

## 2. Chemical Properties—Elemental Analysis by EDS

Amphiboles are nonstoichiometric minerals and often contain substitutional cations in varying amounts. Therefore, precise determination of their chemistry is difficult and positive identification based on chemistry alone is not reliable. This may be particularly pertinent when dealing with asbestos minerals present as minor constituents in mineral samples.

Elemental ratios, which are sometimes used to distinguish between asbestos types, often vary over wide ranges even in standard samples. The presence of gold coating, which would tend to preferentially absorb x-rays from lighter elements more than heavier elements, may make the situation even worse. In view of these ambiguities, and due to inherent practical difficulties in obtaining representative quantitative EDS elemental analyses from submicroscopic fibers, the present Level II and Level III protocols specify the use of only qualitative EDS spectra, which are often very valuable for screening purposes in the identification procedure. For example, in distinguishing between tremolite and actinolite type of amphibole, actinolite usually contains Fe, but tremolite does not.

## 3. Selected Area Electron Diffraction (SAED)

The method of obtaining an SAED pattern of a randomly oriented specimen is usually described in the EM instruction manual. The general directions for using the instrument to obtain and photograph SAED patterns are:

- (1) Select the image magnification for the selected area.
- (2) Bring the desired field of view to the center of the screen.
- (3) Insert the appropriate field-limiting aperture (according to the desired field of view) into the beam path.
- (4) Obtain the sharpest field-limiting aperture shadow.
- (5) Confirm that the desired field of view is in the field-limiting aperture.
- (6) Focus the specimen image; a photograph of the selected area image can be taken.
- (7) Obtain the SAED pattern, remembering to retract the objective lens aperture from the beam path. The SAED pattern will be observed on the fluorescent screen.
- (8) Select the desired camera length (the shorter the length, the better for SAED patterns of asbestos taken at high magnification).
- (9) Focus the SAED pattern sharply. The beam stopper is used to intercept the bright center spot.

- (10) For photography, the illumination is expanded (condenser reduced) after focusing the pattern, so that the pattern becomes barely visible (indistinct). A manual time exposure of approximately 20 to 30 s (maybe more depending on such factors as specimen and film) is required. The beam stopper can be left in place or removed from the beam path 1 to 2 s before closing the shutter. A double exposure of the specimen image and the SAED pattern can be taken if particle-to-particle spacing is adequate.

#### 4. Use of Tilting to Acquire Exact Zone-Axis SAED Patterns

##### Determination of the Tilt Axis—

In the side-entry type electron microscopes, the instrument tilt axis is always fixed. However, the position of the tilt axis on the viewing screen shifts with magnification. Also, there is always an angular rotation between the image and the SAED pattern. It is highly desirable to know the location of the tilt axis on the viewing screen and its relationship vis-a-vis SAED pattern under the operating conditions to make effective use of specimen tilting for obtaining exact zone-axis orientations. The following steps can be used to locate the position of the tilt axis:

- (1) A gold-coated EM grid with a standard asbestos mineral specimen on a polycarbonate replica film is placed in a tilt-rotation or double-tilt holder and inserted at 0° tilt into an aligned TEM set at 100 kV, 100  $\mu$ A, 20,000X magnification, and 20- $\mu$ m camera length operation.
- (2) The image is focused on the fluorescent screen, which is at approximately 16,000X magnification.
- (3) A circular hole in the polycarbonate replica is positioned in the center of the field of view.
- (4) On tilting, the circular feature changes to an ellipse with the major axis unchanged, and indicates the position (direction) of tilt axis at that magnification. The minor axis shows the perpendicular direction to the tilt axis. A high tilt angle defines the tilt axis more accurately than a small tilt angle. Figure A15 illustrates the effect of tilt.
- (5) A double-exposure photograph at 0° tilt and at some high tilt angle, such as 30°, is taken of the focused circular hole for reference.

##### Tilting—for zone-axis SAED Patterns—

Quantitative SAED requires knowledge of crystallography to obtain useful zone axis diffraction patterns from which precise measurements can be made for comparison with known asbestos standards on file. Thus the method of obtaining the visual SAED pattern of randomly oriented specimens, as in Level I and II analysis, is modified for quantitative SAED pattern analysis. It requires tilting of the specimen to align major crystallographic directions with the

electron beam. The zone axis is a line parallel to a set of intersecting crystal planes and nearly parallel to the electron beam. A zone-axis pattern thus gives regular repeat distances and even intensities of spots throughout the pattern.

