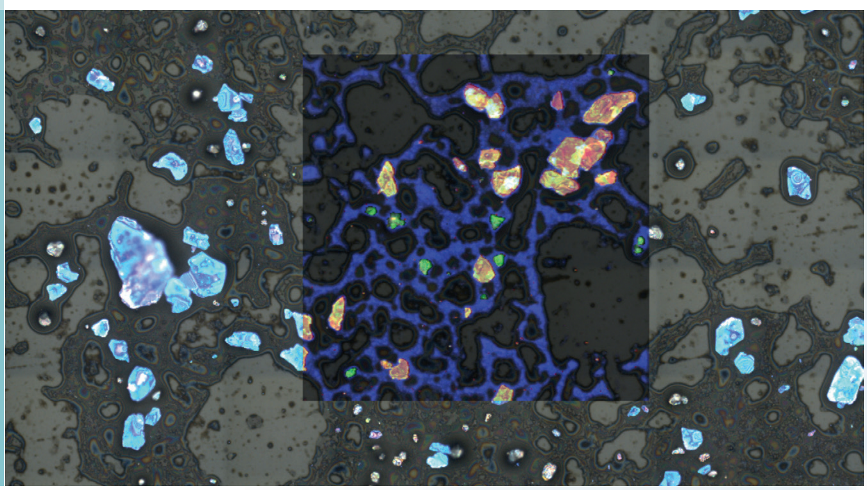
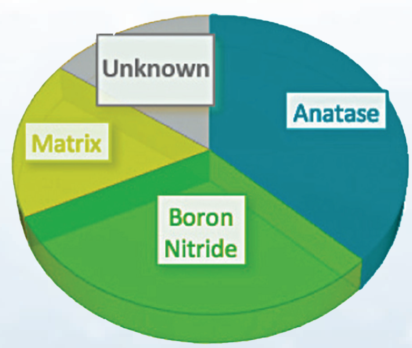
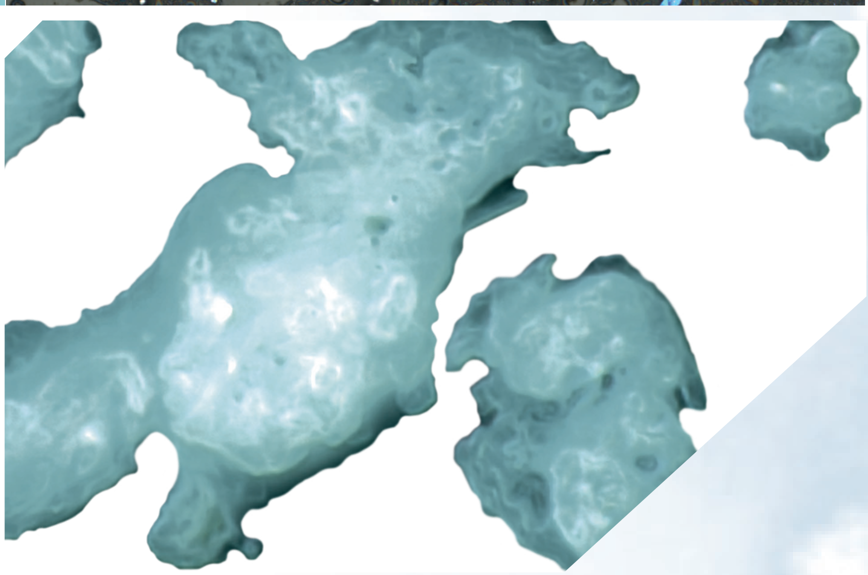
APPLICATION NOTE

ParticleScout for Automated Confocal Raman Imaging Analysis of Microparticles







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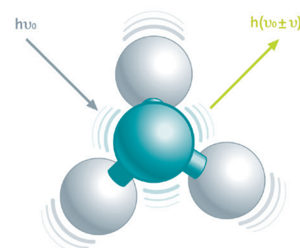
The Raman principle

The Raman effect is based on inelastic scattering of excitation 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 that can be identified in its Raman spectrum.

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 and nondestructive method for analyzing the molecular composition of a sample.



