TWELFTH EDITION

BIOLOGY
LABORATORY MANUAL

Darrell S. Vodopich | Randy Moore

Mc Graw Hill Education

Biology

Laboratory Manual

Twelfth Edition

Darrell S. Vodopich
Baylor University

Randy Moore
University of Minnesota

Mc Graw Hill Education

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Design: *David W. Hash*
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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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Preface

We have designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, safe, easy to perform, and especially appropriate for large classes. Few experiments require more than one class meeting to complete the procedure. Each exercise includes many photographs and illustrations, traditional topics, and experiments that help students learn about life. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you read about these areas, you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined—your future success as a scientist and an informed citizen depends on your ability to seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions—their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTOR

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple, safe, and straightforward experiments are most effective if you interpret the work in depth. Most experiments can be done easily by a student in 2 to 3 hours. Terminology, structures, photographs, and concepts are limited to those that the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance and is not an autotutorial system. We've tried to guide students from observations to conclusions, to help students make their own discoveries, and to make the transition from observation to understanding biological principles. But discussions and interactions between student and instructor are major components of a successful laboratory experience. Be sure to examine the "Questions for Further Study and Inquiry" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad application to their world.

DIGITAL INTEGRATION

As educators, we recognize that today's students are digital learners. Virtually every exercise of this manual is accompanied by tailor-made digital resources, including assignable questions and a variety of high-definition videos, PowerPoint images, and other resources that demonstrate basic techniques, emphasize biological principles, test for understanding, and engage students as they learn biology in the laboratory.

Digital resources are available to instructors at **connect.mheducation.com.** Instructors will want to assign these resources to help students know what they'll be doing, what principles they'll be investigating, and what concepts they'll need to understand before coming to lab.

WHAT'S NEW IN THIS EDITION

Throughout the manual, we have expanded and improved several of the most popular and effective features of previous editions, including

- **Learning Objectives** have been updated to provide an overview of what students will do and learn in the exercise.
- **Procedures** and **Doing Biology Yourself** require students to *do* biology as they apply skills they've learned to develop and study hypotheses about biology.
- **Questions** throughout each exercise encourage students to pause and think about their data and what they've learned in lab.
- **Questions for Further Study and Inquiry** at the end of each exercise help students apply what they've learned to broader topics and issues in biology.
- **Writing to Learn Biology** encourages students to develop their ideas about what they learned in lab.

- **Caution** and **Safety First** icons make students aware of safety issues associated with the procedures they'll use in lab.
- Boxed readings titled **Inquiry-Based Learning** encourage students to apply what they've learned to independently answer questions about intriguing biological topics.
- Updated health-related exercises help students better understand topics such as blood pressure, atherosclerosis, and their risk of cardiovascular disease.
- Several illustrations have been replaced with photographs to provide more realistic images to support the Exercise content.
- Approximately 60 illustrations and photos have been revised.
- Questions within procedures now include lines on which students can write their answers.
- An assignable, updated library of videos and Connect questions helps students prepare for lab and understand the instruments and techniques that will be important for their investigations. Instructors may assign these videos before class time to help ensure that students arrive prepared for lab.

Exercise-Specific Changes

- Exercise 1—Additional explanation provided for both mean and standard deviation
- Exercise 2—Mass, volume, and median are further defined; new illustration in figure 2.3 on measuring the volume of liquid; figure 2.4b has explanatory labels added
- Exercise 3—Additional questions have been added to Procedure 3.6 Using a dissecting microscope
- Exercise 4—Several illustrations have better labels; a new photo is supplied for figure 4.6a *Elodea* cells; figure 4.13 has been redrawn to more directly correlate to the associated photo; a new question is added to Questions for Further Study and Inquiry to compare plant and animal cells
- Exercise 6—Qualitative tests are defined; a new photo has been added to figure 6.2 to explain Benedict's test
- Exercise 7—Clarifying edits made to introductory material
- Exercise 9—Explanations of hypotonic, hypertonic, and isotonic are expanded
- Exercise 10—Steps of Procedures 10.1 and 10.2 are clarified; a new question on experimental design has been added to Questions for Further Study and Inquiry
- Exercise 13—Figure 13.2 caption is expanded
- Exercise 14—Explanation of the structure of chromatids is expanded
- Exercise 15—Labels for figure 15.2 have been added for paternal versus maternal chromosomes; description of the structure of replicated versus nonreplicated chromosomes has been clarified; figure 15.6 is new; figure 15.7 is revised to clarify the state and number of chromosomes in first polar bodies and second polar bodies, and corpus albicans has been labeled and added as a defined term in the text
- Exercise 16—Global prevalence of genetically transformed crops has been updated to 2017 statistics
- Exercise 17—Figure 17.4 has a panel of 3 new photos on sickle cell anemia; figure 17.6 contains improved photos of hairlines
- Exercise 18—Definition of evolution is revised to be more concise; questions about Hardy-Weinberg genetics are expanded for clarity; a new question about the effect of natural selection on sickle cell anemia has been added to Questions for Further Study and Inquiry
- Exercise 19—Figure 19.2 has been revised to better illustrate lineages of human evolution; the term "diastema" has been added and defined; figure 19.4 is relabeled for clarity
- Exercise 20—Procedure 20.4 is expanded to help students design and implement experimental controls.
- Exercise 22—Formula for population growth is revised; data for Figure 22.5 are updated to reflect 2018 predictions; question 6 is expanded to include 2018 population values and growth rates
- Exercise 23—Question 1 is revised to emphasize hypothesis testing; table 23.3 is reorganized to accept handwritten student data
- Exercise 24—Organization of domains and kingdoms is updated to current taxonomy; table 24.1, prokaryotic versus eukaryotic characteristics, is modified for precision; figure 24.2, structure of a bacterial cell, is revised and contains a new photo; explanation of binary fission is expanded to include protein FtsZ and its role in cell separation
- Exercise 25—Explanations of Archaeplastida and the term "protist" are clarified; in table 25.2 the list of chlorophylls diagnostic to each type of algae is updated; figure 25.4 is relabeled to clarify sexual versus asexual reproductive paths; figure 25.8 contains a new photo of Volvox colonies

- Exercise 26—Photomicrograph and illustration of African sleeping sickness blood cells and parasites are revised to clarify their relationship
- Exercise 27—Explanations of fungal sporangiophores and sporangia are expanded; figure 27.13 is modified to better show the diagnostic reproductive structure, ascus; Questions for Further Study and Inquiry has a new question to explain the benefit of fungi to other organisms
- Exercise 31—A learning objective is added on understanding flower structure and function; the explanation of sporogenesis is expanded; a Question for Further Study and Inquiry has been added to help students understand flower parts
- Exercise 32—A new question is added to Questions for Further Study and Inquiry on common leaf morphologies
- Exercise 35—The definition of bioassay is revised
- Exercise 36—Introductions to terms animals, multicellular, ancient, and primitive have been clarified; description of intracellular versus extracellular digestion in poriferans has been clarified
- Exercise 37—Taxonomic hierarchy of the classes and subphyla of flatworms is updated; the groups Neodermata and Turbellaria have been redefined and updated; taxonomy of tapeworms is updated
- Exercise 39—Taxonomy of major arthropod classes has been updated and reorganized to include Chelicerata, Crustacea, Myriapoda, and Hexapoda; table 39.3 has been relabeled to reflect updated arthropod taxonomy
- Exercise 40—The taxonomy of pre-vertebrate groups has been updated; class Actinopterygidii has replaced Osteichthyes; figure 40.21 of amphibian transitional stages is revised
- Exercise 41—Figure 41.2 has revised labeling; figure 41.3 is relabeled to distinguish flat cuboidal and columnar cells more clearly; figure 41.4 is relabeled to show Bowman's capsule more clearly; figure 41.5 is relabeled to more clearly distinguish columnar cells; figure 41.7 has been replaced to better show stratified squamous epithelium; types of connective tissue have been separated into connective tissue proper and special connective tissue
- Exercise 42—Descriptions of the appendicular skeleton and the axial skeleton are added; the number of skull, spine, and rib cage bones has been updated to conventional values; figure 42.2 is new; Figure 42.4 has been replaced with improved images of normal and osteoporotic bone; revisions to Questions for Further Study and Inquiry
- Exercise 43—A new learning objective is added to distinguish between isotonic and isometric contractions; explanations of muscle load, muscle tone, and muscle tension are expanded; figure 43.2 is relabeled to clearly distinguish between flexion and extension; Procedure 43.1 concerning flexion and extension of the forearm has been modified for clarity
- Exercise 44—Descriptions of negative pressure and its role in breathing have been expanded; procedures to distinguish the role of intercostal muscles and breathing are expanded and clarified; Procedure 44.1 has been modified for more consistent chest expansion measurements; typical values for tidal, expiratory, inspiratory, and residual volumes have been provided; directions for measuring breathing rate in Procedure 44.7 are clarified
- Exercise 46—Figure 46.1 has been modified to illustrate fovea centralis; Procedure 46.3 has been modified to accommodate lab partners
- Exercise 47—A new Question 2 has been added; Question 3 has been expanded to provide more examples and practice with terms such as cranial, caudal, lateral, distal, etc.; directions for the skinning and abdominal incision during rat dissection are expanded
- Exercise 48—Descriptions of the thyroid gland and diaphragm are expanded; explanatory questions about the lung structure and heart musculature are expanded
- Exercise 49—Figure 49.4 has been revised and enlarged to better show the structure and cross section of a kidney
- Exercise 50—Distinction has been enhanced between the animal and vegetal poles
- Exercise 51—Directions are enhanced for Procedure 51.1 to examine kinesis in pill bugs; directions are enhanced for Procedure 51.2 to study agonistic behavior in fighting fish, to encourage better creativity by the students in experimental design; a new question has been added to Questions for Further Study and Inquiry
- Appendix II has been updated to include upcoming changes to how a basic unit of the metric system is defined

Teaching and Learning Tools

McGraw-Hill Connect® Biology

McGraw-Hill Connect Biology provides online presentation, assignment, and assessment solutions. It connects your students with the tools and resources they'll need to succeed at **connect.mheducation.com**.

With Connect Biology, you can deliver assignments and quizzes online. A robust set of questions and activities is presented and aligned with this lab manual's learning outcomes. Pre-lab worksheets and Investigation worksheets are also included within Connect. As an instructor, you can edit existing questions and write entirely new questions. Track students' performance—by question, by assignment, or in relation to the class overall—with detailed grade reports. Integrate grade reports easily with Learning Management Systems (LMS), such as Blackboard—and much more.

McGraw-Hill Create™

With **McGraw-Hill Create**, you can easily rearrange exercises, combine material from other content sources, and quickly upload content you have written, such as your course syllabus or teaching notes. Find the content you need in Create by searching through thousands of leading McGraw-Hill textbooks. Arrange your book to fit your teaching style. Create even allows you to personalize your book's appearance by selecting the cover and adding your name, school, and course information. Order a Create book and you'll receive a complimentary print review copy in 3–5 business days or a complimentary electronic review copy (eComp) via e-mail in minutes. Go to **create.mheducation.com** today and register to experience how McGraw-Hill Create empowers you to teach *your* students *your* way.

Laboratory Resource Guide

The *Laboratory Resource Guide* is essential for instructors and laboratory assistants and is available free to adopters of the Laboratory Manual within Connect under the Instructor Resources tab.

Welcome to the Biology Laboratory

Welcome to the biology laboratory! Although reading your textbook and attending lectures are important ways of learning about biology, nothing can replace the importance of the laboratory. In lab you'll get hands-on experience with what you've heard and read about biology—for example, you'll observe organisms, do experiments, test ideas, collect data, and make conclusions about what you've learned. You'll do biology.

You'll enjoy the exercises in this manual—they're interesting and informative and can be completed within the time limits of your laboratory period. We've provided questions to test your understanding of what you've done; in some of the exercises, we've also asked you to devise your own experiments to answer questions that you've posed. To make these exercises most useful and enjoyable, follow these guidelines noted in the next sections.

THE IMPORTANCE OF COMING TO CLASS

Biology labs are designed to help you experience biology firsthand. To do well in your biology course, you'll need to attend class and pay attention. To appreciate the importance of class attendance as it relates to making a good grade in your biology course, examine figure 1, which is a graph showing how students' grades in an introductory biology

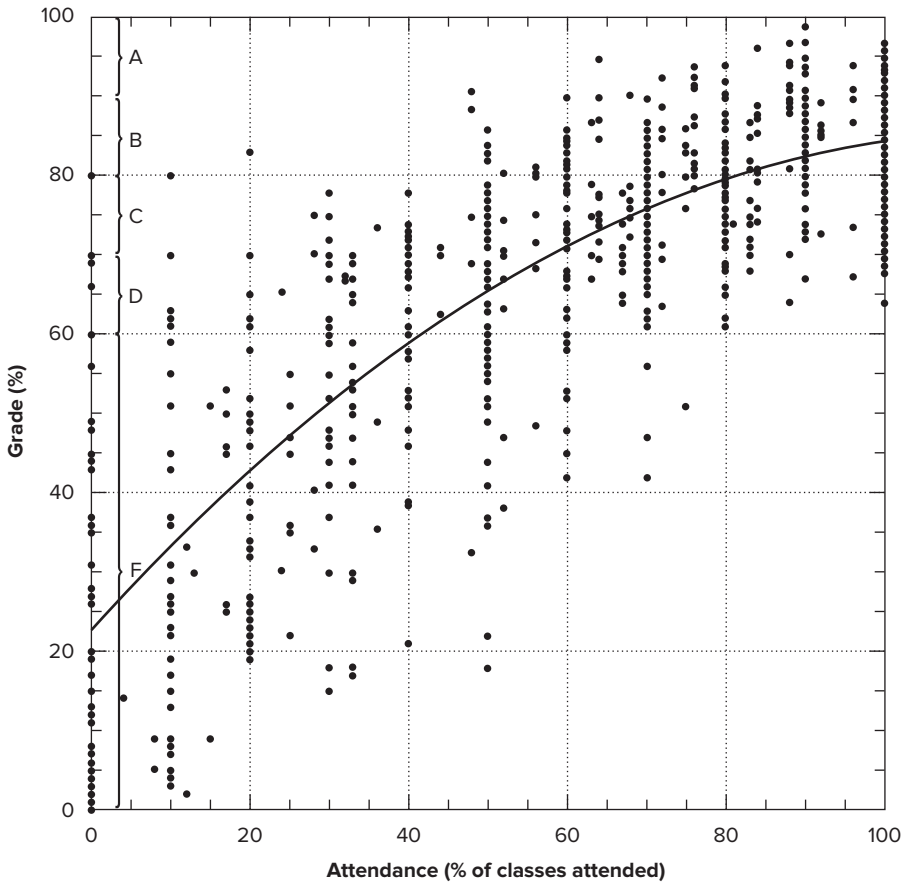

Figure 1 Relationship of students' grades in an introductory biology course to their rates of class attendance.

course correlate to their rates of class attendance. Data are from a general biology class at the University of Minnesota. On page xii, write an analysis of the data shown in figure 1. What do these data mean?

