Designation: E 1951 – 02

Standard Guide for Calibrating Reticles and Light Microscope Magnifications[1]

This standard is issued under the fixed designation E 1951; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers methods for calculating and calibrating microscope magnifications, photographic magnifications, video monitor magnifications, grain size comparison reticles, and other measuring reticles. Reflected light microscopes are used to characterize material microstructures. Many materials engineering decisions may be based on qualitative and quantitative analyses of a microstructure. It is essential that microscope magnifications and reticle dimensions be accurate.

1.2 The calibration using these methods is only as precise as the measuring devices used. It is recommended that the stage micrometer or scale used in the calibration should be traceable to the National Institute of Standards and Technology (NIST) or a similar organization.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 7 Terminology Relating to Metallography[2]

E 112 Test Methods for Determining Average Grain Size[2]

3. Terminology

3.1 *Definitions*—All terms used in this guide are defined in Terminology E 7.

4. Significance and Use

4.1 These methods can be used to determine magnifications as viewed through the eyepieces of light microscopes.

4.2 These methods can be used to calibrate microscope magnifications for photography, video systems, and projection stations.

4.3 Reticles may be calibrated as independent articles and as components of a microscope system.

5. Procedures

5.1 *Nominal Magnification Calculations*:

5.1.1 A calculated magnification, using the manufacturer's supplied ratings, is only an approximation of the true magnification, since individual optical components may vary from their marked magnification. For a precise determination of the magnification observed through an eyepiece, see the procedure describe in 5.5.

5.1.2 For a compound microscope, the total magnification (M_t) of an image through the eyepiece is the product of the objective lens magnification (M_o), the eyepiece magnification (M_e), and, if present, a zoom system or other intermediate lens magnification (M_i). An expression for the total magnification is shown in Eq 1.

$$M_t = M_o \times M_e \times M_i \quad (1)$$

5.1.3 *Example 1*—For a microscope configured with a 10X objective, a 10X eyepiece, and a 1.25X intermediate lens, the total magnification observed through the eyepiece would be calculated as follows.

$$M_t = (10)(10)(1.25) = 125 \quad (2)$$

5.2 *Calibration for Photomicrography Magnifications*:

5.2.1 The magnification of an image can be determined by photographing a calibrated stage micrometer using the desired optical setup. First, photograph the stage micrometer using the desired combination of objective, bellows extension, zoom and intermediate lens, and then measure the apparent ruling length on the photomicrograph. The measurement should be made consistently from an edge or center of one division to the corresponding edge or center of another (see Note 1). By dividing this apparent length of ruling by the known dimension of the micrometer, the magnification of the photomicrograph is determined (see Fig. 1). The accuracy of the calibration is dependent on the accuracy of the calibrated stage micrometer and the scale used to measure the apparent length of the photographed ruling.

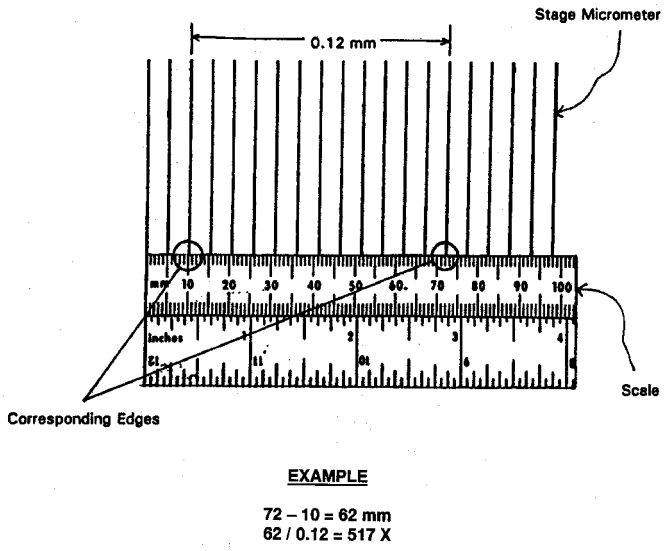
NOTE 1—The choice of using the edge or center of a reticle line depends on the method of manufacture used to produce the measuring device. Some devices are calibrated from center to center while others are measured from one edge to another. Consult with the manufacturer to determine which method should be employed.

