CHAPTER 2: Observing the Microbial Cell

MULTIPLE CHOICE

1. Who proved that stomach ulcers are caused by a bacterium?
   a. Hooke
   b. Marshall
   c. Gram
   d. Jenner
   e. van Leeuwenhoek

   ANS: B DIF: Easy REF: Introduction TOP: VI
   MSC: Remembering

2. The part of the human eye that is most involved in resolving an image is the:
   a. iris
   b. lens
   c. optic nerve
   d. retina
   e. cornea

   ANS: D DIF: Medium REF: 2.1 TOP: I.A
   MSC: Remembering

3. A ball-shaped microbe is referred to as a:
   a. bacillus
   b. coccus
   c. vibrio
   d. strepto
   e. spirochete

   ANS: B DIF: Easy REF: 2.1 TOP: I.C.ii.b
   MSC: Remembering

4. Resolution is the smallest distance by which two objects can be _____ and still be _____.
   a. magnified; seen
   b. separated; distinguished
   c. magnified; separated
   d. distinguished; separated
   e. magnified; distinguished

   ANS: B DIF: Medium REF: 2.1 TOP: I.A.i
   MSC: Understanding

5. 400 nm is equivalent to:
   a. 4.0×10^{-5} m
   b. 4.0×10^{-6} m
   c. 4.0×10^{-7} m
   d. 4.0×10^{-8} m
   e. 4.0×10^{-9} m

   ANS: C DIF: Medium REF: 2.1 TOP: I.A
   MSC: Applying

6. Which of these series arranges microbes from smallest to largest?
   a. virus → bacterium → red blood cell → paramecium
   b. virus → red blood cell → bacterium → paramecium
   c. bacterium → virus → paramecium → red blood cell
   d. bacterium → virus → red blood cell → paramecium
   e. paramecium → red blood cell → bacterium → virus

   ANS: A DIF: Difficult REF: 2.1 TOP: I.C
   MSC: Applying

7. The heating of water when exposed to light is primarily due to:
   a. reflection
   b. refraction
   c. absorption
   d. scattering
   e. fluorescence

   ANS: C DIF: Difficult REF: 2.2 TOP: II.B.i.a
   MSC: Remembering

8. Wavelength interference results in small observed objects (like bacteria) being surrounded by:
   a. a capsule
   b. a membrane
   c. an Airy disk
   d. a dark field
   e. a cell wall

   ANS: C DIF: Medium REF: 2.2 TOP: II.C
   MSC: Remembering

9. What is the most important property that enables a lens to magnify an image?
   a. absorption
   b. fluorescence
   c. reflection
   d. refraction
   e. scattering

   ANS: D DIF: Medium REF: 2.2 TOP: II.C
   MSC: Understanding

10. When two waves are out of phase by _____ wavelength, they produce destructive interference, canceling each other's amplitude and resulting in contrast in the image.
    a. one-tenth of a
    b. one-eighth of a
    c. one-quarter of a
    d. one-half of a
    e. one

    ANS: D DIF: Difficult REF: 2.2 TOP: II.D
    MSC: Understanding

11. Increasing the refractive index of the medium between the object and the objective lens increases:
    a. refraction
    b. reflection
    c. magnification
    d. resolution
    e. wavelength

    ANS: D DIF: Difficult REF: 2.2 TOP: II.D
    MSC: Understanding

12. If a glass slide was submerged in a beaker of immersion oil, the slide would be:
    a. undetectable
    b. brighter than its surroundings
    c. darker than its surroundings
    d. fluorescent
    e. stained

    ANS: A DIF: Medium REF: 2.2 TOP: II.A.ii.a
    MSC: Applying

13. What would happen if a lens had the same refractive index as air?
    a. Light would not pass through the lens.
    b. The image would be magnified more than with a glass lens.
    c. The image would be magnified, but the resolution would be less than with a glass lens.
    d. The image would be magnified, and the resolution would be greater than with a glass lens.
    e. The image would not be magnified.

