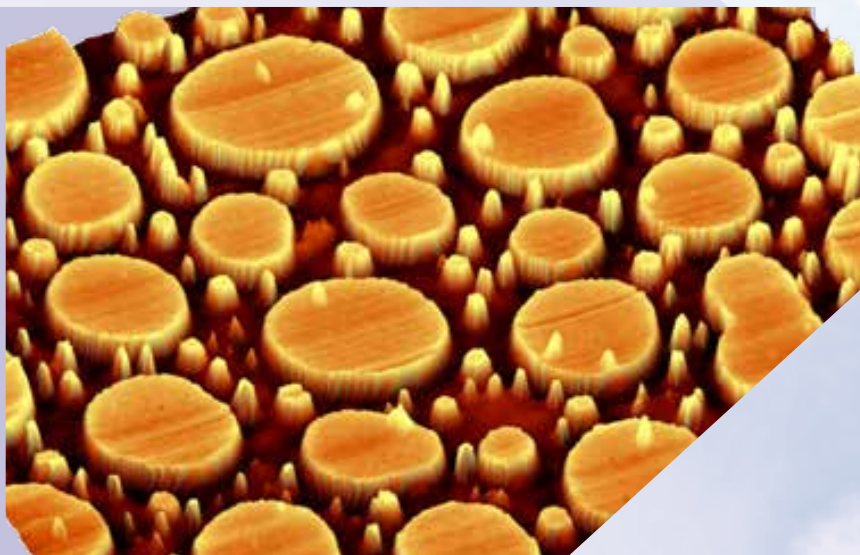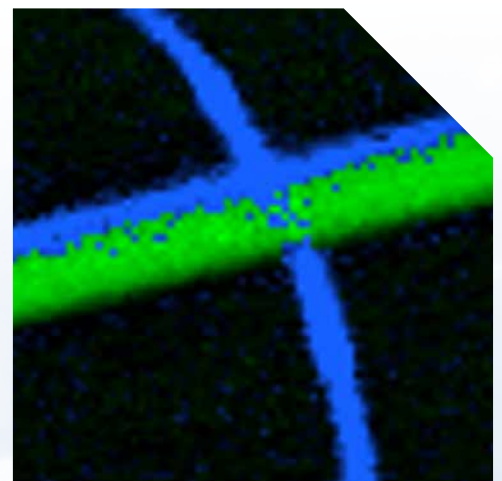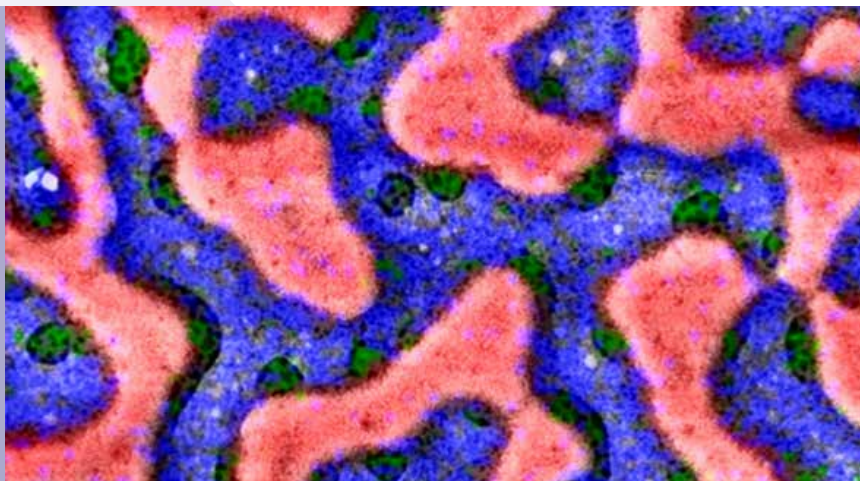APPLICATION NOTE

# Correlative Raman Imaging of Polymeric Materials

Confocal Raman microscopy is a powerful technique for high-resolution, non-destructive and label-free analysis of polymers in two or three dimensions. It can easily be combined with other imaging techniques such as AFM or SNOM.

The Raman principle

The Raman effect is based on the inelastic scattering of light by the molecules of gaseous, liquid or solid materials. The interaction of a molecule with photons causes vibrations of its chemical bonds, leading to specific energy shifts in the scattered light. Thus, any given chemical compound produces a particular Raman spectrum when excited and can be easily identified by this individual "fingerprint."