[1] This guide is under the jurisdiction of ASTM Committee E04 on Metallography and is the direct responsibility of Subcommittee E04.03 on Light Microscopy.

Current edition approved April 10, 2002. Published June 2002.

Originally published as E1951–98. Last previous edition E1951–01.

[2] *Annual Book of ASTM Standards,* Vol 03.01.



NOTE 1—This schematic shows the procedure used to determine the calibrated magnifications of video screens, video printers, projection screens, and photographs.

FIG. 1 Procedure for Determining Calibrated Magnifications

5.2.2 *Example 2*—For a metallograph with a given configuration (50X objective), determine the calibrated magnification of a photomicrograph.

5.2.2.1 A photograph of a stage micrometer was taken (Fig. 1). A rule was overlaid. From one corresponding edge of a division to another, using the rule, a distance of 62 mm was measured over a known distance of 0.12 mm on the photograph of the stage micrometer. Dividing 62 mm by 0.12 mm yields a photographic magnification of 517X.

5.2.3 By photographing a stage micrometer using various combinations of objectives, intermediate lenses, zoom and bellows extensions, a table can be produced which summarizes the possible magnifications of a system.[3] Microscopes incorporating devices allowing continuous magnification ranges (zooms) should not be used for critical measurements, except by including reference photos of traceable reticles taken concurrently with the measured item. Mechanical play in these devices can be a significant source of error.

5.3 *Calibration for Projection Screens, Video Systems, and Video Printers*:

5.3.1 For projection screens that are not also photographic stations and for video monitors, the magnification can be calibrated as follows. Focus an image of a stage micrometer on the screen, and then measure the projected apparent length of the ruling. If convenient, the measurement can be made directly on the screen, or by transferring the apparent length to a scale using pinpoint dividers. It should not be assumed that a video system has the same magnification in the *x* (horizontal) and *y* (vertical) axis. Further, it should not be assumed that the ratio of the magnification in the *x* direction versus the *y* direction is equal to the ratio of the dimensions of an individual pixel in the *x* and *y* directions. The measurement should be made consistently from an edge or center of one division to the corresponding edge or center of another. The magnification is calculated by dividing the measured apparent length by the known dimensions of the micrometer (see Example 2 in 5.2.2 and Fig. 1).

5.3.2 Magnifications of video prints should be calibrated by use of a print or prints of two measuring devices, one placed on each axis of a single print or one placed on opposite axes on two separate prints. This calibration print should be produced at the same magnification as the prints of interest. Exercise care to ensure that the aspect ratio of the object is reproduced accurately in the print, as the *x* and *y* dimensions of the final print can be adjusted independently through the controls provided on some printers.

