

Biospectroscopy at the Manchester Interdisciplinary Biocentre

The Manchester Interdisciplinary Biocentre (MIB) at The University of Manchester (UK), is a large research facility located in central Manchester. The research undertaken in the MIB is said to address a number of grand challenges, including industrial biotechnology, energy and biofuels, and biomedical healthcare. These are realized via four main research themes: biomolecular mechanism and catalysis; synthetic and chemical biology; systems biology; and enabling technologies. This research spotlight focuses on biospectroscopy in the MIB, namely vibrational spectroscopies. This is just one area of research across just three of the many research groups in the MIB, which could be said to exemplify the fundamental and applied aspects of this field, its interdisciplinary nature and also the way it realizes several of the research themes and grand challenges already mentioned, with cutting edge and innovative research.

The Manchester Interdisciplinary Biocentre (MIB) at the University of Manchester opened in 2006 in central Manchester on Princess Street. The MIB is a large research facility ($13,100\text{ m}^2$) initially costing approximately UK£38 million to construct, and home to a wide range of primary research laboratories, computational research laboratories, core technology facilities, lecture theatre, meeting rooms and offices over a total of five floors (FIGURE 1). It can accommodate 600 research staff in up to 75 research groups from diverse fields. The internal architecture reflects the ethos of this interdisciplinary environment with much of the laboratory space being multifunctional and open plan, featuring a range of constantly updating high-tech and high-spec facilities. This has been said to help ensure that the widest possible range of expertise and techniques can be brought to bear on quantitative bioscience problems and to promote interdisciplinary, challenge-orientated bioscience and biotechnology at the highest international level [101]. Research in MIB is said to address a number of grand challenges, including industrial biotechnology, energy and biofuels, and biomedical healthcare. These grand challenges are realized via four main research themes (biomolecular mechanism and catalysis, synthetic and chemical biology, systems biology and enabling technologies), which undertake studies at the molecular, systems and design levels. Of course, much of this research is underpinned by these enabling technologies, and the aim of this spotlight is to focus attention on just one aspect of these, namely **biospectroscopy**, and specifically, vibrational spectroscopy in MIB. This is an area of research across three of the

research groups, which could be said to exemplify the fundamental and applied aspects of this field, its interdisciplinary nature, and the way it realizes several of the research themes mentioned above.

Peter Gardner is head of an internationally recognized biomedical spectroscopy research group within the MIB. This group has focused on the application of **IR spectroscopy** to the study of prostate and other urological cancers, in collaboration with the Paterson Institute for Cancer Research [102] amongst others. In the UK, cancers of the lung, bowel, breast and prostate account for 47% of all cancer deaths, with prostate cancer having the second highest mortality rate in men after lung cancer [103]. The Gardner group at MIB have undertaken FT-IR spectroscopic analysis of prostate tissue and cell lines, from both benign and malignant prostate cancers for several years, starting with a pilot study published in the *Journal of Pathology* in 2003 [1]. It was during this pilot study that they demonstrated the clear differentiation of benign and malignant cells, and the first demonstration of the separation of FT-IR spectra of prostate cancer cell lines derived from different metastatic sites. Within only a few years, further advances and many publications later, the group had honed their biospectroscopy-based diagnostic technique, using FT-IR in combination with chemometric methods.

For example, one of these studies collected a total of 395 IR spectra from 40 prostate cancer tissue biopsies from 39 patients. These were analyzed using PC-DFA, and compared with results from histopathological Gleason tissue scoring, and the less observer-dependent TNM (tumour/node/metastases) classification

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system. Whilst a significant number of spectroscopic studies have been published that have discriminated non-cancerous from cancerous tissue [2], this particular study by the Gardner group demonstrated the more challenging and rigorous task of grading cancerous tissue, showing that IR spectral data can clearly give an indication of the stage of the disease and identify clinically aggressive prostate cancer (**FIGURE 2**) [3]. A recent major breakthrough for this group was understanding the ‘dispersion artifact’ [4], and the subsequent development of the resonant Mie scattering–extended multiplicative signal correction (RMieS–EMSC) algorithm [5]. Indeed, this advance has allowed for IR spectra of single cells to be corrected for scattering distortions, which has impacted and started to change the field of IR spectroscopy, with many groups now using this correction method. An example of this is a recent publication by this group applying the RMieS–EMSC algorithm to a study of renal cells showing that stem-like cells can now be identified using IR spectroscopy [6].

