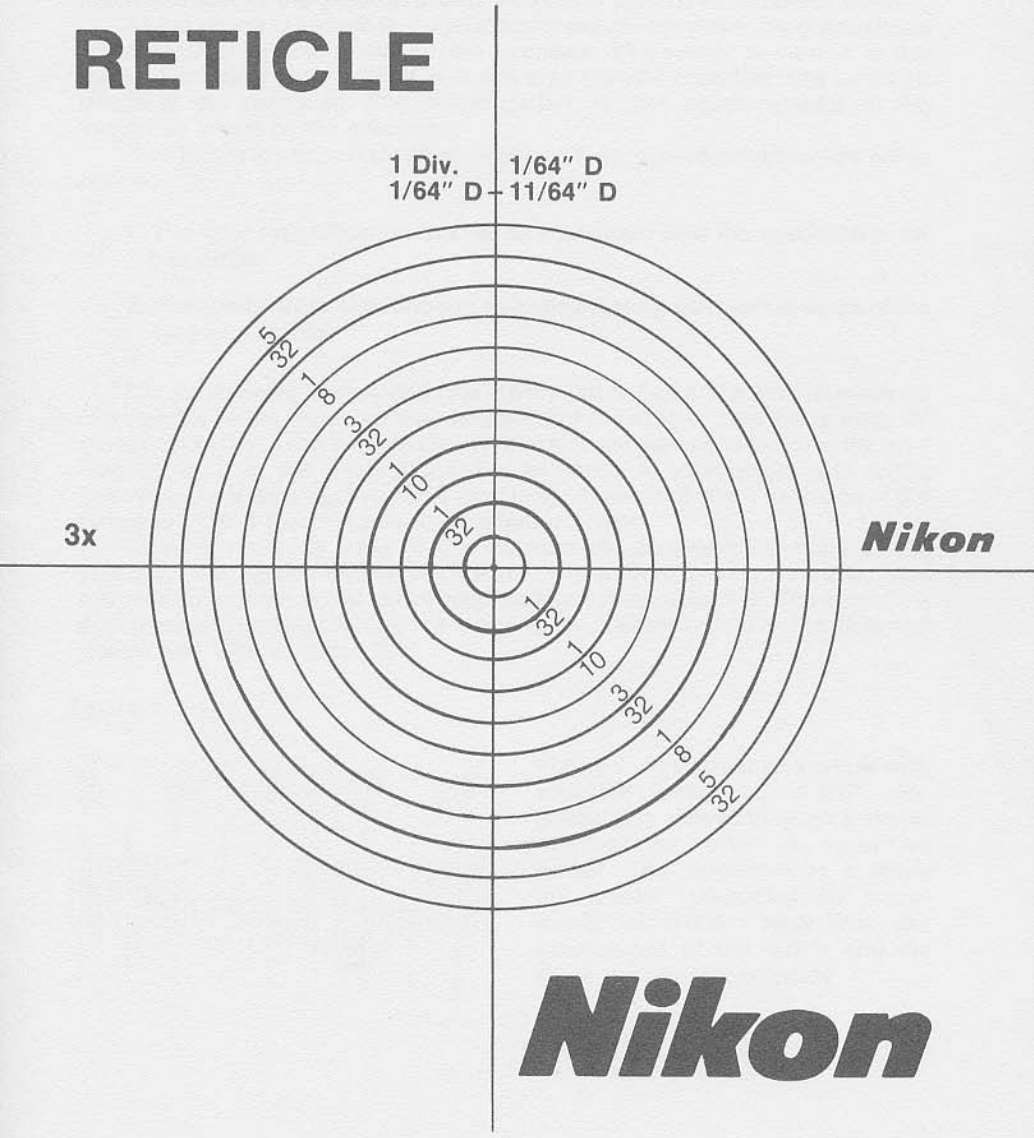
HOW TO USE THE MICROSCOPE EYEPIECE RETICLE



Nikon

USING THE MICROSCOPE EYEPIECE RETICLE

By Ivan Feldman

Few users of microscopes have an understanding of the simple, but necessary, techniques needed for accurate measurement.

Generally, texts on microscopy do not explain how to calibrate and/or measure with a microscope. Errors in magnification and dimensions are easily committed and true magnification is often ommitted entirely or only approximated.

