CHAPTER 2: Observing the Microbial Cell

MULTIPLE CHOICE

1. Who compared "small animals" from his teeth before and after drinking coffee?
   a. Hooke
   b. Fleming
   c. Gram
   d. Jenner
   e. van Leeuwenhoek

   ANS: E DIF: Easy REF: Introduction OBJ: Factual
   TOP: Introduction

2. One _____ is one-thousandth of a millimeter.
   a. micrometer
   b. nanometer
   c. meter
   d. centi
   e. kilo

   ANS: A DIF: Medium REF: 2.1 OBJ: Factual
   TOP: I.A

3. In humans, resolution is achieved by focusing an image on a retina, packed with light-absorbing:
   a. rods
   b. cones
   c. nerves
   d. photoreceptor cells
   e. lenses

   ANS: D DIF: Medium REF: 2.1 OBJ: Factual
   TOP: I.A

4. Having fewer photoreceptors per surface area means higher:
   a. resolution
   b. magnification
   c. refraction
   d. reflection
   e. wavelength

   ANS: A DIF: Difficult REF: 2.1 OBJ: Applied
   TOP: I.A

5. 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 OBJ: Applied
   TOP: I.A.i

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

   ANS: A DIF: Easy REF: 2.1 OBJ: Factual
   TOP: I.C.ii.a

7. All electromagnetic radiation travels through a vacuum at what speed?
   a. 3×10^8 mm/sec
   b. 3×10^8 cm/sec
   c. 3×10^8 m/sec
   d. 3×10^8 ft/sec
   e. 3×10^8 mph

   ANS: C DIF: Difficult REF: 2.2 OBJ: Factual
   TOP: II.A

8. If an object and its surroundings absorb or reflect radiation equally then the object will be:
   a. undetectable
   b. reflected
   c. refracted
   d. radiated
   e. fluoresced

   ANS: A DIF: Medium REF: 2.2 OBJ: Applied
   TOP: II.A.ii.a

9. Which 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 OBJ: Applied
   TOP: II.C

10. What is the key property that enables a lens to magnify an image?
    a. reflection
    b. resolution
    c. frequency
    d. refraction
    e. wavelength

    ANS: D DIF: Medium REF: 2.2 OBJ: Applied
    TOP: II.C

11. Magnification without resolution is known as _____ magnification.
    a. complete
    b. zero
    c. maximum
    d. total
    e. empty

    ANS: E DIF: Easy REF: 2.2 OBJ: Factual
    TOP: II.D

12. 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
    b. one-eighth
    c. one-quarter
    d. one-half
    e. one

    ANS: D DIF: Difficult REF: 2.2 OBJ: Factual
    TOP: II.D

13. An image is magnified when light passes through a refractive material shaped so as to _____ its rays.
    a. absorb
    b. block
    c. concentrate
    d. condense
    e. spread

    ANS: E DIF: Medium REF: 2.2 OBJ: Applied
    TOP: II.D

14. 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: Applied
    TOP: II.D

15. With the 100× lens, the refractive index of light passing through the specimen is maintained by insertion of _____, with a refractive index comparable to that of glass.
    a. immersion oil
    b. water
    c. air
    d. bacteria
    e. stain

    ANS: A DIF: Easy REF: 2.3 OBJ: Factual
    TOP: III.A

16. 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; touch

    ANS: C DIF: Medium REF: 2.3 OBJ: Factual
    TOP: III.A

17. A/An _____ 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 OBJ: Factual
    TOP: III.B.i

18. The total _____ of the microscope is obtained by multiplying the magnification of the ocular lens by that of the objective lens.
    a. resolution
    b. magnification
    c. refraction
    d. reflection
    e. wavelength

    ANS: B DIF: Easy REF: 2.3 OBJ: Applied
    TOP: III.B.i

19. Higher-power lenses require more light and thus an open:
    a. ocular
    b. lens
    c. objective
    d. condenser
    e. diaphragm

    ANS: E DIF: Easy REF: 2.3 OBJ: Applied
    TOP: III.B.i

20. If you are using a microscope with a 10× ocular lens and a 100× objective, what is the total magnification?
    a. 10-fold
    b. 100-fold
    c. 110-fold
    d. 1,000-fold
    e. This is not enough information.

