

# **Investigation of manganese as a radioprotective agent in human cancers**

**by Thomas E. Lockwood**

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**Doctor of Philosophy: Science**  
under the supervision of Dist. Prof. Philip Doble and Dr. David Bishop

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# Certificate of Authorship

I, Thomas Edward Lockwood, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy: Science, in the Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Date:	May 25, 2022



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# List of Publications

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# Abstract

Manganese complexes are effective and catalytic scavengers of  $O_2^{\bullet-}$ , mimicking the behaviour of superoxide dismutase (SOD). These complexes even replace typical SOD in certain extremophile bacteria, providing resistance to huge doses of radiation. Recently, manganese concentrations have been shown to correlate with inferred radiotherapy resistance in human tumours. Radiation plays an important role in the treatment of cancer, but there is still no reliable way to predict an individual tumour's response to therapy. Imaging of manganese in tumours using laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) could provide a novel way of predicting radioresistance and informing treatments, but currently implemented methods have some significant shortcomings. Measurement of manganese using ICP–MS is difficult due to the low concentrations in tissues and high background from polyatomic interferences. Existing LA–ICP–MS image processing software usually falls into one or more of the following categories: hard to use, limited in compatibility or unverifiable due to a closed-source nature. This thesis addresses these shortcomings by developing low-background standards and open-source software for both single cell (sc) and LA–ICP–MS. These new tools are then used to interrogate the metal contents of skin, testis, pancreas and brain tumours, and compare them to the inferred radioresistance of the cancers. Radioresistance is also investigated in a breast cancer cell model using ultraviolet (UV) radiation as a gamma surrogate.

Moulds were used to reproducibly prepare gelatine standards with controllable thickness, and surface characteristics that were improved over traditional methods. To prepare the standards: heated gelatine was pipetted into laboratory-made and commercial moulds, dried, frozen, de-moulded and dehydrated. When compared with traditional gelatine and homogenised tissue standards, the new standards had improved analytical figures of merit. Background signals for transition metals were reduced by removing endogenous elements from the gelatine using a chelating resin.

Open-source LA–ICP–MS imaging software was created using modern design and visualisation philosophies, minimising errors in data interpretation. The new software was compatible with data

collected using ICP–MS from common vendors, and implemented calibration, drift correction, spike filtering, segmentation, overlays and an image calculator. Documentation and a simple graphical user interface eases adoption, contributes to ease of use and maximises productivity.

Transition metals were quantified in tissue microarrays of testis, skin and pancreatic cancer. Concentrations of Mn, Fe, Cu and Zn all correlated with the inferred resistance of the tumours. These correlations also existed in normal testis, skin and pancreatic tissues. In testis tumours with different inferred radiosensitivities, seminoma and nonseminomatous germ cell tumours, only Cu correlated with resistance.

Concentrations of metals were quantified in glioblastoma and meningioma, brain tumours with vastly different patient outcomes. Glioblastoma contained significantly more Cu than benign (grade I) meningioma, but similar concentrations of other transition metals. Previous studies have noted raised serum Cu in patients with glioblastoma and this could be linked to tumour angiogenesis. Concentrations of Mn were increased in grade II over grade I meningioma, but did not reach significance.

To aid in the investigation of single-cellular Mn concentrations software was developed for single particle (sp) and scICP–MS analysis, with multiple methods of thresholding and calibration. The code is open-source, highly vectorised and could process  $10 \times 10^6$  points in 19 ms. The software was demonstrated using the analysis of TiO<sub>2</sub> nano particles in water, LA–spICP–MS of micro-plastics in soil and the measurement of C fixation in algae.

The radioprotective properties of inorganic Mn<sup>2+</sup> were investigated using UV radiation and a breast cancer cell model. Toxicity of MnCl<sub>2</sub> was determined, with a median lethal dose of 1.2 mmol L<sup>-1</sup> in 1 % fetal calf serum (FCS), an order of magnitude lower than in 10 % FCS. Cells were treated with 10 and 100 μmol L<sup>-1</sup> MnCl<sub>2</sub> for 24 h before exposure to 0 to 400 J m<sup>-2</sup> UV-C radiation. Similar viabilities were observed across the range of treatments; Mn had no protective effects. Uptake of Mn from the media was determined using acid digestion and scICP–MS with similar results, although low signal prevented accurate quantification via scICP–MS in untreated cells. Multi-modal distributions of Mn were observed in treated cells, information that was lost when digestion was performed.

The tools created during this thesis will assist in future investigations of manganese and radio-resistance. The primary limitation of imaging Mn via LA–ICP–MS, standards with insufficient limits of detection, has been solved and the new software will accelerate LA and scICP–MS analyses. Further experiments with large sample sizes and radiotherapy patient outcomes are required to definitively conclude if manganese complexes contribute to tumour radioresistance.

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# Abbreviations

<b>ANOVA</b>	analysis of variance	<b>LED</b>	light-emitting diode
<b>BDD</b>	boron-doped diamond	<b>LOD</b>	limit of detection
<b>CD133</b>	prominin-1	<b>MRN</b>	MRE11-RAD50-NBS1
<b>CD30</b>	tumour necrosis factor receptor superfamily member 8	<b>MS</b>	mass spectrometry
<b>CNS</b>	central nervous system	<b>NBT</b>	nitro blue tetrazolium
<b>CRM</b>	certified reference material	<b>NHEJ</b>	non-homologous end joining
<b>CSC</b>	cancer stem cell	<b>NP</b>	nano particle
<b>DAB</b>	3,3'-diaminobenzidine	<b>NSGCT</b>	nonseminomatous germ cell tumour
<b>DMEM</b>	Dulbecco's Modified Eagle Medium	<b>PAM</b>	polyacrylamide
<b>DNA</b>	deoxyribonucleic acid	<b>PBS</b>	phosphate buffered saline
<b>DSB</b>	double-strand break	<b>PFS</b>	progression free survival
<b>FCS</b>	fetal calf serum	<b>PMMA</b>	poly(methyl methacrylate)
<b>FFPE</b>	formalin-fixed paraffin-embedded	<b>PNC</b>	particle number concentration
<b>FITC</b>	fluorescein isothiocyanate	<b>PTFE</b>	poly(tetrafluoroethylene)
<b>GUI</b>	graphical user interface	<b>RGB</b>	red-green-blue
<b>H&amp;E</b>	haematoxylin and eosin	<b>ROI</b>	region of interest
<b>HIF1</b>	hypoxia inducible-factor 1	<b>ROS</b>	reactive oxygen species
<b>HPF</b>	high-power field	<b>RSD</b>	relative standard deviation
<b>HRP</b>	horseradish peroxidase	<b>sc</b>	single cell
<b>ICP</b>	inductively coupled plasma	<b>SEC</b>	size-exclusion chromatography
<b>IHC</b>	immunohistochemistry	<b>SIMS</b>	secondary ion mass spectrometry
<b>LA</b>	laser ablation	<b>SOD</b>	superoxide dismutase
<b>LD<sub>50</sub></b>	median lethal dose	<b>sp</b>	single particle

## *Abbreviations*

**TBS** tris-buffered saline

**TMA** tissue microarray

**TOF** time-of-flight

**UV** ultraviolet

**XOD** xanthine oxidase