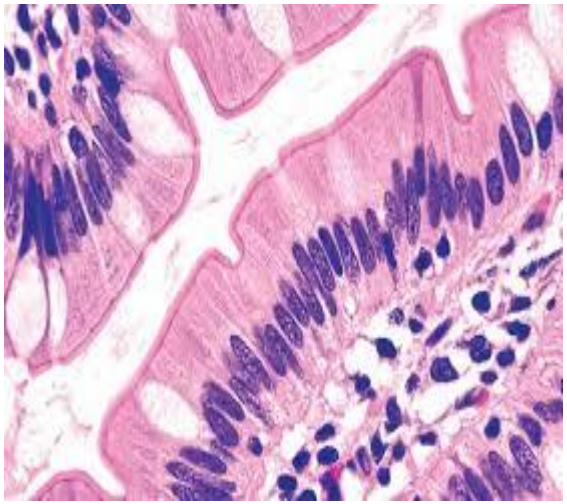
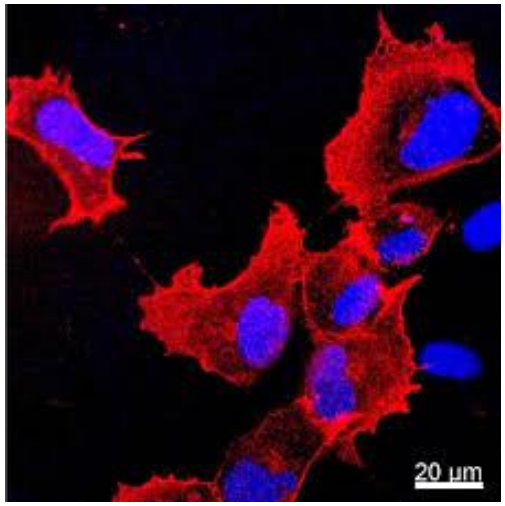
Histological techniques-2





By

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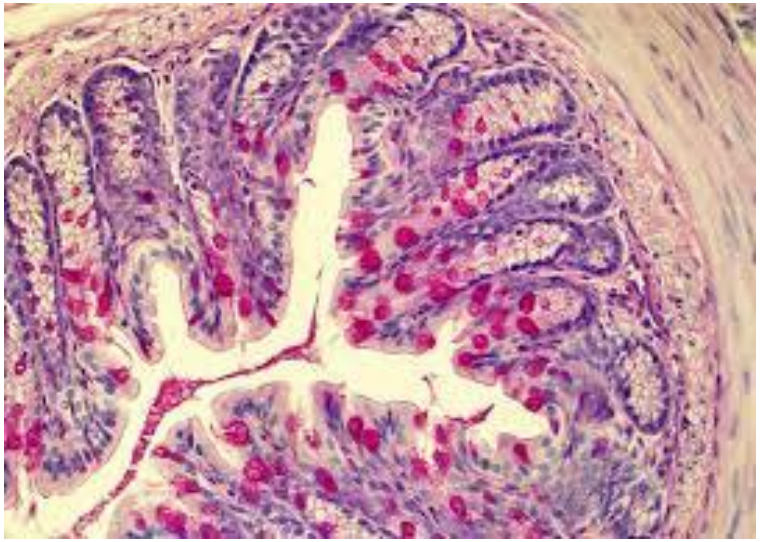
ILos

1. Recognize the basic stains for studying histology.
2. Know the steps of staining of a paraffin section with H&E
3. Describe Special staining technique as histochemistry &immunohistochemistry.

Tissue staining

- Most tissues are colorless , so stains must be used for visualize and distinguish the different parts of cells & tissues under microscope.



Types of stains

I- According to the reaction of stain:	
Acidic (stains basic structures as cytoplasm) ⟶ acidophilia	Eosin stain
Basic (stains acidic structures as nucleus) ⟶ basophilia	Heamatoxylin stain
Neutral	Leishman stain
Physical: dissolves in tissue without any chemical reaction	sudan III for fatty tissues.