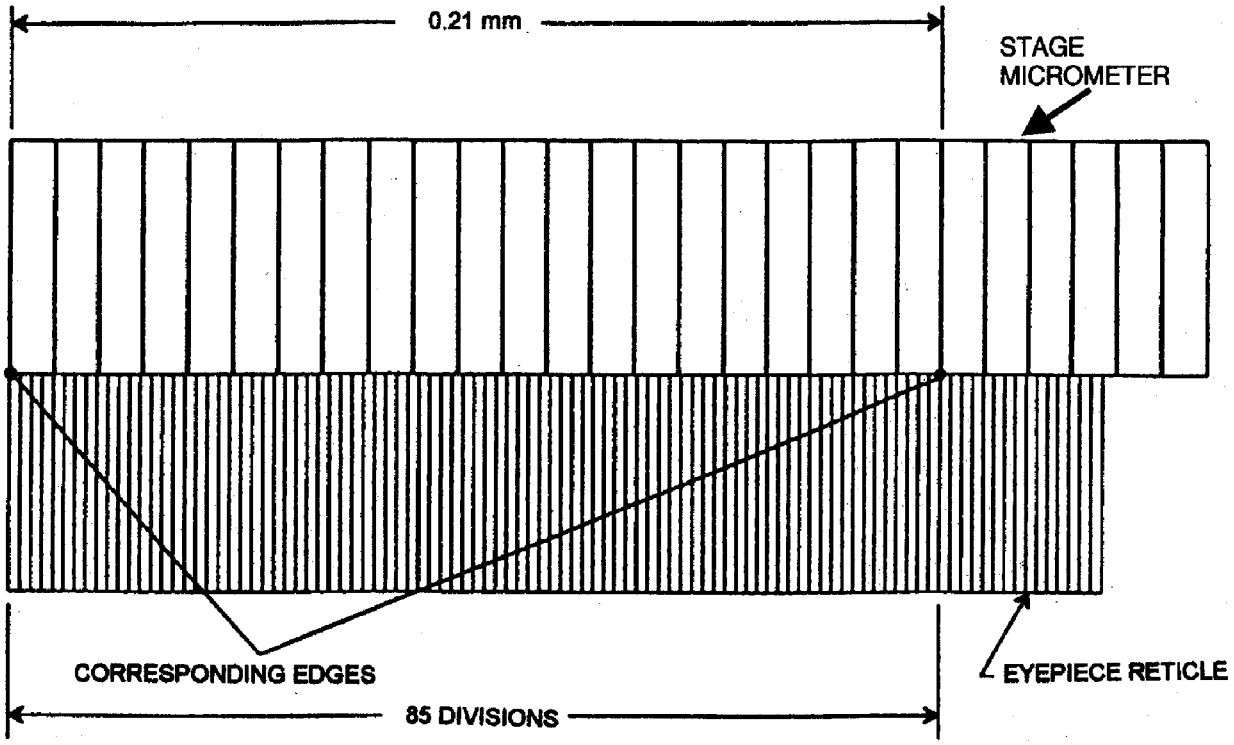
5.3.3 Most high quality video printers will allow some adjustment of the final print dimensions. Major adjustments to magnification should be made by use of intermediate projection lenses or microscope objectives. Increasing magnification by use of video printer controls is not recommended due to the degradation of resolution.

5.4 *Eyepiece Micrometer Calibration*:

5.4.1 To calibrate an eyepiece micrometer reticle, view through the eyepiece an image of a stage micrometer using a given objective and intermediate lens combination. Overlay the eyepiece micrometer image on the stage micrometer image, with one end of each coincident upon one another. The measurement should be made consistently from an edge of one division to the corresponding edge of another (Fig. 2). The eyepiece reticle calibration can be determined by dividing the known length of the stage micrometer by the number of overlaid eyepiece micrometer divisions. This calculation yields a length per division value of the micrometer for a given optical setup.

5.4.2 *Example 3*—For a given microscope configuration

[3] Vander Voort, G. F., Metallography, Principles and Practice, McGraw Hill, New York, NY, 1984, pp. 279-280.



EXAMPLE

(0.21 mm / 85 divisions) (1000 μm / 1 mm) = 2.47 = 2.5 μm / division

NOTE 1—This schematic diagram illustrates the procedure used to calibrate an eyepiece measuring reticle.

FIG. 2 Diagram for Calibrating an Eyepiece Measuring Reticle

(40X objective), determine the length per division value of an eyepiece micrometer.

5.4.2.1 The image of the eyepiece micrometer was aligned with the stage micrometer image (Fig. 2). Eighty-five divisions were counted over a distance of 0.21 mm on the stage micrometer. The length per division can then be calculated as follows.

$$(0.21 \text{ mm} / 85 \text{ divisions})\,(1000 \text{ μm} / 1 \text{ mm}) = 2.47 = 2.5 \text{ μm/division} \quad (3)$$

5.4.3 Repeat the procedure listed above for various objective and intermediate lens combinations to create a table of eyepiece micrometer calibrations.

NOTE 2—In order for the magnification to be consistent from user to user, the eyepiece reticle must be focussed for the user's eyes before focusing the microscope on the image as produced by the objective. Also, the positioning of the reticle in the eyepiece must be repeatable.

NOTE 3—Caution must be observed if both eyepiece tubes are adjustable. Also, change in interpupillary distance may change the magnification, particularly in older microscopes.