Either a double-tilt or a tilt-rotation type specimen holder can be used for obtaining zone-axis patterns. A double-tilt holder is often preferred because tilt-rotation combination involves translational movement of the fiber during tilting, necessitating constant adjustment of the specimen-positioning controls to keep the specimen centered in the SAED aperture. On the other hand, it is much easier to obtain an accurate measure of the degree of tilt and perform systematic tilting with the tilt-rotation specimen holder. It is only necessary to rotate the specimen (fiber) until the tilt axis (as determined earlier) coincides with a major row of spots and then tilt until a major zone axis is parallel to the incident electron beam. Alternately, fiber axis of the fiber can be oriented either parallel or perpendicular to the tilt axis and then further tilting is used to obtain exact zone-axis orientations.

In order to avoid flip-flopping between image and diffraction modes while tilting, a recommended procedure is to defocus the diffraction pattern (the aperture becomes visible and the specimen/fiber can be seen in it) so that a double image of fiber in aperture can be seen with a poorly focused diffraction pattern. The movement of the fiber can then be tracked in relation to the spot pattern during tilting and kept centered in the SAED aperture by use of the specimen-positioning controls (knobs) of the microscope. Sometimes a larger aperture aids in the tracking-pattern recognition process.

An experienced electron microscopist can readily recognize the geometrical features like Kikuchi lines or Laue zones in the SAED pattern and use these to obtain the exact zone-axis SAED patterns. A detailed discussion of Kikuchi patterns and Laue zones and their utility in tilting experiments may be found in any standard text book on electron microscopy. Use of the double-tilt specimen holder is very helpful and less tedious in tilting experiments. However, all laboratories may not have both types of specimen holders available. A skilled microscopist can use either specimen holder without much difficulty. Experience and skill are more important factors in SAED analysis than the type of specimen holder used.

## 5. Characteristics of SAED Patterns Encountered in Asbestos Analysis

Successful application and exploitation of SAED analysis in asbestos analysis needs prior knowledge of the general appearance and distinguishing characteristics of other SAED patterns which are often encountered. The following discussion summarizes some of the observed SAED features of asbestos and other related minerals. This discussion is by no means comprehensive and assumes that the reader is familiar with general crystallography and the nomenclature pertaining to various aspects of SAED patterns.

### Minnesotaite and Stilpnomelane—

These iron-rich non-asbestos layer minerals are often encountered in asbestos analysis of specimens from certain geographic locations. Particulates of these minerals lie near their basal (001) planes. Stilpnomelane and

minnesotaite both possess large superlattices and their commonly observed SAED patterns are easily distinguishable from amphibole patterns. The spacing (in reciprocal space) is about half (for minnesotaite) or less than that for most amphiboles. These minerals can be readily distinguished in Level I or Level II analyses if a gold coating (optional) is applied to the specimen grids. A visual inspection of the number of rows of spots inside the (111) gold ring is sufficient to distinguish minnesotaite and stilpnomelane from amphiboles.

#### Chrysotile—

Due to the cylindrical lattice of chrysotile the SAED pattern is unique. The SAED pattern observed is symmetrical about the cylinder axis,  $x$ , and the spacing of the rows of spots is proportional to  $1/a$ , where  $a$  is 0.53 nm. The most distinguishing features of the pattern are the flared spots of the type (130) which occur in the first layer line. The flaring is due to the cylindrical lattice. A typical EDS spectra shows the presence of only Mg and Si (Figure A11).

#### Amphiboles—Systematic Absences, Twinning, and Double Diffraction—

The most commonly observed row of diffraction spots found in SAED patterns in amphiboles is in the  $y^*$  or  $b^*$  direction, representing the shortest reciprocal spacing between the spots (18.4 Å in real space). There are many strong zone axis orientations containing the  $y^*$  row of spots. The lattice of amosite, crocidolite, tremolite, and actinolite is c-centered, and for such a lattice the  $h + k$  odd spots are absent along the  $y^*$  or  $b^*$  row. In practice, however, weak spots may be present in forbidden positions due to the presence of thin multiple twinning on (100), which cause streaking parallel to  $a^*$ . Often, reciprocal nets from both twins are present in the same SAED pattern. In a twinned crystal, the number of important diffraction nets containing  $b^*$  is doubled, leading to the observation that the diffraction patterns appear insensitive to tilt.

