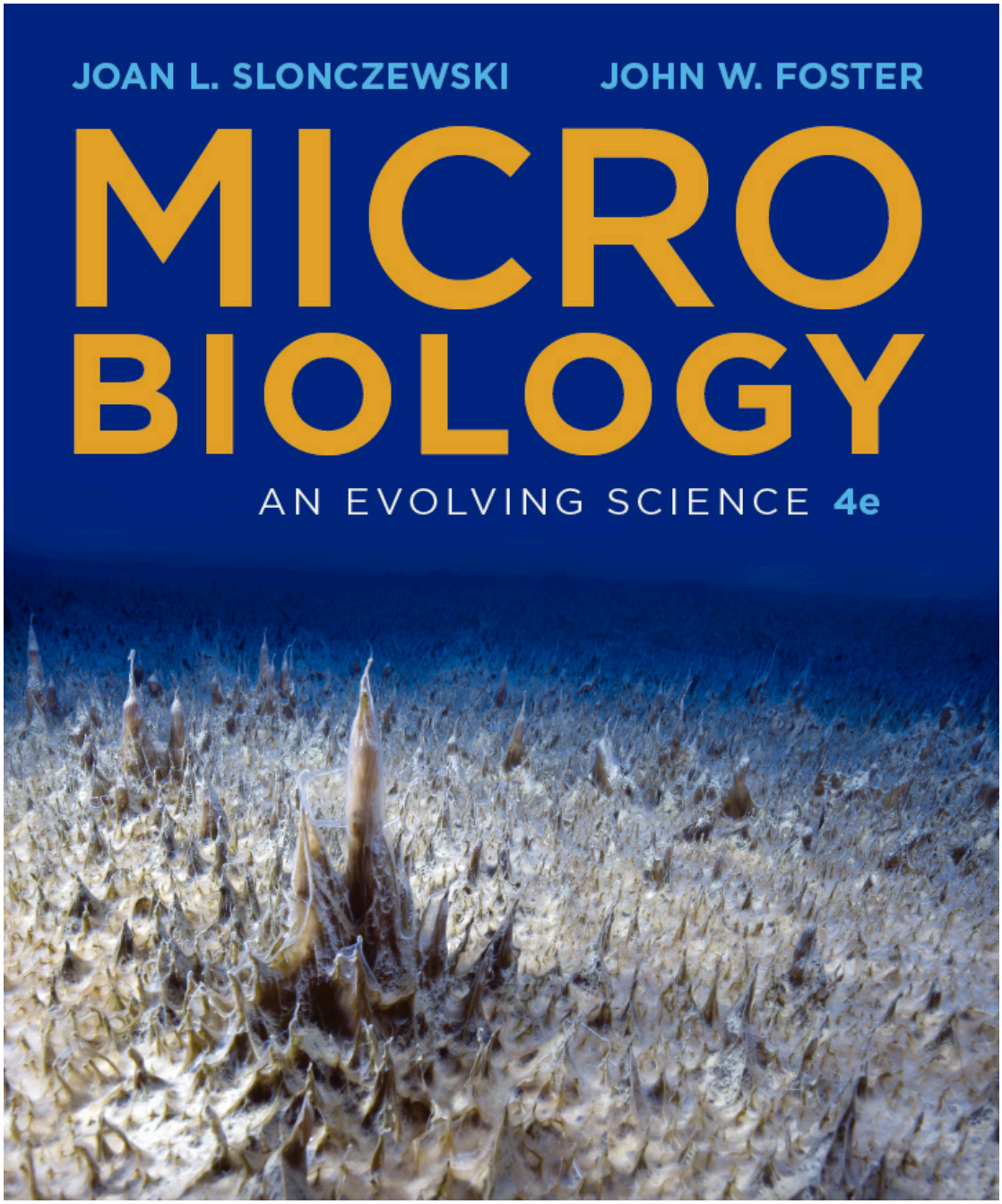
Instructor's Manual

to accompany



Instructor's Manual by Robert Carey, Lebanon Valley College

Chapter 1

Microbial Life: Origin and Discovery

SUMMARY

In some respects, this is the most challenging chapter in the text. This is when you have to throw out some bait to pique students' interest and then set the hook so you can reel them in for the rest of the introduction to the multifaceted realm of microbiology. Emphasis should be placed on the importance and expansiveness of microbiology in students' lives. Throughout the text you will notice two running themes—the human gut microbiome and Antarctic microbiology—that highlight exciting discoveries in medical and ecological microbiology. The two themes are called out throughout each chapter with marginal icons so that they're easy to find, discuss, and incorporate into your lecture.

1.1 From Germ to Genome: What Is a Microbe?

Learning Objectives

- 1.1.a. Recall the definition of a microbe.
- 1.1.b. List examples of microbes.
- 1.1.c. Describe some problems with the definition of a microbe.
- 1.1.d. Explain the implications of microbial genome sequencing.

This section lays the foundation for understanding the term "microbe," encompassing the different forms of "life" it covers. The discussion should define single-celled organisms and multicellular communities. Examples of each of these are found in the text.

Viruses should be mentioned as falling into the category of microbe. It is here that some of the differences between viruses and other microbes should be defined.

The size differential of many of these microbes is also important. The authors give some interesting examples within the text; pay particular attention to Table 1.1. Size and microbial observation will come up again during more detailed discussions of microscopy in Chapter 2.

Genome analysis of sequences from individual microbes or from microbial communities has provided information on how microbes live within their environment and the relatedness between organisms.

Discussion Points

- Discussion of viruses versus microbes can lead to a class discussion as to whether viruses are "alive." A discussion of the characteristics of what we call "life" is useful in this endeavor. This also can dovetail

nicely with a discussion of the origin of life as described within Special Topic 1.1.

- Discuss what can be learned from sequencing individual genomes and from metagenome analysis of environmental samples.
- Discuss the ever-increasing speed at which genomes may be sequenced.
- Introduce the usefulness of metagenomics for learning about organisms that are difficult to culture in the laboratory.

1.2 Microbes Shape Human History

Learning Objectives

- 1.2.a. List both positive and negative impacts that microbes have had on human history.
- 1.2.b. Explain why the microscope is an important tool in the field of microbiology.
- 1.2.c. Identify the contributions of the following individuals: Nightingale, Hooke, van Leeuwenhoek, Pasteur, and Tyndall.
- 1.2.d. Compare and contrast Spallanzani's, Pasteur's, and Tyndall's experiments that tested spontaneous generation.

