Biology 2015 – Evolution and Diversity Lab

Lab 1, Part 1: Microscopy, Bacteria, and Gram Staining

Introduction

At the beginning of lab today you will learn the proper use of light microscopes for both brightfield and phase contrast observations. The basic light microscope is a widely used research and diagnostic tool and, beginning with this week's lab, you'll have plenty of opportunities to hone your microscopy skills throughout the rest of this course. Use the first part of this handout to learn the proper use of the light microscope at your workstation.

Once you are comfortable with how to properly use your microscope you can begin exploring the diversity in the bacteria we have brought in for this week's lab. Bacteria are remarkably numerous and diverse, although most of their diversity is metabolic rather than morphological. However there are some important, basic, morphological features you should be familiar with. These include size, shape, motility and Gram stain reaction. You will be able to observe at least some of the diversity of bacteria for these features in the bacterial cultures provided for this lab.

Microscopy

First, you need to know how to set up a microscope for proper Kohler illumination. When you have set up Kohler illumination on your microscope, you are assured of achieving the best resolution that your microscope can provide - and "resolution" is generally much more important than "magnification" in using a microscope. (Why?)

Setting up and using Kohler illumination

1. Place a prepared **specimen slide,** with the **coverslip** facing upward, onto the **stage** of the microscope. Turn on the microscope's light source (on your microscope, rotate a red "wheel" located on the left side of the base of the instrument). Then turn the **nosepiece** of the microscope until the **10x objective** is in position to observe the specimen on your slide.

2. Find the **condenser.** This is a lens system that is suspended directly underneath the stage. On many microscopes, including yours, there will be a **"turret"** in the condenser system that can be set to several different positions. Look for the label **"BF"** (for "Brightfield") on this turret. If you see another label, such as "DF" or "PH1", rotate the turret until the label "BF" is facing you. *(Note for microscopes other than the Leica microscopes used in this lab: Turret labels may vary from one microscope to another. For example, sometimes "H" is used instead of "BF" to indicate Brightfield. And on some microscopes the upper lens of the condenser must be rotated into the light pathway when you are using the 10x or higher. It must then be rotated out of the light pathway when switching back to lower magnification objectives.)*

3. Find the condenser's focusing knob. It is usually located somewhere to the left of the condenser assembly, and is often black. Use it to move the condenser until its upper lens is close to, but not touching, the bottom of the microscope slide.

4. Find the **field diaphragm** in the base of the microscope (directly below the condenser), and the **condenser diaphragm** on the condenser itself. Adjust both of these so that they are fully opened (move the adjusting levers all the way to your left- see below). On your microscopes, the field diaphragm is adjusted using a small lever that can be moved from left to right. But on other microscopes you may find a knurled ring, or perhaps a "wheel" similar to the wheel that adjusts the light source on your microscope. Most microscopes use a lever to adjust the condenser diaphragm (sometimes also called the condenser 'aperture'), but a few use a knurled ring.

5. Look through the **eyepieces** and focus on the specimen. Most microscopes, including yours, have both an outer 'coarse' focusing knob and an inner 'fine' focusing knob. Yours are white, and are located on the sides of the microscope, near the back. The eyepieces on your microscope have rubber cups that extend upward. These should be folded down if you wear eyeglasses. Once the specimen is in focus when viewed by your right eye, close that eye and focus the specimen for your left eye using the knurled ring near the base of the left eyepiece. (This should only have to be done when you first sit down to use the microscope.) You may need to turn the light intensity down a bit for comfort, and the specimen will probably look 'washed out' at first. Once you have a specimen in focus, you're finally ready to set up the illumination system!

6. First, you'll need to fully close down the field diaphragm. Then, as you look through the microscope, you should be able to see a circle of light against a much darker background. That circle of light is the light coming through the field diaphragm. Use the focusing knob of the **condenser** to focus on the edge of the field diaphragm until it appears sharp **(note that this is not the same as adjusting the focus so, DO NOT USE THE COARSE/FINE FOCUS KNOBS TO ACHIEVE THIS AS DOING SO WILL MESS UP THE MICROSCOPE!).** When you have the condenser close to proper sharpness you'll see that the edge of the diaphragm turns bluish when you move the knob in one direction, and reddish when you move it in the opposite direction. You'll want to stop at about the point where this color changes.

7. When you've got the black edges of the field diaphragm as sharp as possible, you'll probably notice that it is not quite in the center of your **field of view.** There are two **centering knobs** on the condenser that are used to move the opening of the field diaphragm around within the field of view. These knobs are located below the stage near the bottom of the condenser assembly. They are almost always silver-colored and extend towards your right and left sides. Use these knobs to center the opening of the field diaphragm.

