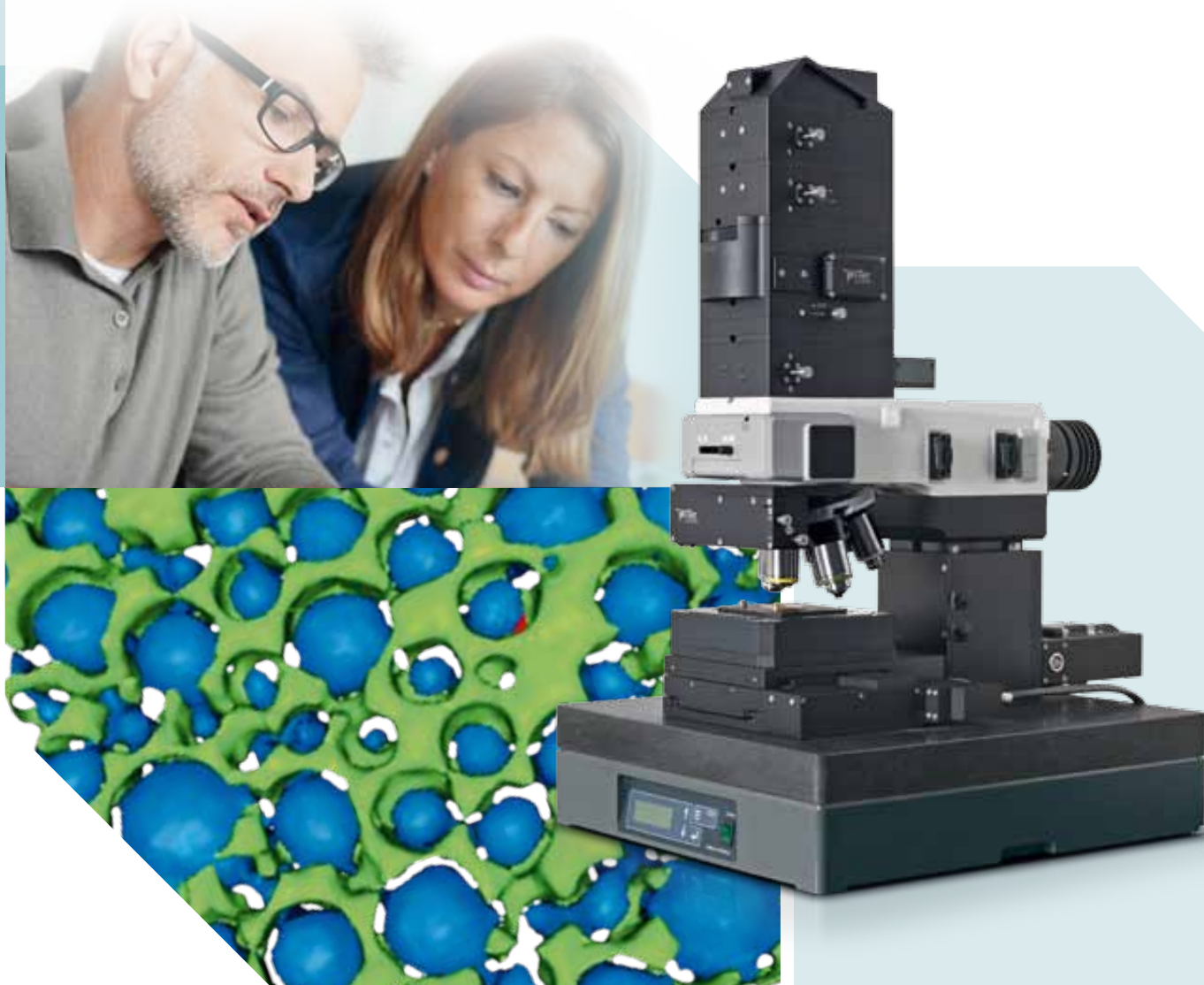


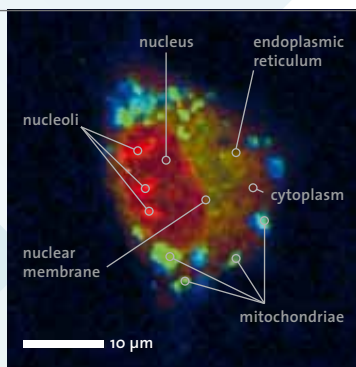
Correlative Raman Imaging  
Raman · RISE · AFM · SNOM

# WITec alpha300 Series

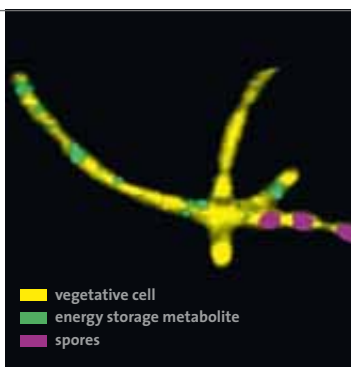
Modular Confocal Raman Microscopy Systems



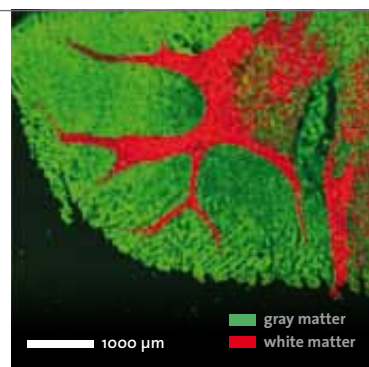
## Life Sciences



Raman image of a cell

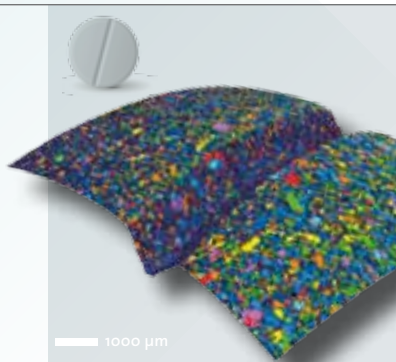


Raman image of *Bacillus cereus*  
© A. Hermelink, Robert-Koch-Institute, Berlin

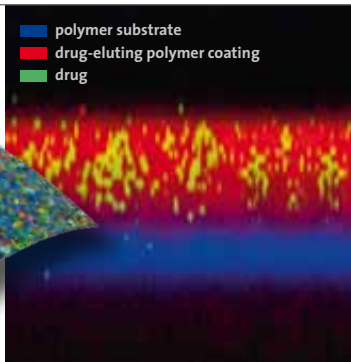


Large-area Raman image of the gray and white brain matter of a hamster brain

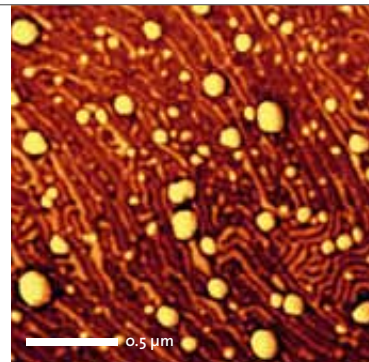
## Pharmaceutics & Cosmetics



TrueSurface™ topographic Raman image of a pharmaceutical tablet

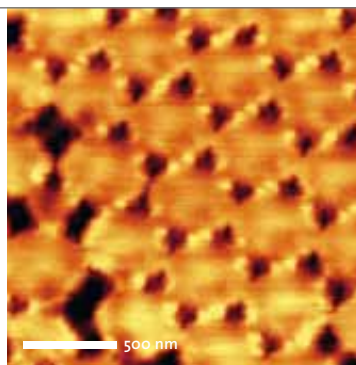


Raman depth-scan image of a drug-eluting stent

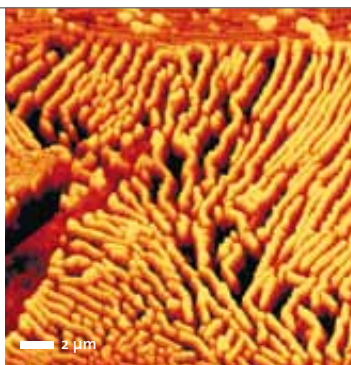


High-resolution AFM phase image of a drug-eluting stent surface showing the polymer substrate structure with the embedded drug particles

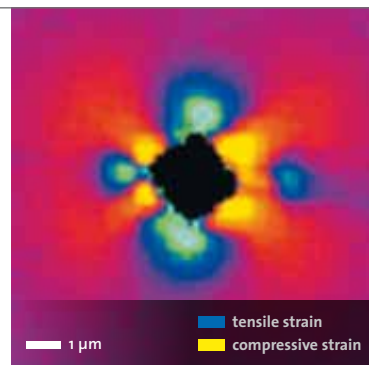
## Materials Science



High-resolution SNOM image of a latex projection pattern

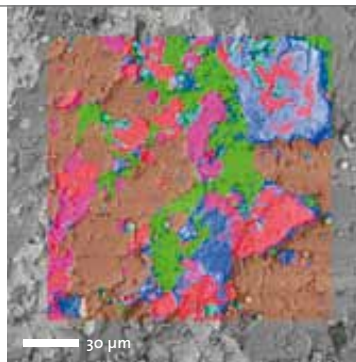


AFM topography image of a steel surface

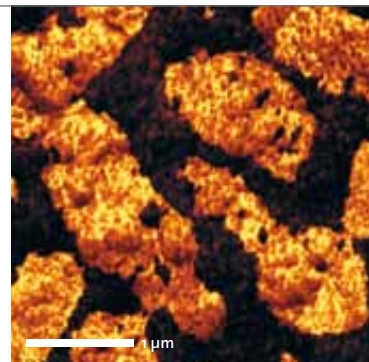
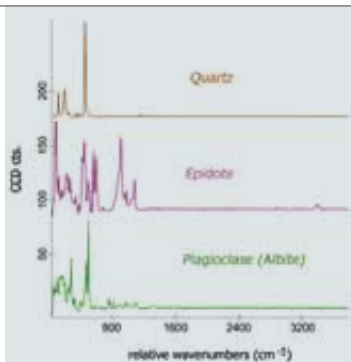


Material stress in silicon imaged via Raman peak-shift analysis

## Geoscience

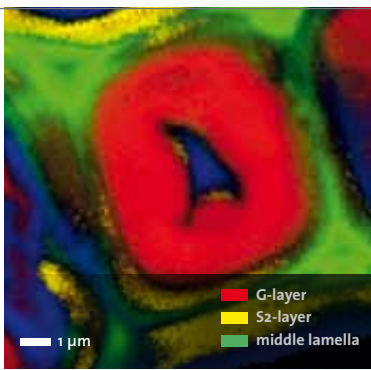


Correlative Raman-SEM (RISE) image of a geological sample with spectra of its molecular compounds

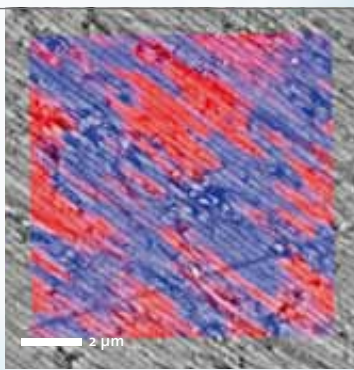


AFM image (Pulsed Force Mode) of fossilized bacteria

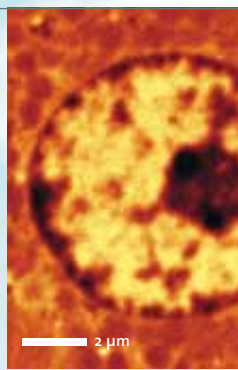




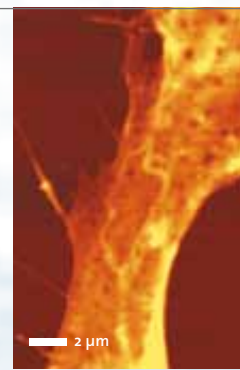
Raman image of a lignified plant cell



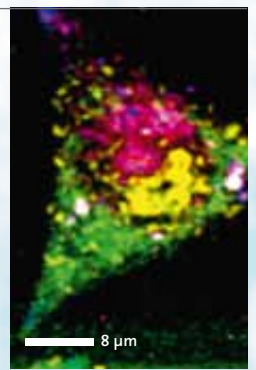
Raman-SEM (RISE) image of a polished cross-section of an abalone shell



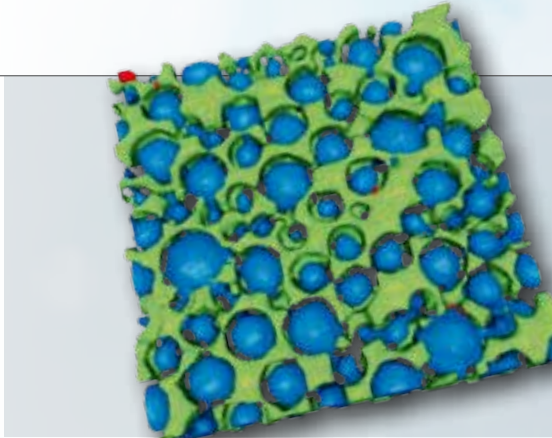
Super-resolution SNOM image of the nucleus of a rat hepatocyte



