centimeter (cm) = 10^{-2} m or 1/100 m

milli (m) = 10^{-3} or 1/1000
millimeter (mm) = 10^{-3} m or 1/1000 m
milliliter (ml) = 10^{-3} l or 1/1000 l

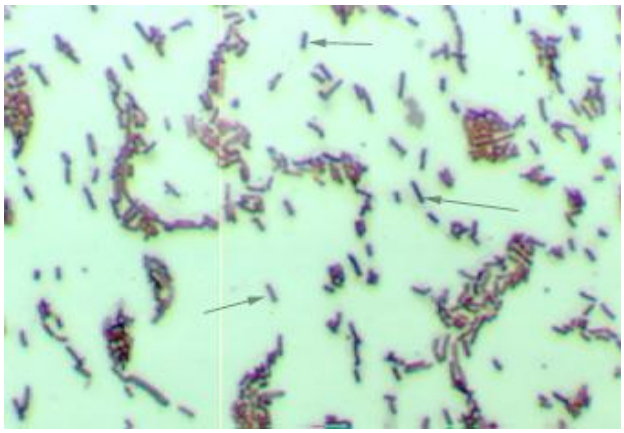
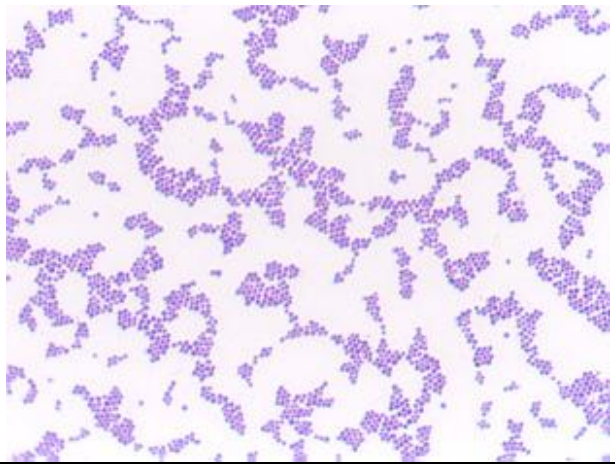
micro (μ) = 10^{-6} or 1/1,000,000
micrometer (μm) = 10^{-6} m or 1/1,000,000 m
microliter (μl) = 10^{-6} l or 1/1,000,000 l

nano (n) = 10^{-9} or 1/1,000,000,000
nanometer (nm) = 10^{-9} m or 1/1,000,000,000 m

In microbiology, we deal with extremely small units of metric length (micrometer, nanometer). The main unit of length is the micrometer (μm) which is 10^{-6} (1/1,000,000) of a meter or approximately 1/25,400 of an inch.

The average size of a rod-shaped (cylindrical) bacterium (see Fig. 1) is 0.5-1.0 μm wide by 1.0-4.0 μm long. An average coccus-shaped (spherical) bacterium (see Fig. 2) is about 0.5-1.0 μm in diameter. A volume of one cubic inch is sufficient to contain approximately nine trillion average-sized bacteria. It would take over 18,000,000 average-sized cocci lined up edge to edge to span the diameter of a dime!

In several labs we will be using pipettes to measure fluid volume in ml.

Fig. 1: A Bacillus or Rod-Shaped Bacterium	**Fig. 2:** A Coccus-Shaped Bacterium
	
Escherichia coli, a rod-shaped bacterium.	*Staphylococcus aureus*, a coccus-shaped bacterium.

F. Using the Microscope (Olympus Model CH-2 Microscope)

1. **Moving and transporting the microscope**

Grasp the arm of the microscope with one hand and support the base of the microscope with the other. Handle the microscope gently, it costs over $1500.

2. Before you plug in the microscope, turn the **voltage control dial** on the right side of the base of the microscope **to 1** (see Fig. 3). Now plug in the microscope and use the on/off switch in the front of the microscope on the base to turn it on. Make sure the entire cord is on the bench top and not hanging down where it could be caught by a leg. **Adjust the voltage control dial to 10** (see Fig. 3).

