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Calibration approaches in laser ablation inductively coupled plasma mass spectrometry for bioimaging applications

Kristina Mervič [a,b], Martin Šala [a], Sarah Theiner [c,*]

[a] National Institute of Chemistry, Hajdrihova 19, 1000, Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000, Ljubljana, Slovenia
[c] Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Strasse 38, 1090, Vienna, Austria

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ABSTRACT

Laser ablation (LA) in combination with inductively coupled plasma mass spectrometry (ICP-MS) is a technically advanced micro-analytical method for direct sampling of solid materials and allows the determination of a majority of elements from the periodic table. In recent years, the technology has undergone major improvements in hardware, software and the methodology, which have led to a significant reduction of the analysis time, higher spatial resolution/image quality, better sensitivity and signal to noise ratios. Reliable and validated quantification procedures remain one of the bottlenecks in LA-ICPMS bioimaging. This review provides a comprehensive overview on different quantification strategies commonly used for bioimaging applications by LA-ICPMS. The advantages and drawbacks of existing quantification approaches in terms of analytical capabilities will be critically discussed and showcases of their application to biological samples will be presented. Recent developments and future perspectives of the field will be discussed.

1. Introduction

Since the introduction of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) for the analysis of solid samples by A. Gray in 1985 [1], it has become an established elemental and mapping technique that is characterized by low limits of detection, high spatial resolution, a wide linear dynamic range and limited sample preparation. The first publication describing the LA-ICPMS analysis of biological sample material was published by Durrant and Ward in 1992 [2]. This feasibility study focused on biological reference materials to demonstrate the potential of the method for multi-element analysis of biological samples. The concept of bioimaging by LA-ICPMS to study the spatial elemental distribution in biological tissue was first introduced by Wang et al., in 1994 [3]. Since then, the range of bioimaging applications for the technique has been expanded towards studies in the fields of metallomics, proteomics, nanotechnology, clinical/medical research and immuno-mass spectrometry imaging/imaging mass cytometry [4–6]. Bioimaging studies by LA-ICPMS have focused on the distribution of drugs or nanoparticles and metal-tagged markers in biological tissue sections and single cells, and on the visualization of the metallome within biological tissue (e.g. in the brain) to compare e.g. diseased vs. non-diseased tissue [6]. Metal homeostasis within biological systems is critical for the immune response, metabolism and intracellular signaling. Moreover, elevated and unregulated concentrations of certain elements have been linked to different diseases. Therefore, dedicated analytical workflows based on elemental imaging are required to quantitative assess their levels within biological samples.

The hardware significantly contributing to the advancements in LA-ICPMS imaging is the introduction of low-dispersion laser ablation cell setups [7,8], providing pulse response durations for a single laser shot of <5 ms [9–12]. This results in better signal to noise ratios, higher throughput and the ability to map at high spatial resolution down to the single-cell level. Understanding of fundamental imaging parameters [13] and the effect of different parameters and imaging strategies have also contributed significantly to the improvement in image quality [14,15]. Beside the laser parameter settings directly connected to the S/N via beam size and dosage, the effects of laser fluence was also studied. It was shown that keeping the energy as low as possible (but still exceeding the ablation threshold) results in lower relative standard deviations [16]. Moreover, the use of higher fluences leads to irregular peak shapes and can result in element-dependent image quality deterioration [17].

One of the main challenges for LA-ICPMS analysis of biological samples is the possibility for accurate, reliable and harmonized quantification. One of the reasons is fractionation that occurs during the

* Corresponding author.
E-mail address: sarah.theiner@univie.ac.at (S. Theiner).

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ablation process, the aerosol transport and the ionization in the ICP source and the potential for signal drifts over the course of an imaging experiment [18,19]. Fractionation is a term describing a wide array of causes that reflect in non-stoichiometric signal response in ICP-MS in comparison with the analyzed material, which can be caused by matrix itself, the wavelength used, the design of the experiment (e.g. down-hole fractionation) etc [20]. Where these phenomena are matrix-dependent, usually matrix-matched standards (laboratory-produced or prepared with reference materials) are required for quantitative analysis by LA-ICPMS. Certified reference materials (CRMs) provide one-point calibration with a high degree of traceability and should match the sample in terms of analyte concentrations as well as chemical composition and physical properties. An example of such CRMs are the NIST SRM 61X series glasses, which are often used for LA-ICPMS calibration. Although they are used for the calibration of different matrices, they are often not well matched to the sample matrix. This is also true for biological samples, so they are rarely used for calibration purposes, but at best as a reference for sensitivity. Therefore, other CRMs are available for biological samples, which are usually provided in the form of freeze-dried powders that are not directly comparable to fresh tissue samples. The limited availability of suitable CRMs for the quantitative LA-ICPMS analysis of different tissue types and single cells have led to the development of a variety of alternative calibration strategies [21–24]. However, the complexity and heterogeneity of biological tissue makes it difficult to closely mimic the sample matrix and to develop appropriate calibration strategies that provide the desired accuracy, precision and robustness. Generally acceptable and harmonized quantification procedures for bioimaging applications by LA-ICPMS have still not been established. In most cases, matrix-matched standards are prepared in-house and undergo internal characterization and validation procedures. For this purpose, the exact elemental concentrations of LA-ICPMS calibration standards are determined by solution-based ICP-MS analysis after acid-assisted digestion. Moreover, their figures-of-merit are reported including linearity of the calibration curve, limits of detection/quantification, precision and long-term stability. Due to the lack of suitable biological CRMs, external and in the ideal case also independent validation procedures are a prerequisite for reliable and accurate quantification workflows for LA-ICPMS bioimaging. Complementary imaging modalities have been used in several studies to benchmark quantitative LA-ICPMS results. Ideally, interlaboratory comparison studies are carried out to prove the validity of newly introduced quantification concepts.

The first protocol for matrix-matched tissue standards was presented by Becker et al., in 2005 [25] and was based on the homogenization of brain tissue samples. These tissue-type standards are often sourced from the same tissue type of the target species (e.g. brain [26], liver [27] or blood [28]), spiked with varying concentrations of the analytes and sectioned to the same thickness as the sample. In this context, the use of polymer-based standards as spin-coated polymer films [29], epoxy resin-embedded standards [30] and printed standards using an inkjet printer [31] have been described. The use of gelatin as matrix for the calibration of elements in soft biological tissue and cells provides several advantages due to the possibility to easily fine-tune the properties of gelatin. Gelatin standards for biological applications by LA-ICPMS have been reported as sections, as micro-arrays [32], filled into defined molds [33] and as (micro-)droplets using manual pipetting [34,35] or a robotic micro-dispensing device [36]. Due to the growing interest in nanotechnology and immuno-mass spectrometry, methods for the quantitative assessment of nanoparticles and metal-conjugated antibodies in biological samples with LA-ICPMS will be critically discussed. A comprehensive overview of different quantification strategies used for biological samples by LA-ICPMS analysis is given in Fig. 1 and Table 1 (summarizes the advantages and disadvantages of the calibration strategies). In terms of quantification concepts for LA-ICPMS analysis, external calibration, isotope dilution analysis, standard addition approaches and semiquantitative analysis as well as internal standardization strategies will be discussed.

1.1. Tissue-type calibration standards

Several LA-ICPMS studies have focused on the development of tissue-type section standards, using homogenates of different tissue types (e.g. liver or brain tissue) that are spiked with varying concentrations of the target analyte and cryo-sectioned to the same thickness as the sample [26,27,37]. The exact concentrations of the analytes within the tissue homogenates are then determined by solution-based ICP-MS analysis following acid-assisted digestion. By matching the material for the standard preparation with the samples, this is considered to eliminate the interferences arising from the sample's matrix. However, the homogenization step can lead to a potential change of the biological material's integrity and the achievement of homogeneous elemental distribution is a must for this type of standards. A general disadvantage associated with section-type calibration standards is the occurrence of thickness inaccuracies and anomalies. The sectioning process and other

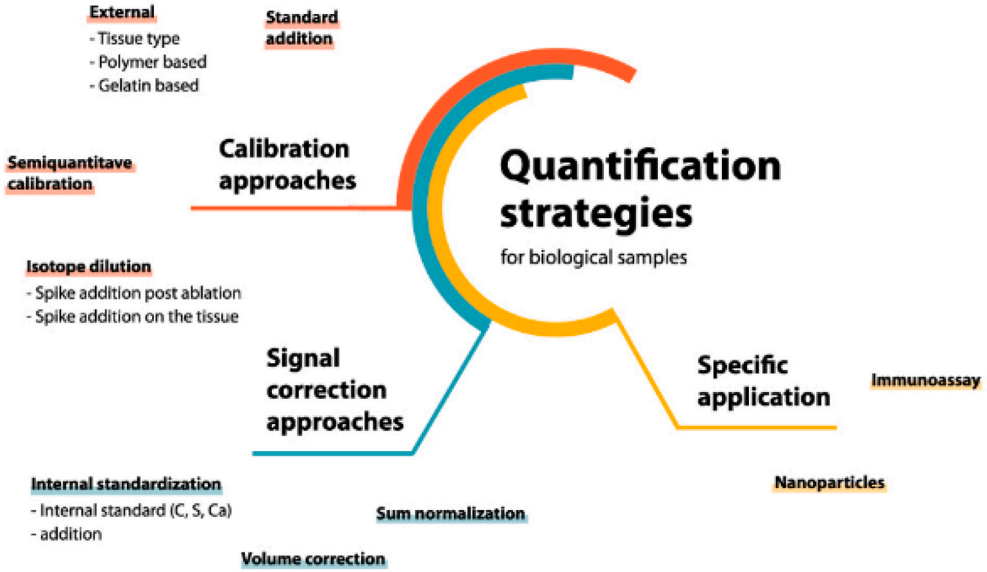

Fig. 1. Overview of different quantification strategies (including signal normalization) used for biological samples by LA-ICPMS imaging.

Table 1
Overview of different quantification and internal standardization concepts for bioimaging applications by LA-ICPMS.

Quantification type	Matrix	Advantages	Disadvantages
External calibration			
Tissue-type sections	Brain, liver kidney, blood	Can be considered as 'true' matrix-matched calibration standards	Access to a microtome Demanding workflow Handling of biological material Homogenization step required Accuracy of the section thickness High elemental background levels Matrix-matching
Polymer-based sections	Polymers	Low elemental background levels Properties of the polymers can be easily modified	Accuracy of the section thickness Access to a microtome Homogenous elemental distribution required
Spin-coating of polymer standards	Polymers	Low elemental background levels Properties of the polymers can be easily modified Can be used as internal standard layer	Matrix-matching Homogenous elemental distribution required
Gelatin sections	Gelatin	No biological material required Low elemental background levels	Access to a microtome Accuracy of the section thickness Homogeneous distribution of the analytes required
Gelatin micro-droplets	Gelatin	Defined volume Low elemental background levels	Availability/access to a micro-array spotter, ink-jet printer No homogeneous distribution of the analytes required
Gelatin micro-arrays	Gelatin	Defined volume Low elemental background levels	Fabrication of micro-arrays No homogeneous distribution of the analytes required
Gelatin molds	Gelatin	Defined thickness and surface roughness Low elemental background levels Good signal precision Easy preparation	Fabrication of molds
Solution-based	Aqueous	Use of aqueous elemental standards	Need to correct for differences in ablation efficiency Requires desolvation
Isotope dilution			
Spike addition post-ablation	–	Traceable to SI units Accurate single-point calibration	Cost intensive Applicable to one analyte
Spike addition on the tissue/cells	Tissue, cells	Traceable to SI units Accurate single-point calibration Matrix-matched	Cost intensive Applicable to one analyte Low count rates per pixel base Applicable for regions of interest
Semiquantification	Gelatin	Simultaneous (semi) quantification of all elements Allows higher concentration ranges	Small number of elements quantified, others semiquantified Applicable to biosamples for now Requires a library of response factors
Standard addition			
Standard addition of droplet standards	Tissue	Can be considered as matrix-matched calibration	High elemental background levels
Internal standardization			
Intrinsic elements (C, P, S, Ca)		Corrects for elemental fractionation and matrix effect Naturally present in sample/standard	Not actual quantification Lack of appropriate elements for IS Matrix-depending partitioning of C
Non-intrinsic elements		Corrects for elemental fractionation, matrix effect and differences in ablation mass	Binding to DNA – not present in the same extent in all cell types
Ablated volume normalized calibration	Any	No need for matrix-matched standards	Additional cost for instrumentation (to determine ablated volume)

preparation steps involved for cryo-sectioned standards can induce artefacts and various types of deformation, which are difficult to control [38]. The main requirement for this type of standards is the quantitative ablation of the standard and the sample (removal of the sample down to the substrate) to ensure that the amount of ablated material is the same. In case the biological sample is heterogenous with regions containing 'softer' and 'harder' material, complete ablation might be difficult to achieve and some residual material from harder parts of the sample can be observed after ablation. These phenomena together with potential thickness inaccuracies between standards and sample can potentially lead to biased LA-ICPMS results. Moreover, another disadvantage for the use of spiked tissue-type standards is the natural presence of endogenous elements contributing to high blank values.

1.2. Tissue-type section standards

The first protocol for matrix-matched tissue standards was presented by Becker et al., in 2005 [25] and was based on the homogenization of brain tissue samples spiked with elemental standard solutions of P, S, Cu and Zn. These tissue-type standards are often sourced from the same tissue type of the target species and subsequently, protocols for standards that are based on brain, liver, kidney or blood as matrix were developed. A general guide for producing matrix-matched standards for assessing the concentrations of trace metals in brain tissue was presented by Hare et al., in 2013 (Fig. 2A) [26]. For this purpose, cortical tissue from sheep brains was homogenized and spiked with varying concentrations of standard solutions of Co, Cu, Fe, Mg, Mn, Sr, Se and Zn. Cryo-sections were prepared and the elemental standard concentrations were determined by solution-based ICP-MS analysis. With the exception of Co and Se, the limits of detection were suitable for quantifying the analytes in a mouse brain sample [26]. The same principle of homogenizing, spiking with elemental standard solutions, freezing and cryo-cutting thin sectioned standards was used as matrix-matched standards for imaging Zn and Mg in rat's brain tissue [39]. True

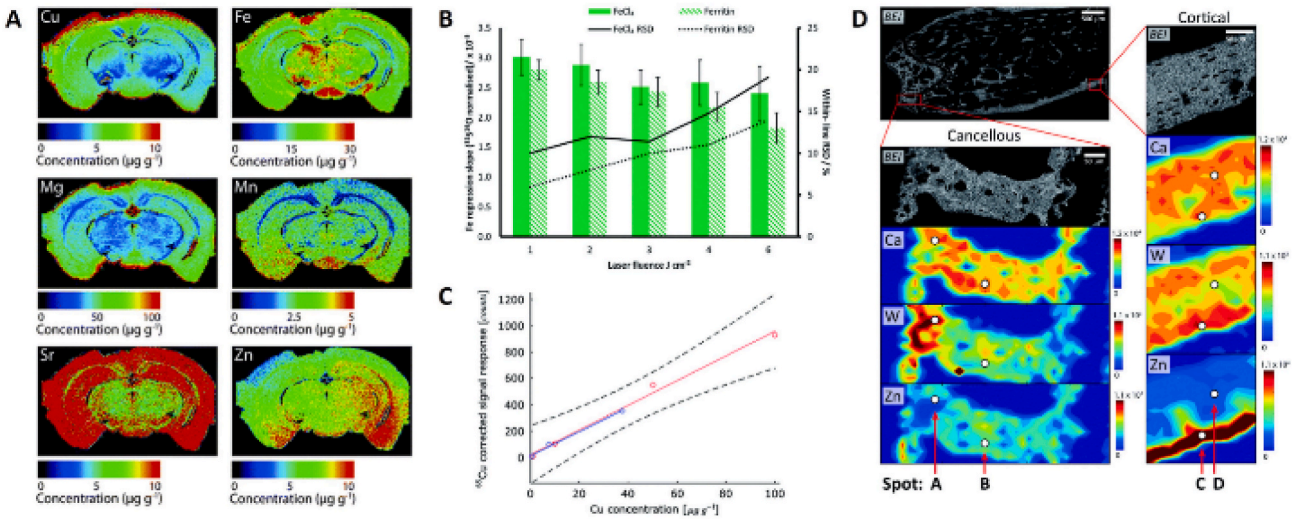

Fig. 2. (A) LA-ICPMS images of the quantitative Cu, Fe, Mg, Mn, Sr and Zn distribution in mouse brain using spiked and homogenized brain tissue standards [26]. (B) Species-dependent sensitivity using either the inorganic spike (Fe) or the species-specific spike (ferritin) in the standard, using different laser fluences [40]. (C) Comparison of calibration curves for spiked sections of liver tissue homogenates and spiked gelatin droplet standards, obtained by LA-ICPMS analysis [37]. (D) LA-ICPMS quantification of Ca, W and Zn deposits in bone samples using hydroxy-apatite collagen standards [44]. Figures adapted from the references [26,37,40,44] with permission from the publishers.

concentrations of Mg and Zn were determined in homogenized tissue by flame atomic absorption spectrometry (FAAS). Furthermore, a methodology for the production of matrix-matched tissue homogenates spiked with Se and Fe was described and its feasibility as calibration standards for quantitative imaging was examined [40]. The effect of elemental species-specific calibration on the quality of quantitative LA-ICPMS results was investigated by comparing tissue-matched standards spiked with inorganic Se and Fe with those spiked with specific-species, i.e. ferritin or selenoproteins (Fig. 2B). Homogeneity of the standards and correlation of the calibration graph slopes were monitored over a laser energy range of 1.0–6.0 J cm^{-2}. For Fe, the slopes were found to agree well, whereas, for Se the choice of the calibrant had an impact on the results [40]. Similarly, the development of durable standards was described for quantitative LA-ICPMS analysis by grinding rat brain tissue and spiking it with aqueous solutions with known concentrations of trace elements (Li, Co, Cr, Cu, Fe, Mn, Pb and Zn). The spiked tissue was then encapsulated in sol-gel pellets by adding it to a prepared xerogel solution. The mixture was poured into molds and dried. The results showed good linearity for all elements, repeatability, homogeneity and long shelf-life due to the stabilization of the standards by the sol-gel matrix [41]. Alternatively, homogenized sheep brain tissue sections mounted on slides were submerged into solutions with varying Fe concentrations rather than spiked with them [42]. In addition, the solutions contained a constant Rh concentration, which was used as an internal standard. To investigate tissue-matched standards for their suitability as calibration standards for quantitative LA-ICPMS imaging, their homogeneity and stability was characterized. Tissue-matched standards showcased homogenous distributions of both Fe and Rh with RSDs of 8.3 % and 4.7 % respectively, stability at room temperature to up to 50 days, and a good linear calibration. The accuracy of the LA-ICPMS data was evaluated against micro-XRF analysis [42]. Tissue-type sections standards based on liver were also employed in the context of cancer research to quantify Ru and Pt concentrations in organs and tumor tissue of mice after treatment with Ru- and Pt-based anticancer compounds [27]. Method validation relied on parallel solution-based analysis of biological samples by ICP-MS after acid-assisted digestion. In another study, the quantitative distribution of Cu in cryo-sections of liver tissue was examined via LA-ICPMS with both matrix-matched thin sections of spiked liver tissue homogenates as well as spiked gelatin droplet standards (Fig. 2C) [37]. No statistical differences were observed between the two approaches. However, low cost, simplicity and availability make spiked gelatin standards the favored choice. Thus, one of the main drawbacks of tissue-type standards is the laborious preparation, which requires access to a cryotome and skilled personnel. Additionally, an accurate estimation of the tissue thickness may be challenging in some cases as well. For single-cell analysis by LA-ICPMS, this approach is unsuitable as the assumption that the volume can be directly derived from the quantitative removal of the irradiated material may underestimate the mass removed, especially for smaller spot sizes. In this case, *a posteriori* surface characterization would be required to precisely define the volume removed [32].

