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A systematic study of high resolution multielemental quantitative bioimaging of animal tissue using LA-ICP-TOFMS

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Methodology for quantitative bioimaging of essential metals including Mg, Mn, Fe, Cu and Zn in tissue cryosections based on the use of high-resolution laser ablation coupled with inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOFMS) has been systematically developed and characterized for the first time. Cryosectioned gelatin standards spiked with all the elements of interest were used for calibration. Analysis under 'no gas' conditions showed satisfactory selectivity and 3-fold improved limits of detection for Mn, Fe and Cu when compared with those when using 2.4 ml min⁻¹ H₂ in the collision/reaction cell of the ICP-TOFMS instrument. Absolute single-pixel limits of detection at 3 μm spatial resolution were in the range of 4–9 femtograms for Mn, Cu and Zn and approximately 40 femtograms for Mg and Fe. Gelatin standards spiked with acidified single-element solutions and with solutions prepared from metal salts were shown to have similar elemental homogeneity. The impact of variable thickness of cryosectioned gelatin on the signal intensity was studied and the linearity of response was established for thicknesses between 10 and 30 μm. The developed method was used for the quantification of essential metals in kidney sections of rats dosed with bis-choline tetrathiomolybdate (TTM) to regulate Cu levels in relation with Wilson's disease as well as in kidney sections of control animals. To achieve this, the developed methodology was extended to also include Mo and detailed distribution of Mo/Cu ratios at cellular to subcellular resolution was obtained, showing fine correlation between these elements in the medulla regions of the kidney.

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Introduction

Metals in the organism may be essential or beneficial as well as non-essential, harmful, or toxic. The role of each metal is governed by bioinorganic chemistry of the organism or a particular cell type.¹ Metal dysregulation is implicated in the pathology of a range of medical conditions including neurodegenerative disorders such as Alzheimer's disease (AD)² and metabolic disorders such as Wilson's disease (WD).³ In cancer, metals play a role in cancer development, for example, in cancer metastasis and also treatments *via* metal-containing drugs.^{4,5} Additionally, metal ions released from metallic implants in the body may be transported and accumulated in organs causing undesirable phenomena.⁶ Therefore, visualizing metals distribution in organs and at cellular levels is critical for understanding the role metals play in the body and how their behaviour might correlate to wider physiological functions.

To better understand how metals function and to discriminate between healthy and diseased tissues as well as to compare data over time and between populations, it is imperative to determine both localization and concentration of metals at

a cellular to sub-cellular resolution. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is the most advanced technique for obtaining spatially resolved information and quantification of metals and many non-metals in thin sections of tissue given its excellent limit of detections (LOD) which are in the range of μg kg⁻¹ to mg kg⁻¹ and fit-for-purpose spatial resolution (μm to <1 μm range). Quantification of elements in animal tissue sections using LA-ICP-MS has associated remaining challenges, of which the heterogeneity of biological samples and the lack of suitable reference materials are significant. Factors such as ablation properties, transport-related phenomena, ionization in the ICP source, signal drift over analysis time and matrix effects need to be addressed for correct quantification.⁷ The possibility to correct for the variability of these factors by using internal standards – either present in the tissue section such as ¹³C or somehow deposited onto the section or the specimen slide is very limited. Despite many approaches proposed so far, no truly universal calibration and quantification protocol exists for the spatially resolved quantification of metals in biological tissues.^{8,9}

One of the main considerations for LA-ICP-MS bioimaging is the maximum imaging speed at which individual pixels of the elemental map are fully resolved. The imaging speed is determined by the highest laser repetition rate available, stability of

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the laser energy, speed of the sample stage movement, washout time within the ablation chamber and the sample transfer line as well as the speed of the mass-spectrometric detection.¹⁰ An ability to completely ablate the full thickness of the specimen without sampling the specimen substrate such as soda-lime glass is another consideration and is addressed by either using higher laser wavelength (typically 266 nm)¹¹ or by carefully controlling laser energy.¹² Development of laser ablation systems in the last few years addressed the imaging speed by providing improved stage control and fast-washout sample transfer solutions.¹³ Commercially available laser ablation systems^{14,15} using low-dispersion (ultrafast) ablation cell geometries are now capable of pulse-resolved imaging at repetition rates of 100 Hz and above.¹⁶