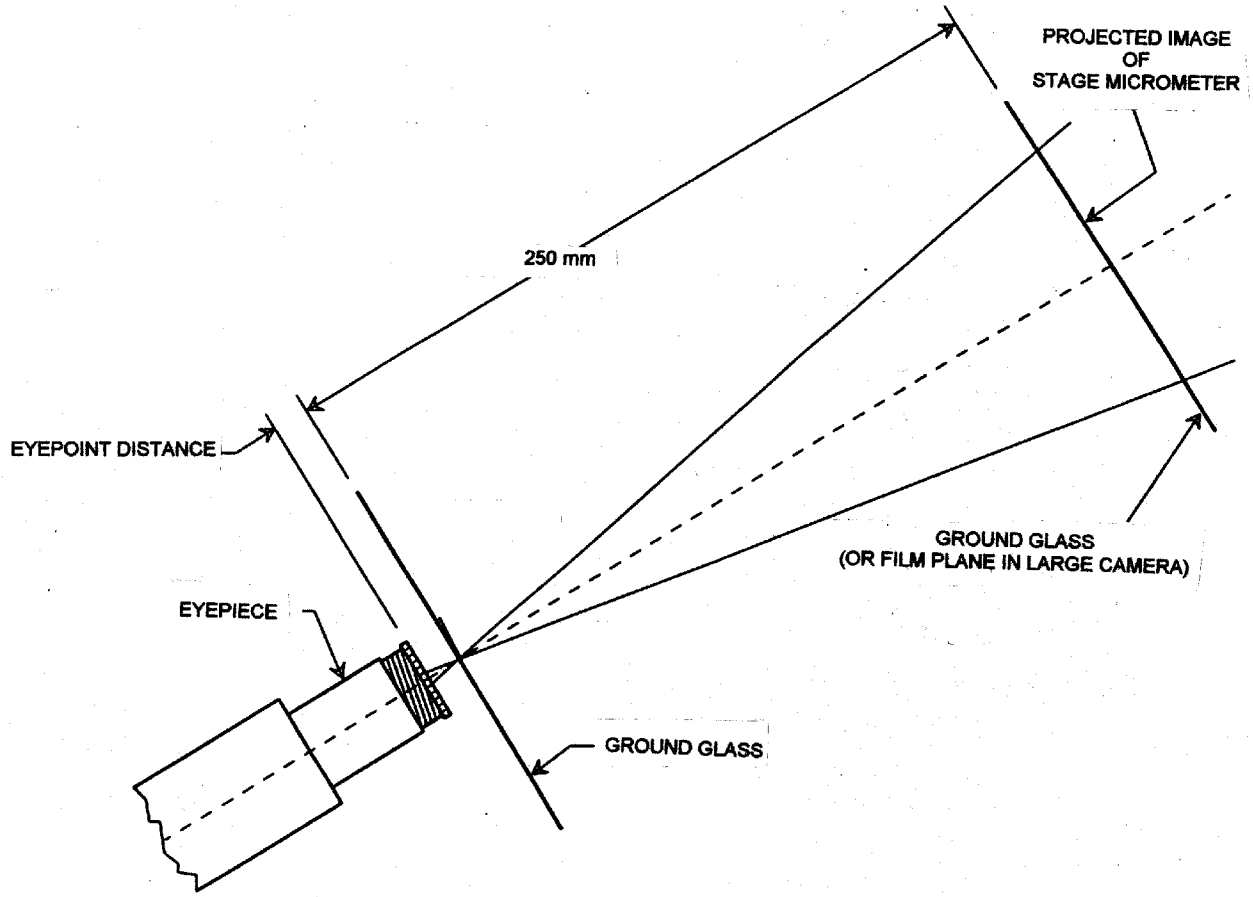
5.5 *Magnification Calibration of Image Viewed Through Eyepieces*:

5.5.1 This procedure will give a calibrated magnification observed through the eyepieces of a particular microscope lens configuration, independent of the user (Fig. 3).

5.5.2 Focus the image of a stage micrometer through the eyepieces. This procedure will require a stage micrometer with high contrast markings.

