

Chapter 2: How We See the Invisible World

* = Correct answer

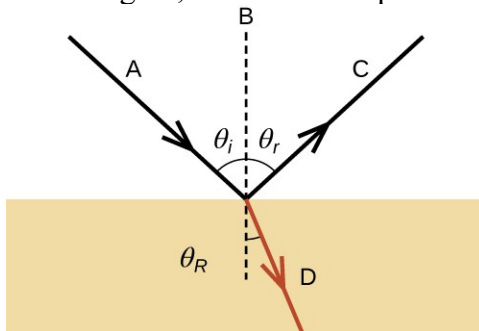
Multiple Choice

1. Which term is used to refer to a wave's rate of vibration?
 - A. amplitude
 - B. frequency*
 - C. trough
 - D. wavelength

Difficulty: Easy

ASM Standard: N/A

2. On the figure, which letter represents a refracted ray of light?



- A. A
- B. B
- C. C
- D. D*

Difficulty: Easy

ASM Standard: N/A

3. On the electromagnetic spectrum, which type of wave has the shortest wavelength?
 - A. cosmic ray*
 - B. infrared ray
 - C. ultraviolet ray
 - D. visible light

Difficulty: Moderate

ASM Standard: N/A

4. Dyes are often used during microscopy to increase which of the following?
 - A. contrast*
 - B. magnification

- C. motility
- D. resolution

Difficulty: Easy
ASM Standard: 32

5. Which of the following was the first to use the term “cells”?
- A. Robert Hooke*
 - B. Robert Koch
 - C. Carolus Linnaeus
 - D. Antonie van Leeuwenhoek

Difficulty: Easy
ASM Standard: N/A

6. Which of the following is the typical magnification of an object viewed using a 10× ocular lens and a 100× objective lens on a brightfield microscope?
- A. 1×
 - B. 10×
 - C. 100×
 - D. 1000×

Difficulty: Moderate
ASM Standard: 32

7. Most modern microscopes have two eyepieces, meaning that they are which of the following?
- A. binocular*
 - B. monocular
 - C. oil immersion
 - D. trinocular

Difficulty: Easy
ASM Standard: 32

8. An annular stop is used for which type of microscopy?
- A. confocal
 - B. darkfield
 - C. fluorescence
 - D. phase contrast*

Difficulty: Easy
ASM Standard: 32

9. Which of the following is the specific name for a stain that colors the background but not the specimen?

- A. acid-fast stain
- B. counterstain
- C. differential stain
- D. negative stain*

Difficulty: Easy
ASM Standard: 32

10. Which type of microscopy would work best for viewing internal cell structures?
- A. atomic force microscopy
 - B. scanning electron microscopy
 - C. scanning tunneling microscopy
 - D. transmission electron microscopy*

Difficulty: Moderate
ASM Standard: 32

11. The Gram-staining procedure is best described as which of the following?
- A. complex staining
 - B. differential staining*
 - C. negative staining
 - D. simple staining

Difficulty: Easy
ASM Standard: 32

12. When there is a substantial difference in refractive index between two materials, which of the following describes what will happen to light passing from one material to the other?
- A. It will fail to be refracted.
 - B. It will undergo a large amount of refraction.*
 - C. It will undergo a small amount of refraction.
 - D. It will undergo a variable amount of refraction.

Difficulty: Moderate
ASM Standard: N/A

13. In microscopy, the focal length refers to which of the following?
- A. a description of the wavelength needed for an image to be in focus
 - B. a measure of the amount of refraction produced by a lens
 - C. a measure of the thickness of the lens needed for an image to be in focus
 - D. the distance to the image point (at which all light entering a lens is parallel)*

Difficulty: Moderate
ASM Standard: 32

14. Which of the following are pigments used in microscopy to absorb and reflect light?

- A. chromatographs
- B. chromophores*
- C. oilophores
- D. photophores

Difficulty: Easy
ASM Standard: N/A

15. Negative staining is the standard approach used to visualize which structure(s)?

- A. capsules*
- B. endospores
- C. flagella
- D. nucleoid regions

Difficulty: Moderate
ASM Standard: 6, 32

16. Which is a standard mordant used in flagella staining?

- A. basic fuchsin
- B. iodine
- C. safranin
- D. tannic acid*

Difficulty: Moderate
ASM Standard: 6, 32

17. Which of the following pairs are common acid-fast staining techniques?

- A. Gram and Kinyoun
- B. negative and Schaeffer-Fulton
- C. Ziehl-Neelsen and Kinyoun*
- D. Ziehl-Neelsen and Schaeffer-Fulton

