



Raman Spectral Imaging – Expanding Capabilities to Fulfill Application Requirements

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Introduction

Raman spectroscopic imaging has increasingly gathered momentum as a method of interest for a wide range of users, including those involved in materials analysis and bio-analysis applications. The relative simplicity and the reliability of modern Raman instrumentation, the higher spatial resolution capabilities and a gradual decline in purchase pricing have continued to attract new and varied users to Raman spectroscopy. Raman spectroscopy can provide the same component identification capability as infrared spectroscopy but without the extensive sample preparation often required for infrared analysis. Raman spectroscopy also offers a 'confocal' capability enabling data collection of subsurface sample spectra for laser transparent samples. The use of Raman for spectral imaging has encountered some restrictions, however, due to the length of time required when obtaining Raman image data for larger sample areas.

Infrared imaging still enjoys an advantage due to lower instrument purchase costs and a longer history as a result of earlier development of the necessary technology. The major obstacles to infrared imaging though, are the restrictions on the spatial resolution for infrared spectroscopy (approximately 2-10 microns) and the requirement to properly prepare the sample to obtain optimal infrared absorption intensities. Ideally, one would use both infrared and Raman imaging techniques for sample analysis, combining the strengths of both analytical methods to exploit the advantages of each method.

The Need for Speed: Methods to Increase Raman Imaging Efficiency

Various methods have been proposed to increase the imaging speed of Raman spectrometer systems while still maintaining the data quality, confocal capability and spatial resolution that can be obtained for 'standard' sample spectra. While no single technology appears to enjoy a superior advantage for increasing the speed of Raman imaging, there are attractive features for each method²⁻³ (and a corresponding buzzword to describe the instrument system).

The Jasco NRS-5000/7000 series of Raman microscopy instruments employs the SPRIntS high-speed imaging system to increase the data collection speed for an imaging sample. The Verti-Scan sample illumination capability of this system provides optimal, reproducible control of the laser illumination spot on the sample, allowing a data collection step-size of less than 50 nanometers while maintaining the confocal capabilities of the instrument. This system provides the ability to obtain high resolution, high speed imaging of standard surface analysis samples, but also provides a 3-D imaging capability that allows confocal imaging of a laser transparent sample in the XYZ format. When combined with the Interval Measurement control software, dynamic imaging experiments for a sample can also be realized, obtaining data in an intensity vs. time format.

This paper will outline the rapid Raman mapping of samples utilizing a sample excitation technology that improves spatial resolution while maintaining the confocal capability of the Raman technique. This mechanism also enables the 3-D mapping of a sample to provide a three dimensional image of component 'inclusions' within a sample matrix. Data will be presented from several samples demonstrating the increased speed and efficiency of this Raman mapping method. We will also discuss the benefits and advantages of this mapping method and outline the capabilities of this instrument system for selected samples.

References

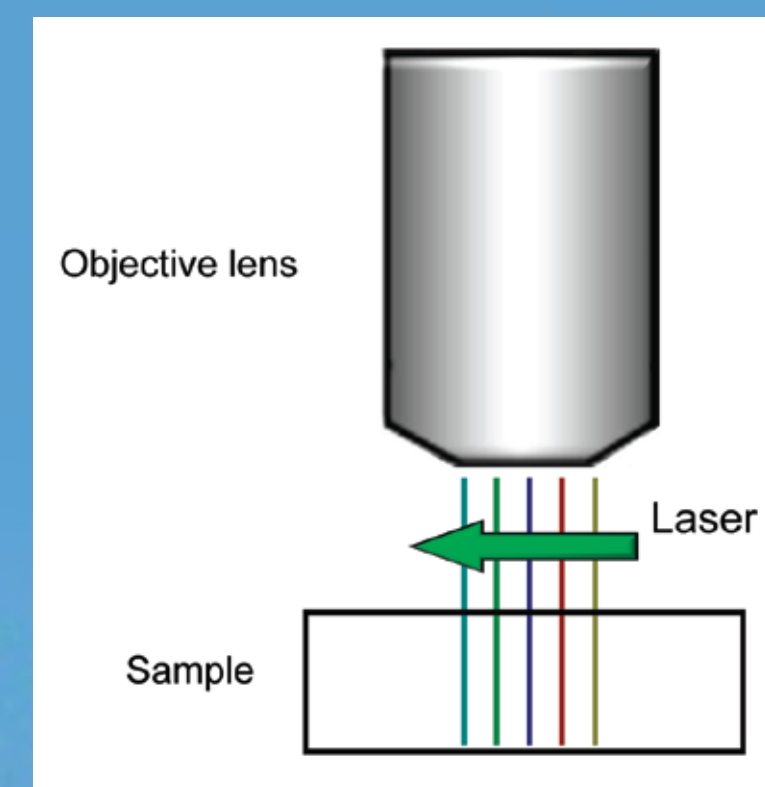
1. R. K. Gilpin and C. S. Gilpin, *Anal. Chem.* **81**, 4679-4694 (2009).
2. P. J. Treado and M. P. Nelson, "Raman Imaging" in *Handbook of Raman Spectroscopy: From the Research Laboratory to the Process Line*, edited by I. Lewis and H. G. M. Edwards, New York, NY: John Wiley and Sons, 2001, pp.191-249.
3. R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, New York, NY: John Wiley and Sons, 2000, pp. 293-331.

