

CHAPTER 2: Observing the Microbial Cell

MULTIPLE CHOICE

1. *Chlamydia trachomatis* transmits infection to a new cell via
- a membrane vesicle.
 - elementary bodies.
 - amoeba.
 - host membranes.
 - the human cell.

ANS: B DIF: Easy REF: 2.Intro
 OBJ: 2.1d Identify the type(s) of microscopy needed to view a particular specimen
 MSC: Remembering

2. The part of the human eye that is MOST involved in resolving an image is the
- iris.
 - lens.
 - optic nerve.
 - retina.
 - cornea.

ANS: D DIF: Easy REF: 2.1
 OBJ: 2.1a Explain how the structure of the human eye dictates the resolution of objects
 MSC: Remembering

3. A ball-shaped microbe is referred to as a
- bacillus.
 - coccus.
 - vibrio.
 - strepto.
 - spirochete.

ANS: B DIF: Easy REF: 2.1
 OBJ: 2.1c Describe and identify bacterial morphologies (e.g., bacilli, cocci, and spirochetes)
 MSC: Remembering

4. Resolution is the smallest distance by which two objects can be _____ and still be _____.
- magnified; seen
 - separated; distinguished
 - magnified; separated
 - distinguished; separated
 - magnified; distinguished

ANS: B DIF: Medium REF: 2.1
 OBJ: 2.1b Differentiate between resolution and detection MSC: Remembering

5. 400 nm is equivalent to
- 4.0×10^{-5} m.
 - 4.0×10^{-6} m.
 - 4.0×10^{-7} m.
 - 4.0×10^{-8} m.
 - 4.0×10^{-9} m.

ANS: C DIF: Medium REF: 2.1
 OBJ: 2.1a Explain how the structure of the human eye dictates the resolution of objects
 MSC: Applying

6. Which of these series arranges microbes from smallest to largest?
- virus → bacterium → red blood cell → paramecium
 - virus → red blood cell → bacterium → paramecium
 - bacterium → virus → paramecium → red blood cell
 - bacterium → virus → red blood cell → paramecium
 - paramecium → red blood cell → bacterium → virus

ANS: A DIF: Difficult REF: 2.1
OBJ: 2.1c Describe and identify bacterial morphologies (e.g., bacilli, cocci, and spirochetes)
MSC: Applying

7. What happens if the wavelength of radiation is larger than the object being viewed?
- a. absorption
 - b. scattering
 - c. no resolution
 - d. refraction
 - e. resolution

ANS: C DIF: Easy REF: 2.2
OBJ: 2.2a Identify what conditions must exist for electromagnetic radiation to resolve an object from neighboring objects or the surrounding medium MSC: Remembering

8. Wavelength interference results in small observed objects (like bacteria) being surrounded by
- a. a capsule.
 - b. a membrane.
 - c. concentric rings.
 - d. a dark field.
 - e. a cell wall.

ANS: C DIF: Difficult REF: 2.2
OBJ: 2.2b Explain the properties of light MSC: Applying

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: Easy REF: 2.2
OBJ: 2.2c Differentiate among absorption, reflection, refraction, and scattering
MSC: Remembering

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
OBJ: 2.2d Explain how lenses magnify images 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
OBJ: 2.2d Explain how lenses magnify images MSC: Understanding

12. If aqueous cytoplasm 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
OBJ: 2.2b Explain the properties of light MSC: Applying

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

ANS: E DIF: Medium REF: 2.2

OBJ: 2.2c Differentiate among absorption, reflection, refraction, and scattering

MSC: Applying

14. The highest useful magnification for a light microscope is about
- 100X.
 - 1,000X.
 - 10,000X.
 - 100,000X.
 - 1,000,000X.

ANS: C DIF: Easy REF: 2.3

OBJ: 2.3a State the factors that influence image quality in bright-field microscopy

