Institution: Sheffield Hallam University

Unit of Assessment: 5 Biological Sciences

Title of case study: Mass Spectrometry Imaging of Biological Tissue

1. Summary of the impact

Clench's research on **M**atrix **A**ssisted **L**aser **D**esorption **I**onisation - **M**ass **S**pectrometry **I**maging (**MALDI-MSI**) technologies has impacted directly on pharmaceutical industry practice regarding studies of drug distribution studies in biological tissues, providing increased information, more rapidly. Companies have benefitted from long-term relationships with **Clench**'s Bioanalysis Research Group and seek its expertise for consultancy purposes. Former members of **Clench's** group hold key positions in industry, implementing and further developing these technologies. **Francese** has had significant success in applying MALDI-MSI to analysis of latent fingermarks for forensic applications benefiting Home Office scientists and crime scene investigation units. Research advances in MALDI-MSI by **Clench** and **Francese** are patented and exploited via licensing.

2. Underpinning research

Clench has a track record of research at Sheffield Hallam University (SHU) (1993-current) in novel applications of mass spectrometry, working with global pharmaceutical companies, to advance techniques applied to distribution of drugs and their metabolic derivatives in biological tissues. Further collaborations in MALDI-MSI, led by **Clench** with colleagues at Bradford, York and Sheffield universities, resulted in highly cited publications.

Clench's initial research, as a senior lecturer at SHU, was funded by Pfizer Global R&D 1999 - 2003 (grant 1) to investigate methods of adapting MALDI-MSI to study the distribution of pharmaceuticals in biological tissues. In 2004, **Clench's** group published the first European paper on MALDI-MSI of pharmaceuticals in Europe and only the second in the world. Co-authored with a Pfizer scientist, this publication (reference 1) detailed a method for studying the distribution of an anti-fungal compound in skin. Further work with Syngenta (Bracknell, UK) identified agrochemicals in plants and led to the application of MALDI-MSI in the horticultural sector (reference 2). Distribution of the bioreductive drug AQ4N and its active metabolite in solid tumours was reported in 2007 in association with researchers at the Institute of Cancer Therapeutics, University of Bradford (reference 3).

Clench received funding from the Engineering and Physical Sciences Research Council (EPSRC) with the Royal Society of Chemistry to support two Analytical Science CASE studentships in collaboration with Applied Biosystems/MDS Sciex, manufacturers of mass spectrometers, to develop instrumentation for MALDI-MSI and to expand the range of applications (grant 2). High repetition lasers and aspects of sample preparation for small molecules were investigated. One key development was the adaptation of a commercial QqTOF mass spectrometer to incorporate a 20kHz Nd:YVO_4 laser; it provided a novel patented method for homogenising the laser beam (Patent GB2460478B, EP2297770B) resulting in higher quality images (reference 4). **Clench** improved the specificity of MALDI-MSI analysis for small molecules and proteins in collaboration with Syngenta and GlaxoSmithKline (GSK) (grants 3, 4). This research enhanced the technique by incorporation of ion-mobility separation (IMS) in conjunction with MALDI-MSI to give increased specificity in images generated by ions of the same *m/z,* based on separation by conformation (reference 5). **Clench** has an on-going collaboration with Waters Corporation in co-development of instrumentation and software for Matrix Assisted Laser Desorption Ionisation Ion Mobility Separation Mass Spectrometry Imaging (MALDI-IMS-MSI).

In 2008, **Clench** was invited by Cancer Research UK, together with Professors Tozer and Paley at the University of Sheffield, to submit an application, which was subsequently funded (£200,654, amount received by SHU) to investigate protein signatures in tumours following vascular-targeted therapies (grant 5). This led to 5 high impact papers including reference 5. Funding was also obtained from the European Cosmetic Agency (grant 6) to use MALDI-MSI to analyse *ex vivo* human skin for the identification of irritants and sensitisers.

