By Walter C. McCrone

Using the microscope...for the nonmicroscopist

LEARNING TO USE the polarized light microscope (PLM) to do all of the things that it can do is a daunting project, comparable to learning how to play the piano or to speak a foreign language. Both pursuits involve considerable hands-on practice. This is not an attractive proposition for today's scientists who much prefer automation for all laboratory operations.

Every day we see and recognize, at sight, thousands of objects, large and small. Everything from the letters and words on this page to particular buildings, a full moon, or the Orion constellation. There are, however, very many important objects too small to see and recognize with the unaided eye. It seems reasonable that if these tiny objects were enlarged microscopically we would learn to recognize many thousands of them. This, indeed, is the basis for microscopy: a 50-µm pollen grain enlarged 350 times will be the size of a dime. The usual PLM enlarges even 7.5-µm (0.0003 in.) objects to dime-size. Nearly all of the objects studied by most microscopists are larger than 7.5 µm—about the size of one of our red blood cells.

We learn to recognize additional objects every day. On seeing something we do not recognize we say, "What's that?" and someone answers, "That's a BCC 025 Avanti (San Jose, CA) lap-top computer." The next time you see one, your brain tells you, "That's a lap-top computer—looks like an Avanti." So, day by day, we add to a vast store of images of objects we learned to identify by word of mouth, a picture, or even a description. There are many published visual aids[1-6] to help the microscopist learn to identify microscopic objects: fibers, pollens, minerals, and metals. To recognize most of these is no more difficult than it is to recognize and remember a new building in town or a new acquaintance.

Many of the tiny objects that we cannot see without a microscope are very distinctive and once seen and identified are easily remembered and recognized on sight. Some similarly distinctive microscopic objects are stellate hairs (*Figure 1*) that grow on tree leaves, diatoms (*Figure 2*), radiolarians, barbed fibers from a feather, airborne particles of Spanish moss, kapok fibers, starch grains, pine pollen (*Figure 3*), mineral wool (*Figure 4*), and wood fibers. No further measurements or tests are required to be certain of their identity.

The microscope is, of course, more than a means for identifying tiny objects. We may wish to look at a known macroscopic object for micro features. A Lincoln penny may have been doubly struck, causing modification of the surface features not visible without magnification. Your finger shows a tiny mark and it

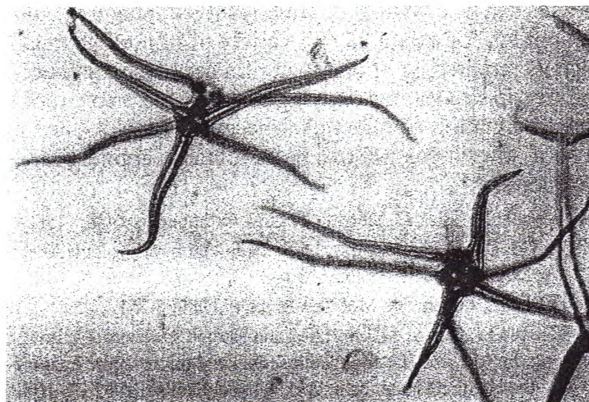

Figure 1 *Maple leaf stellate hairs, 200x.*

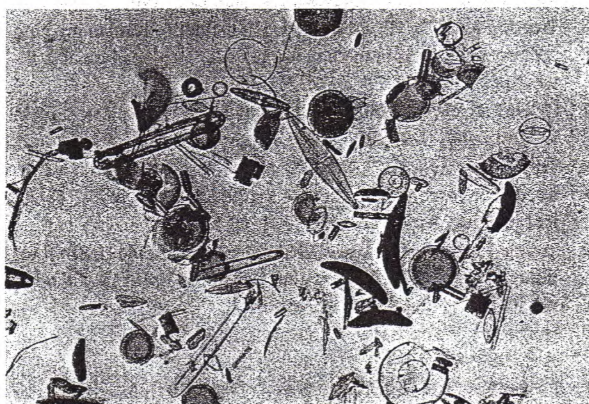

Figure 2 *Diatoms, 80x.*

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hurts when rubbed; the microscope shows a tiny sliver of wood or metal. The signature on a painting may be on top of an earlier varnish layer; this suggests addition of the signature long after the artist named was dead.

