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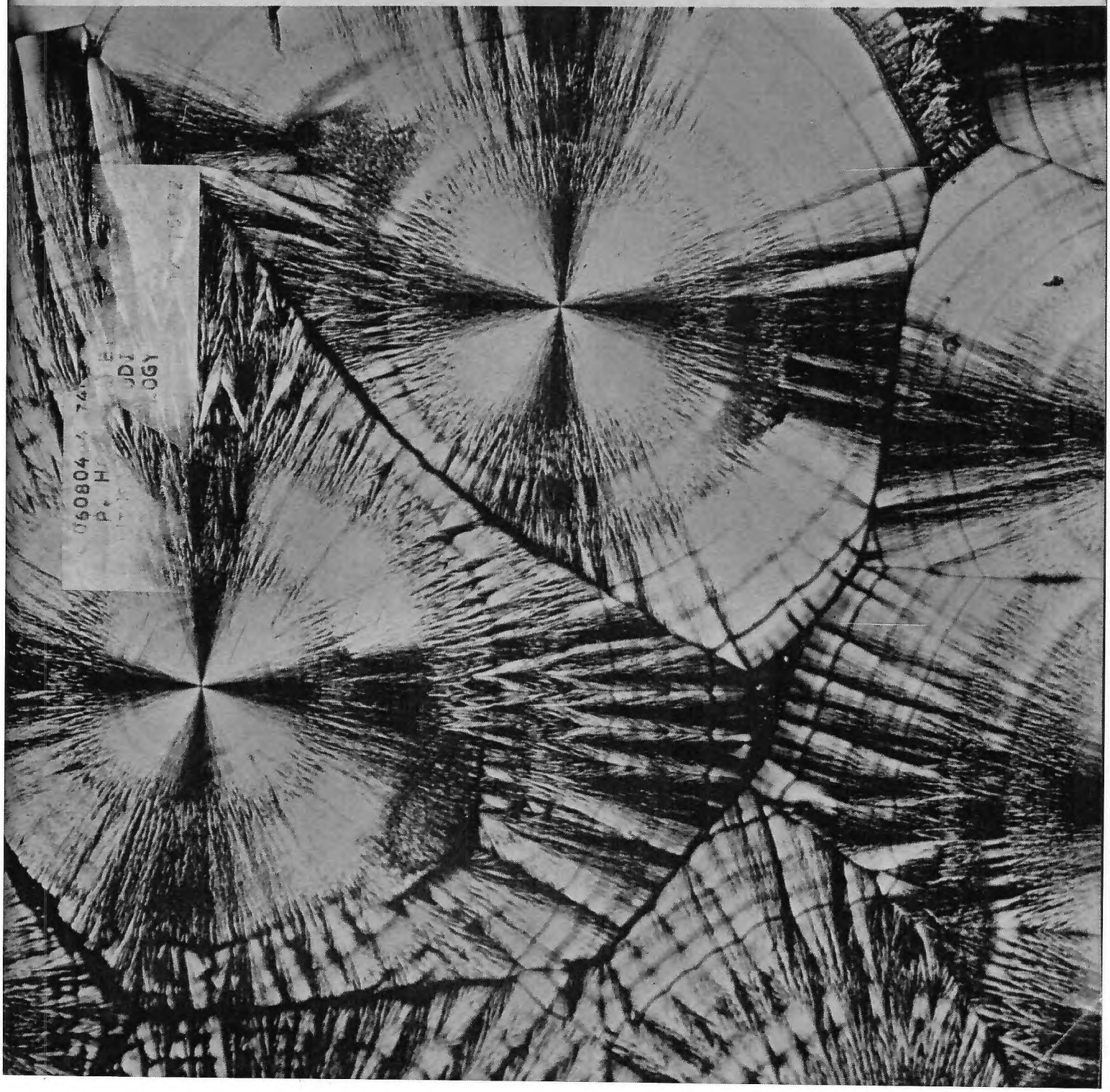
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Asbestos