MSC: Applying

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

ANS: D DIF: Easy REF: 2.3

OBJ: 2.3c Explain the function of the components of a compound microscope

MSC: Remembering

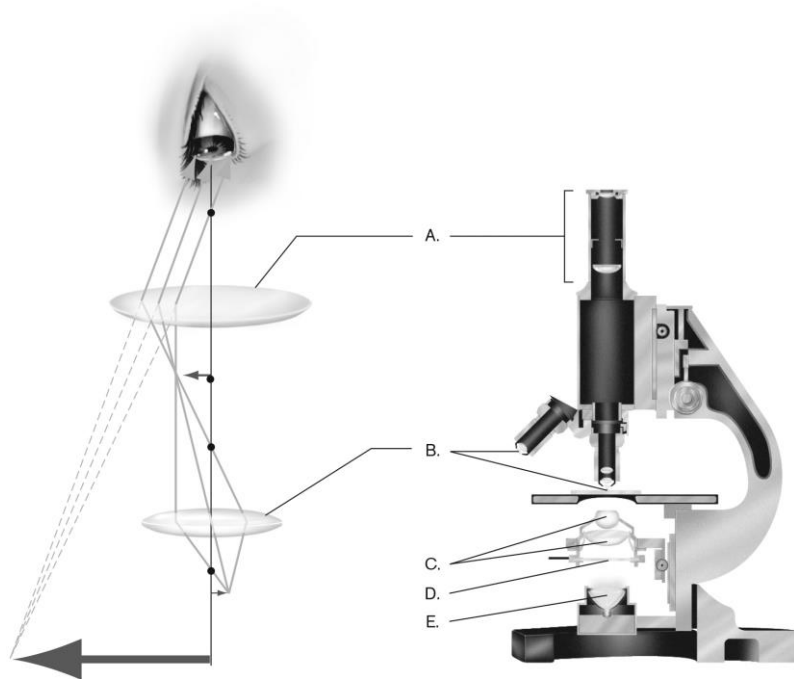
16. Which of these arranges the steps of the Gram stain into the correct order?
- iodine → crystal violet → decolorizer → safranin
 - safranin → decolorizer → crystal violet → iodine
 - crystal violet → decolorizer → iodine → safranin
 - crystal violet → decolorizer → safranin → iodine
 - crystal violet → iodine → decolorizer → safranin

ANS: E DIF: Medium REF: 2.3

OBJ: 2.4g Explain how the structure of the bacterial cell wall relates to its Gram-stain status

MSC: Remembering

17. Label the condenser in the figure below.



- a. A
- b. B
- c. C

- d. D
- e. E

ANS: C

DIF: Easy

REF: 2.3

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron microscopy

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: Easy

REF: 2.3

OBJ: 2.4f Classify stains as simple or differential

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: Easy

REF: 2.3

OBJ: 2.3a State the factors that influence image quality in bright-field microscopy

MSC: Understanding

20. Staining helps to visualize bacteria by increasing the

- a. size of the cells.
- b. motility of the cells.
- c. contrast of the image.
- d. magnification of the image.
- e. aberration of the image.

ANS: C

DIF: Easy

REF: 2.3

OBJ: 2.4e Explain the advantages and disadvantages of fixing and staining specimens

MSC: Remembering

21. One advantage of a wet mount is
- a. low contrast.
 - b. that the organism is still.
 - c. viewing in a natural state.
 - d. high contrast.
 - e. that the sample does not overheat.

ANS: C DIF: Easy REF: 2.3

OBJ: 2.3d Describe the advantages and disadvantages of a wet mount

MSC: Remembering

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

OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status

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

OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status

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

OBJ: 2.3a State the factors that influence image quality in bright-field microscopy

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: Easy REF: 2.3

OBJ: 2.3c Explain the function of the components of a compound microscope

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

OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status

MSC: Analyzing

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

ANS: B DIF: Difficult REF: 2.3

OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status

MSC: Analyzing

28. Which type of microscopy is used to identify the 3-D structure of biofilms?
- phase-contrast
 - dark-field
 - fluorescent
 - bright-field
 - confocal

ANS: E DIF: Easy REF: 2.4

OBJ: 2.4c Describe the advantages of super-resolution imaging

MSC: Analyzing

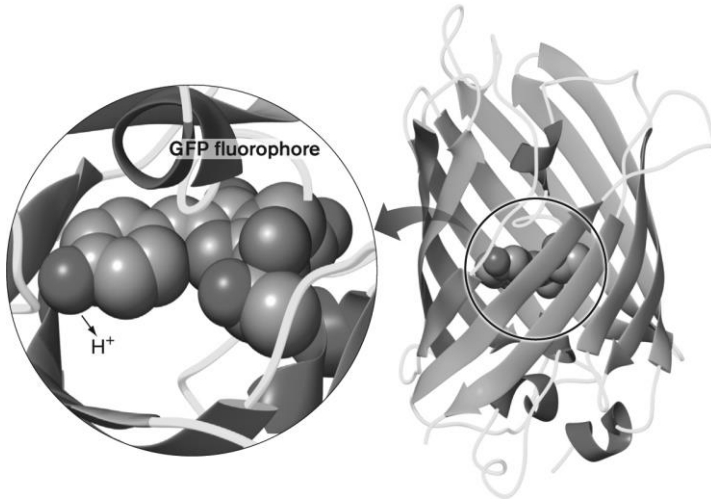
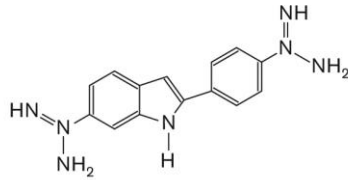
29. Fluorescent microscopy that absorbs light at 260nm would MOST likely fluoresce at
- 100 nm.
 - 200 nm.
 - 260 nm.
 - 400 nm.
 - 800 nm.