AFM image of a cell's surface

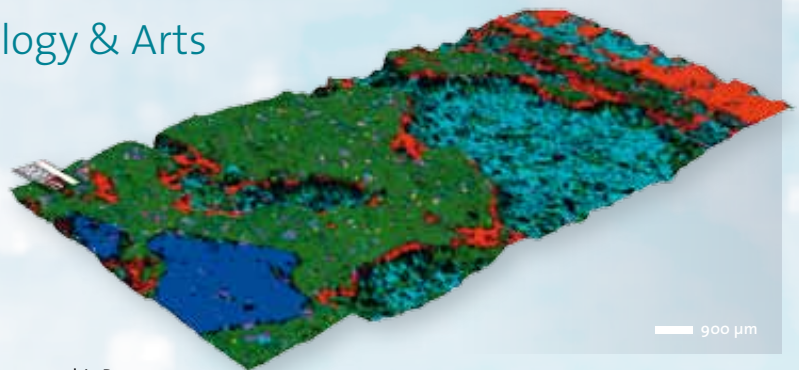


Correlative Raman – GFP-fluorescence image of a eukaryotic cell

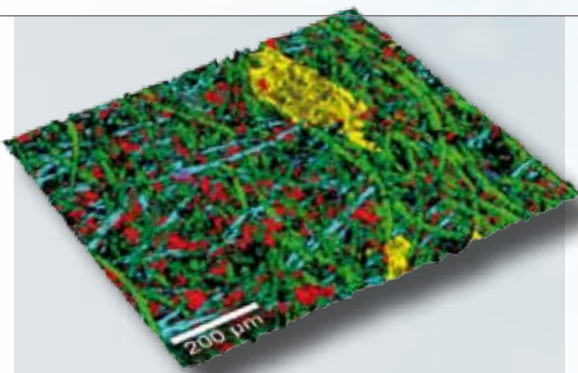


3D Raman volume image of a pharmaceutical emulsion

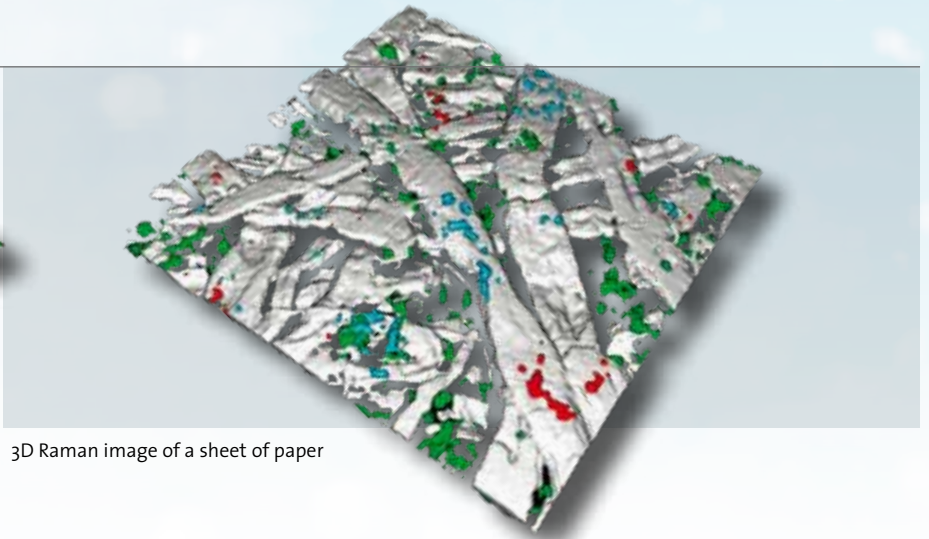
## Archeology & Arts



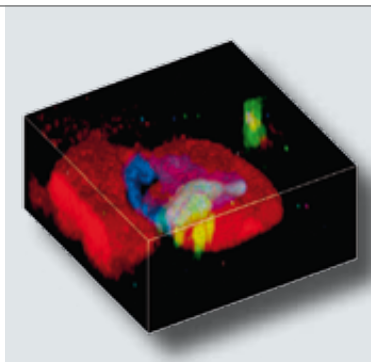
TrueSurface™ topographic Raman image of an ancient wall painting



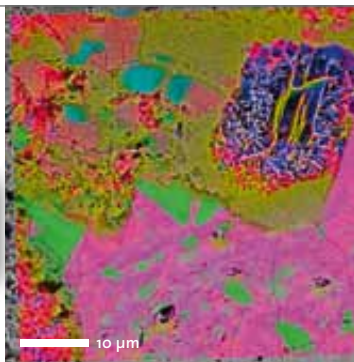
TrueSurface™ topographic Raman image of paper fibers



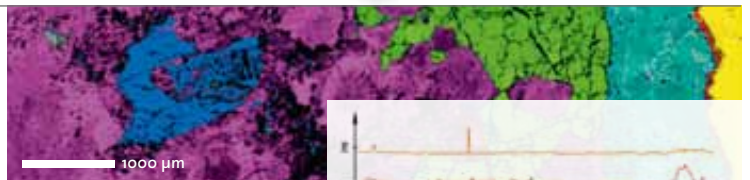
3D Raman image of a sheet of paper



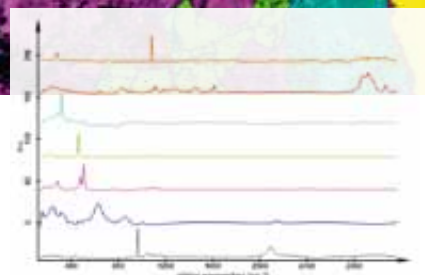
3D Raman image of a fluid inclusion in garnet



Correlative Raman-SEM (RISE) image of hematite and goethite in several crystal orientations



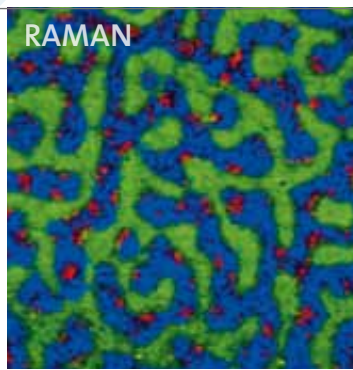
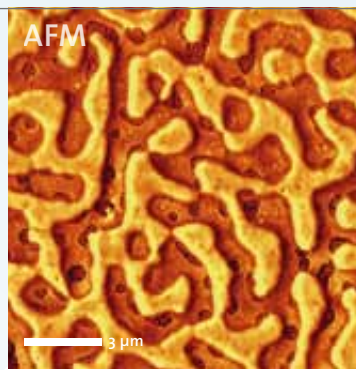
- Plagioclase
- Titanium oxide
- Quartz
- Fluorite
- Organics
- Calcite



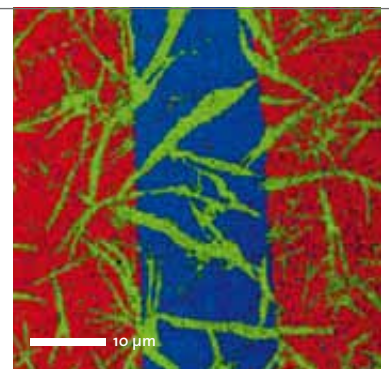
Large-area Raman image of a polished rock section with corresponding spectra  
© C. Heim, Göttingen University



## Polymer Science, Thin Films & Coatings

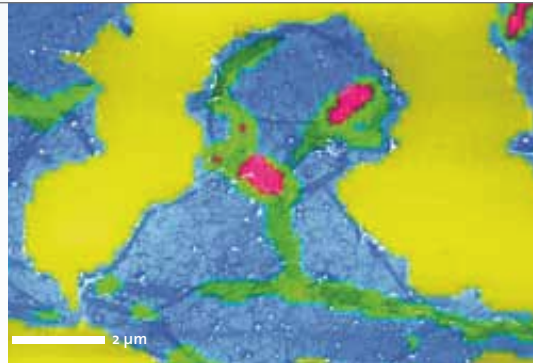


Correlative Raman (right) – AFM (left) images of a three-component polymer blend

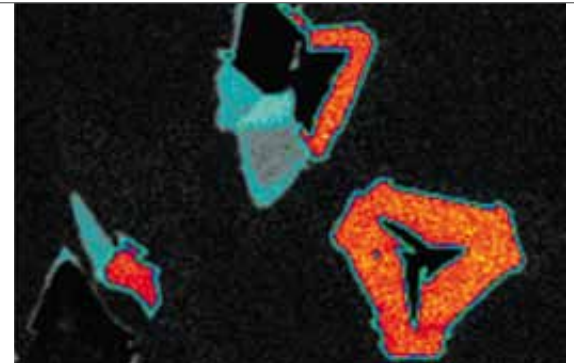


Raman image of a very thin (7.1 nm) PMMA film (red) on glass (blue) with a 4.2 nm alkane contamination layer (green) on top

## Low-Dimensional Materials



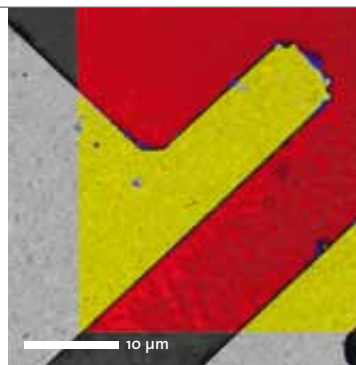
Correlative Raman-SEM (RISE) image of a graphene flake. The colors indicate the graphene layers and wrinkles



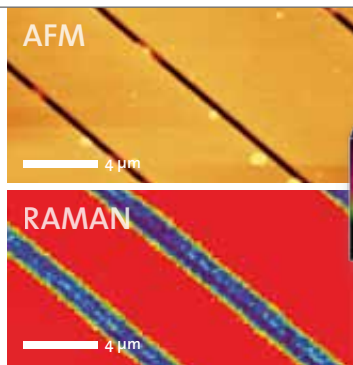
Peak intensity map of MoSe<sub>2</sub> at 19 relative wavenumbers (cm<sup>-1</sup>)

© M. O'Brien, Trinity College, Dublin

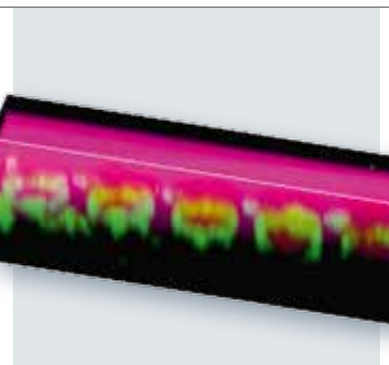
## Photovoltaics & Semiconductors



Correlative Raman-SEM (RISE) image of a gallium arsenide sample (yellow: gold substrate; red: GaAs; blue: residues from production)

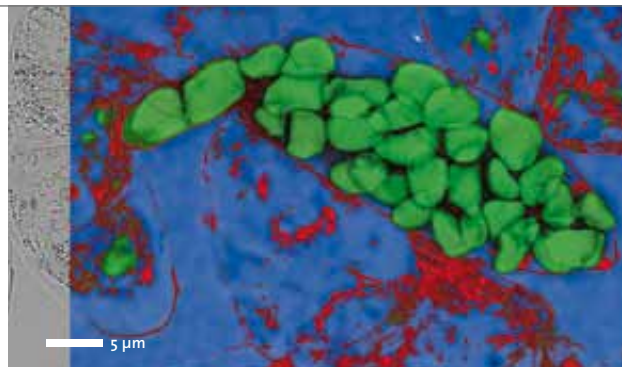


AFM and Raman images of the same sample area on a wafer

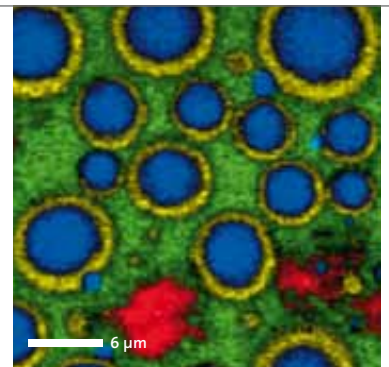


3D confocal Raman image of a GaN layer

## Food Science

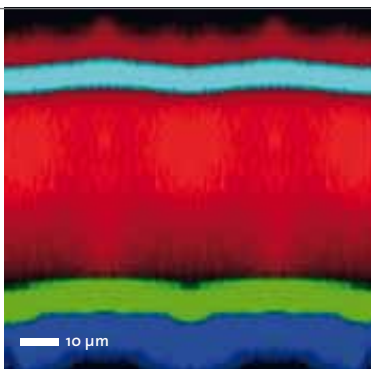


White-light and Raman image overlay of banana pulp

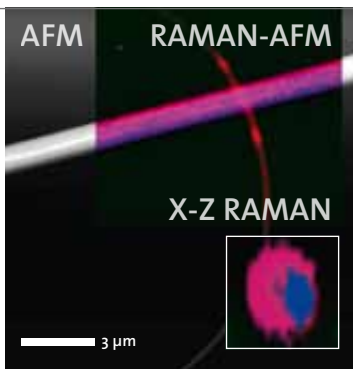


Emulsion used in food production (red and green: fatty matrix; blue: aqueous phase; yellow: emulsifier)

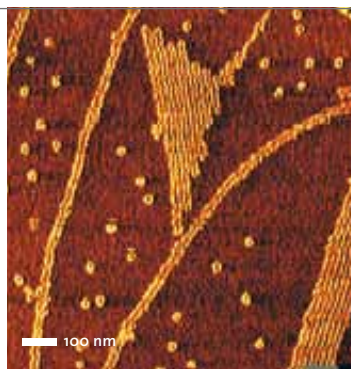




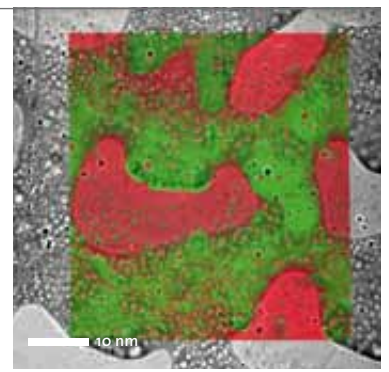
Raman depth-scan image of the inner multi-layer coating of a beverage container