One study reported the use of egg yolk as matrix for the preparation of calibration standards for tissue samples [43]. For this purpose, egg yolk was spiked with the analyte of interest, homogenized, heated up to 90 °C, and cooled down to generate a solid structure similar to tissue. The egg yolk standards were sectioned by cryo-cutting and fixed on a glass slide. In a proof-of-principle study, the quantification method was applied to investigate the delivery of therapeutic cells to the target organs. The quantitative distribution of tumor cells and macrophages labelled with a Tm-complex were tracked in different mouse tissue samples and benchmarked to information obtained by magnetic resonance imaging (MRI) [43].

1.3. Bone and teeth samples

Moreover, several LA-ICPMS studies have also focused on the development of tissue-type calibration standards for hard tissues. In this context, bone was evaluated as matrix for the preparation of matrix-matched calibration standards to quantify tungsten and zinc deposits in bone with LA-ICPMS [44]. Mouse bone samples were dried under vacuum, grounded using a ball mill and spiked with appropriate volumes of multi-element standard solutions. After mixing, the standards were evaporated to dryness, manually homogenized and compressed into a 1.0 mm thick pellet with a manual hydraulic press. The analytical performance (linearity, limits of detection and accuracy) of the pressed bone powder standard was tested alongside hydroxyapatite:collagen (HC) (mimics the composition of bone) and hydroxyapatite (HA) synthesized pressed powder standards (Fig. 2D). While, bone standards showed a good linearity for tungsten ($R^2 > 0.995$), the linearity was less for zinc ($R^2 > 0.90$). The accuracy was tested by quantifying zinc in NIST

SRM 1486 through external calibration and comparing the results to the known reference value of zinc. Both matrix-matched standards (bone and HC pellets) provided accurate quantification of zinc in NIST SRM 1486 without correction for the calcium content [44].

Teeth are another commonly analyzed mineralized tissue as they provide a valuable chronological bioindicator of toxic metal exposure [45] and as they can be used to reveal past human/animal migration. Recently, studies have used various standards from in-house prepared matrix-matched standards made from healthy teeth to synthetic hydroxyapatite (HA) standards doped with the elements of interest to calibrate tooth samples instead of the usual single-point calibration with NIST glass-SRM [46]. One study used matrix-matched standards prepared by grinding healthy teeth, sieving the powder, spiking with known volumes of elemental solutions, and then drying, homogenizing, and pressing into disks [47]. This quantification method, using matrix-matched laboratory standards, proved effective in determining element concentrations in thin cross-sections of both healthy and filled teeth. The use of these standards ensured accurate and precise results in the quantitative determination of Al, Ba, La and Sr in dental tissues with LA-ICPMS [47].

In another study, three synthetic HA materials (i.e., amorphous HA crystals, pelletized HA powder, and pelletized HA powder that was subsequently sintered) were prepared and evaluated for their suitability as external calibration standards for human teeth. The pelletized HA powder showed the best ablation properties compared to the previous materials HA and NIST glass-SRM, especially in tooth analysis. The metals were integrated uniformly, and the calibration curves showed excellent linearity. Detection limits ranged from 0.1 to 2 μg kg^{-1} for Mg, Al, Ni, Cu, Zn, Cd, Ba and Pb [45].

1.4. Hair samples

Spiked hair strands served as matrix-matched tissue standards for external calibration due to the limited availability of certified reference materials from human hair and their powder nature, which makes them not ideal as matrix-matched standards. Therefore, to quantify the distribution of analytes along a single hair strand, element-enriched hair strand standards were prepared in-house by immersing human hair in solutions with different bromine and iodine concentrations [48]. The exact elemental concentrations in the hair standards were determined by digestion followed by pneumatic nebulization (PN-)ICP-MS analysis. The method was successful in achieving homogenous standards and generating highly linear calibration curves [48]. Similarly, As and Pb levels were determined in individual hair strands of leukemia patients by LA-ICPMS [49]. The calibration method was based on matrix-matched hair standards spiked with the analytes of interest and the exact metal concentrations were determined in a portion of the spiked hair standards by ICP-MS analysis [49]. A similar principle was applied to the LA-ICPMS analysis of mummy hair samples, using three contemporary hair samples with known As concentrations as matrix-matched standards [50]. In another study, both metal-enriched hair powder standards and hair strand standards immersed in metal solutions were used for quantification [51]. Method development focused on arsenic with the use of sulphur as internal standard element. While a good linear correlation and LOD was obtained, the binding of As to keratin in hair has proven to be a slow process [51]. In-house prepared spiked hair strands were also used as calibration standards to quantify the platinum concentration along a single hair strand from a patient who had received cisplatin as a cytostatic drug [52]. Complete ablation of the hair cross-section increased the sensitivity of the method. A lownoise-intensity ratio was obtained along the hair strand, and the variation of the Pt signal as a function of the respective cisplatin dose was clearly visible [52]. An alternative calibration strategy for hair analysis by LA-ICPMS was based on spiked keratin films as calibration materials [53]. For this purpose, the keratin films were prepared by extracting keratin from human hair followed by the formation of films through self-assembly, self-aggregation, and cross-linking activities. The doped keratin films showed better recovery and linearity for Pb quantification compared to the traditional method of soaking hair in a solution with the element of interest. This is because hair strands have limited surface area for Pb adsorption and not the right physiology to retain it, whereas keratin films were able to retain high quantities of Pb on their surface [53].

1.5. Polymer-based standards

The use of polymer- and resin-based standards that are spiked with different metal-containing solutions has been described in several LA-ICPMS studies for calibration purposes. The standards are based on materials with characteristics that can be easily modified, however, a homogeneous thickness and elemental distribution are essential in the preparation of polymer-based standards.

For the preparation of polymer-based standards, different strategies were reported in the literature. One early study developed a method based on spin-coating with solutions of polymethylmethacrylate (PMMA, 10 %), xylene (40 %) and chlorobenzene (50 %) that were spiked with organometallic Cu and Zn standards [29]. Ruthenium phthalocyanine dye and an organometallic yttrium standard were added to be used as internal standards. After spin-coating, substrates were baked on a hot plate at 130 °C for 2 min. The thickness of the PMMA thin films was characterized by profilometry and the surface roughness evaluated by atomic force microscopy (AFM). UV–Vis spectroscopy was used to determine the change in concentration of the dye and a ten-fold increase in analyte concentration in the film was observed, compared to the spin-coating solution due to solvent evaporation. The polymer-based calibration standards were evaluated using the elements Cu and Zn and their analytical figures-of-merit were benchmarked to homogenized brain tissue standards that were spiked with the same analytes and prepared as cryo-sections. The proposed approach also allowed for internal standardization and drift correction by employing internal standard elements (Y and Ru) in the underlying thin film. For this purpose, the biological sample was placed on top of the thin film and co-ablation of the tissue sample and the internal standard layer required careful optimization of the laser fluence to enable selective and quantitative/-full ablation of both layers (down to the substrate) [29]. A similar calibration approach was developed that was based on an aqueous soluble polymer (dextran) to increase the number of elements that can be spiked into the polymer standards [54]. For this purpose, a dextran solution was prepared in water and spiked with different amounts of metal solutions (in total 11 elements) and internal standards solution. Solutions were spin-coated onto glass slides and dried with a light N_2 gas stream. The average mass of the polymeric films was determined for each calibration point using an ultra-high precision balance by weighting glass slide before and after film deposition. The film thickness was determined by optical profilometry. The LA-ICPMS quantification method was validated using in-house prepared kidney homogenates spiked with known amounts of metals and applied to clinical human samples [54].

As alternative to spin-coating, another quantification strategy is based on the preparation of polymer-based section standards. One study used a cold-curing resin based on 2-hydroxyethylmethacrylate (Technovit 7100) that was used as infiltration solution and embedding medium. For preparation of the standards, the infiltration solution was spiked with different amounts of platinum(II) acetylacetonate, which is well soluble in organic solvents [30]. Subsequently, dimethyl sulfoxide was added to the infiltration solution to heat up the mixture and initiate the polymerization. After hardening, the resin standards were sectioned to a thickness of 5 μm. The same resin/infiltration solution was also used for embedding of the tissue samples to simulate 'matrix-matching'. The standards were characterized using ICP-MS analysis after microwave-assisted acid digestion to assess the elemental concentrations. As proof-of-principle, the platinum distribution was quantified by

LA-ICPMS at different time intervals in tissues of mice treated with the chemotherapeutic drug cisplatin. The tissue samples (testis, cochlea and kidney) were selected with regard to potential side effects and toxicity of cisplatin-treatment towards these tissue types [30]. In a subsequent study, the same calibration approach was used to quantify platinum in major functional units of testicle, cochlea, kidney, nerve and brain sections from cisplatin-treated mice [55]. Regarding the potential applicability of this standard type for 'hard' biological material, the quantitative platinum distribution was evaluated in tibia samples of mice treated with a platinum(II) anticancer compound [56]. Platinum was quantified in the hard bone samples upon resin-embedding to unravel potential targeting options of platinum chemotherapeutics in the treatment of bone metastases. Platinum levels were set in relation to the phosphorus and calcium distribution and correlative micro-XRF analysis was performed [56].

Recently, a new method for preparing polymeric reference materials for microanalysis using a 3D printing technique was proposed [57]. The elemental standard solutions were doped in polyacrylate resin by mixing with dispersant and then printed, layer by layer using a 3D printing technique at room temperature. The amount of dispersant in the mixture was optimized to achieve homogeneity of the printed polymer reference materials. The homogeneity was evaluated using LA-ICPMS analysis in line scan mode. The results showed that the printed sample was homogeneous on the 50-μm scale, and for some elements also on the 14-μm scale. Furthermore, the mass concentration of the doped elements was determined using ID-ICP-MS and proved to be equivalent to the nominal concentrations determined by the gravimetric method. Overall, compared to conventional preparation methods for polymer calibration standards, the 3D printing approach showed improved concentration accuracy and homogeneity of the prepared standards [57].

1.6. Gelatin-based standards

Gelatin was proposed as biological matrix mimic for the preparation of calibration standards for LA-ICPMS imaging. The main advantages of the use of gelatin are that it is easy to handle, readily available, inexpensive, non-toxic and provides a soft matrix similar to biological tissue. The matrix is considered to partially resemble fibrous protein collagen and its protein content is supposed to match the protein-rich cellular matrix. The fabrication of highly homogenous gelatin standards is not straight-forward and different approaches were used including total ablation, fast drying, the employment of high temperatures during the drying process, printing of gelatin standards and the production of gelatin micro-droplets.

In order to prove the matrix-matching capabilities of gelatin for biological tissue, several studies were performed. For this purpose, Cu quantification was compared using thin sections of spiked liver tissue homogenates and gelatin droplet standards [37]. No statistical differences were obtained between the results using the two calibration approaches and therefore, both were considered suitable for quantitative Cu bioimaging of liver cryo-sections [37]. In another approach, the calibration concept of standard addition on different mouse tissue samples was compared to external standardization based on gelatin micro-droplet standards. Cross-validation revealed consistent quantitative results between the two calibration approaches and matrices [58]. Both studies provided proof that gelatin-based standards could serve as matrix-matched calibrations for bioimaging applications by LA-ICPMS. One LA-ICPMS study evaluated the suitability of gelatin and the cold-curing resin Technovit as standard materials for bioimaging applications by investigating their particle transport and ionization characteristics using an optical particle counter inserted in-line between a 213 nm LA system and the ICP-MS instrument [59]. The size of the particles generated by the gelatin standards was smaller (sizes in the nm-range) than those of the Technovit standards (sizes in the low μm-range) resulting in higher signal intensities during ICP-MS analysis. Increasing the laser fluence resulted in a larger number of μm-sized particles for both materials, whereas the ionization efficiency of gelatin aerosols proved to be superior compared to aerosols produced from Technovit standards [59].

The properties of gelatin can be easily modified and the number and concentrations of analytes can be adjusted. For the latter, it has to be considered that with an increasing number of elements in gelatin as matrix, the gelatin becomes brittle, precipitation can occur and it becomes difficult to handle, especially at higher elemental concentrations. Whereas the working range of tissue-type section standards is determined by the natural abundance of endogenous elements and/or elements artificially introduced during sample preparation, gelatin shows comparably lower elemental background levels. One study compared gelatin standards prepared from a variety of animal sources and presented a method based on chelating resins to remove background metals in gelatin in order to increase the dynamic calibration range [33].

For quantification by LA-ICPMS, gelatin-based calibration standards in form of cryo-sections [60–62], micro-arrays [32], molds [33], bio-printed standards [63] and (micro-)droplets [35,36,64] were described. The use of gelatin sections was introduced as a calibration concept similar to tissue-type section standards. For this purpose, gelatin was doped with the analytes of interest and mixtures of gelatin and aqueous standard solutions were heated to 45–60 °C to ensure homogeneity of the analyte distribution. Gelatin cryo-sections were prepared with the same thickness as the biological samples and ablated using the same laser ablation parameters. Most studies verified the analyte concentrations within the gelatin standards performing acid digestion followed by bulk ICP-MS analysis. Gelatin-based section calibration was employed in several LA-ICPMS bioimaging studies in the medical context. As examples, this type of calibration was used to quantify the gadolinium deposition in the brain of patients that obtained with Gd-based contrast agents [65], to quantify the amounts of Fe, Cu and Zn in the brains of Alzheimer's disease and control mice [62] and to evaluate the iron and copper levels in liver needle biopsy specimens of patients suffering from Wilson's disease [60]. One LA-ICP-TOFMS study evaluated gelatin-based sections for the quantification of Mg, Mn, Fe, Cu and Zn in tissue cryo-sections [66]. The impact of variable thickness of gelatin sections on the signal intensity was studied and gelatin standards spiked with acidified single-element solutions and with solutions prepared from metal salts were compared. The developed method was used for the quantification of essential metals and molybdenum in kidney sections of rats dosed with bis-choline tetrathiomolybdate [66].

Recently, the production of bio-printed gelatin standards using a 3D printing device was proposed [63]. The gelatin was doped with lanthanide up-conversion nanoparticles and the bio-printed gelatin standards were compared with gelatin section standards and proved to be superior with regard to throughput, batch-to-batch repeatability and elemental signal homogeneity at 5 μm pixel size. For comparison and characterization, the thickness of the gelatin sections and the bio-printed gelatin standards was determined using multiphoton fluorescence microscopy. Bio-printing of gelatin standards provided a high level of automation, well-controlled spatial distribution in all three axes and the ability to control the temperature of the printing ink/gelatin and the printer bed [63]. The latter features prevented migration of material within the printed gelatin standards and allowed avoiding the occurrence of coffee-stain effects [67]. In a subsequent study, the same approach was adopted to prepare bio-printed gelatin standards for surface enhanced Raman scattering (SERS) [68]. Gelatin standards were spiked with SERS nanotags, which consisted of gold nanoparticles and a Raman reporter. The developed standards were characterized by single particle ICP-MS analysis and LA-ICP-TOFMS imaging [68].

In order to overcome the limitations associated with section-type standards (e.g. thickness inaccuracy, access to a cryotome and required homogenous distribution of the analytes), different quantification concepts based on gelatin as matrix were developed. For this purpose, a high-density gelatin micro-array was developed and characterized for calibration purposes by LA-ICPMS (Fig. 4C) [32].

Micro-machining of the micro-array was achieved by the laser ablation system itself and the method was cross-validated by synchrotron radiation (SR)-XRF analysis. Since all of the necessary instrumentation is already available in LA-ICPMS labs, the proposed micro-array standards are an attractive alternative to micro-dispensed droplet arrays, where a dedicated micro-droplet dispenser or micro-pipetting system is required. As a case study, the Cu uptake was studied in a model organism resulting from transition metal exposure at the sub-cellular level by LA-ICPMS and SR-XRF analysis [32]. The features of these microarrays could be especially attractive in high-throughput single-cell analysis. Alternatively, gelatin molds were evaluated as LA-ICPMS standards and benchmarked to gelatin sections and homogenized brain tissue standards (Fig. 4D) [33]. Compared to tissue-type standards, they proved to be superior in terms of thickness accuracy, signal precision and robustness for reproducible quantification and with regard to their analytical figures of merit. The latter ones depended on the levels of elements that were naturally abundant in the gelatin, which deviated significantly between different animal sources of gelatin. Therefore, an additional metal extraction step based on various resins was used to reduce the elemental backgrounds in gelatin and to improve the limits of detection [33].

