

MICROTOMY

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MICROTOMY



- Microtome an instrument
- Microtomy the process of cutting thin sections

The histological preparation involves stages -

- 1. Fixation- formaldehyde, Carnoy's fluid and Bouin's fixative.
- 2. Dehydration- increasing concentrations of ethanol
 - replace alcohol with anhydrous system like chloroform, xylol, benzene or toluene
 - ethanol and xylol were taken in 1:1 ratio
 - treatment with pure xylol
- 3. Infiltration- xylol and liquid paraffin ratio 3:1, 1:1 and 1:3
 - fresh molten paraffin
- 4. Embedding block making
- 5. Sectioning trimming
 - fixed to a block holder
 - micron thin sections
 - Meyer's fixative

(white of egg 50 ml, Glycerol 50 ml; Sodium salicylate 1 gm.)

6. Slide preparation

Slide preparation

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Warm the slide
        Xylene - 10 minutes
  100% - 30% alcohol - 5 minutes
     Distilled water - 5 minutes
   Haematoxylin stain - 2 minutes
Distilled water wash - 10 - 15 minutes
 Dip in dilute HCl (if over stained)
  30% - 100% alcohol - 5 minutes
      Eosin stain - 30 seconds
   100% alcohol wash - 5 seconds
         Xylene - 5 minutes
Mount the slide using DPX mountant
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TABLE 1.2. Some Basic and Acid Dyes

COLOR

Basic dyes

Methyl green Green

Methylene blue Blue

Pyronin G Red

Toluidine blue Blue

Acid dyes

Acid fuchsin Red

Aniline blue Blue

Eosin

Orange G Orange

TABLE 1.1. Summary of Hematoxylin and Eosin (H&E) Staining

CELL AND EXTRACELLULAR COMPONENT	STAIN REACTION
Nucleus	
Heterochromatin Euchromatin Nucleolus	Blue Negative Blue
Cytoplasm	
Ergastoplasm	Blue
General cytoplasm	Pink
Cytoplasmic filaments	Pink
Extracellular material	
Collagen fibers	Pink
Elastic fibers ^A	Pink, but not usually distin- guishable from collagen fi- bers
Reticular fibers ^B	Pink, but not usually distin- guishable from collagen fi- bers
Ground substance	Blue, but only if present in large amounts, as in cartilage matrix
Bone matrix (decalci- fied)	Pink
Basement membrane ^B	Pink

^ASpecial staining procedure used for their demonstration, such as one containing resorcin-fuchsin or orcein.

^BSpecial staining procedure used for their demonstration, such as silver impregnation or periodic acid-Schiff (PAS) stain.