application note

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RISE Microscopy - Correlative Ultra-Structural SEM and Molecular Raman Imaging

RISE Microscopy is a novel correlative microscopy technique that combines SEM and confocal Raman Imaging. Through RISE Microscopy ultra-structural surface properties can be linked to molecular compound information. This unique combination opens up new dimensions for more comprehensive sample characterization.

RISE Microscope - Correlative Raman Imaging and Scanning Electron Microscopy

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a high-resolution 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, resulting in an image of the sample's topography, morphology, surface structure and materials orientation. The images are acquired with nano- or micrometer resolutions and magnification ranges of 10 – 10.000x. Due to the large depth of field, it is also possible to generate a 3D appearance of the surface. SEM is a non-destructive method which allows combinations with other imaging techniques.

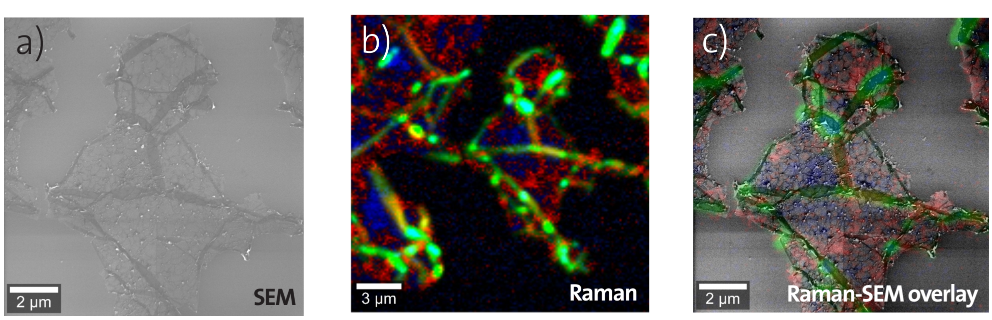
Confocal Raman Imaging

Confocal Raman Imaging is a non-destructive spectroscopic technique for the analysis of molecular compounds in a sample. The Raman effect is exhibited when light interacts with the chemical bonds in a material. Due to vibrations in the chemical bonds this interaction causes a specific energy shift in the back scattered light which appears in a unique Raman spectrum which can be detected. The confocal Raman Imaging technique combines Raman spectroscopy with a confocal microscope. Thus the spatial distribution of the chemical components within the sample are detected and imaged. Additional sample characteristics such as the relative amount of a specific component, stress and strain states, or crystallinity can be further analyzed. 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 (~200 nm). A confocal microscope setup is furthermore characterized by an excellent depth resolution and features the generation of 3D Raman images and depth profiles.

Correlative Raman Imaging and Scanning Electron (RISE) Microscopy

Correlative Raman Imaging and Scanning Electron (RISE) Microscopy combines the advantages of both imaging techniques within one instrument. In this way ultra-structural surface informationis linked to data regarding the sample's molecular compounds.
To switch between the different measuring techniques the sample is automatically transferred and re-positioned. The acquired results can be correlated and the images overlaid.

SEM is often combined with EDX (Energy-dispersive X-ray spectroscopy) to acquire additional information about the chemical characteristics of a sample. In comparison to EDX confocal Raman imaging has the benefit of being able to detect not only the chemical elements but also the molecules and chemical



Example RISE Microscopy application on graphene: a) SEM image of a graphene sample b) Color-coded confocal Raman image. The colors display the graphene layers and wrinkles. Image parameters: 100 µm x 100 µm, 150 x 150 pixels = 22,500 spectra, integration time: 0.08 s/spectrum c) SEM image overlaid with the confocal Raman image.

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compounds of a sample. Confocal Raman imaging reveals, for example, if a carbon atom belongs to diamond, graphite, graphene or if it is part of an organic molecule. Furthermore the spatial distribution of a sample's components can be imaged.

Application Examples of RISE Microscopy

A correlative Raman Imaging and Scanning Electron Microscope (RISE Microscope, TESCAN, Brno, Czech Republic; WITec, Ulm, Germany) was used to investigate a geological diorite sample. Due to the applied non-destructive RISE techniques it was not necessary to stain or fix the sample prior to the measurement procedure. The sample was first imaged with the SEM microscope function in order to analyze the surface structure (figure 1a). After the initial measurement the sample was automatically transferred and re-positioned for confocal Raman imaging within the vacuum chamber of the electron microscope. The same sample area was then recorded in Raman imaging mode. At each image pixel a complete Raman spectrum characterizing of the molecular composition was generated. The acquired SEM and color-coded Raman images were overlaid to compare the ultra-structure with the molecular components (figure 1b). In figure 1c the corresponding Raman spectra are displayed. Each chemical compound can be easily identified by its specific Raman "fingerprint".