At the same time, scanning mass analysers used by the most common ICP-MS instruments introduce restrictions to the number of isotopes that can be detected quasi-simultaneously from sections of a transient ion beam corresponding to individual laser shots. This limitation was a driving force in developing and improving the ICP-MS instrumentation employing time-of-flight (TOF) analysers (ICP-TOFMS), which acquires the entire mass range at short integration times.^{17–19} ICP-TOFMS is well-suited for the analysis of short transient events, such as those generated by single-pulse-resolved laser ablation.²⁰ Although the single-isotope sensitivity for commercially available TOFMS instruments is lower than that obtainable with single-*m/z* monitoring on quadrupole mass spectrometers (QMS), TOFMS surpasses sequential ICP-MS instruments in total ion sensitivity, which is critical for multielemental analysis.²¹ Moreover, modern ICP-TOFMS instruments can record the entire elemental mass range signals at 500 Hz or higher. To achieve artefact-free imaging, triggered acquisition can be employed to enable real-time, spot-resolved multielemental analysis synchronized with the arrival of the aerosol plume of a single laser shot to the ICP-TOFMS.²² The developments in the ICP-TOFMS provide a solid analytical platform for all areas of solid-state analysis and, in particular, for bioimaging where fast multielemental detection is a paramount.^{10,18,23–30}

Quantification strategies for silicate materials in LA-ICP-MS typically include external calibration using one or more certified reference materials (CRMs) with a silicate glass matrix and with the intensity corrected by the intensity of an internal standard (IS). The IS is typically one of the major components of the sample and it may be determined by an independent technique. Using this traditional approach in other areas of solid-state analysis including bioimaging presents a serious challenge since no matrix CRMs are currently available. This is certainly true for most bioimaging applications such as imaging of animal tissue sections and single cells. Chemical elements present in animal tissue at high levels and homogeneously distributed (*e.g.*, C, N, O) are not necessarily suitable for LA-ICP-MS analysis and/or for quantification by an independent non-destructive technique and, so, their potential use as IS is limited.

Several materials have been used as calibration standards in LA-ICP-MS bioimaging: inkjet-printed paper,³¹ commercially available powdered CRMs with animal tissue matrix,^{23,24,32}

spiked agarose film,³³ spiked homogenised tissue,³⁴ and spiked gelatin.³⁵ Of those, the closest matrix-matching was likely achieved by using homogenised tissue and gelatin-based preparations. Both materials can be spiked with the elements of interest at different concentration levels, providing multi-point calibration curves, and can be frozen and sliced to the same thickness as the material to be imaged, to provide direct comparison.^{35–38}

Over the past few years, calibration with spiked gelatin presented as either cryosections^{39–42} or droplets, deposited manually,^{43,44} in microarrays⁴⁵ or using a microspotter^{27,28} or a bioprinter,³⁰ has largely replaced matrix-matched calibration with spiked homogenised tissue in LA-ICP-MS bioimaging. Despite the wide use of gelatin standards, quantification using gelatin cryosections has not yet been studied systematically and applied to single-shot high-resolution imaging. The limits of this quantification strategy, such as the effect of sample heterogeneity at a single-pixel level and the possibility of using different section thickness for the standard and the sample, have not been discussed in detail so far.

This work describes a systematic approach towards spatial multi-element quantification of several essential metals including Mg, Cu, Zn, Mn and Fe in biological tissue using high resolution LA-ICP-TOFMS and calibration with multi-element spiked cryosectioned gelatin standards. The optimal LA-ICP-TOFMS conditions for achieving a compromise between sensitivity and selectivity were investigated. The effect of the spiking solution (*e.g.*, with pH-neutral salts or HNO₃-based solutions) on the elemental homogeneity of the produced standards was studied. The impact of thickness of cryosectioned gelatin on the signal intensity was studied at thicknesses between 10 and 30 μm and the fit-for-purpose linearity of response was established. Finally, the developed methodology was applied to the quantitative imaging of Mg, Mn, Fe, Cu Zn and Mo in kidney samples of the Sprague Dawley rat following bis-choline tetra-thiomolybdate treatment to regulate Cu levels in relation to Wilson's disease.

Experimental

Instrumentation

For all experiments, an imageBIO266 laser ablation system (266 nm, Elemental Scientific Lasers, USA) in combination with a low-dispersion ablation cell in a TwoVol3 ablation chamber was coupled using a 0.8 mm ID PEEK tubing and a Dual Concentric Injector (DCI) to an ICP-TOFMS instrument (icpTOF2R, ToFwerk AG, Switzerland). The tubing length between the laser ablation system and the DCI was kept to a minimum (<15 cm). LA was carried out in a helium atmosphere (99.99% purity, BOC Ltd, UK) and a stream of argon gas (99.99%, BOC Ltd, UK) served as the carrier gas.

