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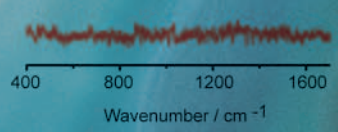

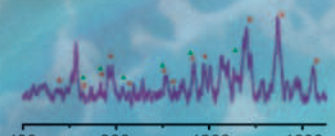

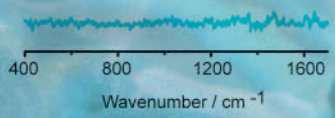

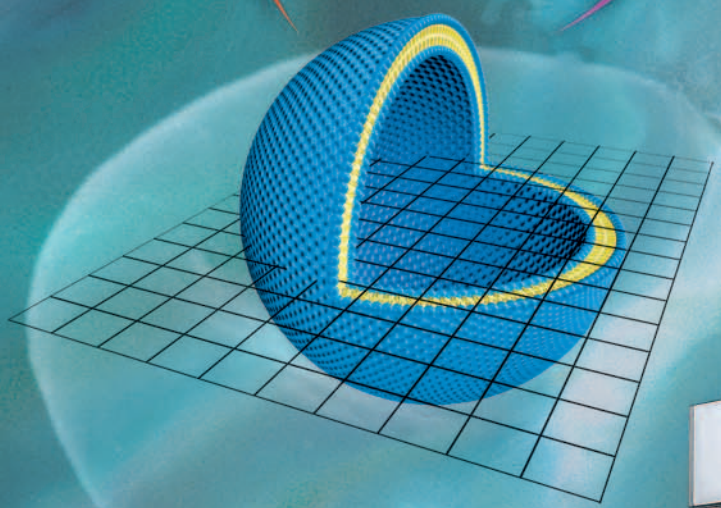

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HORIBA JOBIN YVON

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A New Dimension in Cell Imaging

Combining Chemical and Spatial Information

There are many microscopic imaging techniques used to investigate the complexities of cell structure, including optical microscopy, confocal laser scanning microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM). These techniques provide highly detailed images of the cells, but they fail to characterise the chemistry and composition of the sample under examination. Spectroscopic techniques, however, can do just that, and in particular Raman micro-spectroscopy is fast becoming established for probing cellular biochemistry on the micron scale.

Raman Spectroscopy

Raman spectroscopy is not a new technique, having been first described by C. V. Raman in 1928. Previously the domain of a small number of advanced Physics labs it is only in recent years that Raman has developed into a useful and wide ranging non-destructive analytical tool, providing a fast chemical fingerprint.

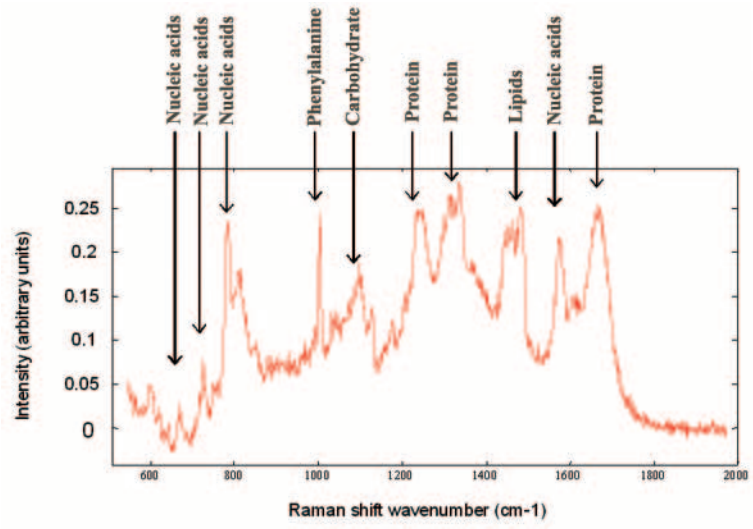
The technique measures the interaction of monochromatic laser light with chemical bonds within the sample, providing an information rich spectrum shedding light on the sample's molecular and structural make up. Since it is a technique using light irradiation, it can be adapted for use on a microscope - the development of the confocal Raman microscope now allows this detailed chemical information to be acquired with microscopic resolution. Thus, the chemistry of individual particles or cells can be quickly interrogated, and mapped images generated. These images contain full spectral content at each pixel, so that distribution of components can be visualised based upon their Raman spectrum. The arrival of instruments featuring inverted microscopes has taken the technique firmly into the realm of the biologist.