The Laboratory for Bioanalytical Spectroscopy [104] is led by Roy Goodacre from the School of Chemistry [105] and this group covers three broad research themes. These include the active development and application of metabolomics technologies for the rapid and accurate characterization of biological systems [7]. This is achieved predominantly via MS-based instrumentation that produces ‘holistic’ whole-organism metabolite data [8] of microorganisms, human and animal biofluids and plant materials [9]. More pertinent to this

article is this group’s active development of vibrational spectroscopic approaches for the analysis of a wide range of complex biological systems, including those already mentioned and other areas such as monitoring of complicated foods matrices [7,10]. These vibrational approaches include mid-IR (FT-IR) spectroscopy (high-throughput scanning, microspectroscopy, chemical imaging, ATR), and **Raman spectroscopy** (including 633, 785 and 532 nm, dispersive Raman, UV resonance Raman [UVR], surface-enhanced Raman scattering [SERS], portable probes, and a fluorescence-suppression Raman system). Finally, the third general theme of this group is their expertise and knowledge in analyzing high-dimensional multivariate data and development of novel chemometric and machine learning techniques, and in the generation of tutorials and standards documents for reporting and best data analysis practises [103,104].

In terms of mid-IR spectroscopy, some recent publications of note from this group include the biomedical application of FT-IR microspectroscopy to dermatological research. This involved collecting IR spectra and constructing chemical maps across extralesional cross sections taken from keloid scars. It was shown that FT-IR microspectroscopy can delineate the edge of scar formation from the keloid scars themselves, and PLS-DA with bootstrapping was used to identify keloid scar from normal surrounding tissue. The authors believe that this proof-of-concept study may have the potential to improve keloid diagnosis and prognosis [11]. An environmental-based study from this group used FT-IR as a metabolite fingerprinting tool for monitoring phenotypic changes within complex bacterial communities capable of degrading phenol. This was achieved by taking multiple samples of activated sludge across a 48-h period and analyzing with FT-IR. The ability for the activated sludge to degrade phenol over extended periods of storage (2–131 days) in the absence of phenol was also investigated using the same approach. An observable reduction in the microbial community’s ability to degrade phenol was accompanied by detectable changes in the IR spectra, said to be related to the cellular phenotype of the microbial community. This study was said to illustrate that FT-IR and chemometrics are able to offer a high-throughput screening approach to assess the metabolic capability, and track the changes, of complex microbial communities from industrial sewage treatment plants to degrade phenol [12]. Another

Key Terms

Biospectroscopy:

Spectroscopic analysis of specimens of tissue, cells or biofluids.

IR spectroscopy: Deals with the IR region of the electromagnetic spectrum; covers a range of techniques, the most common being a form of absorption spectroscopy.

Raman spectroscopy:

Spectroscopic technique used to analyze vibrational, rotational, and other low-frequency modes in a system and which is reliant on inelastic or Raman scattering.



Figure 1. Manchester Interdisciplinary Biocentre.



recent study of note using FT-IR involved the monitoring of recombinant antibody production by mammalian cell cultures. This study demonstrated the ability of FT-IR spectroscopy as a tool for the rapid monitoring of cell cultures for recombinant protein production, as well as its potential as an online technique for the measurement of antibody production in industrial-scale bioreactors [13].

As well as the IR spectroscopy already mentioned in both the Gardner and Goodacre groups, a variety of Raman approaches are also available and there is a thriving Raman spectroscopy research community within the MIB. As already mentioned, the Laboratory for Bioanalytical Spectroscopy has a considerable

amount of Raman equipment and expertise, and a large publication record within this particular field. Within the Goodacre group, of their recent Raman-related publications, those of particular interest include studies related to the human papilloma virus (HPV). Raman microscopic imaging was used to chemically map cervical carcinoma cells to reveal the site of action of the HIV protease inhibitor indinavir, in conjunction with LC-MS analysis of cell fractionation products to confirm the location. The results were said to suggest that the most likely site of action for this compound against HPV were the E6-expressing cells, where indinavir undergoes nuclear accumulation [14]. Another recent study of interest involved the

