Molecular characterization of metastatic ovarian cancer
by MALDI imaging mass spectrometry

A thesis submitted for the degree of

Doctor of Philosophy

as a combination of research narrative and portfolio of scientific publications by

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Discipline of Biochemistry

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December 2011
# Table of contents

Abstract ............................................................................................................................................................ 9  

Declaration ..................................................................................................................................................... 10  

Acknowledgement of help .............................................................................................................................. 11  

Acknowledgements ........................................................................................................................................ 12  

Publications .................................................................................................................................................... 13  

Abbreviations ................................................................................................................................................. 14  

Chapter 1 Manuscript Context ........................................................................................................................ 17  

Chapter 1 MALDI Imaging Mass Spectrometry (MALDI-IMS) — Application of Spatial Proteomics for Ovarian Cancer Classification and Diagnosis ........................................................................................................... 19  

Statement of authorship for chapter 1 ........................................................................................................... 20  

1.1 Abstract ................................................................................................................................................... 23  

1.2 Epidemiology of Ovarian Cancer ............................................................................................................. 23  

1.3 Early Detection of Ovarian Cancer ........................................................................................................... 24  

1.4 Molecular Classification of Ovarian Carcinomas ..................................................................................... 25  

1.5 Application of Proteomics to Ovarian Cancer .......................................................................................... 27  

1.6 Tissue Analysis by Mass Spectrometry .................................................................................................... 28  

1.7 Methods for in Situ MALDI-TOF Analysis of Ovarian Cancer Tissue ..................................................... 32  

1.8 Profiling Cancer Tissues Using MALDI-TOF MS .................................................................................... 35  

1.9 Profiling vs. Imaging ................................................................................................................................ 37  

1.10 Software for Data Analysis ....................................................................................................................... 38
1.11  Automated Sample Preparation for Imaging Cancer Tissues ............................................................. 39
1.12  Peptide Imaging Provides Data Complementary to Protein Imaging .................................................... 41
1.13  Using Histology to Guide Imaging Mass Spectrometry ....................................................................... 42
1.14  Ovarian Cancer Biomarker Discovery Using Imaging Mass Spectrometry ........................................... 43
1.15  Application of Tryptic Digestion to Formalin-Fixed Paraffin Embedded Ovarian Tissues ................... 44
1.16  Conclusions and Future Prospects ....................................................................................................... 46

Thesis synopsis and aims ............................................................................................................................... 47

Chapter 2  Materials and methods ........................................................................................................... 49

2.1  Materials and reagents ....................................................................................................................... 49
  2.1.1  Solvents, chemicals and matrixes .................................................................................................... 49
  2.1.2  Consumable materials ................................................................................................................... 50
  2.1.3  Proteolytic enzymes ...................................................................................................................... 51
  2.1.4  Peptide and protein calibration standards .................................................................................... 51
  2.1.5  Instruments/equipment ................................................................................................................. 52

2.2  Chapter three methods ......................................................................................................................... 53
  2.2.1  Experimental Tissues and Solvents .............................................................................................. 53
  2.2.2  Sample Preparation ....................................................................................................................... 53
  2.2.3  Matrix Deposition – CHIP-1000 .................................................................................................. 54
  2.2.4  Matrix Deposition – ImagePrep .................................................................................................... 54
  2.2.5  MALDI-TOF Imaging Mass Spectrometry and Data Analysis .................................................... 55

2.3  Chapter four methods ............................................................................................................................ 55
  2.3.1  Chemicals and reagents ................................................................................................................. 55
  2.3.2  Tissue source ................................................................................................................................. 55
  2.3.3  Tissue sectioning ........................................................................................................................... 56
  2.3.4  Tryptic digestion and matrix deposition methods (ChIP-1000) .................................................... 56
  2.3.5  Matrix deposition (ImagePrep station) .......................................................................................... 56
2.3.6 MALDI-TOF/TOF imaging mass spectrometry ..............................................................57
2.3.7 Data processing ........................................................................................................58
2.3.8 Haematoxylin and eosin staining ............................................................................58

2.4 Chapter five methods ..................................................................................................58
2.4.1 Chemicals and Reagents .......................................................................................58
2.4.2 Tissue Samples ......................................................................................................59
2.4.3 Section Preparation and Antigen Retrieval .........................................................59
2.4.4 Trypsin Deposition. ChIP-1000 Printing ...............................................................60
2.4.5 ImagePrep Nebulization .......................................................................................60
2.4.6 Matrix Deposition. ChIP-1000 Printing ...............................................................61
2.4.7 ImagePrep Nebulization .......................................................................................61
2.4.8 Imaging MALDI-TOF-MS ...................................................................................61
2.4.9 Imaging Tandem MS ............................................................................................62
2.4.10 Haematoxylin and Eosin Staining ......................................................................62
2.4.11 In Situ Digestion and Extraction .........................................................................63
2.4.12 HPLC and Fraction Collection ..........................................................................63
2.4.13 MALDI-TOF MS for HPLC Fractions .................................................................64
2.4.14 Tandem MS for HPLC Fractions ........................................................................64
2.4.15 HPLC-ESI-Ion Trap MS and MS/MS .................................................................65
2.4.16 HPLC-ESI-LTQ-Orbitrap MS and MS/MS ........................................................65