ANS: D DIF: Difficult REF: 2.4

OBJ: 2.4b Recall the factors that determine the cell specificity of a fluorophore

MSC: Applying

30. What molecule is shown below?



- a. GFP
- b. FM4-64
- c. DNA
- d. RNA
- e. DAPI

ANS: E DIF: Medium REF: 2.4

OBJ: 2.4b Recall the factors that determine the cell specificity of a fluorophore

MSC: Applying

31. What happens to an electron when excited by light at the excitation wavelength?
- a. It remains in the same orbital.
 - b. It moves to a lower energy orbital.
 - c. It moves to a higher energy orbital.
 - d. It emits a photon.
 - e. It is filtered.

ANS: C DIF: Medium REF: 2.4

OBJ: 2.4a Differentiate between the excitation and emission wavelengths

MSC: Understanding

32. 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: 2.4

OBJ: 2.4b Recall the factors that determine the cell specificity of a fluorophore

MSC: Understanding

33. Which of the following is an advantage of using chemical imaging microscopy?
- a. observing an organism in its living environment
 - b. displaying plankton
 - c. measuring pH within a cell
 - d. showing distribution of chemicals in a cell
 - e. pinpointing a protein location

ANS: D DIF: Medium REF: 2.4

OBJ: 2.4d State the function of chemical imaging microscopy MSC: Applying

34. 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: Easy REF: 2.4

OBJ: 2.4b Recall the factors that determine the cell specificity of a fluorophore
MSC: Understanding

35. 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: 2.5

OBJ: 2.5a Describe under what conditions dark-field microscopy is the best choice for viewing specimens
MSC: Remembering

36. 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: 2.5

OBJ: 2.5b Explain the principles behind phase-contrast microscopy
MSC: Applying

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

ANS: B DIF: Easy REF: 2.5

OBJ: 2.5a Describe under what conditions dark-field microscopy is the best choice for viewing specimens
MSC: Analyzing

38. Which type of microscopy gives a “false 3-D image”?
- a. Gram stain
 - b. nuclear magnetic resonance
 - c. scanning electron
 - d. negative stain
 - e. differential interference contrast

ANS: E DIF: Easy REF: 2.5

OBJ: 2.5c State the types of images obtained with differential interference contrast microscopy
MSC: Remembering

39. Which type of microscopy is used to view the internal structures of a specimen?
- a. light
 - b. atomic force
 - c. scanning electron
 - d. transmission electron
 - e. confocal fluorescence

ANS: D DIF: Easy REF: 2.6

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron microscopy
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

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron microscopy
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

OBJ: 2.6c Proposed applications for scanning probe microscopy
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

OBJ: 2.6c Proposed applications for scanning probe microscopy
MSC: Applying

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

ANS: C DIF: Difficult REF: 2.6

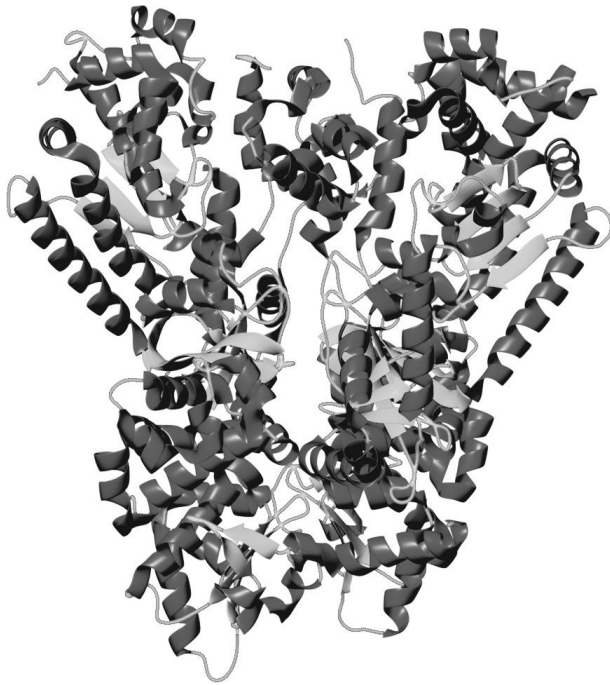
OBJ: 2.6a Compare and contrast light microscopy and electron microscopy
MSC: Applying