The following information will be helpful:

It is essential to understand how the eyepiece reticle can assist you. Graduations on the reticle scale have an absolute value. The *absolute value* is the actual size of the graduations as measured against an absolute scale.

As the reticle is viewed through the microscope eyepiece, the graduations are magnified by the power of the eyepiece. The reticle pattern is in the position of the object plane. It is at this area that the objective lens forms an image of the specimen. The reticle pattern is then superimposed on the magnified image of the specimen.

Two things are important when selecting the eyepiece power for use with a reticle:

1. The total magnification desired to accurately view the specimen to be measured.

2. A magnification high enough to clearly distinguish each division of the eyepiece reticle.

It is essential to understand that the magnification of the eyepiece has *no relationship* to the measurement capability of the reticle. It serves to magnify the reticle so that it can be seen clearly, and also serves as one factor in the total magnification of the microscope. For example: A microscope with a 10x eyepiece and a 3x objective has a total magnification of 30x. The image at the reticle is 3x and then magnified by a factor of 10x.

When a reticle is used in the microscope, an enlarged image of the specimen is projected on the reticle scale. The amount by which the specimen image is enlarged is equal to the magnification of the objective. The size of the specimen is found by *dividing the absolute value of the reticle by the objective power.* See Figures 1 and 2.

Figure 1

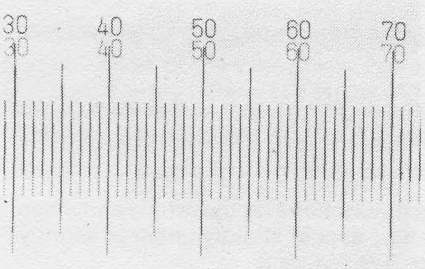


Figure 1 shows eyepiece reticle with absolute value of 0.005" per graduation viewed through a stereo microscope with 1x objective power. The specimen is a stage micrometer graduated in increments of 0.005". Note that the graduations of the reticle and the stage micrometer coincide.

Figure 2.

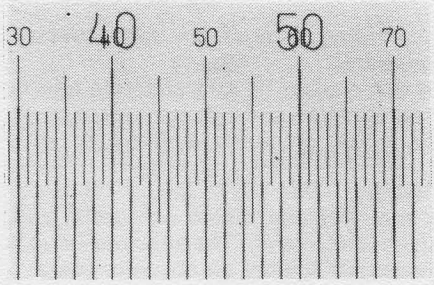


Figure 2 shows the same reticle as in Figure 1. Absolute value of 0.005". per graduation. These can be seen as the thinner lines, viewed through a stereo microscope with 2x objective. The specimen can be seen as the darker, heavier lines, which is a stage micrometer graduated in increments of 0.005". Note that the graduations of the stage micrometer coincide with every second line of the eyepiece reticle. From this it can definitely be determined that the objective power is *exactly* 2x. Now divide the absolute value of the reticle (0.005") by the objective power (2x) and determine that each graduation of the reticle has a value of 0.0025".

To make the measuring process easier, many reticles supplied for microscopes are not of the absolute type, but rather the graduations have been accurately enlarged by a certain factor. For example, a reticle of 0.005" specifically designed for use in a measuring microscope for tool room use, with 3x objective, would have an absolute value of 0.015".

Using the methods so far discussed, accurate measurements may be taken only when the objectives can be adjusted to an exact magnification. Objectives furnished with the Nikon Measurescope are of this type and allow extremely accurate measurements without the use of a stage micrometer.

Stereo microscopes and Research microscopes use objectives which are not adjustable to a precise magnification factor. (The exception is a Zoom Stereo microscope which can be adjusted to exact objective magnification by using an eyepiece reticle and stage micrometer of known value.) While each of these objectives has a stated nominal value, they are not primarily designed for measuring applications. When accurate measurements are to be taken with these instruments, observe the following:

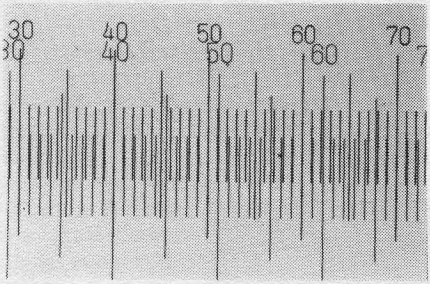
1. Place a stage micrometer with a known value on the stage with the microscope objective in place.