The laser ablation settings and ICP-TOFMS settings were optimized daily to achieve high intensities for ⁵⁹Co⁺, ¹¹⁵In⁺, and ²³⁸U⁺ while keeping the oxide level (based on ²³²Th¹⁶O⁺/²³²Th⁺) below 1% and the laser-induced elemental fractionation (based on ²³²Th⁺/²³⁸U⁺) at 1 ± 0.2. An in-house prepared gelatin-based



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| Laser ablation parameters | |
| Laser fluence | 6 J cm ⁻² |
| Laser spot diameter | 3 μm |
| Laser repetition rate | 100 Hz |
| Laser scan speed | 300 μm s ⁻¹ |
| Ablation gas flow 1 (He) | 0.45 L min ⁻¹ |
| Ablation gas flow 2 (He) | 0.55 L min ⁻¹ |
| ICP-TOFMS parameters | |
| Carrier gas flow | 0.85–0.87 L min ⁻¹ |
| Auxiliary gas flow | 0.8 L min ⁻¹ |
| Cooling gas flow | 14 L min ⁻¹ |
| RF power | 1550 W |
| Collision/reaction cell (CRC) gas | No gas mode: unpressurized cell Hydrogen mode: 2.4 ml min ⁻¹ H ₂ |
| Notch filter ²³² Th ⁺ / ²³⁸ U ⁺ | ⁴⁰ Ar ⁺ , ⁴⁰ Ar ₂ ⁺ 0.85–1.1 |
| ThO ⁺ /Th ⁺ | <1% |
| Monitored isotopes | ²⁴ Mg, ²⁵ Mg, ²⁶ Mg, ⁵⁵ Mn, ⁵⁶ Fe, ⁵⁷ Fe, ⁶³ Cu, ⁶⁵ Cu, ⁶⁴ Zn, ⁶⁶ Zn, ⁶⁸ Zn, ⁹⁵ Mo, ⁹⁸ Mo |

the Igor Pro (Wavemetrics, USA) environment. Software was used to model and subtract the baseline intensities that elevate the total signal on each m/z channel. Following mass calibration, baseline subtraction, and peak integration of isotopes from every TOFMS spectrum, the integrated signal time traces for all m/z channels were exported as comma-separated values (CSV) files using a TwImage programme (Tofwerk AG, Switzerland). Those m/z channels that had overlapping signals of interfering ions (^{55}Mn , ^{56}Fe , ^{57}Fe , ^{65}Cu) were processed by peak fitting, the rest of the channels by peak integration.⁴⁸

Results and discussion

Selectivity and limits of detection

Despite numerous advantages of ICP-MS, spectral interferences greatly affect the selectivity of the ICP-MS detection of many elements. Spectral overlaps, typically singly or doubly charged atomic and molecular ions, depend on the sample composition and the conditions of the plasma. Quadrupole-based ICP-MS instruments are generally not capable of mass separation of these spectral interferences. The mass resolving power (R_m) of the icpTOF2R is in the range of 5000–6000 (reported as $m/\Delta m$, where Δm is the width of the mass peak at full-width half-maximum; FWHM) and is sufficient to resolve many doubly charged atomic and some molecular interferences. As the TOF mass analyzer measures all ion signals within the measurable m/z range, both the identification of interferences and deconvolution of overlapping mass-spectral peaks are possible. For example, Hendriks *et al.*²¹ showed how peak deconvolution increases R_m effectively about 2-fold and so can isolate the signals of $^{31}\text{P}^+$ and $^{68}\text{Zn}^+$ from background species ($^{15}\text{N}^{16}\text{O}^+$, $^{14}\text{N}^{16}\text{OH}^+$, and $^{40}\text{Ar}^{14}\text{N}^{14}\text{N}^+$, respectively).

However, R_m of the icpTOF2R may not be sufficient when abundant molecular ions need to be separated from the isotopes of interest. Another approach to resolve spectral interferences is to use ion-molecule reactions (using a reaction gas such as H_2) or kinetic energy discrimination (using He as a collision gas) in a multipole ion guide. Burger *et al.*⁴⁹ investigated the impact of using H_2 or He as reaction and/or collision gases in the LA-ICP-TOFMS to target ^{40}Ca and ^{80}Se and improved LODs by orders of magnitude.

In the current work, we have investigated the effect of using H_2 in the collision/reaction cell (CRC) on the selectivity and sensitivity of the simultaneous detection of Mg, Mn, Fe, Cu and Zn. The H_2 flow rate (2.4 ml min^{-1}) was selected at the level at which $^{40}\text{Ar}^+$ did not need to be removed from the ion beam by the radiofrequency (RF) quadrupole notch filter. We compared the performance of the fast multi-elemental LA-ICP-TOFMS imaging under the conditions optimal for their selective determination (hydrogen mode) with that under the conditions optimized for the best overall sensitivity (no gas in the CRC).

