**Histology and Its Methods of Study**

## Objectives: Upon completion of this lecture, the student should be able to:

## Describe the components of the tissue and their functions.

## Explain the main steps of tissue preparations.

## Describe the important or the purpose of each step in tissue processing.

## Understand the basics of simple staining

* Describe the H&E staining procedure.

**Introduction**

All living things (or organisms) are built from cells: small, membrane enclosed units filled with a concentrated aqueous solution of chemicals, and equipped with the extraordinary ability to create copies of themselves by growing and then dividing into two cells. Higher organisms, including ourselves, are communities of cells derived by growth and division from a single founder cell. Every animal or plant is a large colony of individual cells, each of which performs a specialized function that is regulated by complicated systems of cell-to-cell communication. Cells, therefore, are the fundamental units of life. **Cell biology**: is the study of cells and their structure, function, and behavior.

**Unity and Diversity of Cells:** cells are not all alike; in fact, they can be wildly different. Here we can summarize some of the cells characteristics:

1. Cells Vary in Appearance and Function.
2. Living Cells All Have a Similar Basic Chemistry.
3. Genes Provide the Instructions for Cell Form, Function, and Complex Behavior

If you cut a very thin slice from a suitable animal tissue and view it using a light microscope, you will see that the tissue is divided into thousands of small cells. The study of tissue properties is called histology.

**Histology**: *is the study of the tissues of the body and how these tissues are arranged to constitute organs*. Tissues are made of two interacting components: **cells** and **extracellular matrix (ECM)**. The main functions of extracellular matrix are:

1- Provide a mechanical support for the cells,

2- Transport nutrients to the cells,

3- Carry away catabolites and secretory products.

Cells produce the extracellular matrix, but it also influenced and sometimes controlled by molecules of the matrix. Many components of the matrix recognized by and attaching to receptors present on cell surfaces which are molecules that cross the cell membranes and connect to structural components of the intracellular cytoplasm. Each of the fundamental tissues (except the central nervous system) is formed by several types of cells and typically by specific associations of cells and extracellular matrix.

**Preparation of Tissues for Study**

It is the preparation of **histological sections or tissue slices** that can be studied with the aid of the light microscope. They must be sectioned to obtain **thin**, **translucent** sections and then attached to glass slides before they can be examined. The ideal microscope tissue preparation should be preserved so that the tissue on the slide has the same structure and molecular composition as it had in the body.

The basic steps used in tissue preparation for histology are:

**1- Fixation**

If a permanent section is desired, tissues must be fixed. Fixation is used to:

* Terminate cell metabolism,
* Prevent enzymatic degradation of cells and tissues by autolysis (self-digestion).
* Kill pathogenic microorganisms such as bacteria, fungi, and viruses.
* Harden the tissue as a result of either cross-linking or denaturing protein molecules.

