"All the νs That's Fit to Print"

The Raman Times

Forecast: Scattered Radiation

Year 26, Issue 2

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Fellgett: "They took advantage of me"

TRIPLEMATE RESPONDS

All that most of us want out of life is an honest advantage, and that's part of the philosophy behind TRIPLEMATE, our new triple spectrograph which tailors Raman spectra for multichannel viewing. To do this, it contains two separate stages. First, a zero-dispersion, double monochromator filters the Rayleigh scatter out of the inelastic scatter, then the latter is passed into a spectrograph that disperses the spectrum, projecting the full spectral feature in one great slice onto the sensitive area of a vidicon or diode array. A turret in the spectrograph has room for three gratings so the frequency range of that spectral slice reaching the detector can be expanded or contracted by selecting a grating of different groove density. An internal microprocessor automatically maintains the central spectral position, regardless of grating selection.

So — what's the advantage? Fellgett [1] was the first to quantify the phenomenon often referred to as the multiplex advantage: improvement in signal to noise achieved by monitoring more than one detection element simultaneously. Each channel of a TV detector is essentially a separate detector: 512 channels for a vidicon, 1024 for a modern diode array. Compare this to a scanning instrument where only one channel is viewed at any instant by the PMT and you can begin to appreciate the advantage of multichannel detection over the traditional photomultiplier. All other things, such as detectivity and resolution, being equal, it would take a scanning instrument 1024 times longer to cover a spectrum than a diode

(Contd. Page 5)

When in the course of scientific events, it becomes necessary to evoke revealing information from recalcitrant samples, our ingenious staff usually rises to the challenge. We'll spare you the duds that seem unavoidable in the development process and proceed directly to our timely techniques.

ACCESSORY GIVES CYTOCHROME A SPIN

Double-beam Raman

Double-beam spectrophotometers are familiar fixtures in many laboratories thanks to their knack for pulling sample spectra out of interfering solvents and background. However, the Raman analog of this device takes a further step into difference spectroscopy. It emerges as a promising probe into the molecular structures responsible for biological and chemical functions (2-6) where subtle peak shifts on the order of 0.1 cm^{-1} often characterize major functional changes.

The SPEX 1475 Difference/Ratio Generator is an accessory that slips into a 1459 Raman Illuminator and operates under control of the DM1 DATAMATE data processor. The split cell (Fig 13) is filled with the two substances to be compared, then spun and sequentially analyzed. While the spectrometer is scanned, DATAMATE's sensors let it know which PMT signals belong to which sample. The two spectra are stored separately, or subtracted in real time.

(Contd. Page 6)

Macro Info From Micro Samples

On the Laser's Edge With MICRAMATE

The bulk of real-world samples are, despite appearances, inhomogeneous conglomerates, though this comes to light only under powerful magnification. Still, the most frustrating aspect of microscopic analyses is the inability to touch what you see. Oh yes, they're there alright, those fragile fibers, crater-like inclusions, or quivering cells. But what is their composition? How does their structure determine their effect on the macroscopic world? To answer these questions without destroying the very things that are under study, the researcher has to tread more carefully than Gulliver through the streets of Lilliput.

One of the most alluring features of Raman spectroscopy is its potential for non-destructive analyses. In most cases, involved sample preparation can be dispensed with. There's no need to grind, pulverize, dissolve, briquet, or otherwise torture your sample. Just place it in the beam (taking care, obviously, that the laser power is below the kindling point) and run the spectrum. Now, if there was only a way to focus that laser beam down to a pinpoint and aim it with surgical accuracy.

The SPEX MICRAMATE *is* that way, an illumination system specifically tailored for Raman investigations on a microscale.

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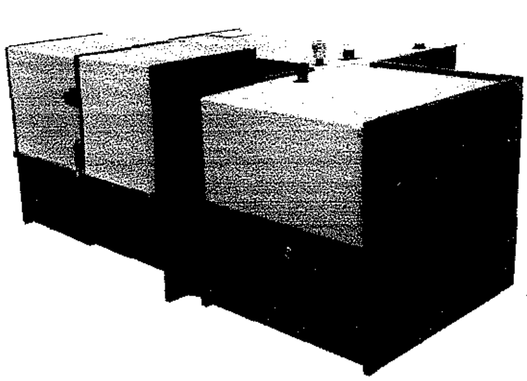
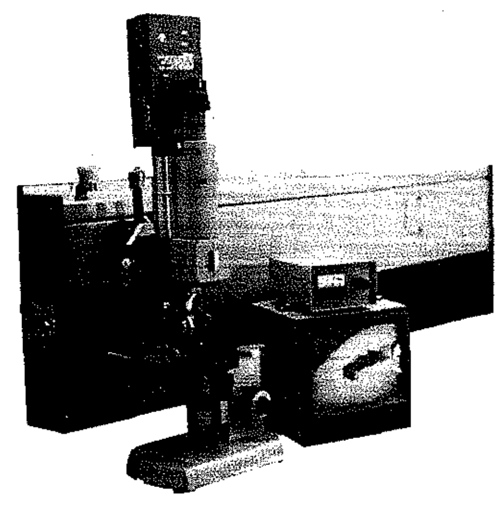


TRIPLE THREAT: TRIPLEMATE, the triple spectrograph ready for multichannel viewing.



TEAMING UP: MICRAMATE mounted on a SPEX 1403 Double Spectrometer.

Continued From Page 1

The MICRAMATE will pick specimens as small as 2 µm in diameter out of the pack. Bolted to the entrance slit of a SPEX laser-Raman system, the MICRAMATE relies on a uniquely modified research-grade microscope to direct and focus the laser beam onto the sample. The scatter that results is collected at 180° and directed into the spectrometer which is then scanned in the normal fashion (Fig 1).

Two objectives are standard with the MICRAMATE: a 10X, 0.22 numerical aperture simplifies coarse adjustment and location with the X-Y translation stage, after which the 40X, 0.95 N.A. is turreted into place for fine tuning and the analysis. To view samples, and at the same time minimize danger to the eyes, the MICRAMATE includes a TV camera and monitor. A neutral-density filter attenuates the laser beam in this mode, automatically swinging out of the way for analyses.

