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Application Note

Biology
Life Sciences
Raman

RA06

Raman Analysis of Single Bacteria Cells

Traditionally, Raman has been a technique of the material scientist, physicist or chemist, but as instrumentation continues to evolve, the power of Raman in biological and medical applications is fast being realised, not least because of the high information content provided and an excellent tolerance for water.

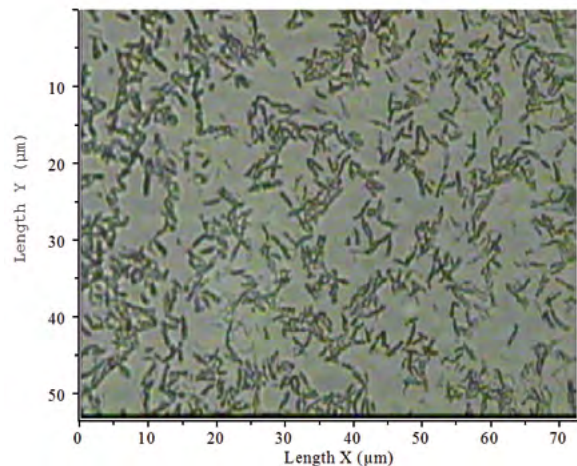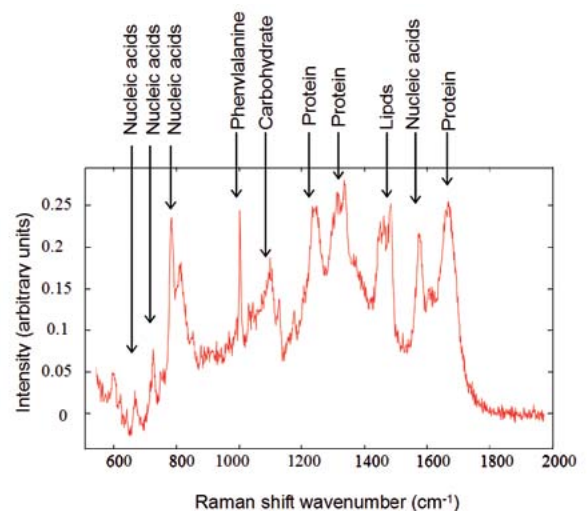*Figure 1: the LabRAM benchtop Raman system*

Figure 2: Above - white light image of bacteria cells obtained with 100x objective. Below - A typical Raman spectrum acquired from a single cell

The ability to identify microbes within a sample remains a significant challenge in environmental and medical research. Since many bacteria cannot be isolated and cultured, traditional analytical techniques based upon cultures present major drawbacks for bacteria analysts. The combination of Raman and microscopy in the confocal Raman microscope instrument such as the LabRAM allows even discrete individual bacteria cells to be successfully analysed in a matter of minutes – without the need for culturing.

Bacteria Analysis

Previous research has shown that Raman microscopy provides a facile method for analysing single bacteria cells.[1] Developing this, the work outlined in this application note involves not only analysing single bacteria cells, but in using the resulting spectra to identify specific bacterial species.[2]

Figure 2 below shows the ease with which single cells can be located with the microscope, yielding a good quality Raman spectrum (acquired with ~8 mW 532nm laser excitation). Tentative assignment of the bands shows contribution from nucleic acids, carbohydrates, proteins and lipids – all of the major building blocks of cells.

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Multivariate Analysis

The large amount of information present in the Raman spectrum creates problems for analysis – each spectrum (see Figure 2 above) comprises overlapping elements from a number of different components. Spectra obtained from individual cells of the same species, and of different species, all show numerous subtle changes, too many to be distinguished by eye.

However, multivariate techniques including principal components analysis (PCA), discriminant functional analysis (DFA) and hierarchical cluster analysis (HCA) allow these many subtle spectral differences to be clarified and used to classify cell types.

Discrimination of Species

Three bacterial species (*Acinetobacter sp.*, ADP1 *E. coli* DH5α and *Pseudomonas fluorescens* SBW25) were studied, with cells taken from three distinct growth stages for each (corresponding to incubations of 4h, 8h, and 22h). Initial multivariate analysis (Figure 3) showed excellent clustering for the three bacterial species, clearing indicating the power of Raman to distinguish the physiological differences between cells of different species.

It is interesting to note that this experiment showed the clustering to be robust to species phenotypic differences, despite temporal differences in cellular physiology during the phases of growth.

