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ARTICLE TYPE

Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS

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Supporting information.

Table S1 Optimised standard operating conditions for LA-ICP-MS

Agilent 7500cs ICP-MS		New Wave UP213 Laser Ablation	
Rf Power	1250 W	Wavelength	213 nm
Cooling gas flow rate	15 l min^{-1}	Repetition frequency	20 Hz
Carrier gas flow rate	1.20 l min^{-1}	Laser energy density	0.11 J cm^{-2} (at 35 %)
Sample depth	4.0 mm	Spot size	65 µm
Scan mode	Peak hopping	Scan rate	15 µm s^{-1}
Dwell time	0.1 s		

Figure S2 shows the $^{13}C/^{12}C$ ratio after background correction across the tissue thickness range, at the different mass resolutions. A decreasing trend in the $^{13}C/^{12}C$ ratio is observed at the lowest mass resolution (W-10% 0.80 amu).

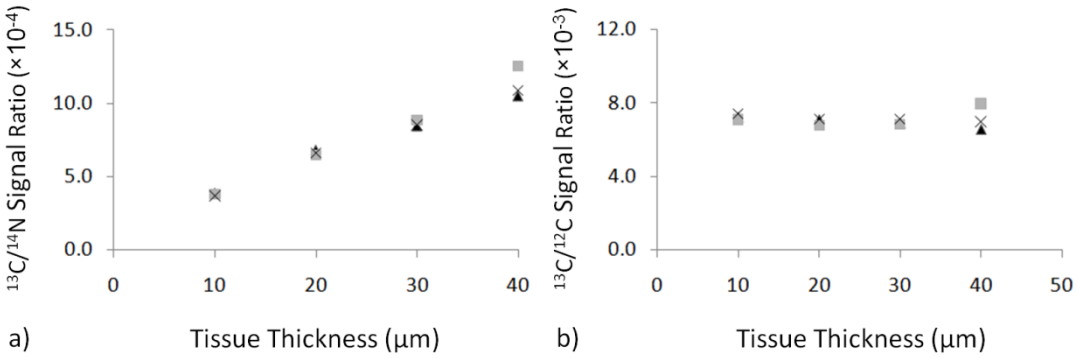

Fig. S2 $^{13}C/^{14}N$ (a) and $^{13}C/^{12}C$ (b) signal ratio as a function of tissue thickness at different mass peak width settings of W-10 % 0.80 amu (▲), 0.65 amu (■) and 0.40 amu (×)

A plot of ^{13}C, ^{59}Co and ^{63}Cu signal intensity as a function of the width of the ablated line scan (Figure S3) shows data typical of the elements investigated in experiments performed in a standard ablation cell and large format cell (LFC).

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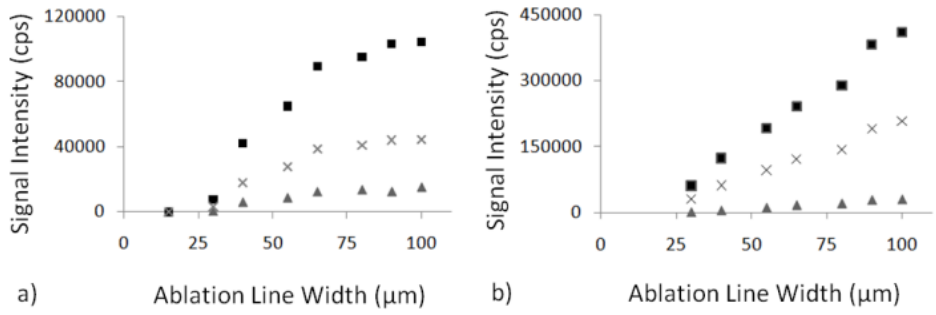

Fig. S3 Plot of ^{13}C (▲), ^{59}Co (■) and ^{63}Cu (×) signal intensity from thin film as a function of ablation line width in (a) a standard ablation and (b) large format cell

Effective normalisation is achieved with ^{13}C if the signal from the sample makes up at least 6 % of the total signal,, as was the case when a 30 µm tissue section was ablated in the LFC.