Raman spectroscopy is a well-established, label-free and non-destructive method for analyzing the molecular composition of a sample.
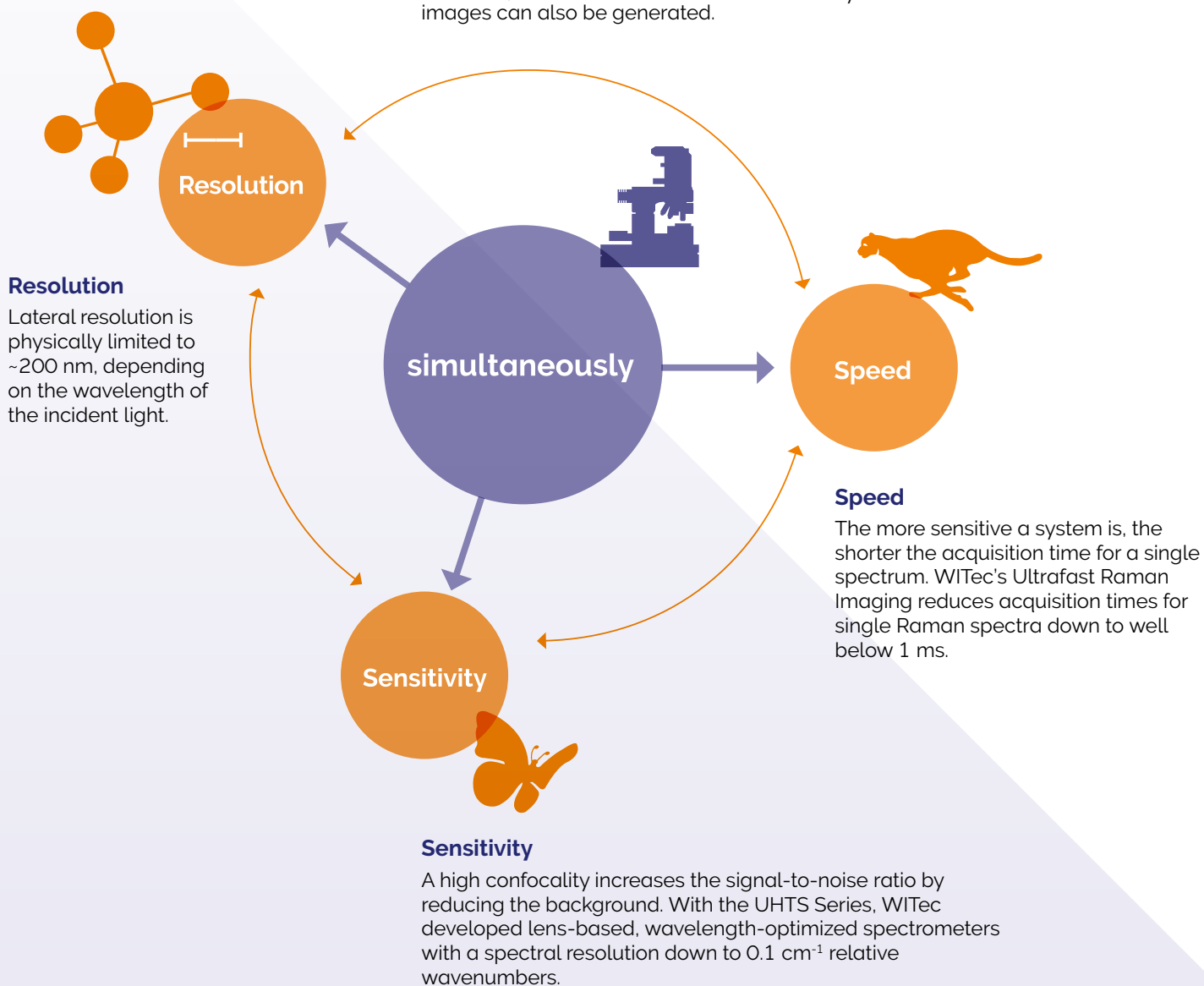
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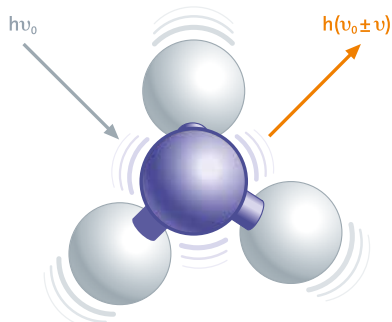
Raman imaging

In Raman imaging, a confocal microscope is combined with a spectrometer and a Raman spectrum is recorded at every image pixel. The resulting Raman image visualizes the distribution of the sample's compounds. Due to the high confocality of WITec Raman systems, volume scans and 3D images can also be generated.

