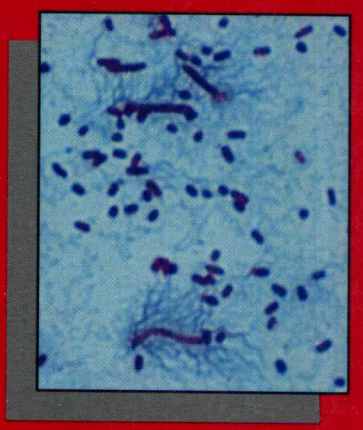
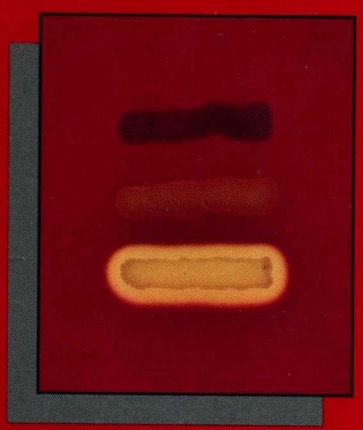
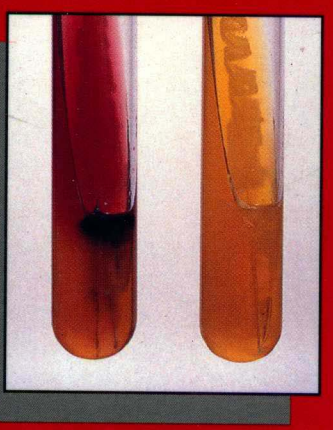
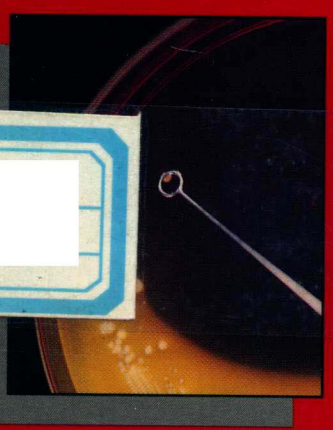
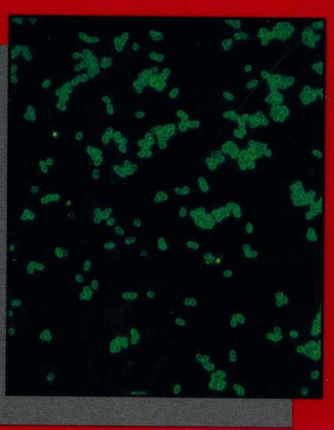
BASIC MICROBIOLOGY TECHNIQUES

Third Edition

Susan G. Kelley
Frederick J. Post

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third edition

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PREFACE

Basic Microbiology Techniques 3rd edition provides students with the necessary skills to work effectively in the exciting field of microbiology. Most students using this manual will be dealing with microbes for the first time. Therefore, exercises have been selected which provide workable experiments of proven success and that are typical procedures used by microbiologists regardless of specialty. The overall objectives of these exercises are to give the student repeated opportunity to practice aseptic technique, to develop isolation procedures, to provide the principles behind many of the biochemical and other procedures used, and to teach laboratory safety. Development of these skills is useful to all students as well as preparing some for advanced courses to give the student the needed practice, each exercise (unless otherwise noted) provides a materials list on a per student basis. Instructors may modify this as needed. No special background courses are prerequisite for students using this manual, although previous experience in biology would be useful.

The range of experiments provided is not intended to be exhaustive. The third edition has been slightly rearranged to provide a greater selection of basic exercises. Although bacteria remain the focus of the manual, other organisms have been included. Students should be aware of how microbes are useful and applied microbiology exercises have been included. The manual is best used for single quarter or semester. The instructor may select the exercises best fitting particular course needs. The manual is not intended to accompany any particular textbook and the authors have used the manual successfully with a number of different texts.

Each exercise has an independent report form with a series of questions focusing on the listed objectives. These questions are intended to help the student but not all answers will be found in the exercise itself. The instructor may wish to discuss these in the laboratory introduction or direct the student to textbooks with the information. An *Instructor's Manual* is available on request.

