Exercise 2-A
MICROSCOPIC TECHNIQUE & EXAMINATION OF MICROORGANISMS

Introduction to Microscopic Technique

Microbiology is the science or study of living organisms too small to be seen with the naked eye. For this reason, a good microscope is an instrument essential to this science. Students in this laboratory will be using microscopes with two lens systems and bright field optics called **compound** or **bright-field microscopes**. A thorough understanding of the basic principles of microscopy and skill in the use of these instruments are necessary to microbiology.

Lenses and Magnification

With the compound microscope, magnification is obtained through the use of two lens systems. The lens system nearest the specimen, called the **objective** lens, magnifies the specimen and produces a real image. The **ocular**, or eyepiece lens system, magnifies the real image yielding a virtual image that is seen by the eye. The total magnification is equal to the product of the ocular magnification and the objective magnification (ocular x objective).

In order to achieve different degrees of magnification, your microscope is equipped with four objective lenses as follows: scanning objective (4X), low-power objective (10X), high-dry objective (45X), and oil-immersion objective (100X). The desired objective is rotated into place by means of a **revolving nosepiece**. Each ocular lens magnifies objects 10 times (10X). The total magnification (ocular x objective) obtained with our compound microscopes is as follows: 40X, 100X, 450X, and 1000X. As magnification is increased, the diameter and depth of the viewing field will be decreased. In order to keep specimens within the viewing field it will be necessary to position them toward the center of the viewing field, and a slight adjustment in focus will be required.

The oil-immersion objective (100X) is to be used only in conjunction with **immersion oil**. The use of oil decreases the **refraction** (bending or scattering) of light rays coming through the observed specimen, thus yielding greater **resolution** and a clearer image. When very small objects (most bacteria) are being observed, it is impossible to obtain a clear image with the oil-immersion lens if immersion oil is not properly applied.

DO NOT USE YOUR OIL-IMMERSION OBJECTIVE WITHOUT OIL!

Do not allow the high-dry objective (45X) to make contact with oil. Oil on this lens will interfere with resolution and prevent accurate observation; it can also cause permanent damage. Always rotate the high-dry objective away from oil droplets applied to slides. Immersion oil left on any objective for an extended period of time (several days - weeks) can seep inside and cause damage. At the end of each laboratory period, check both the high-dry and oil-immersion lenses and thoroughly remove any oil remaining with clean, dry, **lens paper** (optical lens wipes). Please do not use Kleenex, Kimwipes, paper towels, or other random paper products to clean microscope lenses.

PLEASE DO NOT LEAVE OIL ON ANY LENSES!

Focus

Two adjustment knobs are used to focus the lens systems on the specimen. The **coarse adjustment** knob moves the body tube over a greater vertical distance and brings the specimen into approximate focus. The **fine adjustment** knob moves the body tube more slowly for precise final focusing. When observing a new specimen, **ALWAYS** begin focusing using the scanning (4X) or low-power (10X) objective. With these, the **working distance** (distance between the lens and the slide when the specimen is in sharp focus) is great enough that there is no danger of damaging a lens by forcing it into the glass of slides, coverslips or the sub-stage condenser lens. **It also avoids damage to prepared slides.**

Once a specimen is in sharp focus under low power, magnification can be increased by turning the revolving nosepiece and bringing the next highest objective into position. Since the objectives are **parfocal**, only a slight adjustment in focus, using the fine focus adjustment knob, is necessary. Remember that it will be necessary to add a small drop of **immersion oil** to the slide prior to moving the oil immersion lens into position.

DO NOT TURN THE COARSE FOCUS ADJUSTMENT KNOB WHILE USING THE HIGH-DRY OR OIL-IMMERSION LENSES.

Illumination

Proper illumination is essential for the effective utilization of the microscope. Each microscope is equipped with a variable intensity light source, a **sub-stage condenser** lens, and an **iris diaphragm**, all of which require proper adjustment in order to achieve optimum contrast, resolution, and depth of field. Light from the illuminating source passes through the iris diaphragm and the sub-stage condenser lens before entering the objective. The sub-stage condenser (located beneath the stage) acts as a light gathering lens system to concentrate the available light on the specimen. With increased magnification more light is required (because the lens aperture is smaller). Adjusting the variable light source or the iris diaphragm will control the amount of light reaching the sub-stage condenser lens.