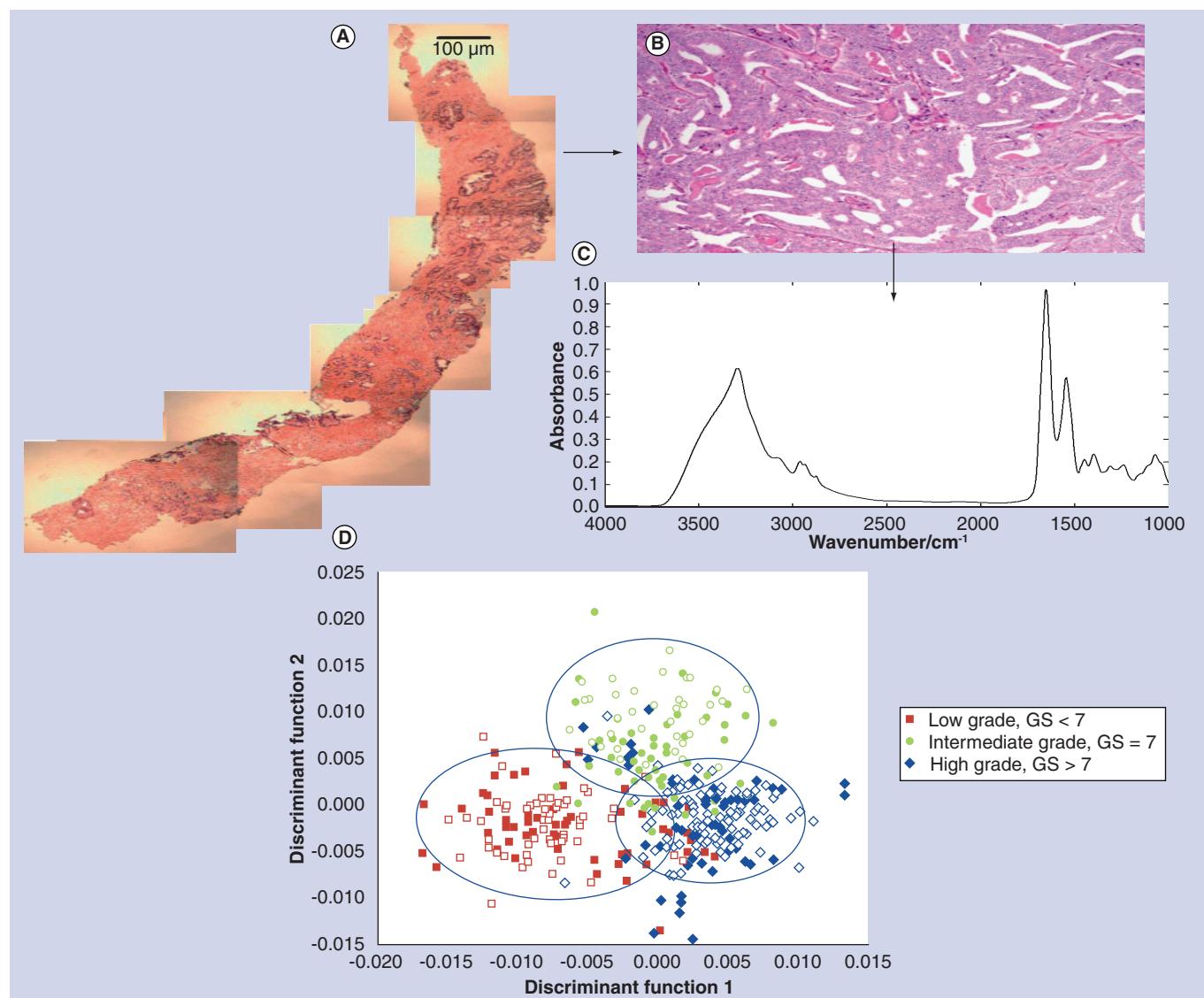


Figure 2. How IR spectroscopy can be used to grade prostate tissue. (A) Prostate needle core biopsy, (B) tissue sample interrogated with IR spectroscopy to give (C) IR spectrum, which, using (D) a supervised method of multivariate analysis, can be assigned as either low, intermediate or high grade of cancer corresponding to a GS of <7 , $=7$ or >7 , respectively. GS:Gleeson Score.



monitoring of succinate dehydrogenase activity isolated from mitochondria by SERS. This was a good example of the ability of SERS to quantitatively monitor enzyme activity in real time [11]. Further investigations of note from this group are some of the SERS and UVRR studies, exemplifying the utility of chemometrics for bacterial identification [15–17].