2. The image of the stage micrometer is superimposed on the eyepiece reticle similar to Figure 1 and 2.

3. If the graduations coincide exactly, as in Figure 1, proceed with the measurements, knowing that each graduation is equal to the stated value of the stage micrometer.

4. In most cases you will find that the graduations on the reticle and stage micrometer do not coincide. They may appear slightly under or slightly over the graduations of the reticle. See Figure 3.

Figure 3.



The eyepice reticle is represented by the top graduations, the stage micromer by the lower.

The stage micrometer has a known value of 0.005" per increment. Note, however, that the graduations of the stage micrometer and reticle do not coincide as they did in Figure 1.

Starting at #40 where both stage micrometer and reticle coincide, the graduation line #50 of the reticle coincides with graduation line #49 of the stage micrometer. Using graduation #40 as a zero point, it takes 10 graduations of the reticle to reach 9 graduation of the stage micrometer. Therefore, the reticle cannot be measuring exactly 0.005" per graduation. Instead, the reticle values are higher than those of the stage micrometer. The value of the reticle must, therefore, be lowered so that it can measure accurately.

In order to determine the exact value of each graduation of the reticle, divide the number of graduations of the stage micrometer by the number of graduations of the reticle. Multiply the answer by the value of the stage micrometer.

In the case of Figure 3:

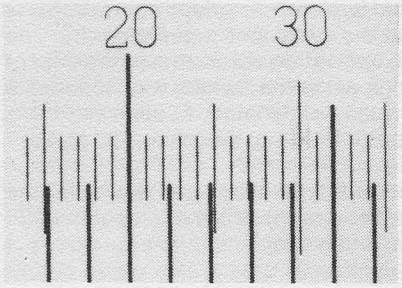
$$\frac{\text{Stage Micrometer Graduations}}{\text{Reticle Graduations}} \times 0.005''$$

$$9/10=0.9 \times 0.005''=0.0045''$$

The result of 0.9 is known as the *calibration constant.* The result 0.0045 is the measuring value of *this* particular reticle used with this particular objective.

In the example of Figure 3, the approximate value of the reticle was known. If the approximate value is not known, you may use the following method:

Figure 4.



The darker lines of Figure 4 are the stage micrometer (0.005" per increment). The smaller number lines are the eyepiece reticle (value unknown).

Starting at #20 of the reticle as the zero point, 12 lines of the reticle coincide with 5 lines of the stage micrometer.

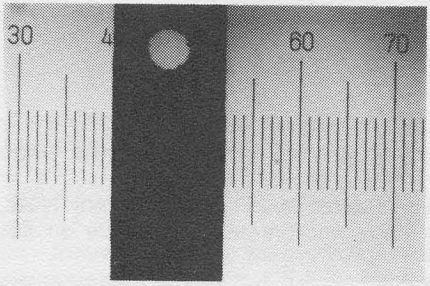
Stage micrometer value = 0.005" x 5 = 0.025".

Eyepiece Reticle = 0.025"÷12 = 0.0021".

The value of each graduation of eyepiece reticle is 0.0021" *when used with this objective only.*

Once the value of the reticle is known, there are two methods that can be used to make accurate measurements with the eyepiece reticle.

Figure 5.



In Figure 5, the specimen (wide black line) is viewed through a 10x eyepiece fitted with a 0.005" reticle using a Stereo microscope with 1x objective. The results of calibration were those illustrated in Figure 3.

Using the graduation #40 as a zero point, observe that the specimen covers exactly 12 graduations.

1. Multiply the calibrated value of the reticle by the number of graduations covered by the specimen.
   0.0045" x 12 = 0.054", or
2. Uncalibrated value of the reticle multiplied by the number of covered by the specimen multiplied by the calibration constant
   0.005" x 12 = 0.06 x 0.9 = 0.054"

Once the calibration constant or calibrated value of a reticle is determined, the stage micrometer need not be used for further measurements. Since the objectives are subject to plus or minus tolerances, this type of calibration must be carried out with *each objective* used with the eyepiece reticle.