In our applications lab, MICRAMATE has already become a permanent fixture. So we'd like to share a few examples of its performance with you. Of course, if your own projects have gone begging for pinpoint resolution, we'd be happy to give your specimen a chance to perform on our X-Y stage.

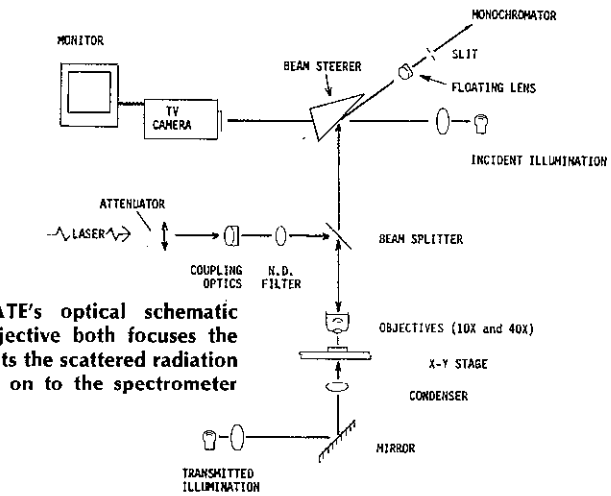


Figure 1 MICRAMATE's optical schematic showing how the objective both focuses the laser beam and collects the scattered radiation which is then passed on to the spectrometer entrance slit.

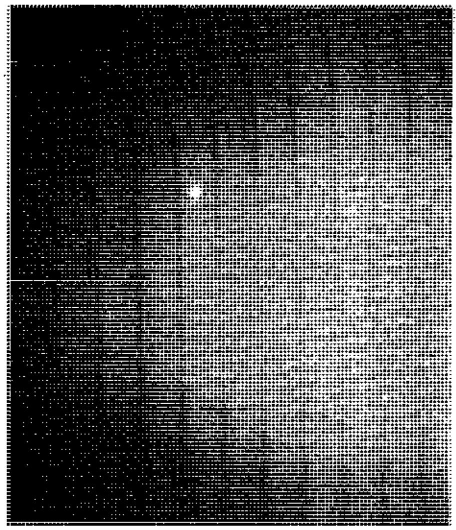


Figure 2 To measure the minimum diameter of the focused laser beam (<2 µm), it was directed to a 150 groove/mm grating.

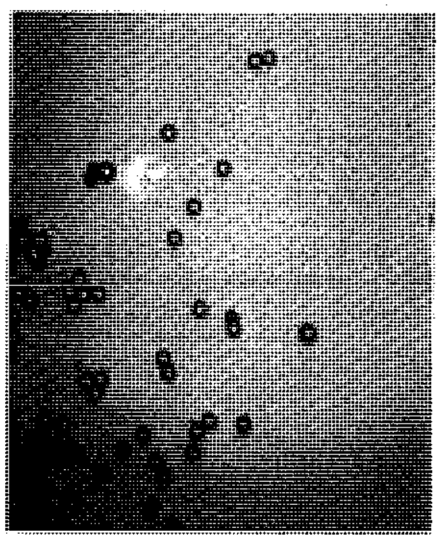


Figure 3 Here the laser isolates a polystyrene sphere just 3 µm in diameter in preparation for a scan of the sample's Raman spectrum.

Getting to the Point

In Fig 2 you'll find a picture of a grating as displayed on MICRAMATE's viewing monitor. The spacing is 150 grooves/mm, demonstrating that the laser beam (white dot) has been tightened down to below 2 µm diameter. We'll be the first to admit that squeezing the excitation beam is not quite equivalent to squeezing out a spectrum, so to find out how minute a particle MICRAMATE could spectrally isolate, we placed calibrated polystyrene spheres of decreasing diameter on the X-Y stage (Fig 3). A single scan of such a 3-µm diameter

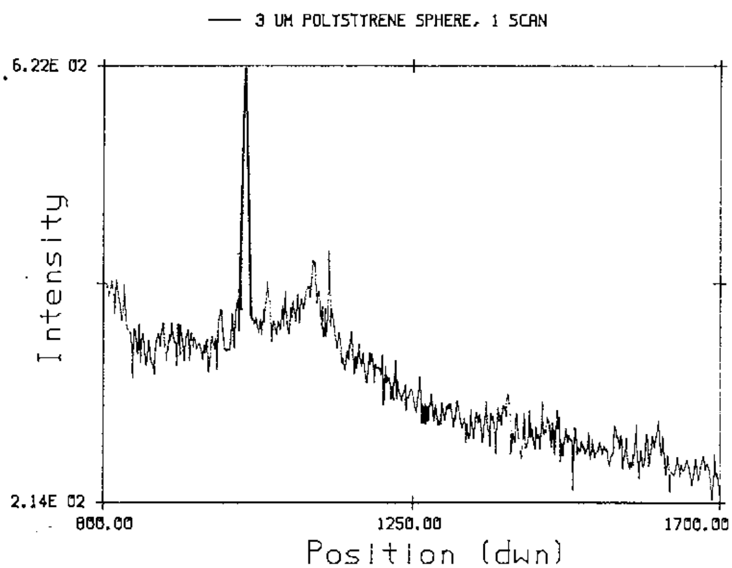


Figure 4 A single scan of the 3 µm polystyrene sphere.

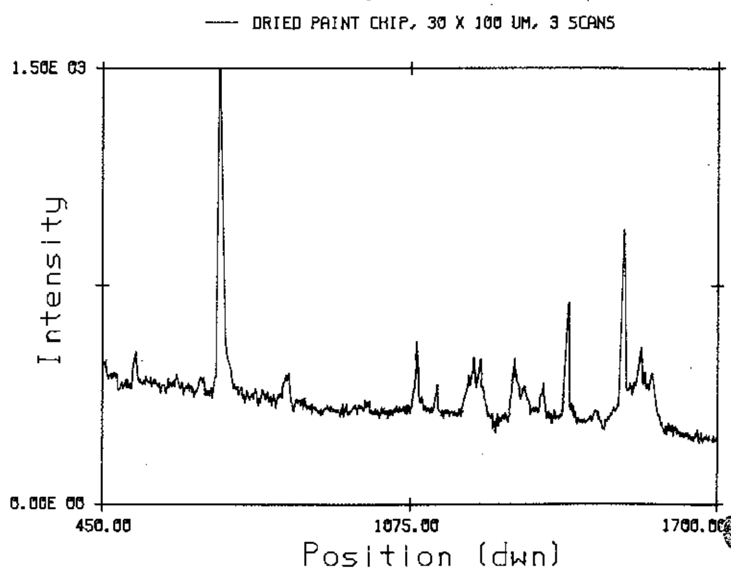


Figure 5 A paint chip with this spectrum would expose a supposed Renaissance portrait as an impostor, no matter how fine the workmanship, for the pigment is a chlorinated phthalocyanine that was not synthesized until the 20th century.