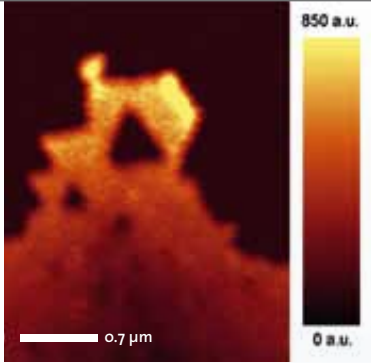
Correlative Raman-AFM image and Raman x-z cross section (inset) of a two-component fiber



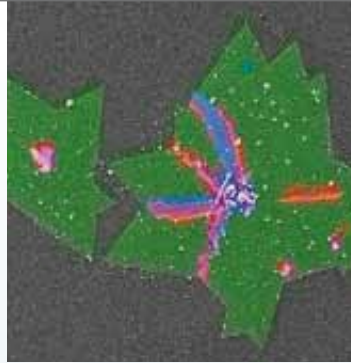
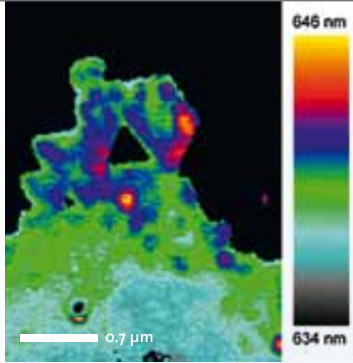
AFM phase image of long chain polymer molecules



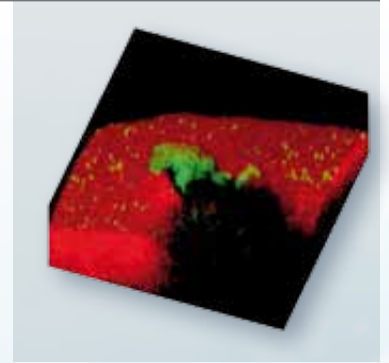
Correlative Raman-SEM (RISE) image of a polymer blend



High-resolution SNOM image of a WS<sub>2</sub> flake (left) and corresponding photoluminescence peak position image (right)

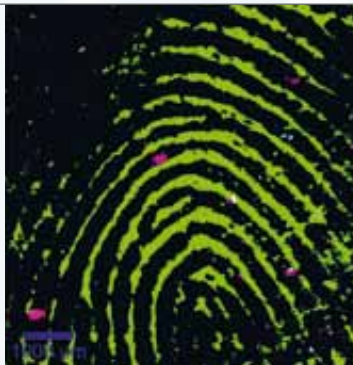


Correlative Raman-SEM (RISE) image of MoS<sub>2</sub> flakes with edges and defects



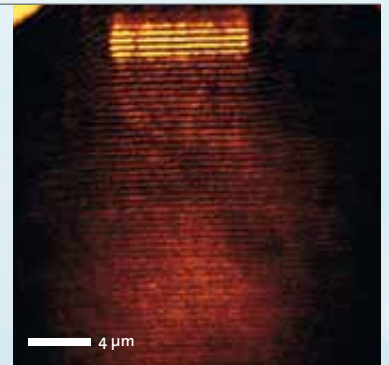
3D Raman image of carbon nanotubes (green) on a polymer matrix (red)

## Forensics

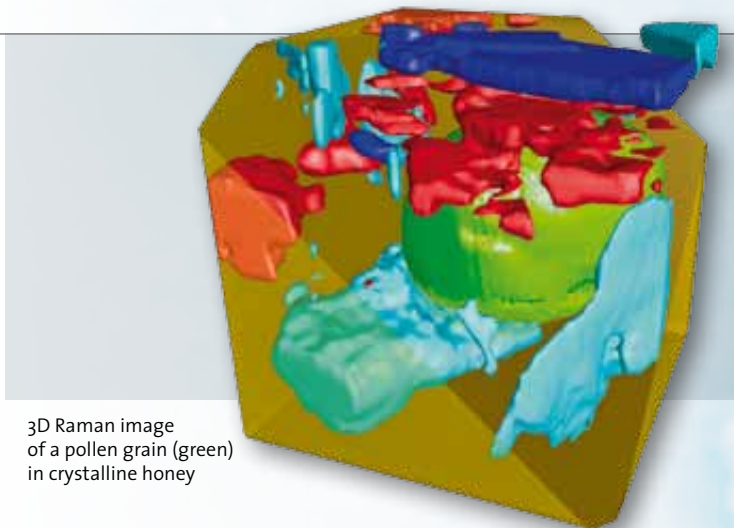


Raman image of a fingerprint with explosive material residues

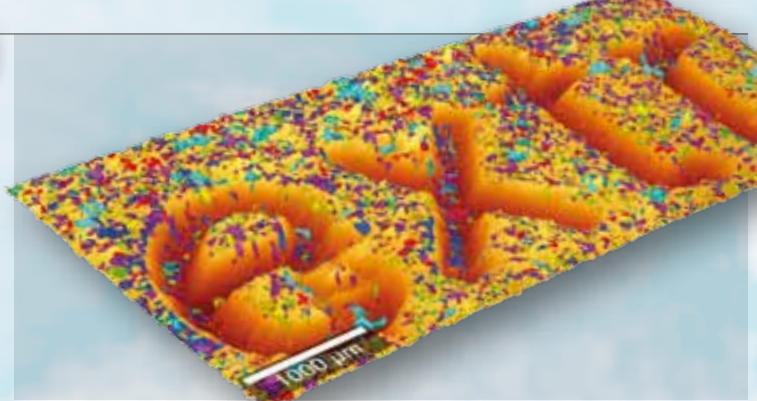
## Nanophotonics



SNOM image of a surface plasmon-polariton wave launched on a nanostructured metal grid



3D Raman image of a pollen grain (green) in crystalline honey



TrueSurface™ topographic Raman image of a dextrose candy

# alpha300 Series The Instruments

# Raman



alpha300 R



alpha300 RA

alpha300 RS

alpha300 A

# SNOM



alpha300 S

# AFM

Pioneering techniques, maximum versatility and ease of operation – that is what WITec, the Raman imaging company, stands for. To meet the most exacting requirements of our customers, WITec continually develops microscopy systems for correlative Raman imaging that fulfill three crucial tasks simultaneously: **they provide the best spatial and spectral resolution along with peerless sensitivity and unmatched speed.**

## Benefits of the WITec alpha300 R series

- Identification and visualization of the distribution of chemical compounds
- Analysis of crystallinity and material stress properties
- Lateral, diffraction-limited spatial resolution down to ~200 nm
- 3D chemical imaging enabled by confocal architecture
- Nondestructive
- No staining or labelling necessary
- Correlative imaging options readily available (i.e. fluorescence microscopy, AFM, SNOM, SEM, TERS)

# Design & Components Modular & Upgradable

01 **OUTPUT COUPLER\***  
primary spectrometer output

02 **OUTPUT COUPLER\***  
outputs for additional spectrometers

03 **LASER INPUT COUPLERS\***  
single & triple wavelength modules  
combinable and stackable for more  
wavelengths

04 **VIDEO CAMERA COUPLER\***  
able to house different  
camera models

05 **KÖHLER WHITE-LIGHT  
ILLUMINATION\***  
with adjustable LED intensity

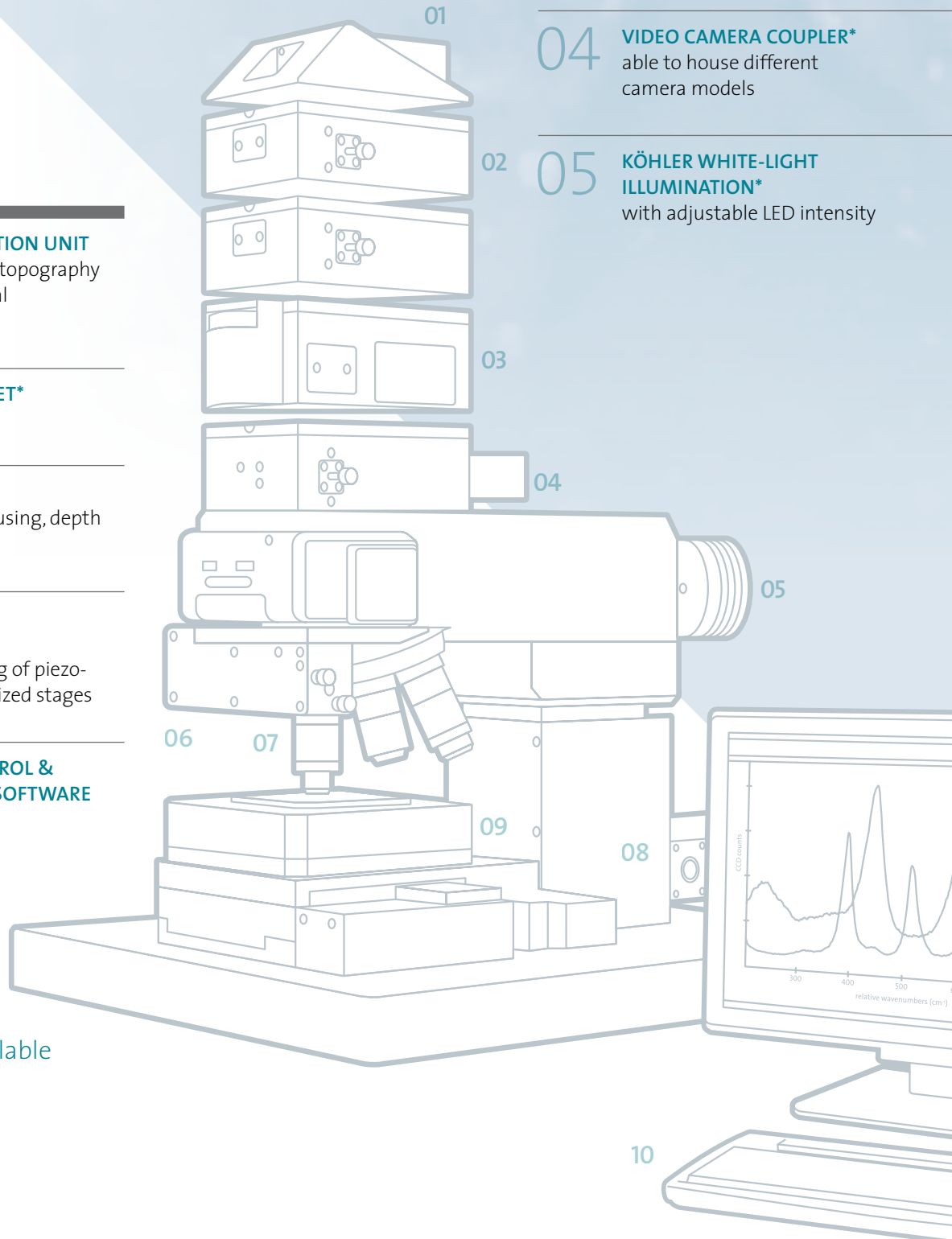
06 **AFM BEAM-DEFLECTION UNIT**  
for cantilever-based topography  
and near-field optical  
measurements

07 **MICROSCOPE TURRET\***  
up to 6 objectives

08 **STEPPER MOTOR**  
for z-movement, focusing, depth  
profiling, 3D stacks

09 **POSITIONING &  
SCANNING STAGES**  
cooperative scanning of piezo-  
driven and/or motorized stages

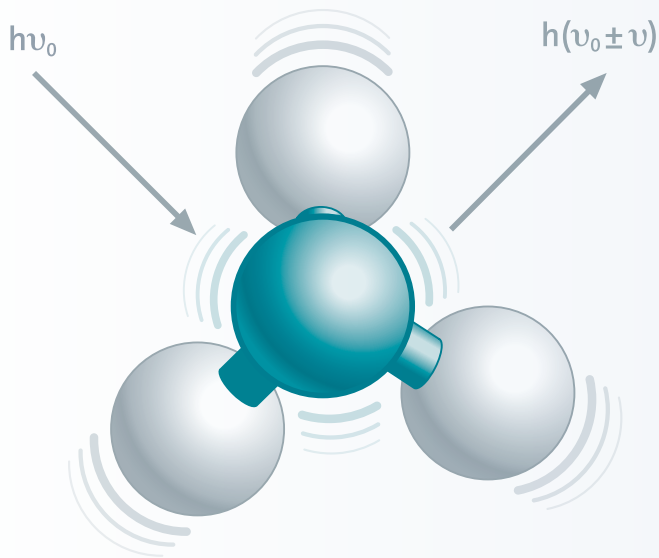
10 **MICROSCOPE CONTROL &  
DATA EVALUATION SOFTWARE**  
WITec Control  
WITec Project  
WITec Project Plus  
WITec TrueMatch



\*motorized options available

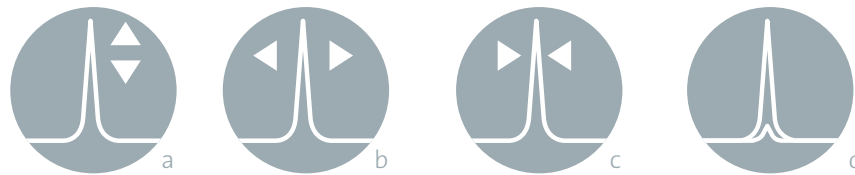


# Confocal Raman Microscopy & 3D Raman Imaging



## 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 can be easily identified by this individual spectral “fingerprint”.