Introduction

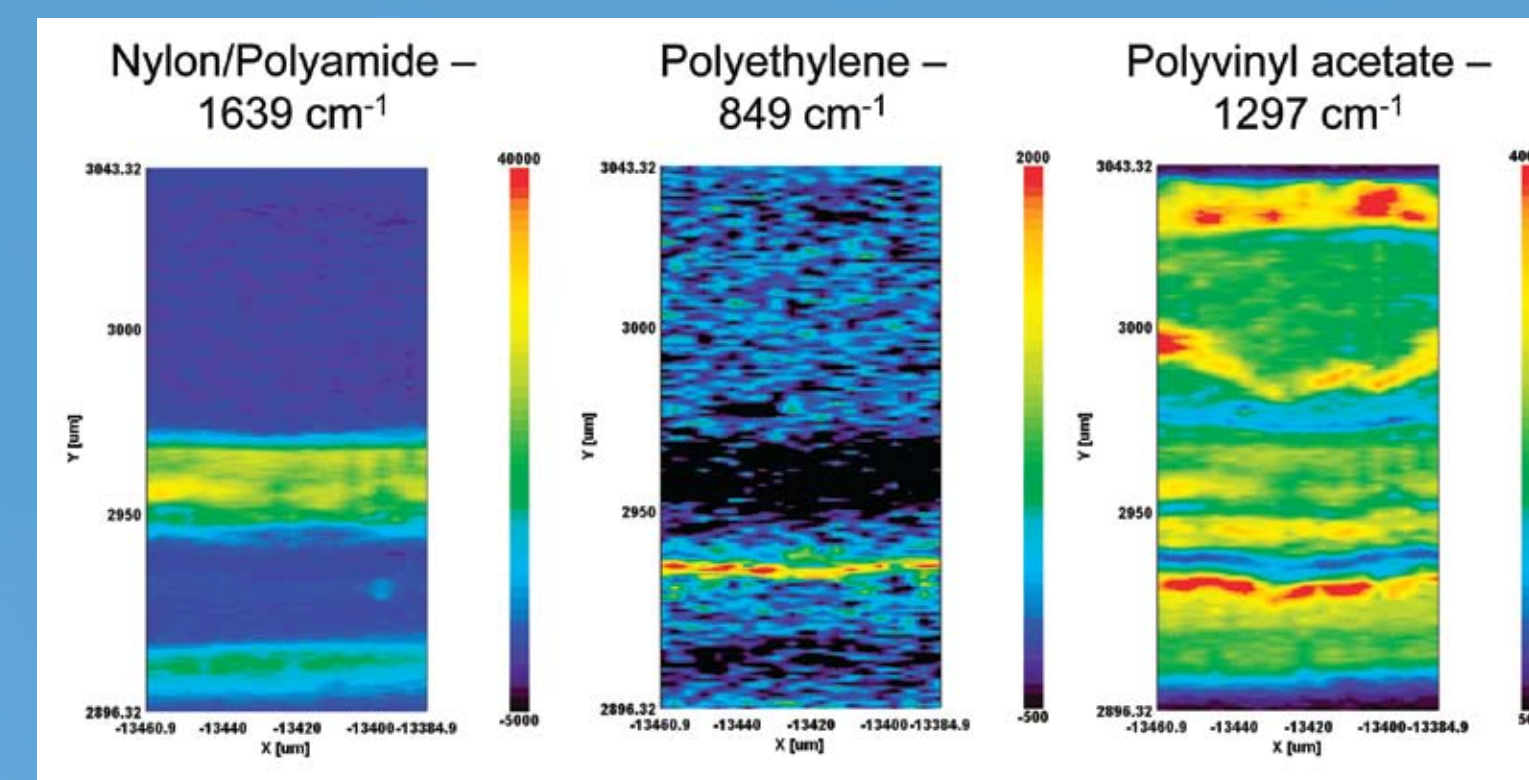
- To increase the speed and efficiency of Raman mapping or 'imaging' applications one could:
 - ✓ Use 'macro' illumination of sample, micro Raman signal collection
 - ✓ Obtain 'bare minimum' signal for strongest peaks of the sample
 - ✓ Reduce spectral/spatial resolution requirements
 - ✓ Use a fast response CCD detector with electron-multiplying to enhance Raman signals
 - ✓ Reduce need to move sample stage for every measurement point

'VertiScan' Sample Excitation

- The laser always irradiates the sample vertically to obtain a Raman image without distortion
- Accurate image information also obtained when using 3-D imaging
- A wide area sample measurement using only laser scanning (50 μm x 50 μm using a 100X objective lens)



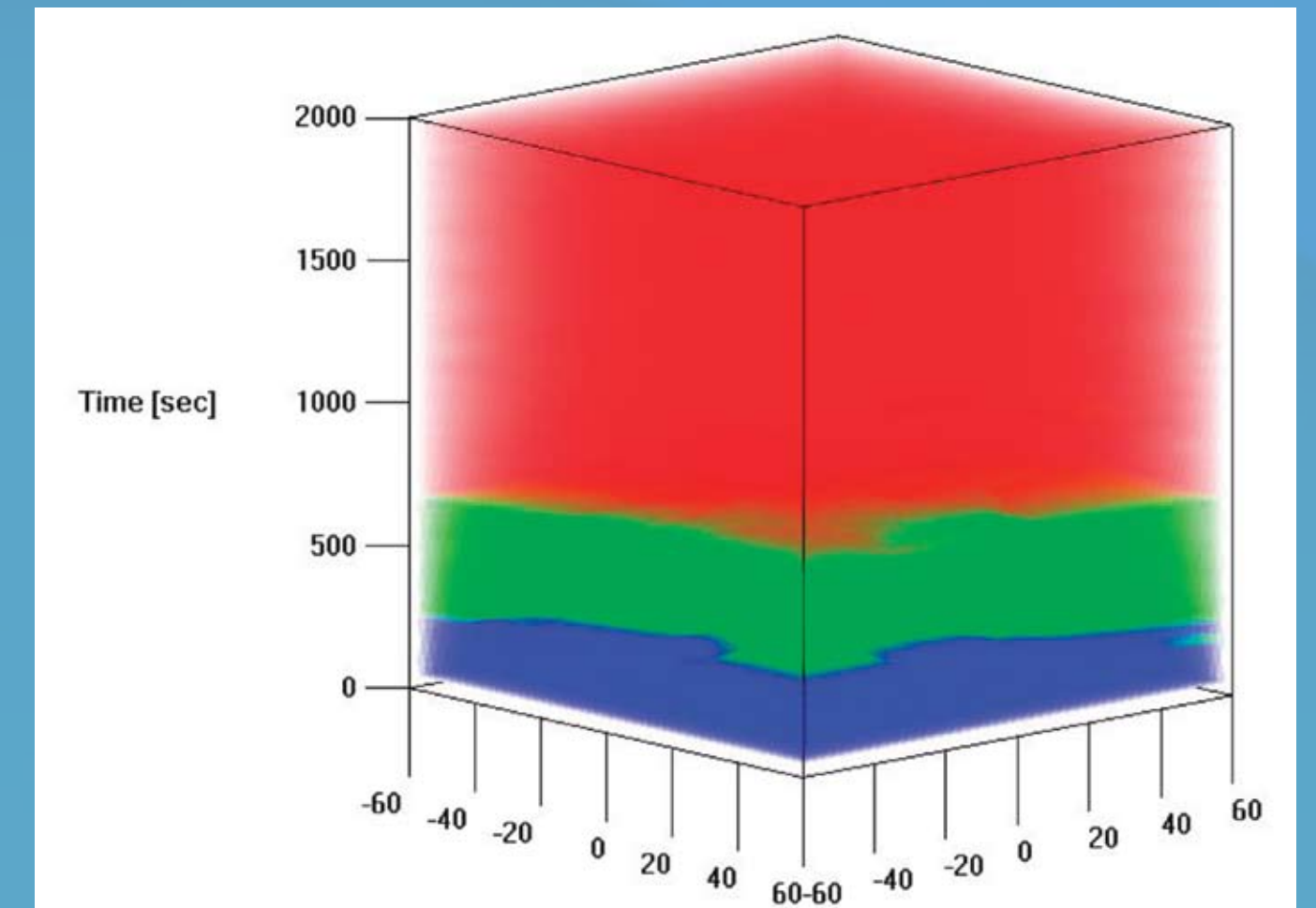
Polymer Laminate – Cross Section Spectral Images Based on Integrated Peak Height



Cyanoacrylate Adhesive Reaction Kinetics

- A cyanoacrylate adhesive was analyzed during the 'drying' process to examine the changes in spectra.
- Raman spectra were collected of a single drop of adhesive as it dried, then analyzed to determine what spectral changes occurred.
- The intensity changes for a peak in the C-H stretching region were analyzed to obtain the rate constants for the reaction process.
- Spectra were collected for a single-point of the glue surface; then a mapping of the glue surface was attempted to obtain data for the reaction time course.