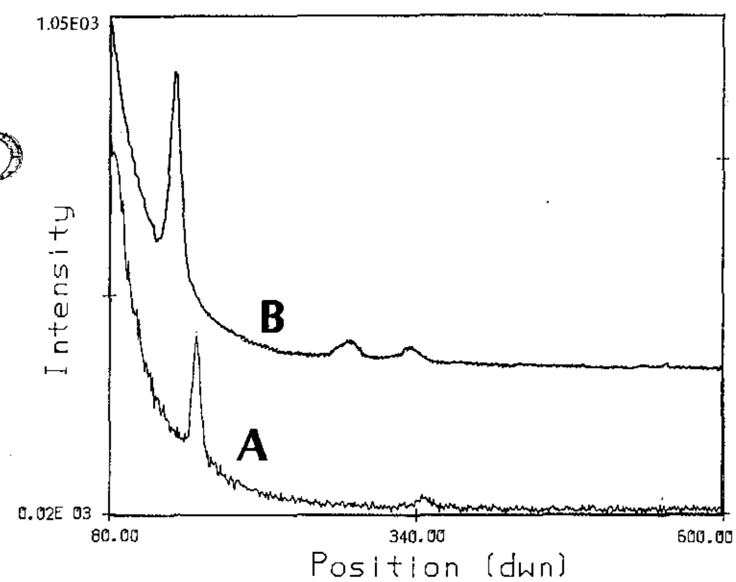


Figure 6 Spectra of 8 μm lead oxide particles on a single sample. MICRAMATE was able to distinguish litharge (A) from surrounding clusters of massicot or amorphous Pb_2O_3 (B).

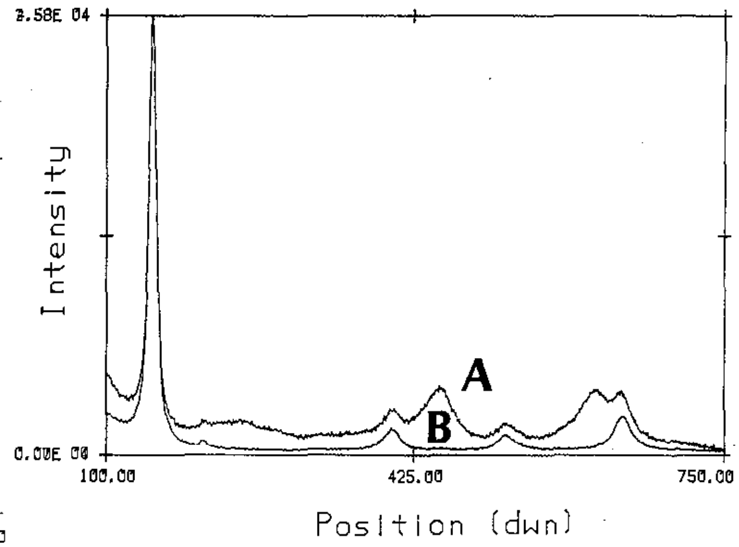


Figure 7 MICRAMATE readily discriminates between crystals of anatase (B) and mixtures of anatase with rutile.

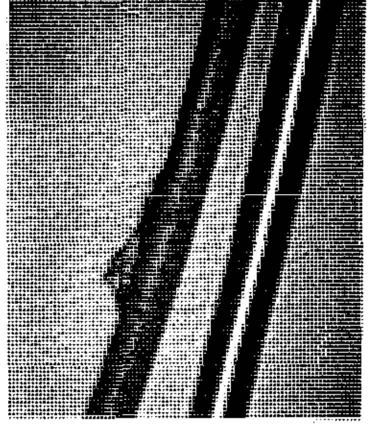


Figure 8 This strand of polyester is marred by a trace of extraneous material that was picked up somewhere between spinneret and loom.

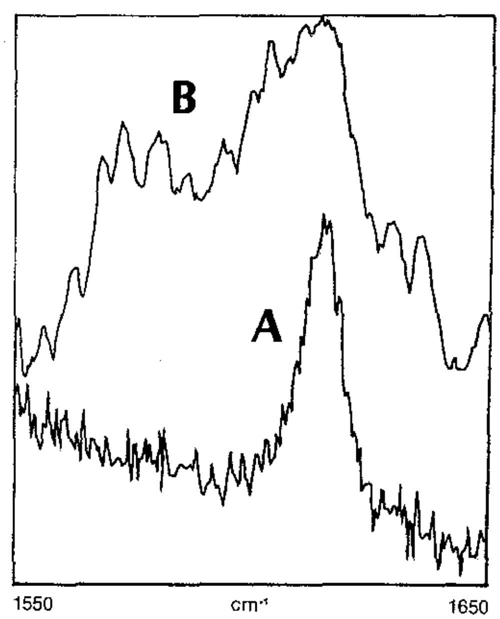


Figure 9 MICRAMATE provided this spectrum of the polyester (A), and when shifted to the contaminant, an incrimating band surfaces at 1578 cm^{-1}.

sphere quickly located the characteristic ring stretch at 1008 cm^{-1} (Fig 4).

Life Mimics Art

As investors scramble for hedges against inflation, dealings in objets d'art can reach frenzied proportions. Consequently, specialized analytical laboratories find themselves pressed into passing judgement over the authenticity of a Ming vase, a parchment scroll, or a Dutch masterpiece — with one rigorous proviso: the object must remain inviolate. If the work is performed on a microscopic scale, however, the unaided eye will detect no difference when a tiny sliver is transported to MICRAMATE's sample stage. Fig 5 presents a spectrum of a paint chip of 30 x 100 μm that was discreetly dislodged from an oil painting. A Reubens it is not, for the spectrum reveals the presence of a synthetic pigment, thalo green, a chlorinated phthalocyanine of recent vintage.

