

# RISE Microscopy: Correlative Raman-SEM Imaging

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## Introduction

Scanning Electron Microscopy (SEM) is a well-established and common method for the analysis of sample surfaces in the nanometer range. However, the analyzing capabilities of SEM are limited to the surface structure. To overcome these limits, SEM is often combined with other analyzing techniques that are suitable for the detection of additional sample characteristics. An example of such an SEM attachment is EDX (Energy-dispersive X-ray spectroscopy), which provides information about the elemental composition of a sample. Although not as frequently used as the well-established EDX-SEM combination, Raman-SEM is becoming increasingly popular for comprehensive sample analysis. Raman spectroscopy can detect the molecular composition. Until now Raman-SEM microscopes enabled the acquisition of single Raman spectra at defined sample positions but did not facilitate the generation of confocal Raman images. By contrast the newly developed correlative RISE (Raman imaging and scanning electron) microscope enables for the first time the acquisition of SEM and confocal Raman images within a single instrument (Figure 1). This article gives an overview of this new correlative imaging technique and provides two example applications.

## Materials and Methods

**SEM** is a high-resolution surface imaging technique that can determine a sample's topography and morphology. In order to generate an image, a focused beam of high-energy electrons scans the sample. Through electron-sample interactions, backscattered and secondary electrons emerge and are detected. These electron signals are commonly used for imaging samples regarding their morphology and topography (secondary electrons) and their contrasts in composition (backscattered electrons). The lateral resolution can be down to about 1 nm [1].

**Confocal Raman imaging** is a spectroscopic technique for the analysis of molecular compounds within a sample. Raman spectroscopy can be used to study solid, liquid, and gaseous samples. The Raman effect is exhibited when light interacts with the chemical bonds in a sample. Vibrations in the chemical bonds cause a specific energy shift in the backscattered light. Those frequency shifts produce a material-specific Raman spectrum. Raman scattering is typically very weak, therefore optimized instrument components for maximum light transmission are essential to obtain high-quality Raman spectra. Besides the chemical components, Raman spectroscopy can measure additional sample characteristics such as the relative amount of a specific component, stress and strain states, or crystallinity. The Raman peak intensity provides information about the relative amount of a specific compound, a peak shift indicates stress and strain states, and the peak width

indicates the degree of crystallinity and allows the microscopist to distinguish between crystalline and amorphous materials [2, 3, 4].



**Figure 1:** The newly developed RISE microscope integrates a SEM and a confocal Raman imaging microscope within a single instrument. Image courtesy of Tescan.

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The confocal Raman imaging technique combines Raman spectroscopy with a confocal microscope. In confocal Raman imaging mode the sample is scanned point-by-point and line-by-line, and only information from the focal plane reaches the detector. In this way information about the spatial distribution of the chemical components within the sample is gathered and imaged. High-resolution confocal Raman microscopes acquire the information of a complete Raman spectrum at every image pixel and achieve a lateral resolution at the diffraction limit of the light employed. A confocal Raman microscope setup is further characterized by a good depth resolution, which facilitates 3D Raman images and depth profiles [5].

**Correlative Raman-SEM imaging** (RISE microscopy, WITec, Ulm, Germany and TESCAN ORSAY Holding, Brno, Czech Republic) combines an SEM and a confocal Raman microscope. The confocal Raman microscope is integrated into the vacuum chamber of the electron microscope. Non-destructive Raman and SEM measurements are consecutively performed at two different positions inside this chamber using an automatic transfer stage. Calibration of the sample position ensures scanning of the same sample area in both SEM and Raman modes. In Raman imaging mode a  $100\times/\text{NA}=0.75$  Zeiss objective is employed. With this objective the sample area is scanned during Raman image acquisition. Instrument control is carried out through the RISE software interface. Image post-processing as well as the correlation and overlay of SEM and Raman images are performed with the same software.

