# Applications of Elemental Bio-Imaging and Development of Novel Quantification Methods

by

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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.

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Christine Austin

29/07/2011

DATE

A little learning is a dang'rous thing;

Drink deep, or taste not the Pierian Spring:

There shallow draughts intoxicate the brain,

And drinking largely sobers us again.

-Alexander Pope, *An Essay on Criticism* (1711)

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# List of Publications and Conference Presentations

1. Austin C., D. Hare, A.L. Rozelle, W.H. Robinson, R. Grimm and P. Doble, *Metallomics*, Elemental bio-imaging of calcium phosphate based crystal deposits in knee samples from arthritic patients, 2009, **1**, (2), 142-147 (Results published in this paper are included in Chapter 2).

2. Rawling T., C.E Austin, D. Hare, P.A. Doble, H.M. Zareie, and A.M. McDonagh, *Nano Research*, Thin Films of Ruthenium Phthalocyanine Complexes, 2009, **2**, (9), 678-687 (Results published in this paper are included in Chapter 4).

3. Austin C., D. Hare, T. Rawling, A.M. McDonagh and P. Doble, *Journal of Analytical Atomic Spectrometry*, Quantification method for elemental bio-imaging by LA-ICP-MS using metal spiked PMMA films, 2010, **25**, (5), 722-725 (Results published in this paper are included in Chapter 3).

4. Austin C., F. Fryer, J. Lear, D. Bishop, D. Hare, T. Rawling, L. Kirkup, A. McDonagh and P. Doble, *Journal of Analytical Atomic Spectrometry,* Factors affecting internal standard selection for quantitative elemental bio-imaging of soft tissues by LA-ICP-MS, 2011, **26**, (7), 1494-1501 (Results submitted in this paper are included in Chapter 4).

5. Austin C., Hare D., Rozelle A.L., Robinson W.H., Grimm R. and Doble P., *International Symposium on Metallomics 2009*, Cincinnati, USA, Elemental bio-imaging of calcium phosphate based crystal deposits in knee samples from arthritic patients. Oral presentation.

6. Austin C., Rawling T., Hare D., McDonagh A., Doble P., *International Symposium on Metallomics 2009*, Cincinnati, USA, Quantification method for elemental bio-imaging by LA ICP-MS using spiked thin films. Oral presentation.

# Abbreviations

| | |
|---|---|
| AFM | Atomic force microscopy |
| ARS | Alizarin red S |
| BCP | Basic calcium phosphate |
| CaP | Calcium phosphate-based |
| CF | Concentration factor |
| CPPD | Calcium pyrophosphate dihydrate |
| CPLM | Compensated polarised light microscopy |
| CPM | Chemical phase mapping |
| CPMM | Calcium phosphate monobasic monohydrate |
| CPP | Calcium pyrophosphate |
| cps | Counts per second |
| CRC | Collision/reaction cell |
| CRM | Certified reference material |
| csv | Comma separated value |
| DRC | Dynamic reaction cell |
| DMF | N,N-Dimethylformamide |
| DMSO | Dimethyl sulfoxide |
| EHDP | $^{14}$C-ethane-1-hydroxy-1,1-disphonate |
| EDS | Energy dispersive spectrometer |
| EDTA | Ethylenediaminetetraaccetic acid |
| EMPA | Electron microprobe analysis |
| ESEM | Environmental scanning electron microscope |
| ETV | Electrothermal vaporisation |
| FAA | Flame atomic absorption |
| FI | Flow injection |
| FIP | First ionisation potential |
| FSXRM | Full spectrum X-ray mapping |
| FTIRM | Fourier transform infrared microscopy |
| HA | Hydroxyapatite |
| HPLC | High pressure liquid chromatography |
| ICP-MS | Inductively coupled plasma-mass spectrometry |
| i.d. | Internal diameter |

| | |
|---|---|
| IDMS | Isotope dilution mass spectrometry |
| IS | Internal standard |
| ISF | Internal standard film |
| KED | Kinetic energy discrimination |
| LA | Laser ablation |
| LFC | Large format cell |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MALDI | Matrix assisted laser desorption/ionisation |
| MRI | Magnetic resonance imaging |
| MSU | Monosodium urate |
| m/z | Mass-to-charge ratio |
| Nd:YAG | Neodymium doped yttrium aluminium garnet |
| NIST | National Institute of Standards and technology |
| OA | Osteoarthritis |
| PA | Pulse/analogue |
| PAA | Poly(acrylic acid) |
| PAGE | Polyacrylamide gel electrophoresis |
| P/B | Peak-to-background ratio |
| PEI | Poly(ethyleneimine) |
| PEO | poly(ethylene oxide) |
| PhCl | Chlorobenzene |
| PIXE | Particle induced X-ray emission |
| PLM | Polarised light microscopy |
| PMMA | Poly(methylmethacrylate) |
| PsA | Psoriatic arthritis |
| PVA | Poly(vinyl alcohol) |
| qMS | Quadrupole mass spectrometer |
| QUT | Queensland University of Technology |
| QXRM | Quantitative X-ray mapping |
| RA | Rheumatoid arthritis |
| RBS | Rutherford backscattering spectrometry |
| REE | Rare earth element |
| RGB | Red, green, blue |