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INTRODUCTION

This manual begins with learning laboratory safety and the proper use of the microscope. An important aspect of the exercises that follow is learning to **be observant**. You should have an idea of what to expect by reading the exercise for the day **before** coming to class. As observations are made, carefully note small variations in shape, color, size, or other aspect. Most of these will be pointed out in class but you must be more critical in your observational ability than ever before. One of the first things you must learn is to "see small". Bacteria, in particular, surprise most students with their minuteness. When you finish this course you should have a better perspective of things you have taken for granted in the past and you may view the world around you with a new insight.

The authors and publisher would appreciate any comments on the use of the manual or its exercises.

LABORATORY SAFETY

GENERAL REQUIREMENTS

1. All materials and clothes other than laboratory manual and notebook are to be kept off and away from the bench.
2. Wear a lab coat or apron. Dyes and other materials CANNOT be removed from clothing.
3. Read each exercise over carefully BEFORE the period so that you know what is to be done and the basic principles involved.
4. Do not begin work until a brief introduction is given by the laboratory instructor. Good laboratory technique hinges on knowing what you are to do. A good policy is to take notes on procedures, modifications and principles in your lab manual.

REGULATIONS

1. Sponge off the bench top with the disinfectant solution provided both BEFORE the class starts and AFTER you have finished for the day. Use the same solution to rinse your hands after washing them with soap and water.
2. Mouth pipetting is **NOT** permitted.
3. On every table will be found pipet holders containing disinfectant. Please keep them clean. They are for dirty pipets ONLY.
4. Keep extraneous materials off the desk top and put all materials away at the end of the day.
5. Use waste baskets or other provided containers.
6. Discard glass in trays and glass test tubes in baskets. DO NOT mix glass and plastics. Plastics, cotton swabs and other disposables are placed in autoclave bags. If appropriate trays, baskets, or bags are not available, see your instructor. DO NOT leave tubes or plates simply lying about.
7. Remove marks from all glassware with xylene available for this purpose.
8. Because the organisms used in this class are potentially pathogenic, aseptic technique is important. Develop proper habits at the beginning. Keep hands and other objects away from your mouth. DO NOT lick labels, NO eating, NO drinking, and NO smoking in the laboratory.
9. Report all accidents such as cuts, burns or spilled cultures to your instructor *immediately*. Students with long hair should be especially cautious around the Bunsen burners.
10. Microscopes should be put away with the low power down and no oil on the lenses. If these are not in proper condition when you take them out, let your instructor know.
11. Media removed from the supply baskets should NOT be returned to the supply baskets under any circumstances.

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_____ _____

Student's Signature *Date*

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UNIT I

Microscopy

Since the first observation of microbes by van Leeuwenhoek about 1680, the microscope has assumed a central role in microbiology. A current definition of a microbe is that a microscope must be used to see the entire organism. Some multicellular animals are included in microbiology because the microscope is the major tool to visualize them (e.g., helminths). This section is intended to introduce the student to a minimum of optics and to the care and handling of the microscope.

1 Care and Use of the Microscope

OBJECTIVES

The student will be able to:

1. identify the parts and respective functions of the microscope.
2. define the terms *total magnification*, *resolving power*, and *working distance*.
3. properly handle and care for the microscope.

Microbiology holds as one of its principle unifying bases the need of a microscope to observe the organisms involved. There are many types of microscopes, such as bright field, phase contrast, interference, electron, differing in manner of construction and details of operation. However, certain principles underlie all microscopes.

MAGNIFICATION results from the use of one or more lenses. In a compound microscope, the objective lens nearest the object magnifies producing a real image. The eyepiece or ocular lens magnifies the real image producing a virtual image seen by the eye. The magnification of a lens is usually expressed in diameters of apparent increase in size or power (e.g., 10X is a ten times increase in size or 10 power). Microscopes generally consist of at least three objective lenses: a low power objective of about 10X, a high power objective of about 40X and an oil immersion lens of about 100X (Figure 1-1). The actual lens magnification depends on the type of microscope and the manufacturer. The total magnification of the microscope is the product of the magnification of the objective and the magnification of the eyepiece.

