

V

The  
PARTICLE ATLAS  
Edition Two

MCCRONE  
DELLY  
PALENIK



# The PARTICLE ATLAS

## Edition Two

*An encyclopedia of techniques for small particle identification*

### Volume V

### Light Microscopy Atlas and Techniques

Walter C. McCrone  
John Gustav Delly  
Samuel James Palenik



**ann arbor science** PUBLISHERS INC.

P.O. BOX 1425 • ANN ARBOR, MICHIGAN 48106

*Type set by SSPA Typesetting, Inc., Carmel, Indiana*  
*Printed by Thomson-Shore, Dexter, Michigan*  
*Bound by John H. Dekker & Sons, Inc., Grand Rapids, Michigan*

*Editorial Assistance by Gail L. Bohnhoff, Jan Carter and Andrée Fé Coers*

Copyright © 1979 by ANN ARBOR SCIENCE PUBLISHERS, INC.  
P.O. Box 1425, Ann Arbor, Michigan 48106, USA

Library of Congress Catalog Card Number: 72-90881  
International Standard Book Number: 0-250-40195-9

Manufactured in the United States of America

# Contents

## VOLUME V: LIGHT MICROSCOPY ATLAS AND TECHNIQUES

FOREWORD .....	1145	2. Determination of $n_D$ , $n_F$ and $n_C$ ....	1160
I. THE LASER		3. Dispersion Staining in the IR and UV.....	1164
RAMAN MICROPROBE .....	1147	D. Conclusion .....	1165
Robert Z. Muggli		IV. REFRACTIVE INDEX LIQUIDS.....	1166
A. Introduction .....	1147	Walter C. McCrone	
B. General Theory .....	1147	A. Introduction .....	1166
C. History and Background .....	1149	B. Cargille Refractive Index Liquids ....	1166
D. Instrumentation .....	1149	1. Safety and Handling.....	1166
E. Data Processing .....	1151	2. Refractive Index Data.....	1167
F. Problems and Solutions .....	1153	V. MICROCHEMICAL REACTIONS IN PARTICLE IDENTIFICATION.....	1175
II. HOFFMAN		Samuel James Palenik	
MODULATION CONTRAST .....	1155	A. Introduction .....	1175
Walter C. McCrone		B. Analytical Approach .....	1176
A. Introduction .....	1155	C. Selected Techniques and Reactions .....	1178
B. Optics .....	1155	1. Inorganic Compounds .....	1178
C. Apparatus .....	1156	2. Organic Compounds .....	1180
D. Applications .....	1157	3. Insoluble Substances .....	1181
III. DISPERSION STAINING .....	1158	4. Complex Substances .....	1182
Walter C. McCrone		D. Summary .....	1184
A. Introduction .....	1158	VI. COLOR PHOTOMICROGRAPHY FOR THE PARTICLE ATLAS.....	1185
B. New Accessories for Dispersion Staining .....	1158	Walter C. McCrone	
1. Multi-Stop Turret Objective.....	1158	A. First Edition .....	1185
2. Dispersion Staining Colors.....	1158	B. Second Edition, Volume II.....	1185
3. A Three-in-One Dispersion Staining Objective .....	1159	C. Second Edition, Volume V.....	1186
C. Extensions in Dispersion Staining Method.....	1160	VII. ORGANIZATION OF THE PARTICLE ATLAS.....	1187
1. Improved Accuracy in Estimating $\lambda_0$ Values.....	1160		

VIII. PHOTOMICROGRAPHS .....	1189	XI. IDENTIFICATION OF ASBESTOS BY POLARIZED LIGHT MICROSCOPY .....	1369
John Gustav Delly		Walter C. McCrone and	
AND DESCRIPTIONS .....	1189	Ian M. Stewart	
Mark E. Andersen, John Gustav Delly,		A. <i>Introduction</i> .....	1369
Lucy B. McCrone, Walter C. McCrone,		B. <i>Sample Preparation</i> .....	1370
Mark Palenik and Samuel James		1. Bulk Samples .....	1370
Palenik		2. Powder Samples .....	1371
		3. Liquid Samples .....	1371
IX. THE DETERMINATION OF GEOGRAPHICAL ORIGIN OF DUST SAMPLES.....	1347	C. <i>Identification of Asbestos</i> .....	1371
Samuel James Palenik		D. <i>Conclusion</i> .....	1378
A. <i>Introduction</i> .....	1347		
B. <i>Techniques</i> .....	1349	XII. PARTICLE ANALYSIS IN THE CRIME LABORATORY.....	1379
1. Preliminary Inspection		Walter C. McCrone	
of the Sample.....	1349	A. <i>Introduction</i> .....	1379
2. Removal from the Substrate.....	1349	B. <i>Fibers</i> .....	1380
3. Separation of Components.....	1349	1. Classification of Fibers.....	1380
4. Identification of Components.....	1349	2. Fiber Sources .....	1380
5. Detailed Examination .....	1350	3. Characterization of	
6. Correlation of the		Individual Fibers .....	1381
Analytical Data .....	1350	C. <i>Glass (Nonfibrous)</i> .....	1390
7. Postulation of a		1. Refractive Index Methods.....	1390
Hypothetical Environment .....	1350	2. Trace Element Methods.....	1392
8. Description or Determination		D. <i>Paint</i> .....	1393
of Geographical Origin.....	1350	E. <i>Soils</i> .....	1394
C. <i>Analysis and Interpretation</i> .....	1351	F. <i>Drugs</i> .....	1395
1. Minerals .....	1351	1. Identification by Crystal	
2. Biological Components .....	1354	Morphology and Optics.....	1395
3. Combustion Products .....	1359	2. Crystallographic Data	
4. Chemicals and		for Antabuse® .....	1395
Industrial Markers .....	1360	3. Identification by	
5. Miscellaneous Components .....	1361	Microcrystal Tests .....	1396
6. Summary .....	1361	4. Identification by	
		Microthermal Methods .....	1397
X. MICROSCOPICAL EXAMINATION OF AIR POLLUTANTS .....	1362	G. <i>Explosives</i> .....	1398
Samuel James Palenik		H. <i>Gunshot Residues (on hands)</i> .....	1399
A. <i>Introduction</i> .....	1362	I. <i>Safe Insulation</i> .....	1400
B. <i>Scope and Applications</i> .....	1362	J. <i>Pencil Lead</i> .....	1400
1. Sampling .....	1363	K. <i>Conclusion</i> .....	1401
2. Qualitative Analyses .....	1363		
3. Quantitative Methods .....	1366		
4. Reporting .....	1366		
C. <i>Summary and Conclusions</i> .....	1368		