BEFORE COMING TO LAB

Watch the lab video. Videos are provided for several of the labs in this manual. Be sure to watch any assigned video associated with the lab you will be completing. These videos will help you know more about what you will be doing, what principles you will be investigating, and what concepts you need to understand before coming to lab.

Read the exercise before coming to lab. This will give you a general idea about what you're going to do, as well as why you're going to do it. Knowing this will not only save time, it will also help you finish the experiments and make you aware of any safety-related issues associated with the lab.

Review any of the lab safety concerns. Before doing any procedures, you'll encounter a section of each exercise titled "SAFETY FIRST" that is marked with its icon:

This icon will warn you of safety concerns (e.g., solvents, acids, bases, hotplates) associated with the work. If you have questions about these safety issues, contact your lab instructor before starting the lab work.

Notify your instructor if you are pregnant, are colorblind, are taking immunosuppressive drugs, have allergies, or have any other conditions that may require precautionary measures. Also, before coming to lab, cover any cuts or scrapes with a sterile, waterproof bandage.

WHEN IN LAB

1. Know what you are going to do. Read and understand the lab before coming to lab.
2. Don't start the exercise until you've discussed the exercise with your laboratory instructor. She or he will give you specific instructions about the lab and tell you how the exercise may have been modified.
3. Work carefully and thoughtfully, and stay focused as you work. You'll be able to finish each exercise within the allotted time if you are well prepared and stay on task.
4. Discuss your observations, results, and conclusions with your instructor and lab partners. Perhaps their comments and ideas will help you better understand what you've observed.
5. Always follow instructions and follow safety guidelines presented by your instructor.
6. If you have questions, ask your instructor.

SAFETY IN THE LABORATORY

Laboratory accidents can affect individuals, classes, or the entire campus. To avoid such accidents, the exercises in this manual were designed with safety as a top priority. You'll be warned about any potentially hazardous situations or chemicals with this image:

When you see this image, pay special attention to the instructions.

The laboratory safety rules listed in table 1 will help make lab a safe place for everyone to learn biology. Remember, it is much easier to prevent an accident than to deal with its consequences.

Read the laboratory safety rules listed in table 1. If you do not understand them, or if you have questions, ask your instructor for an explanation. Then complete table 1 and sign the statement at the bottom of page xii.

BEFORE YOU LEAVE LAB

Put away all equipment and glassware, and wipe clean your work area.

AFTER EACH LABORATORY

Soon after each lab, review what you did. What questions did you answer? What data did you gather? What conclusions did you make?

Also note any questions that remain. Try to answer these questions by using your textbook or visiting the library. If you can't answer the questions, discuss them with your instructor. Welcome to the biology laboratory!

Table 1

Laboratory Safety Rules

Rule	Why is this rule important? What could happen if this rule is not followed?
Behave responsibly. No horseplay or fooling around while in lab.	
Do not bring any food or beverages into lab, and do not eat, drink, smoke, chew gum, chew tobacco, or apply cosmetics when in lab. Never taste anything in lab. Do not put anything in lab into your mouth. Avoid touching your face, chewing on pens, and other similar behaviors while in lab. Always wear shoes in lab.	
Unless you are told otherwise by your instructor, assume that all chemicals and solutions in lab are poisonous, and act accordingly. Never pipette by mouth. Always use a mechanical pipetting device (e.g., a suction bulb) to pipette solutions. Clean up all spills immediately, and report all spills to your instructor.	
Wear safety goggles when working with chemicals. Carefully read the labels on bottles and know the chemical you are dealing with. Do not use chemicals from an unlabeled container, and do not return excess chemicals back to their container. Report all spills to your instructor immediately.	
Unless your instructor tells you to do otherwise, do not pour any solutions down the drain. Dispose of all materials as per instructions from your instructor.	
If you have long hair, tie it back. Don't wear dangling jewelry. If you are using open flames, roll up loose sleeves. Wear contact lenses at your own risk; contacts hold substances against the eye and make it difficult to wash your eyes thoroughly.	
Treat living organisms with care and respect.	
Your instructor will tell you the locations of lab safety equipment, including fire extinguishers, fire blanket, eyewash stations, and emergency showers. Familiarize yourself with the location and operation of this equipment.	
If anything is splashed into your eyes, wash your eyes thoroughly and immediately. Tell your lab instructor what happened.	
Notify your instructor of any allergies to latex, chemicals, stings, or other substances.	
If you break any glassware, do not pick up the pieces of broken glass with your hands. Instead, use a broom and dustpan to gather the broken glass. Ask your instructor how to dispose of the glass.	
Unless told by your instructor to do otherwise, work only during regular, assigned hours when the instructor is present. Do not conduct any unauthorized experiments; for example, do not mix any chemicals without your instructor's approval.	
Do not leave any experiments unattended unless you are authorized by your instructor to do so. If you leave your work area, slide your chair under the lab table. Keep walkways and desktops clean and clear by putting books, backpacks, and so on along the edge of the room, in the hall, in a locker, or in an adjacent room. Keep your work area as clean and uncluttered as possible.	
Don't touch or put anything on the surface of hotplates unless told to do so. Many types of hotplates have no visible sign that they are hot. Assume they are hot.	
Know how to use the equipment in lab. Most of the equipment is expensive; you may be required to pay all or part of its replacement cost. Keep water and solutions away from equipment and electrical outlets. Report malfunctioning equipment to your instructor. Leave equipment in the same place and condition that you found it. If you have any questions about or problems with equipment, contact your instructor.	
Know what to do and whom to contact if there is an emergency. Know the fastest way to get out of the lab. Immediately report all injuries—no matter how minor—to your instructor. Seek medical attention immediately if needed. If any injury appears to be life-threatening, call 911 immediately.	
At the end of each lab, clean your work area, wash your hands thoroughly with soap, slide your chair under the lab table, and return all equipment and supplies to their original locations. Do not remove any chemicals or equipment from the lab.	

Name _____

Lab Section _____

Your lab instructor may require that you submit this page at the end of today's lab.

1. In the space below, write an analysis of the data shown in figure 1.

After completing table 1, read and sign this statement:

2. I have read and I understand and agree to abide by the laboratory safety rules described in this exercise and discussed by my instructor. I know the locations of the safety equipment and materials. If I violate any of the laboratory safety rules, my instructor will lower my grade and/or remove me from the lab.

_____

Signature

_____

Name (printed)

_____

Date

EXERCISE

1

Scientific Method

The Process of Science

Learning Objectives

By the end of this exercise you should be able to:

1. Define science and understand the logic and sequence of the scientific method.
2. Develop productive observations, questions, and hypotheses about the natural world.
3. Calculate the range, mean, and standard deviation for a set of replicate measurements.
4. Design and conduct a controlled experiment to test a null hypothesis.
5. Understand the difference between a hypothesis and a scientific theory.

Please visit connect.mheducation.com to review online resources tailored to this lab.

The word *science* brings to mind different things to different students. To some students, science is a textbook. To others, it's a microscope, a dissected frog, or a course that you take. In fact, science is none of those things. Some definitions are more useful than others, but for biological research a good definition of **science** is *the orderly process of posing and answering questions about the natural world through repeated and unbiased experiments and observations*. This definition emphasizes that science is a process rather than a book, course, or list of facts. Science is not a "thing." It's a way of thinking about and doing things—a way of learning and knowing about the natural world (fig. 1.1).

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Figure 1.1 Science is a process of learning about the natural world. Doing experiments that involve gathering repeated and unbiased measurements (data) is at the heart of testing hypotheses and answering questions.

Our definition also emphasizes that people do science by *asking questions* and then *doing experiments* to answer those questions. Questions and curiosity are part of human nature, and science is a human activity. Like any human task, it takes practice to do science effectively.

Finally, our definition emphasizes that science is a tool for learning about the *natural world*. It is ineffective for moral choices, ethical dilemmas, and untestable ideas. For example, the scientific method cannot tell us if pollution is good or bad. It can tell us the environmental *consequences* of pollution, but whether these consequences are "good" or "bad" is a judgment that we make based on our values or goals, not on science. Although this is an important limitation of the scientific method, science remains one of the most powerful ways of understanding our world.

Question 1

What practices besides science are used among world cultures to learn about the natural world?

The questioning and testing inherent in science systematically sift through natural variation to find underlying patterns. The natural world includes much variation, and learning biology would be relatively easy if simple observations accurately revealed patterns of the natural world. But they usually don't—nature is too complicated to rely solely on simple observation. We can certainly learn much about

our environment just by looking around us, but casual observations are often biased and misleading because nature varies from time to time and from organism to organism. Biologists need a structured and repeatable process for testing their ideas about the variation in nature. Science is that process.

Question 2
What factors might be responsible for variation in measurements of traits such as the heights of 10-year-old pine trees or the kidney filtration rates of 10 replicate lab-mice?

The process of science deals with variation primarily through an organized sequence of steps that maintains as much objectivity and repeatability as possible. Although these loosely organized steps, sometimes called the **scientific method,** vary from situation to situation, they are remarkably effective for research and problem solving. The typical steps in the process of science are:

- Make insightful observations
- Pose and clarify testable questions
- Formulate hypotheses
- Do experiments to gather data
- Quantify the data
- Test the hypotheses
- Refine hypotheses and retest
- Answer the questions and make conclusions

DEVELOPMENT OF OBSERVATIONS, QUESTIONS, AND HYPOTHESES

Make Insightful Observations

Good scientists make insightful observations. But that's not as easy as it seems. Consider these two observations:

Observation 1: There are fewer elk in Yellowstone National Park than there used to be.

Observation 2: The density of elk in Yellowstone National Park has declined during the consecutive dry years since the reintroduction of the native wolf population.

Which of these two observations is stronger and more useful? Both of them may be true, but the second one is much more insightful because it provides a context to the observation that the elk population is declining. It also suggests a relevant factor—that is, the reintroduction of the wolf population—as a productive topic for investigation. It also suggests a relationship between density of the elk population and the variation in the local environment.

Procedure 1.1 Make insightful observations

1. Consider the following two observations.

Observation 1: Fungi often grow on leftover food.

Observation 2: Fungi such as mold and yeast grow more on leftover bread than on leftover meat.

Which of the above observations is more useful for further investigation? Why? _____

_____

_____

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. Briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Record the more insightful of the two observations on Worksheet 1 on page 9.

2. Consider this observation: Pillbugs (sometimes called roly-poly bugs) often find food and shelter where fungi are decomposing leaf litter (fig. 1.2).

For this example we are interested in whether pillbugs are attracted to leaves or to fungi (including yeasts) growing on the leaves' surfaces.

Observation 1: Pillbugs often hide under things.

Propose a more productive observation.

Observation 2: _____

_____

Record Observation 2 on Worksheet 2 on page 10. You may revise this later.

Pose and Clarify Testable Questions

Productive observations inspire questions. Humans think in terms of questions rather than abstract hypotheses or numbers. But phrasing a good question takes practice and experience, and the first questions that capture our attention are usually general. For example, "Which nutrients can yeast most readily metabolize?" is a general question that expands the observation posed in procedure 1.1. This question is broadly applicable and is the type of question that we ultimately want to understand. Enter this as the General Question in Worksheet 1.

Broad questions are important, but their generality often makes them somewhat vague. The best questions for the process of science are specific enough to answer clearly. Therefore, scientists usually refine and subdivide broad questions into more specific ones. For example, a more specific question is "What classes of biological molecules are most readily absorbed and metabolized by yeast?" Enter this as Specific Question 1 in Worksheet 1.

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Figure 1.2 Pillbugs are excellent experimental organisms to test hypotheses about microenvironments, such as those under logs and within leaf litter. Pillbugs are readily available and easily cultured in the lab (10×).

A further clarification might be "Does yeast absorb and metabolize carbohydrates better than it absorbs and metabolizes proteins?" This is a good, specific question because it clearly refers to organisms, processes, and variables that are likely involved. It also suggests a path for investigation—that is, it suggests an experiment. Enter this as Specific Question 2 in Worksheet 1.

Question 3
Consider the questions "What color is your roommate's car?" and "How many legs do cats have?" To answer these questions, would you use the scientific method, or would you rely on observation? Why?

Procedure 1.2 Posing and refining questions

1. Examine the following two questions.

Question 1: Do songbird populations respond to the weather?

Question 2: Do songbirds sing more often in warm weather than in cold weather?

Which of those questions is more useful for further investigation? Why? _____

_____

_____

_____

2. Examine the following general question, and record it in Worksheet 2.

General Question: What influences the distribution of pillbugs?

Propose a specific question that refers to the food of pillbugs as a variable, and record it here and in Worksheet 2. Know that you may revise this later.

Specific Question 1 _____

_____

Propose a more specific question that refers to pillbugs eating leaves, as opposed to pillbugs eating fungi growing on leaves. Record this question here and in Worksheet 2. Know that you may revise this later.

Specific Question 2 _____

_____

Formulate Hypotheses

Well-organized experiments to answer questions require that questions be restated as testable hypotheses. A **hypothesis** is a statement that clearly states the relationship between biological variables. A good hypothesis identifies the organism or process being investigated, identifies the variables being recorded, and implies how the variables will be compared. A hypothesis is a statement rather than a question, and an analysis of your experimental data will ultimately determine whether you accept or reject your hypothesis. Remember that even though a hypothesis can be falsified, it can never be proved true.

Accepting or rejecting a hypothesis, with no middle ground, may seem like a rather coarse way to deal with questions about subtle and varying natural processes. But using controlled experiments to either accept or reject a hypothesis is effective. The heart of science is gathering and analyzing experimental data that lead to rejecting or accepting hypotheses relevant to the questions we want to answer.

In this exercise, you are going to do science as you investigate yeast nutrition and then experiment with food choice by pillbugs. As yeast ferments its food, CO_2 is produced as a by-product. Therefore, we can measure the growth of yeast by measuring the production of CO_2 (fig. 1.3).

A hypothesis related to our question about the growth of yeast might be:

H_0: CO_2 production by yeast fed sugar is not significantly different from the CO_2 production by yeast fed protein.

A related alternative hypothesis can be similarly stated:

H_a: Yeast produces more CO_2 when fed sugar than when fed protein.

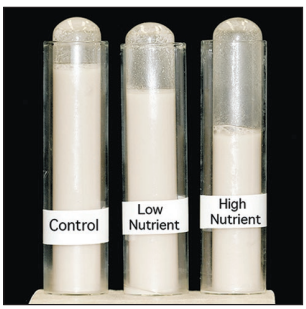

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Figure 1.3 These tubes of yeast are fermenting nutrients provided in solution. The CO_2 produced by the yeast accumulates at the top of the test tubes and indicates that yeast's rate of metabolism. From left to right, the tubes include a control with no added nutrients, a tube with low nutrients, and a tube with high nutrients.

The first hypothesis (H_0) is a **null hypothesis** because it states that there is *no difference*. This is the most common way to state a clear and testable hypothesis. (Your instructor may elaborate on why researchers state and test null hypotheses more effectively than alternative hypotheses.) Researchers usually find it more useful to associate statistical probabilities with null hypotheses rather than with alternative hypotheses. Enter the null hypothesis into Worksheet 1.

