

10. Mount either the whole sample filter or a wedge cut from the sample filter on a clean glass slide.

a. Cut wedges of ca. 25 percent of the filter area with a curved-blade steel surgical knife using a rocking motion to prevent tearing.

b. Place the filter or wedge, dust slide up, on the slide. Static electricity will usually keep the filter on the slide until it is cleared.

c. Hold the glass slide supporting the filter approximately 1 to 2 cm from the glass tube port where the acetone vapor is escaping from the heated flask. The acetone vapor stream should cause a condensation spot on the glass slide ca. 2 to 3 cm in diameter. Move the glass slide gently in the vapor stream. The filter should clear in 2 to 5 sec. If the filter curls, distorts, or is otherwise rendered unusable, the vapor stream is probably not strong enough. Periodically wipe the outlet port with tissue to prevent liquid acetone dripping onto the filter.

d. Using the hypodermic syringe with a 22-gauge needle, place 1 to 2 drops of triacetin on the filter. Gently lower a clean 25-mm square cover slip down onto the filter at a slight angle to reduce the possibility of forming bubbles. If too many bubbles form or the amount of triacetin is insufficient, the cover slip may become detached within a few hours.

e. Glue the edges of the cover slip to the glass slide using a lacquer or nail polish.

Note.—If clearing is slow, the slide preparation may be heated on a hotplate (surface temperature 50°C) for 15 min to hasten clearing. Counting may proceed immediately after clearing and mounting are completed.

Calibration and Quality Control

11. Calibration of the Walton-Beckett graticule. The diameter, d_c (mm), of the circular counting area and the disc diameter must be specified when ordering the graticule.

a. Insert any available graticule into the eyepiece and focus so that the graticule lines are sharp and clear.

b. Set the appropriate interpupillary distance and, if applicable, reset the binocular head adjustment so that the magnification remains constant.

c. Install the 40 to 45 X phase objective.

d. Place a stage micrometer on the microscope object stage and focus the microscope on the graduated lines.

e. Measure the magnified grid length, L_m (μm), using the stage micrometer.

f. Remove the graticule from the microscope and measure its actual grid length, L_a (mm). This can best be accomplished by using a stage fitted with verniers.

g. Calculate the circle diameter, d_c (mm), for the Walton-Beckett graticule:

$$d_c = \frac{L_m \times D}{L_a}$$

Example: If $L_m = 108 \mu\text{m}$, $L_a = 2.93 \text{ mm}$ and $D = 100 \mu\text{m}$, then $d_c = 2.71 \text{ mm}$.

h. Check the field diameter, D (acceptable range $100 \text{ mm} \pm 2 \text{ mm}$) with a stage micrometer upon receipt of the graticule from the manufacturer. Determine field area (mm^2).

12. Microscope adjustments. Follow the manufacturer's instructions and also the following:

a. Adjust the light source for even illumination across the field of view at the condenser iris.

Note.—Kohler illumination is preferred, where available.

b. Focus on the particulate material to be examined.

c. Make sure that the field iris is in focus, centered on the sample, and open only enough to fully illuminate the field of view.

d. Use the telescope ocular supplied by the manufacturer to ensure that the phase rings (annular diaphragm and phase-shifting elements) are concentric.

13. Check the phase-shift detection limit of the microscope periodically.

a. Remove the HSE/NPL phase-contrast test slide from its shipping container and center it under the phase objective.

b. Bring the blocks of grooved lines into focus.

Note.—The slide consists of seven sets of grooves (ca. 20 grooves to each block) in descending order of visibility from sets 1 to 7. The requirements for counting are that the microscope optics must resolve the grooved lines in set 3 completely, although they may appear somewhat faint, and that the grooved lines in sets 6 to 7 must be invisible. Sets 4 and 5 must be at least partially visible but may vary slightly in visibility between microscopes. A microscope which fails to meet these requirements has either too low or too high a resolution to be used for asbestos, tremolite, anthophyllite, and actinolite counting.

c. If the image quality deteriorates, clean the microscope optics and, if the problem persists, consult the microscope manufacturer.

14. Quality control of fiber counts.

a. Prepare and count field blanks along with the field samples. Report the counts on each blank. Calculate the mean of the field blank counts and subtract this value from each sample count before reporting the results.

Note 1.—The identity of the blank filters should be unknown to the counter until all counts have been completed.

Note 2.—If a field blank yields fiber counts greater than 7 fibers/100 fields, report possible contamination of the samples.

b. Perform blind recounts by the same counter on 10 percent of filters counted (slides relabeled by a person other than the counter).

15. Use the following test to determine whether a pair of counts on the same filter should be rejected because of possible bias. This statistic estimates the counting repeatability at the 95% confidence level.

Discard the sample if the difference between the two counts exceeds $2.77(F)s_r$, where F = average of the two fiber counts and s_r = relative standard deviation, which should be derived by each laboratory based on historical in-house data.

Note.—If a pair of counts is rejected as a result of this test, recount the remaining samples in the set and test the new counts against the first counts. Discard all rejected paired counts.

16. Enroll each new counter in a training course that compares performance of counters on a variety of samples using this procedure.

Note.—To ensure good reproducibility, all laboratories engaged in asbestos, tremolite, anthophyllite, and actinolite counting are required to participate in the Proficiency Analytical Testing (PAT) Program and should routinely participate with other asbestos, tremolite, anthophyllite, and actinolite fiber counting laboratories in the exchange of field samples to compare performance of counters.

Measurement

17. Place the slide on the mechanical stage of the calibrated microscope with the center of the filter under the objective lens. Focus the microscope on the plane of the filter.

18. Regularly check phase-ring alignment and Kohler illumination.

19. The following are the counting rules:

a. Count only fibers longer than 5 μm. Measure the length of curved fibers along the curve.

b. Count only fibers with a length-to-width ratio equal to or greater than 3:1.

c. For fibers that cross the boundary of the graticule field, do the following:

1. Count any fiber longer than 5 μm that lies entirely within the graticule area.

2. Count as ½ fiber any fiber with only one end lying within the graticule area.

3. Do not count any fiber that crosses the graticule boundary more than once.

4. Reject and do not count all other fibers.

d. Count bundles of fibers as one fiber unless individual fibers can be identified by observing both ends of a fiber.

e. Count enough graticule fields to yield 100 fibers. Count a minimum of 20 fields. Stop at 100 fields regardless of fiber count.

20. Start counting from one end of the filter and progress along a radial line to the other end, shift either up or down on the filter, and continue in the reverse direction. Select fields randomly by looking away from the eyepiece briefly while advancing the mechanical stage. When an agglomerate covers ca. ¼ or more of the field of view, reject the field and select another. Do not report rejected fields in the number of total fields counted.

Note.—When counting a field, continuously scan a range of focal planes by moving the fine focus knob to detect very fine fibers which have become embedded in the filter. The small-diameter fibers will be very faint but are an important contribution to the total count.