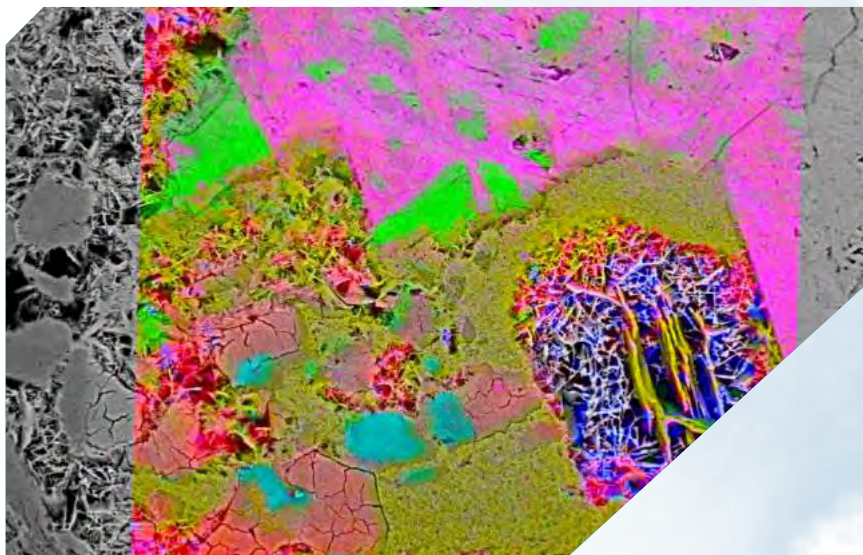
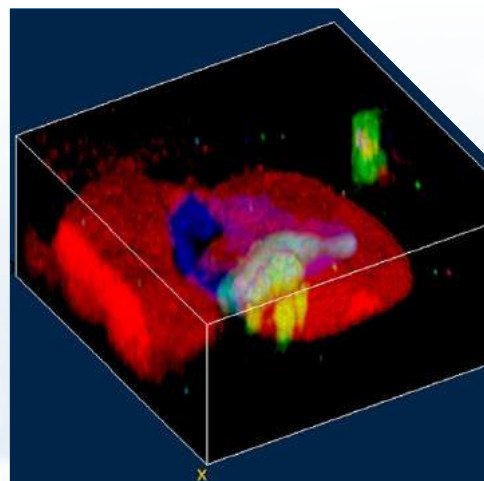
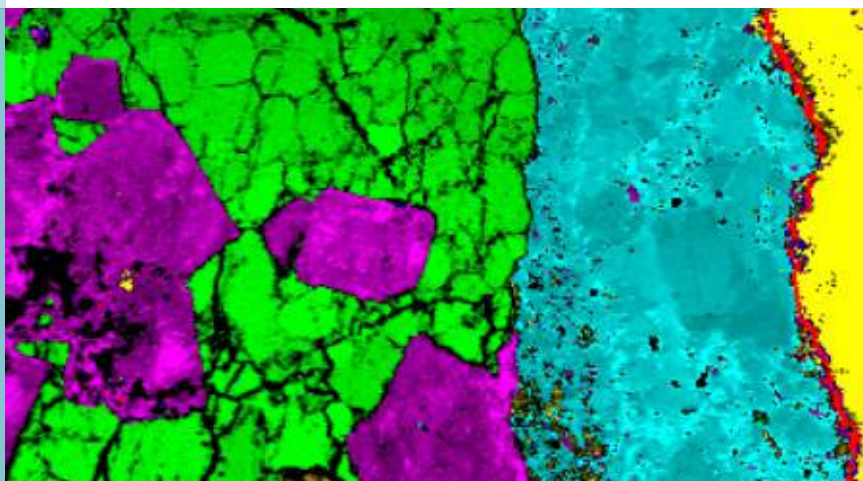


# Confocal Raman Imaging, Correlative Raman-SEM, and Atomic Force Microscopy Geoscience Applications



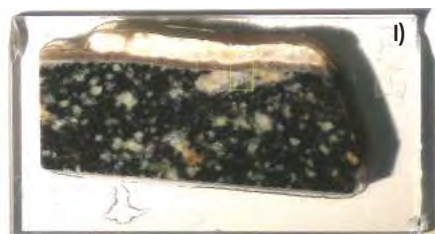
## Confocal Raman Imaging: Solutions in Geoscience

Raman spectroscopy has long been applied in geoscience, for example for the identification and characterization of minerals, or in the observation of mineral phase transitions in high and ultra-high pressure/temperature experiments. In most cases, measurements have been carried out with a micro-Raman setup, i.e. information was obtained from single or multiple points of interest on a sample. This way, few details of the spatial distribution of components, mineral phases or chemical variations could be observed, even though this information may contribute significantly to the understanding of a sample's complexity. By means of Confocal Raman Imaging (CRI), such characteristics can be evaluated from large-area scans on the centimeter scale to the detailed investigations with sub-micron resolution. WITec confocal Raman microscopes of the alpha300 and alpha500 series

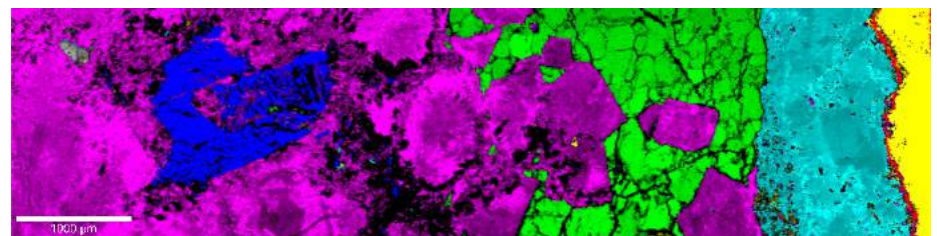
allow for such measurements with very high sensitivity and resolution. CRI is a tool that provides information complementary to data obtained by e.g. electron microprobe (EMP), energy dispersive X-ray analysis (EDX) or secondary ion mass spectrometry (SIMS). In addition to the quantitative, semi-quantitative elemental and isotopic data acquired by these techniques, confocal Raman imaging contributes the visualization of the distribution or molecular information over a defined sample area. Furthermore, considering that most geo-materials are transparent from the NUV to VIS and NIR to some degree, this information can be obtained three dimensionally due to the confocal setup of the microscopes. The following examples highlight some key analytical features of this technique for geoscience applications.