Stored Energy

Lead oxide has a habit of masquerading in a variety of phases. Litharge, the tetragonal guise of PbO, is the most common incarnation and its spectrum is shown in Fig 6A. This particular particle was found huddled among a cluster of other oxide granules, each about 8 μm in diameter. A touch of the microscope's translation stage then brought a neighboring particle under the laser dot to generate its spectrum (Fig 6B). Most likely it is massicot (rhombic PbO) or amorphous Pb_2O_3.

During the normal charge/discharge cycle of a typical storage battery, lead's oxides undergo a similar metamorphosis. The extent and direction of these changes are fundamental to a manufacturer's understanding and quality control of his product.

One Coat — or Two?

A material with a dual personality, titanium dioxide may crystallize in either the primitive (rutile), or body-centered (anatase) form. Because of a superior ability to render a paint, plastic, or paper opaque, rutile is much preferred for pigments. And Raman spectra, which can easily distinguish between the two forms, provide a convenient monitor for quality control.

Dr. Philip Trotter (of Eastman Kodak Research Labs in Rochester, NY) challenged MICRAMATE with TiO_2 crystals approximately 100 μm in diameter. One of these crystals turned out to be almost pure anatase (Fig 7B) while another, nearby, was shown to be a mixture of the two forms (Fig 7A).

Hanging by a Thread

From seat covers to designer blouses, well-woven synthetics begin with strands of thread spun from complex polymers. Unless the material is perfectly uniform, (both chemically and physically) production snarls pose a continuing threat. Fig 8 shows two strands of polyester about 20 μm in diameter. One of them is marred by an extraneous bit of flotsam. When MICRAMATE is alternately focused on this strand and its unidentified blemish, the spectra in Fig 9 result. Note the 1614 cm^{-1} band in Fig 9A that is characteristic of a polyester, while a telltale band appears around 1578 cm^{-1} in Fig 9B and belongs only to the blemish. That information alone might be enough to track down and eliminate the source of the contamination. (Contd. Page 4)

A Piece of the Rock

In our last SPEAKER, we gave you a colorful glimpse of some of the minerals found in Franklin, N.J. At that site, which boasts at least 20 substances found nowhere else on earth, the hunt for new candidates to catalog and christen is eternal. Of course, the easy ones have long since been gleaned, so the search is now concentrated on crystals small enough to have been overlooked by naked eyes. Under MICRAMATE's magnification, a crystal about 25 x 50 μm, isolated from a background matrix of sphalerite, yields the spectrum in Fig 10A. The C-O stretch vibration at 1080 cm^{-1} testifies that a carbonate is lurking inside. Further testimony as to color and x-ray diffraction pattern confirms the conclusion. It's smithsonite, a zinc carbonate. Fig 10B is a spectrum from still another, and even smaller, microscopic crystal measuring about 5 x 20 μm. It was found to be brantite, a calcium arsenate imprisoned in a matrix of calcite and black willemite. Note the symmetric As-O stretch above 800 cm^{-1}

Something to Sneeze at

If you're one of the millions who suffer from hayfever, just the sight of our final example in Fig 11 may be enough to make your eyes water. This 40X magnification of a grain of golden-rod pollen clearly exposes the particle's thorny exterior that is specially adapted to increase stickability to the stigmatic surface of the plant. A glimpse into the interior of this 20-μm subject is supplied by MICRAMATE (Fig 12). The Raman spectrum is predominately due to β-carotene, the pigment responsible for the characteristic color that is the plant's namesake.

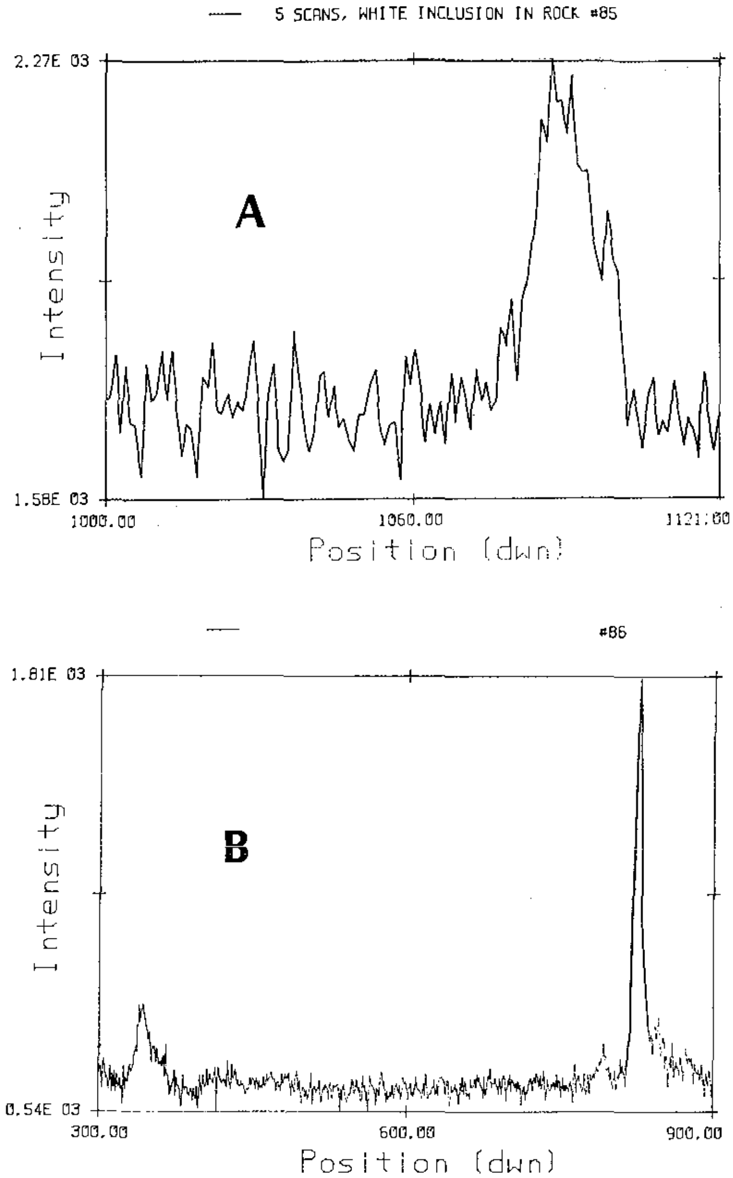


Figure 10 MICRAMATE isolated the spectra of these crystals (smithsonite, A; brantite, B) even while they sat surrounded by matrices of other minerals.

