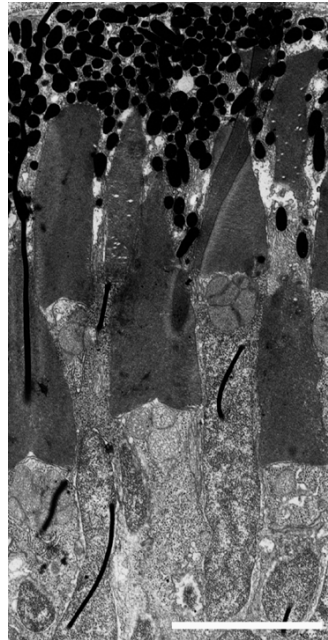


# Primer in Histology



Stephan Neuhaus

Histology – microscopic anatomy studying a thin slice of tissue under the microscope

1. Preparation of Tissue

Fixation  
Processing  
Embedding

2. Sectioning

Types of Microtoms  
Cryosections  
Plasticsections

3. Staining

Standardstaining

# Overview over most commonly used Techniques

<b>Cryosection</b>	tissue can be native or fixed thickness (3 – 30 micrometer) compatible with immunohistochemistry fast
Paraffin	thickness (5 – 30 micrometer) mostly compatible with immunohistochemistry very good tissue preservation
<b>Plastic Section</b>	thickness (1-5 micrometer) rarely compatible with immunohistochemistry great tissue preservation
Ultrathin (Epon)	used for electron microscopy tedious but unsurpassed detail

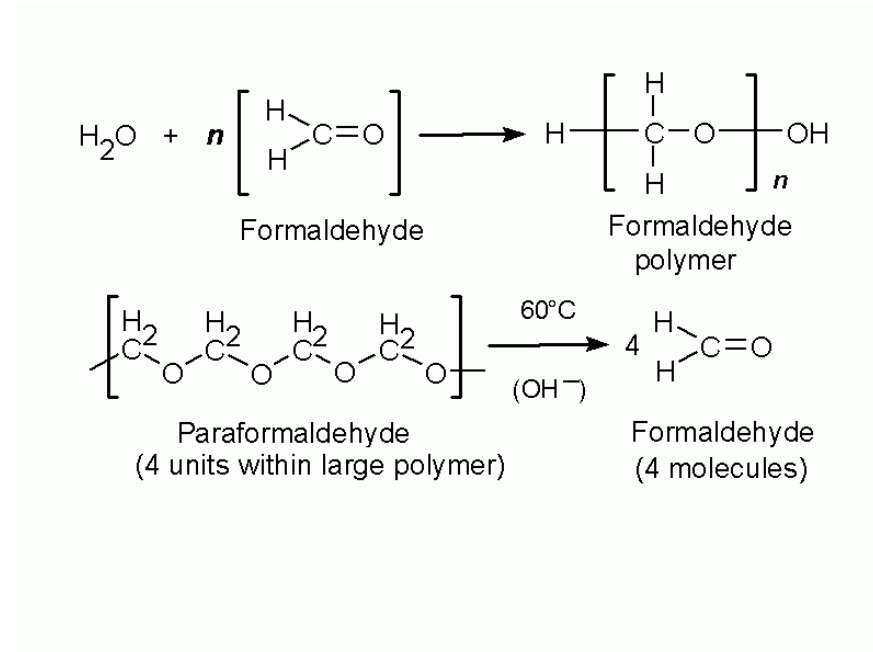
**Fixation** - preservation and hardening the tissue to prepare for processing

Types of Fixations:

