Notes and Practice: Tools of the Biologist

1. Compound Light Microscope:

A compound light microscope has 2 lenses. The first magnifying lens is found in the eyepiece, also known as the ocular. The second magnifying lens is found in the objective.
To determine the total magnification of compound microscope, multiply the magnification of the ocular times the magnification of the objective that is in use. **For example, if a microscope has an ocular with a 10x lens and the low power objective has a magnification of 10x, then the total magnification of the microscope is 100x (10 X 10).**

Complete the following examples:

A compound light microscope has a 5x ocular lens and objectives of 10x and 45x.

a. What is the lowest total magnification the microscope can have? Show your work.

b. What is the highest total magnification the microscope can have? Show all work.

The specimen that you are examining with a compound light microscope is called the **OBJECT.** What you view through the microscope in the **FIELD OF VIEW** is called the **IMAGE.** ***When making a drawing of what you see through the microscope, you always place your drawing of the image in a circle, which represents the field of view.***

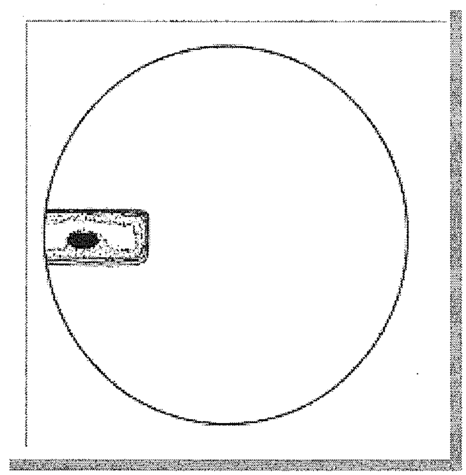
Complete the following questions:

a. Imagine that you are viewing the lowercase letter f through a compound light microscope. Draw the object (the letter f) as it would appear on the slide and then draw the image of this f as it would appear in the microscope.

b. How do the object and the image differ when using a compound light microscope?

c. When looking through the ocular, you decide to move the slide to the left. Which way did the image move? (Remember the image is viewed opposite in comparison to the object.)

d. The diagram below represents a specimen on a slide as seen with the low power objective of a compound light microscope. Explain how the slide should be moved to observe the entire specimen.



When changing objectives on a compound light microscope, the magnification of the microscope is altered. For example, if you change from a low power objective of 10x and move to a high power of objective of 40x, you have increased the magnification of the microscope 4 times and decreased the field of view by the same amount (4x). That means that you see less of the image, but at a greater magnification.

Let's say that you see 20 cheek cells when using the low power objective of 10x. Now you move to the high power objective of 40x. How many cheek cells will you see now?

Increase the magnification 4x

10x ——————→ **40x**

Decrease the field of view 4x

20 cells ——————→ **5 cells (divide 20 by 4)**

If you increase the field of view 4x (10x X 4 = 40x) then you decrease the size of the field of view by the same amount. If you see 20 cells under 10x, then you would see 5 cells (20/4 = 5).

Try the following problems:

a. You count 5 cells when looking at onion cells under high power of 40x. How many cells will you see if you move down to low power of 10x? Show all work.

b. You see 15 amebas (tiny single celled organisms) under low power of 10x. Predict how many cells you will view when viewing them under 50x magnification. Show all work.

c. When increasing the magnification from 10x to 40x, the image gets bigger and takes up more of the field of view (you see less of the image, but more magnified). Describe what happens to the brightness of the field of view when you move from low to high power.

d. Explain why you must center your image under low power before moving to the high power objective.

e. Describe the steps you would take to focus your microscope under low power.

f. Explain why you only use the fine adjustment when focusing under high power.

g. Describe the function of the diaphragm.

MAGNIFICATION increases the size of the image you are viewing in your microscope. **RESOLUTION** or **RESOLVING POWER** describes the ability of the microscope to distinguish between two objects close together. For example, when looking at human cheek cells, the cells are often adjacent or lying on top of each other. A microscope with good **RESOLUTION** enables the viewer to see the boundaries among cells so that he/she can see each cell clearly.

a. Explain why resolving power is the limiting factor of a microscope. In other words, why is resolution more important than power of magnification in a microscope?

When measuring microscopic objects through a compound light microscope, we use **micrometers (µm).** **To convert from millimeters to micrometers, simply move the decimal point three spaces to the right (micrometers are smaller than millimeters). To convert from micrometers to millimeters, move the decimal point three spaces to the left.**

1 millimeter (mm) = 1,000 micrometers (µm)

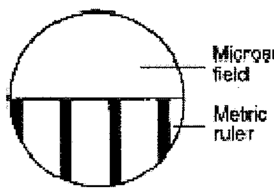
Try the following conversion problems:

a. 45 mm = µm
b. 2,540 µm = mm
c. 65.2 cm = mm = µm

Since we have no ruler to measure with when using the compound microscope, we must "guessimate". In order to estimate the size of an image, you must have the diameter of the field of view.