## I. Large Area Scan: overview and variation

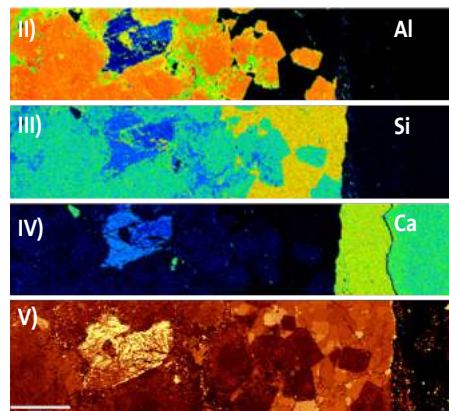
A sample from the deep biosphere of Äspö, Sweden, was studied using the large-area scan mode of an alpha500 R, aiming to characterize secondary cleavage fillings in a 1.8 to 1.4 billion-years-old diorite (sample courtesy of C. Heim and V. Thiel, EMP data courtesy of A. Kronz, Geobiology Group, University of Göttingen, Germany). A section (Fig. 1) was obtained from a drill core from borehole KJ 0052 F01, run by SKB (Swedish nuclear fuel and waste management) and drilled from a tunnel at ~450 m below the surface with a sampling depth in the core of 12 m. A large-area scan of 8 x 2 mm was performed on this polished section with 800 x 200 pixels and an integration time of 36 ms per spectrum using a 532 nm excitation wavelength.



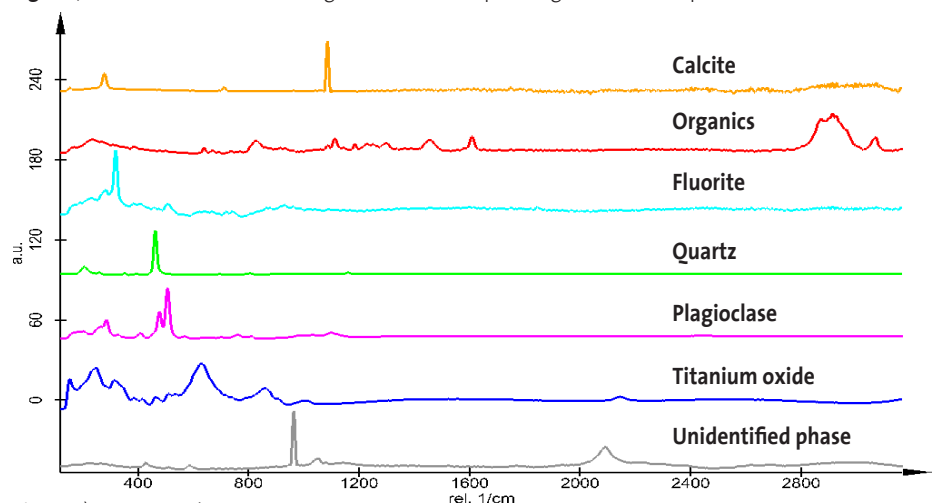
**Fig. 1: I)** Orange rectangle indicates scan position on polished rock section.



**Fig. 2: I)** Combined false color image and the corresponding color-coded spectra.



**Fig. 1: II-IV)** EMP maps showing elemental distribution; V) Corresponding Raman image, integrated intensity 140-300 rel.1 cm.



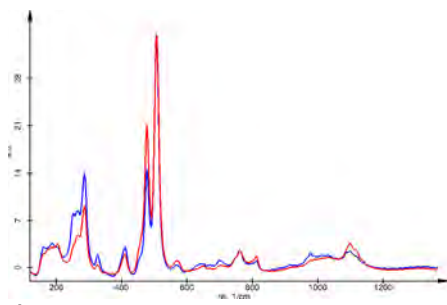
**Fig. 2: II)** Corresponding Raman spectra.

Intensities integrated from 140 to 300 rel. 1 cm are compared to EMP maps of Al, Si and Ca (Fig. 1II-IV), and indicate a similar overall chemical contrast (Fig. 1V) assignment of mineral phases and their gross distribution over the scanned area. The color-coded Raman image (Fig. 2I) and corresponding spectra (Fig. 2II) depict for the general assignment of mineral phases and their gross distribution over the scanned area.

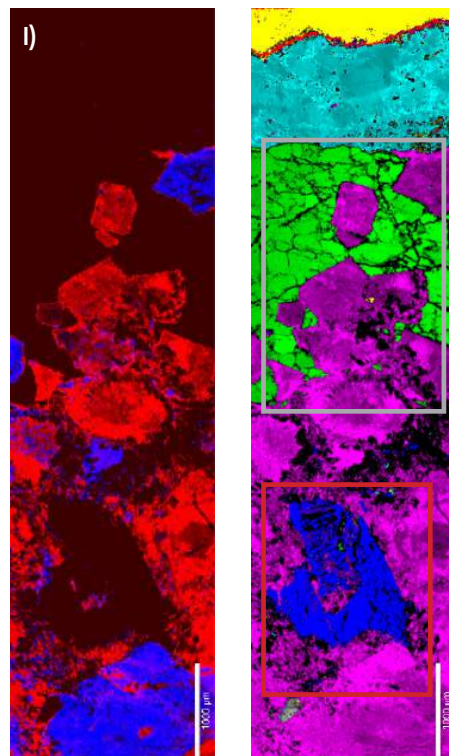
In addition to the mineralogical context information, organic components were identified, spectrally characterized and located, trapped between two generations of hydrothermal precipitates (fluorite and calcite), indicating at which point in time a “deep biosphere” was active within these rocks. Cluster analysis of the data set using the WITec Project software package revealed discrete areas of variation in the mineral phases (Fig. 3).