5.5.3 Determine the position of the eyepoint of the system as follows: (*1*) adjust the lighting on the microscope to a maximum, (*2*) place an opaque or translucent piece of material perpendicular to the light path. A circular projection of the light will appear. (*3*) Move the material away from the eyepiece lens until the size of the circular light beam becomes a minimum. Initially, the size of the beam will decrease until the eyepoint distance is reached, then at a distance greater than the optical eyepoint, the size of the circular projection will increase. (*4*) Note the distance of the eyepoint from the eyepiece lens.

5.5.4 Place an unexposed piece of film or a rigid piece of



NOTE 1—A schematic diagram illustrating the procedure used to determine the magnification observed through the microscope eyepieces.

FIG. 3 Diagram for Magnification Observed Through Microscope Eyepieces

viewing medium, such as ground glass, perpendicular to the light path at a point 250 mm plus the eyepoint distance away from the eyepiece lens. The calibration measurement can then be made directly on the ground glass or on the developed film or resulting print. The calibration is completed by placing the divisions of a rule coincident upon the projected image of the stage micrometer. The alignment should be made consistently from an edge of one division to the corresponding edge of another. (A large-format bellows camera, without lens, may be conveniently used here. If this is done, a film of the image can also be exposed, with the calibration then performed on the developed film.)

5.5.5 Determine the observed magnification by dividing the measured length of the projected section of the stage micrometer by the known length of that section of the stage micrometer.

5.5.6 Repeat this procedure for various objective and intermediate lens combinations to create a table of observable magnifications.

5.5.7 *Example 4*—Determine the magnification viewed through an eyepiece with a microscope configuration consisting of a 10X objective and a 10X eyepiece.

5.5.7.1 Using an overhead transparency, and a rule placed perpendicular to the plane of the eyepiece lens, the eyepoint was determined to be at a distance of 18 mm. Next, a distance of 268 mm was measured perpendicular from the plane of the eyepiece.

5.5.7.2 A viewing medium was fixed at this distance parallel to the plane of the eyepiece lens. The divisions of a rule were placed coincident upon the projected image of the stage micrometer consistently from an edge of one division to the corresponding edge of another. A distance of 89 mm was measured over a known distance of 0.9 mm on the stage micrometer. By dividing the measured length by the known length a calibrated magnification of 99X was determined.

5.6 *Filar Eyepiece Calibration*:

5.6.1 The calibration of a filar measuring eyepiece is similar to that of an eyepiece reticle as illustrated in Fig. 2. The moveable cross-hair in the eyepiece is positioned at an extreme end of a stage micrometer coincident with one micrometer division. The measurement should be made consistently from an edge or the center of one division to another.

5.6.2 For a drum filar eyepiece, note the micrometer drum value. Traverse the cross-hair over as many micrometer divisions as possible that are visible in the central region of the field of view. Note the new micrometer drum value. To obtain the total drum movement, subtract the final drum value from the initial value. The value of each increment on the filar drum is determined by dividing the actual length traversed on the

stage micrometer by the total drum movement. Repeat this procedure for each objective of interest. This calculation is similar to that of determining an eyepiece micrometer calibration (Example 3 in Section 5.4.2.1).

5.6.3 For digital filar eyepieces, a multiplier must by determined for each objective.

5.6.3.1 To determine the value of the multiplier for a specific microscope configuration, set the multiplier to one, and traverse a known distance.

5.6.3.2 The value of the multiplier is determined by dividing the known distance traversed by the value determined by the filar eyepiece.

5.6.3.3 Next, set the instrument to zero, and enter the approximate multiplier into the system. Traverse the stage micrometer as described in the previous section. If the measured distance is incorrect, adjust the multiplier accordingly. Reset to zero, and traverse the stage micrometer again.

5.6.3.4 Repeat these steps until an accurate multiplier has been determined for each objective.

5.6.3.5 *Example 5*—Determine the digital filar eyepiece multiplier for a given microscope configuration. (50X objective). After setting the multiplier to 1, a distance of 250 µm was traversed along an image of a stage micrometer. The filar eyepiece measured 17 698. The multiplier was then determined by dividing 250 by 17 698. The resulting multiplier is 0.01412. Next, the accuracy of the multiplier was checked. First, the system was zeroed, then the multiplier (0.01412) entered, and the distance of 250 µm traversed. The filar eyepiece measured 249.51; therefore, the calibration was complete.