There are two different basic microscope types: the stereomicroscope and the standard mono-objective compound microscope. The stereo is a low-magnification instrument, usually less than 100×, but it yields an erect 3-D image with lots of room for large objects: your hand, for example, to look for that sliver. The other, more often depicted microscope has a single objective and is capable of much higher magnification, e.g., 2500×.

In general, the stereo is not a tool for identifying micro objects, i.e., small objects, but not micro. One can identify mites, pine pollen, cotton fibers, mineral wool or glass wool, hair, and spray paint spheres, i.e., anything distinctive in shape or color and larger than about 10 μm (0.0004 in.).

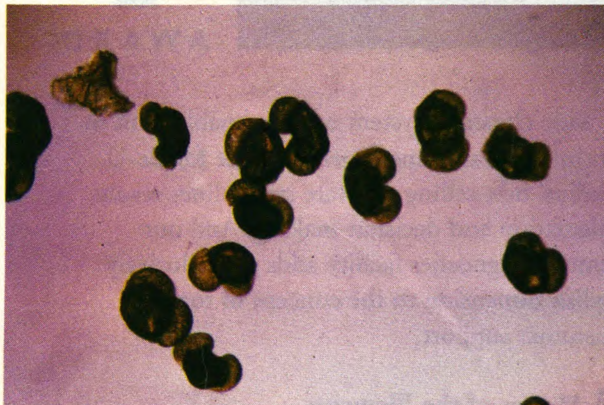

Figure 3 *Pine pollen, 60x.*

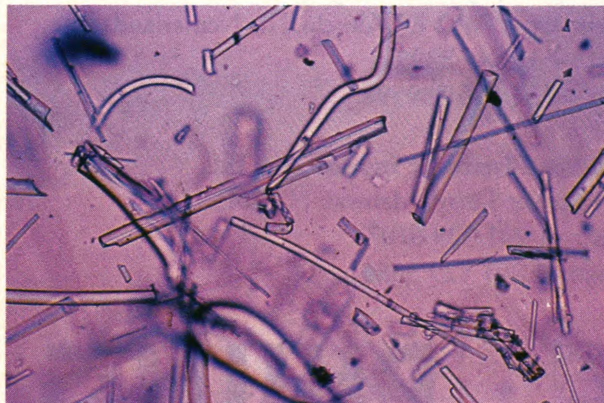

Figure 4 *Mineral wool, 200x.*

The stereo is more suitable for studying the condition of some materials: brush marks on a painting; scratches and other marks on an MS-67 Morgan dollar; corrosion of contacts on an integrated circuit chip; nature of the deterioration of a paint layer in a Rembrandt painting; sharpness of a needle, razor blade, or scalpel; and presence of contaminants in almost anything including paper, paint layers, foods, parenteral solutions, and polymer films.

The stereomicroscope is appropriate for sampling, by removing portions from a mixture or matrix. Examples are removal of tiny, colored paint-layer samples from an old master, different color corrosion products from a metal, specific components of a crime laboratory soil sample, or that sliver from your finger. Many of the objects examined with the mono-objective microscope are prepared first using the stereo. If, for example, I wish to determine what fibers are present in this page of paper, I cut with a razor a tiny submillimeter strip a few millimeters long along the bottom edge. I put this in a drop of water on a microscope slide, then, holding it down with one needle, I lightly scrape individual fibers from the surface while viewing the operation with the stereo. As soon as I have a hundred or more fibers dispersed in the water drop, I add a coverglass and transfer it to the PLM. Actually, this microscope usually has four objectives on a rotating turret, but only one at a time is used.

There are two important types of mono-objective microscopes: biological and the polarized light version, the PLM. The latter is more complex and harder to learn to use but, once learned, is a very powerful research tool.

The PLM is used to make visible the characteristic features of micro objects. The microscopist can then interpret those observations in terms of chemical identification, quality, purity, and relationship to the problem involved. A neophyte may visualize everything a trained microscopist visualizes but not the significance of each observation. What he/she can recognize and interpret, however, still may often solve a problem or help to suggest another approach that would lead to a solution.