The detection of the most abundant isotope of iron (^{56}Fe) by ICP-MS is known to be affected by the $^{40}\text{Ar}^{16}\text{O}^+$ interference. The effect of H_2 in the CRC on the selectivity of ^{56}Fe detection by LA-ICP-TOFMS was investigated. Fig. 1 shows that $^{57}\text{Fe}/^{56}\text{Fe}$ values obtained by LA-ICP-TOFMS at 100 Hz repetition rate for five Fe concentration levels (45–400 mg kg^{-1}) with no gas and with 2.4

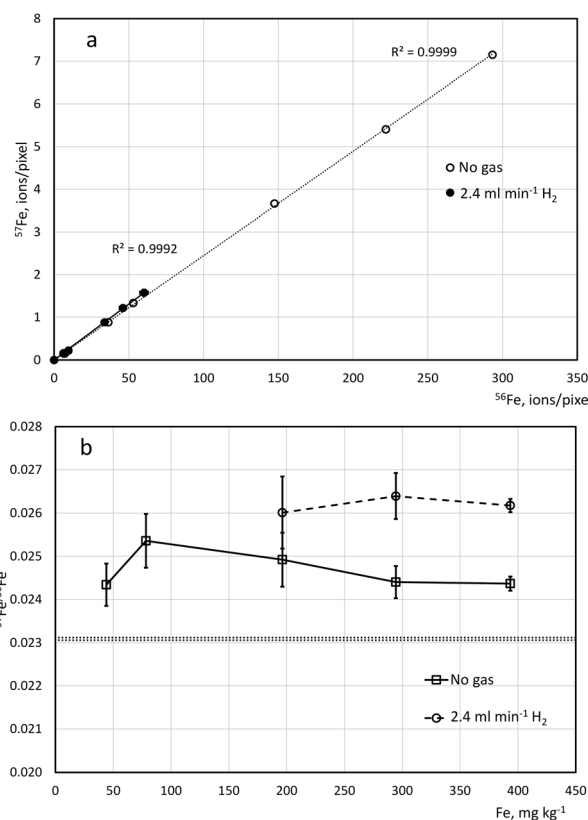


Fig. 1 Relationship between the intensities of ^{57}Fe and ^{56}Fe (a) and the ratio of $^{57}\text{Fe}/^{56}\text{Fe}$ (b) as detected by LA-ICP-TOFMS, under no-gas conditions in the CRC and using H_2 at 2.4 ml min^{-1} , in gelatin-based standards spiked with Fe at five concentration levels (milligrams per kilogram of dry gelatin). Isotope ratios were only calculated for intensities of ^{57}Fe above 1 count per pixel. Ablation was conducted at $3 \mu\text{m}$ spot size at 100 Hz laser repetition rate, isotope ratios calculated after subtracting the blank signal. Errors bars represent standard deviation (1sd) between four individual gelatin sections. The natural range of $^{57}\text{Fe}/^{56}\text{Fe}$ is within the dot lines.

$\text{ml min}^{-1} \text{H}_2$ in the CRC are near the range of natural $^{57}\text{Fe}/^{56}\text{Fe}$ values (0.02306–0.02312).⁵⁰ The deviation of the isotope ratios from the natural value (6% and 13% for unpressurized cell and cell pressurized with H_2 , respectively) is likely to be explained by the mass bias towards the heavier isotope, known for the ICP-TOFMS instruments.^{51,52} The data obtained in this study show that the mass-resolving power of icpTOF2R in combination with peak deconvolution is sufficient for selective low-signal detection of ^{56}Fe . While pressurizing CRC with H_2 has benefits in enabling detection of ^{40}Ca , ^{39}K , and ^{80}Se , it was found to lead to lower single-pixel LOD as the signal suppression due to

Table 2 Single-pixel LOD of the elements in gelatin standards sectioned at $30 \mu\text{m}$ thickness for $3 \mu\text{m}$ spot size at 100 Hz repetition rate in LA-ICP-TOFMS

| LOD, mg kg^{-1} | ^{24}Mg | ^{55}Mn | ^{56}Fe | ^{63}Cu | ^{64}Zn |
|--------------------------------------|------------------|------------------|------------------|------------------|------------------|
| Unpressurized cell | 59 | 3.2 | 27 | 10 | 15 |
| $2.4 \text{ ml min}^{-1} \text{H}_2$ | 123 | 9.2 | 77 | 33 | 17 |



collision/reaction gas led to poorer counting statistics (Table 2). These results suggest that unless there is a need to include elements affected by the notch filter (*e.g.*, Ca and K) it is more beneficial to conduct multielemental LA-ICP-TOFMS bioimaging under unpressurized conditions (no gas in the CRC), especially when using an instrument with higher mass resolution such as icpTOF2R.

Single-pixel LODs for Mg, Mn, Fe, Cu and Zn (Table 2) have been determined based on the analyte signal obtained by laser ablation of unspiked gelatin. Typically, data for 8500–10 500 pixels were collected for each of the 3–4 individual gelatin sections. The same number of replicates applied for the standards used for the calibration curve.

The compound Poisson distribution that describes the low-count TOFMS signals⁵³ is dependent on an empirical detector response curve, and thus cannot be simply calculated. This potentially affects the correct determination of LOD for LA-ICP-TOFMS imaging, especially at higher laser repetition rates. However, since most pixels obtained from 30 μm unspiked gelatin sections at 100 Hz were giving non-zero signal (higher than 1 count per pixel), the so-called “IUPAC approximation”⁵⁴ is deemed to be satisfactory²⁴ for the purposes of calculating LOD:

$$\text{Single-pixel LOD} = (3.29\sigma_B + 2.71)/\text{sensitivity},$$

where LOD is the minimum detectable value and σ_B is the standard deviation of the single-pixel measurement of an unspiked gelatin sample.

