

Using A Drawing Attachment to Project Grids and Scales into the Microscope Field of View

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Introduction

It is often overlooked that microscope drawing attachments are two-way devices. Their primary function is to get an image **out** of the microscope, but they can also be used to project a reference image, grid, or scale **into** the microscope. A projected image usually functions as a substitute for an eyepiece reticle (= reticule, graticule).

This do-it-yourself approach to reticles doesn't produce the best images; contrast is low. If you do a lot of work with a particular reticle, you want the real thing. If you have occasional use for a reticle pattern, or wish to experiment with different designs, or need a unique pattern, then the method described here for projecting reticles is reasonable and cost effective. You can easily construct reticle patterns to try them out. You can build a collection of reticles without tying up eyepieces or emptying your bank account. Diatom mounters can make templates for arrangement patterns.

This article offers pointers on setting up a drawing attachment to project grids and scales, then shows some applications: measuring length and area, counting, comparing, specimen alignment. Reticle images in this article are reduced to hold down file size, but they still have sufficient resolution to be usable; zoom in to see more. You can copy images to the clipboard, or zoom in and do screen captures; either way, you will have a good start on making your own reticles.



Figure 1. Drawing attachments. **A.** On a 1971 Zeiss Stereomicroscope IV (Zeiss catalog image). **B.** On a 1978 Olympus BHAP polarizing microscope (author's microscope). These examples are older models, but drawing attachments are also available for newer and current model microscopes.

Modern Modular Drawing Attachments



Figure 2. Components of the Olympus BH-DA drawing attachment for BH and CH series microscopes.

Figure 2 shows a typical drawing attachment, also called drawing arm, or drawing tube. It projects an image of the drawing paper and pencil into the microscope field of view. It is then a simple matter to trace the outline of what you see (Figure 3). In this article, I will consider the reverse process. A paper print of a grid or scale is placed where the drawing paper is usually positioned, and the projected image is merged with the microscope field of view (Figure 4).

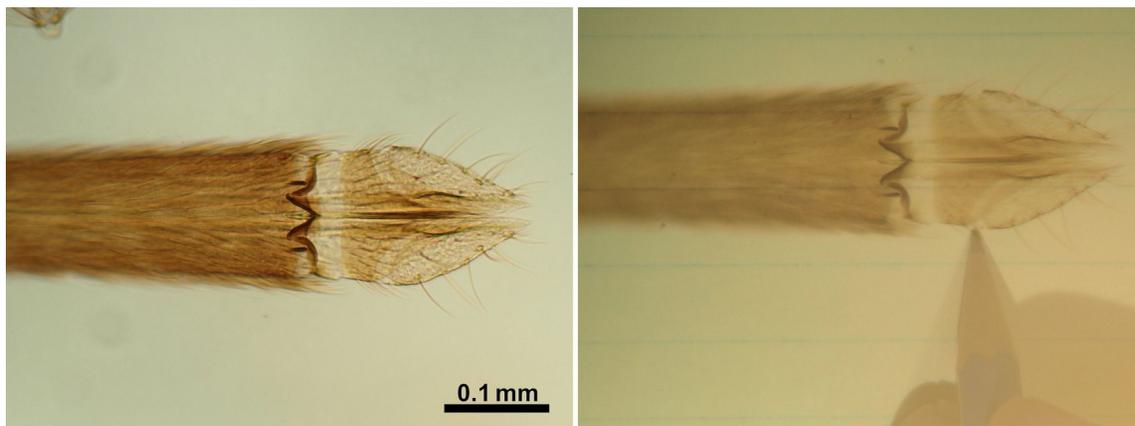
Avoiding Distortion. The drawing arm image is rectilinear; distortion is not a concern as it is with a camera lucida.

Maintaining Image Contrast. A brighter desk lamp on the drawing surface gives better images in the microscope.

Balancing Light. Close the drawing arm sleeve to block external light and see the image with normal contrast (Figure 3A). To superimpose the drawing surface on the microscope field (Figure 3B), open the drawing arm sleeve and adjust the microscope lamp voltage to balance the two images. High contrast images (Figure 4) are difficult to balance; portions will be washed-out or dark. Uniform contrast images are easier to balance.

