The Case for Polarized Light Microscopy*

Walter C. McCrone, McCrone Research Institute

I was one told by a Nobel Laureate in Chemistry that light microscopy was simply a service foundation. By this he meant to class the microscope with computers, gas chromatographs, infrared spectrophotometers, x-ray diffractometers, mass spectrometers, etc. With all due respect to this gentleman and to these other instruments, there is a vital difference between the polarized light microscopes (PLM) and each of these instruments. First, a trained microscopist requires far more training than a qualified operator of, and interpreter of data from these other instruments. Second, there is considerably more basic physical and chemical information observable and measurable with PLM. Third and most important, PLM involves direct examination and study of otherwise invisible tiny objects or object detail.

All other microanalytical instruments yield indirect evidence in the form of a chart recording or digital readout of some single characteristic of the sample. If I want to tell someone how to recognize a specific person as he or she emerges from the 747 jetway, I am best served with a photograph. My eyes instantly observe many visual characteristics of a person never before seen in a photograph and instant recognition of such person is reasonably certain.

Our eyes and brain are designed to give us a set of size, shape, and color information allowing us to recognize at sight thousands of objects: a pencil, banana, spectacles, TV monitor, monarch butterfly, elephant, Boeing 747, etc. There is no more effective analytical tool that the eye. There is, however, a lower size limit for objects to be identified by our unaided eyes. We do not resolve tiny objects smaller that about 0.1 mm.

Here is where the light microscope comes to our aid. Now, we can see, characterize, and identify at sight thousands of objects, some smaller than 1.0 μm: bacteria, micrometeorites, dust as paper fibers, oil soot, quartz, calcite, ragweed pollen, or chrysotile asbestos, contaminant particles in paper, polymer film, parental solutions, etc. All of these and thousands of other particles become familiar to microscopists as they gain experience[1].

A microscopist also identifies aspects of shape, size, and color that indicate purity, decomposition, crystal form, precipitated versus ground, even polymorphic form or hydrate versus anhydrate. Many substances appear in different guises. Iron oxide as Fe_2O_3 may be rust, mineral hematite, yellow ochre, red ochre, burnt sienna, etc.: Fe_3O_4 may be the mineral magnetite, sintered spheres in power plant flyash, or spalled flakes of millscale from steel or wrought iron, etc. Even cellulose fibers or animal hairs are identified down to species by microscopy. DNA may do the same thing given a database but the microscope does the job much faster.

It is often important in dating art objects to determine which of several forms of a given chemical substance, e.g., pigments and media, is present. Cinnabar (HgS) the mineral was used as a pigment by caveman artists 30,000 years ago. Dry process HgS was first used by artists ~ 700 A.D. whereas wet process HgS was first prepared late in the 18th century and its presence in a painting would eliminate Rembrandt, Titian, Leonardo, and all other pre-1970 artists as the artist.

Other microanalytical instruments yield elemental analyses but not the molecular composition. SEM images only the surface of any sample. PLM , on the other hand, includes such a wealth of physical measurements and observations that molecular identification is assured. In addition, the physical condition and purity of that substance is also apparent. This leads to the process of formulation (e.g., precipitation, grinding, weathering, corrosion, etc.), and often, geographical sourcing, or individualization of, e.g., glass as from a window, optical glass, automobile headlight, bottle, etc.; or a human hair from a prime suspect in a murder case. PLM is always a good screening test to eliminate many samples that would otherwise require more expensive and lengthy microanalytical procedures. It is ideal for "needle in a haystack" analysis; again for chrysotile, cocaine, modern pigments in a supposed Rembrandt, contaminants in paper, polymers, IC chips, etc.