XIII. APPLICATION OF PARTICLE STUDY IN ART AND ARCHAEOLOGY.....	1402
Walter C. McCrone	
<i>A. Introduction</i> .....	1402
<i>B. Polarized Light Microscopy</i> .....	1403
<i>C. Sampling</i> .....	1403
1. Minimum Specifications for a Polarizing Microscope.....	1404
2. Desirable Additional Features for a Polarizing Microscope.....	1404
3. Minimum Specifications for a Tungsten Filament Microscope Illuminator .....	1405

4. Minimum Specifications for Stereobinocular Microscopes .....	1405
5. Desirable Additional Features for Stereobinocular Microscopes...	1405
<i>D. Pigment Identification</i> .....	1406
<i>E. Fiber Identification</i> .....	1413
XIV. LITERATURE SURVEY .....	1414
Gail L. Bohnhoff	
INDEX FOR VOLUMES I-V .....	1427

## Foreword

In 1967 the first edition of *The Particle Atlas* appeared. By 1973 rapid developments in particle identification justified the appearance of a second edition in four volumes. Now, it again seems necessary to bring particle analysis techniques up to date with two additional volumes. Volume V is an update for light microscopy while Volume VI performs the same function for electron optical methods. The number of common particle types is increased from 609 in Volumes II and III to 1022 with Volumes V and VI. Color photomicrographs and descriptions of the additional 412 particulate compositions appear in Volume V, and corresponding SEM pictures appear in Volume VI. Most of the 412 substances also have EDXRA patterns, and many of the smallest particles also have TEM pictures and SAED patterns.

Small particles move freely in the wind, and any microscopist can expect to see almost any composition in any sample. We have, therefore, extended each of the categories (pollens, fibers, minerals, etc.) to include more of the most commonly found substances. At the same time we have concentrated on several areas where small particles are most likely to be of interest: industrial contaminants, environmental particles, crime lab trace evidence and art/archaeology. These areas overlap, of course; for instance, art conservators as well as crime lab evidence examiners are interested in paints and fibers; microscopists studying environmental pollution are as interested in industrial particulate effluent as the industrial microscopist who identifies contaminants in his products.

In addition to many new substances, each of the two new volumes endeavors to bring microscopy and particle identification up to date. The second edition included techniques and instruments not yet invented at the time of the first edition, *e.g.*, ion microprobe analysis and automation of various electron optical instruments. Now, again, in 1979 we have Hoffman modulation contrast and the laser Raman microprobe as at least two new approaches to particle study.

Another important new development is the return of many microscopists to the polarizing microscope.

Electron microscopy, especially the SEM, was expected to bury the light microscope—and it very nearly did just that. Most colleges and universities dropped their courses in light microscopy. If it had not been for a very few schools like Cornell and IIT and for mineralogy departments in general, there would have been no college courses in this important area. It began to become apparent about 1970 that TEM, SEM, EMA *et al.* were, at best, expensive ways to solve particle problems and, at worst, they could not solve such problems. Many schools then began to look back at light microscopy as microscopists themselves found they were beginning more and more to dust off and use their old PLM. The manufacturers of polarizing microscopes helped by introducing new stands with excellent optics and improved usability. The sales of light microscopes (and their cost) increased greatly, and the number of polarizing microscopes sold also steadily increased. Since colleges were slow to recognize the changing emphasis, schools like the McCrone Research Institute took on an increasing responsibility for teaching microscopy. In 1978, for example, 48 intensive 3- to 5-day courses were held by the Institute with a total of nearly 700 students. Most of these students, many from crime labs or art conservation labs, are interested in small particles. The largest number of the students, however, were industrial microscopists interested in contamination problems.

We see, in the future, a continuing increase in the application of light microscopy to particle identification problems. At McCrone Associates eleven microscopists now specialize in PLM, whereas only eight microscopists specialize in the electron optical methods. Light microscopy is far from dead in the problem-solving research labs in the world.

This is perhaps the best place to acknowledge the assistance of those who helped produce these two new volumes of the second edition. Those with primary responsibilities either for light micrographs (John Delly) or electron micrographs (John Brown) or writing (Ian Stewart, John Gavrilovic, Anna Teetsov, Skip

Palenik, Walter McCrone, Donald Brooks and Robert Muggli) are acknowledged either as authors or with by-lines on their sections. Gene Grieger and Rich Shimps were also major contributors of the TEM/SAED illustrations. Others who contributed technically were Lucy McCrone, Ralph Hinch, Mark Andersen, Betty Majewski, Mark Palenik, Debbie Piper,

Chuck Bowen, Olga Kist, Joycelyn Blanchette, Beverly Phillips, Dale Johnson and Jim Reineck. The artwork was done by Anita Douthat; organizing, editing, proofing and typing were handled by Cleopatra Brown, Sylvia Graft and Gail Bohnhoff. Finally, Oppenheimer Goldberg was a tower of strength on whom we all relied during the entire project.



# I. The Laser Raman Microprobe

ROBERT Z. MUGGLI

## A. Introduction

The particle analyst expects and is expected to identify both organic and inorganic particles. There is, however, a big difference between these two categories. For example, there is a finite and relatively small number of inorganic compounds, whereas the number of known organic compounds is very large—and the number of possible organic compounds approaches infinity. Furthermore, essentially all inorganic compounds have been characterized by crystallographic methods, including optical crystallography. Organic compounds, on the other hand, are largely uncharacterized in terms of crystallographic properties. They have been identified in the past by elemental analysis followed by classification reactions and the determination of certain physical parameters such as refractive index, density and molecular weight. More recently, infrared absorption and mass spectroscopy have been applied. The current method for complete unknowns is apparently structure determination by x-ray diffraction, which has become a practical procedure since the development of computers. It is now possible, for example, to determine a complicated crystal structure in a few days' time where once this would have been worth a PhD.

In the above discussion we have assumed that a few grams, or at least milligrams, of material are available for analysis. Unfortunately, many times, the particle analyst has only a single particle that must be identified. Until very recently the identification of organic particles was based on their morphological appearance and characteristics. In other words, if they were biological (*e.g.*, starch grains, cellulose fibers, pollen grains, etc.), identification could be quite certain and rapid. A few organic substances—more important than others and, therefore, more carefully studied—can also

be identified by recrystallizing them according to set procedures so that their microscopical appearance will indicate their composition. This can be used for a relatively small number of explosives, drugs and even fewer extraneous organic compounds. None of these, of course, are general methods for single particles of an organic substance.

