Observing Microorganisms through a Microscope

Units of Measurement

- 1 µm = 10^{-6} m = mm
- 1 nm = 10^{-9} m = mm
- 1000 nm = µm
- 0.001 µm = nm

Microscopy: The Instruments

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- In a compound microscope, the image from the objective lens is magnified again by the ocular lens.
- Total magnification = _____
- Resolution: The ability of the lenses to distinguish two points.
- Shorter wavelengths of light provide greater resolution.

- Refractive index: _____
- The light may bend in air so much that it misses the small high-magnification lens.
- Immersion oil is used to keep light from bending.

Brightfield Illumination

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- Light reflected off the specimen does not enter the objective lens.

Darkfield Illumination

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- Light reflected off the specimen enters the objective lens.

Phase-Contrast Microscopy

- Accentuates diffraction of the light that passes through a specimen.

Fluorescence Microscopy

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- Fluorescent substances absorb UV light and emit visible light.
- Cells may be stained with fluorescent dyes (_____).

Confocal Microscopy

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- The laser illuminates each plane in a specimen to produce a three-dimensional image.

Electron Microscopy

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- The shorter wavelength of electrons gives greater resolution.

Transmission Electron Microscopy (TEM)

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- Light passes through specimen, then an electromagnetic lens, to a screen or film.
- Specimens may be stained with heavy metal salts.
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Scanning Electron Microscopy (SEM)

- An electron gun produces a beam of electrons that scans the surface of a whole specimen.
- Secondary electrons emitted from the specimen produce the image.
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Scanning-Probe Microscopy

- Scanning tunneling microscopy uses a metal probe to scan a specimen.
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- Atomic force microscopy uses a metal and diamond probe inserted into the specimen.
- Produces three-dimensional images.

Preparation of Specimens for Light Microscopy

- Smear: A thin film of a solution of microbes on a slide.
- A smear is usually fixed to attach the microbes to the slide and to kill the microbes.

Preparing Smears for Staining

- Live or unstained cells have little contrast with the surrounding medium. However, researchers do make discoveries about cell behavior by observing live specimens.
- Stains consist of positive and negative ions which stain different components.
- In general, when the object you want to see is stained, this is a positive stain.
- Staining the background instead of the cell is called negative staining. The object you are trying to see is not stained.

Simple Stains

- Simple stain:
- A mordant may be used to hold the stain or coat the specimen to enlarge it.

Differential Stains: Gram Stain

- The Gram stain classifies bacteria into gram-positive or gram-negative.
- Gram-positive bacteria tend to be killed by penicillin and detergents.
- Gram-negative bacteria are more resistant to antibiotics.

	Color of Gram–positive cells	Color of Gram–negative cells
Primary stain: Crystal violet		
Mordant: Iodine		
Decolorizing agent: Alcohol-acetone		Colorless
Counterstain: Safranin		

Differential Stains: Acid-Fast Stain

- Cells that retain a basic stain in the presence of acid-alcohol are called acid-fast.
- Non–acid-fast cells lose the basic stain when rinsed with acid-alcohol, and are usually counterstained (with a different color basic stain) to see them.

Special Stains

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- Heat (or time) is required to drive a stain into endospores.
- Flagella staining requires a mordant to make the flagella wide enough to see.

Study Objectives

1. Define the terms: magnification, resolution, and refractive index.

2. What effect does the wavelength of light have on microscopic resolution?

3. Compare and contrast the different types of microscopes: brightfield, darkfield, phase-contrast, fluorescence, and electron (TEM and SEM) microscopy.

4. Differentiate between simple, differential and special staining.

5. Describe 2 reasons why Gram staining an unknown bacterium would be important.