8. While observing your specimen through the eyepieces, continue to open up the field diaphragm until all the corners meet at the edge of the field of view. As you do this, you may need to make additional adjustments in order to keep the opening of the field diaphragm centered in the field of view. Once it is perfectly center, open it up until all the field diaphragm leaflets have just barely passed out of the field of view.

9. Finally, you'll need to adjust the condenser diaphragm. You should make this adjustment while looking through the microscope at the focused specimen. Turn the dial that has "**Ph**" on the left and the number "**2**" on the condenser itself. Slowly close down the condenser diaphragm until you see a barely perceptible darkening of the specimen image. **Most people close the condenser diaphragm until it's opening is too small,** but this will prevent you from obtaining the best resolution from your microscope. Your microscope is now set up for Kohler illumination with the 10x objective. **YOU MUST REPEAT STEPS 6 THROUGH 9 EACH TIME YOU SWITCH OBJECTIVES!** The diaphragms should never be used to adjust the brightness of the light illuminating the specimen. Opening or closing the diaphragms after Kohler illumination has been set up will only reduce the resolution of the microscope. Instead, adjust the light source until its brightness is satisfactory.

Viewing specimens

For many specimens, it's appropriate to begin your observations using the 10x objective. It's often easier to 'find' and focus on your specimen using the 10x objective. And there is less danger of pushing your objective lens into the specimen - breaking your microscope slide - while you're getting it into focus. When you find features that you would like to examine in greater detail, you can rotate the 40x objective into position, **and then you must make those adjustments in steps 6 to 9 above (for Kohler illumination)**, and continue your observations. The total magnification of your microscope is generally found by multiplying the magnification of the objective you're using by the magnification of the eyepiece lenses on your microscope. Eyepieces are most often 10x lenses, so your total magnification when using the 10x objective is about 100x.

Using phase contrast microscopy

Many biological specimens, especially bacteria, single cells or single-celled animals, are almost transparent and so have very low contrast when viewed by Brightfield microscopy. Phase contrast microscopy provides a way of viewing such specimens in much greater contrast. You don't need to know the details of how phase contrast works for this course, but you do need to know how to recognize that a microscope is equipped to use phase contrast. And you do need to know how to properly set up phase contrast.

Phase contrast microscopy uses special objective lenses that are usually marked with the word "Phase", or perhaps the abbreviation "Ph". This marking usually also includes a number so that the whole label would read "Phase 1", or "Ph 2", for examples. There's some variation from one manufacturer to another. If a microscope is equipped to use phase contrast, the condenser will generally also have an adjustable ring or "turret" with several positions labeled "Ph 1", "Ph 2", etc. To use phase contrast microscopy, you simply adjust the turret on the condenser to the position where its 'Ph' label matches the 'Ph' label on the objective lens you're using.

Then you set up Kohler illumination, just as you would do for Brightfield microscopy, with just one exception. **In step 9, you should not close the condenser diaphragm down - instead, leave it fully opened.** (Your microscope even has a "Ph" label at the point where the condenser diaphragm lever is set to fully open!) In phase contrast you will notice that the overall image seen through the microscope is darker than for Brightfield microscopy, but the contrast is much greater.

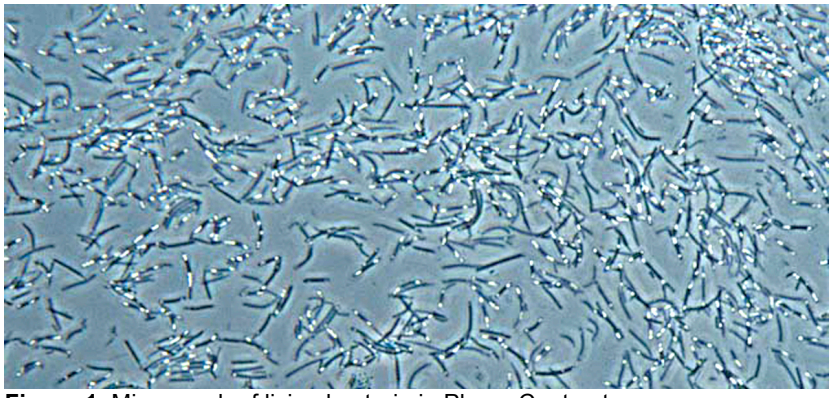


Figure 1. Micrograph of living bacteria in Phase Contrast.

Microscope care

Microscopes are somewhat delicate instruments. Treat them gently. If something that is supposed to move won't move, or if the microscope doesn't seem to be working properly for *any* reason, please *stop* and find an instructor for help. Please use only lens paper to clean the objectives, condenser or eyepiece lenses. Don't use Kimwipes or paper towels! If you need to move a microscope, use both hands - one should be placed under the microscope base, and the other should have a good grip on curved part of the microscope stand. When class is over, please put the lowest power objective in position. Then put the dust cover on the microscope.