II- According to the stained tissue:	
General (H &E)	Special stain

Staining

Routine stains

H&E

Special stains

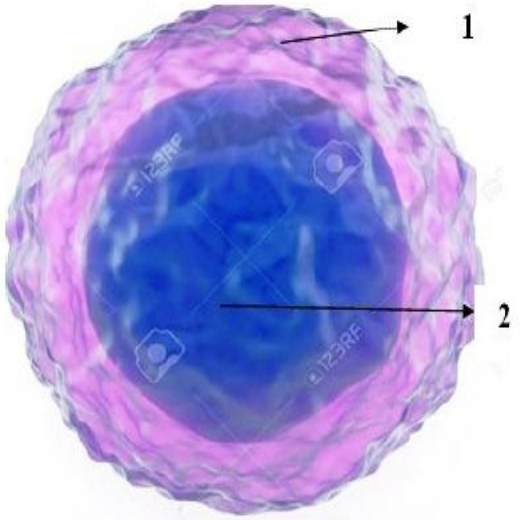
e.g. Tichrome stain

H&E (Routine histological stains)

1-Eosin (E):

red acidic dye (- ve charged)

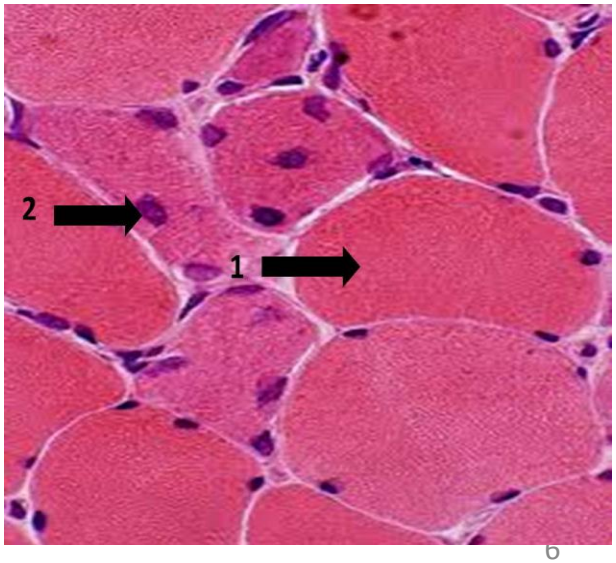
- Stains basic (cationic +ve) components of the cell with a red color e.g. cytoplasm. **Acidophilic structure=red**



2-Hematoxylin (H) :

blue basic dye (+ve charged)

- Stains acidic (anionic –ve) components of the cell with a blue color e.g. nucleus, ribosomes (r-RNA). **Basophilic structure=blue**



Staining of a paraffin section with H&E

- ***Initially,*** the paraffin must be removed, a process called clearing (by xylene).
- After clearing, only the tissue remains adhering to the slide.
- A lot of stains have been recognized, but the two stains most widely used for routine work are **hematoxylin &eosin (H & E).**

Staining of a paraffin section with H&E

1-Identify the upper side by scratching the wax

2-Replace paraffin by xylol

3-Replace xylol by alcohol absolute alcohol 100%

4-Replace alcohol by water (descending alcohol)

