The Development of Raman Spectroscopy as a Tool for Clinical Applications

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Introduction

Raman spectroscopy is an optical technique that measures the chemical composition and molecular structure of a sample. Utilisation of the 'molecular fingerprint' of Raman spectra has proven an effective analytical approach in geology, semiconductors, materials and polymer science fields. The application of Raman spectroscopy and microscopy within biology is rapidly increasing and it has proven to be a valuable analytical tool for various applications. There are potential clinical applications of Raman to in the detection of various cancers, AMR, neurodegenerative disease, renal disorders and the diagnosis of primary immunodeficiency amongst other areas.

The FAST Healthcare NetworksPlus, in collaboration with the NHS England Knowledge Transfer Partnership scheme, held a sandpit event to consider how to advance the clinical application of Raman spectroscopy with a particular emphasis on the detection of antimicrobial resistance (AMR). This report summarises the outcomes of the sandpit and it is divided into three sections: the results of a SWOT[1] analysis, the outcome of a roadmapping exercise and some overall conclusions.

SWOT Analysis

A summary of the SWOT analysis carried out in the sandpit is shown in Fig. 1, and each area is considered in turn here.

Strengths

Raman spectroscopy uses a laser to excite the bonds in a sample and the scattered light therefore contains information about the sample. Therefore, this is an extremely powerful technique as minimal sample preparation is required for a response to be measured in a well-designed system. No 'labelling' of the sample under test is required, and no previous knowledge about the sample is assumed. Therefore, the technique is well-suited to measurements where there is a 'poorly posed problem' being assessed. For example, if there is a concern that a surface is contaminated with some biological molecule, then Raman spectroscopy can test the surface without needing to make any decisions about what type of molecules are being looked for, as would be needed for a specific test. The ability to choose the wavelength of the laser used allows different responses to be measured, which provides significant practical flexibility. It is also known that different biological molecules give very characteristic responses as a function of wavelength and therefore high specificity can be achieved. Furthermore, the technique is non-invasive, fast and a surface can be mapped. Consumables for running a test are also very cheap.

[1] SWOT – strength, weakness, opportunity, threat.

Weaknesses

Like many optical systems, the capital cost of a Raman spectrometer is high, although by engineering a system for a specific clinical application, there is room to ensure that this is kept as low as reasonably possible. The sample volume under test is also very small, as it is only a surface that is being probed by the laser beam and the spot size is usually small. This places an upper limit of sensitivity when low concentrations of a biological molecule are being measured. Although biological molecules have a distinctive spectrum, there are few publicly available databases of spectra that can be accessed for interpretation of Raman spectra from a sample. Finally, the high laser power that is required can cause unintentional sample heating, which can be particularly problematic for biological samples.

Opportunities

A consequence of the lack of current databases of spectra is that there is a real opportunity for a good library of clinically relevant Raman spectra to be created. This could be linked with cloud-based artificial intelligence for analysis of data, with the result that the technique could be used for the identification of very many target biomolecules. There are technical opportunities, including the development of surface-enhanced Raman spectroscopy and correlation of Raman data with other analysis techniques and tools such as genomics.

Threats

The main threat is around the time to clinical adoption of Raman spectroscopy. This is a result of the complexity of clinical samples and the need for good calibration and use standards to ensure the quality of data and the ability to effectively use databases of Raman spectra for analysis. In addition, there are significantly cheaper specific tests available for certain targets.

S	• Low consumable cost • Specificity • Little sample preparation • Insensitive to water • Able to choose length • Label-free and fast • Non-invasive • Spatial mapping	• High capital cost • Sensitivity • Small sample volume • Lack of databases of spectra for bio -molecules & standards for use • Sample heating • Largely surface analysis only	**W**
O	• Can measure many targets • Creation of bio databases • Correlative analysis with other methods • Surface enhancement • Cloud-based AI for spectrum analy -sis • Genomics link • Automation	• Cheaper specific tests • Good calibration required • Complexity of diluted samples • Clinical engagement • Time to adoption	**T**

FIGURE 1. SWOT ANALYSIS OF RAMAN SPECTROSCOPY FOR CLINICAL APPLICATIONS.

Roadmap

In the Roadmapping exercise, groups were asked to consider first the clinical opportunities that Raman spectroscopy might address, followed by the technological developments that would be required to achieve this and finally any other barriers or enablers to uptake. Groups were also asked to suggest a timescale for developments. Figure 2 shows a consolidated summary of the output from all of the groups.

