SERS Analysis of single living lymphocytes

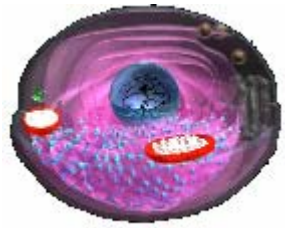
Introduction

Raman microscopy is a technique which has proven highly valuable in a wide range of applications. Within biomedical research, its relevance to highly selective identification of biomolecules including amino acids and proteins, DNA/RNA and chemotherapeutic drugs is fast being realised. However, the efficiency of Raman scattering is low, and collection times can be long, and detection limits high.

Figure 1: the LabRAM INV

Surface enhanced Raman spectroscopy (SERS) can enhance the scattering cross section by several orders of magnitude, allowing species present in very low concentrations to be identified within seconds.

The properties and behaviour of many biomolecules, including a variety of chemotherapeutic drugs, are of strong interest in order to manage and improve disease treatment protocols. Knowledge of the active concentration and location (within the cell) of analytes would greatly improve cancer therapy, since if drugs are administered in high dosage the cellular response is to increase extrusion of drugs from within the cell.



The work outlined within this application note has applied SERS analysis to the identification and localisation of Rhodamine 6G, which, despite not being a chemotherapeutic drug, has the same pathway and intracellular destination as many anthracycline chemotherapeutic drugs, such as doxorubicin and daunorubicin.

Experimental

Lymphocyte cells isolated from blood obtained from healthy donors were washed, centrifuged and seeded out. Cultivation took place in DMEM, with addition of colloidal gold (60 nm in diameter) and Rhodamine 6G (1mM). Immediately prior to analysis any remains of colloids and analyte that did not enter the cells during cultivation were removed, and the cells washed. The cells were analysed in phosphate buffered saline (PBS) at pH 7.4

Raman analysis was carried out on the LabRAM INV microscope system, with 633 nm (5.4 mW) and 785 nm (1.5 mW) laser excitation sources. A typical collection time of 1 s was used to acquire good quality SERS spectra. Mapping was carried out over a 20 µm x 20 µm region with 1 µm step. The mapping was repeated at three depths, 5 µm apart, thus creating a full XYZ Raman image.

