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.

1.1 From Germ to Genome: What Is a Microbe?

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, but pay particular attention to Table 1.1 and Fig. 1.4. 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.”
• Size, again, can be emphasized in a brief discussion on genome size. Sequencing of bacteriophage ΩX174 revealed that much information could be packed into a small genome by making use of overlapping gene sequences.

1.2 Microbes Shape Human History

This section enables students to see how all-encompassing microbes are in their 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, the endosymbiont theory, microscopic observation, 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 parts of Table 1.2 to take students through the development of microbiology.
• Show pictures of van Leeuwenhoek’s microscope and his “animalcules” (Fig. 1.13).
• 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. 1.14).
• Discuss Florence Nightingale’s role in the development of medical statistics and study of the cause of disease (Fig. 1.11).

1.3 Medical Microbiology

Whether or not your course focuses on medical microbiology, students tend to be most 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

• The visuals in figures 1.11A, 1.15C, 1.21A, and 1.21B are useful for helping solidify the information in this chapter, and might even bring some levity to the discussion.
• Discuss Koch’s postulates (Fig. 1.17) and compare and contrast his work with anthrax and tuberculosis. Discuss its limitations for specific diseases such as AIDS.
• Vaccine development for smallpox is a great look at history and development of microbiology (Fig. 1.18).
• 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.
• Some differences between bacteria and viruses are introduced. Fig. 1.21 can be used to illustrate the “simple” structure of a virus.

1.4 Microbial Ecology

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 between microbes and plants and animals that may be familiar to students can be used to begin a discussion of biofilms.

1.5 The Microbial Family Tree

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 hurdles in classification through history. These should include visual observation through microscopy and staining, lifestyle or environmental issues, biochemical activities, and most recently, genomic comparison. Inherent in these discussions should be Lynn Margulis’s, at the time 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 to understanding it as having three domains. Along the way, many organisms have been renamed and reclassified.

Discussion Points

• Discuss the three domains of life, beginning with the simplistic phylogenetic tree shown in Fig. 1.5 and then the more detailed tree in Fig. 1.28.
• Be sure to explain the significance of Lynn Margulis’s endosymbiont theory (Fig. 1.26) in adjusting the family tree.
• Mention should be made of the fine-tuning of the tree, involving different names and placement on the tree, due to the work of Carl Woese (Fig. 1.28).

1.6 Cell Biology and the DNA Revolution

This last section stresses the fact that the amount of knowledge exploded in the twentieth century—more than 90% of our knowledge has been acquired since 1900. 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 include discussions of Griffith and Avery’s experiments on genetic trans-
formation, as well as 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.

The observations and understanding of the transfer of genetic information via bacteria and viruses to other microbes have enabled us to develop the field of recombinant DNA technology. With that ability 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. The field of ethics is a course in and of itself.

**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 of X-ray crystallography in the discovery of the structure of DNA (Fig. 1.31).
- Discussion should cover Griffith and Avery’s genetic transformation experiments.
- Great movies to make an impact on students are *The Double Helix*, starring Jeff Goldblum as Nobel laureate James Watson, and *Protein Synthesis: An Epic on the Cellular Level*, with an introduction by Nobel laureate Dr. Paul Berg.

**Weblinks**

The following weblinks appear in Chapter 1, and on the Microbiology StudySpace student website at microbiology2.com/links.

*The Nobel Prize*

The Nobel Prize website presents the lectures and autobiographies of all Nobel Prize winners, including many who were awarded prizes for advances in microbiology.

http://nobelprize.org/

*National Center for Biotechnology Information*

The National Center for Biotechnology Information (NCBI) provides free access to all published genome sequences.


*Centers for Disease Control and Prevention*

The CDC (Centers for Disease Control and Prevention), in Atlanta, is the U.S. agency for medical information and epidemiology.

http://www.cdc.gov/

**List of Prokaryotic Names with Standing in Nomenclature**

List of prokaryotes and the nomenclatural changes as cited in the Approved Lists of Bacterial Names or validly published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM).

http://www.bacterio.cict.fr/

**American Society for Microbiology**

From the ASM website: “The American Society for Microbiology is the oldest and largest single life science membership organization in the world. Membership has grown from 59 scientists in 1899 to more than 43,000 members today, with more than one third located outside the United States. The members represent 26 disciplines of microbiological specialization plus a division for microbiology educators.”

http://www.asm.org/

**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.


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.