A well-written null hypothesis is useful because it is testable. In our experiment, the null hypothesis (1) specifies yeast as the organism, population, or group that we want to learn about; (2) identifies CO_2 production as the variable being measured; and (3) leads directly to an experiment to evaluate variables and compare means of replicated measurements.

Procedure 1.3 Formulating hypotheses

1. Examine the following two hypotheses:

Hypothesis 1: Songbirds sing more when the weather is warm.

Hypothesis 2: The number of bird songs heard per hour during daylight temperatures above 80°F (27°C) is not significantly different from the number heard per hour at temperatures below 80°F (27°C).

Which of these hypotheses is more useful for further investigation? Why?_____

_____

Which of these hypotheses is a null hypothesis? Why?

_____

2. Examine the following hypothesis.

Hypothesis 1: Pillbugs prefer leaves coated with a thin layer of yeast.

Propose a more effective null hypothesis. Be sure that it is a null hypothesis, that it is testable, and that it includes the parameter you will control in an experiment.

Hypothesis 2 (H_0): _____

_____

Enter your null hypothesis in Worksheet 2.

EXPERIMENTATION AND DATA ANALYSIS: YEAST NUTRITION

Gather Experimental Data

To test our hypothesis about yeast growth, we must design a controlled and repeatable experiment. The experiment suggested by our specific question and hypothesis involves offering sugar such as glucose to one population of yeast, offering protein to another population of yeast, and then measuring their respective growth rates. Fortunately, yeast grows readily in test tubes. As yeast grows in a closed, anaerobic container, it produces CO_2 in proportion to how readily it uses the available food. CO_2 production is easily measured by determining the volume of CO_2 that accumulates at the top of an inverted test tube (fig. 1.3).

Experiments provide data that determine if a hypothesis should be accepted or rejected. A well-designed experiment links a biological response to different levels of the variable being investigated. In this case, the biological response is CO_2 production, which indicates growth. The levels of the variable are sugar and protein. These levels are called **treatments,** and in our experiment they include glucose, protein, and a control. For this experiment the **treatment** (i.e., independent) **variable** being tested is the type of food molecule (i.e., protein, sugar), and the **response** (i.e., dependent) **variable** is the CO_2 production that indicates yeast growth.

An experiment that compensates for natural variation must be well designed. It should (1) include replications, (2) test only one treatment variable, and (3) include controls. **Replications** are repeated measures of each treatment under the same conditions. Replications effectively deal with naturally occurring variation. Usually the more replications, the better. Your first experiment today will include replicate test tubes of yeast, each being treated the same. Good design tests only one treatment variable at a time.

Good experimental design also requires **controls** to verify that the biological response we measure is a function of the variable being investigated and nothing else. Controls are standards for comparison. They are replicates with all of the conditions of an experimental treatment *except the treatment variable*. For example, if the treatment is glucose dissolved in water, then a control has only water (i.e., it lacks only glucose, the treatment variable). This verifies that the response is to glucose and not to the solvent. Controls validate that our results are due only to the treatment variable.

Procedure 1.4 An experiment to determine the effects of food type on yeast growth

1. Label 12 test tubes as C1–C4, G1–G4, and P1–P4. See Worksheet 1.

2. To test tubes C1–C4 add 5 mL of water. These are control replicates.

3. To test tubes G1–G4 add 5 mL of 5% glucose solution. These are replicates of the glucose treatment.

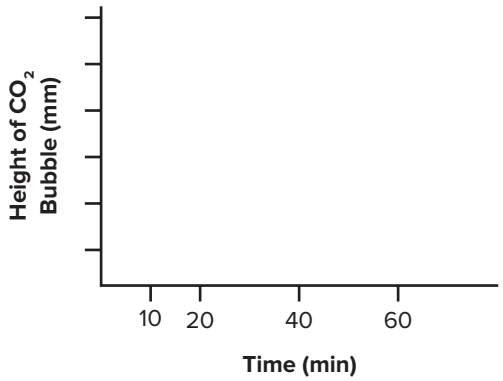
4. To test tubes P1–P4 add 5 mL of 5% protein solution. These are replicates of the protein treatment.

5. Swirl the suspension of yeast until the yeast is distributed uniformly in the liquid. Then completely fill the remaining volume in each tube with the yeast suspension that is provided.

6. For each tube, slide an inverted, flat-bottomed test tube down over the yeast-filled tube. Hold the yeast-filled

tube firmly against the inside bottom of the cover tube and invert the assembly. Your instructor will demonstrate how to slip this slightly larger empty tube over the top of each yeast tube and invert the assembly. If done properly, no bubble of air will be trapped at the top of the tube of yeast after inversion.

7. Place the tubes in a rack and incubate them at 50°C.
8. Measure the height (mm) of the bubble of accumulated CO_2 after 10, 20, 40, and 60 minutes. Record your results in Worksheet 1 and graph them here:



9. When you are finished, clean your work area and dispose of the contents of each tube as instructed by your lab instructor.

Test Your Predictions by Analyzing the Experimental Data

Analysis begins with summarizing the raw data for biological responses to each treatment. The first calculation is the **mean** ($\bar{x}$), which is the average of a set of numbers (e.g., measurements) for replicates of each treatment and the control. That is, the mean is a single number that represents the central tendency of the response variable. Later the mean of each treatment will be compared to determine if the treatments had different effects.

The second step in data analysis is to calculate variation within each set of replicates. The simplest measure of variation is the **range,** which is the highest and lowest values in a set of replicates. A wide range indicates much variation in the data. The **standard deviation (SD),** another informative measure of variation, summarizes variation just as the range does, but the standard deviation is less affected by extreme values. Refer to the box "Variation in Replicate Measures" to learn how to calculate the standard deviation.

Question 4
Even the seemingly simple question "How tall are mature males of the human species?" can be difficult to answer. How would you best express the answer?

Procedure 1.5 Quantify and summarize the data

1. Examine your raw data in Worksheet 1.
2. Calculate the mean of the response variable (CO_2 production) for the four control replicates. To calculate the means for the four replicates, sum the four values and divide by four. Record the mean for the control replicates in Worksheet 1.
3. The CO_2 production for each glucose and protein replicate must be adjusted with the control mean. This ensures that the final data reflect the effects of only the treatment variable and not the solvent. Subtract the control mean from the CO_2 production of each glucose replicate and each protein replicate, and record the results in Worksheet 1.
4. Record in Worksheet 1 the range of adjusted CO_2 production for the four replicates of the glucose treatment and of the protein treatment.
5. Calculate the mean CO_2 production for the four adjusted glucose treatment replicates. Record the mean in Worksheet 1.
6. Calculate the mean CO_2 production for the four adjusted protein treatment replicates. Record the mean in Worksheet 1.
7. Refer to "Variation in Replicate Measures," and calculate the standard deviation for the four adjusted glucose treatment values and for the four adjusted protein treatment values. Record the two standard deviations in Worksheet 1.

Test the Hypotheses

Our hypothesis about yeast growth is tested by comparing the mean CO_2 production by yeast fed glucose to the mean CO_2 production by yeast fed protein. However, only determining if one mean is higher than the other is not an adequate test because natural variation will always make the two means at least slightly different, even if the two treatments have the same effect on yeast growth. Therefore, the means and the variation about the means must be compared to determine if the means are not just different but **significantly different.** To be significantly different, the differences between means must be due to the treatment and not just due to natural variation. If the difference is significant, then the null hypothesis is rejected. If the difference is not significant, then the null hypothesis is accepted. Testing for significant differences is usually done with statistical methods.

Statistical methods calculate the probability that the means are significantly different. But these complex calculations are beyond the scope of this exercise. We will use a simpler method to test for a significant difference between the means of our two treatments. We will declare that two means are significantly different *if the means plus or minus 1/2 of the standard deviation do not overlap.*

Variation in Replicate Measures

Natural variation occurs in all processes of biology. This variation will inevitably produce different results in replicated treatments. One of the most useful measures of variation of values about the mean is **standard deviation.** It's easy to calculate: calculate the mean, calculate the deviation of each sample from the mean, square each deviation, and then sum the deviations. This summation is the sum of squared deviations. For example, data for CO_2 production by yeast in four replicate test tubes might be 22, 19, 18, and 21 mm. The mean is 20 mm.

CO_2 Production (mm)	Mean	Deviation	Deviation^2
22	20	2	4
19	20	−1	1
18	20	−2	4
21	20	1	1
			Sum of squared deviations = 10

The summary equation for the sum of squared deviations is

$$\textbf{Sum of squared deviations} = \sum_{i=1}^{N}(x_i - \bar{x})^2$$

where

N = **total number of samples**

$\bar{x}$ = **the sample mean**

x_i = **measurement of an individual sample**

The summation sign ($\sum_{i=1}^{N}$) means to add up all the squared deviations from the first one ($i = 1$) to the last one ($i = N$).

The sum of squared deviations (10) divided by the number of samples minus one ($4 - 1 = 3$) produces a value of $10/3 = 3.3$ mm^2 (the units are millimeters squared). This is the variance:

$$\textbf{Variance} = \frac{\textbf{sum of squared deviations}}{N - 1}$$

The square root of the variance, 1.8 cm, equals the standard deviation

$$\textbf{SD} = \sqrt{\textbf{Variance}} = \sqrt{3.3} = 1.8$$

The standard deviation is often reported with the mean in statements such as, "The mean CO_2 production was 20 ± 1.8 mm." The standard deviation helps us understand the spread or variation among replicated treatments. For example, if the standard deviation is zero, all of the numbers in the set are the same. A larger standard deviation implies that individual numbers are farther from the mean.

For example, consider these two means and their standard deviations (SD):

$Mean_a = 10$ SD = 5	$Mean_b = 20$ SD = 10
$Mean_a - (½)SD = 7.5$	$Mean_b - (½)SD = 15$
$Mean_a + (½)SD = 12.5$	$Mean_b + (½)SD = 25$

Are $Mean_a$ and $Mean_b$ significantly different according to our test for significance? Yes they are, because 7.5 ↔ 12.5 does not overlap 15 ↔ 25.

Procedure 1.6 Testing hypotheses

1. Consider your null hypothesis and the data presented in Worksheet 1.
2. Calculate the glucose mean − (½)SD and the glucose mean + (½)SD. Record them in Worksheet 1.
3. Calculate the protein mean − (½)SD and the protein mean + (½)SD. Record them in Worksheet 1.
4. Do the half standard deviations surrounding the means of the two treatments overlap? Record your answer in Worksheet 1.
5. Are the means for the two treatments significantly different? Record your answer in Worksheet 1.
6. Is your null hypothesis accepted? Or rejected? Record your answer in Worksheet 1.

Answer the Questions

The results of testing the hypotheses are informative, but it still takes a biologist with good logic to translate these results into the answers of our specific and general questions. If your specific questions were well stated, then answering them based on the results of your experiment and hypothesis testing should be straightforward.

Procedure 1.7 Answering the questions: yeast nutrition

1. Examine the results of hypothesis testing presented in Worksheet 1.
2. Specific Question 2 was "Does yeast absorb and metabolize carbohydrates better than it absorbs and metabolizes proteins?" Enter your answer in Worksheet 1.
3. Does your experiment adequately answer this question? Why or why not? _____

   _____

   _____
4. Specific Question 1 was "What classes of biological molecules are most readily absorbed and metabolized by yeast?" Enter your best response in Worksheet 1.

5. Does your experiment adequately answer Specific Question 1? Why or why not?_____

_____

_____

6. The General Question was "Which nutrients can yeast most readily metabolize?" After testing the hypotheses, are you now prepared to answer this general question? Why or why not? _____

_____

_____

EXPERIMENTATION AND DATA ANALYSIS: FOOD PREFERENCE BY PILLBUGS

In the previous procedures you developed and recorded observations, questions, and hypotheses concerning food preference by pillbugs. Pillbugs may be attracted to dead leaves as food, or they may be attracted to fungi growing on the leaves as food. Leaves dipped in a yeast suspension can simulate fungi growing on leaves. Use the following procedures as a guide to the science of experimentation and data analysis to test the hypothesis you recorded in Worksheet 2.

Procedure 1.8 Design an experiment to test food preference by pillbugs

1. Design an experiment to test your hypothesis in Worksheet 2 about food preference by pillbugs. To do this, specify:

   Experimental setup _____

   _____

   Treatment 1 to be tested _____

   _____

   Treatment 2 to be tested _____

   _____

   Control treatment _____

   _____

   Response variable _____

   _____

   Treatment variable _____

   _____

   Number of replicates _____

   _____

   Means to be compared _____

   _____

2. Conduct your experiment and record the data in Worksheet 2.
3. Analyze your data. Record the control means and adjusted treatment-means in Worksheet 2.
4. Calculate the range and standard deviation for your treatments, and record them in Worksheet 2.
5. Test your hypothesis. Determine if the null hypothesis should be accepted or rejected. Record the results in Worksheet 2.
6. Answer the Specific Question 2, Specific Question 1, and General Question posed in Worksheet 2.

Procedure 1.9 Answering the questions: food preference by pillbugs

1. Examine the results of your hypothesis testing presented in Worksheet 2.
2. Enter your answer to Specific Question 2 in Worksheet 2. Does your experiment adequately answer this question? Why or why not? _____

   _____

   _____

3. Enter your best response to Specific Question 1 in Worksheet 2. Does your experiment adequately answer this question? Why or why not? _____

   _____

   _____

4. After testing the hypotheses, are you now prepared to answer your General Question "What influences the distribution of pillbugs?" Why or why not? _____

   _____

   _____

Question 5
What are some examples of biological theories?

Scientific Theories

Throughout this course you will make many predictions and observations about biology. When you account for a group of these observations with a generalized explanation, you have proposed a scientific theory.

In science, as opposed to common usage, a theory is a well-substantiated explanation of some aspect of the natural world that usually incorporates many confirmed observational and experimental facts. A scientific theory makes predictions consistent with what we see. It is not a guess; on the contrary, a scientific theory is widely accepted within the scientific community—for example, the germ theory claims that certain infectious diseases are caused by microorganisms. Scientific theories do not become facts; scientific theories *explain* facts.

INQUIRY-BASED LEARNING

How do changes in temperature affect the production of CO_2 by yeast?

Observation: Fermentation of nutrients by yeast produces CO_2, and the production rate of this CO_2 can be used to measure growth of the yeast. In this lab you've already investigated how the production of CO_2 is affected by different nutrients (i.e., sugar, protein). Here you'll investigate another variable: temperature.

Question: How is the production of CO_2 by yeast affected by temperature?

a. Establish a working lab group and obtain Inquiry-Based Learning Worksheet 1 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis. Record this hypothesis.

d. Outline on Worksheet 1 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Study and Inquiry

1. Newspaper articles often refer to a discovery as "scientific" or claim that something has been proved "scientifically." What is meant by this description?

2. Experimental results in science are usually reviewed by other scientists before they are published. Why is this done?

3. Have all of our discoveries and understandings about the natural world been the result of applying the scientific method? How so?