Bacteria

Examining bacterial cultures

You should start by observing the bacterial cultures provided. There are a number of bacterial available for you to examine today. If the cultures are successful we will have *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Rhodospirillum rubrum*. Bacteria can be found just about everywhere, and they are pretty much invisible to the naked eye. So it's necessary to follow procedures that minimize the possibility that you'll be observing or studying the 'wrong' bacterium, or the possibility that you'll be growing up a mixture of different bacteria when you really wanted to grow just one! Here's what you should do to obtain a sample of one of the pure cultures for examination by light microscopy (an instructor will demonstrate this procedure):

1. Find the culture you'd like to sample.

2. Take a clean microscope slide, and place a *small* drop of water in its center then use the flame of a Bunsen burner to sterilize a microbiology transfer loop by heating it red hot. Let the loop cool for a few seconds and then use it to remove a ***very small amount*** of bacterial slime from the culture surface. (There are *lots* of bacteria in even the smallest amount of the culture - use too much, and you may have a very hard time seeing the individual bacteria.)

3. Dab your transfer loop a few times in the drop of water on your slide to wash off the bacteria. Then flame your transfer loop one more time, to kill any leftover bacteria.

4. Place a coverslip on top of the drop of water, and your bacteria should be ready to examine and be sure to keep water off the top of the coverslip! Plan to use the **40x objective and phase contrast** to observe the live bacteria in each of the cultures. But remember that it's best to start with the 10x objective to get roughly focused on your microscope slide, and then use the 40x objective to begin observing your bacteria. For an even closer look at the bacteria, you may want to use the 100x objective. Before you use this objective, however, make sure there isn't excess water extending out from underneath your coverslip.

For each of the bacteria, you should observe the general shape of the cells, their approximate size, and their motility. Notice any variability you may observe in the shape or size of the bacteria from any one culture. And take notes on all of this, including the transfer procedure and the names of the bacteria. The cultures provided were selected to allow you to observe a range of bacterial sizes and shapes.

Gram Staining

Bacteria are divided into two groups based on the Gram stain reaction. Gram-positive bacteria stain purple, and Gram-negative bacteria stain pink or red. Hans Christian Gram developed the Gram stain procedure in the late 1800's. You'll be using the procedure to stain two of the bacteria provided today- *Escherichia coli,* and *Bacillus subtilis.* You should probably work in pairs for this exercise, each of you staining a different bacterium. The mechanism by which the Gram stain works have been a controversial issue for many years- read over the account in the paragraph below.

The Gram Stain Mechanism

At this time the exact mechanism of the Gram stain reaction is not yet known. The most prominent current theories are as follows:

(1) The cell wall of a Gram-positive bacterium is composed of a heteropolymer of amino acids and sugars called peptidoglycan. This wall provides a barrier through which the crystal violet- iodine complex cannot pass during decolorization. When this wall is removed enzymatically with lysozyme, Gram positive cells no longer retain the stain complex and become Gram negative. A Gram-negative bacterium contains less peptidoglycan and more lipid than a Gram positive organism. These chemical characteristics cause more effective and rapid removal of dye complex when decolorizer is applied.

(2) There may exist a specific Gram-positive substrate within the cell. All major cellular macromolecules have been implicated, including lipids, carbohydrates, proteins, and nucleic acids. This theory is hard to prove because removal of any of these components from the cell vastly alters the chemistry of the cell wall. However, there is some evidence for a crystal violet- ribonucleic acid-iodine complex in Gram-positive cells.

(3) The real situation may be the combination of above two theories suggesting that a Gram positive bacterium is not only less permeable but also contains a compound that reacts with and holds the stain complex tightly.

Reference:
Bassiri, Eby. Penn State University
http://www.sas.upenn.edu/labmanuals/biol123/Table_of_Contents_files/7-Staining.pdf

The Gram Stain Procedure:

1.Clean a microscope slide with a few drops of 95% ethanol, and wipe the slide dry with a Kimwipe. Take a wax pencil and draw a circle on the center of your slide. This will form a "corral" within which you will place your bacteria and apply your solutions. Label one end of the slide with the name of the culture you are sampling, and place a small drop of water in the center of your wax pencil circle.

2. Now, use a transfer loop to sample either *Escherichia coli* or *Bacillus subtilis.* (Follow the sterilization and sampling procedure you learned earlier.) Transfer only a small amount of the culture to your drop of water, and try to spread it out a bit with the transfer loop. Pass your slide **briefly** over an **alcohol lamp** to warm the slide and help stick the bacteria onto the slide. You may need to repeat this warming of the slide a few times, but be sure not to get the slide too hot - you'll cook the bacteria and they won't look very good after cooking. Your goal is just to help speed up the air-drying of the bacteria onto your slide.
3. Now use just a few drops of crystal violet solution to cover your specimen for 1 minute, and then gently rinse the cells with water. If you were to look at the cells at this point, all of them - whether Gram positive or Gram negative - would be stained purple.

