Prescotts Microbiology 12th edition Willey Solutions Manual

SKU: 9781264088393-SOLUTIONS

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2 Microscopy

CHAPTER OVERVIEW

This chapter provides a relatively detailed description of the bright-field microscope and its use. Other common types of light microscopes are also described. Following this, various procedures for the preparation and staining of specimens are introduced. The chapter continues with a description of the two major types of electron microscopes and the procedures associated with their use. It concludes with descriptions of recent advances in microscopy: electron cryotomography and scanning probe microscopy.

LEARNING OUTCOMES

After reading this chapter, students should be able to:

- relate the refractive indices of glass and air to the path light takes when it passes through a prism or convex lens
- correlate lens strength and focal length
- evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope
- predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen
- create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced
- recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist
- plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible
- compare what happens to Gram-positive and Gram-negative bacterial cells during each step of the Gram-staining procedure
- create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes
- decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography
- distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses
- compare and contrast light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution, and the quality of the image created

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GUIDELINES FOR ANSWERING THE COMPREHENSION CHECK QUESTIONS

As the name of this section implies, these questions range from first-order retrieval of information from the preceding text to application of the content to open-ended questions or scenarios. As such, the answers are either easily obtained from the text or may have many different answers that can be considered valid. For this reason, we do not include these questions in the instructor's manual.

GUIDELINES FOR ANSWERING THE MICRO INQUIRY QUESTIONS

Figure 2.2: ***How would the focal length change if the lens shown here were thicker?***
As you increase the lens thickness, then the focal length would decrease, which in turn means the magnification would be greater. Note that this is true of the convex lenses used in microscopes, not for all lenses.

Figure 2.10: ***What is the purpose of the condenser annulus in a phase-contrast microscope?***
The condenser annulus is used to direct a ring of light to the condenser. The light is then focused by the condenser onto the specimen. The deviated and undeviated light patterns created by the specimen pass through the phase plate to achieve phase-contrast illumination.

Figure 2.13: ***How might the fluorescently labeled antibody used in figure 2.13b be used to diagnose an infectious disease?***
Fluorescent microscopy can be performed on a specimen sample collected from the patient. Because the antibody is specific for the pathogen, an image should only be obtained if the pathogen causing the disease is present. Fluorescently tagged antibodies are commonly used to diagnose certain bacterial infections (e.g., syphilis, chlamydial infections).

Figure 2.17: ***Why is the decolorization step considered the most critical in the Gram-staining procedure?***
The difference between Gram-positive staining cells and Gram-negative staining cells is not absolute, but temporal. Thus, over-decolorizing (too long) causes all cells to appear Gram-negative (because with enough time, even Gram-positive cell walls will lose the crystal violet-iodine complex), and under-decolorizing (too short) causes all cells to falsely appear Gram-positive. So, the decolorization step is technically critical. This is the step where the Gram-positive and Gram-negative cells are first visually different as well.

Figure 2.23: ***Why are all electron micrographs black and white (although they are sometimes artificially colorized after printing)?***
Color in light comes from different wavelengths of the photons, while the electrons used do not have colors. The images are artificially colorized to make them easier to view (see Figure 2.26).

Figure 2.28: ***Compare the magnification of this image with that shown in figure 2.26 (SEM). How many times greater is the magnification shown here?***
Scanning tunneling microscopy has the highest magnification of the image (×2,000,000) followed by transmission electron microscopy (figure 2.24b, ×42,750), while the scanning electron micrograph (figure 2.27, ×15,549) has the lowest magnification.

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GUIDELINES FOR ANSWERING THE ACTIVE LEARNING QUESTIONS

1. ***You have prepared a specimen for light microscopy, stained it using the Gram staining procedure, but failed to see anything when you looked through your light microscope. Discuss the things that you may have done incorrectly.***
Students consider the many steps that must be performed successfully with the Gram stain. There could be issues with insufficient heat fixation, over-vigorous washing, improper staining or destaining, over-decolorization, focusing, etc.