Influence of the spiking solution on the homogeneity of the gelatin standards

Preparing calibration standards for quantitative LA-ICP-MS bioimaging that would be stable, reproducible and homogeneous is a challenging task that has been approached by researchers in a number of ways. Commercially obtained gelatin typically contain very low concentrations of many elements of interest, can be further purified if needed and may be handled in more creative ways than homogenised tissue and so it is often a matrix of choice. As the integrity of gelatin can be compromised by low pH caused by spiking with commercial element stock solution, typically in nitric or hydrochloric acid matrix, spikes prepared from metal salts and/or buffering of the gelatin solution have been proposed.⁵⁵ In this study, the homogeneity of the standards prepared with addition of HNO_3 -based spikes and with the addition of spikes prepared from nitrate salts without acidification was compared. In chemical imaging, the homogeneity of the sample can be described by the value of single-pixel standard deviation of the analyte signal intensity, assuming the noise produced by heterogeneity is larger than the noise produced by the process of measurement. Therefore, the comparison was conducted based on the values of the between-pixel relative standard deviation (RSD) at two different levels of spiking for Mg and Zn, and at three different level for Mn and Cu (Fig. 2).

To eliminate the effect of spurious pixels (caused by *e.g.*, a dust particle or a bubble in gelatin) on the single-pixel

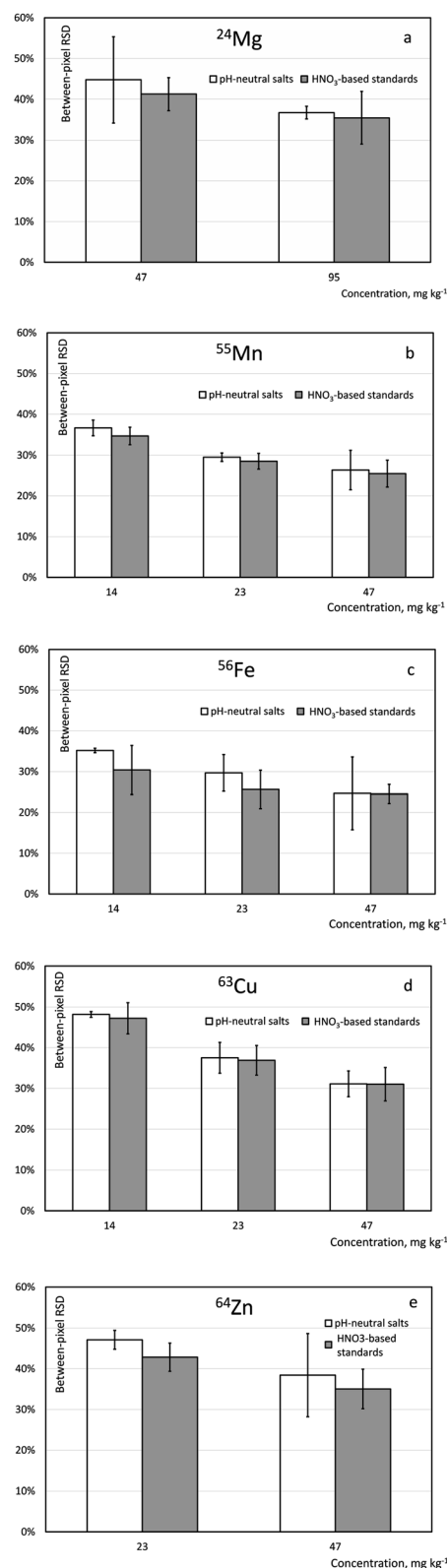


Fig. 2 Single-pixel relative standard deviation (RSD) of the LA-ICP-TOFMS imaging signal for ²⁴Mg, ⁵⁵Mn, ⁵⁶Fe, ⁶³Cu and ⁶⁴Zn (a–e) in gelatin standards spiked at different levels (14–95 milligrams per kilogram of dry gelatin) with pH-neutral salt and HNO_3 -based standard solutions, after removal of outliers. Error bars correspond to 1sd between the average responses obtained from three separate cryosections.