Particle analysts have been searching for some time for a general procedure applicable to picogram particles that would enable them to identify any particle that can be characterized with the light microscope. Most of the particles that concern the particle analyst are airborne or fluidborne and, therefore, are usually less than  $10\text{ }\mu\text{m}$  ( $< ca\text{ }1\text{ ng}$ ) in size.

There is no microanalytical method available today that identifies or at least characterizes such samples, except the newly developed laser Raman microprobe. X-ray diffraction requires a single crystal, at least  $50\text{ }\mu\text{m}$  in its longest dimension, and usually prepared from a larger quantity of material. Infrared absorption routinely requires a few micrograms of material, equivalent to a sphere in excess of  $100\text{ }\mu\text{m}$  in diameter. Even with a Fourier transform system, a  $20\text{-}\mu\text{m}$ -diameter particle may be barely adequate. Mass spectroscopy requires samples larger than those generally available. This, again, brings us back to the laser Raman microprobe and its small particle size analyzing capability.

## B. General Theory

Two laboratories, the National Bureau of Standards (NBS) and the University of Lille in France, have been working on the problem of Raman spectroscopy applied to small particles using laser excitation and a light microscope. Recently, both reported that samples of organic or inorganic particles in the size range from

picogram to nanogram<sup>1,2</sup> can be analyzed by this new technique (Figure 1). Previously, it was reported by Freeman and Landon<sup>3</sup> that Raman spectra could be generated from small samples. Unfortunately, their idea of small was equivalent to a particle about one-half millimeter on edge. A series of papers by Rosasco *et al.*<sup>4-8</sup> and Delhay *et al.*<sup>9,10</sup> have now reported the success of these two groups in the development of the laser Raman microprobe.

Those interested in the theoretical details with respect to Raman spectroscopy should refer to the book by Dolish *et al.*<sup>11</sup> The Raman effect was predicted before it was experimentally observed, and Sir C. V. Raman won the 1930 Nobel Prize for his observation of the now-named Raman shift. Dolish *et al.* state that a classic work in the theoretical interpretation of the Raman effect is that of Placzek.<sup>12</sup>

<sup>1</sup> Rosasco, G. J., and E. S. Etz, "The Raman microprobe: a new analytical tool," *Res./Dev.* **28** (6), 20 (1977).

<sup>2</sup> Delhay, M., and P. Dhamelinourt, "Raman microprobe and microscope with laser excitation," *J. Raman Spectrosc.* **3**, 33 (1975).

<sup>3</sup> Freeman, S. K., and D. O. Landon, "Small sample handling in laser Raman spectroscopy," *Anal. Chem.* **41**, 398 (1969).

<sup>4</sup> Etz, E. S., G. J. Rosasco and W. C. Cunningham, *The Chemical Identification of Airborne Particles by Laser Raman Spectroscopy*, Environmental Analysis, G. W. Ewing, Ed., Academic Press, New York, 1977.

<sup>5</sup> Rosasco, G. J., E. S. Etz and W. A. Cassatt, "The analysis of discrete fine particles by Raman spectroscopy," *Applied Spec.* **29** (5), 396 (1975).

<sup>6</sup> Blaha, J. J., G. J. Rosasco and E. S. Etz, "Raman microprobe characterization of residual carbonaceous material associated with urban airborne particulates," *Applied Spec.* **32** (3), 292 (1978).

<sup>7</sup> Blaha, J. J., and G. J. Rosasco, "Raman microprobe spectra of individual microcrystals and fibers of talc, tremolite and related silicate minerals," *Anal. Chem.* **50** (17), 892 (1978).

<sup>8</sup> Etz, E. S., G. J. Rosasco and J. J. Blaha, *Observation of the Raman Effect from Small, Single Particles: Its Use in the Chemical Identification of Airborne Particulates*, Environmental Pollutants, T. Y. Torbarra *et al.*, Eds., Plenum, New York, 1978, p. 413.

<sup>9</sup> Delhay, M., M. Lerberg and D. Landon, "At the molecular level, the MOLE tells us what, where, when," *Ind. Res.* **69** (April 1977).

<sup>10</sup> Bridoux, M., and M. Delhay, *Time-Resolved and Space-Resolved Raman Spectroscopy*, *Advances in Infrared and Raman Spectroscopy*, Vol. II, Chapter 4, R. J. H. Clark and R. E. Hester, Eds., Heyden, New York, 1976, p. 140.

<sup>11</sup> Dolish, F. R., W. G. Fateley and F. F. Bentley, *Characteristic Raman Frequencies of Organic Compounds*, Wiley-Interscience, New York, 1974.

<sup>12</sup> Placzek, G., *Handbuch der Radiologie*, Vol. VI, Part 6, E. Mark, Ed., Akademische Verlagsgesellschaft, Leipzig, 1934, pp. 209-374 [translation: US Atomic Energy Commission UCRL-256-(L), 1962].

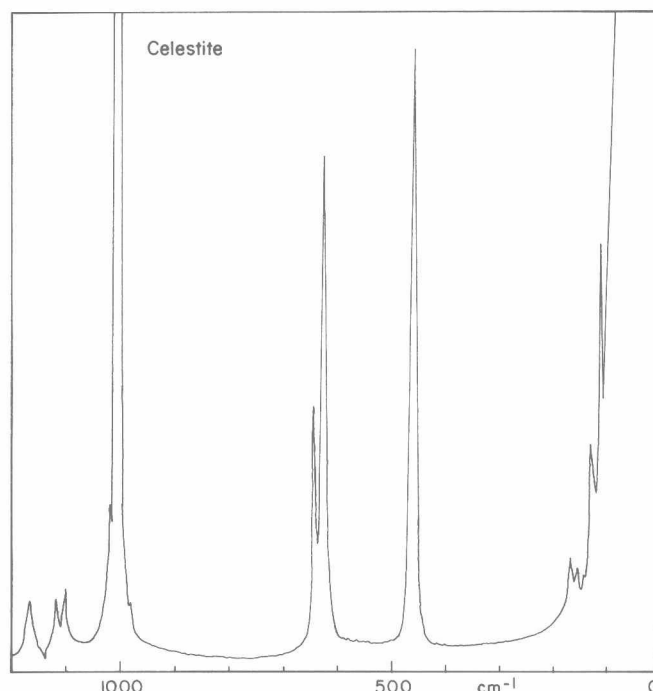


Figure 1. Raman spectrum of single crystal (10  $\mu\text{m}$ ) of celestite ( $\text{SrSO}_4$ ) as determined by MOLE.

The Raman effect is observed as discrete changes in the frequency of light scattered from a sample illuminated by a monochromatic light source. When light impinges on a sample, most of the light is scattered at its original frequency (Rayleigh scattering); however, a few parts per million are scattered with wavelengths of specific frequencies above and below the frequency of the irradiating light. These discrete shifts in frequency are known as Raman shifts.