One gelatin-based calibration strategy is based on the use of (micro-) droplet standards that are either pipetted manually or automated via an ink-jet device or a robotic micro-droplet dispenser. In droplet-based approaches, heterogeneous elemental distributions can occur on the microscale due to the coffee stain and/or Maragoni effect shown in Fig. 3 [69,70].

On the macroscale, this problem can be overcome by quantitative/full ablation of the entire droplet. However, this process can be time-consuming due to the dimensions of each droplet, especially when the droplet deposition is performed manually. For gelatin-based droplets, volumes of around 1–300 μL and droplet areas of up to 4.5–6 mm^2 have been reported, in case manual pipetting was performed by micro-pipettes and a micro-balance was used to record the exact amounts of the deposited droplet standards [35,71]. Reduction of the droplet size can be achieved, e.g. by drying the droplets on a hydrophobic surface. Alternatively, the analysis time can be decreased by ablating a representative part of the droplet, e.g. by ablating a cross-section or by spot analysis, provided that the elemental distributions are homogenous. One LA-ICPMS study systematically evaluated different parameters in the preparation of gelatin droplet standards in order to achieve a homogenous elemental distribution within the droplets and to avoid the occurrence of the coffee stain and/or Maragoni effect [67]. The latter

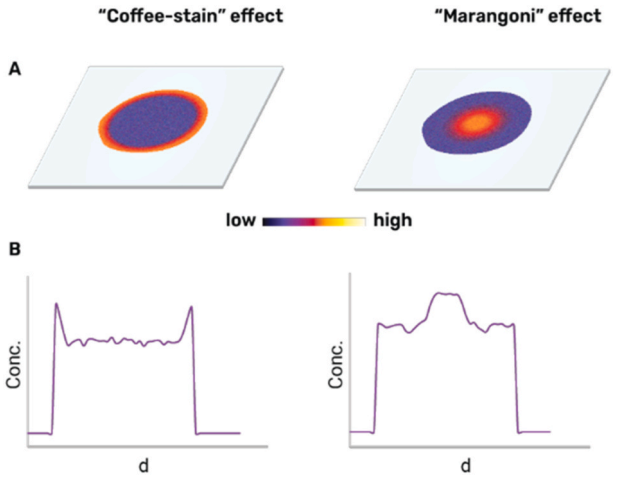

Fig. 3. Scheme of elemental distributions (A) on the surface and (B) in depth laterally through the center of the sample, showing the heterogeneous distribution of elements in the formation of gelatin droplets, which can result from the coffee stain or Maragoni effect.

ones proved to be element dependent and it was shown that the optimization of the drying/setting conditions of gelatin-based droplets was essential to provide homogenous elemental distributions. Increased temperatures improved the three-dimensional homogeneity of elements within the droplets and a drying temperature of 100 °C was recommended by using a mechanical convection oven to ensure a temperature-controlled environment (Fig. 4A) [67]. A subsequent LA-ICPMS study compared the quantitative ablation of entire gelatin-based droplet standards and spot ablation of highly homogenous dried gelatin gels spiked with different analytes [35]. For the second approach, the ablation volume was precisely determined using atomic force microscopy (AFM). Both calibration methods were applied to quantify membranous receptors in breast cancer cell lines using receptor-specific hybrid tracers for fluorescence confocal microscopy and high-resolution LA-ICPMS analysis (Fig. 4E) [35].

Gelatin-based droplet standards were further employed in LA-ICPMS studies in the context of metal-based anticancer drug research. One study addressed the platinum uptake in individual cells in the kidney of monkeys treated with cisplatin to evaluate possible mechanisms for cisplatin-induced nephrotoxicity [64]. In another study, the quantitative platinum distribution was studied by LA-ICPMS in ovarian cancer peritoneal xenografts after intraperitoneal chemoperfusion of oxaliplatin [72]. Quantification was accomplished by the use of gelatin droplet standards spiked with elemental platinum standards. Platinum accumulation was mainly observed in the extracellular matrix and results were correlated with results obtained by SR-XRF analysis [72].

One study developed a single particle detection method based on LA-ICPMS analysis for nanoparticle (NP) analysis in biomaterials [73]. Custom-made gelatin standards containing commercial or synthesized gold NPs of various sizes and with different number concentrations were used to optimize the conditions for the measurement of gold NPs. The optimized LA-ICPMS method was employed to study the size, number concentration and localization of Au NPs in roots of sunflower plants grown hydroponically with Au NPs added [73]. Gelatin droplet standards were evaluated for the quantification of iron oxide nanoparticles in gelatin microspheres containing $CaCO_3$ crystals by laser ablation in combination with ICP-MS/MS tandem technology [74]. Method development focused on resolving interferences ($^{40}Ar^{16}O^+$) on the signal of $^{56}Fe^+$ by relying on chemical resolution using a gas mixture of NH_3 and He. Fe-spiked gelatin droplet standards were prepared on a high-purity single-side polished silicon wafer and used as external calibration standards. Their corresponding Fe concentrations were determined by pneumatic nebulization ICP-MS/MS analysis after acidic digestion [74].

The dispensing of small-sized droplet standards in a precise and automated way can be achieved by the use of a commercial inkjet device or a robotic micro-spotting/arraying system. A modified dosing device based on a commercial ink cartridge was developed for the precise deposition of pL-droplets onto samples [75]. The method was applied to characterize the elemental composition of thin-layered materials via standard addition [75]. Another study describes the use of a MicroFab Jetlab micro-dispensing unit to create a rectangular grid of gold standard solutions [76]. The quantitative uptake of Au nanoparticles and of an Au-peptide cluster into single cells was studied by LA-ICPMS via this approach [76,77]. A micro-array spotter was used to print ICP-MS standard solutions onto nitrocellulose membranes [78] and on target glass slides [79] with volumes in the pL-range. This method was introduced for single-cell analysis by LA-ICP(-TOF)MS to quantify an Ir-DNA intercalator and an mDOTA-Ho dye in adherent 3T3 fibroblast cells [78,79]. A similar approach was employed to study the quantitative uptake of Ag nanoparticles in a multicellular tumor spheroid model by the use of droplet standards based on Ag nanoparticle suspensions [80].

Multi-element calibration standards for LA-ICP-(TOF)MS bioanalysis were introduced based on gelatin micro-droplets that were generated by a micro-spotter (Fig. 4B) [36]. This approach provided the robotic dispensing of droplets with volumes in the pL-range with high precision (around 5–10 %). The small size of the droplets with a diameter of

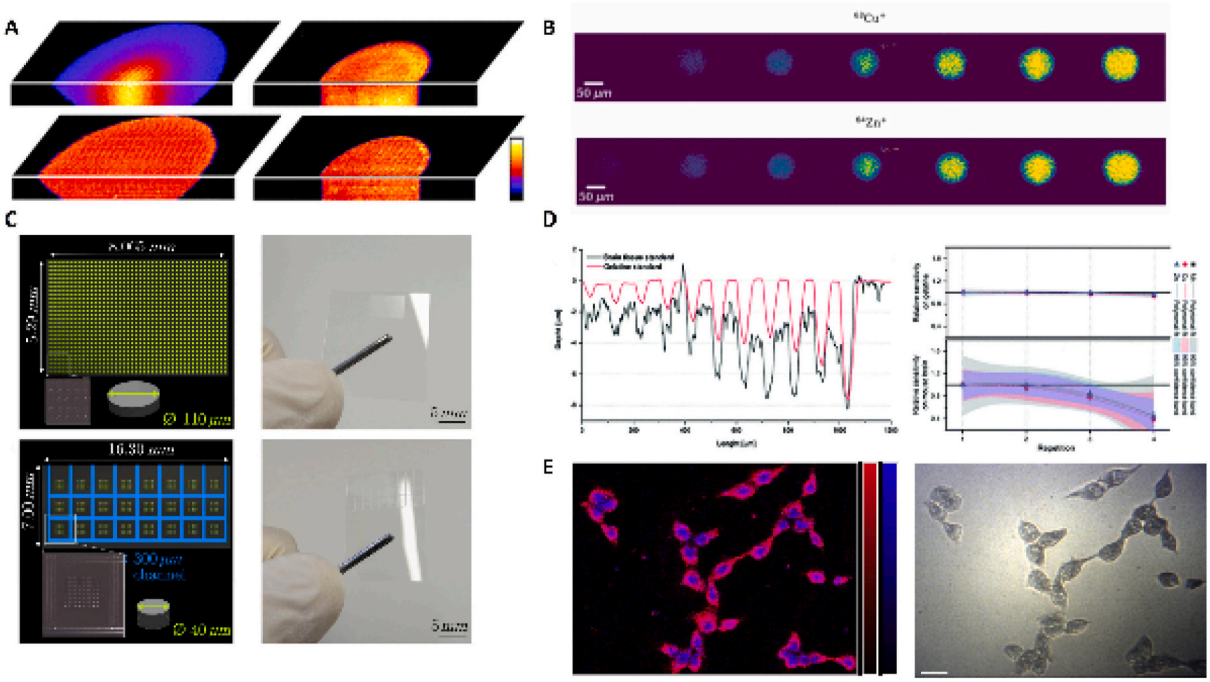

Fig. 4. (A) Effect of drying temperature (room temperature vs. 100 °C) on elemental distributions in gelatin-based droplet standards [67]. (B) Calibration sequence of multi-element gelatin micro-droplet standards prepared by a micro-dispensing device [36]. (C) Micro-array standards based on gelatin for single-cell analysis. The micro-array was machined by the laser ablation system [32]. (D) Profilometry data of standards that were ablated several times with increasing laser power and relative sensitivities for Mn, Cu and Zn derived from repeated ablation of calibration standards [33]. (E) Two channel LA-ICP-MS image (left) and brightfield microscopic image (right) of cells stained with receptor-specific hybrid traces, channels representing ^{89}Y and ^{193}Ir signal response [35]. The Figures were adapted from the references [32,33,35,36,67], with permission from the publishers.

100–200 µm and the use of a low-dispersion LA setup resulted in an analysis time of less than 10 min for a calibration blank and a series of 5 calibration standards. This is in a similar time regimen as a calibration sequence for solution-based ICP-MS analysis. The quantitative LA-ICPMS imaging method was validated by solution-based flow injection ICP-MS/MS analysis [36]. In a subsequent study, the calibration method was further validated by standard addition and isotope dilution approaches [58]. The developed calibration method is applicable to the multi-element quantification of biological samples including single cells and tissue samples by LA-ICPMS analysis [36,81,82]. The gelatin micro-droplet standards are the first ones that are commercially available for bioimaging by LA-ICPMS [83].

1.7. Solution-based quantification/online addition of standards

Alternatively, solid-liquid calibration is also utilized for quantification purposes in LA-ICPMS. Dual flow systems enable simultaneous introduction of the laser ablated material and a nebulized aqueous standard solution. This method entitles mixing of the carrier gas flow coming from the ablation cell with an aerosol generated by nebulization of an aqueous standard solution. Both standards with natural isotopic composition as well as isotopically enriched standards have been used for such experiments. Alternating addition of standard and blank solutions to the sample stream allows for quantification of the analyte concentration in the sample. The method requires correction for the differences in ablation efficiency as well as desolvation of the wet aerosol prior to its mixing with the sample aerosol, when aiming to maintain the advantages of 'dry plasma' conditions. The main advantage of this calibration method is that it enables quantification based on aqueous elemental standards, which are usually readily available in ICP-MS laboratories. However, it cannot account for possible variations in ablation efficiency or altered transport efficiencies [84].

Various solution-based calibration methods for quantitative analysis of biological samples have been developed over the years. In one study, a dual flow of the carrier and nebulizer gas was used to transport the aerosol of the nebulized aqueous standard and that of the ablated brain tissue sample into the ICP source [85]. Aerosols were introduced separately in the injector tube inside a special ICP torch and then mixed in the inductively coupled plasma as shown in Fig. 5. The nebulizer produces wet aerosols affecting the sensitivity and linearity of the calibration curves. The introduction of water in the plasma by nebulization of aqueous standards increases the formation of polyatomic ions, which can interfere with the signal of the analyte isotopes. The effect of water on the calibration curves is different for different isotopes, and variations in the ratio of the slopes of the calibration curves can be observed. The loss of analytes during the nebulization step may also affect the calibration curves. Thus, calibration in dry plasma utilizing matrix-matched standards, i.e. homogenous brain laboratory standards, was also performed. The ratios of the calibration curves slopes obtained

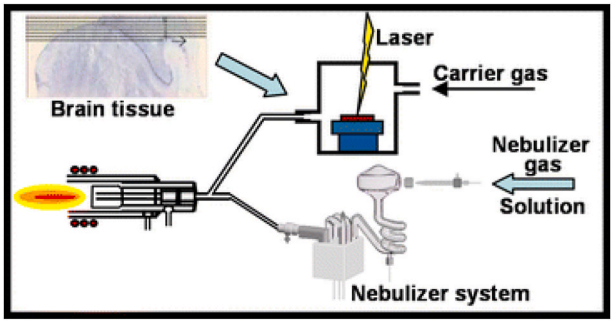

Fig. 5. Schematic of the solution-based calibration approach for the analysis of mouse brain by LA-ICPMS. The laser ablation and the gas nebulizer are coupled separately to the ICP-MS. The aerosols from the nebulizer and LA are simultaneously introduced into the injection tube. The Figure was adapted from Ref. [85] with permission from the publishers.

with the aqueous standards and solid standards approach were applied to correct for the differences of sensitivity among LA-ICPMS and ICP-MS analysis. Generally, it is believed that the sensitivity is lower in the case of LA-ICPMS analysis compared to solution-based ICP-MS analysis. However, this is only the sensitivity of the method *per se*, not taking into account the dilution steps in the process of sample preparation (e.g. during sample digestion). Therefore, sensitivities would need to be evaluated for every sample/nuclide individually. After correcting for differences in sensitivity between ICP-MS and LA-ICPMS analysis, the concentrations of metals in mouse brains determined by calibration with aqueous standards were in agreement with those obtained by calibration with solid standards [85]. Similarly, a method for quantitative analysis was developed using a desolvating nebulizer system (DNS) coupled with LA and on-line internal standardization [86]. The results showed that DNS-ICP-MS signals were much stronger than LA-ICPMS signals for various elements. To correct for the differences in sensitivities, internal standard elements were used to obtain calibration curves. The method was applied to both standard reference materials and biological tissues, and the results were in good agreement with certified values [86].

The solution-based quantification approach was also applied to quantify elements on (external contamination) and in hair samples by LA-ICPMS. In one study, a dual argon flow of the carrier gas and nebulizer gas was used and external calibration was employed via defined standard solutions before analysis of a single hair strand [87]. Again, due to the differences in elemental sensitivities of LA-ICPMS and ICP-MS analysis, correction factors had to be applied. The correction factors were calculated using hair with known analyte concentrations (as measured by ICP-MS). Different essential and toxic metals and iodine were monitored in human and mouse hair, with good linear correlation coefficients of the calibration and limits of detection [87]. In another approach, essential and toxic elements in single hair strands were determined using solution-based calibration in LA-ICPMS with a double focusing sector field mass spectrometer. The method was compared to quadrupole-based ICP-MS (ICP-QMS) and different solution-based quantification strategies such as standard addition, and isotope dilution were employed for the quantification of uranium. A standard addition technique was developed for uranium determination in powdered hair by coupling the laser ablation chamber to an ultrasonic nebulizer. The results showed good agreement between the ablated and digested hair samples for essential and toxic elements, and the technique can be used to compare different components in hair from normal and exposed populations. The method has the potential for isotopic forensics investigation and monitoring after exposure accidents as well as a routine research tool for the assessment of a 'normal' and exposed population [88].

While solution-based calibration in LA-ICPMS can compensate for matrix-related differences and enables quantification based on aqueous standards, it requires correction for differences in ablation efficiencies and in elemental sensitivities between ICP-MS and LA-ICPMS analysis. This is accomplished either by calculating the correction ratio based on calibration curves' slopes obtained via solution standard and solid standard calibrations or by inclusion of an internal standard.

1.8. Isotope dilution approaches for LA-ICPMS analysis

Ideally, quantification procedures that are universally applicable and not laboratory dependent would be favorable for bioimaging applications by LA-ICPMS to facilitate the inter-laboratory comparability of the obtained results. The implementation of isotope dilution mass spectrometry (IDMS) protocols into LA-ICPMS workflows provides an accurate single-point calibration method that is traceable to SI units [89,90]. Isotope dilution analysis (IDA) relies on the principle that a change in the isotopic composition of the analyte of interest is induced by adding a well-defined amount of an isotopically-enriched spike to the sample. The analyte concentration of the sample is determined with high accuracy and precision by measuring the isotopic composition/ratios of the sample, the spike and the mixture/blend (containing the sample and the spike). IDA provides the advantage that the results do not depend on matrix effects and instrument instabilities over the course of an LA-ICPMS experiment due to the measurement of isotope ratios. As disadvantages, IDA requires dedicated isotopically-enriched spike solutions that can be expensive, IDA cannot be applied to monoisotopic elements and the sample preparation can be laborious in comparison to external calibration, which reduces the sample throughput. Moreover, the exact mass/volume of the spike solution added to the sample or the mass flow needs to be known/calculated. The main requirement for isotope dilution analysis is the isotope equilibration between the spike and the sample to ensure that the nuclide of the spike behaves similar to the nuclide of the analyte in the sample. For imaging applications of isotope dilution approaches, this can be a challenging task as a homogenous distribution of the spike is required on the target tissue/cell, while the integrity of the biological sample needs to be preserved. The approximate concentration of the target analyte in the sample has to be already known before adding the spike, as IDA requires an adequate spike/sample ratio (over- or underspiking needs to be avoided) to yield acceptable results without high uncertainties. In the field of bioimaging, IDA in combination with LA-ICPMS has the great potential to validate calibration strategies that provide higher throughput (e.g. matrix-matched external calibration with internal standardization), as would be required for routine medical applications.