When the scanning (4X) and low power (10X) objectives are being used, it is advisable to keep the iris diaphragm closed, since at these magnifications definition and detail are most clear when the light is not too intense. When the oil-immersion objective is being used, the diaphragm must be opened to allow additional light to pass through. During routine observations the sub-stage condenser can be kept in its **highest** position and will require little adjustment.

In order to prevent electric shock or damage to the instrument, be certain the microscope light switch has been turned to the off position before removing the cord from or attaching it to the current source.

Microscope Placement and Transport

In this laboratory, microscopes are numbered and stored in cabinets with corresponding numbers for each instrument. Once assigned a particular microscope, students become responsible for microscope care and proper placement within the cabinet. Do not exchange microscopes without authorization, and notify the instructor at once if there is any malfunction of, or damage to, an assigned instrument.

Always use both hands when transferring a microscope between the cabinet and work area. Carry the instrument at abdomen level with one hand holding the curved arm, and another placed under the base for support. Use extreme care when returning or removing microscopes to/from the cabinets as bumping these fragile, expensive instruments against hard surfaces can cause damage.

Use of the Microscope and Special Precautions

Proper use of the microscope will be demonstrated in class while students have instruments present. Please heed the special precautions listed, and treat all microscopes with care.

1. Avoid dirtying the lenses (fingerprints, mascara, etc.). When microscope lenses become dirty, clean them carefully with clean dry lens paper (optical lens wipes).

2. Never leave a slide on the microscope when it is not in use.

3. Always remove oil from the oil immersion lens after use. **Note**: Oil that has become dry or hardened on a lens may be removed with lens paper slightly moistened with lens cleaner (Windex). **DO NOT** allow oil to contact the 45X lens, and **DO NOT** use liquids to clean it.

4. Before returning a microscope to the cabinet, position the revolving nosepiece with the scanning (4X) objective centered over the stage. This will avoid possible contact between an objective lens and the condenser or glass slide in the event the coarse focus adjustment knob is turned while the microscope is in the cabinet.

5. Keep the mechanical stage clean (free from oil) and dry.

6. Never allow objective lenses to touch glass slides or cover slips.

7. Do not use excessive light intensity when viewing specimens. Excess illumination will "burn out" the image and is likely to cause eyestrain and headaches.

8. Make certain the microscope light switch is in the off position before the power cord is removed from or plugged into a source of electric current.

Other Microscopes available for your use

Students working in this laboratory will have access to microscopes other than the compound microscopes discussed above. These microscopes will not be assigned to specific students, but will be available to the entire class and will be used primarily for demonstration purposes.

Stereomicroscopes - The stereomicroscopes allow students to observe the three dimensional features of bacterial colonies, fungus cultures and other macroscopic materials. These microscopes may be set to magnify objects either 50 or 100 times and allow for the observation of live cultures with or without the preparation of slides.

Videomicroscope - The videomicroscope may be used for brightfield, darkfield or phase-contrast microscopy (see text). Materials may be viewed through the ocular lens or on the video monitor. Note – the high dry objective on the videomicroscope and other Swift instruments magnifies objects 40X instead of 45X.

Digital Videomicroscope – A digital videomicroscope purchased with funds provided through the Sierra College Foundation, Microbiology account can be used to capture computer images of microscopic materials. This instrument provides better resolution at high magnification (1000X) than does the Swift Videomicroscope. Use of this microscope is restricted to trained individuals and requires that specific arrangements be made.

Prepared Slides

Prepared slides containing a variety of different microorganisms are available to students in both slide boxes and on metal trays. When using these materials, please be courteous to others and obtain slides for viewing **one-at-a-time**. Return each slide to its proper location prior to obtaining the next. If immersion oil is applied to a slide surface (necessary for bacteria only), remember to remove this prior to returning the slide to its appropriate box or tray. You may use Kleenex, Kimwipes or paper towels to clean glass slides, but please do not use lens paper.

Note that some slide preparations used in this laboratory are quite thick and subject to damage from contact with long objectives. **DO NOT** attempt to view thick prepared slides with the high-dry (45X) or oil-immersion (100X) lenses since the weight of the objective and nosepiece will break the coverslips and/or otherwise damage these preparations.

