cells were sprayed sequentially into the high-temperature plasma, where the constituents in each cell were atomized, ionized, and detected by ICP-MS in the time-resolved mode. In the mass spectra, the intensity of each transient signal is

Table 2. Applications of spICP-MS analysis

Instrument	Nebulizer	Spray chamber	Dwell time (ms)	Transport efficiency (%)	Size detection limit (nm)	Application	Reference
ICP-QMS Agilent 4500	Babington	–	10	–	Aluminum particles: 30	Colloids in water	47
ICP-QMS Thermo X2	Concentric	Conical with impact bead	10	–	AuNPs: 15	Immunoassay with AuNP tags	57
ICP-QMS Agilent 7500	Concentric	Scott double pass	10	~9	–	AgNPs and AuNPs in water	49
ICP-QMS Thermo X2	Concentric	Conical with impact bead	0.5	0.75	AuNPs: 15	DNA assay with AuNP probes	58
ICP-QMS PerkinElmer NexION 300Q	Concentric (glass)	Cyclonic	10	4–6	15–20	AgNPs and AuNPs in biological tissues	101
MC-ICP-MS Nu Plasma HR	Concentric	Cyclonic	200	–	130	ErNPs in water	52
ICP-QMS Perkin Elmer NexION 350D	Concentric	Cyclonic	0.1	–	20	AuNPs in tomato plants	102
ICP-QMS Thermo iCAP	Microflow concentric	Cyclonic	5	–	56	Lead nanoparticles in game meat	103
ICP-TOFMS TOFWERK icpTOF	Concentric	Cyclonic (quartz)	0.3	–	180	CeO_2 nanoparticles in soils	53
ICP-QMS PerkinElmer NexION 300D	Concentric (PFA-ST)	Cyclonic (glass)	0.05	~9	19	AgNPs in soils	104
ICP-QMS Agilent 7900	Concentric	Cyclonic (quartz)	0.1	3.5	18	SeNPs in yeast	105
ICP-QMS PerkinElmer NexION 300X	Concentric (PFA-ST)	Cyclonic (PC^3 glass)	0.05	3–5	~14	AgNPs in bivalve mollusks	106
ICP-SFMS Nu AttoM ES	Microflow concentric	Desolvation system	0.05	16	AgNPs: 3.5 TiO_2NPs: 12.1	TiO_2 NPs in sunscreen lotions, rainwater, and swimming pool water	51
ICP-QMS PerkinElmer NexION 350	Concentric	Cyclonic	0.05	15	–	Protein analysis with AuNP tags	61
ICP-QMS Agilent 7700x	Concentric	Scott double pass	3	7.8	AgNPs: 23 AuNPs: 16	AgNPs and AuNPs in sewage sludge	107
ICP-TOFMS TOFWERK icpTOF 2R	Concentric	Cyclonic (quartz)	3	7–15	6–311 Dependence on isotopes	Particles in rivers	108
ICP-TOFMS TOFWERK icpTOF 2R	Concentric	APEX Ω desolvation system	0.3	11.6	40 to several hundred Dependence on isotopes	Gunshot residues	109
ICP-TOFMS TOFWERK icpTOF	Concentric	Cyclonic	2	5–7	15–307 Dependence on isotopes	Anthropogenic nanomaterials in urban rain and runoff	110

related to the atomic constituents in a single cell, and the frequency of the transient signals is directly proportional to the number of cells. scICP-MS has been utilized for the analysis of metals in bacteria,[64] trace elements in algae[65,66] and in mammaliam cells,[20,67–70] metal medicines in human cells,[71,72] and nanoparticles in *Tetrahymena*.[73] Using a chemical labeling strategy, the fucose contents in single cells were determined via europium using scICP-MS, achieving a detection limit of 4.2 zmol fucose.[74] Single bacteria metabolically labeled by a lanthanide-encoding were counted and recognized with scICP-MS, providing a new way to identify bacteria and study variability at a single bacterium level.[75]