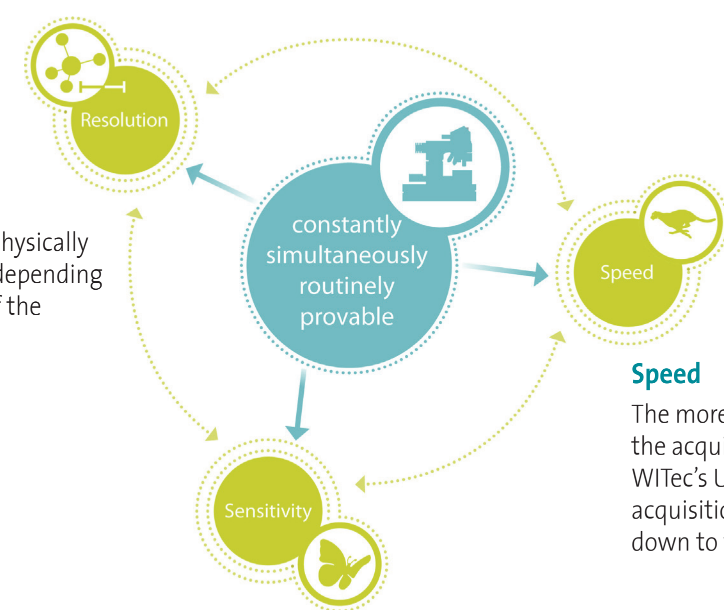
Raman imaging

When Raman spectra are collected at every measurement point using a confocal microscope combined with a spectrometer, a Raman image can be generated that 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 from 2D images from different focal planes.

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 ultrahigh-throughput spectrometer (UHTS). The 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.

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 relative wavenumbers (@633 nm excitation).

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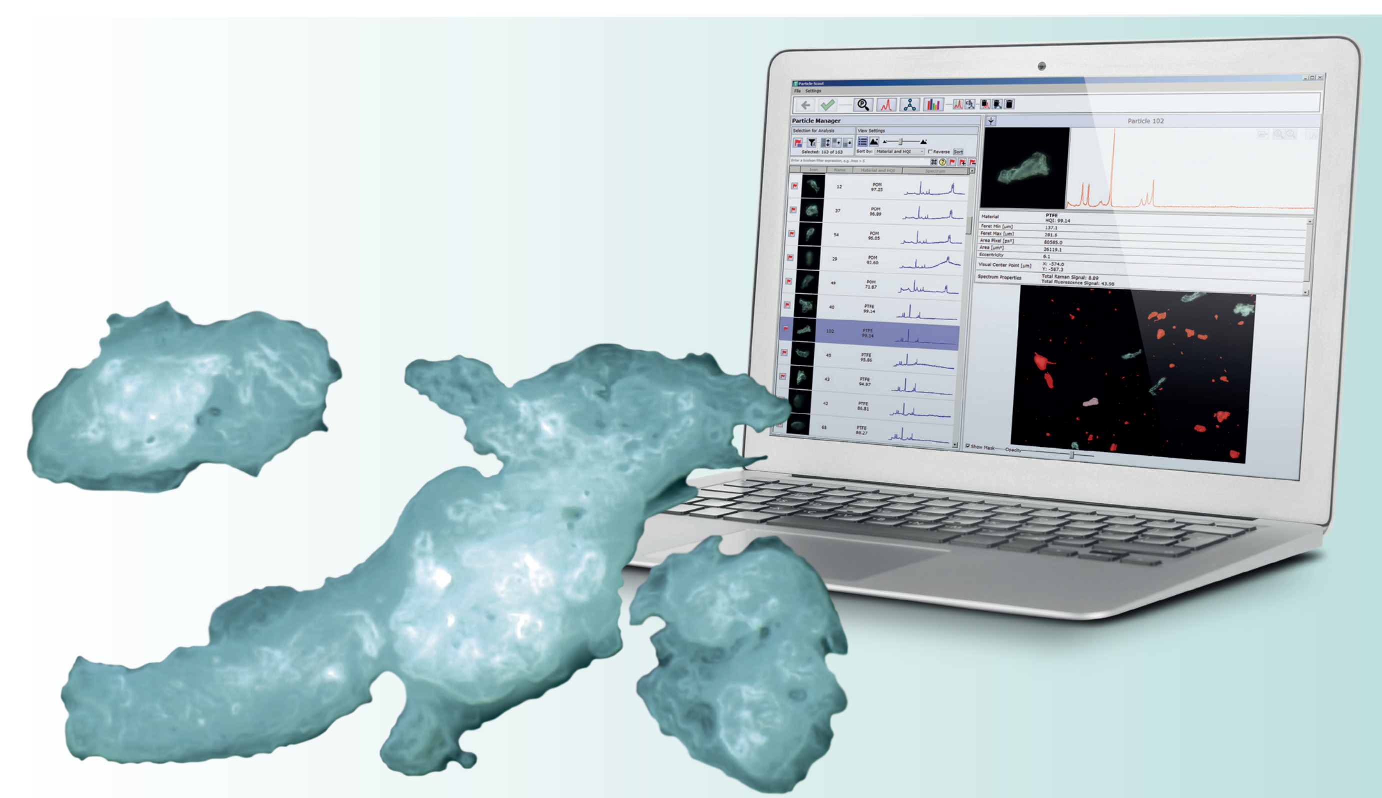
Automated particle analysis with ParticleScout