Francese, who joined SHU in 2008 in her first lectureship post, collaborated with **Clench** on a number of aspects of MALDI-MSI including the adaptation of the laser in mass spectrometers to improve image visualisation (reference 4). **Francese** then went on to adapt the technique of

MALDI-MSI for examination of latent fingermarks, which combined identification of an individual with information on the chemical content of fingermarks (reference 6). This research led to two patented technologies (GB2489215B and GB2493998A, in the UK and pending international applications WO2012120279 and WO2013027011). Eleven peer reviewed papers on the developments of this technique for fingermark analysis have been published and funding from the Home Office (grant 9) obtained to determine the feasibility of incorporating MALDI-MSI into routine fingermark analysis.
Grants 7, 8 and 10 demonstrate GSK's continued support and interest in MALDI-MSI research in the Bioanalysis research group at SHU.

3. References to the research (**Citations Scopus October 1st 2013**)

1. Bunch J, **Clench MR**, Richards DS (2004) Determination of pharmaceutical compounds in skin by imaging matrix-assisted laser desorption/ionisation mass spectrometry. Rapid Communications in Mass Spectrometry, 18, 3051-3060. **(66 citations)**.
DOI: 10.1002/rcm.1725
2. Mullen AK, **Clench MR**, Crosland S, Sharples KR (2005) Determination of agrochemical compounds in soya plants by imaging matrix-assisted laser desorption/ionisation mass spectrometry. Rapid Communications in Mass Spectrometry, 19, 2507-2516. (**28 citations**). DOI: 10.1002/rcm.2078
3. Atkinson SJ, Loadman PM , Sutton C, Patterson, LH, **Clench MR** (2007) Examination of the distribution of the bioreductive drug AQ4N and its active metabolite AQ4 in solid tumours by imaging matrix-assisted laser desorption/ionisation mass spectrometry. Rapid Communications in Mass Spectrometry, 21, 1271-1276. (**44 citations).** DOI: 10.1002/rcm.2952
4. Trim PJ, Djidja MC, Atkinson SJ, Oakes K , Cole LM, Anderson DMG, Hart PJ, **Francese S**, **Clench MR** (2010). Introduction of a 20 kHz Nd:YVO4 laser into a hybrid quadrupole time-of-flight mass spectrometer for MALDI-MS imaging. Analytical and Bioanalytical Chemistry, 397, 3409-3419. (**21 citations**). DOI: 10.1007/s00216-010-3874-6.
5. Cole LM, Djidja M-C, Bluff J, Claude E, Carolan VA, Paley M, Tozer GM, **Clench MR** (2011) Investigation of protein induction in tumour vascular targeted strategies by MALDI MSI. Methods, 54 (4), 442-453 (**10 citations**). DOI: 10.1016/j.ymeth.2011.03.007. **Clench,** REF 2, Output 2.
6. Wolstenholme R, Bradshaw R, **Clench** MR, **Francese** S. (2009). Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. Rapid Communications in Mass Spectrometry, 23 (19), 3031-3039. (**34 citations**).
DOI: 10.1002/rcm.4218. **Francese** REF 2 output 4.

Key grants awarded to Clench for MALDI-MSI

1. 1999–2003 - Pfizer Global R&D (£52,770). Application of MALDI/MS to the analysis of small molecules in biological tissues.
2. 2004-2008 - EPSRC/RSC Two Analytical Science CASE Studentships in collaboration with Applied Biosystems/MDS Sciex (£114,000). Imaging Matrix Assisted Laser Desorption Ionisation Mass Spectrometry.
3. 2005-2008 - BBSRC CASE Award Syngenta (£56,600). Imaging MALDI-MS for the Examination of The Uptake and Distribution of Xenobiotics in Plants.
4. 2006-2009 - BBSRC CASE Studentship GSK S3093 (£61,500). Imaging MALDI-MS for Direct Drug Distribution Analysis.
5. 2008-2013 - Cancer Research UK (£200,654K). Magnetic resonance and matrix assisted laser desorption mass spectrometry imaging for progressing the development of tumour vascular targeted drugs.
6. 2008-2012 - COLIPA European Cosmetic Association (£253,041K). Using human skin models to assess for chemical sensitivities using a combined tissue imaging mass spectrometry approach together with immunological measurements.
7. 2009-2012 - MRC Case studentship with GSK (STU029065) (£79,300). The application of MALDI Mass spectrometry and related techniques for imaging drug disposition in asthma, COPD and other respiratory disease models.
8. 2011 - 2014 - Steifel (GSK) STU/10024881 (£78,500K). Examination of pharmacotoxicological dynamic responses by mass spectrometry imaging and profiling.

