Raman's renaissance at 75

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Amidst the bustle of the 23,000 PITTCON® 2003 attendees, Raman spectroscopy quietly celebrated its 75th birthday. While it was used extensively throughout the 1930–1950s, in more recent years, this vibrational spectroscopy tool has been quietly developing in the shadow of its counterpart, FTIR. In her presentation for the prestigious Waters Symposium, Dr. Fran Adar of **Jobin Yvon, Inc.** (Edison, NJ), traced both the roots of this technology and its current rise to prominence.*

Raman spectroscopy has a lot to celebrate in its 75th year. Easier to own and simpler to use, new systems such as the LabRam (**Jobin Yvon**) can be unpacked and up and running within an hour, using the standard 110-V current available in any laboratory.

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Gone, also, is the need for liquid nitrogen for the detector and water cooling for the laser. Since current lasers are lower power and air cooled and detectors are typically air cooled, liquid nitrogen is only necessary in the state-of-the art FTIR–Raman hybrids. Today's systems combine simplified site location with easy signal acquisition and processing, making protocol development and implementation routine, even for a newcomer to Raman. Enjoying a higher sensitivity than ever before in its history, Raman spectroscopy probes those ranges masked by the broad O–H and N–H peaks in conventional FTIR, making it ideal for wet samples and the amines, which are so important for today's protein analyses, as well as a wide variety of metallic ion, hydration, corrosion, and polymer analyses. Fast signal collection tracks kinetics and takes chemical analysis beyond fingerprinting into the real world of processes.

Historical challenges

The Raman signal is often less than 1/10millionth as bright as the excitation signal and is often overwhelmed by scatter or fluorescence from impurities.

*Details of Dr. Adar's presentation are now available at www.JyHoriba.com/Raman75.htm.

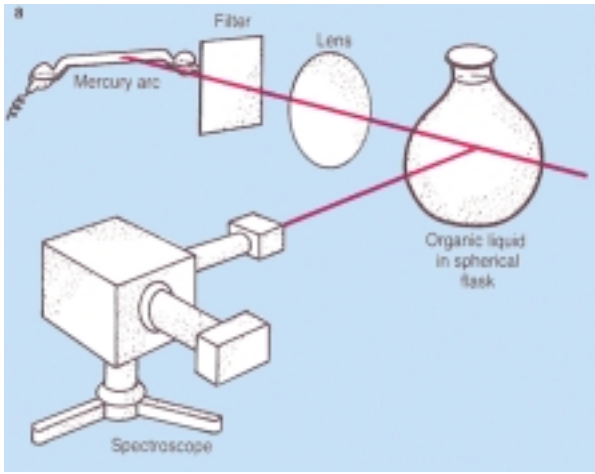

Figure 1 *C.V. Raman's spectrograph, 1928. (Picture courtesy of American Chemical Society,* C&E News *1999; Jan:103.)*

However, using filtered sunlight as a source, a simple prism spectrograph isolates the Raman effect and just his eye as the detector (*Figure 1*), C.V. Raman first detected this tell-tale elastic scatter of energy from key molecular bonds in 1928. His work earned him the prestigious Nobel Prize two years later.

Using Raman in the early days presented challenges from both sample and equipment. Not only is the faint Raman signal nearly difficult to detect, any flash of light from a particle in the solution could ruin the photographic plate used as detectors in the early systems. To ensure that the sample was physically clean and contained no fluorescing impurities, chemists first synthesized the sample and then conducted multiple distillations, often taking up to three months to complete. On the equipment side, a high index of refraction prisms (up to 35 mm on a side) was used to disperse the spectrum. To ensure their optical purity and freedom from strain, these prisms were cooled from the glass state, often for up to a year. While the advent of lasers helped Raman's cause by boosting the excitation power, these systems required a source of three-phase, 220-V power as well as elaborate plumbing for the laser's water cooling system. These experimental and equipment issues made Raman expensive and rare, used by only a few experts.