Controlling Grid Size. When a projected grid needs to be a particular size, such as 0.1 mm squares at the image plane, a stage micrometer on the microscope stage is used as reference to adjust projected grid size:

- **Coarse adjustment:** change the projection eyepiece to one with a different magnification (2.5X, 3.3X, 5X).
- **Fine adjustment:** use the drawing arm zoom control.
- **If you have to:** raise or lower the grid with respect to the drawing arm to change projected grid size.
- **If all else fails:** make another computer print of the grid with a size better suited to your optical configuration.



A

B

Figure 3. Drawing attachment used in the normal fashion – to make a drawing. **A.** Normal view with a 10X objective. The three-dimensional subject doesn't focus all in one plane, making it a good candidate for a drawing or for image stacking. **B.** paper and pencil projected onto the image. The subject is the proboscis of a female *Culex pipiens* mosquito from a Watson & Sons Ltd. slide dating from the early 20th Century (Bracegirdle, 1998 for dating the slide). It looks like a rock drill bit to me!

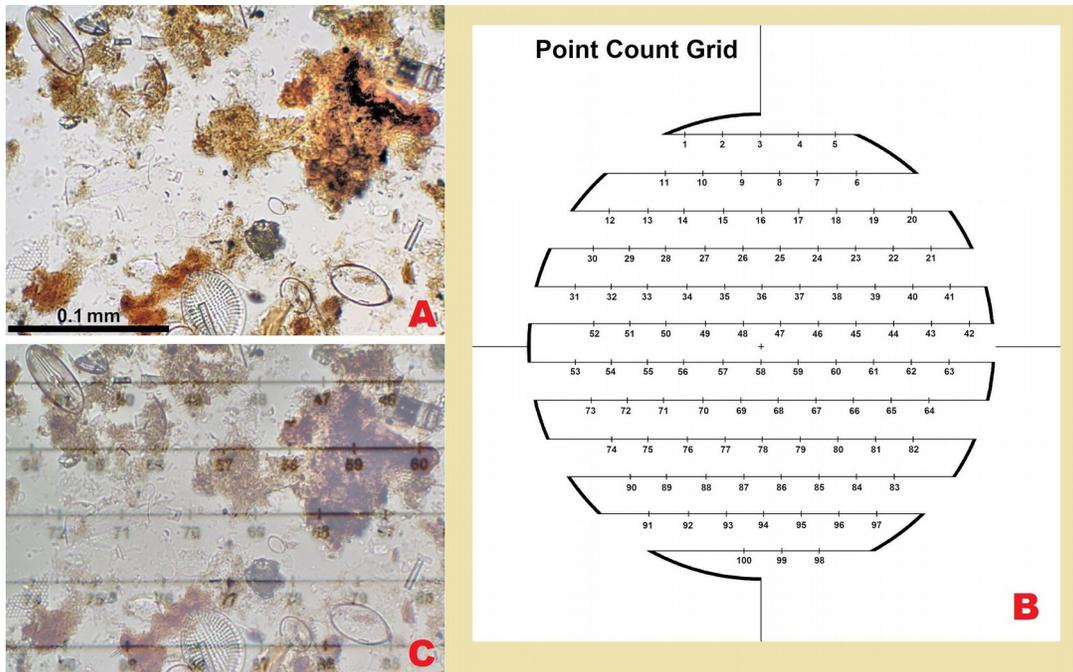


Figure 4. Drawing attachment used in a reverse fashion – to project a grid into the microscope. The sample is a whole rock sediment smear used to determine relative proportions of components. The sample is anchor mud from 100 feet depth in Moira Sound, Prince of Wales Island, southeast Alaska. In a few million years, after the organic debris has distilled off, this will make a nice diatomite. **A.** Normal view with a 20X objective. **B.** Point count grid; a 3 inch diameter circle. **C.** Grid projected onto the field of view by the drawing attachment. This small-sensor camera sees only a cropped portion of the visual field; when viewing through the eyepieces, you see the full circle of the reticle.

Bonus Feature: photograph your grid. An eyepiece reticle is not on the same optical path as the camera; a photograph does not include eyepiece reticle lines. A grid projected by a drawing attachment *is* on the camera's optical path; it is your option to include or exclude the grid from a photograph. Including the grid provides documentation for points counted.

