

Raman imaging of difficult surfaces

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For those of us who have enjoyed battling with the issues of getting good data from Raman experiments over the years, the newer capabilities around Raman imaging have added a whole additional order of magnitude of complexity around delivering good data. Better array detectors and our vendors' concentration on optimising signal-to-noise has dramatically improved the total time of an analysis, making Raman imaging a viable analytical and diagnostic tool.

Modern Raman microscopy equipment designed around standardised OEM supplied microscopes has taken much of the risk out of standard measurements. For higher dimension 3D analyses, achieving consistent and reproducible results is still a significant challenge. These challenges must be overcome to realise the potential of 3D Raman imaging as a reliable standard laboratory experiment. Being sure that you have strategies in place to deliver Raman intensities that are correct and comparable is essential if you want to exploit chemometric analyses on the 2D imaging data, not to mention using intensity data for 3D analyses and data interpretation using confocal Raman microscopy.

Simpler 2D image analyses of a uniform samples

The "simplest" sample for Raman imaging experiments, when compared to single-point Raman experiments, would probably be something as flat as a silicon wafer that could also be mounted perfectly flat (see Figure 1).

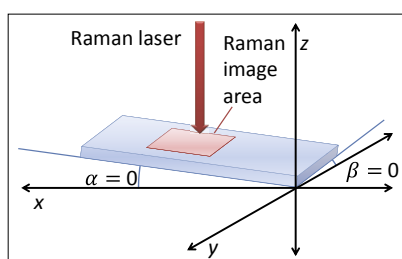


Figure 1. Simplistic diagram of the perfect world scenario for Raman imaging, a completely flat sample mounted with no angle between the x–y axes of the sample stage and the axes defining the image area to be scanned.

Why? Well it is simple—focus once and wherever you move the sample for the next measurement in your data array there should (instrument stability and sample temperature issues aside) be no need to re-adjust.

Interestingly the "issue" of the need to adjust the laser focus for the best signal was once a critical factor in a decision taken several years ago to reject a vendor from a bidding process. Here a perfectly good health and safety based decision around the protection of the user from potentially hazardous laser radiation saw them design the adjusting screws for the laser focus/sample position behind the laser interlocks. So just when the instrument's operator needed the laser on—when trying to adjust the sample to get the best signal out of the instrument—they would open the sample compartment to access the adjusting screws and the laser would cut out! Very safe I am sure—but a real show-stopper when you want to ensure simplicity of operation for laboratory staff who are not specifically Raman

specialists. This type of issue is also not fantastic for system stability or source longevity if your strategy for protection of the user requires a breach of the safety interlocks to switch off the laser rather than simply blocking the beam path.

One simple solution to the "non-flat but planar sample", where α and/or β are no longer zero, is the ability to use the Raman microscope and computerised sample table to take and store a series of measurements at different points around the image to be scanned. The sample position and the correct settings for the best Raman signal are then available in the control computer. By taking enough data points to map the major changes, the computer can then interpolate between the points keeping the laser optimally focused across the whole sample image area. This is a rather time-consuming manual approach, but does deliver the desired results and can cope with surfaces which are not only at an angle but which are not flat. However, you need to store increasingly larger numbers of individual measurements to calibrate the adjustments required by the instrument.

Samples of increasing surface complexity

Unfortunately, most of the real world in which we live and conduct our analyses is not flat. So if we want to deliver more than just spectra of a single point from samples with uneven irregular surfaces, we need to be creative in how we conduct the analyses. Figure 2 shows an irregular surface and how much the laser focus needs to move up and down to

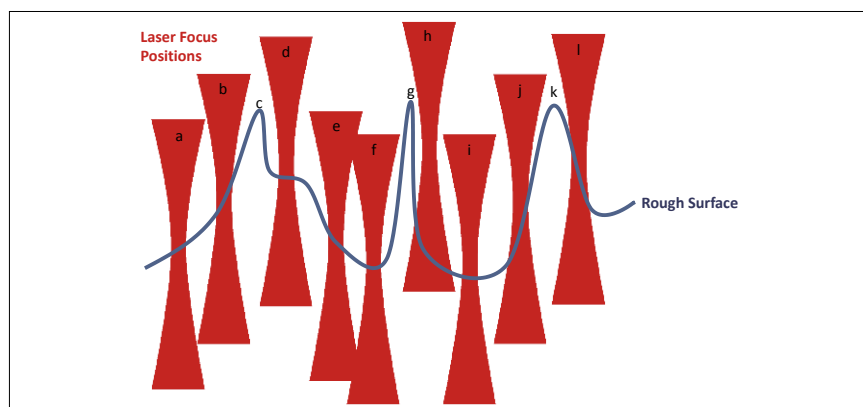


Figure 2. Laser focus movement to track changes in the height of features in a rough sample. The letters are reference points for further discussion in the text.

try to deliver comparable spectra across the whole scan. Now obviously this is in 2D, but it is easy enough to imagine the additional dimension of added complexity when imaging. If we look at the individual laser focus positions, a and b will pretty much give representative spectra, c shows a potential feature which would, if created by an impurity in the layer, be incorrectly represented in the Raman image. Positions d–f, i, j and l should give good data but again g and k represent missed features, and h will show weaker Raman peaks than are actually present, as the Raman scattered signal is not being generated from the laser focus. This will also lead to a loss of lateral reso-

lution in the data we can obtain from our Raman image.

