**Study of Microbial Structure:**

**Microscopy and Specimen Preparation**

**Fill in the Blank Questions**

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

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

**True / False Questions**

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

**Multiple Choice Questions**

4. Which of these microscopes can be used to create high-resolution three-dimensional images of cells?
A. differential interference contrast
B. dark field
C. phase-contrast
**D.** confocal

5. 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.

6. A 30× objective and a 20× ocular produce a total magnification of
A. 230×.
B. 320×.
C. 50×.
**D.** 600×.

7. A 45× objective and a 10× ocular produce a total magnification of
A. 900×.
B. 55×.
**C.** 450×.
D. 145×.

8. 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

9. 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

**True / False Questions**

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

**Fill in the Blank Questions**

11. The \_\_\_\_\_\_\_\_\_\_ is the distance between the specimen and the objective lens when the specimen is in focus.
**working distance**

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

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

14.  In order to view a specimen with a total magnification of 400×, a \_\_\_\_\_\_\_\_\_\_ objective must be used if the ocular is 10×.
**40×**

**True / False Questions**

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

16. 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**

17. Resolution decreases when the wavelength of the illuminating light decreases.
**FALSE**

18. Immersion oil is used to prevent a specimen from drying out.
**FALSE**

19. 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**

20. Immersion oil increases the amount of light passing through a specimen and entering the objective lens.
**TRUE**

**Multiple Choice Questions**

21. 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.

22. 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

23. 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

24. 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.

25.  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.

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

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

28.  In the Gram-staining procedure, the decolorizer is
A.  iodine.
B.  safranin.
C.  crystal violet.
**D.**  ethanol or acetone.

29. In the Gram-staining procedure, the counterstain is
A. iodine.
**B.** safranin.
C. crystal violet.
D. alcohol.

30. In the Gram-staining procedure, the mordant is
**A.** iodine.
B. safranin.
C. crystal violet.
D. alcohol.

31. 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

32. 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

33. 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

34. 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

35. 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

36. 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

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

38. The Schaeffer-Fulton procedure is used to stain
A. flagella.
B. fat deposits.
**C.** endospores.
D. DNA of chromosomes.

**True / False Questions**

39. Gram staining divides bacterial species into roughly two equal groups.
**TRUE**

40. Negative staining facilitates the visualization of bacterial capsules which are intensely stained by the procedure.
**FALSE**

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

42. Mordants increase the binding between a stain and specimen.
**TRUE**

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

**Fill in the Blank Questions**

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

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

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

47. 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**

**Multiple Choice Questions**

48. The Gram-staining procedure is an example of:
A. simple staining
B. negative staining
**C.** differential staining
D. fluorescent staining

**True / False Questions**

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

**Multiple Choice Questions**

50. 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

**True / False Questions**

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

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

53. 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**

**Fill in the Blank Questions**

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

**Multiple Choice Questions**

55. 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.

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

57. 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.

**Fill in the Blank Questions**

58. \_\_\_\_\_\_\_\_\_\_ 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**

59. The \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ microscope is capable of atomic resolution of specimens, even when they are immersed in water.
**Scanning tunneling**

60. The designer of the first transmission electron microscope, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, was awarded the 1986 Nobel Prize in physics.
**Ernst Ruska**

**Multiple Choice Questions**

61. 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.

**True / False Questions**

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