The potential of IDA for the accurate quantification of metal-binding proteins by gel electrophoresis (GE) in combination with LA-ICPMS detection was demonstrated in several studies [91,92]. For the absolute quantification of transferrin, species-specific isotope dilution was performed based on the use of an isotopically enriched ^{57}Fe-transferrin complex to quantify natural transferrin in human serum samples. The developed ID method was compared to external calibration and validated using a serum reference material [93]. In one study, isotopically enriched ^{64}Cu, ^{68}Zn-superoxide dismutase (SOD) was prepared to quantify natural SOD in spiked liver extracts using polyacrylamide gel electrophoresis (PAGE) in combination with LA-ICPMS detection [94]. Species-unspecific isotope dilution was applied to quantify transferrin and albumin in human serum by non-denaturing (native) GE and LA-ICPMS detection of the sulphur signal. Spike addition was achieved by immersing the protein strips with a^{34}S-enriched spike solution after gel electrophoresis and the method was validated using a serum reference material [95].

Different strategies for the addition of the isotopically-enriched spike solution have been described for LA-ICPMS bioimaging experiments. It has to be taken into account that in some of the described approaches, the condition of isotopic equilibration between the spike and the sample is not entirely fulfilled. As a consequence, IDA is no longer an absolute or fully traceable quantification method. This is for example the case, when the isotope equilibrium is obtained in the ion source only (e.g. by addition of the spike as solution post-ablation). One approach was based on a solid-spiking procedure, where powders were spiked with isotopically-enriched standards, dried, homogenized and pressed into the form of a pellet [96]. This ID method was applied to analyze coal samples, soils and sediments by LA-ICPMS [97–99]. The isotope dilution procedures were validated using commercially available solid reference materials and it was shown that the accuracy and uncertainty of the results could be improved in comparison to external calibration. As disadvantage, the sample preparation process for the production of pressed pellets is time-consuming and laborious. Moreover, due to the steps of homogenization, mixing and pressing, it is evident that the solid ID-LA-ICPMS approach is not suitable for biological samples (tissue sections and cells) as the integrity and cell arrangement/spatial information would get lost. For bioimaging applications of isotope dilution in combination with LA-ICPMS, the addition of the spike aerosol can be achieved online after the ablation cell through a micro-nebulizer and spray chamber. This method was applied to study the quantitative accumulation of platinum in rat kidneys after cisplatin perfusion using

quadrupole- and TOF-based LA-ICPMS detection [100,101]. The same approach was used to evaluate the quantitative uptake of platinum in a multicellular tumor spheroid model upon treatment with cisplatin. Validation of the ID method was accomplished by external calibration using gelatin-based standards and confocal fluorescence microscopy imaging of the spheroids [102]. One study reported on online double IDA for the quantification of Fe in homogenized sheep brain as model sample by LA-ICPMS [103]. The isotopically enriched ^{57}Fe spike solution was introduced post-ablation using a total consumption nebulizer. An estimation of the measurement uncertainty was performed, demonstrating that the mass of spike, the measured ratio of the standard blend and the mass of the calibrant were the factors with the greatest contribution to the overall uncertainty. In addition, external calibration with internal standardization was performed as comparison. For this calibration approach, the main contributing factors for the measurement uncertainty were the uncertainty in the linear least square regression and the signal variation [103].For the addition of the spike solution post-ablation, it has to be taken into account that variations occurring during processes in the laser ablation cell are not accounted for. Alternatively, the use of IDMS for imaging approaches requires a homogeneous distribution of the isotopically-enriched spike onto the sample surface for spatial analysis. One strategy was based on the automatic deposition of the spike solution on the sample surface by the use of a commercial inkjet printer. The quantitative uptake of cisplatin, carboplatin and oxaliplatin in mice kidney was studied via this strategy and compared to external calibration [104]. For single-cell analysis, an ID-LA-ICPMS protocol was developed based on a micro-array of single cells and the precise deposition of a known picolitre droplet of an enriched isotope solution onto each cell in the array with a commercial inkjet printer [105]. Single cells containing the analyte and dispensed droplets containing the spike were simultaneously ablated. The method was applied to evaluate the quantitative uptake of silver nanoparticles into macrophages at the single-cell level, as a proof-of-concept [105]. In another LA-ICPMS study, a micro-spotting device was used to deposit pL-volume droplets (containing a platinum-enriched spike solution) onto tissue sections of mice that were treated with a platinum-based drug. The absolute platinum quantity was obtained for μm-sized regions of interest in tissue samples, as defined by the extension of the deposited pL-volume droplet. Isotope dilution analysis was performed to quantify platinum in regions of interest in tumor tissue, mouse liver and spleen and the results were compared to external calibration using gelatin micro-droplet standards [58]. One study used a strategy, where isotopically enriched spike solutions were pipetted onto tissue sections (encircled with a silicon grease pen to create a barrier for the spike droplet) [106]. Isotope exchange/equilibration parameters were optimized and the ID approach was compared to external calibration by homogenous in-house prepared standards. The method was applied to the quantitative LA-ICPMS imaging of Fe, Cu and Zn in mouse brain of Alzheimer's Disease and correlative micro-XRF analysis was performed [106]. A recent ID-LA-ICPMS study used an electrospray-based coating device (ECD) to evenly distribute a known amount of the isotopically-enriched spike solution (^{65}Cu and ^{67}Zn) on mouse brain sections [107]. The mass of the spiked isotopes and the tissue sections on the slides was calculated by weighing them on an analytical balance. As proof-of-principle, the quantitative ID method was applied to quantify Cu and Zn in Alzheimer's disease mouse brain sections and validated by acid digestion and solution-based ICP-MS analysis [107].

1.9. Semiquantification approaches for LA-ICPMS imaging

The wider use of AI in all scientific areas will definitely leave its mark also in analytical chemistry, although the advancement in this field are so far limited, but has been already discussed in the recent review by Pan et al. [24] With more and more laboratories having access to ICP-TOFMS systems and the mantra of 'measure all nuclides all the time', the requirement to 'quantify all nuclides all the time' is a direct consequence. The advances made in the quantification with the use of gelatin standards have made it easier, however, it is practically impossible to fabricate calibration standards that include all the elements. Metarapi et al. have produced different sets of gelatin-based micro--droplet standards cumulatively including 72 elements; single elements standards were kept in acidic media and were not always compatible [108]. From these standards, a library of response factors was constructed and was later used in semiquantitative calibrations. The method was evaluated in two steps, (i) bootstrapping with gelatin standards and (ii) using real murine tissue thin slices. In the first step, a certain number of the elements (out of 48 elements) was chosen to serve as standards for the semiquantitative approach. The other elements were predicted by semiquantitative calibration; this was repeated (*in silico*) for a million times. The difference (the 48 elements had defined known concentrations) was plotted against the number of elements, and it was established that for the suitable accuracy, between 10 and 15 elements should be used (Fig. 6). In the second part, a similar exercise was done on real tissue sections, but only a handful of times were tested. The developed semi-quantification approach was based on 10 elements as calibration standards and provided the determination of 136 nuclides of 63 elements, with errors below 25 %, and for half of the nuclides, below 10 %. Also, a web application was developed and is free to use with all the needed instructions available in the paper electronic supporting information. Until this study, the term 'semiquantitative approach' was used mainly in the cases, where non-matrix matched standards were used; a concept that should not be mistaken with the described approach [108].

1.10. Internal standardization strategies

Internal standards are frequently used to correct for measurement deviations caused by matrix effects, elemental fractionation and instrumental drift in quantitative LA-ICPMS analysis. Ideally, an internal standard (IS) also accounts for variations in the mass ablated and transported to the ICP-MS. An effective IS for LA-ICPMS analysis should therefore match the following criteria, (i) a similar behavior to the analyte during the ablation process, transport and in the ICP, (ii) a homogenous distribution in the sample and the standard, and (iii) a similar atomic mass and/or first ionization potential as the analyte [109], with the proximity of the atomic mass being the most important factor. In literature, different strategies and elements/isotopes have been proposed for internal standardization in bioimaging experiments by LA-ICPMS. Signal normalization in LA-ICPMS analysis makes use of elements that are either intrinsically present in the biological sample or of non-intrinsic elements that are introduced by different approaches (e.g., by placing a thin layer containing the IS beneath or on top of the sample).

1.11. Carbon

The potential of carbon (^{13}C) as 'universal' internal standard for elemental mapping of biological samples by LA-ICPMS was long time under discussion in literature. Due to the presence of carbon and its (apparent) homogeneity in biological matrices, its suitability as internal standard to correct for potential signal drift and variations in the ablated mass of biological samples was extensively evaluated. An excellent overview on the topic regarding the factors affecting internal standard selection for quantitative elemental bioimaging of soft tissues by LA-ICPMS, was written by Austin et al. and it gives the conditions to be met, for ^{13}C to be used as an internal standard, despite of its drawbacks [109]. Importantly, Guenther et al. observed that carbon showed a matrix-dependent partitioning into carbon-containing gaseous species and carbon containing particles [110]. Trace element analytes were exclusively transported as the particulate phase. The matrix-dependent formation of gaseous carbon species can lead to inaccuracies of the target analyte and the IS in the ablation cell and tubing. The formation of the gaseous phase leads to broader or double peaks and thus to a

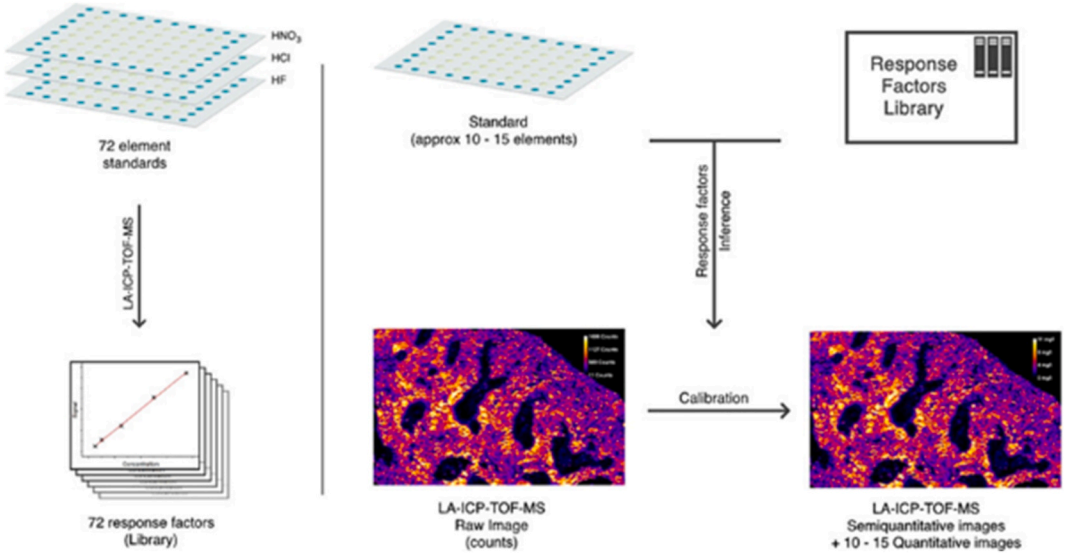

Fig. 6. Workflow for semi-quantitative analysis by LA-ICP-TOFMS. Calibration standards are based on multi-element gelatin micro-droplets [108].

distortion of the results. Further studies have shown that during laser ablation of gelatin (representative of biological samples) with higher laser fluence, an element-specific (C, As, Zn, Se, etc.) gaseous phase is formed, leading to double peaks that cause smearing effects and affect the accuracy of quantification [16,17]. Moreover, ^{13}C shows a background signal in the ICP-MS without ablation due to its presence in the atmosphere and possible impurities from the argon gas. Carbon may not be uniformly distributed in biological samples due to differences in tissue water content. Also, the atomic mass and/or first ionization potential varies significantly to many analytes.

Plant tissues have proven to be specifically difficult to analyze by LA-ICPMS, because of their relatively large size, varied structure and problems with ensuring the flatness and consequently, the focus over the entire sample area [111]. Therefore, ^{13}C has been used as an IS in several LA-ICPMS studies of plants to compensate for the effects of referencing on a loss of laser focus, overlapping layers of leaf tissue and cell damage within the imaged leaf tissue. One LA-ICPMS study assessed C, Mg, P, Ca, and Rb as potential candidates for internal standardization to study the Zn and Cd distribution in leaves of the hyperaccumulator *Arabidopsis helleri* [112]. Signal normalization with ^{13}C was used alongside calibration with cellulose-matrix matched standards to study five different tree species sampled in the area of Daejeon, Korea, and their accumulation rates of metal pollutants in their rings by LA-ICPMS. In order to determine the average concentration of metals in each annual ring, the surfaces of wood cores were analyzed using line scan mode. The results showed that the hardness and properties of the wood varied depending on the tree species and age. To correct for baseline shift of ICP-MS and differences in matrix ablation efficiencies, as well as changes in wood density during the ablation process, ^{13}C was used as an internal standard [113]. Quantitative LA-ICPMS analysis was used to map Ag, Mn and Cu in soybean leaves cultivated in the absence or in the presence of silver nanoparticles in comparison to silver nitrate [114]. The study evaluated suitability of the isotopes ^{12}C and ^{13}C in comparison to ^{28}Si and ^{31}P as IS. Based on the highest precision obtained and the highest homogeneity in the sample surface (verified through the LA images), ^{13}C was found to be the best internal standard [114]. Another LA-ICPMS study performed quantitative mapping of metallic pollutants in sweet basil. The experiment involved the cultivation of sweet basil in a nutrient solution spiked with 100 and 1000 ng mL^{-1} of Cs, followed by determination of the Cs distribution in the leaves using lab-synthesized standard pellets and ^{13}C as an internal standard. This work compared elemental images of the control leaf with the same matrix to the images of the leaf being analyzed, as the element analytes were transported solely as particulate phase from the carbon-containing gaseous species and carbon-containing particles during laser ablation [115]. Interestingly, one study used ^{13}C internal standard for the investigation of the As content in Andean mummy hair, while hairs are made of sulphur rich protein α-keratin and sulphur would therefore be a more appropriate choice [116].

1.12. Other intrinsic elements

Other elements than carbon that are usually present in the biological samples (e.g. phosphorus, sulphur and calcium) can be prone to segmentation to specific compartments and thus inappropriate to be used as IS. The suitability of the isotope ^{34}S as IS was evaluated in different LA-ICPMS biological studies. Sulphur was used as internal standard in an LA-ICPMS study for human hair and nail investigations, where a method was developed to analyze Mn, Cu, Zn, Sr, Y, Pb and U for human biomonitoring [117]. A similar LA-ICPMS study used sulphur as internal standard in the analysis of human hair and nails to determine the element concentrations and isotope ratios of 8 elements. Some isotope ratio changes reflect actual changes in diet, physiology, living area while other changes were attributed to the weathering and external contamination [118]. Leopard seal whiskers were used as a tool for trace element biomonitoring (Hg, Pb, Cd, and Se) and higher Hg burden was observed in comparison to previous studies [119]. A common element that is used an internal standard in LA-ICPMS studies on bone and teeth is ^{43}Ca, as it is homogenously distributed and does not suffer from the same problems as carbon [120–122]. With regard to phosphorus, it was shown that it can be used to compensate for differences in cell thickness and density in a multicellular tumor spheroid model. This approach together with single-cell resolution enabled to detect the quantitative platinum uptake in the different cell compartments of the spheroid model upon treatment with oxaliplatin [123].

1.13. Non-intrinsic elements

There are different strategies described for the use of non-intrinsic element for signal normalization approaches in LA-ICPMS studies. One study reported on the direct elemental analysis of whole blood samples by LA-ICPMS by taking advantage of the possibility to add an element of choice to the blood sample before ablation in a cryogenic cell. Rhodium was selected as internal standard and a low LOD and good accuracy and precision were obtained [124]. For section-based biological samples, another approach for internal standardization without the use of

intrinsic elements was based on adding a thin polymer or metal layer containing internal standard elements beneath the sample. Spin-coating of a PMMA layer doped with yttrium and ruthenium as IS elements onto glass slides provided a thin film, where the sample was placed on top [29]. Co-ablation of the tissue sample and the internal standard layer required careful optimization of the laser fluence to enable selective and quantitative/full ablation of both layers down to the substrate. The IS (Y and Ru) from the polymer layer were compared to ^{13}C in terms of precision and accuracy (quantified values were compared to results obtained by SN-ICPMS analysis). The use of ^{13}C as IS improved precision most likely due to higher background counts, whereas the quantification was less accurate as compared to Y and Ru as IS elements. Signal normalization to Y and Ru resulted in less precision but improved the accuracy of quantification by LA-ICPMS [29]. In one study, a gelatin-layer that was doped with thulium as IS was placed between the glass support and the tissue. Matrix-matched standards were prepared from rat kidney tissue spiked with uranium as target analyte to quantify U concentrations in renal tissue of rats [125].

Alternatively, the internal standard can be deposited on top of the biological sample section. This methodology can be based on sputtering techniques for the homogenous deposition of an IS layer on the tissue surface. LA-ICPMS studies used this strategy by employing gold as internal standard element in the thin films [126,127]. Gold poses several advantages as IS as its first ionization potential is comparable with elements such as zinc or copper. There are no background signals of gold in biological tissue and the mass of ^{197}Au is unlikely to be influenced by any spectral interference. It could be shown that gold standardization compensated instrumental drifts, matrix related ablation differences and day-to-day signal changes [126,127]. Automated deposition of an internal standard layer on top of the sample/tissue surface can be also achieved by the use of an inkjet printer [128–130]. The ink was spiked with the internal standard element (e.g. In and Ir) and printed onto the samples. It was shown that the homogenous distribution of the IS was further improved by coating the samples by gelatin. This approach proved to be effective to overcome day-to-day variations and instrumental drifts [131].

One internal standardization approach introduces the internal standard into the sample by different (labeling) strategies. One study developed a method based on iodination of fibroblast cells and tissue sections. Iodine was employed as an elemental dye with localization in the cell nuclei and lower iodination of the surrounding cytoplasm [132]. The use of iodine as an internal standard to correct for tissue inhomogeneities in LA-ICPMS was investigated for the simultaneous detection of two tumor markers in breast cancer tissue. Additionally, lanthanide background resulting from glass ablation was corrected for by Eu standardization.

One internal standardization strategy for quantitative LA-ICPMS bioimaging used an iridium-based intercalator that is commonly employed in imaging mass cytometry [133]. The Ir-based intercalator binds to the DNA of cells and therefore, it allows visualizing single cells in tissues based on their nuclei and to perform single-cell segmentation. In this study, its potential as internal standard was evaluated as it offers the advantage that it does not only correct for drift and matrix effects but also for differences in the ablated mass. As disadvantage, the metal intercalator binds to DNA, which is confined in the cell nucleus and might not be present to the same extent in all cell types. Therefore, a spatial resolution was employed in the study that was greater than the size of a single cell [133].