Key Grants awarded to Francese for MALDI-MSI

9. 2011-2014 - Home Office Ref: HOS/11/017 (£38,250). Integration of MALDI MSI in to the Home Office operational fingermark examination workflow.

10. 2012-2016 - BBSRC Case studentship with GSK (BBSRC BB/K501104/1) (£124,173). Using ion mobility mass spectrometry to investigate the distribution and effect of dosed compounds on endogenous molecules.

4. Details of the impact

Clench and **Francese's** research on mass spectrometry imaging has impacted upon the pharmaceutical industry specifically with GSK collaborators (source 1), whilst training of staff from Unilever by **Clench's** group at Sheffield Hallam University has facilitated knowledge transfer of the technique into industry. Four technologies have been patented with regards to mass spectrometry techniques and applications, in the UK, EU, USA and China, two of which are licensed to commercial partners, Elforlight Ltd (source 2) and Consolite Forensics. Technique development work by the Bioanalysis group has changed practice within the pharmaceutical industry. Specifically, a sustained professional relationship with GSK since 2006, with a succession of CASE studentships (4), has enabled the group to influence the methodology used to assess drug distribution in animal models. By allowing faster tracking of label-free drugs with higher spatial resolution than previously obtained, this collaborative work has speeded up the drug discovery pipeline. More recent work by **Francese** with the Home Office to embed MALDI mass spectrometry analysis of fingerprints into routine forensics is gaining impact. **Francese** is working with West Yorkshire Police to provide information on fingerprint and non fingerprint forensic evidence, which is feeding into their crime scene investigations. The technology developed by **Francese** is reported in the new 2014 manual of fingerprint development techniques, edited by the Home Office, as one of the technologies with strong potential to be adopted by practitioners. Comments from (source 3) confirms the impact of the technique in forensic investigations:

'The impact that the MALDI-MSI research can have in fingerprint and forensic science include increased detections, adding contextual evidence to cases and providing higher specificity in substance identification than existing processes'.

'MALDI-MSI offers significant potential to enhance contextual information that can be obtained from fingermarks, in particular the presence and location of contaminants of operational interest that may be present in marks. It offers other potential operational benefits such as revealing additional ridge detail that has not been detected by other chemical/physical techniques, and the separation of overlapping marks from different donors'

'Recent work to confirm that marks have been deposited in human blood also offers an improvement in specificity over existing methods, which only determine whether proteins or haem is present. This new method may add confidence to conclusions drawn and overcome arguments often raised about substances that may give 'false positives' with existing reagents'.

The primary paper (reference 1) reporting findings from the initial project on MALDI-MSI of small molecules in collaboration with Pfizer Global R&D, led to the adoption of the technique by pharmaceutical companies following personal interaction of **Clench** with scientists from companies including GSK, Syngenta, Quotient Bioresearch Ltd and instrument manufacturers Waters Corporation (Source 4). **Clench** has collaborated with Waters Corporation since 2006 and the company has invested ~£350K p.a. in MALDI Imaging since then. A senior manager at Waters Corporation (Source 4) commented that the company sells 5-10 million pounds worth of MALDI systems per annum of which 30% are for MALDI imaging. He also commented *'in the early days* (of mass spectrometry imaging) *your help was vital in getting us off the ground'*). Specifically in the UK, GSK introduced the MALDI-MSI instrumentation at its Stevenage site (source 1). **Clench**'s investigations of agrochemical distribution in plants using MALDI-MSI resulted in him participating in a BBC programme "*Afterlife - The Strange Science of Decay*", assessing N^{13} uptake of decayed plant material in new plant growth by MALDI-MSI. The programme, which was broadcast by BBC4 on 06/12/2011 and repeated on 06/08/2012, attracted viewing figures of 613,000 and 234,000 respectively (source 7). **Clench's** research on the combination of ion mobility separation with MALDI-MSI has been carried out in collaboration with Waters Corporation, Manchester, one of the world's leading instrument manufacturers. This significant collaboration started in 2006 and is on-going; Waters Corporation sponsored the Discovery & Life Science Webinar Series 2013(04/09/2013) in which **Clench** discussed progress in MALDI-MSI of biological tissue and **Francese** discussed multi-informative analysis of latent fingermarks by MALDI-MSI (300 participants) (source 6). **Clench's** work was, and is, a major contributory factor in Waters Corporation's decision to invest in development of MALDI-IMS-MSI (source 4).