Physical: heat, microwave, freeze-drying

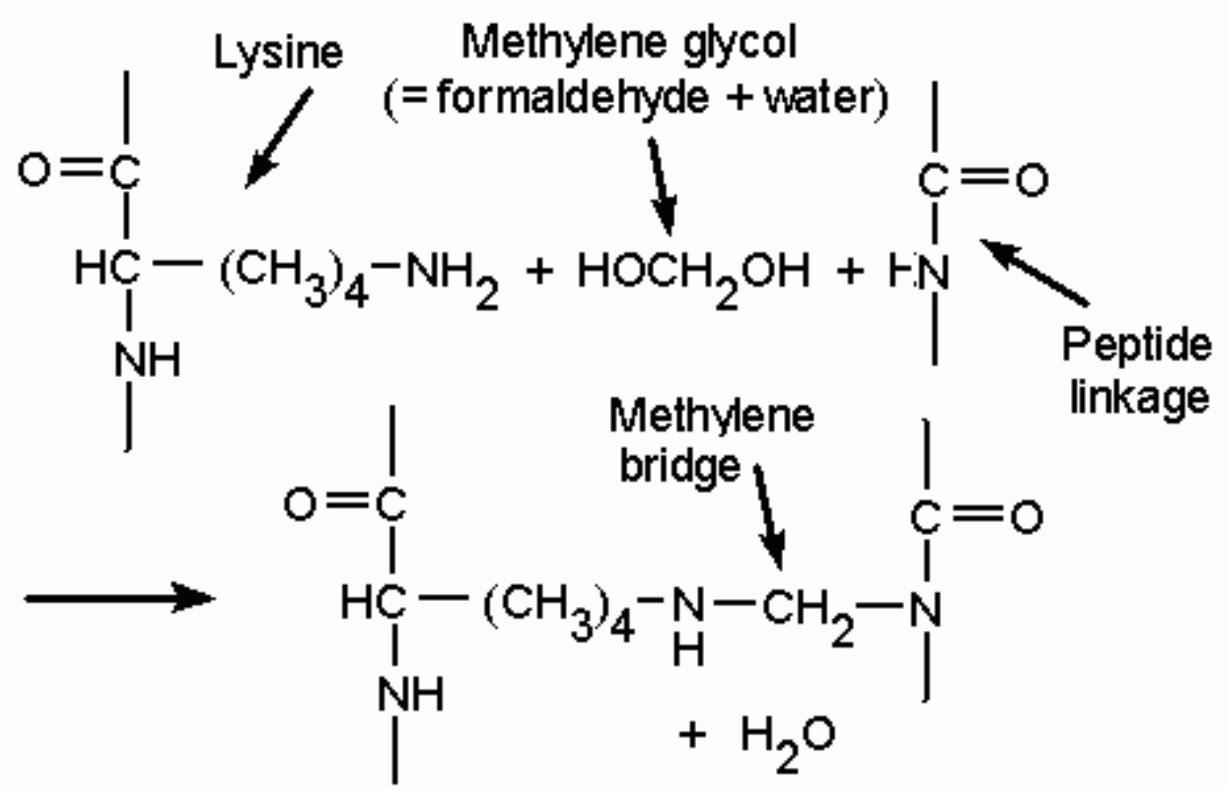
Chemical: Coagulation (Alcohols, Acids (picric and trichloroacetic acid))  
Crosslinking (para-formaldehyde, glutaraldehyde, osmium tetroxide)

The most common used fixating agent is **para - formaldehyde**

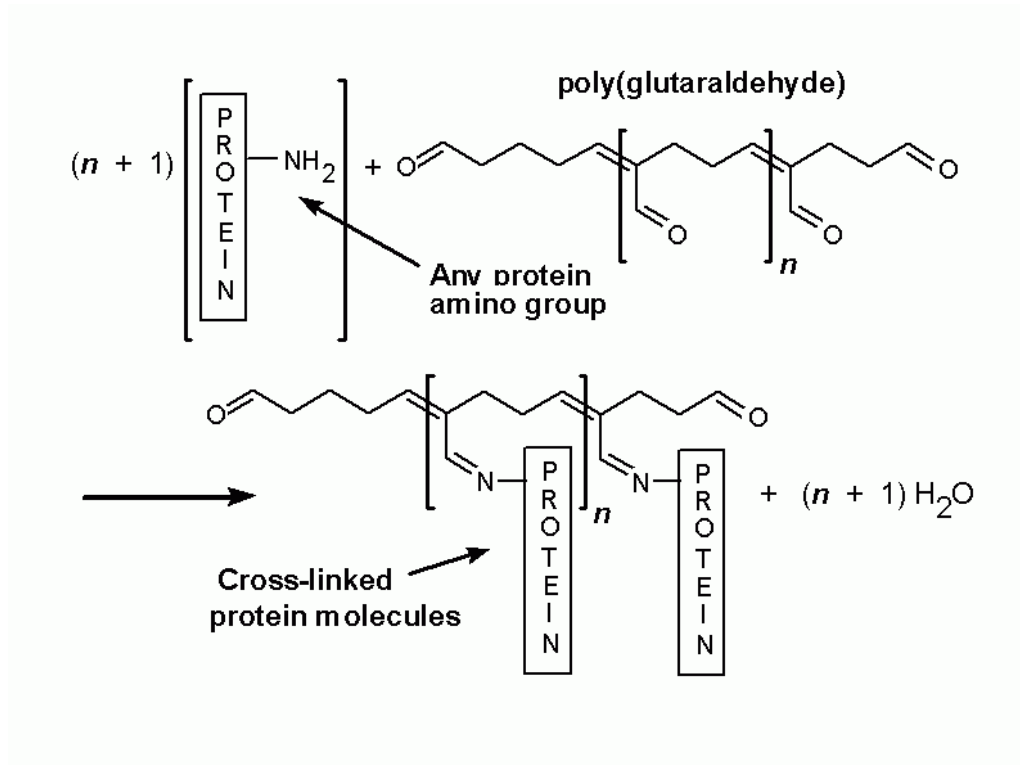


+ penetrates tissue well and fast  
compatible with immunohistochemistry

consider: fixation is reversible  
cross linking takes much more time than penetration



# glutaraldehyde fixation



- + penetrates tissue very well and crosslinks fast
- mostly incompatible with immunohistochemistry