For example, in the diagram below, a millimeter ruler is used to measure the diameter of the field of view of a compound microscope. Each division is equal to 1 millimeter (mm). Therefore, the field of view is about 3.5 mm or 3,500 μm.

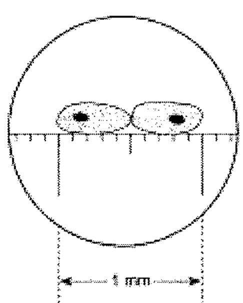
Finding the Size of a Microscope Field of View



In the pictured field of view at the left, it can be observed that there are approximately 3 1/2 divisions equal to a length of 3.5 mm. Therefore this field of view is equal to 3.5 mm or 3,500 micrometers.

Now let's estimate the size of two cheek cells viewed under low power as illustrated in the example below. In this problem, you are given a measurement reference that the two cheek cells measure 1 mm or 1,000 . Toμm find the size of one cell, divide 1,000/2. The size of one cell is 500 μm.

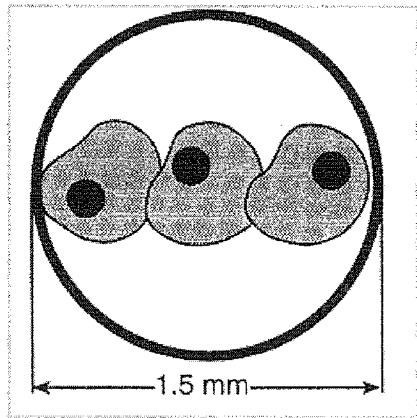
Finding the Size of Multiple Cells in a Field of View



The two cells in this field take up a field of view of one millimeter. Therefore, the size of the specimen is equal to 1 mm/2 cells or 0.5 mm per cell. There is 500 micrometers in 0.5 mm., so the average size of each cell is 500 micrometers

Now, let's see how you do. Estimate the size of the nucleus inside one of the cells in the problem above. (Hint: how many nuclei can fit into 1,000 μm?). Show all work in the space below.

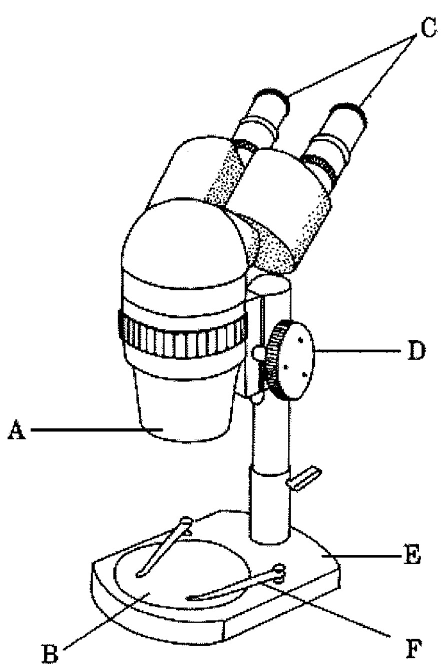
Here is another practice problem:
The diagram below shows three cells in the field of view of a microscope. The diameter of the field of view is 1.5 millimeters.



What is the approximate diameter of each cell? Show all work in the space below.

2. **Dissecting Microscope:**

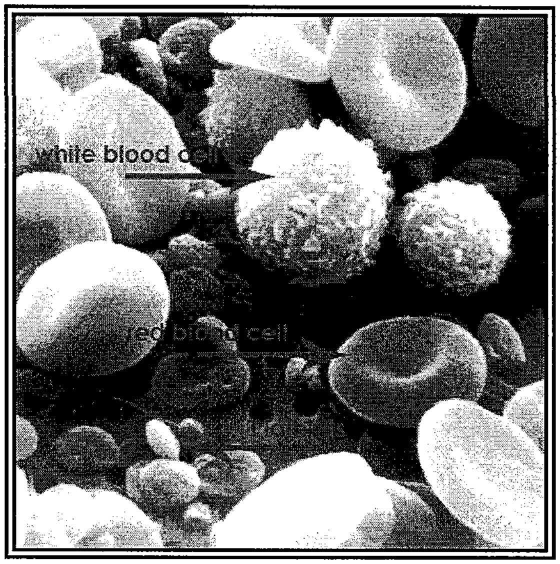
Below is a diagram of a typical dissecting microscope. This microscope is used to examine small, opaque specimens (often living ones). Dissecting microscopes are binocular. This means they form a 3-D image of the specimen. The image and the object are in the same orientation so when you move the specimen to the left, the image moves to the left as well.