In a second application example RISE Microscopy was performed on a microtome slide of hamster brain tissue. In the SEM image the white and gray brain matter can be distinguished by their structural differences. Overlaying the SEM with the color-coded Raman image reveals a more precise view of the distribution of the gray and white matter (figure 2a). Both tissues can be clearly distinguished by their typical Raman spectra (figure 2b).

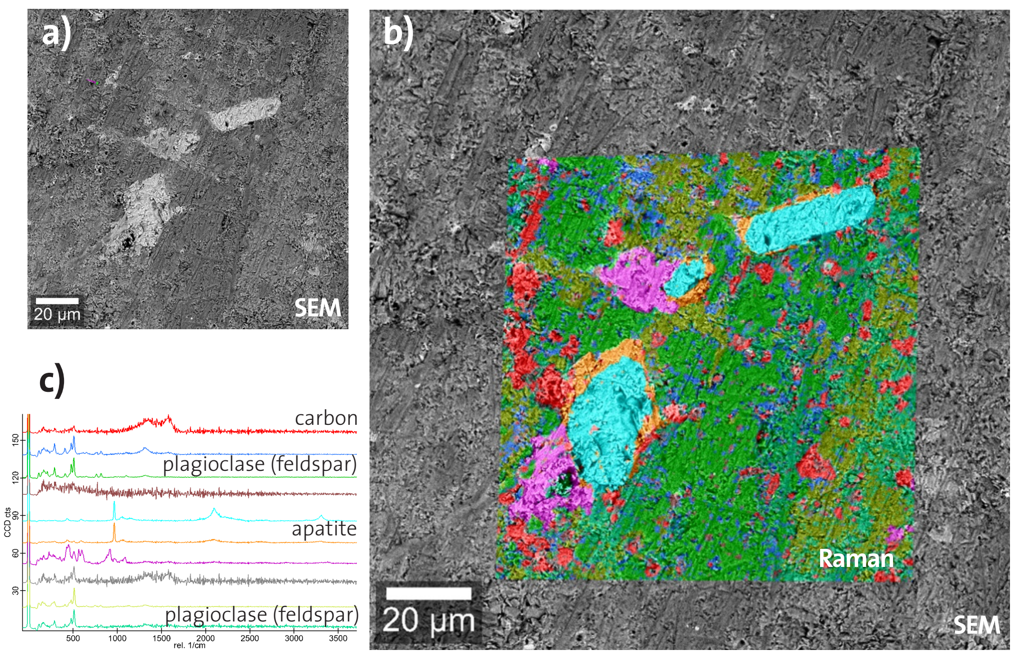

Fig. 1: a) SEM Image of a geological diorite sample b) SEM image overlaid with the Raman image. The different colors in the Raman image illustrate the various molecular compounds. Raman image: 100 µm x 100 µm, 300 x 300 pixels = 90,000 spectra, integration time: 34 ms/spectrum. c) The corresponding color-coded Raman spectra display each molecular component of the sample.

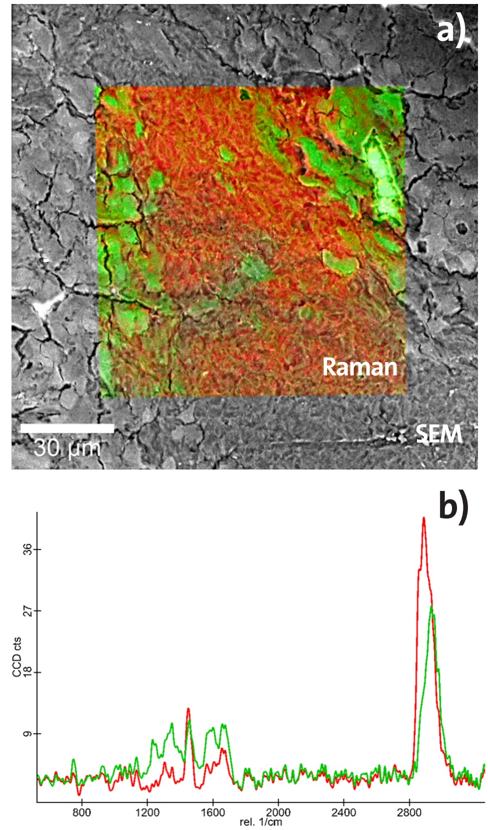

Fig. 2: a) Raman-SEM image overlay of a hamster brain tissue sample. In the color-coded Raman image the white brain matter is shown in Green and the gray brain matter in Red. 100 µm x 100 µm, 300 x 300 pixels = 90,000 spectra, integration time: 50 ms/spectrum b) The corresponding Raman spectra reveal the different spectral characteristics of the white and gray brain matter.

Conclusion

Through RISE Microscopy it is possible to link ultra-structural surface with molecular compound information. This combination enables new possibilities in comprehensive sample characterization. RISE Microscopy pairs ease-of-use with exceptional analytic benefit and is therefore suited to a large variety of applications such as surface and materials science, nanotechnology, polymer science, geo sciences, life science, and in pharmaceutical industry.