2.5 Chapter six and seven methods .................................................................................66
2.5.1 Chemicals and Reagents .....................................................................................66
2.5.2 Tissue Samples .....................................................................................................66
2.5.3 Section Preparation and Antigen Retrieval .........................................................67
2.5.4 In situ tryptic digestion .........................................................................................67
2.5.5 Deposition of internal calibrants .........................................................................67
2.5.6 Matrix deposition ..................................................................................................68
2.5.7 MALDI-TOF-MS imaging data acquisition ........................................................68
4.5.1 Tryptic peptide MALDI imaging mass spectrometry ................................................................. 106
4.5.2 Tryptic peptide imaging using a ChIP-1000 ........................................................................... 107

4.6 Application of tryptic peptide imaging to ovarian cancer ....................................................... 113

4.7 Concluding remarks ................................................................................................................ 115

4.8 Supplementary Information .................................................................................................... 116

Chapter 5 Manuscript Context ........................................................................................................ 119

Chapter 5 Citric Acid Antigen Retrieval (CAAR) for Tryptic Peptide Imaging Directly on Archived Formalin-Fixed Paraffin-Embedded Tissue ........................................................................ 121

Statement of authorship for chapter 5 .................................................................................... 122

5.1 Abstract ................................................................................................................................. 124

5.2 Introduction .......................................................................................................................... 124

5.3 Results and Discussion .......................................................................................................... 127

5.3.1 Citric Acid Antigen Retrieval Provides Access to the Proteome of Archived Ovarian Cancer Tissue. 127

5.3.2 Citric Acid Antigen Retrieval Utilized Alongside Automated Nebulization. .......................... 132

5.3.3 Tandem MS Acquisition from Tissue Sections. .................................................................... 141

5.4 Concluding Remarks. ............................................................................................................ 147

5.5 Acknowledgment ................................................................................................................... 147

5.6 Supporting information ........................................................................................................... 148

Chapter 6 Evaluation of internal calibrants for tryptic peptide MALDI-IMS data ...................... 173

6.1 Introduction ........................................................................................................................... 173

6.2 Results and Discussion ......................................................................................................... 175

6.2.1 Automated generation of ion maps, mass deviation maps and grouped peak lists .............. 175

6.2.2 Characterizing m/z deviation across tissues using internal calibrants .............................. 180
6.2.3 Use of internal calibrant peptides to reduce in situ m/z deviation ............................................. 182

6.3 Concluding Remarks ......................................................................................................................... 192

Chapter 7 – Characterization of peritoneal ovarian cancer metastasis by tryptic peptide imaging mass spectrometry .......................................................................................................................... 193

7.1 Introduction........................................................................................................................................... 193

7.2 Results and Discussion .......................................................................................................................... 196

7.2.1 Tryptic peptide MALDI-IMS analysis of ovarian cancer metastases ............................................ 196

7.2.2 Linking peak groups to histology using IonMapper generated signal to noise maps ............... 197

7.2.3 Peptide identification and the application of k-means clustering ............................................... 203

7.2.4 Matching clusters to histology .................................................................................................... 208

7.2.5 Cancer peak group features – Hamming distance clustering results ........................................ 210

7.2.6 Cancer features – Cosine distance clustering results ............................................................... 216

7.2.7 Combining peak group features from Hamming and cosine distance results .......................... 220

7.2.8 Peritoneal stroma features ......................................................................................................... 220

7.2.9 Identification of cancer and stroma-specific peak groups by LC-coupled mass spectrometry ... 226

7.2.10 Improved workflow for characterization of ovarian peritoneal metastasis ........................... 235

7.3 Supplementary Information ................................................................................................................ 241

Chapter 8 Concluding remarks ............................................................................................................... 253

8.1 Aim one ............................................................................................................................................... 253

8.2 Aim two ............................................................................................................................................... 254

8.3 Aim three ............................................................................................................................................ 254

8.4 Aim four ............................................................................................................................................. 255

8.5 Conclusions and future prospects ..................................................................................................... 256

References ............................................................................................................................................... 259
Abstract

Imaging mass spectrometry (IMS) is a novel technology which measures the spatial distribution of drugs, lipids, peptides and proteins across tissue sections by application of mass spectrometry (MS) directly to the section surface. Several hundred analytes can be measured across a tissue in a single IMS experiment, without the need for antibodies and without prior knowledge of tissue composition or structure. In the context of human cancers, the molecular information collected by IMS approaches has been used to grade cancers and predict patient survival. IMS is thus a potentially technology capable of providing valuable complementary information to classical histology and immuno-histochemistry.