Anyone looking at a particulate sample can say, "This sample is almost 100% fibers." With a bit more experience: "These fibers are natural and not man-made." At successive stages of experience the microscopist adds, "These fibers are wood fibers," "These fibers are softwood," "....chemically pulped rather than mechanically," and, eventually "....Loblolly Pine." "These are also pine pollen grains," and finally, "This product would be dangerous to use as intrawall building insulation. It is flammable and has resulted in spontaneous combustion." Even a neophyte could reach all but the final conclusion by looking at fiber and pollen photomicrographs in the CD-ROM edition of the Particle Atlas[1] or other literature sources.[2-6]

be repeated ad infinitum with any other solvents and the same original substance (as small as 1–2 μm). In 1 hr the solubility of any subnanogram particle can be determined in 20–30 different solvents and the sample is still there.

5) The solubility of polymer films is determined a little differently. A cm-square of polymer on a microscope slide is placed on the microscope stage and brought into focus with a 5× objective. Place a tiny droplet of a possible solvent on the top surface of the film. This is best obtained by picking a drop from a larger drop on a dropper tip by passing a clean metal needle tip through the drop of solvent and placing the tiny droplet on the film by contact. It is best (but not necessary) to watch the solvent action (or lack thereof) with crossed polars after rotating the stage and film to the brightest color. Any solvent action will change this color. The drop should be small enough to evaporate in less than 30 sec, and a second liquid can be used on the same film. In the absence of crossed polarizers, the polymer film with the drop of solvent can be viewed with the stereomicroscope. A needle tip exploring the drop of solvent on the polymer surface will discover softened polymer if it is soluble. After the solvent evaporates, surface examination of the spot will show no change (insoluble), or semifluid viscous liquid, or a dried crater-like spot, all indicating solubility.

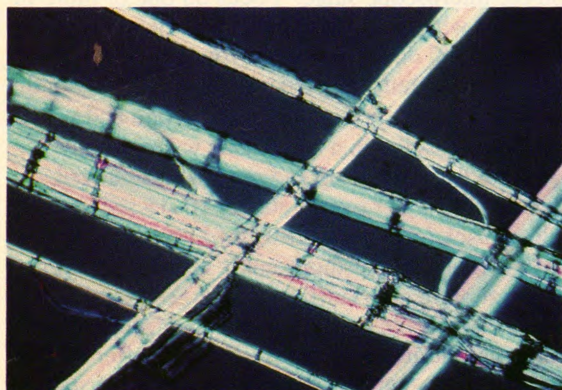

Figure 8 *Flax fibers showing nodes characteristic of bast fibers, 200x, crossed polars.*

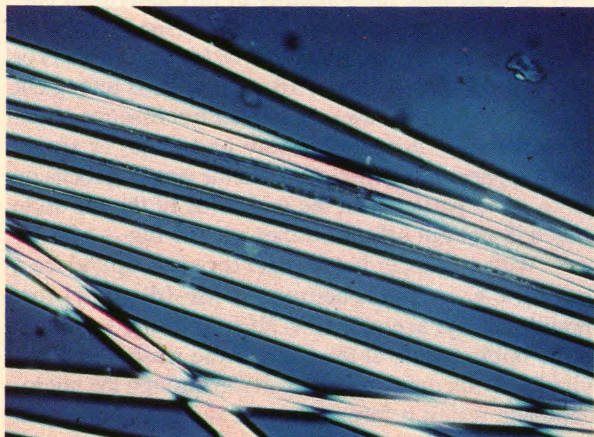

Figure 9 *Polyethylene fibers with smooth, circular cross sections, 200x, crossed polars.*

6) Is this fiber natural or man-made? Natural fibers are relatively short and usually tapered on, at least, one end. They always have some variations in cross section (diameter and shape) along their length: cotton is twisted, hairs have scales, and bast fibers have markings (nodes) shaped like I, V, or X along the length (*Figure 8*). Silk, although long and smooth like man-made fibers, has crossover marks from other silk fiber impressions on underlying fibers during formation of the cocoon by the silkworm.

Man-made fibers, on the other hand, can be miles long with little or no change along the length (*Figure 9*). They may show many very tiny black dots—these are TiO_2, a delustering pigment, that are only present in man-made fibers, although they are not always present.

7) During a canoe trip up a rock-walled canyon in Southern France, I noticed many black streaks and strips of black stain up the rock face and removed a sample with a knife blade and placed it in a small plastic bag. Once back in my laboratory, examination at 100× surprisingly showed no black material, only green alga and a red-brown fungal substance. Being a mixture of two complementary colors, they together absorbed all white light and therefore appeared to be black.