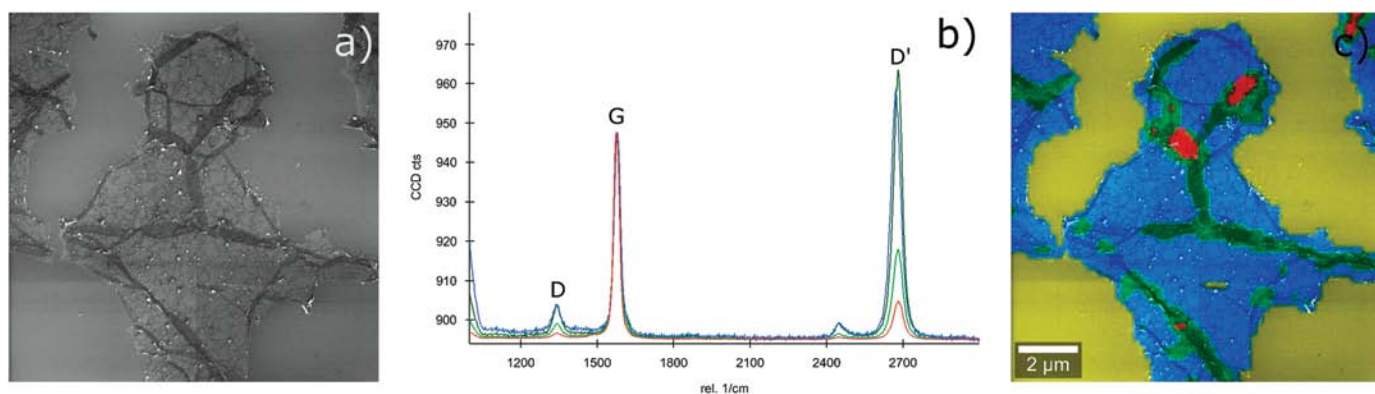
**Sample preparation.** A CVD (chemical vapor deposition) graphene film on a silicon wafer was mounted on a SEM stub and inserted in the SEM chamber for further examination in high-vacuum mode without any further preparation (no conductive carbon or gold coating was applied). Multi-layer graphene films have a thickness of only a few atomic layers of carbon. Such thin films can be imaged best in secondary electron SEM mode because secondary electrons have a low penetration depth resulting in a stronger detectable image contrast.

In a second sample, polymer solutions were prepared from poly-methyl metacrylate (PMMA) and poly-styrene (PS) by dissolving 10 mg of the polymer in 1 ml toluene. Blends were prepared by mixing 2 ml of the PMMA toluene solution and 1 ml of PS toluene solution. A droplet of the solution was placed

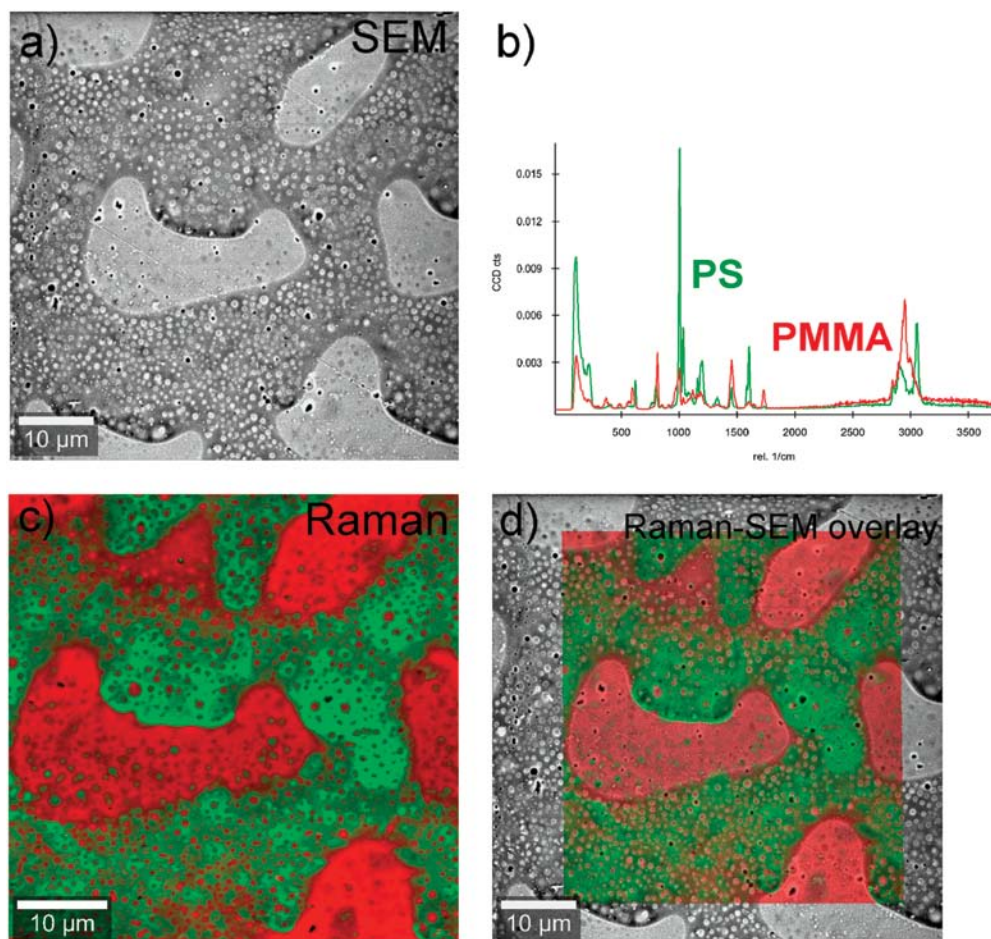
on a clean glass cover slide and dried in ambient conditions. A blend film with a thickness of several hundred nm is formed on the glass substrate after the evaporation of the solvent. The glass substrate with the blend film was mounted on a SEM stub and inserted in the SEM chamber without any further sample preparation or coating. To allow uncoated specimens for Raman analysis it is beneficial to measure the samples in low voltage SEM mode or gas atmosphere such as nitrogen. This is another difference to EDX analysis that needs relatively high voltage to excite the X-rays. The SEM analysis was performed by detecting the backscattered electrons. Backscattered electrons are largely elastically scattered, and heavier atoms deflect more electrons producing a stronger signal.

