Preparation of samples for histological examination by light and electron microscopy

Sampling

- Purpose: obtain specimen of cells or tissue
- Size: 0.5-1 cm^3 for light microscopy, ~1 mm^3 for electron microscopy
- Methods: *biopsy* (excision, puncture, curettage, ascites, and aspiration) or *necropsy*

Fixation

- Purpose: stabilize cell and tissue structures by denaturing them. This is necessary, since freshly removed tissues are
  - chemically unstable
  - will dry up and shrink
  - suffer from hypoxia and bacteria
  - will autolyse (degrade via own enzymes)
- A good fixative must
  - preserve the structure well
  - quickly penetrate into the tissue block
  - not interact negatively with the staining
- Most commonly used substances include Formaldehyde for LM (12-24h); Glutaraldehyde for EM (1-3h).
- Ethanol, organic acids, inorganic acids, heavy metal salts, or compounds are also used
- Excess fixative is rinsed off with water
- Water is removed via ascending series of ethanol
- To allow embedding medium to enter, ethanol is cleared with a solvent miscible in both (ex: xylen)

Embedding

- Purpose: give firm texture to the sample to allow thin cutting
- Hard tissues (like dental or bone) require softening by acid (decalcification), or grinding to thin specimens
- Infiltration: tissue is placed into molten embedding medium (typically paraffin for LM, epoxide for EM)
- Hardening at room temperature
- Stable method: Formalin-Fixed-Paraffin-Embedded (FFPE). Many applications.

Cutting

- Microtomes are used to precisely control thickness
- Types: sliding, rotary, cryotomes, ultramicrotomes
- Cryostat: rotary microtome in freezing box; used to cut frozen tissue without embedding
- Thickness: 5-10 µm (light microscopy), 70-100 nm (electron microscopy)

Staining

- Before starting, one must affix sections to microscopic glass using albumin or gelatin
- Purpose: cells and their contents are usually colorless and thus invisible without staining for light microscopy. Electron microscopy uses heavy metals instead of staining for visualization
- Chromophilic compounds have high affinity for dyes, chormophobic ones do not.
- Basophilic components take up basic dyes (ex: nucleic acids); acidophilic components take up acidic dyes (cytoplasm, ionized proteins). Eosinophilic compounds take up eosin (ex: collagen)
- Routine staining visualizes all tissue components; special staining visualizes a particular structure.
- Mounting: attach cover slip with transparent adhesive over stained sample on slide

Routine Stain	cell nucleus	cytoplasm	collagen	erythrocytes	muscle fibers	Examples
Hematoxylin-Eosin (HE)	blue	pink-red	pink	red	red	Muscle
Hematoxylin-Eosin-Saffron (HES)	blue	pink-red	yellow	red	red	Esophagus
Azocarmine G-Aniline blue-orange G (AZAN)	red	red	blue	orange	red	Umbilical cord

Special Stains	Stained Structures
Gomori impregnation	reticular and nerve fibers - black
Oil red	lipids - red
Orcein, Resorcin, Fuchsin (elastic)	elastic fibers - brown/red-brown
Periodic acid Schiff (PAS) Reaction	polymers incorporating sugars - red
Pappenheim	blood cells
Schmorl	fine bone structures
Sudan black	lipids - blue-black
Toluidin blue/alcian blue	cartilage, bone, ECM - blue

References

Mescher, A. and Junqueira, L., 2018. *Junqueira's basic histology*. New York: McGraw-Hill, pp. 1-4.