Molecular Microspectroscopy: Where are we and where are we going

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Molecular microspectroscopy is the application of infrared or Raman spectroscopy, combined with light microscopy, for chemical analysis on the microscopic scale. For over a decade infrared microspectroscopy (ISM) has been an expanding technology both scientifically and commercially. Micro-Raman spectroscopy has a long history of scientific accomplishments with limited commercial success.[1] Both of these techniques give spectral data that can be related to the bonding, crystalline state, isotopic content and molecular orientation of a material, but not its elemental composition. In this regards, molecular microspectroscopy compliments the elemental analyses performed with electron microscopes using x-ray emission spectroscopy. Resolving molecular chemistry of microscopic domains is the essence of molecular microspectroscopy.[2]

Raman spectroscopy is experiencing renewed interest because of several advances in optics and photonics. Near-infrared lasers combined with Fourier transform spectrometers greatly reduced fluorescence interference, making Raman spectroscopy practical for many samples.[3] The development of filters with high rejection of the Rayleigh scattered radiation simplifies the optical design of Raman systems.[4] These filters are playing an important role in micro-Raman. CCD and CID detector technology, lasers, acusto-optical tuned filters (AOTF), liquid-crystal tuned filters (LCTF) and imaging spectrometers are contributing to a proliferation of new micro-Raman systems.[5-6]

The advantages of micro-Raman spectroscopy are sub-micron spatial resolution, visible or near-infrared excitation and an extended spectral range for the analysis of both inorganic and organic molecules. Its disadvantages are that Raman scattering is very weak and many materials are naturally fluorescent, which interferes with Raman spectral measurement.

IMS has rapidly developed into a mature analytical discipline, achieving both scientific and commercial success.[7] Infrared spectroscopy is a fundamental instrumental method for chemical analysis with large data bases for qualitative analysis and established methods for quantitative analysis. IMS instrumentation is available as accessory microscopes for infrared spectrometers or as dedicated IMS systems. There are over five thousand IMS systems world wide, with sixty percent of these in the US. Chemicals, coatings, electronics, pharmaceuticals, plastics, rubber and textile industries are major users of IMS technology. Forensic laboratories have established IMS as an essential tool for trace evidence analysis. Clearly IMS has established itself as a valuable technique for molecular microanalysis.

The future holds the promise of spectral imaging. Spectral imaging is recording the infrared or Raman spectrum of an array of points simultaneously. With a spectral image it should be possible to point out any morphological feature within this image and instantaneously display the infrared or Raman spectrum of that feature. In addition, by selecting certain spectral features, an image can be constructed wherein the contrast reflects the distribution of specific chemical components. With current technology, chemical distributions are recorded by sequentially analyzing individual points. These methods are limited by the fact that the data collection time per point is several seconds to as much as a few minutes. If an array detector sensitive to the mid-infrared region were available that had 256 x 256 elements then, in theory, the data collection time for the array of 65,536 elements would be no longer than the data collection time for for a single point. We look forward to seeing these detectors readily available at reasonable prices and the computational power expanded to rapidly process the large arrays of data. These chemical imaging IMS systems will then reach our goal of relating what we see to its composition.

An imaging Raman System (Renishaw[8]) is commercially available which uses filter technology combined with a CCD detector. This system combines filter technology for imaging with a point analysis system for high-resolution Raman spectroscopy. Other imaging Raman systems have been constructed from LCTF and AOTF. These systems are pointing the way to the future.

We are entering a new era of molecular microspectroscopy where relating chemistry to the micro-structure is of major importance. In the past decade, the users of molecular microspectroscopy have been primarily analytical chemists. Their major concern was chemical analysis; imaging was only a means to locate microscopic samples for spectral analysis. The era of spectral imaging is important to biologist and materials scientist who need to supplement their studies of micro-structure with detailed molecular analysis. The technology of combining imaging and chemistry has evolved over several decades with many major accomplishments. We are at the threshold of new advances, with new challenges and new opportunities. ■

1. G. Rosasco, E. S. Etz, and W. A. Cassatt, *Appl. Spectrosc.*, 29(1975), 396
2. C. D. Arvanitopolos and J. L. Koenig, Appl. Spectrosc., 50(1996), 1
3. *Foruier Transform Raman Spectroscopy from Concept to Experiment,* D. B. Chase and J. F. Rabolt, Eds (Academic Press, New York, NY, 1994)
4. M. Futamata, *Appl. Spectrosc.*, 50(1996), 199
5. I. Kurtz, R. Dwelle and P. Katska, *Rev. Sci. Instrum.* 58(1987), 11
6. P. J. Treado, I. W. Levin and E. N. Lewis, Appl. Spectrosc., 48(1940), 607
7. *Practical Guide to Infrared Microspectroscopy,* H. J. Humecki, Ed., (Marcel Dekker, Inc. New York, NY, 1995)
8. Renishaw Inc., Schamburg, IL.

Reprinted from Proceedings, Microscopy and Microanalysis '96

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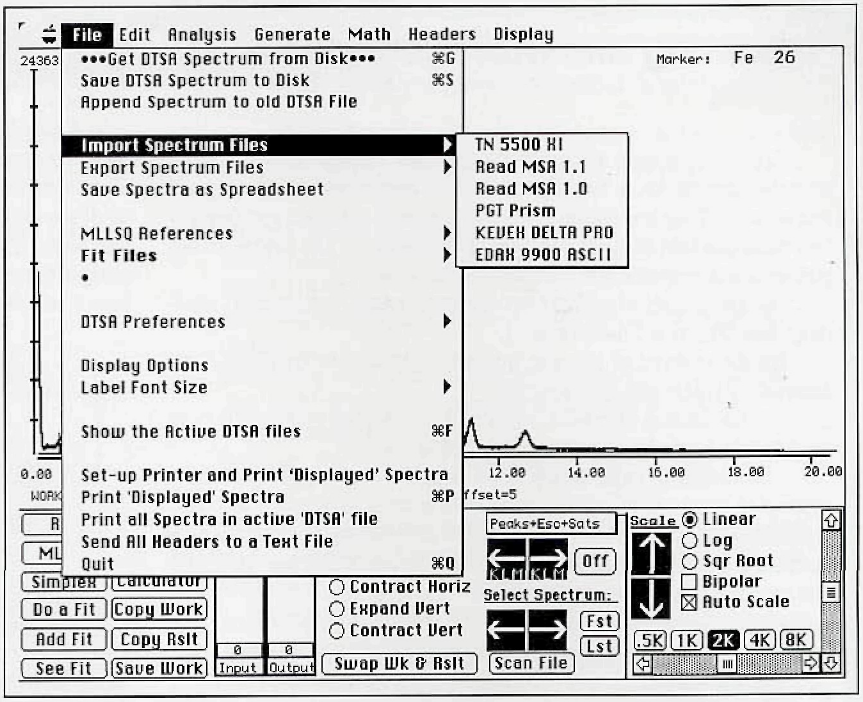

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