High-resolution measurements of particles are of great interest in many fields of application. WITec's ParticleScout is an analysis tool for the alpha300 Raman microscope series that surveys, categorizes, analyzes, quantifies and identifies particles over even large sample areas. Automated routines sort particles and acquire their Raman spectra, generating a report that provides a detailed overview of the sample.

Pollen, dust, flour, metal flakes and pigments in paints, titanium dioxide in sunscreen and toothpaste, fat crystals in food emulsions – these and many more substances in our daily lives contain or consist of microparticles. Recently, the public and scientific community have directed their attention toward microplastic particles in the environment.

Confocal Raman microscopy is ideally suited to finding, classifying and identifying microparticles because not only does it yield images with a resolution down to 300 nm, but with Raman vibrational spectroscopy the chemical components of a sample can be identified. It is a nondestructive method that requires little, if any, sample preparation. A Raman microscope can generate high-resolution images that show both the structural features and distribution of molecules within a sample.

However, Raman spectroscopic imaging is not yet widely applied to microparticle analysis. The challenge in Raman microparticle analysis lies in automating the detection of individual particles and classifying those of interest by size or shape before determining their chemical compositions. For such analyses, WITec has developed ParticleScout. This tool enables measurements that proceed from a white light or dark field sample overview to particle detection, acquisition of Raman spectra, post-processing of spectra and chemical identification to creating a final report. During this process the user can define the criteria according to which the particles shall be investigated, such as area, perimeter, minimum/maximum Feret diameter, elongation or equivalent diameter and many other parameters.



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How to identify and classify microplastic particles with ParticleScout