RESOLVING POWER is the ability of a lens or a microscope to distinguish between two closely adjacent points. The theoretical resolving power of a microscope is rarely achieved due to lens aberrations and the fact that extraordinary performance on the microscope by the user is required. The resolving power is a function of the wavelength of light used and the numerical aperture of the lens. Numerical aperture refers to the ability of a lens to gather light and is expressed by the relation

$$NA = i \sin \theta$$

where θ is one half the angle of the entering cone of light and i is the index of refraction of the medium along the light path. The index of refraction for air is 1.0. In order to increase the light gathering ability of the lens, something with an index of refraction greater than 1.0 must be placed between the object and the lens. Immersion oil serves this purpose having an index of refraction nearly identical with the glass of the lens, about 1.56.

Resolving power can be calculated as follows:

$$d = \frac{\lambda}{2(NA)}$$

where d = diameter of the smallest observable object (μm), λ = wavelength of light (μm), NA is numerical aperture (unitless) and 2 results from the use of a condenser below the stage to collect oblique light to illuminate the specimen.

The resolution should be better as wavelength decreases but is limited by several factors. The human eye is most sensitive to green light and "sees" best at about 500 nm. Green or blue green filters are often used to aid in this. Photography works best at ultraviolet wavelengths and special lenses are used. For optimum resolving power, in addition to an expert microscopist, oil must be interposed between the condenser and the bottom of the slide. This is not usually done in the teaching laboratory.

WORKING DISTANCE is the space between the objective and the specimen when the latter is in focus. The higher the magnification, the shorter this distance. The shorter the working distance, generally speaking, the greater the amount of light required to see the specimen. The diaphragm of the condenser should be almost completely open when used with the oil immersion lens.