What About A Camera Lucida?

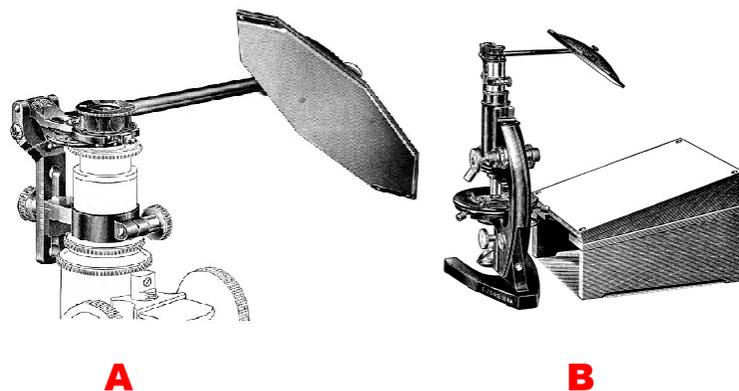


Figure 5. Abbe camera lucida as illustrated in **A.** the 1927 and **B.** the 1934 Zeiss catalogs.

The camera lucida is the old-school approach to drawing attachments. It can be used – with effort – to project a reference grid into the microscope. A camera lucida is not model-specific like a drawing attachment, and can be used on various models of microscope. This advantage is offset by a number of inconveniences. It is somewhat tricky to balance the lighting using the neutral density filters built into the apparatus. The field of view is narrow and dark. Viewing is a one-eyed squint; eye strain is a common side effect. It is an inconvenient fit to an inclined eye-tube. You often need to tilt the paper for a rectilinear view as in Figure 5B. The bottom line: it is probably more trouble than it is worth to try to use a camera lucida for reticle grid projection.

Making Grids and Scales

With the basic equipment described, let us look at some reticle designs. The catalogs of reticle manufacturers are good references to see the many designs in common use. [Pysler-SGI](#), for example, illustrates 77 reticle designs. Homemade reticles can be computer drafted as bitmap or vector images and printed at 10% to 20% of their original size to match the scale you need. On my own equipment, a 3 inch diameter reticle 3/4 fills the microscope field of view. How reliable are these home-made reticles? Some researchers tape transparent patterns onto their computer screen to make measurements; the home-made projection grids are at least that good. In fact, they are a little better because they can be size-adjusted.

Reticles For Measuring and Counting

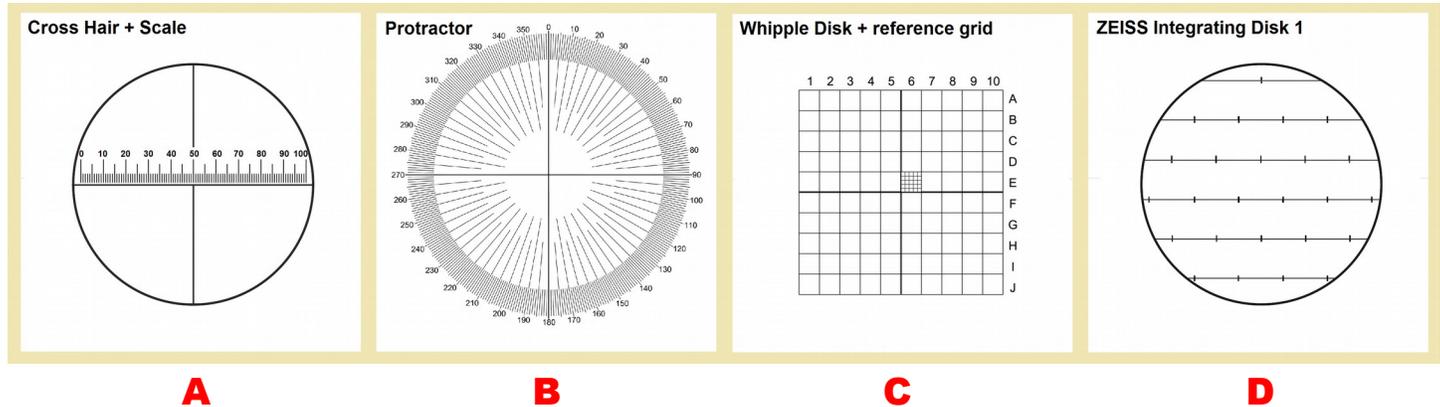


Figure 6. Reticles for measuring and counting.