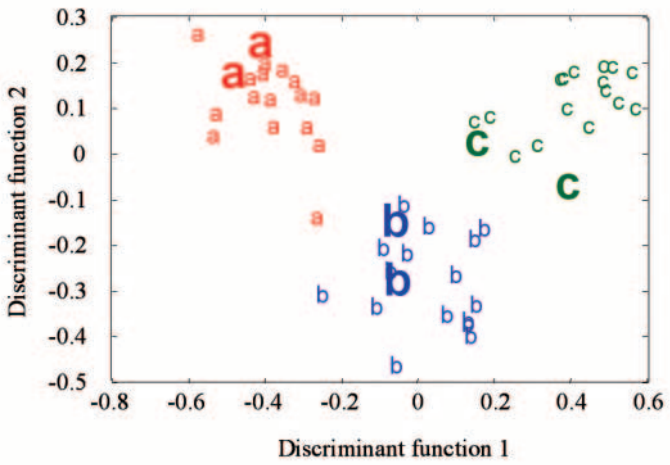
Fingerprinting bacteria

The chemical fingerprinting capability of Raman has been fully exploited by researchers at the Centre for Ecology and Hydrology (CEH) in Oxford, UK, who have used the confocal performance to analyse single bacteria cells [1].

Three bacterial species (Acinetobacter sp., ADP1 *E. coli* DH5α and Pseudomonas fluorescens SBW25) were studied. A typical Raman spectrum obtained from a single cell (in the order of 1–2 µm in size) is shown in Figure 1. Tentative assignment of the bands shows contribution from nucleic acids, carbohydrates, proteins and lipids – all of the major building blocks of cells. Multivariate analysis of data obtained from the three species gives excellent clustering, clearly indicating the power of Raman to distinguish the subtle physiological differences between species.

Further work by the CEH researchers has shown the ability of Raman microanalysis to distinguish growth stage, and to identify and quantify uptake of isotope labeled nutrients. Additionally, they have presented evidence for the application of Raman spectroscopy as a bioassay for pollutant bioavailability and toxicity [1, 2]. The ability to image these single cells using Raman (Fig. 1) has also been demonstrated, leading the way to future investigations of chemical distribution within individual bacteria.





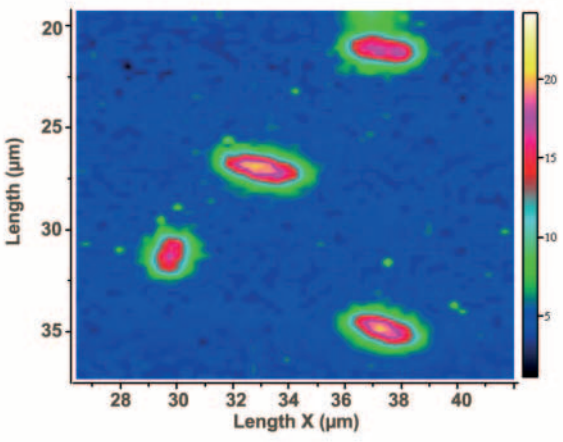


Fig. 1: (left) A typical Raman spectrum acquired from a single cell. (top right) Multivariate analysis of spectra from three bacterial species shows good discrimination between species. For each species letters in normal typeface indicate training data, and letters in bold typeface indicate testing data. (bottom right) A Raman mapped image of four bacteria cells.

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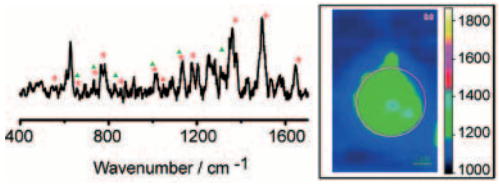

Fig. 2: SERS spectra from a lymphocyte incubated with both Rhodamine 6G and gold colloids. The image to the right is the intensity map of the central intracellular layer. Raman bands from rhodamine 6G (●) and intracellular components (▲) are labelled.

The potential interest for this type of bacteria analysis cannot be underestimated. Most methods of identification require single cells to be cultured, but it is estimated that more than 99 % of bacteria from the natural environment cannot be cultured. Raman microscopy, however, can assist in the fast identification of unculturable degraders of special pollutants, with a spectrum obtained in the matter of minutes.

Enhanced results

An interesting variant of Raman is the technique of surface enhanced Raman scattering (SERS), which can enhance spectral intensities by several orders of magnitude. The enhancement is provided through interaction of the sample with a suitably nanoscale roughened SERS active surface (typically gold or silver), and allows species present in extremely low concentrations to be identified within seconds.

Researchers from Chalmers University of Technology, AstraZeneca and Göteborg University in Sweden [3] used SERS to investigate the intracellular localisation of chemotherapeutic drugs in single living lymphocytes. Their aim was to more fully understand the properties, behaviour and localisation of these biomolecules, and thus improve on current disease treatment protocols. The lymphocyte cells were cultured in the presence of colloidal gold (to give the surface enhancement) and Rhodamine 6G (a model drug compound with the same pathways in the lymphocytes as anthracycline drugs such as doxorubicin and daunorubicin).