## Results and Discussion

**Investigation of a graphene film.** Graphene films are stable, thermally and electrically conductive molecules. These characteristics account for the great interest in graphene for many applications in electronics, photovoltaics, and the development of new materials. An area of  $12\times 12\ \mu\text{m}^2$  was first imaged in SEM mode. The graphene surface was analyzed, and defects such as wrinkles and stratifications were detected (Figure 2a). Further characterization of the film defects by EDX would not be beneficial because the detectable carbon X-ray signal from such thin films is not sufficient. The graphene film was analyzed instead in Raman imaging mode. A hyperspectral data set was generated containing the information of thousands of Raman spectra. The main spectral components of the data set were identified by the RISE software. The resulting Raman spectra provide information for a more detailed characterization of the graphene film: differences in the peak width and peak intensity can be observed among the spectra. Those spectral differences arise from the defects in the graphene film. On the basis of the differences in the D'-peak intensities of Figure 2b, several graphene layers can be distinguished from each other. In the corresponding color-coded Raman image, these layers are represented by different colors revealing areas with single-layered graphene (shown in blue) and areas with multi-layered graphene (shown in green and red). The silicon substrate is shown in yellow. The Raman image consists of  $90\times 90$  pixels yielding 8,100 Raman spectra,



**Figure 2:** RISE microscopy of a CVD graphene film in high vacuum. (a) Secondary electron SEM image of the graphene film revealing defects in the surface structure. (b) Main Raman spectra identified by differences in the peak width and intensity. (c) Corresponding color-coded, confocal Raman image. The colors show the graphene layers and wrinkles (blue indicates a single layer, whereas green and red areas are multi-layer). The silicon substrate is shown in yellow. Image parameters: 8,100 Raman spectra were collected in less than 7 minutes.



**Figure 3:** RISE microscopy of a PMMA-PS polymer blend in 20 Pa of nitrogen. (a) The SEM image of the polymer blend shows different surface topography within the sample. (b) Raman spectra for PS (green spectrum) and PMMA (red spectrum) can be clearly distinguished and identified. (c) The color-coded confocal Raman image reveals the distribution of the two polymers within the blend. Green: PS, red: PMMA. (d) Raman-SEM image overlay demonstrating the correlation between surface structure and the appearance of the two different polymers.

and the total acquisition time of the complete Raman image was less than 7 minutes. By overlaying the SEM and Raman images, information from both imaging techniques can be correlated (Figure 2c).

**Analysis of a PMMA-PS-polymer blend.** For the investigation of the polymer blend, some measurement parameters were adjusted to the sample's requirements. For the SEM analysis a small working distance to the sample (~ 8 mm), a pressure of nitrogen of 20 Pa, and an accelerating voltage of 10 kV were chosen. The SEM image reveals two separate areas distinguished by their topographical differences: parts of the blend had a smooth surface, whereas other parts exhibited a fine two-phase structure (Figure 3a). However, from the SEM image alone it was not possible to make a clear statement about the identity of the blend components. An EDX analysis would not distinguish the different phases because the same elements are present in each phase. However, when analyzed in confocal Raman imaging mode, the two polymers can be identified by their specific Raman "fingerprint." Thus two distinct spectra identify PMMA and PS (shown in red and green in Figure 3b, respectively). The hyperspectral Raman image with the same color-coding reveals the distribution of the two components

within the sample (Figure 3c). In some parts of the Raman image a mixture of the colors of the two polymers is apparent, although it is known that the polymers do not mingle. A reason for this could be that those areas contain relatively small polymer phases, which cannot be spatially resolved by the Raman imaging system. The confocal Raman microscope as part of the RISE microscope provides an experimentally proven lateral resolution around 360 nm and a depth resolution around 1.5 μm. As a consequence, the colors of the areas that contain particles much smaller than the spatial resolution appear mingled in the Raman image. The Raman image shows an area of 50 × 50 μm<sup>2</sup> and consists of 300 × 300 pixels resulting in 90,000 Raman spectra. The integration time was 50 ms per spectrum. The overlay of the SEM and the Raman images links the structural characteristics of the polymer blend with the appearance of PMMA and PS (Figure 3d).

## Conclusion

This article describes the RISE microscopy technique, a new imaging method for comprehensive analyses of structural and chemical sample characteristics.

Though well-established as stand-alone techniques, combined Raman-SEM analysis provides a novel capability by correlating the information from both measurements. Two example applications of RISE microscopy were demonstrated: graphene layers and a two-phase polymer. RISE microscopy provides new sample information and complements other well-established correlative SEM imaging techniques. Thus RISE microscopy is suited to applications in surface science, materials science, nanotechnology, polymer science, geo science, life science, and pharmaceutical research.

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