ANS: E DIF: Medium REF: 2.2 TOP: II.C
MSC: Applying

14. The highest useful magnification for a light microscope is about:
    a. 100X
    b. 1,000X
    c. 10,000X
    d. 100,000X
    e. 1,000,000X

    ANS: C DIF: Medium REF: 2.2 TOP: II.D
    MSC: Applying

15. A(n) _____ acts to vary the diameter of the light column in a light microscope.
    a. condenser
    b. objective
    c. ocular
    d. diaphragm
    e. lens

    ANS: D DIF: Easy REF: 2.3 TOP: III.B.i
    MSC: Remembering

16. Which of these arranges the steps of the Gram stain into the correct order?
    a. iodine → crystal violet → decolorizer → safranin
    b. safranin → decolorizer → crystal violet → iodine
    c. crystal violet → decolorizer → iodine → safranin
    d. crystal violet → decolorizer → safranin → iodine
    e. crystal violet → iodine → decolorizer → safranin

    ANS: E DIF: Medium REF: 2.3 TOP: III.E.ii.a
    MSC: Remembering

17. When Gram stained, most eukaryotes appear:
    a. colorless
    b. purple
    c. pink
    d. green
    e. black

    ANS: C DIF: Medium REF: 2.3 TOP: III.E.ii.a
    MSC: Remembering

18. Malachite green is commonly used to stain:
    a. eukaryotic cells
    b. Gram-negative cells
    c. Gram-positive cells
    d. bacterial endospores
    e. acid-fast cells

    ANS: D DIF: Medium REF: 2.3 TOP: III.E.ii.c
    MSC: Remembering

19. As lens strength increases, the light cone _____ and the lens must be _____ the object.
    a. narrows; nearer to
    b. narrows; farther from
    c. widens; nearer to
    d. widens; farther from
    e. widens; touching

    ANS: C DIF: Medium REF: 2.3 TOP: III.A
    MSC: Understanding

20. Staining helps to visualize bacteria by:
    a. increasing the size of the cells

b. increasing the motility of the cells
c. increasing the contrast of the image
d. increasing the magnification of the image
e. increasing the aberration of the image

ANS: C DIF: Medium REF: 2.3 TOP: III.D.iii
MSC: Understanding

21. In a proper Gram stain, positive cells are stained by:
a. crystal violet only
b. safranin only
c. both crystal violet and safranin
d. neither crystal violet nor safranin
e. not enough information has been provided to know

ANS: C DIF: Medium REF: 2.3 TOP: III.E.ii.a
MSC: Understanding

22. Which two components of the Gram stain form a complex that is retained by Gram-positive cells?
a. crystal violet and iodine
b. safranin and iodine
c. crystal violet and safranin
d. alcohol and safranin
e. alcohol and iodine

ANS: A DIF: Medium REF: 2.3 TOP: III.E.ii.a
MSC: Understanding

23. Which of the following is best visualized using a negative stain?
a. Gram-negative cell wall
b. acid-fast cell wall
c. capsule
d. endospores
e. flagella

ANS: C DIF: Medium REF: 2.3 TOP: III.E.ii.d
MSC: Understanding

24. Which of these numeric aperture and light combinations would give the best resolution?
a. numeric aperture = 0.8, wavelength = 600 nm
b. numeric aperture = 0.8, wavelength = 500 nm
c. numeric aperture = 1.0, wavelength = 700 nm
d. numeric aperture = 1.0, wavelength = 600 nm
e. numeric aperture = 0.8, wavelength = 400 nm

ANS: E DIF: Difficult REF: 2.3 TOP: III.A
MSC: Applying

25. What is the total magnification of a light microscope when using a 25X ocular and 40X objective lens?
a. 15X
b. 65X
c. 400X
d. 1,000X
e. 1,200X

ANS: D DIF: Medium REF: 2.3 TOP: III.B.i
MSC: Applying

26. What is the best explanation for a Gram-positive bacterium appearing pink after performing a Gram stain?
a. The crystal violet was left on for too long.
b. The iodine was left on for too long.

c. The decolorizer was left on for too long.
d. The safranin was left on for too long.
e. The stain was properly performed.

ANS: C DIF: Difficult REF: 2.3 TOP: III.E.ii.a
MSC: Analyzing

27. What is the best explanation for a Gram-negative bacterium appearing purple after performing a Gram stain?
a. The safranin was not applied.
b. The decolorizer was not applied.
c. The iodine was not applied.
d. The crystal violet was not applied.
e. The stain was properly performed.

