

## Chapter 02 Microscopy

### Fill in the Blank Questions

1. The \_\_\_\_\_ is the point at which a lens focuses parallel beams of light.

**focal point**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.01.02 Correlate lens strength and focal length*

*Section: 02.01*

*Topic: Microscopy*

2. The \_\_\_\_\_ is the distance between the center of a lens and the point at which it focuses parallel beams of light.

**focal length**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.01.02 Correlate lens strength and focal length*

*Section: 02.01*

*Topic: Microscopy*

### True / False Questions

3. Light rays are refracted (bent) when they cross the interface between materials with different refractive indices.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.01.01 Relate the refractive indices of glass and air to the path light takes when it passes through a prism or convex lens*

*Section: 02.01*

*Topic: Microscopy*

### Multiple Choice Questions

4. Confocal microscopes exhibit improved contrast and resolution by

A. illumination of a large area of the specimen.

**B.** blocking out stray light with an aperture located above the objective lens.

C. use of light at longer wavelengths.

D. use of ultraviolet light to illuminate the specimen.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

5. A 30× objective and a 20× ocular produce a total magnification of

A. 230×.

B. 320×.

C. 50×.

**D.** 600×.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

6. A 45× objective and a 10× ocular produce a total magnification of

- A. 900×.
- B. 55×.
- C. 450×.**
- D. 145×.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

7. A microscope that exposes specimens to ultraviolet, violet, or blue light and forms an image with the light emitted at a different wavelength is called a \_\_\_\_\_ microscope.

- A. phase-contrast
- B. dark-field
- C. scanning electron
- D. fluorescence**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.02.03 Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced*

*Section: 02.02*

*Topic: Microscopy*

8. Immersion oil can be used to increase the resolution achieved with some microscope lenses because it increases the \_\_\_\_\_ between the specimen and the objective lens.

- A. optical density
- B. refractive index**
- C. optical density and refractive index
- D. neither optical density nor refractive index

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.01.01 Relate the refractive indices of glass and air to the path light takes when it passes through a prism or convex lens*

*Section: 02.01*

*Topic: Microscopy*

## True / False Questions

9. A substage condenser is used to focus light onto the specimen, which increases the resolution of a light microscope.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

## Fill in the Blank Questions

10. The \_\_\_\_\_ is the distance between the specimen and the objective lens when the specimen is in focus.

**working distance**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

11. The useful magnification of a light microscope is limited by the \_\_\_\_\_ of the light source being utilized.

**wavelength**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

12. The special dyes used in fluorescence microscopy that absorb light at one wavelength and emit light at a different wavelength are called \_\_\_\_\_.

**fluorochromes**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.02.03 Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced*

*Section: 02.02*

*Topic: Microscopy*

13. In order to view a specimen with a total magnification of 400×, a \_\_\_\_\_ objective must be used if the ocular is 10×.

**40×**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

### **True / False Questions**

14. Confocal microscopes, in combination with specialized computer software, can be used to create three-dimensional images of cell structures.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.03 Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced*

*Section: 02.02*

*Topic: Microscopy*

15. A light microscope with an objective lens numerical aperture of 0.65 is capable of allowing two objects 400 nm apart to be distinguished when using light with a wavelength of 420 nm.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

16. Resolution improves when the wavelength of the illuminating light decreases.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

17. Immersion oil is used to prevent a specimen from drying out.

**FALSE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

18. It is possible to build a light microscope capable of 10,000× magnification, but the image would not be sharp because resolution is independent of magnification.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

19. Immersion oil increases the amount of light entering the objective lens.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*

*Section: 02.02*

*Topic: Microscopy*

### **Multiple Choice Questions**

20. If the objective lenses of a microscope can be changed without losing focus on the specimen, they are said to be

A. equifocal.

B. totifocal.

**C. parfocal.**

D. optifocal.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.01 Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope*

*Section: 02.02*

*Topic: Microscopy*

21. An instrument that magnifies slight differences in the refractive index of cell structures is called a (n) \_\_\_\_\_ microscope.

- A.** phase-contrast
- B. electron
- C. fluorescence
- D. densitometric

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.03 Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced*

*Section: 02.02*

*Topic: Microscopy*

22. The instrument that produces a bright image of the specimen against a dark background is called a (n) \_\_\_\_\_ microscope.