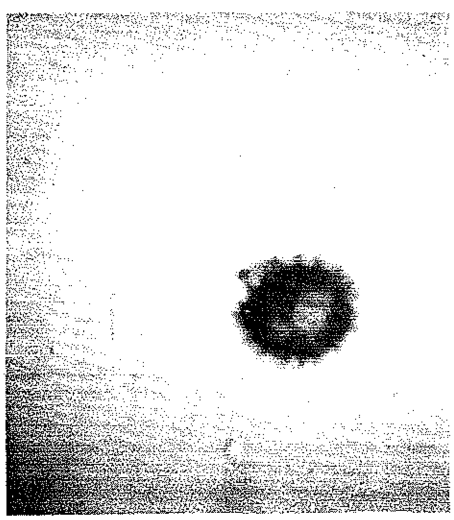


Figure 11 A notorious allergen, this speck of golden-rod pollen measures 20 μm from spine to spine.

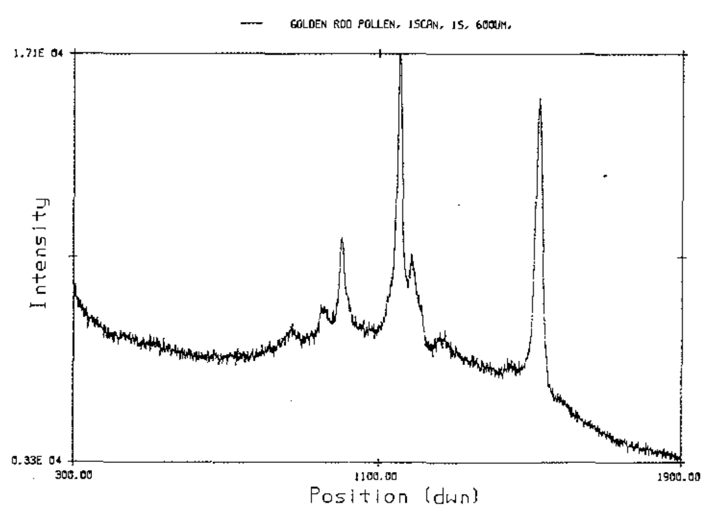
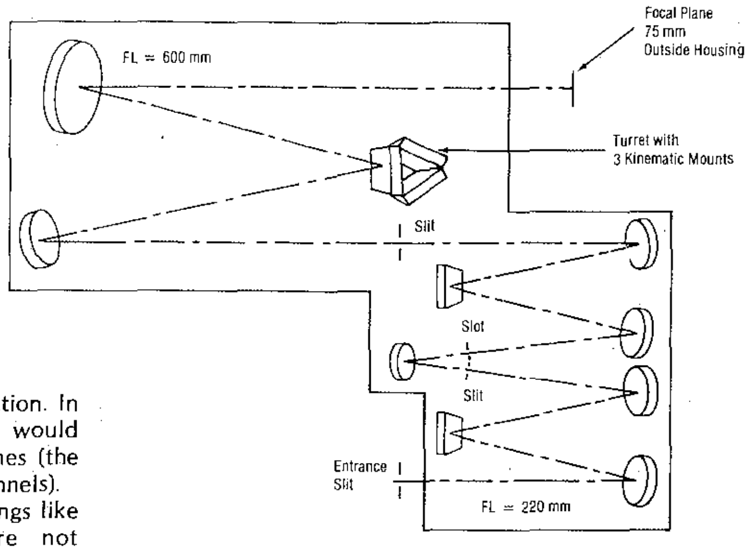


Figure 12 MICRAMATE reveals that the pollen contains a substantial amount of β-carotene

TRIPLEMATE



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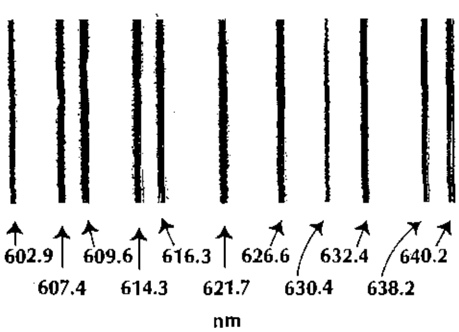
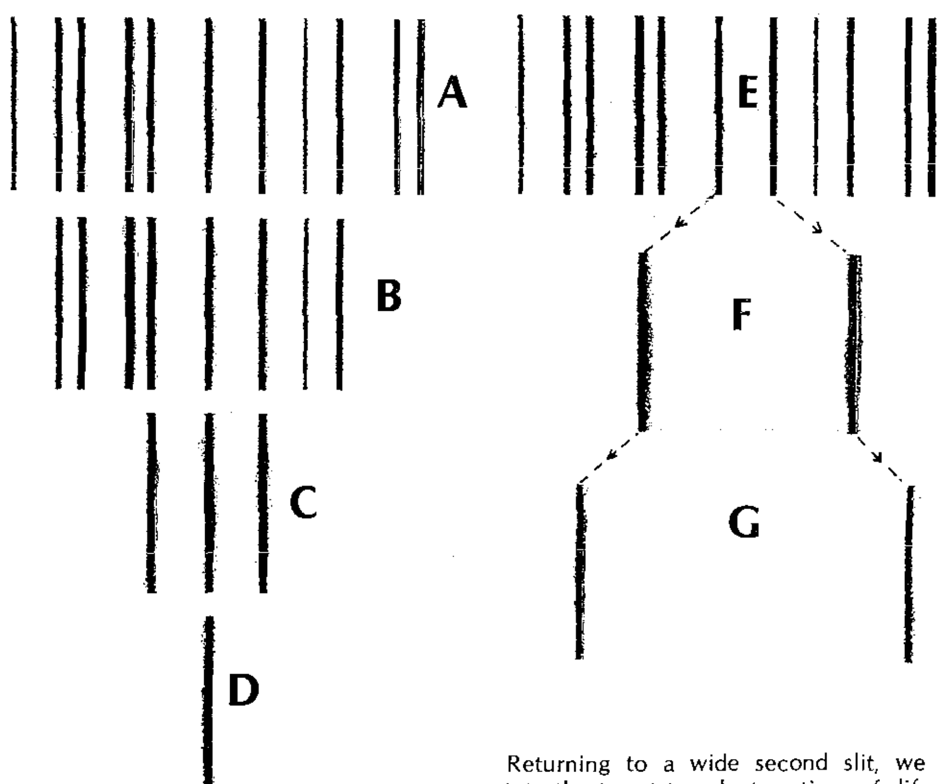
array measures in a single integration. In this ideal case, signal to noise would therefore be improved by 32 times (the square root of the number of channels).

