Confocal Raman Microscopy:
True Surface and 3D Raman Imaging

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Abstract: Confocal Raman microscopy combines extremely sensitive Raman spectroscopy with high resolution optical microscopy, leading to chemical images with diffraction limited resolution. High confocality in Raman imaging always results in high focus sensitivity and this can make measurements difficult with rough inclined samples. Aim of this paper is to present solutions for true confocal Raman imaging on microscopic flat and inclined samples.

Introduction: Recently after its invention, confocal microscopy has been used to reconstruct three-dimensional images of micro-objects by using a spatial pinhole to eliminate out-of focus light in specimens thicker than the focal plane. Raman spectroscopy on the other hand is known to be used to unequivocally determine the chemical composition of a material. The confocal Raman microscope combines the chemically sensitive Raman spectroscopy with high resolution confocal optical microscopy. The discrimination of out of focus information used in confocal microscopy is particularly beneficial for confocal Raman imaging since it reduces the volume from which the Raman spectrum is collected. This leads to a diffraction limited resolution in chemical imaging of samples [1-2]. However, the high confocality always results in high focus sensitivity. Therefore, Confocal Raman imaging of rough opaque samples was so far very challenging and time consuming, due to the inability to keep the samples in focus.
The true surface confocal Raman imaging method combines confocal Raman imaging and optical profilometry techniques. An integrated profilometer is used to acquire topographic scans of several square millimeters, similar to very large AFM topographic images. The coordinates of this large topographic image are used to trace the surface contours while acquiring the confocal Raman image. Therefore, topographic and diffraction limited Raman images of heavily inclined and rough samples can be obtained in one instrument without further preparation.

Principle of operation of true surface confocal Raman imaging: Figure 1 shows the principle of operation of the topographic sensor. It uses the principle of chromatic aberration to record the surface topography of a sample. A white light point source is focused onto the sample with a hyperchromatic lens assembly, a lens system with a good point mapping capability, at a strong linear chromatic error. Every color has therefore a different focal distance. The light reflected from the sample is collected with the lens and focused through a pinhole onto a spectrometer. As only one specific color is in focus at the sample surface, only this light can pass through the confocal pinhole. The detected wavelength is therefore related to the surface topography. Scanning the sample in the XY plane (up to 50 x 100 mm) reveals a topographic map of the sample. This map can then be followed in a subsequent Raman image so that the Raman laser is always kept in focus with the sample surface (or at any distance below the surface). Depending on the type of sensor used, a lateral resolution of 10-25 µm and a vertical resolution of 40-120 nm can be achieved at a measurement range of 1-3 mm and a working distance of 10-16 mm.
The topographic coordinates from the profilometer measurement are used to perfectly follow the sample surface in confocal Raman imaging mode. The result is an image revealing chemical properties at the surface of the sample, even if this surface is rough or inclined.

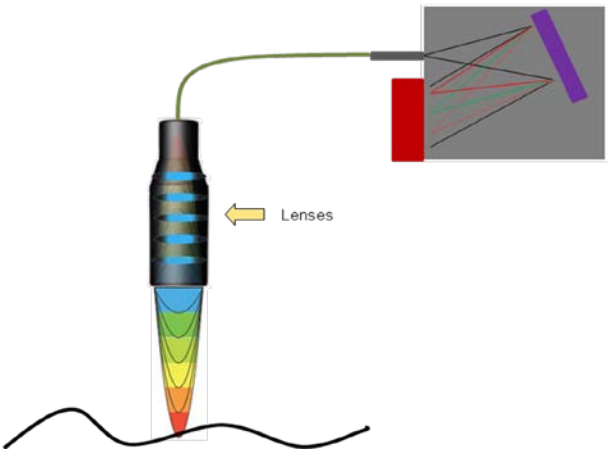

Fig. 1. Principle of operation of the topographic sensor (image adapted from: με (http://www.micro-epsilon.de)
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Measurement example: Fig. 2 (left) shows the topography of a pharmaceutical tablet with a surface roughness on the order of several hundred micrometers. The True Surface Raman image overlayed onto the 3D sample surface is presented in figure 2(right). This image shows the distribution of API (red) in the various excipients (green and blue color).

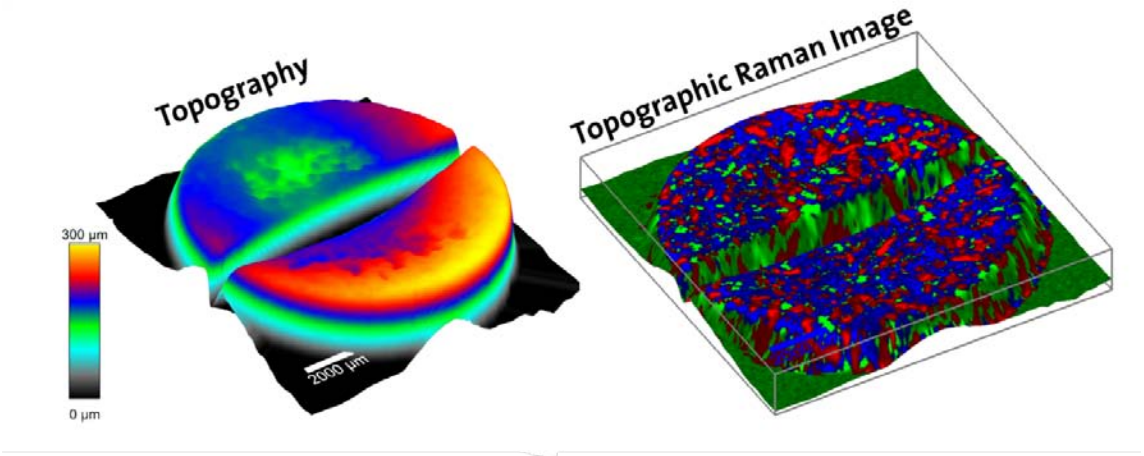

Fig. 2. Topography (left) and True Surface Raman image (right) of a pharmaceutical tablet.

Summary: The aim of this contribution is to explain the principle of the True Surface Confocal Raman Microscopy and to show its application in various fields of applications.

References

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