Both Raman and the infrared spectra are based on molecular bond bending and stretching; however, the selection rules are somewhat different. There are certain bands that are either Raman-active or infrared-active, but not both; the Raman spectrum is, in general, more complex than an infrared spectrum. The infrared spectrum is largely the result of the interaction of the chemical bonds within the molecule. This is true of Raman spectra as well; however, molecular positioning, as in different polymorphic forms of a compound, will affect the Raman spectra much more than it will affect infrared spectra (Figures 2-4). Thus, although more information can be obtained from Raman spectra than from infrared spectra, interpretation becomes more difficult. For example, the various crystal forms of  $\text{SiO}_2$  will give different Raman spectra but very similar infrared spectra.

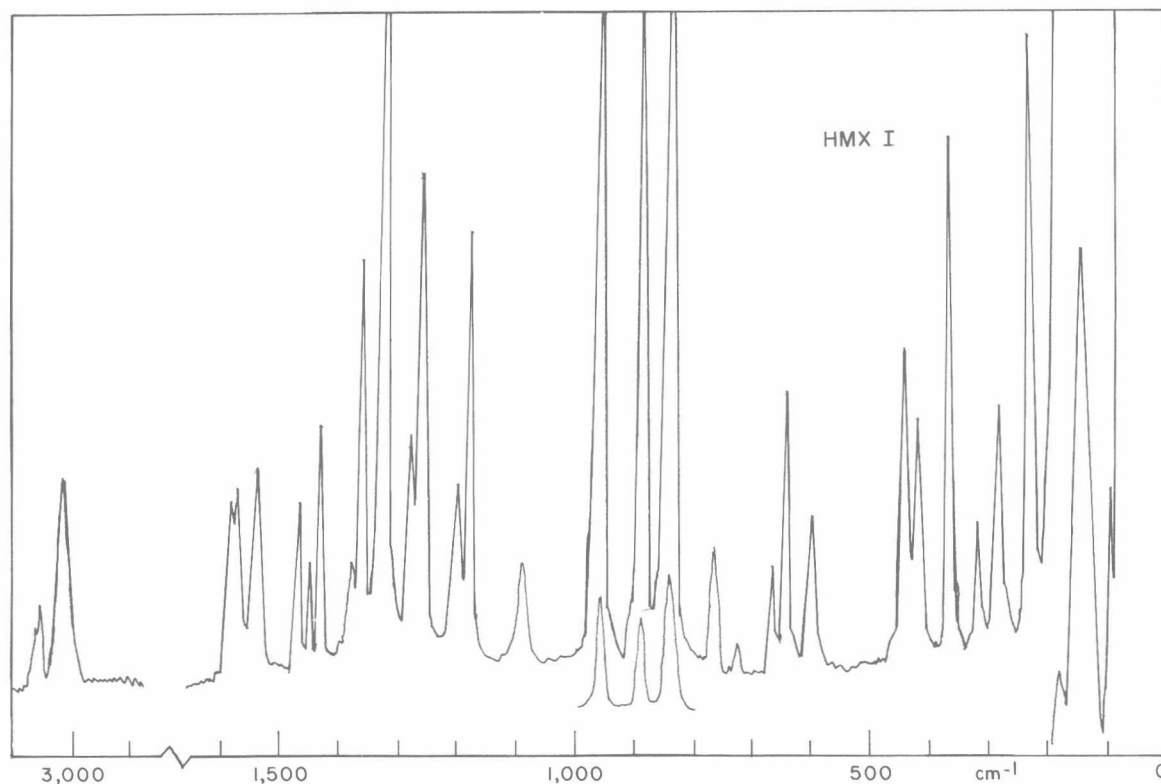


Figure 2. Micro Raman spectrum of a particle of  $\alpha$ -octahydro,1,3,5,7-tetranitro-1,3,5-tetrazocine (HMX I, the room temperature stable phase).

### C. History and Background

Traditionally, the production of Raman spectra has been a rather tedious procedure. In most cases, a mercury vapor lamp was used as the exciting source with photographic film as the detector. A mercury vapor lamp produces several wavelengths of light and, in order to readily interpret a Raman spectrum, a single wavelength of light must be used. Filtering to obtain a single wavelength of light reduces the intensity of the radiation and, consequently, the intensity of the scattered radiation. Inasmuch as the scattered Raman intensity is about  $10^{-6}$  times the intensity of the irradiating energy, the exposures were many hours long. Other difficulties include fluorescence, which can obscure the Raman spectra, and absorption of the exciting radiation by colored samples. In spite of all these difficulties Raman spectroscopy has been used quite extensively since the 1930s. However, with the development of the laser and modern detectors, Raman spectroscopy has finally come into its own. By proper choice of laser wavelength for the incident radiation, the effects of absorption and fluorescence can be

minimized. Modern detectors coupled with double-grating spectrometers allow one to effectively sort and record the very weak Raman scattered radiation. Because of the difficulties encountered in previous recorded spectra, samples have been traditionally large. As modern technology advanced, sample size diminished until, currently, liquid samples with a volume of a few nanoliters can be analyzed with the laser Raman spectrometer.

### D. Instrumentation

Recently, two laser Raman microprobes have appeared in the literature. These two microprobes are: MOLE (Figure 5), based on the designs of Dr. Delhaye and his group<sup>2</sup> in France, and the instrument described by Drs. Rosasco and Etz<sup>1</sup> of the NBS. The two instruments use the same type of spectrometer and detector as well as similar lasers for illumination. The optical path, however, is different in the two instruments. The optics for illuminating the sample and collecting the scattered radiation in MOLE are those of a commercially available microscope. In the NBS