Huq, Anwar, Mohammed Yunus, Syed S. Sohelb, Abbas Bhuiyab, Michael Emche, et al. 2010. Simple sari cloth filtration of water is sustainable and continues to protect villagers from cholera in Matlab, Bangladesh. *mbio* 1:e00034.
Chapter 1

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, for their insecticidal activity, and for 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 key 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 on liquid and 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 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 gone to 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. 37)

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 oxygen is used up, certain microbes ferment their carbon sources to produce ethanol or acetate. If oxygen is replaced, what do you think happens to the ethanol or acetate?

ANS: Many microbes, including yeast and enteric bacteria (such as Escherichia coli), possess the ability to oxidize carbon sources either using oxygen gas (O₂) or using the carbon source as oxidant for itself, a process called fermentation. Most products of fermentation, such as ethanol and acetate, cannot be fermented further to yield energy. But if oxygen once again becomes available, the microbe can then use it to oxidize the fermentation products to CO₂, a process that does yield energy.

3. Why do you think so many environmental microbes cannot be cultured in laboratory broth or agar media?

ANS: Think about what kinds of substances microbes have access to in “wild” environments. 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 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 underrecognized 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. The Part I interview of Rita Colwell describes how she and Anwar Huq devised an inexpensive way for Bangladeshi villagers to prevent cholera. What historical discoveries in microbiology, both medical and environmental, laid the foundation for their approach? **ANS:** The work of Huq and Colwell was based on a century of study of *Vibrio cholerae* through traditional methods of pure culture (invented by Koch) and microscopy (visual and EM, developed by many microscopists), as well as more recent approaches of biofilm microbiology (discovered by Colwell and others). Study of metabolism was important to show that *V. cholerae* could digest chitin, a major polymer of the copepod exoskeleton. These findings contributed to the hypothesis that *V. cholerae* could be growing in association with copepods. Finally, Florence Nightingale’s foundation of medical statistics enabled Huq and Colwell to design a study showing a declining incidence of cholera associated with sari-cloth water filtration.
CHAPTER 2  Observing the Microbial Cell

SUMMARY

This chapter introduces microbial observation. Students can be taken through its history from van Leeuwenhoek’s observation of “wee animalcules” to the most recent development of scanning probe microscopy to visualize live molecules at incredible amounts of magnification.

All forms of microscopy are discussed with examples of what has been observed with 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

This section introduces all the generic terms used in microscopy. It is imperative that students understand detection, resolution, and magnification and how they are related. It is also important to understand the size range 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.
• Fig. 2.6 shows three examples of the use of light microscopy 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.
• Fig. 2.7 serves to reinforce this concept by showing size ranges observable with each technique and a photograph to illustrate the point.

2.2 Optics and Properties of Light

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 (ultraviolet and X-rays) as well as longer wavelengths (such as microwaves and radiowaves). 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

• Fig. 2.8 presents the electromagnetic spectrum, which is useful for discussing the relationship between wavelength and frequency.
• After explaining Fig. 2.8, you may want to ask the students to discuss why the resolution obtained through fluorescence microscopy is greater than that obtained through light microscopy.
• Fig. 2.12 illustrates how to use a lens and the principle of how diffraction leads to magnification.

2.3 Bright-Field Microscopy

Bright-field microscopy is the method that most students 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 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 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 Fig. 2.17. Students should be introduced to the operation and terms relevant to bright-field microscopy,
- Fig. 2.16 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. It is illustrated in Figs. 2.22 and 2.23.

2.4 Dark-Field, Phase-Contrast, and Interference 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. As such, no stains are needed, and hence we can observe living cells. There is a short discussion about interference microscopy. Its strong point is that it allows the best definition of shape, but it does not work well with organisms with a low refractive index.

Discussion Points

- The physics of the spider light stop condenser system in dark-field microscopy is illustrated in Fig. 2.26.
- Studying motility is possible using dark-field microscopy. An excellent illustration of this is shown in Fig. 2.27. You can show this figure and follow up with Thought Question 2.6 to provoke class discussion.
- Fig. 2.29 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.