4. Next, place a few drops of iodine solution on the cells for 1 minute. And, once again, gently rinse the cells with water. The iodine will form complexes with the crystal violet that's inside the cells, and all the cells will remain purple-colored.

5. Now, find a dropper bottle containing 95% ethanol. Slowly drop the ethanol onto your cells, holding the slide at an angle over a sink so that the ethanol drops flow over your cells and off the end of the slide. Be patient at this step, but stop rinsing when the ethanol dripping from the slide is no longer colored blue. The ethanol will dehydrate the cells,

shrinking them somewhat and shrinking their walls. When the pores in the thick walls of Gram-positive bacteria shrink, the iodine-crystal violet complexes are trapped inside the cells. Gram-positive bacteria will remain purple after this stage. Gram-negative bacteria have thin walls that do not trap the purple stain inside. Gram-negative bacteria become colorless at this stage.

6. Allow the slide to air dry a bit. Now cover your cells with a few drops of safranin O stain solution for 1 minute. The safranin acts as a "counter-stain", staining the colorless Gram negative bacteria pink or red, and leaving the Gram positive bacterial purple.

7. Rinse the slide in water, blot off excess water from around your cells, and allow the slide to dry. You should mount the slide on the stage of your microscope (you won't need a cover slip this time), and focus and center your cells under the 40x objective using Brightfield illumination. You'll probably see some cell debris and bits of agar on your slide, and these often stain pink or red. Be sure to find the bacteria! Gram-negative bacteria should look pink or red; Gram-positive bacteria should look purplish. Record your results in your notebook.

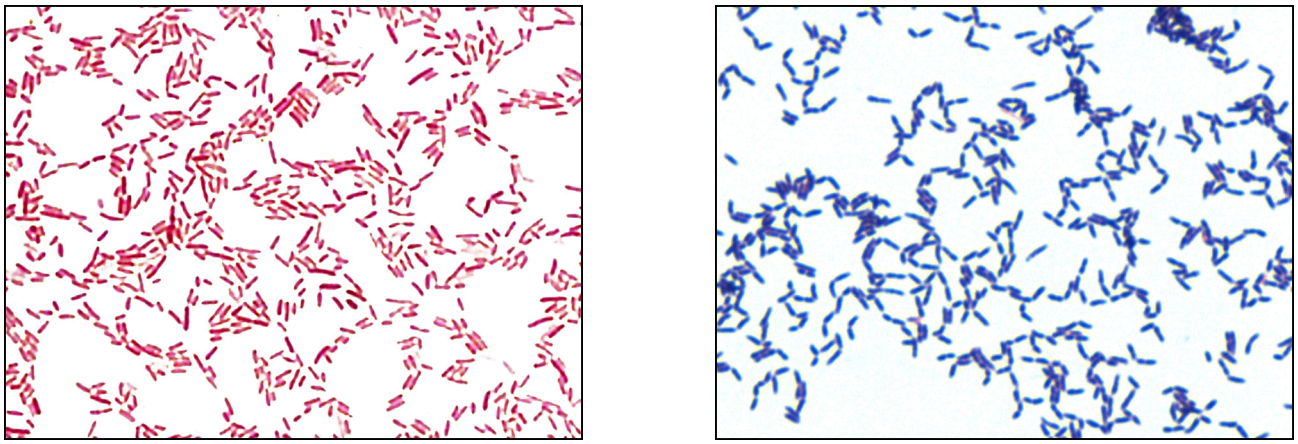


Figure 2. Micrographs of Gram-negative (left) and Gram-positive (right) bacteria. Note that the more bacteria you place on the slide, the less likely you are to see a result that is this clean. Also, notice that on each slide the bacteria show some variability in size and in intensity of staining.

Lab 1, Part 2: Cyanobacteria

Cyanobacteria

Cyanobacteria were once universally known as "blue-green algae" and they were grouped with the eukaryotic algae. Then, in the 1970's it was discovered that they are actually prokaryotes. The term "cyanobacteria" refers to what is evidently a monophyletic group within the bacteria. It's interesting that both their earlier name and their current one refer to the generally blue-green color that characterizes the group. In one of today's specimens you'll see the difference between the 'green' color of typical land plants, and the 'blue-green' color of the cyanobacteria.