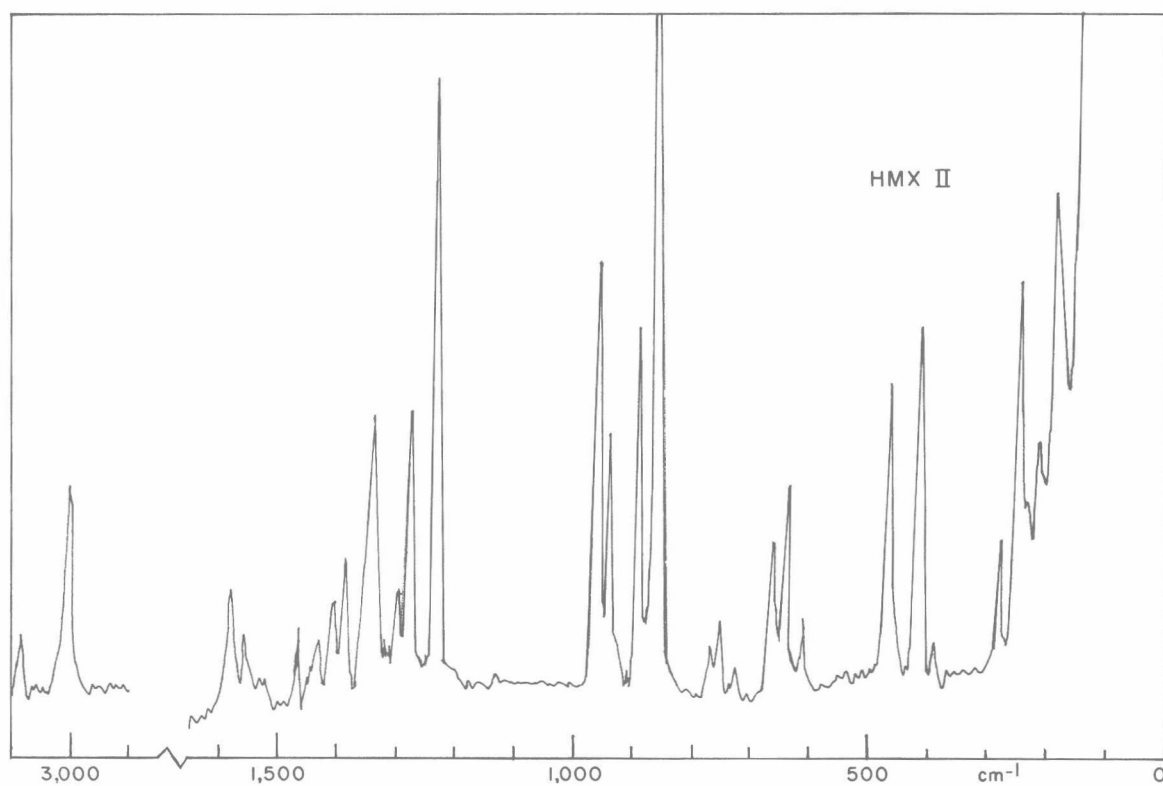


Figure 3. Micro Raman spectrum of a particle of HMX II.

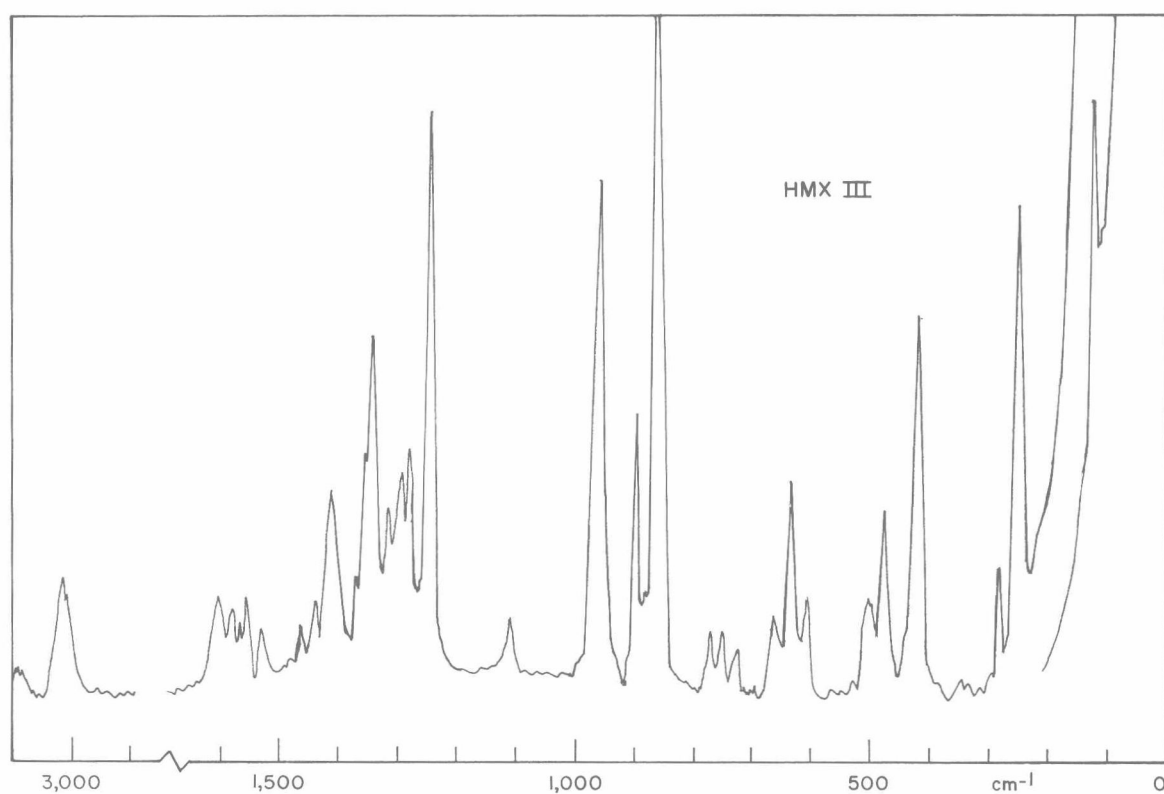


Figure 4. Micro Raman spectrum of a particle of HMX III.

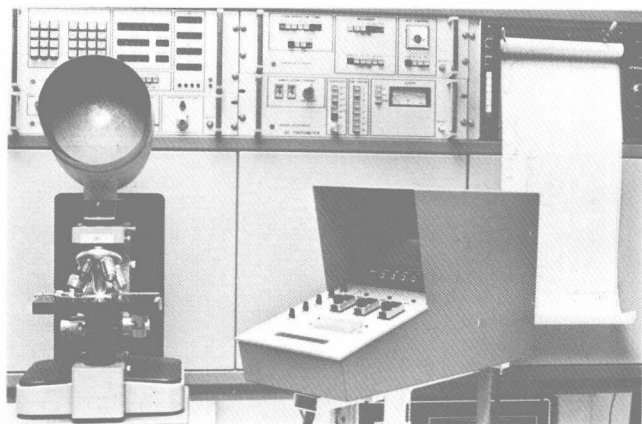


Figure 5. Photograph of MOLE.

instrument a vibration-isolation table and rigid optical bench are used to support the optical components (Figures 6 and 7). MOLE is designed to illuminate the sample either by annular or point illumination with a variety of microscope objectives, whereas the NBS instrument is designed to image the light from the laser onto an inlet pinhole to minimize stray radia-

tion and then focus this light on the sample with a microscope objective. The only nonstandard portion of the NBS instrument is the ellipsoidal collection mirror which was built to Block Engineering specifications. The NA of the collection mirror is approximately 0.86 and, thus, it provides effective collection of the scattered light. A variety of objectives can be used with MOLE, and the collection efficiency of the objectives is generally of the same order of magnitude as that of the ellipsoidal mirror.

