

# MACS Lab, Inc.

## TECHNICAL NOTES

### **Comparison of TEM Asbestos Fiber counting and Asbestos counting using PCM 7400 Method**

**by Jim Richards**

A considerable amount of misunderstanding exists regarding the comparison of TEM analysis of airborne asbestos fibers and that of Method 7400<sup>1</sup>. No direct correlation is possible for several reasons.

We must consider resolution of the microscopes because this is the central issue with the problem of comparison. Resolution is the ability to distinguish the presence of two objects at some distance. For example, if there are two tennis balls positioned 1 inch apart and placed on top of a box 100 yards away can you see both? If not then at what distance do they have to be separated to see them as two distinct objects? This is the simple definition of resolution.

The resolution of a microscope can be calculated by using this formula<sup>2</sup>:

$$\text{Minimum distance between two points} = \frac{(0.61) \times \text{wavelength of light used}}{\text{Numerical Aperture}}$$

The Numerical Aperture (N.A.) is  $= i \sin \theta$

where:  $\theta$  = one-half the angle of the cone of light entering the objective

$i$  = the refractive index of the medium in which the objective is used

Typical N.A. for PCM lenses are around 0.66 but never more than 1 because air has a refractive index of 1 and is the medium in which the objective lens is working.

Using the above formula one can calculate the maximum resolution of an optical microscope. If 530 nanometer light is used (the approximate wavelength of the green filter used in PCM 7400 analysis) the *maximum resolution* of the microscope would be 0.49 micrometers (microns).

Following Method 7400 a test slide must be analyzed to check the calibration of the microscope. The method requires the Health and Safety Executive / National Physical Laboratory (abbreviated HSE-NPL) Mark II Phase Shift Test Slide be used.. HSE-NPL is a part of the government of the United Kingdom. This test slide has 7 groups of 20 lines each and they are arranged from left to right. The lines are of increasingly smaller width as the slide is moved to the right. According to Method 7400, a microscope must resolve the first three sets of lines, partly resolve the next three and the remaining two (6 and 7) must be invisible. The microscope is adjusted so that the above conditions are met. It is obvious to the observer that the length of all the lines is the same, however, the width becomes increasingly thin as one looks to the right. As the linewidth becomes increasingly smaller the lines eventually become invisible.

In the TEM electrons are used instead of photons. The wavelength of visible light photons is in the order of 400 to 700 nanometers. The wavelength of a 100 KeV electron is 0.00370 nanometers<sup>3</sup>. This is 143,000 times smaller than the wavelength of the light used in the Phase Contrast Microscope. Due to that fact that electron lenses are much poorer than lenses used for visible light we find that the TEM actually has a maximum resolution of 0.2 nanometers<sup>4</sup> which is 2,500 times less than the optical microscope.

Therefore the TEM can easily resolve fibers that are much smaller than an optical microscope is capable.

Fibers are defined by Method 7400 as having an aspect ratio of 3:1 or greater<sup>1</sup>. In the real world most asbestos fibers have aspect ratios much greater than 3:1. For example, a 10 micron fiber when examined in the TEM may have a width of only 0.1 micron giving an aspect ratio of 100:1. This situation is quite common. It is rare to find fibers with aspect ratios much less than 20:1. Herein lies the problem.

When one attempts to observe a fiber of 8 or 10 microns in a Phase Contrast Microscope and that fiber has an aspect ratio of 100:1, the fiber will be invisible. That is because the width of the fiber is 4 to 5 times smaller than the theoretical optical resolution of the microscope. However, the fiber can easily be seen in the TEM. In fact the viewing screen of a TEM operated at 20,000X has a diameter of 5 microns. Therefore a fiber with a length of 10 microns and a width of 0.49 microns (the limit of optical resolution) would occupy about 10% of the width of the viewing screen field of view. This fiber would be invisible in the Phase Contrast Microscope.

#### References:

- 1 Method 7400, NIOSH Manual of Analytical Methods.
- 2 Optics, 3rd Edition, Eugene Hecht, Addison-Westley Publishing, 1998, ISBN 0-201-83887-7
- 3 Electron Diffraction and Optical Diffraction Techniques, B.E.P.Beeston, Robert W. Horne and Roy Markham, Cambridge University, 1990, ISBN 0 444 10411 9
- 4 Transmission Electron Microscopy, Second Edition, Ludwig Reimer, Springer-Verlag, 1989, ISBN 0-540-50499-0