

3D Confocal Raman Imaging of Transparent and Opaque Samples

U. Schmidt*, J. Yang**, W. Liu**, A. Jauss*, and T. Dieing*

* WITec GmbH, 89081 Ulm, Germany

** WITec Instruments, Maryville, TN 37804

Recently after its invention, confocal microscopy has been used to reconstruct three-dimensional images from micro-objects by using a spatial pinhole to eliminate out-of focus light in specimens that are thicker than the focal plane. Raman imaging benefits from the confocal setup, since it reduces the volume from which the Raman spectrum is collected, leading to a diffraction limited resolution in chemical imaging of samples [1-2]. The latest spectroscopic detector technology combined with a high-throughput confocal microscope recently allowed significant improvements in sensitivity allowing acquisition times for a single Raman spectrum to be reduced to 0.7 milliseconds [3]. Using such a sensitive setup can also be an advantage when performing measurements on delicate and precious samples requiring the lowest possible levels of excitation power. Time resolved investigations of fast dynamic processes can also benefit from such ultrafast spectral acquisition times.

In a first example, the ultrafast confocal Raman imaging capabilities of the alpha300 R were used to analyze an oil-alkane-water emulsion - a transparent sample - three dimensionally. In a volume of $30 \times 30 \times 11.5 \mu\text{m}^3$, 23 confocal Raman scans were acquired at different z-positions leading to 23 Raman images each consisting of 150×150 pixel (22 500 spectra). The total acquisition time for one image was 60 s resulting in 23 min for the acquisition of the complete stack (517 000 Raman spectra). Fig. 1 shows a 3D image of the distribution of the three compounds within the analyzed sample volume (green: oil, red: alkane, blue: water).

Confocal Raman imaging of rough opaque samples was thus far very challenging and time consuming, due to the inability to keep the samples in focus. The topographic Raman imaging method allows confocal Raman imaging guided by the surface topography obtained by an integrated profilometer. Large area topographic coordinates from the profilometer measurements can be precisely correlated with the large area confocal Raman imaging data. This allows true surface Raman imaging on heavily inclined or rough surfaces, with the true surface held in constant focus, while maintaining highest confocality. Fig. 2a shows the topography of a pharmaceutical tablet with a surface topography on the order of several hundred micrometer. In Fig. 2b the true surface Raman image is presented overlaid onto the 3D sample surface. This image shows the distribution of API (red) in the various excipients (green and blue color).

The aim of this contribution is to show how 3D Raman imaging can be applied in various fields of applications such as pharmaceuticals, biology, and material sciences and lead to a better understanding of microscopic samples.

References

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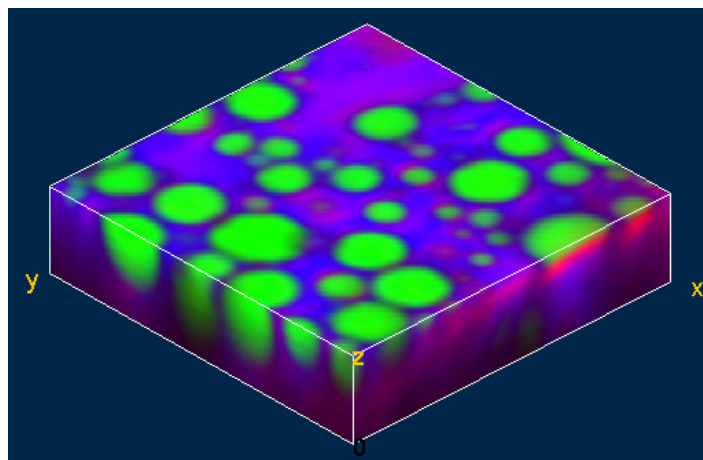


FIG. 1. Three dimensional reconstructed image of the distribution of the oil, alkane and water in a body lotion; green: oil, red: alkane, blue: water, $30 \times 30 \times 11.5 \mu\text{m}^3$ scan range, $150 \times 150 \times 23$ pixel, (517 500 spectra), total acquisition time of the stack: 23 minutes.

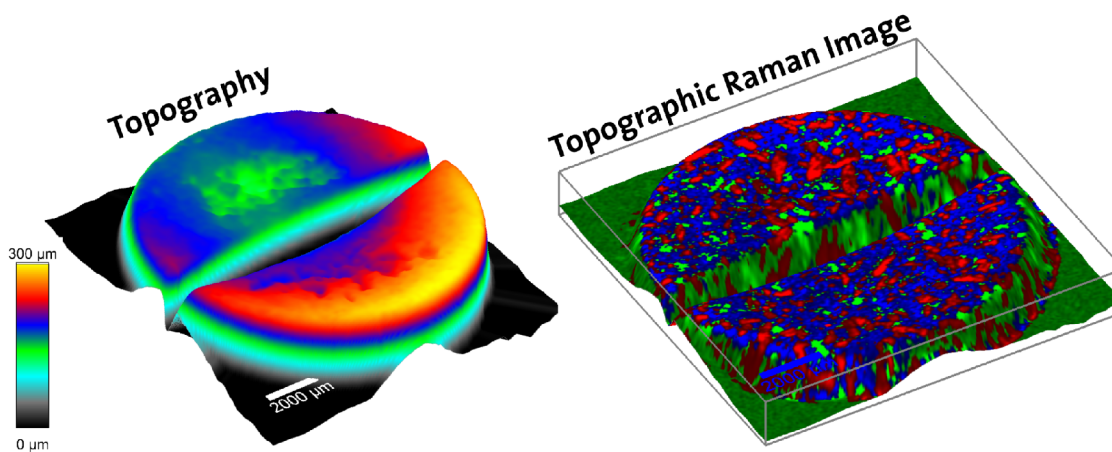


FIG. 2. Topography and true Surface Raman image of a pharmaceutical tablet.