

Chemical Imaging without Dyeing

Confocal Raman Microscopy in Bio-Medical and Pharmaceutical Research

In the Life Sciences and Bio-medical Research, the localisation of chemical compounds within cells or tissues is one of the most important and difficult tasks. The development of novel pharmaceutical forms such as drug delivery coatings or capsules also requires as much information as possible about the chemical and structural composition of the new devices. An often employed technique for these kinds of studies is fluorescence microscopy, where the sample must be treated with specific dyes before the images can be acquired using a dye-specific excitation laser. Whenever such a staining is not appropriate, which often leads to the sample being useless for further studies, Confocal Raman Microscopy plays an important role in non-destructively imaging the chemical properties while requiring only minimal sample preparation, if any.



The Raman Effect and its Application in Microscopy

In Raman spectroscopy, a vibrational quantum state is excited within a molecule, leading to an energy shift between the incident light and the back-scattered light. This energy shift is unique to each molecule and allows the chemical identification of compounds within a sample. By integrating a sensitive Raman spectrometer within a state-of-the-art microscope setup, Raman microscopy with a spatial resolution down to 200 nm laterally and 500 nm vertically can be achieved using visible light excitation. With this setup, a complete Raman spec-

trum at each image pixel is acquired, typically taking between 50 and 100 ms and leading to Raman images consisting of tens of thousands of spectra. From this multi-spectrum file, an image is generated by integrating over a certain Raman line in all spectra or by evaluating the various peak properties such as peak-width, min/max analysis, or peak position. Due to the confocal arrangement, even depth profiling and 3-D imaging are possible if the sample is transparent.

Imaging of Living Cells

With the high resolution imaging of cells or tissues, sample preparation is often

complex and time consuming due to mounting, microtome cutting or staining procedures. As Confocal Raman Microscopy requires only minimal sample preparation, it is now possible to efficiently image the chemical properties of living cells in their physiological surroundings without inflicting damage. In this experiment, epithelial rat cells were investigated with the WITec CRM 200 Confocal Raman Microscope using a 60x water-immersion objective (Nikon, NA=1,0) and a 532 nm frequency-doubled Nd:Yag laser (10 mW). In total, 10,000 spectra were acquired at an acquisition time of 100 ms per spectrum. The scan range was 40 x 40 μm .

Fig. 1 shows one of the 10,000 spectra in which proteins and lipids can be identified by their characteristic Raman bands. For the generation of images, it is either possible to analyse dedicated peaks as described above, or to apply fit procedures using basis spectra of the chemical compounds of interest. Such basis spectra can be generated by acquiring the spectrum of a pure substance or by averaging the spectra of a specific area in the image using the integrated software package which additionally optimises the signal to noise ratio. Fig. 2 (left) shows three images obtained after different fit procedures corresponding to the mitochondriae (1), the endoplasmic reticulum (2), and the nucleoli (3) regions. Afterwards, these images were colour coded with the software and combined into one image (Fig. 2 left). Using this method, a "colour-labelled" image can be created without dyeing the sample, clearly showing the different parts of the

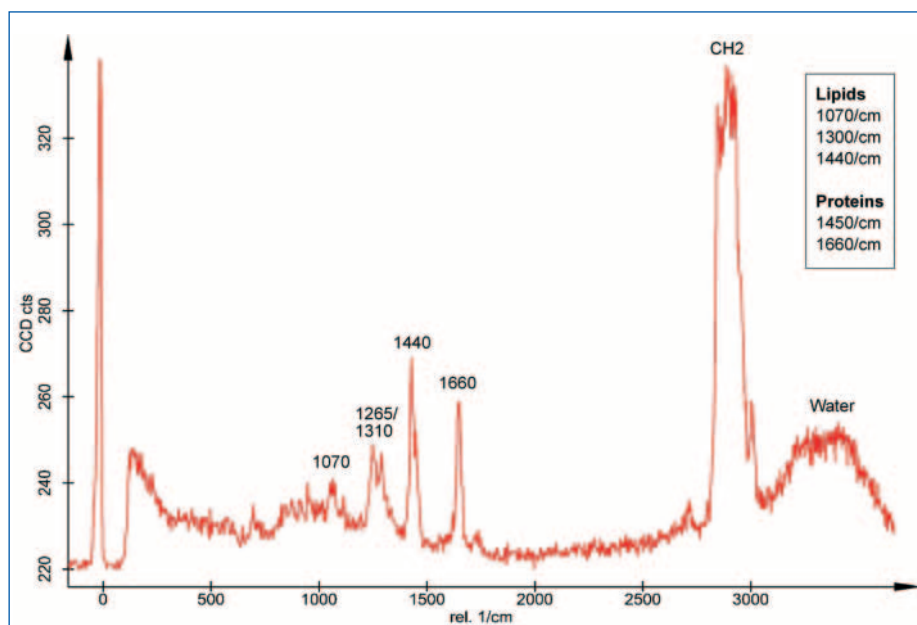


Fig. 1: One of the 10,000 Raman spectra of a cell with the cell-characteristic peak positions.