ANS: B DIF: Difficult REF: 2.3 TOP: III.E.ii.a
MSC: Analyzing

28. A useful application of dark-field optics is the study of bacterial:
a. motility
b. surfaces
c. interiors
d. shape
e. structure

ANS: A DIF: Medium REF: 2.4 TOP: IV.A
MSC: Remembering

29. Which of the following techniques are based upon wave interference?
a. X-ray diffraction and phase contrast microscopy
b. phase contrast and dark-field microscopy
c. bright-field and dark-field microscopy
d. X-ray diffraction and atomic force microscopy
e. scanning and transmission electron microscopy

ANS: A DIF: Medium REF: 2.4 TOP: IV.A | IV.B
MSC: Understanding

30. In which type of microscopy do dust particles interfere the most?
a. bright-field microscopy
b. dark-field microscopy
c. phase-contrast microscopy
d. interference microscopy
e. fluorescence microscopy

ANS: B DIF: Easy REF: 2.4 TOP: IV.A.iii
MSC: Understanding

31. Which of the following would be best suited to observe the motility of microbial cells?
a. Gram stain
b. nuclear magnetic resonance
c. scanning electron microscopy
d. negative stain
e. phase-contrast microscopy

ANS: E DIF: Medium REF: 2.4 TOP: IV.B
MSC: Understanding

32. DAPI is a dye that is commonly used in _____ microscopy.
a. bright-field
b. dark-field
c. phase contrast
d. confocal
e. fluorescence

ANS: E DIF: Medium REF: 2.5 TOP: V.B.i
MSC: Remembering

33. A fluorophore used in fluorescence microscopy that absorbs light at 260 nm would most likely fluoresce at:
a. 100 nm
b. 200 nm
c. 260 nm
d. 400 nm
e. 800 nm

ANS: D DIF: Difficult REF: 2.5 TOP: V.A
MSC: Understanding

34. The fluorophore DAPI specifically binds:
a. the cytoplasm
b. the cell wall
c. protein
d. RNA
e. DNA

ANS: E DIF: Easy REF: 2.5 TOP: V.B.i
MSC: Understanding

35. The aromatic groups of the fluorophore DAPI associate exclusively with the:
a. cell wall
b. base pairs of DNA
c. flagella
d. cell membrane
e. pili

ANS: B DIF: Medium REF: 2.5 TOP: V.B.i
MSC: Understanding

36. Fluorescence microscopy using labeled antibodies is referred to as:
a. immunofluorescence
b. autofluorescence
c. confocal microscopy
d. phase-contrast microscopy
e. dark-field microscopy

ANS: A DIF: Easy REF: 2.5 TOP: V.B.ii
MSC: Understanding

37. Which of the following can be used to localize proteins in a microbial cell?
a. DAPI and immunofluorescence
b. acridine orange and green fluorescent protein (GFP) fusions
c. DAPI and acridine orange
d. GFP fusions and immunofluorescence
e. DAPI and GFP fusions

ANS: D DIF: Difficult REF: 2.5 TOP: V.B.ii | V.B.iii
MSC: Understanding

38. Which of these techniques can be used to localize the DNA sequence at the origin of replication in a bacterial cell?
a. fluorescence microscopy
b. phase contrast
c. X-ray diffraction
d. atomic force microscopy
e. cryo-EM

ANS: A DIF: Difficult REF: 2.5 TOP: V.B.iv
MSC: Understanding

39. Which form of microscopy is used with DNA microarrays to observe differences in gene expression?
    a. light microscopy
    b. atomic force microscopy
    c. scanning electron microscopy
    d. transmission electron microscopy
    e. confocal fluorescence microscopy

    ANS: E DIF: Difficult REF: 2.5 TOP: V.C
    MSC: Understanding

40. The knife used to cut embedded specimens for observation by transmission electron microscopy is called a:
    a. crystallographer
    b. microtome
    c. grid
    d. polymer
    e. scalpel

    ANS: B DIF: Easy REF: 2.6 TOP: VI.B.i
    MSC: Remembering

41. Atomic force microscopy measures _____ between a probe and an object to map the three-dimensional topography of a cell.
    a. hydrogen bonds
    b. covalent interactions
    c. van der Waals forces
    d. pH changes
    e. magnetic interactions

    ANS: C DIF: Easy REF: 2.6 TOP: VI.D.ii
    MSC: Remembering

42. Which type of microscopy is particularly useful to study the surfaces of live bacteria?
    a. atomic force
    b. scanning electron
    c. transmission electron
    d. dark-field
    e. bright-field

    ANS: A DIF: Easy REF: 2.6 TOP: VI.D.ii
    MSC: Remembering

43. Transmission electron microscopy commonly has a resolution of _____ times the highest resolution possible for light microscopy.
    a. 10
    b. 100
    c. 1,000
    d. 10,000
    e. 1,000,000