Mass cytometry, a specific scICP-MS using a TOF analyzer, was originally designed to analyze single cells labeled with lanthanide isotopes.[76] Instead of the fluorophores in traditional flow cytometry analysis, enriched isotopes are labeled with single cells as reporters and are analyzed by mass cytometry. More than 40 parameters can be determined in single cells using mass cytometry because many stable isotopes are available as reporters without any spectral overlap.[77] This post-fluorescence technique is believed to be the next platform for flow cytometry, allowing simultaneous analysis of the cell surface and intracellular proteins, signaling components, cell cycle state, cell viability, and nucleic acids (mRNA and DNA) in a single cell.[77] Table 3 shows the recent applications of scICP-MS analysis in single cell suspensions.

Table 3. Applications of scICP-MS analysis

Instrument	Nebulizer	Spray chamber	Dwell time (ms)	Transport efficiency (%)	Sample flow rate (μL min^{-1})	Application	References
ICP-SFMS **Thermo Element 1**	Microflow concentric	Scott double pass	4	–	200	Uranium in bacteria	64
ICP-QMS **Agilent 7500a**	V-groove	Scott double pass	10	0.6	400	Trace elements in algal cells	65
ICP-QMS **Agilent 7500a**	Microflow concentric	Custom-made single pass	10	3.0	20	Determination of Bi drugs in single *Helicobacter pylori* cells	72
ICP-QMS **Thermo X7**	Concentric	Conical with impact bead	5	3.1	250	Determination of quantum dots in single cells	111
ICP-QMS **Agilent 7500a**	Concentric	Single pass	0.05–10	~100	10	Highly efficient single cell analysis of microbial cells	19
ICP-QMS **Thermo X7**	Microflow concentric	Conical with impact bead	5	3.1	200	Quantification of Gd@C82(OH)22 and cisplatin in HeLa and 16HBE cells	71
ICP-QMS **PerkinElmer NexION 300D**	Concentric (PFA-ST)	Cyclonic (quartz)	5	1	320	Determination of Fe, Cu, Zn, Mn, P, and S in HeLa and A549 and 16HBE	17
ICP-TOFMS **Fluidigm CyToF2**	Concentric	Single pass	0.013	–	45	Quantification of AgNPs in human T-lymphocytes	112
ICP-QMS **Agilent 7700**	Parallel path (EnyaMist)	Single pass	10	25	10	Cisplatin in human ovarian carcinoma cells (A2780)	113
ICP-QMS **Thermo XII**	Microflow concentric	–	5	2.96	30	Quantification of Fe, Pt in HepG2 cells with a droplet-splitting microchip	67
ICP-QMS **PerkinElmer NexION 300D**	Microflow concentric	Single pass (heated)	4	2	10	Quantification of Mn, Fe, Co, Cu, Zn, P, and S in HeLa and A549 and 16HBE cells.	20
ICP-QMS **PerkinElmer NexION 350D**	Concentric (Meinhard)	Single pass (Asperon)	0.05	31.33	283	Quantification of Au NPs in freshwater algae	66
ICP-QMS **Agilent 7500a**	Concentric (quartz)	Scott double pass	10	4	5	Quantification of Au NPs in MCF-7 cells	114
ICP-QMS **Agilent 7900**	Concentric (MicroMist)	Scott double pass	10	0.02–0.03	300	Quantification of Ag NPs in single cells	115
ICP-MS/MS **Thermo iCAP-TQ**	Concentric (MicroMist)	Cyclonic	1	25	10	Quantification of Cu in individual spores	116
ICP-QMS **PerkinElmer NexION 300X**	Concentric (PFA)	Single pass (Asperon)	0.05	9.9	19.4	Evaluation of As uptake and lipid profile changes from cells	117
ICP-TOFMS **Fluidigm CyTOF**	Concentric	Single pass	–	–	30	Quantification of Au NPs in *Tetrahymena thermophila*	73
ICP-MS/MS **Agilent 8900**	Concentric （Quartz）	Scott double pass	1	–	200	Intracellular antagonism of Cu2+ against Cd2+ in different cells	70
ICP-QMS **PerkinElmer NexION 300D**	Microflow concentric	Single pass	0.1	12	40	Quantification of AuNPs in individual HepG2 cells	118
ICP-TOFMS **Fluidigm CyTOF**	Concentric	Single pass	–	–	30	Quantification of Pb in human erythrocytes	69
ICP-QMS **PerkinElmer NexION 300D**	Concentric (Meinhard)	Single pass (Asperon)	0.05	30–40	15–20	Quantification of AgNPs in yeast cells	119
ICP-QMS **PerkinElmer NexION 2000**	Concentric	Single pass (Asperon)	0.10	15-36	20	Counting and recognizing single bacteria via lanthanide encoding	75
ICP-QMS **PerkinElmer ELAN DRC-II**	Micronebulizer (Homemade)	None (Direct infusion)	10	>70	13	An oil-free passive microfluidic system for high transport efficiency of single cells	21