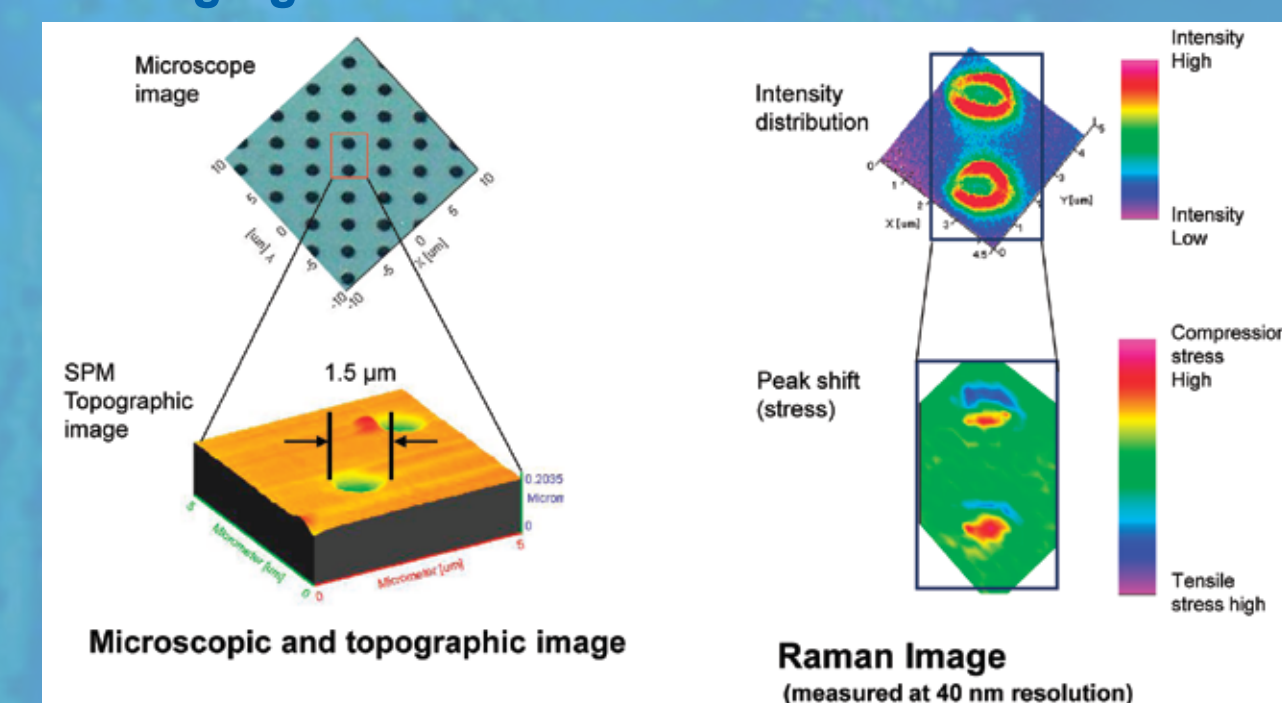
CyanoAcrylate Adhesive – 3-D Mapping Data



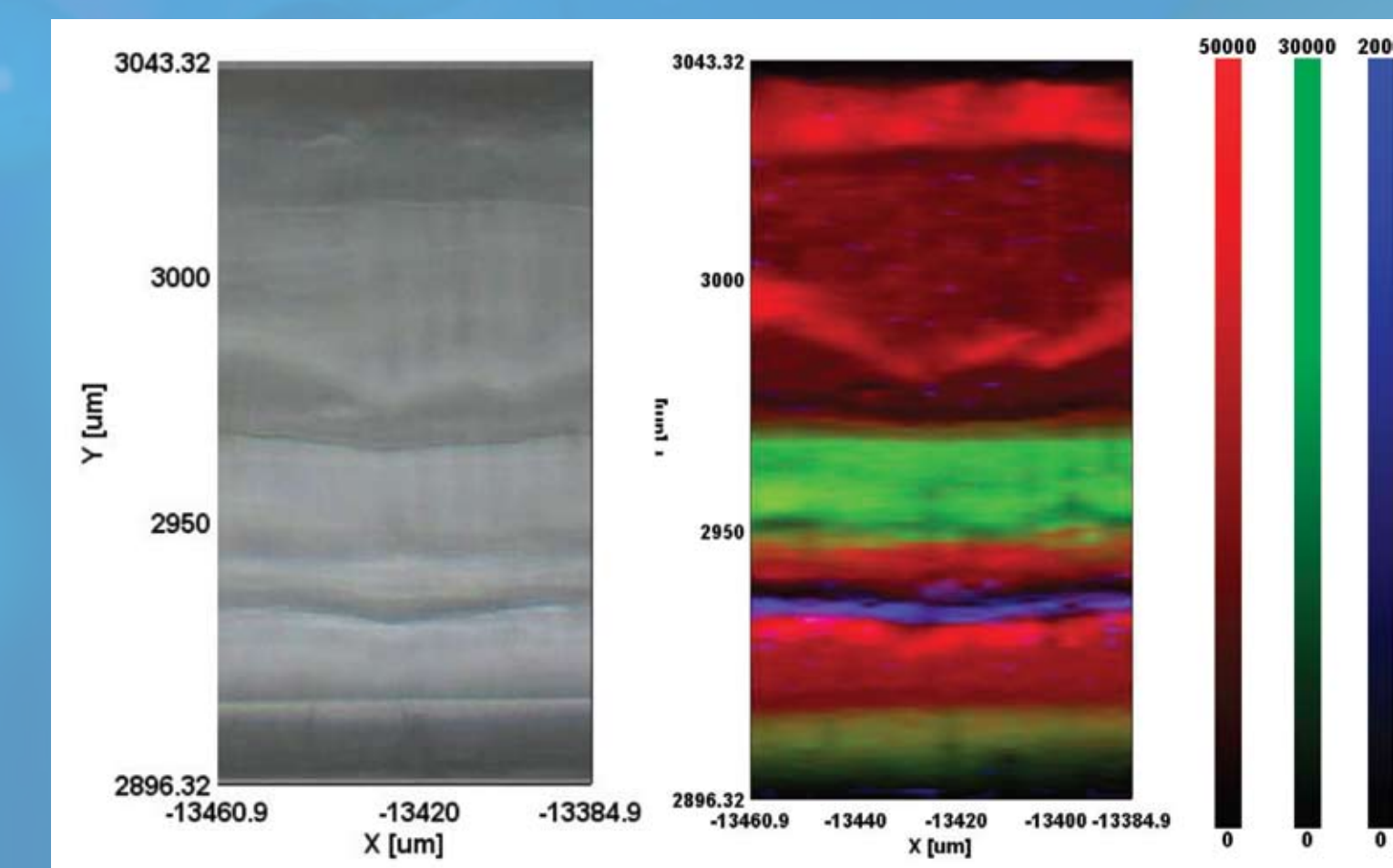
SPRIntS High Speed Imaging

The SPRIntS and VertiScan functions can provide an imaging measurement with a minimum 30 nm spatial resolution, without the use of an auto-stage.