This is illustrated by the plagioclase phase, in which two distinct spectra show different distributions within the sample regions identified as plagioclase (Fig. 3I). The rutile phase is represented by the blue and yellow spectra and corresponding regions, an unidentified titanium-containing phase and its variation is represented by the red and green spectra and corresponding regions (Fig. 3II).

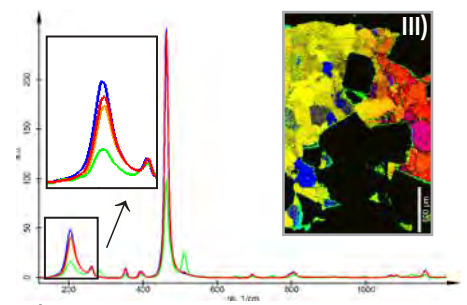
Four distinct regions were identified for the quartz phase based on variations in relative peak intensity (Fig. 3 III), note the discrete edges around plagioclases.



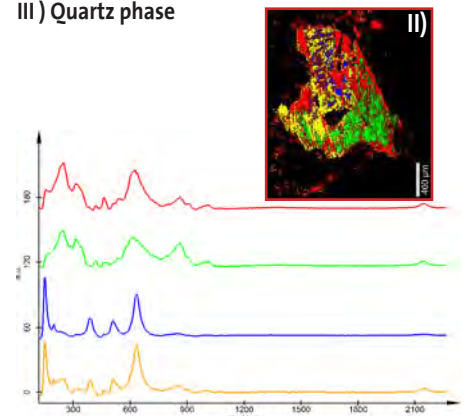
I) Plagioclase phase



**Fig. 3:** Differentiation of variations in I) the plagioclase phase: two individual spectra show distinct distribution; II) the rutile/Ti-phases: two different materials in two distinguishable configurations; III) the quartz phase: distinct regions are reflected in the variation of relative peak intensities in the spectra, note edges around plagioclases.



III) Quartz phase



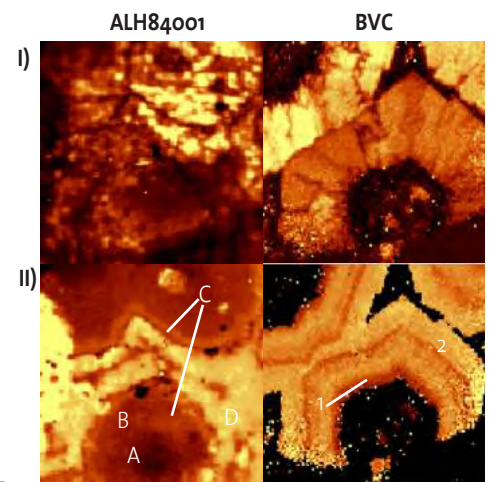
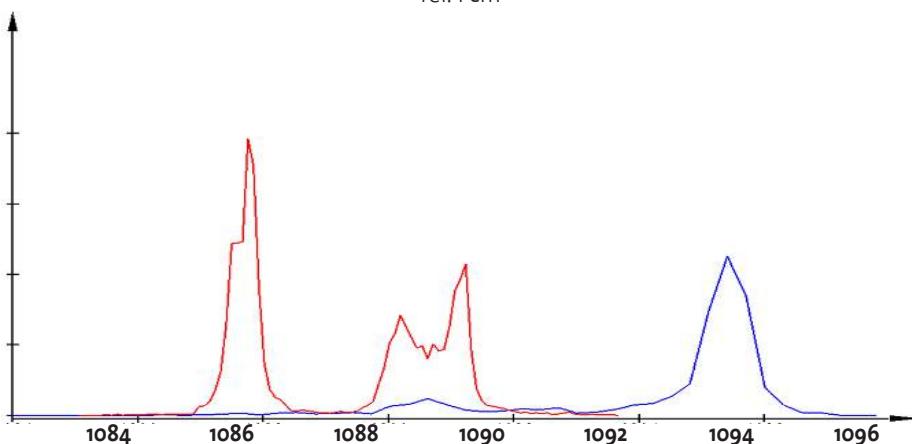
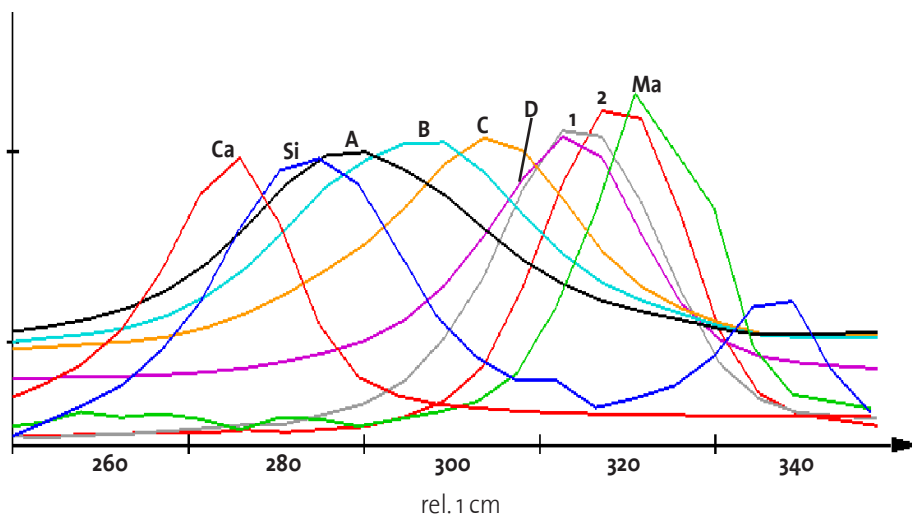
II) Rutile phase