When making precise measurements using a Stereo Zoom microscope, it will be necessary to use a stage micrometer *each time the objective zooming ring is moved.* Although the zoom ring is graduated with the nominal objective powers, it is impossible to return the zoom control to exactly the same position that would be necessary for precise measuring purposes.

Microscope Eyepieces

A microscope eyepiece is designed to further magnify the primary image formed by the microscope objective, and also limit the field of view.

In Nikon's revolutionary new CF Optical System, chromatic aberration has been corrected in both the objectives and the eyepieces independently. The result is a dramatic reduction of chromatic aberration across the entire field of view. The orange coloring around the fringe of the field which was noticeable in the compensating system has been virtually eliminated. Eyepieces of this type, when used with a reticle, exhibit no color fringing; therefore, 100 percent of the field can be utilized.

There are a number of other conventional-type eyepieces available (the Huygenian and the Ramsden being the most common). Many microscopes still offer Huygenian-type eyepieces. However, when eyepiece reticles are used, a Ramsden or Kellner eyepiece should be used.

The Huygenian eyepiece is of simple construction, consisting of two plano-convex lenses mounted with convex sides towards the objective. The lens nearest the eye is known as the eye lens, and the one closer to the objective is known as the field lens. This type of eyepiece is also uncorrected, giving a blue fringe to the edge of the field and is best suited for low-power achromatic objectives. The principal image is formed between the two lenses, making it inconvenient for use with a reticle whose accuracy is affected by the aberrations of the eye lens alone.

The Ramsden eyepiece is of construction similar to the Huygenian, except that the field lens has the plane side nearest the objective. The diaphragm is located below the lens system. This type of eyepiece has the advantage of imparting less distortion to scales and lines than the Huygenian; and therefore, its main use is for micrometry, as the reticle is placed on the field diaphragm.

The Kellner eyepiece is an improved Ramsden ocular with an achromatic doublet for the eye lens, allowing the chromatic aberration of the field lens to be more fully corrected. The eyepiece has a high eye point, useful to spectacle wearers, but does suffer from some distortion. As the lower focal plane is below the field lens, any aberrations will effect the primary image and eyepiece reticle equally. The main use of the Kellner eyepiece, therefore, is in measurements with the microscope.

The compensating eyepiece is generally constructed of two seperate lenses, one or both of which are compound. This eyepiece may be recongized by the color of the fringe around the inside edge of the diaphragm when daylight is viewed through the eyepiece. Ordinary eyepieces show a blue fringe, while compensating eyepieces show a yellow, orange, or red fringe.

The chromatic difference of magnification common to all high-power objectives can be corrected by using a compensating eyepiece. This eyepiece not only corrects for the chromatic difference of magnification introduced by the objectives but is also designed to correct image curvature to some extent. The compensating eyepiece should not be used with low-power achromatic objectives, because color aberration may be introduced. With the Nikon CF Optical System, it is no longer necessary to use compensating eyepieces.

Filar Micrometer Eyepieces

The use of a Filar Micrometer eyepiece is one of the most precise and accurate means of measurement. For occasional measurments the eyepiece reticle serves a useful purpose; but for greater precision and accuracy, the filar eyepiece is absolutely essential.

The Filar Micrometer eyepiece is fitted with a drum which activates a vertical cross hair which travels across a fixed vernier scale. Some filar eyepieces have a graduated scale incorporated with the cross hair and both move simultaneously. There are two types of Nikon Filar Micrometer eyepieces available:

1. External Reading Inch System used on Measuring Microscopes.

Eash division on the calibrated outside drum represents an absolute travel of 0.005" of the internal crossline. The outside drum has 50 divisions; therefore, each complete rotation of the drum is equal to 0.025".

The non-rotating portion of the drum is divided into 16 divisions, each representing 0.025". The total travel of the filar micrometer is 0.400".

The outer knurled knob of the micrometer drum also rotates and acts as a zero setting device. The internal crossline will move and may be positioned at any desired portion of the specimen. This feature allows measurements to always begin with a zero reading on the micrometer drum.