In some cases SAED patterns can contain spots from both twin individuals which overlap. However, not all the spots present in the composite SAED patterns are generated by the overlapping nets; some spots may be present because of double diffraction where a diffracted beam from one twin becomes the transmitted beam when it enters the other twin.

The purpose of the above discussion is to point out that although many complications exist in the analysis of SAED patterns, these can be overcome; in a good goniometric tilting stage most amphiboles can be identified by SAED analysis.

#### Amosite—

The nearest reciprocal lattice section to the (100) direct lattice plane in amosite is (301)\* and it is also the most commonly observed section. Due to the presence of the thin (100) twins, this section closely resembles (100)\*.

Typical EDS spectra from amosite fibers (Figure A11) show mainly Si and Fe with smaller amounts of Mg and Mn. Mn is frequently observed as a substitutional cation in amosite.

### **Crocidolite—**

Most of the commonly observed patterns are asymmetrical and cannot be indexed easily. However, they all show rows of spots separated by a reciprocal repeat (R) corresponding to the fiber axis (0.53 nm).

The main elements observed in typical EDS analysis are Mg, Si, Ca, and Fe. Na, which is usually present in crocidolite, may not be detected in gold-coated specimens because of absorption, or because of overlapping secondary peaks from the copper grid.

### **Tremolite-Actinolite—**

Tremolite and actinolite show a variety of SAED patterns which have very similar appearances. In actinolite some of the Mg is replaced by Fe, with the result that interplanar d-spacings of actinolite are slightly larger than tremolite. In both tremolite and actinolite, the main elemental constituents are Mg, Si, and Ca. Actinolite also contains some Fe.

### **Anthophyllite—**

Even though anthophyllite has an orthorhombic crystal structure, its commonly observed patterns are similar to the monoclinic amphiboles. Anthophyllite fibers dehydrate more easily in an electron beam and are, therefore, more difficult to study.

EDS elemental analysis shows the main constituents to be Si and Mg with a small amount of Fe.

## **6. Determination of Camera Constant and SAED Pattern Analysis**

As mentioned earlier, a thin film of gold is evaporated on the specimen EM grid to obtain zone-axis SAED patterns superimposed with a ring pattern from the polycrystalline gold film. Since d-spacings corresponding to identifiable gold rings are known, these can be used as an internal standard in measuring unknown d-spacings on an SAED pattern from a fiber. The precision of measurement is as good as the quality of the photograph (or negative) and usually the measurements should be in the order of 0.1-0.2 nm with an angular tolerance of 0.5-1.5 degrees. The measurements can be made by several methods: manually with a ruler, with a mechanical aid, or a densitometer, etc. The patterns can be read directly on the developed negative or on an enlarged non-glossy print.

In practice, it is desirable to optimize the thickness of the gold film so that only one or two sharp rings are obtained on the superimposed SAED pattern. Thicker gold film would normally give multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown fibrous particulates. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings are unnecessary on zone-axis SAED patterns.

## **7. Determination of Camera Constant Using Gold Rings**

An average camera constant using multiple gold rings can be determined as explained below. However, in practice, in most cases determination of the

average camera constant is not necessary and thicker gold films are not desirable. The camera constant, CC, is 1/2 the diameter, D, of the rings times the interplanar spacing, d, of the ring being measured and is expressed as:

$$CC(\text{mm-Å}) = \frac{D(\text{mm})}{2} \times d(\text{Å})$$

The value of d for each ring can be obtained from the JCPDS file.

