LABORATORY 1

Microscope and Simple Stains

OBJECTIVES

- To learn or review the parts and proper use of the microscope
- To understand the use of different objectives for magnification
- To perform a wet mount and heat fixed-simple stain of bacteria
- To identify bacterial cell morphology (shapes and arrangements)

MATERIALS

Compound microscope
Cultures of bacteria (bacilli and cocci)
Hay infusion (pond water with hay incubated in sunlight for 3-4 days) microscope slides
Prepared stained smears of spiral organisms
Saline
Methylene blue
Safranin
Congo red
Toothpicks

BACKGROUND

It is important for microbiology students to be familiar with the compound microscope, as this is the instrument for viewing bacteria. The instructor will review the important parts of the microscope, as shown in the figure, and also cover proper care and use of the scope.

Light is used to illuminate the specimen on the slide, which is often stained to increase contrast and facilitate viewing. The path of light through the lenses of the scope (shown in figure) results in an enlargement, or magnification, of the image generated. It also results in an inversion of the image. The power of the objective and ocular lenses determines the degree of magnification of the image. Total Magnification = objective X ocular (e.g., If the ocular is 10X and the low power objective is 10X, then total magnification of the image is 100X).

As the magnifying power of the lens increases, the working distance decreases. This is the space between the objective lens and the slide. It is important to consider this distance to avoid damage to objectives or slides.

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Resolving power, or resolution, is the ability of a lens to distinguish two nearby points as separate and distinct. It is the capacity of a microscope to differentiate detail. Increased magnification without adequate resolution does not provide additional information in the image. The resolution is dependent upon the numerical aperature (NA) of the objective lens. The NA is an optical value stamped on the lens by the manufacturer and is a function of the light gathering capacity of the lens. The greater the NA, the better the resolution of the microscope using that lens.

$$\text{RP (resolving power)} = \frac{\text{wavelength of light}}{\text{2 x NA (numerical aperture)}}$$

Example: 550 nm (average light wavelength) ÷ (2 x 0.25) = 1100 nm

Thus the smallest distance between points on an object that can clearly be distinguished with that 10X objective is 1.1 micrometer, or 1100 nm.

Light scattered by the specimen is diffracted in many directions. Light traversing through the different substances of air, glass, and water also results in refraction, or bending of the light. Therefore, light from the specimen may not all enter the objective lens, depending on the degree of refraction and the size of the lens aperture, or opening. Higher-power lenses have smaller apertures. Immersion oil is used to reduce this refraction of light, allow more light to enter the lens, and thereby generate a brighter, clearer image at high magnification. This is because the refractive index of the oil is the same as the glass of slide and lens, so no light is refracted before entering the lens (see figure). Oil must be put only on the oil objective and must be cleaned off after each use!

CARE AND HANDLING OF THE MICROSCOPE

1. Each student is assigned one scope for the entire term. It is used by several other lab sections, also. Before each use, examine the microscope carefully and report any unusual condition or damage.

2. Always use both hands to carry the microscope–one holding the arm, one under the base.

3. Keep the oculars, objectives, and condenser lenses clean. Use dry lens paper only to wipe them. See instructor if this is not sufficient for cleaning.

4. Carefully turn the nosepiece to change objectives, and watch while changing to avoid damage to the objective or slide.

5. At the end of each microscope session, remove slide from the stage, wipe away the oil on the immersion objective, and place the scanning objective in vertical position.

6. Carefully return the microscope to its cabinet.

Staining is used to increase the contrast between bacteria and the slide in order to clearly visualize the cells. This can be accomplished by using a positively charged stain that interacts with the cell surface to make the cells darker than the background. It can alternatively be done by staining the background, using a negatively charged stain that is repelled by the cell surface. These two staining procedures are quick and are used to verify the presence of bacteria and to determine the cell morphology.