Natural Infusions

Natural infusions are samples containing a wide variety of living microorganisms representing those typical of natural habitats, e.g., ponds, streams, etc. Wet mounts can be prepared from these materials by placing a small quantity of liquid plus visible debris on a clean glass slide. By placing one edge of a clean cover slip near the border of the water droplet and then tipping it over until it rests on the slide surface, air is pushed out and observations can be made without the distraction of bubble edges. Depending on the sources used, natural infusions may contain representatives from all microbial groups, as well as some multicellular animals.

Procedure:

1. Obtain a letter "e" slide and make a variety of observations designed to increase your understanding of microscope optics and the operation of the mechanical stage.
   a. Place the letter "e" slide in the stage clips with the "e" right-side-up and then observe the letter as it appears through the scanning (4X) objective.
   b. Move the letter "e" slide from side to side, and then forward and back using the mechanical stage knobs to determine how these operate.
   c. Increase the magnification to 10X and then 40X, and notice how these increases in magnification influence what can be observed. Record your observations.

2. Obtain a slide containing crossed threads and make some observations designed to increase your understanding of depth of field.
   a. Observe the threads using the scanning (4X) objective and note how many threads are in focus at one time.
   b. Increase the magnification to low power (10X) and then high dry (45X) and repeat the observation. Note variations in the number of threads in focus at one time.

3. Obtain a prepared slide showing two forms of diatoms and make some observations designed to increase your understanding of the effects of light intensity.
   a. Place the slide on the stage, close the iris diaphragm and attempt to locate the diatoms using the low power (10X) objective.
   b. Focus on the diatoms, and then increase and decrease the light intensity by opening and closing the iris diaphragm. Record how changes in light intensity influence the virtual images of the specimens being observed.

4. Obtain a prepared slide containing three types of bacteria in a single mixed smear and make some observations designed to increase your appreciation for the effects of immersion oil.
   a. Place the slide on the stage and observe the bacteria with your low power (10X) objective. Focus on a mass of bacteria and then position the mass at the center of the viewing field.
   b. Increase magnification to 100X and observe the bacterial mass without applying immersion oil to the slide surface. Attempt to focus the image and notice the degree of resolution obtainable. Note also the location of the cell mass relative to center.
   c. Apply a drop of immersion oil to the slide surface, swing the 100X objective into the oil and repeat your observations. Notice how decreasing light refraction influences resolution and record this information.
   d. Align a single bacterium with a portion of the ocular micrometer, and record the number of units required to accurately represent this cell's diameter and length. The unit value for each ocular division will be determined during exercise 2-C.

5. Prepare one or more wet mounts of the various infusion samples provided and observe them using the low power (10X) and high dry (45X) objectives. Practice making adjustments in magnification, focus and illumination and observe again how these influence the virtual images being observed. **Note** – You can quickly gain a working familiarity with the operation of the mechanical stage by trying to "follow" a swimming microbe around the slide.

6. Compare some of the organisms present in the wet mount to the illustrations in the syllabus and try to determine which groups the various organisms belong to (bacteria, algae, protozoa, or fungi). Note specific characteristics that tend to distinguish the various groups of organisms.

Questions:

1. What is microbiology?

2. How many objective lenses does your microscope have, and what is their power of magnification (alone and when used in combination with the ocular lens)? _____
   _____

3. When changing from low power to oil immersion, it will be necessary to _____ (increase or decrease) the amount of light used in order to see clearly.

4. If you wish to keep a specimen within the field of view as you increase magnification, you must position it where within the viewing field? _____ Why is this so?
   _____

5. You should always begin focusing using the _____ objective lens. Why is this so? _____

6. Why is it not advisable to use the coarse focus adjustment knob when observing specimens with the oil immersion lens? _____
   _____

7. Why should the high-dry objective be rotated away from a drop of oil on a slide or cover slip rather than through it? _____

8. What happens to depth of field as magnification is increased? _____

Exercise 2-B
MICROSCOPIC MEASUREMENT

Introduction

Microscopic objects or organisms can be measured using a microscope that is equipped with an **ocular micrometer** (figure 2.1). This is simply a disc of glass with equally spaced lines or divisions etched on its surface. When an ocular micrometer is in place, ruled lines appear within the field of view. The actual distance between these lines is arbitrary, and changes as magnification changes (as objectives are changed). In this laboratory students will be using stage micrometers having both US (inch) and metric scales; however, only the metric scale will be used for measuring microorganisms. Students not familiar with the metric system of measurement may want to review this information online or in a textbook.

