Electronic Supplementary Material (ESI) for Analyst.

Supporting information:

Gelatin gels as multi-element calibration standards in LA-ICP-MS bioimaging: fabrication of homogeneous standards and microhomogeneity testing

For the preparation of a homogeneous 10 % (*m*/*v*) gelatin calibration standard containing 20 mg kg^{-1} of the desired element, 1 g of gelatin was weighed, 8.98 mL of ultrapure water added (MilliQ, Millipore) and dissolved at 55 °C to obtain a clear solution. Subsequently, 20 µL of a multi-element standard (As, Be, Cd, Cr, Gd, Hg, Ni and Pb, 1000 g L^{-1} ICP-grade, CertiPUR, Merck, Germany) was added to the solution, thoroughly mixed and degassed in an ultrasonic bath to avoid the evolution of bubbles during the drying step at higher temperatures. Drops of approximately 20 µL were carefully deposited on a hot glass microscope slide, covered with a petri dish and dried for 1 h in a mechanical convection oven at 100 °C, resulting in circular deposits with a diameter of ca. 5 mm and a height of ca. 0.1 mm. This optimized procedure yielded the most homogeneous gelatin calibration standards with regards to elemental lateral and depth distribution; other standards used in this work were prepared by changing the type of gelatin, the element concentration and the drying/setting temperature. Measurements were performed using a nanosecond excimer laser ablation system (193 nm ArF*; Analyte G2, Teledyne Photon Machines Inc., Bozeman, MT) interfaced with a quadrupole ICP-MS instrument (Agilent 7900x, Agilent Technologies, Santa Clara, CA). Ablation took place in a HelEx II two-volume ablation cell, and helium (carrier gas flow rate, cup = 0.5 L min^{-1}; cell = 0.3 L min^{-1}) was used to transport the ablated material from the ablation cell to the ICP-MS. 0.8 L min^{-1} of argon was added as makeup for carrier gas before feeding it to the torch. Gelatin standards were ablated using the following LA-ICP-MS operating conditions: laser beam (square mask), 80 µm; scanning speed, 400 µm s^{-1}; repetition rate, 25 Hz; fluence, 0.40 J cm^{-2}; acquisition time, 0.020 s (per element). For surface mapping of the gelatin drop adjacent line scans were made whereas for depth mapping multiple passes on the same line were performed. For optimization 6 different gelatins were used: three from porcine skin (bloom strengths 60, 180 and 300), two from bovine skin (bloom strengths 75 and 225) and one from cold water fish skin (the molecular weight of the gelatin is 60 kDa, roughly equivalent to high bloom strength (225-325) gelatins). All the gelatins were purchased at Sigma-Aldrich, USA. After optimization the gelatin from porcine skin, type A, with bloom strength 300 was used.

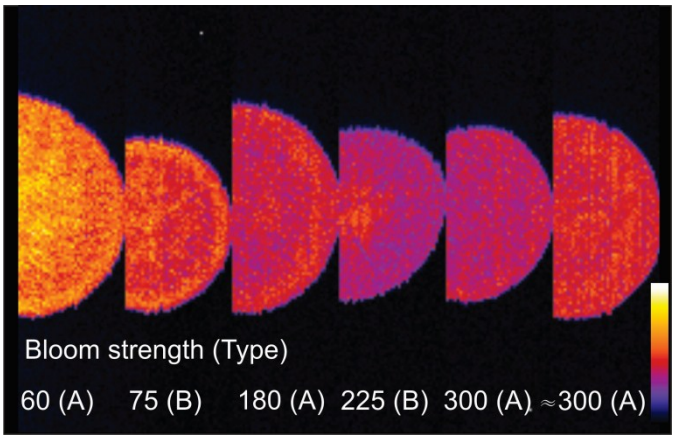

Fig. S1 Distribution of As on the surface of dried gelatin drops (only half drops were ablated) for various bloom strengths and types. All gelatins have been prepared as 10 % *m*/*v* solutions, deposited on a hot glass slide and dried at 60 °C. Although this temperature is not optimal regarding the drying/setting conditions, it clearly shows the effect of bloom strength on elemental distribution. The gelatin at the far right is cold water fish skin and therefore an exact bloom strength cannot be given.

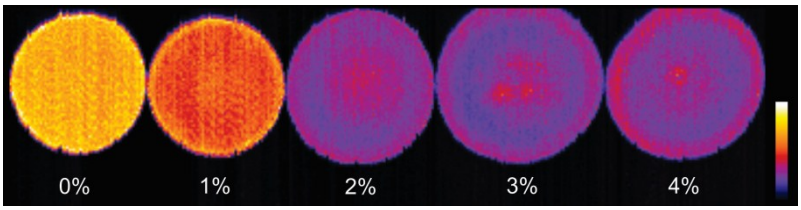

Fig. S2 Distribution of Gd on the surface of the dried gelatin drop (bloom strength 300, type A) as a function of increasing contents of HNO_3 in the initial gelatin solution.