Of course, in the real world things like resolution and detectivity are not equalizable, but rather optimizable, and so TRIPLEMATE does not automatically obsolete all scanning systems. What it does do is provide a three-fold benefit: it saves time, it permits operation with a pulsed laser, and it reduces the chance of sample degradation by shortening exposures to high-powered laser beams.

Once whetted in this manner, the researcher's appetite for our TRIPLEMATE has made this instrument an instant success. Yet to a few others, a clear understanding of its principles has remained an elusive delicacy that could only be tasted after resorting to a full-blown setup complete with laser, illuminator, and multichannel analyzer. Because of this, demonstrations have mostly been confined to our applications lab. Now, however, we think we've devised a solution as portable as it is evocative. Here's how it's done. Instead of a laser-activated sample, a neon lamp is placed at the entrance port, a TV camera at the exit, and we're on our way.



This 40-nm spectral segment is achieved by setting both TRIPLEMATE stages to 621.7 nm and cranking open the center slit of the filter stage to maximize the bandpass. The resolution, established by the entrance slit to the spectrograph stage, is 9cm^{-1}. In this case, the filter stage is fitted with a pair of 600 groove/mm gratings, while a 300 grove/mm grating does the dispersing in the spectrograph.



The second series of photos shows how a decrease in the width of the second slit in the filter stage gradually limits the bandpass of radiation flowing into the spectrograph. The outer lines to both sides of the central wavelength progressively disappear as the bandpass is tightened from 40 to 0.13 nm, A to D. Since the filter stage operates in the subtractive dispersion mode, the light that reaches the spectrograph is homogenized, i.e. undispersed.

Returning to a wide second slit, we rotate the turret to select gratings of different groove densities and, consequently, different dispersions. Notice how the range of frequencies reaching the detector is truncated by switching from a 300 (E) to a 1200 (F) to an 1800 (G) groove/mm grating. At the same time, the line separation has increased by factors of 4 and 6 respectively. Resolution correspondingly improves to 1.5 cm^{-1}. Note particularly how, after all this, the 621.7 and 626.6 nm lines remain centered on the screen — thanks to TRIPLEMATE's proficient microprocessor.

Now, to the advantages inherent in the TRIPLEMATE, think of grafting on a MICRAMATE: wouldn't this be the ULTIMATE in Raman performance?

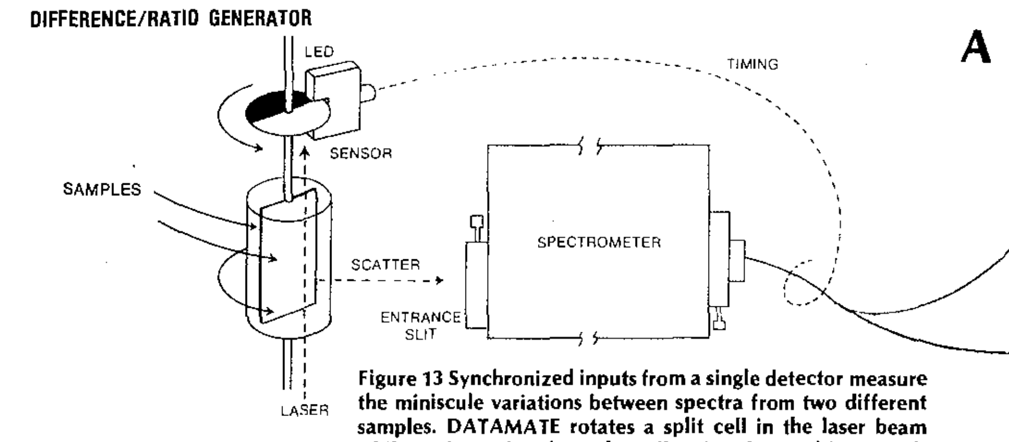


Figure 13 Synchronized inputs from a single detector measure the miniscule variations between spectra from two different samples. DATAMATE rotates a split cell in the laser beam while timing pulses from the cell assign the resulting signals to their respective samples.

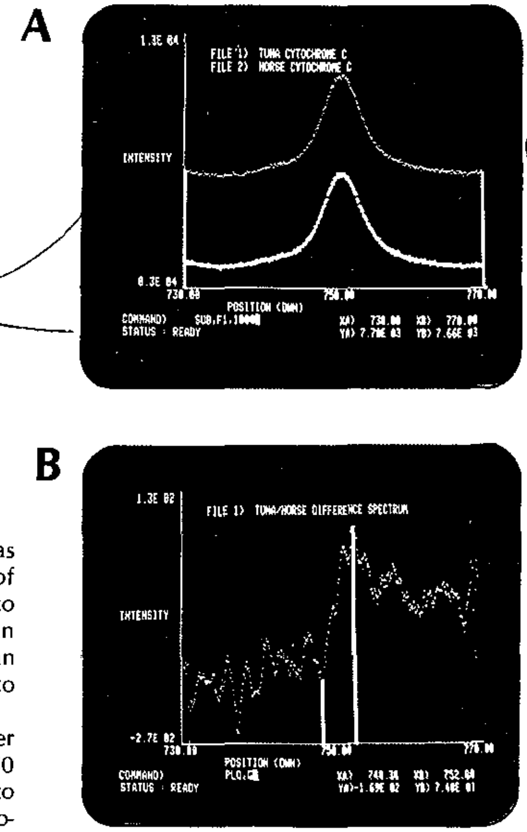


Figure 14 The 1475 Difference/Ratio Generator locates a peak shift of 0.1 cm^{-1}— better than the repeatability of the spectrometer itself.