- A. phase-contrast
- B. electron
- C. bright-field
- D.** dark-field

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.02.03 Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced*

*Section: 02.02*

*Topic: Microscopy*

23. As the magnification of a series of objective lenses increases, the working distance

- A. increases.
- B.** decreases.
- C. stays the same.
- D. cannot be predicted.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.01.02 Correlate lens strength and focal length*

*Section: 02.01*

*Topic: Microscopy*

24. Prior to staining, smears of microorganisms are heat-fixed in order to
- A. allow eventual visualization of internal structures.
  - B. ensure removal of dust particles from the slide surface.
  - C. attach it firmly to the slide.**
  - D. create small pores in cells that facilitates binding of stain to cell structures.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.01 Recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

25. Acid-fast organisms such as *Mycobacterium tuberculosis* contain \_\_\_\_\_ constructed from mycolic acids in their cell walls.
- A. proteins
  - B. carbohydrates
  - C. lipids**
  - D. peptidoglycan

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 02.02 Bacteria have unique cell structures that can be targets for antibiotics, immunity and phage infection.*

*ASM Topic: Module 02 Cell Structure and Function*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Mycobacteria*

*Topic: Preparing Microscopy Specimens*

26. In the Gram-staining procedure, the primary stain is
- A. iodine.
  - B. safranin.
  - C. crystal violet.**
  - D. alcohol.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

27. In the Gram-staining procedure, the decolorizer is

- A. iodine.
- B. safranin.
- C. crystal violet.
- D.** ethanol or acetone.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

28. In the Gram-staining procedure, the counterstain is

- A. iodine.
- B.** safranin.
- C. crystal violet.
- D. alcohol.

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Identifying Microorganisms*

*Topic: Preparing Microscopy Specimens*

29. In the Gram-staining procedure, the mordant is

- A.** iodine.
- B. safranin.
- C. crystal violet.
- D. alcohol.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

30. After the primary stain has been added but before the decolorizer has been used, gram-positive organisms are stained \_\_\_\_\_ and gram-negative organisms are stained \_\_\_\_\_.

- A.** purple; purple
- B. purple; colorless
- C. purple; pink
- D. pink; pink

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

31. After the decolorizer has been added, gram-positive organisms are stained \_\_\_\_\_ and gram-negative organisms are stained \_\_\_\_\_.

- A. purple; purple
- B.** purple; colorless
- C. purple; pink
- D. pink; pink

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

32. After the secondary stain has been added, gram-positive organisms are stained \_\_\_\_\_ and gram-negative organisms are stained \_\_\_\_\_.

- A. purple; purple
- B. purple; colorless
- C. purple; pink**
- D. pink; pink

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

33. If the decolorizer is left on too long in the Gram-staining procedure, gram-positive organisms will be stained \_\_\_\_\_ and gram-negative organisms will be stained \_\_\_\_\_.

- A. purple; blue
- B. purple; colorless
- C. purple; pink
- D. pink; pink**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

34. If the decolorizer is not left on long enough in the Gram-staining procedure, gram-positive organisms will be stained \_\_\_\_\_ and gram-negative organisms will be stained \_\_\_\_\_.

- A.** purple; purple
- B. purple; colorless
- C. purple; pink
- D. pink; pink

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

35. Which of the following is considered to be a differential staining procedure?

- A. Gram stain
- B. Acid-fast stain
- C.** Both Gram stain and Acid-fast stain

D. Leifson's flagella stain

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Identifying Microorganisms*

*Topic: Preparing Microscopy Specimens*

36. Basic dyes such as methylene blue bind to cellular molecules that are
- A. hydrophobic.
  - B. negatively charged.**
  - C. positively charged.
  - D. aromatic.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

### **True / False Questions**

37. Gram staining divides bacterial species into two groups based on differences in cell wall structure.

**TRUE**

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 02 Cell Structure and Function*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Bacterial Cellular Morphology*

*Topic: Preparing Microscopy Specimens*

38. Negative staining facilitates the visualization of bacterial capsules that are intensely stained by the procedure.

**FALSE**

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 02.03 Bacteria and Archaea have specialized structures (e.g. flagella, endospores, and pili) that often confer critical capabilities.*

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 02 Cell Structure and Function*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Bacterial Cellular Morphology*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

39. Negative staining with India ink can be used to reveal the presence of capsules that surround bacterial cells.

**TRUE**

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 02.03 Bacteria and Archaea have specialized structures (e.g. flagella, endospores, and pili) that often confer critical capabilities.*

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 02 Cell Structure and Function*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Bacterial Cellular Morphology*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

40. Mordants increase the binding between a stain and specimen.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

41. In order to stain flagella so that they may be readily observed by light microscopy, it is usually necessary to increase their thickness.