MOLE has an additional feature not available on the NBS instrument (Figure 8). Using an image intensifier, TV camera and monitor system, one can select a specific Raman frequency and image those regions of the sample that are associated with that specific frequency (Figures 9–13). This is a very useful technique as it allows one to distinguish a variety of materials in a field of view based on the Raman scattering frequencies of those specific materials. The spatial resolution in MOLE is approximately  $1\text{ }\mu\text{m}$ . Although the NBS instrument lacks this imaging capability, it appears to have slightly better signal-to-noise ratio and hence better sensitivity.

### E. Data Processing

These two microprobes, as last, make the examination of small samples for chemical bond information a reality. It was pointed out above that, prior to the utilization of micro Raman techniques, structural information could be derived by infrared spectroscopy only on much larger samples. Both infrared and Raman spectroscopy yield molecular structure information on samples that cannot be fully identified. That is, the presence of individual features such as carbonyl, amino, ester and olefin linkages can be identified by these techniques. How they are grouped together in the ultimate molecular structure is not precisely defined.

There are tables both for organic as well as inorganic compounds correlating specific spectral bands with particular structural features.<sup>11, 13, 14</sup> In addition, there are some small collections of spectra available with which one can compare spectra on a fingerprint basis. Since relatively few organic compounds have been studied by x-ray diffraction or described crys-

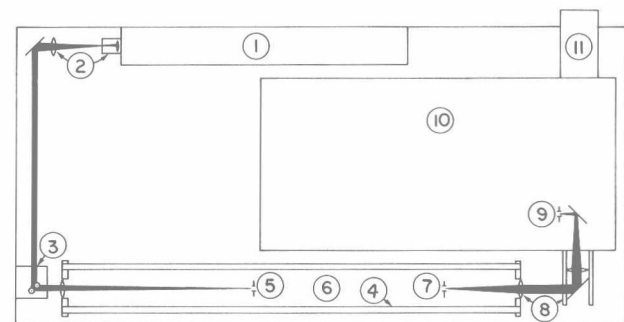


Figure 6. Schematic showing components of NBS Raman microspectrophotometer: 1—laser; 2—beam expander; 3—beam director and Pellin-Broca prism; 4—fore optical rail system; 5—entrance pinhole; 6—focusing, viewing and collection optics (Figure 7 for detail); 7—exit pinhole; 8—monochromator optics; 9—entrance slit; 10—double monochromator; 11—photomultiplier tube.

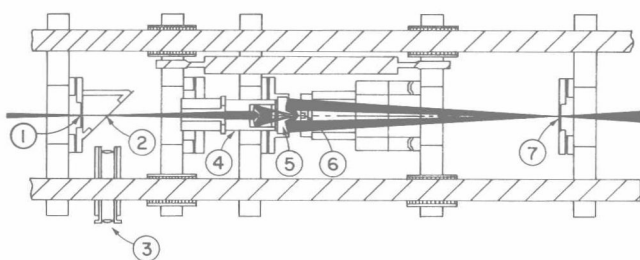


Figure 7. Detail of focusing, viewing and collection optics: 1—entrance pinhole; 2—beam splitter; 3—viewing ocular; 4—reflecting microscope objective; 5—ellipsoidal collection mirror; 6—sample substrate; 7—exit pinhole.

<sup>13</sup> White, W. B., *Structural Interpretation of Lunar and Terrestrial Minerals by Raman Spectroscopy, Infrared and Raman Spectroscopy of Lunar and Terrestrial Minerals*, Chapter 13, C. Karr, Jr., Ed., Academic Press, New York, 1975, p. 299.

<sup>14</sup> Ross, S. D., *Inorganic Infrared and Raman Spectra*, McGraw-Hill, New York, 1972.



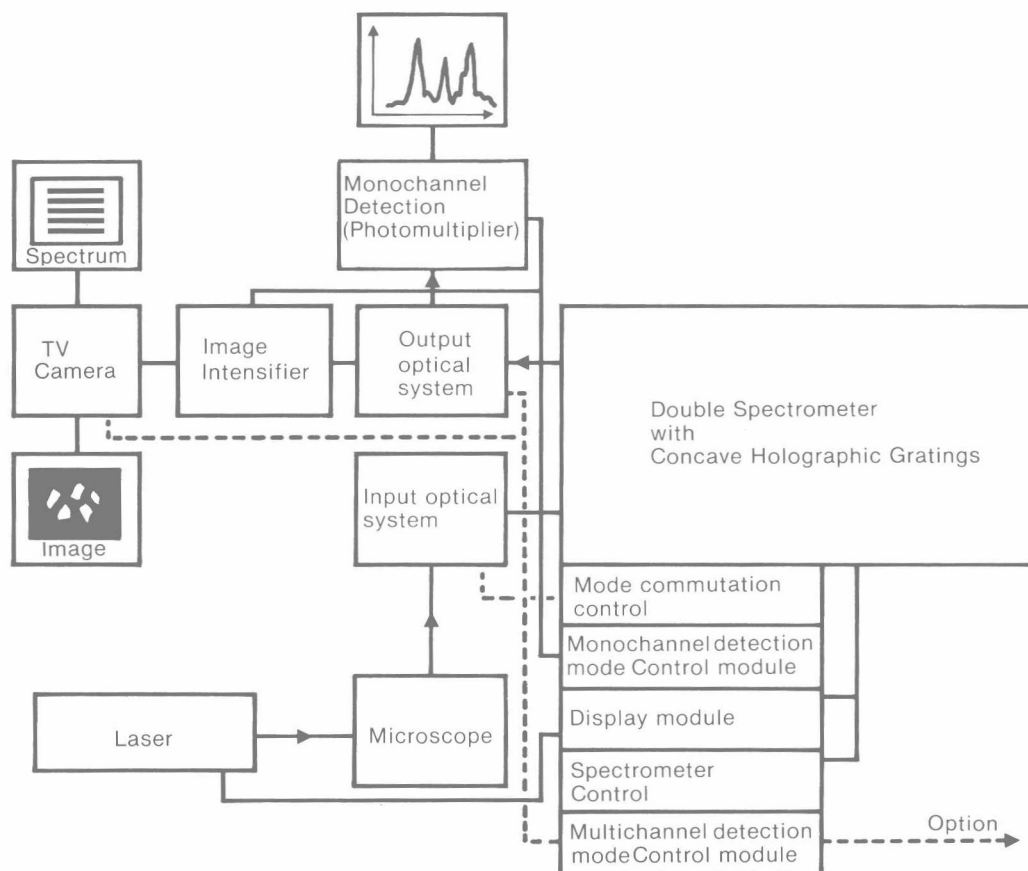


Figure 8. Interrelation of MOLE components.

