LAB 1
Introduction to Microscopy

I. Ubiquity of Microorganisms
II. Microscopy

I. UBIQUITY OF MICROORGANISMS

Microorganisms are ubiquitous; that is, they are present nearly everywhere. In this lab you will try to isolate bacteria and other microorganisms from various sources using different types of media.

TERMS AND DEFINITIONS

Culture media (medium, singular): solution of nutrients required for the growth of bacteria.

Agar: a carbohydrate derived from seaweed used to solidify a liquid medium.

Tryptic Soy Agar (TSA): a rich solid medium containing a digest of casein (the principal milk protein) and soy products. It is an all-purpose medium that supports the growth of many diverse organisms.

Tryptic Soy Broth (TSB): a rich liquid medium containing a digest of casein and soy products. It is a general-purpose medium that supports the growth of organisms that are not exacting in their food requirements.

Colony: a visible population of microorganisms originating from a single parent cell and growing on a solid medium.

PROCEDURE: (EACH STUDENT) Collect 2 TSA plates and 1 TSB tube from the side bench.

1. Moisten a sterile swab with sterile water (see bottle on your bench). Using this swab, collect a sample from any surface or object (e.g. doorknobs, shoes, drinking fountain, a strand of hair, various body parts, etc.). Try whatever interests you and be creative.

2. After the sample has been collected, inoculate a Tryptic Soy Agar (TSA) plate by gently rolling the swab over the surface of the agar. Discard the used swab into a biohazard container.

3. Label the bottom of the plate with your name, lab section #, date, and the source of the sample. Write on the outer edge so that the markings won't interfere with observing the colonies growing on the plate.

4. Inoculate a second Tryptic Soy Agar plate by the following procedure. Open the lid of the plate, place it close to your mouth and cough hard 3 times onto the plate. Place the lid back on. Correctly label your plate.

5. Inoculate a tube of Tryptic Soy Broth by removing the cap, putting your thumb over the top of the tube, and inverting the tube several times. Replace the cap. Label the tube with your name and lab section using a piece of tape. **Do not write directly on the cap or tube**.

6. Incubation: After inoculating culture media with microorganisms, it is usually incubated at a temperature that most closely mimics the organisms' natural environments.
   *a. TSA swabbed plate - room temperature (RT)
   *b. TSA cough plate - body temperature--37°C incubator
   c. TSB tube - RT

***Plates are always incubated in an inverted position (agar side up).** There are only a few exceptions to this rule that you will see later in the course.

II. MICROSCOPY

In this exercise you will become familiar with a bright field microscope that you'll be using throughout the semester.

TERMS AND DEFINITIONS

Microscope: a device for magnifying objects that are too small to be seen with the naked eye.

a. Simple microscope: single lens magnifier
b. Compound microscope: employs two or more lenses

Parfocal: the objective lenses are mounted on the microscope so that they can be interchanged without having to appreciably vary the focus.

Resolving power or resolution: the ability to distinguish objects that are close together. The better the resolving power of the microscope, the closer together two objects can be and still be seen as separate.

Magnification: the process of enlarging the size of an object, as an optical image.

Total magnification: In a compound microscope the total magnification is the product of the objective and ocular lenses (see figure below). The magnification of the ocular lenses on your scope is 10X.