Difficulty: Moderate
ASM Standard: 32

18. Which is the primary stain used in the Ziehl-Neelsen technique?

- A. acid-alcohol
- B. carbolfuchsin*
- C. crystal violet
- D. methylene blue

Difficulty: Moderate
ASM Standard: 32

19. Which is the correct order of steps in Gram staining?

- A. crystal violet stain, ethanol decolorization, Gram's iodine mordant, safranin counterstain

- B. crystal violet stain, Gram's iodine mordant, ethanol decolorization, safranin counterstain*
- C. ethanol stain, crystal violet rinse, safranin counterstain, Gram's iodine mordant
- D. safranin stain, Gram's iodine mordant, ethanol decolorization, crystal violet counterstain

Difficulty: Easy
ASM Standard: 32

20. It is important to fix bacterial smears on a slide for which of the following reasons?
- A. to attach them to the slide*
 - B. to cause swelling of the cell to make interior structures more visible
 - C. to enhance the uptake of stains
 - D. to provide a nutrient medium to sustain the bacteria

Difficulty: Easy
ASM Standard: 32

21. Which of the following is not commonly used for chemical fixation?
- A. acetic acid
 - B. ethanol
 - C. glutaraldehyde
 - D. iodine*

Difficulty: Moderate
ASM Standard: 32

22. Which of the following is not involved in preparing a bacterial slide for Gram staining?
- A. allowing the sample to dry on the slide
 - B. fixing the specimen to the slide
 - C. placing a smear on the slide
 - D. preparing a wet mount*

Difficulty: Moderate
ASM Standard: 32

23. Which of the following most specifically refers to stains with positively charged chromophores?
- A. acid-fast dyes
 - B. acidic dyes
 - C. basic dyes*
 - D. positive dyes

Difficulty: Moderate
ASM Standard: 32

24. Which of the following most specifically refers to a stain that dyes a specimen rather than the background?

- A. counterstain
- B. mordant
- C. negative stain
- D. positive stain*

Difficulty: Moderate
ASM Standard: 32

25. Which of the following describes the difference between the Ziehl-Neelsen and Kinyoun acid-fast staining techniques?
- A. The Ziehl-Neelsen technique uses a counterstain and the Kinyoun technique does not.
 - B. The Ziehl-Neelsen technique uses an acidic dye and the Kinyoun technique does not.
 - C. The Ziehl-Neelsen technique uses heat and the Kinyoun technique does not.*
 - D. The Ziehl-Neelsen technique uses two dyes and the Kinyoun technique does not.

Difficulty: Moderate
ASM Standard: 32

26. Which of the following described the difference between gram-positive and gram-negative cells that causes them to stain differently after Gram staining?
- A. Gram-positive cells have a mycolic acid layer in their cell walls that holds in stain, whereas gram-negative cells lack this layer.
 - B. Gram-positive cells have capsules and gram-negative cells have S layers.
 - C. Gram-positive cells have thicker cell membranes than gram-negative cells.
 - D. Gram-positive cells have thicker peptidoglycan layers in their cell walls than do gram-negative cells.*

Difficulty: Moderate
ASM Standard: 7, 32, 34, 36

27. Which of the following would not potentially cause a gram-positive cell to appear gram-negative after Gram staining?
- A. counterstaining with crystal violet instead of safranin*
 - B. exposing the cells to ethanol for too long
 - C. using older bacterial cultures
 - D. using safranin as a primary stain

Difficulty: Moderate
ASM Standard: 32, 34, 36

28. Which of the following does not cause the probe to move up and down above the specimen in atomic force microscopy?
- A. chemical bonding
 - B. electrostatic forces
 - C. van der Waals forces
 - D. variations in current*

Difficulty: Difficult
ASM Standard: 32

29. Quantum tunneling of electrons is important in the mechanism underlying which microscope type?
- A. AFM
 - B. SEM
 - C. STM*
 - D. TEM

Difficulty: Moderate
ASM Standard: 32

30. Which of the following is the chemical in some bacterial cells' walls that makes them resist decolorization after acid-fast staining?
- A. acetic acid
 - B. mycolic acid*
 - C. peptidoglycan
 - D. phospholipid

Difficulty: Moderate
ASM Standard: 32

31. Gram staining is useful for bacterial identification. Which of the following is another way it is most useful?
- A. Knowing Gram staining results allows a researcher to know which organisms a bacterium can infect.
 - B. Knowing Gram staining results allows a researcher to select the appropriate growth medium for optimal bacterial growth.
 - C. Knowing Gram staining results is helpful in choosing an effective antibiotic.*
 - D. Knowing Gram staining results is helpful in understanding a bacterium's metabolic requirements.

