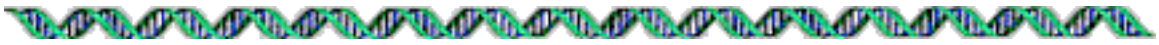
Quantitative Microscopy



A. Objectives

Become familiar with

1. Calibrating your ocular micrometer;
2. Measuring field of view;
3. Determining the actual size of a specimen seen through the microscope

B. Before coming to lab

1. Read Appendix D: Units and measures and this laboratory exercise;
2. Work through the metric conversion exercise (turn in at the beginning of this lab);
3. Review the introduction to microscopy, lab 3;

C. During this and the following lab

1. Obtain a compound microscope and illuminator.
2. Calibrate your ocular micrometer (see Part E, Tool 3 below). Report values for one ocular unit at 4X, 10X, and 40X in your lab notebook. Calculate the size of the **field of view** (the circular portion of the slide you can see through the scope) under each objective lens and complete the table "Objective lens data".
3. Copy or paste the table on objective lens data (see below) into your notebook and fill out the columns. Report all measurements in micrometers.
4. Familiarize yourself with the dissecting scopes.
5. Scan the worksheet in Part F to see if you need to investigate some of the answers in this lab (e.g., do you know which lenses are parfocal?)

D. After lab

1. Review this lab by filling out the worksheet in Part F.
2. Write one paragraph in your lab notebook in which you evaluate the organization of your laboratory notes and the results for labs 1 through 4. For example, how well did you organize your data? What could you do better next time to make it easy for you or anyone interested in your experiments to read your notes? Do your drawings comply with the instructions in lab 3, Part E, Tool 2?

E. Microscopist's toolbox 2

Tool 3: Calibrating the ocular micrometer

Your microscopes have built-in ocular micrometers to measure objects on slides. Our ocular micrometers have 100 subdivisions. Before you can use the micrometer, you have to calibrate it against a ruler of known dimensions, the stage micrometer. Calibrations have to be repeated for each objective lens. To calibrate

1. Place a stage micrometer on the microscope stage, and using the lowest magnification (4X), focus on the grid of the stage micrometer.