# Processing - preparation for embedding medium

## **Cryosections:**

Freezing problem; ice crystals destroy tissue

Tissue needs to be equilibrated in freeze protection solution

usually sucrose (30%) or mixture of sucrose and embedding medium

## **Plastic Sections:**

Tissue needs to be dehydrated by alcohol series

Dehydrated tissue gets infiltrated with embedding medium (JB4 solution A)

**Embedding** - harden tissue for sectioning  
and orient to enable section plane of choice

**Cryosections:**

equilibrated tissue is embedded in O.C.T./ tissue tek  
(polyvinyl alcohol, polyethylene glycol)  
shock frozen in liquid nitrogen before transferring to cryostat

**Plastic Sections:**

Polymerization of equilibrated tissue by adding catalyst (JB4: solution B)

Tricky part is to keep orientation of sample during the hardening process

# Sectioning

- prepare tissue slices and stick them onto slides for staining and viewing

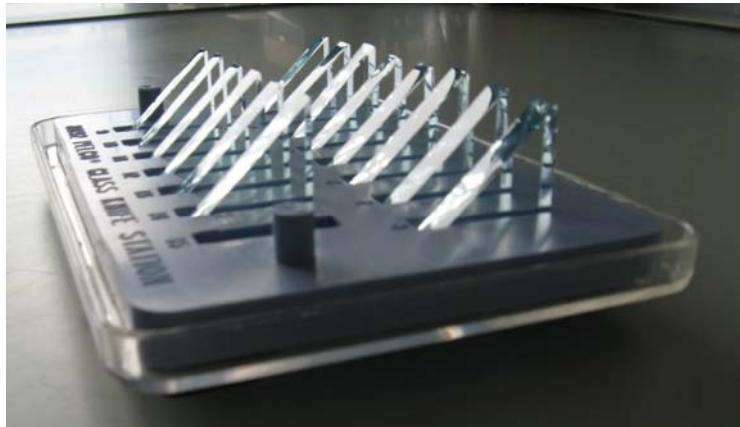
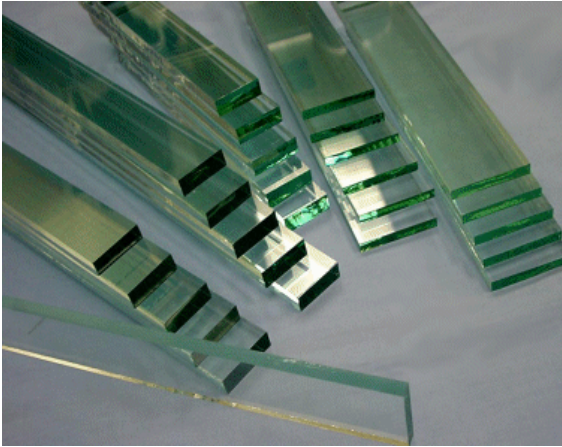


# Cryosections

- tissue is glued with O.C.T. onto holder
- metal knives or disposable blades are used
- block is trimmed and cut in cryostat
- sections are taken up by coated glass slide and dried on heatplate

# Plastic Sections

- tissue block is chucked into holder
- metal or glass knives are used
- block is trimmed and cut in microtom
- sections transfered to drop of water to be stretched
- dried on heat block onto coated slide





# Staining

- visualize specific feature of tissue

## **Standard Histology**

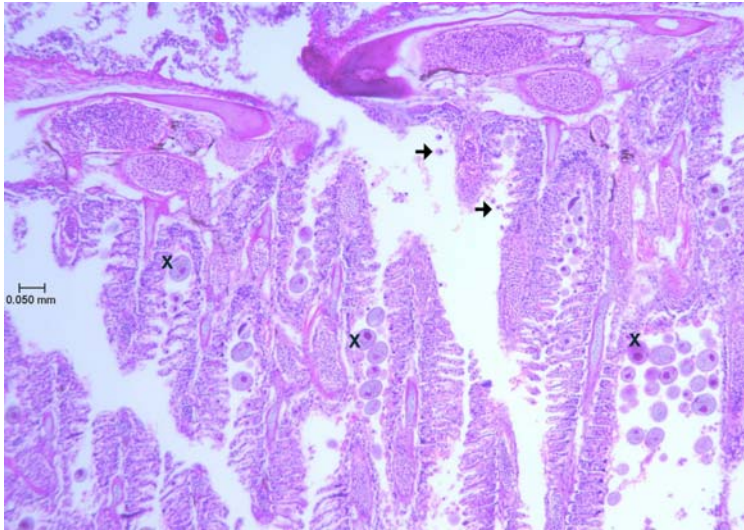
hematoxylin & eosin stain (H&E),  
Richardson, Methylene Blue

## **Specific Stains**

hundreds to choose from

## **Immunohistochemistry**

mostly on cryosections or post-stained plastic sections



Velvet Disease

