INTRODUCTION TO THE MICROSCOPE

The microscope which you will use is a compound, monocular, light microscope. The word compound refers to the fact that the light passes through two or more lenses before it reaches the eye. Monocular (mono—one, ocular—eyepiece) simply means there is only one eyepiece (number one on your drawing). A light microscope refracts (bends) visible light in order to magnify.

Care of the Microscope:

Since the microscope is an expensive piece of equipment, certain procedures must be followed in caring for it:

1. The microscope should be carried with one hand firmly on the arm (No. 3) and the other hand under the base (No. 6).

2. The microscope should be placed in a position on the table where is not likely to be knocked off.

3. Lenses are easily scratched, therefore, they require proper cleaning.
   a. Blow away grit which may be on the ocular (No. 1).
   b. Wipe lightly with lens paper. Never touch the lens with anything except lens paper.
   c. For cleaning objectives, repeat a and b.
   d. Never remove the ocular or the objective from the microscope.
   e. If dark blurred areas still appear in microscope field of view and they are not on the slide, ask the instructor for assistance.

4. The rest of the microscope (not the lenses) may be wiped clean with a paper towel.

Parts of the Microscope:

You are now ready to get your microscope. Go to the microscope cabinet and get the microscope you have been assigned. CARRY IT CORRECTLY back to your seat and use the drawing (Figure 1) to locate the following parts of the microscope:

1. Ocular—this microscope has a 10X ocular (this will be of importance later).

2. Body tube—maintains proper distance between ocular and other lenses.
3. Arm—holds lenses in correct position; also serves as handle.

4. Coarse adjustment—large knob. Turn the coarse adjustment knob and observe what happens. This adjustment is used only on LOW POWER, NEVER on high power.

5. Fine adjustment—small knob. Turn this knob and observe what happens. Nothing? Turn it again and watch the stage (No. 11) very closely. This adjustment is used to bring the object into sharpest focus. It is the only adjustment used on high power.

6. Base—simply a structure which serves as a platform on which the other parts of the microscope sit.

7. Light source—electric light used to generate light.

8. Switch—turns light on and off.

9. Iris diaphragm—regulates the amount of light which strikes the subject. It is usually better to use too little light instead of too much light.

10. Condenser—focuses the light which passes through the iris diaphragm on to the object. The condenser on your microscope is permanently fixed, therefore, no adjustments can be made.

11. Stage—platform where mechanical stage is located. Your microscope slide should be fitted between the arms of the mechanical stage with the subject on the slide placed over the opening in the stage.

12. Low power objective lens—this lens is smaller than the other two; it has the number 10 on it, and is further indicated by a blue band around the lower part of objective.

13. High power objective lens—this lens has the number 40 and a red band. Only the fine adjustment should be used to focus this objective.

14. High dry objective lens—this lens has the number 60 on it and an orange band located around the lower end. Only the fine adjustment should be used to focus this objective.

15. Revolving nosepiece or turret—this device holds the objectives and revolves allowing one to change objectives.

Mechanical stage—the slide is placed between the arms of this structure and is moved left and right by the upper knob and forward and backward by the lower knob (the one nearest you).
Using the Microscope:

In order to use the microscope, you must have something to look at, consequently, you need to know how to prepare a slide.

The first slide you will prepare is called a dry mount.

1. Get one microscope slide.

2. Place on the center of the slide the letter "e" in the correct position.

3. On top of the letter "e" place a cover slip.

4. Now you are ready to use the microscope.

5. Be sure the low power objective is in position.

6. Roll stage all the way up with the coarse adjustment.

7. Turn on the light.

8. Place the slide on the microscope stage with the letter "e" over the center of the hole in the stage.

9. Look through the ocular and move the slide around until you see the piece of paper which has the letter "e" on it.

10. Now that you have found the paper, turn the coarse adjustment away from you until it comes into focus. You may need to adjust the iris diaphragm to adjust the amount of light.