5-Stain in haematoxylin

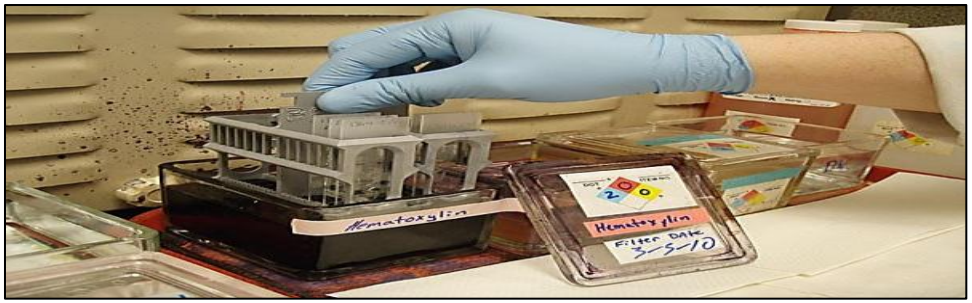
6-Wash in tap water

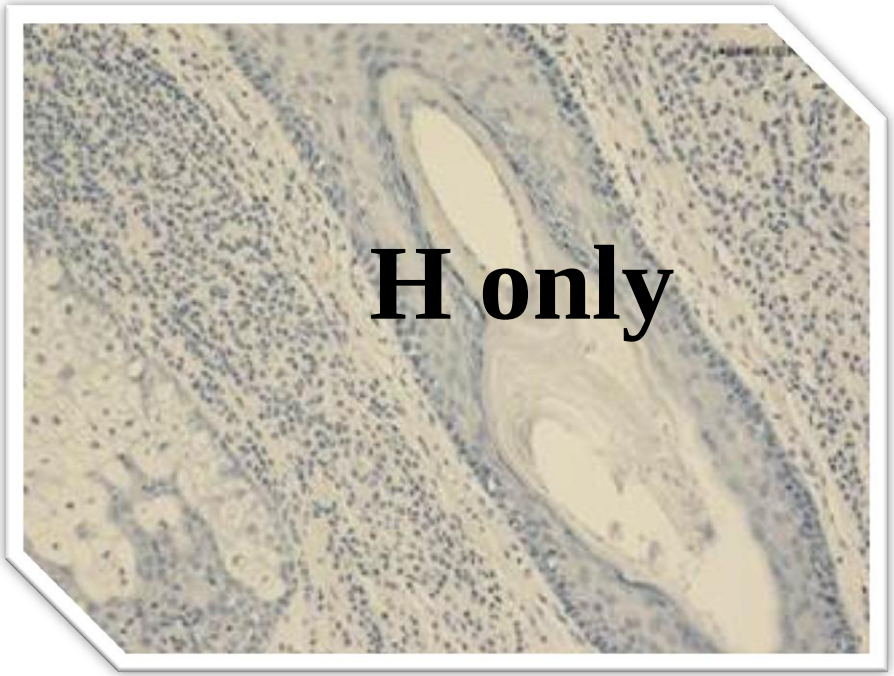
7-Counter-stain in eosin

8-Dehydrate in ascending grades of alcohol

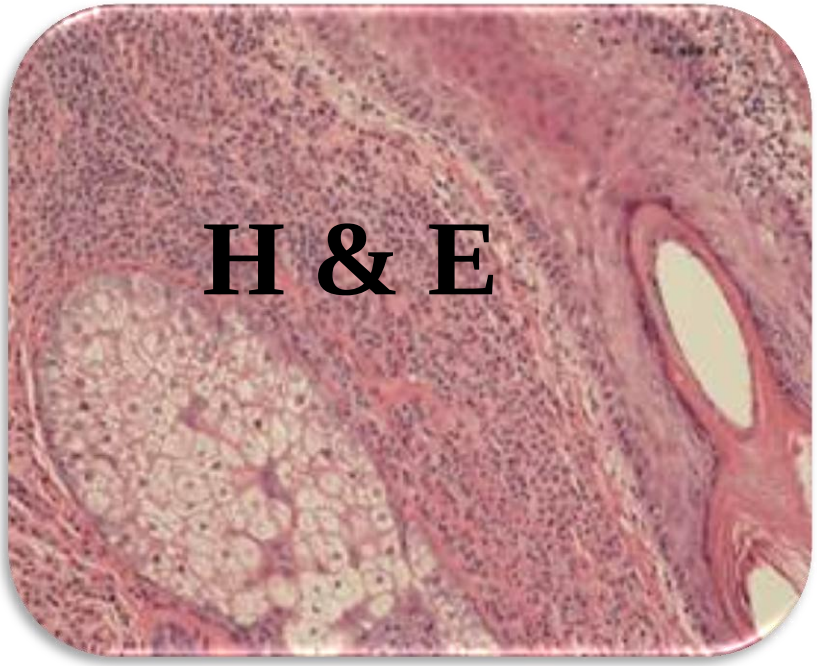
9-Clear in xylol

10-Mount in Canada balsam & cover with cover slip




H only


E only

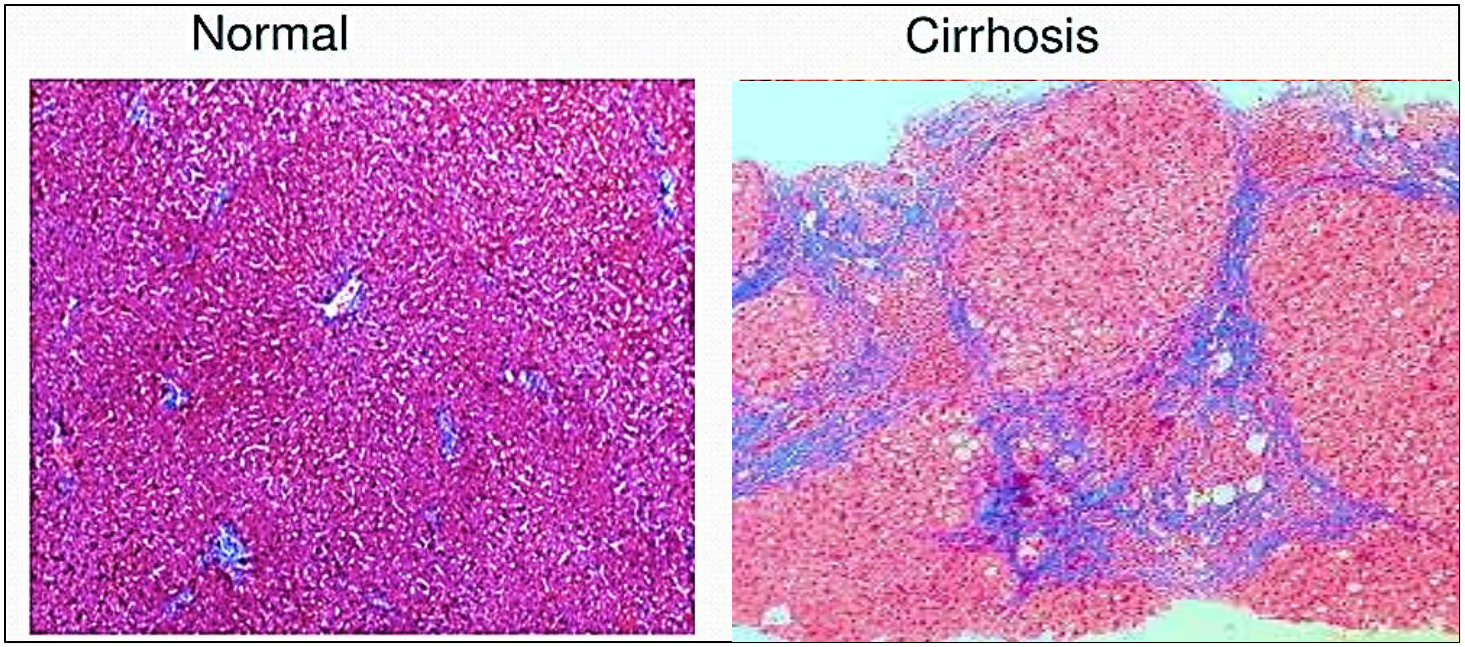

