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Immunohistochemistry: Imaging tissues and cells

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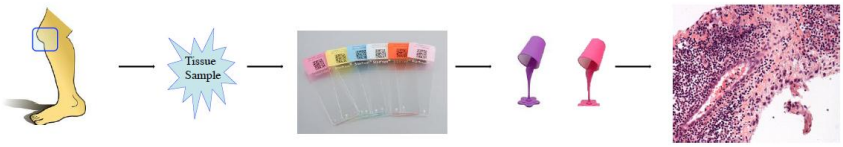
Histology is an essential tool in a medical or research laboratory. It is the study of the microscopic structure and anatomy of cells and tissues of animals and plants and is performed by examining a thin slice (section) of tissue using an Optical or Fluorescent microscope. The ability to visualise or differentially identify microscopic features using a microscope can be enhanced using histological stains such as Haematoxylin & Eosin



There are five steps in preparing tissue for histological examination:

1mm —
1cm ———————
A micron is one tenth of a mm

Fixation

The tissue is stored in a solution that will kill any bacteria that may make the tissue rot!
The most common fixatives used are buffered formalin or 4% formaldehyde in buffered saline

Dehydration & Clearing

The dehydration step is to remove all of the water and fixative from the tissue with ethanol. The solvent xylene is then used to infiltrated the tissue causing it to become more transparent (clearing).

Embedding

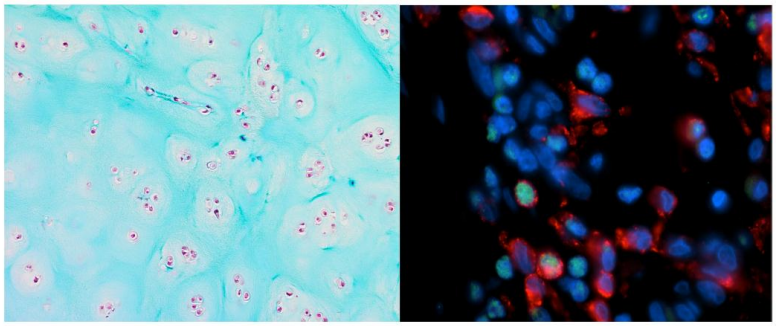
The tissue is transferred to a bath of wax at 58 - 60°C, this allows molten paraffin to mix into the xylene fully infiltrating the tissue. The next step is to orientate the tissue and embed it in a mould with molten paraffin then it is cooled it quickly to form a solid block of wax.

Sectioning

The tissue is now ready to be cut into extremely thin slices (sections). A microtome is used to cut the embedded tissue into sections. As the microtome moves the block of tissue up and down it passes over a knife that cuts the block into sections usually about 4 or 5 microns thick.

Mounting & Staining

The paraffin sections are transferred to a water bath at approximately 40°C to allow them to expand and straighten out. The sections are collected onto positively charged slides and are left to dry out. The slides can now be stained.



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