Cyanobacteria are the only prokaryotes capable of oxygenic photosynthesis. They appear in the fossil record when the earth was young, around **3.5 billion** years ago! For at least 2 billion years they were the *only* significant primary producers on earth. And over that time their photosynthesis was responsible for the early increase in gaseous oxygen on earth that produced banded iron formations and red beds, and lead to the evolution of aerobic respiration.

Eukaryotes eventually acquired oxygenic photosynthesis when some of them acquired cyanobacteria through endosymbiosis (now these are cell chloroplasts). Cyanobacteria are still common, widespread and important today in both aquatic and terrestrial communities.

Some cyanobacteria are especially important because they can also 'fix' atmospheric nitrogen. They are capable of converting atmospheric N2 to NH3 - ammonia (or NH_4^+, the ammonium ion, in solution) - the first step in making atmospheric nitrogen available to other organisms. There are both free-living and symbiotic cyanobacteria that are important in nitrogen fixation, and you'll get to see examples of both in today's lab. Some of the most important nitrogen-fixing cyanobacteria have specialized cells called **heterocysts** (sometimes called "heterocytes") that fix nitrogen. But other cyanobacteria are able to fix nitrogen, at least under some circumstances, without the specialized cells.

Cyanobacteria can be *unicellular* (some unicellular species are referred to as *colonial* when multiple cells are held together by the polysaccharide substance called mucilage). Cyanobacteria can also be *filamentous* (see picture at right), which is sometimes referred to as a type of multicellularity. Numerous species are also *symbionts* with plants or fungi.


Filaments of *Ocillatoria* observed under a microscope.

Cyanobacteria were traditionally been classified into 5 taxonomic sections; sections I and II include unicellular species, while sections III to V filamentous taxa. Current phylogenetic work is showing that many of these groups are not monophyletic. To simplify the learning process, we will follow the section classification system as we examine the representative species in lab today. It is important to keep in mind that these sections are based on cyanobacteria morphology and do not reflect the evolutionary history of the groups.

We will examine specimens representing sections I, III, IV, and V. All sections are described below:

I. Unicellular, reproduce by binary fission or budding.
II. Unicellular, reproduce by multiple fission only ***(we do not have a representative for section II)***
III. Filamentous with no specialized cells or branches.
IV. Filamentous with specialized cells but no branches.
V. Filamentous with specialized cells and branches.

General guidelines for today's work with cyanobacteria

Living cultures of the following genera are available today: ***Merismopedia***, ***Gloeocapsa, Oscillatoria, Spirulina, Anabaena, Nostoc, and Fischerella.*** We also ordered ***Eucapsis*** and ***Gloeotrichia***. I am not sure which species we will get for each of these so I have to wait to update the handout until tomorrow morning.

You should make slides of each of these cyanobacteria by placing a drop of the culture on a microscope slide and covering it with a coverslip. Try not to get too much material under your coverslip, especially for the filamentous organisms, as the tangle of material may prevent you from finding important characteristics of the organisms.

You'll probably find that phase contrast microscopy, using the 10X and 40X objectives, will be most useful in observing these organisms. After you have each genus in focus at 40X, and in phase contrast, you should try observing the cells by brightfield microscopy as well. This will give you a better impression of the color of each organism. (All you should have to do is move the condenser turret to the brightfield position, and adjust the condenser diaphragm.)

One thing to keep in mind as you examine these cyanobacteria is that most of them have been grown up in culture, and the culture conditions may not be similar to the natural conditions in which these organisms might be found in the wild. The difference is most likely to be seen in organisms that produce specialized cells in nature. The specialized cells you might expect to see in cyanobacteria include **heterocysts** and **akinetes.**

Akinetes are specialized cells, often larger than 'ordinary' cells of the organism and with thicker walls that function as reproductive structures capable of withstanding harsh conditions. Akinetes may be the cells that overwinter in a pond, or that can withstand desiccation when a vernal pool dries up in late summer. We don't expect you to see akinetes, but they might be present today in samples of *Anabaena*. And you should give a bit of thought as to why the specialized heterocysts and akinetes of cyanobacteria might not be found in cultures received from biological supply houses!

You should be able to find heterocysts in *Nostoc, Anabaena* and probably in *Fischerella.* In *Nostoc* you should be able to find heterocysts in filaments collected from the bottom of the culture container. We have two species of *Anabaena.* One is the symbiont of *Azolla* plants growing 'wild' in our greenhouse. It will have heterocysts. The other is a free-living species.