Ovarian cancers have the highest mortality of any gynaecological cancer. The high mortality results from late diagnosis due to the asymptomatic nature of ovarian malignancies. Advanced stage ovarian tumours will shed cancer cells into the abdominal cavity, where they subsequently implant into the peritoneum and form metastatic tumour nodules. Despite invasive surgery and adjuvant chemotherapy, there is a large increase in patient morbidity following peritoneal metastasis. Compounding this issue further is the absence of reliable grading systems for ovarian cancer and a subsequent lack of individualized treatments for specific cancer sub-types. As a result of the potential ability to grade tumours and provide patient prognoses based on IMS data, the molecular composition of ovarian metastatic tumours was investigated by IMS.

The novelty of IMS required set up of a robust and reproducible workflow. Methods were thus optimized for IMS analysis of both frozen and formalin-fixed paraffin-embedded (FFPE) ovarian tumour tissue. Subsequently it was shown that optimization of available antigen retrieval and tryptic digest methods for accessing FFPE tissues could achieve higher tryptic peptide signal to noise at a better spatial resolution than methods available in the literature. As such, a complete tryptic peptide IMS workflow was developed alongside liquid chromatography (LC) and MS/MS based peptide identification. In conjunction with this workflow, methods for improving the matching of IMS peptides to LC-MS/MS identified peptides using internal calibrants and development of an in-house software tool were described.

As a result of the work presented in this thesis, a complete tryptic peptide IMS workflow which could be applied to virtually any cancer tissue was developed. The application of this workflow, and exploratory k-means clustering, to ovarian peritoneal metastases showed that key tryptic peptides could be found which distinguish cancer tissue from the surrounding peritoneal stroma. This represented the first step in characterizing these metastatic tumours at the molecular level. The results in this thesis are a precursor to future work which will validate these peptide markers and develop a classification system for metastatic ovarian cancers based on patient survival and response to chemotherapy.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Ove Johan Ragnar Gustafsson and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Ove Johan Ragnar Gustafsson

..........................................................
Acknowledgement of help

I would like to acknowledge the following people for their help during my thesis research:

All co-authors for the scientific research articles included in this thesis, in particular for their roles in evaluating manuscripts, which can be an arduous task. I would like to extend a special thanks to my supervisors Dr. Peter Hoffmann and Professor Shaun McColl, who were co-authors on and evaluated all manuscripts and thesis chapters.

A/Prof. Martin Oehler for his constant support of MALDI-IMS as a collaborator, contributor to project design and implementation as well as providing human ovarian tissue samples for the research presented in this thesis.

A/Prof. Inge Koch and Prof. Steve Marron for their optimization and implementation of k-means clustering, the results of which are presented in chapter seven.

James Eddes for his assistance in writing the software required to process and present the data in chapters six and seven.

Carmela Ricciardelli, Miranda Ween and Noor Lokman for their assistance with technical questions and tissue processing after surgeries.

Dr. Fergus Whitehead for his assistance annotating the metastatic tumour sections in chapter seven.

Prof. Mark Baker for arranging the loan of the ChIP-1000 instrument to the Adelaide Proteomics Centre.
Acknowledgements

Most acknowledgements finish with a profound statement, something along the lines of…..wise men can’t jump…….ok so that's not profound at all but you know what I mean. I’ve never followed trends so I’m going to start mine with a joke. I don’t know the reference but obviously the joke is not mine.

A cation runs into a bar screaming “I’ve lost my electron, I’ve lost my electron!”. The bartender calms the ion down and asks “Are you sure?” The ion replies, “I’m positive.”

Now that the giggles have subsided…….When I started my Honors year in 2007, one of my supervisors asked me why I chose science. The answer now, as I write the final parts of my doctoral thesis, is thankfully the same as it was then, which I take to mean that I probably made the right choice. The answer I gave was that I will never be happy as part of the status quo. I want to be a force for change in the world, no matter how small my contribution. I have many people to thank for their support, friendship and help over the past three years. It would take many pages to properly thank everyone who has contributed to my life so I will have to settle for those which have had the greatest impact.

The Adelaide Proteomics Centre has been my home away from home for four years. Most important to my PhD experience at the centre have been my supervisors, Peter Hoffmann and Shaun McColl, who have always been supportive, patient and willing to discuss new methods and experiments.