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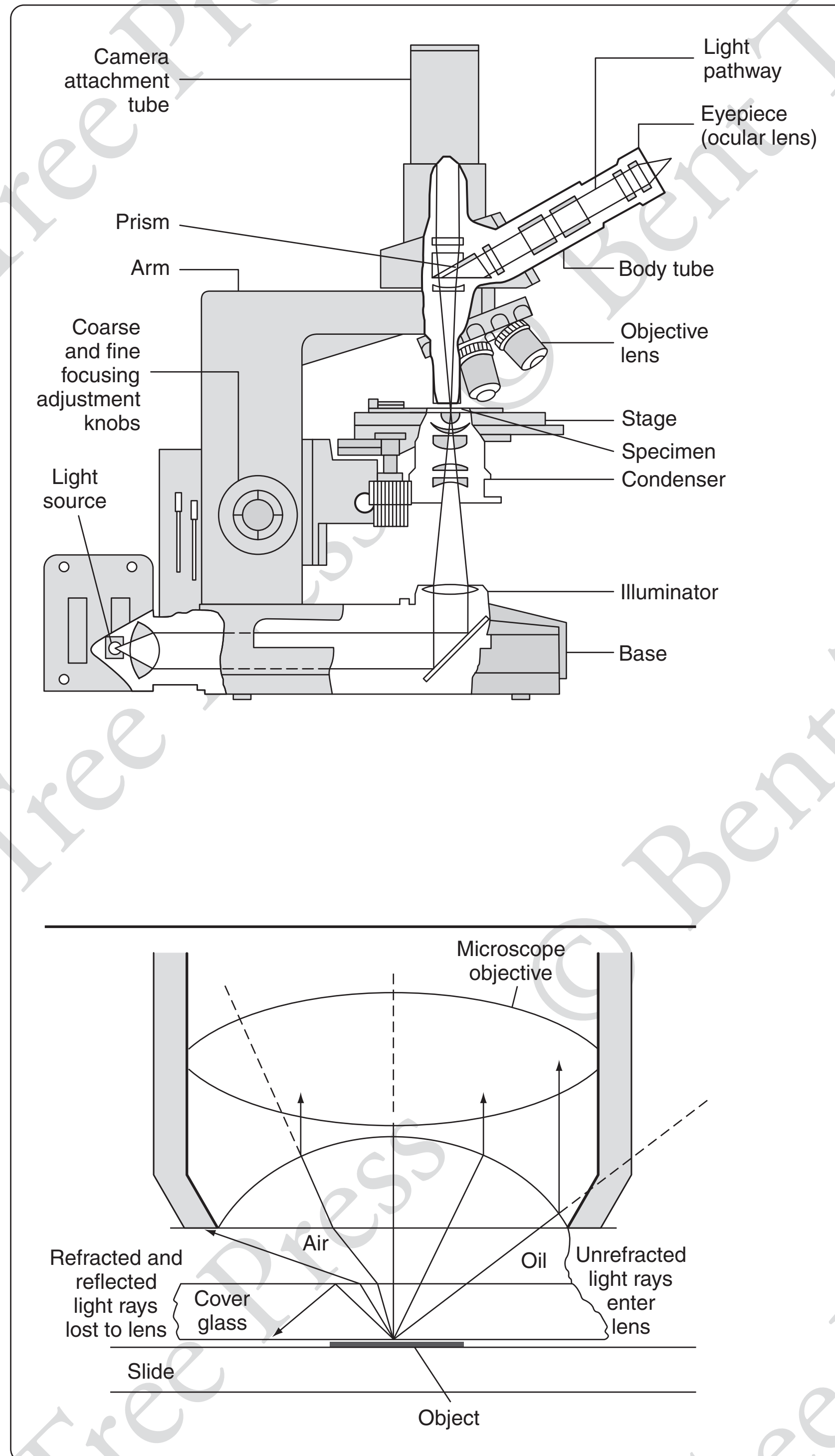

Bacterial cells are classified based on cell morphology. This includes cell shape and grouping. The three common bacterial cell shapes are cocci, bacilli, and spirals, as shown in the figure. Less common bacterial shapes include square and star. The cocci and bacilli are frequently grouped in particular arrangements, as a result of remaining attached through cell division. These groupings are designated with terms such as staphylo-, strepto-, palisade, etc.