## II. High resolution: spectral and spatial information

High spectral and spatial resolution is necessary to observe chemical variations when they occur in micron-sized features. The spectral resolution is required to distinguish minor compositional changes, and the spatial resolution allows the depiction of such changes at the required (sub-)micron level. This is illustrated by small pancake-shaped carbonate structures, so-called globules, from the martian meteorite ALH84001 and a terrestrial analogue from Svalbard (mantle peridotite xenolith from the Bockfjorden volcanic complex, BVC) (see Steele et al., 2007 for further description). Figure 4 shows the

results of confocal Raman imaging studies undertaken with a high spectral resolution setup of the carbonate composition in the ALH84001 and the BVC xenoliths. Images in row I of Fig. 4 show the overall carbonate distribution with relative intensity integrated over the peaks around  $1090\text{ cm}^{-1}$  (spectra showing the peak center in Fig. 4 row I). Various carbonate reference samples were measured to demonstrate chemical variation, including calcite (Ca), siderite (Si) and magnesite (Ma) (Fig. 4, spectra row II). The images in row II of this figure represent shifts of the carbonate  $\sim 330\text{ cm}^{-1}$  peak and clearly

delineate discrete zones of chemical variation separated from one another on the (sub-)micron scale (the lighter colors show a peak shift toward higher wavenumbers). The letters A, B, C and D represent distinct chemical zones in the carbonate globule of the martian meteorite, the numbers 1 and 2 show zoning in the BVC carbonates. The conclusions drawn from these observations contribute further to understanding the processing of volatile and biologically relevant compounds ( $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) on Mars and illustrate the importance of high spectral and spatial resolution in confocal Raman microscopy studies (for a detailed discussion see Steele et al., 2007).



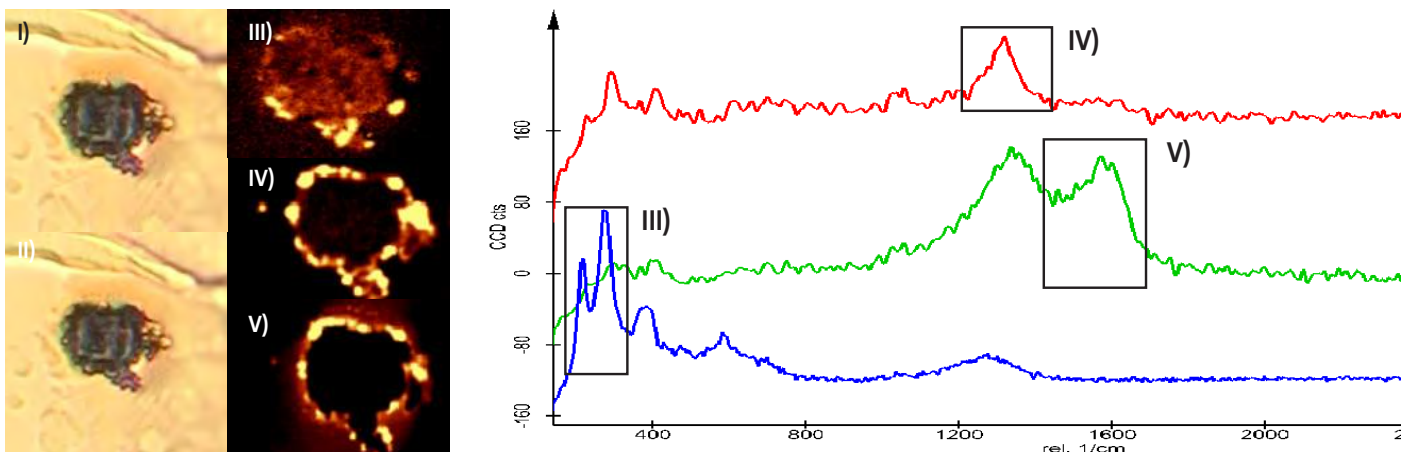
**Fig. 4:** Carbonate composition in ALH84001 and BVC xenoliths. Images in row I show the carbonate distribution integrated over the  $\sim 1090\text{ cm}^{-1}$  peak, the spectra in row I show the center distribution of the carbonate signal for these images. Images in row II represent peak center shifts of the carbonate  $\sim 330\text{ cm}^{-1}$  peak and corresponding spectra (light color = higher wavenumber). Scale bar ca.  $20\text{ }\mu\text{m}$ . Images courtesy of Meteoritics and Planetary Sciences; from Steele et al., 2007.

### III. High sensitivity: revealing hidden details

Valuable compositional and structural information may be contained even in the smallest sample volumes. In order to uncover such information it is not only a requirement to be able to spectrally and spatially resolve chemical features at the highest resolution, it is furthermore essential to work at the highest possible sensitivity. Confocal Raman microscopes of the WITec alpha series provide this level of sensitivity due to the highest possible throughput of Raman-shifted photons from the sample surface to the detector. This allows for the analysis of very small and delicate samples without the risk of damaging them. In geosciences such materials can, for example, be aerosols or interstellar dust particles (IDPs).

Figure 5 shows a collage of confocal Raman images of an IDP (Fig. 5I; for further detail see Toporski et al., 2004). The combined false-color image in II shows that distinct regions for the components shown in images III to V can be separated from one another. Further unidentified mineral phases (Fig. 5III) are separated from hematite/carbon-bearing phases (Fig. 5IV) and hematite phases (Fig. 5V) and could be associated with distinct regions on the sample. To allow for such differentiation and to enable the separation of labile components and at the same time avoid sample alteration due to excessive excitation laser energy, high sensitivity is a prerequisite.