By Walter C. McCrone and Ian M. Stewart

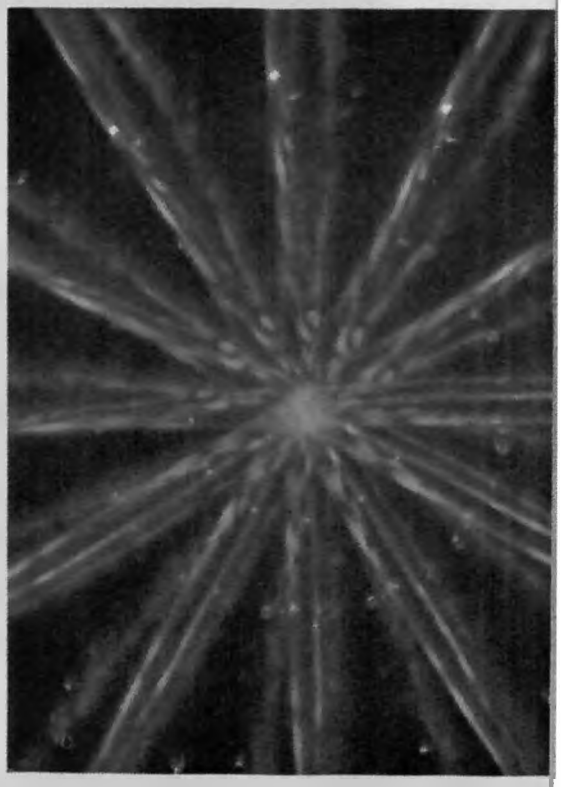
IN MAY 1968, Dr. Irving J. Selikoff of the Mount Sinai Hospital in New York established his position as the Rachel Carson of the industrial hygiene world. A series of *New Yorker* articles at that time was based largely on Dr. Selikoff's experiences in the study of asbestosis, lung and stomach cancer, mesothelioma, and other causes of death related to exposure to asbestos. His careful analyses of mortality of industrial workers in known asbestos fiber contaminated areas are convincing arguments that asbestos is one of the most deadly industrial hygiene hazards.

The reputation of large industrial concerns has not always been good when faced with evidence that their plant environments may be contributing to worker disabilities, if not death. However, largely through Dr. Selikoff's effort, followed up by the FDA, Department of Labor, and other interested parties, most responsible companies are now doing their best to minimize, if not eliminate, asbestos as a health hazard.

The microscopist has an important role in this program to control asbestos as a contaminant in the air we breathe, the fluids we drink, and the food we eat. He must be able to identify asbestos fibers and quantify their size and number. There are problems because there are other fibrous substances confusingly similar to asbestos, and there are several different types of asbestos (*Table 1*), differing in optical properties and composition and possibly in the degree of their hazard to humans.

Analytical methods must be sensitive enough for the identification of single fibers. Of course, only a microscope and a trained microscopist can do this. There are methods, e.g., x-ray diffraction and DTA, that identify minute percentages of asbestos in

Dr. McCrone is Scientific Adviser and Mr. Stewart is Manager, Electron Optics Group, McCrone Associates, Inc.



Chrysotile fiber bundle.