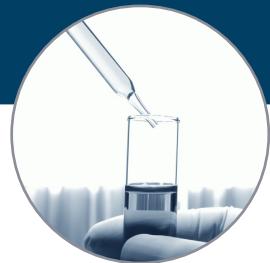
The Laboratory for Bioanalytical Spectroscopy is not the only group undertaking research using Raman spectroscopy in the MIB. Ewan Blanch heads a group whose central theme of research is the application of Raman spectroscopies to study the structure and behavior of biological molecules. This research utilizes some novel spectroscopic approaches to studying biological systems, including conventional Raman spectroscopy, Raman optical activity (ROA), SERS and the new technique of surface-enhanced Raman optical activity (SEROA). There are of course several areas of research within this group, and these include studying the mechanisms of protein folding and misfolding (using novel 2D Raman and 2D ROA correlation techniques), Raman characterization of RNA structure, shear-induced unfolding of proteins, machine learning optimization of SERS (in collaboration with Roy Goodacre), development of the new SEROA technique [18], which may provide a new tool for nanotechnology, and finally, developing a suite of bioinformatics tools to incorporate into an online Biological Raman Database (BRD), which will complement other protein structure databases.

Recent publications from this group include the application of Raman and ROA for the characterization of secondary and tertiary structure in carbohydrate. This study showed that both Raman and ROA spectroscopies are able to characterize higher order structure in the glycosaminoglycan hyaluronan. This was said to demonstrate that these vibrational spectroscopies can make a major impact on our understanding of glycan and polysaccharide structure, which is currently very limited [19]. Another study involved the accurate determination of protein secondary structure content from Raman and ROA spectra. Linear regression was developed for the analysis of spectra of proteins in order to determine their secondary structure contents (e.g., α -helix and β -sheet) with a significantly high degree of accuracy, to within 1–2% of Protein Data Bank (PDB) structures [106]. Moreover, it was noted that as vibrational spectroscopies are measurable for most, if not all, proteins, then this analytical

tool greatly strengthens the complementarity of these methods to those mainstream structural biology techniques, such as x-ray crystallography and NMR spectroscopy [20].

After many years of debate and conjecture, this group and others have recently demonstrated that shear forces in fluids can perturb protein structure. Using Raman spectroscopy, the role of protein structural parameters (size and secondary structure content) and fluid-flow shear parameters were investigated, to determine the sensitivity of proteins to shear-induced unfolding [21]. A very recent study by this group used a hydrogel polymer as a reproducible surface for SEROA. As biomolecules are almost always chiral, it has been said that ROA has proven to be an incisive probe of both biomolecular structure and behavior. It is known, however, that ROA is currently limited in its applicability by the need for high sample concentrations, with several researchers attempting to circumvent this potential obstacle using surface enhancement from metal surfaces, which has proven to be difficult. This group have only very recently demonstrated SEROA as a chiroptical technique for the first time, by using a hydrogel polymer forming polyacrylic acid to control colloid aggregation [22].

This spotlight has focused on biospectroscopy at the MIB, and readily illustrates the breadth of expertise and the fundamental and applied nature of the research across a broad range of themes and applications, using a range of the spectroscopic techniques and expertise available in this interdisciplinary research institute. However, it would be an omission not to mention the other forms of biospectroscopy, such as the NMR work of Andrew Almond's group amongst others [23], or indeed the many forms of biospectrometry, such as the state-of-the-art MS, chromatographic and bioinformatics/chemometrics techniques employed by Douglas Kell [24] and Roy Goodacre's groups [8] that are also available within this dynamic research environment. Moreover, it would also be a serious omission not to mention the Photon Science Institute (PSI) at The University of Manchester, another one of the University's main research institutes [107]. The PSI is another world-class interdisciplinary research institute with a wealth of expertise and sophisticated laser equipment in state-of-the-art laboratory facilities, whose aim is to pursue and focus photon science research across many disciplines such as energy research, nanoscience, life sciences and medicine.



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