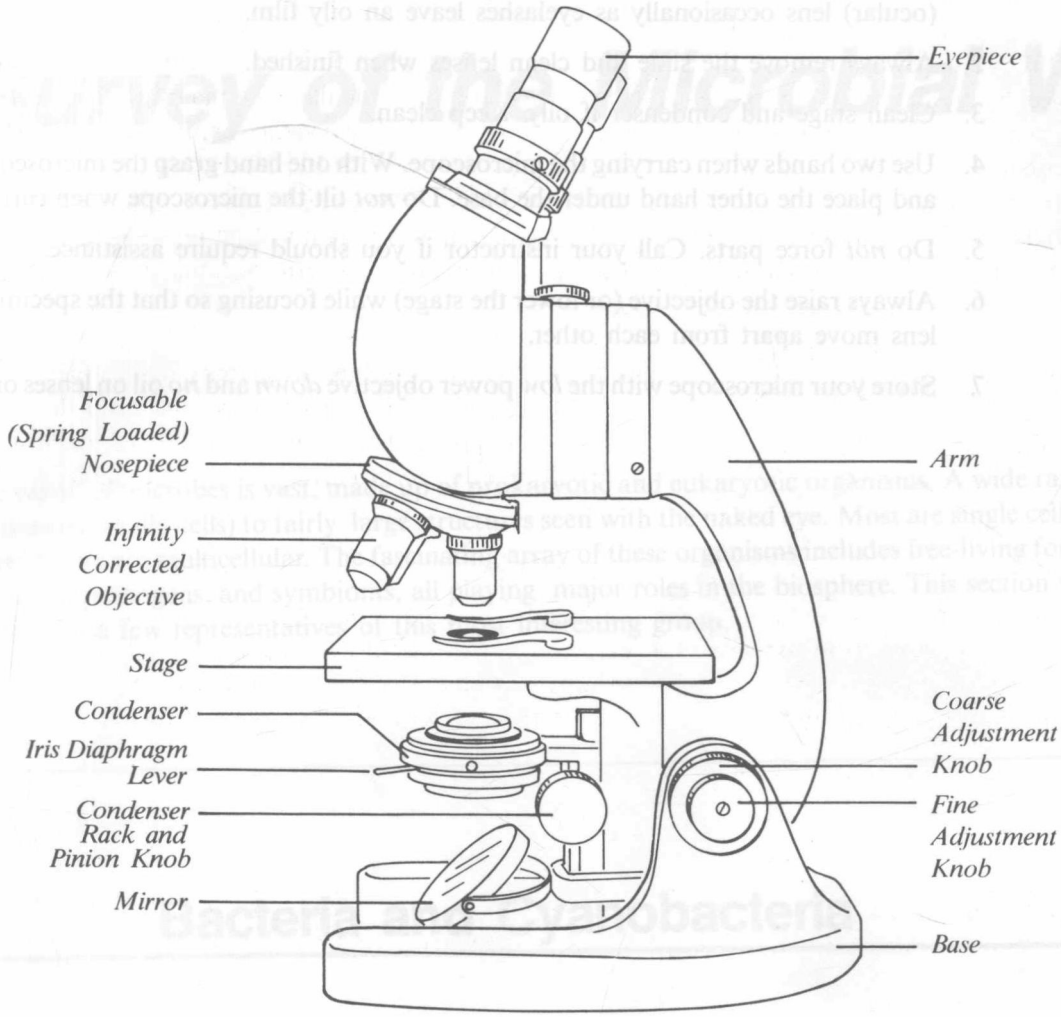
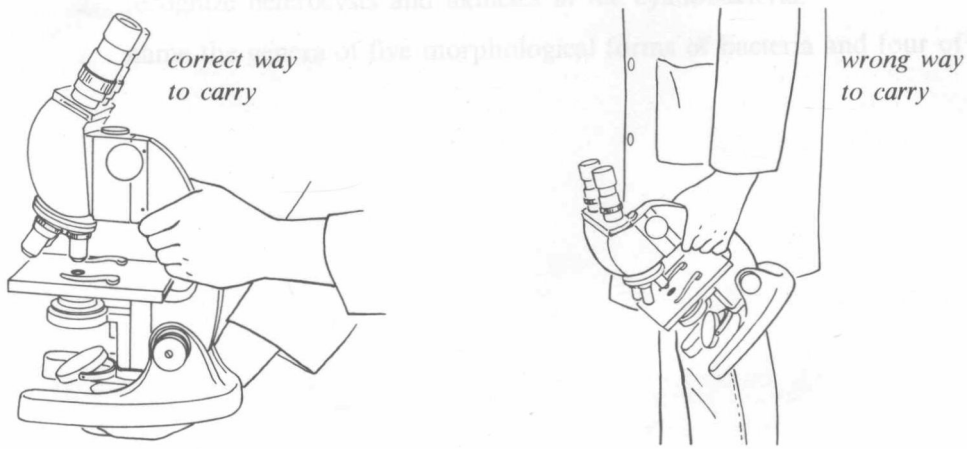


Figure 1-1. Compound light microscope



PRECAUTIONS

1. Clean the lenses with lens paper only (not a paper towel or Kleenex). Clean top eyepiece (ocular) lens occasionally as eyelashes leave an oily film.
2. Always remove the slide and clean lenses when finished.
3. Clean stage and condenser if oily. Keep clean.
4. Use two hands when carrying the microscope. With one hand grasp the microscope arm and place the other hand under the base. Do *not* tilt the microscope when carrying it.
5. Do *not* force parts. Call your instructor if you should require assistance.
6. Always raise the objective (or lower the stage) while focusing so that the specimen and lens move apart from each other.
7. Store your microscope with the *low* power objective *down* and *no* oil on lenses or stage.

UNIT II

Survey of the Microbial World

The world of microbes is vast, made up of prokaryotic and eukaryotic organisms. A wide range of sizes from viruses (not really cells) to fairly large structures seen with the naked eye. Most are single cells but a few of the larger forms are multicellular. The fascinating array of these organisms includes free-living forms, saprophytes, parasites, pathogens, and symbionts, all playing major roles in the biosphere. This section will introduce the student to a few representatives of this most interesting group.

2 Bacteria and Cyanobacteria

OBJECTIVES

The student will be able to:

1. name some of the morphological forms of bacteria and cyanobacteria.
2. recognize heterocysts and akinetes in the cyanobacteria.
2. name the genera of five morphological forms of bacteria and four of cyanobacteria.