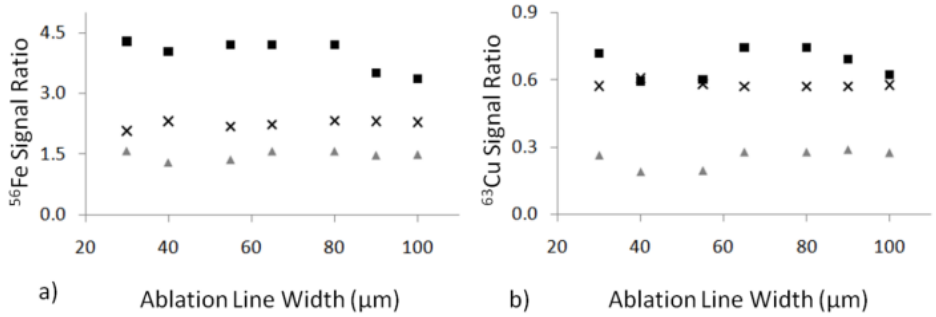

Fig. S4 Normalised ^{56}Fe (a) and ^{63}Cu (b) signal intensity ratios in tissue (30 µm thick) as a function of ablation line width in the LFC. Analytes in tissue are normalised to ^{13}C (▲), ^{59}Co (■) and ^{85}Rb (×)

Elements representing a wide mass range were monitored by analysing the NIST 612 CRM every 3.5 hrs during the course of an elemental bio-imaging experiment imaging mouse brains. The drift in signal intensities was observed to be more pronounced for light elements than heavy elements and was also greater in the first half of the experiment (0 – 7.5 hrs). Normalisation to an IS close in mass gave the best reduction in variation (Table S5). Normalisation to ^{13}C was not as effective as another element close in mass. This may be due to the different sources of signal drift for ^{13}C compared to the other analytes.

Table S5 Change in normalised signal intensity at regular intervals during an elemental bio-imaging experiment.

Time (hrs)	$^{7}Li/^{9}Be$	$^{24}Mg/^{13}C$	$^{7}Li/^{59}Co$	$^{59}Co/^{66}Zn$	$^{66}Zn/^{13}C$	$^{85}Rb/^{89}Y$	$^{7}Li/^{85}Rb$	$^{208}Pb/^{209}Bi$	$^{7}Li/^{208}Pb$	$^{59}Co/^{208}Pb$	$^{208}Pb/^{13}C$
0	8.74	5.40	1.14	7.30	0.547	0.630	0.966	0.710	2.13	1.86	2.15
4	8.70	5.73	0.874	6.31	0.764	0.689	0.637	0.746	1.13	1.29	3.73
7.5	8.36	5.06	0.812	6.38	0.720	0.694	0.599	0.749	0.923	1.14	4.04
11	8.52	5.45	0.831	6.29	0.774	0.660	0.601	0.745	1.05	1.26	3.87
14.5	8.29	4.29	0.722	7.10	0.608	0.669	0.527	0.781	0.896	1.24	3.48
RSD (%)	2.36	10.7	18.2	7.27	14.7	3.84	25.9	3.40	42.0	21.1	21.9

The ability of selected IS elements to compensate for non-uniform sample thickness was also assessed based on average RSDs from line scans ablated at various omega lens settings (Figure S6). The data was not background corrected as the focus of this experiment was to assess how well an IS element compensated for a variation in sample thickness across a line scan. Optimal analyte/IS pairs generally produced RSDs of less than 10 % across the different omega lens settings. Only ^{24}Mg and $^{56, 57}Fe$ had optimal analyte/IS pairs produce

larger RSDs of around 20 %. ^{89}Y was identified as an ideal IS for the heavy analytes (^{85}Rb and ^{197}Au) and $^{52, 53}Cr$ and ^{59}Co for the mid-mass analytes ($^{56, 57}Fe$, ^{63}Cu and ^{66}Zn). Normalisation of ^{24}Mg to ^{52}Cr consistently produced the lowest % RSD at each omega lens setting. This may be due to both ions having C-based interferences ($^{12}C_2^+$ and $^{40}Ar^{12}C^+$). Alternate isotopes for both elements (^{25}Mg and ^{53}Cr) should be considered in samples with a high variation of C content.