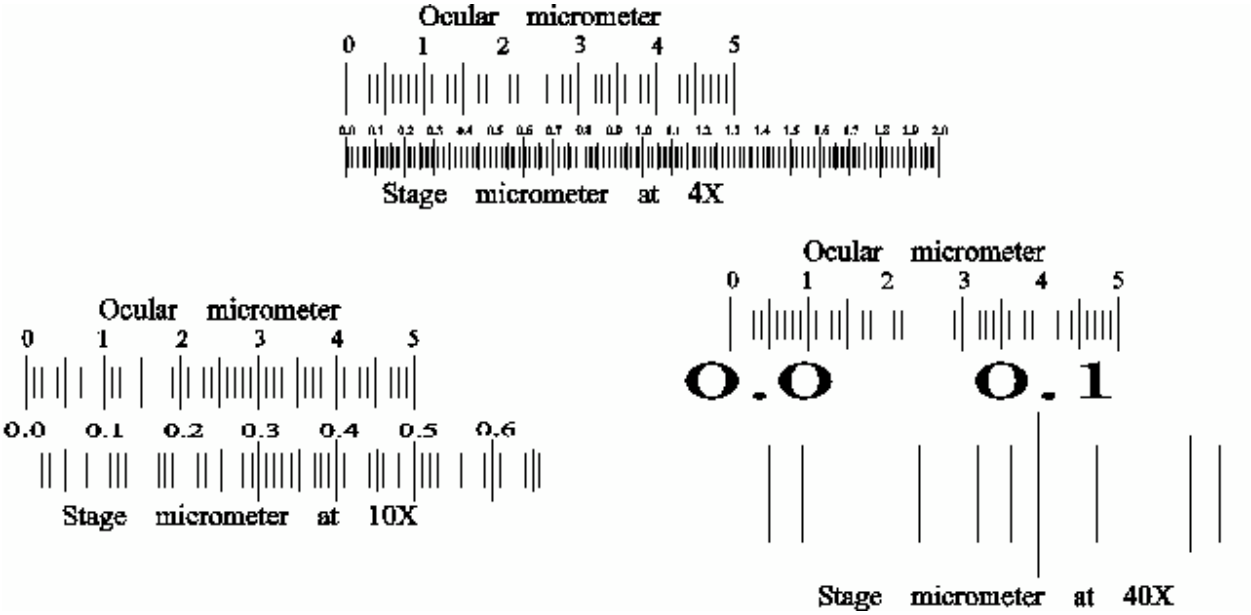


Figure 1: Stage and ocular micrometers superimposed at different magnifications (reprinted with permission from Heidcamp, H.W. Online Cell Biology Laboratory Manual)

2. Rotate the ocular micrometer by turning the appropriate eyepiece. Move the stage until you superimpose the lines of the ocular micrometer upon those of the stage micrometer. With the lines of the two micrometers coinciding at one end of the field, count the spaces of each micrometer to a point at which the lines of the micrometers coincide again (Fig. 1).
3. Since each division of the stage micrometer measures 0.01 mm, and since you know how many ocular divisions are equivalent to one stage division, you can now calculate the number of micrometers in each space of the ocular scale. For example, if 20 spaces on the ocular micrometer equal 0.16 mm on the stage micrometer then 1 space on the ocular scale at that magnification equals:
   0.16/20 =0.008 mm = 8 microns
4. Repeat for 10X and 40X. Record your observations.

Tool 5: Using a dissecting (stereoscopic) microscope

In dissecting scopes, rather than passing through the specimen, light is bounced off the surface of the object. Therefore, dissecting scopes allow the observation of thicker and opaque objects as well as larger living organisms that would be altered by wet mount preparation. Magnification, however, usually does not exceed 30X (using a 10X ocular and 3X objective lens).

To use:

1. Center your specimen on the stage.
2. Illuminate your specimen with the light source provided.
3. Use the adjustment knob to focus.

F. Worksheet

1. How does changing from the 4X to the 40X objective lens affect

a. The light intensity you need
b. The size of the field of view
c. The size of the object
d. The working distance
e. The depth of field

2. Which of the objective lenses are parfocal (that means that little refocusing is required when moving from one lens to another)?

3. How does closing the substage iris diaphragm affect
   a. Image contrast
   b. Resolution
   c. Image brightness

4. Why does the ocular micrometer have to be calibrated for each objective lens?

5. Define depth of field.

6. What do the markings on the objective lenses mean?

7. Name three functions of cover slips.

8. What variations did you expect to find in nuclear diameters observed in different tissues? What did you discover from your observations? What factors do you think limit organelle (or cellular) size and shape?

9. Define resolution. What factors limit the resolving power of a microscope?

10. Why would the use of a stain such as acidified methyl green improve the viewing of cellular structures? Do you think the use of stains can improve resolution?

11. Which objective lens should you use when first viewing a slide?

12. For your microscope: What is the total magnification when you use the 40X objective lens?

13. The resolution of a microscope depends on N.A. Is the resolution better with a high or low N.A.?

14. At 40X, 10 stage units correspond to 50 ocular units. How many mm is one ocular unit? How many μm?

	Stage units	**Ocular units**	**mm/ocular unit**	**Converted to μm**
40x	10	50		

15. A plant structure measures 3,000 μm at 4X. How many μm will it measure at 10X? What is it's real length? Assume you are using a microscope with a 10X ocular and no head magnification.

16. Using your 4X objective, you measure the diameter of the field of view to be 3 mm. At 10X, the length of a letter "e" takes up half the width of the diameter of your field. The oculars on your microscope are 10X. What is the size of the letter?

17. When would you use a dissecting scope rather than a compound microscope?

Table of objective lens data **Microscope # ____**

	Scanning lens	Low power lens	High dry lens
Objective lens magnification			
Eyepiece magnification			
Binocular head magnification (if any)			
Numerical aperture			
Total magnification			
Resolving power (at 550 nm)			
Field of view			
Working distance			