1.14. Ablated volume normalized calibration

In LA-ICPMS analysis, quantification usually relies on matrix-matched standards to solve elemental fractionation problems, where internal standardization is commonly used to correct for instrumental drift, and for non-dissectible samples, differences in ablated mass are corrected for. However, it is difficult to ensure a homogeneous distribution of the internal standard and a uniform laser light absorptivity is challenging in practice. Therefore, a new approach has been developed employing an ablation volume normalization method, in which the ablated volume is used to correct for ablation differences. The approach is based on measuring the volume after ablation with profilometry followed by normalization of the LA-ICPMS signal to the ablated volume. The first LA-ICPMS study regarding volume-corrected signals focused on differences in ablation rates within and between samples and standards by normalizing elemental maps based on the ablation volume per pixel measured by optical profilometry [134]. This approach moves from mass-to-mass concentrations to mass-to-volume concentrations and improves the accuracy of 2D LA-ICPMS mapping, as demonstrated using a decorative glass with different elemental concentrations. In a subsequent study, a variety of materials were investigated, including glasses, carbonates, gelatins, plants and zircon materials. Both, “hard” and “soft” materials were considered, serving as calibration standard and sample. A cross-calibration was performed by analyzing each material against each other in bulk analysis mode using a non-matrix-matched calibration by ablation volume normalization for validation of the method. The obtained concentrations in the different materials consistently matched the certified values regardless of the standard material used for calibration, highlighting the effectiveness of transferring calibration standards to materials with clearly defined elemental compositions. In particular, gelatin and glass showed good compatibility and versatility for calibration purposes [135]. One important aspect regarding the use of mass fractions (ppm, ppb etc.) and concentrations (g mL^{-1}, µg $µL^{-1}$ etc.) was also addressed in this study. Converting units from concentrations (actually the amount measured in the volume, either determined by a measurement procedure or with the sample preparation procedure, *e.g.* by preparation of thin sections of desired thickness) to mass fractions requires knowledge on the density of the sample. Considering the complexity of biological specimen, the density will normally not be homogenous across all of the sample and therefore, quantitative LA-ICPMS results should be presented as concentrations.

1.15. Applications of quantification procedures by LA-ICPMS

1.15.1. Quantification in immuno-mass spectrometry imaging by LA-ICPMS

LA-ICPMS is also employed for immuno-mass spectrometry imaging (iMSI), where the spatial expression of biomolecules is targeted in tissue sections following immunostaining procedures. A typical workflow including sample preparation and immuno-labelling for iMSI using LA-ICPMS analysis is shown in Fig. 7A [136]. Various elemental labeling strategies for antibodies have been developed for the application of multiplex immunoassays in combination with LA-ICPMS detection [136–138]. Metal tags include single lanthanide complexes such as DOTA, polymeric tags containing several lanthanides (e.g., MaxPar® metal-conjugated reagents), metal-coded affinity tags (MeCATs), nanoparticles, fluorescent Au or Ag nanoclusters (NCs) and quantum dots [139–144].

In imaging mass cytometry, high-resolution LA-ICP-TOFMS (using a CyTOF instrument) is employed to perform multi-parametric characterization of cell populations in tissue samples [145]. Whereas IMC is used purely in a qualitative way e.g., for cell phenotyping, several approaches were developed in the context of iMSI by LA-ICPMS for the quantification of analytes/biomolecules after metal-labeling strategies. One of the challenges associated with accurate quantification is a non-controllable antibody labeling chemistry. The binding efficiency of multiplexed staining can be affected by a number of factors including epitope blocking and other forms of steric hindrance [136]. To control the labeling degree, one study developed a novel antibody labeling technique that resulted in one label per antibody [146]. The labeling method was based on the use of small antibody-binding C2 domains that were modified with conventional MeCATs. Via this strategy, it was

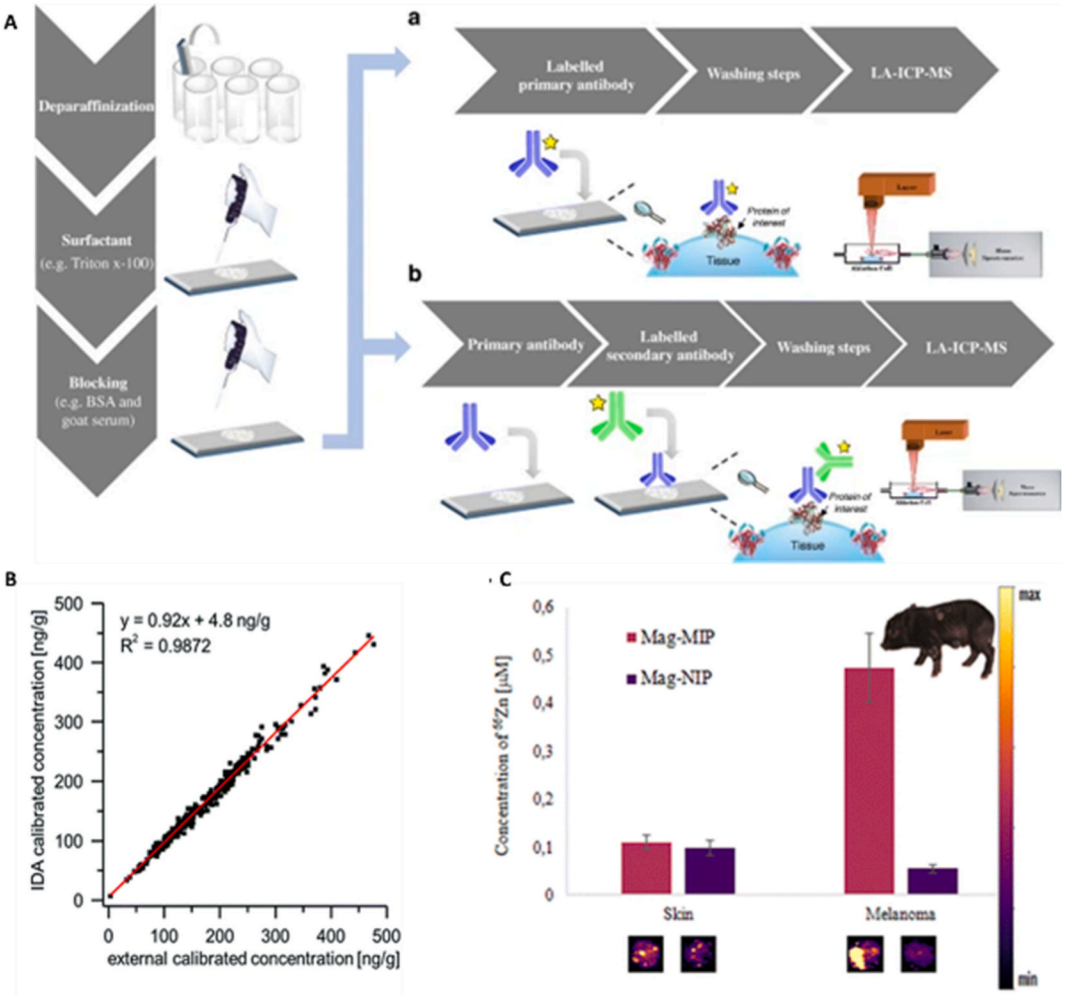

Fig. 7. (A) Workflow for immuno-mass spectrometry bioimaging with LA-ICPMS [136]. (B) Correlation of the Yb concentration determined by on-line isotope dilution analysis (IDA) and external calibration for quantification of enriched metal labels used in immuno-mass spectrometry imaging [148]. (C) Comparison of ^{66}Zn levels in healthy and melanoma skin of minipigs determined by molecularly imprinted polymers (MIPs)-LA-ICPMS [149]. The Figures were adapted from the references [136,148,149], with permission of the publishers.

possible to quantify the amount of introduced lanthanide ions into the sample and the molar amount of the antibody that was bound to the target protein. An LA-ICPMS-based western blot immunoassay was used to evaluate the applicability of six C2-tagged antibodies. Quantification of the labelled antibody–antigen complexes was achieved by the use of house-made calibration membranes [146]. One LA-ICPMS study investigated the effects of multiplexing on reproducible binding using metal-conjugated antibodies for different muscle proteins in murine quadriceps sections [147]. The average concentrations of the lanthanide analytes were determined in a series of sections upon individual and multiplexed immunostaining with the respective metal-conjugated antibodies. This reproducibility study revealed no significant differences between the individual and multiplexed application of the antibodies [147].

LA-ICPMS imaging in combination with gelatin-based mold standards was used to study the quantitative localization of dystrophin in muscle sections [150]. For this purpose, Gd-labelled anti-dystrophin antibodies were employed, where Gd was quantified as a proxy for the relative expression of dystrophin. The method was validated in murine and human skeletal muscle sections following k-means clustering segmentation and applied to patients suffering from Duchenne muscular dystrophy [150]. Online IDA was used to quantify elemental labels routinely used in imaging mass cytometry [148]. In this case, the protocol utilized enriched isotopes for the respective metal-tagged antibodies, which allowed quantification by IDA using LA-ICPMS imaging. The sample aerosol (containing the enriched isotope from the sample) was mixed with a wet aerosol from an aqueous standard containing the analyte (usually a lanthanide) with a natural isotopic abundance. The method was applied to quantify Yb-labelled anti-tyrosine hydroxylase in cryo-section of mouse brain. The mass flow parameters were calculated by ablation of a ^{172}Yb-spiked matrix-matched standard. IDA was compared with external calibration using gelatin standards and proved to be more robust for quantification as the isotope ratios were not influenced by signal drifts or plasma fluctuations (Fig. 7B) [148]. One LA-ICPMS study used molecularly imprinted polymers (MIPs) to target metallothionein, *i.e.* a metal-containing protein (Zn and Cd) [149]. Iron oxide magnetic particles were exploited as carrier of the polymeric layer and due to the agglomeration of the particles in the presence of an external magnet, the MIPs were capable of isolating metallothionein from a tissue sample. As proof-of-principle, the prepared particles were employed for the quantification of metallothionein in melanoma and healthy skin of melanoma-bearing minipigs. Significantly higher amounts of Zn were found in tumor tissue than in healthy skin (Fig. 7C) [149].

Nanoparticles are also commonly used as labels for quantitative determination of biomolecules by LA-ICPMS imaging. One study addressed the quantitative imaging of amyloid beta (Aβ) peptide in the brain, which is important for Alzheimer's disease (AD) research and

drug development [151]. The Aβ antibody was labelled with AuNPs to produce AuNPs-anti-Aβ conjugates that could bind to Aβ in brain sections. Quantitative imaging of Aβ was achieved by measuring Au concentrations using homogenised matrix-matched brain sections standards as external calibrants. The stoichiometric ratios between the metal conjugates and Aβ were optimized, and the efficiency of the immuno-reaction after labelling was investigated. By considering the molar relationship between AuNPs and Anti-Aβ, as well as the ratio of Anti-Aβ to Aβ, Aβ was quantitatively mapped in the brain by LA-ICPMS. The method accurately indicated the location and concentration of Aβ aggregation and was consistent with traditional immunohistochemical staining. The use of AuNPs improved the sensitivity and intuitiveness of the method due to the increased signal from Aβ [151]. In another study, a method for quantitative bioimaging of specific proteins in biological tissues using antibody-conjugated gold nanoclusters (AuNCs) and LA-ICPMS detection was described [152]. The distribution of metallothioneins (MT1/2 protein isoforms) in human retina tissue sections was successfully determined as proof-of-concept. AuNCs were conjugated to the selected antibody and provided high amplification for detection of MT1/2 distribution in the neurosensory retina layers. Elemental images of ^{197}Au were quantified using gelatin matrix-matched standards and converted to quantitative 2D images of MT1/2 concentration. The results obtained with LA-ICPMS were validated by comparison with measurements performed with a commercial ELISA kit. The developed strategy can be extended to other antibodies and metal markers and opens new possibilities for quantitative imaging of proteins in various biological tissues [152].

A recent LA-ICPMS study introduced matrix-matched standards that mimic the matrix of cultured cells by using the same cell line of the sample to create laboratory standards [153]. For this purpose, single-cell laboratory standards based on cells supplemented with Au nanoclusters (NCs) were developed. The cell standards were characterized by ICP-MS and LA-ICPMS analysis. A single biomarker strategy using Au NCs as specific antibody labels was employed for the analysis of selected proteins in individual cells by LA-ICPMS. Quantitative data for the proteins in the cells using the proposed matrix-matched calibration and LA-ICPMS analysis were successfully corroborated with commercial ELISA kits [153].

1.15.2. Quantification strategies for nanoparticle analysis with LA-ICPMS

The increasing use of nanoparticles (NPs) in various fields, e.g., biology, medicine, consumer products, energy production, etc., raises safety concerns, especially with regard to their effects on human health through inhalation and dermal exposure. NPs have different uptake mechanisms than dissolved species, which warrants thorough investigations. As a result, the number of studies on NPs and the development of dedicated measurement methods have greatly increased. The advent of nanotechnology has transformed the field of single-molecule analysis, enabling the detection of nanoparticles with remarkable sensitivity and resolution. LA-ICPMS is an effective technique for monitoring and quantifying nanoparticles in biological tissues. It offers high spatial resolution, allowing precise determination of the spatial distribution of nanoparticles in a sample, and serves as a valuable technique for monitoring NP-labelled antibodies. Several calibration strategies have been explored for the analysis/imaging of nanoparticles and the indirect detection of biomolecules using labelled nanoclusters in tissues, cells and other biological samples by LA-ICPMS. However, a reliable and accurate quantification of NPs by LA-ICPMS is challenging due to the lack of suitable standards and uncertainties associated with matrix effects. Most LA-ICPMS studies on NPs rely on standards that are prepared from elemental standard solutions. They offer the advantage that they are comparably easy to produce and to characterize (in most laboratories they have been already developed and exist based on in-house procedures), and that they can cover a reasonable mass range. However, it is still inconclusive whether the ablation behaviour of elemental standards, their transport to the ICP-MS and atomization and ionization in the ICP is consistent with those of NPs. Standards prepared from NP suspensions require thorough characterization with regard to NP stability and number of particles, they usually only cover a mass range with a NP number $> 10^3$ and NP agglomeration can occur, which impacts the NP number within the standard. Concepts for quantification of NPs by LA-ICPMS are based on the use of frozen and spiked brain tissue, spiked organic materials (e.g., gelatin or agarose), or dried residues on substrates, each strategy having its own capabilities and limitations depending on the type of sample, matrix, and target element or nanoparticle.

In recent years, single particle-inductively coupled plasma mass spectrometry (SP-ICPMS) has transformed the field of nanometallomics [154]. The method allows for differentiation between dissolved and particulate metal signals and enables quantification of the number and size of nanoparticles. However, SP-ICPMS is limited to the analysis of NPs in solution. To address this issue, one study demonstrated the use of solid samples by LA-ICPMS for localized NP analysis in biomaterials. LA-SP-ICPMS conditions, such as laser fluence, beam size, and dwell time were optimized to minimize NP degradation, peak overlap, and interference from dissolved gold and silver nanoparticles in a series of studies (Fig. 8A) [73,155,156]. A data processing algorithm was developed to extract the NP number concentration and size from the measurements. As a proof-of-concept, a cross-section of a sunflower root sample exposed to gold NPs was successfully imaged under the optimized LA-SP-ICPMS conditions, demonstrating the potential for localized NP characterization [73]. In addition, it was observed that the use of a low laser fluence (< 1 J cm^{-2}) is critical to avoid NP degradation and to ensure reliable results. The degradation of gold NPs (AuNPs) during laser ablation was studied and guidelines were provided for selecting the optimal laser fluence for NP analysis in gelatin, a matrix that mimics biological tissue. AuNPs with known sizes and narrow size distribution were used to monitor the measured NP size at different laser fluences. Optical profilometry was used to accurately measure the amount of material ablated at different laser fluences and provide a more accurate estimate of NP degradation [155]. In another study, advanced data processing and visualization techniques in LA-SP-ICPMS were used to image sunflower roots exposed to ionic silver (Ag^+). Detailed multiplexed images were obtained showing the uptake and transformation of Ag^+ into silver nanoparticles (AgNPs) within the root cross sections. The size of the biosynthesized AgNPs was found to be influenced by the reducing power of specific root compartments. Various visualization strategies were used to show the spatial distribution of Ag^+ and AgNPs, focusing on the number and size of individual AgNPs in selected root regions. Statistical analysis of AgNP distribution was also performed [156].

A ‘single-cell isotope dilution analysis’ (SCIDA) approach was developed to analyze metal NPs in single cells using LA-ICPMS (Fig. 8B) [105]. The principles of SCIDA were demonstrated using macrophage cells as a model to study the uptake of Ag NPs at the single-cell level. A microfluidic technique was used to place single cells in an array, and each cell was dispensed with a precise picoliter drop of an enriched isotope solution using a commercial inkjet printer. Accurate quantification of Ag NPs in single cells was achieved by isotope dilution LA-ICPMS analysis. The average Ag mass of 1100 single cells closely matched the average of the cell population analyzed by solution-based ICP-MS. The detection limit for Ag NPs in single cells was 0.2 fg Ag per cell [105]. Another method to quantify Au NPs in single cells was based on matrix-matched calibration standards from dried residues of picoliter droplets generated by an inkjet printer [77]. The distribution of the gold mass in single cells exposed to NIST Au NPs showed a lognormal distribution. The average measurement agreed well with the measurement from an aqua regia digested Au NP solution. The limit of quantification was determined to be 1.7 fg Au [77].