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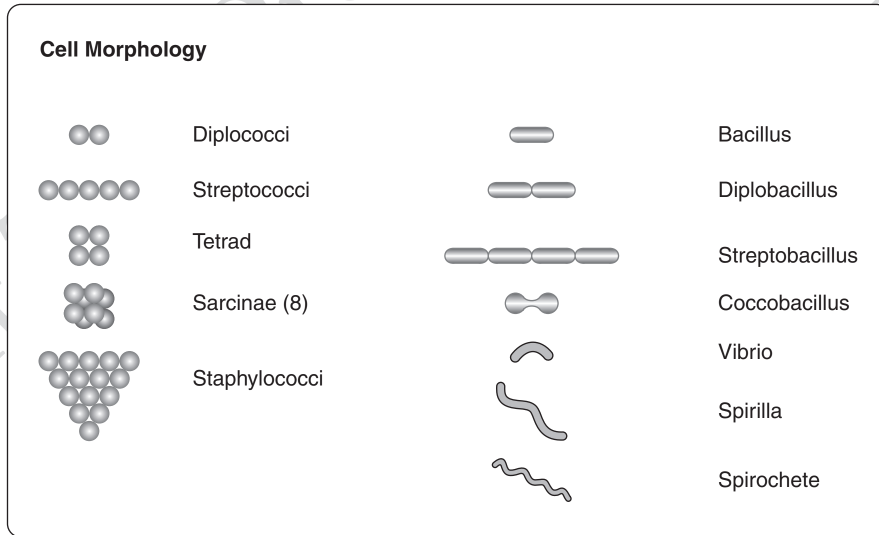

PROCEDURE A: SMEAR PREPARATION FROM PLATE OR BROTH

Numerous staining procedures begin with a smear preparation.

1. Begin with a clean, dry slide (use a cleaning powder, such as BonAmi, with water) and avoid fingerprints. Mark slide on top with wax pencil.

2. Place a loopful (not a large drop) of saline on the slide.

3. With a sterile, cooled inoculating loop, pick up a very small amount of bacterial culture (touch an agar colony) and mix into the saline on the slide, making a smear about the size of a dime.

4. Allow the smear to air dry. A thin white film should be visible.

5. Heat fix the slide by passing it rapidly through a flame three times. The slide will get warm but not hot. This kills the bacteria and makes them adhere (fix) to the slide, so they are ready for staining.

6. If using a broth culture, no loopful of saline is necessary. Just spread a loop or two of culture on the clean slide and allow to air dry. Then heat fix and stain as usual. As always, be sure to sterilize the loop before and after each use!

PROCEDURE B: THE SIMPLE STAIN

1. Place the slides on the staining rack over the sink and flood the bacterial smear with Methylene blue or Safranin. Leave on for 2 minutes.

2. Wash each slide gently with water, draining off the excess. Lay the slide down on a paper towel and fold the towel over the slide to blot (not rub) the slide to dry the smear. Allow the slide to air dry (a few seconds).

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3. Position on microscope, find and focus the smear at low power, then switch to high dry. Turn nosepiece to move objective sideways and add immersion oil to smear, then turn oil immersion objective into oil droplet. Bacteria are routinely viewed with the high power oil immersion lens to obtain the required detail for determining cell morphology.

4. Lab partners should use different stains, to allow comparison of each organism with each staining solution.

5. Repeat the process of smear preparation and staining with some material scraped from the surface of your teeth and around the gums.

6. Also observe the prepared slide(s) of spiral organisms (spirilla or spirochetes).

PROCEDURE C: NEGATIVE STAIN

1. Place a drop of Congo red stain on one end of a clean slide. Obtain bacterial culture and mix into the drop of stain.

2. Using the edge of a second clean slide, spread the drop of bacteria and stain across the first slide in a smear, feathering the edge at one end.

3. Allow the stain to air dry. When dry, observe a thin area of the smear under oil immersion as before. Cells will be unstained in a background of stain or ink.

