



77 Elbo Lane, Mt. Laurel, NJ 08054  
(856) 762-2000 • (800) 648-1151 • FAX (888) 882-0680  
www.origio.com

## Calibration for Manual Analysis

### Calculating the F factor

The factor F is a calibration derived using a variable obtained from a specific microscope that you have in your lab. The calculation must be performed for each microscope and magnification used.

Once you have calibrated your microscope and obtained the factor we call F: you can use that F factor for all samples analyzed on that microscope.

The formula for the calculation of the F factor is:

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

F = the factor determined for each microscope, magnification and Standard Count depth (20um)

T = the chamber thickness (depth in microns)  
If you are using the 20um Standard Count, Then insert the number 20 in this slot.

D = the distance across a single box of the reticle. (in microns)

*\*\*The only measurement that you need to perform is to determine D, the distance across a single box. See the following page for using the stage micrometer and calculating D.*

Remember that in your calculation for the **Factor F**, **D** is squared in the formula because the area that the sperm are in takes into account width x length x height. A simplified expression of this formula is:

Area = D x D x the Standard Count size.

$$F = \frac{1,000,000}{\text{Area}} \quad \text{OR} \quad F = \frac{1,000,000}{T \times D}$$

If, for example

$$D = 47 \text{ microns} \quad F \frac{1,000,000}{20 \times 47 \times 47} = 22.6$$
$$T = 20 \text{ microns}$$

## How to Calculate the D (distance) by using the Stage Micrometer

1. Install the reticle in the microscope eyepiece or replace the microscope eyepiece with the auxiliary eyepiece, which has the reticle within. Your view through the eyepiece should be as in Figure 2.

\*\*\*If you have any questions regarding the installation of your reticle, please contact one of our account/technical representatives.

2. Place the stage micrometer on the stage of the microscope. Your view through the eyepiece should be as in Figure 3.

3. Line up the boxes in the reticle such that one of the larger lines on the stage micrometer are equivalent to the right edge of the reticle.

4. Each section between the larger lines is equivalent to 100um. Each section between the medium lines is equivalent to 50um and between the smallest of lines it is equivalent to 10um.

5. Determine the number of microns across all ten boxes of the reticle. To calculate the distance across one box, divide the total distance measured by the number 10.

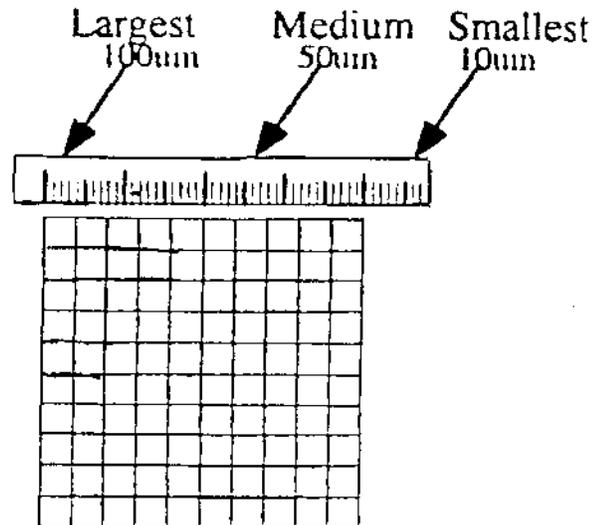
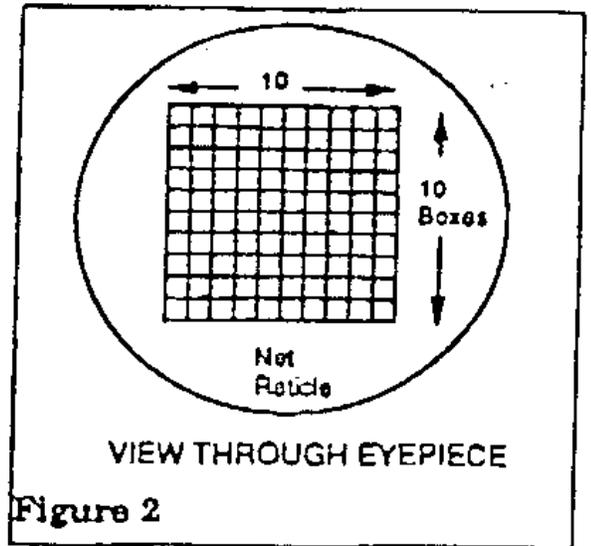


Figure 3

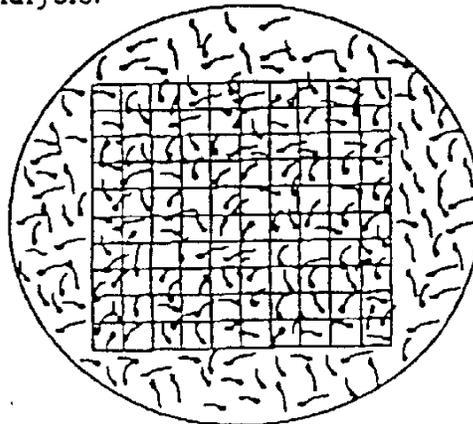
## Calculating Sperm Concentration

The Formula for calculation of sperm concentration is:

$$C = N \times F$$

Where      C = sperm concentration  
              N = average number of sperm per box  
              F = Factor

We recommend counting at least 100 to 200 sperm, if the concentration permits. Select a field for analysis from the center of the Standard Count Chamber. Because there is no permanent grid etched on the chamber, you may select any field you wish and as many as you require for your analysis.



### Calculating (N)

The most important parameter is to keep track of the number of boxes that have been counted. To obtain the average number of sperm per box (N), divide the total number of sperm counted by the total number of boxes within which the sperm were counted.

$$\frac{\text{\# of sperm}}{\text{\# of boxes}} = \text{avg. sperm/box}$$

## Important Information:

Every time you change the magnification, a new F factor must be calibrated. The area delineated by the grid pattern in the eye piece must be calibrated for each magnification to be used with Standard Count.

All microscopes must be calibrated separately. Because the actual magnification obtained from different microscopes varies, even when identical optics and manufacturers are compared.

## Determining the Percent Motility

The percent motility of the sample may be obtained along with the value of N using a slight modification of the above procedure.

1. Count only the motile sperm in the boxes and record that number.
2. Recount the same boxes, this time only counting the non-motile sperm.
3. Add your results from Step 1 and Step 2, which equals the total number of sperm counted.
4. Divide the number in Step 1 (the motile sperm) by the number in Step 3 (total sperm counted) and multiply by 100. This is the percent motility.

$$\frac{\text{\# of motile sperm}}{\text{\# of motile + non-motile sperm}} = \% \text{ Motility}$$