- (a) Measure the diameters (two perpendicular locations) of the gold rings in mm as precisely as possible (see Figure A16).
- (b) Measure as many distinct rings as possible to minimize systematic errors.
- (c) Example: if the measured values in mm are  $D_1, D_2, D_3, D_4,$  and  $D_5$ , these will represent, respectively, d-spacings of

$$\frac{4.079}{\sqrt{3}}, \frac{4.079}{2}, \frac{4.079}{\sqrt{8}}, \frac{4.079}{\sqrt{11}}, \text{ and } \frac{4.079}{\sqrt{12}} \text{ Å}$$

- (d) The camera constants will be:

$$CC_1 = \frac{D_1}{2} \times \frac{4.079}{\sqrt{3}} = \frac{D_1}{2} \times 2.355$$

$$CC_2 = \frac{D_2}{2} \times \frac{4.079}{2} = \frac{D_2}{2} \times 2.04$$

$$CC_3 = \frac{D_3}{2} \times \frac{4.079}{\sqrt{8}} = \frac{D_3}{2} \times 1.442$$

$$CC_4 = \frac{D_4}{2} \times \frac{4.079}{\sqrt{11}} = \frac{D_4}{2} \times 1.23$$

$$CC_5 = \frac{D_5}{2} \times \frac{4.079}{\sqrt{12}} = \frac{D_5}{2} \times 1.178$$

- (e) The camera constant for the SAED pattern is the average of  $CC_1, CC_2, CC_3, CC_4,$  and  $CC_5$ . Table 2 presents an example of camera-constant determination.

TABLE 2. DETERMINATION OF CAMERA CONSTANT (EXAMPLE)

Ring No.	D <sub>1</sub> readings (mm)	Mean D <sub>1</sub> (mm)	d-spacing, d <sub>1</sub> (Å)	Camera constant C <sub>1</sub> = D <sub>1</sub> /2 x d <sub>1</sub>
1	23.0, 22.0	22.5	2.355	26.5
2	27.4, 27.6	27.5	2.04	28.0
3	37.8, 38.2	38.0	1.44	27.4
4	44.6, 45.4	45.0	1.23	27.7

  

Mean Value of Camera Constant	=	$\frac{\sum C_1}{n}$	=	$\frac{26.5 + 28.0 + 27.4 + 27.7}{4}$	=	27.4 (mm-Å)
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### 8. Measurement of d-Spacings and Interplanar Angles

The gold film, because of its small, randomly oriented crystallites, produces a ring pattern superimposed on the SAED pattern from the fibers. The diameters of the gold rings correspond to known values of d-spacings, and this provides an internal standard to correct for inherent uncertainties present due to variations in instrumental and/or operating conditions. Since the d-spacings of interest on SAED patterns are usually the ones that lie closest to the center spot (transmitted beam), a camera constant measured from the first gold ring in the direction of measurement of d-spacings will usually give better accuracy in computed spacings than the use of an average camera constant. This method will account for any distortions in the symmetry of the gold ring pattern. The zone-axis SAED pattern usually has several rows of spots within the circular pattern of the gold rings. These rows of spots contain information about the two sets of planes in the crystal structure and the angle between them. The following procedure outlines the steps necessary to obtain the distances between planes (d-spacings) and the corresponding interplanar angle,  $\theta$  (see Figure A17):

- (1) From the spot pattern, determine the row with spots most closely spaced, and designate this as a horizontal row. Draw a fine line to show the row through the origin, and designate this the zeroth row. Draw fine lines to show the first and succeeding horizontal rows. For a few horizontal rows, measure the mean spacing between adjacent spots (or the minimum vector):

$$X_1 = \frac{\text{Distance between spots } m \text{ units apart}}{m}$$

where  $m$  is chosen as an optimum number to minimize measurement errors. The mean horizontal spot distance,  $X$ ,

equals the summation of  $X_1$  divided by the number,  $n$ , of rows measured. The  $d$ -spacing in  $A$  corresponding to this vector is the camera constant divided by  $X$ , and is labeled  $d_2$ . Table 3 presents an example of spot spacing measurement within a horizontal row.