Table 1 **Asbestos**

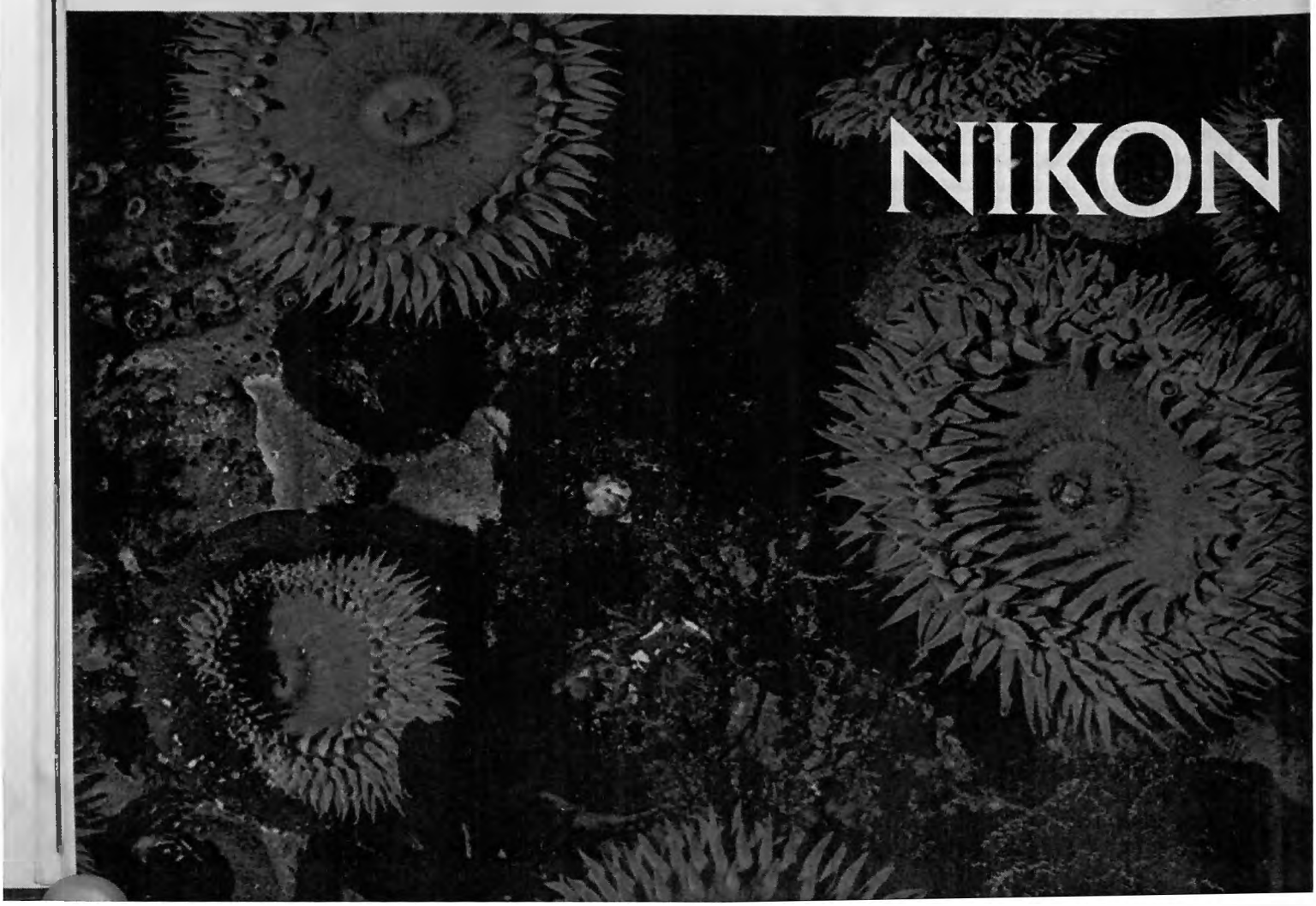
	Serpentine	Amphiboles				
	CHRYSOTILE	ACTINOLITE	TREMOLITE	ANTHOPHYLLITE	AMOSITE	CROCIDOLITE
Composition	$3MgO \cdot 2SiO_2 \cdot 2H_2O$	$2CaO \cdot 4MgO \cdot FeO \cdot 8SiO_2 \cdot H_2O$	$2CaO \cdot 5MgO \cdot 8SiO_2 \cdot H_2O$	$7MgO \cdot 8SiO_2 \cdot H_2O$	$5 \cdot 5FeO \cdot 1 \cdot 5MgO \cdot 8SiO_2 \cdot H_2O$	$Na_2O \cdot Fe_2O_3 \cdot 3FeO \cdot 8SiO_2 \cdot H_2O$
Specific gravity	2.36–2.5	3.03–3.5	2.9–3.2	2.85–3.5	2.6–3.0	3.0–3.45
Crystal system	monoclinic	monoclinic	monoclinic	orthorhombic	monoclinic	monoclinic
Refractive indices	1.49–1.57	1.62–1.68	1.60–1.65	1.60–1.66	1.66–1.70	1.69–1.71

