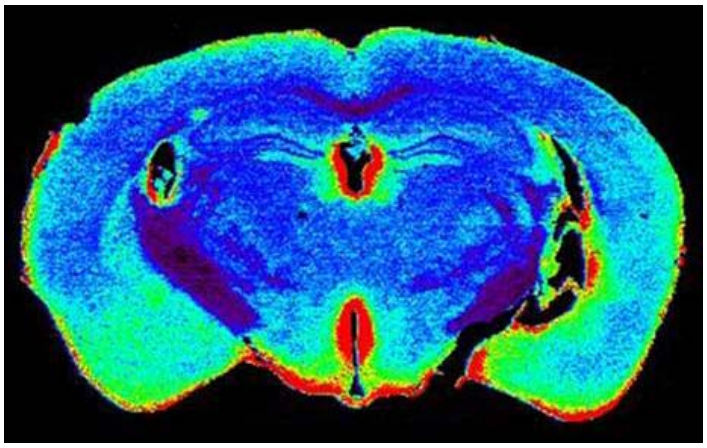
Instrumental considerations and applications of elemental bio-imaging

by Jessica Lear



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Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

Jessica Lear

For Peter and Phyllis

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- Lear, J., Hare, D., Adlard, P., Finkelstein, D., & Doble, P. (2012). Improving acquisition times of elemental bio-imaging for quadrupole-based LA-ICP-MS. *Journal of Analytical Atomic Spectrometry, 27*(1), 159-164.

- Lear, J., Hare, D., Fryer, F., Adlard, P., Finkelstein, D., & Doble, P. (2012). High-resolution elemental bio-imaging of Ca, Mn, Fe, Co, Cu and Zn employing LA-ICP-MS and hydrogen reaction gas. *Analytical Chemistry*, DOI: 10.1021/ac301156f

- Lear, J., Veasey, S. C., Zhu, Y., Doble, P., Hare, D., Wang, S., et al. (2012). Long-term Intermittent Hypoxia Results in Cobalt Accumulation in the Brain: Implications for Vitamin B12 in Obstructive Sleep Apnea. Sleep, Under Review.

- Austin, C., Fryer, F., Lear, J., Bishop, D., Hare, D., Rawling, T., Kirkup, L., McDonagh, A., & Doble, P. (2011). Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS. *Journal of Analytical Atomic Spectrometry, 26*(7), 1494-1501.

- Hare, D., Lear, J., Bishop, D., Beavis, A., & Doble, P. (2012). Protocol for production of matrix-matched brain tissue standards for imaging by laser ablation-inductively coupled plasma-mass spectrometry. *Analytical Methods,* Under Review.

- Article in preparation by Paul Adlard and David Finkelstein of the Mental Health Research Institute (MHRI), Victoria; *PBT2: A Novel Neuroprotective Agent that Enhances Cognition in Ageing*

List of conference presentations

- Lear, J., Hare, D., & Doble, P. (2009). *Elemental bio-imaging of trace elements in neurological disorders: Improving the technique*. Paper presented at the 17th Annual RACI R&D Topics Conference.

- Lear, J., Hare, D., & Doble, P. (2011). *Elemental Bio-imaging: Can acquisition times be improved?* Paper presented at the 3rd International Symposium on Metallomics.

Abbreviations

µg	Micrograms
µm	Micrometres
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AGE	Advanced glycation end-product
Ar	Argon
As	Arsenic
Aβ	Beta-amyloid
BBB	Blood brain barrier
BuChE	Butyrylcholinesterase
C	Carbon
Ca	Calcium
ChEI	Cholinesterase inhibitor
CNS	Central nervous system
Co	Cobalt
CPS	Counts per second
CQ	Clioquinol
CRM	Certified reference material
Cu	Copper
EBI	Elemental bio-imaging
Fe	Iron
g^{-1}	per gram
H	Hydrogen
He	Helium
HNO_3	Nitric acid
ICP	Inductively coupled plasma
K	Potassium
KO	Knockout
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LFC	Large format cell
m/*z*	Mass –to-charge ratio
MALDI	Matrix-assisted laser desorption/ionization
Mg	Magnesium