## Additional sample information from Raman spectra:

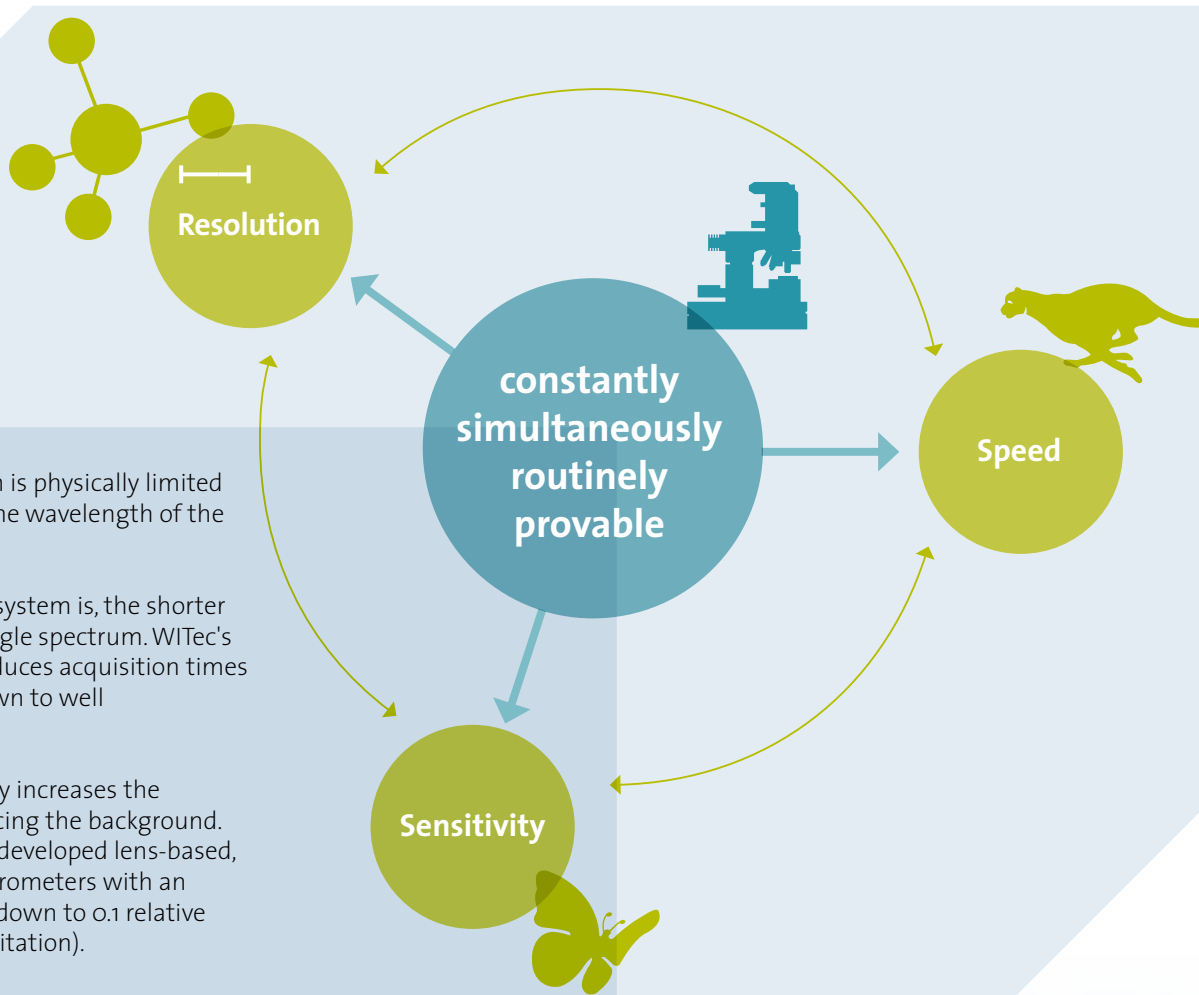
- Peak intensity: Quantity of a specific compound
- Peak shift: Identification of stress and strain states
- Peak width: Degree of crystallinity
- Polarization state: Crystal symmetry and orientation

## 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.



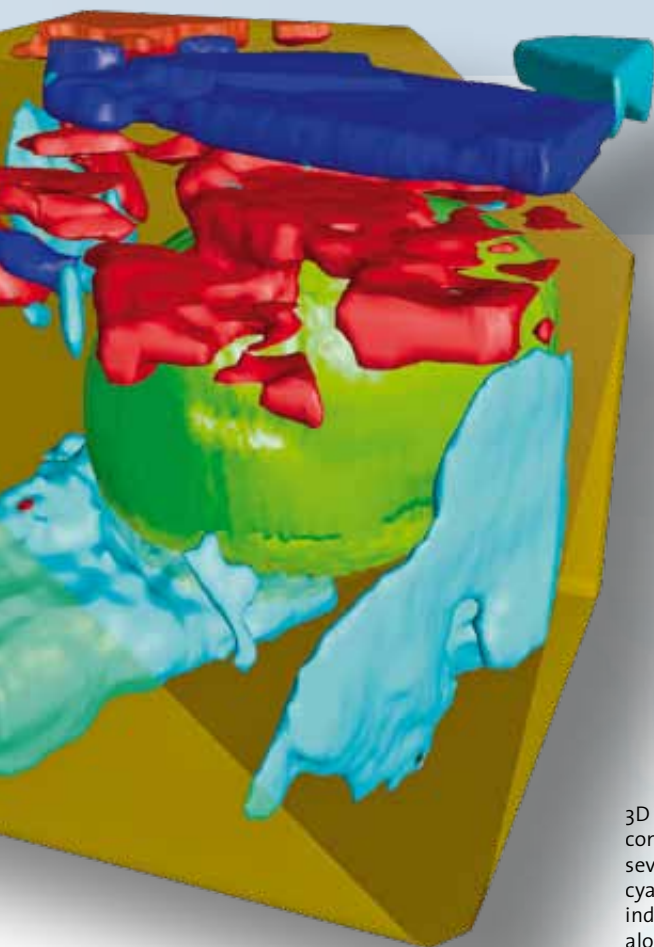




**Resolution:** Lateral resolution is physically limited to ~200 nm, depending on the wavelength of the incident light.

**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 an ultimate spectral resolution down to 0.1 relative wavenumbers (@633 nm excitation).



3D Raman image of a honey droplet containing a pollen grain (green) and several crystalline phases (red, blue, cyan), reconstructed from 50 individual 2D Raman images acquired along the z-axis.

### 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 system (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.

# alpha300 R Raman Microscope

The alpha300 R is a confocal Raman microscopy system available in several configurations with different features to fulfill a wide variety of customer requirements.

Whether you want to perform micro-Raman mapping or high-end 3D Raman imaging, as a Raman newcomer or an experienced user, in a scientific setting or an industrial laboratory, WITec offers the perfect Raman imaging solution for you.

## Key features and benefits

- True confocality, ideally suited to depth profiling and 3D Raman image generation
- Lateral resolution limited only by physical law
- Spectral resolution down to 0.1 relative wavenumbers (@633 nm excitation)
- Focus stabilization compensates for thermal and mechanical variations during long-term measurements
- Laser wavelength selectable from UV to NIR
- Throughput-optimized UHTS spectrometers with a variety of focal lengths
- Fast Raman Imaging™ and Ultrafast Raman Imaging with motorized or piezo-driven scanning stages
- 3D images and depth profiles with motorized or piezo-driven scanning stages





# alpha300 access

The alpha300 access is a high-quality confocal micro-Raman system that provides an entry point to WITec's technology.

## Key features and benefits

- Class-leading capability within challenging budget and procurement environments
- High-performance Raman mapping
- Exceptional spectral quality provided by the WITec UHTS Raman spectrometer series
- High-quality and ultra-precise optical components
- WITec Raman and imaging know-how
- The future of Raman spectroscopy through upgradeability



## Raman lab arrangement

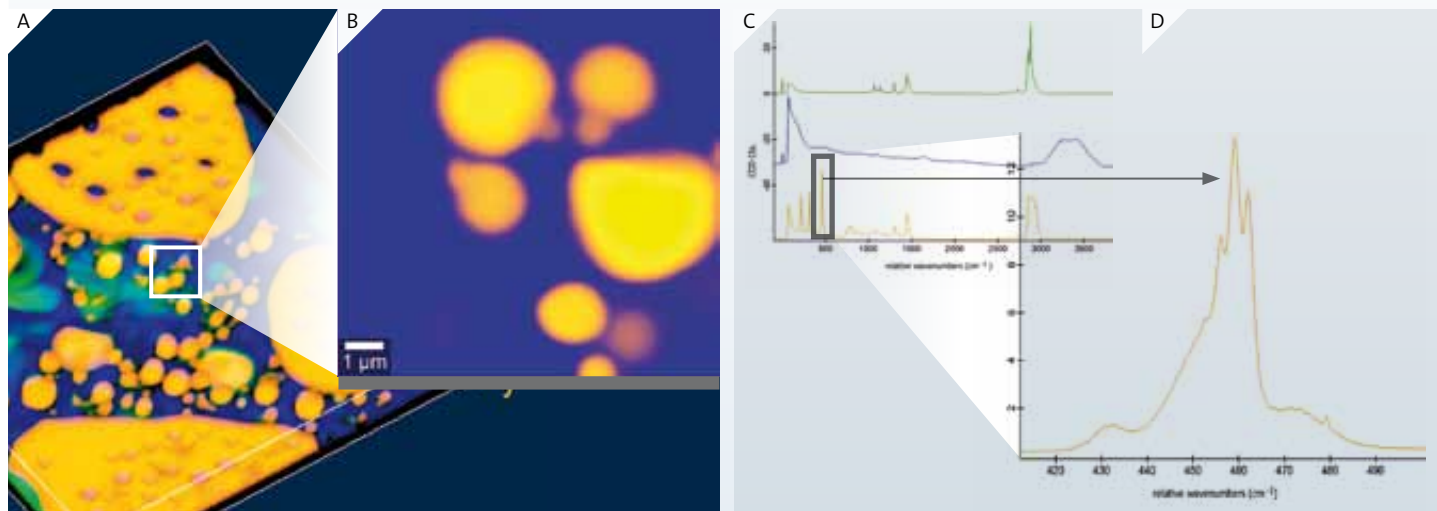


# apyron™ Raman Microscope

As a member of the WITec microscope family, *apyron*™ sets the benchmark for automated Raman imaging systems with excellent imaging quality, outstanding spectral and spatial resolution, ultrafast acquisition times and exceptional signal sensitivity. It is a highly-automated, push-button instrument. An extensive set of default and advanced measurement parameters in combination with motorization and a powerful software environment allow the user to obtain optimal results with unprecedented ease.



## High spatial and spectral resolution simultaneously: CCl<sub>4</sub> in an emulsion



**(A)** 3D confocal Raman image of an emulsion with corresponding Raman spectra shown in **(C)** green: alkane; blue: water; yellow: carbon tetrachloride + oil. Image parameters: 200 x 200 x 20 pixels, 100 x 100 x 10 μm<sup>3</sup> scan range, 0.06 s integration time per spectrum, 532 nm excitation wavelength. **(B)** Zoom-in image with high spectral resolution. **(D)** Due to the high spectral resolution of the spectroscopic system, the CCl<sub>4</sub> band at 460 relative wavenumbers (cm<sup>-1</sup>) can be resolved at room temperature into three peaks that reflect the isotopic variations of the molecules.



# alpha300 Ri

## Inverted Confocal Raman Microscope

The alpha300 Ri turns chemical imaging upside down. Its inverted beam path preserves all the functionality of WITec's alpha300 R microscopes while introducing a new angle in access and handling. The ability to view and investigate samples from below is a great advantage when working with aqueous solutions or oversized samples. Studies in life sciences, biomedicine, pharmaceuticals and geosciences in particular will benefit from the consistency and flexibility provided by the geometry of the alpha300 Ri. Many accessories and upgrade possibilities developed for the alpha300 series are compatible with the Ri version.

Bulky samples that would be challenging to investigate underneath a conventional microscope objective turret can be accommodated by placing them on the stage of the alpha300 Ri.

An inverted beam path allows liquid samples to be placed on the fixed plane of the stage for quick and repeatable measurements.

The motorized sample stage also facilitates the mounting of environmental enclosures and other accessories.



# alpha300 A

## Atomic Force Microscope

The WITec alpha300 A atomic force microscope integrated with a research-grade optical microscope provides superior optical access, easy cantilever alignment and high-resolution sample survey.

Using optical pre-inspection by means of various illumination and detection techniques (e.g. bright field, dark field, polarization, fluorescence, etc.), the user can conveniently determine the area of interest for the AFM measurement. By simply rotating the microscope turret, the user can switch between conventional microscopy and AFM modes quickly and easily.

The WITec AFM objective provides a direct view of both sample and cantilever for straightforward and precise cantilever tip positioning. The alpha300 A system includes an extremely linear and precise capacitive feedback-controlled scanning stage featuring TrueScan™ for exceptional accuracy over the entire scan range.