**TRUE**

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 02.03 Bacteria and Archaea have specialized structures (e.g. flagella, endospores, and pili) that often confer critical capabilities.*

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 02 Cell Structure and Function*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Bacterial Cellular Morphology*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

## **Fill in the Blank Questions**

42. The procedure in which a single stain is used to visualize microorganisms is called \_\_\_\_\_ staining.

**simple**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

43. \_\_\_\_\_ is the process by which internal and external structures of cells and organisms are preserved and maintained in position.

**Fixation**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.01 Recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist*

*Section: 02.03*

*Topic: Bacterial Cellular Morphology*

*Topic: Preparing Microscopy Specimens*

44. Thin films of bacteria that have been air-dried onto a glass microscope slide are called \_\_\_\_\_.

**smears**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

45. A procedure that divides organisms into two or more groups depending on their individual reactions to the same staining procedure is referred to as \_\_\_\_\_ staining.

**differential**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

## **Multiple Choice Questions**

46. The Gram-staining procedure is an example of \_\_\_\_\_.

A. simple staining

B. negative staining

**C.** differential staining

D. fluorescent staining

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

## **True / False Questions**

47. The Gram-staining procedure is widely used because it allows rapid identification of a microorganism with little additional testing.

**FALSE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.03.02 Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible*

*Section: 02.03*

*Topic: Identifying Microorganisms*

*Topic: Preparing Microscopy Specimens*

### **Multiple Choice Questions**

48. Regions of a specimen with higher electron density scatter \_\_\_\_\_ electrons and, therefore, appear \_\_\_\_\_ in the image projected onto the screen of a transmission electron microscope.

A. more; lighter

**B.** more; darker

C. fewer; darker

D. fewer; lighter

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

### **True / False Questions**

49. Because transmission electron microscopy uses electrons rather than light, it is not necessary to stain biological specimens before observing them.

**FALSE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

50. Scanning electron microscopes bombard specimens with a stream of electrons; however, the specimen image is produced by electrons that are derived from atoms of the specimen itself rather than by the electrons used to bombard the specimen.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

51. It was possible to view viruses only after the invention of the electron microscope because they are too small to be seen with a light microscope.

**TRUE**

*ASM Objective: 02.01 The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).*

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 02 Cell Structure and Function*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

## Fill in the Blank Questions

52. An electron microscope uses \_\_\_\_\_ lenses to focus beams of electrons onto a specimen.

**magnetic**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Section: 02.04*

*Topic: Microscopy*

## Multiple Choice Questions

53. Scanning electron microscopy is most often used to reveal

**A.** surface structures.

B. internal structures.

C. both surface and internal structures simultaneously.

D. either surface or internal structures, but not simultaneously.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

54. Small internal cell structures are best visualized with a
- A. light microscope.
  - B. dark-field microscope.
  - C. transmission electron microscope.**
  - D. flagellar microscope.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Bacterial Cellular Morphology*

*Topic: Microscopy*

55. In transmission electron microscopy, spreading a specimen out in a thin film with uranyl acetate, which does not penetrate the specimen, is called
- A. freeze-etching.
  - B. simple staining.
  - C. shadow staining.
  - D. negative staining.**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

## **Fill in the Blank Questions**

56. \_\_\_\_\_ breaks frozen specimens along lines of greatest weakness, often down the middle of lipid bilayer membranes so that they may be observed by transmission electron microscopy.

**Freeze-etching**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

57. The \_\_\_\_\_ microscope is capable of atomic resolution of specimens, even when they are immersed in water.

**Scanning tunneling**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.05.01 Distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses*

*Section: 02.05*

*Topic: Microscopy*

58. The designer of the first transmission electron microscope, \_\_\_\_\_, was awarded the 1986 Nobel Prize in physics.

**Ernst Ruska**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 1. Remember*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: History of Microbiology*

*Topic: Microscopy*

**Multiple Choice Questions**

59. Atomic force microscopes use a scanning probe that maintains a fixed distance from the surface of the specimen. It is useful for specimens that
- A.** do not conduct electricity well.
  - B. have extremely uneven surfaces.
  - C. both do not conduct electricity well and have extremely uneven surfaces are correct.
  - D. neither do not conduct electricity well nor have extremely uneven surfaces is correct.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.05.01 Distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses*

