Chapter 2

A Brief Journey to the Microbial World

I. Seeing the Very Small

- 2.1 Some Principles of Light Microscopy
- 2.2 Improving Contrast in Light Microscopy
- 2.3 Imaging Cells in Three Dimensions
- 2.4 Electron Microscopy
2.1 Some Principles of Light Microscopy

- Compound light microscope uses visible light to illuminate cells
- Many different types of light microscopy:
  - Bright-field
  - Phase-contrast
  - Dark-field
  - Fluorescence

2.1 Some Principles of Light Microscopy

- Bright-field scope (Figure 2.1a)
  - Specimens are visualized because of differences in contrast (density) between specimen and surroundings (Figure 2.2)
- Two sets of lenses form the image (Figure 2.1b)
  - Objective lens and ocular lens
  - Total magnification = objective magnification × ocular magnification
  - Maximum magnification is ~2,000×
Figure 2.1a A light microscope

- Ocular lenses
- Objective lens
- Stage
- Condenser
- Focusing knobs
- Light source
- Specimen on glass slide

Figure 2.2 Bright-field photomicrographs of pigmented microorganisms

(a) 

(b)
2.1 Some Principles of Light Microscopy

- **Resolution**: the ability to distinguish two adjacent objects as separate and distinct
  - Resolution is determined by the wavelength of light used and numerical aperture of lens
  - Limit of resolution for light microscope is about 0.2 μm
2.2 Improving Contrast in Light Microscopy

- Improving contrast results in a better final image
- Staining improves contrast
  - Dyes are organic compounds that bind to specific cellular materials
  - Examples of common stains are methylene blue, safranin, and crystal violet

![Animation: Microscopy & Staining Overview](image)

![Animation: Staining](image)

---

**Figure 2.3** Staining cells for microscopic observation

- **I. Preparing a smear**
  - Spread culture in thin film over slide
  - Dry in air

- **II. Heat fixing and staining**
  - Pass slide through flame to heat fix
  - Flood slide with stain; rinse and dry

- **III. Microscopy**
  - Place drop of oil on slide; examine with 100x objective lens
2.2 Improving Contrast in Light Microscopy

- Differential stains: the **Gram stain**
- Differential stains separate bacteria into groups
- The Gram stain is widely used in microbiology (Figure 2.4a)
  - Bacteria can be divided into two major groups: gram-positive and gram-negative
  - Gram-positive bacteria appear purple and gram-negative bacteria appear red after staining (Figure 2.4b)

© 2012 Pearson Education, Inc.

---

**Figure 2.4a** The Gram stain - Steps in the procedure

**Step 1**
Result: All cells purple

Flood the heat-fixed smear with crystal violet for 1 min

**Step 2**
Result: All cells remain purple

Add iodine solution for 1 min

**Step 3**
Result: Gram-positive cells are purple; gram-negative cells are colorless

Decolorize with alcohol briefly — about 20 sec

**Step 4**
Result: Gram-positive (G⁺) cells are purple; gram-negative (G⁻) cells are pink to red

(a)

Counterstain with safranin for 1–2 min

Marmara University - Enve 303 Env. Eng. Microbiology - Prof. BARIŞ ÇALLI

© 2012 Pearson Education, Inc.
2.2 Improving Contrast in Light Microscopy

- **Phase-Contrast Microscopy**
  - Invented in 1936 by Frits Zernike
  - Phase ring amplifies differences in the refractive index of cell and surroundings
  - Improves the contrast of a sample without the use of a stain
  - Allows for the visualization of live samples
  - Resulting image is dark cells on a light background (Figure 2.5)
2.2 Improving Contrast in Light Microscopy

- **Dark-Field Microscopy**
  - Light reaches the specimen from the sides
  - Light reaching the lens has been scattered by specimen
  - Image appears light on a dark background (Figure 2.5)
  - Excellent for observing motility

![Figure 2.5](Image) Cells visualized by different types of light microscopy. The same field of cells of the baker's yeast *Saccharomyces cerevisiae* visualized by (a) bright-field microscopy, (b) phase-contrast microscopy, and (c) dark-field microscopy. Cells average 8–10 µm wide.
2.2 Improving Contrast in Light Microscopy

- **Fluorescence Microscopy**
  - Used to visualize specimens that *fluoresce*
    - Emit light of one color when illuminated with another color of light (Figure 2.6)
  - Cells fluoresce naturally (*autofluorescence*) or after they have been stained with a fluorescent dye like DAPI
  - Widely used in microbial ecology for enumerating bacteria in natural samples
2.3 Imaging Cells in Three Dimensions

- **Confocal Scanning Laser Microscopy (CSLM)**
  - Uses a computerized microscope coupled with a laser source to generate a three-dimensional image (Figure 2.8)
  - Computer can focus the laser on single layers of the specimen
  - Different layers can then be compiled for a three-dimensional image
  - Resolution is 0.1 µm for CSLM

---

**Figure 2.8** Confocal scanning laser microscopy

(a) Confocal image of a microbial biofilm community cultivated in the laboratory. The green, rod-shaped cells are *Pseudomonas aeruginosa* experimentally introduced into the biofilm. Other cells of different colors are present at different depths in the biofilm.

