

# Basic Microscopy

## Ocular Micrometer Calibration

Microorganisms are measured in micrometers or microns. To measure organisms under the microscope, a calibrated ruler that is etched on a glass disk is used. This is called an ocular micrometer. The distance between each engraved line is called an ocular division or O.D. The other component to measure microorganisms is the stage micrometer. It is used to determine the exact distance measured by an ocular distance.

The lines in the stage micrometer are exactly ten microns apart. Step 1: Remove the eyepiece and insert the ocular micrometer into a 10X eyepiece. The ocular micrometer is divided into ocular divisions. Step 2: Place the calibrated stage micrometer slide on the stage above the light source. Using both the coarse and fine adjustment knobs on the microscope, focus until a sharp view is obtained on the engraved lines of the micrometer.

Step 3: Now adjust the field so the 0 line of the ocular micrometer scale is exactly superimposed upon the 0.0 line of the stage micrometer scale. Step 4: Without moving the stage micrometer, locate the point as far to the extreme right as possible, where a line on the ocular micrometer is superimposed over a line on the stage micrometer.

Step 5: Count the number of ocular division that are in the actual distance measured on the stage micrometer to the point where the lines of both the ocular micrometer and stage micrometer are superimposed on each other. Step 6: Count the number of ocular divisions on the ocular micrometer between the 0 line and the superimposed line to the far right.

Divide the distance determined in Step 5 by the number of ocular divisions in Step 6 and multiply by 1000 to give the ocular micrometer units in microns. Repeat Steps 3 through Step 6 for each objective on the microscope. If at any time the ocular micrometer is moved to a different microscope or a new objective is added to the microscope, the ocular micrometer calibration procedure must be completed again.