



## **Histology:**

is the study of the tissues of the body and how these tissues are arranged to constitute organs. **Histology** involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ.

Tissues are made of two interacting components: **cells and extracellular matrix (ECM)**. The ECM consists of many kinds of molecules, most of which form complex structures, such as collagen fibrils and basement membranes. The main functions of ECM to supports the cells and transporting nutrients to the cells, and carrying away their wastes and secretory products.

## **PREPARATION OF TISSUES FOR STUDY:**

The most common procedure used in the study of tissue is the preparation of histological sections or tissue slices that can be examined under the light microscope. tissues are examined via a light that is transmitted through the tissue . Because most tissues and organs are too thick for light to pass through them ,they must be sectioned to obtain thin, translucent sections and then attached to glass slides before they can be examined .



## **The basic steps used in tissue preparation for histology:**

### **1. Tissue fixation**

Slide preparation begins with fixation of your tissue specimen. This is a crucial step in tissue preparation, and its purpose **is to prevent tissue autolysis and putrefaction**. For best results, your biological tissue samples should be transferred into fixative immediately after collection. Although there are many types of fixative, **most specimens are fixed in 10% neutral buffered formalin**. The optimum formalin-to-specimen volume ratio should be at least 10:1 (e.g., 10ml of formalin per 1cm<sup>3</sup> of tissue). This will allow most tissues to become adequately fixed within 24-48 hours.

### **2. Specimen transfer to cassettes**

After fixation, specimens are trimmed using a scalpel to enable them to fit into an appropriately labelled tissue cassette. Specimens should not be so big that they fill the cassette. The filled tissue cassettes are then stored in formalin until processing begins.

### **3. Tissue processing**

Processing tissues into thin microscopic sections is usually done using a paraffin block, as follows:



- *Dehydration* is the first step, which involves immersing your specimen in increasing concentrations of alcohol to remove the water and formalin from the tissue.
- *Clearing* is the next step, in which an organic solvent such as xylene is used to remove the alcohol and allow infiltration with paraffin wax.
- *Embedding* is the final step, where specimens are infiltrated with the embedding agent – usually paraffin wax. The tissue becomes surrounded by a large block of molten paraffin wax . Once the block solidifies, it provides a support matrix that allows very thin sectioning.
- **4. Sectioning**

Your tissue specimen is now ready to be cut into sections that can be placed on a slide. Wax is removed from the surface of the block to expose the tissue. Blocks are chilled on a refrigerated plate or ice tray for 10 minutes before sectioning. A microtome is used to slice extremely thin tissue sections off the block in the form of a ribbon. The microtome can be pre-set to cut at different thicknesses, but most tissues are cut at around 5  $\mu\text{m}$ .

Once cut, the tissue ribbons are carefully transferred to a warm water bath. Here they are allowed to float on the surface, and can then be scooped up onto a slide placed under the water level. Slides should be

clearly labelled, and then allowed to dry upright at 37°C for a few hours to gently melt the excess paraffin wax .

## 5. Staining

Most cells are transparent, and appear almost colourless when unstained. Histochemical stains (typically haematoxylin and eosin) are therefore used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate. Following staining, a cover slip is mounted over the tissue specimen on the slide, using optical grade glue, to help protect the specimen.



Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition. Copyright © McGraw-Hill Education. All rights reserved.



Source: Anthony L. Mescher: Junqueira's Basic Histology: Text and Atlas, 15th Edition.

## The microtome