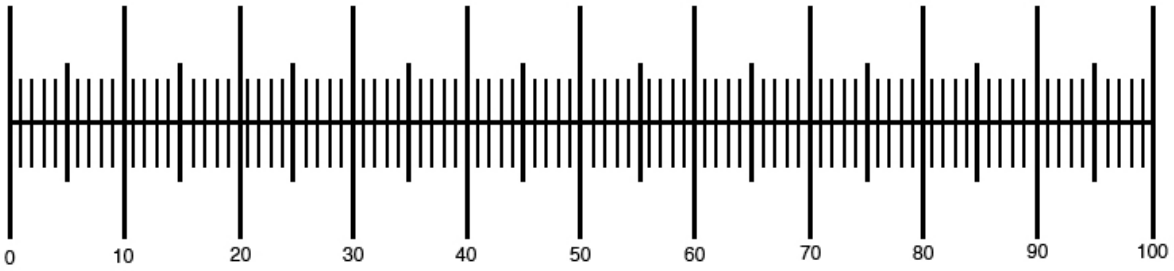


Fig. 2.1 - Ocular Micrometer: **The distance between the lines is arbitrary, and changes when different objectives are being used.**

The actual distance between the lines of the ocular micrometer can be **calibrated** using a **stage micrometer** (Fig. 2.2) on which is etched parallel lines spaced a known distance apart. The exact distance between these lines is indicated on the micrometer itself.

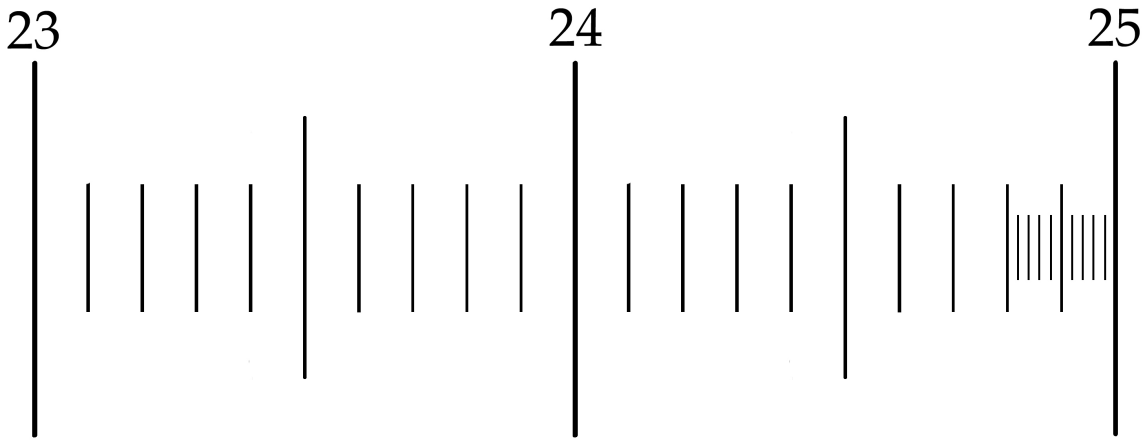


Fig. 2.2 - Stage Micrometer: **On the metric scale, lines 1 - 25 are spaced exactly 1mm (1000μm) apart, the lines between 24 and 25 are spaced 0.1mm (100μm) apart, and the smallest lines (far right) are spaced 0.02mm apart. Note - 0.02 mm = 20 micrometers (20μm) = 20 microns = 20μ.**

In order to calibrate the ocular micrometer, it is necessary to superimpose the two scales (ocular and stage micrometers) within the same field of view and then to determine the number of ocular divisions that coincide with one division (minimal distance) on the stage micrometer. This procedure (calibration) must be repeated for each objective. Below (Figure 2.3) we see a representation of the stage and ocular micrometers, as they would appear when using the oil immersion objective. **It is not necessary to use immersion oil when viewing the lines of the stage micrometer with the 100X lens.**