LA-ICPMS was used to gain insight into the spatial distribution and penetration behavior of NPs in complex three-dimensional tissue models. In one study, collagen-rich microstructures were produced in

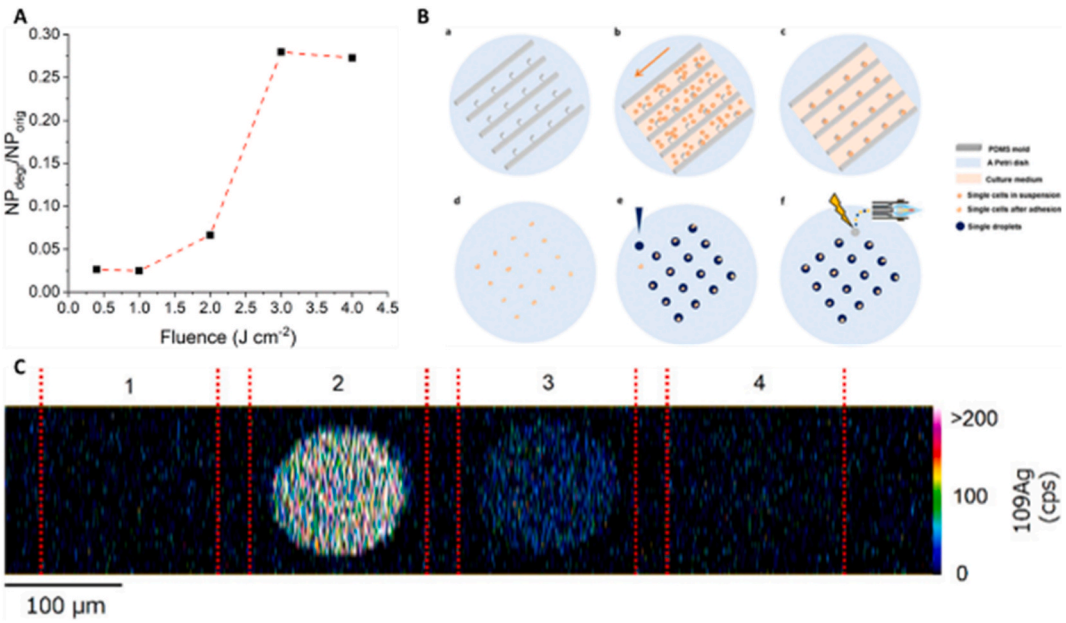

Fig. 8. (A) Influence of the laser fluence on the degradation of gold nanoparticles in gelatin using LA-ICPMS analysis [155]. (B) A schematic diagram of single-cell isotope dilution analysis with LA–ICPMS for the quantification of nanoparticles in single cells [105]. (C) Image of Ag NP calibration spots representing 50 (1), 3500 (2), 350 (3) and 35 (4) fg of Ag NPs [80]. The Figures were adapted from the following references [80,105,155], with permission of the publishers.

multicellular fibroblast spheroids (MCSs) to serve as a three-dimensional tissue analog for studying Ag NPs penetration [157]. LA-ICPMS imaging of the thin sections showed the distribution of Ag NPs along with other elements (Ag, P, Cu, Zn, and Br), indicating the localization of the particles. The distribution correlated with the presence of specific elements and was predominantly located in the outer edge of the spheroid model corresponding to proliferating cells [157]. In a subsequent study, the interactions of Ag NPs and the distributions of intrinsic minerals and biologically relevant elements were studied within thin sections of the MCS, using LA-ICP-(TOF)MS analysis [80]. Matrix-matched calibration standards were designed and printed using a non-contact piezo-driven array spotter with an Ag NP suspension and multi-element standards (Fig. 8C). The method allowed the detection of Ag, Mg, P, K, Mn, Fe, Co, Cu, and Zn in the femtogram range, which is sufficient for the determination of intrinsic minerals in thin MCS sections. After a 48h incubation period, Ag NPs were found to be concentrated in the outer rim of the MCS and undetectable in the core. Quantitative measurement of the total mass of Ag NPs in a thin section using LA-ICP-TOFMS imaging was consistent with results obtained by ICP sector field mass spectrometry in liquid mode after acid-assisted digestion. This approach demonstrates the potential of LA-ICP-TOFMS for spatially resolved nanoparticle imaging and elemental analysis in complex biological samples such as MCS [80].

Moreover, one strategy was developed that combines the concept of bioprinting for the preparation of gelatin-based multi-element standards and LA-ICP-TOFMS analysis [63]. Lanthanide up-conversion nanoparticles were incorporated into a gelatin matrix to produce the bioprinted calibration standards. The bioprinting approach showed higher throughput, better repeatability, and better elemental signal homogeneity compared to manual cryo-sectioning of standards. Bioprinting reduced inter-batch variability and analysis time because multiple standards were printed simultaneously. The bioprinted calibration standards remained stable for two months with proper storage [63]. The suitability of gelatin-printed calibration standards were additionally validated for the absolute quantitation of nanoparticles in Surface-Enhanced Raman Scattering (SERS) by developing a new 2D quantitation model [68]. SC-ICPMS was used to characterize the absolute concentration of the SERS nanotags. Gelatin-based calibration standards containing gold nanoparticles and a Raman reporter were prepared using a novel printing approach. The standards were further characterized using LA-ICP-TOFMS to assess the distribution and response of the nanotags. The LA-ICP-TOFMS analysis demonstrated the homogeneous distribution of nanotags and a linear relationship between the gold concentration and the response [68].

One recent study described a new approach for the preparation of quantitative standards for NPs that is based on particulate standards [158]. AuNP standards were prepared via micro-nano fabrication on an ITO (indium tin oxide) glass substrate. The fabrication process included coating a PMMA layer on the substrate followed by E-beam lithography to create an array of holes and a set of coordinate grids. Three different AuNPs (one synthesized and two commercial AuNPs) were used to prepare the calibration standards. The suspension of each AuNP was spread and dried on the fabricated PMMA layer. After the removal of PMMA, only the AuNPs located in the holes and the lines of the coordinate system were left on the ITO slide. The method allowed the preparation of AuNP standards with high accuracy and precision, covering a wide mass range. The approach highlights the importance of using particulate rather than ion standards for accurate quantification of NP, as Au ions and AuNPs exhibit different signal transduction efficiencies during LA-ICPMS analysis. However, the method has not yet been applied specifically to biological samples [158].

2. Conclusions and outlook

Initially, the development of elemental calibration standards for bioimaging applications by LA-ICPMS was based on manual fabrication and elaborate multi-step procedures, that required skilled personnel, in most cases, the handling of biological material and microtome cutting. The latter one can induce possible thickness inaccuracies of the standards that are biasing the results. Nowadays, state-of-the-art quantification is more and more relying on reliable, automated and most importantly, reproducible and operator-independent processes that are based on spin-coating, inkjet printers and robotic micro-droplet dispensing systems. Automation of the standard production by these processes can drastically increase the throughput to generate calibration standards. This makes these approaches interesting for mass production of standards and potential commercialization. With regard to the matrix of the standards, there is an increasing tendency observable towards the

fabrication of calibration standards that are based on materials (e.g., polymers and gelatin) with properties and characteristics that are easy to control, modify, and fine-tune for a specific application. These more 'universal' biological matrices can mimic the biological sample and can overcome some of the problems associated with tissue-type standards, such as handling of biological material and the background abundance of endogenous elements within the standards, potentially affecting the calibration range and limits of detection. There is still a requirement for certified materials such as NIST 61X glasses that are specific for bio-imaging applications by LA-ICPMS.

In comparison to the described progress in the development of matrix-matched standards for elemental quantification in tissues and cells, the quantification of nanoparticles is understandably still in its initial stages, regarding only recent interest in the field. The standardization of the NP size usually relies on a single-point calibration and therefore, multi-point calibration standards are required for the development of the field. Similarly, the quantification of metal-conjugated antibodies is in its beginning, but the more often the methodology is used, the more reliable matrix-matched standards will emerge.

With the upcoming of ICP-TOFMS technology, all nuclides are always measured all the time and therefore, highly multiplexed standards and/or semiquantitative approaches are required to be able to quantify all nuclides of interest.

CRediT authorship contribution statement

Kristina Mervič: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Martin Šala:** Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Sarah Theiner:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

There are no conflicts of interest to declare.

Data availability

No data was used for the research described in the article.

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References

[1] A.L. Gray, Solid sample introduction by laser ablation for inductively coupled plasma source mass spectrometry, Analyst 110 (5) (1985) 551–556.
[2] S.F. Durrant, N.I. Ward, Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) for the multielemental analysis of biological materials: a feasibility study, Food Chem. 49 (3) (1994) 317–323.
[3] S. Wang, R. Brown, D.J. Gray, Application of laser ablation-ICPMS to the spatially resolved micro-analysis of biological tissue, Appl. Spectrosc. 48 (11) (1994) 1321–1325.
[4] D. Pozebon, G.L. Scheffler, V.L. Dressler, Recent applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for biological sample analysis: a follow-up review, J. Anal. At. Spectrom. 32 (5) (2017) 890–919.
[5] T.J. Stewart, Across the spectrum: integrating multidimensional metal analytics for in situ metallomic imaging, Metallomics 11 (1) (2019) 29–49.
[6] P.A. Doble, R.G. de Vega, D.P. Bishop, D.J. Hare, D. Clases, Laser ablation–inductively coupled plasma–mass spectrometry imaging in biology, Chem. Rev. 121 (19) (2021) 11769–11822.
[7] S.J.M. Van Malderen, A.J. Managh, B.L. Sharp, F. Vanhaecke, Recent developments in the design of rapid response cells for laser ablation-inductively coupled plasma-mass spectrometry and their impact on bioimaging applications, J. Anal. At. Spectrom. 31 (2) (2016) 423–439.
[8] A. Gundlach-Graham, D. Günther, Toward faster and higher resolution LA–ICPMS imaging: on the co-evolution of LA cell design and ICPMS instrumentation, Anal. Bioanal. Chem. 408 (11) (2016) 2687–2695.
[9] T. Van Acker, S.J.M. Van Malderen, T. Van Helden, C. Stremtan, M. Šala, J.T. van Elteren, F. Vanhaecke, Analytical figures of merit of a low-dispersion aerosol transport system for high-throughput LA-ICP-MS analysis, J. Anal. At. Spectrom. 36 (6) (2021) 1201–1209.
[10] C. Neff, P. Becker, D. Günther, Parallel flow ablation cell for short signal duration in LA-ICP-TOFMS element imaging, J. Anal. At. Spectrom. 37 (3) (2022) 677–683.
[11] H.A.O. Wang, D. Grolimund, C. Giesen, C.N. Borca, J.R.H. Shaw-Stewart, B. Bodenmiller, D. Günther, Fast chemical imaging at high spatial resolution by laser ablation inductively coupled plasma mass spectrometry, Anal. Chem. 85 (21) (2013) 10107–10116.
[12] M. Šala, V.S. Šelih, C.C. Stremtan, J. Teun van Elteren, Analytical performance of a high-repetition rate laser head (500 Hz) for HR LA-ICP-QMS imaging, J. Anal. At. Spectrom. 35 (9) (2020) 1827–1831.
[13] J.T. van Elteren, V.S. Šelih, M. Šala, Insights into the selection of 2D LA-ICP-MS (multi)elemental mapping conditions, J. Anal. At. Spectrom. 34 (9) (2019) 1919–1931.
[14] J.T. van Elteren, M. Šala, D. Metarapi, Comparison of single pulse, multiple dosage, and 2D oversampling/deconvolution LA-ICPMS strategies for mapping of (ultra)low-concentration samples, Talanta 235 (2021) 122785.
[15] C. Neff, P. Keresztes Schmidt, P.S. Garofalo, G. Schwarz, D. Günther, Capabilities of automated LA-ICP-TOFMS imaging of geological samples, J. Anal. At. Spectrom. 35 (10) (2020) 2255–2266.
[16] A. Jerše, K. Mervič, J.T. van Elteren, V.S. Šelih, M. Šala, Quantification anomalies in single pulse LA-ICP-MS analysis associated with laser fluence and beam size, Analyst 147 (23) (2022) 5293–5299.
[17] T. Van Helden, K. Mervič, I. Nemet, J.T. van Elteren, F. Vanhaecke, S. Rončević, M. Šala, T. Van Acker, Evaluation of two-phase sample transport upon ablation of gelatin as a proxy for soft biological matrices using nanosecond laser ablation – inductively coupled plasma – mass spectrometry, Anal. Chim. Acta 1287 (2024) 342089.
[18] S.E. Jackson, Calibration strategies for elemental analysis by LA-ICP-MS, in: P. Sylvester (Ed.), Laser Ablation–ICP–MS in the Earth Sciences - Current Practices and Outstanding Issues, vol. 40, 2008.
[19] H.P. Longerich, D. Günther, S.E. Jackson, Elemental fractionation in laser ablation inductively coupled plasma mass spectrometry, Fresen. J. Anal. Chem. 355 (5) (1996) 538–542.
[20] S. Zhang, M. He, Z. Yin, E. Zhu, W. Hang, B. Huang, Elemental fractionation and matrix effects in laser sampling based spectrometry, J. Anal. At. Spectrom. 31 (2) (2016) 358–382.
[21] I. Konz, B. Fernández, M.L. Fernández, R. Pereiro, A. Sanz-Medel, Laser ablation ICP-MS for quantitative biomedical applications, Anal. Bioanal. Chem. 403 (8) (2012) 2113–2125.
[22] A. Limbeck, P. Galler, M. Bonta, G. Bauer, W. Nischkauer, F. Vanhaecke, Recent advances in quantitative LA-ICP-MS analysis: challenges and solutions in the life sciences and environmental chemistry, Anal. Bioanal. Chem. 407 (22) (2015) 6593–6617.
[23] D. Hare, C. Austin, P. Doble, Quantification strategies for elemental imaging of biological samples using laser ablation-inductively coupled plasma-mass spectrometry, Analyst 137 (7) (2012) 1527–1537.
[24] H. Pan, L. Feng, Y. Lu, Y. Han, J. Xiong, H. Li, Calibration strategies for laser ablation ICP-MS in biological studies: a review, TrAC, Trends Anal. Chem. 156 (2022) 116710.
[25] J.S. Becker, M.V. Zoriy, M. Dehnhardt, C. Pickhardt, K. Zilles, Copper, zinc, phosphorus and sulfur distribution in thin section of rat brain tissues measured by laser ablation inductively coupled plasma mass spectrometry: possibility for small-size tumor analysis, J. Anal. At. Spectrom. 20 (9) (2005) 912–917.
[26] D.J. Hare, J. Lear, D. Bishop, A. Beavis, P.A. Doble, Protocol for production of matrix-matched brain tissue standards for imaging by laser ablation-inductively coupled plasma-mass spectrometry, Anal. Methods 5 (8) (2013) 1915–1921.
[27] A.E. Egger, S. Theiner, C. Kornauth, P. Heffeter, W. Berger, B.K. Keppler, C. G. Hartinger, Quantitative bioimaging by LA-ICP-MS: a methodological study on the distribution of Pt and Ru in viscera originating from cisplatin- and KP1339-treated mice, Metallomics 6 (9) (2014) 1616–1625.
[28] J.A.T. Pugh, A.G. Cox, C.W. McLeod, J. Bunch, B. Whitby, B. Gordon, T. Kalber, E. White, A novel calibration strategy for analysis and imaging of biological thin sections by laser ablation inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 26 (8) (2011) 1667–1673.
[29] C. Austin, D. Hare, T. Rawling, A.M. McDonagh, P. Doble, Quantification method for elemental bio-imaging by LA-ICP-MS using metal spiked PMMA films, J. Anal. At. Spectrom. 25 (5) (2010) 722–725.
[30] O. Reifschneider, C.A. Wehe, I. Raj, J. Ehmcke, G. Ciarimboli, M. Sperling, U. Karst, Quantitative bioimaging of platinum in polymer embedded mouse organs using laser ablation ICP-MS, Metallomics 5 (10) (2013) 1440–1447.
[31] M. Bonta, H. Lohninger, V. Laszlo, B. Hegedus, A. Limbeck, Quantitative LA-ICP-MS imaging of platinum in chemotherapy treated human malignant pleural mesothelioma samples using printed patterns as standard, J. Anal. At. Spectrom. 29 (11) (2014) 2159–2167.