8) Forensic investigators often have the problem of comparing particles from a crime scene with particles from a suspect's clothing. It is not essential, although perhaps somewhat less satisfying, that the particles be identified. If they are distinctive and relatively rare, it is convincing to be able to say the two samples are identical in all microscopical features and very likely to have a common origin. In one such case, very unusual gray particles found on a rape-murder suspect matched identical particles from the rape scene.

9) Are these two compounds identical? Put a poppy-seed quantity of one component (A) under a coverslip on a slide and heat it. If it melts, add a two-poppy-seed quantity of the other component (B) at the edge of the coverslip and melt it (it runs by capillarity under the coverslip into contact with A). Heat a bit more to melt half of A, then place it on the microscope stage and watch A grow toward B. If the A crystals grow into and through B melt without stopping, then A = B. If A≠ B, then A will stop, B will nucleate independently, and grow to the zone of that which will usually crystallize as a eutectic. In *Figure 10*, TNT stopped growing from the left and a new compound grew along the TNT crystal-front followed by another compound growing from the right. It soon stopped, leaving a band of eutectic melt between the two crystal fronts; this melt slowly crystal-

lized as a eutectic. This is a typical addition-compound formation in which TNT and mononitronaphthalene (MNN) are melted in contact with each other. Surely, such a series of steps during cooling proves beyond a doubt that TNT and MNN are two different compounds, and it took only 3 min.

10) Elemental analyses for anions or cations are readily carried out by PLM. Submicrogram quantities of the test substance and the chemical reagent are placed in two separate droplets close together on a microscope slide. A narrow glass tip is used to draw a bridge of solution from one drop to the other. Diffusion and convection slowly mix the two solutions and soon lead to precipitation of reaction products characteristic of the ion with that reagent. *Figure 11* shows drusy crosses characteristic of reaction between zinc ions and potassium mercuric thiocyanate. The test result after mixing a drop containing a sodium salt and the reagent, uranyl acetate, is shown in *Figure 12*.

A very unusual and unexpected microchemical test is shown in *Figure 13*. A fallout particle from the first H-bomb test at Bikini (1951) was placed in a drop of water on a microscope slide and immediately dissolved with concurrent production of typical rhomb-shaped crystals of calcite. I thought I was only testing for the solubility of H-bomb fallout in water. A little thought gave this answer: Bikini atoll is coral, alias $CaCO_3$; on vaporization, the calcium ions form CaO, which crystallizes in the atmosphere on cooling as cubic CaO crystals. These are the fallout particles; they are water soluble and react with CO_2 in the water to form the insoluble calcite crystals. All water drops, I learned for the first time, exist as a solution of carbonic acid, H_2CO_3.

I hope that one, at least, of these examples may induce a few venturesome bench scientists to give the microscope a chance to solve some of their problems. The more one uses the microscope the more useful it

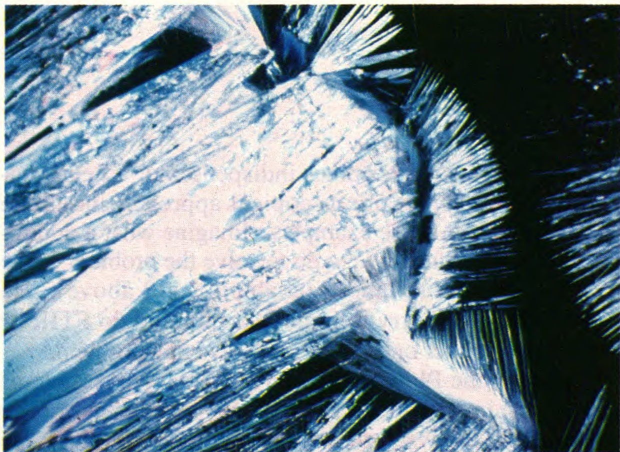

Figure 10 *A mixed fusion of TNT and mononitronaphthalene showing an addition compound and eutectic proving nonidentity, 200x, crossed polars.*

Figure 11 *Microchemical test for Zn showing drusy crosses with potassium mercuric thiocyanate, 160x.*