5.6.4 For more specific calibration procedures for digital filar eyepieces, see the manufacturer's instruction manual.

5.7 *Grain Size Comparison Rectile Calibration*:

5.7.1 A grain size reticle consists of a series of figures, representing various grain sizes, which allow an operator to conveniently determine the grain size of a specimen according to Test Methods E112 comparison method (Fig. 4). The following calibration method ensures that the figures represent an accurate ASTM grain size number, G, as defined in Test Methods E112.

5.7.2 This method is applicable to both an eyepiece that has the entire selection of grain sizes visible at once, and to a turret eyepiece which displays only a segment of the entire reticle in the field of view.

5.7.3 All grain size comparison graphics are calibrated for size by the number of grain sections per unit area. The reticle figure area for a single grain size must be measured, then calculated for the equivalent area on the specimen surface. To make this calculation, the microscope system magnification from the specimen to the reticle plane must be determined. Also, the number of grain sections in the reticle figure area must be counted.

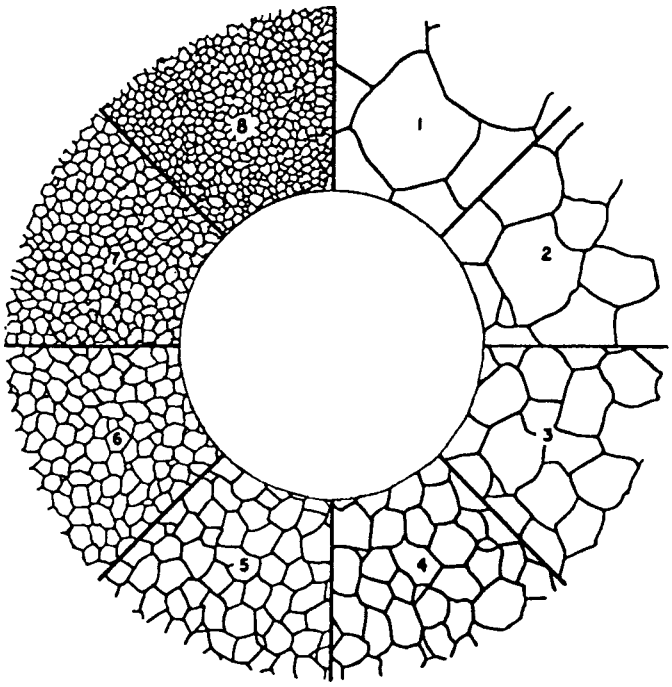
5.7.4 *Calibration Procedure Steps*:

5.7.4.1 Locate two pairs of easily identifiable points in the grain size eyepiece reticle figure, then remove the reticle from the eyepiece.

5.7.4.2 Measure the absolute distances, d_1 and d_2, between the point pairs, preferably by observation of the reticle on a stereomicroscope with a previously calibrated eyepiece micrometer reticle. A schematic diagram illustrating this procedure can be seen in Fig. 5.

