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Application Note

Biology
Life Sciences
Raman

RA08

SERS for Intracellular Imaging

The non-destructive nature of Raman spectroscopy coupled with its high information content can provide scientists with essential information to answer fundamental questions concerning intracellular pharmacokinetics:

- **drug location**
- **intracellular kinetics**
- **the nature of interaction between drugs and their pharmacological targets**
- **drug concentration in subcellular regions**

By coupling Raman spectroscopy with microscopy, it is possible to obtain full spectral information with a spatial resolution in the order of 1 µm. However, Raman is a weak phenomenon, and its sensitivity is not always ideal, particularly when analysing what are often relatively low concentration of intracellular components.

Surface Enhanced Raman Scatter (SERS)

Despite Raman scatter's inherent low sensitivity, it is possible to increase sensitivity by using resonance and surface enhancement effects. Increases in sensitivity can be by many orders of magnitude, improving from 10^{-3}M for spontaneous (normal) Raman, to 10^{-5}M for resonance Raman, and up to 10^{-12}M for surface enhanced Raman scatter (SERS). Common metals used for SERS include gold and silver, and these can either be used in the form of a nano-scale roughened surface onto which the sample is adsorbed, or as a colloid suspension

Intracellular Measurements

In the work described here, cells have been incubated with a silver colloid in order to provide SERS enhancement within the cell, and the subsequent Raman spectroscopy has allowed the distribution of intracellular drug components to be analysed.

Comparison has also been made with bulk solutions, in order to confirm that adsorption onto the metal has no effect on the Raman spectrum other than enhancement.

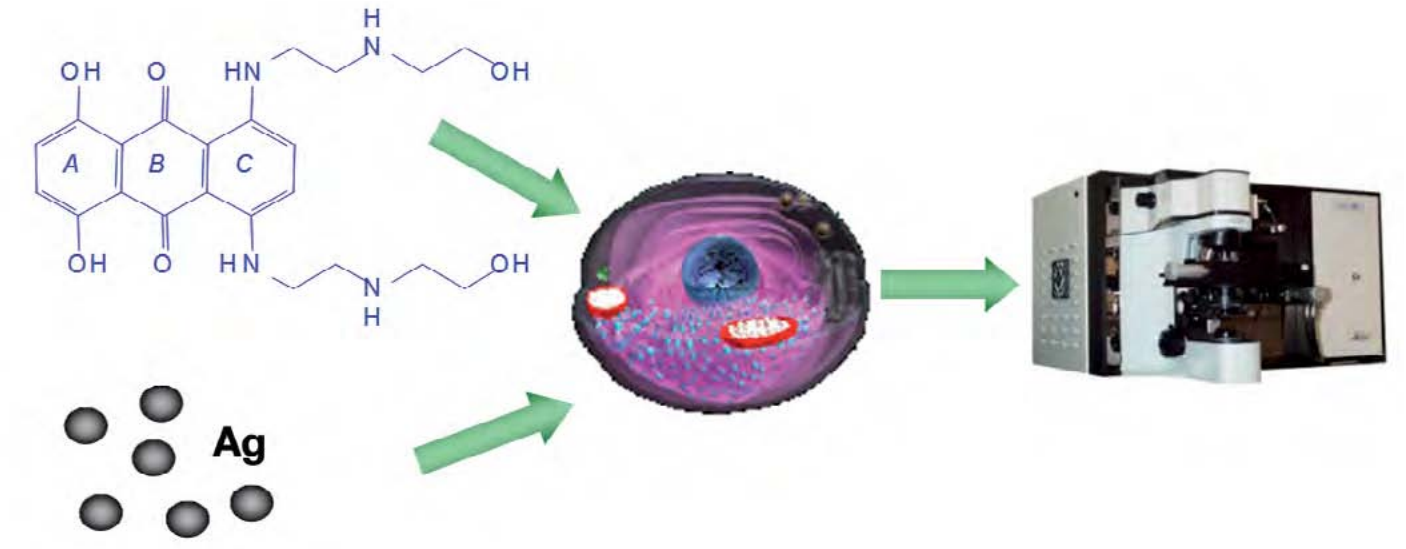

Figure 1: Schematic of intracellular SERS experiment showing the chemical structure of mitoxantrone

The drug studied here is mitoxantrone (Figure 1), which is an anthraquinone anticancer drug structurally related to DNA-anthracycline antibiotics. It is extremely potent, and has been used to treat many types of cancer.

Initial work focussed on analysing the drug in solution, in order to obtain reference spectra.