4. Suppose that you hear that two means are *significantly* different. What does this mean?

5. Can means be different but not significantly different? Explain your answer.

6. How can science be used to address "big" issues such as climate change?

7. Some people dismiss evolution by natural selection as being "only a theory." Biologists often respond that yes, evolution *is* a scientific theory. What does this mean?

8. A hallmark of a scientific theory is that it is falsifiable. What does this mean, and why is it important?

Worksheet 1 The Process of Science: Nutrient Use by Yeast

OBSERVATION _____

QUESTIONS

General Question: _____

Specific Question 1: _____

Specific Question 2: _____

HYPOTHESIS H_0: _____

EXPERIMENTAL DATA: Nutrient Use by Yeast

	Treatments					Treatments Minus Control $\bar{x}$	
Replicate	**Control CO_2 Production (mm)**	**Replicate**	**Glucose CO_2 Production (mm)**	**Replicate**	**Protein CO_2 Production (mm)**	**Glucose CO_2 Production Adjusted for the Control $\bar{x}$**	**Protein CO_2 Production Adjusted for the Control $\bar{x}$**
C1	_____	G1	_____	P1	_____	_____	_____
C2	_____	G2	_____	P2	_____	_____	_____
C3	_____	G3	_____	P3	_____	_____	_____
C4	_____	G4	_____	P4	_____	_____	_____

Control $\bar{x}$ = _____
Glucose $\bar{x}$ = _____
Glucose range = _____ − _____
Glucose SD = _____

Protein $\bar{x}$ = _____
Protein range = _____ − _____
Protein SD = _____

TEST HYPOTHESIS

Glucose $\bar{x}$ − (½)SD = _____

Glucose $\bar{x}$ + (½)SD = _____

Protein $\bar{x}$ − (½)SD = _____

Protein $\bar{x}$ + (½)SD = _____

Do the half standard deviations surrounding the means of the two treatments overlap? Yes _____ No _____

Are the means for the two treatments significantly different? Yes _____ No _____

Is the null hypothesis accepted? _____ or rejected? _____

ANSWER QUESTIONS

Answer to Specific Question 2 _____

Answer to Specific Question 1 _____

Answer to General Question _____

Worksheet 2 The Process of Science: Food Preference by Pillbugs

OBSERVATION _____

QUESTIONS

General Question: _____

Specific Question 1: _____

Specific Question 2: _____

HYPOTHESIS H_0: _____

EXPERIMENTAL DATA: Food Preference by Pillbugs

Treatments						Treatments Minus Control $\bar{x}$	
Replicate	**Control**	**Replicate**	**Treatment 1**	**Replicate**	**Treatment 2**	**Treatment 1 Adjusted for the Control $\bar{x}$**	**Treatment 2 Adjusted for the Control $\bar{x}$**
1	_____	1	_____	1	_____	_____	_____
2	_____	2	_____	2	_____	_____	_____
3	_____	3	_____	3	_____	_____	_____
4	_____	4	_____	4	_____	_____	_____

Control $\bar{x}$ = _____
Treatment 1 $\bar{x}$ = _____
Treatment 1 range = _____ − _____
Treatment 1 SD = _____

Treatment 2 $\bar{x}$ = _____
Treatment 2 range = _____ − _____
Treatment 2 SD = _____

TEST HYPOTHESIS

Treatment 1 $\bar{x}$ − (½)SD = _____

Treatment 1 $\bar{x}$ + (½)SD = _____

Treatment 2 $\bar{x}$ − (½)SD = _____

Treatment 2 $\bar{x}$ + (½)SD = _____

Do the half standard deviations surrounding the means of the two treatments overlap? Yes _____ No _____

Are the means for the two treatments significantly different? Yes _____ No _____

Is the null hypothesis accepted? _____ or rejected? _____

ANSWER QUESTIONS

Answer to Specific Question 2 _____

Answer to Specific Question 1 _____

Answer to General Question _____

EXERCISE

2

Measurements in Biology

The Metric System and Data Analysis

Learning Objectives

By the end of this exercise you should be able to:

1. Understand the difference between accuracy and precision in measurements.
2. Identify the metric units used to measure length, volume, mass, and temperature.
3. Measure length, volume, mass, and temperature in metric units.
4. Convert one metric unit to another (e.g., grams to kilograms).
5. Use measures of volume and mass to calculate density.
6. Practice the use of simple statistical calculations such as mean, median, range, and standard deviation.
7. Analyze sample data using statistical tools.

Please visit connect.mheducation.com to review online resources tailored to this lab.

Every day we're bombarded with numbers and measurements. They come at us from all directions, including while we're at the supermarket, gas station, golf course, and pharmacy, as well as while we're in our classrooms and kitchens. Virtually every package that we touch is described by a measurement.

Scientists use a standard method to collect data as well as use mathematics to analyze measurements. We must measure things before we can objectively describe what we are observing, before we can experiment with biological processes, and before we can predict how organisms respond, adjust to, and modify their world. Once we have made our measurements, we can analyze our data and look for variation and the sources of that variation. Then we can infer the causes and effects of the biological processes that interest us.

ACCURACY AND PRECISION

Scientists strive to make accurate, precise measurements. The **accuracy** of a group of measurements refers to how closely the measured values agree with the true or correct value. In contrast, the **precision** of a group of measurements refers to how closely the measurements agree with each other. That is, precision is the degree to which the measurements produce the same results, regardless of their accuracy. Scientists strive to make measurements that are accurate and precise.

Question 1

a. Can measurements be accurate but not precise? Explain.

b. Can measurements be precise but not accurate? Explain.

To help you check your answers, consider an analogy involving shooting arrows at a bull's-eye target (fig. 2.1). In this analogy, each arrow (represented by a dot on the target) would represent a measurement. Accuracy would be the

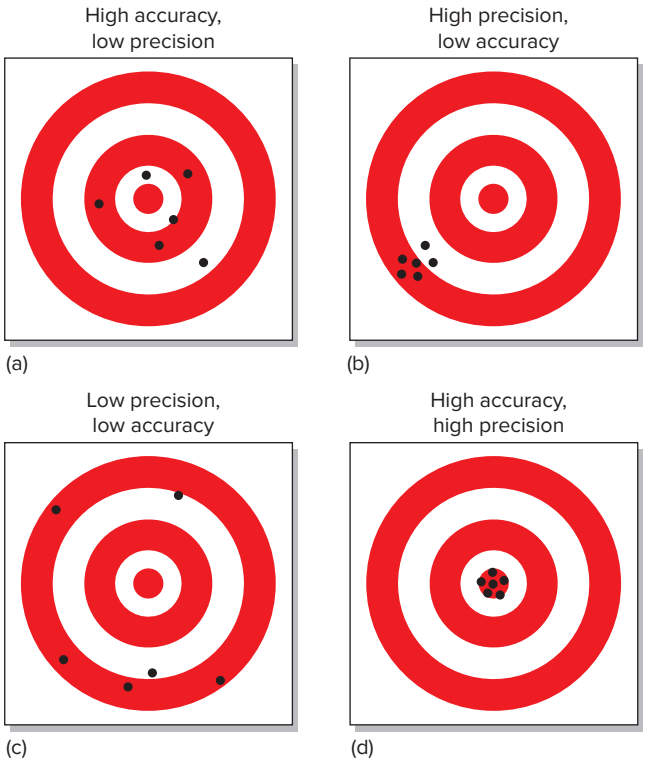


Figure 2.1 Precision and accuracy. Measurements can be (*a*) accurate but not precise, (*b*) precise but not accurate, (*c*) neither precise nor accurate, or (*d*) both precise and accurate.

closeness of the arrows to the center of the target; arrows closest to the bullseye would be most accurate. Precision would be the size of the cluster of arrows, regardless of how close they are to the center of the target.

THE METRIC SYSTEM

Scientists throughout the world use the **metric system** to make measurements. The metric system is also used in everyday life virtually everywhere except the United States. With few exceptions (e.g., liter bottles of soda), most measurements in the United States use the antiquated English system of pounds, inches, feet, and so on. Check with your instructor about bringing to class common grocery store items with volumes and weights in metric units or examining those items on display.

The most modern form of the metric system is called the International System of Units (abbreviated SI). Metric measurement is used worldwide in science to improve communication in the scientific community. Scientists make all of their measurements in the metric system; they do not routinely convert from one system to another. When scientists have mixed metric units with English units, the results have often been confusing, and have sometimes been disastrous. For example, in 1999, the $125-million Mars Climate Orbiter was approaching Mars to study the planet's climate. Lockheed Martin Astronautics, which built the spacecraft, gave NASA critical flight information in English units, but software aboard the orbiter expected the data in metric units. As a result, the orbiter was sent into, rather than safely above, the Mars atmosphere, where it disintegrated.

The following conversions will help give you a sense of how some common English units are related to their metric equivalents:

1 inch = 2.5 centimeters
1 foot = 30 centimeters
1 yard = 0.9 meter
1 mile = 1.6 kilometers
1 fluid ounce = 30 milliliters
1 pint = 0.47 liter
1 quart = 0.95 liter
1 gallon = 3.8 liters
1 cup = 0.24 liter

To learn more about these conversions, see Appendix II.

This exercise will introduce you to making metric measurements of length, mass, volume, and temperature. During this lab, you should spend your time making measurements, not reading background information. Therefore, *before lab*, *read this exercise carefully to familiarize yourself with the basic units of the metric system*.

Metric units commonly used in biology include:

meter (m)—the basic unit of length
liter (L)—the basic unit of volume
kilogram (kg)—the basic unit of mass
degrees Celsius (°C)—the basic unit of temperature

Unlike the English system with which you are already familiar, the metric system is based on units of ten. This simplifies conversions from one metric unit to another (e.g., from kilometers to meters). This base-ten system is similar to our monetary system, in which 10 cents equal a dime, 10 dimes equal a dollar, and so forth. Units of 10 in the metric system are indicated by Latin and Greek prefixes placed before the base units:

Prefix (Latin)		***Division of Metric Unit***	
deci	(d)	0.1	10^{-1}
centi	(c)	0.01	10^{-2}
milli	(m)	0.001	10^{-3}
micro	(μ)	0.000001	10^{-6}
nano	(n)	0.000000001	10^{-9}
pico	(p)	0.000000000001	10^{-12}
Prefix (Greek)		***Multiple of Metric Unit***	
deka	(da)	10	10^{1}
hecto	(h)	100	10^{2}
kilo	(k)	1000	10^{3}
mega	(M)	1000000	10^{6}
giga	(G)	1000000000	10^{9}

Hints for Using the Metric System

1. Use decimals, not fractions (e.g., 2.5 m, not 2½ m).
2. Express measurements in units requiring only a few decimal places. For example, 0.3 m is more easily manipulated and understood than 300000000 nm.
3. When measuring pure water, the metric system offers an easy and common conversion from volume measured in liters to volume measured in cubic meters to mass measured in grams: 1 mL = 1 cm^3 = 1 g.
4. The metric system uses symbols rather than abbreviations. Therefore, do not place a period after metric symbols (e.g., 1 g, not 1 g.). Use a period after a symbol only at the end of a sentence.
5. Do not mix units or symbols (e.g., 9.2 m, not 9 m 200 mm).
6. Metric symbols are always singular (e.g., 10 km, not 10 kms).
7. Except for degrees Celsius, always leave a space between a number and a metric symbol (e.g., 20 mm, not 20mm; 10°C, not 10° C).
8. Use a zero before a decimal point when the number is less than one (e.g., 0.42 m, not .42 m).

Thus, multiply by

0.01	to convert centimeters to meters
0.001	to convert millimeters to meters
1000	to convert kilometers to meters
0.1	to convert millimeters to centimeters

For example, there are 10 millimeters per centimeter. Therefore, to convert 62 centimeters to millimeters,

$$62 \text{ cm} \times \frac{10 \text{ mm}}{\text{cm}} = 620 \text{ mm}$$

In these conversion equations, the units being converted *from* (in this case, centimeters) cancel out, leaving you with the desired units (in this case, millimeters). Also note that when units are converted to *smaller* units, the number associated with the new units will *increase*, and vice versa. For example, 620 meters = 0.620 kilometer = 620,000 millimeters = 62,000 centimeters.

Question 2
Make the following metric conversions:

1 meter = _____ centimeters = _____ millimeters
92.4 millimeters = _____ meters = _____ centimeters
10 kilometers = _____ meters = _____ decimeters
82 centimeters = _____ meters = _____ millimeters
3.1 kilograms = _____ grams = _____ milligrams
281 milliliters = _____ liters = _____ deciliters
35 millimeters = _____ centimeters = _____ meters

Length and Area

The **meter** (m) is the basic unit of length. Units of area are squared units (i.e., two-dimensional) of length.

$1 \text{ m} = 100 \text{ cm} = 1000 \text{ mm} = 0.001 \text{ km} = 1 \times 10^{-3} \text{ km}$
$1 \text{ km} = 1000 \text{ m} = 10^3 \text{ m}$
$1 \text{ cm} = 0.01 \text{ m} = 10^{-2} \text{ m} = 10 \text{ mm}$
$470 \text{ m} = 0.470 \text{ km}$
$1 \text{ cm}^2 = 100 \text{ mm}^2$ (i.e., $10 \text{ mm} \times 10 \text{ mm} = 100 \text{ mm}^2$)

To help you appreciate the magnitudes of these units, here are the lengths and areas of some familiar objects:

Length	
Housefly	0.5 cm
Diameter of penny	1.9 cm
Diameter of baseball	7.4 cm
Soda can	12.2 cm
Toyota Camry	4.7 m
Mt. Everest	8848 m

Area	
Credit card	46 cm^2
Total skin area of adult human male	1.8 m^2
Ping-pong table	4.18 m^2
Surface area of human lungs	80 m^2
Football field (goal line to goal line)	4459 m^2
Central Park (New York City)	3.4 km^2

Procedure 2.1 Make metric measurements of length and area

Most biologists measure lengths with metric rulers or metersticks.

1. Examine intervals marked on the metric rulers and metersticks available in the lab.
2. Make the following measurements. Be sure to include units for each measurement.

Length of this page _____
Width of this page _____
Area of this page (Area = Length × Width) _____
Your height _____
Thickness of this manual _____
Height of a 200-mL beaker _____
Height of ceiling _____
Height of your chair _____
Length of your cell phone _____

Question 3
What are some potential sources of error in your measurements?

Volume

Volume is the space occupied by an object. Units of volume are cubed (i.e., three-dimensional) units of length. The liter (L) is the basic unit of volume.

$1 \text{ L} = 1000 \text{ cm}^3 = 1000 \text{ mL}$
$1 \text{ L} = 0.1 \text{ m} \times 0.1 \text{ m} \times 0.1 \text{ m}$
$1 \text{ cm}^3 = 0.000001 \text{ m}^3$

To help you appreciate the magnitudes of these units, here are the volumes of some familiar objects:

Chicken egg	60 mL
Coke can	355 mL
One breath of air	500 cm^3

Scientists often measure volumes with pipets and graduated cylinders. Pipets are used to measure small volumes, typically 25 mL or less. Liquid is drawn into a pipet using a bulb or pipet pump (fig. 2.2). Never pipet by mouth.