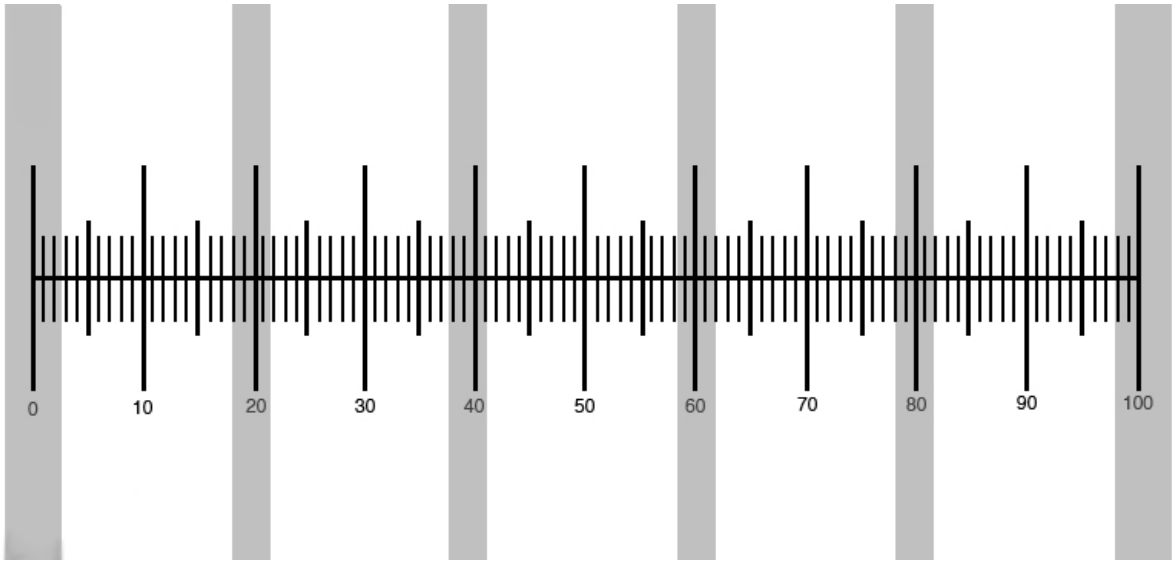


Fig. 2.3 - Ocular micrometer superimposed on stage micrometer: **In this case the lines of the stage micrometer appear much broader than those of the ocular micrometer due to the increased magnification. The exact distance between ocular divisions is 0.001mm or 1 micrometer (1μm = 1 micron or 1μ).**

Once the ocular micrometer has been calibrated, the stage micrometer is removed, and the ocular scale can be used to measure microorganisms simply by counting the units or graduations superimposed on the object being viewed, and multiplying this number by the known distance between the graduations.

Procedure:

1. Set the revolving nosepiece so that you are viewing the stage with your low power (10X) objective. Mount the stage micrometer on the microscope stage, make certain you are viewing the metric scale (numbers not preceded by decimal points), and center it within your field of view (numbers right-side-up and reading left to right).

2. Notice that the ocular micrometer will move if the ocular lens is turned since it is fixed within the eyepiece. Rotate the ocular micrometer until the two scales are super-imposed one upon the other (both right-side-up) and then bring both scales into focus to the extent possible.

3. Adjust the position of the stage micrometer so that numbered lines are aligned with the left hand (zero), and right hand (10 or 100) ends of the ocular micrometer. When calibrating the low-power (10X) objective, the lines of the ocular micrometer should coincide with numbered lines on the stage micrometer at both ends.

4. Calculate the number of micrometers (microns) in each space or graduation of the ocular scale by dividing the number of stage micrometer units by the number of ocular micrometer units visible within the coinciding space. When using the low power (10X) objective, the calculation will be 1000μm/100 ocular units = X μm per ocular unit. Ocular micrometers with numbers 1-10 still have 100 divisions. Record your findings on the worksheet page.

5. Switch to the scanning (4X) lens and repeat the calibration process. Align the left hand end (zero) of the ocular micrometer with a stage micrometer numbered line, and then note the ocular value aligning with the next numbered line. Divide stage value (1000μ) by ocular value and record the information on the worksheet page.

6. Move the stage micrometer so that what appears to be the right hand end (lines 24-25) is in view, switch to the high dry (45X) lens and align the left hand end (zero) of the ocular micrometer with the center of line 24. Note that lines marking 100 micrometer divisions are now visible on the stage micrometer. Calibrate the ocular micrometer as before (stage units/ocular units) and record your findings.

7. Move the stage micrometer so that the **smallest** divisions are centered in your viewing field and switch to the oil immersion (100X) lens (do not apply immersion oil to the stage micrometer). Align ocular unit values (0, 20, 40, 60, 80, 100) with the **centers** of stage micrometer lines as shown above (Fig. 2.3). At this magnification, each unit (space) visible on the stage micrometer equals 20 micrometers (20μm = 20 microns or 20μ) therefore, since one unit on the stage micrometer equals 20 units on the ocular scale, each unit within the ocular micrometer equals one micron (1μ). Record this information on the worksheet page.

