Microscopes and Prokaryote Diversity

Learning Objectives
Building on the learning objectives from your lab syllabus, you will be expected to:
1. Be able to recognize and correctly classify all organisms covered;
2. Be able to describe the characteristics of each organism;
3. Be able to recognize and correctly identify everything listed in bold face type;
4. Be able to recognize and correctly identify typical prokaryotic and eukaryotic cells;
5. Be able to correctly make a wet-mount slide;
6. Be able to correctly operate a compound microscope such that you can clearly see these organisms at high magnification.

Pre-Lab Activity
1) Review the parts and use of a microscope from the BIOL 1107 lab handout – Compound Microscope and Cell Structure and Function
2) Use your text book (Biological Science 6th edition by Freeman) to review the general structure of prokaryotic cells. Fill in the table below to contrast them to typical eukaryotic cells.

<table>
<thead>
<tr>
<th></th>
<th>present in all cells</th>
<th>present only in (some or all) prokaryotes</th>
<th>present only in (some or all) eukaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nucleus</td>
<td></td>
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<td></td>
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<tr>
<td>ribosomes</td>
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<td></td>
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<tr>
<td>cell membrane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flagella made of flagellin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>flagella made of microtubules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cytoskeleton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>membranous organelles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cell wall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>divide by mitosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>divide by binary fission</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Your review of these two major types of cells should show you that the prokaryotes are structurally more simple than the eukaryotes. Their diversity is due to their metabolic capabilities (their genes), not their form.
Introduction
As a group, the prokaryotes possess numerous metabolic pathways that are not found in the eukaryotes. For example: many can 'fix' nitrogen (convert nitrogen gas into ammonia ions for use in building proteins and other molecules); others can use light energy to drive their metabolism, but they don't necessarily use chlorophyll or release oxygen; and, some are actually poisoned by the presence of oxygen gas.

As our understanding of these organisms increased, it became obvious that they are not all closely related. Current classification systems place the prokaryotes in two Domains, and all of the eukaryotes in another. Again, this underscores their extreme amounts of difference, both when compared to each other and when compared to the eukaryotes.

In this lab, we will briefly survey the structural variability and some of the metabolic diversity of these groups. As you work your way through these activities keep in mind that when you are viewing live samples you have microscopic organisms suspended in solution. Under these conditions a phenomenon known as Brownian movement can be observed. Brownian movement refers to the random movement of small solid particles (or microorganisms) as they are continuously jostled by molecules of solution.

Activity 1: Shapes of prokaryotic cells
Use the prepared slide to observe these three major cell shapes found in prokaryotes:
round or spherical shape = coccus
rod shaped = bacillus
spiral = spirillum

Note that the cells may remain attached, forming colonies, which may take various forms, including filaments or clumps.

Draw what you observe in the spaces below

Magnification ______ X  Magnification ______ X  Magnification ______ X
Activity 2: Domain Bacteria

The majority of prokaryotes belong to this Domain, including all those known to cause human diseases as well as many that are either beneficial or harmless. Members of the Domain Bacteria occupy diverse habitats, including on and in other organisms, fresh and salt water and soil. Many form mutually beneficial relationships with eukaryotes, including ourselves.

A. Yogurt-forming bacteria

The beneficial bacterial include some used for food processing - in this case, partially digesting the proteins and other solids of milk, transforming it into yogurt. Two genera should be present: Lactobacillus and Streptococcus. (Note: Streptococcus includes some notorious pathogens, but S. lactis is a beneficial species.)

Prepare a wet-mount slide by mixing a tiny dab of yogurt with a drop of water and then adding a coverslip. The mixture should have clear areas; it should not be entirely opaque. Observe under progressively higher magnification to locate the bacteria, which will be floating in between the areas of coagulated protein and other milk solids. Draw what you observe in the space below.

What shape is Lactobacillus? ________________________________________________________________

What shape is Streptococcus? ________________________________________________________________

Note the random vibration of these cells. What is it's source? ______________________________________

B. Rhodospirillum rubrum is an example of the purple non-sulfur bacteria. This is one of several groups of bacteria that use chlorophyll-like molecules to capture light energy, but photosynthesize without producing O₂.

Reminder: O₂ is generated by photosynthesis only when chlorophyll a uses water as a source of H⁺ and electrons. These bacteria don’t generate O₂ because they use different pigments, and molecules other than water as sources of electrons.
Make a wet-mount of this culture - do not add any additional liquid. Draw your observations in the space below.