This section enables students to see how all-encompassing microbes are in our world and how that became apparent to us over time. There are certain basic concepts that should be covered, including, but not limited to, the origin of microbes, microscopic observation, the role of microbes in fermentation, spontaneous generation, and germ theory. There is a great story in the text regarding each concept, depending on what type of teaching approach you use.

Historical events from Table 1.2 give students a feel for the large expanse of time over which our microbial knowledge has been obtained. The focus of your course will determine which microbial approach will be taught. Do, however, make a point of emphasizing the importance of the "golden age" of microbiology in laying the groundwork for the rapid acquisition of knowledge in the twentieth century and the beginning of the twenty-first.

Discussion Points

- Use Special Topic 1.1 to discuss the origin of life and the relation of early life to modern microbes.
- Use parts of Table 1.2 to take students through the development of microbiology.
- Show pictures of van Leeuwenhoek's microscope and his "animalcules" (Fig. 1.12).
- Discuss and draw pictures of Redi's spontaneous generation experiments with raw meat and maggots.
- Discuss and show Pasteur's use of swan-neck flasks to disprove spontaneous generation (Fig. 1.13).
- Discuss Florence Nightingale's role in the development of medical statistics and the study of the causes of disease (Fig. 1.10).

1.3 Medical Microbiology

Learning Objectives

- 1.3.a. Describe what constitutes a pure culture and how to obtain one.
- 1.3.b. List Koch's postulates.
- 1.3.c. Assess some of the practical obstacles in applying Koch's postulates.
- 1.3.d. Recall the contributions of various individuals to the discovery and implementation of vaccination.
- 1.3.e. Compare the roles of immunization, antiseptics, and antibiotics in human disease treatment and prevention.
- 1.3.f. Describe how viruses were discovered.

Whether or not your course focuses on medical microbiology, students tend to be very interested in this topic. This section discusses the unfolding of the germ theory of disease, including the discovery of viruses. Discussion should extend from the debate concerning spontaneous generation through the development of Koch's postulates.

Depending on your teaching style, there are great stories attached to the development of pure culture, vaccines, the discovery and use of antiseptics and antibiotics, and the isolation and identification of viruses.

Discussion Points

- Discuss Koch's postulates (Fig. 1.17) and compare and contrast his studies of anthrax and tuberculosis. Discuss postulates' limitations for specific diseases such as AIDS.
- Vaccine development for smallpox provides a great look at the history and development of microbiology (Fig. 1.18). Discussion of modern anti-vaccination movements may stimulate student interest in this section.
- Alexander Fleming's accidental discovery of penicillin (Fig. 1.20) is a useful lead-in to discussion of the importance of antibiotics, the need to search for new antibiotics, and antibiotic abuse.
- Discuss viruses and contrast them with bacteria in the context of disease-causing agents.

1.4 Microbial Ecology

Learning Objectives

- 1.4.a. List Winogradsky's contributions to microbial culture technique.
- 1.4.b. Define what distinguishes lithotrophs from other organisms.
- 1.4.c. Explain the role of microbes in geochemical cycling, especially that of nitrogen.
- 1.4.d. Compare the roles of animal endosymbionts and plant endosymbionts.

This section introduces enrichment culture technology through discussions of the use of Winogradsky's column to isolate lithotrophs. Winogradsky's ecosystems allowed the isolation of microbes able to exist on inorganic materials. These microbes play an important role in geochemical cycling, and the interconversion of inorganic and organic forms of nitrogen, sulfur, phosphorous, and other minerals.

Endosymbionts come up again in this section because they are widespread in all ecosystems. Many of these endosymbionts exist as microbial communities attached to surfaces in biofilms.

Discussion Points

- A discussion of Winogradsky's column (Fig. 1.22) covers the topics of enrichment culture, lithotrophs, and geochemical cycling (Fig. 1.23).
- The role of microbes in addressing the current ecological crisis facing our planet can be introduced here.
- The endosymbiotic relationships among microbes, plants, and animals may be familiar to students and can be used to begin a discussion of biofilms.

1.5 The Microbial Family Tree

Learning Objectives

- 1.5.a. Explain why microbes can be challenging to classify taxonomically.
- 1.5.b. Outline how microbial classification has changed over time.
- 1.5.c. Appraise endosymbiosis as an explanation for mitochondria and chloroplasts.
- 1.5.d. Describe the importance of DNA sequencing in classification.
- 1.5.e. Compare and contrast archaea, bacteria, and eukaryotes.

Many people have family members interested in generating a family tree. It is often quite difficult to fill in all the pieces, although some families are able to go back many generations. Imagine trying to make a family tree of all living organisms.

Briefly discuss the process of microbial classification through history, including the difficulty in using traditional concepts of species to describe microbes. These discussions should also include: lifestyle or environmental issues, the history of visual observation through microscopy and staining, biochemical activities, and genomic comparison, which is the newest point of interest. Inherent in these discussions should be Lynn Margulis's initially controversial endosymbiont theory and Carl Woese's use of rRNA as a "molecular clock." Together, these innovations caused us to alter our belief that the tree of life had five kingdoms and instead to understand it as having three domains. Along the way, many organisms have been renamed and reclassified.

Discussion Points

- Discuss the three domains of life using the tree in Figure 1.28.
- Be sure to explain the significance of Lynn Margulis's endosymbiont theory (Fig. 1.26) in the understanding of eukaryotic origins.
- Mention should be made of horizontal gene transfer and the continuing development of our understanding of microbial evolutionary history.

1.6 Cell Biology and the DNA Revolution

Learning Objectives

- 1.6.a. Describe the roles of the electron microscope and the ultracentrifuge in advancing our knowledge of cell structure and function.
- 1.6.b. Explain how studies on microbes fostered our knowledge of DNA function and enhanced DNA technology.
- 1.6.c. State some uses of microbes in medicine and industry.

This last section stresses the use of more modern technologies such as electron microscopy and DNA sequencing to greatly enhance our understanding of microbes. The advent of electron microscopy has allowed us to visualize the internal structures of microbes. Svedburg developed the technique of ultracentrifugation to study the properties of macromolecular cellular components. These technologies led Mitchell and Moyle to propose the chemiosmotic hypothesis. Cells were no longer thought of as just "bags of enzymes."