    ANS: D DIF: Medium REF: 2.3 OBJ: Applied
    TOP: III.B.i

21. Which is the counterstain in the Gram stain procedure?
    a. crystal violet
    b. methylene blue
    c. malachite green
    d. safranin
    e. Gram's iodine

    ANS: D DIF: Easy REF: 2.3 OBJ: Factual
    TOP: III.E.ii.a

22. Which of the following staining processes requires crystal violet?
    a. acid-fast stain
    b. antibody stain
    c. negative stain
    d. Gram stain
    e. spore stain

    ANS: D DIF: Easy REF: 2.3 OBJ: Factual
    TOP: III.E.ii.a

23. Gram's iodine is the _____ in the Gram staining procedure.
    a. primary stain
    b. counterstain
    c. decolorizer
    d. negative stain
    e. mordant

    ANS: E DIF: Easy REF: 2.3 OBJ: Factual
    TOP: III.E.ii.a

24. Eukaryotes stain:
    a. Gram-neutral
    b. Gram-positive
    c. Gram-negative
    d. blue
    e. no color

    ANS: C DIF: Medium REF: 2.3 OBJ: Factual
    TOP: III.E.ii.a

25. Which of the following is a true statement about Gram staining?
    a. The Gram stain differentiates between the three domains.
    b. In a Gram-negative cell, the crystal violet–iodide complex is retained by multiple layers of peptidoglycan.
    c. The outer membrane is disrupted by the decolorizer.
    d. Human cells appear Gram-positive.
    e. The mordant is used so that Gram-negative cells may be observed.

    ANS: C DIF: Medium REF: 2.3 OBJ: Applied
    TOP: III.E.ii.a

26. The _____ in the Gram stain process binds to the crystal violet, generating a complex that is held more tightly within the cell.
    a. mordant
    b. safranin
    c. alcohol
    d. bacteria
    e. slide

    ANS: A DIF: Medium REF: 2.3 OBJ: Applied
    TOP: III.E.ii.a

27. Which of the following is a negative stain?
    a. acid-fast stain
    b. endospore stain
    c. antibody stain
    d. simple stain
    e. capsule stain

    ANS: E DIF: Easy REF: 2.3 OBJ: Factual
    TOP: III.E.ii.d

28. X-ray diffraction and phase contrast microscopy both involve:
    a. wave interference
    b. observation of living specimens
    c. differential stains
    d. simple stains
    e. shadowing.

    ANS: A DIF: Difficult REF: 2.4 OBJ: Applied
    TOP: IV.A | IV.B

29. Observations of bacterial flagella during motility are best suited to:
    a. bright-field microscopy
    b. dark-field microscopy
    c. SEM
    d. TEM
    e. NMR

    ANS: B DIF: Easy REF: 2.4 OBJ: Applied
    TOP: IV.A.ii

30. 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 OBJ: Applied
    TOP: IV.A.ii

31. 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 OBJ: Conceptual
    TOP: IV.A.iii

32. Which form of microscopy is based on the difference in refractive index between cell components and the surrounding medium?
    a. bright-field
    b. dark-field
    c. phase contrast
    d. confocal
    e. fluorescence

    ANS: C DIF: Medium REF: 2.4 OBJ: Applied
    TOP: IV.B

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

    ANS: D DIF: Difficult REF: 2.4 OBJ: Conceptual
    TOP: IV.D.i

34. When light is absorbed by an object and emitted at a longer wavelength, it is referred to as:
    a. fluorescence
    b. magnification
    c. reflection
    d. refraction
    e. radiation

    ANS: A DIF: Easy REF: 2.5 OBJ: Applied
    TOP: V.A

35. Fluorescence requires excitation and emission at different:
    a. refractive indexes
    b. wavelengths
    c. contrasts
    d. refractions
    e. densities

    ANS: B DIF: Easy REF: 2.5 OBJ: Applied
    TOP: V.A

36. In fluorescence microscopy, incident light is absorbed by the specimen and reemitted at a _____ energy, resulting in a _____.
    a. lower; longer wavelength
    b. lower; shorter wavelength
    c. higher; longer wavelength
    d. higher; shorter wavelength
    e. higher; higher contrast

    ANS: A DIF: Difficult REF: 2.5 OBJ: Applied
    TOP: V.A

37. 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 OBJ: Factual
    TOP: V.B

38. The fluorophore acridine orange specifically binds:
    a. cytoplasm
    b. cell wall
    c. protein
    d. RNA
    e. DNA

    ANS: E DIF: Easy REF: 2.5 OBJ: Factual
    TOP: V.B.i

39. The use of antibodies linked to fluorophores is known as:
    a. fluorescence
    b. immunofluorescence
    c. X-ray diffraction
    d. atomic force microscopy
    e. cryo-EM

    ANS: B DIF: Easy REF: 2.5 OBJ: Factual
    TOP: V.B.ii

40. Which form of microscopy is used with DNA microarrays to observe differences in gene expression?
    a. light microscopy
    b. atomic force microscopy
    c. SEM
    d. TEM
    e. confocal fluorescence microscopy

    ANS: E DIF: Difficult REF: 2.5 OBJ: Applied
    TOP: V.C

41. Which of the following is true of transmission electron microscopy but NOT scanning electron microscopy?
    a. The specimen is usually fixed and embedded.
    b. The embedded specimen is cut into thin sections with a microtome.
    c. The specimen is stained with heavy metal.
    d. The specimen is viewed as three-dimensional.
    e. The requirement for a vacuum precludes the viewing of live organisms.