I am forever grateful to the crew of the proteomics centre, including Mark Condina, Megan “Retallicka” Penno, James Eddes, Sandra Hack, Florian “Florider” Weiland, Karina “Kaz” Martin, Tomas “Charlie Brown” Koudelka, Yin Ying Ho and Chris “Vinnie” Cursaro. Without you all this thesis would not be possible and the past three years would have been a lot less fun. I also want to thank my close circle of friends. You know who you are (at least you should) and you also know how much friends mean in my life.

The support of parents is often unseen and even more often taken for granted. Thank you to my parents. I love you both and am forever in your debt for providing everything I ever needed to reach my goals. Finally I want to thank my beautiful fiancé Tanja. She has been the most important person in my life over the past seven years and her unwavering support during my PhD has been a blessing. Convincing such an amazing woman that I’m worth her time remains my greatest achievement.

Ove Johan Ragnar Gustafsson

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Publications

Directly related to thesis:


Arising from thesis:

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>ANI</td>
<td>Aniline</td>
</tr>
<tr>
<td>3-AP</td>
<td>3-acetyl-pyridine</td>
</tr>
<tr>
<td>AR</td>
<td>Antigen retrieval</td>
</tr>
<tr>
<td>AWM</td>
<td>Abundance weighted mean</td>
</tr>
<tr>
<td>CAAR</td>
<td>Citric acid antigen retrieval</td>
</tr>
<tr>
<td>CCTV</td>
<td>Close circuit television</td>
</tr>
<tr>
<td>CHCA</td>
<td>α-cyano-4-hydroxycinnamic acid</td>
</tr>
<tr>
<td>ChIP-1000</td>
<td>Chemical inkjet printer 1000</td>
</tr>
<tr>
<td>CID</td>
<td>Collision induced dissociation</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo dalton</td>
</tr>
<tr>
<td>2-DE</td>
<td>Two dimensional electrophoresis</td>
</tr>
<tr>
<td>2,5-DHB</td>
<td>2,5-dihydroxybenzoic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immuno-sorbent assay</td>
</tr>
<tr>
<td>EOC</td>
<td>Epithelial ovarian carcinoma</td>
</tr>
<tr>
<td>ESI</td>
<td>Electro-spray ionization</td>
</tr>
<tr>
<td>EIOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FA</td>
<td>Formic acid</td>
</tr>
<tr>
<td>FFPE</td>
<td>Formalin-fixed paraffin-embedded</td>
</tr>
<tr>
<td>FIGO</td>
<td>International federation of gynecology and obstetrics</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width half maximum</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and eosin</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyeraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>HFIP</td>
<td>1,1,1,3,3,3-Hexafluoro-2-propanol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>hnRNP</td>
<td>Heterogeneous nuclear ribonucleoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IHC</td>
<td>Immuno-histochemistry</td>
</tr>
<tr>
<td>IMS</td>
<td>Imaging mass spectrometry</td>
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<tr>
<td>IPA</td>
<td>Isopropanol</td>
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<tr>
<td>IT</td>
<td>Ion trap</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium tin oxide</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
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<tr>
<td>LCM</td>
<td>Laser capture micro-dissection</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography – mass spectrometry</td>
</tr>
<tr>
<td>LTQ</td>
<td>Linear trap with quadrupole</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix assisted laser desorption/ionization</td>
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<tr>
<td>MALDI-IMS</td>
<td>Matrix assisted laser desorption/ionization imaging mass spectrometry</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>mL</td>
<td>Milli litre</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>nL</td>
<td>Nano litre</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Non small cell lung cancer</td>
</tr>
<tr>
<td>OCT</td>
<td>Optimal cutting temperature polymer</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PIC</td>
<td>Percentage intensity contribution</td>
</tr>
<tr>
<td>pmol</td>
<td>Pico mole</td>
</tr>
<tr>
<td>png</td>
<td>Portable network graphic</td>
</tr>
<tr>
<td>POS</td>
<td>Percentage of spectra (within a cluster)</td>
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<tr>
<td>ppm</td>
<td>Parts per million</td>
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<tr>
<td>PTM</td>
<td>Post translational modification</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>SA</td>
<td>Sinapinic acid</td>
</tr>
<tr>
<td>SIMS</td>
<td>Secondary ion mass spectrometry</td>
</tr>
<tr>
<td>SLSC</td>
<td>Standard light scatter curve</td>
</tr>
<tr>
<td>SNAP</td>
<td>Sophisticated numerical annotation procedure</td>
</tr>
<tr>
<td>STS</td>
<td>Soft tissue sarcoma</td>
</tr>
<tr>
<td>TOF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFE</td>
<td>2,2,2-trifluoroethanol</td>
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</table>