- (2) The perpendicular distance between two adjacent horizontal rows is similarly measured. This interrow spacing,  $Z$ , is the mean separation between horizontal rows, and equals the distance between a number of rows divided by the number of spaces. This distance is an additional vector for comparison that coincides with the slant vector,  $d_1$ -spacing, when angle  $\theta_{1,2}$  is  $90^\circ$ . The row-spacing ( $R$ ) equals the camera constant divided by  $Z$ . Table 3 presents an example of perpendicular spacing between horizontal rows; Figure A17 illustrates spot and row spacing.
- (3) To obtain the  $d_1$ -spacing and corresponding angle  $\theta_{1,2}$ , a perpendicular is drawn to the zeroeth horizontal row through the origin. A line is drawn to the first spot to the right of the perpendicular in the first row and extended through the succeeding rows. This line, called the slant vector, forms the acute angle  $\theta_{1,2}$ . The mean spacing,  $Y$ , between spots on the slant vector can be measured by dividing the maximum distance between spots by the number of spaces between them, or by calculating from the interrow spacing:

$$Y = \frac{R}{\sin \theta_{1,2}}$$

The  $d$ -spacing in  $A$  corresponding to this vector is the camera constant,  $CC$ , divided by  $Y$  and labeled  $d_1$ .

$$d_1(A) = \frac{CC \times \sin \theta_{1,2}}{R} = \frac{CC}{Y}$$

Figure A18 illustrates the relationship of  $d_1$ ,  $d_2$ ,  $\theta_{1,2}$  and  $R$ . In some cases, the interplanar angle  $\theta_{1,2}$  may be more than  $90$  degrees (not shown in Figure A18).

Summary of Data from Each SAED Pattern:

- (a) The camera constant,  $CC$ , as determined from the gold rings, normalizes the distances on the SAED pattern regardless of such factors as magnification and tilting.

TABLE 3. DETERMINATION OF SPOT SPACINGS (EXAMPLES)

Reading	Separation (mm)	Units	Mean spacing, $\bar{X}_1$ (Å)
Spot spacing within a horizontal row, $d_2$ :			
1	49	16	3.006
2	42.7	14	3.05
3	—	—	—
			3.028 = Mean

$$d\text{-spacing} = \frac{27.4}{3.028} = 9.05 \text{ Å}$$

Perpendicular spacing between horizontal rows,  $R$ :

1	43	8	5.0375
—	—	—	—
—	—	—	—
			5.0375 = Mean

$$d\text{-spacing, } R = \frac{27.4}{5.0375} = 5.44 \text{ Å}$$

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Note: It is preferable that the camera constant values used in computing d-spacings are measured from the first one or two gold ring diameters in the direction of d-spacing measurement.

(b) The parameters of interest are:

- d-spacing of spots in a horizontal row:  $CC/X = d_2$
- d-spacing of spots in the slant vector:  $CC/Y = d_1$
- angle  $\theta_{1,2}$  formed between a horizontal row and slant vector
- d-spacing corresponding to row separation as an additional parameter of interest:  $CC/Z = R$ .

It should be noted that the use of camera constant in the form used here in calculating  $d_1$ ,  $d_2$ , and  $R$ , which are measured in reciprocal space on SAED patterns, automatically converts the calculated numbers into real space spacings, which are then compared to those from a suitable standard file.

### 9. Identification of Unknown Fibers

Unknown d-spacings ( $d_1$  and  $d_2$ ), interrow spacing ( $R$ ), and interplanar angles ( $\theta$ ) measured from zone-axis SAED patterns of unknown fibers are compared with corresponding known values tabulated in JCPDS powder diffraction files, or those computed using lattice parameters and crystal structures of candidate asbestos minerals, or with the values contained in an internally developed file from standard specimens of candidate minerals. Table 4 is an example of the IITRI standards file (Jones et al., 1981). Figures A19 to A22 are examples of zone-axis SAED patterns.

Unknowns are matched as closely as possible to the file parameters for positive identification. However, considerable care and competent judgment are required in Level III confirmatory analysis. For example, amphiboles are usually nonstoichiometric minerals, and thus a perfect match may not be possible between the d-spacings and interplanar angles determined from unknown fibers and those available from standard minerals. JCPDS Powder Diffraction files do not list interplanar angles. Since amphiboles have low-symmetry crystal structures, tabulated values of d-spacings and interplanar angles would be extensive and very expensive to generate, and to get an accurate match may not be possible because these tables are derived assuming certain lattice parameters which may not be the same as those of the unknown fibers being analyzed. Given these inherent uncertainties, it would seem that use of internally developed SAED files consisting of several readily accessible orientations (by virtue of natural habit of amphibole fibers) from standard amphibole species could eliminate a lot of tedious unnecessary work and yet provide reliable data for comparison and identification of unknown fibers.