The bacteria and cyanobacteria are the prokaryotic cell types among the microbes. The bacteria are the smallest cell types averaging less than one micrometer in diameter. The cyanobacteria average slightly larger. Morphologically the bacteria are fairly limited in types (Figures 2-1, 2-2) and classification is only partially based on shape. The cyanobacteria are more varied with a number of specialized cells such as heterocysts and akinetes found in some. Extensive branching occurs in morphologically more advanced forms of both groups. The cyanobacteria also contain chlorophyll *a* (only) and combined with accessory pigments often colors the cells a green, blue green or brown. The color may be seen in larger forms under the microscope. Chlorophyll *a* does not occur in the bacteria being replaced by bacteriochlorophylls of several varieties. Although colonies are often colored, pigments in bacteria cannot be seen in cells under the microscope.

This exercise is intended to show only a few of the many morphological forms of bacteria and cyanobacteria occurring in nature. It also provides an opportunity to practice use of the microscope as described in Exercise 1. It should be noted that few if any intracellular structures can be seen in either group.

Some cyanobacteria exhibit motility of a gliding type, also found in a few bacterial groups; otherwise they are nonmotile. Gliding occurs **only** when a cell or filament is in contact with a surface (e.g., a coverslip or slide). **Akinetes** are special reproductive cells formed by some cyanobacteria. **Heterocysts** are rounded specialized cells often appearing very refractile and in which nitrogen fixation takes place. These structures are usually observed in filamentous form under low available nitrogen conditions. The location of these in the filament is useful in taxonomy of the filamentous forms.