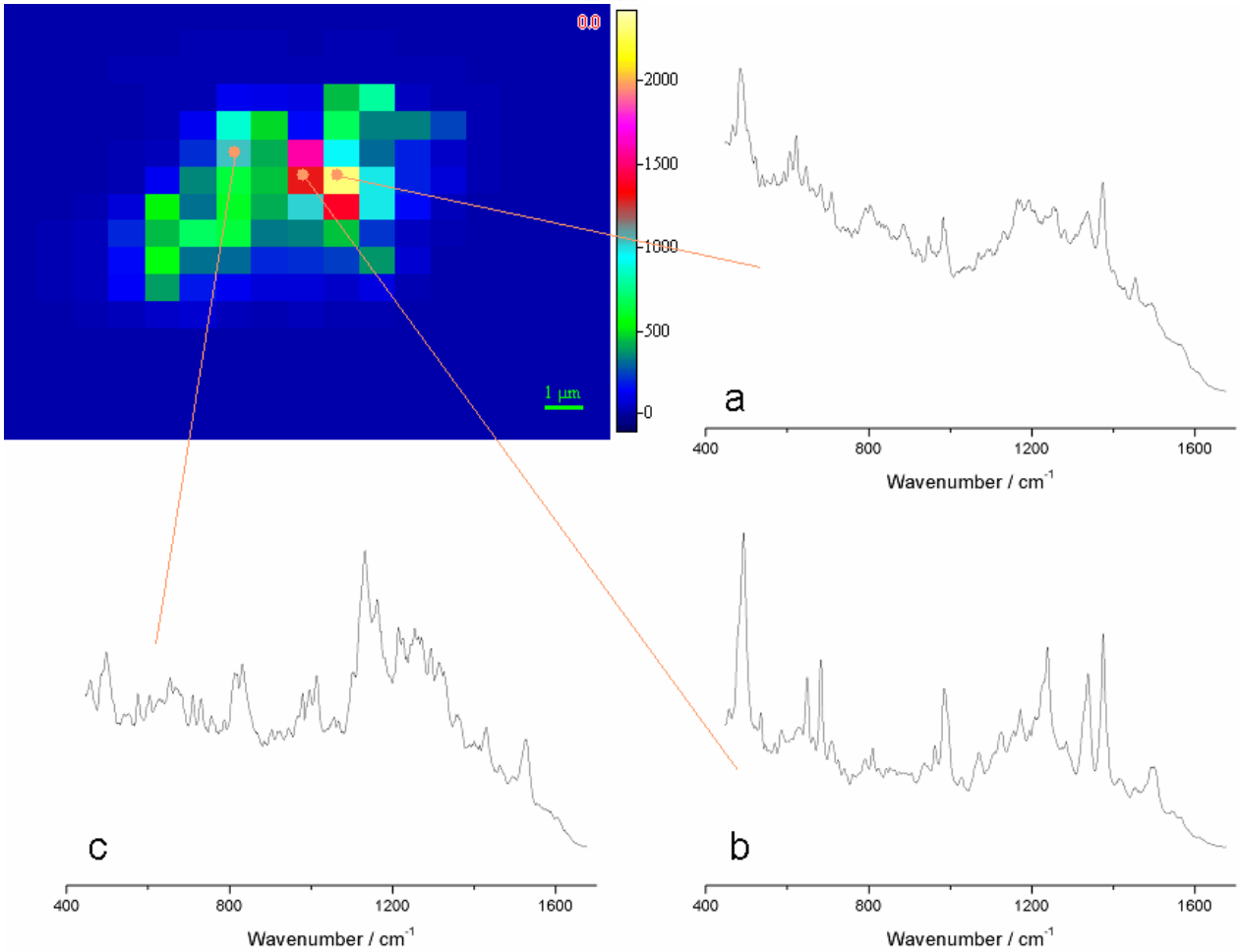


***Figure 2**: A surface enhanced Raman image of a lymphocyte incubated only with gold colloids. The mapped area represents the middle layer from within the cellular volume and the colour scale represents the sum of intensities of all measured wavenumbers.*

Results

Initial measurements were made on lymphocytes which had been incubated solely with gold colloids (see Figure 2). Observed spectral features differed across the cell, but species such as the nucleotides thymine, adenine and guanine, and amino acids tyrosine and phenylalanine can be identified.

With successful intracellular SERS analysis demonstrated, the second step was to examine the feasibility of simultaneously detecting an introduced analyte with the intracellular components.

Mapped images from above and below the cell showed no, or very weak, spectral bands from either native components or rhodamine 6G (see Figure 3 on following page. In the central mapping region surface enhanced signal from both of these groups could be identified, including the xanthene ring deformation bands (610 cm-1 and 770 cm-1) as well as the intense bands due to ring stretching (1360 cm-1, 1510 cm-1, 1650 cm-1).

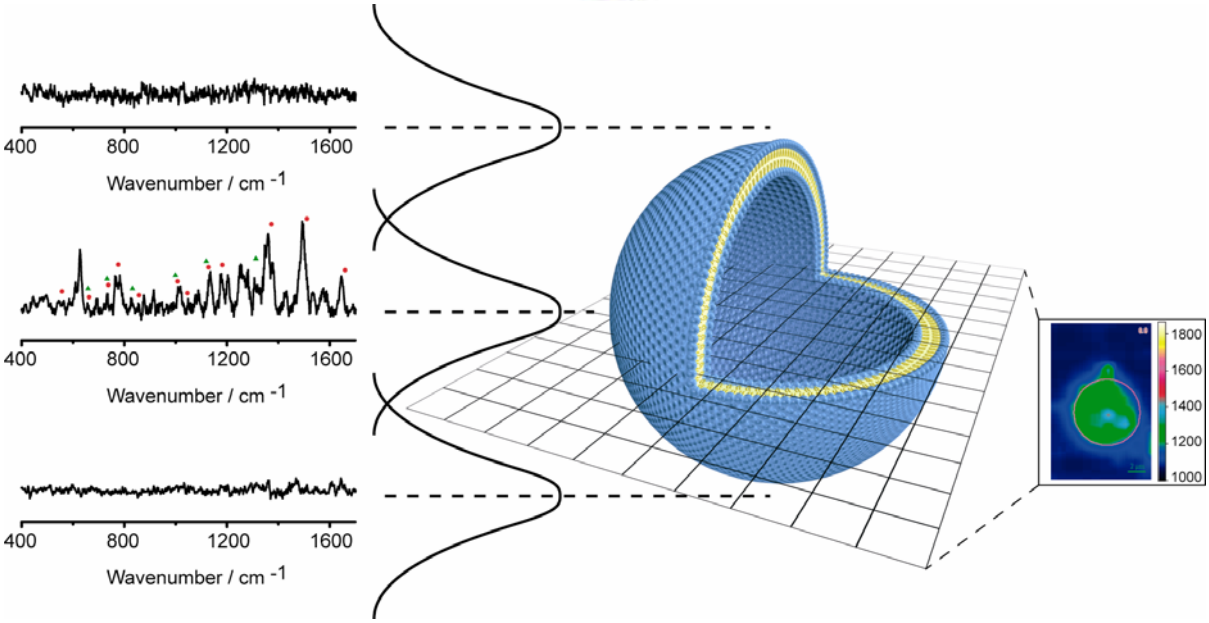


***Figure 3**: Surface enhanced Raman spectra from three different layers, 5 µm apart, of a lymphocyte incubated with both rhodamine 6G and colloids, where the middle layer was within the cell. The image to the right is the intensity map of the intracellular layer. A spectrum from this image contains Raman bands from rhodamine 6G (●), and contribution from intracellular components (▲)*

Multivariate analysis of the results from a number of mapping experiments allowed the signal of rhodamine 6G to be extracted from the image spectra. Principal Components Analysis (PCA) made it possible to separate covariant features (for example, Raman spectra of different compounds). Two principal components were calculated, which together explained over 90% of the total variation. The first principal component (pc1) contained a mean variation in the data set, whilst the second component (pc2) expressed the variation of the rhodamine 6G content in the cell.

