Goals:
1) recognize and correctly classify all organisms covered;
2) describe the characteristics of each organism;
3) recognize and correctly identify everything listed in **bold** face type;
4) recognize and correctly identify typical prokaryotic and eukaryotic cells;
5) correctly make a wet-mount slide;
6) correctly operate a compound microscope such that you can clearly see these organisms at high magnification.

**Before coming to lab:**
1) review the parts and use of a microscope (Biol 1107 lab handout);
2) use your text book 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>
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<tr>
<td>nucleus</td>
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<td>ribosomes</td>
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<td>cell membrane</td>
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<tr>
<td>flagella made of flagellin</td>
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<tr>
<td>flagella made of microtubules</td>
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<tr>
<td>cytoskeleton</td>
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<tr>
<td>membranous organelles</td>
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<tr>
<td>cell wall</td>
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<tr>
<td>divide by mitosis</td>
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<td>divide by binary fission</td>
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</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 \( a \) 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.

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.

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, 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.

Working with a partner, use the salt solution to prepare wet-mount slides of a few of the sample organisms available. How does each organism react to this concentration of salt?
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 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.

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.

What shape(s) is/are present?

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

*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 $O_2$.

Reminder: $O_2$ is generated by photosynthesis only when chlorophyll *a* uses water as a source of $H^+$ and electrons. These bacteria don’t generate $O_2$ 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.

Describe their motion. Is a flagellum visible?

Which term describes the shape of these cells?

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.

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.

**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.

**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.

**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.

**Comparison of Prokaryotic and Eukaryotic cells.**

Crush a small piece (approximately 1 mm²) of the floating water fern *Azolla* on a slide and make a wet-mount slide. This fern has specialized pockets on the underside of its 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.

Then, make a slide with some of the mixed cultures. 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.