The discussions in Section 3.1 on the challenges and solutions in spICP-MS analysis can also be applied to scICP-MS. In addition, other issues should be considered in scICP-MS analysis. First, the sample preparation of single cells should be performed with additional caution to maintain the cell integrity and avoid elemental losses. The buffer solutions used for the cell sample preparation should be compatible with ICP-MS because organic solvent or inorganic salts often have adverse effects. In mass cytometry, fixation and permeabilization are usually required. However, these treatments may lead to the loss of naturally occurring metals in the cells, which restricts their application in metallomics. Second, the behaviors of single cells in the plasma, such as transport efficiency and atomization-ionization efficiencies, are different from those of standard solutions. Therefore, the intracellular elements usually exhibit different behaviors from the calibration solutions. For example, Li *et al.* found a 30% lower sensitivity for uranium in single bacteria, compared to the uranium solution.[64] The development of calibration methods and certified standard materials of single cells will ensure that scICP-MS is an accurately quantitative method.

Unlike solution-based scICP-MS, LA-ICP-MS enables *in situ*

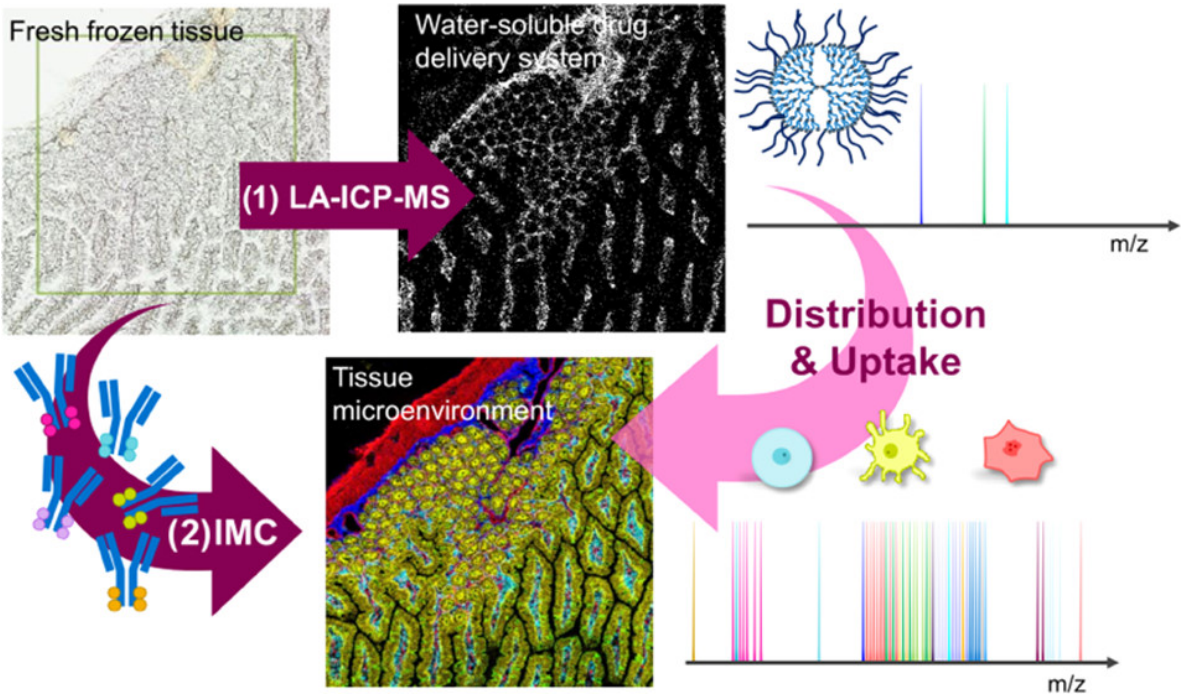


Fig. 1 A new strategy for imaging both metals and proteins in tissue sections. The strategy involves two consecutive imaging acquisitions from the same tissue section with LA-ICP-MS analysis followed by tissue staining and a standard IMC analysis (Reprinted with permission from Strittmatter N *et al.* Anal. Chem. 2021, 93:3742. Copyright 2022 American Chemical Society.)[100]

analysis of single cells. A single cell is ablated by laser shots, and the resulting aerosol is introduced and analyzed by ICP-MS. The transport efficiency of the aerosols is high, nearly 100% under the optimal conditions, and is independent of the cell size. In addition, aerosols are more easily ionized compared with intact cells, making quantitative analysis of single cells possible. However, some obstacles must be overcome to enable wider applications of LA-ICP-MS for single cell analysis. The first obstacle is the low analytical throughput. In many applications reported in the literature, single cells are manually targeted under a microscope and then analyzed by LA-ICP-MS.[78–80] The entire process is typically slow and time consuming. To improve the analytical throughput, Zheng *et al.