11. Move the slide until you see the letter "e".

12. Draw the position of the letter "e" as it appears through the microscope.

13. While looking through the microscope move the slide to the right. Which way did the letter "e" appear to go?

14. Now move the slide away from you. Which way did the letter "e" appear to go?

15. What can you conclude from questions 12, 13, and 14?

16. Now you are ready to go to the high power objective.
17. While still on low power place a portion of the letter "e" in the center of the field of view, and be sure you have it in sharp focus.

18. Grasp the objectives on revolving nosepiece and turn it until the high power objective (red band) clicks into position. It looks as if the objective is going to hit the slide, but it will not if you had it in focus on low power.

19. Adjust the light, if necessary, by opening or closing the iris diaphragm.

20. While looking through the ocular turn the FINE ADJUSTMENT (#5) from you until you get the sharpest focus. You will not have to turn the fine adjustment very much. If you do not see some of the ink of the letter "e" go back to low power and re-center and re-focus and then go to high power and try it again.

21. Repeat this exercise until you are proficient at using the microscope.

**Main rules to follow:**

1. Be sure not to use too much light (iris diaphragm controls amount of light).

2. Always find subject on low power before going to high power.

3. Never use coarse adjustment on high power.

4. Always store microscope properly:
   a. Low power objective in down position.
   b. Stage in high position.
   c. Cord neatly rolled.
   d. No slide left on microscope.
   e. Dust cover on microscope.

5. Keep both eyes open as you study materials under the microscope.

**Magnification and field view:**

The magnification of a microscope can be determined very simply. The ocular of a microscope has a number on it. Our microscope has 10X ocular. You will also see a number on the three objectives: 10 on the lower power objective, 40 on the high power objective, and 60 on the high dry objective. The magnification (power) is determined by simply multiplying the ocular by the objective being used. The "X" on the ocular will help you remember this.
For Example: Ocular X Objective = Magnification
Low power 10X 10 = 100
High power 10X 40 = 400
High dry 10X 60 = 600

What would be the magnification if the ocular was 20X and the objective was 10X?

Field of view refers to the portion of the slide you see with the microscope. You will notice that the field of view is greatly reduced when the high power objective is being used.

Determine the diameter of the low power field of view:

Lay a clear plastic ruler which is marked in millimeters across the stage so that the inner edge of one vertical line is just visible at the left edge of the field, and the horizontal line is across the diameter of the field. The distance of the second line is 1 mm. Estimate the distance between the second vertical line and right of the field. How many millimeters, in diameter, is your low power field? Change this to micrometers (1mm = 1000 micrometers). Now using the known diameter of the field of view of the low power objective, you can determine the diameter of the field of view of the high power objective. This is done by dividing the diameter of the field of view of low power by 4.0. The factor 4.0 is used because the diameter of the low power is 4.0 times greater than the diameter of the high power field of view.

Thus far you have looked at prepared slides. We will now have you make a slide of your own epithelial cells.

1. Obtain a microscope slide from the tray on your table and clean the slide if it is dirty by washing with soapy water and a good rinse.

2. Place a small drop of water in the center of the slide.

3. Using a clean toothpick gently scrape the inside of your cheek. Stir the material from your cheek onto the drop of water on the slide.

4. Gently place a cover slip on the drop of liquid and look at the cells on low power with your microscope. Hint - it is usually helpful to reduce the light when looking for unstained cells. How much cell detail can you see?

5. Now, without removing the slide from the microscope, place a small drop of Methylene blue stain (little dropper bottles on the table) on the edge of the coverslip so that the stain moves under the coverslip and mixes with the cells. It may take a minute or two to let the stain diffuse into the area you are looking at.

6. Now look at the same cells again with the stain added. How much detail can you see now?
7. Note the light staining area around a darker staining area. This is the cytoplasm of the cell. The dark staining region in the center is called the nucleus.

8. Now clean up. Remove the slide, clean it, and replace it in the slide box.

9. Wipe up and clean your area. Put your microscope back into the proper slot in the cabinet.