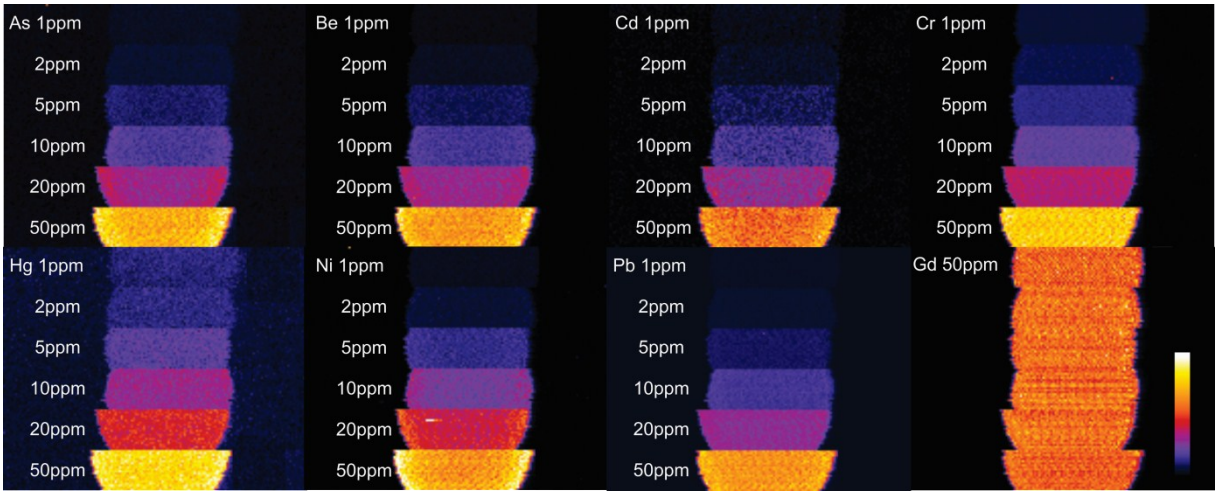

Fig. S3 Distribution images for eight elements in gelatin drops/films calibration standards ranging from 1 to 50 mg kg^{-1}; the acquisition time per element was 20 ms and the beam size was 80 µm (square mask).

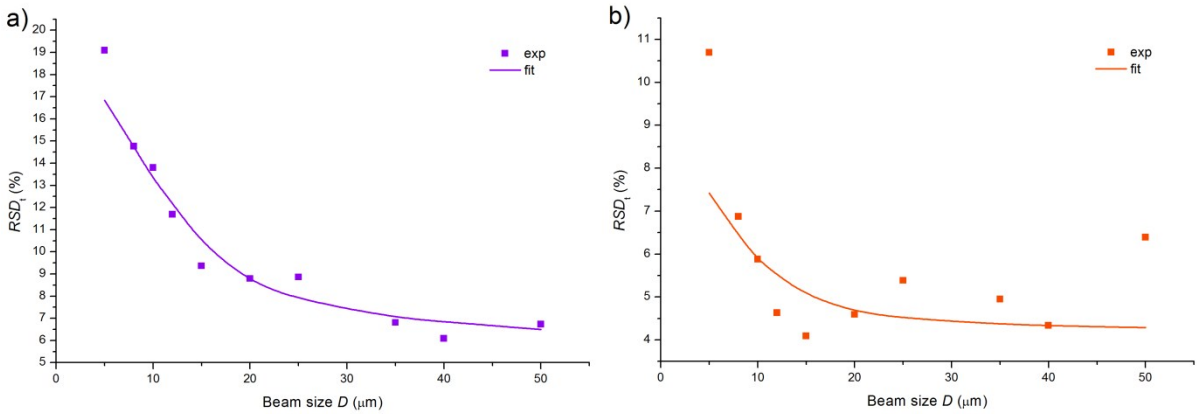

Fig. S4 Microhomogeneity testing of multi-element calibration standards by ablating the standards using different beam sizes and thus different pixel sizes in the elemental images. Presented is the goodness of fit for As (a) and Cr (b) when fitting the experimental standard deviation data SD_t in the elemental images for the respective beam sizes based on the pixel intensity values A using Eqs. [1] and [2].

Table S1 Calibration graph characteristics for element standards in gelatin, prepared according to the above optimized procedure (detailed LA-ICP-MS operating conditions can be found in the ESI).

Nuclide	LOD (µg g^{-1})	R^2	Concentration range (µg g^{-1})
As-75	1.3	0.9998	1-50
Be-9	0.9	0.9968	1-50
Cd-111	1.9	0.9911	1-50
Cr-52	0.2	0.9992	1-50
Hg-201	3.5	0.9868	1-50
Ni-60	0.2	0.9935	1-20
Pb-208	0.04	0.9998	1-50