    ANS: C DIF: Difficult REF: 2.6 TOP: VI.A
    MSC: Understanding

44. Which of the following would be most appropriate to visualize viral particles being assembled inside an infected bacterial cell?
    a. dark-field microscopy
    b. atomic force microscopy
    c. fluorescence microscopy
    d. scanning electron microscopy
    e. transmission electron microscopy

    ANS: E DIF: Medium REF: 2.6 TOP: VI.A | VI.B
    MSC: Understanding

45. A microscopic structure that is interpreted incorrectly is a/an:
    a. microtome
    b. crystal
    c. shadow
    d. antibody
    e. artifact

ANS: E DIF: Easy REF: 2.6 TOP: VI.C.i
MSC: Understanding

46. Unlike transmission electron microscopy, cryo-electron microscopy:
    a. requires making thin slices of the sample to be viewed
    b. does not require staining with heavy metals
    c. may be used to view living tissues
    d. uses a weaker electron beam
    e. can provide a color image of the microbial cell

    ANS: B DIF: Medium REF: 2.6 TOP: VI.D
    MSC: Understanding

47. The digitally combined images of cryo-EM can achieve resolution comparable to that of:
    a. scanning electron microscopy
    b. transmission electron microscopy
    c. interference microscopy
    d. X-ray crystallography
    e. dark-field microscopy

    ANS: D DIF: Difficult REF: 2.6 TOP: VI.D.i
    MSC: Understanding

48. Which of the following techniques can visualize bacteria without focusing electromagnetic radiation?
    a. cryo-electron microscopy
    b. phase-contrast microscopy
    c. dark-field microscopy
    d. atomic force microscopy
    e. X-ray diffraction

    ANS: D DIF: Medium REF: 2.6 TOP: VI.D.ii
    MSC: Understanding

49. The spots recorded on film during X-ray diffraction analyses are due to:
    a. artifacts
    b. scattering
    c. wave interference
    d. absorption
    e. fluorescence

    ANS: C DIF: Medium REF: 2.7 TOP: VII.A.i
    MSC: Understanding

50. Which of these techniques would provide the best resolution of an enzyme's structure?
    a. scanning electron microscopy
    b. transmission electron microscopy
    c. cryo-EM
    d. X-ray diffraction analysis
    e. atomic force microscopy

    ANS: D DIF: Medium REF: 2.7 TOP: VII.A.i
    MSC: Understanding

SHORT ANSWER

1. List and describe three common shapes of bacteria.

    ANS:
    Bacilli (bacillus in the singular) are rod-shaped bacteria. Cocci (singular, coccus) are spherical-shaped bacteria. Spirochetes are tightly coiled spirals or corkscrew-shaped bacteria.

    DIF: Easy REF: 2.1 TOP: I.C.ii MSC: Remembering

2. Microbes were detected long before the invention of the microscope. How could this be?

   ANS:
   Detection is the ability to observe the presence of an object, such as when we detect a group of bacteria in a culture tube or growing on a surface like a food product. Even though we can detect the group, we can't resolve individual cells without the magnification afforded by microscopes.

   DIF: Easy REF: 2.1 TOP: I.B.i MSC: Understanding

3. Are all bacilli *Bacillus*? Explain.

   ANS:
   No. *Bacillus* refers to a particular genus of organisms that are commonly found in the soil. Although they are rod-shaped, the members of this genus are not the only bacteria that have this cellular morphology. The term bacillus refers to any rod-shaped microbe, which means that not all bacilli belong to the genus *Bacillus*.

   DIF: Easy REF: 2.1 TOP: I.C.ii MSC: Understanding

4. If your eyes had photoreceptors packed as closely as an eagle's (about eight times greater than humans), would you be able to resolve a virus (100 nm in size) using a light microscope? Why or why not?

   ANS:
   No. Although your resolving power would be much improved, the light microscope's power will still be limited by the wavelengths of light that you can see (roughly 400 nm for human eyes). Objects less than 400 nm cannot be resolved by light in the visible spectrum.

   DIF: Medium REF: 2.1 TOP: I.A MSC: Applying

5. Describe three conditions that are necessary for electromagnetic radiation to resolve an object.

   ANS:
   There must be contrast between the object and its surroundings. The wavelength of the radiation must be equal to or smaller than the size of the object. The detector must have sufficient resolution for the given wavelength.