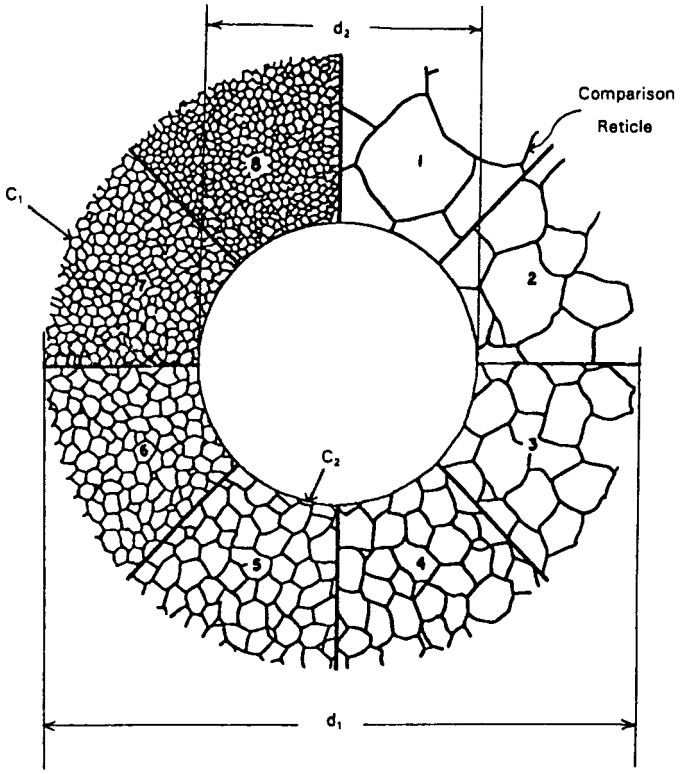
5.7.4.3 Make enlarged photographic prints of each of the grain size sectors from the reticle (or from a contact-printed film negative of the reticle). The print enlargement should be such that all grain sections in the smallest-size sector can be easily counted.

5.7.4.4 Count the grains present in each sector. Count each



NOTE 1—A schematic diagram representing a grain size comparison reticle.

FIG. 4 Representation of Grain Size Comparison Reticle



NOTE 1—A schematic diagram illustrating the measurements described in 5.7.4.2 and 5.7.4.5.

FIG. 5 Diagram of Measurements

grain completely included in the sector, N_{inside}, as one, and count each grain intercepted by the borders of the sector, $N_{intercepted}$, as one half. See Fig. 6.

5.7.4.5 Determine the absolute area of each grain size sector from its photograph by geometric techniques, using the absolute length between the points measured in 5.7.4.2 as the measurement basis.

NOTE 4—Example: Many grain size reticles consist of eight 45° sectors of a circle, C_1, concentric with a smaller, central, image-free circle, C_2. One-eighth of the difference between the two circular areas is the area of each grain sector. The areas of these circles can be determined using the diameters ($A = (\pi / 4) d^2$) illustrated in Fig. 5.

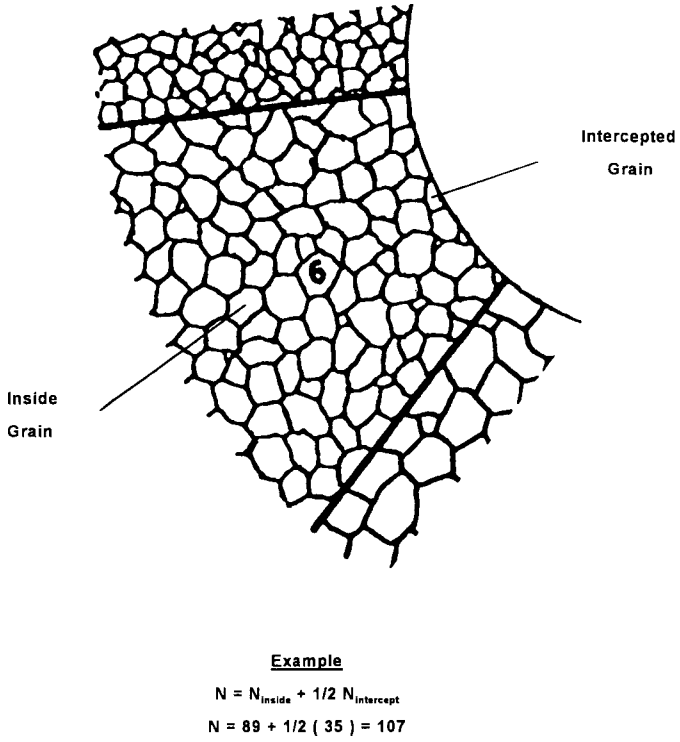
5.7.4.6 Divide the measured area of each sector by the corresponding grain counts as determined for that particular sector in 5.7.4.4. The results will represent the average grain areas for each sector on the reticle.