* designed a microwell array to trap single cells, which were analyzed by LA-ICP-MS using a grid pattern, greatly improving the analytical thoughput.[81] Löhr *et al.* used a piezo-acoustic spotter to produce a single cell array for high-throughput LA-ICP-MS analysis. Under the optimal parameters, a single cell occupancy of >99%, high throughput of up to 550 cells per hour, and high cell recovery (>66%) were achieved.[82]

The second obstacle is the lack of commercially available reference materials for single cells; thus, it is difficult to achieve accurate and reliable quantification with LA-ICP-MS and to compare the results with those from different laboratories. Many in-house standards have been developed to overcome this obstacle. Wang *et al.* used the dried residues of picoliter droplets as the single cell standards for LA-ICP-MS analysis.[80] Van Malderen *et al.* added a range of concentrated Cu standard solutions to gelatin and used microfabrication techniques to prepare a high-density microarray gelatin standards.[83] Besides the in-house standards for external calibration, isotope dilution calibration was developed in which each cell is dispensed with a known picoliter droplet of an enriched isotope solution using an inkjet printer and then analyzed using isotope dilution LA-ICP-MS.[84]

Spatial metallomics. The spatial organization of cells and tissues is closely related to biological functions, and understanding the spatial context is critical for life sciences research.[85] When cells are dissociated from tissues, the spatial context is lost. To address this matter, techniques for spatially resolved multi-omics have been rapidly developing in recent years. For example, spatial transcriptomics was selected by *Nature Methods* as the Method of the Year 2020;[86] a high-spatial-resolution multi-omics sequencing technique named DBiT-seq was developed for co-mapping mRNAs and proteins in tissue sections.[87] In terms of metallomics, it is also necessary to understand the localization, speciation, and metabolism of the metals and metalloids at a subcellular level and study their connections and interactions with the genes, proteins, or metabolites in the spatial dimension, *i.e. spatial metallomics*. In combination with other spatial omics, spatial metallomics will

provide profound insights into not only cellular phenotypes but also the basic chemicals underlying these cellular properties.