[32] S.J.M. Van Malderen, E. Vergucht, M. De Rijcke, C. Janssen, L. Vincze, F. Vanhaecke, Quantitative determination and subcellular imaging of Cu in single cells via laser ablation-ICP-mass spectrometry using high-density microarray gelatin standards, Anal. Chem. 88 (11) (2016) 5783–5789.
[33] M.T. Westerhausen, T.E. Lockwood, R. Gonzalez de Vega, A. Röhnelt, D.P. Bishop, N. Cole, P.A. Doble, D. Clases, Low background mould-prepared gelatine standards for reproducible quantification in elemental bio-imaging, Analyst 144 (23) (2019) 6881–6888.
[34] D. Gholap, J. Verhulst, W. Ceelen, F. Vanhaecke, Use of pneumatic nebulization and laser ablation–inductively coupled plasma–mass spectrometry to study the distribution and bioavailability of an intraperitoneally administered Pt-containing chemotherapeutic drug, Anal. Bioanal. Chem. 402 (6) (2012) 2121–2129.
[35] T. Van Acker, T. Buckle, S.J.M. Van Malderen, D.M. van Willigen, V. van Unen, F. W.B. van Leeuwen, F. Vanhaecke, High-resolution imaging and single-cell analysis via laser ablation-inductively coupled plasma-mass spectrometry for the determination of membranous receptor expression levels in breast cancer cell lines using receptor-specific hybrid tracers, Anal. Chim. Acta 1074 (2019) 43–53.
[36] A. Schweikert, S. Theiner, D. Wernitznig, A. Schoeberl, M. Schaier, S. Neumayer, B.K. Keppler, G. Koellensperger, Micro-droplet-based calibration for quantitative elemental bioimaging by LA-ICPMS, Anal. Bioanal. Chem. 414 (2022) 485–495.
[37] M. Costas-Rodríguez, T. Van Acker, A.A.M.B. Hastuti, L. Devisscher, S. Van Campenhout, H. Van Vlierberghe, F. Vanhaecke, Laser ablation-inductively coupled plasma-mass spectrometry for quantitative mapping of the copper distribution in liver tissue sections from mice with liver disease induced by common bile duct ligation, J. Anal. At. Spectrom. 32 (9) (2017) 1805–1812.
[38] K.-A. Dorph-Petersen, J.R. Nyengaard, H.J.G. Gundersen, Tissue shrinkage and unbiased stereological estimation of particle number and size, J. Microsc. 204 (3) (2001) 232–246.
[39] K. Jurowski, M. Szewczyk, W. Piekoszewski, M. Herman, B. Szewczyk, G. Nowak, S. Walas, N. Miliszkiewicz, A. Tobiasz, J. Dobrowolska-Iwanek, A standard sample preparation and calibration procedure for imaging zinc and magnesium in rats' brain tissue by laser ablation-inductively coupled plasma-time of flight-mass spectrometry, J. Anal. At. Spectrom. 29 (8) (2014) 1425–1431.
[40] K. Billimoria, D.N. Douglas, G. Huelga-Suarez, J.F. Collingwood, H. Goenaga-Infante, Investigating the effect of species-specific calibration on the quantitative imaging of iron at mg kg−1 and selenium at μg kg−1 in tissue using laser ablation with ICP-QQQ-MS, J. Anal. At. Spectrom. 36 (5) (2021) 1047–1054.
[41] H. Sela, Z. Karpas, H. Cohen, Y. Zakon, Y. Zeiri, Preparation of stable standards of biological tissues for laser ablation analysis, Int. J. Mass Spectrom. 307 (1) (2011) 142–148.
[42] J. O'Reilly, D. Douglas, J. Braybrook, P.W. So, E. Vergucht, J. Garrevoet, B. Vekemans, L. Vincze, H. Goenaga-Infante, A novel calibration strategy for the quantitative imaging of iron in biological tissues by LA-ICP-MS using matrix-matched standards and internal standardisation, J. Anal. At. Spectrom. 29 (8) (2014) 1378–1384.
[43] O. Reifschneider, K.S. Wentker, K. Strobel, R. Schmidt, M. Masthoff, M. Sperling, C. Faber, U. Karst, Elemental bioimaging of thulium in mouse tissues by laser ablation-ICPMS as a complementary method to heteronuclear proton magnetic resonance imaging for cell tracking experiments, Anal. Chem. 87 (8) (2015) 4225–4230.
[44] C.R. VanderSchee, D. Frier, D. Kuter, K.K. Mann, B.P. Jackson, D.S. Bohle, Quantification of local zinc and tungsten deposits in bone with LA-ICP-MS using novel hydroxyapatite–collagen calibration standards, J. Anal. At. Spectrom. 36 (11) (2021) 2431–2438.
[45] M.T. Westerhausen, M. Bernard, G. Choi, C. Jeffries-Stokes, R. Chandrajith, R. Banati, D.P. Bishop, Preparation of matrix-matched standards for the analysis of teeth via laser ablation-inductively coupled plasma-mass spectrometry, Anal. Methods 15 (6) (2023) 797–806.
[46] M.L. Praamsma, P.J. Parsons, Calibration strategies for quantifying the Mn content of tooth and bone samples by LA-ICP-MS, Accred Qual. Assur. 21 (6) (2016) 385–393.
[47] A. Hanć, A. Olszewska, D. Barałkiewicz, Quantitative analysis of elements migration in human teeth with and without filling using LA-ICP-MS, Micochem. J. 110 (2013) 61–69.
[48] D.L.R. Novo, T. Van Acker, J. Belza, F. Vanhaecke, M.F. Mesko, Laser ablation-ICP-mass spectrometry for determination of the concentrations and spatial distributions of bromine and iodine in human hair, J. Anal. At. Spectrom. 37 (4) (2022) 775–782.
[49] R. Luo, X. Su, W. Xu, S. Zhang, X. Zhuo, D. Ma, Determination of arsenic and lead in single hair strands by laser ablation inductively coupled plasma mass spectrometry, Sci. Rep. 7 (1) (2017) 3426.
[50] S. Byrne, D. Amarasiriwardena, B. Bandak, L. Bartkus, J. Kane, J. Jones, J. Yañez, B. Arriaza, L. Cornejo, Were Chinchorros exposed to arsenic? Arsenic determination in Chinchorro mummies' hair by laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS), Microchem. J. 94 (1) (2010) 28–35.
[51] U. Kumtabtim, A. Matusch, S. Ulhoa Dani, A. Siripinyanond, J. Sabine Becker, Biomonitoring for arsenic, toxic and essential metals in single hair strands by laser ablation inductively coupled plasma mass spectrometry, Int. J. Mass Spectrom. 307 (1) (2011) 185–191.
[52] D. Pozebon, V.L. Dressler, A. Matusch, J.S. Becker, Monitoring of platinum in a single hair by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) after cisplatin treatment for cancer, Int. J. Mass Spectrom. 272 (1) (2008) 57–62.
[53] P. Cheajesadagul, W. Wananukul, A. Siripinyanond, J. Shiowatana, Metal doped keratin film standard for LA-ICP-MS determination of lead in hair samples, J. Anal. At. Spectrom. 26 (3) (2011) 493–498.
[54] C. Arnaudguilhem, M. Larroque, O. Sgarbura, D. Michau, F. Quenet, S. Carrère, B. Bouyssière, S. Mounicou, Toward a comprehensive study for multielemental quantitative LA-ICP MS bioimaging in soft tissues, Talanta 222 (2021) 121537.
[55] C. Köppen, O. Reifschneider, I. Castanheira, M. Sperling, U. Karst, G. Ciarimboli, Quantitative imaging of platinum based on laser ablation-inductively coupled plasma-mass spectrometry to investigate toxic side effects of cisplatin, Metallomics 7 (12) (2015) 1595–1603.
[56] B. Crone, L. Schlatt, R.A. Nadar, N.W.M. van Dijk, N. Margiotta, M. Sperling, S. Leeuwenburgh, U. Karst, Quantitative imaging of platinum-based antitumor complexes in bone tissue samples using LA-ICP-MS, J. Trace Elem. Med. Biol. 54 (2019) 98–102.
[57] T. Gao, T. Ren, Y. Zhou, P. Song, S. Wang, The production of polymer reference materials for microanalysis with high homogeneity by a 3D printing method, J. Anal. At. Spectrom. 38 (4) (2023) 893–901.
[58] A. Schweikert, S. Theiner, M. Šala, P. Vician, W. Berger, B.K. Keppler, G. Koellensperger, Quantification in elemental bioimaging - evaluation of different calibration strategies enabled by micro-droplets, Anal. Chim. Acta 1223 (2022) 340200.
[59] R. Niehaus, M. Sperling, U. Karst, Study on aerosol characteristics and fractionation effects of organic standard materials for bioimaging by means of LA-ICP-MS, J. Anal. At. Spectrom. 30 (10) (2015) 2056–2065.
[60] O. Hachmöller, M. Aichler, K. Schwamborn, L. Lutz, M. Werner, M. Sperling, A. Walch, U. Karst, Element bioimaging of liver needle biopsy specimens from patients with Wilson's disease by laser ablation-inductively coupled plasma-mass spectrometry, J. Trace Elem. Med. Biol. 35 (2016) 97–102.
[61] M. Cruz-Alonso, B. Fernandez, A. Navarro, S. Junceda, A. Astudillo, R. Pereiro, Laser ablation ICP-MS for simultaneous quantitative imaging of iron and ferroportin in hippocampus of human brain tissues with Alzheimer's disease, Talanta 197 (2019) 413–421.
[62] J. Liu, L. Zheng, X. Wei, B. Wang, H. Chen, M. Chen, M. Wang, W. Feng, J. Wang, Quantitative imaging of trace elements in brain sections of Alzheimer's disease mice with laser ablation inductively coupled plasma-mass spectrometry, Microchem. J. 172 (2022) 106912.
[63] K. Billimoria, Y.A.D. Fernandez, E. Andresen, I. Sorzabal-Bellido, G. Huelga-Suarez, D. Bartczak, C. Ortiz de Solórzano, U. Resch-Genger, H.G. Infante, The potential of bioprinting for preparation of nanoparticle-based calibration standards for LA-ICP-ToF-MS quantitative imaging, Metallomics 14 (12) (2022).
[64] T. Van Acker, S.J.M. Van Malderen, M. Van Heerden, J.E. McDuffie, F. Cuyckens, F. Vanhaecke, High-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging of cisplatin-induced nephrotoxic side effects, Anal. Chim. Acta 945 (2016) 23–30.
[65] S. Fingerhut, A.-C. Niehoff, M. Sperling, A. Jeibmann, W. Paulus, T. Niederstadt, T. Allkemper, W. Heindel, M. Holling, U. Karst, Spatially resolved quantification of gadolinium deposited in the brain of a patient treated with gadolinium-based contrast agents, J. Trace Elem. Med. Biol. 45 (2018) 125–130.
[66] S. Strekopytov, K. Billimoria, H. Goenaga-Infante, A systematic study of high resolution multielemental quantitative bioimaging of animal tissue using LA-ICP-TOFMS, J. Anal. At. Spectrom. 38 (3) (2023) 704–715.
[67] M. Šala, V.S. Šelih, J.T. van Elteren, Gelatin gels as multi-element calibration standards in LA-ICP-MS bioimaging: fabrication of homogeneous standards and microhomogeneity testing, Analyst 142 (18) (2017) 3356–3359.
[68] A.A. Leventi, K. Billimoria, D. Bartczak, S. Laing, H. Goenaga-Infante, K. Faulds, D. Graham, New model for quantifying the nanoparticle concentration using SERS supported by multimodal mass spectrometry, Anal. Chem. 95 (5) (2023) 2757–2764.
[69] R.D. Deegan, O. Bakajin, T.F. Dupont, G. Huber, S.R. Nagel, T.A. Witten, Capillary flow as the cause of ring stains from dried liquid drops, Nature 389 (6653) (1997) 827–829.
[70] T. Kajiya, D. Kaneko, M. Doi, Dynamical visualization of "coffee stain phenomenon" in droplets of polymer solution via fluorescent microscopy, Langmuir 24 (21) (2008) 12369–12374.
[71] K. Kysenius, B. Paul, J.B. Hilton, J.R. Liddell, D.J. Hare, P.J. Crouch, A versatile quantitative microdroplet elemental imaging method optimised for integration in biochemical workflows for low-volume samples, Anal. Bioanal. Chem. 411 (3) (2019) 603–616.
[72] C. Carlier, B. Laforce, S.J.M. Van Malderen, F. Gremonprez, R. Tucoulou, J. Villanova, O. De Wever, L. Vincze, F. Vanhaecke, W. Ceelen, Nanoscopic tumor tissue distribution of platinum after intraperitoneal administration in a xenograft model of ovarian cancer, J. Pharm. Biomed. Anal. 131 (2016) 256–262.
[73] D. Metarapi, M. Šala, K. Vogel-Mikuš, V.S. Šelih, J.T. van Elteren, Nanoparticle analysis in biomaterials using laser Ablation−Single Particle−Inductively coupled plasma mass spectrometry, Anal. Chem. 91 (9) (2019) 6200–6205.
[74] T. Van Acker, E. Bolea-Fernandez, E. De Vlieghere, J. Gao, O. De Wever, F. Vanhaecke, Laser ablation-tandem ICP-mass spectrometry (LA-ICP-MS/MS) imaging of iron oxide nanoparticles in Ca-rich gelatin microspheres, J. Anal. At. Spectrom. 34 (9) (2019) 1846–1855.
[75] F. Kuczelinis, J.H. Petersen, P. Weis, N.H. Bings, Calibration of LA-ICP-MS via standard addition using dried picoliter droplets, J. Anal. At. Spectrom. 35 (2020) 1922–1931.
[76] J. Zhai, Y. Wang, C. Xu, L. Zheng, M. Wang, W. Feng, L. Gao, L. Zhao, R. Liu, F. Gao, Y. Zhao, Z. Chai, X. Gao, Facile approach to observe and quantify the αIIbβ3 integrin on a single-cell, Anal. Chem. 87 (5) (2015) 2546–2549.

[77] M. Wang, L.-N. Zheng, B. Wang, H.-Q. Chen, Y.-L. Zhao, Z.-F. Chai, H.J. Reid, B. L. Sharp, W.-Y. Feng, Quantitative analysis of gold nanoparticles in single cells by laser ablation inductively coupled plasma-mass spectrometry, Anal. Chem. 86 (20) (2014) 10252–10256.

[78] K. Löhr, H. Traub, A.J. Wanka, U. Panne, N. Jakubowski, Quantification of metals in single cells by LA-ICP-MS: comparison of single spot analysis and imaging, J. Anal. At. Spectrom. 33 (9) (2018) 1579–1587.

[79] K. Löhr, O. Borovinskaya, G. Tourniaire, U. Panne, N. Jakubowski, Arraying of single cells for quantitative high throughput laser ablation ICP-TOF-MS, Anal. Chem. 91 (18) (2019) 11520–11528.

[80] A. Arakawa, N. Jakubowski, G. Koellensperger, S. Theiner, A. Schweikert, S. Flemig, D. Iwahata, H. Traub, T. Hirata, Quantitative imaging of silver nanoparticles and essential elements in thin sections of fibroblast multicellular spheroids by high resolution laser ablation inductively coupled plasma time-of-flight mass spectrometry, Anal. Chem. 91 (15) (2019) 10197–10203.

[81] A. Schoeberl, M. Gutmann, S. Theiner, M. Schaier, A. Schweikert, W. Berger, G. Koellensperger, Cisplatin uptake in macrophage subtypes at the single-cell level by LA-ICP-TOFMS imaging, Anal. Chem. 93 (49) (2021) 16456–16465.

[82] M. Schaier, S. Theiner, D. Baier, G. Braun, W. Berger, G. Koellensperger, Multiparametric tissue characterization utilizing the cellular metallome and immuno-mass spectrometry imaging, JACS Au 3 (2) (2023) 419–428.

[83] https://metallomic.com/(accessed 8/August/2023).

[84] C. O' Connor, B.L. Sharp, P. Evans, On-line additions of aqueous standards for calibration of laser ablation inductively coupled plasma mass spectrometry: theory and comparison of wet and dry plasma conditions, J. Anal. At. Spectrom. 21 (6) (2006) 556–565.

[85] D. Pozebon, V.L. Dressler, M.F. Mesko, A. Matusch, J.S. Becker, Bioimaging of metals in thin mouse brain section by laser ablation inductively coupled plasma mass spectrometry: novel online quantification strategy using aqueous standards, J. Anal. At. Spectrom. 25 (11) (2010) 1739–1744.

[86] G. Zhang, Q. Li, Y. Zhu, Z. Wang, Solution-based calibration strategy for laser ablation–inductively coupled plasma–mass spectrometry using desolvating nebulizer system, Spectrochim. Acta, Part B 145 (2018) 51–57.

[87] V.L. Dressler, D. Pozebon, M.F. Mesko, A. Matusch, U. Kumtabtim, B. Wu, J. Sabine Becker, Biomonitoring of essential and toxic metals in single hair using on-line solution-based calibration in laser ablation inductively coupled plasma mass spectrometry, Talanta 82 (5) (2010) 1770–1777.

[88] H. Sela, Z. Karpas, M. Zoriy, C. Pickhardt, J.S. Becker, Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), Int. J. Mass Spectrom. 261 (2) (2007) 199–207.

[89] P. Rodríguez-González, J.M. Marchante-Gayón, J.I. García Alonso, A. Sanz-Medel, Isotope dilution analysis for elemental speciation: a tutorial review, Spectrochim. Acta, Part B 60 (2) (2005) 151–207.

[90] K.G. Heumann, Isotope-dilution ICP–MS for trace element determination and speciation: from a reference method to a routine method? Anal. Bioanal. Chem. 378 (2) (2004) 318–329.

[91] A. Sussulini, J.S. Becker, Combination of PAGE and LA-ICP-MS as an analytical workflow in metallomics: state of the art, new quantification strategies, advantages and limitations, Metallomics 3 (12) (2011) 1271–1279.

[92] J. Chen, R. Wang, M. Ma, L. Gao, B. Zhao, M. Xu, Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS)–based strategies applied for the analysis of metal-binding protein in biological samples: an update on recent advances, Anal. Bioanal. Chem. 414 (24) (2022) 7023–7033.

[93] I. Konz, B. Fernández, M.L. Fernández, R. Pereiro, A. Sanz-Medel, Absolute quantification of human serum transferrin by species-specific isotope dilution laser ablation ICP-MS, Anal. Chem. 83 (13) (2011) 5353–5360.

[94] C.L. Deitrich, S. Braukmann, A. Raab, C. Munro, B. Pioselli, E.M. Krupp, J. E. Thomas-Oates, J. Feldmann, Absolute quantification of superoxide dismutase (SOD) using species-specific isotope dilution analysis, Anal. Bioanal. Chem. 397 (8) (2010) 3515–3524.

[95] L. Feng, D. Zhang, J. Wang, D. Shen, H. Li, A novel quantification strategy of transferrin and albumin in human serum by species-unspecific isotope dilution laser ablation inductively coupled plasma mass spectrometry (ICP-MS), Anal. Chim. Acta 884 (2015) 19–25.

[96] M. Tibi, K.G. Heumann, Isotope dilution mass spectrometry as a calibration method for the analysis of trace elements in powder samples by LA-ICP-MS, J. Anal. At. Spectrom. 18 (9) (2003) 1076–1081.

[97] J. Terán-Baamonde, A. Carlosena, R.M. Soto-Ferreiro, J.M. Andrade, A. Cantarero-Roldán, S. Muniategui-Lorenzo, A solid-spiking matrix matched calibration strategy for simultaneous determination of cadmium and chromium in sediments by isotope dilution laser ablation inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 35 (3) (2020) 580–591.

[98] B. Fernández, F. Claverie, C. Pécheyran, O.F.X. Donard, Solid-spiking isotope dilution laser ablation ICP-MS for the direct and simultaneous determination of trace elements in soils and sediments, J. Anal. At. Spectrom. 23 (3) (2008) 367–377.

[99] S.F. Boulyga, J. Heilmann, T. Prohaska, K.G. Heumann, Development of an accurate, sensitive, and robust isotope dilution laser ablation ICP-MS method for simultaneous multi-element analysis (chlorine, sulfur, and heavy metals) in coal samples, Anal. Bioanal. Chem. 389 (3) (2007) 697–706.

[100] O.B. Bauer, C. Köppen, M. Sperling, H.-J. Schurek, G. Ciarimboli, U. Karst, Quantitative bioimaging of platinum via online isotope dilution-laser ablation-inductively coupled plasma mass spectrometry, Anal. Chem. 90 (11) (2018) 7033–7039.