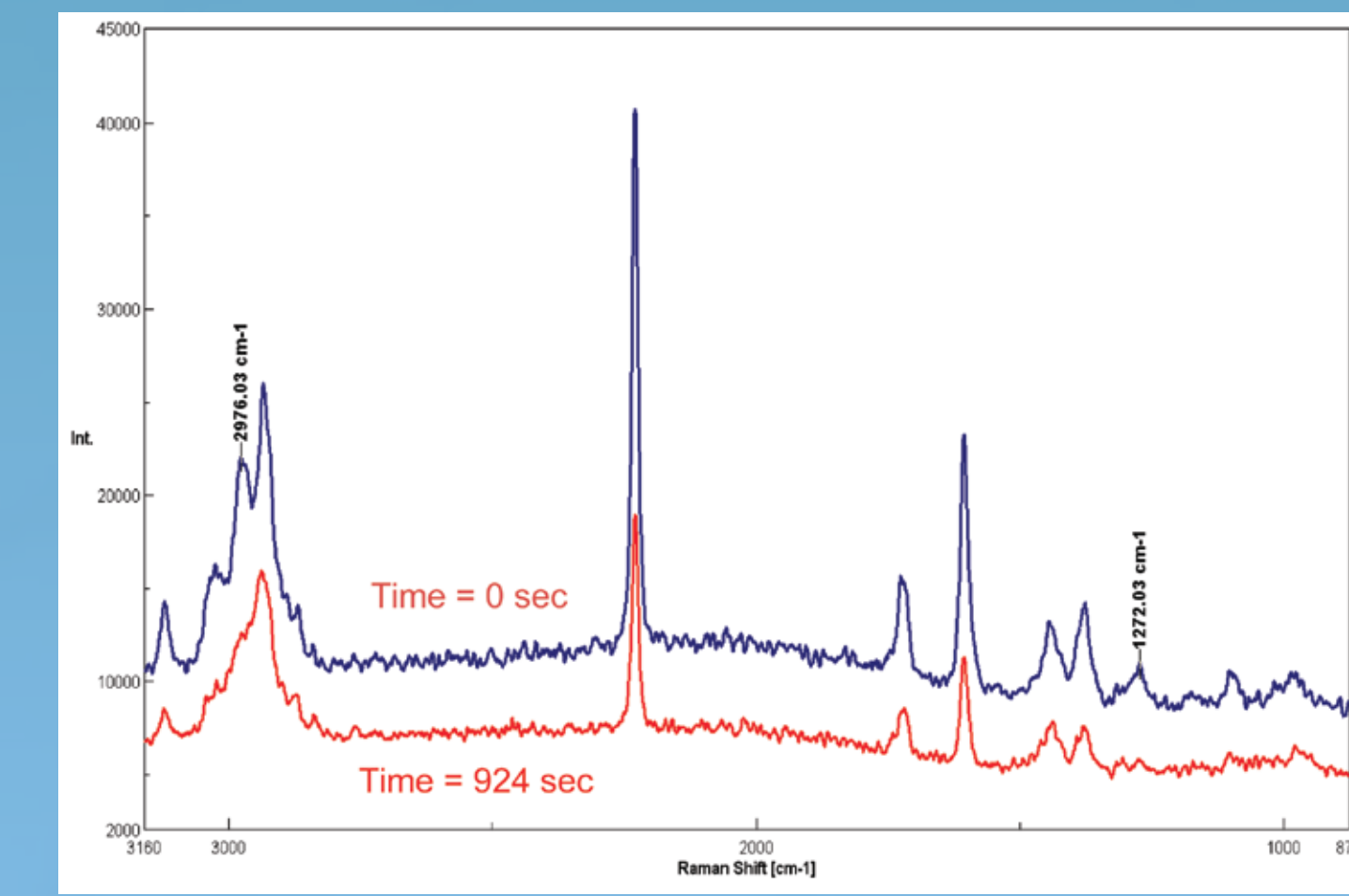
Raman imaging of small area at sub-micron scale



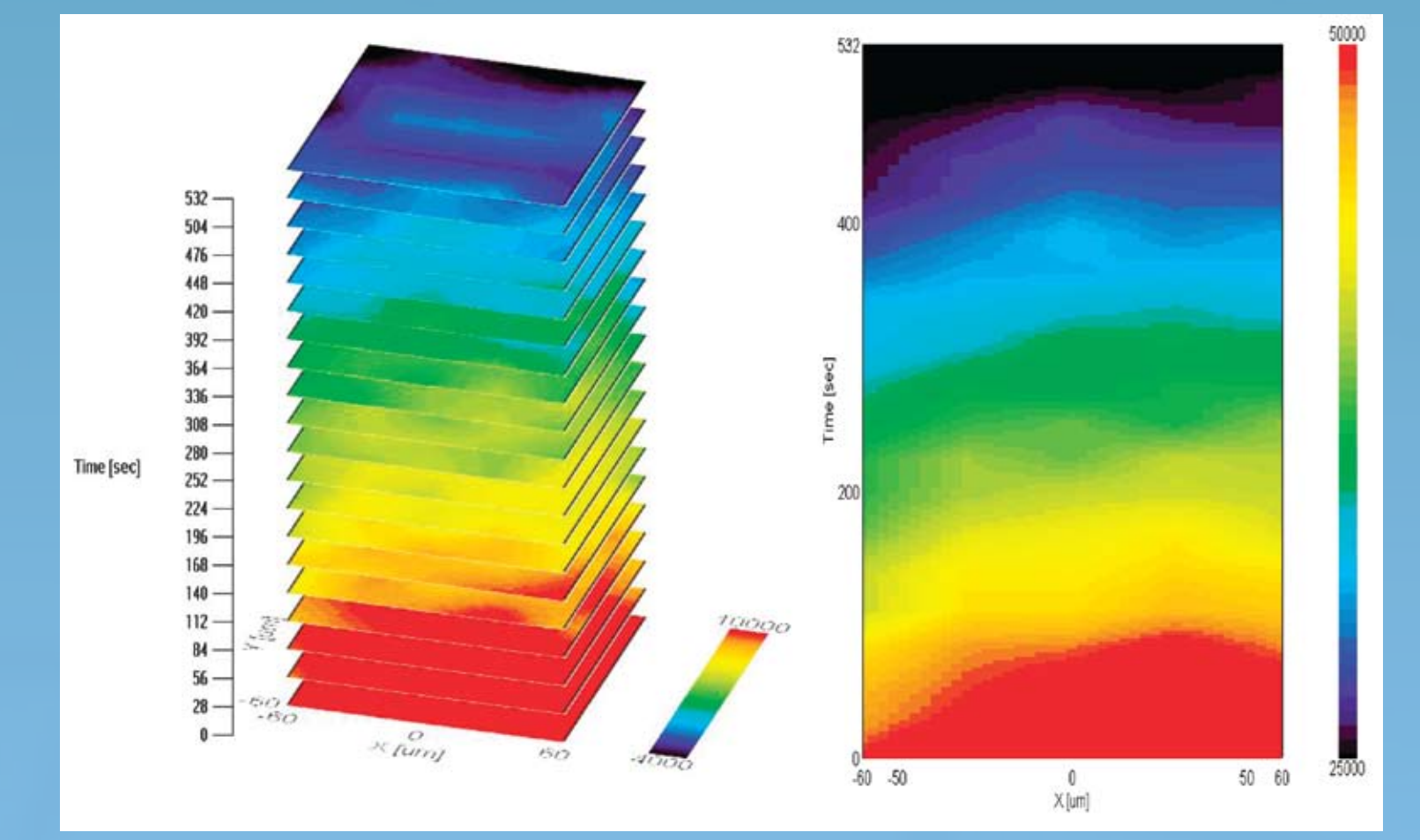
Polymer Laminate – Image Overlay of Individual Laminate Spectral Images