Cross Hair + Scale. Figure 6A is the basic reticle everyone uses: a scale for measuring length. With the drawing arm, you can trace the image of a stage micrometer to make a direct-reading scale for that optical combination. Measurements are faster because a computational step is eliminated; there is no need to convert eyepiece units to actual length. Reticle scales are the fastest way to measure length, but results have medium precision. For higher precision, use an eyepiece filar micrometer or computer software such as the free [ImageJ](#) microscopy software from the National Institutes of Health.

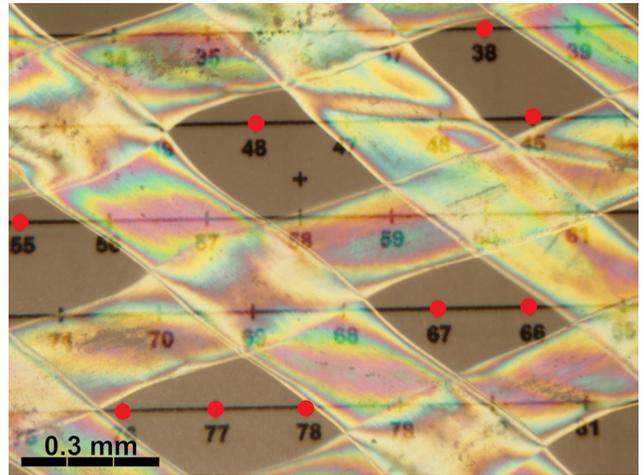
Protractor. Figure 6B shows a reticle for measuring angles. You don't need to draw this pattern; a draftsman's plastic protractor can be placed below the drawing attachment and used as-is. Again, the reticle scale provides a medium precision measurement, probably no better than the nearest degree. Higher precision is given by a goniometer eyepiece, a calibrated rotating stage, or computer software such as ImageJ.

Whipple Disk. Figure 6C is an example from the large family of square grid reticles. Grids have many uses. They help you keep your place when systematically examining a field of view. If rows and columns are labeled, the grid can act as a finder to point out areas of interest. Counts can be made of objects within grid squares. Statistical point counts can be made of objects at grid line intersections. If the grid has a known size, it can be used to measure lengths and areas.

The Whipple disk was designed for drinking water analysis and is used with a slide that provides a cell of known depth such as a Sedgewick-Rafter cell. Known cavity depth and known area of reticle squares give a known volume in which objects can be counted. This is much like a hemacytometer blood cell counter, but on a larger scale for larger particles. For more on hemacytometer counts, see Robert Pavlis' [Micscape article](#).

Zeiss Integrating Disk 1 (Figure 6D). This was formerly provided by Zeiss as an eyepiece reticle; it is also known as a Hennig ("Henning" in some catalogs) grid after its developer. Variants on this format often have more than 25 points. Any grid can be used as a point counter; the 10x10 Whipple disk in Figure 6C allows 121 grid points to be counted. Grids are used to select random points in the sample to build up a count of the sample's components. Randomness is obtained from a uniform grid by placing the grid randomly over a field of view. As multiple fields of view are counted, the count becomes more accurate and representative of the sample as a whole. Figure 7 is an example of point counts used to compute percent of total sample.

Figure 7. Estimating percent composition. The subject is a Smith, Beck, & Beck slide from 1865-1880 (Bracegirdle, 1998 for dating the slide). It is an open weave fabric of fine horsehair viewed in cross-polarized light with a 4X objective. A point count grid (Figure 4B) is projected onto the field of view by the drawing attachment. Points that fall between threads are highlighted in red. There are 9 such points from a total of 35; an initial estimate is that 9/35, or 25.7%, of the fabric is open space.



As a check, open spaces were measured in ImageJ and found to be 25.3% of the image. It is only luck that this count is so close to the correct value. A slight shift of the grid gives 8 or 10 hits instead of 9. Counting 10 fields (350 points) gives a more reliable value. Still, this is a good showing for the point count method, which is a fraction of the effort needed to measure each open space.