*Section: 02.05*

*Topic: Microscopy*

### **True / False Questions**

60. Scanning tunneling electron microscopes create a three-dimensional image of specimens at atomic level resolution.

**TRUE**

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 2. Understand*

*Learning Outcome: 02.05.01 Distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses*

*Section: 02.05*

*Topic: Microscopy*

### **Multiple Choice Questions**

61. If immersion oil was replaced with water, what would happen?
- A. The refractive index would increase, improving resolution.
  - B.** The refractive index of water would be greater than air but less than oil, improving resolution less than oil.
  - C. The refractive index of water would be less than that of air, decreasing resolution.
  - D. There would be no difference.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*  
*ASM Topic: Module 08 Microbiology Laboratory Skills*  
*Blooms Level: 4. Analyze*  
*Learning Outcome: 02.02.02 Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen*  
*Section: 02.02*  
*Topic: Microscopy*

62. As the resolution of a microscope system improves, the size of the smallest object that can be seen clearly
- A. is larger.
  - B.** is smaller.
  - C. is not affected.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*  
*ASM Topic: Module 08 Microbiology Laboratory Skills*  
*Blooms Level: 3. Apply*  
*Section: 02.02*  
*Topic: Microscopy*

63. If you forgot to heat fix a smear before doing a Gram stain, which of the following might occur?
- A. The stains would not adhere to the bacteria.
  - B.** The smear may not adhere to the slide.
  - C. The decolorization step of the Gram stain would not work properly.
  - D. Gram-positive and Gram-negative bacteria would both stain purple.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*  
*ASM Topic: Module 08 Microbiology Laboratory Skills*  
*Blooms Level: 4. Analyze*  
*Learning Outcome: 02.03.01 Recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist*  
*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*  
*Section: 02.03*  
*Topic: Microscopy*  
*Topic: Preparing Microscopy Specimens*

64. A specimen has been prepared for viewing with a transmission electron microscope, using uranyl acetate as a negative stain. The area stained by the uranyl acetate will be \_\_\_\_\_ electron dense compared to specimen itself.

- A.** more
- B. less
- C. equally

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.04.01 Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Section: 02.04*

*Topic: Microscopy*

*Topic: Preparing Microscopy Specimens*

65. If you forgot the decolorization step while performing a Gram stain, which outcome would you expect?

- A. Gram-positive bacteria would stain pink.
- B.** Gram-negative bacteria would stain purple.
- C. Gram-negative bacteria would be unstained.
- D. Gram-positive bacteria would be unstained.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

66. If you forgot to apply the safranin counterstain while performing a Gram stain, which outcome would you expect?

- A. Gram-positive bacteria would stain pink.
- B. Gram-negative bacteria would stain purple.
- C. Gram-negative and Gram-positive bacteria would be unstained.
- D.** Gram-negative bacteria would be unstained.

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 4. Analyze*

*Learning Outcome: 02.03.03 Compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure*

*Section: 02.03*

*Topic: Preparing Microscopy Specimens*

67. Which type of microscopy would be preferred for creating a three dimensional view of the distribution and arrangement of flagella on a bacterial cell surface?

- A. Bright-field microscopy
- B.** Scanning electron microscopy
- C. Fluorescence microscopy
- D. Transmission electron microscopy

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Learning Outcome: 02.05.02 Evaluate light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution, and the quality of the images created*

*Section: 02.04*

*Section: 02.05*

*Topic: Microscopy*

68. Which type of microscopy would be preferred for showing fine internal detail of the eukaryotic organelles?

- A. Bright-field microscopy
- B. Scanning electron microscopy
- C. Fluorescence microscopy
- D.** Transmission electron microscopy

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.04.02 Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography*

*Learning Outcome: 02.05.02 Evaluate light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution, and the quality of the images created*

*Section: 02.04*

*Section: 02.05*

*Topic: Microscopy*

69. You are researching the structure of a transmembrane protein. Which type of microscopy would provide you the best view of this protein?

- A. Bright field microscopy
- B. Scanning electron microscopy
- C. Transmission electron microscopy
- D.** Atomic force microscopy

*ASM Objective: 08.01 Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).*

*ASM Topic: Module 08 Microbiology Laboratory Skills*

*Blooms Level: 3. Apply*

*Learning Outcome: 02.05.01 Distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses*

*Learning Outcome: 02.05.02 Evaluate light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution, and the quality of the images created*

*Section: 02.05*

*Topic: Microscopy*