CyanoAcrylate Adhesive – Raman Spectra at Different Reaction Times

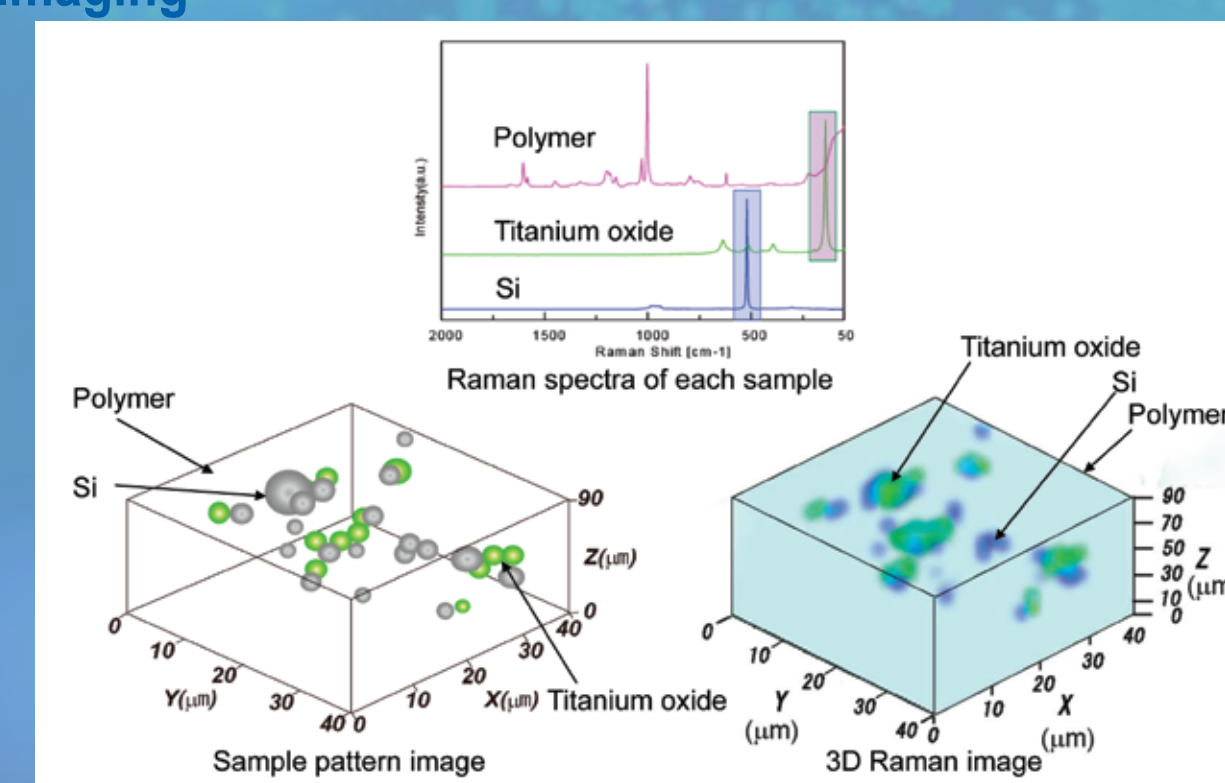


CyanoAcrylate Adhesive – Mapping Data Analysis Figures

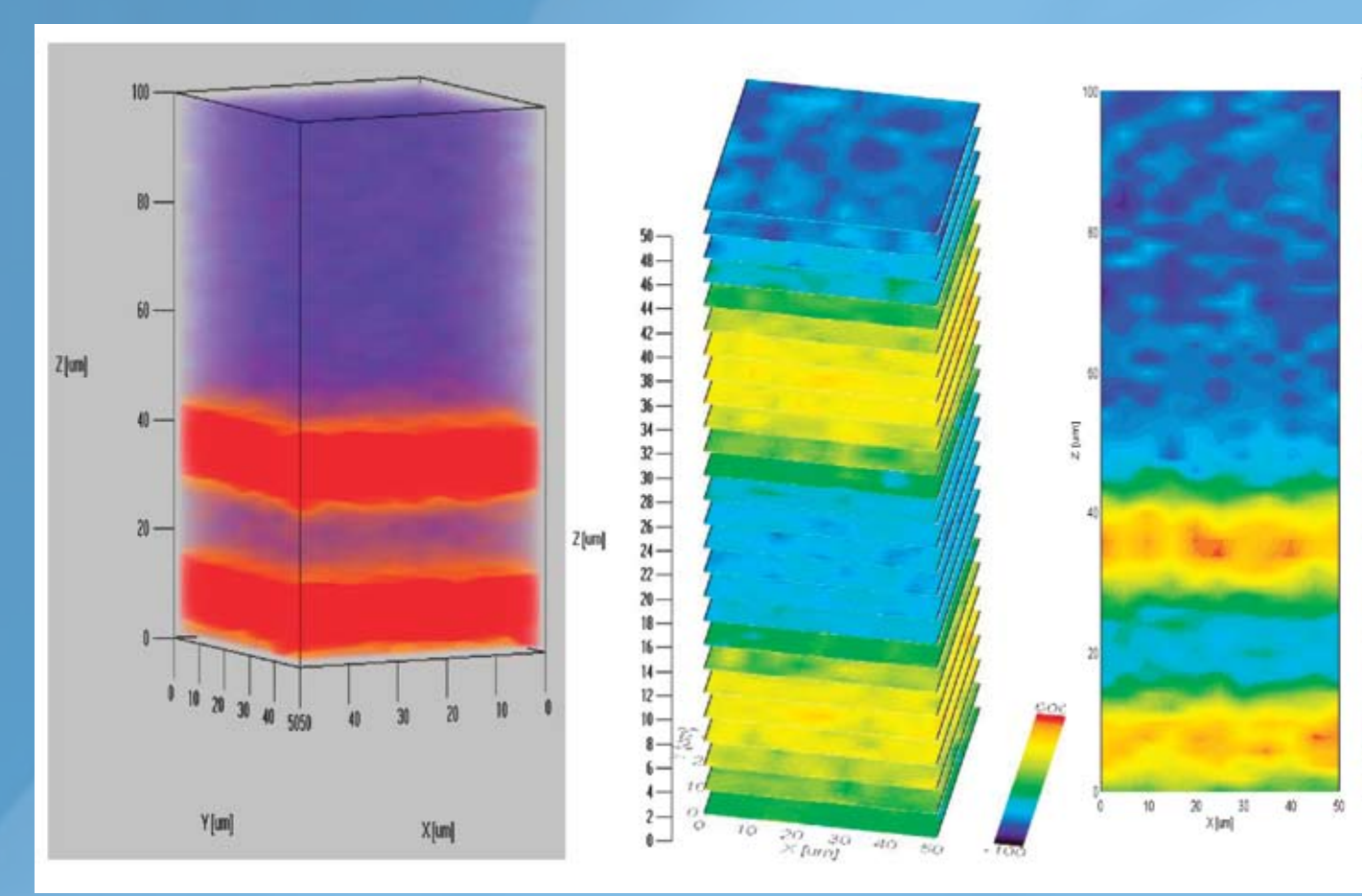


The instrument system can acquire depth imaging data from a sample using the confocal capability of the Raman spectrometer and create a 3-D image from the Raman intensity data. Multilayer sample analysis is also possible using this feature.