Volume Estimation, Stereology

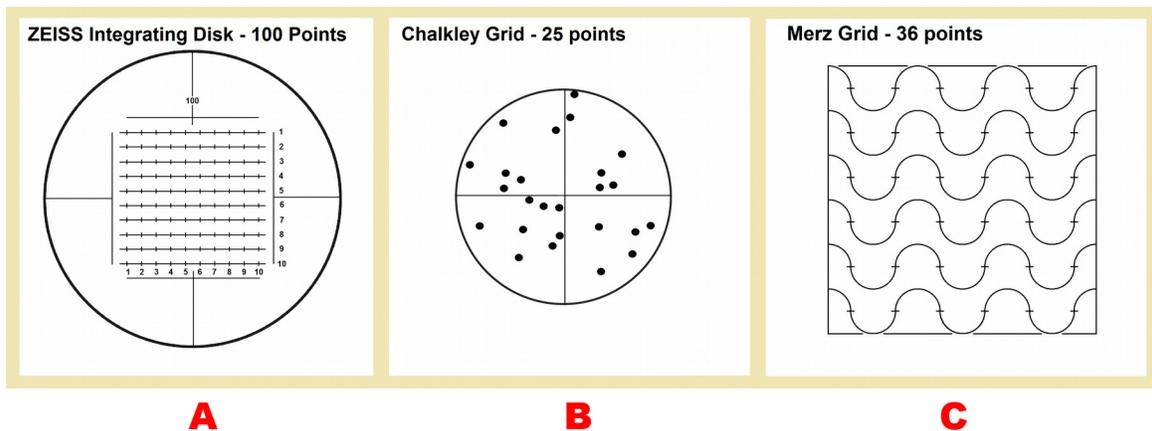


Figure 8. Stereological grids. These are point count grids from which area is estimated and volume is inferred.

Stereology can be described as making three-dimensional inferences from two-dimensional measurements. A 3-D volume appears as a 2-D area on a thin-section. A 2-D membrane appears as a 1-D line. The science of stereology has developed some sophisticated techniques in recent decades, but the very first technique – the Delesse Principle of 1847 – still has utility. It asserts that a homogeneously distributed component has the same fraction of total volume as its fraction of total area in random sections. The basic data of stereology are tabulations of point counts. If a component fits the assumptions of the model and comprises 28% of the counts, then:

$$28\% \text{ of counts} = 28\% \text{ of cross-sectional area} = 28\% \text{ of volume}$$

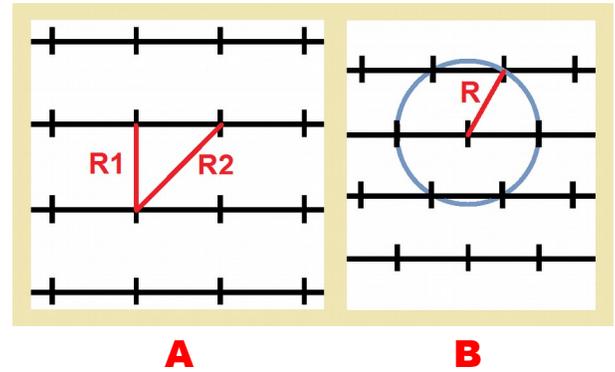
Simple, but a good starting point. del Cerro and Triarhou published a [Micscape article](#) on the Delesse Principle.

Since stereology is based on counting, any of the grids described earlier can be used. Figure 9 shows some additional grids used for stereological measurements.

Zeiss 100-Point Integrating Disk. Figure 8A is a development from the “Zeiss integrating eyepiece 1” (Figure 6D) to include a higher density of grid points. Whether you use a 25 point grid or a 100 point grid is largely personal preference. Some authors recommend 25 points for biological cells as this requires examination of more fields and gives a better overview of the entire sample. Those working with smaller dispersed particles tend to favor a 100 point grid.

Custom Point Count Grid (Figures 4B and 7). This 100-point grid is my own design and is the one I use most often for rock matrix and microfossil counts. The broken circle draws the eye along and keeps you on course while counting. The numbers have no role in the counts, but they help you keep track of your location, and show you in which direction you are scanning. The staggered points give a more uniform field coverage with all points the same distance apart (Figure 9).