With incubation only with gold colloids (ie, no model drug compound) species such as thymine, adenine, guanine, tyrosine and phenylalanine were identified. With the analyte (Rhodamine 6G) introduced, both intracellular components and analyte could be observed in the spectrum (Fig. 2 and cover). Multivariate analysis allowed the intracellular and analyte components to be separated, despite significant spectral overlap.

This work represents just the beginning in what are likely to be key investigations into the intracellular activity of drugs, continuing the vital development of specific targeted treatments.

From cells to tissue

SERS is a technique which has also been used by Prof. Manfait and co-workers at the Université de Reims, France, for analysing the intracellular interactions of anti-cancer drug mitoxantrone [4].

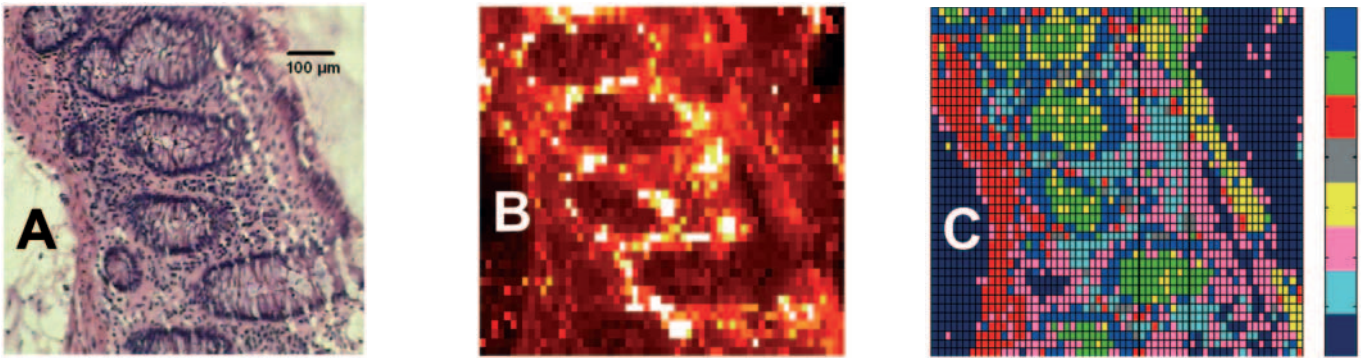

Fig. 3: Analysis results for human colonic tissue section (A) optical image of histopathologic HE stained tissue, (B) Raman mapped image illustrating intensity of the Amide I band, and (C) pseudo-colour Raman maps created by using K-means cluster analysis in which each cluster (consisting of similar spectra) is assigned to one colour

However, as well as studying these fundamental properties, work has also focused on disease diagnosis of tissue [5], where the ability of Raman to pick up even very subtle changes in tissue biochemistry allows healthy and diseased tissue to be accurately distinguished.

In Figure 3, Raman mapped imaging of healthy colonic tissue is illustrated. It is immediately clear that the visible structural features of the tissue coincide with the chemical information provided by the Raman. Cluster analysis of the Raman data allows spectra of similar profile to be grouped and displayed as a single unit, allowing specific tissue types to be located. This technique makes use of the large information content present even in just one spectrum, which will give an indication of the presence of the various chemicals present within the tissue, such as DNA, RNA, protein, lipid and carbohydrate. Comparison between data obtained for healthy and diseased tissue enables scientists to not only learn more on the biochemical changes caused by the cancer, but also categorise tissue according to disease state.

Conclusions

There are many fast optical imaging techniques available to the cell biologist which are widely used to analyse biological specimens. Emerging micro-spectroscopic techniques such as Raman microscopy open up an exciting new dimension for imaging, by combining spatial and spectroscopic information.

Through the ability to investigate real cellular biochemistry it is possible to rapidly learn more about chemical processes, interactions, and behaviour, down to the single cell level.

References

[1]Huang W. E. *et al.*: Analytical Chemistry 76, 4452 (2004)

[2]Singer A. C. *et al.*: Journal of Microbiological Methods 60, 417–422 (2005)

[3]Eliasson C. *et al.*: Spectrochimica Acta A, 61, 755–760 (2005)

[4]Nabiev I. *et al.*: Journal of Physical Chemistry 99, 1608–1613 (1995)

[5]Manfait M. *et al.*: Biomedical Spectroscopy, Mahadevan-Jansen A. and Pupppels G. J., Editors, SPIE 3918, 153–160 (2000)

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