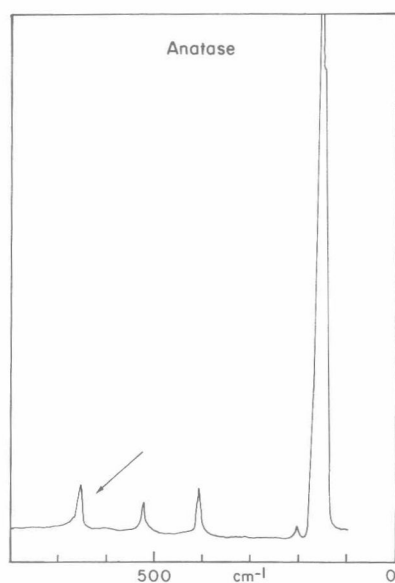
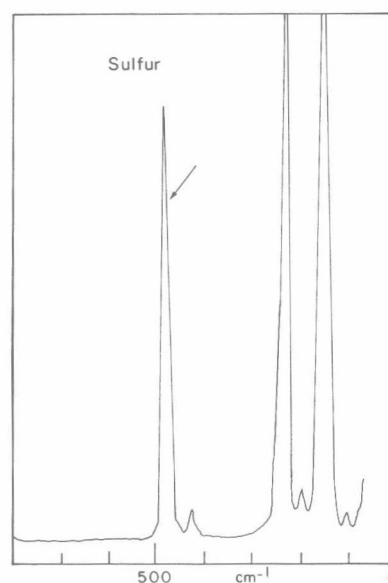
Figure 9. Raman spectrum of anatase ( $\text{TiO}_2$ ); the arrow indicates the imaging frequency in Figure 13.

Figure 10. Raman spectrum of sulfur (S); the arrow indicates the imaging frequency in Figure 12.

tallographically, one often isolates and characterizes small organic particles only to be unable to proceed further in their identification. Chemical spot tests such as those reported by Feigl are, of course, of some value, but with the introduction of the laser Raman

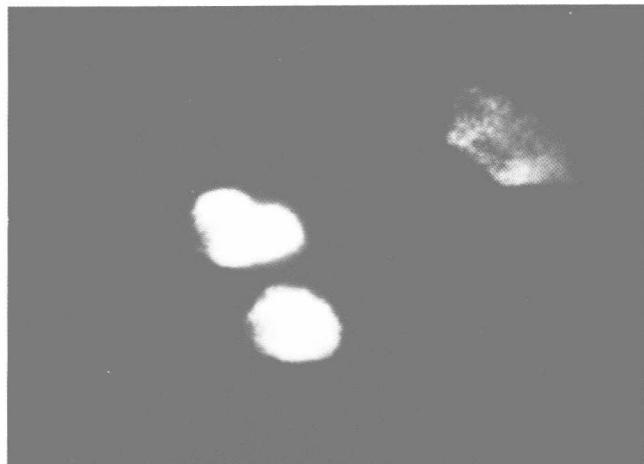


Figure 11. Photomicrograph of a mixture of anatase and sulfur.

microprobe we are now able to "fingerprint" specific organic materials. The spectra may also yield considerable information about the chemical structure of that material even when the published fingerprint is not available for comparison.

#### F. Problems and Solutions

One of the major problems with MOLE is the energy absorbed by the sample, which may lead to burnup or evaporation. However, successful analysis of organic materials, such as methacrylate polymers which readily depolymerize with heat, demonstrates that this technique can be utilized with many somewhat thermally or photolytically unstable samples. The difficulties listed above are still problems, in particular the interference due to fluorescence or absorption of light. The effect of the color of the sample can be minimized by irradiating with light of a wavelength not absorbed. As has been pointed out, the Raman lines appear with specific shifts in frequencies between the irradiation wavelength and the observed Raman bands, and hence Raman spectra are the same no matter what wavelength of light is used to irradiate the sample. In addition, it is often possible, if the sample is dyed or pigmented, to separate the pigment or dye from some small portion of the sample under



Figure 12. MOLE image of sulfur utilizing  $474\text{ cm}^{-1}$  Raman line for image formation.

study and determine the Raman spectrum from that portion of the sample. For example, if a droplet of solvent is placed on a black polystyrene polymer and the substrate on which the polymer rests is tilted slightly, some reasonably clear polymer solution can

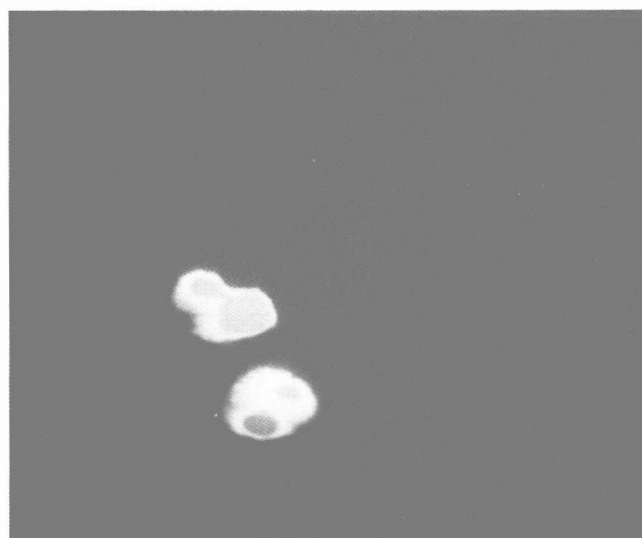


Figure 13. MOLE image of anatase utilizing  $640\text{ cm}^{-1}$  Raman line for image formation.

emerge from the original sample and, upon evaporation of the solvent, the more or less unpigmented polymer area can be studied. Not only has the sample been partially separated from its pigment, but it is in intimate thermal contact with the substrate and, there-

fore, not as susceptible to degradation by the energy of the illuminating beam.