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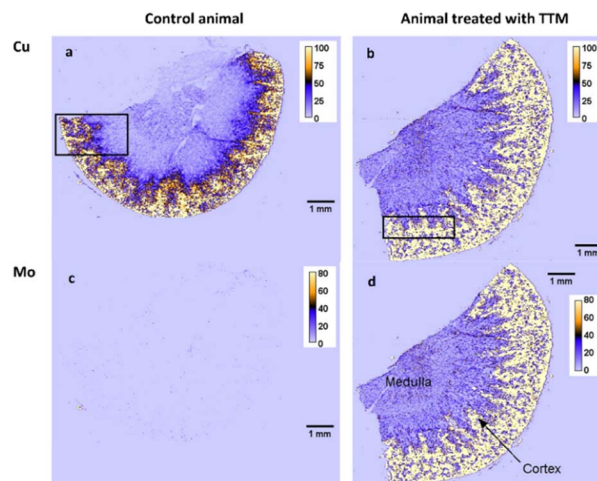


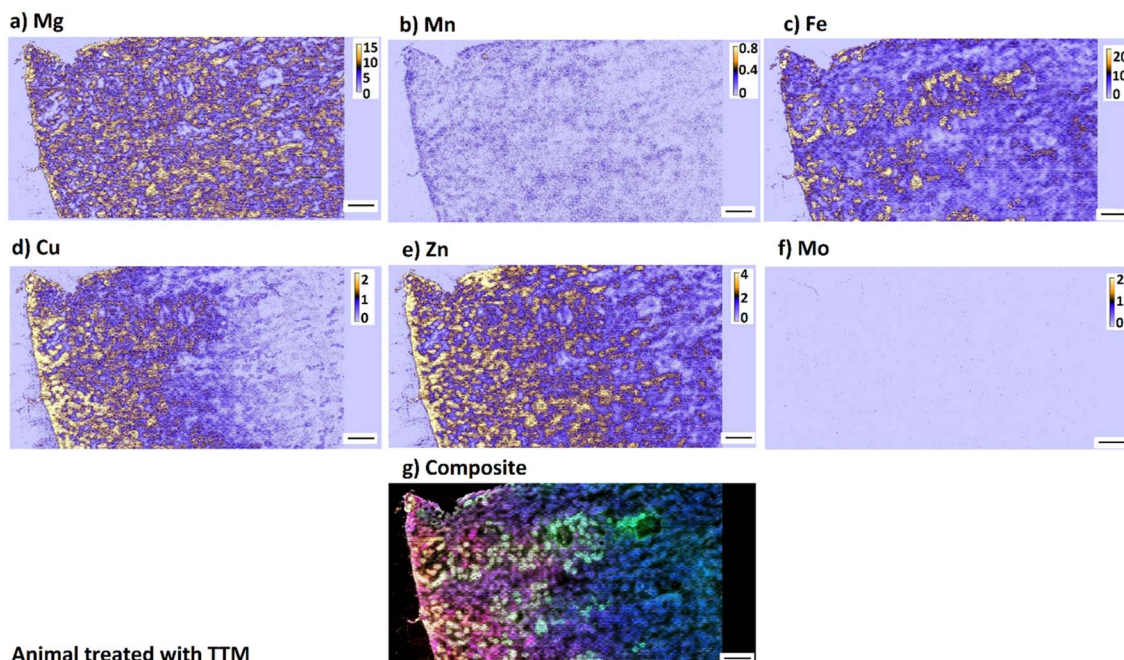
Fig. 5 Distributions of Cu (a and b) and Mo (c and d) in rat kidney taken from a control animal (a and c) and from a rat given 60 mg per kg per day TTM (b and d), imaged at pixel size $18 \times 20 \mu\text{m}$. Colour scale is in fg per pixel. Black rectangle approximately indicated the area used for high-resolution imaging ($3 \times 3 \mu\text{m}$) in the consecutive section (see Fig. 6).

Calibration curve slope expressed in absolute amount of analyte (e.g., femtograms) can be directly used to calculate the amount of corresponding analyte in each individual pixel of the tissue section. When comparing tissues of different thicknesses, analyses performed at different pixel sizes or other reasons require expression in mass fraction (mg kg^{-1}), the conversion can be performed using known or assumed densities of gelatin and tissues samples at the time of sectioning. When the linearity range of the sectioning protocol is established, like in this study, the thicknesses of gelatin and tissues sections may be different. The typical water content in rat tissues is *ca.* 75%,⁵⁹ much lower than in gelatin standards (90%); therefore, it can be argued that the dry thickness of tissue cryosectioned at 4 μm setting is not too dissimilar from the dry thickness of gelatin cryosectioned at 10 μm and, therefore, within the range of LA-ICP-TOFMS linear response established in this study.