(b) Confocal image of a filamentous cyanobacterium growing in a soda lake. Cells are about 5 µm wide.
2.4 Electron Microscopy

- Electron microscopes use electrons instead of photons to image cells and structures (Figure 2.9)
- Two types of electron microscopes:
  - Transmission electron microscopes (TEM)
  - Scanning electron microscopes (SEM)
2.4 Electron Microscopy

- **Transmission Electron Microscopy (TEM)**
  - Electromagnets function as lenses
  - System operates in a vacuum
  - High magnification and resolution (0.2 nm)
  - Enables visualization of structures at the molecular level (Figure 2.10a and b)
  - Specimen must be very thin (20–60 nm) and be stained

**Figure 2.10a** Electron micrographs. (a) Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). Note the DNA forming the nucleoid. The cell is about 0.8 µm wide.
2.4 Electron Microscopy

- **Scanning Electron Microscopy (SEM)**
  - Specimen is coated with a thin film of heavy metal (e.g., gold)
  - An electron beam scans the object
  - Scattered electrons are collected by a detector and an image is produced (Figure 2.10c)
  - Even very large specimens can be observed
  - Magnification range of 15×–100,000×
II. Cell Structure and Evolutionary History

- 2.5 Elements of Microbial Structure
- 2.6 Arrangement of DNA in Microbial Cells
- 2.7 The Evolutionary Tree of Life

2.5 Elements of Microbial Structure

- All cells have the following in common:
  - Cytoplasmic membrane
  - Cytoplasm
  - Ribosomes
2.5 Elements of Microbial Structure

- Eukaryotic vs. Prokaryotic Cells
  - *Eukaryotes* (Figures 2.11b and 2.12c)
    - DNA enclosed in a membrane-bound nucleus
    - Cells are generally larger and more complex
    - Contain organelles
  - *Prokaryotes* (Figures 2.11a and 2.12a and b)
    - No membrane-enclosed organelles, no nucleus
    - Generally smaller than eukaryotic cells
Figure 2.12c Electron micrograph of sectioned eukaryotic cells

(c) Eukarya

Eukaryote

- Cytoplasmic membrane
- Nucleus
- Cell wall
- Mitochondrion

© 2012 Pearson Education, Inc.
Marmara University - Enve 303 Env. Eng. Microbiology - Prof. BARIŞ ÇALLI

Figure 2.11a Internal structure of prokaryotic cells

(a) Prokaryote

- Cytoplasm
- Nucleoid
- Ribosomes
- Plasmid
- Cell wall
- Cytoplasmic membrane

© 2012 Pearson Education, Inc.
2.5 Elements of Microbial Structure

• **Viruses**
  – Not considered cells
  – No metabolic abilities of their own
  – Rely completely on biosynthetic machinery of infected cell
  – Infect all types of cells
  – Smallest virus is 10 nm in diameter
2.6 Arrangement of DNA in Microbial Cells

- **Genome**
  - A cell’s full complement of genes
- Prokaryotic cells generally have a single, circular DNA molecule called a *chromosome*
  - DNA aggregates to form the nucleoid region (Figure 2.14)
  - Prokaryotes also may have small amounts of extra-chromosomal DNA called plasmids that confer special properties (e.g., antibiotic resistance)
2.6 Arrangement of DNA in Microbial Cells

- Eukaryotic DNA is linear and found within the nucleus
  - Associated with proteins that help in folding of the DNA
  - Usually more than one chromosome
  - Typically two copies of each chromosome
  - During cell division, nucleus divides by mitosis
  - During sexual reproduction, the genome is halved by meiosis

- Escherichia coli Genome
  - 4.64 million base pairs
  - 4,300 genes
  - 1,900 different kinds of protein
  - 2.4 million protein molecules

- Human Cell
  - 1,000× more DNA per cell than *E. coli*
  - 7× more genes than *E. coli*
2.7 The Evolutionary Tree of Life

- **Evolution**
  - The process of change over time that results in new varieties and species of organisms

- **Phylogeny**
  - Evolutionary relationships between organisms
  - Relationships can be deduced by comparing genetic information in the different specimens
  - Ribosomal RNA (rRNA) is excellent for determining phylogeny
  - Relationships visualized on a phylogenetic tree (Figure 2.16)

![Figure 2.16 Ribosomal RNA (rRNA) gene sequencing and phylogeny. (a) DNA is extracted from cells. (b) Many identical copies of a gene encoding rRNA are made by the polymerase chain reaction](image-url)
2.7 The Evolutionary Tree of Life