No need for compromises

The Raman effect is extremely weak, so every Raman photon is important for imaging. Therefore WITec Raman imaging systems combine an exceptionally sensitive confocal microscope with an ultra-high throughput spectrometer (UHTS). Precise adjustment of all optical and mechanical elements guarantees the highest resolution, outstanding speed and extraordinary sensitivity – simultaneously!

This optimization allows the detection of Raman signals of even weak Raman scatterers and extremely low material concentrations or volumes with the lowest excitation energy levels. This is an unrivaled advantage of WITec systems.

Resolution

Lateral resolution is physically limited to ~200 nm, depending on the wavelength of the incident light.

Resolution

simultaneously

Speed

Sensitivity

Speed

The more sensitive a system is, the shorter the acquisition time for a single spectrum. WITec's Ultrafast Raman Imaging reduces acquisition times for single Raman spectra down to well below 1 ms.

Sensitivity

A high confocality increases the signal-to-noise ratio by reducing the background. With the UHTS Series, WITec developed lens-based, wavelength-optimized spectrometers with a spectral resolution down to 0.1 cm^{-1} relative wavenumbers.

Correlative Raman-AFM-SNOM imaging of a polymer mixture

A three-component polymer blend was studied by using a combination of Atomic Force Microscopy (AFM), Scanning Near-field Optical Microscopy (SNOM) and confocal Raman imaging.

While AFM operating in AC Mode recorded topographic information and local mechanical characteristics, SNOM detected optical properties with resolution far below the diffraction limit and Raman imaging revealed the molecular composition of the sample. Correlated AFM – SNOM – confocal Raman imaging, provides deep insight into the chemical and physical properties of polymer mixtures on a sub-micrometer scale.

The phase separation of a thin film blend of 1:1:1 polysterene (PS), styrene-butadiene-rubber (SBR) and ethyl-hexyl acrylate (EHA) was analyzed with a WITec alpha300 RAS microscope featuring all three analysis methods integrated within one instrument. The sample, spin-coated onto glass, remained in place throughout all measurements, allowing for images to be correlated. Evaluation of the acquired data and generation of depth profiles was accomplished with the WITec Software Suite.

The topography of the sample measured with AFM reveals a three-level structure (Figure 1A). The simultaneously recorded phase image (Figure 1B) shows a fine netlike texture at the lowest topographic level, material containing small spheres in the intermediate layer and an amorphous pattern in the uppermost substance. The SNOM image acquired from the same sample section indicates that the thinnest areas of the samples are opaque (Figure 1C). These areas contain EHA only, as can be seen in the Raman image (Figure 1E) that was generated from the Raman spectra (Figure 1D). The uppermost features of the sample appear to be PS, which is known to form spheres. The perfect correlation of topography, phase separation, SNOM and molecular composition is illustrated by the overlay of the AFM phase and Raman images (Figure 1F).
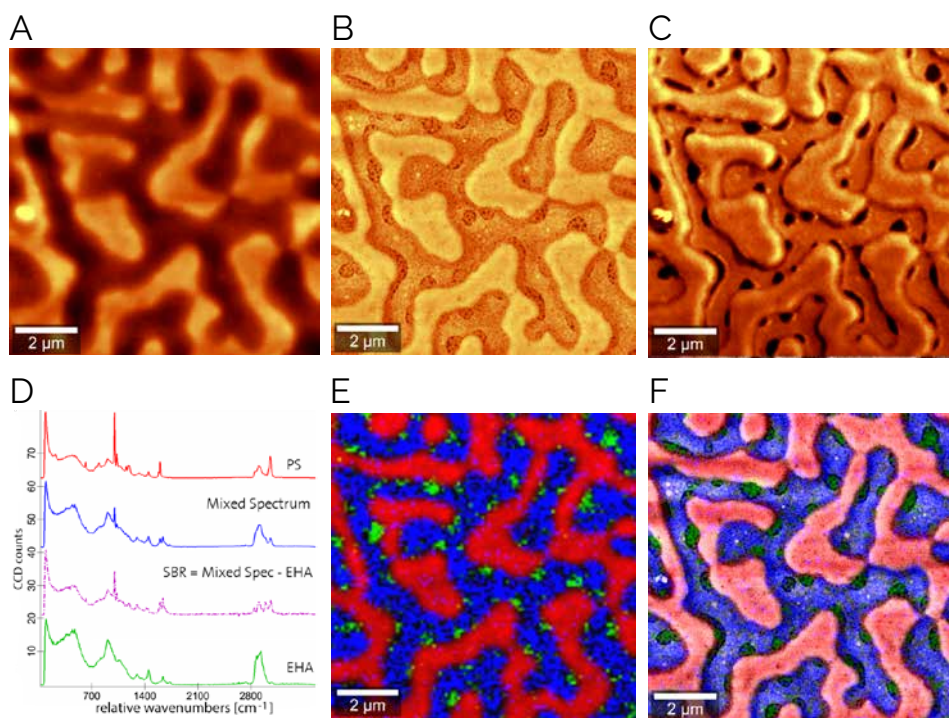