A history of the DNA revolution should also be discussed, emphasizing the discovery of the double-helical structure of DNA. Watson and Crick are names almost everyone is familiar with, but Franklin and Wilkins also played important roles. The book *The Double Helix*, by James Watson, or the movie *The Double Helix*, starring Jeff Goldblum as Watson, are interesting multimedia offerings.

Molecular techniques for the manipulation of DNA have led to the generation of recombinant proteins, DNA sequencing, and other biotechnology and bioinformatics advances. With those abilities comes extreme transformation in medicine and industry, leading to many dangers and ethical questions. These were first discussed at an Asilomar Conference, where scientists met to restrict and regulate their own field of research.

Discussion Points

- Introduce electron microscopy as a means to visualize internal cell structure.
- Discuss the development of ultracentrifugation by Svedberg and the significance of Svedberg units and ribosome "size."
- Discuss the role that Rosalind Franklin and X-ray crystallography had in the discovery of the structure of DNA (Fig. 1.31).
- Discussion should cover Griffith and Avery's genetic transformation experiments.
- DNA sequencing (Fig. 1.32) is among the many important technological advances brought about by

modern cellular and molecular microbiology.

eTOPICS

eTopics are supplementary sections that explore additional material in depth. They are available to students within the ebook, which is packaged free of charge with every new print copy, and within the coursepacks, which are provided for download on the instructor's resource site, wwnorton.com/instructors.

1.1 An Interview with Rita Colwell: The Global Impact of Microbiology

eTopic 1.1 contains an interview with Rita Colwell, a microbiologist famous for her work on *Vibrio cholerae*, the causative organism of cholera. It discusses her laboratory work as well as her work in such roles as director of the National Science Foundation.

1.2 Clifford W. Houston: From Aquatic Pathogens to Outer Space—An Interview

eTopic 1.2 contains an interview with Clifford Houston, a microbiologist from the University of Texas Medical Branch. The interview touched on his career path and his most important contributions to the field of microbiology, including the development of techniques to detect toxins from streptococci and *Salmonella*.

RECOMMENDED READINGS

The following readings are presented at the end of the textbook chapter as resources for further exploration of the topics discussed in Chapter 1.

Albers, Sonja-Verena, Patrick Forterre, David Prangishvili, and Christina Schleper. 2013. The legacy of Carl Woese and Wolfram Zillig: From phylogeny to landmark discoveries. *Nature Reviews. Microbiology* **11**:713–719.

Blaser, Martin, Peer Bork, Claire Fraser, Rob Knight, and Jun Wang. 2013. The microbiome explored: Recent insights and future challenges. *Nature Reviews. Microbiology* **11**:213–217.

Blount, Zachary D., Christina Z. Borland, and Richard E. Lenski. 2008. Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli. Proceedings of the National Academy of Sciences USA* **105**:7899–7906.

Breitbart, Mya, Luke R. Thompson, Curtis A. Suttle, and Matthew B. Sullivan. 2007. Exploring the vast diversity of marine viruses. *Oceanography* **20**:135–139.

Brock, Thomas D. 1999. *Robert Koch: A Life in Medicine and Bacteriology.* ASM Press, Washington, DC.

Dinc, Gulten, and Yesim I. Ulman. 2007. The introduction of variolation "A La Turca" to the West by Lady Mary Montagu and Turkey's contribution to this. *Vaccine* **25**:4261–4265.

Dubos, Rene. 1998. *Pasteur and Modern Science.* Translated by Thomas Brock. ASM Press, Washington, DC.

Fleishmann, Robert D., Mark D. Adams, Owen White, Rebecca A. Clayton, Ewen F. Kirkness, et al. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**:496–512.

Gann, Alexander, and Jan Witkowski. 2010. The lost correspondence of Francis Crick. *Nature* **467**:519–524.

Hesse, Wolfgang. 1992. Walther and Angelina Hesse—early contributors to bacteriology. *ASM News* **58**:425–428.

Leuf, Birgit, Kyle R. Frischkorn, Kelly C. Wrighton, Hoi-Ying N. Holman, Giovanni Birarda, et al. 2015. Diverse uncultivated ultra-small bacterial cells in groundwater. *Nature Communications* **6**:6372.

Maddox, Brenda. 2002. *The Dark Lady of DNA.* Harper Collins, New York.

Margulis, Lynn. 1968. Evolutionary criteria in Thallophytes: A radical alternative. *Science* **161**:1020–1022.

Raoult, Didier. 2005. The journey from *Rickettsia* to Mimivirus. *ASM News* **71**:278–284.

Sherman, Irwin W. 2006. *The Power of Plagues.* ASM Press, Washington, DC.

Thomas, Gavin. 2005. Microbes in the air: John Tyndall and the spontaneous generation debate. *Microbiology Today* (Nov. 5): 164–167.

Ward, Naomi, and Claire Fraser. 2005. How genomics has affected the concept of microbiology. *Current Opinion in Microbiology* **8**:564–571.

ANSWERS TO REVIEW QUESTIONS (P. 34)

1. Explain the apparent contradictions in defining microbiology as the study of microscopic organisms or as the study of single-celled organisms.

   ANS: Most single-celled organisms require a microscope to be viewed, but not all. Some species are actually large enough to see with the naked eye. Many microbes form multicellular communities that render them visible. Two examples of these are mushrooms and biofilms. There are also some multicellular organisms that are microscopic but do not fall into the microorganism category.

2. What is the genome of an organism? How do genomes of viruses differ from those of cellular microbes?

   ANS: The genome of an organism is the total genetic information contained in the organism's chromosomal DNA. For most cellular organisms it contains all the information necessary for the organism's self-replication. The genome of a virus is not always DNA. Furthermore, the viral genome does not contain all the information needed for self-replication. It relies on the cell machinery and its genome typically contains information to take over host cell processes to generate more virus particles.

3. Under what conditions might microbial life have originated? What evidence supports current views of microbial origin?

ANS: The early Earth environment was composed mainly of highly reduced compounds. Living cells may have formed from spontaneous reactions sparked by UV absorption or electrical discharge. Miller found that when reduced compounds were subjected to an electrical discharge, several amino acids were observed. Oró did a similar experiment and found the production of adenine. There is still debate as to where the first cells came from. Some scientists believe life has an extraterrestrial origin.

4. List the ways in which microbes have affected human life throughout history.
   ANS: Probably the first thing that will come to mind is a microbe's disease-causing properties. Microbes have been used in food production, mining, insecticidal activity, and antibiotic production, to name just a few uses. We also rely on organisms to cycle compounds such as carbon and nitrogen.