The rise of infrared and fall of Raman

IR analysis also faced challenges during its early

years. Producing long-wavelength sources and long-wavelength detectors was challenging. However, these technical problems were soon resolved, making IR easier to use. With the development of Fourier transform interferometers for infrared measurement, IR became a truly robust technique. Commercial vendors began to sell more and invested their profits in R&D. Soon IR became the instrument of choice for analytical chemists. While physicists and other material scientists were interested in pursuing Raman, analytical chemists were less comfortable with lasers or with Raman as a technology.

To sway the balance further, graduate schools often taught that Raman was difficult to do because of fluorescence and the faint signal. Throughout the analytical community, experience with Raman was rare and often negative. Raman gained a reputation as being difficult to use and, because it was laser based, had high utility requirements and was hazardous. Since so many samples fluoresced, masking the Raman signal, few chemists saw its applicability.

The addition of microscopy has caused the major renaissance and acceptance of Raman spectroscopy.

Technology advances drop the barrier

Ironically, Raman finally got its big break in the late 1980s, when its interferometric-based Fourier transform version (FT-Raman) was proven by Bruce Chase at **DuPont** (Wilmington, DE) and John Rabolt, then at **IBM** (San Jose, CA). Using a near-infrared (NIR) laser, FT-Raman resolved many of the fluorescence problems. More importantly, it was easier to use. However, its lower sensitivity compared to grating-based dispersive Raman limited its application. FT-Raman did, however, act as a catalyst and reawakened interest in Raman spectroscopy; dispersive Raman spectroscopy was a sleeping giant waiting in the wings.

Over the years, the core system has undergone many changes. Light sources evolved from filtered sunlight to arc lamps to water-cooled lasers. However, while lasers provided a significant increase in excitation energy, they also required complex plumbing for their cooling system, which always presented the risk of serious leaks in the laboratory. Today's compact, air-cooled NIR lasers are easier to install, have lower cost of maintenance, and present no risk of leakage.

Prisms were replaced first by ruled gratings and then holographic gratings. The grating's higher angular dispersion and larger useful aperture significantly improved sensitivity and accuracy. Since the grating's dispersion could be well-characterized mathematically if the entry angle was well known, the exact wavelength would be known. Furthermore, holographically recorded gratings reduced stray light levels by orders of magnitude, totally eliminating the smear and aberrations known as grating "ghosts"—artifacts of a particular ruling engine—and producing clean, clear spectra.

Photographic plates quickly replaced the human eye, which was used as Raman's original detector. In the late 1930s, plates were replaced by photomultiplier tubes (PMTs). While PMTs offered low background noise, they suffered from low quantum efficiency. The solution was to integrate for long periods to collect a single spectrum.

However, Raman instruments were still too big and too slow. For example, to characterize the orientation in a fiber, a Raman system needed at least three spectra, each integrated for 90 min or more. The early 1980's saw the advent of multichannel detectors such as the imaging PMT and photodiode array, which reinstated the multiplexing of the photographic plate and added the advantages of an electronic detector. These detectors provided good signal-to-noise ratios, wavelength response, and dynamic range, coupled with digital storage and manipulation.

Today's systems use charge-coupled devices (CCDs). Their 2-D arrays and rapid signal processing expanded Raman's capabilities from 1-D line scanning to full 2- and 3-D mapping, while considerably decreasing the time to collect spectra to a few seconds instead of the 90 min mentioned earlier.

The new detector technology prompted a major redesign to take advantage of the detector sizes. Originally, double monochromators were used, which provided an 18-mm field of view. A typical system, using an argon laser with an 1800-g/mm grating, produced spectral coverage of 150 cm^{-1}. Replacing the standard, exit slit and PMT detector with the larger intermediate slits and a multichannel focal plane detector degraded spectral low-frequency performance. In the new CCD systems, spectral resolution is limited by the dispersion over two pixels. Depending on the system used, current resolutions typically fall between 2 and 20 cm^{-1}.

Both spectral coverage and resolution are affected by the focal length of the spectrograph, groove density of the grating, wavelength of the laser, and pixel size in the detector. The problem was how to design a system that would produce very high resolution with good stray light rejection. The solution came in the form of triple spectrograph systems, such as the T64000 (**Jobin Yvon**). Using three spectrographs in tandem, the T64000 uses a series of dispersion and refocusing steps to produce extremely clear, high-resolution spectra (1 cm^{-1}/pixel), even at low frequencies. These high-end, powerful systems allow measurements very close to the laser line (<5 cm^{-1}) and can be tuned to any laser excitation line.