Figure 9. A staggered grid has more uniformly spaced intersections than a square grid. **A.** Square grid intersections are at two distances from one another. **B.** A staggered grid based upon equilateral triangles has all adjacent points the same distance apart. The uniform spacing can simplify math for uses such as area calculation, but is not important when doing random point counts.



Chalkley Point Array. The pattern in Figure 8B was devised by cancer researcher H. Chalkley in the 1940s. It has 25 points randomly distributed in the viewing field. This design was formerly available as a Zeiss eyepiece reticle, and can now be had from various reticle manufacturers. One use of this reticle is to count how many of the 25 randomly located points coincide with the material of interest. This so-called “Chalkley count” is sometimes taken to define thresholds of significant vs. insignificant concentrations.

Merz Grid (“Mertz” in some catalogs). The reticle pattern in Figure 8C is not as complicated as it first appears. If you concentrate on the tic marks where counts are made, you see that it is just a 6x6 square grid of 36 points with semicircular connecting lines. Merz grids are useful in situations where straight line grids don't work well. In an image with strong linear features (Figure 10), straight grid lines parallel to structure are hard to see. If the periodicity of the structure matches the spacing of the grid, you can end up in a situation where counting points are all hits or all misses – not a properly random sampling situation. If you must use a square grid with a specimen having periodic structure, the grid should be rotated at an angle to the structure as was done with the horsehair slide in Figure 7. The Merz grid with its curved grid lines cuts across structure at any orientation. Stereological methods that measure intercept length along grid lines can be used. The Merz grid is widely used by medical researchers to estimate volumes and surface areas. The NIH ImageJ software can print a Merz grid on top of any digital image.

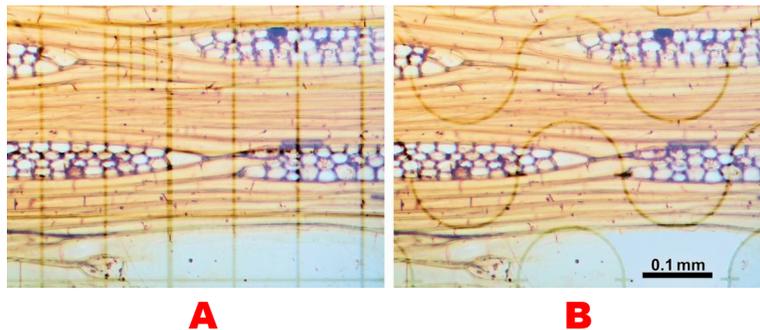


Figure 10. Grids projected onto a linear structure. The subject is a Victorian mount of mahogany wood sections by an unidentified maker. **A.** A square grid doesn't work well for this subject and orientation because horizontal grid lines are hard to see, and because periodic structure can align with the grid giving non-random sampling. **B.** The Merz “wavy line” grid cuts across structure and is easier to use.