Graduated cylinders are used to measure larger volumes. To appreciate how to make a measurement accurately, pour 40–50 mL of water into a 100-mL graduated cylinder, and observe the interface between the water and air. This interface, called the **meniscus**, is curved because of surface tension and the adhesion of water to the sides of the cylinder. When measuring the liquid in a cylinder such as a graduated

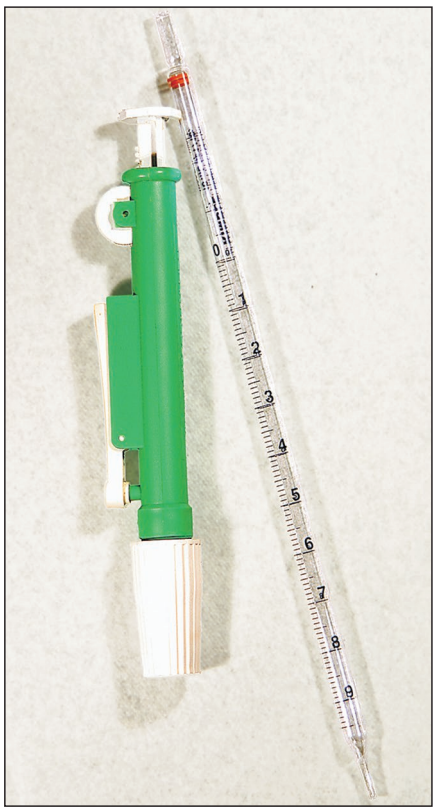


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Figure 2.2 A pipet is used to extract and dispense volumes of liquid. A suction device (shown in green on the left) draws fluid into the pipet, and graduated markings on the pipet allow precise measurement of a fluid's volume. Never use your mouth to suck fluid into a pipet.

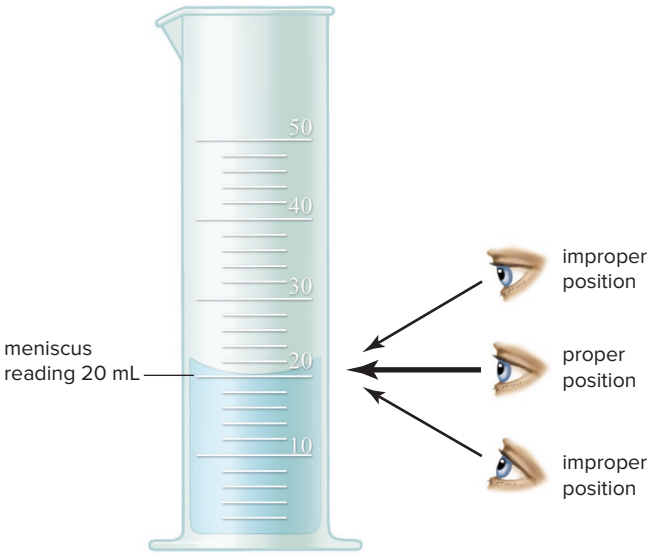

Figure 2.3 When measuring the volume of liquid in a graduated cylinder, always position your eye at the bottom of the meniscus. The correct volume is 20 mL.

cylinder, always position your eyes level with the meniscus and read the volume at the lowest level (fig. 2.3).

Procedure 2.2 Make metric measurements of volume

1. Biologists often use graduated cylinders to measure volumes. Locate the graduated cylinders available in the lab to make the following measurements. Determine what measurements the markings on the graduated cylinder represent. Be sure to include units for each measurement.
2. Measure the volume (in mL) needed to fill a cup (provided in the lab). _____
3. Measure the volume (in L) needed to fill a gallon. _____

Procedure 2.3 Measure the volume of a solid object by water displacement

1. Obtain a 100-mL graduated cylinder, a thumb-sized rock, and a glass marble.
2. Fill the graduated cylinder with 70 mL of water.
3. Gently submerge the rock in the graduated cylinder. Notice that the volume of the contents rises.
4. Carefully observe the meniscus of the fluid and record its volume.
5. Calculate and record the volume of the rock by subtracting the original volume (70 mL) from the new volume.

   Rock volume _____
6. Repeat steps 2–5 to measure and record the volume of the marble.

   Marble volume _____

Biologists use pipets to measure and transfer small volumes of liquid from one container to another. The following procedure will help you appreciate the usefulness of pipets.

Procedure 2.4 Learn to use a pipet

1. Add approximately 100 mL of water to a 100-mL beaker.
2. Use a 5-mL pipet with a bulb or another filling device provided by your instructor to remove some water from the beaker.
3. Fill the pipet to the zero mark.
4. To read the liquid level correctly, your eye must be directly in line with the bottom of the meniscus.
5. Release the liquid into another container.

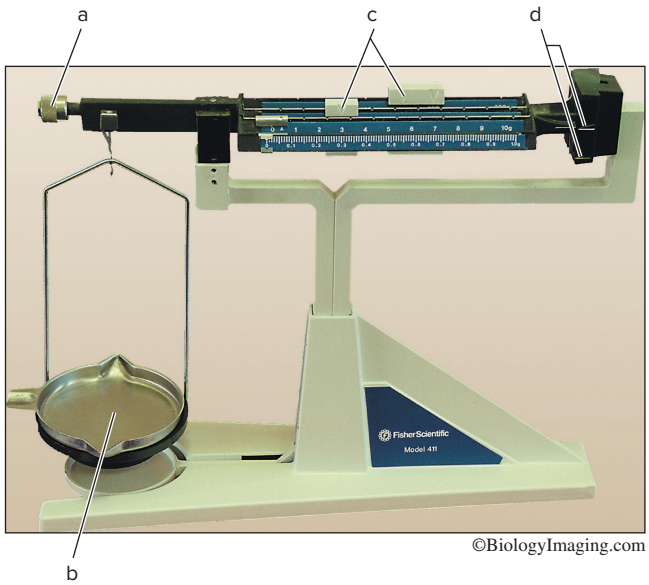

Figure 2.4 Biologists use balances to measure mass. (A) The parts of a triple-beam balance include the (*a*) zero-adjustment knob, (*b*) measuring pan, (*c*) movable masses on horizontal beams, and (*d*) balance marks. (B) A top-loading balance has a measuring pan, a power switch, and a zero calibration ("tare") button.

Question 4
What volume of liquid did you measure?

Mass

The **kilogram** (kg) is the basic unit of mass.[1] A kilogram is approximately equal to the mass of 1000 cubic centimeters (cm^3) of water at 4°C. Similarly,

$$\mathbf{1\ kg = 1000\ g = 10^3\ g} \qquad \mathbf{1\ mg = 0.001\ g = 10^{-3}\ g}$$

Note that the kilogram is the only base-unit of SI that includes a prefix ("kilo," symbol "k") as part of its name (see Appendix II). Here are the approximate masses of some familiar objects:

Housefly	12 mg	9V battery	40 g
Hummingbird	1.6 g	Human heart	300 g
Ping-pong ball	2.45 g	Basketball	0.62 kg
Quarter	6.25 g		

Biologists usually measure mass with a top-loading balance or a triple-beam balance (fig. 2.4). Locate the triple-beam balances or top-loading electronic balances in the lab. Triple-beam balances get their names from their three horizontal beams. Suspended from each of the three beams are movable masses. Each of the three beams of the balance is marked with graduations: the closest beam has 0.1-g graduations, the middle beam has 100-g graduations, and the farthest beam has 10-g graduations.

Procedure 2.5 Make metric measurements of mass

1. Before making any measurements, clean the weighing pan and move all of the suspended weights to the far left. The balance marks should line up to indicate zero grams; if they do not, turn the adjustment knob until they do. Measure the mass of an object by placing it in the center of the weighing pan and moving the suspended masses until the beams balance. The mass of the object is the sum of the masses indicated by the weights on the three beams.
2. If you're using an electronic balance, turn on the balance and let it warm up for 5 minutes. Wait until the display reads 0.0 g; if the display does not read 0.0 g, press the "tare" button to reset the display to 0.0 g. If you are weighing an object such as a coin or pencil, place the object on the measuring pan. After the display has stabilized, read and record the object's mass.
3. If you are weighing a liquid, powder, or similar specimen, place an empty beaker (in which you will place the liquid) or a piece of weighing paper (on which you will place the powder) on the balance's measuring pan. After the display has stabilized, press the "tare" button to reset the display to 0.0 g. Place the liquid in the beaker (or the powder on the weighing paper). After the display has stabilized, read and record the mass.

1 *Remember that mass is not necessarily synonymous with weight. Mass measures an object's* **potential** *to interact with gravity, whereas weight is the force exerted by gravity on an object. Thus, a weightless object in outer space has the same mass as it has on earth.*

4. Measure the masses of the following items. Be sure to include units for each measurement.

Penny _____

Paper clip _____

Pencil _____

Rock (used in procedure 2.3) _____

100-mL beaker (empty) _____

100-mL beaker containing 50 mL of water _____

Question 5

a. **Density** is mass per unit volume. Use data that you've gathered to determine the density of water at room temperature.

Density of water = (mass/volume) = _____

b. What is the density of the wooden pencil? Does it float? Why?

c. What is the density of the rock? Does it sink? Why?

Temperature

Temperature is the measure of the kinetic energy of molecules—that is, the amount of heat in a system. Biologists measure temperature with a thermometer calibrated in degrees Celsius (°C). The Celsius scale is based on water freezing at 0°C and boiling at 100°C. You can interconvert °C and degrees Fahrenheit (°F) by using the formula 5(°F) = 9(°C) + 160. Here are some typical temperatures:

−20°C	temperature in a freezer
−18°C	mixture of ice and salt
0°C	water freezes
4°C	temperature in a refrigerator
22°C	room temperature
30.6°C	butter melts
37°C	human body temperature
40°C	a hot summer day
50°C	hottest day on record in Phoenix, AZ
71°C	flash pasteurization of milk
75°C	hot coffee
100°C	water boils
260°C	broiler temperature

Procedure 2.6 Make metric measurements of temperature

1. Obtain a thermometer in the lab. Handle the thermometer with care. If it breaks, notify your instructor immediately.

Significant Figures

Let's suppose that you're measuring the length of a bone, as shown in figure 2.5. How would you record this length—as 8 cm? 8.3 cm? 8.33 cm? 8.33333 cm? To answer this question, you need to know something about significant figures.

Significant figures are the number of figures required to record a measurement so that only the last digit in the number is in doubt. For example, if the ruler you're using is calibrated only in centimeters and you find that the object you're measuring is between 8 and 9 cm long (fig. 2.5), then you should estimate your measurement only to a tenth of a centimeter. That is, a measurement of 8.3 cm is acceptable, but 8.33 is not because it implies a precision that did not exist in the equipment you used to make the measurement. If, however, your ruler was calibrated in millimeters, then 8.33 cm would be acceptable. Remember this: When recording measurements, include all of the digits you are sure of plus an estimate to the nearest one-tenth of the next smaller digit.

Here are some other guidelines for using the correct number of significant figures in your measurements:

When adding or subtracting measurements, the answer should have no more precision than the measurement having the least number of significant figures. For example, suppose the air temperature in an incubator drops from 8.663°C to 8.2°C. This is a difference of 8.663°C – 8.2°C = 0.5°C, not 0.463°C. If the second temperature reading had been 8.200°C, then the correct answer would have been 0.463°C.

When converting measurements from one set of units to another, do not introduce precision that is not present in the first number. For example, 8.3 cm = 83 mm, not 83.0 mm.

When manipulating two measurements simultaneously, the precision of the final measurement should not exceed that of the least number of significant figures. For example, the calculation for the mass of 17.2 mL of water is 17.2 mL × 0.997821 g mL^{-1} = 17.2 g, not 17.162521 g.

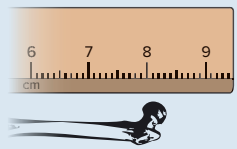

Figure 2.5 How long is this bone? 8 cm? 8.3 cm? 8.33 cm?

Rounding Numbers

Do not change the value of the last significant digit if that digit is followed by a number that is less than 5. For example, if two significant figures are required, 6.449 rounds to 6.4, 66.449 rounds to 66, 66.641 rounds to 67, and 6.591 rounds to 6.6. Here is how an original measurement of 49.5149 rounds to various numbers of significant figures:

Five significant figures:	49.515
Four significant figures:	49.51
Three significant figures:	49.5
Two significant figures:	50
One significant figure:	50

Statisticians disagree on what to do when the number following the last significant figure is exactly 5, as in 89.5 (and, in this case, the precision is limited to two significant figures). Some round the measurement to the higher number, while others claim that doing so introduces bias into the data. You can decide which approach to take, but be consistent.

2. Determine the range of the temperatures that can be measured with your thermometer by examining the scale imprinted along the barrel of the thermometer.

3. Measure the following temperatures:

Room temperature	_____ °C
Cold tap water	_____ °C
Hot tap water	_____ °C
Inside refrigerator	_____ °C

UNDERSTANDING NUMERICAL DATA

Statistics offer a way to organize, summarize, and describe data—the data are usually samples of information from a much larger population of values. Statistics and statistical tests allow us to analyze the sample and draw inferences about the entire population. Consequently, the use of statistics enables us to make decisions even though we have incomplete data about a population. Although this may seem unscientific, we do it all the time; for example, we diagnose diseases with a drop of blood. Decisions are based on statistics when it is impossible or unrealistic to analyze an entire population.

Let's say that you want to know the mass of a typical apple in your orchard. To obtain this information, you could analyze one apple, but how would you know that you'd picked a "typical" sample? After all, the batch from which you chose the apple may contain many others, each a little different. You'd get a better estimate of "typical" if you increased your sample size to a few hundred apples, or even to 10,000. Or, better yet, to 1,000,000.

The only way to be certain of your conclusions would be to accurately measure all the apples in your orchard. This is impossible, so you must choose apples that *represent* all of the other apples—that is, you must be working with a *representative sample*. A statistical analysis of those sample apples reduces the sample values to a few characteristic measurements (e.g., mean mass). As you increase the size of the sample, these characteristic measurements provide an ever-improving estimation of what is "typical."

There are a variety of software programs that perform statistical analyses of data; all you have to do is enter your data into a spreadsheet, select the data that you want to analyze, and perform the analysis. Although these software packages save time and can increase accuracy, you still need to understand a few of the basic variables that you'll use to understand your numerical data. We'll start with the mean and median:

The **mean** is the arithmetic average of a group of measurements. Chance errors in measurements tend to cancel themselves when means are calculated for relatively large samples; a value that is too high because of random error is often balanced by a value that is too low for the same reason.

The **median** is, after arranging the measurements from the smallest to the largest, the middle value that divides the set of measurements into two subsets of equal size. If there are an even number of measurements, the median is the mean of the two middle values.