Table 2 **Dispersion staining colors for asbestos**

Asbestos type	Refractive index liquid	Dispersion staining color	
		Parallel	Perpendicular
Chrysotile	1.560	light blue	magenta
Anthophyllite	1.610	blue-green	golden yellow
Amosite	1.670	red magenta	golden yellow
Crocidolite	1.700	magenta	blue magenta

small samples, but only the microscopist can hope to identify individual fibers. Fortunately, the crystallographic properties of the different types of asbestos are sufficiently distinct to enable certain identification. If the fibers are thicker than about 0.5 μm, light microscopy suffices; smaller fibers require the electron microscope.

Identification of the larger fibers by light microscopy is based on refractive index measurement using a polarizing microscope. The easiest way to apply refractive index measurement to asbestos fiber identification is by use of dispersion staining, an optical staining procedure. This is based on annular or central screening of the objective aperture in common with specific refractive index media, such as the Cargille liquids. Under these conditions each type of asbestos shows two characteristic dispersion staining colors, one for the light vibration direction parallel, and another perpendicular, to the fiber length (but only in one particular refractive



index liquid). *Table 2* shows the colors obtained with several kinds of asbestos when mounted in appropriate Cargille refractive index liquids. Different colors or no colors are observed if other than the specified liquids are used for each particular asbestos sample. Finding small percentages of very fine asbestos fibers requires careful application of the method. In particular, intense Kohler illumination with carefully centered substage and objective apertures is essential. A careful microscopist should, however, be able to detect 0.01% of >0.5 μm-thick fibers by this technique. Light microscopy is ideal for rapid identification of asbestos in most powdered samples: dust, mortar, insulation or wallboard, mineral samples such as talc, etc. When the presence of asbestos in a liquid medium, such as a water supply, soft drink, beer, or parenteral solution is suspected, one must assume most of the fibers, if not all, will be too fine to observe by light microscopy. One turns, then, to the transmission electron microscope (*Figure 1*). The transmission electron microscope (TEM) is superior to the scanning electron microscope for two reasons. Smaller fibers can be observed and identified by TEM, and selected area electron diffraction (SAED) is more dependable for identification of asbestos than the elemental analysis capability of the SEM.



Figure 1 *Model 200KV transmission electron microscope.*

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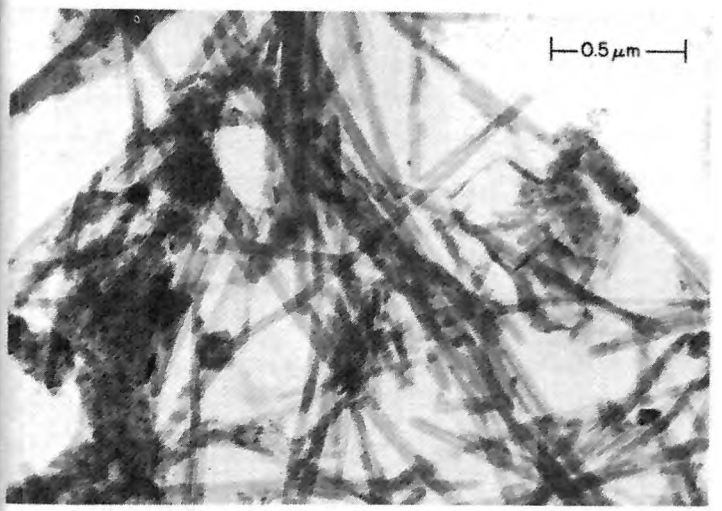


Figure 2 *Chrysotile fibers filtered from a water supply, 30,000X.*



Figure 3 *Selected area electron diffraction pattern for a single chrysotile fiber.*

A sample of water of known volume is filtered through the finest membrane filter. A section of filter approximately 2–3 mm^2 is then placed particle side down on a previously carbon-coated electron microscope grid, and the membrane filter is dissolved using acetone in a Soxhlet extractor. The sample grids are examined in our laboratory with a JEM 200 electron microscope, using an accelerating voltage of 150 kv at a magnification of 17,000X on the M3 range of the instrument. This magnification is chosen to focus the intermediate lens aperture in line with the specimen plane; thus, a SAED pattern of the fiber is obtained with no other adjustments to the microscope. In this way it is possible to spot check the diffraction pattern of individual fibers rapidly (*Figures 2* and *3*).

