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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.

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

[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.

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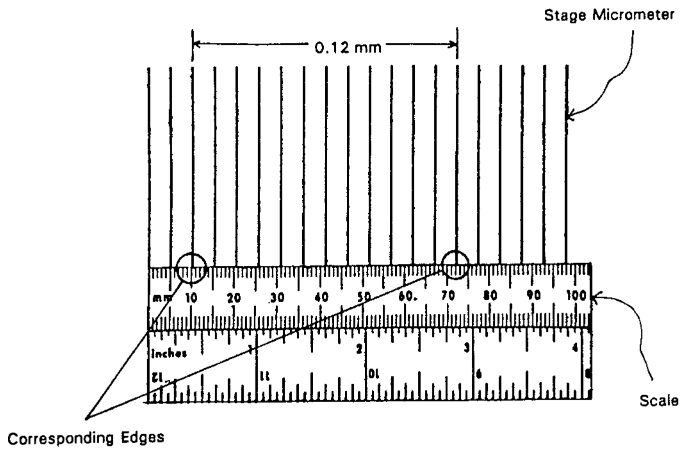
[2] *Annual Book of ASTM Standards*, Vol 03.01.



EXAMPLE

72 - 10 = 62 mm

62 / 0.12 = 516 X

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

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 same *x:y* ratio exists on the screen as in pixel representation. 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 Section 5.1.3 and Fig. 1).

5.3.2 Magnifications of video prints should be calibrated by use of a print of two measuring devices, one placed on each axis of the print. 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.

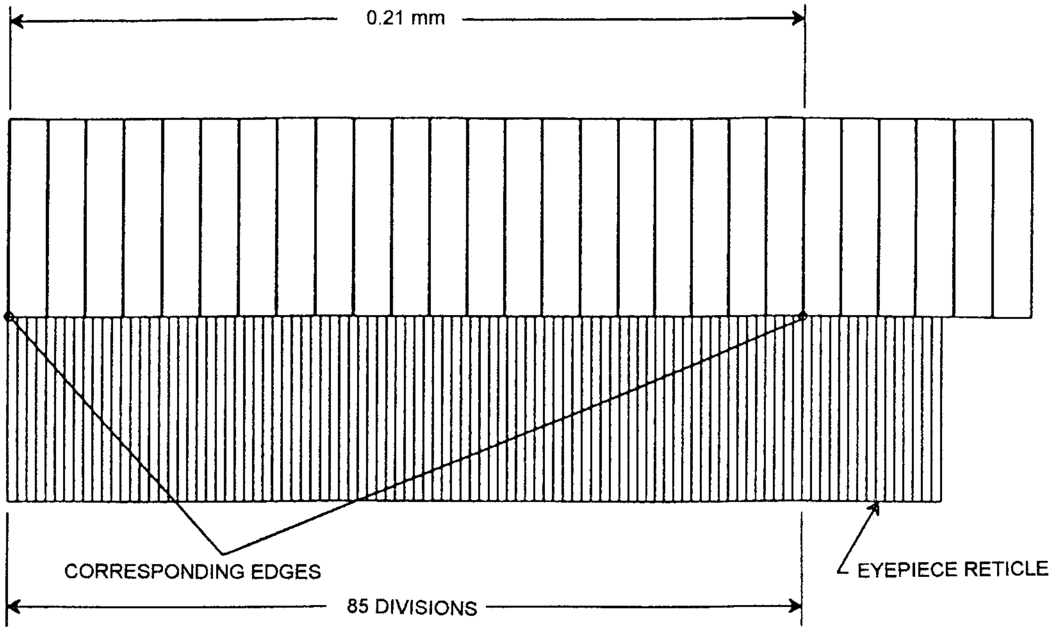
5.4.2 *Example 3*—For a given microscope configuration (40X objective), determine the length per division value of an eyepiece micrometer.

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.

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

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

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



EXAMPLE

$$(0.21 \text{ mm} / 85 \text{ divisions})(1000\ \mu m / 1 \text{ mm}) = 2.47 = 2.5\ \mu m / \text{division}$$

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

FIG. 2 Diagram for Calibrating an Eyepiece Measuring Reticle

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 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.

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