Holographic notch filters were the final piece in the Raman puzzle. When coupled with CCDs in the early 1990s, they opened the door for very simple, but powerful, single spectrograph Raman systems. Acting like optical gates, notch filters reflect the excitation from

the laser to the sample, then transmit the resulting Raman signal to the detector. Their excellent background suppression characteristics made it possible to observe lines as close as 30 cm^{-1} to the frequency of the laser excitation, depending on how the type of filter was used and how it was mounted. For most applications, these easy-to-use systems provide higher throughput and the optimum analytical setup, making Raman a serious contender in the analytic market.

Microscopy: Zeroing in and boosting signal

The addition of microscopy has caused the major renaissance and acceptance of Raman spectroscopy. First, microscopes facilitate the collection of the Raman signal from a smaller, more defined sampling volume. Second, the high numerical aperture of microscopic optics increase signal collection tenfold. Finally, it also solves many of the fluorescence problems. To find a good target area, users just hunt for a spot that does not glow. Raman users have also found that if the sample was exposed to laser for a while, the fluorescence would bake out. The microscope focuses the beam to 1 µm versus 100 µm in a conventional spectrograph, quenching the fluorescence 10,000× faster.

Adding confocal apertures to the microscope enhanced the signal-to-background ratio still further by physically blocking the fluorescence. This step was especially important because fluorescence is emitted from a large volume in the sample, while the Raman signal comes specifically from the illuminated spot.

Computer growth: An enabling technology

As with most analytical instrumentation, the dramatic growth in the area of high-powered desktop computers has had a significant impact on Raman's development over the past 30 years. Modern software provides full control, either manually or automatically, over data acquisition; peripheral functions, such as external triggers or heating stages; and multiple imaging modes. These advances opened the door for full acquisition control and sophisticated spectral analysis. Gone is the guesswork. Once the spectrum is acquired, it is automatically displayed in the correct wavelength units. New, easy-to-use graphic user interfaces (GUIs) present an array of manipulations from simple arithmetic to user-friendly baseline subtraction, band fit, curve-smoothing functions, and multivariate analysis. Manipulations in the computer rather than by eye increase both sensitivity and ease by 2–3 orders of magnitude.

New software also opens a variety of options in the transition from simple spectra to 2- and 3-D chemical mapping. For a particular species, the spectroscopist can map intensity, line width, or peak position. Alternatively, the software can provide full spectrum model point-by-point (*Figure 2*).

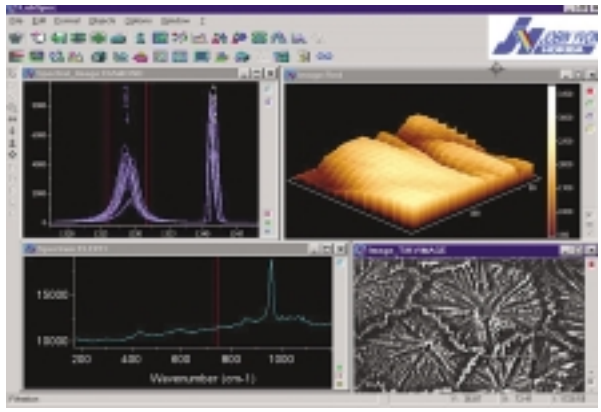

Figure 2 *Clockwise from lower left: single-line Raman spectrum, overlaid Raman spectra, Raman map, and brightfield microscopy image; objective magnification: 50 ×. (Image courtesy of* ***Jobin Yvon****.)*

Summary

Today's Raman microprobes are sleek, functional, and easy to use. Whether as a complement to FTIR or stand alone system, they lower important analytical barriers to advanced analyses of biological, chemical, pharmaceutical, and polymeric materials and processes. As shown by Dr. Adar's presentation, Raman microprobes have energetically and thoughtfully taken advantage of the many technological innovations since those early observations of C.V. Raman 75 years ago to become the fastest-growing analytical spectroscopic technique.

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