This has also been demonstrated on particles returned from comet 81P/Wild2 by the NASA Stardust spacecraft (Rotundi et al., 2008). The study of small, delicate and unstable samples further benefits from CRI being nondestructive, so that samples can subsequently be investigated using other more invasive micro-analytical techniques (e.g. SIMS, ToFSIMS, TEM, etc.).



**Fig. 5:** Image collage of an IDP; I) video image; II) combined false color image of III) - V), colors as in the spectra; III) relative intensity distribution resulting from integration over the two peaks around  $200 - 300 \text{ cm}^{-1}$  (blue spectrum); IV) relative intensity map of combined hematite / carbon D band phases ( $1280 - 1400 \text{ cm}^{-1}$ ; red spectrum); V) relative intensity map of hematite phases (green spectrum).

## IV. 3D analysis: unveil the information trapped inside

Fluid inclusions were discovered in ultra-high pressure (diamond-grade) garnets from the Kokchetav Massif in northern Kazakhstan (samples and data courtesy of Andrey Korsakov, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia). Characterization of the inclusion contents regarding composition and petrographic context in this case may provide information on micro-thermometry. Due to confocality, CRI allows for the non-destructive and noninvasive analysis of such features. The garnet was polished so that the inclusion remained intact some tens of microns below the surface (Fig. 6I). As can be seen in Fig. 6IIb, the garnet shows significant variation in relative peak intensities across the scanned area. The spectra in Fig. 6IIa are normalized to the peak near 900 rel. 1 cm. Differences are apparent between the red and the magenta spectra (peaks near 850 and 360 rel. 1 cm).

The orange spectrum possibly represents a Carbonate phase in direct proximity to, or as part of, the inclusion. This variation is confirmed in the depth scan (Fig. 6IIIa and b). Here too, spectra are normalized to the peak near 900 rel. 1 cm, differences are apparent between the red and the magenta spectra (peaks near 850 rel. 1 cm and near 360 rel. 1 cm; Fig. 6IIa). Interestingly, the aqueous phase showed significant variation, which can clearly be seen from the spectra shown in Figs. 6IIc and IIIc, both for the X-Y and the X-Z scans. Variations may be attributed to the presence of H<sub>2</sub>O in liquid and gaseous phases, or the presence of hydrous silicate phases. Changes in garnet peak intensities appear only around the inclusion. This may either indicate a differential stress regime attributable to the influence of the fluid inclusion, or may be due to slight variations in the garnet composition adjacent

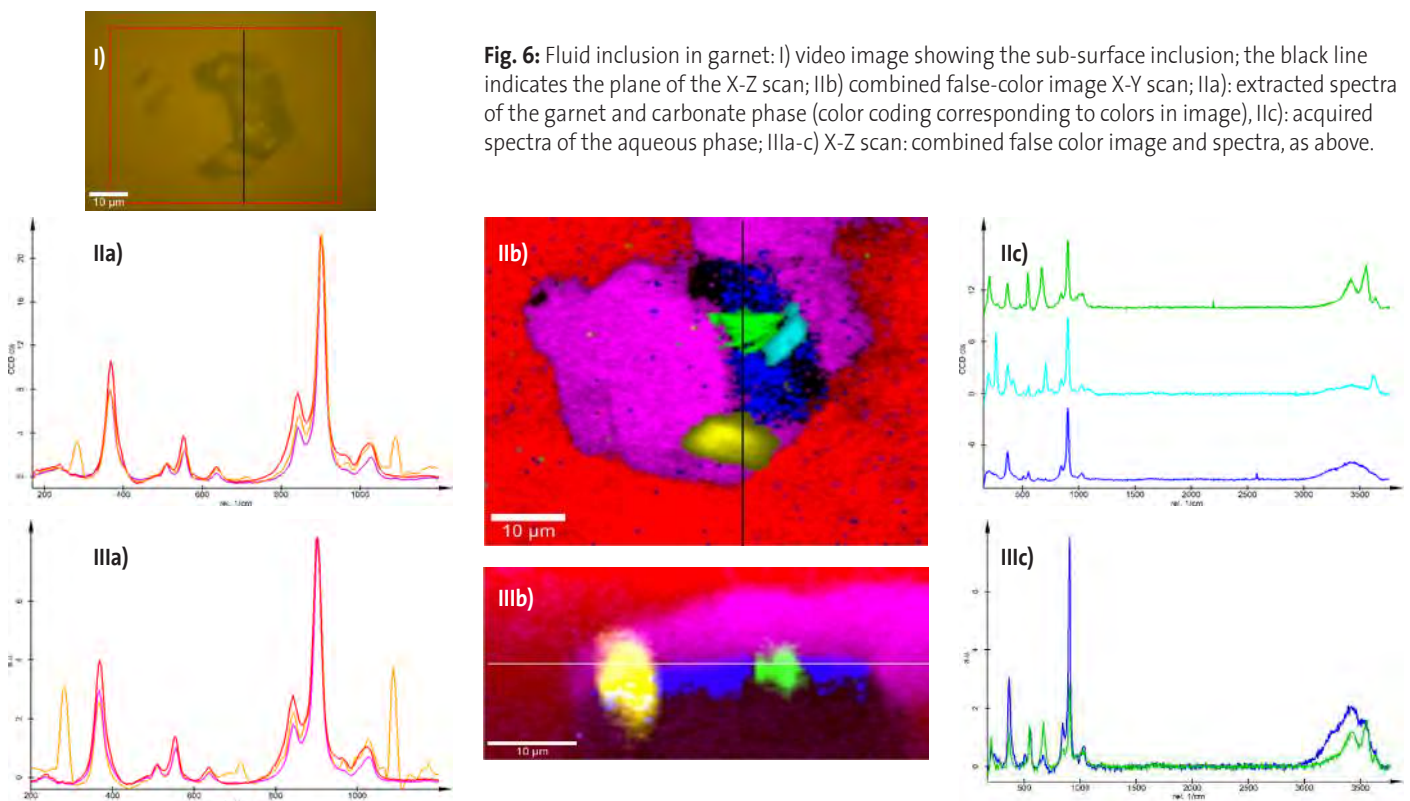
to the inclusion.