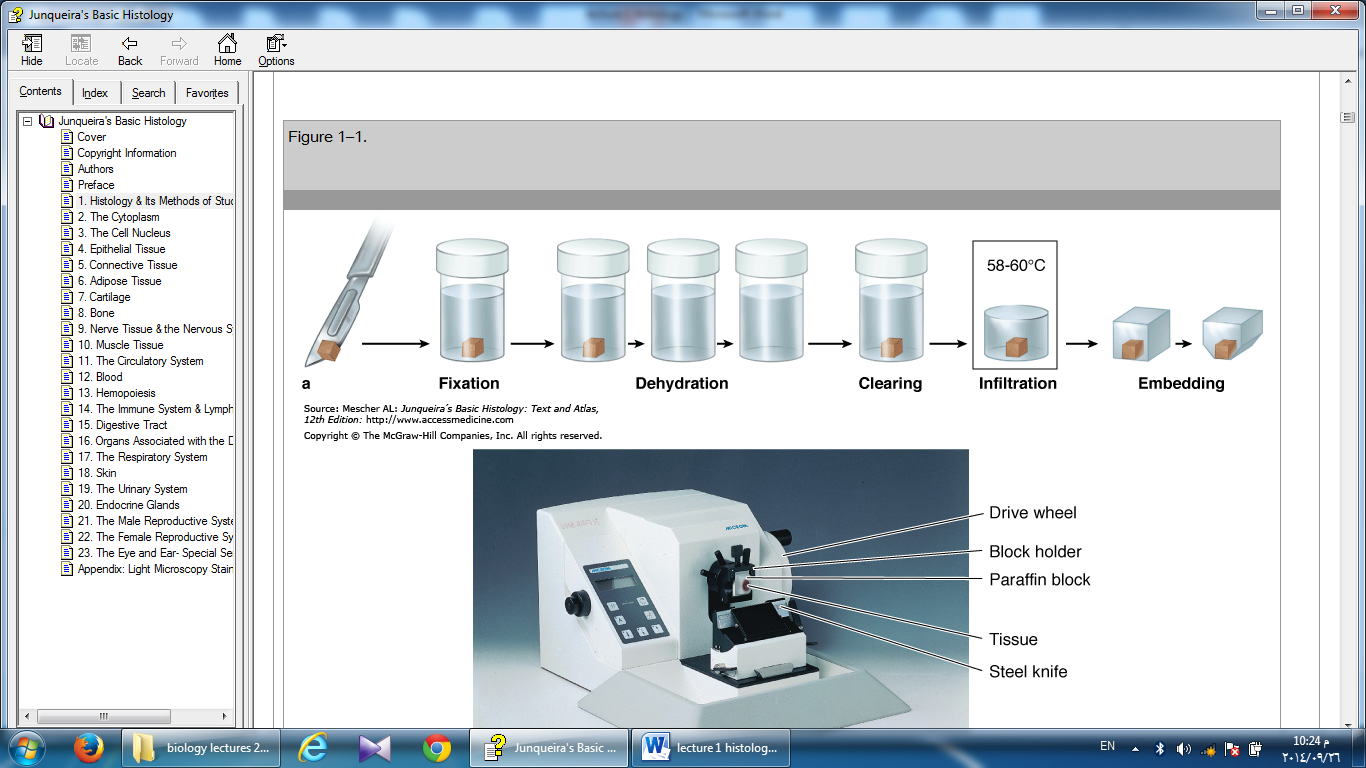
One of the best fixatives for routine light microscopy is formalin, a buffered isotonic solution of 37% formaldehyde.

Due to high resolution afforded by the electron microscope, a double fixation procedure, using a buffered glutaraldehyde solution followed by a second fixation in buffered osmium tetroxide, is a standard procedure in preparations for fine structural studies. The effect of osmium tetroxide is to preserve and stain lipids and proteins.

***Fixation by freezing***

It involves the submission of the tissues to rapid freezing. A freezing microtome (cryostat) is then used to section the frozen block with tissue. This method allows the rapid preparation of sections and it is also effective in the

Figure 1-1: steps of tissue preparation

histochemical study of very sensitive enzymes or small molecules and useful when structures containing lipids are to be studied (no xylene).

**2- Embedding & Sectioning**

Tissues are usually embedded in a solid medium to facilitate sectioning. Embedding substances gives a rigid consistency to the tissue. Embedding materials include paraffin and plastic resins. Paraffin is used routinely for light microscopy; resins are used for both light and electron microscopy. It involves two main steps: **dehydration** and **clearing**. The water is first extracted by bathing tissue successively in a graded series of mixtures of ethanol and water, usually from 70% to 100% ethanol (dehydration). The ethanol is then replaced with other solvent, xylene. As the tissues are infiltrated with this solvent, they generally become transparent (clearing). Once the tissue absorbs the solvent, it is placed in melted paraffin in an oven, typically at 52–60°C. The heat causes the solvent to evaporate, and the spaces within the tissues become filled with paraffin. The tissue together with its impregnating paraffin hardens after removal from the oven. The hard blocks containing the tissues are then placed in the microtome and are sliced by the microtome's steel or glass blade into sections 1 to10 micrometers thick, (micrometer (1um) = 1/1,000 of a millimeter (mm) = 10–6 m). The sections are floated on water and then transferred to glass slides to be stained.