The experimental conditions used allowed adsorption to the SERS agent without detectable perturbations of the drug (MXT) or of the molecular interactions within the drug/DNA complex (MXT+ctDNA). On the basis of these experiments and on band assignments by comparing results obtained through resonance Raman and SERS measurements, it has been possible to infer a preferential intercalation of rings A and B of the chromophore with the DNA double helix, while ring C remains outside (Figure 2).

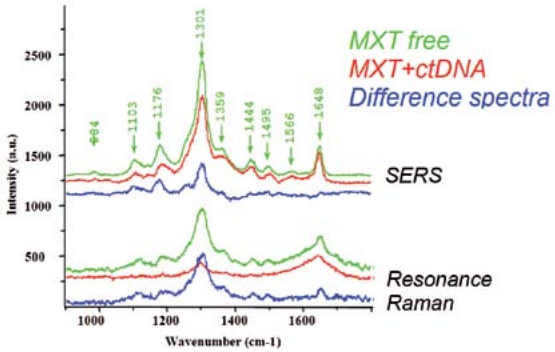

Figure 2: Comparative SERS and resonance Raman spectra of model complexes in solution obtained with 514.5nm excitation. Data have been offset for clarity.

The 1300 cm^{-1} band is the strongest feature in the spectrum, and has been assigned to the ring stretching mode coupled with C-O stretching motions. This band is very sensitive to the C-O group in ring A of the mitoxantrone molecule, and shows a decrease in intensity upon complexation. There is also evident a small shift in the ratio of intensity at 1308 and 1207 cm^{-1}.

To record a Raman mapped image from a single K562 cell, a group of cells were treated with 1 µM drug concentration for 1 hr at 37 °C and washed twice with buffer by centrifugation. The treated cells were then incubated with pre-aggregated silver colloid for 15 minutes. Aggregates which had not penetrated the cells were eliminated by successive washing in the buffer. After treatment of the cell population with the drug and incubation with colloids, an individual cell was selected under the microscope and a Raman mapped image was generated.

Comparative analysis of the spectra from model complexes in solution and within the cell confirms that data obtained from either *in vivo* or *in vitro* sampling is comparable. As the figure shows excellent signal to noise can be achieved for the intracellular measurements, on account of the surface enhancement (Figure 3).

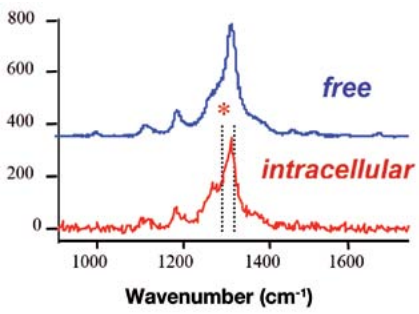

Figure 3: Spectra free and intracellular mitoxantrone. Data have been offset for clarity.

Note that the intracellular spectrum exhibits a decrease of the 1308/1270 cm^{-1} band ratio. A similar effect was also observed in the reference spectra of MXT+ctDNA obtained through *in vitro* measurements (see Figure 2), indicating the MXT analysed intracellularly to be bound rather than free.

The conventional white light image and the confocal Raman image of a treated cancer cell are shown below. The spectral image is constructed using the area of the band in the 1296-1306 cm^{-1} region.

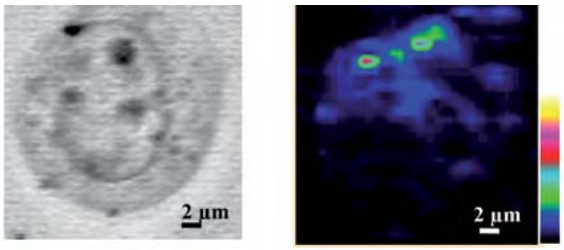

Figure 4: White light image (left) and Raman mapped image showing intensity at 1296-1306cm-1 (right)

The white light image clearly shows the presence of silver colloids (dark spots) inside the cell. In the spectral image, the regions of high intensity correspond to the regions where the colloids can be found in the cell.

Summary

Raman image has been obtained with confocal SERS microspectroscopy from a single, live K562 cancer cells treated with an anticancer drug mitoxantrone, at a concentration of 10-7 M. SERS spectra have been recorded from the inside of the cell in which silver colloidal particles have been introduced to generate the SERS effect.

The potential of confocal SERS microspectroscopic imaging at the single cell level is very important for the selective analysis of drugs inside these cells.

Acknowledgement

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