2. ***What type of microscopy and stain (if appropriate) would you use to visualize each of the following? (There may be more than one correct answer.) Be sure to explain your answer. Mycobacterium tuberculosis (which causes tuberculosis), microbes in pond scum, Staphylococcus aureus from a skin wound, measles virus, bacterial flagella, Borrelia burgdorferi (the spirochete that causes Lyme disease), and isolated bacterial ribosomes.***
Possible answers for each example include:

Mycobacterium tuberculosis – Acid-fast staining on a bright-field microscope to visualize the difficult to stain mycolic acid in the cell walls of *M. tuberculosis.*

Microbes in pond scum – Dark-field microscope, phase-contrast microscope, differential interference contrast (DIC) microscope, and any other microscope that can visualize live microbes.

Staphylococcus aureus from a skin wound – Many options possible - Bright-field microscope with simple staining or Gram staining, Confocal microscopy for biofilm observation, TEM for high resolution visualization, and more.

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Measles virus – TEM or electron cryotomography to have the magnification needed to visualize viruses.

Bacterial flagella – Flagella stain using a bright-field microscope to thicken the flagella so they are large enough to be visualized or electron microscopy.

Borrelia burgdorferi – Dark-field microscope to visualize the unique shape. Other possibilities that allow for visualization of a bacterial cell will also work.

Isolated bacterial ribosomes – Electron microscope or scanning tunneling microscope to produce large enough magnification to visualize bacterial ribosomes.

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3. ***Stimulated emission depletion (STED) microscopes were first developed in the early 21st century. They are modifications of confocal microscopes that use two highly focused beams of light rather than one, as in typical confocal microscopy. The first beam uses a wavelength that excites the fluorophore with which a specimen is labeled. This creates a fluorescent spot where the fluorophore is located. The second beam is a hollow cone. It strikes the outermost edges of the fluorescent spot illuminated by the first beam. The wavelength used by the second beam returns the fluorophore to its ground state, thus only the center of the fluorescent spot is visible. The result is that the resolution of STED microscopes is greater and objects smaller than 200 nm can be resolved. If you could use STED microscopy, what questions in biological science would you want to ask and why would you ask these questions? You may want to refer to figures 2.15 and 2.19 to help formulate your answer.***

This is opinion, so there may be various answers. The answer should discuss the benefit of improved resolution to questions related to the observation of individual micro-organisms that would otherwise be impossible to resolve with confocal microscopy. Students should provide and discuss support for the biological significance of each proposed question.

4. ***In response to the COVID-19 pandemic, a group of Chinese scientists used cryo-EM to visualize the human cell receptor ACE-2 alone and interacting with the receptor-binding domain of the virus spike protein. What advantage did cryo-EM provide as compared to scanning or transmission EM? List at least three reasons this information is important in terms of understanding disease caused by the virus and the development of treatments and a vaccine.***

Read the original paper: Yan, R. et al. 2020. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*. 367:1444-1448. DOI: 10.1126/science.abb2762.

Cryo-EM allows for the use of TEM to visualize molecules that have been rapidly frozen to retain their 3-D shape. Since cryo-EM uses TEM, it has better resolution than scanning EM but with the advantage of preparing the specimen in such a way that the 3-D shape is maintained. Knowing the 3-D structure of the spike protein is important for the following reasons:

(1) To properly identify the receptor it is capable of binding. Knowing the receptor the virus uses can help understand which cells of the body are susceptible to infection by the virus, which is essential knowledge for understanding disease progression.
(2) To identify possible therapeutic targets. Understanding the interactions between the spike protein and the receptor it binds to can lead to identification of potential therapeutic targets that can block this interaction.
(3) To identify essential information for vaccine production. Knowing the structure of the spike protein and its receptor can aid in the development of vaccines that can produce an immune response to and memory of the spike protein.

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