Because of the large energy input to the sample, contact to the substrate is quite important. When the samples are fairly large, one can spread out the beam and, thus, the flux on any area of the sample is decreased, thereby minimizing degradation. However, as the sample size is decreased, the flux on the sample must be increased in order to obtain the Raman spectrum within a few minutes. If the sample is in intimate contact with a substrate having good heat conductivity, degradation or loss of sample is diminished. One way, as pointed out above, is to dissolve the sample and let it re-form on the substrate. A sapphire substrate is often used with samples particularly sensitive to heat because its good heat conductivity minimizes sample destruction. When the sample is less thermally sensitive, it can often be analyzed directly on a nonfluorescent glass slide. Other substrates found to be useful are lithium fluoride and sodium chloride. Not only do these have essentially no Raman spectra, but they are readily obtainable materials. A disadvantage of the sapphire substrate is that its strong spectral lines can completely mask lines from the sample.

The irradiance levels, the heating and the minimum particle sizes that generally can be analyzed with Raman spectroscopy have been discussed elsewhere.<sup>5</sup> It has been calculated that, if one fully illuminates a 1- $\mu\text{m}$  particle with the necessary laser power (approximately 100 mW under typical conditions), one should then obtain Raman spectra. In fact, this has been demonstrated by the production of spectra from particles in that size range.

The spectra of polystyrene particles in the 4 to 9  $\mu\text{m}$  size range and of a polyvinylchloride particle about 3  $\mu\text{m}$  in diameter have been published.<sup>5</sup> This shows that usable spectra can be obtained from samples of a size approaching the lower limit of resolution of the light microscope. By extending Raman spectroscopy to the analysis of microscopic particles, it has been assumed that all physical phenomena underlying the Raman scattering of light from samples of macroscopic dimensions apply equally to Raman scattering from particles approaching dimensions comparable to the wavelength of the exciting radiation. Fortunately, work to date has shown that the spectra of

small particles and bulk samples are essentially the same.<sup>5</sup>

The effects of the presence of thermally conductive coatings such as aluminum, copper and gold have been studied.<sup>4</sup> It has been shown that when particles are coated with these materials, laser-induced heating of particles is generally increased. Such films are apparently thick enough to absorb heat but not thick enough to be good conductors. Transparent films on particles have been used to aid in the improvement of the thermal contact of the particle with the substrate and to prevent contamination of the surface of the particle with, for example, water vapor. It has also been shown that a thin film of nonfluorescing hydrocarbon oil can successfully prevent the absorption of water by hygroscopic materials. In addition, when the material from which one hopes to produce Raman spectra is not water-soluble, we have shown that the input intensity of the laser energy can be increased up to 5 or 10 times if the sample is immersed in water. Evidently, when a sample is affixed to a substrate such as sapphire, the rate of thermal conduction through the sample is slow enough so that heating can still be observed. However, when the sample is immersed in water, the point at which the laser beam impinges on the sample is in contact with liquid water. Water is a good absorber of heat and yields poor Raman spectra; therefore, it minimizes thermal degradation of a water-insoluble substance while not producing interfering Raman lines.

The preparation of samples for micro Raman spectroscopy can be quite simple. The sample is placed on a substrate and analyzed. No vacuum chambers, sample coatings or special sectioning are necessary. If fluorescence is present, it can usually be minimized by irradiating the sample for a time; normally, this will cause the fluorescence to "decay." It is often possible to find micro areas of a sample which show little or no fluorescence, a technique that is not possible with a standard Raman spectrometer.

Thus, micro laser Raman microprobes can be used to identify previously unidentifiable particles. In addition, the imaging capabilities of MOLE allow determinations to be made of the size and distribution of specific components of a composite mixture.

## II. Hoffman Modulation Contrast

WALTER C. McCrone

### A. Introduction

A common problem in microscopy is object contrast. If the substage aperture has to be closed in order to see the object, the resolving power is severely reduced. Particle microscopists often attempt to maintain high resolution and at the same time enhance contrast by mounting their samples in Aroclor®. This excellent mountant has a conveniently high refractive index, usually sufficiently different from the particles to permit use of a full condenser aperture and, hence, obtain maximum resolution.

Often, however, particle microscopists resort to a contrast enhancement technique such as darkfield, Zernike phase contrast (Volume I, p. 39) or Nomarski differential interference contrast, DIC, (Volume I, p. 35). Such methods increase the sensitivity of refractive index measurement besides making all particles more distinctly visible. On occasion, the mounting medium is not a matter of choice, and particle contrast cannot then be enhanced by choosing mountants of very different refractive index. Particles on a membrane filter, for example, are difficult to see unless the filter structure is cleared by immersing the filter and particles in a liquid matching the filter in refractive index. This is seldom the best liquid in which to study the particles. Counting asbestos fibers, usually chrysotile with indices near 1.55, is not easily done when the clearing liquid has to have an index of 1.51. Usually, in this situation, microscopists have turned to phase contrast to make the fibers easier to size and count.

### B. Optics

A new contrast enhancement method with advantages over other methods has been developed by

Hoffman and Gross<sup>15-19</sup> since Volumes I-IV of *The Particle Atlas* appeared in 1973. Now termed Hoffman modulation contrast (HMC), this new system can be adapted easily to most microscopes. It enhances contrast without the halos characteristic of phase contrast and without apparent loss of resolution. The image, like DIC, is "three-dimensional" and permits optical sectioning of an object with little or no interference from detail above or below best focus. Also, like DIC, the effect is directional, and both detect phase gradients by converting opposite gradients into lighter or darker images compared to the background. The means by which this is accomplished is very different, however, for the two procedures. Nomarski, in his DIC system, uses a modified Wollaston prism to produce two sheared rays of light, separated by only about 1  $\mu\text{m}$ , passing through the object space. Recombining these two rays with a second Wollaston prism, after passage through the object, introduces interference with resulting darker borders on one side of the object and lighter borders on the other side. This produces a shadow effect and the three-dimensional appearance.

Hoffman and Gross, on the other hand, accomplish similar results by quite different optical principles. Figure 14 shows schematically the components required to convert a brightfield microscope for modulation contrast. A system of apertures below the substage condenser is conjugate with a matching system

<sup>15</sup> Hoffman, R., and L. Gross, *Nature* **254**, 586 (1975).

<sup>16</sup> Hoffman, R., and L. Gross, *Appl. Optics* **14**, 1169 (1975).

<sup>17</sup> Hoffman, R., and L. Gross, *J. Micros.* **110**, 205 (1977).

<sup>18</sup> Hoffman, R., and L. Gross, Chapter in *Microstructural Science*, Vol. 4, E. W. Filer *et al.*, Eds., American Elsevier, New York, 1977, p. 287.

<sup>19</sup> Hoffman, R., *Amer. Lab.* (April, 1978).