5. Summarize the key experiments and insights that shaped the controversy over spontaneous generation. What questions were raised, and how were they answered?
   ANS: Spontaneous generation means that life arises spontaneously, without parental organisms. In the 1600s, Redi showed that maggots appearing on decaying meat were actually the offspring of flies. When flies could not gain access to the meat, no maggots were observed. In the 1700s, Spallanzani sterilized liquid broth and showed that no organisms could grow unless the medium was inoculated. Proponents of spontaneous generation argued that there was no growth due to lack of oxygen.

   In the 1800s, Pasteur created swan-necked flasks to illustrate that it was not the lack of oxygen that had prevented growth in Spallanzani's experiments. In the late 1800s, Tyndall recognized the presence of heat-resistant spores in some boiled media that resulted in the growth of microbes.

6. Explain how microbes are cultured in liquid and on solid media. Compare and contrast the culture methods of Koch and Winogradsky. How did their different approaches to microbial culture address different questions in microbiology?
   ANS: Liquid and solid media can be identical, with the exception that agar has been added to solidify the medium in the latter case. A solid medium allows for an organism to be isolated in "pure culture." Koch used a defined solid medium to isolate the causative agent of tuberculosis. This allowed the isolation of organisms that fed on organic materials.

   Winogradsky studied microbes in their natural habitats. He was able to isolate organisms that fed solely on inorganic materials. The Winogradsky column is still used in labs today as a form of enrichment culture to isolate organisms of a desired nature. Koch was able to design synthetic media to culture and isolate specific organisms. Winogradsky used nature's own enrichment culture in the form of a column to isolate organisms with very special traits.

7. Explain how a series of observations of disease transmission led to the development of immunization to prevent disease.
   ANS: In Turkey, in the early 1700s, it was found that fluid from smallpox pustules could be used to immunize other people. In some cases, however, individuals contracted serious disease and became contagious. In the late 1700s, in England, Jenner used matter from cowpox lesions to immunize against

smallpox. It had been recognized that milkmaids contracted cowpox and became mildly sick, but were then seemingly immune to smallpox. Pasteur then used attenuated, or weakened, viruses for immunization. Ultimately, it was discovered that one can use simply a molecular component of a pathogen to generate immunity.

8. Summarize key historical developments in our view of microbial taxonomy. What attributes of microbes have made them challenging to classify?
   ANS: Microscopy allowed for the visualization of microorganisms. Through the development of staining techniques and more sensitive forms of microscopy, we were able to begin categorizing organisms. With the advent of the analysis of various metabolic pathways, we were able to further categorize them. Ultimately, with the ability to sequence genomes, or partial genomes, taxonomic classification has reached new levels. Some organisms have been renamed or moved to different locations on the phylogenetic tree. Many organisms are difficult to culture; in fact, only a very small percentage of organisms have been identified and sequenced.

9. Explain how various discoveries in "natural" bacterial genetics were used to develop recombinant DNA technology.
   ANS: Griffith first observed transformation when some material from dead bacteria caused previously harmless bacteria to turn into a deadly form. Approximately 15 years later, Avery identified the transforming material as DNA. It was found that organisms contained restriction endonucleases that cut DNA at specific sequences. These enzymes have been used to cut and paste DNA to make recombinant DNA for genetic transfer of information between organisms. Viruses, in their entirety or in part, may be used to transfer information into an organism.

ANSWERS TO END-OF-CHAPTER THOUGHT QUESTIONS (P. 34)

1. How do the Earth's microbes contribute to human health? Include examples of environmental microbes outside the human body, as well as microbes associated with the human body.
   ANS: Humans, like all animals, require oxygen to breathe, as well as organic foods such as carbohydrates and proteins. The sole source of oxygen in our environment is phototrophic bacteria and plants. Other environmental bacteria, such as intestinal bacteria, form essential amino acids and vitamins that our own bodies cannot synthesize. In our digestive tract, and in our skin, bacteria produce defensive molecules such as short-chain fatty acids that inhibit the growth of disease-causing bacteria.

2. When space scientists seek evidence of life on Mars, why do you think they expect to find microbes rather than creatures like the "alien monsters" often depicted in science fiction?
   ANS: On Earth, microbes grow throughout all biospheres. Microbes even live in some of the harshest environments on Earth such as the Atacama Desert and in Antarctica. These environments are closer to a Martian habitat than any other found on Earth. Therefore, scientists hypothesize that potential life on

Mars would be similar to the life found in these harsh Earth environments, meaning they would likely be microbial in nature.

3. Why do you think so many environmental microbes cannot be cultured in laboratory broth or agar media?
   ANS: Microbes in "wild" environments have access to many different kinds of substances. For example, plant roots exude complex polysaccharides, proteins, and vitamins, any of which may be needed for a given microbe to grow. Also, metabolism may require syntrophy, that is, growth in the presence of another species. The second species may metabolize a substance released by the first as a waste product. Another aspect of environment is the nature of a surface. For instance, some species of *Streptococcus* bacteria require a tooth enamel surface to grow, and thus they need to grow as part of a tooth biofilm.

4. Outline the different contributions to medical microbiology and immunology of Louis Pasteur, Robert Koch, and Florence Nightingale. What methods and assumptions did they have in common, and how did they differ?
   ANS: Louis Pasteur discovered the principle of chirality of biological molecules. Chirality, or handedness, determines whether a substrate can be used by enzymes. Pasteur also discovered the principle of immunization by an attenuated pathogen such as the causative agent of diphtheria. He made this discovery as the result of inoculation with a culture that was accidentally aged. Pasteur also showed that microbes could not grow in the absence of preexisting microbes. However, he never tested microbial sources containing heat-resistant spores.

   Robert Koch developed key techniques of plate culture and Koch's postulates for identifying the causative agent of a disease. While Pasteur had profound insights about the properties of microbes, Koch was a more methodical investigator, and the key procedures that he developed (plate culture and Koch's postulates) are still in use today.

   Florence Nightingale was not aware of the nature of microbes as individual cells, but she was aware of the role of contagion in disease transmission. She also addressed the question of disease transmission on the scale of populations, rather than individuals. Unlike Koch and Pasteur, she developed and applied mathematical approaches of statistics to draw conclusions about the spread of microbial disease.