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To demonstrate the acuity of the 1475, we duplicated some of the work of D. L. Rousseau [4] on cytochrome c, a small hemeprotein that assists electron transport in the respiratory chain. Fig 14 represents spectra from the 750 cm^{-1} bands of tuna and horse-heart cytochrome c (A) as well as the difference between them (B).

Assuming a Lorentzian line shape, the shift is calculated from the equation

$$\Delta\nu = I_d \Gamma / 2.61\ I$$

where I_d is the peak-to-valley intensity of the difference spectrum, I the intensity of the 750 cm^{-1} band, Γ its FWHM, and Δν the frequence shift.

In agreement with Rousseau's results, our data revealed a shift of 0.14 ± 0.04 cm^{-1}, a measurement that is more precise than the specified repeatability of the spectrometer. The paradox is easily resolved. Instead of generating a full spectrum, then repeating it for a second sample, the two spectra are generated on a point-by-point basis in a single scan.

Repeating the experiment with horse-heart cytochrome c in both halves of the split cell produced no observable shift, demonstrating that the instrument was performing properly. Quite clearly, the technique opens up areas of research previously inaccessible to earlier Raman techniques.

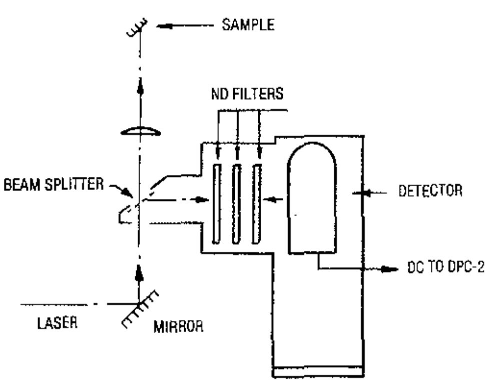

Figure 15 Laser radiation is intercepted by the beam splitter and diverted into the Ratiometer. The resulting output of the device is introduced into the DC channel of a DPC2 photometer or DM1 DATAMATE.

Source Fluctuations

While the intensity of a commercial gas laser may be remarkably stable, that of dye and uv lasers is often subject to change without notice. This flicker can easily smother even a healthy Raman signal, unless a 1464 Ratiometer is there to monitor the output.

Designed around a small photometer sensitive to radiation between 200 to 650 nm, the 1464 reference detector plugs into the DATAMATE or DPC2 Digital Photometer. Placed in the optical path of a SPEX Illuminator (Fig 15), the photometer intercepts about 2% of the laser beam and

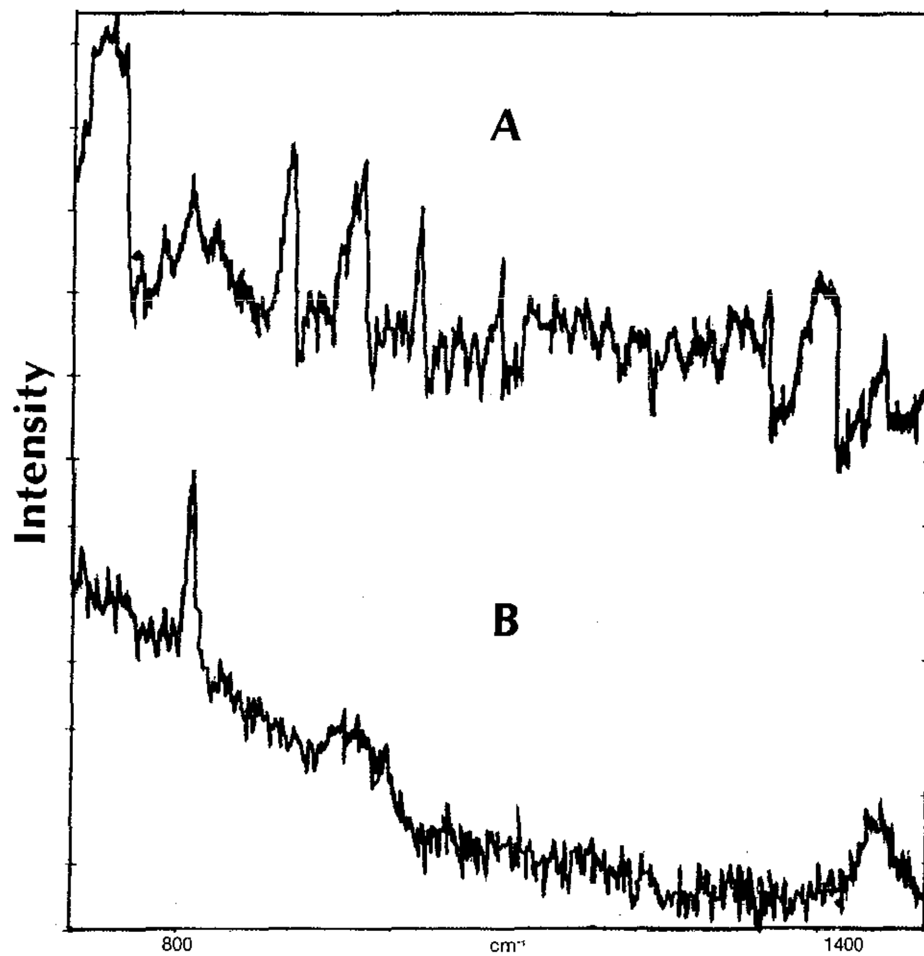

Figure 16 The Raman spectrum of PMMA (A) is indecipherable until the Ratiometer is connected to compensate for fluctuations in the output of the exciting dye laser (B).

converts it to a DC signal which passes to the DPC2 or DM1. There the normal Raman signal from the spectrometer's photomultiplier is scaled by the 1464's output to produce the intensity-compensated signal.