In practice, SAED analysis combined with qualitative EDS analysis may help resolve certain cases where a close match in d-spacings and interplanar angles is not possible. For difficult specimens or SAED patterns of controversial nature, a second opinion may be necessary, especially if a legal case is involved.

TABLE 4. COMPARISON OF d-SPACINGS FROM SAED FILE  
AND POWDER DIFFRACTION FILE (EXAMPLE)

Amphibole type	Zone axis	Internal Standard File Data				Powder Diffraction File Data (1975)		
		d <sub>1</sub> (Å)	d <sub>2</sub> (Å)	θ (deg)	Interrow spacing, R (Å)	d <sub>1</sub> (Å)	d <sub>2</sub> (Å)	File index no.
Amosite	[100]	5.3	9.14	90.0	5.3	5.22	9.20	17-725
	[30 $\bar{1}$ ]	1.79	9.26	84.0	—	1.76	9.20	17-725
	[101]	4.88	9.23	74.0	5.17	4.84	9.20	17-725
	[ $\bar{1}$ 01]	4.14	9.11	78.0	4.21	4.10	9.20	17-725
	[ $\bar{3}$ 10]	5.22	5.13	95.0	—	5.22	5.12	17-725
Crocidolite	[100]	5.22	8.97	90.0	5.22	5.20	9.02	19-1061
	[101]	4.94	9.05	75.0	5.19	5.89	9.02	19-1061
	[ $\bar{1}$ 10]	4.79	8.19	79.0	5.23	4.89	8.40	19-1061
	[30 $\bar{1}$ ]	1.75	8.97	83.5	—	1.76	9.02	19-1061
	[ $\bar{3}$ 10]	5.12	5.12	96.0	—	—	—	19-1061
Tremolite	[100]	5.04	9.03	90.0	—	5.07	8.98	13-437
	[101]	4.83	9.03	75.0	—	4.87	8.98	13-437
	[ $\bar{2}$ 0 $\bar{1}$ ]	2.59	8.97	80.5	—	2.59	8.98	13-437
	[30 $\bar{1}$ ]	1.72	8.98	83.5	—	1.69	8.98	13-437
Anthophyllite	[100]	—	—	90.0	5.24	5.28	8.90	9-455
	[ $\bar{1}$ 42]	4.56	4.56	60.0	—	4.50	4.50	9-455

## SECTION 7

### ARCHIVAL SAMPLES

#### DISCUSSION OF PROTOCOL

Samples that have been collected on filter substrates other than polycarbonate, or that have been collected without regard to filter loading levels, are referred to as archival samples. These samples were usually collected for other analytical objectives, such as for optical microscopy or gravimetric analysis, for defined sampling periods without regard to concentration levels in the air, or for collection of particles larger than 10  $\mu\text{m}$  in diameter. Such samples were historically collected, and are of value and interest in determining the presence of asbestos fibers and/or structures. Filter substrates designated as archival samples include glass fiber filters; cellulose or modified paper filters; cellulose ester filters; other organic polymeric membranes, such as polystyrene, nylon, and polyvinyl chloride; and all overloaded organic polymeric membrane filters.

The purpose of the preparation step is to transfer particles from a filter surface to an EM grid with a minimum of distortion in morphology and size distribution. The nature of non-polycarbonate filter substrates or particle loading makes it sometimes necessary to transfer a satisfactory quantity of particles to a polycarbonate filter prior to transfer to the EM grid. At present, only transfer to an EM grid from a polycarbonate filter has been standardized.

A modified preparation technique is recommended for archival samples, followed by the analytical methodology using Level I, Level II, or Level III effort--with the understanding that these samples will indicate the presence of asbestos, and secondarily the number, size, distribution, and morphology. The results from sample to sample are less precise due to problems in standardizing the preparation procedures used for archival samples.

The archival filter samples are prepared for analysis based on the information sought, type of filter material, and particle loading on the filter. The various preparation techniques for these filters include:

- (1) Individual particle picking and/or reverse washing of the filter, with subsequent filtration of the filtrate using a polycarbonate filter.
- (2) Collapsing the membrane filter structure by exposure to solvent vapor (surface fusion), to produce a more uniform substrate for replication and grid transfer.