5. Outline the different contributions to environmental microbiology of Sergei Winogradsky and Martinus Beijerinck. Why did it take longer for the significance of environmental microbiology to be recognized, as compared with pure-culture microbiology?
   ANS: Sergei Winogradsky was the first to report microbes oxidizing sulfur, nitrogen, and iron instead of organic energy sources. He studied microbes in complex natural habitats such as wetlands, using the Winogradsky column for enrichment culture. Martinus Beijerinck discovered nitrogen fixation, and revealed the nitrogen-fixing symbiosis between rhizobia and plants. He also discovered viruses as filterable agents that infected plants. Beijerinck discovered the first known form of anaerobic respiration, involving reduction of sulfate.

   The work of both Winogradsky and Beijerinck was under-recognized in their time because they did

not study human disease, and because they studied organisms mainly in mixed culture. Koch's development of pure culture became the "gold standard" for defining reproducible experiments in microbiology, but ironically it delayed understanding of systems such as multispecies biofilms, in which pure culture is not possible.

6. What kinds of evidence support the common ancestry of life from cells with RNA chromosomes? Could cells with RNA chromosomes exist today? Why or why not?

   ANS: RNA has the ability to code information like DNA as well as the ability to catalyze reactions like a protein enzyme can. This has led to the hypothesis that the earliest cells used RNA as both their genome and as the catalysts for their metabolism. Cells with RNA chromosomes would be unlikely to exist today. This is because DNA transmits information with greater fidelity than RNA, and protein enzymes are far more efficient catalysts than RNA. Thus, an "RNA-based" cell would be at a great competitive disadvantage compared to modern cells.

Chapter 2

Observing the Microbial Cell

SUMMARY

This chapter introduces microbial observation. Optics are discussed in general, as are the principles of all forms of microscopy.

Types of microscopy are discussed with examples of what has been observed using each technique. The physics behind the use of electromagnetic radiation and lenses is discussed in each case.

This introduction to visualizing cells and cellular components will progress to Chapter 3, which describes how visual observation was used to reveal details about cell structure and function.

2.1 Observing Microbes

Learning Objectives

- 2.1.a. Explain how the structure of the human eye dictates the resolution of objects.
- 2.1.b. Differentiate between resolution and detection.
- 2.1.c. Describe and identify bacterial morphologies (e.g., bacilli, cocci, and spirochetes).
- 2.1.d. Identify the type(s) of microscopy needed to view a particular specimen.

This section introduces all the generic terms used in microscopy. It is imperative that students understand detection, resolution, and magnification as well as how they are related. It is also important to understand the size ranges of organisms. It is here that the major forms of observation, from light microscopy to X-ray crystallography, can be introduced.

Discussion Points

- Resolution is an extremely important concept. Using a real-life example to illustrate resolution, discuss how you may be able to see a person at a distance in a photo, but you will not be able to resolve enough features to determine the person's identity, even if you enlarge the photo. This concept is further illustrated in Figure 2.3.
- Figure 2.6 shows three examples of the use of light microscopy (LM) and three examples of the use of scanning electron microscopy (SEM). The difference in the level of resolution and detail between the two forms is very evident.
- Figure 2.7 serves to reinforce this concept by showing size ranges observable with each technique and a photograph to illustrate the point. Combine the discussion of this figure with a discussion of Figure 2.4

so that the students will understand which microscopy techniques are potentially appropriate for observing different cells.

2.2 Optics and Properties of Light

Learning Objectives

- 2.2.a. Identify what conditions must exist for electromagnetic radiation to resolve an object from neighboring objects or the surrounding medium.
- 2.2.b. Explain the properties of light.
- 2.2.c. Differentiate among absorption, reflection, refraction, and scattering.
- 2.2.d. Explain how lenses magnify images.

This section continues to introduce the physical properties of light, its interaction with objects, and optics. Visible light is one portion of the spectrum of electromagnetic radiation, which includes shorter wavelengths (such as ultraviolet and X-rays) as well as longer wavelengths (such as microwaves and radio waves). For the processes discussed in this chapter, the focus will be on the portion of the spectrum from visible light toward the ultraviolet end of the spectrum.

Light travels as a wave, which can be absorbed, reflected, refracted, or scattered by an object. Each of these processes plays a role in one or more forms of microscopy. In particular, the role that refraction plays in magnification should be explained.

Discussion Points

- Figure 2.8 presents the electromagnetic spectrum, which is useful for discussing the relationship between wavelength and frequency.
- Figures 2.9 and 2.10 show the interaction of light with matter. This can lead into discussions of magnification.
- Figure 2.11 illustrates how to use a lens and the principle of how diffraction leads to magnification.
- Figure 2.12 gives a good explanation of the optics of resolution.

2.3 Bright-Field Microscopy

Learning Objectives

- 2.3.a. State the factors that influence image quality in bright-field microscopy.
- 2.3.b. Label the parts of a compound microscope.
- 2.3.c. Explain the function of the components of a compound microscope.
- 2.3.d. Describe the advantages and disadvantages of a wet mount.

- 2.3.e. Explain the advantages and disadvantages of fixing and staining specimens.
- 2.3.f. Classify stains as simple or differential.
- 2.3.g. Explain how the structure of the bacterial cell wall relates to its Gram-stain status.

Bright-field microscopy is the method that most students will have the opportunity to use in introductory labs. The physics and operation of the microscope should be discussed at some level. With this should come an explanation of the reason behind the use of oil (Fig. 2.14) with the 100X lens and how to calculate total magnification.

In bright-field microscopy the cell is observed as a dark object (absorbing light) against a bright background (transmitting light), hence the name. The contrast between a cell, which is predominantly water, and its environment, which is commonly water, is usually minimal. Consequently, the contrast has to be enhanced by fixing and staining the specimen prior to observation. Many different stains are used, each providing distinct information about a specimen. The most widely used staining procedure used in microbiology is the Gram stain. A more detailed discussion of cell envelope differences appears in Chapter 3.

Discussion Points

- The microscope and its optics are diagrammed in Figure 2.15. Students should be introduced to the operation and terms relevant to bright-field microscopy.
- Figure 2.13 is an excellent illustration for relating the optics of resolution (from Figure 2.12) to the increased resolution generated by greater magnification.
- Figure 2.14 illustrates the importance of using immersion oil with the 100X lens.
- It is important to emphasize the difference between a simple stain and a differential stain.
- Different staining processes are mentioned, but it is most important to discuss the Gram stain. This type of staining is illustrated in Figures 2.20, 2.21, and 2.22.