SECTION I. Unicellular, Colonial Cyanobacteria

Gloeocapsa

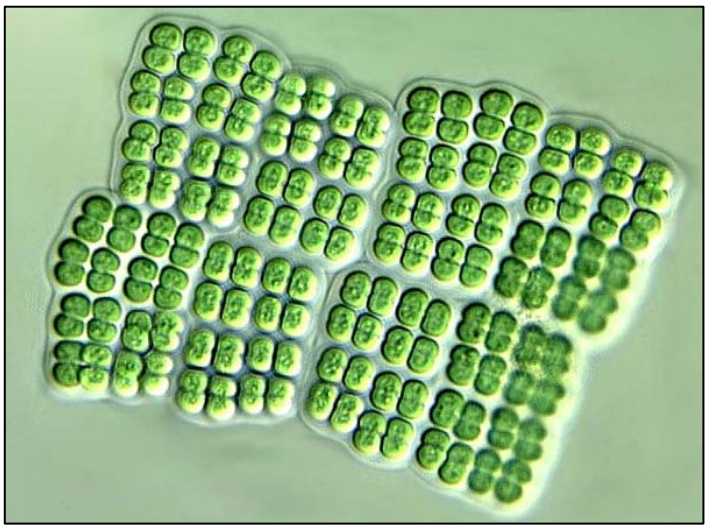
Gloeocapsa is a unicellular cyanobacterium that is considered to be colonial. In this culture, you will probably find individual cells, along with pairs of cells and groups of four cells. *Gloeocapsa* doesn't usually form large colonies, though its smaller colonies may sometimes 'clump' together.

You should closely examine your *Gloeocapsa* colonies. Can you offer any explanation as to why the colonial growth habit, in either of these forms, might be advantageous for unicellular cyanobacteria?

A colony of *Gloeocapsa* cells. This is a fairly average size colony for the cultures you will see in lab. Clumps of these colonies may be any times larger, and can be hard to study by light microscopy. This particular micrograph offers good hints as to how the colonies grow.

Merismopedia

The cells of *Merismopedia* are organized into 'sheets' that sometimes contains many cells. *Merismopedia* is considered 'colonial' because the sheets really are aggregates of single cells, not tissue-like structures. Examine the organism, and, as usual, draw/describe the size, shape, and arrangement of cells in the sheets. Make a second slide in the dilute India ink suspension provided (or simply add a drop of India ink at the edge of your coverslip) and record what you see. A mucilaginous or gelatinous 'sheath' surrounds most cyanobacteria. In some cases, as is for *Merismopedia*, it can be quite thick.



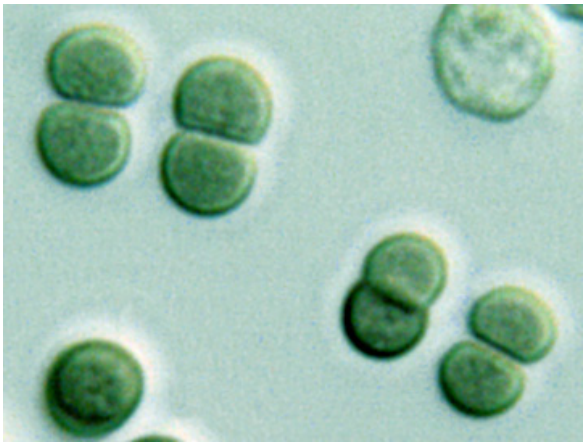
Several colonies of the *Merismopedia* sp.

As you examine *Merismopedia,* you'll notice the remarkably 'geometric' organization of the cells into rows and columns.

From what you see in these colonies, can you suggest what might be involved in how these colonies form?
colonial growth habit, in either of these forms, might be advantageous for unicellular cyanobacteria?

Eucapsis

Eucapsis are free floating cyanobacteria found in in bogs and swamps. Most species are known from acidic and cold (stenothermal) swamps, peaty pools, raised bogs and clear lakes (mainly in temperate to subpolar zones). There are also species described from volcanic soils as well as slightly alkaline waters (e.g., swamps with aquatic plants, channels, lakes, pools) of tropical and warmer areas of the temperate zone.



Eucapsis

SECTION II. Unicellular, reproduce by multiple fission only.

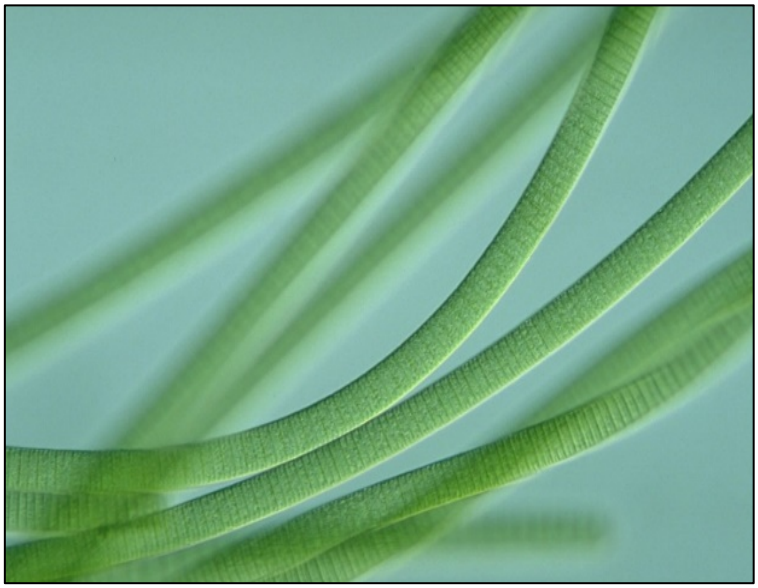
We do not have any examples of Section II to examine.