The total number of asbestos fibers is related to a definite area of the filter, and hence a definite volume of sample, by counting fibers over a known number of TEM grid squares (having known areas). The length and width of each asbestos fiber is recorded. Interpolation from the screen scribed at half-centimeter intervals allows an accuracy of measurement on the screen of approximately ± 0.05 cm, or about ± 34 nm. Measurements of the individual fibers are read into a tape recorder; this permits the operator to devote full attention to the screen, precludes duplicate counting of single fibers, prevents missing fibers, and maintains the operator's dark accommodation.

The recorded data are transcribed onto punch-tape and computer processed to give listings of the length, width, and aspect ratio of the fibers, together with a computed mass of each fiber calculated on the basis of density D and dimensions L and W ($D \times L \times W^2$). Density values for the different asbestos types are given in Table 1. Also presented in the computer print-out is the number of fibers per liter of water, the size distribution of the fibers based on length and width, and the distribution of fibers by aspect ratio. The program automatically assigns the longest dimension to the fiber length and excludes all fibers with an aspect ratio below three.

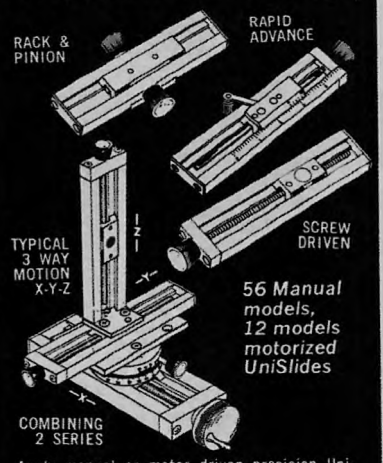
This procedure, designed by Ian Stewart of our laboratory, has been applied with excellent success to a number of water samples containing large numbers of amphiboles per liter but very low weight percentages. Most of these samples show no asbestos fibers by light microscopy. It is therefore important to specify the method of analysis when stating analytical results. The same sample of talc could be analyzed by different methods with very different results, e.g.:

1. X-ray diffraction shows no chrysotile or amphiboles.

2. A phase-contrast method (similar to the NIOSH procedure to determine the number of fibers per milliliter of air) shows 1500 fibers greater than $5\mu m/m^3$ of sample.

3. The scanning electron microscope with energy dispersive x-ray analyzer shows about 10^4

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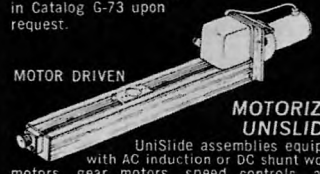


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fibers/liter suspected of being amphibole asbestos.

4. Dispersion staining shows 150 anthophyllite fibers/gram of sample.

5. Transmission electron microscopy shows 5×10^6 anthophyllite fibers/liter of sample.

The AEI Scientific Apparatus, Ltd. EMMA-4 (*Figure 4*) only recently installed in our laboratory will undoubtedly be the ideal instrument for the detection and identification of very fine asbestos fibers. A combination transmission electron microscope and electron microprobe analyzer (EMA), it enables the microscopist to see the smallest fiber and to identify it by diffraction pattern and elemental analysis. Although a given sample mounted on a TEM grid can be examined successively by TEM and EMA, the advantage of being able to examine many particles in a sample with assurance of one-to-one correspondence of particle size, shape, diffraction pattern, and elemental analysis is obvious. We have already applied this instrument to some asbestos samples with excellent results, and expect it will be the instrument of choice for such samples in our laboratory.

The monitoring of asbestos as a contaminant in our environment is an excellent example of one important problem solved by microscopy. The importance of choosing the proper analytical tool and having a well-trained operator is also emphasized by this application to asbestos detection and identification.

Figure 4 *EMMA-4, a combined transmission electron microscope and electron microprobe analyzer.*