5.7.4.7 The system magnification must now be calibrated. Observe a stage micrometer through the reassembled grain size reticle eyepiece. Record the apparent distance between the two points located in 5.7.4.1. Repeat this for each optical set-up where the grain size eyepiece will be used. Divide each apparent distance between the points by the absolute distance determined in 5.7.4.2. The result is the system magnification to the reticle for that optical set-up.

5.7.4.8 Calculate the ASTM grain size number, G, shown by each reticle sector, using the following Eq 4. The average grain area of the reticle is measured in mm^2.

$$G = 3.3219 \log_{10} \left[\frac{\text{system magnification}^2}{\text{average grain area of reticle}} \right] - 2.9542 \qquad (4)$$

5.7.5 *Example 6*—Calibrate the ASTM grain size number for the sector labeled six on the comparison reticle.



NOTE 1—A schematic diagram illustrating the counting of the number of grains per sector.

FIG. 6 Counting the Number of Grains Per Sector

5.7.5.1 Absolute distances were measured in Fig. 5 (d_1= 92 mm and d_2= 43 mm). Next, the number of grains in sector 6 were counted (Fig. 6). Eighty-nine inside and thirty-five intercepted grains were measured for a total of 107 grains. Continuing, the absolute area of the sector was determined. Based on diameters of 92 mm and 43 mm, the sector area was calculated as follows.

$$\begin{aligned} \text{sector area} &= 1/8\ (\text{area}_{C1} - \text{area}_{C2}) \\ &= 1/8\ (\pi / 4\ (92)^2 - \pi/4\ (43)^2) \\ &= 649.4\ \text{mm}^2 \end{aligned} \quad (5)$$

5.7.5.2 The average grain area of the sector is then 649.4 mm^2 divided by 107 grains, or 6.1 mm^2. Also, using the procedure described in 5.7.4.7, the system magnification was determined to be 52X. Finally, using Eq. 4 listed previously, the grain size represented in Sector 6 can calculated to be 5.8.

5.7.5.3 Arrange the answers in a table, with columns for the grain size sector numbers and rows for the various optical set-ups.

6. Precision and Bias

6.1 No round robin testing has been done to establish precision or bias estimates for these procedures. Such data will be incorporated when available.

6.1.1 Listed below are considerations for some of the procedures. Also, it should be recognized that in any system where zoom lens settings or bellows draw changes are used, their repeatability is open to question unless fixed mechanical stops are used.

6.2 The value for calculated magnifications in 5.1 is dependent upon the accuracy of the marked magnifications for the objective, eyepiece and any intermediate lens used. For more precise work the procedures listed in 5.5 should be used.

6.3 The precision of the film calibration method in 5.2 and the direct screen measurement in 5.3 is influenced by parallax when measuring the image, and by the accuracy of markings on the scale used. An engraved machinists scale of high quality should be used. The results of a direct screen measurement of video systems can also be influenced by geometrically distorted pictures due to imperfections in the camera or monitor. Changes in magnification may occur on some video monitors as the monitor controls are adjusted. Also, the dimensions of the pixels of the camera might not match those of the monitor, further reducing the precision.

6.4 Calibration of an eyepiece micrometer with a stage micrometer image per 5.4 is straightforward. The most likely error is in estimating fractions of a division, when the two images do not coincide perfectly at both ends of the measured distance.

6.5 Grain size reticle calibration performed according to 5.7 is tedious, but uncomplicated. The measurements in 5.7.4.2 and 5.7.4.7 are subject to the same error noted in 6.4.

NOTE 5—Grain size reticles are used according to the comparison procedure in Test Methods E 112, in place of the wall charts or transparency overlays. Many commercial reticles do not use the ASTM graphics for their reticle illustrations. They often display a tighter range of grain section areas than the log-normal distribution of grain areas found in an actual cross-section of ferrite microstructure. Further, the commonplace 8-sector reticle allocates only about one-tenth of the field of view to each grain size, thus reducing the available standard area, compared to the ASTM graphics. Both of these facts, if applicable, should be considered when estimating grain size with comparison reticles.

7. Keywords

7.1 calibration; filar micrometer; grain size; magnification; microscope; photography; reticle; stage micrometer; video systems