Introduction to Histology

Dr. Heba Kalbouneh
Associate Professor of Anatomy and Histology
The name "Histology" is derived from the Greek word for a tissue "Histos", and "-logos" = “the study of”

It is tightly bounded to molecular biology, genetics, immunology and other basic sciences
Cells are the basic unit of structure and function in living things.

Organs made up of tissues that work together to perform a specific activity.

Tissues made up of cells that are similar in structure and function and which work together to perform a specific activity.

Systems are groups of two or more organs that work together to perform a specific function for the organism.

Levels of organisation
<table>
<thead>
<tr>
<th>Week no.</th>
<th>Theory</th>
<th>Practical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Overview of histology</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>2</td>
<td>Cell overview</td>
<td>Microscopes and Microtechniques</td>
</tr>
<tr>
<td>3</td>
<td>Epithelium-1</td>
<td>Cell review</td>
</tr>
<tr>
<td>4</td>
<td>Epithelium-2</td>
<td>Epithelium-1</td>
</tr>
<tr>
<td>5</td>
<td>Epithelium-3</td>
<td>Epithelium-2</td>
</tr>
<tr>
<td>6</td>
<td>Connective tissue-1</td>
<td>Revision- Quiz</td>
</tr>
<tr>
<td>7</td>
<td>Connective tissue-2</td>
<td>Connective tissue-1</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td><em>Midterm exam</em></td>
</tr>
<tr>
<td>9</td>
<td>Adipose tissue</td>
<td>Connective tissue-2</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Cartilage</td>
<td>Adipose tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td>11</td>
<td>Bone-1</td>
<td>Cartilage</td>
</tr>
<tr>
<td>12</td>
<td>Bone-2</td>
<td>Bone-1</td>
</tr>
<tr>
<td>13</td>
<td>Muscle tissue</td>
<td>Bone-2</td>
</tr>
<tr>
<td>14</td>
<td>Nervous tissue-1</td>
<td>Muscle tissue</td>
</tr>
<tr>
<td>15</td>
<td>Nervous tissue-2</td>
<td>Nervous tissue</td>
</tr>
</tbody>
</table>
Suggested Histology Reference

Junqueira’s Basic Histology
Text and Atlas
13th edition
By Anthony L. Mescher
History

- **Bichat** is the first anatomist who defined the term “TISSUE” without the use of microscope

Units used in microscopy

- One millimeter = 1000 micrometer (µm)
- One micrometer = 1000 nanometer (nm)
Microtechniques

- Tissue preparation for microscopic examination
- There are different methods but the basic principles are similar
  
  Hardening and sectioning of the tissue
  
  Examples: paraffin and freezing techniques
Microtechniques

1. Fixation
2. Dehydration
3. Clearing
4. Impregnation (infiltration)
5. Embedding
6. Section cutting
7. Staining
8. Mounting
Fixation: Exposing the tissue to chemical agents called fixatives i.e paraformaldehyde
Dehydration

The process to remove the water by using a graded series of alcohol
Then the tissue can be filled with the paraffin or other embedding agent
Clearing

- Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium. i.e Xylene
Embedding
Sectioning
Flattened paraffin sections
Mounting

- The process to place (mount) the tissue sections on the adhesive coated glass slides
Freezing technique

- Tissues are frozen using liquid nitrogen
- Frozen tissues are sectioned by cryostat
- It is faster and preserve tissue components

The quality of the section is poor with more artifacts
While paraffin technique produces intact tissue with less artifacts
Staining techniques

- The stain is a chemical substance which reacts with certain tissue components producing a color
  1. Ordinary stains
  2. Immunohistochemistry and Immunocytochemistry
  3. Hybridization techniques
Immunohistochemistry

- Rely on the use of antibody directed against molecule of interest, usually protein
- The antibody is usually labeled with a colored substance
Direct method-
primary antibody only

Goat anti-actin labeled with 594
Indirect method – primary and secondary antibodies

- Goat anti-actin
- Donkey anti-goat labeled with 488

Goat anti-actin
Donkey anti-goat labeled with 488
Hybridization techniques

- To detect and localize the presence or absence of specific DNA sequences on chromosomes
- To detect and localize specific RNA targets (e.g., mRNA) in cells
- A small oligonucleotide which is complementary to the target DNA/RNA sequence is used (e.g., fluorescent probes)
- Can be applied to tissue sections, smears or chromosomes
Hybridization techniques
Microscopy

- Light Microscopy
  ✓ Phase contrast
  ✓ Interference
  ✓ Fluorescence
  ✓ Polarizing

- Electron Microscopy
  ✓ Transmission EM
  ✓ Scanning EM
Light microscopy

- The basic functional unit consists of a tube; having an objective lens at one end and an ocular lens at the other.
- The objective lens enlarges the image of the object in the direction of the ocular lens.
- The ocular lens further magnifies this image toward the observer’s eye.
- The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses.
Phase Contrast Microscopy