Difficulty: Difficult
ASM Standard: 7, 23

32. Which of the following is not true regarding capsule staining?
- A. Capsules are typically stained using basic dyes.*
 - B. Capsule staining does not require heat fixation in advance.
 - C. Capsule staining requires careful selection of a stain that can stain the capsule itself.
 - D. Capsule staining typically involves negative staining.

Difficulty: Difficult
ASM Standard: 7, 23

33. Because endospores are difficult to stain, which of the following is generally needed?
- A. Heat must be used to make the endospore take up stain.
 - B. Mycolic acid must be added to encourage the endospore to take up stain.
 - C. Negative staining must be used to stain endospores because they do not absorb stain.*
 - D. The cells must be cooled to make the endospore more brittle prior to staining.

Difficulty: Difficult
ASM Standard: 7, 32

34. When endospores are stained using the Schaeffer-Fulton method, which of the following correctly describes how they appear after staining?
- A. The endospore appears green within a pink cell, because only the endospore retained the primary stain.*
 - B. The entire cell appears green because the endospore surrounds all the vegetative material.
 - B. The entire cell appears pink because the endospore surrounds all the vegetative material.
 - C. The entire cell appears pink except for a clear area representing the endospore.

Difficulty: Moderate
ASM Standard: 6, 7, 32

35. Specimens for TEM are prepared by doing which of the following?
- A. dehydrating the specimen in ethanol and then coating it in a material that will repel electrons
 - B. sputter coating with a substance such as gold to repel electrons
 - C. using an ultramicrotome to cut very thin sections from samples embedded in plastic resin*
 - D. using chemical fixation, followed by standard staining techniques such as the Gram stain

Difficulty: Difficult
ASM Standard: 32

True/False

36. In fluorescence, emitted photons have the same amount of energy as absorbed photons.
Answer: False

Difficulty: Easy
ASM Standard: N/A

37. A microscope with very high magnification and low resolution would allow you to clearly see small structures.
Answer: False

Difficulty: Easy
ASM Standard: 6

38. The difference between a simple microscope and a compound microscope is that the latter only has one lens.

Answer: False

Difficulty: Easy

ASM Standard: N/A

39. Flagella staining is very useful for diagnostic purposes in medical settings.

Answer: False

Difficulty: Moderate

ASM Standard: 7, 32

40. The transmission electron microscope and scanning electron microscope produce very similar images, but the former can show three-dimensional external structures.

Answer: False

Difficulty: Moderate

ASM Standard: 6, 32

41. Differential interference contrast microscopy produces three-dimensional images of specimens.

Answer: True

Difficulty: Moderate

ASM Standard: 32

42. During specimen preparation for transmission electron microscopy, the specimens are dehydrated in alcohol and embedded in plastic resin.

Answer: True

Difficulty: Difficult

ASM Standard: 32

43. In atomic force microscopy, the movement of the probe tip is determined using Hooke's law of elasticity.

Answer: True

Difficulty: Difficult

ASM Standard: 32

Matching

44. Match each term with the best definition.

- | | |
|--------------|---|
| A. amplitude | i. electromagnetic radiation within certain parameters |
| B. frequency | ii. the height of a wave peak or depth of a wave trough |

- C. visible light iii. the rate of vibration of a wave
D. wavelength iv. the distance between two wave peaks
Answers: A. ii., B. iii., C. i., D. iv.

Difficulty: Easy
ASM Standard: N/A

45. Match each term with the best definition.

- | | |
|-----------------|--|
| A. absorbance | i. Light waves change direction when they enter a new medium. |
| B. diffraction | ii. Two waves interact. |
| C. interference | iii. A wave travels through something. |
| D. refraction | iv. A material captures light energy. |
| E. transmission | v. Light bends or scatters as it interacts with objects or openings. |
- Answers: A. iv., B. v., C. ii., D. i., E. iii.