### References:

Rotundi A. and 19 other authors (2008) Combined micro-Raman, micro-Infrared, and field emission scanning electron microscopy analysis of comet 81P/Wild2 particles collected by Stardust. *Meteoritics and Planetary Science*, **43**, 367-397.

Steele A., Fries M., Amundsen H., Mysen B., Fogel M., Schweizer M and Boctor N. (2007) Comprehensive imaging and Raman spectroscopy of carbonate globules from Martian meteorite ALH84001 and a terrestrial analog from Svalbard. *Meteoritics and Planetary Science*, **42**, 1549-1566.

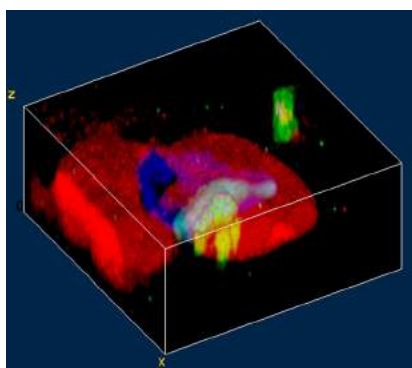
Toporski J., Fries M., Steele A., Nittler L. and Kress M. (2004) Sweeping the skies: Stardust and the origin of our Solar System imaging and Microscopy, **04/2004**, 38-40.



**Fig. 6:** Fluid inclusion in garnet: I) video image showing the sub-surface inclusion; the black line indicates the plane of the X-Z scan; IIb) combined false-color image X-Y scan; IIa): extracted spectra of the garnet and carbonate phase (color coding corresponding to colors in image), IIc): acquired spectra of the aqueous phase; IIIa-c) X-Z scan: combined false color image and spectra, as above.

## 3D imaging of a garnet

Fluid inclusions in rock samples are quite common but vary widely in their dimensions. In the following, a fluid inclusion in garnet was observed. The scan range was  $60 \times 60 \times 30 \mu\text{m}^3$ . The fluid inclusion (water) is displayed in blue, the garnet in red, calcite in green and mica in cyan (Fig. 7).

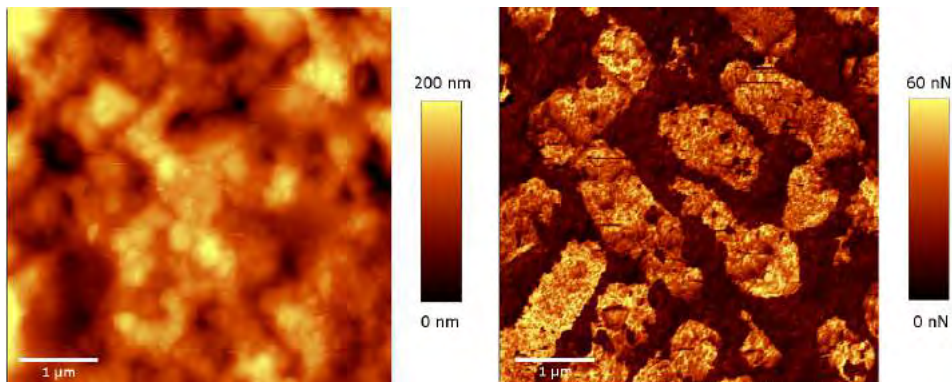


**Fig. 7:** 3D Confocal Raman image of a fluid inclusion in garnet

## Atomic Force Microscopy on geological samples

Atomic Force Microscopy (AFM) is a non-destructive imaging technique for surface characterization on the nanometer scale. Besides high-resolution topography images, local material properties such as adhesion or stiffness can be investigated by analyzing the tip-sample interaction forces.

AFM is combineable with confocal Raman imaging in one instrument.