- Comparative rRNA sequencing has defined three distinct lineages of cells called **domains**:
  - *Bacteria* (prokaryotic)
  - *Archaea* (prokaryotic)
  - *Eukarya* (eukaryotic)

- *Archaea* and *Bacteria* are NOT closely related (Figure 2.17)
- *Archaea* are more closely related to *Eukarya* than *Bacteria*
- Eukaryotic microorganisms were the ancestors of multicellular organisms (Figure 2.17)
III. Microbial Diversity

- 2.8 Metabolic Diversity
- 2.9 Bacteria
- 2.10 Archaea
- 2.11 Phylogenetic Analyses of Natural Microbial Communities
- 2.12 Microbial Eukarya

© 2012 Pearson Education, Inc.

2.8 Metabolic Diversity

- The diversity in microbial cells is the product of almost 4 billion years of evolution
- Microorganisms differ in size, shape, motility, physiology, pathogenicity, etc.
- Microorganisms have exploited every conceivable means of obtaining energy from the environment

© 2012 Pearson Education, Inc.
2.8 Metabolic Diversity

- **Chemoorganotrophs**
  - Obtain their energy from the oxidation of organic molecules (Figure 2.18)
  - Aerobes use oxygen to obtain energy
  - Anaerobes obtain energy in the absence of oxygen

- **Chemolithotrophs**
  - Obtain their energy from the oxidation of inorganic molecules (Figure 2.18)
  - Process found only in prokaryotes

- **Phototrophs**
  - Contain pigments that allow them to use light as an energy source (Figure 2.18)
  - Oxygenic photosynthesis produces oxygen
  - Anoxygenic photosynthesis does not produce oxygen
2.8 Metabolic Diversity

- All cells require carbon as a major nutrient
  - **Autotrophs**
    - Use carbon dioxide as their carbon source
    - Sometimes referred to as *primary producers*
  - **Heterotrophs**
    - Require one or more organic molecules for their carbon source
    - Feed directly on autotrophs or live off products produced by autotrophs
2.8 Metabolic Diversity

- Organisms that inhabit extreme environments are called **extremophiles**
- Habitats include boiling hot springs, glaciers, extremely salty bodies of water, and high-pH environments

2.9 Bacteria

- The domain *Bacteria* contains an enormous variety of prokaryotes (Figure 2.19)
- All known pathogenic prokaryotes are *Bacteria*
- The *Proteobacteria* make up the largest phylum of *Bacteria*
  - Gram-negative
    - Examples: *E. coli*, *Pseudomonas*, and *Salmonella*
- Gram-positive phylum united by phylogeny and cell wall structure
  - Cyanobacteria are relatives of gram-positive bacteria
2.9 Bacteria

- Many Other Phyla of Bacteria
  - Green sulfur bacteria and green nonsulfur bacteria are photosynthetic
  - *Deinococcus* is extremely resistant to radioactivity
  - Chlamydia are obligate intracellular parasites
2.10 *Archaea*

- Two Phyla of the Domain *Archaea*
  - *Euryarchaeota* (Figure 2.28)
    - *Methanogens*: degrade organic matter anaerobically, produce methane (natural gas)
    - *Extreme halophiles*: require high salt concentrations for metabolism and reproduction
    - *Thermoacidophiles*: grow in moderately high temperatures and low-pH environments

- *Crenarchaeota* (Figure 2.28)
  - Vast majority of cultured *Crenarchaeota* are hyperthermophiles
  - Some live in marine, freshwater, and soil systems
2.11 Phylogenetic Analyses of Natural Microbial Communities

- Microbiologists believe that we have cultured only a small fraction of the Archaea and Bacteria
- Studies done using methods of molecular microbial ecology, devised by Norman Pace
  - Microbial diversity is much greater than laboratory culturing can reveal
2.12 Microbial *Eukarya*

- Eukaryotic microorganisms include algae, fungi, protozoa, and slime molds (Figure 2.32)
  - Protists include algae and protozoa
    - The algae are phototrophic (Figure 2.33a)
    - Protozoa NOT phototrophic (Figure 2.33c)
  - Fungi are decomposers (Figure 2.33b)
  - Algae and fungi have cell walls, whereas protozoa and slime molds do not
**Figure 2.33a** Microbial Eukarya - Algae

![Image](Image)

**Figure 2.33b** Microbial Eukarya - Fungi

![Image](Image)
2.12 Microbial *Eukarya*

- Lichens are a mutualistic relationship between two groups of protists (Figure 2.34)
  - Fungi and cyanobacteria
  - Fungi and algae
- Microbial eukaryotes are comprehensively discussed in Chapter 20
Figure 2.34 Lichens. (a) An orange-pigmented lichen growing on a rock, and (b) a yellow-pigmented lichen growing on a dead tree stump.