PRINCIPLES OF MICROSCOPY

1. Compare the low power (10X) and high power (40X) objective lenses of your microscope. Do the stated magnifications accurately reflect the comparative resolving capabilities of these lenses?

The stated magnifications suggest that the 40X lens is 4 times better than the 10X lens (i.e., 40/100). The numerical apertures, however, indicate that the 40X lens has 3X the resolving power as compared to the 10X lens (i.e., 0.65/0.22). See the Abbe formula on page 35 of the lab manual.

2. a. What is the theoretical resolving power (resolution) of your 40X lens when used with blue light? Blue light has a wavelength of 0.45 μm. b. Ultraviolet light has a wavelength of about 0.25 μm. If the same lens could be used and the UV light could be detected (by photographic film, for example) what would be the theoretical resolution?

Abbe formula: $R = \dfrac{0.61\lambda}{\text{N.A.}}$ for blue light $R = \dfrac{0.61 \times 0.45\ \mu m}{0.65} = 0.42\ \mu m = 420\ nm$

for ultraviolet light $R = \dfrac{0.61 \times 0.25\ \mu m}{0.65} = 0.23\ \mu m = 230\ nm$

If U.V. light could be used, resolution would improve significantly.

3. A synthetic fiber has a width that occupies 1/9 of the diameter of your field of view at 400X. What is the width of this fiber in mm?

Diameter of view at 400X is 0.45 mm (see table in Principles of Microscopy Laboratory, Exercise #2 of this lab manual).

0.45 mm x 1/9 = 0.05 mm = 50 μm

4. A long filamentous alga has a width of 10 μm. How much of the field of view (what percent of the width) is occupied by this filament at 100X?

Diameter of view at 100X is 1.8 mm or 1800 μm
10 μm/1800 μm x 100 = 0.55% = 0.6%

5. An animal cell has a radius of 10 μm. How much of the field of view will this cell occupy at 400X? Answer in terms of the percent <u>area</u> that is occupied.

area = πr^2, at 400X, diameter of view is 0.45 mm --- radius is 0.225 mm or 225 μm
area of field of view = π (225 μm)^2 = 3.14 x (225 μm)^2 = 158962.5 μm^2
area of cell = π (10 μm)^2 = 3.14 x 100 μm^2 = 314 μm^2

314 μm^2/158962.5 μm^2 x 100 = 0.20% = 0.2%