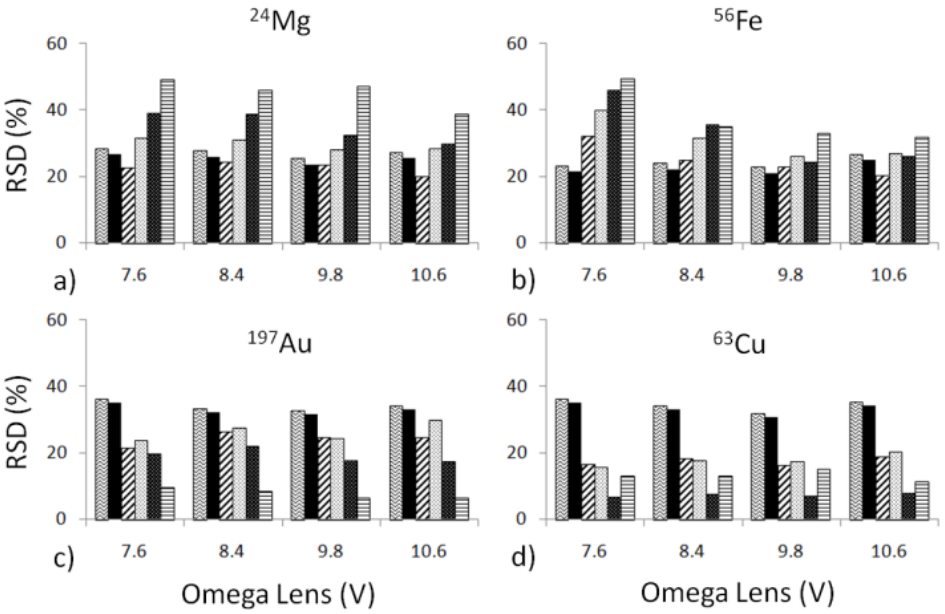

Fig. S6 Line scan RSDs at each omega lens setting produced from ablation of thin film. ^{24}Mg (a), ^{56}Fe (b), ^{197}Au (c), and ^{63}Cu (d) with no IS (▨) and normalised to ^{13}C (■), ^{52}Cr (▨), ^{53}Cr (▨), ^{59}Co (▩) and ^{89}Y (▤) ISs.

Signal stability across different cell sampling locations was assessed in the standard cell and LFC. Sampling positions are labelled in Figure S7.

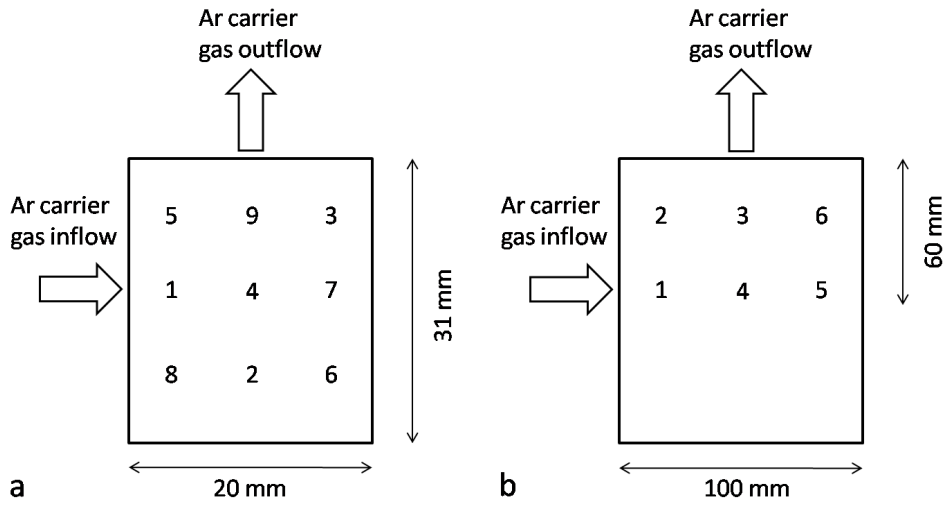

Fig. S7 Ablation cell sampling locations for (a) standard cell and (b) large format cell

The signal instability across different sampling locations is illustrated by a few selected elements in Figure S8 which shows the deviation of sampling position ratios from the ideal. A ratio of each location to the cell centre should ideally be 1 if all sampling locations are independent of cell coordinates. The plots show little difference between the ISs used for normalisation, though a larger difference is observed at position 1 between analyte/IS combinations. The most likely reason is the proximity of the Ar carrier gas inlet causing turbulence and unreliable mass transport.