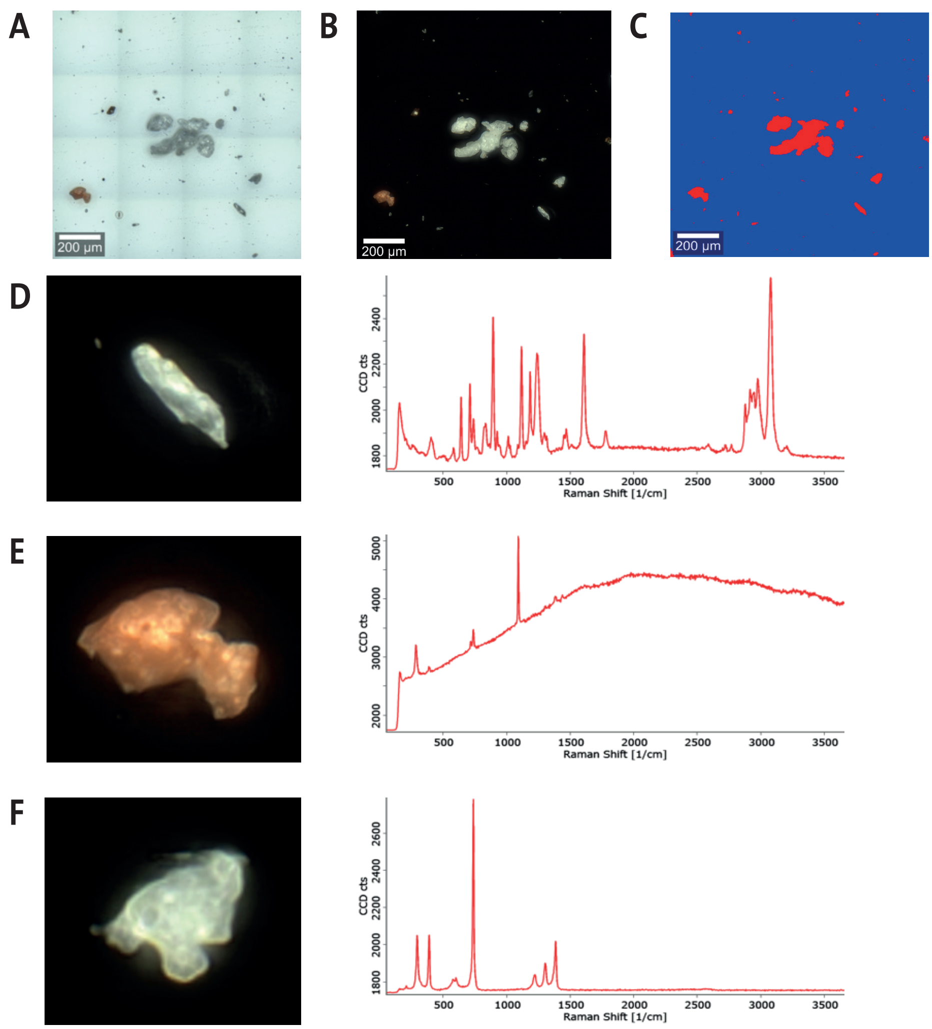
A variety of microplastic particles were mixed and then analyzed with an alpha300 Raman microscope equipped with ParticleScout.

First, bright field (A) and dark field (B) images of a 1 mm x 1 mm area were acquired. Then particles were automatically detected according to predefined features and represented in the form of a two-color image (C).

As conventional Raman imaging of large areas would also include much of the empty space surrounding the sparsely distributed particles, the software automatically records spectra of selected particles only, thus greatly accelerating the workflow of the measurement. Focus stacking yields more sharply defined particle outlines.

From each selected particle, a Raman spectrum was acquired (D-F). After processing the spectra (i.e. background subtraction) the chemical identities of the particles were determined.

Finally, a report (Table 1) was generated, identifying the materials of the particles as well as their sizes and quantities within the sample.



Large-area bright field (A) and dark field (B) views of a mixture of microplastic particles were generated by image stitching. The particles were automatically identified and displayed as a single two-color mask (C). From every particle, a single Raman spectrum was acquired (E-F). After processing the spectra, the chemical compositions of the individual particles were identified using TrueMatch.

All data are represented in Table 1, indicating the quantity, size and molecular composition (polymer type) of particles in the sample.

PS: polystyrene; POM: polyoxymethylene; PET: polyethylene terephthalate; PC: polycarbonate; PTFE: polytetrafluoroethylene.

Table 1

	Sum	5-10 µm	10-20 µm	20-50 µm	60-100 µm	> 100 µm
PS	89	47	12	8	17	5
POM	59	34	12	8	4	1
PET	217	106	70	20	17	4
PC	87	18	45	17	7	0
PTFE	913	417	297	103	77	19
Unknown	150	45	78	8	19	0
Sum	1515	667	514	164	141	29