Comparison Reticles

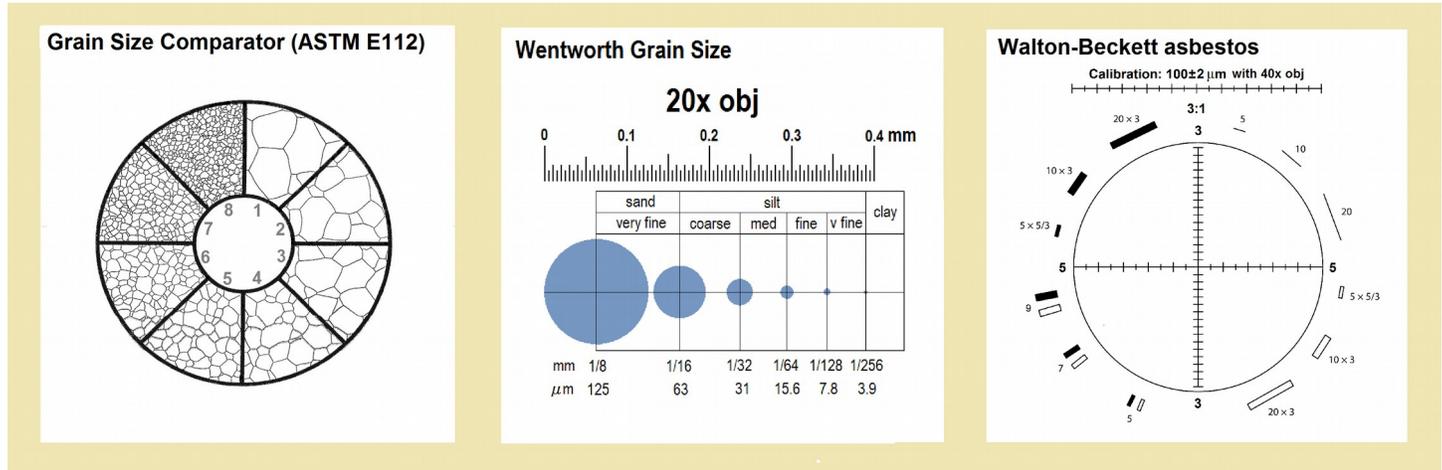
**A****B****C**

Figure 11. Examples of comparison reticles for assigning particles to size classes.

Comparison reticles assign particles to descriptive classes, often on the basis of size, shape, or packing of particles. Another use for comparison reticles is to check tolerances of processed materials. Comparison reticles determine the sizes of sand grains, aerosol sprays, smoke particles, seeds, paint, and many other particulate materials.

Home-made projection reticles that involve size comparison should include a reference scale for length calibration. The projected image of that scale must be adjusted while comparing to a stage micrometer so the projected grid has the correct size for direct-reading. The process was described on page 2 of this article.

Metallurgical Grain Size Comparator. Figure 11A is one of several comparison reticles used by metallurgists. This one is for etched polished surfaces of steel alloys. ASTM publication E1951 documents the calibration of this reticle; ASTM publication E112 covers its use. I don't have a need for this particular reticle, but have always considered it to be a good model for reticle designs that classify other characteristics: geological grain size, grain shape, grain rounding, grain packing, porosity, etc.

Wentworth Grain Size. Figure 11B is a pattern of my own design. Once the projected image is size-adjusted with a stage micrometer, it provides a direct-reading scale for clastic grain size classification. There is a different version of this reticle for each objective magnification: 4X, 10X, 20X, and 40X. This reticle is an example from the family of patterns called “globe and circle reticles”; others of this type include Patterson, Porton, British Standard, Fairs, and Matthews.

The Wentworth scale is used by geologists to describe the size of sedimentary particles; it covers a full range of sizes from boulders through clay. Boundaries between classes begin at 1 mm (the boundary between “very coarse sand” and “coarse sand”) and increase by doubling to 2, 4, 8, etc. mm for the larger size classes. Successive finer grained boundaries halve to 1/2, 1/4, 1/8, etc. mm.

Walton-Beckett Asbestos Reticle (Figure 11C). First, a disclaimer: the Walton-Beckett pattern in this article is strictly for demonstration. Public health protocols for detecting and measuring asbestos are exacting and strict. Reticles must be certified for commercial use. Reticles must be calibrated frequently, and the every-day stage micrometer must itself be calibrated by a reference stage micrometer. A home-made projection pattern would never be used in a commercial asbestos lab (even though the size-adjustable reticle has the same or better accuracy as a fixed commercial reticle).

The Walton-Beckett reticle is used with a biological microscope and a 40X phase contrast objective to examine airborne dust captured on an air filter. Any fibrous (thread-like) particle in the correct size range is assumed to be asbestos – not correct, but this assumption allows the counting to be done by a minimally trained technician rather than by an optical mineralogist. The reticle is designed for counting fibers, and for the rapid determination of fiber length, breadth, and aspect ratio. For more on asbestos sampling and analysis, see the World Health Organization manual [here](#).