[101] O.B. Bauer, O. Hachmöller, O. Borovinskaya, M. Sperling, H.-J. Schurek, G. Ciarimboli, U. Karst, LA-ICP-TOF-MS for rapid, all-elemental and quantitative bioimaging, isotopic analysis and the investigation of plasma processes, J. Anal. At. Spectrom. 34 (4) (2019) 694–701.

[102] S. Marković, K. Uršič, M. Cemazar, G. Sersa, B. Starešinič, R. Milačič, J. Ščančar, High spatial resolution imaging of cisplatin and Texas Red cisplatin in tumour spheroids using laser ablation isotope dilution inductively coupled plasma mass spectrometry and confocal fluorescence microscopy, Anal. Chim. Acta 1162 (2021) 338424.

[103] D.N. Douglas, J. O'Reilly, C. O'Connor, B.L. Sharp, H. Goenaga-Infante, Quantitation of the Fe spatial distribution in biological tissue by online double isotope dilution analysis with LA-ICP-MS: a strategy for estimating measurement uncertainty, J. Anal. At. Spectrom. 31 (1) (2016) 270–279.

[104] I. Moraleja, M.L. Mena, A. Lázaro, B. Neumann, A. Tejedor, N. Jakubowski, M. M. Gómez-Gómez, D. Esteban-Fernández, An approach for quantification of platinum distribution in tissues by LA-ICP-MS imaging using isotope dilution analysis, Talanta 178 (2018) 166–171.

[105] L.-N. Zheng, L.-X. Feng, J.-W. Shi, H.-Q. Chen, B. Wang, M. Wang, H.-F. Wang, W.-Y. Feng, Single-cell isotope dilution analysis with LA–ICP–MS: a new approach for quantification of nanoparticles in single cells, Anal. Chem. 92 (21) (2020) 14339–14345.

[106] L. Feng, J. Wang, H. Li, X. Luo, J. Li, A novel absolute quantitative imaging strategy of iron, copper and zinc in brain tissues by Isotope Dilution Laser Ablation ICP-MS, Anal. Chim. Acta 984 (2017) 66–75.

[107] J. Liu, L. Zheng, Q. Li, L. Feng, B. Wang, M. Chen, M. Wang, J. Wang, W. Feng, Isotope dilution LA-ICP-MS for quantitative imaging of trace elements in mouse brain sections, Anal. Chim. Acta 1273 (2023) 341524.

[108] D. Metarapi, A. Schweikert, A. Jerše, M. Schaier, J.T. van Elteren, G. Koellensperger, S. Theiner, M. Šala, Semiquantitative analysis for high-speed mapping applications of biological samples using LA-ICP-TOFMS, Anal. Chem. 95 (19) (2023) 7804–7812.

[109] C. Austin, F. Fryer, J. Lear, D. Bishop, D. Hare, T. Rawling, L. Kirkup, A. McDonagh, P. Doble, Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS, J. Anal. At. Spectrom. 26 (7) (2011) 1494–1501.

[110] D.A. Frick, D. Günther, Fundamental studies on the ablation behaviour of carbon in LA-ICP-MS with respect to the suitability as internal standard, J. Anal. At. Spectrom. 27 (8) (2012) 1294–1303.

[111] P.d.S. Moreau, M.A.Z. Arruda, Direct analysis of tree rings using laser ablation-ICP-MS and quantitative evaluation of Zn and Cu using filter paper as a solid support for calibration, J. Anal. At. Spectrom. 37 (4) (2022) 795–804.

[112] M. von Bremen-Kühne, H. Ahmadi, M. Sperling, U. Krämer, U. Karst, Elemental bioimaging of Zn and Cd in leaves of hyperaccumulator Arabidopsis halleri using laser ablation-inductively coupled plasma-mass spectrometry and referencing strategies, Chemosphere 305 (2022) 135267.

[113] J.Y. Kim, J. Park, J. Choi, J. Kim, Determination of metal concentration in road-side trees from an industrial area using laser ablation inductively coupled plasma mass spectrometry, Minerals 10 (2) (2020) 175.

[114] K. Chacón-Madrid, M.A. Zezzi Arruda, Internal standard evaluation for bioimaging soybean leaves through laser ablation inductively coupled plasma mass spectrometry: a plant nanotechnology approach, J. Anal. At. Spectrom. 33 (10) (2018) 1720–1728.

[115] J.A. Ko, N. Furuta, H.B. Lim, Quantitative mapping of elements in basil leaves (Ocimum basilicum) based on cesium concentration and growth period using laser ablation ICP-MS, Chemosphere 190 (2018) 368–374.

[116] D. Amarasiriwardena, M. Ahmed, B. Arriaza, Environmental arsenic exposure by ancient Andeans: measurement of as in mummy hair using LA-ICP-MS, J. Archaeol. Sci. Rep. 48 (2023) 103883.

[117] Y.-N. Chan, J.T.-S. Lum, K.S.-Y. Leung, Development of a comprehensive method for hair and nail analysis using laser ablation-inductively coupled plasma-mass spectrometry, Micochem. J. 188 (2023) 108503.

[118] K. Rodiouchkina, I. Rodushkin, S. Goderis, F. Vanhaecke, Longitudinal isotope ratio variations in human hair and nails, Sci. Total Environ. 808 (2022) 152059.

[119] P. Charapata, C.T. Clark, N. Miller, S.S. Kienle, D.P. Costa, M.E. Goebel, H. Gunn, E.S. Sperou, S.B. Kanatous, D.E. Crocker, R. Borras-Chavez, S.J. Trumble, Whiskers provide time-series of toxic and essential trace elements, Se:Hg molar ratios, and stable isotope values of an apex Antarctic predator, the leopard seal, Sci. Total Environ. 854 (2023) 158651.

[120] M. Arora, A. Reichenberg, C. Willfors, C. Austin, C. Gennings, S. Berggren, P. Lichtenstein, H. Anckarsäter, K. Tammimies, S. Bölte, Fetal and postnatal metal dysregulation in autism, Nat. Commun. 8 (1) (2017) 15493.

[121] A.L. Wright, E.T. Earley, C. Austin, M. Arora, Equine odontoclastic tooth resorption and hypercementosis (EOTRH): microspatial distribution of trace elements in hypercementosis-affected and unaffected hard dental tissues, Sci. Rep. 13 (1) (2023) 5048.

[122] A.-F. Maurer, P. Barrulas, A. Person, J. Mirão, C. Barrocas Dias, O. Boudouma, L. Segalen, Testing LA-ICP-MS analysis of archaeological bones with different diagenetic histories for paleodiet prospect, Palaeogeogr. Palaeoclimatol. Palaeoecol. 534 (2019) 109287.

[123] S. Theiner, S.J.M. Van Malderen, T. Van Acker, A. Legin, B.K. Keppler, F. Vanhaecke, G. Koellensperger, Fast high-resolution laser ablation-inductively coupled plasma mass spectrometry imaging of the distribution of platinum-based anticancer compounds in multicellular tumor spheroids, Anal. Chem. 89 (23) (2017) 12641–12645.

[124] F. Li, X. Lei, H. Li, H. Cui, W. Guo, L. Jin, S. Hu, Direct multi-elemental analysis of whole blood samples by LA-ICP-MS employing a cryogenic ablation cell, J. Anal. At. Spectrom. 38 (1) (2023) 90–96.

[125] N. Grijalba, A. Legrand, V. Holler, C. Bouvier-Capely, A novel calibration strategy based on internal standard–spiked gelatine for quantitative bio-imaging by LA-ICP-MS: application to renal localization and quantification of uranium, Anal. Bioanal. Chem. 412 (13) (2020) 3113–3122.
[126] I. Konz, B. Fernández, M.L. Fernández, R. Pereiro, H. González, L. Álvarez, M. Coca-Prados, A. Sanz-Medel, Gold internal standard correction for elemental imaging of soft tissue sections by LA-ICP-MS: element distribution in eye microstructures, Anal. Bioanal. Chem. 405 (10) (2013) 3091–3096.
[127] M. Bonta, H. Lohninger, M. Marchetti-Deschmann, A. Limbeck, Application of gold thin-films for internal standardization in LA-ICP-MS imaging experiments, Analyst 139 (6) (2014) 1521–1531.
[128] S. Hoesl, B. Neumann, S. Techritz, M. Linscheid, F. Theuring, C. Scheler, N. Jakubowski, L. Mueller, Development of a calibration and standardization procedure for LA-ICP-MS using a conventional ink-jet printer for quantification of proteins in electro- and Western-blot assays, J. Anal. At. Spectrom. 29 (7) (2014) 1282–1291.
[129] S. Hoesl, B. Neumann, S. Techritz, G. Sauter, R. Simon, H. Schlüter, M. W. Linscheid, F. Theuring, N. Jakubowski, L. Mueller, Internal standardization of LA-ICP-MS immuno imaging via printing of universal metal spiked inks onto tissue sections, J. Anal. At. Spectrom. 31 (3) (2016) 801–808.
[130] I. Moraleja, D. Esteban-Fernández, A. Lázaro, B. Humanes, B. Neumann, A. Tejedor, M.L. Mena, N. Jakubowski, M.M. Gómez-Gómez, Printing metal-spiked inks for LA-ICP-MS bioimaging internal standardization: comparison of the different nephrotoxic behavior of cisplatin, carboplatin, and oxaliplatin, Anal. Bioanal. Chem. 408 (9) (2016) 2309–2318.
[131] B. Neumann, S. Hösl, K. Schwab, F. Theuring, N. Jakubowski, Multiplex LA-ICP-MS bio-imaging of brain tissue of a parkinsonian mouse model stained with metal-coded affinity-tagged antibodies and coated with indium-spiked commercial inks as internal standards, J. Neurosci. Methods 334 (2020) 108591.
[132] C. Giesen, L. Waentig, T. Mairinger, D. Drescher, J. Kneipp, P.H. Roos, U. Panne, N. Jakubowski, Iodine as an elemental marker for imaging of single cells and tissue sections by laser ablation inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 26 (11) (2011) 2160–2165.
[133] D.A. Frick, C. Giesen, T. Hemmerle, B. Bodenmiller, D. Günther, An internal standardisation strategy for quantitative immunoassay tissue imaging using laser ablation inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 30 (1) (2015) 254–259.
[134] K. Mervič, J.T. van Elteren, M. Bele, M. Šala, Utilizing ablation volume for calibration in LA-ICP-MS mapping to address variations in ablation rates within and between matrices, Talanta 269 (2024) 125379.
[135] K. Mervič, M. Šala, V. Šelih, J.T. van Elteren, Non-matrix-matched calibration in bulk multi-element laser ablation – inductively coupled plasma – mass spectrometry analysis of diverse materials, Talanta 271 (2024) 125712.
[136] M. Cruz-Alonso, A. Lores-Padín, E. Valencia, H. González-Iglesias, B. Fernández, R. Pereiro, Quantitative mapping of specific proteins in biological tissues by laser ablation–ICP-MS using exogenous labels: aspects to be considered, Anal. Bioanal. Chem. 411 (3) (2019) 549–558.
[137] S. Theiner, K. Loehr, G. Koellensperger, L. Mueller, N. Jakubowski, Single-cell analysis by use of ICP-MS, J. Anal. At. Spectrom. 35 (9) (2020) 1784–1813.
[138] L. Mueller, A.J. Herrmann, S. Techritz, U. Panne, N. Jakubowski, Quantitative characterization of single cells by use of immunocytochemistry combined with multiplex LA-ICP-MS, Anal. Bioanal. Chem. 409 (14) (2017) 3667–3676.
[139] J. Pisonero, D. Bouzas-Ramos, H. Traub, B. Cappella, C. Álvarez-Llamas, S. Richter, J.C. Mayo, J.M. Costa-Fernandez, N. Bordel, N. Jakubowski, Critical evaluation of fast and highly resolved elemental distribution in single cells using LA-ICP-SFMS, J. Anal. At. Spectrom. 34 (4) (2019) 655–663.
[140] C. Giesen, T. Mairinger, L. Khoury, L. Waentig, N. Jakubowski, U. Panne, Multiplexed immunohistochemical detection of tumor markers in breast cancer tissue using laser ablation inductively coupled plasma mass spectrometry, Anal. Chem. 83 (21) (2011) 8177–8183.
[141] E. Valencia, B. Fernández, M. Cruz-Alonso, M. García, H. González-Iglesias, M. T. Fernández-Abedul, R. Pereiro, Imaging of proteins in biological tissues by fluorescence microscopy and laser ablation-ICP-MS using natural and isotopically enriched silver nanoclusters, J. Anal. At. Spectrom. 35 (2020) 1868–1879.
[142] B. Paul, D.J. Hare, D.P. Bishop, C. Paton, V.T. Nguyen, N. Cole, M.M. Niedwiecki, E. Andreozzi, A. Vais, J.L. Billings, L. Bray, A.I. Bush, G. McColl, B.R. Roberts, P. A. Adlard, D.I. Finkelstein, J. Hellstrom, J.M. Hergt, J.D. Woodhead, P.A. Doble, Visualising mouse neuroanatomy and function by metal distribution using laser ablation-inductively coupled plasma-mass spectrometry imaging, Chem. Sci. 6 (10) (2015) 5383–5393.
[143] L. Waentig, N. Jakubowski, S. Hardt, C. Scheler, P.H. Roos, M.W. Linscheid, Comparison of different chelates for lanthanide labeling of antibodies and application in a Western blot immunoassay combined with detection by laser ablation (LA-)ICP-MS, J. Anal. At. Spectrom. 27 (8) (2012) 1311–1320.
[144] D.P. Bishop, N. Cole, T. Zhang, P.A. Doble, D.J. Hare, A guide to integrating immunohistochemistry and chemical imaging, Chem. Soc. Rev. 47 (11) (2018) 3770–3787.
[145] Q. Chang, O.I. Ornatsky, I. Siddiqui, A. Loboda, V.I. Baranov, D.W. Hedley, Imaging mass cytometry, Cytometry 91 (2) (2017) 160–169.
[146] S. Kanje, A.J. Herrmann, S. Hober, L. Mueller, Next generation of labeling reagents for quantitative and multiplexing immunoassays by the use of LA-ICP-MS, Analyst 141 (23) (2016) 6374–6380.
[147] M.G. Mello, M.T. Westerhausen, P. Singh, P.A. Doble, J. Wanagat, D.P. Bishop, Assessing the reproducibility of labelled antibody binding in quantitative multiplexed immuno-mass spectrometry imaging, Anal. Bioanal. Chem. 413 (21) (2021) 5509–5516.
[148] D. Clases, R. Gonzalez de Vega, P.A. Adlard, P.A. Doble, On-line reverse isotope dilution analysis for spatial quantification of elemental labels used in immunohistochemical assisted imaging mass spectrometry via LA-ICP-MS, J. Anal. At. Spectrom. 34 (2) (2019) 407–412.
[149] K. Veverkova, K. Pavelicova, M. Vlcnovska, M. Vejvodova, V. Horák, V. Kanicky, V. Adam, T. Vaculovic, M. Vaculovicova, Detection of metallothionein as melanoma marker by LA-ICP-MS combined with sample pretreatment by magnetic particles coated with an imprinted polymeric layer, J. Anal. At. Spectrom. 38 (2023) 1618–1625.
[150] D.P. Bishop, M.T. Westerhausen, F. Barthelemy, T. Lockwood, N. Cole, E. M. Gibbs, R.H. Crosbie, S.F. Nelson, M.C. Miceli, P.A. Doble, J. Wanagat, Quantitative immuno-mass spectrometry imaging of skeletal muscle dystrophin, Sci. Rep. 11 (1) (2021) 1128.
[151] X. Gao, H. Pan, Y. Han, L. Feng, J. Xiong, S. Luo, H. Li, Quantitative imaging of amyloid beta peptide (Aβ) in Alzheimer's brain tissue by laser ablation ICP-MS using gold nanoparticles as labels, Anal. Chim. Acta 1148 (2021) 238197.
[152] M. Cruz-Alonso, B. Fernandez, M. García, H. González-Iglesias, R. Pereiro, Quantitative imaging of specific proteins in the human retina by laser ablation ICPMS using bioconjugated metal nanoclusters as labels, Anal. Chem. 90 (20) (2018) 12145–12151.
[153] A. Lores-Padín, B. Fernández, M. García, H. González-Iglesias, R. Pereiro, Real matrix-matched standards for quantitative bioimaging of cytosolic proteins in individual cells using metal nanoclusters as immunoprobes-label: a case study using laser ablation ICP-MS detection, Anal. Chim. Acta 1221 (2022) 340128.
[154] M.D. Montaño, J.W. Olesik, A.G. Barber, K. Challis, J.F. Ranville, Single Particle ICP-MS: advances toward routine analysis of nanomaterials, Anal. Bioanal. Chem. 408 (19) (2016) 5053–5074.
[155] D. Metarapi, J.T. van Elteren, M. Šala, Studying gold nanoparticle degradation during laser ablation–single particle-inductively coupled plasma mass spectrometry analysis, J. Anal. At. Spectrom. 36 (9) (2021) 1879–1883.
[156] D. Metarapi, J.T. van Elteren, M. Šala, K. Vogel-Mikuš, I. Arčon, V.S. Šelih, M. Kolar, S.B. Hočevar, Laser ablation-single-particle-inductively coupled plasma mass spectrometry as a multimodality bioimaging tool in nano-based omics, Environ. Sci.: Nano 8 (3) (2021) 647–656.
[157] A. Arakawa, N. Jakubowski, S. Flemig, G. Koellensperger, M. Rusz, D. Iwahata, H. Traub, T. Hirata, High-resolution laser ablation inductively coupled plasma mass spectrometry used to study transport of metallic nanoparticles through collagen-rich microstructures in fibroblast multicellular spheroids, Anal. Bioanal. Chem. 411 (16) (2019) 3497–3506.
[158] W. Luo, F. Dong, M. Wang, T. Li, Y. Wang, W. Dai, J. Zhang, C. Jiao, Z. Song, J. Shen, Y. Ma, Y. Ding, F. Yang, Z. Zhang, X. He, Particulate standard establishment for absolute quantification of nanoparticles by LA-ICP-MS, Anal. Chem. 95 (15) (2023) 6391–6398.