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Three Dimensional Hyperspectral Imaging using Confocal Raman Microscopy

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Similar to confocal laser scanning microscopy (CLSM), a confocal Raman microscope illuminates each point of the specimen and collects Raman signal through a confocal hole allowing depth discrimination. Unlike CLSM, a confocal Raman microscope also discriminates the signal spectrally. The result is Raman hyperspectral imaging where each point of the space – lateral and axial – yields Raman spectra to provide spatially resolved chemical compositions and Raman images to provide the spatial distribution of chemical compounds.

Spectral resolution, which is critical to chemical information, is determined by the laser wavelength, the entrance slit width, the focal length of the monochromator, the grating and to a certain extent the sample. Spatial resolution, which is critical to spatial information, depends on the sample, the laser wavelength, the numerical aperture of objective lens and the confocal hole diameter. A typical confocal Raman microscope can achieve < 1 cm^{-1} spectral resolution, < 0.5 μm lateral spatial resolution and < 2 μm axial spatial resolution with a visible laser.

One additional element of Raman hyperspectral imaging is the size/volume of data acquired – one data set is composed of hundreds or thousands of spectra or images. The amount of data makes it possible for statistical assessment of data while making it impossible for individual inspection of each data point. It is imperative to employ methodical and numerical analyses followed by spectroscopic and micrographic validation and verification for scientifically correct and meaningful results. Visualization of results becomes important as well due to the amount and variety of information that can be extracted and presented.

In this paper, a few selected examples of two and three dimensional Raman images will be presented to demonstrate various aspects of confocal Raman hyperspectral imaging – spatially resolved chemical information, spatial distribution of chemical compounds, application of numerical analyses and visualization of results. We will show the power and importance of confocal Raman hyperspectral imaging for complete characterization of many samples.

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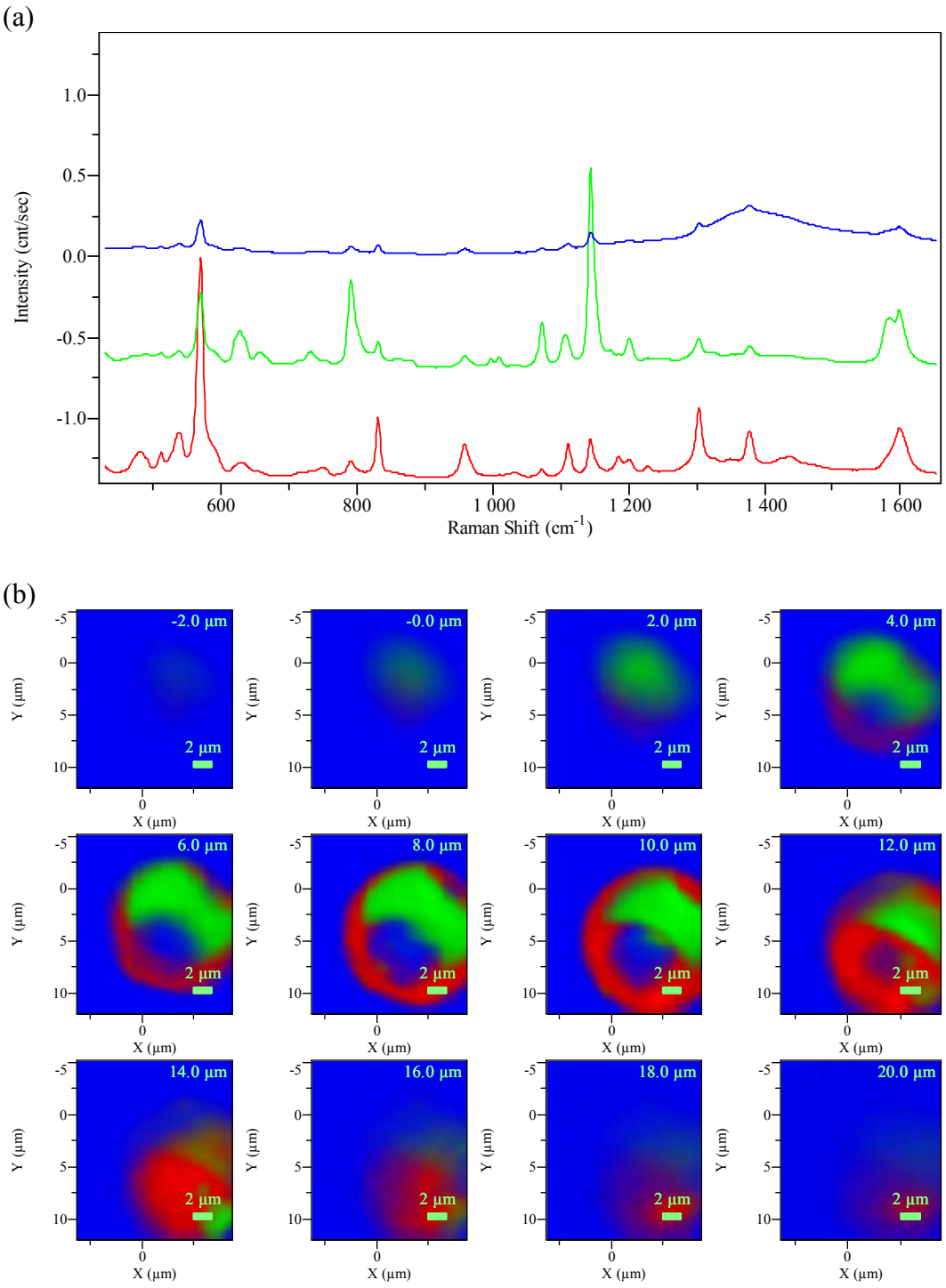

Figure 1. Confocal Raman hyperspectral imaging results from a thermopastic bead, processed by direct classic least square regression. (a) Models that are statistically representative of spectral species present and (b) score images (color-coded to models) that represent the spatial distribution of identified spectral species. Numbers marked in each score image is the relative height of the sample.

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