H & E

Special stains

- **value:**

-Special stains answer specific questions like *what type of cells and tissues*

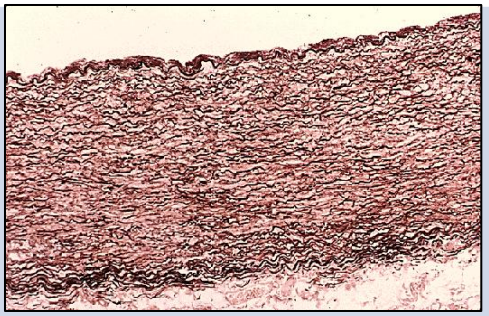
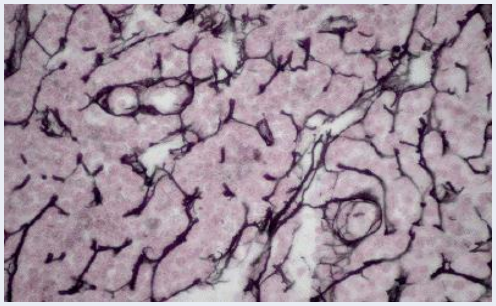
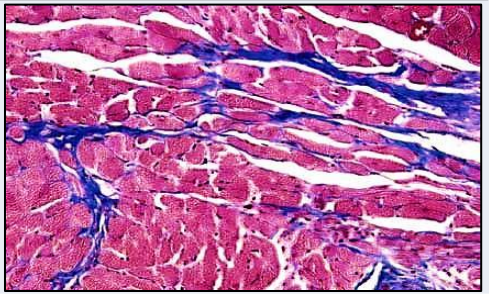
-Used in the diagnosis of medical diseases like Tichrome stain in case of Liver Cirrhosis