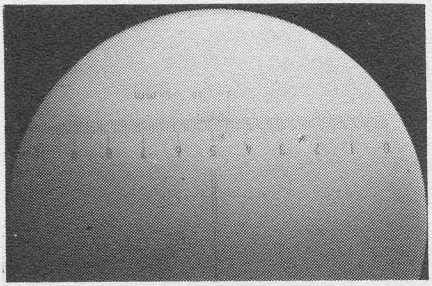
The internal moving crossline and scale is divided into an absolute value of 0.025" per division. Calibration can be done easily by using a stage micrometer with reference to the graduations of the internal scale.

Since the actual measuring value is found by dividing the absolute value of the micrometer drum (0.0005") by the objective magnification, the following table will give the value of the micrometer divisions and the internal scale divisions. The magnification of the objectives must be accurate to obtain these results.

OBJECTIVE	*MICROMETER DIVISION*	*INTERNAL SCALE DIVISION*
1x	0.0005"	0.025"
3x	0.00016"	0.0083"
5x	0.0001"	0.005"
10x	0.00005"	0.0025"
20x	0.000025"	0.00125"
40x	0.0000125"	0.000625"
100x	0.000005"	0.00025"

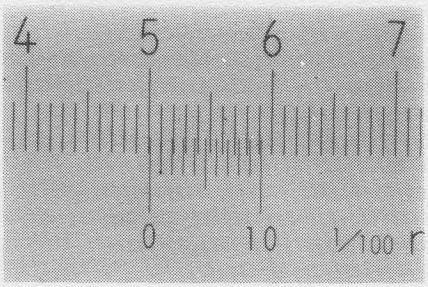
Internal Reading Metric System for All Research-type Microscopes. All measurements on this CF Filar Micrometer are taken through the eyepiece. The outside drum of this eyepiece is not graduated and only serves to move the reference line and main scale across the fixed vernier scale.

Figure 6.



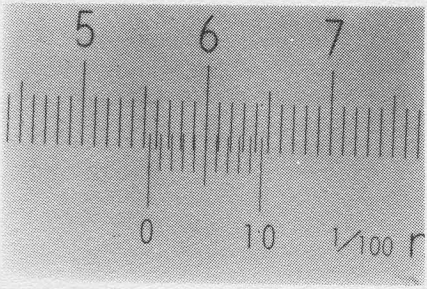
The main scale has a total travel of 10 millimeters. The scale is also divided into 100 graduations with each division representing 0.1 millimeter. The small fixed vernier scale located directly under the main scale is divided into 10 divisions, making possible measurements of 0.01 millimeter. These values are absolute and will change proportionately according to the power being used.

Figure 7.



View through filar micrometer eyepiece with reading of 5.01 millimeters.

Figure 8.



View through filar micrometer eyepiece with reading of 5.55 millimeters.

Since this filar micrometer eyepice is used with objectives with a stated *nominal* value, it is important to always calibrate this filar micrometer with a stage micrometer.

The Nikon Metric Filar Micrometer eyepiece has a unique feature which makes calibration with different objectives relatively easy. The lower portion of the eyepiece has a graduated ring which when rotated optically changes the effective tube length so that the stage micrometer can coincide exactly with a portion of the internal scale of the filar. With most other filar micrometers, the chances of exactly super imposing the filar scale to the stage micrometer would depend on the accuracy of the objective lens.

The following table will give you the units of measurements that you can obtain with different objectives:

OBJECTIVE	*MAIN SCALE*	*VERNIER SCALE*
1x	0.1mm	0.01mm
2x	0.05mm	0.005mm
2.5x	0.04mm	0.004mm
4x	0.025mm	0.0025mm
5x	0.020mm	0.0020mm
10x	0.010mm	0.0010mm
12.5x	0.008mm	0.0008mm
15x	0.0066mm	0.00066mm
20x	0.005mm	0.0005mm
30x	0.0033mm	0.00033mm
40x	0.0025mm	0.00025mm
60x	0.0016mm	0.00016mm
100x	0.001mm	0.0001mm

There are a few microscope accessories that change the effective magnification of the objectives. For example, when using a teching head or D.I.C. attachment, there is a factor of 1.25x to be considered A 10x objective is effectively a 12.5x objective when used with these attachments. An Epi-Fluorescent Illuminator has a factor of 1.5x; and, therefore, the same 10x objective has an effective magnification of 15x instead of 10x.

