Microtome

A **microtome** (from the Greek *mikros*, meaning "small", and *temnein*, meaning "to cut") is a tool used to [cut](http://en.wikipedia.org/wiki/Cutting) extremely thin slices of material, known as sections. Important in science, microtomes are used in [microscopy](http://en.wikipedia.org/wiki/Microscopy), allowing for the preparation of samples for observation under transmitted light  or electron  radiation. Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to [electropolishing](http://en.wikipedia.org/wiki/Electropolishing" \o "Electropolishing) and [ion milling](http://en.wikipedia.org/wiki/Focused_ion_beam).

Microtomes use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. **Steel blades are used to prepare sections of animal or plant tissues for light microscopy**[**histology**](http://en.wikipedia.org/wiki/Histology).

**Glass knives are used to slice sections for light microscopy and to slice very thin sections for**[**electron microscopy**](http://en.wikipedia.org/wiki/Electron_microscopy).

**Industrial grade diamond knives are used to slice hard materials such as bone, teeth and plant matter for both light microscopy and for electron microscopy**.

**Applications**

The most common applications of microtomes are:

* **Traditional**[**Histology**](http://en.wikipedia.org/wiki/Histological)**Technique**: tissues are hardened by replacing water with [paraffin](http://en.wikipedia.org/wiki/Paraffin_wax). The tissue is then cut in the microtome at thicknesses varying from 2 to 50 µm. From there the tissue can be mounted on a microscope slide, stained with appropriate aqueous dye(s) after prior removal of the paraffin, and examined using a light microscope.
* [**Cryosectioning**](http://en.wikipedia.org/wiki/Frozen_section_procedure)**Technique:** water-rich tissues are hardened by freezing and cut in the frozen state with a reezing microtome or microtome-[cryostat](http://en.wikipedia.org/wiki/Cryostat); sections are stained and examined with a light microscope.
* [**Electron Microscopy**](http://en.wikipedia.org/wiki/Electron_Microscopy)**Technique**: after embedding tissues in epoxy resin, a microtome equipped with a glass or gem grade diamond knife is used to cut very thin sections (typically 60 to 100 nanometer). Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope. This instrument is often called an *ultramicrotome*.
* **Botanical Microtomy Technique**: hard materials like wood, bone and leather require a sledge microtome. These microtomes have heavier blades and cannot cut as thin as a regular microtome.
* [**Spectroscopy**](http://en.wikipedia.org/wiki/Spectroscopy)**(especially**[**FTIR**](http://en.wikipedia.org/wiki/FTIR)**or**[**Infrared spectroscopy**](http://en.wikipedia.org/wiki/Infrared_spectroscopy)**) Technique:** thin polymer sections are needed in order that the infra-red beam will penetrate the sample under examination. It is normal to cut samples to between 20 and 100 µm in thickness. For more detailed analysis of much smaller areas in a thin section, FTIR [microscopy](http://en.wikipedia.org/wiki/Microscopy) can be used for sample inspection.

**Microtome types**

**Sledge microtome**

[](http://en.wikipedia.org/wiki/File:Sledge_microtome.jpg)

A sled microtome.

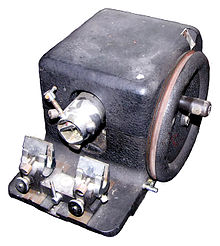
A sledge microtome is a device where the sample is placed into a fixed holder (shuttle), which then moves backwards and forwards across a knife.

 By adjusting the angles between the sample and the microtome knife, the pressure applied to the sample during the cut can be reduced.

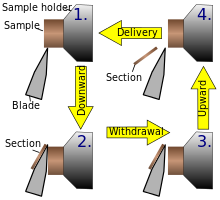
Typical applications for this design of microtome are of the preparation of large samples, such as those embedded in paraffin for biological preparations.

Typical cut thickness achievable on a sledge microtome is between 1 and 60 µm.

**Rotary microtome**

[](http://en.wikipedia.org/wiki/File:Microtome-1.jpg)

This instrument is a common microtome design. This device operates with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position.