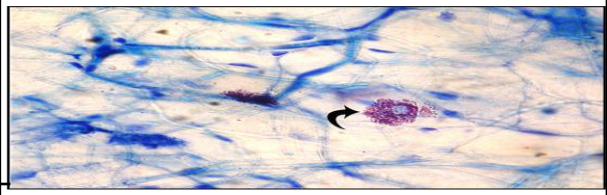
Some special stain for organic compound

Carbohydrates	- Periodic acid–Schiff (PAS) (carbohydrates & mucin/ magenta colour)
	- Best carmine (red Glycogen)
Lipids	• Oil Red O
	• Sudan III (orange)
	• Sudan black (black)
	• osmic acid (black)
Blood	- Giemsa stain
Nucleic acids	• Methyl Green Pyronin
Collagen	- Van Gieson
Connective tissue	• Masson trichrome (3 stains used in combination to stain different tissues Components) e.g. **collagen fibers** stained blue

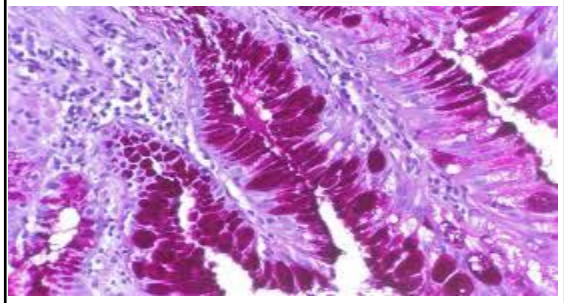
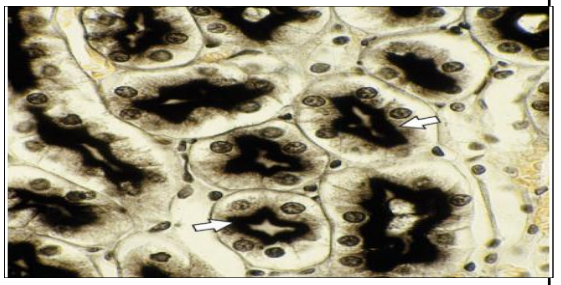
Some special stain for fibers

Elastic fibers	Orcein stain (brown) (wall of aorta)	
Reticular fibers	Silver stain	
Neurofilaments	Silver stain	
Collagen fibers	-Van Gieson -Masson trichrome	

Special staining techniques used for medical diagnosis

Technique	Explanation	Example
Vital stain	-Staining living tissue inside the body by non toxic dyes -Done by injecting the dye into living animal prior to examine the tissue.	Trypan blue stain (Macrophages) 
Supravital stain	Staining living tissues outside the body.	Brilliant cresyl blue staining reticulocytes
Metachromatic stain	Staining the tissues with a color different from the original color of stain	Toluidine blue for Mast cells 
Polychromatic stain	Staining the tissues with multiple colors in spite of using a single stain.	Geimsa stain for blood.

Special staining techniques used for medical diagnosis

Technique	Explanation	Example
Histochemictry	-technique **selectively identify & demonstrate the distribution of chemical compounds or enzymes** within & between the cells. principle: enzyme of interest in a cell or tissue converts its substrate → colored product -specific reagent when added to the tissue usually produce insoluble coloured (for L/M) or electron-dense (for E/M) compounds that enable the localization of specific substance.	Periodic acid –Schiff (PAS) for Mucine  -alkaline phosphatase enzyme 

Special staining techniques used for medical diagnosis

Immuno-histochemistry

-using specific antibodies to check for their antigens in a sample of tissue.