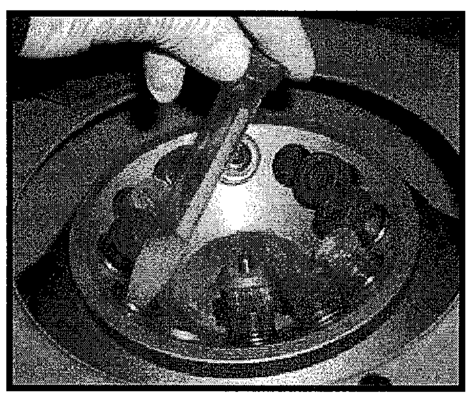
A: Objective
B: Stage
C: Paired Eyepieces or Oculars
D: Knob for Adjustment of Focus
E: Foot and Stand
F: Stage Clips

3. Scanning Electron Microscopes:

Compound light microscopes cannot provide the high magnifications needed to see very microscopic specimens. Electron microscopes use electrons and magnets These electromagnets are used to bend an electron beam which is used to produce an image on a screen. The electron beam also has high resolution, so the image that is produced is very detailed with greater clarity. Below is a picture of a scanning electron image of a drop of human blood.

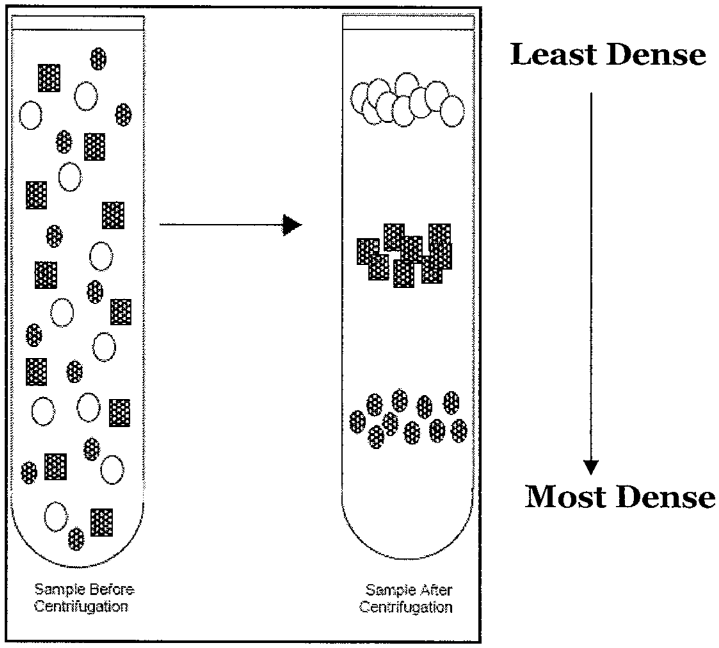


4. Ultracentrifuges



Centrifugation is one of the most important and widely applied research techniques in biochemistry, cellular and molecular biology, and in medicine. A sample let's say of your blood is loaded into a test tube and spun at a very high rate of speed. Particles in the sample solution will eventually settle at their given densities. At the end of the process, the plasma, white blood cells, red blood cells, and platelets would be suspended at different levels in the test tube according to their relative densities. If a lab tech just wanted to look at your red blood cells, they could easily separate them from the rest of the blood cell layers in the test tube. (see picture below)

Separation of Particles in a Solution by Centrifugation



5. Stains:

Stains are used in microscopy to enhance the structures of a given specimen for better viewing under a the microscope. For our purposes, we will use iodine (Lugol's solution) to stain plant cells and methylene blue for animal cells.

Image 1

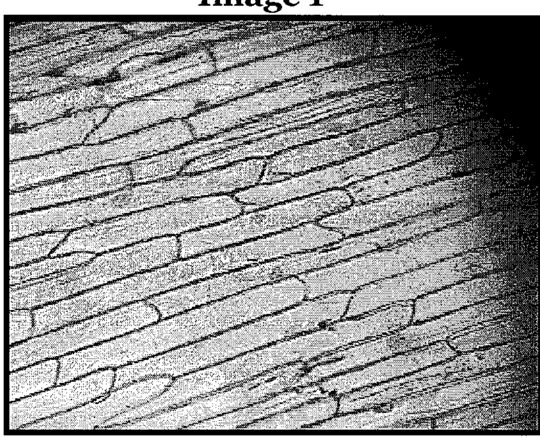


Image 2