

Exp. 6. Microtomy and steps in microtomy

Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to electropolishing and ion milling. Microtome sections can be made thin enough to section a human hair across its breadth, with section thickness between 50 nm and 100 μm .

PRINCIPLE: microtome is a sectioning instrument that allows the cutting of extremely thin slices of a material known as section . microtome are used in microscopy , allowing for the preparation of sample for observation under transmitted light or electrons radiation

TYPES OF MICROTOMES –

- 1.) Rotary microtome.
- 2.) Sliding or Base Sledge Microtome.
- 3.) Cambridge rocking microtome.

STEPS FOR PROCESSING TISSUES:

1. After fixing in Bouin's fluid for 12-24 hours, wash well in tap water and then in distilled water. Remove as much of picric acid as possible by repeated washing.
2. Dehydrate the tissues in 30%, 50%, 70%, 90% and absolute alcohol, 4-6 hours in each grade. You can leave the tissue in 70% alcohol for any length of time and process a few pieces at a time.
3. Clear in xylene for 15-30 minutes. Small sized tissues are better as they become transparent quickly.

Embedding in wax

Pour molten wax (50-60 $^{\circ}\text{C}$ range) in the bottle containing the tissues. Put in the incubator for 12-18 hours. Incubator should be set at 58-60 $^{\circ}\text{C}$ temperature, preferably at 60 $^{\circ}\text{C}$. Keep filtered molten wax in beakers at the same melting range in the incubator. After infiltration of wax into the tissues for 12-18 hours make the tissue blocks.

Two L-shaped brass metallic pieces are put on the glass plate smeared with glycerin. Pour the molten wax into the cavity formed by connecting together the two metallic pieces and transfer the material in the middle of the wax-containing cavity. Remove the wax block after setting and drying. Trim the paraffin blocks to proper size and keep in paper covers to be used later.

Equipment required

1. Honning stone. Adair Dutt Co. Pvt. Ltd. India, 21, Asaf Ali Road, New Delhi-110001.
2. Leather-stropper with wooden base, from the same company.

3. Microtome machine. Westwox Optik model MT 1090. Precision Rotary Microtome.
4. Microtome knife. American Optical from USA.
5. Machine oil. We use Usha tailoring machine oil, 3-in-one.
6. Haematoxylin stain for microscopy LOBA CHEMIE.
7. Eosin (soluble in alcohol) BDH or E. Merck Company.
8. Microslides, cover slips of different sizes.
9. DPX mountant.
10. Basic fuchsin (Para Rasaniline). Sigma chemical Co. USA.

Some hints while cutting sections

Cut at 7 μ m thickness.

Prepare water bath at 58 or 60 $^{\circ}$ C.

Smear albumen (Mayer's albumen:-White albumen, 50 ml; glycerin, 50 ml; sodium salicylate, 1.0 gram) on the slide. It will fix sections on the slide.

Put a few drops of 30% alcohol on the albumen smeared slide.

Cut the paraffin ribbon into bits containing 5 or 6 sections and put ribbon-bits on the slide with alcohol.

Put the ribbon into the water bath. The ribbon will completely stretch in the water bath without melting. Lift it with the same slide coated with Mayer's albumen.

Rest the slide at an angle of 45 $^{\circ}$ C against a solid surface. Water under the material will get drained out in 6-8 minutes.

Put the slides in the incubator overnight (24 hours) at temperature of 56-58 $^{\circ}$ C.

Remove the slides next day and store them in a slide box as long as required.

Prepare the staining set in staining jars that contain grooves for holding slides. Six slides in 3 sets can be processed at a time. The procedure is as follows:

1. Downgrading or Hydration. Xylene deparaffinization followed by 5 minutes each in absolute alcohol, 90%, 70%, 50% and 30% alcohol grades and distilled water.

2. Staining nuclei. In Harris haematoxylin 30-60 seconds depending on the tissues. Then dip the slides in acid water 0.5% (0.5 ml HCL in 100 ml of water) for one or two dips. Immediately put in tap water and wash for bluing of the nuclei in a glass trough with grooves.

3. Upgrading or dehydration. It is done by putting the slide for 5 minutes each in distilled water, 30%, 50%, 70% and 90% (2 changes) alcohol grades.

4. Cytoplasmic staining. Two dips in Eosin for 10 seconds each and wash for 3-5 minutes in 90% alcohol and then two changes of 5 minutes each in absolute alcohol. Then two changes of 5-10 minutes each in xylene for clearing. Then mount in DPX.

Keep the DPX mounted slides in the enamel tray overnight in the incubator for quick drying. Take out the slides next day and store in a slide box for examination under microscope at leisure.