2.4 Fluorescence Microscopy and Super-Resolution Imaging

Learning Objectives

- 2.4.a. Differentiate between the excitation and emission wavelengths.
- 2.4.b. Recall the factors that determine the cell specificity of a fluorophore.
- 2.4.c. Describe the advantages of super-resolution imaging.
- 2.4.d. State the function of chemical imaging microscopy.

In fluorescence microscopy, the object absorbs light at one wavelength and then emits the light at a longer, visible wavelength. The wavelength that is emitted determines the color that is observed. A fluorophore is the fluorescent molecule used to stain the specimen. Some fluorophores have affinity for a certain component

of a cell. Fluorophores can be attached to antibodies or DNA for use in microscopic analysis.

In confocal microscopy a laser beam is used to excite a fluorophore and generate a three-dimensional image.

Discussion Points

- Figure 2.24A shows a fluorophore at the molecular level, and Figure 2.24B shows the absorption and emission spectra for a fluorophore.
- Discuss the specificity and usefulness of chemical affinity, immunofluorescence, gene fusion, and DNA hybridization. Understanding these techniques is necessary before studying Chapter 3.
- Figure 2.27B illustrates the use of fluorophores to observe the replisome and origin of DNA replication in bacterial cells.
- Figure 2.28 shows how super-resolution imaging of fluorescent microscope images may be used to observe the movement of molecules within cells.

2.5 Dark-Field and Phase-Contrast Microscopy

Learning Objectives

- 2.5.a. Describe the conditions under which dark-field microscopy is the best choice for viewing specimens.
- 2.5.b. Explain the principles behind phase-contrast microscopy.
- 2.5.c. State the types of images obtained with differential interference contrast microscopy.

Dark-field microscopy allows the detection of entities that are too small to be resolved with bright-field microscopy. You should emphasize the difference between detection of an object and resolution of an object's precise shape and size. Scattered light from the object is detected by the use of a condenser lens containing a "spider light stop." It can be used in the study of motility because it allows detection of bacterial flagella, which are too narrow to be resolved by bright-field microscopy.

Phase-contrast microscopy allows observation based on differences in refractive indexes between the cytoplasm, the medium, and subcellular entities. It employs an annular ring in the optics. This produces dramatic visual differences between objects having only a small difference in refractive index. Therefore, no stains are needed, and hence we can observe living cells.

Discussion Points

- The physics of the spider light stop condenser system in dark-field microscopy is illustrated in Figure 2.32.
- Studying motility is possible using dark-field microscopy. An excellent illustration of this is shown in

Figure 2.33. You can show this figure and follow up with Thought Question 2.7 to provoke class discussion.

- Figure 2.35 shows how the specimen and phase plate each shift the light wave by one-fourth of a wavelength, resulting in a total difference of one-half wavelength. This increases the contrast, enabling the visualization of live microbes with phase-contrast microscopy. A resulting image can be seen in Figure 2.34.

2.6 Electron Microscopy, Scanning Probe Microscopy, and X-Ray Crystallography

Learning Objectives

- 2.6.a. Compare and contrast light microscopy and electron microscopy.
- 2.6.b. Identify images obtained via scanning electron microscopy and transmission electron microscopy.
- 2.6.c. Propose applications for scanning probe microscopy.
- 2.6.d. State what kind of information is obtained from X-ray crystallography analysis.
- 2.6.e. Explain the methods and benefits of using cryo techniques in cryo-electron microscopy and cryocrystallography.

Electron microscopy is based on magnification using a beam of electrons as the radiation source. Electrons traveling in a voltage potential exhibit a wave property, analogous to the wave property of light rays. The wavelength of the electron beam is much smaller than that of light; for this reason, much smaller dimensions can be resolved by electron microscopy than by light microscopy.

The two major forms of electron microscopy are transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The electron microscope has parts analogous to those of a light microscope. In electron microscopy, the radiation source is an electron beam rather than visible light, and the lenses are magnets rather than glass. In both TEM and SEM, samples can be stained with heavy metal. In TEM, the electron beam is transmitted through the thin section of a stained specimen, revealing internal structure. In SEM, the electron beam is reflected off the surface of the stained specimen, and a picture of the surface of the specimen is obtained.

In cryo-electron microscopy and cryo-electron tomography, the flash-frozen samples are unstained, resulting in high-resolution images.

Atomic force microscopy is a method that measures van der Waals forces between the electron shells of adjacent atoms on the cell surface and the tip of the probe. It allows the study of surfaces of live bacteria in water solution.

X-ray crystallography allows studies of structures at the molecular level. X-ray crystallography has revealed the structure of many molecules. The X-ray crystallography of DNA by Rosalind Franklin, which helped reveal the double-helical nature of DNA, is probably the most widely discussed example.

Discussion Points

- Discuss sample preparation for TEM and SEM microscopy and the possibility of artifact introduction in the process.
- Discuss cryo-EM and how sample preparation leaves the specimen in a form that should closely resemble the viable form.
- Use Figure 2.38 to compare and contrast light microscopy and TEM.
- Discuss sample preparation for TEM, including fixation, embedding, sectioning, and staining.
- Figure 2.39A, which illustrates the structure of the SEM, can be used as an aid in the discussion of the relevant physics.
- Discuss the need for shadowing a surface of a specimen for SEM.
- Figure 2.40 contains an image of flagellar motors generated by TEM. This might help students understand the kinds of structures that could be visualized with TEM as opposed to light microscopy.
- Figures 2.43 and 2.44 describe cryo-electron tomography and include a viral structure inferred from the use of this technique.
- Figure 2.45 illustrates the principle of atomic force microscopy.
- Figure 2.46 shows the principle behind X-ray diffraction and the data observed in a particular case.
- X-ray crystallography requires a crystallized specimen and therefore amounts to looking at a static picture.
- X-ray data analysis can provide information about model molecular structure, as illustrated by Figure 2.48.

PROCESS ANIMATIONS

The following process animation expands upon the art from the textbook. The process animations can be accessed through the Digital Resources website, digital.wwnorton.com/microbio4, as well as through the ebook and in the coursepacks, which are provided for download on the instructor's resource site, wwnorton.com/instructors.

- **Microscopy: Optics and Properties of Light**

This animation depicts the concepts of magnification and resolution that are described in this chapter using the figures from the text. The structure and functioning of compound microscopes are also discussed at some length.

Animation Discussion Question 1: Discuss Airy discs and how they are related to resolution.

