

قسم التقنيات الاحيائية

جامعة الانبار

المادة: الاحياء المجهرية

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المرحلة: الثانية

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Part 2 Observing Microorganisms Through a Microscope Units of Measurement

1. The standard unit of length is the meter (m).
2. Microorganisms are measured in micrometers, μm (10^{-6} m), and in nanometers, nm (10^{-9} m).

Microscopy: The Instruments

1. A simple microscope consists of one lens; a compound microscope has multiple lenses.

Light Microscopy

Compound Light Microscopy

2. The most common microscope used in microbiology is the compound light microscope (LM).
3. The total magnification of an object is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens.
4. The compound light microscope uses visible light.
5. The maximum resolution, or resolving power (the ability to distinguish two points) of a compound light microscope is 0.2 μm ; maximum magnification is 2000X.
6. Specimens are stained to increase the difference between the refractive indexes of the specimen and the medium.
7. Immersion oil is used with the oil immersion lens to reduce light loss between the slide and the lens.
8. Brightfield illumination is used for stained smears.
9. Unstained cells are more productively observed using darkfield, phase-contrast, or DIC microscopy.

Darkfield Microscopy

10. The darkfield microscope shows a light silhouette of an organism against a dark background.
- II. It is most useful for detecting the presence of extremely small organisms.

Phase-Contrast Microscopy

12. A phase-contrast microscope brings direct and reflected or diffracted light rays together (in phase) to form an image of

- the specimen on the ocular lens.
13. It allows the detailed observation of living organisms.

Differential Interference Contrast (DIC) Microscopy

14. The DIC microscope provides a colored, three-dimensional image of the object being observed.
15. It allows detailed observations of living cells.

Fluorescence Microscopy

16. In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet light source.
17. The microorganisms appear as bright objects against a dark background.
18. Fluorescence microscopy is used primarily in a diagnostic procedure called fluorescent-antibody (FA) technique, or immunofluorescence.

Confocal Microscopy

19. In confocal microscopy, a specimen is stained with a fluorescent dye and illuminated with short-wavelength light.
20. Using a computer to process the images, two-dimensional and three-dimensional images of cells can be produced.

Two-Photon Microscopy

21. In TPM, a live specimen is stained with a fluorescent dye and illuminated with long-wavelength light.

Scanning Acoustic Microscopy

22. Scanning acoustic microscopy (SAM) is based on the interpretation of sound waves through a specimen.
23. It is used to study living cells attached to surfaces such as cancer cells, artery plaque, and biofilms.

Electron Microscopy

24. Instead of light, a beam of electrons is used with an electron microscope.
25. Instead of glass lenses, electromagnets control focus, illumination, and magnification.
26. Thin sections of organisms can be seen in an electron micrograph produced using a transmission electron microscope.

Magnification:
10,000-100,000X. Resolving
power: 2.5 nm.

27. Three-dimensional views of the surfaces of whole microorganisms can be
Obtained with a Scanning electron microscope

Magnification:
1000-10,000X. Resolving
power: 20 nm.

Scanned-Probe Microscopy

28. Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) produce three-dimensional images of the surface of a molecule.

Preparing Smears for Staining

- 1. Staining means coloring a microorganism with a dye to make some structures more visible.**
- 2. Fixing uses heat or alcohol to kill and attach microorganisms to a slide.**
- 3. A smear is a thin film of material used for microscopic examination.**
- 4. Bacteria are negatively charged, and the colored positive ion of a basic dye will stain bacterial cells.**
- 5. The colored negative ion of an acidic dye will stain the background of a bacterial smear; a negative stain is produced.**

Simple Stains

- 6. A simple stain is an aqueous or alcohol solution of a single basic dye.**
- 7. It is used to make cellular shapes and arrangements visible.**
- 8. A mordant may be used to improve bonding between the stain and the specimen.**

Differential Stains

- 9. Differential stains, such as the Gram stain and acid-fast stain, differentiate bacteria according to their reactions to the stains.**
- 10. The Gram stain procedure uses a purple stain (crystal violet), iodine as a mordant, an alcohol decolorizer, and a red counterstain.**
- II. Gram-positive bacteria retain the purple stain after the decolorization step; gram-negative bacteria do not and thus**

- appear pink from the counterstain.
12. Acid-fast microbes, such as members of the genera *Mycobacterium* and *Nocardia*, retain carbolfuchsin after acid-alcohol decolorization and appear red; non- acid-fast microbes take up the methylene blue counterstain and appear blue.

Special Stains

13. Negative staining is used to make microbial capsules visible.
14. The endospore stain and flagella stain are special stains that color only certain parts of bacteria.

Q1/1-I. Fill in the following blanks.

- a. 1 M.m ----- m
b. 1----- 10⁻⁹ m
c. 1M.m ----- nm

2. Which type of microscope would be best to use to observe each of the following?
- a stained bacterial smear
 - unstained bacterial cells: the cells are small, and no detail is needed
 - unstained live tissue when it is desirable to see some intracellular detail
 - a sample that emits light when illuminated with ultraviolet light
 - intracellular detail of a cell that is 1 μ m long
 - unstained live cells in which intracellular structures are shown
3. Calculate the total magnification of the nucleus of a cell being observed through a compound light microscope with a 10X ocular lens and an oil immersion lens.
4. The maximum magnification of a compound microscope is (a)----- ; that of an electron microscope, (b)-----.
The maximum resolution of a compound microscope is (c)----- ; that of an electron microscope, (d) -----.
One advantage of a scanning electron microscope over a transmission electron microscope is (e)-----.
5. Why is a mordant used in the Gram stain? In the flagella stain?
6. What is the purpose of a counterstain in the acid-fast stain?
7. What is the purpose of a decolorizer in the Gram stain? In the acid-fast stain!

References': 1- Microbiology an introduction TWELFTH EDITION.

Gerard. Torture.2016.

2- Microbiology an introduction TENTH EDITION. Gerard.
Tortora.2010.