Difficulty: Moderate
ASM Standard: N/A

46. Match each type of microscope with a common use for that microscope type.

- | | |
|-------------------|--|
| A. brightfield | i. commonly used clinically; helpful in distinguishing living from dead cells, finding molecules within a cell, and for immunostaining |
| B. darkfield | ii. widely used and easily available; used for examining common protists, bacteria, and tissues; stains are often needed |
| C. fluorescence | iii. uses an annular stop, refraction, and interference to produce relatively high-resolution images without stains |
| D. phase contrast | iv. used to examine an image produced with light that hits a specimen indirectly owing to the use of an opaque light stop; often used to view living microorganisms without stains |
| E. two-photon | v. often used to penetrate thick specimens, such as biofilms, with long-wavelength light |

Answers: A. ii., B. iv., C. i., D. iii., E. v.

Difficulty: Difficult
ASM Standard: 32

47. Match each type of microscopy with the best description.

- | | |
|-------------------------------------|---|
| A. atomic force microscopy | i. measures changes in current as a probe is moved at a constant height over a specimen |
| B. scanning electron microscopy | ii. measures variations in the height of a probe moving over the specimen; height variations occur to maintain a constant current |
| C. scanning tunneling microscopy | iii. yields images from electrons produced by an electron beam hitting the surface of a sputter-coated specimen |
| D. transmission electron microscopy | iv. produces images from electrons that pass through a very thin specimen, allowing internal structures to be visualized |

Answers: A. ii., B. iii., C. i., D. iv.

Difficulty: Moderate
ASM Standard: 32

48. Match the staining procedure with the chemicals used.

- | | |
|--------------------|--|
| A. acid-fast stain | i. crystal violet, iodine, ethanol, safranin |
| B. capsule stain | ii. tannic acid or potassium alum, pararosaniline or basic fuchsin |
| C. endospore stain | iii. carbolfuchsin, acid alcohol, and methylene blue |
| D. flagella stain | iv. malachite green, safranin |
| E. Gram stain | v. India ink or nigrosin, sometimes with positive staining |

Answers: A. iii., B. v., C. iv., D. ii., E. i.

Difficulty: Moderate
ASM Standard: 32

49. The choices describe possible scenarios and outcomes from Gram staining with standard stains (e.g., crystal violet and safranin). Match the scenario with the most likely outcome.

- | | |
|---|---|
| A. A gram-negative cell is stained, but the ethanol step is skipped. | i. The cell will probably appear pink, which will not correctly indicate its cell wall structure. |
| B. A gram-positive cell with a severely damaged cell wall is stained. | ii. The cell will appear pink. |
| C. A healthy gram-negative cell is stained. | iii. The cell will appear purple/violet. |
| D. A healthy gram-positive cell is stained. | iv. The cell will probably appear purple/violet, which will not correctly indicate its cell wall structure. |

Answers: A. iv., B. i., C. ii., D. iii.

Difficulty: Difficult
ASM Standard: 6, 32

Fill in the Blank

50. Compared with air, water has a(n) _____ refractive index.

Answer: higher

Difficulty: Easy
ASM Standard: N/A

51. The _____ electron microscope produces high-resolution images of three-dimensional surface structures of sputter-coated specimens.

Answer: scanning

Difficulty: Moderate
ASM Standard: 32

52. Robert Hooke published _____, in which he described his observations of cork via compound microscopes.

Answer: *Micrographia*

Difficulty: Easy

ASM Standard: N/A

53. _____ microscopy uses an opaque stop, is useful for viewing living organisms without using stains, and produces images with a dark background.

Answer: Darkfield

Difficulty: Moderate

ASM Standard: 32

54. Acid-fast bacteria have _____ in their cell walls that retain carbolfuschin stain.

Answer: mycolic acids

Difficulty: Moderate

ASM Standard: 6, 7, 32

55. In acid-fast staining, cells that are not acid-fast usually appear _____.

Answer: blue

Difficulty: Moderate

ASM Standard: 32

56. During preparation of specimens for scanning electron microscopy, specimens are often sputter coated with _____ or a similar material.

Answer: gold

Difficulty: Moderate

ASM Standard: 32

57. Negative staining of capsules is commonly done using India ink or _____.

Answer: nigrosin

Difficulty: Moderate

ASM Standard: 6 32

58. An acid-fast staining technique similar to the Ziehl-Neelsen technique is called the _____ technique.

Answer: Kinyoun

Difficulty: Moderate

ASM Standard: 32

59. When an object is relatively small relative to a wavelength of light, diffraction is _____ than if an object is relatively large.