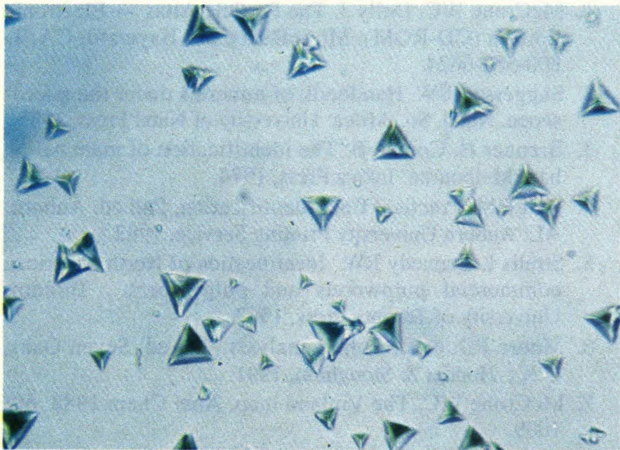

Figure 12 *Crystals characteristic of a test for sodium using uranyl acetate as a reagent, 160x.*

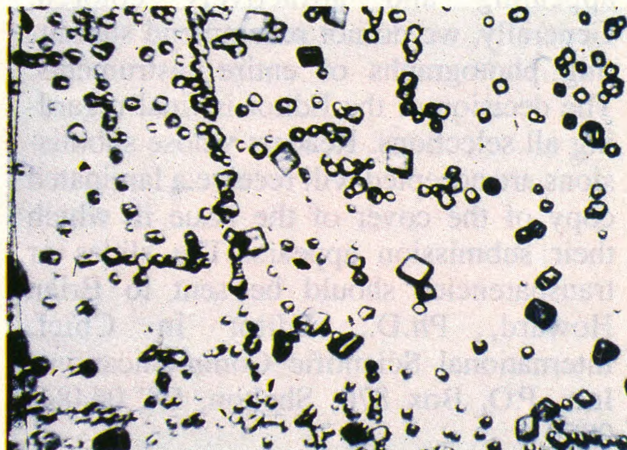

Figure 13 *A particle of H-bomb fallout in a water drop precipitated these calcite crystals, 200x.*

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becomes; it soon becomes indispensable. The advantage of the direct microscopical approach should be apparent from these examples. Imagine what a nonmicroscopist would have to do to solve the problem of the red-spotted drug tablets (example no. 2 above). He would probably try to remove a spot and do FTIR or HPLC. Neither one is likely to solve this problem.

Often, the PLM (in trained hands) solves problems (almost) impossible to solve by any other means. The Vinland Map was proven to be a modern map by the very thin yellow ink line along and under the black ink lines and by the presence of pigment titanium oxide (available only in the 20th century) in a form only identifiable microscopically. This pigment differs from any other TiO_2 by its 0.3 μm average diameter. Similarly, the Turin shroud (it need no longer be capitalized) shows two common artist's pigments, submicrometer iron oxide and mercuric sulfide particles as the only colored components of the shroud image.[7] These two pigments, red ochre and vermilion, respectively, in a collagen tempera medium detected by light microscopy, were unrecognized by any of the techniques used by the group of 30 scientists who had an equal opportunity to study the 30-plus tape samples used by the light microscopist who reported in 1980 a slightly-prior-to-1356 date[8] for the shroud before the carbon-14 date of 1325 ±65 years announced by three different laboratories in 1988.[9]

References

1. McCrone WC, Delly J. The Particle Atlas — Electronic Edition (CD-ROM). MicroDataware, Hayward, CA; 1-800-582-6624.
2. Saggerson EW. Handbook of minerals under the microscope. Natal, So. Africa: University of Natal Press, 1986.
3. Brenner H, Coman B. The identification of mammalian hair. Melbourne: Inkata Press, 1974.
4. Hall DM. Practical fiber identification, 2nd ed. Auburn, AL: Auburn University Printing Service, 1982.
5. Strelis I, Kennedy RW. Identification of North American commercial pulpwoods and pulp fibers. Toronto: University of Toronto Press, 1967.
6. Moore PD, et al. Pollen analysis, 2nd ed. Seven Oaks, U.K.: Hodder & Stoughton, 1991.
7. McCrone WC. The Vinland map. Anal Chem 1988; 60: 1009.
8. McCrone WC. The shroud of Turin: blood or artist's pigment. Accounts of Chem Res 1990; 32: 77–83.
9. Damon PE, et al. Nature 1989; 337: 611.