3. **Adjusting the eyepieces**

These microscopes are binocular, that is, they have 2 ocular lenses (eyepieces; see Fig. 4). To adjust them, first find the proper distance between your eyes and the eyepieces by closing one eye and slowly moving your head toward that eyepiece until you see the complete field of view - about 1 inch away. Keep your head steady and both eyes in the same plane. Now open the other eye and gradually increase the distance between the eyepieces until it matches the distance between your eyes. At the correct distance you will see one circular field of view with both eyes.

4. **Positioning the slide**

Place the slide specimen-side-up on the stage so that the specimen lies over the opening for the light in the middle of the stage. Secure the slide **between** - not under- the slide holder arms of the mechanical stage (see Fig. 3). The slide can now be moved from place to place using the 2 control knobs located under the stage on the right of the microscope (see Fig. 3).

5. **Adjusting the illumination**

a. Adjust the **voltage** by turning the **voltage control dial** located in the rear, right-hand side of the microscope base (see Fig. 3).. For oil immersion microscopy (1000X) set the light on 9 or 10. At lower magnifications less light will be needed.

b. Adjust the **amount of light coming through the condenser** using the **iris diaphragm lever** located under the stage in the front of the microscope (see Fig. 3). Light adjustment using the iris diaphragm lever is critical to obtaining proper contrast. For oil immersion microscopy (1000X), the iris diaphragm lever should be set almost all the way open (to your left for maximum light). For low powers such as 100X the iris diaphragm lever should be set mostly closed (to your right for minimum light).

c. The **condenser height control** (the single knob under the stage on the left-hand side of the microscope; see Fig. 4) should be set so the condenser is all the way up.

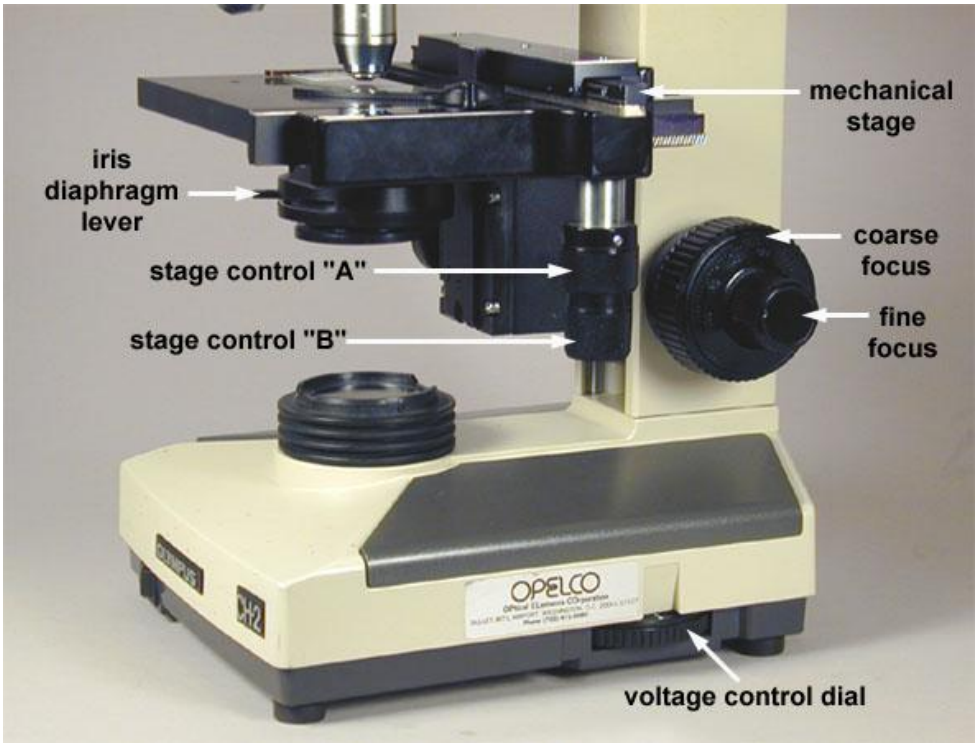
6. **Obtaining different magnifications**