Figure 2-1. Oil immersion view of various morphological types of bacteria

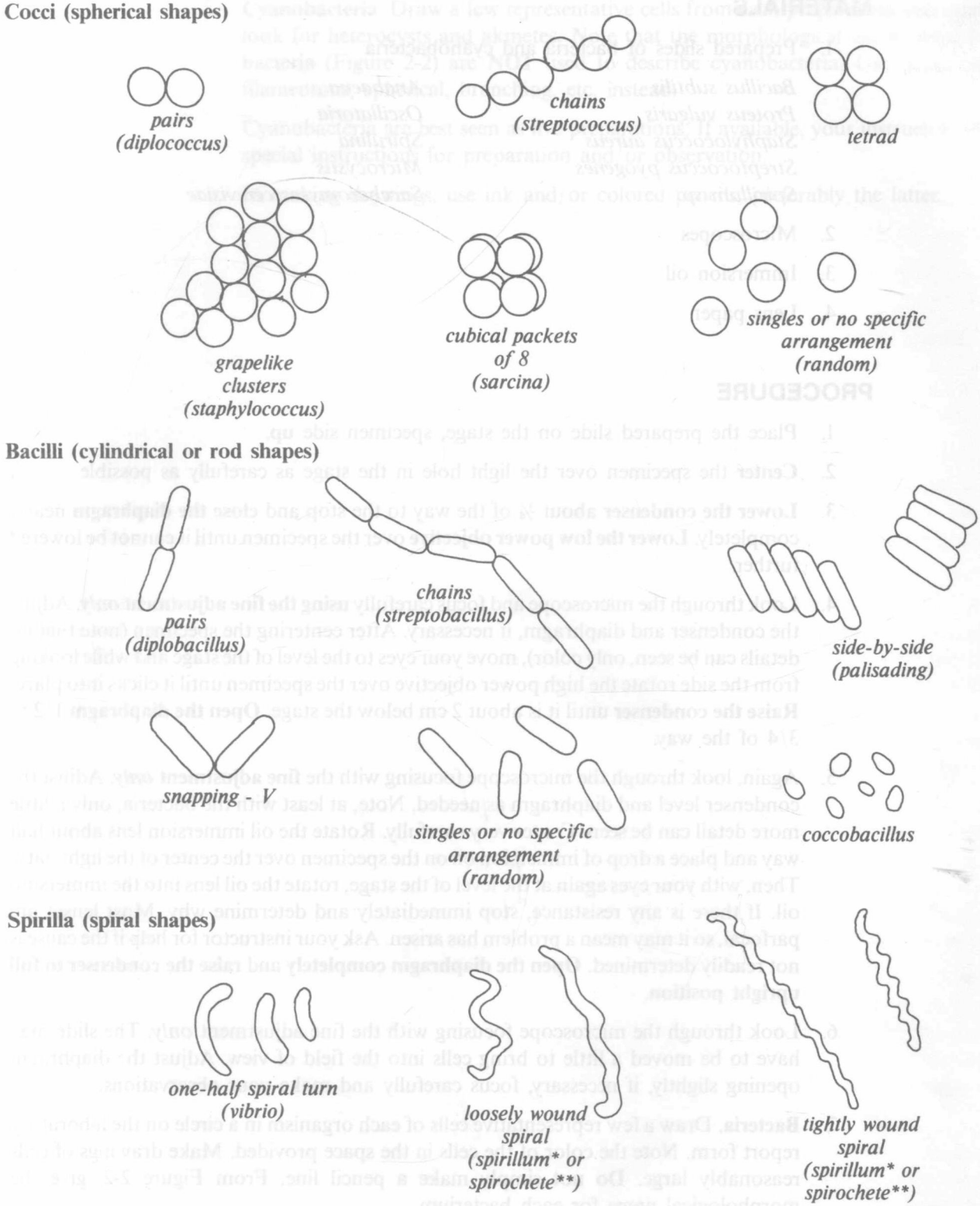


Figure 2-2. Bacterial shapes and arrangements (morphology)
(Note: These terms are not used for cyanobacteria)

MATERIALS

1. Prepared slides of bacteria and cyanobacteria

Bacillus subtilis	*Anabaena*
Proteus vulgaris	*Oscillatoria*
Staphylococcus aureus	*Spirulina*
Streptococcus pyogenes	*Microcystis*
Spirillum sp.	*Saccharomyces cerevisiae*

2. Microscopes
3. Immersion oil
4. Lens paper

PROCEDURE

1. Place the prepared slide on the stage, specimen side **up.**
2. **Center** the specimen over the light hole in the stage as carefully as possible.
3. **Lower the condenser** about ¾ of the way to the stop and **close the diaphragm** nearly completely. **Lower the low power objective** over the specimen until it cannot be lowered further.
4. Look through the microscope and focus carefully **using the fine adjustment *only***. Adjust the condenser and diaphragm, if necessary. After centering the specimen (note that no details can be seen, only color), move your eyes to the level of the stage and while looking from the side rotate the high power objective over the specimen until it clicks into place. **Raise the condenser** until it is about 2 cm below the stage. **Open the diaphragm** 1/2 to 3/4 of the way.
5. Again, look through the microscope focusing with the **fine adjustment *only***. Adjust the condenser level and diaphragm as needed. Note, at least with the bacteria, only a little more detail can be seen. Center very carefully. Rotate the oil immersion lens about half way and place a drop of immersion oil on the specimen over the center of the light path. Then, with your eyes again at the level of the stage, rotate the oil lens into the immersion oil. If there is any resistance, stop immediately and determine why. Most lenses are parfocal, so it may mean a problem has arisen. Ask your instructor for help if the cause is not readily determined. **Open the diaphragm completely** and **raise the condenser to full upright position**.
6. Look through the microscope focusing with the fine adjustment *only*. The slide may have to be moved a little to bring cells into the field of view. Adjust the diaphragm opening slightly, if necessary, focus carefully and make your observations.
7. **Bacteria**. Draw a few representative cells of each organism in a circle on the laboratory report form. Note the color of the cells in the space provided. Make drawings of cells reasonably large. **Do not** simply make a pencil line. From Figure 2-2, give the morphological name for each bacterium.

8. **Cyanobacteria**. Draw a few representative cells from each preparation and especially look for heterocysts and akinetes. Note that the morphological names used for the bacteria (Figure 2-2) are **NOT** used to describe cyanobacteria. Use terms such as filamentous, spherical, branching, etc. instead.

   Cyanobacteria are best seen as live preparations. If available, your instructor will give special instructions for preparation and/or observation.

   In making drawings, use ink and/or colored pencils, preferably the latter.