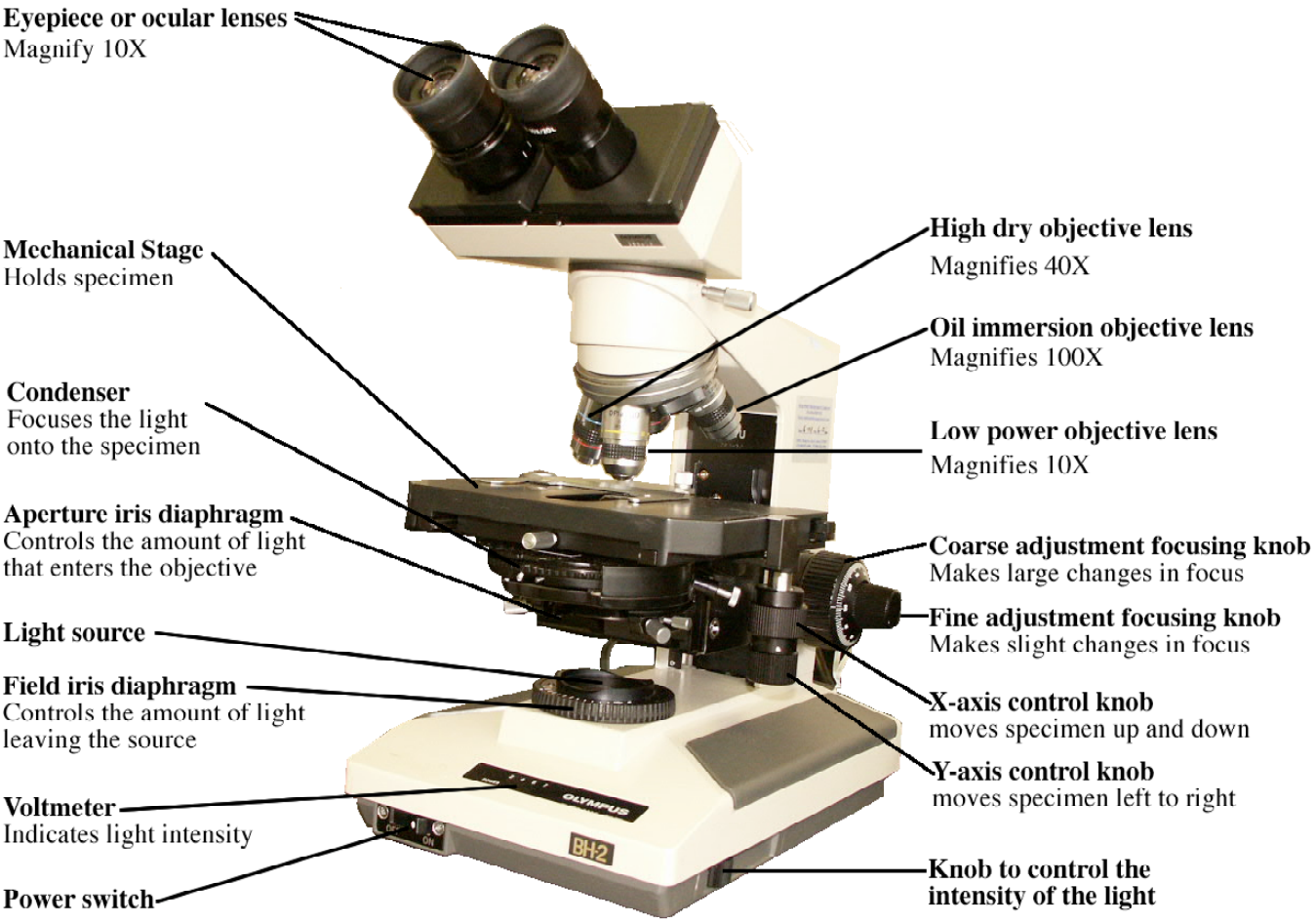
Objective lens × Ocular lens = Total magnification

For example:	low power:	(10X)(10X) = 100X
	high dry:	(40X)(10X) = 400X
	oil immersion:	(100X)(10X) = 1000X

Immersion Oil: Clear, finely detailed images are achieved by contrasting the specimen with their medium. Changing the refractive index of the specimens from their medium attains this contrast. The **refractive index** is a measure of the relative velocity at which light passes through a material. When light rays pass through the two materials (specimen and medium) that have different refractive indices, the rays change direction from a straight path by bending (refracting) at the boundary between the specimen and the medium. Thus, this increases the image's contrast between the specimen and the medium.

One way to change the refractive index is by staining the specimen. Another is to use immersion oil. While we want light to refract differently between the specimen and the medium, we do not want to lose any light rays, as this would decrease the resolution of the image. By placing immersion oil between the glass slide and the oil immersion lens (100X), the light rays at the highest magnification can be retained. Immersion oil has the same refractive index as glass so the oil becomes part of the optics of the microscope. Without the oil the light rays are refracted as they enter the air between the slide and the lens and the objective lens would have to be increased in diameter in order to capture them. Using oil has the same effect as increasing the objective diameter therefore improving the resolving power of the lens.

MICROSCOPE COMPONENTS



MICROSCOPE CARE

1. Never slide a microscope across a bench surface. Always carry a microscope with both hands. One hand should be placed on the arm and the other should support the base.
2. Microscopes should be cleaned both before and after use. Use ONLY lens paper and lens cleaner. Kleenex, paper towels and even Kimwipes can scratch the lenses.
3. ONLY use oil when using the100X oil immersion lens. DO NOT get oil on the other objective lenses.
4. Store microscopes with the 10X (low power) objective lens in position or such that the region lacking a lens is in position. Turn the light intensity all the way down.
5. DO NOT wrap the cord around the microscope. Instead, fold the cord and place it between the arm and the stage or beneath the stage.
6. Use the course adjustment focusing knob to lower the stage towards the light source. DO NOT crank down on the knob!
7. Replace the dust cover before putting the microscope away.

PROCEDURE: (EACH STUDENT) The primary objective of this exercise is to gain experience in using the bright field microscope. You will observe the microbial life present in pond water and hay infusions as you practice working with the microscope.

1. Place a slide on the lab bench frosted side **up**. The frosted section should feel rough.

2. Draw a line on the slide with a Sharpie marker. This line will be used to help you focus.

3. Choose to observe either the pond water or hay infusion. Collect some of the chosen sample using a transfer pipette. Be sure to stir it and then pick up some of the "gunk" from the bottom of the jar. Put a drop of the sample next to the line on the slide.