Figure 1: Correlative, high-resolution AFM – SNOM – confocal Raman imaging of a 1:1:1 mixture of PS, SBR and EHA

(A, B) Using AFM in tapping mode the sample's topography (A) and its phase image (B) were measured simultaneously. **(C)** With SNOM the transparency of the same area was determined with the dark spots being opaque. **(D, E)** Concurrently with the AFM and SNOM measurements the Raman spectra (D) were recorded for each pixel of the area and displayed as a false color image (E). PS (red), EHA (green), SBR (violet), mixed spectrum of SBR and EHA (blue). **(F)** Overlay of the AFM phase and Raman images.

All correlated structural, physical and chemical data lead to the conclusion that the EHA forms the lowermost layer on the microscope slide. It is covered by a layer of SBR as indicated by the blended (EHA-SBR) Raman spectrum. PS spheres are submerged in and protrude out of this double-layer polymer film.

The combination of AFM, SNOM and confocal Raman imaging in a single instrument enables easy-to-use, non-destructive, physical and chemical characterization of heterogeneous materials at very high resolution.

Characterization of polymer blends with correlative microscopy: Confocal Raman imaging and Atomic Force Microscopy

Polymers play an essential role in modern materials science. Due to their widely varying mechanical and chemical properties they are used in almost every field of application and remain important for developing new materials with demanding requirements. Thus, knowledge of the morphology and chemical composition of heterogeneous polymeric materials on a sub-micrometer scale is crucial.

Certain properties, however, are difficult to study with conventional characterization techniques, which are often not able to chemically differentiate materials with sufficient spatial resolution and without damaging, staining or otherwise treating them.

Correlative microscopy comprised of Atomic Force Microscopy (AFM) and confocal Raman imaging opens new avenues for the analysis of advanced polymeric materials when a detailed understanding of physical and chemical properties is required. While AFM records topographic information and local mechanical characteristics along and perpendicular to the surface of a sample with high spatial resolution, Raman imaging reveals its molecular composition.