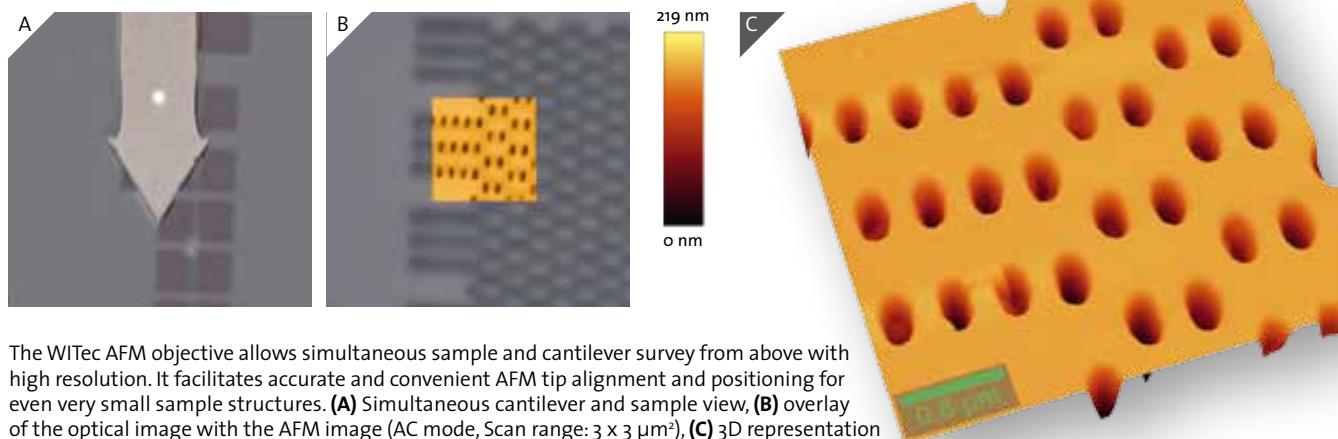
### Key features and benefits

- Surface characterization on the nanometer scale
- Optical and AFM combination
- Nondestructive imaging
- Minimal, if any, sample preparation
- Ease of use in air and liquids
- Combinable with confocal Raman imaging and scanning near-field optical microscopy (SNOM)
- TrueScan™ controlled piezo-driven scanning stages with capacitive feedback loops:
  - 30 x 30 x 10  $\mu\text{m}^3$
  - 100 x 100 x 20  $\mu\text{m}^3$
  - 200 x 200 x 20  $\mu\text{m}^3$

### AFM modes

- AC Mode
- Contact Mode
- Digital Pulsed Force Mode™ (DPFM)
- Electrostatic Force Microscopy (EFM)
- Kelvin Probe Microscopy
- Lateral Force Microscopy (LFM)
- Lift Mode™
- Magnetic Force Microscopy (MFM)
- Nanomanipulation/Lithography

### Simultaneous cantilever and sample view for easy determination of the measurement position

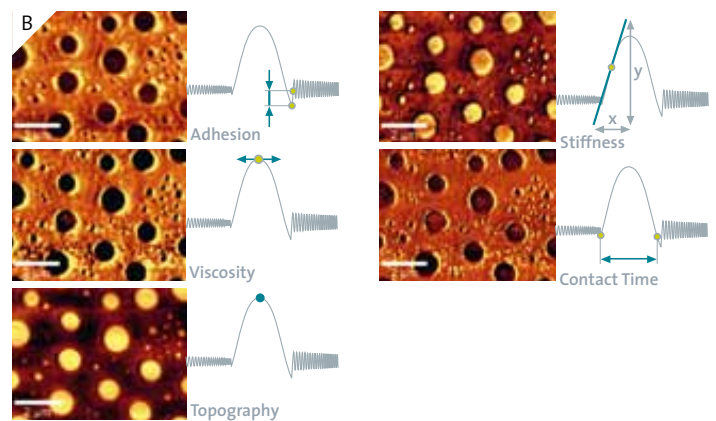
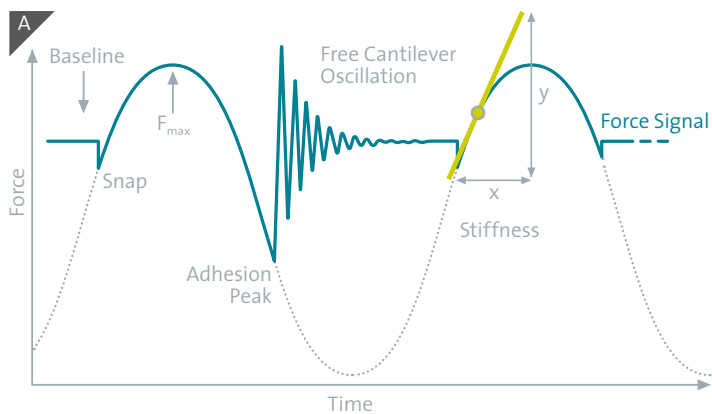


The WITec AFM objective allows simultaneous sample and cantilever survey from above with high resolution. It facilitates accurate and convenient AFM tip alignment and positioning for even very small sample structures. **(A)** Simultaneous cantilever and sample view, **(B)** overlay of the optical image with the AFM image (AC mode, Scan range: 3 x 3  $\mu\text{m}^2$ ), **(C)** 3D representation of the AFM image from **(B)**.





## Imaging of surface properties with DPFM



## Digital Pulsed Force Mode (DPFM)

Pulsed Force Mode (PFM) is a non-resonant, intermittent contact mode for atomic force microscopy that allows the characterization of material properties such as adhesion, stiffness and viscosity along with the sample topography. Additionally, lateral forces are virtually eliminated. Therefore high-resolution mapping of delicate samples in air and liquids is easily achievable while maintaining a scanning speed comparable to contact mode AFM. In contrast to most other intermittent contact techniques, the perpendicular forces on the sample (introduced by the AFM tip) are controlled by a feedback loop.

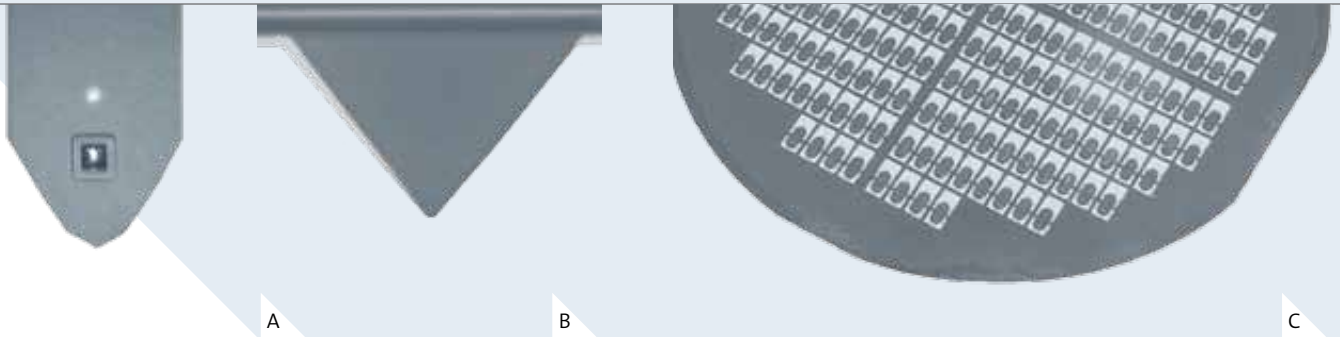
(A) The PFM electronics induces a sinusoidal modulation of the z-piezo of the AFM with an amplitude of 10 – 500 nm at a user-selectable frequency of between 100 Hz and 2 kHz, what is far below the resonant frequency of the cantilever. A complete force-distance cycle is carried out at this rate, resulting in the force signal as shown in the figure. (B) An ethyl-hexyl-acrylate/polystyrene blend (EHA/PS) was spin-coated onto a glass substrate. Various surface properties were analyzed and imaged simultaneously using DPFM. Dark areas in the images correspond to low values.

# alpha300 S

## Scanning Near-field Optical Microscope

Super-resolution microscopy down to 60 nm can be achieved with the WITec scanning near-field optical microscope (SNOM) alpha300 S. SNOM, as compared to other optical super-resolution techniques such as STED or STORM is not dependent on fluorescent tags or specialized laser excitation sources. The operation of the system is straightforward as various measurement procedures such as a high-speed automatic cantilever approach and adjustment can be controlled through the intuitive software.

The alpha300 S combines the advantages of SNOM, confocal microscopy and AFM in a single instrument. By rotating the objective turret one can easily switch between the different modes. One field that can benefit immensely from this technique is nanotechnology research, in particular nanophotonics and nano-optics. In life sciences and materials research, SNOM permits optical detection of the most miniscule surface structures of transparent as well as opaque samples.



**(A)** Video camera top view of a SNOM sensor and sample, **(B)** side view of a cantilever pyramid, **(C)** SNOM cantilever wafer.

Comparison of optical resolution between confocal microscopy **(D)** and scanning near-field optical microscopy **(E)** on a latex projection pattern.

### Cantilever SNOM-sensors

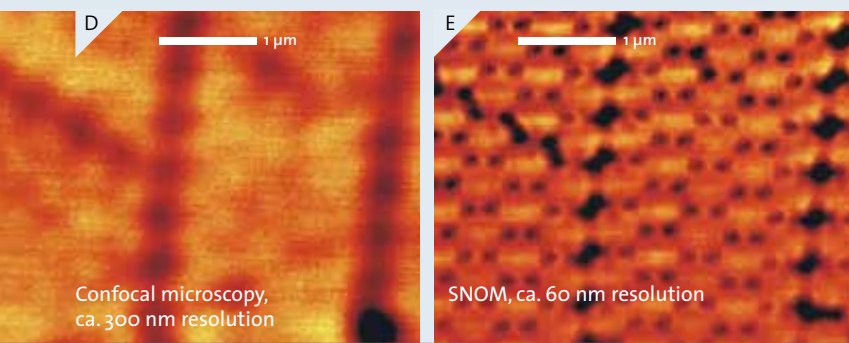
The alpha300 S uses unique, patented, high-quality micro-fabricated SNOM sensors consisting of a silicon cantilever with a hollow pyramid tip. The SNOM aperture is at the apex of the pyramid. The laser light used for optical imaging is focused into the back of the tip and then onto the sample. Due to the wide opening angle of the pyramid, the transmission coefficient is much higher than that of fiber probes with the same aperture diameter. An established and proven manufacturing process

produces tips with apertures of varying sizes to meet customers' individual requirements. Cantilever SNOM sensors are, unlike fiber tips, very robust and flexible in the z-direction and allow the beam deflection technique to precisely control the tip-sample distance. All of these innovative characteristics make the handling of probes during near-field measurements very user-friendly for the most reliable optical imaging available beyond the diffraction limit.

## Key features and benefits

- Spatial resolution beyond the diffraction limit (ca. 60 nm laterally)
- Unique patented SNOM sensors
- Ease of use in air and liquids
- Nondestructive, label-free imaging technique provides super-resolution microscopy with minimal, if any, sample preparation
- Upgradeable with confocal Raman imaging for correlative microscopy and near-field Raman imaging
- Three techniques always integrated within one instrument: confocal microscopy, AFM and SNOM

## Super-resolution microscopy: SNOM





# Correlative Raman Imaging Made Easy

WITec's alpha300 microscope series was designed with inherent flexibility and configurability to address users' individual requirements. This not only involves easy and cost-effective upgrade and extension options, but also the possibility to add other microscopy techniques to the instrument.

As no single imaging approach can reveal every feature of a sample, a more comprehensive understanding can be achieved by using several techniques and correlating the results.

This advantage is particularly pronounced when using methods that deliver images of the morphology of a sample while also determining its molecular composition.

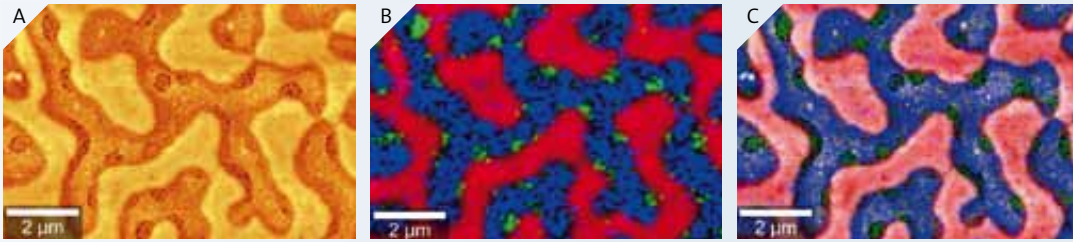
WITec's modular product line incorporates nearly all scanning probe and optical microscopy techniques. Each microscope model can always be equipped with the functionality of another variation of the alpha300 family either as a built-in feature or as a later upgrade. The WITec hardware and software environment is used for all new features and upgrades, ensuring the best possible compatibility and ease of use.

## Switching microscopy modes by rotating the objective turret

**Scanning Probe Microscopy (SPM) mode for AFM or SNOM:**  
The SPM cantilever is held at the end of the objective's arm. The cantilever is aligned with an integrated highly-precise inertial drive.

**Optical modes:** Rotating the turret provides access to the optical modes, allowing high-resolution confocal optical and chemical imaging to be seamlessly linked with AFM or SNOM measurements without transferring the sample.





### Raman-AFM

By combining the materials analysis capability of confocal Raman imaging with the ultrahigh topographic and lateral resolution of an AFM, the chemical properties of a sample can be easily correlated with its surface characteristics.

The well-established alpha300 RA was the first integrated Raman-AFM system on the market and it continues to set the benchmark for combined instrument configurations.

The alpha300 RA incorporates the features of the alpha300 R for chemical imaging along with the alpha300 A for atomic force microscopy.

