Calculating the Sperm Concentration

$$C = N \times F$$

Calculate the sperm concentration by using the above formula

If the concentration permits, we recommend counting at least 100-200 sperm from a field selected in the center of the Standard Count chamber.

1. **Calculate (N)** by dividing the total number of sperm counted by the number of boxes counted. Obviously, you must keep track of the number of boxes counted in order to perform this calculation.

$$N = \frac{\text{\# of Sperm}}{\text{\# of Boxes}}$$

2. **The factor (F)** is a calibration factor designed to compensate for the optical variation that is experienced from microscope to microscope, even those of the same model and manufacturer. Once a specific microscope is calibrated and the factor F is derived, you can use that value F for all samples analyzed with the same magnification on that specific microscope.

$$F = \frac{1{,}000{,}000}{T \times D2}$$

F = the calibration factor determined for each microscope, magnification, and Standard Count chamber depth.
T = the chamber depth (in microns). For the Standard Count, you would use the number 20.
D = the distance across a single box of the reticle (in microns)

3. **Calculate (D)** by using a Stage Micrometer. To achieve this:

1. Fit the reticle into the microscope eyepiece, insuring that it is firmly in place and parallel to the optical plane.
2. Place the stage micrometer on the microscope stage.
3. Line up the stage micrometer so that one of the larger lines is imposed upon the right edge of the reticle matrix. The divisions on the stage micrometer are 100um (distance between the large lines), 50um (distance between the secondary lines) and 10um (distance between the smallest lines).
4. Measure the distance across all 10 boxes of the reticle.
5. To calculate the distance across a single box in the reticle matrix, divide the distance obtained in step # 4 by 10.

4. **Incorporate the value (D)** into the formula, and derive the factor F.
Remember that the value D is squared in the formula to account for width x length.
Assuming that the value of D is 25 microns and that you are using a Standard Count with a 20 micron chamber depth, the factor F would then be equal to

$$F = \frac{1{,}000{,}000}{20 \times 252}$$

$$F = 80$$

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