1	Area:	SOP NO:
2	Prepared /Review Date:	Page No:

OCULAR and STAGE MICROMETERS

Size is one of the most important physical features employed in the identification and characterization of an organism. The exact size of a microorganism can only be determined by utilizing a calibrated **ocular micrometer**. An ocular micrometer (Fig. 2-1) is a glass disc on which a series of uniformly spaced lines has been inscribed. The ocular micrometer is placed in one of the eyepieces of the microscope; however, the distance between the etched lines depends upon the objective lens used to view the specimen. In order to determine the precise distance between the lines of an ocular micrometer, it must be calibrated with a **stage micrometer** (Fig. 2-2). The inscribed lines on a stage micrometer are exactly 0.01 mm (or 10 μm) apart.

In order to calibrate the ocular micrometer for a particular objective lens, the ocular and stage micrometers are superimposed, and the number of ocular graduations per stage

micrometer graduation is determined. Figure 2-3 indicates that six ocular micrometer graduations fit between two stage micrometer graduations; therefore, one space of the ocular micrometer is equal to 10 µm/6 or 1.66 µm. In order to determine the dimensions of an organism, the number of graduations occupied by the organism (Fig. 2-4) is counted and multiplied by the distance between graduations. For example, if an organism occupied the space of seven graduations, this particular dimension would be 7 x 1.66 or 11.6 µm.

Very seldom will an exact number of ocular micrometer lines fit between two stage micrometer lines. If that is the case, the number of stage micrometer lines is divided by the total number of ocular micrometer lines in order to determine one ocular graduation. **EXAMPLE**: 25 graduations on the ocular micrometer precisely match 4 graduations on the stage micrometer.
Remembering that the graduations of the stage micrometer are 10 µm apart, one ocular graduation = 40/25 = 1.6 µm.

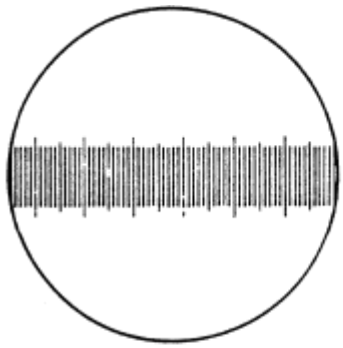

Figure 2-1. Ocular micrometer.

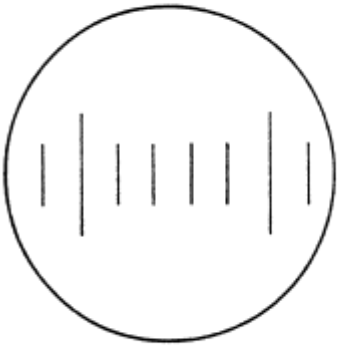

Figure 2-2. Stage micrometer.

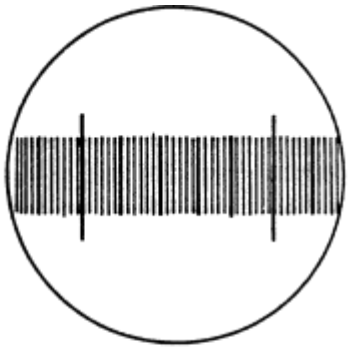

Figure 2-3. Ocular micrometer superimposed on stage micrometer.

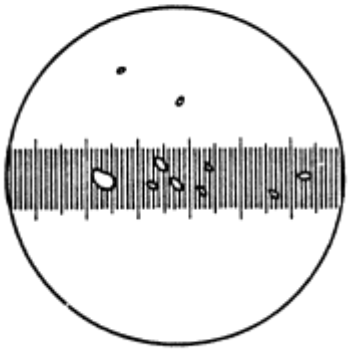

Figure 2-4. Use of the ocular micrometer to measure organisms.

Material:

1. Ocular micrometer
2. Stage micrometer
3. Prepared slides of bacteria

Procedure:

1. Obtain a stage micrometer from the instructor. Place the stage micrometeron the stage of the microscope.

2. Rotate one of the microscope eyepieces until the lines of the ocular micrometer are parallel with those of the stage micrometer.
3. Match the lines on the left edges of the two micrometers by moving the stage micrometer so that the graduations of the ocular micrometer are superimposed over those of the stage micrometer.

4. Determine the number of ocular micrometer spaces that fall within a given number of stage micrometer spaces.

5. Calculate the distance between each ocular graduation by using the following formula:

1 ocular micrometer space (μm) = $\frac{\text{x spaces on the stage micrometer}}{\text{y spaces on the ocular micrometer}}$

6. Repeat the procedure for the 10X and 40X objectives and record results. **Estimate** the calibration of the 100X oil-immersion objective lens by dividing the value for the 10X objective by 10.

Results:

4X objective	
10X objective	
40X objective	
100X objective	