

Raman Imaging of Samples with Uneven or Rough Surfaces using Confocal Microscopy with Surface Scan Imaging (SSI)

Introduction

A confocal Raman microspectrometer is an excellent choice for analysis of materials with high spatial resolution - in the order of submicrons. This makes it possible to characterize and image minute components such as APIs or bulking agents and foreign materials as small as 1 μm , as well as extremely thin layers. However, to achieve the high spatial resolution it is necessary to use objective lenses with high magnification, resulting in a depth-of-field that is extremely narrow; any slight deviation in the focus at the measurement surface greatly affects the intensity of the measured spectrum. As a result, imaging measurement of a sample with an uneven or a sloping surface may result in poor measurement unless the objective lens is refocused at each measurement point. This results in extremely long measurement times.

To make extremely fast measurement in a short time JASCO has developed Surface Scan Imaging (SSI). SSI is used to assess the surface shape of the sample as height information of the stage prior to measurement and performs Raman measurement while scanning the stage according to this surface shape information. Using SSI, even a sample with a very uneven surface can now be measured to give good Raman spectra with high S/N at all data points.

Keywords

Raman, Surface Scan Imaging, SSI, Confocal Raman Imaging

Comparison of SSI with Conventional Autofocus Techniques

Conventionally, Raman imaging measurement of a sample with surface roughness was performed with autofocus (AF) was carried out at each measurement point (figure 1-a), or XYZ 3D imaging measurement was carried out (figure 1-b).

SSI (figure 1-c) calculates the sample height (irregularity) information from a multifocus* image and performs imaging measurement while scanning the stage based on this information. Although it also depends on measurement conditions and sample shape, the time required for Raman imaging measurement is greatly shortened compared with the conventional method.

* The multifocus image (all-focus image) is an image obtained when the stage is scanned in the Z direction to acquire multiple observation images with different focal points, and each layer that is in focus is extracted and a 'single in-focus image' is synthesized for the entire field of view. This multifocus image allow the details the entire surface shape of the sample to be identified prior to measurement.



NRS-4100

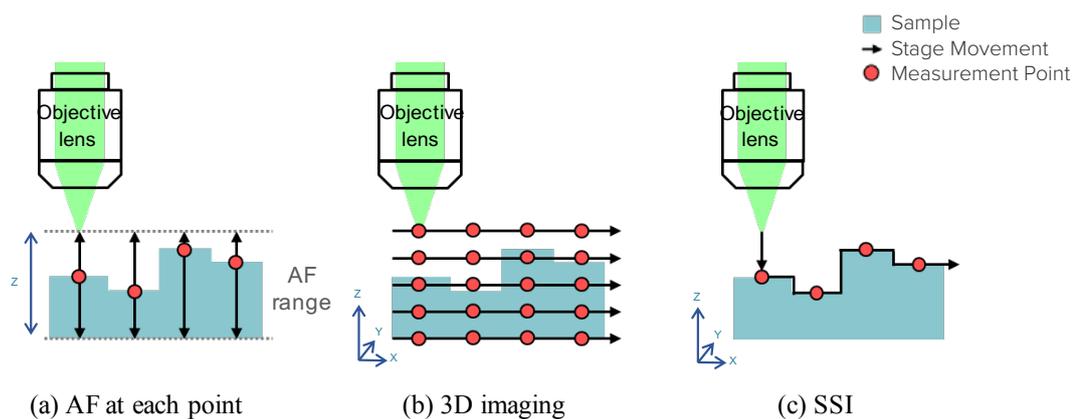


Figure 1. Uneven surface sample measurement.

Raman Imaging Measurement of a Pharmaceutical Tablet using SSI

Imaging analysis of the uneven part of the surface of a tablet (concave and convex difference: approximately 170 μm) was measured using SSI. The measurement parameters are as follows:

Measurement Conditions	
Instruments	NRS-4500 Laser Raman spectrometer
Excitation Wavelength	532 nm
Objective Lens	20x (long working distance type)
Interval	10 μm
Measurement Points	54 x 28 points
Exposure Time	0.5 second

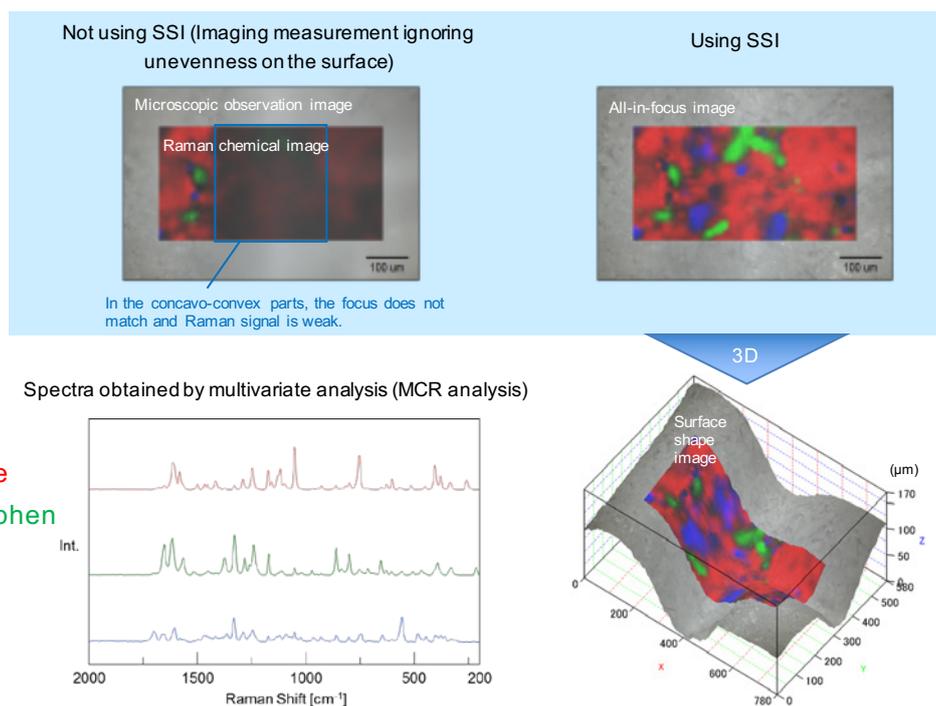


Figure 2. Measurement results.

When measurement is performed following the surface shape by using SSI, a clear Raman chemical image can be obtained because the focus matches at each point. By overlapping the Raman chemical image on the 3D surface shape image, the quantitative result and shape information of the concavo-convex part of the sample can be checked simultaneously.

Conclusion

Using SSI, Raman imaging measurement of a sample with surface unevenness and inclination can be performed easily and quickly. In addition to the tablet measurement shown here, SSI makes it easier to analyze a wide range of samples with surface roughness including rock and concrete, DLC coating, resins, foods, medicines, fertilizer granules etc.