The median is less sensitive to extreme values than is the mean. To appreciate this, consider a sample consisting of 14 leaves having the following lengths (all in mm):

80 69 62 74 69 51 45 40 9 64 65 64 61 67

The mean length is 58.6 mm. However, none of the leaves are that length, and most of the leaves are longer than 60 mm. In biology, the mean is usually preferred to the median when reporting descriptive statistics.

Consider these sets of data:

1 3 5 7 9 – the mean and median are both 5

1 3 5 7 14 – the median is 5, but the mean is 6

1 3 5 7 34 – the median is 5, but the mean is 10

Question 6

a. Does the mean always describe the "typical" measurement? Why or why not?

b. What information about a sample does a mean *not* provide?

Determine the median by arranging the measurements in numerical order:

9 40 45 51 61 63 64 64 65 67 69 69 73 80

The median is between the seventh and eighth measurements: 64 mm. In this sample, the mean differs from the median.

Question 7

a. What is responsible for this difference between the mean and median?

b. How would the median change if the 9-mm-long leaf was not in the sample?

c. How would the mean change if the 9-mm-long leaf was not in the sample?

d. Consider these samples:

Sample 1: 25 35 32 28

Sample 2: 15 75 10 20

What is the mean for Sample 1? _____

What is the mean for Sample 2? _____

In most of the exercises in this manual, you'll have time to make only one or two measurements of a biological structure or phenomenon. In these instances, a mean may be the only descriptor of the sample. However, if your class combines its data so that there are many measurements, you'll need to know how to do a couple of other calculations so that you understand the variation within your sample.

Variability

As you can see, the samples in Question 7d are different, but their means are the same. Thus, the mean does not reveal all there is to know about these samples. To understand how these samples are different, you need other statistics: the range and standard deviation.

The **range** is the difference between the extreme measurements (i.e., smallest and largest) of the sample. In Sample 1, the range is $35 - 25 = 10$; in Sample 2 the range is $75 - 10 = 65$. The range provides a sense of the variation of the sample, but the range can be artificially inflated by one or two extreme values. Notice the extreme values in the sample of leaf measurements previously discussed. Moreover, ranges do not tell us anything about the measurements between the extremes.

Question 8

a. Could two samples have the same mean but different ranges? Explain.

b. Could two samples have the same range but different means? Explain.

The **standard deviation** indicates how measurements vary about the mean. The standard deviation is easy to calculate. Begin by calculating the mean, measuring the deviation of each sample from the mean, squaring each deviation, and then summing the deviations. This summation results in the **sum of squared deviations.** For example, consider a group of shrimp that are 22, 19, 18, and 21 cm long. The mean length of these shrimp is 20 cm.

Sample Value	*Mean*	*Deviation*	*(Deviation)²*
22	20	2	4
19	20	−1	1
21	20	1	1
18	20	−2	4

Sum of Squared Deviations = 10

The summary equation for the sum of squared deviations is:

$$\textbf{Sum of squared deviations} = \sum_{i=1}^{N}(x_i - \bar{x})^2$$

where

N = **total number of samples**

$\bar{x}$ = **the sample mean**

x_i = **measurement of an individual sample**

This formula is simple. The summation sign ($\sum_{i=1}^{N}$) means to add up all the squared deviations from the first one ($i = 1$) to the last one ($i = N$). The sum of squared deviations (10) divided by the number of samples minus one ($4 - 1 = 3$) produces a value of $10/3 = 3.3$ cm^2 (note that the units are centimeters squared). This is the **variance:**

$$\textbf{Variance} = \frac{\textbf{sum of squared deviations}}{N - 1}$$

The square root of the variance, 1.8 cm, equals the **standard deviation (SD):**

$$\textbf{SD} = \sqrt{\textbf{Variance}} = \sqrt{3.3} = 1.8$$

The standard deviation is usually reported with the mean in statements such as, "The mean length of the shrimp was 20 ± 1.8 cm."

The standard deviation helps us understand the spread or variation of a sample. For many distributions of measurements, the mean ± 1 SD includes 68% of the

measurements, whereas the mean ± 2 SD includes 95% of the measurements.

Procedure 2.7 Gather and analyze data statistically

1. Use a meterstick or tape measure to measure your height in centimeters. Record your height here: _____ cm
2. Record your height and gender (male or female) on the board in the lab.
3. After all of your classmates have reported their heights, calculate the following:

   Size of sample

   All classmates _____

   Male classmates _____

   Female classmates _____

   Mean height

   All classmates _____

   Male classmates _____

   Female classmates _____

   Median height

   All classmates _____

   Male classmates _____

   Female classmates _____

   Range

   All classmates _____ to _____

   Male classmates _____ to _____

   Female classmates _____ to _____

   Standard deviation

   All classmates _____ ± _____

   Male classmates _____ ± _____

   Female classmates _____ ± _____

If there is sufficient time, obtain a newspaper that advertises cars, groceries, or other common commodities. Choose one example (e.g., new cars) and determine its average price (e.g., determine the average price of a new car).

Question 9

a. What does your calculation tell you?

b. What are the limitations of your sample?

Your instructor may ask you to do other statistical tests, such as Student's *t*, chi-square, and analysis of variance (ANOVA). The type of test you'll do will depend on the amount and type of data you analyze, as well as the hypotheses you are trying to test.

INQUIRY-BASED LEARNING

How much do the areas and shapes of leaves vary?

Observation: Leaves, which are the primary photosynthetic organ of most plants, are adapted for absorbing light. This involves exposing large surface areas to the environment.

Question: How do the surface area and shape of leaves vary on different parts of plants?

a. Establish a working lab group and obtain Inquiry-Based Learning Worksheet 2 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. If leaves are not available from outdoor plants (e.g., during winter), use the plants provided by your instructor that were grown indoors. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 2 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Study and Inquiry

1. What are the advantages and disadvantages of using the metric system of measurements?

2. Why is it important for all scientists to use a standard system of measures rather than the system that may be most popular in their home country or region?

3. Do you lose or gain information when you use statistics to reduce a population to a few characteristic numbers? Explain your answer.

4. Suppose that you made repeated measurements of your height. If you used good technique, would you expect the range to be large or small? Explain your answer.

5. Suppose that a biologist states that the average height of undergraduate students at your university is 205 cm plus or minus a standard deviation of 17 cm. What does this mean?

6. What does a small standard deviation signify? What does a large standard deviation signify?

7. Is it possible to make a perfectly precise measurement? Explain.

8. When in our everyday lives do we *not* want precise measurements?

EXERCISE

3

The Microscope

Basic Skills of Light Microscopy

Learning Objectives

By the end of this exercise you should be able to:

1. Identify and explain the functions of the primary parts of a compound microscope and dissecting (stereoscopic) microscope.
2. Carry and focus a microscope properly.
3. Use a compound microscope and dissecting microscope to examine biological specimens.
4. Prepare a wet mount, determine the magnification and size of the field of view, and determine the depth of field.

Please visit connect.mheducation.com to review online resources tailored to this lab.

Many organisms and biological structures are too small to be seen with the unaided eye (fig. 3.1). Biologists often use a light microscope to observe such specimens. A **light microscope** is a coordinated system of lenses arranged to produce an enlarged, focusable image of a specimen. A light microscope **magnifies** a specimen, meaning that it increases its apparent size. Magnification with a light microscope is usually accompanied by improved **resolution,** which is the ability to distinguish two points as separate points. Thus, the better the resolution, the sharper or crisper the image appears. The resolving power of the unaided eye is approximately 0.1 mm (1 in = 25.4 mm), meaning that our eyes can distinguish two points that are 0.1 mm apart. A light microscope, used properly, can improve resolution as much as 1000-fold (i.e., to 0.1 µm).

The ability to discern detail also depends on **contrast,** which is the difference between the lightest and darkest parts of an image. Therefore, many specimens examined with a light microscope are stained with artificial dyes that increase contrast and make the specimen more visible.

The invention of the light microscope was profoundly important to biology because it was used to formulate the cell theory and study biological structure at the cellular level. Light microscopy has revealed a vast new world to the human eye and mind (fig. 3.2). Today, the light microscope is the most fundamental tool of many biologists.

THE COMPOUND LIGHT MICROSCOPE

Study and learn the parts of the typical compound light microscope shown in figure 3.3. A light microscope has two, sometimes three, systems: an illuminating system, an imaging system, and possibly a viewing and recording system.

Illuminating System

The illuminating system, which concentrates light on the specimen, usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a lightbulb located

Caring for Your Microscope

Microscopes are powerful tools for understanding biology. However, they're also expensive and fragile and require special care. When you use your microscope, always do the following to ensure optimal performance and care:

- Always carry your microscope upright with both hands—one hand under the base and the other around the microscope's arm (fig. 3.3).
- Always begin by cleaning the ocular and objective lenses with lens paper.
- Always start your examinations with the low-power objective in place.
- If you shift to the high-power objective, rotate the objective into place carefully. Never force the objective lens into place. If the objective lens contacts the slide, stop and restart your examination with the low-power objective lens.
- After shifting to the high-power objective, always use only the fine adjustment to focus the image.
- When you've completed your work with the microscope, clean the lenses with lens paper, wrap the electrical cord securely around the microscope's arm, and return your microscope to its storage area.

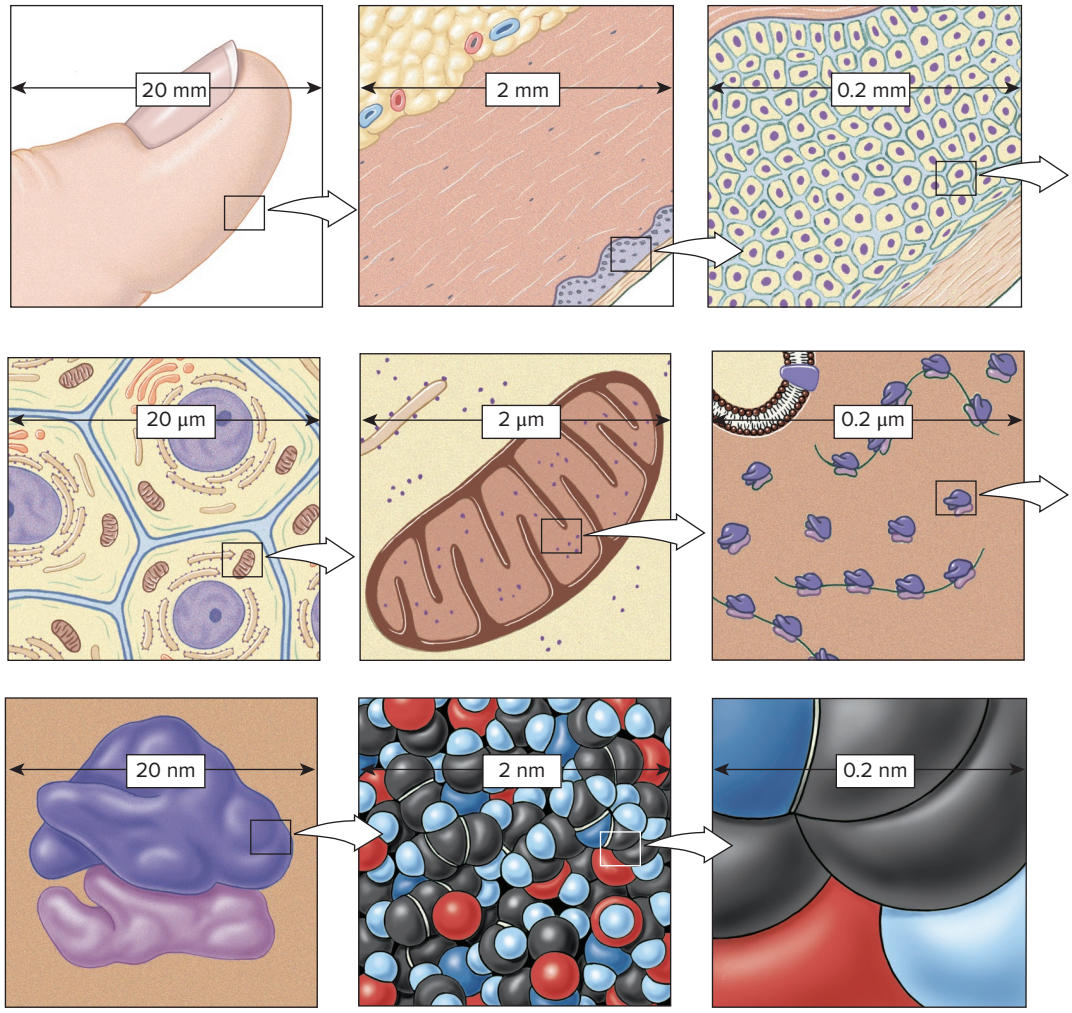

Figure 3.1 The size of cells and their contents. This diagram shows the size of human skin cells, organelles, and molecules. In general, the diameter of a human skin cell is about 20 micrometers (µm), of a mitochondrion is 2 µm, of a ribosome is 20 nanometers (nm), of a protein molecule is 2 nm, and of an atom is 0.2 nm.

at the base of the microscope. The light source illuminates the specimen by passing light through a thin, almost transparent part of the specimen. The **condenser lens,** located immediately below the specimen, focuses light from the light source onto the specimen. Just below the condenser is the **condenser iris diaphragm,** a knurled ring or lever that can be opened and closed to regulate the amount of light reaching the specimen. When the condenser iris diaphragm is open, the image will be bright; when closed, the image will be dim.

Imaging System

The imaging system improves resolution and magnifies the image. It consists of the objective and ocular (eyepiece) lenses and a body tube. The objectives are three or four lenses mounted on a revolving nosepiece. Each objective is a series of several lenses that magnify the image, improve resolution, and correct aberrations in the image. The most common configuration for student microscopes includes four objectives: low magnification (4×), medium magnification (10×), high magnification (40×), and oil immersion (100×). Using the oil immersion objective requires special instructions, as explained in Exercise 24 to study bacteria. To avoid damaging your microscope, do not use the oil immersion objective during this exercise.

The magnifying power of each objective is etched on the side of the lens (e.g., 4×). The **ocular** is the lens that you look through. Microscopes with one ocular are **monocular** microscopes, and those with two are **binocular** microscopes. Oculars usually magnify the image 10 times. The **body tube** is a metal casing through which light passes to the oculars. In microscopes with bent bodytubes and inclined oculars, the body tube contains mirrors and a prism that redirect light to the oculars. The **stage** secures the glass slide on which the specimen is mounted.

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Figure 3.2 "Egad, I thought it was tea, but I see I've been drinking a blooming micro-zoo!" says this horrified, proper 19th-century London woman when she used a microscope to examine her tea. People were shocked to learn that there is an active, living world too small for us to see.