2.5 Fluorescence 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

- Fig. 2.31A shows a fluorophore at the molecular level, and Fig 2.31B 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.
- Fig. 2.33 illustrates the use of two fluorophores to observe cells during the sporulation process.
- Figs. 2.34 and 2.35 are microscopic observations by confocal microscopy.

2.6 Electron Microscopy

Electron microscopy is based on magnification using a beam of electrons as the radiation source. Electrons traveling in a voltage potential contain 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 microscope (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.

**Discussion Points**

- Discuss sample preparation for TEM and SEM microscopy and the possibility of artifact introduction in the process. Use Figs. 2.40 and 2.41 to compare the resulting images.
- Discuss cryo-EM and how sample preparation leaves the specimen in a form that should closely resemble the viable form.
- Use Fig. 2.38 to compare and contrast light microscopy and TEM.
- Discuss sample preparation for TEM, including fixation, embedding, sectioning, and staining.
- Fig. 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.
- Discuss the effects of artifacts in microscopy. For example, Fig. 2.43 demonstrates a few examples that involve artifacts originally identified as nanobacteria.
- Fig. 2.44 and Figs. 1 and 2 in Special Topic 2.1 can be used to discuss how three-dimensional visualization can be achieved.

### 2.7 Visualizing Molecules

The previous discussions have all concerned resolving microbes or subcellular structures. The discussion now moves to the techniques of X-ray crystallography, which allows study at the molecular level. X-ray crystallography has revealed the structure of many molecules. Probably the most widely discussed is the X-ray crystallography of DNA done by Rosalind Franklin, which has been used to elucidate the double-helical nature of DNA.

**Discussion Points**

- Fig. 2.45 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 to model molecular structure, as illustrated in Fig. 2.47.

### Process Animations

The following Process Animation for Chapter 2 can be found on the Instructor’s Resource Disc, and at the Microbiology StudySpace student website at microbiology2.com/animations.

- Microscopy

### Weblinks

The following weblinks appear in Chapter 2, and on the Microbiology StudySpace student website at microbiology2.com/links.

#### Molecular Expressions: Exploring the World of Optics and Microscopy

The Molecular Expressions website features photo galleries that explore the fascinating world of optical microscopy. Molecular Expressions offers one of the Web’s largest collections of color photographs taken through an optical microscope (commonly referred to as “photo-micro-graphs”).

http://www.microscopy.fsu.edu/

#### Videos of Swimming Bacteria

Howard Berg at the Rowland Institute, Harvard, maintains a collection of on-line videos of many kinds of motile bacteria, including *E. coli*, *Salmonella*, *Rhodobacter*, *Pseudomonas*, *Mycoplasma*, and more. Includes flagellar, twitching, gliding, and *Mycoplasma* motility.

http://www.rowland.harvard.edu/labs/bacteria/index_movies.html

#### Protein Data Bank: Research Collaboratory for Structural Bioinformatics Protein and Nucleic Acid Databases

The RCSB Protein Database provides a variety of tools and resources for studying the structures of biological macromolecules and their relationships to sequence, function, and disease.

http://www.rcsb.org/pdb/home/home.do

#### Biomolecules at Kenyon

Molecular tutorials by undergraduate students on anthrax lethal factor and other proteins, as well as instructions to write your own tutorials.

http://biology.kenyon.edu/BMB/biomolecules.htm
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. Define 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 wave front 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.

Scattering: This occurs when a portion of the wave front 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).

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 wave front.

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

**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.


**Answers to Review Questions (p. 70)**

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. Define 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 wave front 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.

Scattering: This occurs when a portion of the wave front 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).

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 wave front.

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 pixilated 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, which passes through the diaphragm, the condenser lens, the specimen, the objective lens, and 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 preparation 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 the difference between transmission and scanning electron microscopy and 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 (3-D) 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. 71)**

1. Explain what features of bacteria you can study by: light microscopy; fluorescence microscopy; scanning EM; transmission EM.
   **ANS:** Light microscopy shows the overall shape of bacterial cells. In a stained specimen, light microscopy can...
3. Explain why artifacts appear, 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.