4. With your forceps, pick up a coverslip and place it on top of the sample. Avoid bubbles by putting the cover slip down at an angle.

5. View the sample using your microscope:
    a. Lower the microscope stage a little in order to secure the prepared slide onto the stage using the spring-loaded slide holder.
    b. Turn on the main power switch and adjust the light until the Voltmeter reads 2. Be certain that both the field iris diaphragm and the aperture iris diaphragm are open and that the condenser is set to 0.
    c. Position the 10X objective lens directly above the focus line on the slide. Use the coarse adjustment knob to bring the stage as close to the 10X lens as is possible. Now use the fine adjustment knob to back the stage away until the line comes into focus (*Note - If you see more than one circle of light when looking in the oculars, move these lenses until only one circle of light is present).
    d. Move the slide to view the sample. Try to identify some of the organisms. You may move the high dry objective into place to get a better look at the tiny microbes, but please do not use the oil immersion lens (100X) at this point.

6. Draw some of the organisms you observed in the results section for Lab 1. When you have finished observing the slide, remove it from the mechanical stage and discard the coverslip in the glass disposal container. Wash and save your microscope slide.

7. Wash the slides under tap water and store them in a slide box provided in your drawer. **Do not** place the slides back into the original box.

8. Every student should have 5 slides in a slide box for future use. Using the colored tape on the lab bench, label your box with your name and lab section.

9. Before you put your microscope away, **ALWAYS** do the following:
    a. Turn off the power and place the 10X objective lens or region lacking a lens into position. Turn the light intensity all the way down.
    b. Clean each and every lens (objectives and oculars) with **LENS PAPER** and cleaning solution. Never, never, never use any other kind of tissue or paper towel.
    c. If using oil, clean up any spilled oil present on other parts of the microscope.
    d. Unplug the microscope, fold the electric cord and place it behind the stage--**DO NOT** wrap cord around the arm of the microscope.
    e. Replace the dust cover on the microscope and carefully put the microscope in the cabinet with the **ARM FACING OUT**.

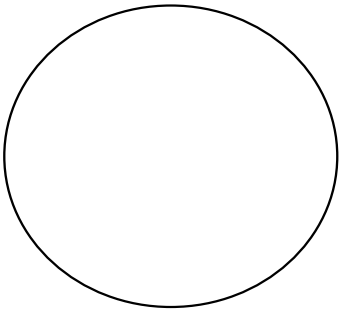
NOTES

LAB 1 RESULTS

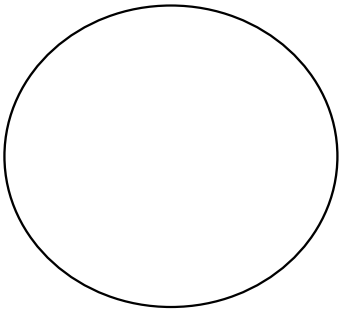
I. UBIQUITY OF MICROORGANISMS

1. Draw the colonies observed on the TSA plates.
   Note: You will be able to make these observations during LAB 2, AFTER the plates have been incubated.

TSA Swab Plate



TSA Cough Plate



2. In your own words describe the **Colony Morphology and Color** of 3 different colonies from either of the TSA plates.

   Colony 1 _____
   _____

   Colony 2 _____
   _____

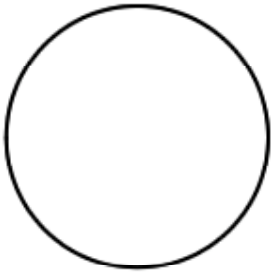
   Colony 3 _____
   _____

3. Does the nutrient broth tube show growth of bacteria? _____
   Based on the amount of turbidity, estimate the number of bacteria/mL present in the nutrient broth.
   _____

II. MICROSCOPY

1. Draw a few of the organisms that you observed

Sample Viewed: _____



Total Magnification: _____

QUESTIONS:

1. Use the method presented in lecture to determine the approximate size of at least two organisms viewed in either the pond water or the hay infusion.

_____

_____

2. Complete the following table.

Microscope Type	**Function**
Bright field	
Microscope Part	**Function**
Aperture Iris diaphragm	
Condenser	
Low power objective lens	
High-dry objective lens	
Oil immersion lens	
Ocular lens	
Mechanical stage	
Coarse Adjustment Knob	
Fine Adjustment Knob	

3. Differentiate between resolving power and magnifying power of a lens.

_____

_____

_____

4. What is meant by the term parfocal? _____

_____

5. What factors are influenced by the iris diaphragm? _____

_____

6. Which objective lens focuses closest to the slide? _____

7. How is total magnification of a compound microscope determined? _____

8. Describe the purpose of using immersion oil with the 100X objective lens?

_____

_____