Microscope Calibration

Why is it important to calibrate each microscope?

Not one microscope is alike. Assessing the same sample with different microscopes will yield in different results unless a correction factor has been applied. This correction factor is constant for every microscope. So once the correction factor (**F**) has been determined, you will not have to do it again with that microscope, with that magnification. This procedure must be followed for each microscope, for each magnification. This factor **F** will help you to determine the exact and correct sperm concentration.

The calibration:

In order to determine **F**, you will need an eyepiece reticle and a stage micrometer.

- Insert the eyepiece reticle (or use the auxiliary eyepiece provided with the microscope which has a reticle in it). Figure 1 shows your view through eyepiece.
- Following put the stage micrometer on the stage of the microscope. Looking through the eyepiece, your view should look approximately like figure 2.
- Line up the stage micrometer in such a way that the "0" is exactly lined up with the edge of the reticle. The larger lines account for 100 μm (numbered in figure 2).
- Measure the distance of all ten boxes of the reticle and divide this distance by 10. This distance will represent "**D**" in the following formula:

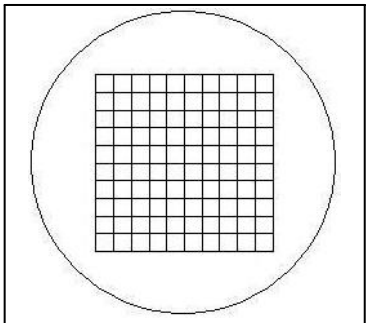

Figure 1: *Schematic drawing of the view through the eyepiece with reticle.*

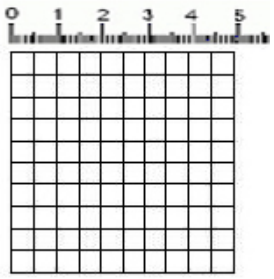

Figure 2: *Schematic drawing of the view of the stage micrometer through the reticle.*

The correction factor:

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

F = correction factor
D = distance of reticle
T = chamber depth of Leja standard count chamber

Example according figure 2 (this might be different for your microscope):

D = 50 μm
T = 20 μm

$$F = \frac{1{,}000{,}000}{20 \cdot 50^2} = 20$$

The only measurement that needs to be done is to measure "**D**", the distance across a single box. **T** is known since this is the chamber depth. The "**F**" factor is the correction factor that allows proper calculation of the volume per box so correct sperm concentrations can be determined.

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