- It uses a lens system that produces visible images from transparent objects
- The structures appear lighter or darker relative to each other
- The light changes its speed and direction when passing in different media
- Useful in tissue culture
Fluorescence Microscopy

- Uses ultraviolet light
- When certain fluorescent substances are irradiated with ultra violet light, it emits light
- They appear as shiny particles on a dark background
- Placed in dark room
Confocal Microscopy

- Uses laser beams
- the laser can be moved (scanned) across the specimen as well as down into the specimen, it can produce 3D images
- Can be used in living and cultured cells and tissue sections
Electron Microscopy

- Uses electron beams instead of light
- Provides the highest resolution of subcellular structures
- Electromagnets to focus the electrons (versus glass lenses to focus the light)
- Detect by fluorescent screen or photographic emulsion
- Requires ultrathin sections (0.02-0.1 µm)
- Uses hard epoxy resin for embedding
- Ultrathin sections are produced by ultramicrotome (Diamond or Glass knives)
Types

- Transmission EM
  - Views the ultrastructural details in shades of gray
  - The bright areas of the images are unstained (the electrons passed through the sample) and the darker regions are areas which have taken up stain and either absorbed or scattered the electrons

- Scanning EM
  - Provides information about the surface of a specimen
  - Samples are coated with a gold-carbon film. The electron beam is then scanned across the specimen surface and the electrons that are reflected off of the surface are captured by the detector
  - Views only the structure as a 3D image
<table>
<thead>
<tr>
<th></th>
<th>Light microscope</th>
<th>Electron microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image</td>
<td>Color images</td>
<td>black and white images</td>
</tr>
<tr>
<td>Images produced by</td>
<td>Visible light rays</td>
<td>Electron beam</td>
</tr>
<tr>
<td>Magnification</td>
<td>up to 1500x but a wider field of view and easier orientation</td>
<td>Up to 2,000 000x</td>
</tr>
<tr>
<td>Resolution</td>
<td>Resolving power to 0.25µm</td>
<td>Resolving power to 0.1nm</td>
</tr>
<tr>
<td>Time</td>
<td>Frozen sections can yield an image within 20 minutes</td>
<td>One day at least</td>
</tr>
<tr>
<td>Section thickness</td>
<td>Ranges from 1-30 µm</td>
<td>Ranges from 0.02-0.1 µm</td>
</tr>
<tr>
<td>Specimen placed on</td>
<td>Glass slide</td>
<td>Copper mesh</td>
</tr>
</tbody>
</table>
Histology is a two dimensional study of a three dimensional reality.
Types of Tissue Sections (1)

**Longitudinal section**
- tissue cut along the longest direction of an organ

**Cross section**
- tissue cut perpendicular to the length of an organ

**Oblique section**
- tissue cut at an angle between a cross & longitudinal section
Types of Tissue Sections (2)

- Would you classify the egg sections as longitudinal, cross, or oblique sections?
- How would the egg look if sectioned in the other two planes?

Practice at home.
• Slices 1 & 5 miss the yolk/cell nucleus

• Cell nucleus is smaller in sections 2 & 4
• **Image A** is a cross section of elbow macaroni, resembling a blood vessel, piece of gut, or other tubular organ.

• **Image B** is a longitudinal section of a sweat gland. Notice what a single slice could look like.
**Stains .. examples**

<table>
<thead>
<tr>
<th>Standard stain (dye)</th>
<th>H &amp; E (Hematoylin &amp; Eosin); Specialized stains include PAS, Ag, Aldehyde fuchsin, Orcein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAS</strong></td>
<td>detects glycogen, glycoproteins, glycolipids and mucins in tissues</td>
</tr>
<tr>
<td>2 steps; CHO are oxidized with periodic acid to aldehyde groups. The Schiff reagent reacts with aldehyde groups to form a deep red-reaction (magenta) product</td>
<td></td>
</tr>
<tr>
<td><strong>Aldehyde fuchsin</strong></td>
<td>stains elastic fibres &amp; β-cells islets of pancreas</td>
</tr>
<tr>
<td><strong>Orcein</strong></td>
<td>stains elastic fibres dark brown</td>
</tr>
<tr>
<td><strong>Silver stain</strong></td>
<td>stains reticular fibres (type III collagen)</td>
</tr>
</tbody>
</table>
Basophilia

Basophilic structures are stained by basic dyes:

• Basic dyes are positive
• Basophilic structures are negative (ex. DNA, RNA, ribosomes, RER)

Basophilic = Blue
Acidophilia

Acidophilic structures are stained by acid dyes:

- Acid dyes are negative
- Acidophilic structures are positive (ex. Proteins, collagen, cytoplasm)

Eosinophilic = Pink