The samples remained in place during all measurements, allowing the resulting images to be correlated. The WITec Software Suite enables a comprehensive evaluation of the acquired data and the generation of depth profiles.
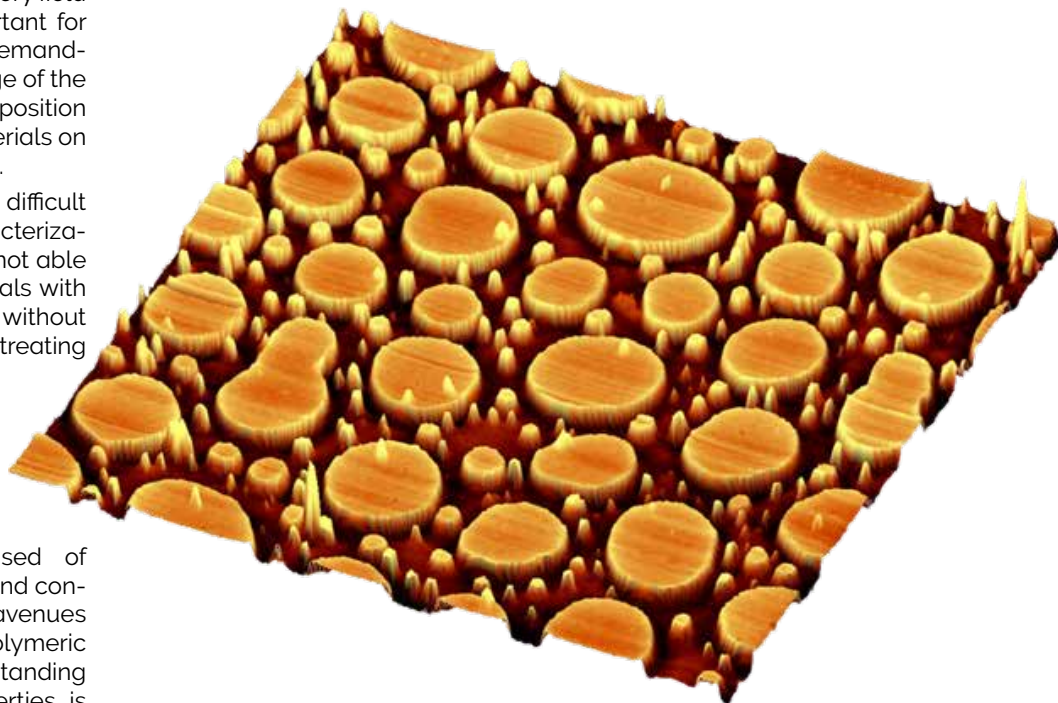

Figure 2: AFM image of a PMMA-SBR blend
Scan area 20 x 20 x 0.03 µm³

Thin films of a mixture of two polymers were studied. Polymethylacrylate (PMMA) and styrene-butadiene-rubber (SBR) were mixed and spin coated onto a glass cover slip. A WITec alpha300 RA microscope was used for imaging.

The mixture was analyzed with AFM in the AC Mode (Figure 2). The topographic image reveals spherical structures which protrude from the surrounding material. Their chemical nature was determined by their Raman spectra (Figure 3).
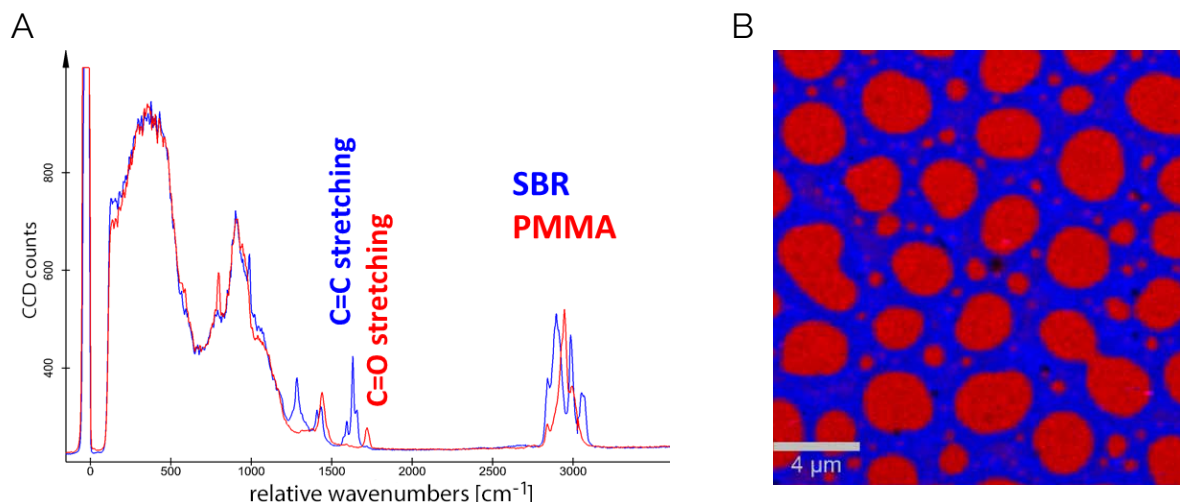
A

B

Figure 3: Raman image of a PMMA-SBR blend

(A) Raman spectra of PMMA (red) and SBR (blue). PMMA was identified by its typical C=O stretching band and SBR molecules by their C=C double bond stretching band. **(B)** Confocal Raman image. PMMA (red) forms round structures in the surrounding SBR (blue).