The following table will assist in determining the nominal value of different measuring reticles at various common objective powers. Each reticle must be calibrated to each objective for precise measurements.

#75950
Eyepiece Reticle 0.1" length, 100 graduations:

Objective Power	*Value p/grad.*
1x	0.001"
2x	0.005"
3x	0.00033"
4x	0.00025"
5x	0.0002"
10x	0.0001"
40x	0.000025"

#75952
Eyepiece Reticle 0.6" length, 200 graduations:

Objective Power	*Value p/grad.*
1x	0.003"
2x	0.0015"
3x	0.001"
4x	0.0075"
5x	0.0006"
10x	0.0003"
40x	0.000075"

#75951
Eyepiece Recticle 0.4" length 200 graduations:

Objective Power	*Value p/grad.*
1x	0.002"
2x	0.001"
3x	0.0066"
4x	0.0005"
5x	0.0004"
10x	0.0002"
40x	0 00005"

#75956
Eyepiece Reticle 0.5" length, 100 graduations:

Objective Power	*Value p/grad.*
1x	0.005"
2x	0.0025"
3x	0.00166"
4x	0.00125"
5x	0.001"
10x	0.005"
40x	0.000125"

#75955
Eyepiece Reticle 0.5" length 50 graduations:

Objective Power	*Value p/grad.*
1x	0.010"
2x	0.005"
3x	0.0033"
4x	0.0025"
5x	0.002"
10x	0.0010"
40x	0.00025"

#75957
Eyepiece Reticle 0.6" length, 40 graduations:

Objective Power	*Value p/grad.*
1x	0.015"
2x	0.0075"
3x	0.005"
4x	0.00375"
5x	0.003"
10x	0.0015"
40x	0.000375"

#75960
Eyépiece Reticle 5mm length, 100 graduations:

Objective Power	*Value p/grad.*
1x	0.05mm
2x	0.025mm
3x	0.0166mm
4x	0.0125mm
5x	0.01mm
10x	0.005mm
40x	0.00125mm

#75961
Eyepiece Reticle 10mm length, 100 graduations:

Objective Power	*Value p/grad.*
1x	0.10mm
2x	0.5mm
3x	0.33mm
4x	0.25mm
5x	0.20mm
10x	0.01mm
40x	0.025mm

#75962
Eyepiece Grid Reticle 1mm x 1mm 100 squares:

Objective Power	*Value p/grad.*
1x	0.10mm
2x	0.05mm
3x	0.033mm
4x	0.025mm
5x	0.02mm
10x	0.010mm
40x	0.0025mm

#75963
Eyepiece Grid Reticle 0.5mm x 0.5mm 100 squares.

Objective Power	*Value p/grad.*
1x	0.5mm
2x	0.25mm
3x	0.16mm
4x	0.125mm
5x	0.10mm
10x	0.05mm
40x	0.0125mm

Mounting a Reticle in the Eyepiece

The correct position of the reticle in the eyepiece is easily found, since in most oculars, a diaphragm or field stop is located in the ocular focal plane. To insert a reticle, unscrew the bottom retainer of the CFW 10x or CFW 15x eyepiece. Insert a *21mm reticle.* The reticle will seat in its proper position. Care should be given to orientation of the reticle so that the numbers of the reticle can be seen in their correct position when viewed through the eyepieces. Reinsert the retaining ring and tighten. The reticle can be focused in the eyepiece by rotating the top of the eyepiece in the normal manner.

A simple reticle holder is available for mounting recticles in the stereo microscope eyepieces. A *24mm* reticle is cemented into the reticle holder. The entire assembly is then inserted from the bottom of the eyepiece barrel and moved towards the eye lens until proper focus is obtained. The reticle holder will maintain its position due to spring tension of the holder on the sides of the eyepiece barrel. The focus point of the reticle can easily be changed to accommodate the user's eye by moving the entire assembly in or out.

Before using this reticle holder, the diaphragm of the stereo eyepiece must be removed to allow the reticle holder to slip into the eyepiece tube.

Reticles used with measuring microscopes are supplied in a small reticle holder which easily drops into the optical head just below the eyepiece. These eyepieces have a diopter adjustment so that a clear image of the reticle can always be obtained. Measuring microscopes require a 24mm reticle.