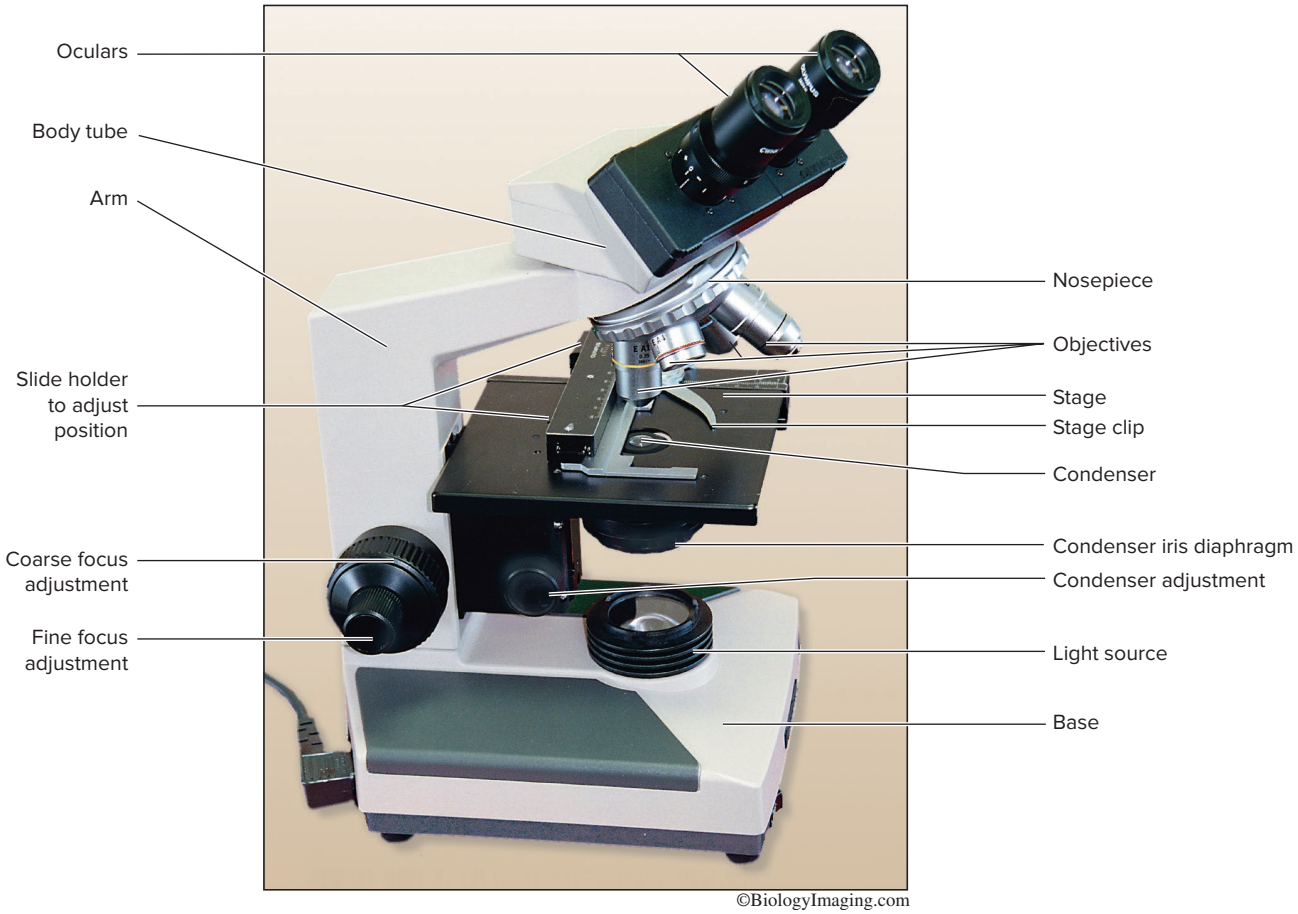

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Figure 3.3 Major parts of a compound light microscope.

A Summary of How to Use a Compound Light Microscope

1. Place the specimen on the microscope's stage.
2. Rotate the low-power objective into place. Center the specimen below the objective.
3. Look through the oculars while using the coarse adjustment to focus on the specimen. Center the area of the specimen that you want to examine.
4. Slowly rotate the high-power objective into place. Look through the oculars while you use the fine adjustment to focus on the specimen.
5. If you "lose" your specimen when you switch from low power to high power, retrace the previous steps, paying special attention to placing the specimen in the center of the field of view.

Viewing and Recording System

The viewing and recording system, if present, converts radiation to a viewable and/or permanent image. The viewing and recording system usually consists of a camera or video screen. Most student microscopes do not have viewing and recording systems.

USING A COMPOUND MICROSCOPE

Although the maximum magnification of light microscopes has not increased significantly during the last century, the construction and design of light microscopes have improved the resolution of newer models. For example, built-in light sources have replaced adjustable mirrors in the illuminating system, and lenses are made of better glass than they were in the past.

Your lab instructor will review with you the parts of the microscopes (and their functions) you will use in the lab (fig. 3.3). After familiarizing yourself with the parts of a microscope, you're now ready for some hands-on experience with the instrument. The videos at the website associated with this manual (connect.mheducation.com) will be especially useful for helping you understand how to properly use your microscope.

Procedure 3.1 Use a compound microscope

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base. Place your microscope on the table in front of you.

Do not use paper towels or Kimwipes to clean the lenses of your microscope; they can scratch the lenses. Clean the lenses only with lens paper.

2. Plug in the microscope and turn on the light source.
3. If it isn't already in position, rotate the nosepiece until the lowest-power (4×) objective is in line with the light source. (The 4× objective is often called the "scanning objective" because it enables users to scan large areas of a specimen.) You'll feel the objective click into place when it is positioned properly. *Always begin examining slides with the lowest-power objective.*
4. Locate the coarse adjustment knob on the side of the microscope. Depending on the type of microscope that you're using, the coarse adjustment knob moves either the nosepiece (with its objectives) or the stage to focus the lenses on the specimen. Only a partial turn of the coarse adjustment knob moves the stage or nosepiece a relatively large distance. *The coarse adjustment should only be used when you're viewing a specimen with the 4× or 10× objective lens.*
5. If your microscope is binocular, adjust the distance between the oculars to match the distance between your pupils. If your microscope is monocular, keep both eyes open when using the microscope. After a little practice you will ignore the image received by the eye not looking through the ocular.
6. Focus a specimen by using the following steps:
   a. Place a microscope slide of newsprint of the letter *e* on the horizontal stage so that the *e* is directly below the lowest-power objective lens and is right side up. It should be centered over the hole in the stage.
   b. Rotate the coarse adjustment knob to move the objective within 1 cm of the stage (1 cm = 0.4 in).
   c. Look through the oculars with both eyes open.
   d. Rotate the coarse adjustment knob (i.e., raising the objective lens or lowering the stage) until the *e* comes into focus. If you don't see an image, the *e* is probably off center. Be sure that the *e* is directly below the objective lens and that you can see a spot of light surrounding the *e*.
   e. Focus up and down to achieve the crispest image.
   f. Adjust the condenser iris diaphragm so that the brightness of the transmitted light provides the best view.
   g. Observe the letter, then rotate the nosepiece to align the 10× objective to finish your observation. Do not use the oil immersion objective.

Question 1

a. As you view the letter *e,* how is it oriented? Upside down or right side up?

b. How does the image move when the slide is moved to the right or left? Toward you or away from you?

c. What happens to the brightness of the view when you go from 4× to 10×?

Magnification

Procedure 3.2 Determine magnification

1. Estimate the magnification of the *e* by looking at the magnified image on lowest magnification (4×), and then at the *e* without using the microscope.
2. Examine each objective and record the magnifications of the objectives and oculars of your microscope in table 3.1.
3. Calculate and record in table 3.1 the total magnification for each objective following this formula:

$$\mathbf{Mag_{Tot} = Mag_{Obj} \times Mag_{Ocu}}$$

where

$\mathbf{Mag_{Tot}}$ **= total magnification of the image**
$\mathbf{Mag_{Obj}}$ **= magnification of the objective lens**
$\mathbf{Mag_{Ocu}}$ **= magnification of the ocular lens**

For example, if you're viewing the specimen with a 4× objective lens and a 10× ocular, the total magnification of the image is 4 × 10 = 40×. That is, the specimen appears 40 times larger than it is.

4. Slowly rotate the high-power (i.e., 40×) objective into place. *Be sure that the objective does not touch the slide!* If the objective does not rotate into place without touching the slide, do not force it; ask your lab instructor to help you. After the 40× objective is in place, you should notice that the image remains near focus and that the field-of-view has gotten smaller. Most light microscopes are **parfocal,** meaning that the image will remain nearly focused after the 40× objective lens is moved into place. Most light microscopes are also **parcentered,** meaning that the image will remain centered in the field of view after the 40× objective lens is in place.
5. You may need to readjust the iris diaphragm because the high-magnification objective allows less light to pass through to the ocular.
6. To fine-focus the image, locate the **fine adjustment knob** on the side of the microscope. Turning this knob changes the specimen-to-objective distance slightly and therefore makes it easy to fine-focus the image. **Use only the fine adjustment when using the 40× (or higher) objective.**

Never use the coarse adjustment knob to focus an image on high power.

Question 2

a. How many times is the image of the *e* magnified when viewed through the high-power objective?

b. If you didn't already know what you were looking at, could you determine at this magnification that you were looking at a letter *e*? How?

Determine the Size of the Field of View

The **field of view** is the area that you can see through the ocular and objective (fig. 3.4). Knowing the size of the field of view is important because you can use it to estimate the size of an object you are examining. The field of view can be measured with ruled **micrometers** (fig. 3.5). An **ocular micrometer** is a small glass disk with thin lines numbered and etched in a row. It was put into an ocular on your microscope so that the lines superimpose on the image and

Table 3.1

Total Magnifications and Areas of Field of View (FOV) for Three Objectives

Objective Power	Objective Magnification	×	Ocular Magnification	=	Total Magnification	FOV Diameter (mm)	FOV Area (mm²)	Measurement (mm) for 1 Ocular Space
4×	_____	×	_____	=	_____	_____	_____	_____
10×	_____	×	_____	=	_____	_____	_____	_____
40×	_____	×	_____	=	_____	_____	_____	_____

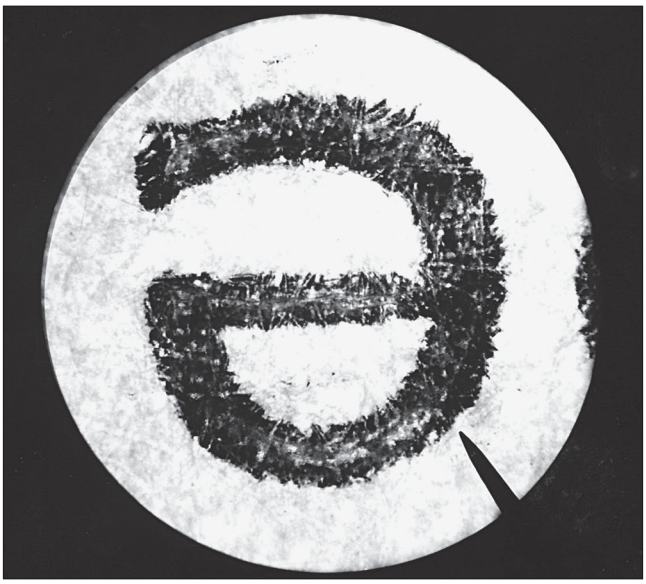

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Figure 3.4 The circular, illuminated field of view of a compound light microscope. Shown here is the letter *e* from newsprint that is magnified 40 times (i.e., 40×).

allow you to measure the specimen. Before you can use the micrometer you must determine for each magnification the apparent distance between the lines on the ocular micrometer. This means that you must calibrate the ocular micrometer by comparing its lines to those lines on a standard ruler called a **stage micrometer.** A stage micrometer is a glass slide having precisely spaced lines etched at known intervals.

Procedure 3.3 Use a stage micrometer to calibrate the ocular micrometer, and determine the size of the field of view

1. Rotate the ocular until the lines of the ocular micrometer parallel those of the stage micrometer (fig. 3.5).
2. Align lines at the left edges (0 lines) of the two micrometers by moving the stage micrometer (fig. 3.5).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. Record the values here.

***y* ocular spaces = *x* stage spaces**

***y* = _____**

***x* = _____**

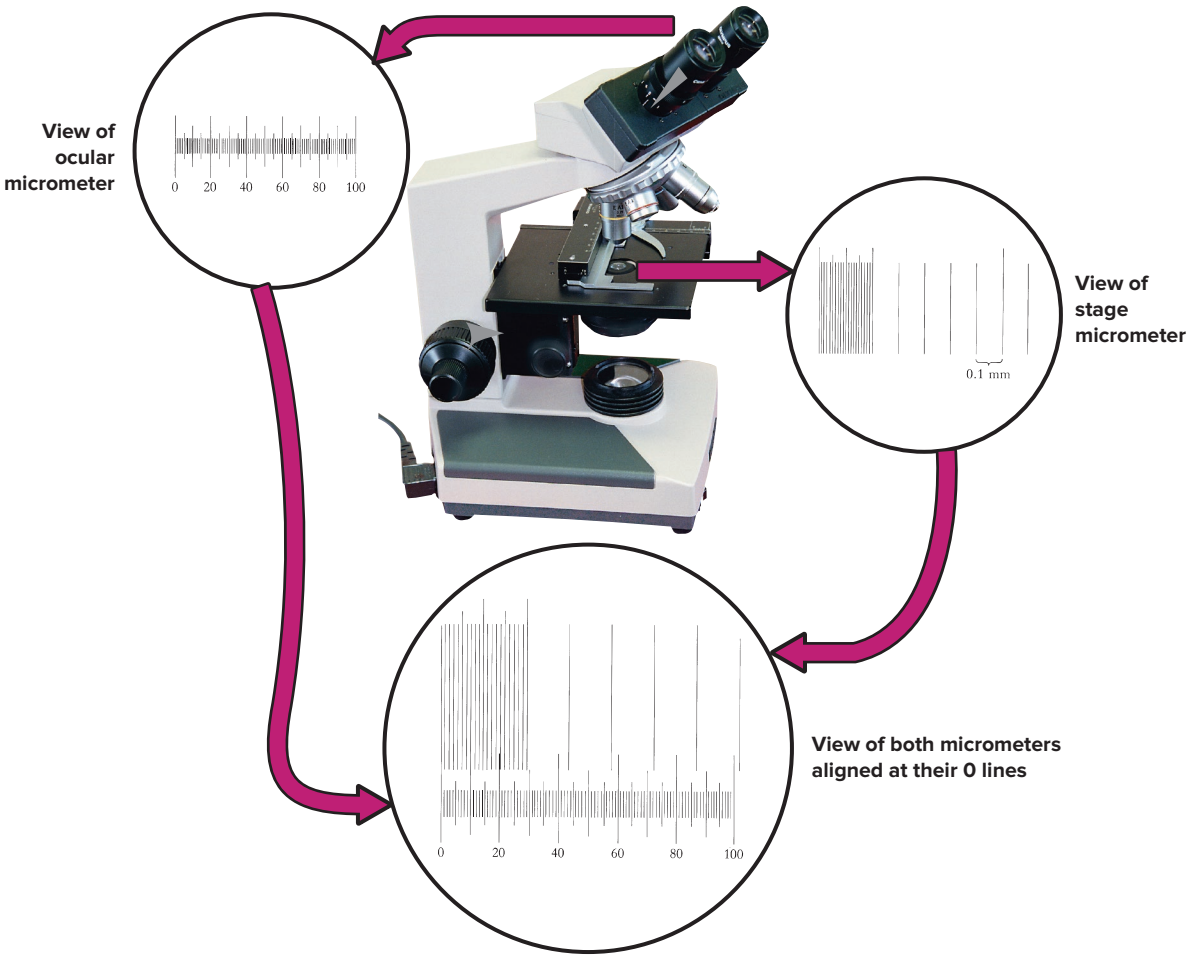

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Figure 3.5 Stage and ocular micrometers. Micrometers are used to calibrate microscopes and measure the size of specimens.