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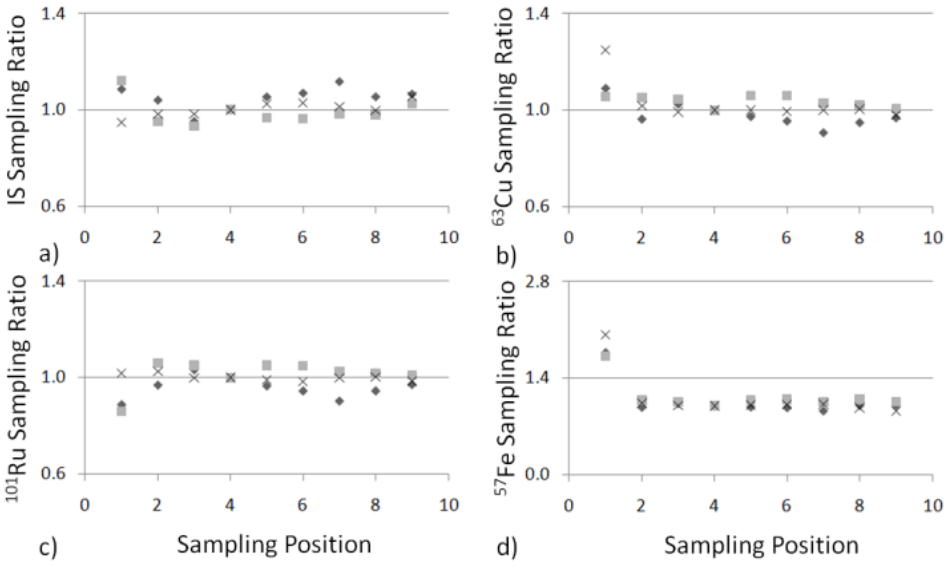

Fig. S8 Signal instability from ablation of a thin film across sampling locations in a standard ablation cell. In a) signal intensities of elements used as ISs at each sampling location are normalised to position 4 to give a sampling ratio (^{13}C (◆), ^{53}Cr (■) and ^{89}Y (×)). For (b-d) analyte/IS ratios at each sampling location are normalised to position 4 to give a sampling ratio: b) ^{63}Cu with normalisation, c) ^{101}Ru with normalization and d) ^{57}Fe with normalisation to ^{13}C (◆), ^{53}Cr (■) and ^{89}Y (×).

Signal stability across different sampling locations was also assessed in the large format cell. Comparable signal stability was observed between the standard cell and LFC but stability of ^{66}Zn and $^{65, 57}$Fe signals was drastically improved in the LFC. Additionally, signal intensities were more reproducible in the LFC than the standard cell; position 1 in the LFC was generally found to give the lowest signal intensity, and positions 5 and 6 the highest signal intensities across the cell for all analytes at all flow rates investigated.

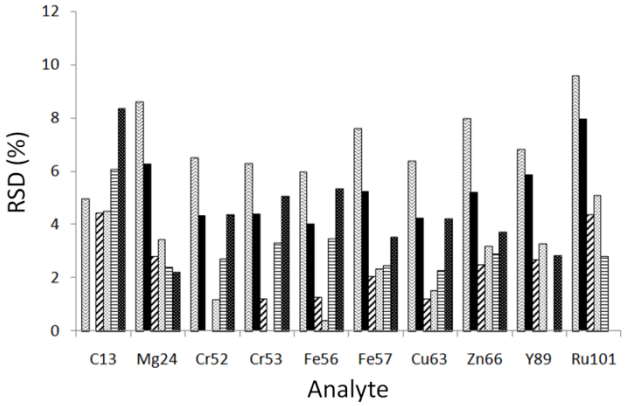

Fig. S9 Comparison of raw (▨) and IS normalised signal stability (% RSD) of analytes in LFC from ablation of a thin film. Analytes are are normalised to ^{13}C (■), ^{52}Cr (▨), ^{53}Cr (▩), ^{89}Y (▤) and ^{101}Ru (▩) ISs.

Notes and references

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