The Raman spectra of the molecules in the island-like structures contain a band at 1735 cm^{-1} relative wavenumbers (Figure 3A, red). This is associated with C=O double bond stretching, identifying the substance as PMMA. The area between the spheres consists of SBR. It was identified by a Raman band at 1640 cm^{-1} relative wavenumbers that is characteristic for the C=C double bonds of the SBR molecules (Figure 3A, blue). The distribution of the polymer was visualized by Raman imaging (Figure 3B).

To determine the thickness of the polymer blend, the film was scratched with a razor blade along the black line indicated in the AFM image (Figure 4A). The depth profile recorded along this line indicates that the PMMA islands are up to 70 nm high and protrude from the SBR film that is 35 nm high (Figure 4B).
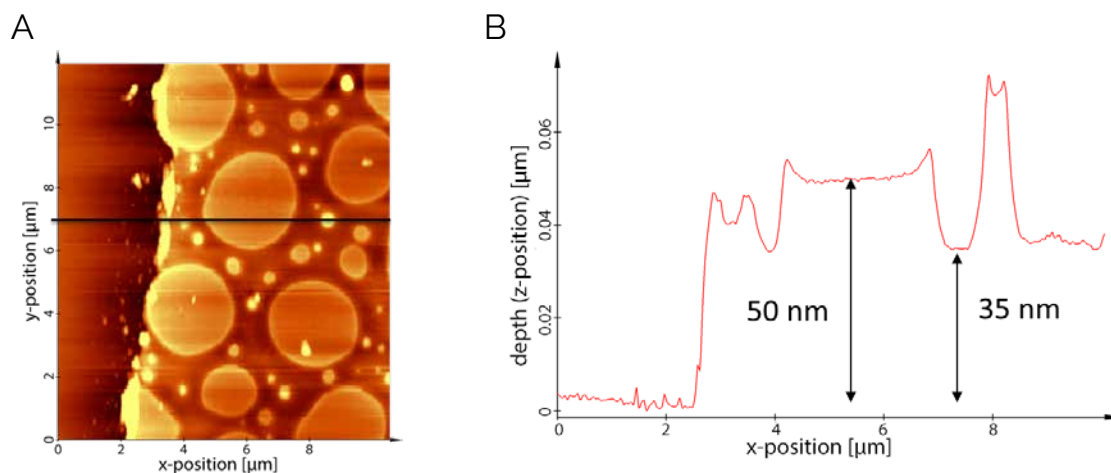
A

B

Figure 4: AFM image and depth profile of a PMMA-SBR mixture

(A) AFM image of the PMMA-SBR blend spin-coated on glass. The film was scratched with a sharp razor along the black line and a depth-profile was measured. **(B)** Depth profile along the black line in (A).

Image parameters: scan range 10 x 12 x 0.6 µm³.

Confocal Raman imaging for depth profiling of polymer films and coatings

A full analysis of a heterogenous polymer blend not only includes the two-dimensional arrangement of its individual components but also their distribution in the third dimension. A comprehensive, three-dimensional characterization of polymers such as films and coatings can be achieved with confocal Raman microscopy. Films and coatings play an important role in many fields of application such as food packaging, drug delivery and medical devices or adhesives.