SECTION III. Filamentous (with no specialized cells nor branches).

The filaments of cyanobacteria are also called **trichomes**. As you examine each of the filamentous genera, take note of the size and shape of the cells that make up the trichomes. Look for dead cells with an 'empty' appearance. Each of the filamentous species you'll see in lab today has the ability to reproduce by fragmentation and the formation of **hormogonia** - short filaments released from the parent filament after the programmed death of adjacent cells.

Oscillatoria

This genus can be found in both freshwater and saltwater. Some members can be found in hot springs, and others are terrestrial. The ends of the filaments often have a characteristic shape that you should look for.



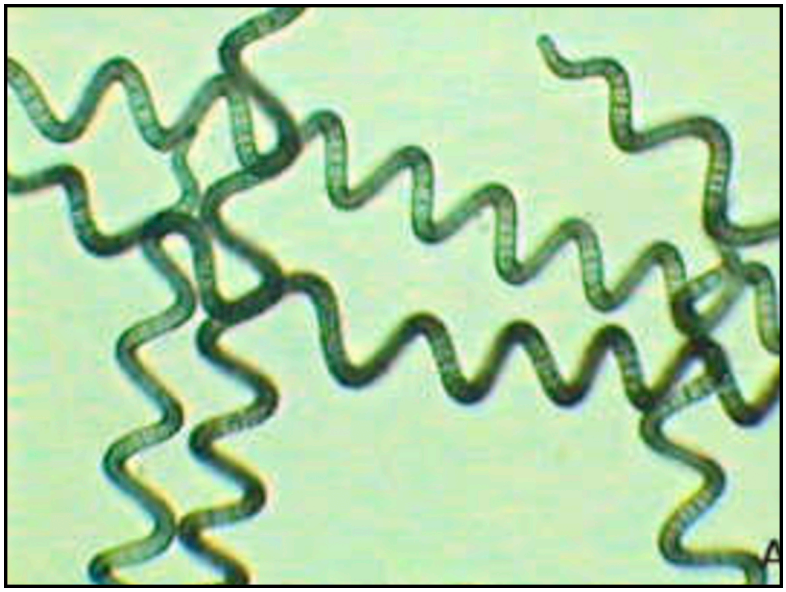
Several filaments of *Oscillatoria.*

Be a little patient when observing this genus, and look for the characteristic motility for which it is named. This movement is easiest to see if you watch individual filaments that stick out from a clump of filaments. When you first observe a clump you may not see much happening, but after a couple of minutes in the bright light of the microscope you may start to see individual filaments begin to move back and forth. If nothing is happening after a couple of minutes, you may want to switch to brightfield illumination -the more intense light may get the filaments going.

The movement you see is the so-called "gliding motility". Filaments that are not part of clumps may glide along their axes. If you see hormogonia in *Oscillatoria* you may see them gliding as well. The mechanism for gliding motility is not well understood.

Spirulina

Spirulina is mass-produced as a dietary supplement and is used as a food source in both South America and Africa. However, a large number of the health benefits ascribed to it are anecdotal with little, if any, rigorous scientific support. Of the nearly 50 known species, the most widely used is *Anthrospira plantensis*. It is available in tablet, flake, and powder form for human consumption and is available for use as a feed supplement for agricultural use. As with all dietary supplements in the U.S., there are no industry-wide regulations governing its production or purity.



Filaments of *Spirulina sp.* Filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix.

SECTION IV. Filamentous with specialized cells (but no branches).

The taxa in this category may produce both of the specialized cell types mentioned earlier in the handout - heterocysts and akinetes. You'll see heterocysts in both of the species you'll examine today, but probably won't see akinetes.

