Institution: The University of Manchester		
Unit of Assessment: 8 (Chemistry)		
Title of case study: DOSY and Pure Shift NMR: from changed practice in the chemical, pharmaceutical and scientific instrument industries to a multimillion-pound new food ingredient		
Period when the underpinning research was undertaken: 2000 – 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Gareth Morris	Professor (1998 – present), Reader (1994 – 1997)	1994 – present
Mathias Nilsson	Professor (2018 – present), Reader (2013 – 2018), Lecturer (2012), Research Fellow (2007 – 2012), PDRA (2005 – 2007)	2013 – present
Ralph Adams	Research Fellow (2014 – present), PDRA (2010 – 2014)	2010 – present
Period when the claimed impact occurred: 1 August 2013 – 31 July 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Pioneering research at The University of Manchester (UoM) has driven major advances in nuclear magnetic resonance (NMR) methodology, resulting in pulse sequences and analysis software that are incorporated in over 90% of modern liquid state NMR spectrometers worldwide – a USD1,000,000,000 *per annum* market. These advances have changed practice and capacity in industry, through the introduction of new products and processes. Diffusion-ordered spectroscopy (DOSY) is having significant economic impact: one food industry company (Givaudan) reports sales growth of >GBP100,000,000 in this REF period as a direct result of a new flavour component found using DOSY. Pure shift methods are routinely used by major companies (*e.g.* Syngenta), and are now a standard feature on spectrometers supplied by all major manufacturers.

2. Underpinning research

The rapid analysis of solutions by spectroscopic means is a very important chemical function. NMR spectroscopy has revolutionised the practice of chemistry by allowing the fast and non-destructive identification of unknown chemical structures in solution. NMR equipment is an essential requirement for any serious chemical laboratory. NMR is unique among spectroscopic methods in its versatility and flexibility, partly as a consequence of the quantum mechanics underlying the phenomenon of magnetic resonance and partly because the instrumentation used has highly flexible computer control, allowing new techniques to be implemented simply by modifying the software.

Two powerful and complementary methods that have been developed at the UoM (a) exploit differences in diffusion rates between mixture components to distinguish their characteristic NMR spectra (DOSY), and (b) greatly enhance the resolution and chemical specificity of those spectra (pure shift NMR). The underpinning research has significantly advanced the knowledge and understanding of these NMR techniques and their applications in determining the compositions and chemical structures of complex samples.

The key to mixture analysis using NMR is to be able to distinguish between signals from different species. One distinctive characteristic of a molecule (determined by its size) that can be measured with NMR is its diffusion coefficient, and extending previous work by Charles Johnson, the UoM researchers developed methods to produce very high resolution DOSY spectra, separating the signals of different mixture components according to size [1-4]. The power of DOSY was greatly increased by the researchers' introduction of broadband homonuclear decoupling ("pure shift") methods, which can increase spectral resolution by nearly a factor of 10 [2, 5, 6].

UoM's introduction of "matrix-assisted DOSY" [3] extended the method to allow the analysis of mixtures in which species diffuse at the same rate, for example those of isomers, by exploiting differential strengths of interaction with a slowly-diffusing matrix.

The pure shift methods [2, 5, 6] that were initially developed to boost the performance of DOSY have proven to be of great importance in their own right, and are now widely used across the whole field of high resolution NMR. Pure shift NMR has found application in many areas of science, including organic chemistry, natural products, pharmaceuticals, drug discovery and metabolomics.

The key improvements in analytical methodology that these two families of methods provide are a greatly improved ability to distinguish the signatures of individual chemical components in chemical mixtures, and a major improvement in the ability to infer structural information from those signatures.

UoM researchers have been actively developing DOSY methods for over 20 years, matrix-assisted DOSY since 2009, and pure shift NMR since 2007. The impacts described here relate only to the period from 1 August 2013 onwards.

3. References to the research

Based on the research described above, Morris was elected as a Fellow of the Royal Society (2014) and Nilsson and Adams were awarded the BRSG/NMRDG Award for Excellent Contribution to Magnetic Resonance by an Early Career Researcher in 2010 and 2018 respectively. Citations are from Scopus, and accurate as of 5 March 2021. UoM authors are highlighted in bold.