Francese's research on fingermark analysis attracted significant media attention (sources 8 and

9). Since her initial paper in 2009, **Francese** further developed the methodology to aid in the identification of sexual assault victims and to analyse fingermarks recovered from crime scenes by standard forensic techniques. She is funded by the Home Office (2011-2014) (grant 9) to convert this research into a validated method (source 2). **Francese** co-developed a powder discharge device (patent pending PCT/GB2013/051838) (with Reed, SHU), which has been licensed to Consolite Forensics for further development, to enhance the company's sales in the forensics market. The initial work was funded through the EPSRC *Bridging the Gap* award to SHU. **Francese** was featured on: The One Show, BBC1, 24/04/2012 (viewing figures 4,170,000), Fingerprint item; Science in Action, BBC Radio World Service, 26/01/2012, http://www.bbc.co.uk/programmes/p00n0f3d; and covered in national, local and specialist press, including The Daily Mail, Yorkshire Post, Science Daily, Police Oracle and The Engineer.

Clench and **Francese** provide expert advice and consultancy to industry through the Unit's analytical services commercial group for MALDI-MSI, including: Unilever R & D (£21K), Procter and Gamble (£15K), Croda Chemicals (£43K), and Alk-Albello (Denmark) (£19K), (income 2008 - 10/2013). The scale of external impact of the MALDI-MSI work at SHU was evidenced by three Mass Spectrometry open days held on: 29/07/2009, 35 Delegates (10 Industry, 21 Universities, 4 Other, which includes NHS, Government laboratory staff); 14/07/2011, 111 Delegates (44 Industry, 53 Universities, 14 Other), 10/04/2013, 107 Delegates: 42 Industry, 60 Universities, 5 Other. The last of these open days was organised in conjunction with the British Mass Spectrometry Society (source 5).
Two scientists trained in MALDI-MSI by **Clench** at SHU are now exploiting this expertise, one at Novartis, Basel, Switzerland and a second at instrument manufacturer Shimadzu, Manchester, UK, the latter as the Global MALDI Applications Specialist. **Clench's** first PhD student at SHU (completed in 2005) in MALDI-MSI was appointed as Head of MALDI-MSI at the National Physical Laboratory in 2013.
In 2007/8 **Clench** was appointed as the British Mass Spectrometry Society (BMSS) Lecturer in recognition of his research expertise in MALDI-MS; he currently leads the Imaging Group within the BMSS. The award enabled **Clench** to share his expertise through a lecture series in the UK on MALDI-MSI. Francese shared her expertise in MALDI-MSI with attendees at the International Symposium on Pharmaceutical and Biomedical Analysis in Bologna, Italy in 2013 (source 10). Further demonstrating the impact and importance of **Clench**'s expertise, and the interest in the technique by the healthcare community in the EU, he was appointed UK member of the Management Committee of EU COST Action BM1104 "*Mass Spectrometry Imaging: New Tools for Healthcare Research*" (2011-2015).

5. Sources to corroborate the impact

Source 1. Principal Research Analyst, GlaxoSmithKline, Stevenage
Source 2. Technical Director, Elforlight Ltd, Daventry
Source 3. Research Scientist, Home Office Centre for Applied Science and Technology, St Albans
Source 4. Senior Manager, Waters Corporation, Manchester
Source 5. Chairman British Mass Spectrometry Society and Associate Principal Scientist, AstraZeneca, Macclesfield
Source 6. Biocompare Webinar, sponsored by Waters Corporation 4/9/2013: Clench and Francese https://waters.omnovia.com/registration/52751363643813 300 participants signed up for live event).
Source 7. "After Life: The Science of Decay" http://www.bbc.co.uk/programmes/b012w66t
Source 8. "Fingerprint breakthrough offers new forensic evidence" http://www.bbc.co.uk/news/technology-14386520
Source 9."Pioneering Fingermark Technology Uses Mass Spectrometry Imaging to Provide Crime Scene Investigators With Key Extra Details" http://www.sciencedaily.com/releases/2011/07/110729175002.htm
Source 10. Speaker profile and abstract for Francese from International Symposium on Pharmaceutical and Biomedical Analysis, 2013. http://www.pba2013.org/speakers.html