It is certainly important for a university to support a strong basic research program and to be world leaders in one or more areas of research; ideally to develop programs, staff, and students leading to world recognition in the form of awards, especially Nobel prizes. The world in all of its facets of activity depends on the results of fundamental research for progress. At the same time, there is need for training of chemists who will be faced in the real world with technical problems. What are the black (green, red, blue) specks in this shortening, parenteral drug, solution, paper coating, paint layer, glass, polymer film, etc., etc.? Why is this dye batch off-color, this explosive unusually sensitive, this IIC chip failure rate so high? Or this compound suddenly showing a melting point 20°C above specs, this powdered drug caking, this emulsion breaking, and this polypropylene fiber showing such low breaking strength? What is clogging the filter in the astronauts space-walking suit? What are the tiny particles on the returning space shuttle windows or the source of this black powder on the outside window sills of the White House? Here is the clothing, worn by a suspect in the Oklahoma bombing - where does he live and what is his occupation? Why is this chocolate less smooth and creamy in one's mouth than Hershey's? Why is our drug product, scopolamine, less effective than that made by Ciba-Geigy? Could this painting have been painted by Giorgione[2]? Was this linen cloth the shroud of Christ[3]? Was this map showing part of North America drawn before Columbus came to these shores[4]? This is a very short sample of problems most of which I have faced and solved using the polarized light microscope. Many I could not have solved without that venerable tool; some could not be solved using any other instrument or techniques and all would have taken much longer if finally successful.

I have had several very interesting microscopical projects on explosives. As part of the harnessing of universities to aid in the war effort (This was 1942), Cornell was a center for work with explosives under the aegis of George Kistiakowski and the National Defense Research Committee of the Office of Scientific Research and Development. The U.S. was gearing up to produce very large quantities of a new high explosive, far more powerful than TNT. I discovered that this explosive (RDX) contained a by-product (HMX) that threatened to scuttle the whole program because it possessed a readily obtained polymorph that was much too sensitive to impact or friction. I then discovered the HMX had four different polymorphic forms, one of which (Form I) was safe to produce[5]. Working closely with a pilot plant in Kingsport, Tennessee, I was able to convince the engineers that their lives and that of many others depended on slower recrystallization of the final RDX/HMX product in order to ensure formation only of Form I. I am happy to report that Holston Ordinance in Kingsport produced many millions of pounds of RDX/HMX during the war and since without incident.

During that same period, I also observed a unique behavior of TNT as it crystallizes from the melt on a microscope slide[6]. Two groups of crystals growing toward each other but oriented at right angles to each other meet in steps along a grain boundary and both then proceed to grow into and through the other - even though they are identical phases. After partially recovering from astonishment, I decided the growing TNT crystals must be highly strained but anisotropically so, in effect, the ends of the crystals were more stable thermodynamically than the sides. This intergrowth was a mechanism for release of the stress. Later I heard that the fabricators of the atom bomb (the U-235 one, not the plutonium type) were having a problem with the spherical TNT outer shell. The cast TNT, after precision machining to shape, changed dimensions after a few weeks by a few tenths of a percent. This opened up cracks between the carefully machined pentagonal blocks of TNT, an intolerable situation. This, I decided, was due to my observed annealing process that takes place slowly at room temperature. I was able to tell them by way of Dr. Kistiakowsky that if they annealed the cast TNT for 4-5 hours at ca. 70°C before machining into blocks, no further change in dimensions would occur.

This and other projects kept me at Cornell for two years of "Post-Doc" before I had to look for an organization willing to hire a "chemical microscopist." Another project that illustrates the versatility of the light microscope is described briefly here taken from the more detailed coverage in IV.C. of "Fusion Methods." Waxes in lubricating oils crystallize on cooling and often cause solidification of automobile crankcase oil in cold winter weather[7]. I found, microscopically, that

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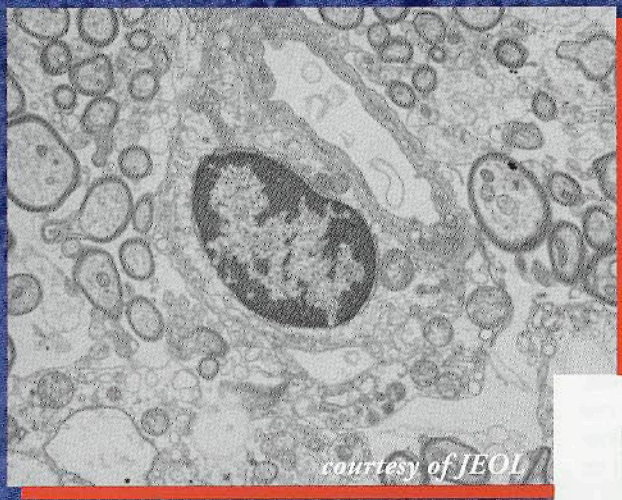