    ANS: B DIF: Medium REF: 2.6 OBJ: Applied
    TOP: VI.A | VI.B

42. Transmission electron microscopy commonly has a resolution of _____ times the highest resolution possible for light microscopy.
    a. ten
    b. one hundred
    c. one thousand
    d. ten thousand
    e. one million

    ANS: C DIF: Difficult REF: 2.6 OBJ: Applied
    TOP: VI.A

43. In which one of the following types of microscopy is the specimen shadowed with heavy metal?
    a. atomic force microscopy
    b. SEM
    c. TEM
    d. X-ray diffraction
    e. dark-field

    ANS: B DIF: Medium REF: 2.6 OBJ: Factual
    TOP: VI.A.ii

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

    ANS: B DIF: Easy REF: 2.6 OBJ: Factual
    TOP: VI.B.i

45. Fixatives and heavy atom stains used in EM can introduce _____ into an image.
    a. colors
    b. resolution
    c. refraction
    d. artifacts
    e. fluorescence

    ANS: D DIF: Easy REF: 2.6 OBJ: Factual
    TOP: VI.C

46. 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 OBJ: Factual
    TOP: VI.C.i

47. In _____, because the frozen sample remains hydrated, the biological molecules retain the same conformation as in solution.
    a. cryo-EM
    b. TEM
    c. SEM
    d. fluorescence microscopy
    e. laser confocal microscopy

    ANS: A DIF: Easy REF: 2.6 OBJ: Applied
    TOP: VI.D.i

48. 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: Factual
    TOP: VI.D.ii

49. Which type of microscopy is particularly useful to study the surfaces of live bacteria?
    a. atomic force
    b. SEM
    c. TEM
    d. dark-field
    e. bright-field

    ANS: A DIF: Easy REF: 2.6 OBJ: Applied
    TOP: VI.D.ii

50. Which technique uses interference patterns from crystallized macromolecules to determine structure at atomic resolution?
    a. SEM
    b. TEM
    c. cryo-EM
    d. X-ray diffraction analysis
    e. atomic force microscopy

    ANS: D DIF: Medium REF: 2.7 OBJ: Factual
    TOP: VII.A.i

SHORT ANSWER

1. What does the phrase “eagle-eyed” mean? Describe why it is scientifically accurate.

   ANS:
   Eagle-eyed means sharp-sighted. Eagles’ eyes can resolve things eight times as small or eight times as far away as humans’ eyes because eagles’ photoreceptors are much more closely packed.

   DIF: Medium REF: 2.1 OBJ: Conceptual TOP: I.A

2. Why is it possible to detect microbes but NOT resolve them?

   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. Even though we can detect the group, we can’t resolve individual cells without magnification.

   DIF: Easy REF: 2.1 OBJ: Applied TOP: I.B.i

3. List and describe three common shapes of bacteria.

   ANS:
   Bacilli (singular, bacillus) 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: Factual TOP: I.C.ii

4. Explain when you would use *Bacillus* versus bacillus.

   ANS:
   Bacillus refers to a genus of organisms. A genus name is always capitalized and italicized. 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: Applied TOP: I.C.ii

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

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

   DIF: Medium REF: 2.2 OBJ: Factual TOP: II.A.ii

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

   ANS:
   1. Absorption. Light energy is absorbed by an object. 2. Reflection. Wave front bounces off of object at angle equal to its incident angle. 3. Refraction. Bending of light when it enters a substance that slows its speed. 4. Scattering wave front interacts with object of smaller dimension than the wavelength.

   DIF: Medium REF: 2.2 OBJ: Applied TOP: II.B.i

7. Compare and contrast the radiation source, the lenses, and the image-capturing device in light and TEM.

   ANS:
   The radiation source for light microscopy is a light, whereas for EM it is an electron source or tungsten filament. The lenses in the light microscope are glass, whereas magnets are used in EM. 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 EM is called the projection lens. The image-capturing device for light is the human eye, or sometimes a camera. The image-capturing device for EM is a fluorescent screen.

