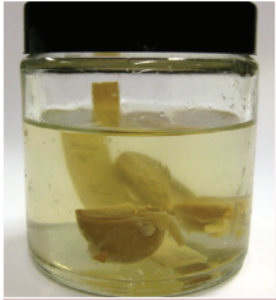
1 Preparation of tissues for histology

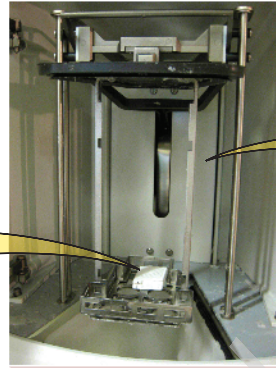
(a) Fixation



First the tissue is placed in fixative and allowed to fix

(b) Dehydration, clearing and wax impregnation

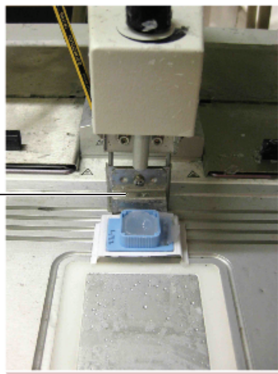
Next, the tissue is trimmed and placed in a cassette (the two halves of which are shown here)



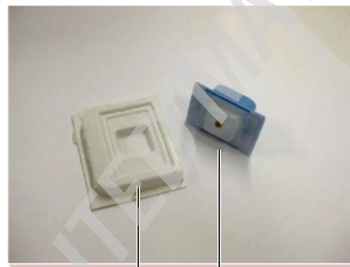
The holder is placed in a basket in the automatic processor

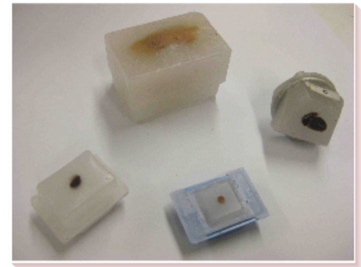
The processor transfers the tissue through a series of alcohol solutions of increasing strength, and then into a clearing agent (xylene) and finally into molten wax to complete the wax impregnation process

(c) Embedding



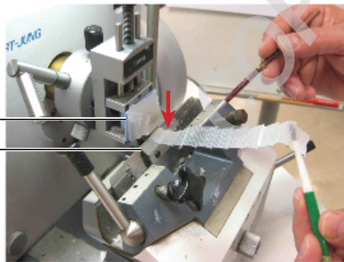
The tissue is transferred to a mould, and hot wax is dispensed into the mould



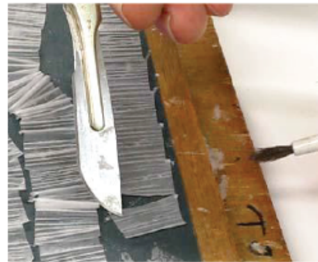


Blocks come in all shapes and sizes, depending on the size of the tissue

(d) Sectioning



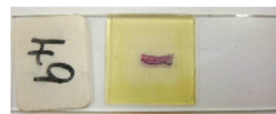
The block is moved up and down (red arrow) and moved incrementally forward (toward the user) to cut sections. Serial sections emerge in a long ribbon, and are picked up with brushes



Single sections are picked up, floated on the surface of hot water, which removes the folds, and then transferred onto a glass slide

(e) Staining

The unstained section on the slide



The final slide after staining and mounting

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Histology is the study of tissues and their appearance.

Histos is Greek for 'web or tissue', and ***logia*** is Greek for 'branch of learning'.

Anatomists first used the word 'tissue' to describe the different textures of parts of the body, as they were being dissected.

Today, histology and pathology (the study of diseased tissues) are routinely used in hospitals and research laboratories to study the organization of tissues and the cells within them.

Sectioning and preparing tissue for staining

To study the structures of cells and their organization within tissues, tissues have to be fixed and 'sectioned' (or cut), stained with dyes, and then observed with the light microscope. This is carried out in the following stages (see Fig. 1).

Fixation

A chemical solution containing a fixative at pH 7.0 is added to the tissue (Fig. 1a). The most commonly used fixative is formaldehyde at a concentration of 4%. (Commonly, dilutions are made from a stock of Formalin, i.e., 37% or 40% formaldehyde.) Formaldehyde binds to and cross-links some proteins, and denatures others, but does not interact well with lipids. The overall effect is to harden the tissue and inactivate enzymes, preventing the tissue from degrading.

Dehydration

In order for sections to be cut, the tissue has to be embedded in wax. However, wax is not soluble in water. Therefore, the water in the tissue has to be removed and eventually replaced with a medium in which wax *is* soluble. This is achieved by, first, sequentially replacing the water with alcohol, placing the tissue in a series of solutions that contain increasing concentrations of alcohol, ending at 100% (Fig. 1b). This process is carried out gradually in order to minimize tissue damage. The tissue must then be 'cleared' before it can be embedded in wax.

Clearing

Next, the section is placed in an organic solvent such as xylene or toluene, which replaces the alcohol. Wax is not soluble in alcohol. The clearing agents are so-called, because the tissue often looks completely clear when it is immersed in clearing agent. Finally, the tissue is impregnated with hot wax (Fig. 1b), which is soluble in this type of organic solvent.

Embedding

The tissue is placed in warm paraffin wax in a mould (Fig. 1c). On subsequent cooling, the wax hardens, and tissue slices can now be cut.

Sectioning

Sections (slices) about 10 to 20 microns (μm) thick are cut using a microtome (Fig. 1d).

Mounting

The wax sections are laid onto a glass microscope slide (Fig. 1e).

Staining

To see detail, the components of the tissue have to be stained. However, the stains that are used are all aqueous. Therefore, the wax has to be dissolved and replaced with water (rehydration), for the stains to be able to penetrate the tissue section. The sections are therefore placed in decreasing concentrations of alcohol, ending up at 0% alcohol (water).

A number of different stains can be used but the most common is hematoxylin & eosin (see Chapter 2).

Dehydration and mounting

The stained specimen is once again dehydrated, before placing it into mounting medium dissolved in xylene. Finally, a coverslip is placed on top of the sample to protect it, and the slide can be viewed on the microscope.

Other types of sectioning

Frozen sections

The tissue is rapidly frozen, fixed, and slices cut using a cryostat, before staining.

Semi-thin sections

The tissue is embedded in epoxy or acrylic resin, which has different properties to wax, and allows thinner sections (less than 2 μm) to be cut.

Sections in electron microscopy

See Chapter 4.