8. Having calibrated the ocular micrometer, remove the stage micrometer, and replace it with a prepared slide containing *Paramecium*. Determine the dimensions (diameter and length), of a number of specimens, and record your measurements in microns.

9. Compare the size of the *Paramecium* to that of the bacterium measured earlier in exercise 2-A (procedure step #4, section d.) Then practice measuring live microorganisms using your low power (10X) and high dry (45X) lenses.

Questions:

1. What is the significance of calibration and how is it accomplished in this laboratory?

2. Why must the ocular micrometer be recalibrated for each new objective used?

3. In figure 2.3 above, what is the absolute length in microns covered by 5 ocular divisions?

4. Does the scanning (4X) lens actually magnify objects 4 times?

5. Approximately, what is the size of a *Paramecium* (diameter and length)?

6. What is the size of a rod-shaped bacterium (diameter and length)?

7. What type of cell does a *Paramecium* have, and what type does a bacterium have? Which cell type (eukaryotic or prokaryotic) is generally larger?

Exercise 2-C
OBSERVATION AND SCIENTIFIC ILLUSTRATION

Introduction

In this laboratory students will be expected to observe a variety of different microorganisms, and to be able to recognize these organisms during laboratory examinations. This will require careful observations with attention to the structural details distinguishing the various microbial groups. One of the best ways to insure the observation of necessary details is to draw/sketch individual organisms as accurately as possible. For this reason, students will be expected to complete and to turn in a number of scientific illustrations.

In some laboratory classes, students are required to use only hard leaded pencils or India ink for their illustrations, and drawings are made in black and white rather than in color. This is because students are more likely to notice structural details if they concentrate on shape, size and texture rather than color. It is important to remember that the colors associated with prepared slides are not characteristic of the organisms being observed but are dependent upon the type of stain or dye used in the preparation process.

The type of paper required for illustrations is variable, but to some extent is dependent upon the type of media used. Some types of paper snag and bleed when ink is applied with a fine-tipped pen and are not suitable for pen and ink illustrations.

Scientific illustrations are not expected to be works of art. Although aesthetic design may be a consideration in your work, detail and accuracy are more important. Drawings must be made large enough to show the necessary details, but at least one inch of margin must be allowed at all boarders. Scientific illustrations (unlike other art forms) typically include a number of labels. These are traditionally placed outside the objects being represented, are printed horizontally, and are usually connected to specific structural details by single straight lines (Fig. 2.4). If more lengthy descriptions are required, these should be included at the bottom of the page or on a separate sheet of paper.