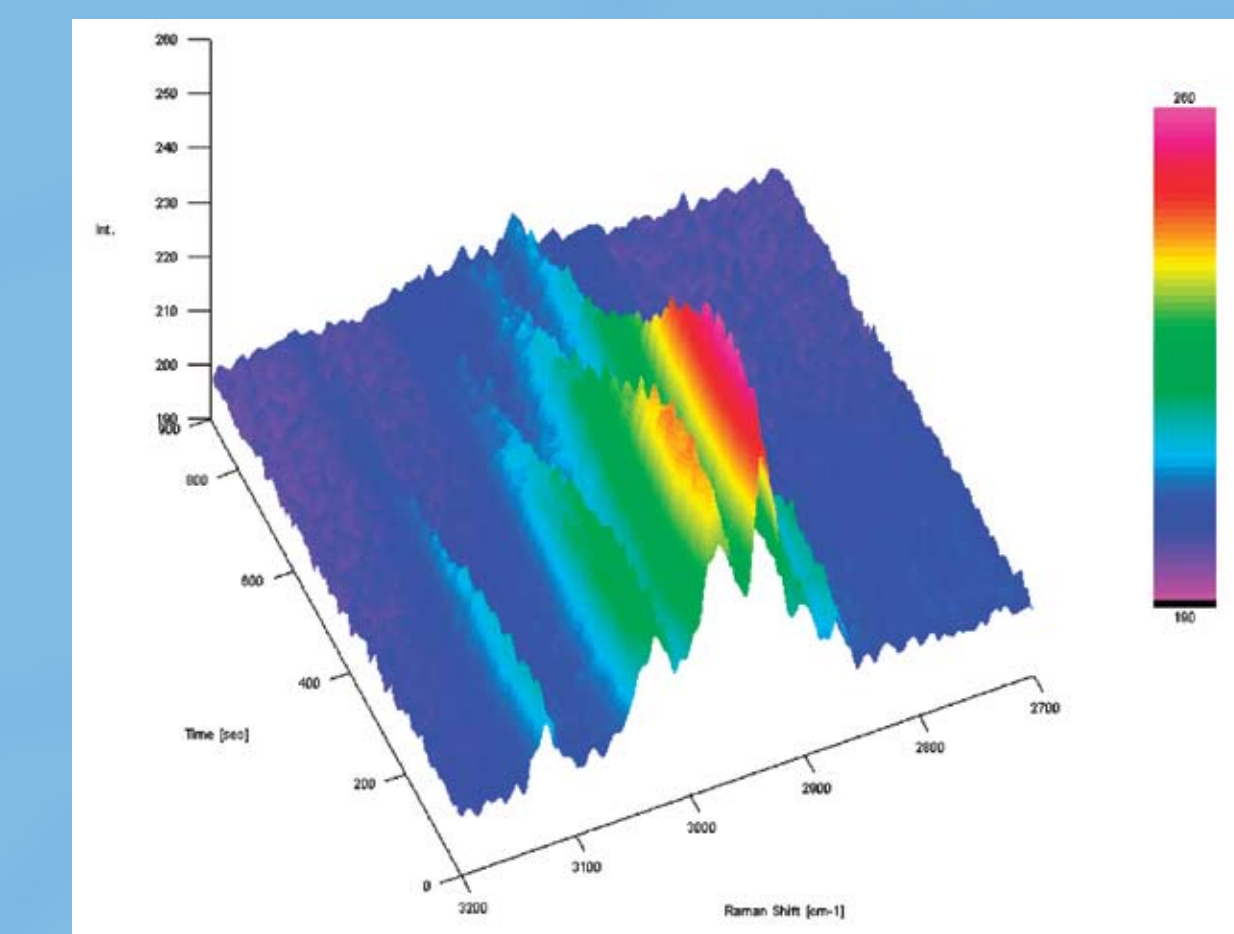
3-D Imaging



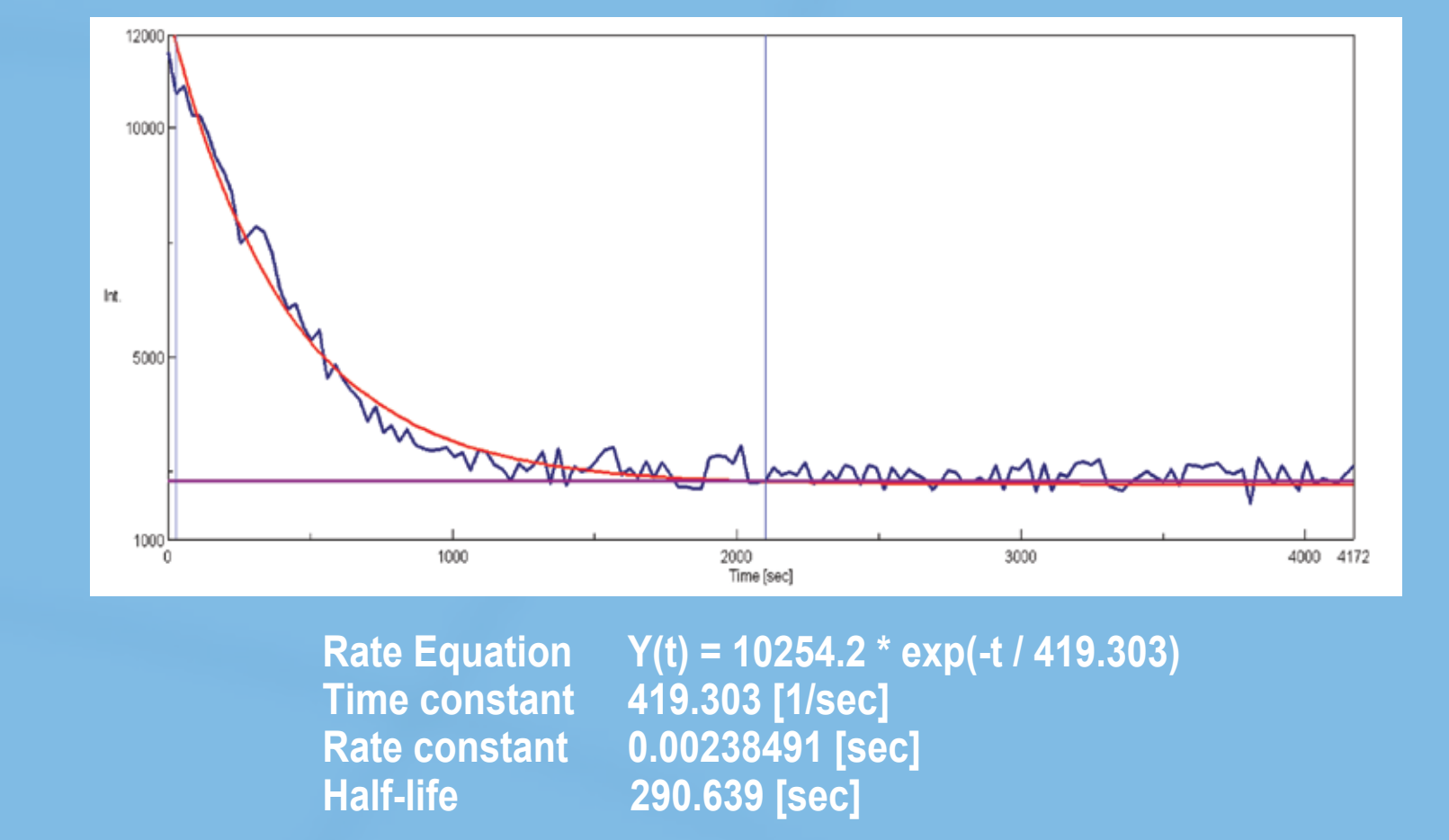
Polymer Laminate – 3-D Image Representations (1639 cm⁻¹)