The smallest space on a stage micrometer = 0.01 mm, so

$$y \text{ ocular spaces (mm)} = x \text{ stage spaces} \times 0.01$$

$$1 \text{ ocular space (mm)} = (x/y) \times 0.01$$

4. Calculate the distance in millimeters between lines of the ocular micrometer. For example, if the length of 10 spaces on the ocular micrometer equals the length of seven spaces on the stage micrometer, then

$$y = 10$$

$$x = 7$$

$$10 \text{ ocular spaces (mm)} = 7 \text{ stage spaces} \times 0.01 \text{ mm}$$

$$1 \text{ ocular space (mm)} = (7 \times 0.01 \text{ mm})/10$$

$$1 \text{ ocular space (mm)} = 0.007 \text{ mm}$$

$$1 \text{ ocular space} = 7 \ \mu\text{m}$$

Therefore, if a specimen spans eight spaces on your ocular micrometer with that objective in place, that specimen is 56 μm long.

5. Calibrate the ocular micrometer for each objective on your microscope. Record in table 3.1 the diameter of the field of view (FOV) for each objective. Also record for each objective lens in table 3.1 the measurement (mm) for 1 ocular space. You can use this information in future labs as you measure the sizes of organisms and their parts.
6. Calculate the radius, which is half the diameter.
7. Use this information to determine the area of the circular field of view with the following formula:

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

8. Record your calculated FOV areas in table 3.1.

Alternate Procedure 3.3 Use a transparent ruler to determine the size of the field of view

1. Obtain a clear plastic ruler with a metric scale.
2. Place the ruler on the stage and under the stage clips of your microscope. If your microscope has a mechanical stage, ask your instructor how to place the ruler to avoid damage. *Carefully rotate the nosepiece to the objective of lowest magnification.*
3. Slowly focus with the coarse adjustment, and then with the fine adjustment, until the metric markings on the ruler are clear.
4. Align the ruler to measure the diameter of the circular field of view. The space between each line on the ruler should represent a 1-mm interval.
5. Record in table 3.1 the diameter of this low-magnification field of view. Also calculate the radius, which is half the diameter.
6. The ruler cannot be used to measure the diameters of the field of view at medium and high magnifications because the markings are too far apart. Therefore, these diameters must be calculated using the following formula:

$$\text{FOV}_{\text{low}} \times \text{Mag}_{\text{low}} = \text{FOV}_{\text{high}} \times \text{Mag}_{\text{high}}$$

where

FOV_{low} = **diameter of the field of view of the low-power objective**

Mag_{low} = **magnification of the low-power objective (Be consistent and use the magnification of the objective, not total magnification.)**

FOV_{high} = **diameter of the field of view of the high-power objective**

Mag_{high} = **magnification of the high-power objective**

For example, if 3.0 mm is the diameter of the field of view for a 4× low-power objective, then what is the diameter of the field of view of the 40× high-power objective?

$$3.0 \text{ mm} \times 4 = \text{FOV}_{\text{high}} \times 40$$
$$0.30 \text{ mm} = \text{FOV}_{\text{high}}$$

7. Calculate and record in table 3.1 the diameters of the field of view for the 10× and 40× magnifications.
8. Calculate and record in table 3.1 the circular area of the field of view for the three magnifications by using the following formula.

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

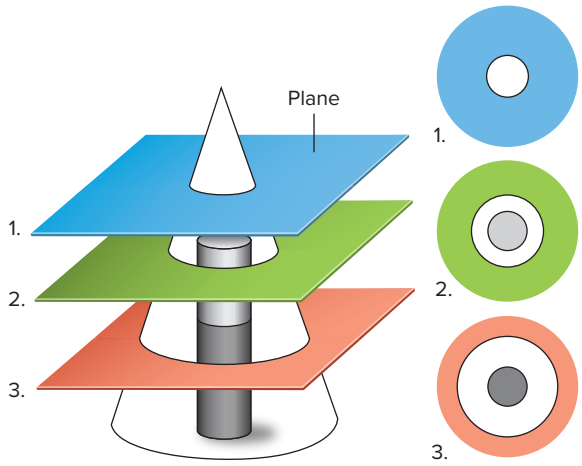
Question 3

a. Which provides the largest field of view, the 10× or 40× objective?

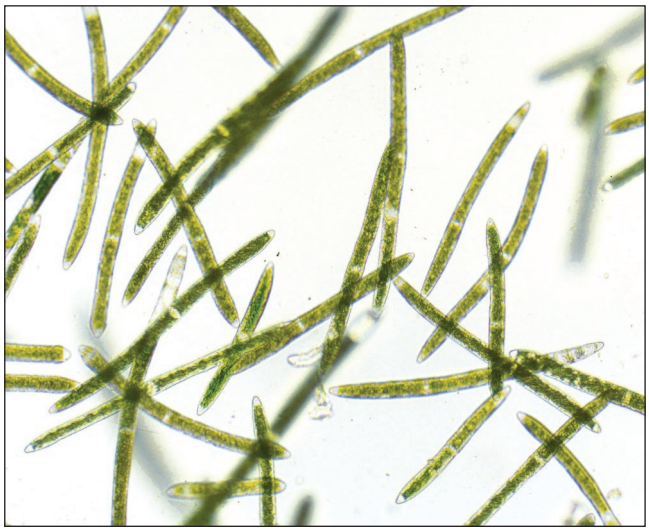
b. How much more area can you see with the 4× objective than with the 40× objective?

c. Why is it more difficult to locate an object starting with the high-power objective than with the low-power objective?

d. Which objective should you use to initially locate the specimen? Why?



(a)



(b) ©BiologyImaging.com

Figure 3.6 How focusing at different planes of a specimen would produce three different images. (*a*) Focusing up and down when you view a specimen can help you to understand its three-dimensional structure. (*b*) A thin depth of field is apparent in this 100× image of cells of *Closterium*, a green alga. The upper and lower layers of cells are out of focus, while the midlayer of cells is within the thin depth of field and is clearly focused.

Determine the Depth of Field

Depth of field is the thickness of the object in sharp focus (fig. 3.6). Depth of field varies with different objectives and magnifications.

Procedure 3.4 Determine the depth of the field of view

1. Using the low-power objective, examine a prepared slide of three colored threads mounted on top of each other.
2. Focus up and down and try to determine the order of the threads from top to bottom. The order of the threads will not be the same on all slides.
3. Re-examine the threads using the high-power objective lens.

Question 4

a. Are all three colored threads in focus at low power?

b. Can all three threads be in focus at the same time using the high-power objective?

c. Which objective, high or low power, provides the greatest depth of field?

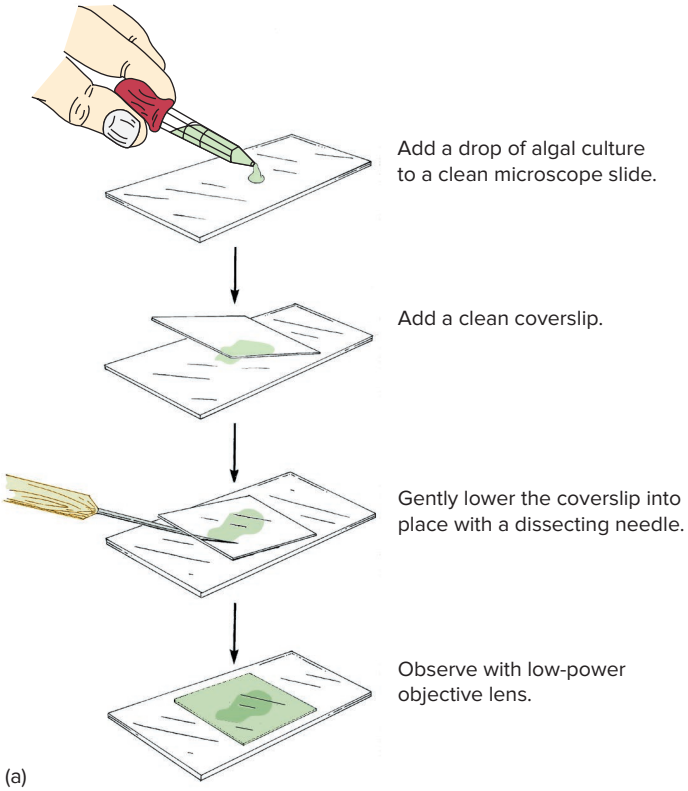
Preparing a Wet Mount of a Biological Specimen

Procedure 3.5 Prepare a wet mount of a biological specimen

1. Place a drop of water containing algal cells from a culture labeled "Algae" on a clean microscope slide.
2. Place the edge of a clean coverslip at an edge of the drop at a 45° angle; then slowly lower the coverslip onto the drop so that no air bubbles are trapped (fig. 3.7). (Your instructor will demonstrate this technique.) The coverslip holds the specimen in place and prevents the lens of an objective from contacting the water and the specimen. This fresh preparation is called a **wet mount** and can be viewed with your microscope.
3. Experiment with various intensities of illumination. To do this, rotate the 4× objective into place and adjust the condenser iris diaphragm to produce the least illumination. Observe the image; note its clarity, contrast, and color. Repeat these observations with at least four different levels of illumination. The fourth level should have the diaphragm completely open.
4. Repeat step 3 for the 10× and 40× objectives.

Question 5

a. Is the image always best with the highest illumination?



(a)

(b) ©BiologyImaging.com

Figure 3.7 (*a*) Preparing a wet mount of a biological specimen. (*b*) A wet mount of pond water will often include the common cyanobacterium *Oscillatoria* (200×). See also figures 3.6 and 25.1–25.4.

b. Is the same level of illumination best for all magnifications?

c. Which magnifications require the most illumination for the best clarity and contrast?

5. Examine your preparation of algae, and sketch in the following field of view the organisms that you see. Don't mistake air bubbles for organisms! Air bubbles appear as uniformly round structures with dark, thick borders.

6. Prepare a wet mount of some newly hatched brine shrimp (*Artemia,* which are popularly referred to as "sea monkeys") and their eggs. Sketch in the following field of view what you see. Use your calculations for the diameter of the field of view to estimate the length of the shrimp.

Approximate length of the shrimp: _____

Question 6

a. Why is it important to put a coverslip over the drop of water when you prepare a wet mount? That is, what are the functions of a coverslip?

b. Approximately how long and wide is a brine shrimp?

Practice

For practice using your microscope, prepare some wet mounts of pond water or a hay infusion to view the diversity of protozoa and algae (fig. 3.8). If the protozoa are moving too fast for you to examine carefully, add a drop of methylcellulose (often sold commercially as Proto-Slo) to your sample. (The

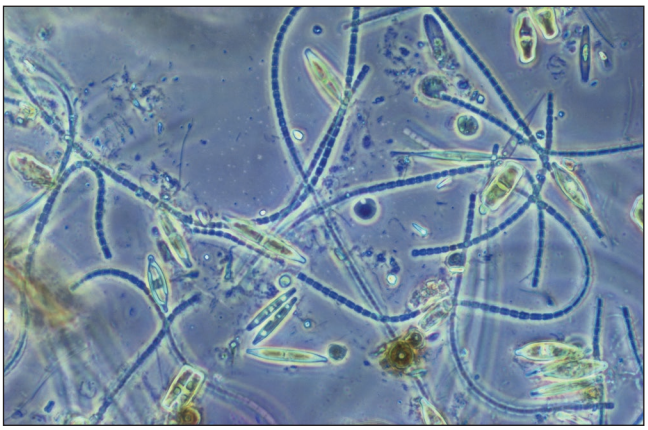


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Figure 3.8 The diversity of organisms in pond water (200×).

methylcellulose will slow the movement of the protozoa.) Also examine the prepared slides available in the lab. You'll examine these slides in more detail in the coming weeks, so don't worry about their contents. Rather, use this exercise to familiarize yourself with the microscope. Also prepare wet mounts of the cultures available in the lab and sketch the organisms that you see. When you've finished, turn off the light source, cover your microscope, and store the microscope in its cabinet.

THE DISSECTING (STEREOSCOPIC) MICROSCOPE

A **dissecting (stereoscopic) microscope** offers some advantages over a compound microscope. Although a compound microscope can produce high magnifications and excellent resolution, it has a small **working distance,** which is the distance between the objective lens and specimen. Therefore, it is difficult to manipulate a specimen while observing it with a compound microscope. Specimens that can be observed with a compound microscope are limited to those thin enough for light to pass through them. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope.

A dissecting microscope provides a much larger working distance than does a compound microscope. This distance is usually several centimeters (compared to a centimeter or less for a compound microscope), making it possible to dissect and manipulate most specimens. Also, most specimens for dissection are too thick to observe with transmitted light from a light source below the specimen. Therefore, many dissecting microscopes use a light source above the specimen; the image you see is formed from reflected light.

Dissecting microscopes are always binocular (fig. 3.9). Each ocular views the specimen at different angles through one or more objective lenses. This arrangement provides a three-dimensional image with a large depth of field. This is in contrast to the image in a compound microscope, which is basically two-dimensional. However, the advantages of a stereoscopic microscope are often offset by lower resolution and magnification than a compound microscope. Most dissecting microscopes have magnifications of 4× to 50×.

Procedure 3.6 Use a dissecting microscope

1. Carry the dissecting microscope to your desk by grasping the microscope's arm with one hand and placing your other hand under the microscope's base.
2. Use figure 3.9 to familiarize yourself with the parts of your microscope.
3. Use your dissecting microscope to examine the organisms available in the lab. Sketch some of these organisms.
4. Use a ruler to measure the diameter of the field of view with your dissecting microscope at several levels of magnification.

Question 7

a. What is the area of the field of view when you use the lowest magnification of your dissecting microscope? What about when you use the highest magnification?

b. Place a microscope slide of the letter *e* on the stage. As you view the letter *e,* how is it oriented?

c. How does the image through a dissecting microscope move when the specimen is moved to the right or left? Toward you or away from you?

d. How does the direction of illumination differ in dissecting as opposed to compound microscopes?

e. What are some examples of biological specimens that would be best examined with a dissecting microscope instead of a compound microscope?

A COMPARISON OF COMPOUND AND DISSECTING MICROSCOPES

Complete table 3.2 comparing magnification, resolution, size of the field of view, and depth of field of a dissecting microscope and a compound microscope. Use the terms *high, low,* or *same* to describe your comparisons.

Question 8

What other differences are there between compound and dissecting microscopes?