*For TERS options, please see page 20.*

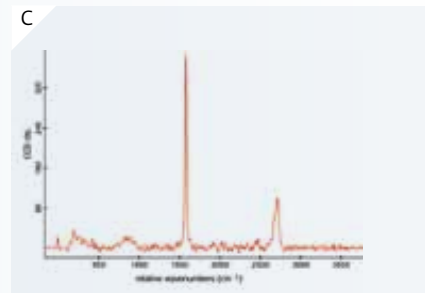
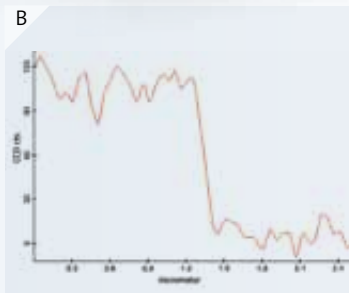
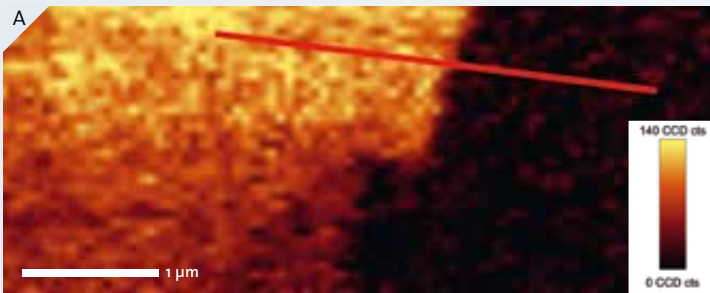
### Correlative, high-resolution AFM-confocal Raman imaging of a polymer mixture

- (A) The sample's phase image was created using AFM in tapping mode.
- (B) Raman spectra were recorded at each image pixel of the area and displayed as a false color image. Red: polystyrene, green: ethyl-hexyl-acrylate (EHA), violet: styrene-butadiene-rubber (SBR), blue: mixed spectrum of SBR and EHA.
- (C) The overlay of the AFM phase and the Raman image identifies and locates the sample's components.

### Raman-SNOM

The alpha300 RS combines Raman characterization and imaging with scanning near-field optical microscopy for optical imaging with resolution beyond the diffraction limit. It combines all features of the alpha300 S and alpha300 R instruments.

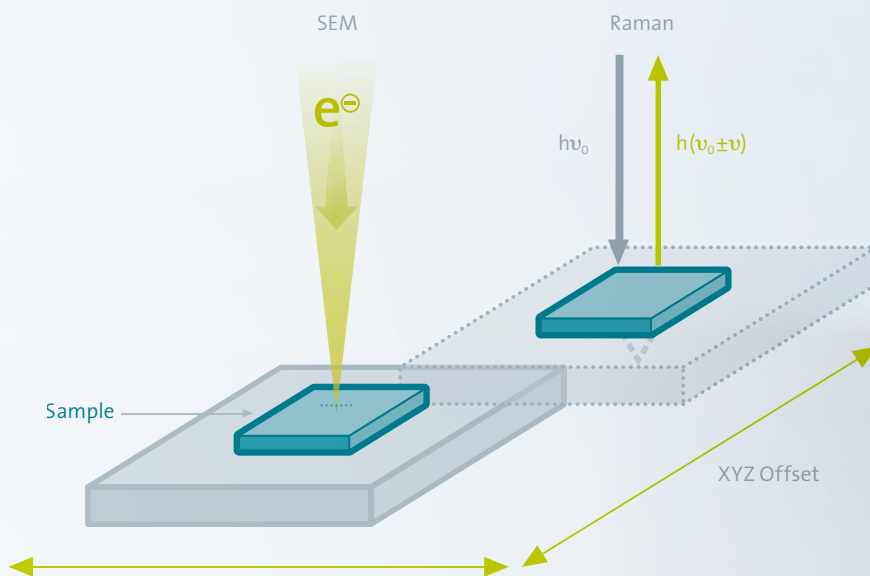
alpha300 R and alpha300 S



(A) Near-field Raman image of the G-Band intensity of graphene. (B) The graph shows the G-band intensity along the red line and reveals the measurable signal variations between the small sample and the substrate. (C) Corresponding Raman spectrum obtained by the Raman-SNOM measurement.

# RISE™ – Raman Imaging and Scanning Electron Microscopy

Confocal Raman imaging and scanning electron microscopy (SEM) are techniques that are ideally suited to correlating structural and chemical information.



## Principle of RISE™ microscopy

Samples are automatically transferred from one measuring position to the other within the vacuum chamber of the combined Raman-SEM instrument, streamlining the workflow and drastically improving ease of use.





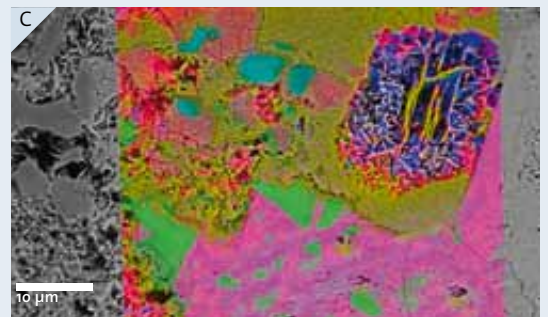
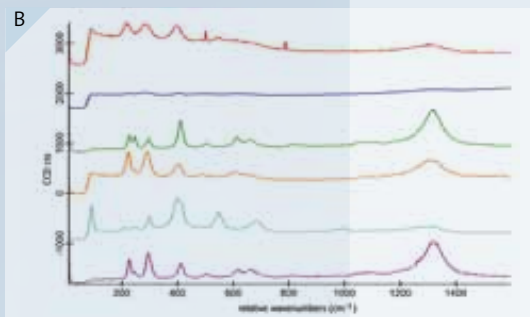
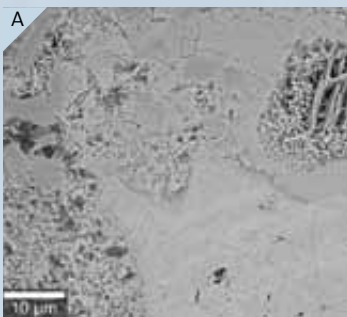
### Key features and benefits

RISE™ microscopy systems combine all features of a stand-alone SEM and a WITec research-grade confocal Raman imaging microscope within one instrument to provide:

- Quick and convenient switching between Raman and SEM modes
- Automated sample transfer from one measuring position to the other within the vacuum chamber
- An integrated software interface for user-friendly measurement control
- Easy correlation of experimental results and image overlay
- SEM and Raman imaging capabilities without compromise
- A truly confocal optical path
- Research-grade optical imaging

The sample remains inside the vacuum chamber during both the SEM and Raman measurements to ensure seamless correlative microscopy.

### Application iron mineralogy



**(A)** In the SEM image a piece of hematite ( $\text{Fe}_2\text{O}_3$ ) displays some structural characteristics. **(B)** Hematite and goethite ( $\text{FeO}(\text{OH})$ ) in several crystal orientations were identified from their Raman spectra. Crystal forms of hematite are depicted in red, blue, green, orange and pink and those of goethite in light blue and cyan. From the spectra, a Raman image was generated. **(C)** Correlation of Raman and SEM data resulted in the RISE™ image.

# Fiber-Based Beam Delivery Facts and Benefits

WITec's modular design relies on efficient beam delivery via optical fiber. Long-term investments in research and development have yielded an exceptional understanding of fiber-coupling mechanisms, enabling benefits unattainable with more conventional methods.

- Virtually lossless energy transmission
- Fibers provide a diffraction-limited point light source for the highest confocality and spatial resolution
- Pre-configured and pre-aligned fiber coupling units guarantee long-term stability and user-friendliness without the need for further adjustment
- Polarization direction of the light is maintained for the most intricate polarization-dependent measurements
- Lasers and spectrometers can be mounted far away from the microscope, allowing flexible and compact system footprints that can alleviate thermal or vibrational disturbances



# Modular Components & Accessories

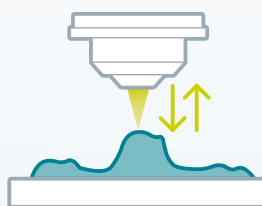
## Beam Path Options

### Excitation and detection options

The WITec alpha300 microscope series features several beam path geometries. The upright microscope enables measurements of opaque samples while the inverted microscope (p. 13) is ideal for experiments on transparent samples. Side-illumination options are also available. The modularity and inherent flexibility of WITec's unique alpha300 microscope systems provide optimized instrumentation for many different types of experiments.

### alpha300 R

Excitation & detection from above



### alpha300 Ri

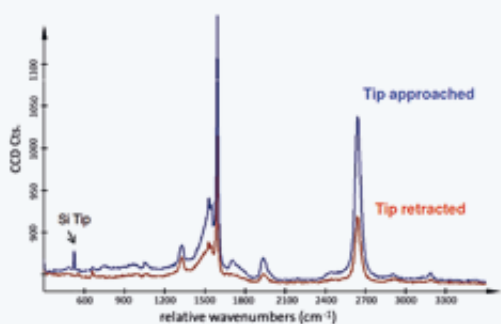
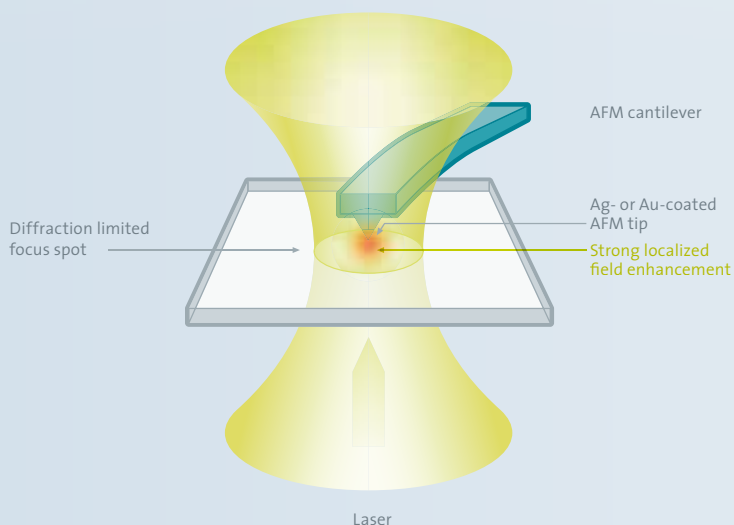
Excitation & detection from below



### WITec TERS module for the alpha300 series

Tip-Enhanced Raman Spectroscopy (TERS) enables the acquisition of chemical information with a lateral resolution far below the diffraction limit. The TERS tip illumination can be performed from either above, below or from the side. The WITec alpha300 microscope series accommodates all excitation approaches and TERS measurements can be carried out alongside Raman and AFM investigations with the same integrated instrument.

### TERS working principle



alpha300 TERS experiment on a carbon nanowire: The enhancement of the spectral intensity in TERS-mode is clearly visible.



# Electronics & Software

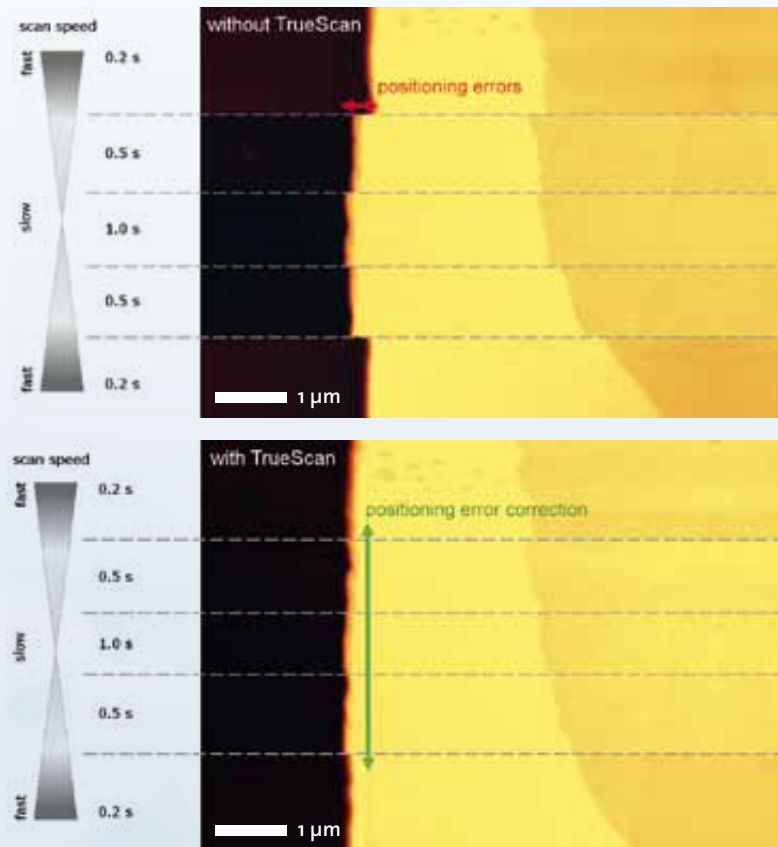
## alphaControl

alphaControl is a patented digital microscope controller based on the system-on-a-chip concept. The digital component of the system is entirely realized within one Field Programmable Gate Array (FPGA). This architecture results in speed, flexibility, accuracy, expandability and precise timing, while simplifying operation of the various microscope configurations and extensions from a single hardware device.

## Key features and benefits

- TruePower™ for precise and absolute laser power adjustment for up to four lasers
- Automated high-speed cantilever approach
- High-speed frequency scan
- TrueScan™ lag error correction
- Automated light intensity adjustment
- High-speed overload protection for photon-counting detectors
- Two built-in fully digital lock-in amplifiers (real amplitude and real phase up to 500 KHz)
- Fully digital feedback-control
- USB 3.0 interface
- Digital piezo controller interface
- Real-time rotation matrix and real-time translation for XYZ scans
- Precise timing
- Modular and upgradable system

## TrueScan™



TrueScan™ is an integrated scanning engine that corrects dynamic positioning errors. As the piezo-driven scanning stage moves the sample forward and backward during the measurement the stage's inertia leads to lag errors. This effect increases with scanning speed. While the incorporated capacitive feedback system determines the actual position of the stage, TrueScan™ can resolve the discrepancy between its actual and intended position, allowing high-resolution lag-free AFM imaging at high scanning speeds.

