

Laboratory methods

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Staining methods

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Histological stains

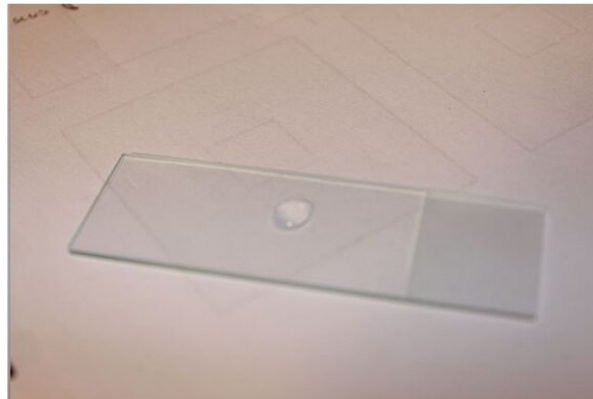
- paraffin blocks are cut into sections, which are **translucent**
= allow us to see shadow structures only (with the exception of pigments)
- we need to **dye** the sections / samples to see the tissues
 - = **orthochromatic stains** (does not change colour on binding to a target)
 - = **metachromatic stains** (change colour on binding to a target)
 - = e.g. toluidin blue stains nuclei blue (orthochemical), but granules purple (metachromatic)



Histological stains

Staining of histological slides

- usually sections of **paraffin** blocks
= or frozen section or tissue in another fixative



histological slide without staining
(section of paraffin block bind to the glass)

Histological stains

Staining of histological slides

- requires following of the protocol:

Histological stains

Staining of histological slides

- requires following of the protocol:

1) binding

= section of the paraffin block (containing tissue sample) is bound to the glass
(via glair or gelatine)

2) removal of wax

= removing the paraffin with **xylene** and **ethanol baths** (declining concentration)

= reverse procedure compared to the embedding the tissue into paraffin blocks

Histological stains

Staining of histological slides

- requires following of the protocol:

3) staining itself

= various dyes can be used, even combined

= application of stains can be **successional** (consecutive), or **simultaneous** (at once)