While the resolution of two-dimensional Raman imaging is limited by diffraction, the quality of depth resolution relies on the confocality of the microscope. WITec confocal Raman microscopes combine a highly sensitive confocal microscope and a high-transmission Raman spectroscopy system. The WITec Software Suite can extract a variety of properties from a single measurement such as peak width, center of mass or peak position of certain Raman lines. For depth profiling measurements, the focal plane can be moved in the z-direction when performing either x-z scans or generating x-y image stacks in the z-direction. Here, the microscope's capabilities are demonstrated by imaging the inner polymer coating of an orange juice container and of polymer layers on paper.
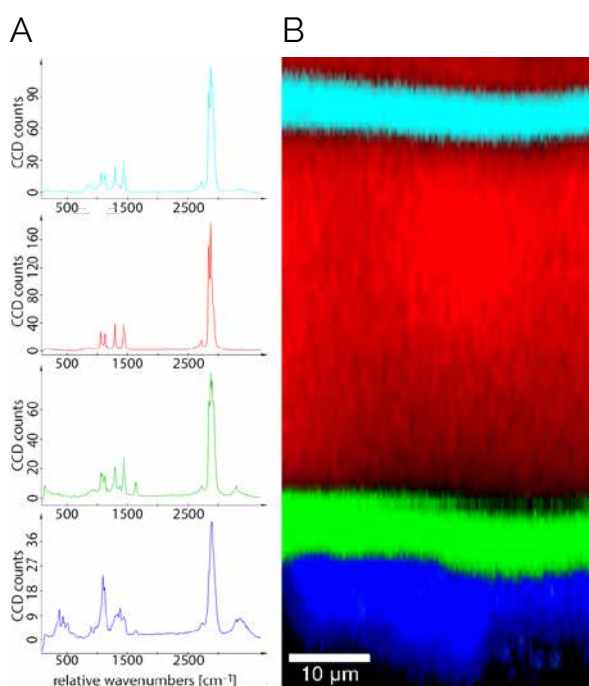
Figure 5: Raman imaging of the inner coating of an orange juice container.

(A) Recorded Raman spectra. **(B)** Corresponding Raman image.

The inner plastic film of a paper beverage container was investigated by performing an x-z scan. Within the acquired multispectrum file, four distinct spectra were observed, each representing a specific chemical compound within the beverage container coating (Figure 5A). The Raman image generated from the spectra shows that the coating is about 80 µm thick and consists of five layers of different thicknesses made up of four compounds (Figure 5B).
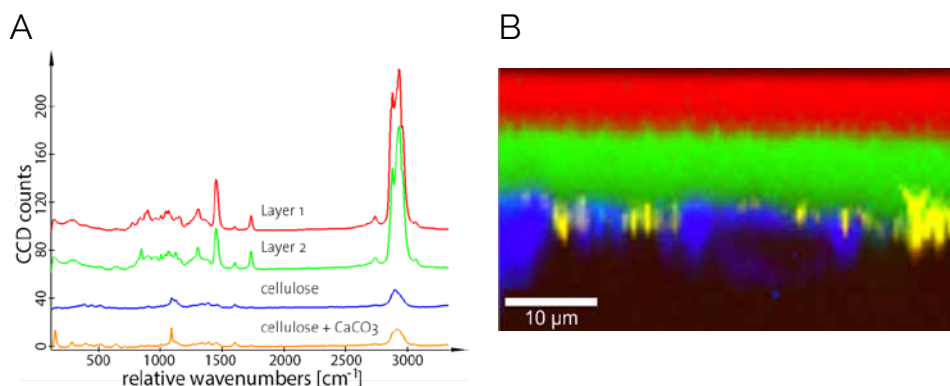
Figure 6: Raman imaging of adhesive polymeric layers on a paper substrate

(A) Recorded Raman spectra. **(B)** Raman depth profile. Image parameters: 50 x 40 µm², 150 x 50 pixels (= 7500 spectra), 0.1 sec/spectrum acquisition time. The two different polymers (red and green) form separate layers on the paper (blue, yellow).

In another experiment, confocal Raman depth scans provided insight into the layered structure of polymers that were applied to the surface of paper in order to make it adhesive.

The two polymers and the paper (consisting of cellulose) were identified by their Raman spectra (Figure 6A). The Raman depth scan (Figure 6B) shows that both polymers are immiscible and form a sharp interface with each other and with the paper beneath.

Correlative AFM/Raman Microscopy of bioinspired nanofibers

Natural fibers display many intriguing qualities such as extreme tearing strength, high flexibility and elasticity, self-crimping and varying chemical properties or morphologies that bio-engineers would like to copy for the design of synthetic materials.

Several methods have been developed to produce artificial fibers, one of which is electrospinning. This technique uses a strong electric field to overcome surface tension to produce a spinning jet from solutions or melts. Thus, electrospinning results in continuous artificial nanofibers with diameters down to the nanometer range. The fibers can be made of one or more materials.

The experimental conditions and solvents used have a great impact on the morphology of the resulting fibers, which is why the products need to be investigated thoroughly.