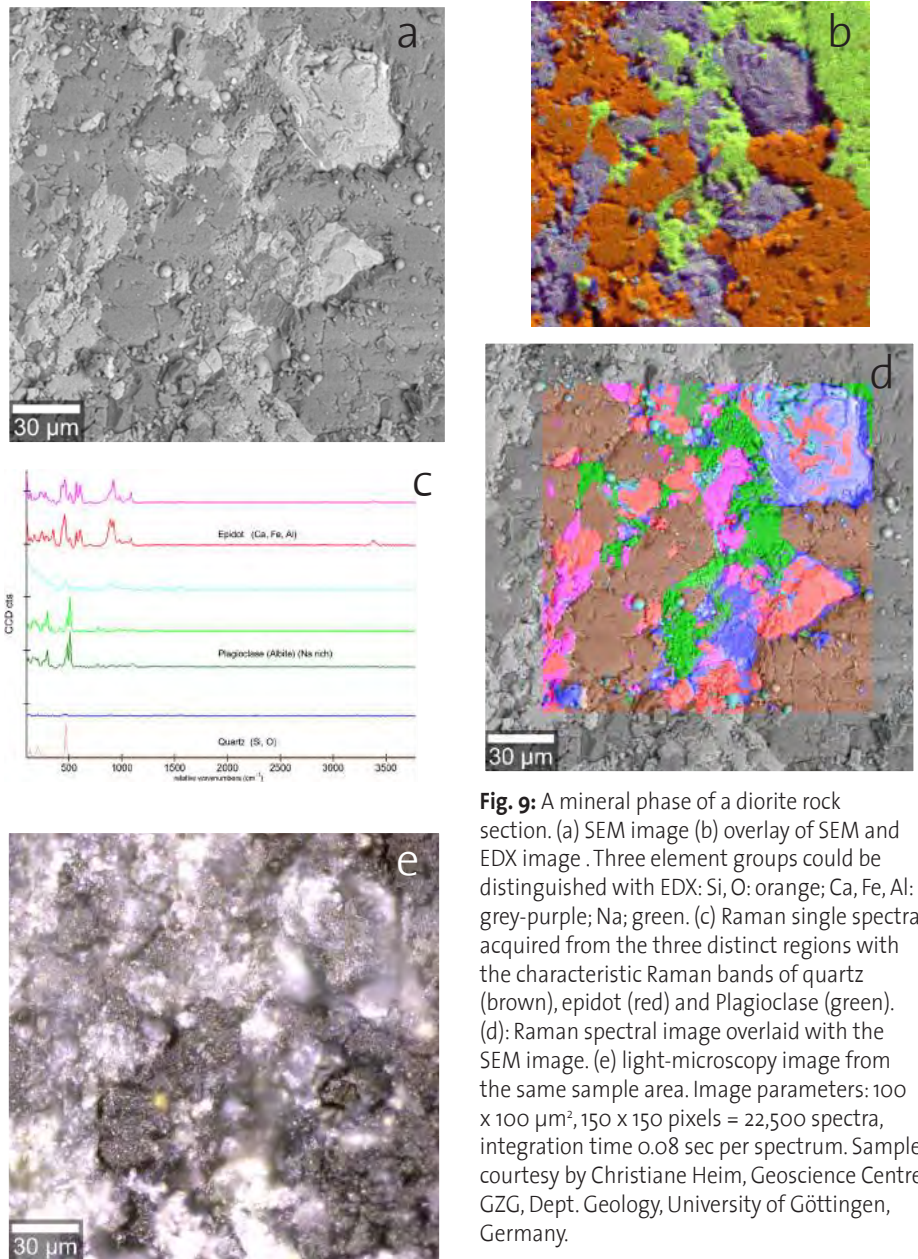
**Fig 8:** Left: Topography AFM image of silicified bacteria. Please notice: cell structures can not be recognized from the topography. Right: The Digital Pulsed Force Mode of the Atomic Force Microscope image shows the different adhesion levels and reveals the bacteria cell structures.

## RISE imaging of geological samples

Correlative imaging techniques gain more and more importance in many fields of applications due to the fact that more characteristics of a specimen can be analyzed with one instrument. The efficient workflow of correlative microscopy techniques is an additional advantage that saves time and money. RISE Microscopy is the combination of confocal Raman Imaging and Scanning Electron Microscopy. It incorporates the sensitivity of the non-destructive, spectroscopic Raman technique along with the atomic resolution of electron microscopy in one instrument. Due to an intelligent positioning system RISE Microscopy enables diffraction limited confocal Raman imaging from exactly the same sample area as the SEM image.

A mineral phases of a rock section from a drill core was analyzed in the next example. The dominant rock type is Diorite. The sample had no need to be treated or vaporized but was only sectioned with a diamond saw under water. This reduces the risk of contamination of the surface, which is of great importance not only for geological drill cores but also for objects derived from the deep sea.

Fig. 9a shows the SEM image of a small sample area using the back-scattered electron detector of the SEM. The same area was analyzed using EDX (Fig. 9b). The distribution of elemental groups indicates the presence of three distinct minerals. Single Raman spectra could be acquired from the three different areas (Fig. 9c) three of which show the characteristic Raman bands for quartz, epidote and plagioclase. This is in good agreement with the elemental composition obtained by EDX. Raman spectra of the three primary minerals were evaluated using cluster analysis. In addition to these spectra, four others were detected, two of which could indicate different grain orientations within the phases of epidote and plagioclase respectively. The spatial distribution of the



**Fig. 9:** A mineral phase of a diorite rock section. (a) SEM image (b) overlay of SEM and EDX image. Three element groups could be distinguished with EDX: Si, O: orange; Ca, Fe, Al: grey-purple; Na: green. (c) Raman single spectra acquired from the three distinct regions with the characteristic Raman bands of quartz (brown), epidote (red) and Plagioclase (green). (d): Raman spectral image overlaid with the SEM image. (e) light-microscopy image from the same sample area. Image parameters: 100 x 100 µm<sup>2</sup>, 150 x 150 pixels = 22,500 spectra, integration time 0.08 sec per spectrum. Sample courtesy by Christiane Heim, Geoscience Centre GZG, Dept. Geology, University of Göttingen, Germany.

minerals is shown in the color coded RISE image (Fig. 9d) where the colors of the Raman image match the colors of the spectra. Not only mineral phase distribution could be detected but also small grains within a mineral phase. Fig. 9e shows the white light image acquired from the same sample area as the SEM image. It clearly emphasizes that high resolution optical images require proper focusing, whereas