(1) **Pelta, M. D.**; **Morris, G. A.**; **Stchedroff, M. J.**; Hammond, S. J. A one-shot sequence for high-resolution diffusion-ordered spectroscopy. *Magn. Reson. Chem.* **2002**, *40*, S147. DOI: 10.1002/mrc.1107 (191 citations)

(2) **Nilsson, M.**; **Morris, G. A.** Pure shift proton DOSY: diffusion-ordered H-1 spectra without multiplet structure. *Chem. Commun.* **2007**, *9*, 933. DOI: 10.1039/B617761A (143 citations)

(3) **Evans, R.**; Haiber, S.; **Nilsson, M.**; **Morris, G. A.** Isomer Resolution by Micelle-Assisted Diffusion-Ordered Spectroscopy. *Anal. Chem.* **2009**, *81*, 4548. (56 citations)

(4) **Nilsson, M.** The DOSY Toolbox: A new tool for processing PFG NMR diffusion data. *J. Magn. Reson.* **2009**, *200*, 296. DOI: 10.1021/ac9005777 (143 citations)

(5) **Aguilar, J. A.**; Faulkner, S.; **Nilsson, M.**; **Morris, G. A.** Pure Shift ^{1}H NMR: A Resolution of the Resolution Problem? *Angew. Chem.-Int. Edit.* **2010**, *49*, 3901. DOI: 10.1002/anie.201001107 (189 citations)

(6) **Foroozandeh, M.**; **Adams, R. W.**; **Meharry, N. J.**; Jeannerat, D.; **Nilsson, M.**; **Morris, G. A.** Ultrahigh-Resolution NMR Spectroscopy. *Angew. Chem.-Int. Edit.* **2014**, *53*, 6990. DOI: 10.1002/anie.201404111 (169 citations)

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Clearing the undergrowth: new NMR techniques for high dynamic range mixtures (**Morris, Nilsson, and Adams**), GBP623,173, January 2018-February 2021 (EPSRC: EP/R018790/1); **Ultrahigh resolution NMR: citius, altius, fortius (Morris and Nilsson),** GBP728,577, September 2016-August 2019 (EPSRC: EP/N033949/1);
New Paradigms for Pure Shift NMR (Nilsson and Morris), GBP367,650, January 2015-December 2017 (EPSRC: EP/M013820/1);
Improving NMR Resolution and Sensitivity - Simultaneously? (Morris, Nilsson, and Adams), GBP425,908, March 2014-February 2017 (EPSRC: EP/L018500/1);
Advanced NMR methods for Formulation Analysis (Nilsson and Morris), GBP99,121, September 2011-February 2015 (EPSRC Industrial CASE award; Syngenta);
New NMR tools for impurity analysis (Morris and Nilsson), GBP92,161, September 2012-February 2016 (EPSRC Industrial CASE award; AstraZeneca);
Pure Shift NMR (Morris), GBP31,500, Apr 2012-Sep 2013 (Agilent Foundation);
Pure Shift Proton NMR: A Resolution of the Resolution Problem? (Morris and Nilsson), GBP482,450, September 2010-February 2014 (EPSRC: EP/I007989/1);

Matrix-Assisted DOSY (Nilsson and Morris), GBP332,000, September 2010-August 2013 (EPSRC: EP/H024336/1);
New methods for mixture analysis by liquids NMR (Nilsson), GBP701,000, September 2007-March 2012 (EPSRC: EP/E05899X/1);
Diffusion-Ordered NMR Spectroscopy: Solving the Overlap Problem (Morris and Nilsson), GBP302,000, May 2007-August 2010 (EPSRC: EP/E057888/1);
Advances in Diffusion-Ordered NMR Spectroscopy (Morris), GBP184,424, May 2004-April 2007 (EPSRC: GR/S90751/01);
Diffusion-Ordered NMR Spectroscopy (Morris), GBP164,533, December 1998-May 2002 (EPSRC: GR/M16863/01).
DOSY and pure shift NMR underpin a recent EPSRC Strategic Equipment Fund award (**Adams** PI, **Morris** Co-I) of GBP1,698,226, September 2020 – August 2023 (EPSRC: EP/V007580/1)

4. Details of the impact

Context

Nuclear magnetic resonance is one of the most important and powerful analytical tools used by chemists, and finds application in a wide range of other fields. It is able to determine, for example, the composition of a mixture, the structures of complex molecules, and the mobilities of these molecules in solution. However, to direct the complex and precisely-timed sequences of radiofrequency and magnetic field pulses that are used in an NMR spectrometer to measure these different properties of substances is a science in itself. The impact described in this case relates to the development and commercial exploitation of two such families of pulse sequences developed at Manchester, known respectively as DOSY and pure shift NMR, that are able to analyse complex mixtures in a unique manner. They have proved vital to industrial research and product development and has led to a significant shift in practice and capacity in industrial research.