| | |
|---|---|
| ROI | Region of interest |
| RSD | Relative standard deviation |
| RSF | Relative sensitivity factor |
| RT | Room temperature |
| RuPc | Ruthenium phthalocyanine |
| SAM | Self assembled monolayer |
| S/B | Signal-to-background ratio |
| SEM | Scanning electron microscopy |
| SFMS | Sector field mass spectrometry |
| SIMS | Secondary ion mass spectrometry |
| SN | Solution nebulisation |
| STEM | Scanning transmission electron microscopy |
| SXRF | Synchrotron-based X-ray fluorescence |
| TDS | Total dissolved solids |
| TEM | Transmission electron microscopy |
| TOF | Time of flight |
| W-10 % | Peak width at 10 % of the peak height |
| WDS | Wave dispersive spectrometer |
| XAS | X-ray absorption spectroscopy |
| XRD | X-ray diffraction |
| XRM | X-ray mapping |
| ZAF | Atomic number, absorption and fluorescence |

# Abstract

This thesis investigates an elemental bio-imaging technique that may be used to detect calcium phosphate-based (CaP) crystals in cartilage. CaP crystals are associated with osteoarthritis and define a subset of other arthritides. It is not yet known if these crystals play a direct role in disease conception/progression or are markers of joint damage. Improving our understanding of the processes involved in crystal formation and their relationship to arthritis may lead to the identification of therapy targets or biomarkers, enabling the development of effective treatments or early detection and monitoring of the disease. This is hindered by the small size and complex biological matrix which make crystal detection difficult using traditional technologies. Greater understanding may be achieved through the application of novel technologies, such as those described in this thesis, to crystal detection in biological materials.

Metallomics is an emerging field first defined in the early 2000's. It is the study of free metals and metal containing species; their interactions, transformations and functions in biological systems. The study of metals is of great importance since many metals play essential roles in maintaining physiological functions or cause toxicity in organisms. Spatially resolved elemental maps offer unique insights into the role of metals at the tissue and cellular level. The production of element distribution maps is termed elemental bio-imaging.

Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) is an elemental bio-imaging technique capable of providing elemental maps, increasingly applied to the study of metals and non-metals in biological samples. LA-ICP-MS offers the benefits of direct multi-element analysis of solid samples with minimal sample preparation, high sensitivity and detection of trace, minor and major elements. In this study LA-ICP-MS was applied to the detection and identification of calcium phosphate-based (CaP) crystals in human cartilage and synovial fluid samples.

The LA-ICP-MS elemental bio-imaging method was developed to detect and identify CaP crystals in cartilage and synovial fluid. The analysis of Ca is hindered by interfering species in the mass spectrum (e.g. $^{40}Ar^{+}$, $^{12}C^{16}O_2^{+}$). Two methods of interference reduction were investigated to improve Ca detection: cool plasma and collision/reaction cell (CRC). The CRC method gave the best improvements in signal-to-background ratios, detection limits and

isotope ratio accuracy. The affect of Ca ($^{44}Ca_2^+$) and Sr-based ($^{88}Sr^{2+}$) interferences on Sr and Ca isotope signal intensities was also investigated. Both elements produced a negligible effect on the respective analyte signal intensities.

Development of a new quantification procedure was undertaken to further improve the LA-ICP-MS imaging method by defining a scale for easy crystal detection. Current quantification procedures are time consuming and laborious. Thin polymer films spiked with analytes and prepared by the spin coating technique were validated using tissue standards and finally used to quantify cartilage sections stained for CaP crystals. The films were prepared from a solution containing 10 % PMMA, 40 % xylene and 50 % chlorobenzene. The new quantification procedure also enabled the inclusion of multiple internal standards (IS) by placing the tissue sample on top of the film. Factors affecting the efficacy of ISs were also investigated. A close match in mass was the dominating factor in selecting optimal analyte/IS pairings and ablation cell design was also identified as an important factor in IS selection. For soft tissue analysis, $^{13}C$ was found to be an effective IS but an element closer in mass to the analyte provided better signal compensation.

The developed LA-ICP-MS elemental bio-imaging technique was successfully applied to the detection of CaP crystals in cartilage. This is the first study to show the correlation between CaP crystals and Sr and therefore this technique may provide new insights into the processes involved in crystal generation and their relationship to arthritis.