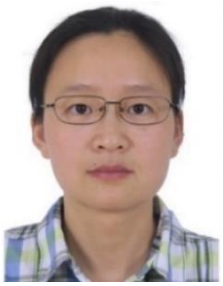
Although there is no specific technique for spatial metallomics thus far, LA-ICP-MS is one of the most promising tools owing to its unique characteristics, as described in Section 2. LA-ICP-MS was used for the elemental imaging of biological tissues in 1994.[88] Since then, LA-ICP-MS has been applied in the imaging of metal contrast agents[89] and trace elements[90]; however, most of these are limited to a few metals or metalloids and are not combined with other spatial omics techniques. With rapid advances in laser ablation systems and ICP-TOF-MS instruments, the new generation of LA-ICP-MS can provide the capabilities of fast imaging speed, high spatial resolution, and full mass spectral scan,[41] making it more suitable for spatial metallomics. Quantitative imaging can also be achieved using the appropriate matrix-matched standard materials, either certified standard materials[91] or in-house standards.[92,93] Finally, many open-source or commercial software packages have been developed to process and reconstruct the enormous spatial imaging data from LA-ICP-MS.[94,95]

Owing to these advances, new methods have been developed. Three-dimensional (3D) quantitative imaging of metals and metalloids has been realized by imaging successive slices of a sample with LA-ICP-MS and reconstructing the images into three dimensions. This method has been applied to the 3D modelling of metallomes in a wide range of biological samples, such as single cells[96] and mouse brains.[97] LA-ICP-MS can image biomolecules in tissues in combination with immunohistochemistry methods, which is usually called imaging mass cytometry (IMC) by life scientists.[98] Biomolecules in tissue sections are stained with metal probes and then imaged via the probes using LA-ICP-MS.[98] Giessen *et al.* simultaneously imaged 32 proteins and protein modifications in tissue sections at a subcellular resolution with LA-ICP-TOF-MS.[99] The success of IMC demonstrates that LA-ICP-MS could become a spatial multi-omics platform. The distributions of metals and proteins in the same tissue section were obtained using a strategy of two consecutive imaging acquisitions with LA-ICP-MS analysis followed by tissue staining and a standard IMC analysis (Fig 1).[100] The LA-ICP-MS technique may be further expanded to image mRNAs or DNAs once suitable metal probes are developed, realizing spatial multi-omics analysis using the same LA-ICP-MS instrument.

CONCLUSIONS

ICP-MS was commercialized approximately 40 years ago and has since become a versatile tool for both routine analysis and pioneering research. The development of ICP-MS is progressing towards higher sensitivity, better detection limits, faster acquisition speed, *in situ* analyses, and automatic data processing. The ICP-MS application also greatly expands to many aspects of metallomics, such as single particle analysis, single cell analysis, and spatial metallomics. It must be emphasized that a single technique cannot meet all the challenges of metallomics; thus, comprehensive strategies should be developed, such as the integration of metal determination with ICP-MS, metal-complex identification with biological mass spectrometry, and speciation characterization with synchrotron X-ray absorption spectroscopy. As with other omics research, metallomics is both a method-driven and data-driven science. An enormous amount of data acquired from various instruments must be handled and interpreted. Thus, a bioinformatics approach is crucial and urgently required in the future. With the rapid development of instrumentation and methodology, ICP-MS-based techniques will evolve further and play a dominant role in metallomics research.

AUTHOR INFORMATION



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The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (11975251 and 11875268) and the fund of the State Key Laboratory of Environmental Chemistry and Ecotoxicology (KF2020-19).

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