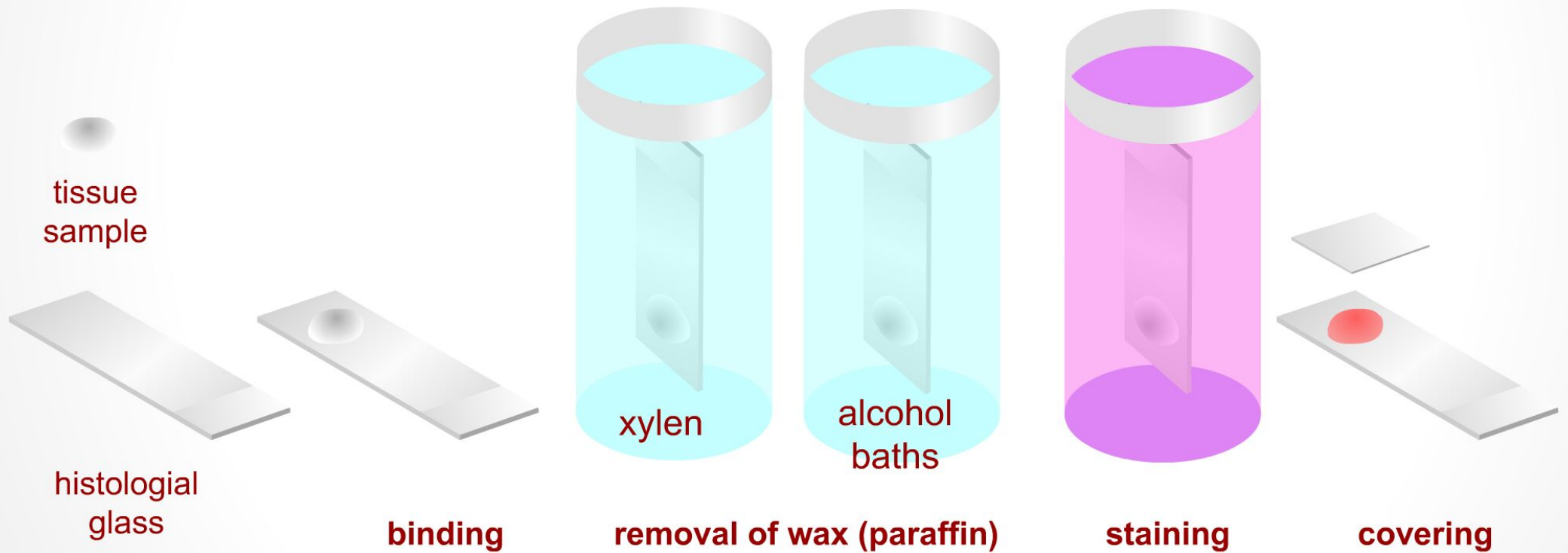
4) covering the slide

= tissue section must be covered with **cover-glass** to endure intact

= mechanical protection + creates optically homogenous environment

Histological stains

Staining of histological slides



Histological stains

Classification of histological dyes

1) Basic dyes

= combination of several dyes to enhance tissue contrast **in general**

= H&E, trichrome, Weigert van Gieson, Heidenhain's hematoxylin...

2) Special dyes

= numerous dyes to highlight **particular** structures within tissue (fibres, organelles)

= impregnation, neurohistochemical methods.....

- however the golden standard remains to be the
hematoxylin-eosin staining
(for centuries)

Basic stains

H&E (hemotoxylin-eosin staining)

- **standard** method

 - = the routine is to stain every tissue with H&E

 - = stains the tissue in **purple-to-blue** and **pink-to-red** fashion

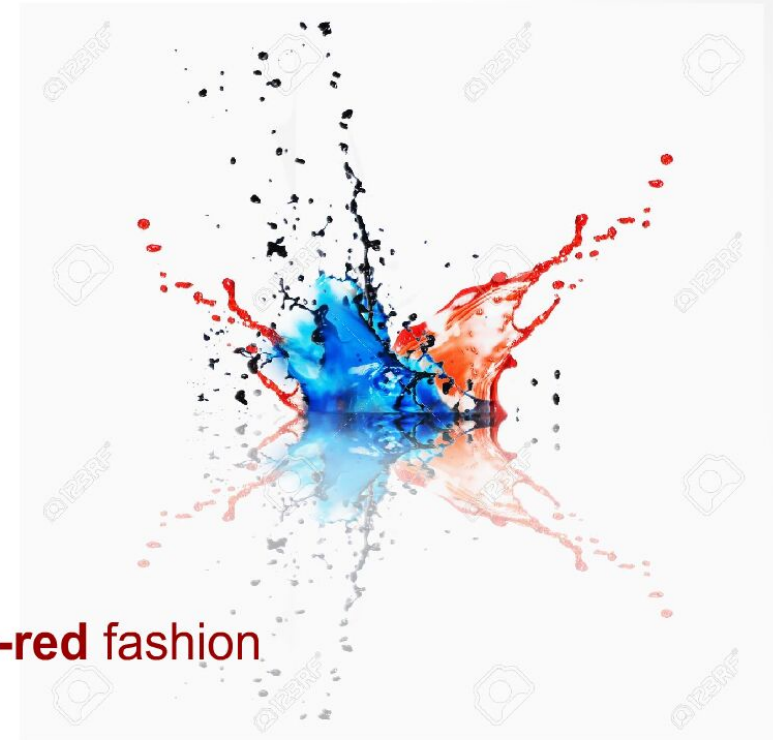
- **hematoxylin** is a basic dye

 - = stains **acid (= basophilic)** components of cells and tissues (nucleic acids, i.e. nucleus, nucleolus, ribosomes; acid mucopolysaccharides = cartilagineous matrix...)

- **eosin** is an acid dye

 - = stains **basic (= acidophilic, eosinophilic, oxyphilic)** components of cells and tissues (mainly proteins = extracellular fibres, cytoplasm, muscles...)

- affinity to both dyes is called **amphophilia** (polychromaticity, heterophilia)



Basic stains

Trichrome stainings

- several methods based on combination of **3 dyes**

= **basic dye** (hematoxylin) + **acid dye** (acid fuchsin, orange G) +

acid dye highlighting collagen (light green, methyl / water blue....)