Pathway to impact

The initial impetus for our developments came from an industrial collaboration with Pfizer Global Research, and led to dedicated processing software, which was initially shared widely but informally with other users. The subsequent development of DOSY software, both open source and licensed for the proprietary operating system of the major NMR manufacturer Varian (subsequently Agilent), gave both the opportunity to exploit the intellectual property generated, and an effective vehicle for disseminating the results to a wide range of users, including many industrial research organisations. Key contributors to this impact were the Department policy of maintaining shared high-resolution NMR facilities, and the assistance of UMIP (University of Manchester Intellectual Property management agent) in negotiating the licensing of DOSY software.

In parallel to licensing proprietary code, the researchers made open-source DOSY processing code available through the DOSY Toolbox (and very recently through the General NMR Analysis Toolbox, GNAT, which has had >1,200 downloads since its release in October 2018). UoM's development of pure shift NMR, initially as an adjunct to DOSY but now very widely used in its own right, has been the subject of several workshops at international conferences (EUROMAR, SMASH); a September 2017 workshop held in Manchester attracted 66 delegates, 11 from abroad and 8 from industry. The workshop materials have been downloaded from the researchers' website over 1,000 times.

In collaboration with the major NMR equipment manufacturer Bruker, the researchers integrated pure shift and DOSY experimental techniques into their commercial software, with the result that many of these experiments are now available on all Bruker spectrometers in a dedicated University of Manchester section of the Bruker user library. The researchers also assisted the other major manufacturer, JEOL, to implement our methods in their software.

Reach and significance of impact

This work has changed the way that NMR is used in the chemical industries, including pharmaceuticals, agrochemicals and food. It led to the discovery of a new flavour ingredient that generated sales well in excess of GBP100,000,000 in the REF period, was key to

maintaining sales of a fungicide worth >GBP100,000,000, and has supported growth in a billion-dollar scientific instrument market.

The principal impacts associated with DOSY and pure shift NMR in this period are:

Driving growth and innovation at NMR instrument manufacturers
The integration of UoM's DOSY and pure shift NMR techniques into major manufacturers Bruker and JEOL's spectrometers has brought both companies significant benefits. Bruker and JEOL both include the techniques in their standard offerings of NMR experiments on current-generation, high-resolution spectrometers [A, B]. Bruker specifically highlight that "…*processing software for diffusion experiments is now an essential part of our Dynamics Centre software suite*", and that "…*pure shift methods have become an indispensable part of our standard experiment library and are used routinely by our customers*" [A]. The improvements in analytical problem-solving for NMR spectrometer customers achieved by DOSY and pure shift methods have also brought financial benefits to Bruker and JEOL. Bruker confirm, "*the wide uptake of usage will definitely have contributed to our global NMR sales (around $400 million per year)*" [A], whilst JEOL say that the techniques "*have contributed to our instrumentation global annual sales of $900M*" [B].

Bruker and JEOL both acknowledge the significant advantages that adopting DOSY and pure shift techniques bring to their customers [A, B]. As Bruker note, these techniques "*have significantly improved the ability of our end users to analyse mixture samples efficiently, and* in extremis *to solve otherwise intractable analytical problems*" [A]. For Bruker, DOSY NMR techniques have spurred them to further technical innovation including special probes (DiffBB and BBO) with 17 T m^{-1} gradient strength and −40 to 150 °C temperature ranges, as well as a new amplifier (GREAT60), all of which further enhance the usefulness of UoM's DOSY techniques [A].