The final magnification is a product of the 2 lenses being used. The **eyepiece or ocular lens** magnifies **10X**. The **objective lenses** (see Fig. 3) are mounted on a turret near the stage. The small **yellow-striped lens** magnifies **10X**; the **blue-striped lens** magnifies **40X**, and the **white-striped oil immersion lens** magnifies **100X**.

Final magnifications are as follows:

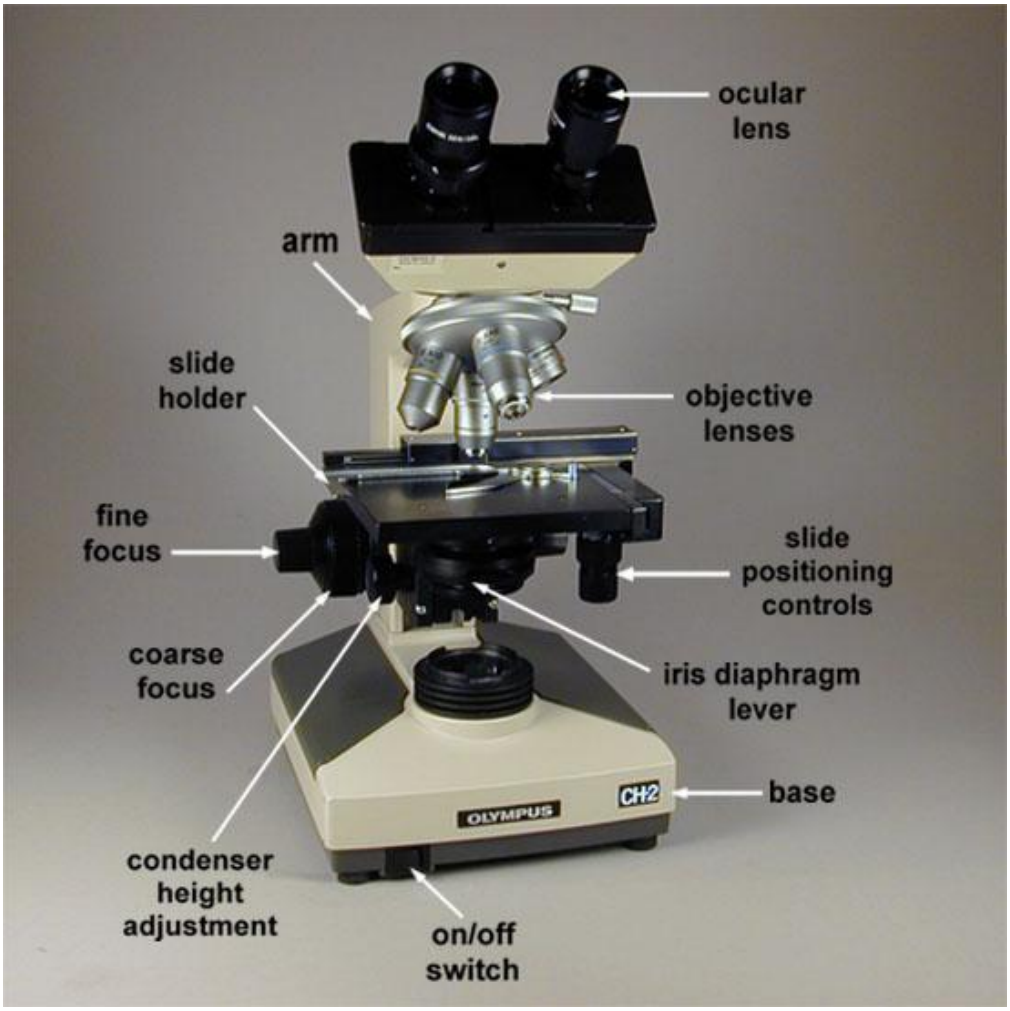
ocular lens	X	objective lens	=	total magnification
10X	X	4X (red)	=	40X
10X	X	10X (yellow)	=	100X
10X	X	400X (blue)	=	400X
10X	X	100X (white)	=	1000X