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: 2.3

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron microscopy
MSC: Understanding

45. Which of the following methods is used to generate the structure shown below?



- a. scanning probe microscopy
- b. scanning electron microscopy
- c. transmission electron microscopy
- d. confocal microscopy
- e. X-ray crystallography

ANS: E DIF: Easy REF: 2.6

OBJ: 2.6d State what kind of information is obtained from X-ray crystallography analysis

MSC: Understanding

46. Cryo-electron microscopy (cryo-EM) is differentiated from transmission electron microscopy because it
- 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: Easy REF: 2.6

OBJ: 2.6e Explain the theory of using cryo techniques in cryo-electron microscopy and cryocrystallography MSC: Analyzing

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

OBJ: 2.6e Explain the theory of using cryo techniques in cryo-electron microscopy and cryocrystallography MSC: Analyzing

48. Which of the following techniques can visualize bacteria without focusing electromagnetic radiation?
- a. cryo-electron microscopy
 - d. atomic force microscopy

- b. phase-contrast microscopy
- c. dark-field microscopy
- e. X-ray diffraction

ANS: D DIF: Medium REF: 2.6
OBJ: 2.6c Proposed applications for scanning probe microscopy
MSC: Analyzing

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
OBJ: 2.6d State what kind of information is obtained from X-ray crystallography analysis
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
OBJ: 2.6d State what kind of information is obtained from X-ray crystallography analysis
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
OBJ: 2.1c Describe and identify bacterial morphologies (e.g., bacilli, cocci, and spirochetes)
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 such as a food product. Even though we can detect the group, we cannot resolve individual cells without the magnification afforded by microscopes.

DIF: Easy REF: 2.1
OBJ: 2.1a Explain how the structure of the human eye dictates the resolution of objects
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

OBJ: 2.1c Describe and identify bacterial morphologies (e.g., bacilli, cocci, and spirochetes)

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

OBJ: 2.1a Explain how the structure of the human eye dictates the resolution of objects

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: Easy REF: 2.2

OBJ: 2.2a Identify what conditions must exist for electromagnetic radiation to resolve an object from neighboring objects or the surrounding medium

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 an object at an angle equal to its incident angle. (3) Refraction: light bends when it enters a substance that slows its speed. (4) Scattering: a wavefront interacts with an object of smaller dimensions than the wavelength.

DIF: Medium REF: 2.2

OBJ: 2.2b Explain the properties of light

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 OBJ: 2.3f Classify stains as simple or differential
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. Carbol-fuchsin 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 OBJ: 2.3f Classify stains as simple or differential
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
OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status
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
OBJ: 2.3g Explain how the structure of the bacterial cell wall relates to its Gram-stain status
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

OBJ: 2.3c Explain the function of the components of a compound microscope

MSC: Understanding

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

ANS:

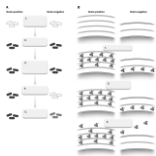
Stains are used to increase the contrast between an object and its surroundings, so as to make it visible. The stains used in light microscopy are usually charged and interact with different cellular components. Positively charged dyes bind to negatively charged cell surfaces. They also are colored, 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

OBJ: 2.3e Explain the advantages and disadvantages of fixing and staining specimens

MSC: Understanding

13. The figure below shows the Gram-staining process. Describe what is happening at step number 3 relative to bacterial cell walls.



ANS:

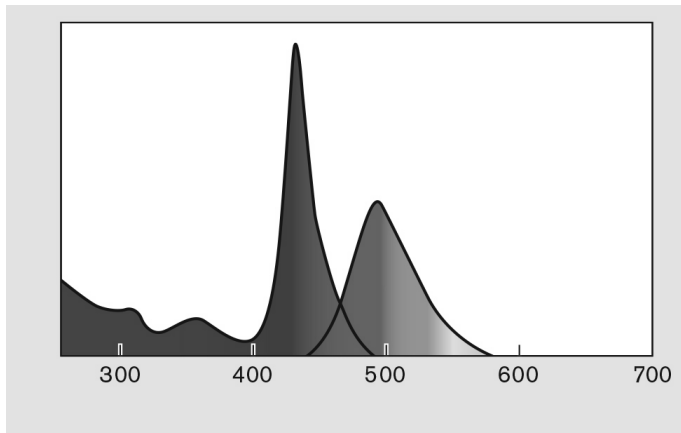
Iodine is added and binds to Gram-positive cells with greater affinity than Gram-negative cells. This is due to a greater concentration of peptidoglycan in the Gram-positive cell walls.