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Polarized Light Microscopy, Continued:

the wax crystallizes as long, interlocking ribbons. These ribbons have two low and one high refractive index indicating parallel orientation of the paraffin-chain molecules making up the wax crystals. The polarized light microscope was used to determine the orientation of the straight-chain paraffin wax molecules by noting that the high index, corresponding to the lengthwise direction of the molecules, was perpendicular to the flat surface of the ribbons. This suggested that very small quantities of an appropriate additive to the oil might be absorbed along the edges of the ribbons to slow the crystallization rates on those faces and force the ribbons to grow shorter and thicker to change the crystal shape. Decyl to dodecyl methacrylates were chosen as additives to the oil. The paraffinic chains were absorbed on the paraffinic side faces of the ribbons. There they interfered with the normal growth of the ribbons. A few tenths of a percent of these additives were sufficient to make the wax crystals more equant in shape and to flow with the still mobile oil at temperatures of -20° F. Without the polarized light microscope, these pour-point depressants could have been invented only by Edisonian research - trying hundreds of substances blindly until, hopefully, one would work.

Microscopy is a way of thinking as one observes directly the way matter behaves under the influence of time, temperature changes, solvents, physical or chemical action, etc. I regard non-microscopical tools and techniques as "10-foot pole" methods in contrast to "being there when and where the action is" when the microscope is used.

We are presently working on research projects that promise to add two more techniques and instruments to the list of continuing microscopical developments. One is an automated "Abbe" refractometer (invented by Don Bloss of VPI) capable of automatically and simultaneously measuring the three refractive indices of a single tiny crystal in less than two minutes. This would take 1-3 hours manually and is no longer done by anyone, possibly excepting only me. I hope mineralogists and others will resume use of PLM and this instrument for the identification of minerals rather than going directly to the electron microprobes, etc. Another instrument we have developed to the prototype stage is an electron microprobe fitted to a polarized light microscope stage so that polarized light microscopy and electron dispersive spectroscopy (EDS) can be done without remounting tiny subnanogram particles. We hope to commercialize both of these soon.

The McCrone Research Institute has taught more than 20,000 industrial, governmental and university chemists the use of the polarized light microscope since 1960. With a staff of about 15 in Chicago, we teach nearly 1,000 students in nearly 100 different courses every year. Most of our students come from industry - scientists who had never heard of PLM in college and now find they need it. We also teach courses on-site at laboratories anywhere in the world. Half of the 24 students in my 1995 Cornell summer class in PLM came from the chemistry department and half from seven other Cornell departments. This illustrates the diversity of areas in which microscopy is needed - essentially any area of materials or biological science.

Important advances in other fields await application to light microscopy. This has happened many times in the past as new materials such as better optical glasses are developed and along with better understanding of diffraction, fluorescence, and interference of light beams. Imaging techniques, especially video-enhanced contrast procedures have already given us excellent light microscope images of object detail 10X smaller than the theoretical diffraction limit (20 nm). Microscopists interested in improved light microscope images should read carefully monthly trade magazines such as *R&D, Microscopy Today,* and *The Microscope* for possible new ways of improving microscope images.

Is there funding for new projects in microscopy? Yes(!) as shown by our own experience; most recently with the National Institute of Justice which supports our current project to combine an electron microprobe with a polarized light microscope in an instrument affordable by the nation's crime labs. The experience of Gary Valaskovic is gaining the support of NSF for his NSOM Ph.D. research at Cornell. We have also had support in the past for a variety of projects. Some for the development of dispersion staining as a rapid and more dependable means of characterizing and identifying low percentages of tiny particles of asbestos, explosives, pigments, street drugs, etc. Support came also for the development of ways to study complex phase diagrams involving physical chemistry: polymorphism, and composition diagrams involving hydrates, solvates, and other addition compound systems especially those also involving polymorphism.