# WITec Suite *FIVE*

## Powerful Software Environment

Experimental setup, instrument control and data analysis are made easy with WITec's software tools.

- Sophisticated data acquisition, evaluation, post-processing and image generation for confocal Raman microscopy, AFM and SNOM.
- The software architecture and graphical user interface offer an integrated and consolidated functionality incorporating every technique and measurement mode.
- Suitable for all experience levels and user requirements through an individually-adjustable user interface.

### Control *FIVE*

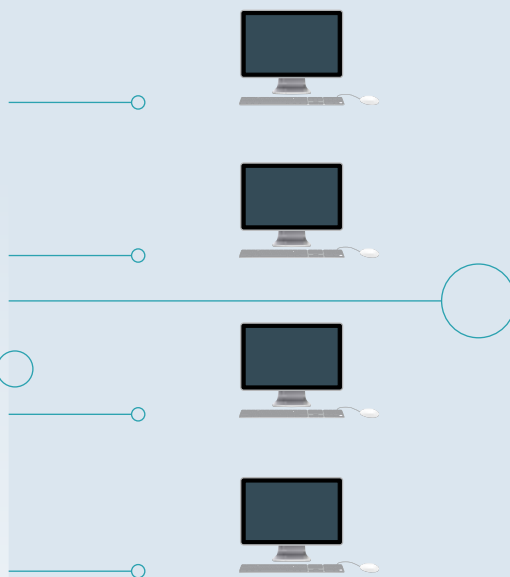


WITec microscope family with alphaControl

#### WITec Control *FIVE*

- Centralized experiment and instrument settings
- Acquisition of imaging and/or spectroscopy data
- On- and offline data analysis and image processing

### Project *FIVE*



#### WITec Project *FIVE*

- Offline data and image processing
- Licensed for an unlimited number of users

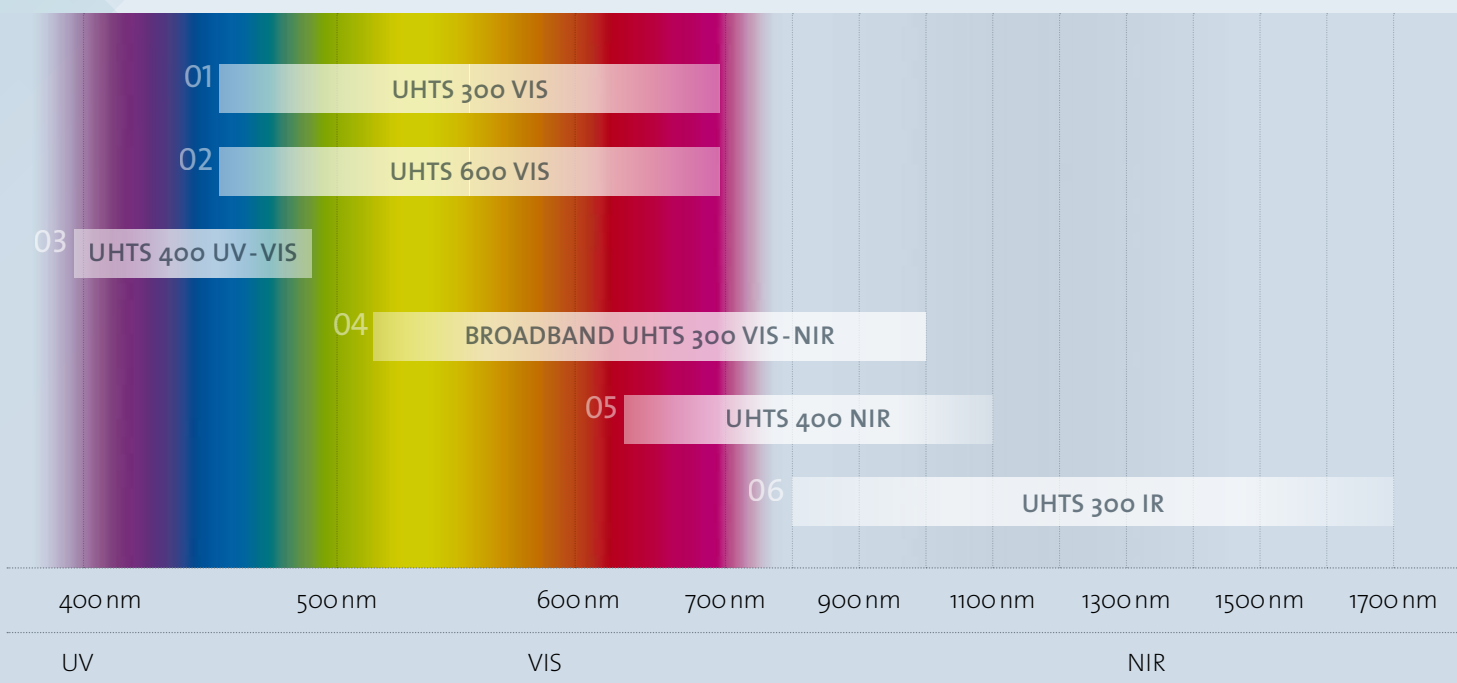
### Project *FIVE+*



#### WITec Project *FIVE+*

- Advanced offline data and image processing
- Chemometric post-processing features and superior data analysis tools: Cluster Analysis, Principal Component Analysis, Spectral Demixing, Advanced Spectral Fitting, Image Correlation and many more
- Single user license

# Modular Components & Accessories



## Spectroscopy solutions: The UHTS series

The WITec Ultra-High Throughput Spectrometer (UHTS) series consists of exceptionally flexible and precise devices that meet the demands of cutting-edge confocal Raman imaging.

Varied excitation sources and scattering experiments require specialized optics for optimal throughput. The WITec UHTS design approach takes this into account by employing a series of lens-based on-axis spectrometers for a range of excitation wavelengths.

Spectrometers of the UHTS series allow more than 70% total transmission for high-speed and high-resolution Raman imaging. They feature an optical fiber port and a motorized triple-grating turret. Customers can choose from among several focal lengths and gratings to match individual requirements in terms of spectral range or resolution.

## Sample positioning and scanning stages

WITec offers manual, stepper motor-driven and piezo-actuated stages to accommodate a wide variety of samples and applications.

- Manual translation stage for accurate sample positioning
- Motorized translation stages for large-scale positioning, mapping and imaging
- Piezo-driven scanning stages with closed-loop operation for AFM and high-resolution Raman measurements

The stages feature several different travel ranges for the highest flexibility.

## Heating & cooling stages

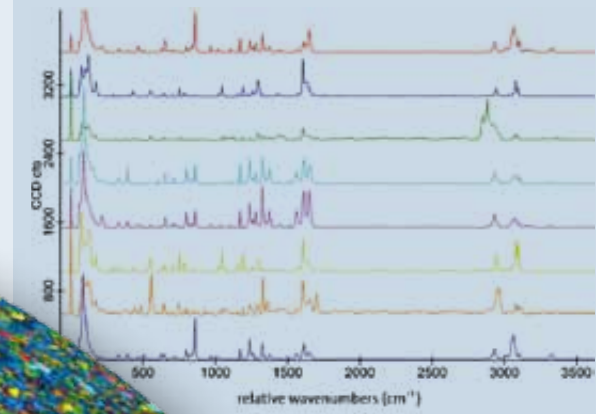
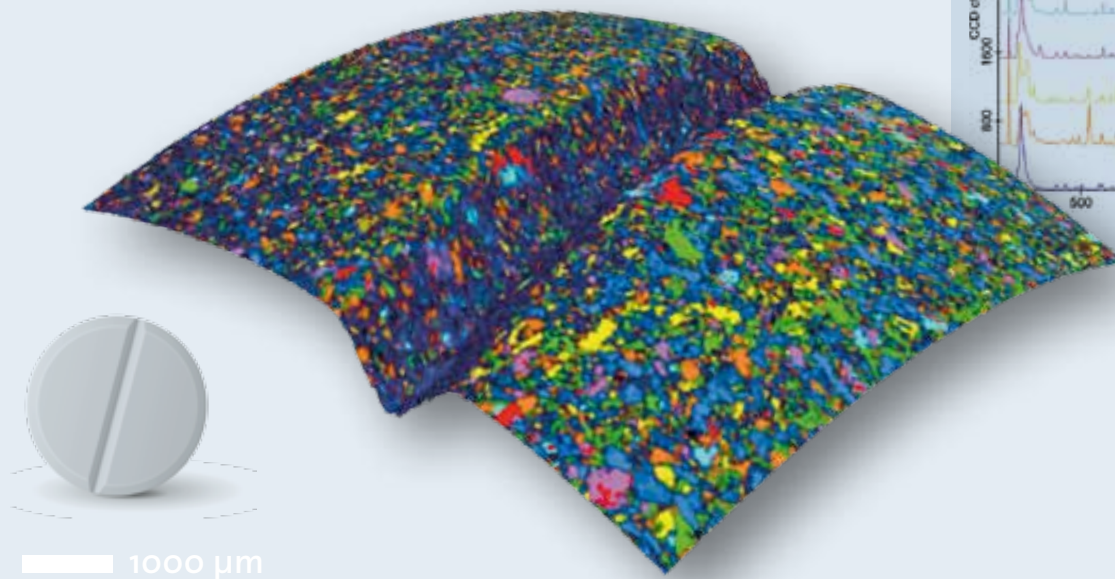
For experiments requiring an accurate temperature control, the alpha300 series can be equipped with heating and cooling stages with ranges from -269°C up to 1500°C (4 K - 1773 K) for AFM and high-resolution Raman measurements.

## Sample mounting stages

A selection of mounting stages is available for securing samples of various shapes and environmental requirements. Microscope slides, Petri dishes, NUNC™ flasks and cylindrical samples such as stents can all be accommodated. The integration of third-party cryostats is available upon request.



# TrueSurface™



## TrueSurface™ microscopy

WITec's patented TrueSurface™ microscopy option enables confocal Raman imaging guided by surface topography. With TrueSurface™ chemical characterization on rough, inclined or irregularly-shaped samples can be carried out precisely because the sensor actively monitors the sample surface and keeps it in focus. This topographic imaging module also compensates for variations during measurements with long integration times. TrueSurface™ pioneered by WITec uses an advanced optical profilometer integrated within the instrument to provide one-pass simultaneous acquisition of both topographic and molecular information.

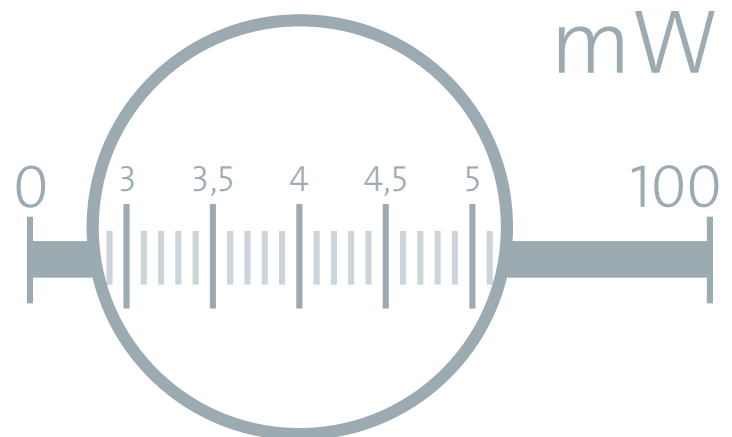
## Laser power adjustment with TruePower™

TruePower is a new experience in laser power adjustment for confocal Raman imaging measurements. The absolute laser power delivered to the microscope is measured and adjusted with an accuracy of 0.1 mW. A laser shutter shields the sample from the laser light and opens only during Raman analysis with an optimized power level to avoid any sample degradation. TruePower is the only laser power regulation system on the market to provide such accuracy.