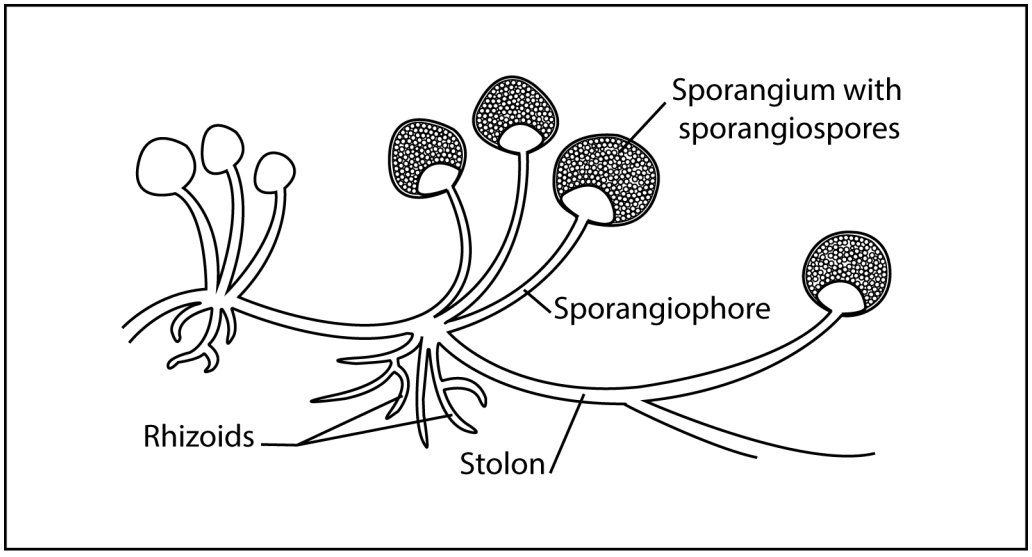


Fig. 2.4 - An Example of Scientific Illustration

In this laboratory illustrations representing various stain techniques must be made in color (colored pencils are recommended, but other drawing tools may be used and are equally suitable), and all drawings must be made large enough to accurately indicate the morphology of the subject being represented. Illustrations to be turned in must be made on standard-sized (8 ½ X 11), white, unlined paper or botany paper, and must accompany the written report materials they represent. Remember that the objective of assigning scientific illustrations in this class is to encourage accurate observation of the materials being studied. Make a sincere attempt to record what is observed, and label all illustrations as neatly as possible.

Procedure:

1. Select an interesting region of one of the wet mounts prepared from the natural infusions provided (Exercise 2-A, procedure step #5), and observe it with your 10X objective.

2. On the worksheet provided (Part 2-C), make a simple scientific illustration representing the organism types visible.

3. Record the size of each organism type as indicated, and label each one as accurately as possible.

4. Increase your magnification to 45X and repeat steps 1 through 3.

Note – Specific illustrations will be assigned in association with various exercises. Make all of these illustrations according to the guidelines presented above. Although most graded illustrations will be returned to you, some may be kept for display purposes.

Name_____ **Lab Section**_____

WORKSHEET
Exercise 2
Microscopic Technique, Measurement, and Illustrations

Goals:

_____
_____
_____

Data and Results:

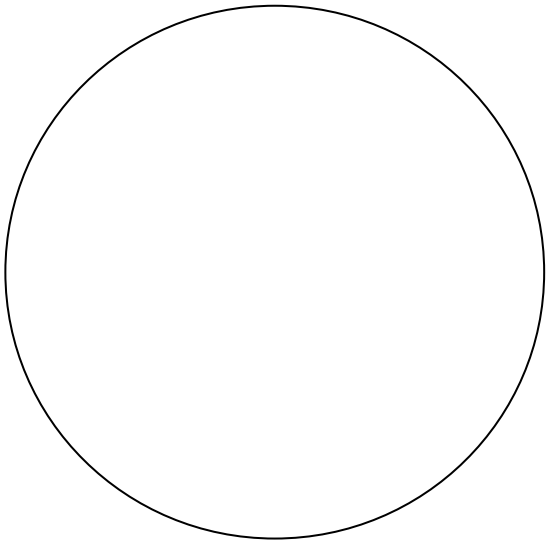
Ex. 2-B: Ocular Micrometer Calibration

Microscope number: _____

Objective	Stage Measurement (µm)	Ocular Measurement (ocular divisions or units)	µm/unit
Scanning			
Low Power			
High Dry			
Oil Immersion			

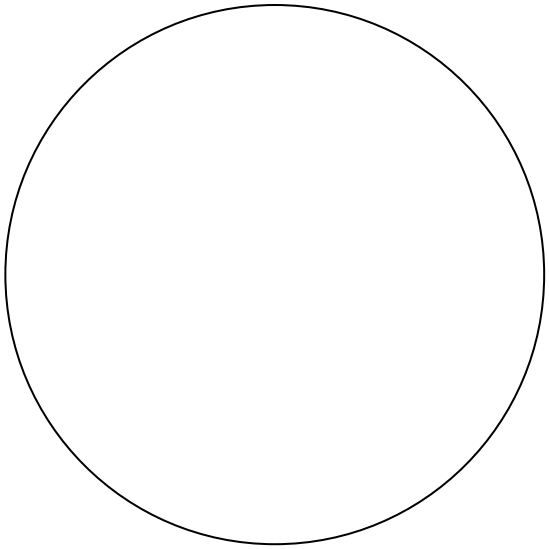
Ex. 2-C: Observations and Scientific Illustration

1. **Specimen of your choice, viewed with the scanning, low power, or high dry objective. Measure one cell.**



Specimen:
Total Magnification:
Length: units x µm/unit = µm
Width: units x µm/unit = µm
Notes:

2. Specimen of your choice, viewed with the oil immersion objective. Measure one cell.



Specimen:			
Total Magnification:			
Length:	units x	μm/unit =	μm
Width:	units x	μm/unit =	μm
Notes:			