Is there a place for teaching microscopy in the universities? Yes! Our annual courses at Cornell, New York University, Illinois Institute of Technology and University of Illinois (Chicago) have been consistently well subscribed. Awareness of the scope of microscopical applications in science and technology will help to continue the parade of accomplishments of this threatened but still eminently useful research tool. ■

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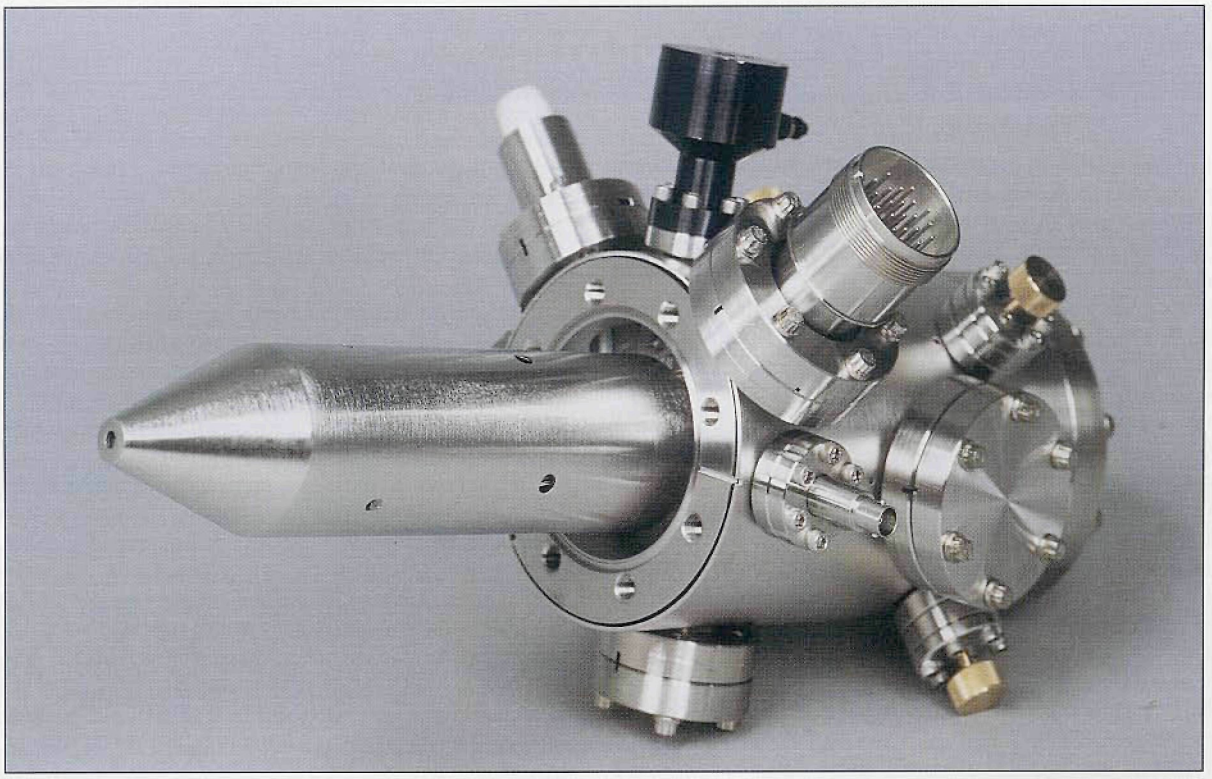
* *An abbreviated version of an extended paper that appeared in American Laboratory, June 1996.*



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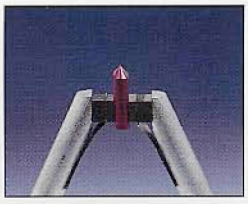

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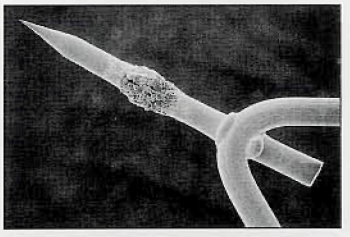

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