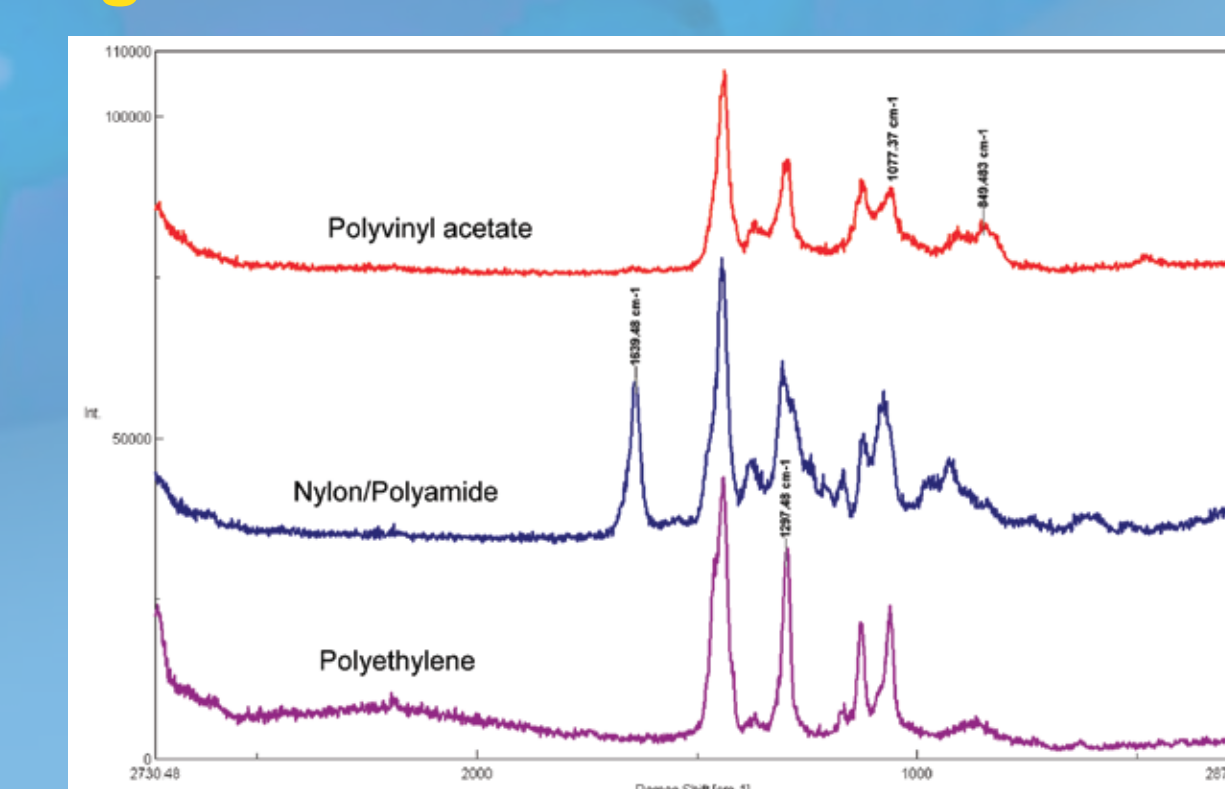
Cyanoacrylate Adhesive – Single-point, 3-D Spectra, C-H Stretching Region