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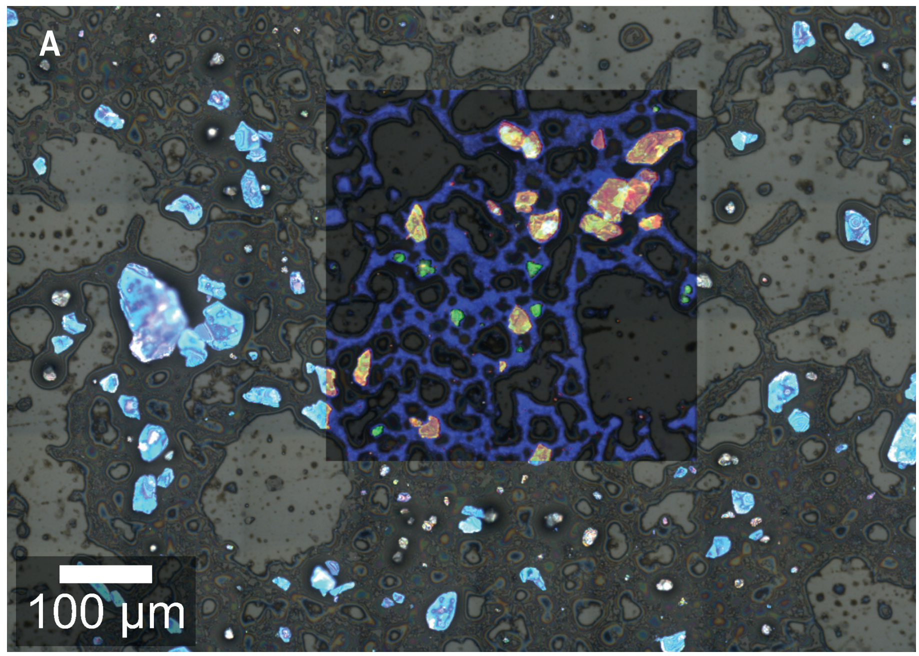
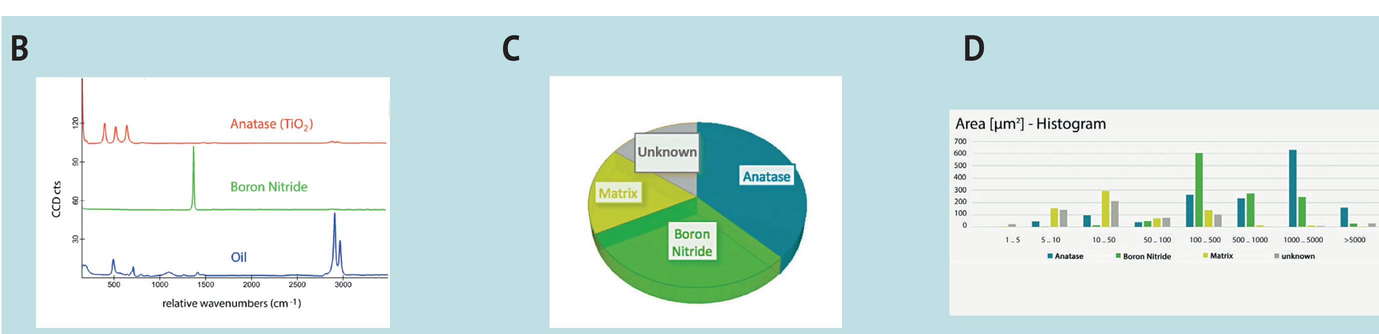
Microparticles in a cosmetic cream

Particle analysis is also valuable for the investigation of pharmaceutical and cosmetic products. Here, its capabilities are demonstrated on a cosmetic peeling cream sample. For imaging, an alpha300 R microscope equipped with ParticleScout was used. First a survey of a large area was generated by the stitching together of optical bright field images by an automated routine. 3941 particles were located and categorized according to their physical shape and size using Boolean filters. From these particles, Raman spectra were acquired. The resulting Raman image was overlaid onto the bright field image (A).



With the seamlessly-integrated TrueMatch software, the Raman data were processed and the components could be identified by referencing the Raman database information. Chemical analysis of the Raman spectra (B) revealed anatase (a mineral form of titanium dioxide) and boron nitride particles in an oil matrix. Further evaluation of the results determined the quantitative prevalence of the sample components (C) and also the distribution of chemical compounds correlated to particle size (D).

In extended analyses, particles could also be linked to parameters such as area, perimeter, bounding box, Feret diameter, aspect ratio, equivalent diameter, spherical equivalent volume and others. As particle classification, image processing and analysis of Raman spectra are executed within one platform, ParticleScout offers an effective solution for automated, comprehensive investigations of particles.



Particles in a cosmetic peeling cream.

A: Optical bright field image overlaid with the confocal Raman image.

B: Corresponding Raman spectra of the molecular components in the sample.

C: Pie chart of the quantitative compound distribution in the sample.

D: Correlation between chemical characteristics and particle size.

WITec Microscopes

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