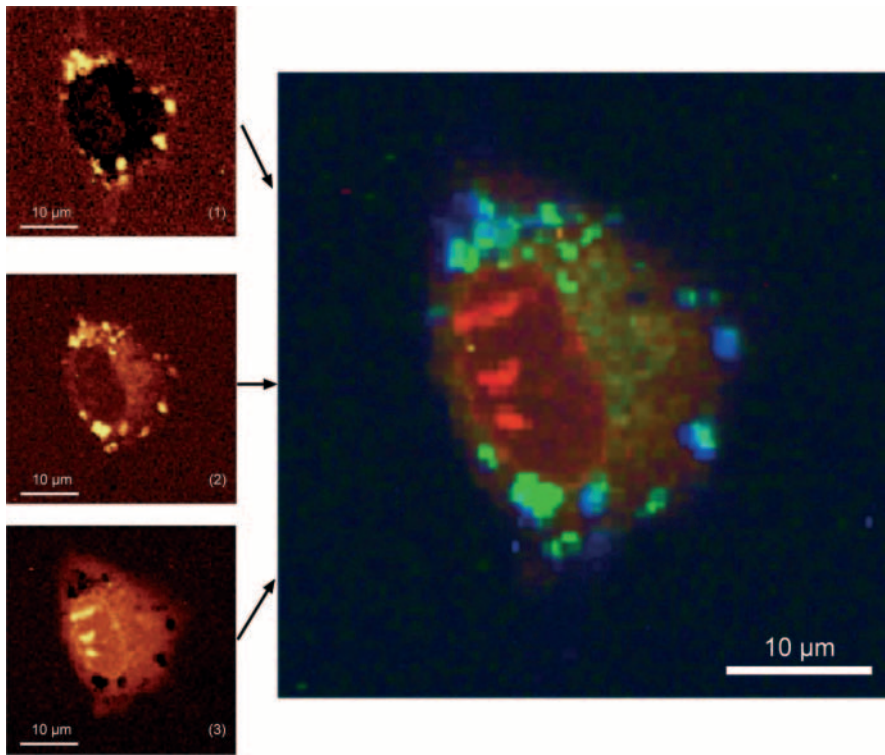


Fig. 2: Left: images obtained by fitting basis spectra to the measured spectra of the complete measurement. (1) Mitochondriae, (2) Endoplasmatic Reticulum, (3) Nucleoli.

Right: colour coded image of the different regions. In order to generate this image, it was not necessary to dye the sample.

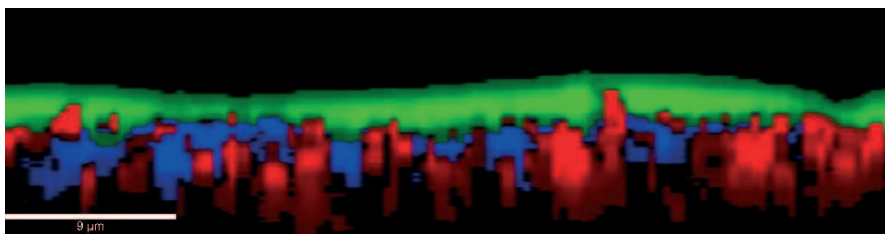


Fig. 3: Depth scan of a tablet coated with a thin film (green) for easier swallowing. The drug and the excipients are protected under the film (red and blue).

cell. In the future, the influence of physiological properties, metabolites or drug distributions in cells or tissues may play an increasingly vital role in the imaging of biological materials.

Pharmaceuticals

In pharmaceutical research, the imaging of drug distribution in various pharmaceutical forms is often used when developing new drug delivery systems. These can be, for example, drug delivery coatings, tablets, capsules, biomedical films or ointments. With its depth profiling capabilities, confocal Raman microscopy is an excellent tool for visualising the drug distribution in a variety of such substrates. Due to its high chemical sensitivity, even amorphous and crystalline drug components can be distinguished. The example in Fig. 3 shows a depth scan (x-z) of a capsule surface, showing

clearly that the tablet is coated with a 1.8 µm thick film to protect the drug and the excipients underneath and for easier swallowing.

Acknowledgement

Witec would like to thank Dr. A. Rück, Institute for Lasertechnology, Ulm, for providing the living cell sample.

Harald Fischer, Andrea Jauss

Witec GmbH

Hoervelsinger Weg 6 · 89081 Ulm · Germany

Tel. +49 700 94832 366 · Fax +49 700 94832 329

harald.fischer@witec.de · www.witec.de