A score plot (see Figure 4) showed that spectra could be divided into three classes, corresponding to rhodamine 6G and intracellular components (class 1) and intracellular components only (class 3). Class 2, in the middle of the score plot, contained spectra without any spectral band information.

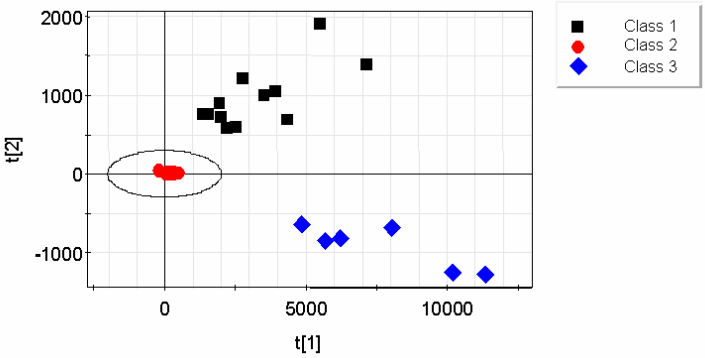


***Figure 4**: A score plot of the first two principal components of the PCA model based on the mapped lymphocyte in Figure 2. Class 1 - rhodamine 6G and intracellular components, class 2 - no spectral information, class 3 - only intracellular components.*

The loading vector of pc2 contains spectral features of rhodamine 6G over the whole spectral range (see Figure 5a). A reference spectrum of rhodamine 6G (Figure 5b) is inserted for comparison, with the main spectral features marked.

Intracellular contributions were found in later principal components, which can be viewed simultaneously when looking at the residuals after two principal components. Within the residuals, spectral contributions from DNA and nucleotides are clearly visible. Importantly, there is no contribution from rhodamine 6G, suggesting that multivariate quantification of the analyte may be feasible using just the second principal component.

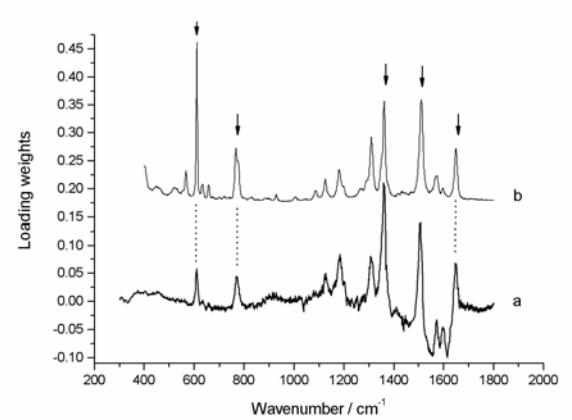


***Figure 5**: The loading vector of the second principal component (a) of the PCA model. A reference spectrum of rhodamine 6G (b) is included for comparison*

Conclusions

It has been shown that the analyte rhodamine 6G can be successfully measured and identified within single living cells using surface enhanced Raman microscopy. Principal components analysis for covariant features in the spectra allowed the identification of rhodamine 6G despite extensive spectral overlap from the cellular matrix.

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Key Reference

Charlotte Eliasson, Anders Lorén, Johans Engelbrektsson, Mats Josefson, Jonas Abrahamsson and Katarina Abrahamsson, Surface-Enhanced Raman Scattering Imaging of Single Living Lymphocytes with Multivariate Evaluation, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2005, **61**, 755-760

HORIBA JOBIN YVON

France : HORIBA Jobin Yvon S.A.S., 231 rue de Lille, 59650 Villeneuve d'Ascq. Tel : +33 (0)3 20 59 18 00, Fax : +33 (0)3 20 59 18 08. Email : raman@jobinyvon.fr www.jobinyvon.fr
USA : HORIBA Jobin Yvon Inc., 3880 Park Avenue, Edison, NJ 08820-3012. Tel : +1-732-494-8660, Fax : +1-732-549-2571. Email : raman@jobinyvon.com www.jobinyvon.com
Japan : HORIBA Ltd., JY Optical Sales Dept., 1-7-8 Higashi-kanda, Chiyoda-ku, Tokyo 101-0031. Tel: +81 (0)3 3861 8231, Fax: +81 (0)3 3861 8259. Email: raman@horiba.com
Germany: +49 (0) 6251 84 75-0 **Italy:** +39 02 57603050 **UK:** +44 (0)20 8204 8142
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