Answer: larger

Difficulty: Difficult
ASM Standard: 32

Short Answer

60. Why is the resolution of a microscope image important?

Sample Answer: A high-resolution image is clear because you can distinguish between points. If the image is low resolution, then it is fuzzy and individual structures are more difficult to see.

Difficulty: Easy
ASM Standard: 32

61. Who is believed to have developed the first microscopes that allowed microbes to be viewed?

Sample Answer: Antonie van Leeuwenhoek is believed to have developed the first microscopes that allowed microscopes to be viewed.

Difficulty: Easy
ASM Standard: N/A

62. What is the name of the lens that focuses light from a microscope light source onto the specimen on the stage?

Sample Answer: The lens that focuses light on the slide is called the condenser.

Difficulty: Easy
ASM Standard: N/A

63. Would you use immersion oil at high magnification or low magnification?

Sample Answer: Immersion oil is used to view specimens at high magnification with a light microscope and only with objective lenses that are designed for oil immersion.

Difficulty: Easy
ASM Standard: 32

64. What is the difference between fluorescence and phosphorescence?

Sample Answer: In both fluorescence and phosphorescence, a material absorbs energy from light and then emits it again at a lower energy level. However, there is a delay between light absorbance and the emission of lower energy photons in phosphorescence that does not occur in fluorescence.

Difficulty: Difficult
ASM Standard: N/A

65. Which lens will produce an image with greater resolution: one with a higher numerical aperture or one with a lower numerical aperture? Why?

Sample Answer: A higher numerical aperture produces an image with greater resolution, because more light can be gathered by the lens.

Difficulty: Moderate

ASM Standard: 32

66. How does immersion oil improve the resolution of a microscope image?

Sample Answer: Immersion oil has a similar refractive index to glass. When light passes from the specimen into air, it is refracted and some is lost rather than passing through the objective. When there is oil between the specimen and the objective lens, more light enters the objective lens rather than being lost, and the image resolution is greater.

Difficulty: Moderate

ASM Standard: N/A

67. For what type of microscopy is an opaque light stop used, and what is its function?

Sample Answer: In darkfield microscopy, an opaque stop is placed between the light source (illuminator) and the condenser lens. The stop blocks light from the illuminator from hitting the specimen directly. This means light does not hit the specimen directly and only refracted or reflected light reaches the objective, producing a bright specimen on a dark background.

Difficulty: Moderate

ASM Standard: N/A

68. Why is it sometimes advantageous to avoid using stains when viewing microorganisms?

Sample Answer: Most stains or the process of staining can kill the specimen. It is often advantageous to view microorganisms without stains when one wishes to observe them alive (e.g., to observe their motility).

Difficulty: Moderate

ASM Standard: N/A

69. What is another name for differential interference contrast microscopy?

Sample Answer: Differential interference contrast microscopy is also called Nomarski optics.

Difficulty: Moderate

ASM Standard: N/A

70. What are some common fluorochromes used in fluorescence microscopy?

Sample Answer: Some common fluorochromes are Texas red, FITC, DAPI, and acridine orange.

Difficulty: Moderate

ASM Standard: 32

71. In immunofluorescence, what are secondary antibodies used for?

Sample Answer: Secondary antibodies bind to primary antibodies, which bind to a an antigen (i.e., a protein, sometimes as part of a pathogen) in indirect immunofluorescence assays.

Difficulty: Moderate

ASM Standard: 32

72. Why is it important to embed specimens in plastic resin during their preparation for TEM?

Sample Answer: TEM specimens must be very thin for internal structures to be visible. An ultramicrotome is used to cut thin sections for this purpose. By embedding the specimens in plastic before cutting them, it is easier to prevent them from being damaged and compressed while being cut. Ethanol is used to help the plastic resin penetrate the cell.

Difficulty: Difficult

ASM Standard: 32

73. Why can electron microscopes achieve higher resolutions than light microscopes?

Sample Answer: Resolution is higher when there are shorter wavelengths. The beams of electrons used by electron microscopes have shorter wavelengths than the light waves used by light microscopes.

Difficulty: Difficult

ASM Standard: 32

74. Why do microscopes have rheostats? What is adjusted by adjusting a microscope's rheostat?

Sample Answer: A microscope rheostat is used to adjust the amount of light on the specimen. This is important to have the optimal amount of light to view the specimen. Having too much or too little light makes the specimen difficult to see clearly.