High resolution images reveal a high level of detail which is not accessible when imaging of the whole organ at low resolution (Fig. 5) is performed and thus provide information on the relationship between Mo and Co as well as between endogenous elements (*e.g.*, Cu and Fe, or Cu and Zn) at cellular level. The medulla region of the kidney, which accumulates metals at higher levels (Fig. 6), was chosen for high-resolution imaging in this work.

| Isotope | ²⁴ Mg | ⁵⁵ Mn | ⁵⁶ Fe | ⁶³ Cu | ⁶⁴ Zn | ⁹⁸ Mo |
|---|------------------|------------------|------------------|------------------|------------------|------------------|
| Absolute LOD (fg) | 1.7 | 0.14 | 1.52 | 0.28 | 0.33 | 0.25 |
| LOD (mg kg ⁻¹), 4 μm-thick kidney section | 44.7 | 3.8 | 40.2 | 7.3 | 8.8 | 6.6 |

Control animal



Animal treated with TTM

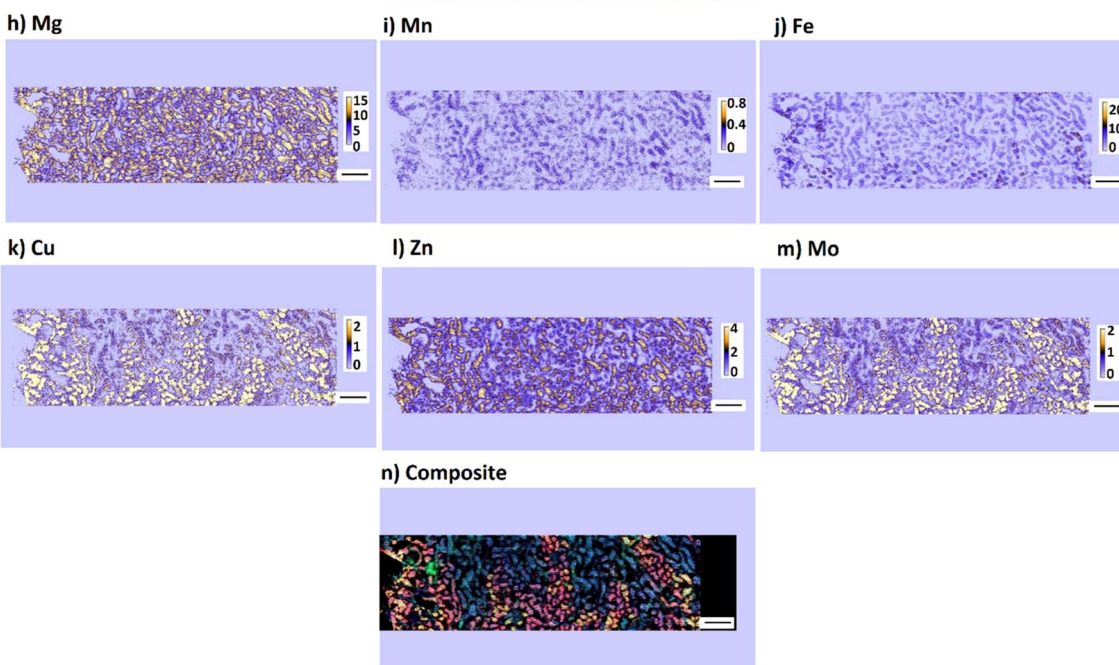


Fig. 6 Distribution of Mg, Mn, Fe, Cu, Zn and Mo (a–f, h–m), and a composite image (g, n) (red = Cu, green = Fe, blue = Mg) in rat kidney taken from a control animal (a–g) and from a rat given 60 mg per kg per day TTM (h–n) imaged at 3 μm resolution. The area imaged is approximately indicated in the whole-organ image in Fig. 5. Colour scale is in fg per pixel. Scale bar corresponds to 200 μm . The isotopes measured are ^{24}Mg , ^{55}Mn , ^{56}Fe , ^{63}Cu , ^{64}Zn and ^{98}Mo .

Results from high-resolution imaging confirmed that, in animals treated with TTM, enrichment in Mo correlates with the enrichment in Cu. This is probably related to the mechanism of de-coppering action of TTM which involves a formation of a ternary complex with Cu and blood albumin that is then excreted with urine.⁶⁰ However, the distribution of Mo/Cu ratio in individual pixels was found to depend greatly on the

resolution chosen (Fig. 7). While lower resolution images give the impression of the near-normal distribution of the Mo/Cu ratio, high-resolution images reveal a more complex pattern that suggests bimodal distribution. The histograms of Mo/Cu distribution at 3 μm spot size calculated separately for high-Mo concentrations ($>64 \text{ mg Mo kg}^{-1}$) and for low-Mo concentrations ($\text{LOD} < \text{Mo} < 64 \text{ mg kg}^{-1}$) show two distinctly different