Magnification ______ X

Describe their motion. _____________________________________________________________
Is a flagellum visible? ___________________________________________________________
Which term describes the shape of these cells? ________________________________________

C. Typical Photosynthetic bacteria – the cyanobacteria
These bacteria are the only prokaryotic group which does 'typical' photosynthesis, using chlorophyll a and releasing oxygen from water. They are the closest relatives of the eukaryotic chloroplast. They also include species with some of the largest prokaryotic cells and some which show limited cooperation between cells and specialization of function.

*Oscillatoria* has the cells arranged in elongate filaments. Make a wet-mount slide from the culture and observe. The genus name describes the characteristic motion of these filaments, which may not begin until they have been illuminated for a few minutes. What do you observe? _____________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

Using the prepared slide, note the variation in cell size possible in this group. Also, look for evidence of binary fission - a cell that is incompletely divided by a wall.
Draw this organism at 10X and 40 X

Using the prepared slide, note the variation in cell size possible in this group. Also, look for evidence of binary fission - a cell that is incompletely divided by a wall.
Draw this organism at 10X and 40 X
*Gloeocapsa* has the cells separate, but temporarily held together by a secreted gelatinous matrix. Make a wet-mount slide from the culture. You should be able to observe this matrix using only control of light intensity and iris diaphragm aperture. If this is difficult, add a drop of india ink to the slide and re-observe. Your slide box also includes a prepared slide of this genus.

Draw this organism at 10X and 40X. Be sure to label the gelatinous matrix.

![Diagram](attachment:diagram_gloeocapsa.png)

*Anabaena* is another filamentous genus, which will have occasional cells that are enlarged and have thickened end walls. These large cells are **heterocysts**, which fix nitrogen. Make a wet-mount slide from this culture and observe, looking carefully for the heterocysts.

Draw this organism at 10X and 40X being sure to find and include a heterocyst. Be sure to label the heterocyst.

![Diagram](attachment:diagram_anabaena.png)

*Nostoc* is similar to *Anabaena*, but differs strongly in the amount of gelatinous matrix which it secretes. When growing on moist soil, it actually forms macroscopic spheres. Observe both the macroscopic material and the prepared slide which is set up as a demo.

Draw this organisms at 10X and 40X.

![Diagram](attachment:diagram_nostoc.png)
Activity 3: Domain Archaea

The best known archaeans have highly specialized habitats (including extremes of temperature, pressure and pH) and thus can be quite difficult to culture. Among the easiest to culture are the halophiles (salt-lovers), who typically thrive in solutions of 10 - 40% salt. For comparison, seawater is typically around 3.5% salt.

Observe both the culture and demo slide (made using a salt solution of a known concentration) of *Halobacterium salinarum*. These organisms are aerobic heterotrophs and can generate ATP from absorbed organic molecules. However, oxygen is often not readily available in their environment (oxygen’s solubility in water declines with increasing salinity), and they have an alternative method of ATP generation. They use a purple pigment, bacteriorhodopsin (a light driven proton pump), which absorbs light energy and generates ATP. Note that this is NOT photosynthesis, because it does not result in the production of carbohydrates from carbon dioxide.

Activity 4: Comparison of Prokaryotic and Eukaryotic cells.

Crush a small piece (approximately 1 mm$^2$) of the floating water fern *Azolla* on a slide and make a wet-mount slide. This fern has specialized pockets on the underside of it’s leaves which are normally colonized by *Anabaena*. This is a mutually beneficial symbiosis. Since both organisms are photosynthetic, what could they be providing for the other?

Compare the plant cells and their chloroplasts to the associated prokaryotic cells of *Anabaena*. Note the relative size of these cells and the presence or absence of visible structures in cells. Also compare both the shade of green color of these cells and the location of the pigments.

Draw *Azolla* at 10X and 40X

In the same spaces above draw *Anabaena* at 10X and 40X making an attempt to show the size differences between *Anabaena* and *Azolla* in these drawings.
Make a wet mount slide with some of the mixed culture sample. These contain several types of prokaryotic cells - mobile and not, large and small, and of various shapes - as well as a variety of eukaryotes.

Describe the diversity that you see - how many different organisms are present? Be certain that you can reliably distinguish obvious prokaryotes from obvious eukaryotes.