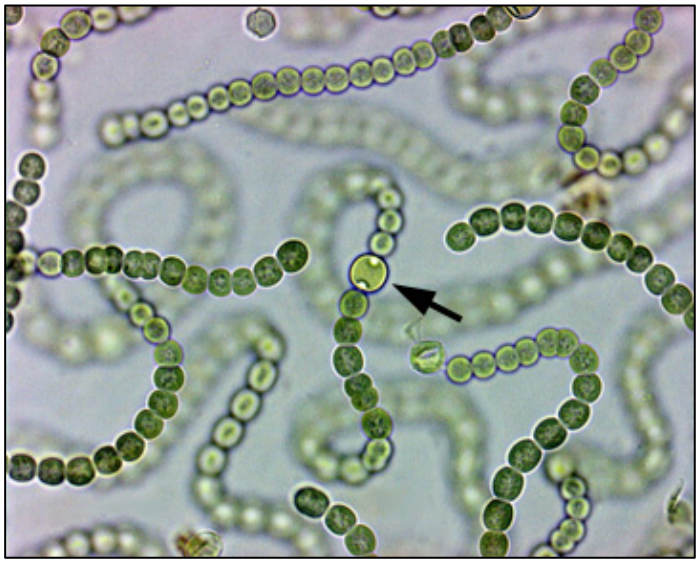
Heterocysts, compared to the ordinary cells of a filament, will often be somewhat larger, will tend to be roughly spherical or barrel-shaped, and will tend to look relatively 'empty'. Under phase contrast illumination, many heterocysts will tend to have a translucent yellow or golden appearance. The heterocysts you see today will never appear to be the same green color you see in the ordinary cells of a filament. Why?

Both the genera you'll see today occur as free-living filaments. But as part of our simplification of this lab, we're showing you a colonial form of *Nostoc,* and a symbiotic form of *Anabaena.* However, we'll give you directions for seeing the filaments of both genera, and these filaments look essentially the same as those of the species that form only free-living filaments.

Nostoc

Nostoc is found in fresh water or in moist terrestrial habitats. It can be found where springs exit from rock cliffs, or in wet meadows, or near the edges of shallow lakes or ponds. The *Nostoc* you'll see today will be filamentous. During the spring *Nostoc* filaments form fairly large colonies composed of filaments surrounded by and enclosing a gelatinous matrix.

If available, you should observe the large colonies we have set out on the lab bench. For the filamentous *Nostoc* you should use a transfer pipet to sample the water at the very bottom of the container we received from the supplier. A drop of this water should contain filaments that should show the typical morphology of the *Nostoc* cells, and should also contain heterocysts.



Nostoc filament with arrow pointing to heterocyst. Under phase contrast they appear yellow or golden.

Anabaena

Anabaena is found in fresh water, and in nature you may find heterocysts, akinetes and hormogonia in addition to the ordinary cells. One of the species you'll see today is a symbiont in the leaves of the aquatic fern *Azolla.* Its filaments look much like the filaments of free-living species, and you will see heterocysts. *Cylindrospermum* (not shown) is another heterocystous cyanobacterium that we have available for you to examine today.

Anabaena showing a heterocyst.

Gloeotrichia

Gloeotrichia grows primarily on the bottom sediments of lakes. It uses the phosphorus on the bottom of the lake to grow and then floats into the water column, where it can re-release the phosphorus, leading to worse algal blooms.

Some species produce toxins that can be harmful to humans and pets. Their presence in the Northeast has increased in the past few decades, and blooms have been seen on a number of Maine lakes. We need to know which of our lakes are affected by *Gloeotrichia* and if it exists in high enough numbers to be a major concern.

Gloeotrichia

SECTION V. Filamentous, Branched Cyanobacteria

Fischerella

Fischerella is found in terrestrial habitats. It can also be found as a symbiont in lichens. (You'll study lichens in Week 9.) This genus has more morphological complexity than you find in most prokaryotes. *Fischerella's* filaments are generally dimorphic. You will notice uniseriate filaments (the cells are in a 'single file') similar to others you've already seen today, and the tips of larger filaments are also often uniseriate. But you will also find multiseriate filament regions in which the thickness of a filament at any point is made up of several cells that result from three-dimensional cell divisions- the kind that produce three-dimensional 'tissues' in other organisms. You'll notice, also, that the shapes of the individual cells in uniseriate and multiseriate regions are different.

Filaments of *Fischerella*. A multiseriate filament extends in an approximately horizontal arc across the micrograph. A number of uniseriate branch filaments extend, mostly 'downward', from the larger filament. If you carefully examine the micrograph, you'll see that the branch filaments form following a cell division in a cell of the multiseriate filament.

True branching occurs in *Fischerella.* The basal cell of each branch forms as a 'daughter cell' resulting from the division of a cell in the parent filament. *Fischerella* can form heterocysts, although we have not generally seen them in this course.

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Important terms

Akinete – a thick-walled, single-celled, non-motile, asexual, resting spore formed by the thickening of the parent cell wall, and usually germinating directly into a new filament.

Heterocyte – enlarged nitrogen-fixing cell occurring along the filaments in some cyanobacteria.

Hormogonium – a portion of a filament in many cyanobacteria that becomes detached as a reproductive body.