



Microscope Calibration

Why is it important to calibrate each microscope?

Since no two microscopes are identical, assessing the same semen sample with different microscopes may yield different results. To adjust for differences between microscopes, a correction factor should be applied to ensure accurate sperm concentrations are obtained. The correction factor (**F**) must be determined for each objective used with the microscope.

Calibration Procedure:

To determine **F**, you will need an eyepiece reticle and a stage micrometer.

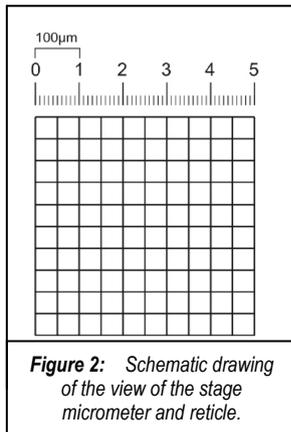


Figure 2: Schematic drawing of the view of the stage micrometer and reticle.

1. Insert the eyepiece reticle (or use the auxiliary eyepiece provided with the microscope which includes a built-in reticle). Figure 1 shows the view through the eyepiece.
2. Position the stage micrometer of the microscope stage so that the "0" value is lined up exactly with the edge of the reticle (see figure 2). The distance between the longer, numbered lines equals 100 μm .
3. Measure the distance between the left and the right edges of the reticle and divide this value by 10 (the number of squares in the reticle, see figure 2) to determine the distance (width) of an individual box. Mark this value as "**D**".

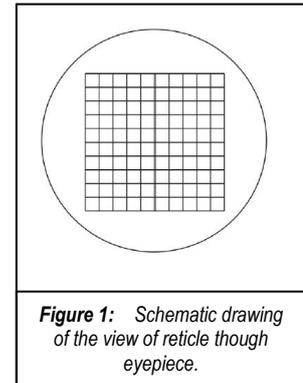


Figure 1: Schematic drawing of the view of reticle through eyepiece.

For example: in figure 2, the distance between the left edge and the right edge of the reticle is 500 μm .

$$\text{Thus: } D = \frac{500 \mu\text{m}}{10} = 50 \mu\text{m} .$$

To calculate the correction factor (**F**), use the following formula:

F = correction factor

D = width of individual box

T = chamber depth of Leja slide

$$F = \frac{1,000,000}{T \times D^2}$$

For example: using the 20 μm Leja® slide and **D** as determined in step 3:

$$F = \frac{1,000,000}{T \times D^2} = \frac{1,000,000}{20 \times 50^2} = 20$$

The correction factor **F** allows proper calculation of the volume of semen per individual reticle box so the correct sperm concentration can be calculated.