How well the scheme works can be appreciated from Fig 16A, a Raman spectrum of polymethylmethacrylate (PMMA) taken with dye-laser excitation at 602 nm. Laser fluctuations render the spectrum indecipherable. Yet repeating the experiment with the 1464 Ratiometer in place produces the recognizable features of the polymer (B)

Clear Solutions

Because Raman bands are notoriously weak, the search for more efficient sample illuminators rarely loses momentum. The Spex 1443V External Resonating Cavity (ERC), for example, has earned a well-deserved reputation for enhancing the Raman signals from gases by multipassing the laser beam through the sample. Now we've successfully applied the ERC to clear liquids.

Normally, the ERC mounts in the Raman Illuminator so that its mirrors, above and below the gas cell, form an optical cavity, threading the laser beam through a central focus point as many as 13 times. To adapt the scheme to liquids, an ordinary 5-mm-pathlength cuvette replaces the gas cell (Fig 17 B). To inhibit the disrupting effects of refraction on the multi-passed beam, the cuvette is mounted on its side.

As a gauge of the ERC's performance, an 0.1% aqueous solution of sodium sulfate was scanned in three configurations. First, the sample occupied the 1459 Illuminator, though the double-pass and scatter-collection mirrors were blocked (Fig 17A). Next, the two mirrors were uncovered. Finally, the ERC was enlisted.

Peak areas tell the whole story (Fig 18). Note particularly the broad band due to a bending motion of the water molecule at 1650 cm^{-1} and the sharp $SO_4^{=}$ line at 982 cm^{-1}. The collection and double-pass mirrors more than tripled the signal (B), whereas the ERC boosted this by an additional factor of two (A).

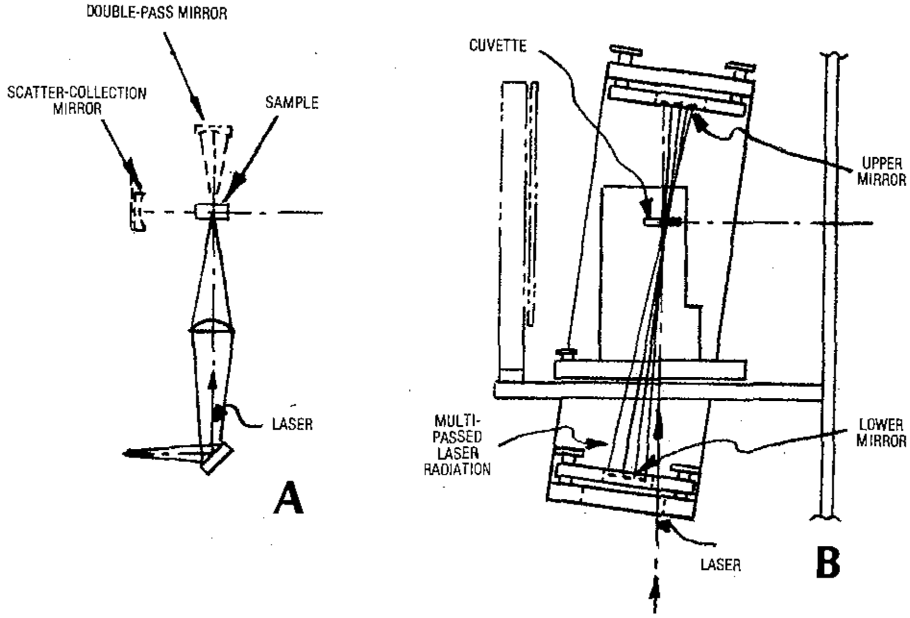

Figure 17 The 1459 Raman Illuminator (A) and the 1443V External Resonating Cavity (B).

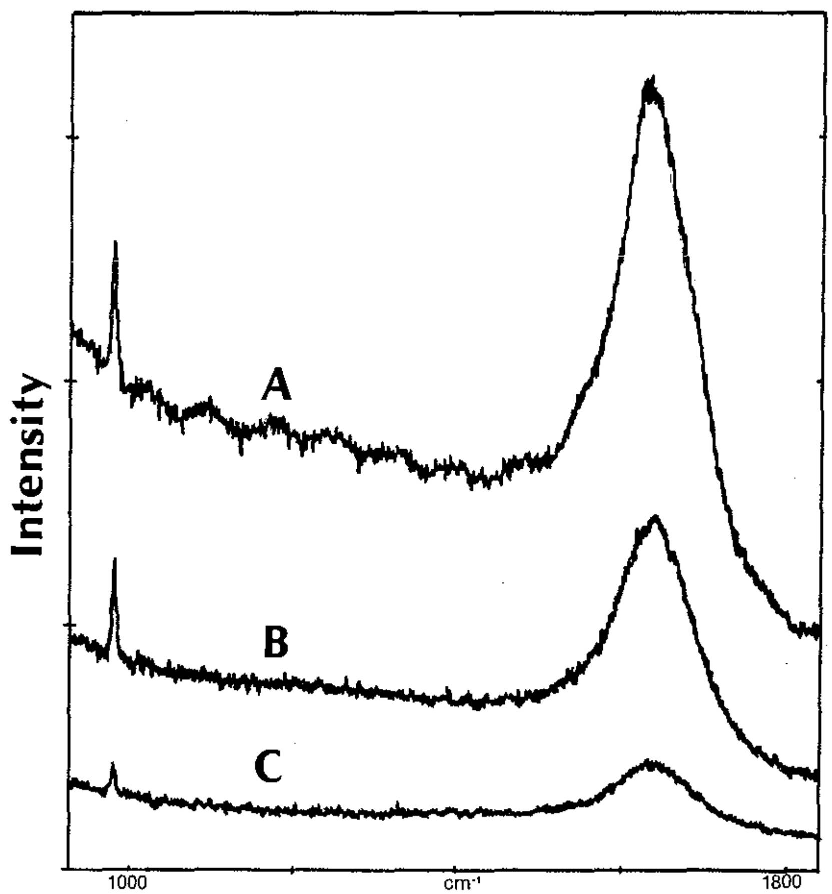

Figure 18 The ERC (A) boosts the Raman signal of this sodium sulfate sample by a factor of six over unaided illumination (C). The addition of a double-pass and scatter-collection mirror (B) provided a three-fold increase.

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