   DIF: Medium REF: 2.2 TOP: II.A.ii MSC: Remembering

6. List and briefly describe four ways that light interacts with objects.

   ANS:
   (1) Absorption: light energy is absorbed by an object. (2) Reflection: a wavefront bounces off of an object at an angle equal to its incident angle. (3) Refraction: bending of light when it enters a substance that slows its speed. (4) A scattering wavefront interacts with an object of smaller dimensions than the wavelength.

   DIF: Medium REF: 2.2 TOP: II.B.i MSC: Remembering

7. Compare and contrast a simple stain (like methylene blue) with the Gram stain. What information about a microbial sample can be collected with each?

   ANS:

Both staining procedures colorize bacterial cells, thereby increasing the sample's contrast and improving resolution. A simple stain will color all microbial cells uniformly. This allows one to record the relative size, shape, and arrangement of any cells present. The Gram stain is a differential stain. In addition to size, shape, and arrangement, this procedure allows one to determine if the cells have a Gram-positive (purple) or Gram-negative (pink) cell wall structure.

DIF: Medium REF: 2.3 TOP: III.E.i MSC: Understanding

8. List three different differential stains used in microbiology. What can be detected with each?

ANS:
The most common differential stain is the Gram stain. This procedure allows one to distinguish between cells having one membrane (Gram-positive) and two membranes (Gram-negative). Another common differential stain is the acid-fast stain. Carbolfuchsin stains the mycolic acid–containing acid-fast cells of the genus *Mycobacterium*. The endospore stain is a differential stain that stains endospores with malachite green. Negative staining and antibody staining are also included in the text.

DIF: Medium REF: 2.3 TOP: III.E.ii MSC: Understanding

9. What color are Gram-positive and Gram-negative cells when properly Gram stained? For each step of the Gram stain procedure, predict the colors of a Gram-positive or Gram-negative cell if that step were omitted during staining. Explain your reasoning.

ANS:
Properly Gram stained Gram-positive cells are purple and Gram-negative are pink.

(1) Skipping primary stain (crystal violet): Gram-positive and Gram-negative would both be pink. No crystal violet–iodide complex would be formed in the Gram-positive wall. All cells would be decolorized and take on the color of safranin.

(2) Skipping mordant (iodine): Gram-positive and Gram-negative would both be pink. No crystal violet–iodine complex would be formed in the Gram-positive wall. All cells would be decolorized and take on the color of safranin.

(3) Skipping decolorizer (alcohol): Gram-positive and Gram-negative would both be purple. The crystal violet–iodine complex would remain in all cells. Although safranin still binds, the purple color is so much more intense that the pink of the safranin cannot be seen.

(4) Skipping secondary stain (safranin): Gram-positive cells would be purple. The Gram-negative cells would be colorless. The dye complex will be removed from the Gram-negative cells, but they will be difficult to see since the counterstain was not applied.

DIF: Medium REF: 2.3 TOP: III.E.ii.a MSC: Understanding

10. Why do some bacteria appear purple after being Gram stained, while others appear pink?

ANS:
Gram-negative cells have a few layers of peptidoglycan cell wall and an outer lipopolysaccharide membrane. Gram-positive organisms have several layers of peptidoglycan and no outer membrane. The multiple layers of peptidoglycan retain the crystal violet–iodine complex, so appear purple. Gram-negative cells do not retain the crystal violet because there are few layers of peptidoglycan and the outer membrane is disrupted by the decolorizer.

DIF: Medium REF: 2.3 TOP: III.E.ii.a MSC: Understanding

11. Compare and contrast the radiation sources, lenses, and image-capturing devices used in light microscopy and transmission electron microscopy.

ANS:
The radiation source for light microscopy is a light, whereas for electron microscopy it is an electron source or tungsten filament. The lenses in the light microscope are glass, whereas magnets are used in electron microscopy. The lenses have similar functions and are arranged in the same order in both types of microscopy. Light microscopy uses a condenser lens, whereas the lens in electron microscopy is called the projection lens. The image-capturing device for light is the human eye, or sometimes a camera. The image-capturing device for electron microscopy is a fluorescent screen.