This demonstrates that whatever growth stage a particular cell is in, Raman provides a facile method for species identification.

a *Acinetobacter sp*
b *ADP1 E.coli DH5α*
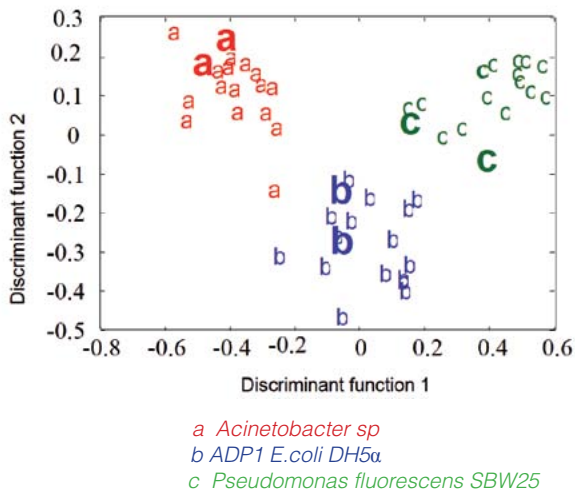c *Pseudomonas fluorescens SBW25*

Figure 3: 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.
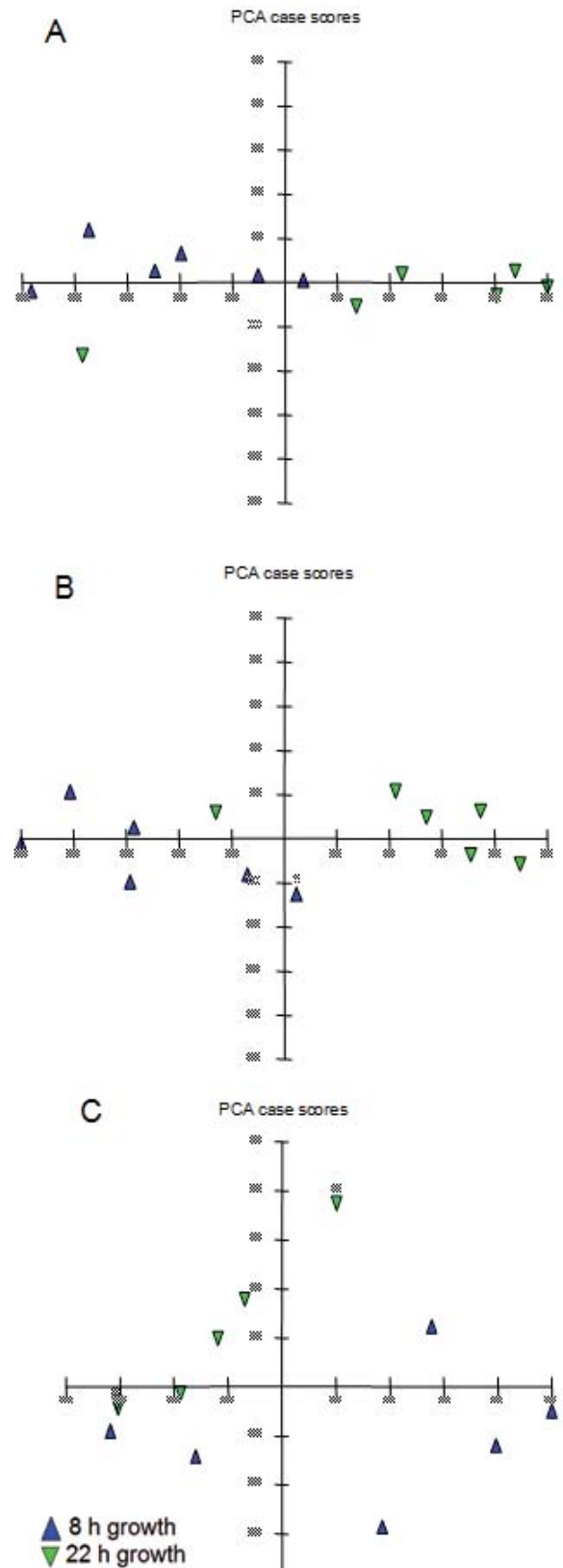

Figure 4: PCA analysis of exponential (8h) and stationary phases (22h) of Acinetobacter sp. ADP1 (A), E.coli DH5α (B) and Pseudomonas fluorescens SBW25 (C).

Discrimination of Growth Phases

Separate PCA analysis of spectra from one species highlighted changes in the relative concentrations of cellular components in the different growth phases (4h, 8h and 22h incubations). As illustrated in Figure 4, for all three strains there was some degree of separation between the exponential (8h) and stationary (22h) growth phases. Analysis into the cause of the differentiation revealed that the ratio of RNA to protein, lipid and carbohydrate was higher in the exponential phase than in the stationary phase.