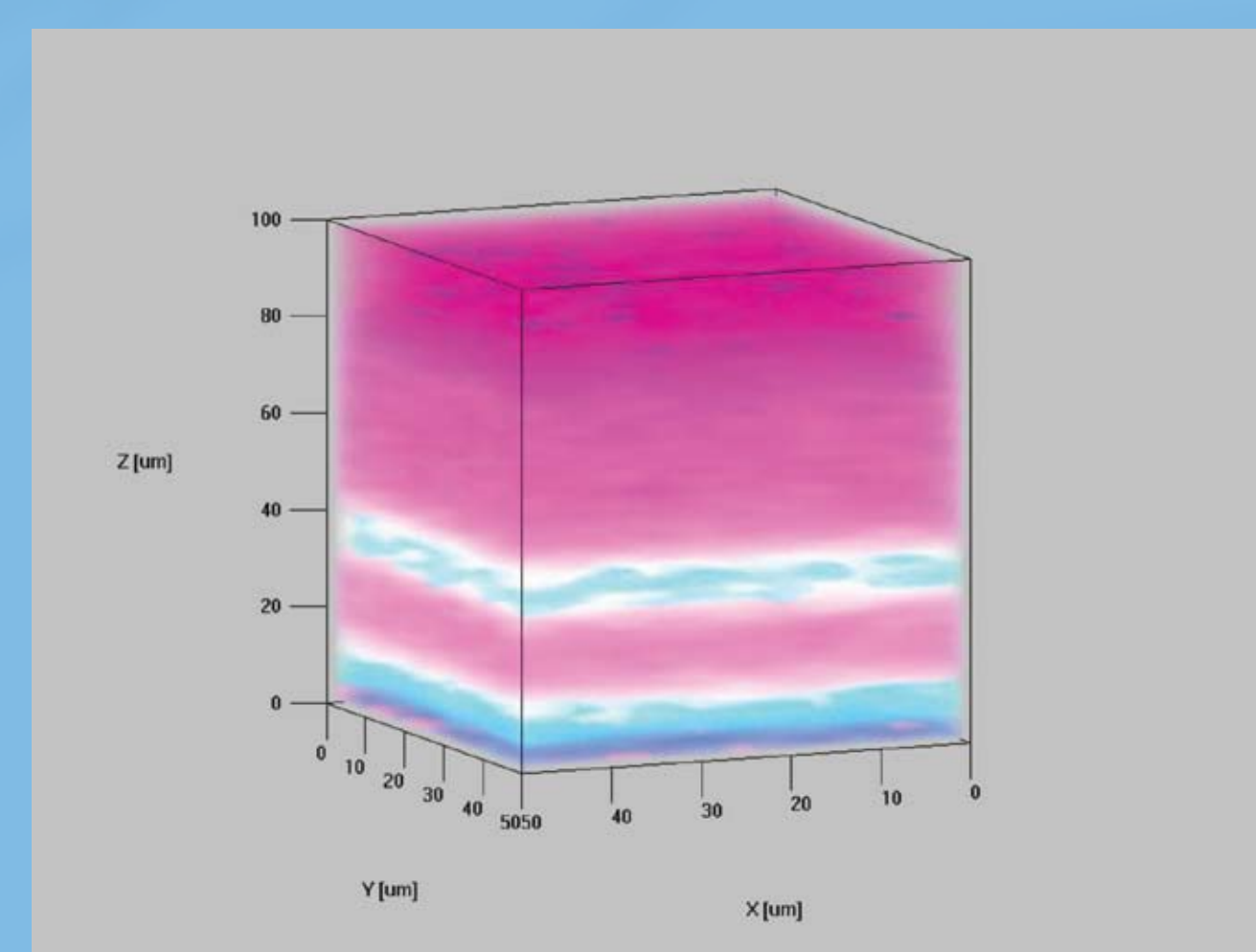
CyanoAcrylate Adhesive – Mapping Data Kinetics Analysis



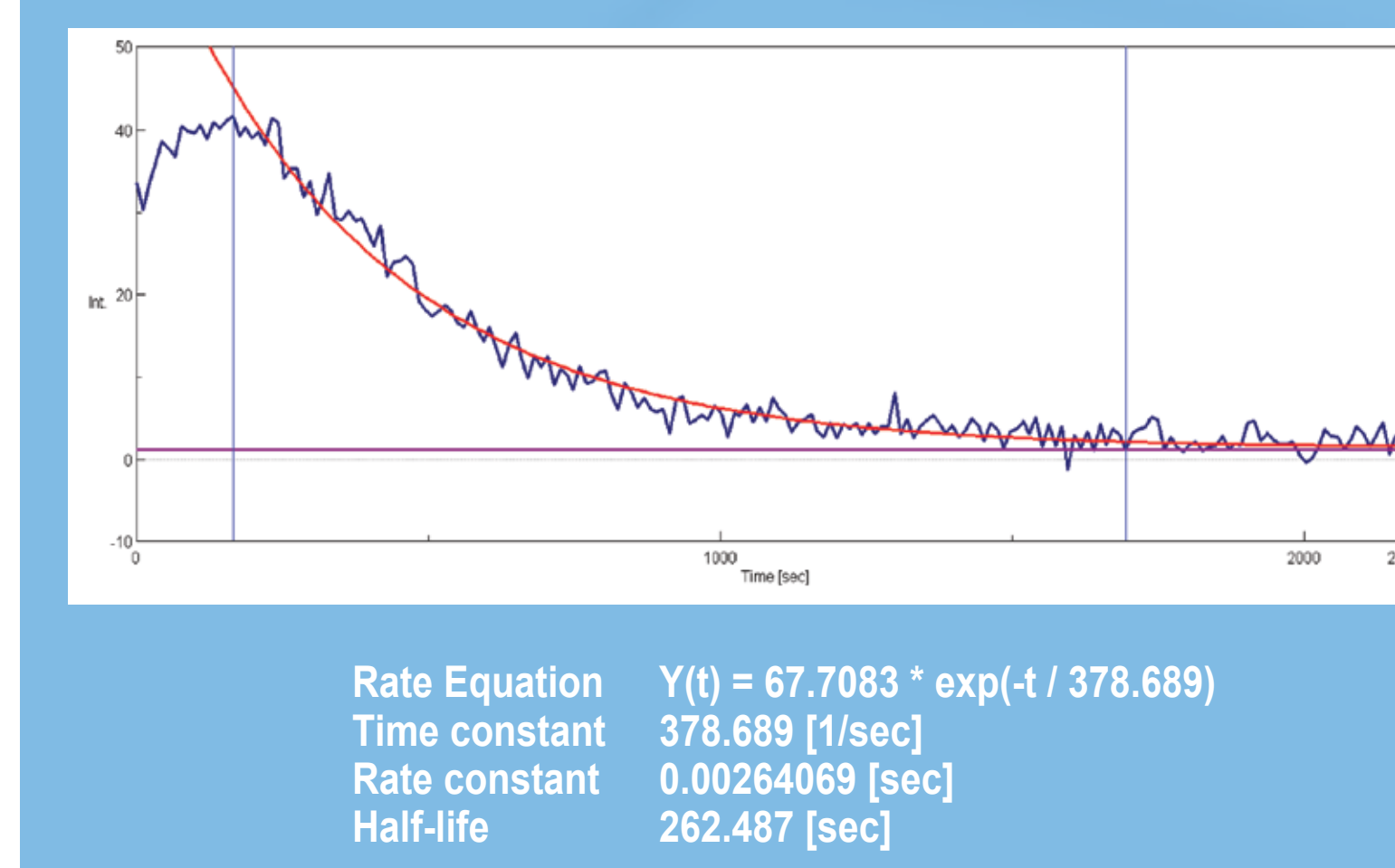
A polymer laminate of multiple layers is comprised of 3 primary polymer components – data can be collected using a 'cross-section' of the laminate



Polymer Laminate – 3-D Composite Image



CyanoAcrylate Adhesive – Single-Point Kinetics Analysis



Conclusions

- The NRS-5000/7000 series instruments offer the SPRIntS and VertiScan features to provide a rapid imaging capability, even when using a manual stage, without:
 - ✓ Confocal distortion
 - ✓ Loss of spectral or spatial resolution
 - ✓ Losses in sensitivity
- 3-D confocal imaging can be obtained using the VertiScan feature, ensuring XYZ imaging without spectral or optical distortion.
- Rapid data collection provides '3-D' spectral mapping of sample surface versus time to obtain reaction rate data of a cyanoacrylate adhesive drying process.
- Further experiments needed to quantify the reaction-rate data for various cyanoacrylate adhesives.