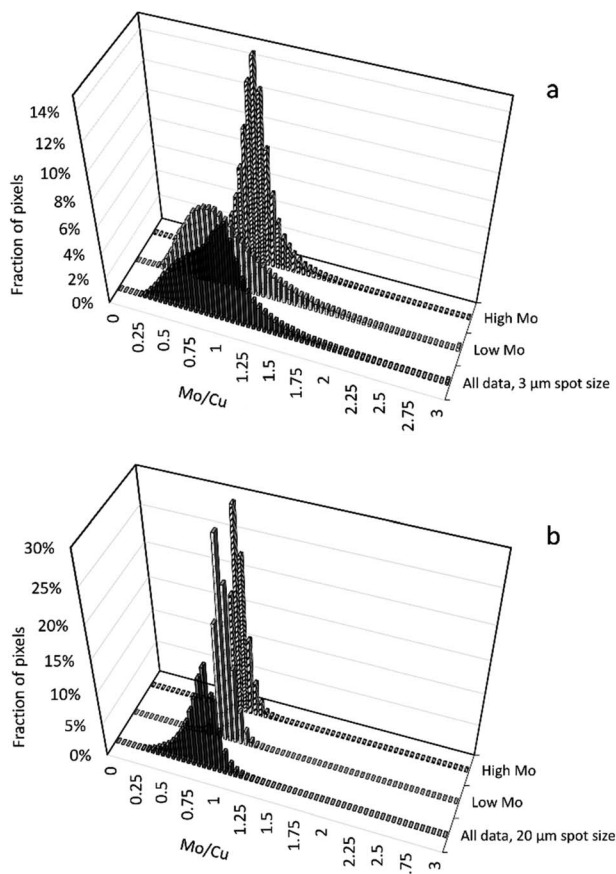


Fig. 7 Histograms of Mo/Cu ratio distribution in single pixels of a kidney section taken from a rat given 60 mg per kg per day bis-choline tetrathiomolybdate (TTM) imaged at $3 \times 3 \mu\text{m}$ (a) and $20 \times 18 \mu\text{m}$ pixel size (b). Data for low Mo concentration ($<64 \text{ mg kg}^{-1}$) and high Mo concentration ($>64 \text{ mg kg}^{-1}$) are shown separately.

patterns with the means of Mo/Cu equal to 0.90 and 0.76, correspondingly. In contrast, Mo/Cu distribution at $20 \mu\text{m}$ spot size does not show visible bimodality.

The mammalian kidney is one of the most complex organs in the body containing at least sixteen different highly specialized epithelial cell types. The number of specialized endothelial cells, immune cells, and interstitial cell types might be even larger.⁶¹ The interplay between different cell types is critical for kidney function. Single-cell metallomics including high-resolution elemental imaging can complement single-cell techniques that involve transcriptomics, metabolomics, and proteomics, which provide new opportunities to classify cells in the kidney and understand their functions. Elemental imaging at the cellular to subcellular level can help to study, for example, the selective toxicity that can be seen following exposure to nephrotoxic substances, that is linked to the differences in response of the cells throughout the nephron of the kidney. Imaging at low-micrometre voxel size allows for determining metal concentrations not only in individual cells, but potentially at the level of organelles such as mitochondria that are typically $1\text{--}2 \mu\text{m}$ in length and contain 20–80% of the cell's iron, copper, and manganese imported for their redox activities,

primarily for electron transport.⁶² Mitochondrial damages and dysfunction are recognized as a leading factor to many chronic and acute renal diseases⁶³ and an inorganic chemical insight into the state of the mitochondrial network would support the characterisation of nephrotoxic and aging effects by other methods.

Conclusions

A systematic approach towards quantitative bioimaging Mg, Cu, Zn, Mn and Fe in animal tissue using LA-ICP-TOFMS has been described in this paper for the first time. This involves the use of cryosectioned gelatin standards spiked with the elements of interest for calibration. Results from careful optimisation of instrumental parameters to achieve optimal limits of detection (in the low $\mu\text{g g}^{-1}$ range) with a fit-for-purpose isotopic selectivity revealed that with ICP-TOFMS and a resolution power of approximately 6000 there is no need for gaining extra selectivity (*e.g.*, by using collision/reaction gases) for the investigated elements in the biological matrix. The homogeneity of gelatin standards spiked with nitric acid solutions was found comparable with that of pH-neutral salt solution making the use of HNO_3 -based standards preferable due to the ease of preparation and the lower blank contributions. The linear response obtained between the signal intensity of all investigated elements and the gelatin thickness under constant laser fluence and repetition rate suggested the use of gelatin cryosections of a single thickness as feasible calibrants for tissue samples sectioned at a range of thicknesses. Finally, the high-resolution quantitative imaging method developed in this paper was extended to include Mo and applied to obtain quantification of Mg, Mn, Fe, Cu, Zn and Mo in the medulla regions of rat kidney from rats administered with a de-coppering Mo-based drug used for treatment of Wilson's disease. The spatially resolved elemental data obtained at cellular and subcellular resolution, provided invaluable information on the relationship between drug-related and endogenous elements, *e.g.*, through Mo/Cu ratio monitoring.

Author contributions

S. Strekopytov: conceptualization, methodology, investigation, formal analysis, validation, visualization, writing – original draft, writing – review & editing. K. Billimoria: conceptualization, methodology, investigation, writing – review & editing. H. Goenaga Infante: conceptualisation, methodology, resources, supervision, writing – review & editing, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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