Short Term (up to 2 years)

There was a clear feeling that Raman spectroscopy could be fairly quickly applied to a variety of fairly basic, but important, clinical scenarios. The detection of antimicrobial-resistant pathogens emerged alongside drug resistance in patient treatment, which is unsurprising as this was the initial focus of the workshop. However, it was also noted that a robust technique for the identification of sepsis is a high clinical priority, in particular the ability to differentiate between bacterial and viral forms of sepsis. Opportunities for Raman to be used in more broad disease classification was also noted.

To achieve this, there is a need to develop reference materials and calibration techniques for Raman spectroscopy to allow confidence in the quantitative output from instrumentation. There are also no standards currently for how clinical samples should be prepared for analysis, and these are also urgently required if there is to be clinical confidence in the results obtained.

Other issues that were highlighted in the short term follow on from the basic technology developments. These include the development of standards for how tests using Raman spectroscopy are actually performed, and the agreement in the community of what quantitative measurement parameters should be used for the different specific biomolecular targets that each clinical scenario is focussed on detecting.

Finally, the critical issue of reimbursement was discussed. The development of clinical Raman tools ultimately requires industry investment, potentially both in capital cost of equipment and in the running of tests as a service. It was not clear how this would be reimbursed by the NHS.

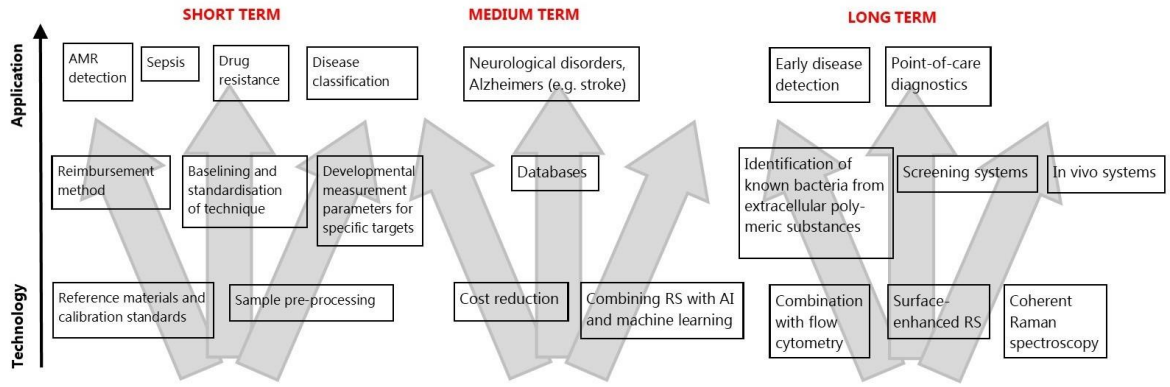

FIGURE 2. RESEARCH ROADMAP FOR THE CLINICAL APPLICATION OF RAMAN SPECTROSCOPY.

Medium Term (3 to 5 years)

In the clinical application of Raman spectroscopy, it was felt that this timescale should allow the development of a tool for detecting neurological disorders, such as Alzheimer's disease (where there are benefits of early detection) and strokes (where diagnosis can be difficult).

Key technological developments in this period should focus on reducing the cost of the Raman spectroscopy as a clinical tool. This was seen as essential to open up new application areas in the long-term. The spectra obtained from Raman spectroscopy of biomolecules are complex, and there was a strong feeling that artificial intelligence (AI) and machine learning (ML) could be applied to the interpretation to allow more complex problems to be addressed.

Associated with this is the need for comprehensive databases of Raman spectra of a diversity of biomolecules which could be used either by humans looking to match spectra, but also by AI and ML systems. It was not clear where ownership of such a database would lie (equipment manufacturers, NHS) or the cost model for accessing such a database.

Long Term (More than 5 years)

In the long term, it was felt that Raman spectroscopy could be applied to the rapidly growing area of point-of-care diagnostics, if the cost structure could be developed to allow this (see Medium Term). There were also opportunities in early disease detection, including conditions such as cancer. Early detection is seen as a national priority for the future of the NHS to reduce cost of treatment and improve quality of life in an ageing population.

In this time scale, it should be possible to make some significant technological improvements to Raman spectroscopy. This includes combination with flow cytometry (which is an emerging

technique in its own right) and the development of both Surface-Enhanced Raman Spectroscopy (SERS) and Coherent Raman Spectroscopy for clinical applications.

Early detection of certain diseases could be made more likely if it becomes possible to identify bacteria from extracellular polymeric substances. It was also felt that by this time, Raman spectroscopy could be developed into a tool for screening of certain diseases. In-vivo systems might also be possible by this stage.

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