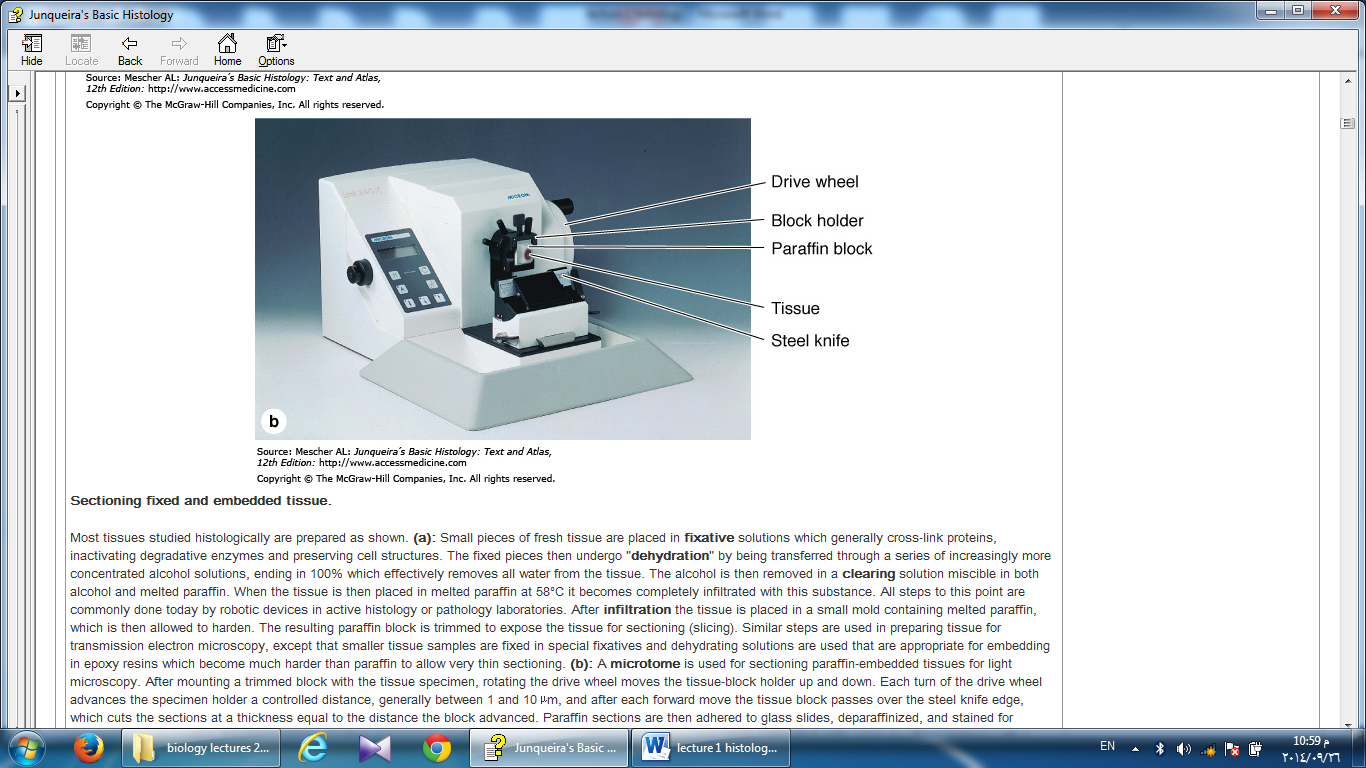


Figure 1-2: The microtome

**3- Staining**

Sections must typically be stained or dyed because most tissues are colorless. Most of these dyes behave like acidic or basic compounds and have a tendency to form electrostatic (salt) linkages with ionizable radicals of the tissues. Tissue components with a net negative charge (anionic) stain more readily with basic dyes and are termed basophilic; cationic components, such as proteins with many ionized amino groups, have affinity for acidic dyes and are termed acidophilic. Basic dyes like toluidine blue, alcian blue, and methylene blue and Hematoxylin stain the nucleic acids, glycosaminoglycans, and acid glycoproteins. Acid dyes (eg, orange G, eosin, acid fuchsin) stain the acidophilic components of tissues such as mitochondria, secretory granules, and collagen. Combination of hematoxylin and eosin (H&E) is used most commonly. Hematoxylin stains DNA of the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of cartilage) blue. In contrast, eosin stains other cytoplasmic components and collagen pink.

**Medical applications**

Biopsies are tissue samples removed during surgery or routine medical procedures. In the operating room or medical center, biopsies are fixed in vials of formalin for later processing and microscopic analysis in a pathology laboratory. If results of such analyses are required before the medical procedure is completed, for example to know whether a growth is malignant before the patient is closed, a much more rapid processing method is used. The biopsy is rapidly frozen in liquid nitrogen, preserving cell structures and at the same time making the tissue hard and ready for sectioning. The frozen sections are placed on slides for rapid staining and microscopic examination by a pathologist.

H.W: DNA and Polysaccharides identification (PAS reaction)