Raman shift caused by ^{13}C addition

Raman spectra of *P. Fluorescens* SBW25 grown in 100% $^{13}C_6$ glucose revealed a number of peaks shifted to lower wavenumber compared with samples grown in 100% ^{12}C (Figure 5). PCA analysis revealed a trend from low to high percentage of ^{13}C-glucose on the second principal component axis. Further analysis showed that the major contributors to the observed spectral differentiation were the protein and nucleic acid signals – more than likely to be due to the fact that these two constituents represent ~75% of the total cellular biomass.
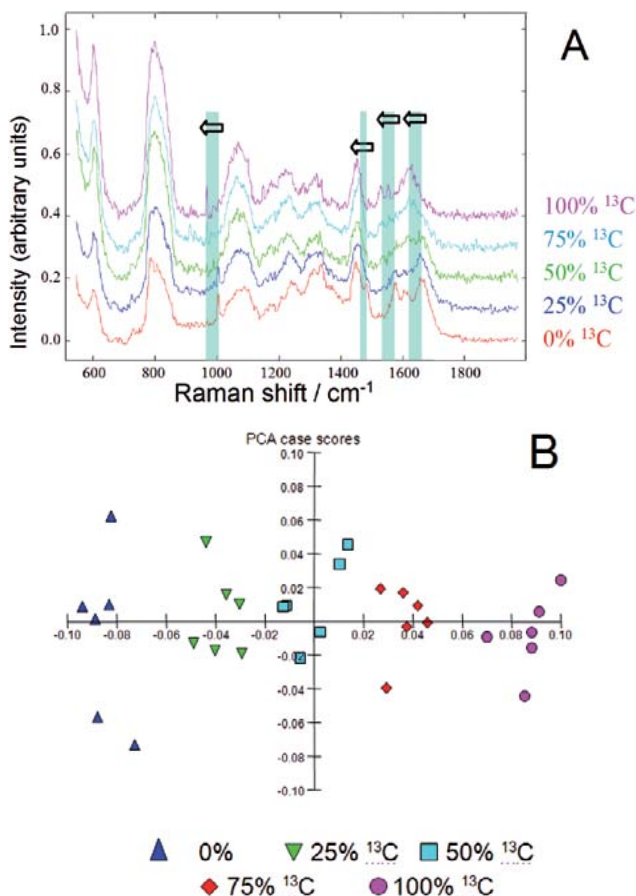
Figure 5: (A) Wavenumber shifts apparent with increasing percentages of ^{13}C (B) PCA analysis of spectra of P. Fluorescens SBW25 incubated with different percentages of $^{13}C_6$-glucose

Raman Mapping of Bacteria Cells

The true confocal operation of the LabRAM Raman microscope allows excellent spatial discrimination to be achieved – an important consideration when analysing single bacteria cells in the order of 1-2 µm in size.

Mapping experiments (Figure 6) demonstrate that even single bacteria cells can be clearly imaged despite their small size.
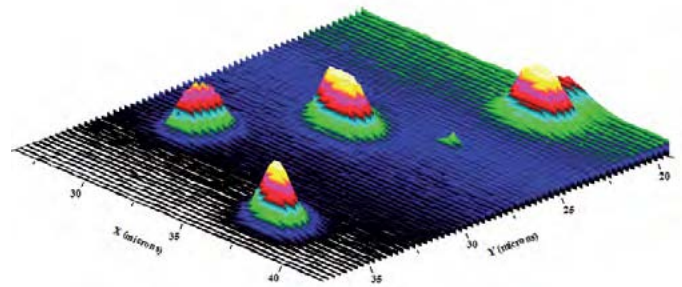
Figure 6: Raman mapped image of bacteria cells.

Conclusions

Raman microscopy has been used to interrogate single bacteria cells – with multivariate analysis it has been shown that different species and growth phases can be quickly distinguished. Cells grown with differing amounts of ^{13}C enriched nutrients showed Raman bands shifted to lower wavenumber due to the heavy isotope. With further multivariate analysis the cells can be classified according to the percentage uptake of ^{13}C.

Acknowledgements

Key References

1. K. Christian Schuster, Ingo Reese, Eva Urlaub, J. Richard Gapes, and Bernhard Lendl, Multidimensional Information on the Chemical Composition of Single Bacterial Cells by Confocal Raman Microspectroscopy, Anal. Chem., 2000, 72, 5529

2. Wei E. Huang, Robert I. Griffiths, Ian P.Thompson, Mark J. Bailey, and Andrew S. Whiteley, Raman Microscopic Analysis of Single Microbial Cells, Anal. Chem., 2004, 76, 4452

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