= collagen fibers can be differentiated from other extracellular matrix / muscle cells

1) blue trichrome stainings

= AZAN, Mallory's trichrome stain

2) green trichrome stainings

= green Masson's trichrome stain





























3) yellow trichrome stainings

Basic stains

Weigert van Gieson

- also a common method to highlight collagen fibres
 - = **iron hematoxylin** (stains nuclei in dark blue) + **picrofuchsin** (stains mucles in yellow and collagen fibres in red)

Basic stains

Staining	Intracellular space			Extracelullular space		
	nuclei	cytoplasm	muscle cells	collagen	reticulin	elastic
H&E		 				
Blue trichrome		  				
Green trichrome						
Yellow trichrome						
Weigert van Gieson						
Heidenhain's hematoxylin						

Basic stains

Cytology stains

- differentiate cells in smear preparations in **cytology**
= due to the staining of nuclei and cytoplasm of cells (not tissues)

1) Pappenheim stain

- = staining of dry and fixated **blood smears**
- = **May-Grünwald staining** (eosin, metylen blue, metanol, glycerin) +
Giemsa-Romanowski staining (eosin, azur A, metylen blue and purple)

2) Papanicolaou stain

- = differentiate cells in smear preparations from bodily secretions in **gynecology**
- = **Harris's hematoxylin** (nuclei) + **orange G and polychrome EA50** (cytoplasm)
- = differentiates maturation of exfoliated keratinocytes of squamous epithelium

Special stains

- numerous dyes to highlight **particular** structures within tissue
= fibres, organelles...

1) Impregnation stains

= demonstration of microscopic structures by adding a mixture containing metal compounds (Ag, Os = black; Au = ruby)

- 1) Gömöri-Lillie's staining (reticular fibres)
- 2) Golgi's staining (neurones)
- 3) Horteg's staining (microglia)
- 4) Cajal's staining (astrocytes)
- 5) Cajal's staining modified by Penfield (astrocytes)
- 6) Holmes's staining (neurofibrils of neurons)
- 7) Impregnation of myelin sheaths of axones

Special stains

2) Neurohistopathological stainings

= highlights structures of **nerve system** (also impregnation can be used)

1) Nissl staining

= stains tigroid (Nissl substance) of neurons in purple

2) Luxol fast blue staining

= used to observe myelin (bluish)

3) Neurone stainings

= several stains, nowadays replaced with IHC (neuronal markers)

4) Glia staining

= several stains, nowadays replaced with IHC (GFAP)

Special stains

3) Elastic atains

= demonstration of **elastic fibres and membranes** within vessels, skin..

= for better orientation, other stains are added (hematoxylin, indigo carmine...)

1) Orcein

= dark brown

2) Resorcin-fuchsin

= dark purple or blue

Special stains

4) Polysaccharide dyes

= mainly **glycogen** and **mucosubstances** (glycolipids, glycoproteins, mucin)

1) PAS (Periodic acid–Schiff stain)

= both glycogen and mucus stained magenta colour (including mycotic structures)

2) PAS diastase stain

= "PAS D", used in combination with diastase (salivary enzyme that breaks down glycogen)

3) Mucicarmine stain

= identifies mucin within cells (glandular differentiation) or matrix ("mucin pools")

4) Alcian blue stain (AB)

= blue staining of acidic polysaccharides such as glycosaminoglycans in cartilage, mucopolysaccharides, sialylated glycocalyx of cells etc.

= can be used in combination as AB-PAS

Special stains

5) Staining of mikroorganisms

= bacterias, fungal organisms...

1) Gram staining

= differentiates 2 groups of bacteria based on their different cell wall constituents
(G+ blue, G- red)

2) Ziehl-Neelsen staining

= used to identify acid-fast organisms (*mycobacterium tuberculosis* red)

3) Grocott staining

= identification of carbohydrates in fungal mikroorganisms (black)

4) Warthin-Starry staining

= *helicobacter pylori* and *campylobacter jejuni* black

Special stains

6) Staining of anorganic substances

= **mineral** substances within human body

1) Perls Prussian / Berlin blue staining

= detects the presence of iron in tissue (blue)

= stains mostly iron in the ferric state which includes ferritin and hemosiderin, rather than ferrous state

2) Von Kossa stain

= illustrates mineralization such as Calcium and Potassium in tissues (black)

3) Rubeanic acid stain

= stained copper substances in black

Special stains

7) Amyloid stain

= pathologic extracellular protein, which can be stored in many organs with their destruction (amyloidosis)

1) Congo red staining

= apple-green birefringence of Congo red stained preparations under polarized light is indicative of the presence of amyloid fibrils (red in light microscope without polarizing filter)

Thank you.



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