

Laboratory methods

Histological stainings. Light
microscopy. Enzymatic
histochemistry

FIXATION METHODS

- Fixation methods can be grouped in two types:
- **physical**
- and **chemical** methods.

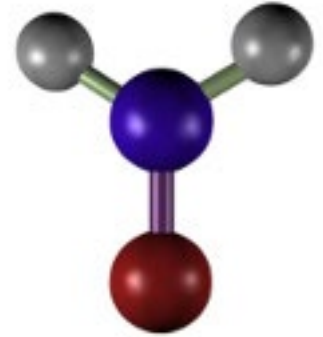
FIXATION METHODS

- **Physical**
- very quick freezing
- heating
- when a very quick fixation is required

FIXATION METHODS

- **Chemical**
 - use aqueous solutions containing fixative substances that form bridges between tissular molecules and it results in the immobilization of the tissular compounds and prevents degradation of the tissue sample.
 - There are two common methods for chemical fixation: **immersion and perfusion.**

FIXATIVES



- **Formaldehyde**
- a very good structural preservation for tissues,
- conserves tissues during long periods of time,
- produces small tissue retractions,
- is compatible with most techniques and histological staining procedures,

RESIN EMBEDDING

- Very thin sections, less than 100 nanometers,
- very hard material using ultramicrotomes,
- embedding the samples in resins.
- Epoxy resins are the most common embedding medium for electron microscopy studies.
- similar to that described for paraffin embedding,
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STAINING

- At light microscopy, most tissues are colorless, excepting those containing some pigments like hemoglobin in the blood or melanin in the epidermis.

GENERAL STAINING

- Dyes are colored substances with affinity for specific molecules of tissues, so they get attached to the tissue and provide color.
- It allows visualizing cells and extracellular matrix to be studied with light microscopes.
- Staining is usually done in tissue sections and cell smears.
- The most common sections for staining are those obtained from paraffin embedding and frozen tissues.

HISTOCHEMISTRY

- The goal of the histochemistry techniques is to detect specific molecules in tissue sections, and therefore it is possible to study their distribution "in situ", that is in the tissue.
- These molecules can not be readily distinguished by general staining techniques. The tissue has to be treated to reveal the molecule we are interested in.
- Histochemical techniques can be divided in two groups: **chemical reactions and histo-enzymology.**