PROCEDURE D: WET MOUNT

1. Obtain a clean slide and coverslip. Pipette a small drop of hay infusion liquid onto the center of the slide. Carefully place a coverslip on top of the drop. Avoid any pieces of hay or dirt in your drop, or the coverslip will not lie flat.

2. Center the coverslipped slide on the microscope stage. Observe the microbes present with different dry objectives. Do not use oil immersion on the wet mount.

3. Draw a sample of the organisms you observe. Make note of any movement. The microbes will include protozoa and algae. Can you identify any of these?

4. Wash slide with Bon Ami after use; discard coverslip in broken glass container at the front of the class.

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REPORTING

1. For each organism, report the cell morphology (shape and arrangement) and make a drawing of your stains and prepared slides. Each of the drawings made should be labeled with the objective power, the total magnification, and the RP or resolution, assuming 550 nm average wavelength of white light.

2. How were the two simple stains different for each organism? Which provides the sharper view?

3. What types of organisms do you observe from the biofilm on your teeth?

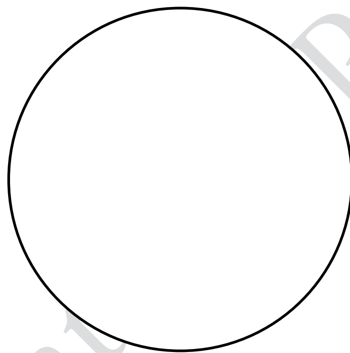
4. Draw a sample of all organisms you observe in the wet mount and make note of any movement.

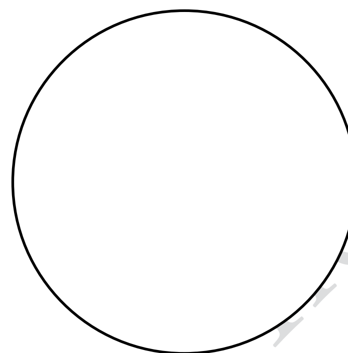
5. Why is oil used on the 97X objective?

6. What is the advantage of parfocal microscope objectives?

7. How did the negative stain compare with the simple stain for clarity?

IN FUTURE LAB EXERCISES, ALL DRAWINGS FROM THE MICROSCOPE MUST BE LABELED WITH THE TOTAL MAGNIFICATION!





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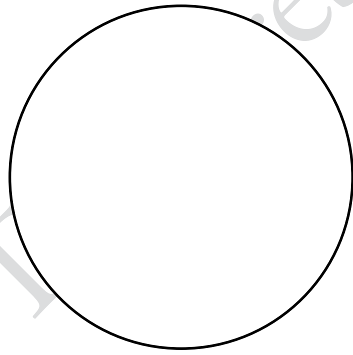

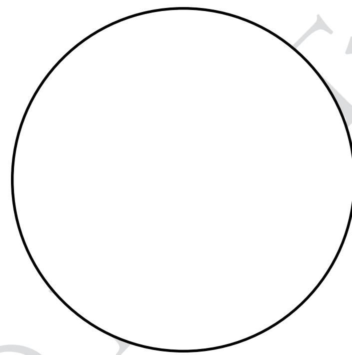

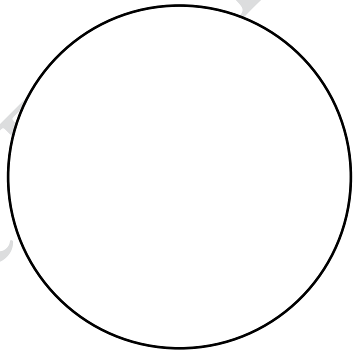

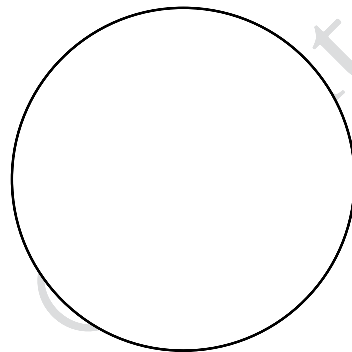

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