Quantitative immuno-mass spectrometry imaging by laser ablation-inductively coupled plasma-mass spectrometry

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Abstract

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is an elemental bio-imaging technique that combines high sensitivity and spatial resolution of elements with quantification in two or three dimensions. LA-ICP-MS has recently been applied to detection and quantification of non-elemental analytes (eg. proteins) in tissue sections using immunohistochemical methods. This approach uses molecular probes such as antibodies, tagged with reporter elements such as the lanthanides which are not found naturally in biological samples. Analyses based on these methods is known as immuno-mass spectrometry imaging (iMSI) and has the potential for application to currently refractive research questions in cell and organ biology and new diagnostic platforms.

The drawbacks of commonly used matrix-matched tissue standards were addressed by development of novel facile methods for the preparation of moulded gelatin standards. Surface roughness and robustness were compared against cryo-sectioned gelatin and homogenised brain tissue standards. The moulded standards had significantly higher accuracy, precision and reproducibility and were easier to prepare. Additionally, background metals in gelatin were removed using chelating resins to increase the dynamic calibration range and to improve limits of analysis.

The resolution of LA-ICP-MS elemental bio-imaging is usually constrained by the diameter of the laser spot size and is often not adequate to explore in situ sub-cellular distributions of elements and proteins in biological tissue sections. Super-resolution reconstruction is a method used for many imaging modalities, combining multiple lower resolution images to create a higher resolution image. This thesis describes a super-resolution reconstruction method for LA-ICP-MS imaging by ablating consecutive layers of a biological specimen with offset orthogonal scans. Layer-by-layer image reconstruction was extended to the third dimension without the requirement of image registration across multiple sections. Quantitative super-resolution reconstruction provided superior image clarity and fidelity in two- and three-dimensions.

These methods were applied to the development of iMSI for both antibody and aptamer probes to quantify and localise dystrophin in muscle, and myelin basic protein in brain. Quantification of dystrophin is challenging by conventional methods and is central to development of Duchenne muscular dystrophy treatments. Samples were stained with a gadolinium labelled anti-dystrophin antibody and analysed by LA-ICP-MS. Normal mouse and normal human samples were found to have ~700 and ~300 parts per billion gadolinium respectively with under 20% relative standard deviation. The results improved on current methods and met FDA guidelines. The feasibility of using aptamers for iMSI was confirmed, which may enable analysis of challenging targets.

Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of 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 information sources and literature used are indicated in the thesis.

This research is supported by an Australian Government Research Training Program

Signature of Student:

Production Note:
Signature removed prior to publication.

Date: 31/07/19

Dedicated to difficulty

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List of Publications

Refereed journal publications

[1] Mika T. Westerhausen*, David P. Bishop*, Annette Dowd, Jonathan Wanagat, Nerida Cole & Philip A. Doble, "Super-Resolution Reconstruction for Two-and Three-Dimensional LA-ICP-MS Bioimaging", Analytical Chemistry, October 2019.

[2] Mika T. Westerhausen*, Thomas E. Lockwood *, David P. Bishop, , Raquel Gonzalez de Vega, Anna Röhnelt, Nerida Cole, Philip A. Doble & David Clases, "Low background mould-prepared gelatine standards for reproducible quantification in elemental bio-imaging", The Analyst, October 2019.

[3] Mika Westerhausen*, David P. Bishop*, Nerida Cole, Elizabeth Gibbs, Rachelle Crosbie-Watson, Florian Barthelemy, Stan Nelson, Carrie Miceli, Philip A. Doble, and Jonathan Wanagat, "Quantitative immuno-mass spectrometry imaging of skeletal muscle dystrophin", Article in preparation, Jan 2020.

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Conference oral presentations

Mika T Westerhausen, David Bishop, Nerida Cole, Philip Doble, "Orthogonal Anisotropic Acquisition LA-ICP-MS for 2D and 3D Super Resolution Reconstruction Bio-Imaging", Asia-Pacific Winter Conference on Plasma Spectrochemistry (APWC), 2017, Matsue, Japan

Mika T. Westerhausen, David P. Bishop, Nerida Cole, Jon Wanagat & Philip Doble, "Super-resolution reconstruction for bio-imaging by LA-ICP-MS", Euorpean Winter Conference on Plasma Spectrochemistry (EWCPS), 2019, Pau, France

Mika T. Westerhausen, David P. Bishop, Nerida Cole, Jon Wanagat & Philip Doble, "3D Super-resolution reconstruction for bio-imaging by LA-ICP-MS", Conference on Laser Ablation, 2019, Maui-Hawaii, USA

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Acronyms & Abbreviations

2D Two Dimensional

3D Three Dimensional

AP Alkaline Phosphatase

AR Anisotropic Ratio

CRM Certified Reference Material

DAPI 4′,6-diamidino-2-phenylindole

DMD Duchenne's Muscular Dystrophy

EBI Elemental Bio-Imaging

EDTA Ethylenediaminetetraacetic acid

FDA Food and Drug Administration

FFPE Formalin-Fixed Paraffin-Embedded

FITC Fluorescein isothiocyanate

FM Fluorescence Microscopy

HRP Horseradish Peroxidase

ICP-MS Inductively coupled plasma mass spectrometry

IHC Immunohistochemistry

LA-ICP-MS Laser Ablation Inductively Coupled Plasma Mass Spectrometry

LIBS Laser-induced breakdown spectroscopy

LOD Limit of Detection

LOQ Limit of Quantification

lPIXE Ion Particle induced X-ray emission

MALDI Matrix Assisted Laser Desorption/Ionization

MeCAT Metal-coded affinity tags

MBP Myelin Basic Protein

MC Multicollector

Mdx Mutation Dystrophin X-Chromosome

MRI Magnetic Resonance Imaging

PBS Phosphate Buffered Saline

PMMA Poly(methyl methacrylate)

ppb Parts per billion

ppm Parts per million

PTFE Polytetrafluoroethylene

RLTV Richardson-Lucy Total Variance

RNA Ribonucleic acid

SCN-DOTA 2-(4-isothiocyanatobenzyl)-1,4,7,10-

tetraazacyclododecane-1,4,7,10-tetraacetic acid

SIMS Secondary Ion Mass Spectrometry

SN-ICP-MS Solution Nebulisation Inductively Coupled Plasma Mass Spectrometry

SRR Super Resolution Reconstruction

ssDNA single stand Deoxyribonucleic acid

TBS Tris Buffered Saline

TCEP Tris(2-carboxyethyl)phosphine

TOF Time of flight

WT Wildtype

XFM X-Ray Fluorescence Microscopy

XPS X-ray photoelectron spectroscopy