

# The Impact of Spatial Sampling Density in Raman Imaging

**HORIBA**JOBIN YVON

Eunah Lee, Fran Adar, and Andrew Whitely, HORIBA Jobin Yvon, Inc.

*Raman mapping has the potential to combine the sensitivity and selectivity of vibrational spectroscopy with the high spatial resolution of a confocal microscope. Selecting an optimum spatial sampling density optimizes data collection time with respect to the feature size of the mapped area.*

Chemical images produced by hyperspectral cubes of data has opened up significant potential for the application of Raman spectroscopy. Raman imaging takes advantage of the excellent sensitivity and specificity of the Raman technique, especially when high spectral resolution is available. Because of the confocality, depth profiling and optical sectioning are easy to implement, adding an additional dimension of information to the Raman map.

One largely overlooked aspect of point mapping technology is its flexibility. The area, shape and the spacing between spatial data points are all customizable to fit the characteristics of the sample and the goal of the project.

## Experimental Conditions

An  $800 \times 800 \mu\text{m}^2$  area of an over-the-counter (OTC) analgesic tablet was measured using spatial sampling steps of 100, 50, 25, and 10 mm. Raman image data sets were obtained using the LabRAM Aramis (Horiba Jobin-Yvon, Edison, New Jersey). The excitation laser was the 632.8 nm line of a He/Ne laser. The magnification of the objective lens was  $\times 10$ . At each spatial point, a Raman spectrum was measured with two 500 ms exposures, over the range of  $496\text{--}2281 \text{ cm}^{-1}$  at the average spacing of  $1.852 \text{ cm}^{-1}/\text{pixel}$ .

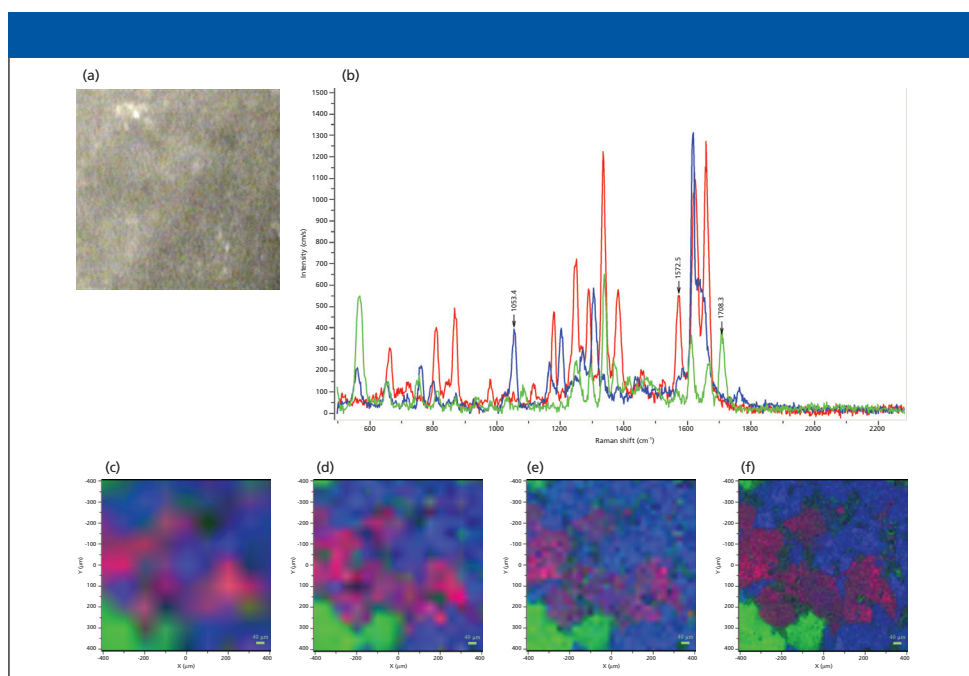
Polynomial baselines were removed from each Raman imaging data set using LabSpec 5 (Horiba Jobin-Yvon) before the integrated intensities of three bands at  $1572.5$ ,  $1708.3$ , and  $1053.4 \text{ cm}^{-1}$  were assigned respectively to red, green and blue for visualization. The criterion for selecting these bands to represent the three major chemical components was to minimize the overlap between bands.

## Results

The spatial elements in this sample are of the order of 100 mm. To identify the chemical species of the major components and their overall distribution, the coarse grid Raman image over a large area (Figure 1c), which took a few minutes to measure, provides sufficient information to determine a region of interest for mapping. As the data point spacing decreases, the data collection time increases and the fine spatial features are revealed. The fine grid Raman image, which took several hours to record, illustrates the chemical distribution in this sample, with details of the morphology of the crystals. Of particular interest is the lack of any features in the black and white TV image that can be correlated with the chemical information.

## Conclusion

In this study it was shown that by first recording a low density, large Raman image, one is able to select a region in which a hyperspectral cube can be used to construct a high quality map.



**Figure 1:** (a) A bright field image; (b) representative spectra; (c–f) Raman images recorded with 100, 50, 25, and 10 mm/pt, respectively.

**HORIBA Jobin Yvon Inc.**

3880 Park Avenue

Edison, NJ 08820

Tel. (732) 494-8660, Fax. (732) 549-5125

www.jobinyvon.com