Food Analysis with Confocal Raman Microscopy

Confocal Raman microscopy is a powerful tool for analyzing the chemical composition of heterogeneous samples on the sub-micrometer scale. In this application note it is demonstrated that the distribution of various chemical compounds in a banana, instant gravy thickener, emulsions, a candy and chocolate products could be successfully visualized, leading to a more comprehensive understanding of the product and the production process.
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.

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.

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.

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).

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.
In the food industry, various ingredients, additives and bio-polymers such as emulsifiers, stabilizers, carbohydrates or thickeners are commonly used in order to optimize the texture or the flavor of food. The distribution and microstructure of the ingredients strongly determine the properties of the final product. Therefore, research and development as well as quality control require powerful analytical tools for studying the distribution of the various compounds in the food. Raman imaging has proven to be a valuable technique for food analysis (1-5).


**Fig. 1** 3D Raman image of a honey droplet. This image was constructed from 50 individual 2D confocal Raman images acquired along the z-axis. It shows a pollen grain (green) and several crystalline phases of sugars (red, blue, cyan).

**Fig. 2** Raman image of banana pulp. An overlay of a confocal Raman image on a white-light image. Green: starch; red: carotinoids; blue: water. Image parameters: 400 x 300 μm², 1200 x 900 pixels, integration time: 2 ms/spectrum, excitation: 532 nm.
Confocal Raman Imaging of Chocolate

Here, white chocolate was investigated with Raman imaging (Fig. 3). One can clearly see a distinct phase separation in the chocolate. Sucrose (blue) and additive particles (red) are embedded in a fatty matrix (green). The size of the sucrose particles vary between 0.65 and 10 μm.

The combination of surface analysis and Raman spectral acquisition enables topographic Raman imaging on rough and uneven samples, such as a sugar bar (Fig. 4). With the TrueSurface™ option, Raman spectra are acquired from precisely along a surface, or at a set, user-defined distance from a surface. This makes the distribution of chemical components within the sample visible in three dimensions. Rough, inclined or irregularly-shaped samples can be investigated with the same ease as standard samples. As the TrueSurface™ sensor actively monitors and maintains a set distance between the objective and sample surface, its closed-loop, one-pass operation can compensate for any variations during measurements with long integration times. This keeps the measurement area in focus at all times and produces sharp chemical Raman images with sub-micrometer resolution.

In the Raman analysis, seven compounds were identified by their spectra (Fig. 4b). Using the TrueSurface™ option, their distribution a very rough surface and even along the company’s imprint was visualized (Fig. 4a).
**Emulsions and Fat-spreads**

Fat-spreads such as butter or margarine are water-in-oil emulsions. The microstructure of fat-spreads determines properties such as suppleness, texture and spreadability. These traits as well as stability depend on the fat crystal network at the interface and the emulsifier and are strongly influenced by the production process. Therefore, manufacturers of emulsions and fat-spreads analyze their products in detail to understand the composition-process-structure-function relationships. For this purpose, confocal Raman imaging is a very valuable technique.

Fig. 5 shows the results of a confocal Raman study of an emulsion containing a fatty matrix, an aqueous phase and the emulsifier E476 polyglycerol polyricinoleate (PGPR). This emulsifier decreases the friction between solid particles, i.e. in chocolate. On the basis of their spectral characteristics (Fig. 5a), the ingredients of the emulsion were imaged (Fig. 5b). It is obvious that PGPR (yellow) forms aggregates at the interface between the water droplets (blue) and the fatty matrix (green and red).

In a study from the Dutch Unilever company (4), van Dalen et al. describe the development of the hyperspectral data analysis of Raman images of fat spreads including data-pre-processing and multivariate curve resolution (MCR). With confocal Raman imaging they not only localized the molecular compounds in fat spreads but could also relate the microstructure of spreads to their production processes. The results (Fig. 6) show that water forms droplets in a continuum of sunflower oil, stabilized at the interface by an emulsifier (monoglycerides) and lipids in the crystalline phase. The lipid crystalline phase (solid fat) is also found in the continuous phase of the emulsion, forming a network between the different water droplets. The image on the right shows the competition/co-crystallisation between the solid fat and emulsifier at the droplet interface.

The authors of the study conclude: “This method can be applied to a wide range of different food emulsions such as butter, margarine, mayonnaise and salad dressings.”
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 budget-conscious microscopes through 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.