ANS: Airy discs are formed by the alternating pattern of constructive and destructive interference of light waves due to refraction through a lens. The size of the Airy disc is determined by the quality of the lens and the wavelength of light, with shorter wavelengths producing

smaller discs. Structures may only be distinguished from one another by our eyes if their Airy discs do not overlap.

Animation Discussion Question 2: What is the purpose of the condenser lens of a compound microscope?

ANS: The condenser lens focuses the light on a small area of the specimen slide. This allows for more efficient illumination of the sample.

eTOPICS

eTopics are supplementary, stand-alone sections that explore additional material in depth. They are available to students within the ebook and within the coursepacks, which are provided for download on the instructor's resource site, wwnorton.com/instructors.

2.1 Molecular "Snapshots": Chemical Imaging

eTopic 2.1 discusses nanoscale secondary ion mass spectrometry (NanoSIMS). The chemical and physical bases of this technique are discussed along with some examples of its utility for answering biological questions. This technique is also discussed in Special Topic 2.1.

2.2 Confocal Microscopy

eTopic 2.2 discusses confocal laser scanning microscopy. The physics underlying this technique are discussed and some example images are shown.

RECOMMENDED READINGS

The following readings are presented at the end of the textbook chapter as resources for further exploration of the topics discussed in Chapter 2.

Altindal, Tuba, Suddhashil Chattopadhyay, and Xiao-Lun Wu. 2011. Bacterial chemotaxis in an optical trap. *PLoS ONE* **6**:e18231.

Chiu, W., M. L. Baker, W. Jiang, and Z. H. Zhou. 2002. Deriving folds of macromolecular complexes through electron cryomicroscopy and bioinformatics approaches. *Current Opinion in Structural Biology* **12**:263–269.

Graumann, Peter L., and Richard Losick. 2001. Coupling of asymmetric division to polar placement of replication origin regions in *Bacillus subtilis*. *Journal of Bacteriology* **183**:4052–4060.

Jiang, W., J. Chang, J. Jakana, P. Weigele, J. King, et al. 2006. Structure of epsilon15 bacteriophage reveals genome organization and DNA packaging-injection apparatus. *Nature* **439**:612–616.

Komeili, A., Z. Li, D. K. Newman, and G. J. Jensen. 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**:242–245.

Lucic, Vladan, Friedrich Förster, and Wolfgang Baumeister. 2005. Structural studies by electron tomography: From cells to molecules. *Annual Review of Biochemistry* **74**:833–865.

Matias, Valério R. F., Ashruf Al-Amoudi, Jacques Dubochet, and Terry J. Beveridge. 2003. Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. *Journal of Bacteriology* **185**:6112–6118.

Murphy, Douglas B. 2001. *Fundamentals of Light Microscopy and Electronic Imaging.* Wiley-Liss, Hoboken, NJ.

Popescu, Aurel, and R. J. Doyle. 1996. The Gram stain after more than a century. *Biotechniques in Histochemistry* **71**:145–151.

Ptacin, Jerod L., Steven F. Lee, Ethan C. Garner, Esteban Toro, Michael Eckart, et al. 2010. A spindle-like apparatus guides bacterial chromosome segregation. *Nature Cell Biology* **12**:791–798.

Tocheva, E., Z. Li, and G. Jensen. 2010. Electron cryotomography, p. 213–232. *In* Lucy Shapiro and Richard M. Losick (eds.), *Cell Biology of Bacteria: A Subject Collection from Cold Spring Harbor Perspectives in Biology.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ANSWERS TO REVIEW QUESTIONS (P. 73)

1. What principle defines an object as microscopic?

   ANS: An object is microscopic if we cannot see it clearly without magnification. The fact that it is microscopic to us is based on our eyes' inherent properties. The size at which something becomes visible depends on the resolution of our eyes.

2. Explain the difference between detection and resolution.

   ANS: Detection of an object simply means that it can be observed. Resolution means the smallest distance at which objects become distinguishable from one another. We can observe a bacterial colony containing thousands of bacteria, but we cannot resolve each bacterium. To resolve or distinguish the individual cells requires magnification with an instrument having increased resolution.

3. How do eukaryotic and prokaryotic cells differ in appearance under the light microscope?

   ANS: Eukaryotic cells are generally larger, and their internal compartmentalized structure can be resolved. Prokaryotic cells tend to be smaller so they can be detected, but internal details are generally too small to be resolved.

4. Explain how electromagnetic radiation carries information and why different kinds of radiation can resolve different kinds of objects.

   ANS: Electromagnetic radiation is a form of energy propagated as waves associated with electrical and magnetic fields. Visible light, ultraviolet light, X-rays, and gamma rays all travel as waves; visible light has the longest wavelength and gamma rays have the shortest. The shorter the wavelength of the energy, the greater is the resolving power.

5. Describe how light interacts with an object through absorption, reflection, refraction, and scattering.

   ANS: *Absorption:* A photon's energy is absorbed by the object and usually converted to a different form of electromagnetic radiation. In bright-field microscopy, when a specimen absorbs light, it is observed as a dark spot against a bright field.

   Reflection: Reflection occurs when a wavefront is redirected from the surface of an object at an angle equal to its incident angle. It is used in the optics of a microscope.

   Refraction: Light bends when it enters an object (such as glass) with a higher refractive index than air. The speed and direction of the light change, resulting in a wider emerging wavefront.

   Scattering: This occurs when a portion of the wavefront is converted to a spherical wave originating from the object. Special optics can use scattered light to detect microbial shapes smaller than the wavelength of light (dark-field microscopy).

6. Explain how refraction enables magnification of an image.

   ANS: When light passes through a refractive material that is shaped to spread the light waves, the image is magnified. When an object is placed within the focal plane of a lens, the light rays from the object are bent by the lens and converge at the opposite focal point. The light rays continue from the focal point and generate an inverted but magnified image of the object.

7. Explain how magnification increases resolution and why empty magnification fails to increase resolution.

   ANS: When an image is magnified by lenses with increased resolution, the distances between parts of the image are enlarged, enabling us to resolve finer details. Empty magnification occurs when details of an image are enlarged in proportion to the entire object. An example of empty magnification is enlarging a pixelated photo. No more detail will be gained; each pixel will simply be enlarged in proportion to the overall picture. Nothing will be gained except size.

8. Explain how angle of aperture and resolution change with increasing lens magnification.

   ANS: The greater the angle of aperture of the lens, the better is the resolution. With a lower magnification lens, the angle of aperture is small, the specimen is farther away, and there is a wide Airy disk. With a higher magnification lens, the angle of aperture is larger, the specimen must be closer to the lens, and there is a narrow Airy disk.