-Localization & staining specific proteins by using their specific antibodies the (antigen-antibody reaction) (Highly specific binding of AB to their Ag)

-Gives you a specific location.

- then Localize specific Ag in cell or tissue sections by labeled AB
- labeled AB: The antibodies are usually linked to a marker such as an enzyme or a florescence dye or radioactive element or colloidal gold (for EM).
- the reaction is visualized by identification of the marker under microscope.

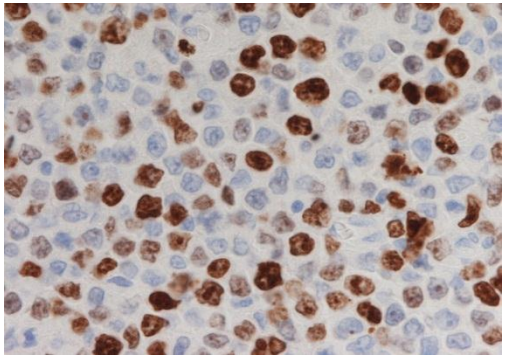
Types:

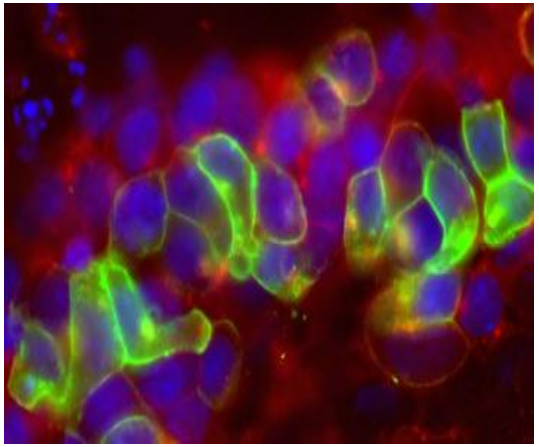
-Direct Method

-Indirect Method (2 steps)

Any macromolecule Such as:

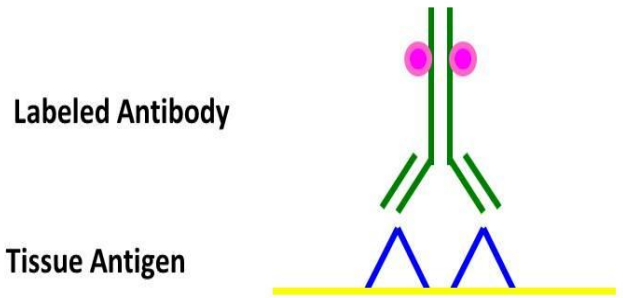
-Cytoplasmic
-Nuclear
-Lipids
-Proteins



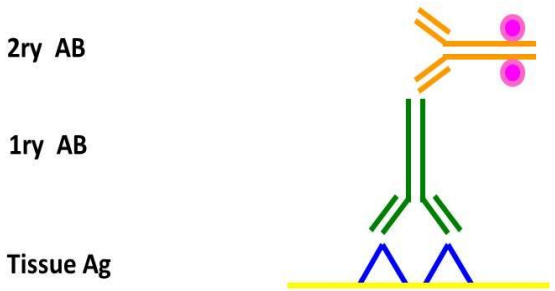


Immunohistochemistry

Direct Method



Indirect Method (2 steps)



Direct method	indirect method
• Fast to get results • Labeling intensity is low	• More sensitive • Getting results takes longer

TEST YOURSELF!

When a tissue section was stained purple-red in color by a blue dye, this is called?

A-vital staining.

B-metachromatic staining.

C-supravital staining.