[](http://en.wikipedia.org/wiki/File:Microtome_principle.svg)

Principle of sample movement for making a cut on a rotary microtome

**Cryomicrotome**

[](http://en.wikipedia.org/wiki/File:Cryostat_microtome.jpg)

**A cryomicrotome.**

For the cutting of frozen samples, many rotary microtomes can be adapted to cut in a liquid nitrogen chamber, in a so-called cryomicrotome setup. The reduced temperature allows for the hardness of the sample to be increased, such as by undergoing a glass transition, which allows for the preparation of semi-thin samples.] However the sample temperature and the knife temperature must be controlled in order to optimise the resultant sample thickness

**Ultramicrotome**

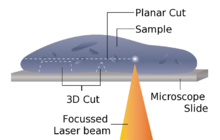
[](http://en.wikipedia.org/wiki/File:Microtome-ultras.jpg)

A ribbon of ultrathin sections prepared by room temperature ultramicrotomy, floating on water in the boat of a diamond knife used to cut the sections. The knife blade is the edge at the upper end of the trough of water.

**Vibrating microtome**

The vibrating microtome operates by cutting using a vibrating blade, allowing the resultant cut to be made with less pressure than would be required for a stationary blade. The vibrating microtome is usually used for difficult biological samples. The cut thickness is usually around 30-500 µm for live tissue and 10-500 µm for fixed tissue.

**Laser microtome**

[](http://en.wikipedia.org/wiki/File:Laser-microtome-schematic.png)

A conceptual diagram of laser microtome operation.

The [laser](http://en.wikipedia.org/wiki/Laser) microtome is an instrument for contact free slicing. Prior preparation of the sample through embedding, freezing or chemical [fixation](http://en.wikipedia.org/wiki/Fixation_(histology)) is not required, thereby minimizing the artifacts from preparation methods. Alternately this design of microtome can also be used for very hard materials, such as bones or teeth as well as some ceramics. Dependent upon the properties of the sample material, the thickness achievable is between 10 and 100 µm.

**Embedding**

It is the casting or blocking of tissue section, which involves the enclosure of the tissue in the infiltration medium used for processing, and then allowing the medium to solidify. The infiltrating medium is selected according to the embedding media that will be used.

**EMBEDDING MEDIA**

Infiltrating and embedding media must fill all spaces within the tissue to support cellular components adequately during microtomy. Density of the hardened medium should approach that of the densest tissue component otherwise section deformation will result. The matrix must be elastic enough to recover sectioning deformation, and plastic enough to facilitate thin sectioning. Tissue-medium adhesion is enhanced if the embedding matrix has a fine uniform crystalline morphology which intimately contacts the tissue. Viscosity and melting point of the infiltration medium partly determine the duration and temperature of processing conditions.

**Embedding tissues in paraffin wax**

Tissues are embedded by placing them in a mold filled with melted embedding medium which is then allowed to solidify. Embedding requirements and procedures are essentially the same for all waxes

At the completion of processing, tissues are held in clean paraffin wax which is free of solvent and particulate matter.

Requirements for embedding are as follows:

• a supply of clean, filtered paraffin wax held at 2-4°C above its melting point.

• a cold plate to rapidly cool the wax.

• a supply of molds in which to embed the tissues.

**General Embedding Procedure**

METHOD

1) Open the tissue cassette, check against worksheet entry to ensure the correct number of tissue pieces are present.

2) Select the mold, there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax.

3) Fill the mold with paraffin wax.

4) Using warm forceps select the tissue, taking care that it does not cool in the air; at the same time.

5) Place the tissue in the mold according to the side to be sectioned. This side should be facing down against the mold. A small amount of pressure may be used in order to have more even embedding.

6) Chill the mold on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept flat.

7) Insert the identifying label or place the labeled embedding ring or cassette base onto the mold.

8) Add more paraffin into the mold to fill the cassette and mold.

9) Cool the block on the cold plate.

10) Remove the block from the mold.

11) Cross check block, label and worksheet