9. Summarize the optical arrangement of a compound microscope.

   ANS: A compound microscope has a light source at the bottom; light passes through the diaphragm, the condenser lens, the specimen, the objective lens, the ocular lens, and then ultimately reaches the eye.

10. Explain how to focus an object and how to tell when the object is in or out of focus.

    ANS: Most microscopes are parfocal, so it is easiest to focus with a low-power objective first, since it generates a greater depth of field. It is possible then to rotate the higher-power lens into view and then perform only a minor adjustment of focus. An object is in focus when its edge appears sharp and distinct

from the background.

11. Explain the relative advantages and limitations of wet mount and stained preparations for observing microbes.

    ANS: The advantage of wet mount preparation is that the specimens may be observed in their natural state without any introduced artifacts. A major disadvantage, however, is that most cells are transparent and there is little or no contrast between the specimen and its background. For this reason, detection and resolution are minimal. To stain a specimen, it must be fixed and then stained. Fixing kills the cells and the stains introduce contrast, allowing the microbe to be observed. However, the fixation or the staining process can introduce artifacts.

12. Explain the significance (and limitations) of the Gram stain for bacterial taxonomy.

    ANS: The Gram stain is a key tool for chemical identification of species in the clinical laboratory. It is used to categorize cells as either Gram-positive, if they retain the crystal violet stain, or Gram-negative, if they do not. The nature of their cell walls determines which category they fall into. The Gram stain differentiates between two major bacterial taxa, Proteobacteria (Gram-negative) and Firmicutes (Gram-positive). Some organisms have very different cell walls and cannot be distinguished by the Gram stain.

13. Explain the basis of dark-field, phase-contrast, and fluorescence microscopy. Give examples of applications of these advanced techniques.

    ANS: Dark-field microscopy uses a "spider light stop" in the condenser lens to detect light scattered by an object. This allows objects to be observed as spots of light in a dark background. This is exceptionally useful for studying bacterial motility. Samples must be very clean because even small dust particles will be observed.

    In phase-contrast microscopy, an annular ring is used. This allows both refracted light from the specimen and the outer cone of transmitted light to be detected. The waves are out of phase, which ultimately results in regions of darkness within the specimen. This technique is particularly useful for eukaryotic organisms, which contain many intracellular components.

    Fluorescence microscopy takes advantage of the fact that some compounds fluoresce. Chlorophyll, for example, is a cellular compound that fluoresces, so chlorophyll-containing microbes may be observed directly with a fluorescence microscope. Most of the time, however, it is necessary to use some sort of stain that fluoresces (a fluorophore) to observe cells or cellular components. DAPI, for example, binds to DNA and fluoresces. One can also attach a fluorophore to an antibody for immunofluorescence studies.

14. Explain how super-resolution imaging enables tracking of intracellular molecules.

    ANS: When a fluorophore emits light, the light emission is not uniform. There is a peak intensity surrounded by light of less intensity. Using computer techniques, the location of peak light emission can be estimated to a much greater precision than is possible with the naked eye. This allows for the tracking of something like a molecule, which is too small to resolve with light microscopy and without the use of

these super-resolution techniques.

15. Explain the difference between transmission and scanning electron microscopy, including the different applications of each.

    ANS: In TEM, the specimens are fixed, embedded, and then cut into thin sections prior to staining. The electron beam is transmitted through the thin sections and the stains increase the contrast within the cell. One can compile data from sequential sections to obtain a composite like a three-dimensional (3D) model of a specimen. In SEM, the specimen is shadowed with a heavy metal. The electron beam then is deflected off the surface of the specimen, allowing observation of peaks and valleys. This generates a type of relief map of a surface. It is also possible to look at an inner surface by subjecting the specimen to freeze-fracture prior to shadowing.

ANSWERS TO END-OF-CHAPTER THOUGHT QUESTIONS (P. 74)

1. Explain what features of bacteria you can study by: (a) light microscopy; (b) fluorescence microscopy; (c) scanning EM; (d) transmission EM.

    ANS: Light microscopy shows the overall shape of bacterial cells. In a stained specimen, light microscopy can reveal a particular aspect of a cell, such as a Gram-positive cell wall. Fluorescent microscopy can show the position of subcellular parts, even a single molecule such as a protein bound to a DNA origin sequence. The shape of the protein, however, is only detected, not resolved. Scanning EM resolves details of the surface contours of bacteria. Transmission EM reveals many subcellular structures, such as ribosomes and DNA fibers, as well as isolated cell parts such as the flagellar motor.

2. Explain how resolution is increased by magnification. Why can't the details be resolved by your unaided eye? Explain why magnification reaches a limit. Why can it not go on resolving greater detail?

    ANS: The resolution of the human eye is limited by the distance between photoreceptor cells in the retina. As an image is magnified, the distance between light rays originating from the object increases. The rays carry information, including the distance between points of an object. Eventually the rays diverge enough that they can be resolved by the retina. Magnification, however, is limited by the wavelength of light. If the details of an object are smaller than the wavelength of light, then the width of the Airy disk interference pattern exceeds the size of the details (Fig. 2.14). As the light rays diverge, the Airy disk only expands and fails to show any smaller details.

3. Explain why artifacts appear in microscopic images, even with the best lenses. Explain how you can tell the difference between an optical artifact and an actual feature of an image.

    ANS: Every lens has an edge. At the edge, the light rays deviate from the parabolic focus. Thus, all lenses cause artifacts arising from aberrations. In addition, some parts of the specimen are always out of focus. An object outside the focal plane may look blurred, or it may appear as a ring with a bright center. To tell whether the appearance of an image is characteristic of the object, or whether it arises from the optics, try focusing up and down. If a ring-shaped feature disappears as the object appearance sharpens,

then the ring shape was an artifact of the optical system.

4. How can "detection without resolution" be useful in microscopy? Explain with specific examples.
   ANS: Detection without resolution is useful in dark-field microscopy. In dark-field, the curve of a flagellum can be detected even though the protein filament is narrower than the wavelength of light, so it is possible to see the flagella rotating on a living bacterium. In fluorescence microscopy, the position of tiny subcellular structures such as the DNA-replicating apparatus can be detected without resolution. While the fluorescent "blob" appears much larger than the structure, its position within the cell is nonetheless accurate and can show how the structure functions within the cell.