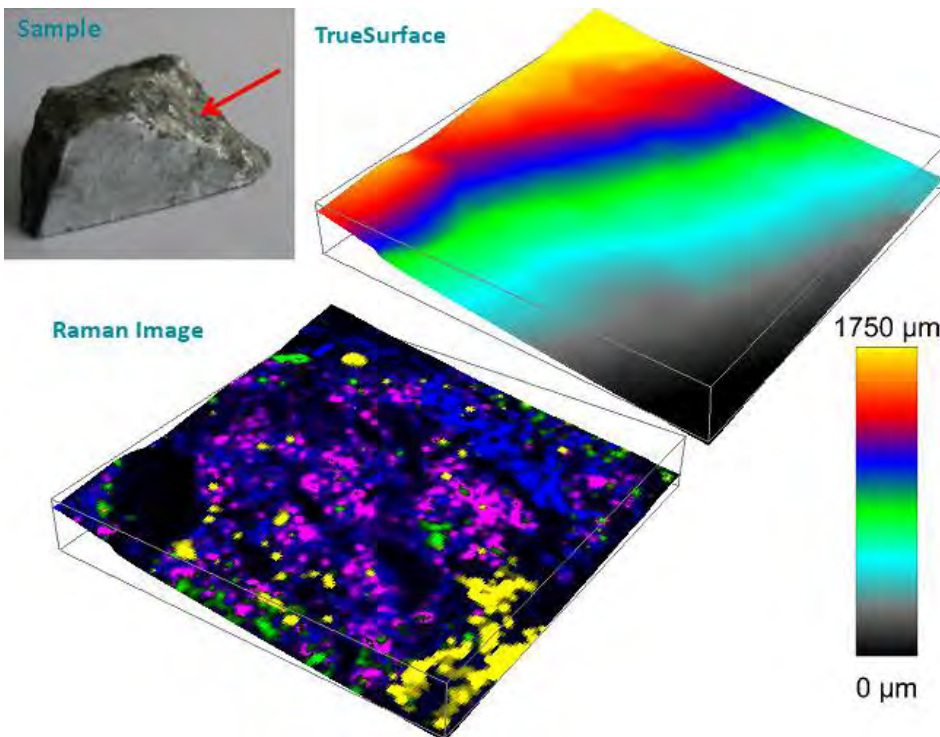
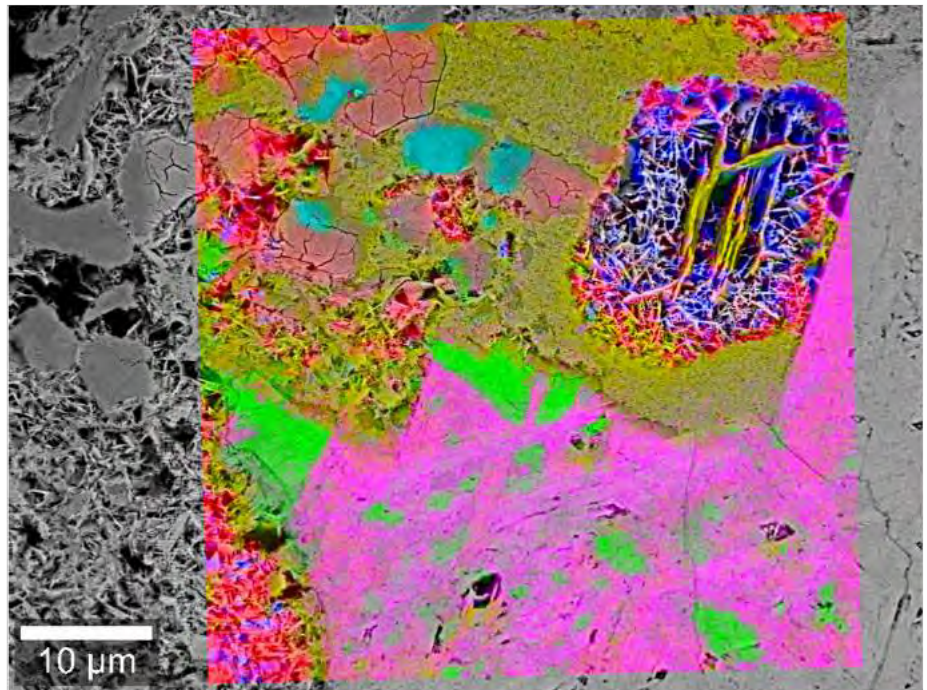
SEM is completely insensitive to surface roughness, which is why it is difficult to retrieve the sample area when using two stand-alone instruments.



## RISE imaging of hematite

A piece of hematite ( $\text{Fe}_2\text{O}_3$ ) was analyzed first with the SEM, then with the integrated confocal Raman microscopy function of the RISE microscope. SEM admittedly depicts some structural characteristics, but cannot differentiate between the oxides presented in the the hematite. However, the Raman spectra indicate the occurrence of several crystal forms of hematite and of goethite ( $\text{FeO}(\text{OH})$ ). The distribution of these minerals is shown in the Raman image that has been overlaid onto the SEM image.

**Fig. 10:** Raman-SEM image of hematite. The sample consists of several crystal forms of hematite (red, blue, green, orange, pink) and of goethite (light blue, cyan). The Raman image was overlaid onto the SEM image. Raman image parameters:  $50 \times 50 \mu\text{m}^2$ ;  $150 \times 150$  spectra, integration time: 150 ms/spectrum, 10 mW laser power.



## Topographic confocal Raman Imaging with TrueSurface Profilometry

The TrueSurface Microscopy option enables confocal Raman imaging guided by surface topography in a one-pass simultaneous operation. 3D chemical characterization on rough, inclined or irregularly-shaped samples can be carried out precisely along or at a set distance from a surface without requiring sample preparation.

Figure 11 shows Raman imaging on an inclined and rough rock sample. An area of  $2 \times 2 \text{ mm}^2$  was investigated, the position is marked with a red arrow. The height profile was recorded simultaneously with the Raman image and illustrates the incline sample plane. The confocal Raman image shows the chemical sample information overlaid on the height profile.

## WITec Product Portfolio



The WITec product portfolio includes imaging systems for Raman, AFM and SNOM analysis as single technique solutions as well as correlative imaging configurations (e.g. Raman-AFM, Raman-SEM).

All WITec microscopes are high-quality modular systems with exceptional optical throughput, unparalleled signal sensitivity and outstanding imaging capabilities.

Their various specifications range from advanced though budget-conscious microscopes to high-end instruments at the very cutting edge of available technology.

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 equipment, allowing our customers to keep pace with future challenges.