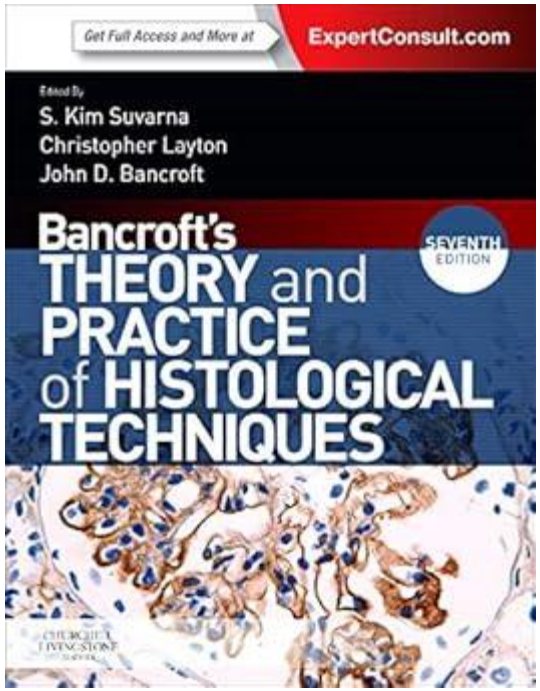
D-acidophilic staining.

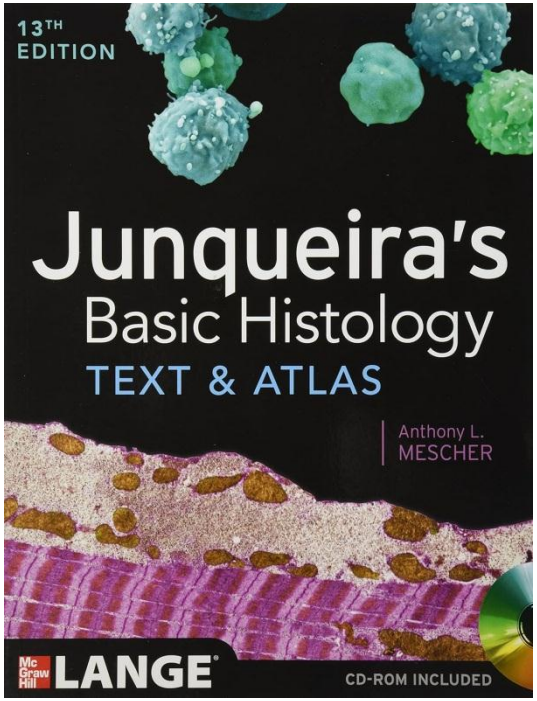
Which of the following techniques is used to localized a special enzyme in a tissue?

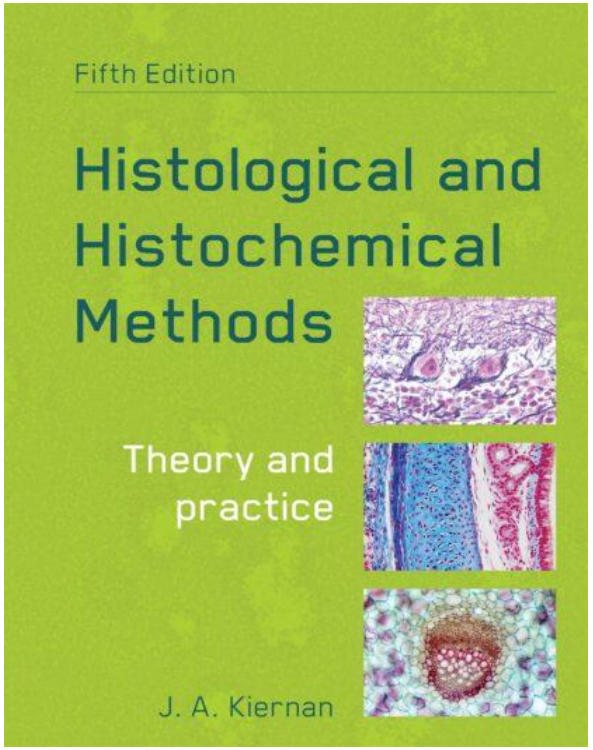
1. Routine microscopic examination (LM & E/M)
2. Immunocytochemistry
3. Autoradiography
4. Cell & Tissue Culture

References

Text books







Web sites

www.histology-world.com

https://www.bbc.co.uk/bitesize/articles/zrp3ydm#znkd96f

Thank you