Here, we show a correlative analysis of atomic force microscopy and confocal Raman imaging of two-component fibers. They were produced by scientists at the Bayreuth Center for Colloids and Interfaces at Bayreuth University (Germany) and consist of silk from the silkworm Bombyx mori and polylactic acid. The fibers were spun from side-by-side nozzles as described by Peng et al. [1].

Microscopy was performed with a WITec alpha300 RA confocal Raman microscope. This instrument is equipped with AFM and Raman imaging capabilities, so that the sample does not need to be moved between the measurements. AFM was conducted in AC mode with Pulsed Force Mode. Raman imaging was performed with a 100x/NA 0.9 objective, 532 nm excitation laser, and a WITec UHTS300 spectrometer equipped with a 600 lines/mm grating. Data analysis was carried out with the WITec Software Suite.

The topography of two nanofibers was investigated with the high-resolution AFM capability of the microscope (Figure 7A). Two cross section measurements were performed as indicated. One fiber was roughly 900 nm thick whereas the other was only about 100 nm thick (Figure 7B).
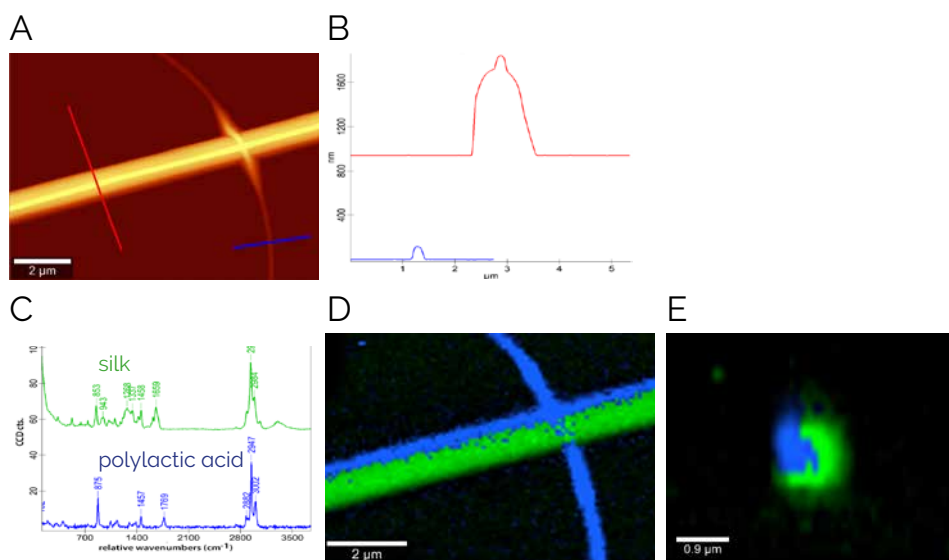

Figure 7: Correlative AFM - Confocal Raman imaging of fibers

(A) Topography of two fibers. **(B)** Height profile at the red and blue positions marked in (A). **(C)** Raman spectra of silk (green) and polylactic acid (blue). **(D)** Confocal Raman image of the fiber. Note that at a right angle to the two-component fiber lies a smaller fiber consisting of polylactic acid only. **(E)** Depth scan along the z-axis of the two-component fiber.

The chemical nature of each fiber was determined by Raman analysis. Silk and polylactic acid could be easily distinguished by their Raman spectra (Figure 7C).

To generate the confocal Raman image (Figure 7D), complete Raman spectra were acquired at each image pixel. The color-coded image (silk in green, polylactic acid in blue) shows the side-by-side distribution of the two components along the axis of the thicker fiber. The second fiber consists of polylactic acid only.

A depth scan along the z-axis of the side-by-side fiber was performed and underlines the aligned, parallel positioning of the fibers' components (Figure 7E).

This data and other examples [2] illustrate that high-resolution AFM and Raman imaging is a superb combination for the precise morphological and chemical characterization of two-component nanofibers.

References

[1] Peng et al. (2016). Two-in-one composite fibers with side-by-side arrangement of silk fibroin and poly (l-lactide) by electrospinning. *Macromolecular Materials and Engineering*, 301(1), 48-55.

[2] Gernhardt et al. (2018). Tailoring the morphology of responsive bioinspired bicomponent fibers. *Macromolecular Materials and Engineering*, 303(1), 1700248.

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