DIF: Easy REF: 2.3

OBJ: 2.4g Explain how the structure of the bacterial cell wall relates to its Gram-stain process

MSC: Understanding

14. The figure below displays absorption and emission spectra for a fluorophore. Describe the difference between absorption and emission and how this is reflected in the figure.



ANS:

The highest peak at a higher energy level is absorption. This is when the photon is absorbed by the electron. The lower peak represents emission, which is when the electron loses energy and drops to a lower orbital.

DIF: Easy REF: 2.4

OBJ: 2.4a Differentiate between the excitation and emission wavelengths

MSC: Understanding

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

ANS:

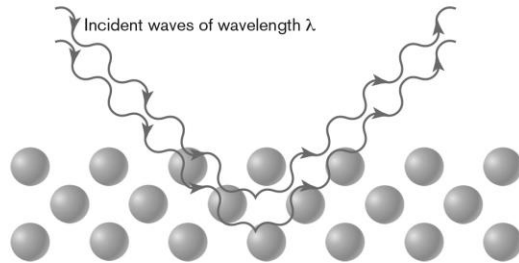
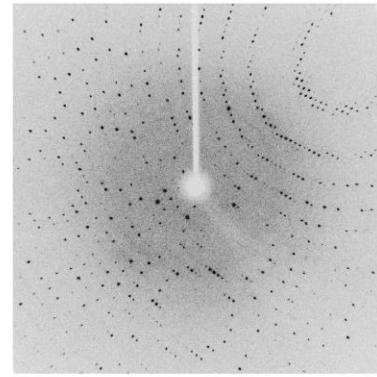
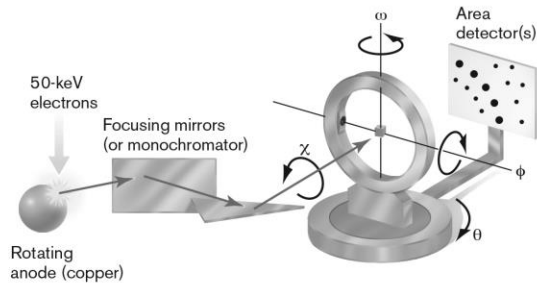
A fluorophore is a fluorescent molecule that can be used to stain a specimen for observation with a fluorescence microscope. Some fluorophores, such as DAPI, have affinity for certain cell chemicals. Antibodies can be labeled with fluorescent dyes and reacted with specific targets in immuno- fluorescence.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

DIF: Difficult REF: 2.4

OBJ: 2.4b Recall the factors that determine the cell specificity of a fluorophore

MSC: Understanding

16. Using the figure below, explain how the visualization of molecules occurs through X-ray crystallography.



ANS:

The X-ray beam focuses on a crystal and is rotated over all the angles and provides a diffraction pattern. The intensity of the diffraction is recorded; this pattern is analyzed using a model of individual molecules.

DIF: Difficult REF: 2.6

OBJ: 2.6d State what kind of information is obtained from X-ray crystallography analysis

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, as color is defined by visible light, not electrons. 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

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron microscopy

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 an electron beam would quickly destroy a living specimen.

DIF: Easy REF: 2.6

OBJ: 2.3b Identify images obtained via scanning electron microscopy and transmission electron

microscopy 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-electron microscopy 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

OBJ: 2.6e Explain the theory of using cryo techniques in cryo-electron microscopy and cryocrystallography MSC: Understanding

20. Explain why components from thermophilic bacteria take less X-ray damage than other bacteria.

ANS:

There is always unavoidable deterioration of specimens under the exposure of X-rays. However, thermophilic bacteria have more stable crystals than homologous crystals growing at moderate temperatures. Since the crystals are more stable, they can better withstand X-ray exposure.

DIF: Medium REF: 2.6

OBJ: 2.6e Explain the theory of using cryo techniques in cryo-electron microscopy and cryocrystallography MSC: Understanding