DIF: Difficult REF: 2.3 | 2.6 TOP: III.B | VI.A MSC: Understanding

12. Why are stains used in microscopy? Compare and contrast the stains used in light versus electron microscopy.

ANS:
Stains are used to increase the contrast between an object and its surroundings, so as to make it visible. The stains used in light microscopy are usually charged and interact with different cellular components. Positively charged dyes bind to negatively charged cell surfaces. They also are colored, so they impart color to a cell or its components. The stains used for electron microscopy are heavy metals or salts, which increase the density of certain components, again increasing contrast. In electron microscopy, the image of the microbe is always black and white.

DIF: Difficult REF: 2.3 | 2.6 TOP: III.D | VI.B MSC: Understanding

13. Name two types of microscopy that are suitable for directly studying bacterial motility. What interaction of light with the microbe is most important for each of these techniques?

ANS:
Either dark-field or phase-contrast microscopy could be used. In dark-field microscopy, the condenser contains an opaque disk held by three “spider legs” across an open ring. No light travels directly up through the specimen, so the only light that reaches the eye is light that is scattered by objects on the slide. This scattered light allows detection of objects that are too small to be resolved by light rays. Phase-contrast microscopy exploits differences in refractive index between cell components and transforms them into differences in intensity of transmitted light due to wave interference.

DIF: Medium REF: 2.4 TOP: IV.A | IV.B MSC: Understanding

14. If you are interested in studying the localization of a protein in a bacterial cell, what techniques would provide you with the best information?

ANS:
Fluorescence microscopy can be used to study protein localization. One method would be to use fluorescently tagged antibodies to detect the proteins using immunofluorescence microscopy. Another possibility would be to make green fluorescent protein fusions with the protein of interest. These hybrid proteins would fluoresce wherever they are in the cell.

DIF: Difficult REF: 2.5 TOP: V.B MSC: Understanding

15. Define a fluorophore and give three examples of how it can be used to label cells.

ANS:

A fluorophore is a fluorescent molecule that can be used to stain a specimen for observation with a fluorescence microscope. Some fluorophores, such as DAPI, have affinity for certain cell chemicals. Antibodies can be labeled with fluorescent dyes and reacted with specific targets in immunofluorescence. Short sequences of DNA attached to a fluorophore can be used to hybridize and label target DNA.

DIF: Difficult REF: 2.5 TOP: V.B MSC: Understanding

16. Archaea and Bacteria differ in the genetic sequences of their ribosomal RNA genes. How can this difference be used to microscopically differentiate between members of these domains?

ANS:
Short DNA sequences that are homologous to either the Bacterial or Archaeal sequences can be conjugated to fluorophores that emit different wavelength light. These probes will anneal to the complementary DNA in the corresponding cells in a sample. When viewed using a fluorescence microscope, the archaeal and bacterial cells will have different colors. This is referred to as fluorescence in situ hybridization, or FISH, analysis.

DIF: Difficult REF: 2.5 TOP: V.B.iv MSC: Applying

17. Most electron micrographs in microbiology textbooks are in color. Is this normal for an electron micrograph? Why or why not?

ANS:
Electron micrographs are not naturally colored. The original image is produced when the electrons bombard a fluorescent screen. The resultant image is processed by a computer to appear as black and white with intensities in the entire range of grays in between. These images are later colorized using computer software (like Photoshop) to improve the aesthetics and provide additional information.

DIF: Difficult REF: 2.6 TOP: VI.A MSC: Understanding

18. Give a few reasons why living organisms may not be observed by transmission electron microscopy (TEM) or scanning electron microscopy (SEM).

ANS:
In TEM, the specimens are fixed and embedded into a polymer for sectioning. The specimen is then stained with heavy metal to increase contrast. In SEM, the entire organism is shadowed with heavy metal prior to observation. Most importantly, however, the entire optical column of the EM must be maintained under vacuum, and a living specimen would be quickly destroyed by an electron beam.

DIF: Easy REF: 2.6 TOP: VI.A | VI.B MSC: Understanding

19. Describe three methods of sample preparation for electron microscopy. Which method would cause the fewest artifacts? Why?

ANS:
(1) Samples can be embedded in a polymer and cut into thin sections with a microtome, then coated with a heavy metal. (2) Samples can be sprayed onto a copper grid, then treated with a heavy metal. (3) Samples may be flash frozen for cryo-electron microscopy. Cryo-EM will cause the fewest artifacts. When using this technique, the cells are not fixed or artificially stained. Instead, the cells are flash frozen—leaving the cell components still hydrated and closest to their original state.

DIF: Medium REF: 2.6 TOP: VI.B MSC: Understanding