Fooling the automated chemometric analysis

One really good development has been the automated or semi-automated analysis of Raman images. Principal component analysis can seek out chemical differences in the sample under analysis or variations in the distribution of specific components across the sample image. Clearly, for these advanced techniques to work error free, the array of data generated by the spectrometer needs to show variations due to the sample itself and not variations due to laser intensity at

the sample from unwanted and potentially unrecognised de-focusing. Such a mistake could, for example, lead to a particular defect in a sample being incorrectly diagnosed.

This effect can be far worse when the next step up the complexity chain is required: when Raman depth measurements are being carried out on transparent or semi-transparent samples. Confocal Raman imaging is a very exciting technique. Here, the laser focus is deliberately scanned down through a sample and the scattered photons collected which can, for example, map the distribution of specific pharmaceutical active ingredients throughout a tablet. When combined with multi-dimensional chemometric analysis, this technique can give superb results in studies around the mode of drug delivery or the reproducibility of mixing in a new manufacturing line, to name two simplistic examples. Neil Everall pointed out a while ago¹ that it was necessary to be worried about signals being observed from areas below (outside) the supposed area of the main laser focus, but if we are unsure where the actual surface of the sample really is located relevant to the laser focus we similarly cannot say later from what depth below the surface our spectra are being collected.

One interesting approach to avoiding the tedious job of manually measuring individual calibration points for the geometry of rough samples has been the integration of precise optical profilometer data into the Raman microscope's capabilities (Figure 3).²

Although this requires additional equipment over and above the normal Raman imaging system, it provides a robust, simple-to-operate and reproducible methodology for approaching Raman imaging and confocal Raman 3D imaging of samples with difficult surface structures.

References

1. Neil Everall, "The influence of out-of-focus sample regions on the surface specificity of confocal Raman microscopy", *Appl. Spectrosc.* **62**, 591–598 (2008). doi: <http://dx.doi.org/10.1366/000370208784658057>
2. Figure 3 adopted with permission from images supplied by Harald Fischer, WITec GmbH, Lise-Meitner-Str. 6, 89081 Ulm, Germany with thanks.

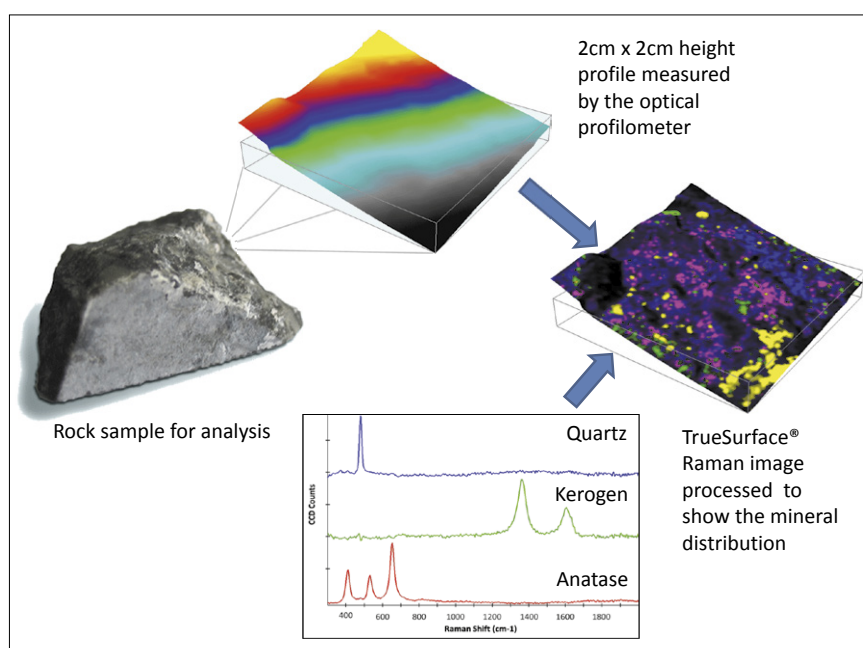


Figure 3. The use of optical profilometer data used to control the Raman imaging microscope to deliver better results from difficult surfaces.²