Alignment Patterns

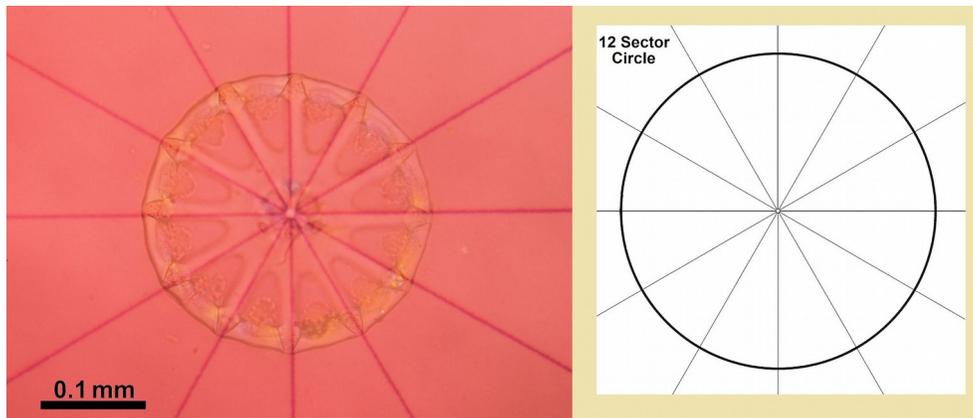


Figure 12. Alignment pattern. Holothurian wheel sclerite from the San Diego Trough, 1277 meters depth, viewed with a 10X objective. The reticle is a guide for arranging additional specimens around the central specimen in a symmetrical pattern. Polarized light and a variable retardation filter (see my earlier Micscape [article](#)) help balance the relative brightness of object and alignment pattern.

The last application I will mention is the use of alignment patterns to arrange specimens for a permanent mount. Diatoms, and diatom-sized objects come to mind. There is a vast published literature on the topic of mounting diatoms. Some past *Micscape* articles include those by Steve Beats ([article](#)), Raymond Hummelink ([article](#)), and Frithjof Sterrenburg ([article](#)).

There are several ways to project alignment patterns into the microscope field of view. A reticle can be placed at one of the conjugate image planes shown in Figure 13: the eyepiece diaphragm, the specimen plane, or the field diaphragm. The drawing attachment offers a fourth method, and it has some advantages. Patterns are easy to construct in a computer graphics program, they are easily printed on ordinary computer paper, and they are a convenient size. Although the pattern is opaque, it can be used in a standard transmitted light microscope that has high magnification lenses available.

A drawing attachment has a practical inconvenience which it shares with eyepiece reticles and field diaphragm projections: the specimen slide is moved during construction, and the specimens lose their registration with the pattern. The exception to this rule is a microfilmed alignment pattern fastened to the construction slide as described by Steve Beats. In this case, the alignment pattern moves with the specimens and holds registration. The drawing attachment is a close second in this regard. The alignment pattern does not automatically remain registered as the construction slide moves, but the pattern is easily shifted on the drawing surface to re-establish registration.

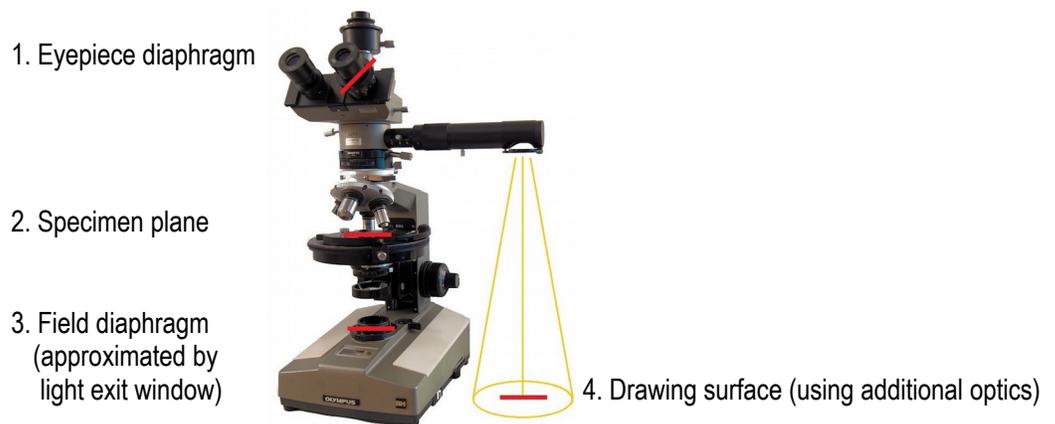


Figure 13. Conjugate image planes in a system adjusted for Köhler Illumination. Objects at the red lines are all in focus at the same time.

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