   DIF: Difficult REF: 2.3 | 2.6 OBJ: Conceptual TOP: III.B | VI.A

8. Describe the lens system of a compound microscope including the location of each lens and the purpose of each.

   ANS:
   The condenser lens is above the light source and functions to concentrate light rays up through the specimen. The objective lenses are immediately above the specimen and the function is to magnify the specimen. A typical light microscope has objective lenses that magnify 10×, 40×, and 100×. The ocular lens is located in the eyepiece and also magnifies the specimen. A typical ocular lens magnifies 10×.

   DIF: Easy REF: 2.3 OBJ: Applied TOP: III.B.i

9. 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 usually are 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 EM are heavy metals or salts, which increase the density of certain components, again increasing contrast. In EM, the image of the microbe is always black and white.

   DIF: Difficult REF: 2.3 | 2.6 OBJ: Conceptual TOP: III.D | VI.B

10. Briefly explain why or how fixatives and stains used in microscopy may introduce artifacts. How might this be determined?

    ANS:
    A specimen may be fixed in many different ways depending on the type of microscopy, but two examples are heat and chemical fixation. Heat can denature certain cellular components and chemicals can also alter structural integrity. Most stains are charged and interact with various cellular components. These interactions may also lead to alteration in structural characteristics. Sometimes it can be determined if something is an artifact by comparing the results using different forms of microscopy.

    DIF: Difficult REF: 2.3 OBJ: Conceptual TOP: III.D

11. Compare and contrast simple and differential stains. Give examples of each.

    ANS:
    A simple stain is used to increase contrast in order to visualize a specimen. Methylene blue, for example, will stain all cells equally. A differential stain allows one to distinguish between cell types or characteristics. The Gram stain procedure is a differential stain. It can be used to distinguish between Gram-positive and Gram-negative cells.

    DIF: Medium REF: 2.3 OBJ: Applied TOP: III.E

12. Briefly state the steps in the Gram stain procedure. What would happen if you accidentally forgot to use the counterstain?

    ANS:
    The Gram stain procedure involves four steps:
    1. the primary stain—crystal violet
    2. the mordant—Gram's iodine
    3. the decolorizer—ethanol
    4. the counterstain—safranin
    If you left out the counterstain step it would be difficult to detect any Gram-negative microbes that were present in the specimen.

    DIF: Medium REF: 2.3 OBJ: Conceptual TOP: III.E.ii.a

13. Why do some bacteria appear purple after being Gram stained and other appear red?

    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: Conceptual TOP: III.E.ii.a

14. Explain how the modified condenser in dark-field microscopy is used to make small microbes visible. Give one advantage and one disadvantage of dark-field microscopy.

    ANS:
    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. Advantages include the ability to detect live organisms without staining, detection of very small organisms that can't be seen with bright-field microscopy, and ability to visualize motility of microbes. Disadvantages are that shapes of objects can't be easily resolved and particulates may be mistaken for organisms.

    DIF: Medium REF: 2.4 OBJ: Applied TOP: IV.A

15. Explain phase-contrast microscopy and give one advantage and one disadvantage of phase-contrast microscopy.

    ANS:
    Phase-contrast microscopy exploits differences in refractive index between cell components and transforms them into differences in intensity of transmitted light. Advantages are that live cells can be viewed and the organelles of eukaryotes are visible. A disadvantage is that it is less effective for organisms whose cytoplasm as a low refractive index.

    DIF: Medium REF: 2.4 OBJ: Applied TOP: IV.B

16. Define a fluorophore and give three examples of how they 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 to and label target DNA.

    DIF: Difficult REF: 2.5 OBJ: Applied TOP: V.B

17. How does laser scanning confocal microscopy produce images?

    ANS:
    A laser beam is focused onto the specimen and scanned across it in two planes at right angles to each other. The laser beam excites a fluorophore and both the excitation and emitted light are focused together. This results in images with very high resolution.

    DIF: Medium REF: 2.5 OBJ: Applied TOP: V.C

18. Give a few reasons why living organisms may NOT be observed by TEM or 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 OBJ: Conceptual TOP: VI.A | VI.B

19. Why is it that a photographic image from an electron microscope is black and white?

    ANS:
    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.

    DIF: Difficult REF: 2.6 OBJ: Conceptual TOP: VI.A

20. Describe three methods of sample preparation for electron microscopy.

    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-EM.

    DIF: Medium REF: 2.6 OBJ: Applied TOP: VI.B