Mn	Manganese
MPAC	Metal-protein attenuation compound
MPO	Myeloperoxidase
MS	Mass spectrometry
Nd:YAG	Neodymium-doped yttrium aluminium garnet
NFT	Neurofibrillary tangles
Ni	Nickel
NIST	National Institute of Standards and Technology
NMDA	*N*-methyl-D-aspartate
O	Oxygen
P	Phosphorous
Pb	Lead
PBT-2	A second generation CQ derivative
PD	Parkinson's disease
Rh	Rhodium
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SRM	Standard reference material
s^{-1}	per second
Se	Selenium
SN-ICP-MS	Solution nebulisation inductively coupled plasma mass spectrometry
TOF	Time-of-flight
Zn	Zinc
ZnT3	A synaptic zinc transporter
α_2M	α_2-Macroglobulin

Abstract

Elemental Bio-Imaging (EBI) is an established application of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) that determines spatial distributions of *in situ* trace element concentrations in thin sections of biological tissues. This project provides an examination of instrumental considerations relating to method development and refinement, and the utilisation of amended methods for the analysis of element concentration distributions in specific biological samples.

The first instrumental consideration examined improving the methods used for matrix-matched tissue standard preparation. The main disadvantage to the previously proposed tissue standard preparation was a decrease in the homogeneity of sectioned tissue as the concentrations of spiked elements increased. The decrease in homogeneity was due to the use of relatively low concentration (1000 µg mL^{-1}) certified single element standard solutions. Substituting the low concentration solutions for higher concentration solutions, prepared using soluble metal salts, allowed for smaller quantities of solution to be spiked into the tissue. This increased homogeneity and the ability to spike a greater number of analytes into each tissue standard.

The appropriateness of increasing laser scan speed for the reduction of total experimental analysis time was also examined. EBI experiments normally employ scan speeds where the distance traversed in one second is equal to or less than the diameter of the laser beam. Consequently, data for a higher-resolution (pixel size = 15 µm x 15 µm) image of a 5 mm x 5 mm tissue section can take upwards of 30 hours to acquire. Appropriate laser scan speeds may be calculated by consideration of the relationship between laser scan speed, laser spot diameter and the total scan cycle of the quadrupole mass analyser. A simple method to

calculate the laser scan speeds capable of reducing the acquisition time by up to a factor of five whilst maintaining dimensional integrity of the image is presented in this thesis.

Two applications were developed utilising increased laser scan speeds. Both applications were related to the study of the neurodegenerative disorder, Alzheimer's disease, and examined particular variables and their effects on the distribution of metals in the brain. The first application examined the effectiveness of a new drug, PBT-2, on the redistribution of elements in mouse brains with the zinc transporter-3 gene removed. Results indicated PBT-2 had little effect on the distribution of ^{66}Zn, ^{63}Cu and ^{56}Fe in the midbrain. The second application examined the effect of intermittent hypoxia and a diet of advanced glycation end-products on element distribution in the brain. The most intriguing results were seen in the images for ^{59}Co. A 100-fold elevation in the concentrations of ^{59}Co was observed between mice exposed to intermittent hypoxia, those in a control environment and wild-type mice not exposed to the experiment's settings.

An examination into the use of H_2 as a reaction gas was conducted. The improvement of the analytical performance of imaging experiments was considered for a range of masses with spectral interferences, including the $^{40}Ar^{16}O^+$ spectral interference on $^{56}Fe^+$. At low (< 1.0 mL min^{-1}) H_2 flow rates, greater spectral interference due to H^+ adducts were observed for ^{57}Fe than with the reaction mode off. At higher flow rates, up to 3.0 mL H_2 per minute, the spectral interferences were reduced leading to an improvement in the limits of analysis for masses with O- and N-based polyatomic interferences.

EBI is typically performed using spatial resolutions of 30 µm x 30 µm and above. Higher resolution imaging is desirable for many biological applications. As a culmination of the project, the combination of the use of improved matrix-matched tissue standards with the use of the reaction cell and increased image acquisition speeds was employed. The

combination of improved parameters resulted in a high quality image of the area of the brain including the substantia nigra being prepared at a resolution of 6 µm x 6 µm for ^{56}Fe.