Fig. 3: An Olympus CH-2 Microscope



Iris diaphragm lever: moving the lever to the left increases the light; moving the lever to the right decreases the light.
Stage control "A": moves the mechanical stage holding the slide forward and backward.
Stage control "B": moves the mechanical stage holding the slide left and right.
Coarse focus: turning the knob away from you raises the stage; turning the knob towards you lowers the stage.
Fine focus: turning the knob away from you raises the stage; turning the knob towards you lowers the stage.

Fig. 4: An Olympus CH-2 Microscope



7. **Focusing from lower power to higher power**

a. Rotate the **yellow-striped 10X objective** until it locks into place (total magnification of 100X).

b. Turn the **coarse focus control** (larger knob; see Fig. 3) all the way **away from you** until it stops.

c. Look through the eyepieces and turn the **coarse focus control** (larger knob) **towards you** slowly until the specimen comes into focus.

d. Get the specimen into sharp focus using the **fine focus control** (smaller knob; see Fig. 3) and adjust the light for optimum contrast using the iris diaphragm lever.

e. If higher magnification is desired, simply rotate the **blue-striped 40X objective** into place (total magnification of 400X) and the specimen should still be in focus. (Minor adjustments in fine focus and light contrast may be needed.)

f. For maximum magnification (**1000X or oil immersion**), rotate the blue-striped 40X objective slightly out of position and place a **drop of immersion oil** on the slide. Now rotate the **white-striped 100X oil immersion objective** into place. Again, the specimen should remain in focus, although minor adjustments in fine focus and light contrast may be needed.

Directions for focusing directly with oil immersion (1000X) without first focusing using lower powers will be given in Laboratory 1.

8. **Cleaning the microscope**

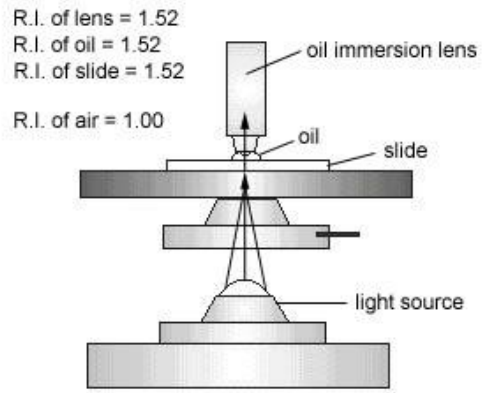
Clean the exterior lenses of the eyepiece and objective before and after each lab using **lens paper** only. (Paper towel or kim-wipes may scratch the lens.) **Remove any immersion oil from the oil immersion lens before putting the microscope away.**

9. **Reason for using immersion oil**

Normally, when light waves travel from one medium into another, they bend. Therefore, as the light travels from the glass slide to the air, the light waves bend and are scattered similar to the "bent pencil" effect when a pencil is placed in a glass of water. The microscope magnifies this distortion effect. Also, if high magnification is to be used, more light is needed.

Immersion oil has the same refractive index as glass and, therefore, provides an optically homogeneous path between the slide and the lens of the objective. Light waves thus travel from the glass slide, into glass-like oil, into the glass lens without being scattered or distorting the image (Fig. 5). In other words, **the immersion oil "traps" the light and prevents the distortion effect that is seen as a result of the bending of the light waves.**

Fig. 5: The Oil Immersion Lens



The immersion oil has the same refractive index as the glass lens and the glass slide. Because the light waves follow a homogeneous path, there is no distortion.