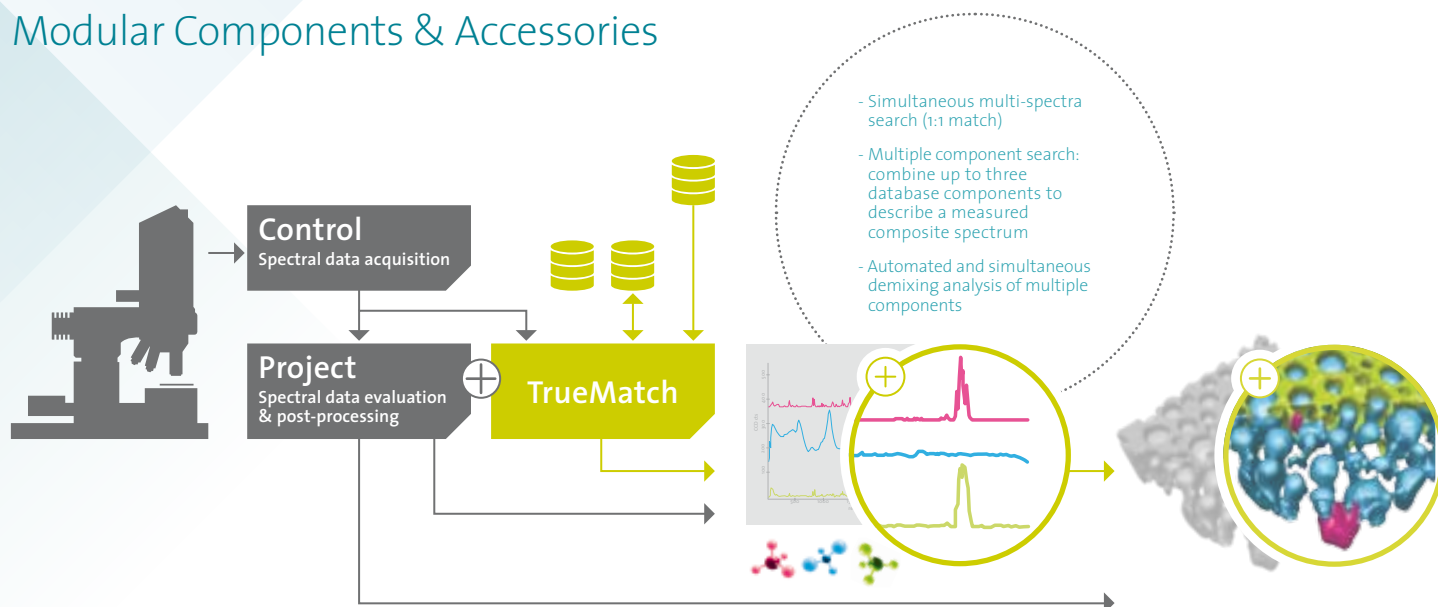
- Optimal laser power determination for the preservation of delicate samples and reproducibility in measurement conditions
- Power series measurements enabled by making power-induced spectral changes quantifiable
- Automated documentation of laser settings
- Retrofittable for most WITec imaging systems

## Laser sources

Excitation laser sources for Raman, SNOM and photoluminescence imaging can be individually chosen and attached in concert to the microscope via optically-matched fiber coupling. Available laser wavelengths range from the UV to the NIR to fulfill a variety of experimental excitation requirements. Switching between different lasers is a matter of simply rotating a wheel in the coupling unit or by a click in the software.



## Modular Components & Accessories



### TrueMatch™ Raman spectral database management

TrueMatch™ is a powerful and innovative software module for identifying the components of a sample and for accessing and developing Raman spectral databases. With this technology, existing databases containing representative Raman spectra of common materials or substrates can be searched and referenced. It also allows users to create their own catalog of spectra pertinent to their research. TrueMatch™ is fully integrated with the WITec Suite *FIVE* software environment and is a perfect complement to its data evaluation and post-processing capabilities.

### LabVIEW interface

The optional LabVIEW interface provides access for alphaControl in order to design and control individual measurement procedures with LabVIEW.

### RayShield coupling unit for Raman imaging at low wavenumbers

The RayShield coupler includes a specialized narrow band filter set which is optimally aligned for the detection of Stokes and anti-Stokes Raman lines close to the Rayleigh line (ca. 10 cm<sup>-1</sup>) while maintaining an ideal Rayleigh shielding.

### Anti-Stokes extension

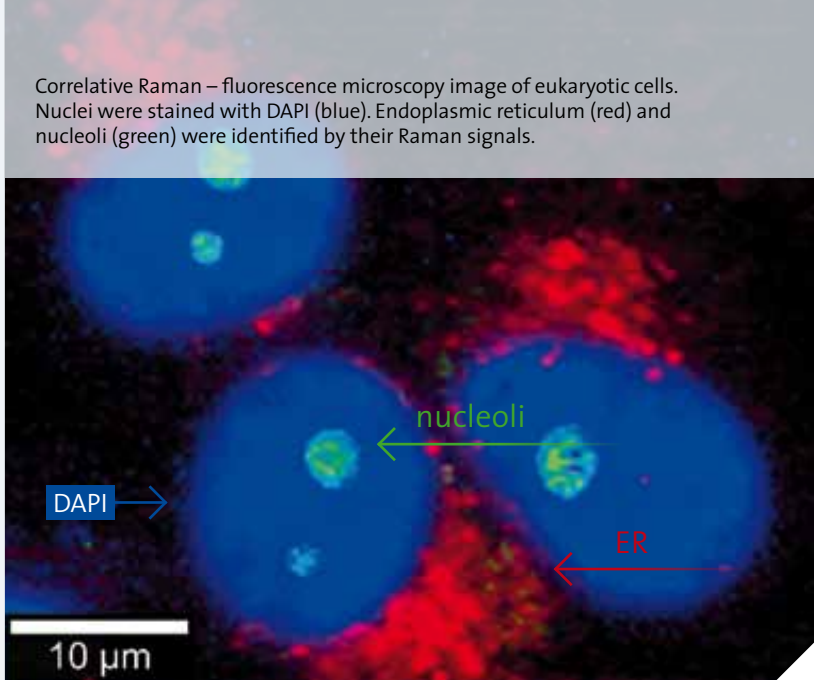
A wavelength-optimized filter unit for the detection of anti-Stokes Raman signals is available as an option for advanced Raman imaging experiments.

### Polarization module

Polarization dependent measurements can be used to analyze molecular orientations and geometries of materials. The module is comprised of an excitation wavelength specific polarization kit for rotating the polarization of the laser light and an analyzer for filtering information of a certain polarization.

### StrobeLock

StrobeLock is a WITec extension that enables time-correlated single photon counting measurements of unprecedented accuracy. The available imaging modes include fluorescence lifetime imaging microscopy (FLIM).



Correlative Raman – fluorescence microscopy image of eukaryotic cells. Nuclei were stained with DAPI (blue). Endoplasmic reticulum (red) and nucleoli (green) were identified by their Raman signals.

### Enclosure & rigid support frame

For maximum reduction of environmental interference such as acoustic or vibrational noise, the WITec support frame and enclosure system greatly contributes to achieving exceptional imaging results. The enclosure can be configured as an air-tight option to allow control of the gas-phase during experiments. Equipped with an interlock, safety regulations (e.g. laser class I requirements) can be easily accommodated.

### Objectives

A wide range of objectives for various microscopy techniques are available to meet virtually any experimental requirement.

### Imaging in liquids

Imaging in liquids can be easily performed by using a water immersion objective in optical mode or the liquid imaging extension for AFM and SNOM modes. A specialized cantilever holder and inertial drive allows operation in fluids (e.g. in Petri dishes).

### Customized solutions

If your application requires other components, WITec looks forward to discussing your individual requirements and helping to get your experiment up and running. The inherent modularity of our product line allows additional high-quality parts and detectors to be easily integrated.

### AFM & SNOM cantilevers

WITec provides several types of high-quality AFM-cantilevers for various AFM imaging modes. WITec is the only company capable of producing highly reliable SNOM cantilever sensors with apertures ranging from 60 nm to 100 nm. Each SNOM cantilever is individually tested before shipment.

### AC (Tapping™) mode for AFM & SNOM

AC mode for WITec microscope systems allows state-of-the-art resonant intermittent-contact AFM and SNOM imaging specifically tailored for soft and delicate samples. Acoustic AC mode enhances the imaging capabilities of contact mode AFM on such samples, eliminating lateral forces and delivering optimized resolution.

### DaVinci nanolithography

Advanced nanotechnology often requires accurate and reliable nano-manipulation or nanolithography tools for precise surface structuring. The WITec DaVinci nanolithography package allows these kinds of experiments in AFM as well as in optical modes with an integrated laser shutter control.

### Featured light microscopy techniques

The alpha300 series offers a selection of sample survey and data acquisition methods such as: fluorescence contrast imaging, DIC, dark field microscopy, petrography and polarization-dependent measurements.

### Photoluminescence detection

For the detection of photoluminescence spectra beyond 1.1 μm, an InGaAs detector can be easily integrated with the spectroscopic setup. Raman experiments can be extended to acquire additional information in the near-infrared photoluminescence regime (or for 1064 nm Raman measurements).



Since its founding in 1997, the Raman imaging company WITec has established itself as a market leader in the fields of Raman microscopy and correlative Raman-AFM, Raman-SNOM and Raman-SEM (RISE) microscopy. WITec's innovative spirit has kept the alpha300 microscope series at the forefront of the Raman imaging market since it initially revolutionized the field and established Fast Raman Imaging™ as a standard technique.

Ongoing development of the first truly confocal Raman imaging system continues to enable the setting of benchmarks in sensitivity, speed and spectral and spatial resolution. As reflected in WITec's maxim "Focus Innovations" our success is based on continually introducing new technologies and a commitment to maintaining customer satisfaction through high-quality, flexible and innovative products.

## Awards



2008

- R&D 100 for the alpha500



2011

- PITCON Editors' Gold Award for TrueSurface™ Microscopy



- R&D 100 for TrueSurface™ Microscopy



- Microscopy Today Innovation Award for TrueSurface™ Microscopy



2012

- Photonics Prism Award Winner (TrueSurface™ Microscopy)



2015

- Photonics Prism Award 2015 Winner (RISE™ Microscopy)

- Achema Innovation Award 2015 (*apyron*™ automated Raman imaging system)

## WITec milestones

1997

- SNOM system with unique cantilever SNOM sensors
- Pulsed Force Mode

1999

- Confocal Raman Microscope (WITec CRM 200) for fast 3D Raman imaging

2003

- WITec Mercury 100 Atomic Force Microscope
- Digital Pulsed Force Mode
- World's first integrated Raman microscope/AFM combination

2006

- Modular alpha300 series with FPGA-based control unit alphaControl

2008

- alpha500 series for large-area and automated multi-point measurements

2010

- TrueSurface™ Microscopy for confocal microscopy along with large-area optical profiling

2014

- RISE™ Microscopy: First integration of Raman Imaging and Scanning Electron Microscopy for correlative Raman-SEM Imaging

2015

- Automated Raman imaging system *apyron*™

2016

- alpha300 series design evolution
- alpha300 *access*

2018

- TrueSurface™ redefined: innovation in one-pass profilometer-guided Raman imaging
- Inverted confocal Raman microscope alpha300 *Ri*
- TrueMatch integrated Raman spectral database software

*To be continued ...*



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WITec K.K., Kanagawa

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**WITec South East Asia**

WITec Pte. Ltd., Singapore

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**WITec Offices Europe**

Barcelona, Lyon, London

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# WITec Product Line

The WITec product portfolio includes imaging systems for Raman, AFM and SNOM analyses as single technique solutions as well as correlative imaging configurations (Raman-AFM, Raman-SNOM, Raman-SEM). All WITec microscopes are high-quality modular systems with exceptional optical throughput, unparalleled signal sensitivity and outstanding imaging capabilities.

The common thread throughout is that all systems are based on the same hardware and software architecture. Whenever required it is possible to simply upgrade any system, even the most basic, with additional features and components, allowing our customers to keep pace with future challenges.

## alpha300 R



The WITec alpha300 R is a research-grade microscope combined with an ultrahigh-throughput spectrometer (UHTS) for Raman imaging with exceptional performance in speed, sensitivity, confocality and optical and spectral resolution.

## alpha300 A



The WITec alpha300 A atomic force microscope provides nanoscale surface characterization with an integrated research-grade optical microscope for easy cantilever alignment and high-resolution sample survey.

## alpha300 S



The alpha300 S is a super-resolution scanning near-field optical microscope (SNOM) that combines the advantages of SNOM, confocal microscopy and AFM in a single instrument. It uses unique micro-fabricated SNOM cantilever sensors for spatial resolution well beyond the diffraction limit.



## apyron™

The WITec *apyron*™ is an intuitive motorized microscope system for Raman spectroscopy and chemical imaging. The push-button principle of the system has the advantage of drastically reducing the time required to become familiar with the operation of the instrument, which greatly accelerates the initiation of measurements and increases the rate of sample turnover. With the *apyron*'s straightforward interface the user can concentrate solely on their experiment.



## alpha300 RA



The alpha300 RA incorporates the features of the alpha300 R along with those of the alpha300 A. This instrument enables correlative Raman-AFM imaging for a more comprehensive understanding of samples.

## alpha300 RS



The alpha300 RS facilitates confocal Raman imaging in combination with scanning near-field optical microscopy for optical imaging with super-resolution down to 60 nm. This microscope combines all features of SNOM and confocal Raman imaging and many AFM modes.

# WITec Product Line

## alpha300 Ri

The inverted beam path of the alpha300 Ri preserves all the functionality of the standard alpha300 R microscope while introducing a new angle in access and handling. The ability to view and investigate samples from below is a great advantage when working with aqueous solutions and oversized samples.



## alpha300 *access*



The alpha300 *access* is a micro-Raman single-spot analysis and mapping microscope. It was specifically engineered for budget-conscious customers with high demands on instrument performance. It offers outstanding spectral quality, optical throughput and signal sensitivity. It is fully compatible with and can be upgraded by adding alpha300 R series components.

## RISE™ Microscopy



RISE™ microscopy is a novel correlative microscopy technique that integrates SEM and confocal Raman imaging. With RISE™ microscopy ultra-structural surface properties can be linked to molecular compound information. The instruments combine all features of an alpha300 R confocal Raman imaging microscope with a stand-alone SEM.





**WE TAKE CARE**  
WITec uses environmentally friendly printed materials. While this policy is only a small contribution to a healthy environment, we at WITec believe that focusing on details can effect positive change in the world.

## WITec alpha300 Series



**alpha300 S:** Scanning  
Near-field Optical Microscope

**apylon™:** Automated  
Confocal Raman Microscope

**access:**  
Micro-Raman System

**alpha300 A:**  
Atomic Force Microscope

**alpha300 R:**  
Confocal Raman Microscope

**alpha300 Ri:** Inverted  
Confocal Raman Microscope

**RISE™:** Raman Imaging –  
Scanning Electron Microscope

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