# Module:
# Techniques in Neuroscience

## Week 3
## Immunohistochemistry: Preserving and studying cells of the brain

## Topic 1
## An introduction to immunohistochemistry – Part 2 of 4

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We can process the tissues by hand, but more commonly - and while you have many, many tissues to process - you will use a tissue processor. There are several types of them. The two common ones are a dip and dunk machine, for example, the one shown here, which has 12 stations in it. The specimens are placed in a basket and loaded onto the processor. After predetermined times in each station with agitation the basket is automatically transferred from graded alcohols to either xylene or Histo-Clear, and then to melted paraffin wax before being removed for embedding in fresh molten paraffin wax. This is also called a dip-and-dunk processor.

This is an example of an enclosed tissue processor. Tissues are loaded into a chamber and the processing reagents are sequentially pumped in and out under vacuum to increase processing efficiency. This can be used as a high throughput processor. Once tissues have been infiltrated with paraffin wax, we then need to embed them. Commonly, you will use a paraffin wax embedding station, as shown here. So once they've been processed on the machine, for example, or by hand, the specimens are transferred to a molten wax tray on the embedding station. Tissues are then placed in metal moulds filled with molten wax and oriented optimally before placing on the cold plate to set the wax.

This image shows an operator embedding a piece of tissue in molten wax which is placed in a metal mould. The in-set shows a high power of the set wax block being taken out of the metal mould.

In this processing system, plastic cassettes are used to enclose each piece of tissue while processing. When embedding, the lids are removed and discarded. The tissues are then placed in the metal moulds, as mentioned earlier, and the plastic base of the cassette is placed on top. This plastic cassette will also have a unique number written on it for that specimen.

Once set, the block plus plastic base is removed. This plastic base is rigid and can be fixed into the vice of a microtome so that the block is firmly held in place while sectioning the block of tissue.

Once we have mounted our block of wax embedded tissue onto the microtome we need to section it. Sectioning is the process of cutting thin slices from the sample, which are required for microscopic examination. There are a number of devices available for sectioning and we will go through them.

Most common is a benchtop rotary microtome. This rotary microtome is used to cut sections from paraffin embedded tissues, and the thickness of the section conventionally ranges between three and 10 microns. A sledge microtome can alternatively be used.

Unlike any other type of sectioning, paraffin wax sections can be cut as ribbons, as is seen on the image. Once sections are cut, they are floated onto water in a bath, maintained at about approximately 40 degrees centigrade. This softens the wax surrounding the tissue sections so it expands allowing the tissue sections themselves to become flat. Sections may then be separated to be mounted onto slides; individually, or mounted as ribbons. This slide shows a ribbon of sections floating out on warm water in a water bath. The ribbon of sections can be seen from which two sections have been detached by hand, by the operator. One of them is being mounted onto a microscope slide.

Another type of microtome with which to cut sections is the vibratome. This is used for samples embedded in softer media not paraffin wax, such as agarose or gelatine, and 50 to 500 micron sections can be cut from these. The sections are cut by a blade vibrating at high lateral speed but advancing slowly through the sample. Sections are collected and are stained as free floating sections or can be mounted onto slides.

This slide shows images of sections that have been cut by the vibratome, so they're vibratome sections. On the left hand side, the sections have been cut but, they have not yet been stained. The sections in the dish on the right hand side are sagittal sections of rat brain, stained using cresyl fast violet, and are ready to mount onto slides and cover slip for microscopical examination.

The image here is of a cryostat. A cryostat is used for sectioning of snap-frozen tissues. Sections are cut using a refrigerated cabinet maintained at approximately -20 degrees centigrade, and it contains a rotary microtome. Cryostat cut sections are usually in the range of eight to 50 microns.

Cut sections are usually mounted onto slides and can be immediately fixed if the tissue has been frozen as fresh tissue, or of the sections can be stored for later use. Thus, you will have to store them at either -20, for short term, or -80, for long term storage.

Because the tissue is snap-frozen, the vitreous water is hard enough to act as the embedding medium for cutting sections. However, one may use an embedding medium, if required. This is particularly useful for delicate or very small samples. One uses a specialised embedding medium, such as the Optimal Cutting Compound - OCT - which freezes at the same density as most soft tissues.

This is an example of another type of microtome. This is a sliding microtome and it's commonly used to section frozen samples without the need for a relatively more expensive cryostat. This machine is fitted to the bench top and the tissue is kept frozen by blasting it with CO2 gas, for example, or cardice - that is, solid CO2.

The sliding microtome can produce 15 to 200 micron sections which are stained as free floating sections.