Changing practice in the food, pharmaceutical, and agrochemical industries
UoM's DOSY and pure shift techniques are now used widely across the chemical industries. They have made significant contributions to the way many companies in the food, pharmaceutical and agrochemical industries perform chemical analysis and characterisation. This change derives from the need in the chemical industries to solve analytical problems with increased speed, precision and detail. A Senior Technical Expert in Crop Protection at international agrochemicals giant Syngenta emphasises the importance of NMR to industry, saying "*Chemical analysis is important throughout the research and development process and this importance is growing because modern environmental and safety regulations demand identification and quantification of molecules at lower and lower levels. NMR remains Syngenta's primary method of structure elucidation*" [C]. Pharmaceutical company Arcinova's Head of Drug Product and Scientific Direction confirms, "…*it is evident that molecular level characterisation of both drug substances and drug products is pivotal throughout pharmaceutical development for optimisation purposes and also for reducing the time to commercialisation*" [D]

DOSY NMR experiments are available to all researchers at Syngenta's Jealott's Hill International Research Centre, their largest R&D site, with >800 active researchers [C]. Specific impacts of DOSY and pure shift at Syngenta include:

- Identifying a xenobiotic metabolite in a highly impure sample, by separating metabolite resonances from contaminants; this was required to maintain multi-million pound sales of a key fungicide product [C]
- Measuring diffusion, in multiple solvents, of a fungicide with sales >GBP100,000,000, helping to "…*optimize and reduce waste from the production process of this fungicide*." [C]
- "*Understanding of how a crop protection product partitioned within a spray mix*", crucial to understanding the mechanism of action of the formulation and previously impossible [C]

Both large and small pharmaceutical companies have also embraced the use of DOSY and pure shift methods. Small pharmaceutical company Arcinova, for example, has fully integrated the techniques into their work, saying "*The techniques developed at Manchester*

are routinely implemented in our own Laboratories and, as a SME [Small and Medium-sized Enterprise]*, we have recently invested >£200,000 in upgrades to our magnetic resonance instrumentation for enabling the highest quality data generation and processing*". [D] Specific impacts of DOSY and pure shift at Arcinova include:

- Characterising monomer, dimer and trimer impurities in a pharmaceutical product used for advanced cancer treatment, since commercialised and now used globally [D]
- Assessing physical stability of a liposomal product to determine whether it met technical requirements for use in humans; this product is now commercially available [D]

The international pharmaceutical giant AstraZeneca has also integrated DOSY and pure shift NMR techniques into their research, describing them as representing "…*a step function improvement in our ability to analyse our samples by NMR.*" [E]. The methods are widely used in the AstraZeneca analysis toolbox, and are "…*playing an important part in the development of both small and medium sized drugs (e.g. oligonucleotides)*" [E]. The benefits to AstraZeneca's business are characterised by a Principal Scientist in Pharmaceutical Technology & Development, who says "*…the understanding that is derived from these experiments helps expedite the drug development process, ultimately allowing us to deliver medicines to patients more effectively.*" [E].

Mestrelab Research, who produce the NMR processing software Mnova, illustrate the breadth of uptake across the chemical industries. Mestrelab's software, which has 150,000 users globally, incorporates specific processing algorithms for DOSY and pure shift NMR experiments [F]. Mestrelab's Managing Director credits the UoM NMR techniques with contributing to their software's success, saying, "*We have no doubt that incorporating processing for pure shift and DOSY methods has contributed to establishing a market-leading position for our Mnova software.*" [F]

Delivering financial benefits to the chemical industries

Alongside the more general benefits derived from the enhanced problem-solving that DOSY and pure shift techniques provide, companies have developed new products directly from their use. As an example, Givaudan, a major international flavours and fragrances company, used DOSY to discover a compound that only occurred in trace amounts in food extracts. This compound positively influences taste perception and is widely applicable to a range of flavour products; sales between 2014 and 2018 generated CHF120,000,000 (approximately GBP100,000,000) [G]. Givaudan emphasise the value of these NMR techniques for companies, stating "*We are optimistic that the newly developed DOSY techniques and the newly deveped* (sic) *processing toolbox will also help us in finding the next "golden bullet" and can give Givaudan a clear competitive advantage*." [G].

5. Sources to corroborate the impact

[A] Letter from a Principal Scientist, Bruker, 27 March 2020
[B] Letter from the Head of NMR Business Development Europe, JEOL, 13 May 2020
[C] Letter from a Senior Technical Expert, Crop Protection, Syngenta, 23 August 2020
[D] Letter from the Head of Drug Product and Scientific Direction, Arcinova, 31 May 2020
[E] Letter from a Principal Scientist, Pharmaceutical Technology & Development, AstraZeneca, 15 September 2020
[F] Letter from the Managing Director, Mestrelab Research, 30 March 2020
[G] Letter from a Senior Research Investigator, Natural Ingredient Discovery, Givaudan, 19 March 2018