Difficulty: Difficult

ASM Standard: 32

Brief Essay

Essay Question Rubric

RATING	Failing	Below Average	Competent	Advanced
Criteria for evaluation	Answer does not provide an argument. Answer contains inaccuracies. Writing is poor and contains numerous grammatical mistakes and	Answer fails to provide examples to support an argument. Writing is poor and grammatical errors are common. Answer is somewhat incoherent.	Answer provides an argument with one or two examples that support it. Writing is acceptable for the college level but may contain one or two grammatical	Answer clearly provides an argument with two or more excellent examples that support it; student makes the argument clearly and eloquently. Answer is well organized

	misspellings.		mistakes or misspellings.	and free of grammatical errors and misspellings.
POINT VALUE	0	1	2	3

Assume rating/grading scale for the question ranges from 0 to 3 points.

75. Explain how direct and indirect immunofluorescence assays differ, including an explanation of why indirect immunofluorescence is more commonly used.

Answer: Student answers will vary but should include the following. Direct immunofluorescence involves the use of an antibody that is attached to a fluorochrome. When the antibody binds to an antigen, the fluorochrome is visible under the fluorescence microscope and allows the location of the antigen to be visualized. In indirect immunofluorescence, a primary antibody lacking a fluorochrome binds to an antigen. Then, a secondary antibody containing a fluorochrome binds to the primary antibody. Because more than one secondary antibody may bind to a primary antibody, indirect immunofluorescence increases sensitivity and can make it easier to visualize the specimen. This is an important reason to choose indirect immunofluorescence even though direct immunofluorescence requires fewer steps.

Difficulty: Moderate
ASM Standard: 32, 36

76. You prepare a slide of a bacterial sample by placing bacteria on a slide and examining it under a brightfield microscope. However, it is difficult to see the bacteria because the contrast is low. What are at least two ways you could increase the contrast?

Answer: Student answers will vary but should include the following. There are several ways you could increase the contrast. You could add a stain, choosing one that would likely stain the structures or cells of interest. You could also use a different form of microscopy, such as darkfield or phase contrast. Another option is to adjust the amount of light on the slide using the diaphragm and/or rheostat. Other options are also possible.

Difficulty: Moderate
ASM Standard: 32

77. Explain some advantages of two-photon microscopy, and explain why this type of microscopy is not more commonly used.

Answer: Student answers will vary but should include the following. Two-photon microscopy is especially valuable for penetrating thicker materials, such as biofilms. It also uses lower-energy light that is less damaging to living tissues than some other forms of microscopy. This means it can be used to obtain high-resolution images of living organisms and tissues. However, the technology is still not widely used because it is very expensive.

Difficulty: Moderate
ASM Standard: 32

78. There is some debate over who invented the microscope. Explain why this debate exists and what factors help some individuals get more credit for their roles than others.

Answer: Student answers will vary but should include components of the following. Multiple people worked on early microscopes, including Galileo. Antonie van Leeuwenhoek and Robert Hooke receive considerable credit for their microscopes and observations. However, it is possible that Hans Janssen and Zaccharias Janssen should receive more credit. There is considerable uncertainty about their exact work. A major reason for this is that Leeuwenhoek and Hooke published their work. Because the Janssens did not, much less is known about their work.

Difficulty: Difficult
ASM Standard: 31

79. Many microbes live in biofilm communities and understanding these communities is important for a variety of reasons. For example, understanding biofilm formation within wounds and medical devices is important for improving patient outcomes. What are ways that light microscopy, confocal microscopy, fluorescence microscopy, electron microscopy, and two-photon microscopy can be used to study biofilms?

Answer: Student answers will vary but should include components of the following. Although light microscopes are easily available, biofilm architecture is difficult to examine under light microscopy because biofilms are too thick to easily examine throughout their structure with light microscopes without cutting and disrupting the biofilm. Confocal microscopy can be helpful in providing three-dimensional images of thicker structures, such as biofilms, but still have lower resolution than two-photon microscopes. Additionally, fluorescent stains can be used with confocal microscopy to enhance visibility with these three-dimensional images. Even without the depth of confocal microscopy, fluorescence microscopy is helpful in identifying the locations of specific molecules or cells. Electron microscopy allows high magnifications, but the specimens are distorted and can't be viewed alive. Two-photon microscopes are especially useful because thick biofilm specimens can be viewed without distortion, but these microscopes are too expensive for general use.

Difficulty: Difficult
ASM Standard: 32

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