

Histological techniques-2



By

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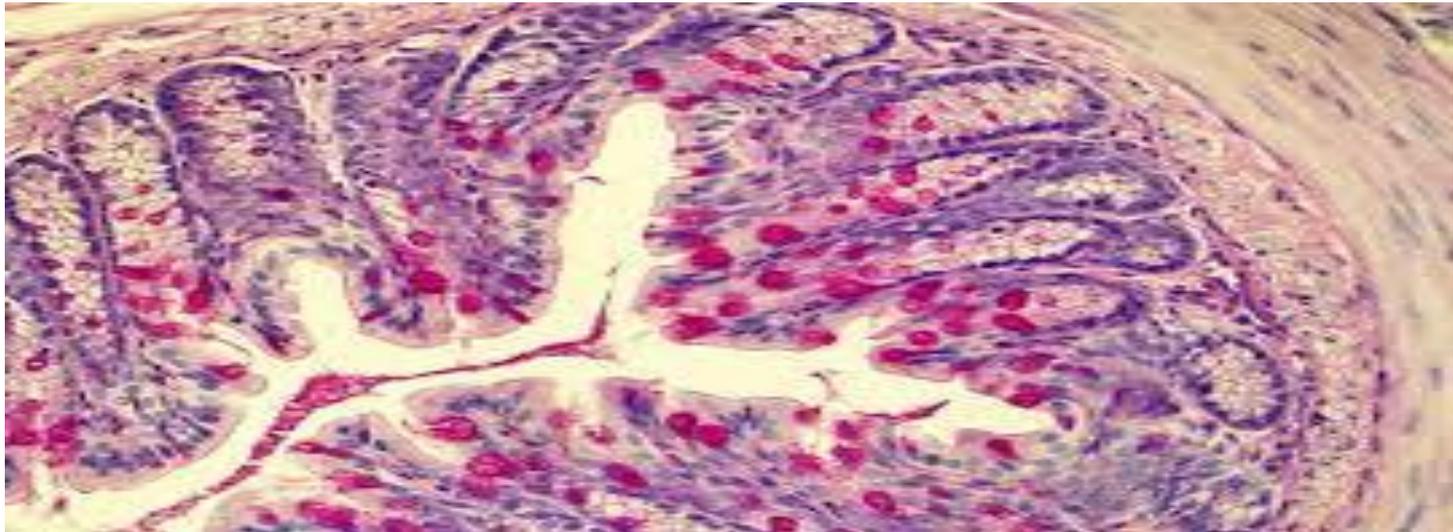
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Learning outcomes

1. Recognize the **basic stains** for studying histology.
2. Describe the **steps of staining** of a paraffin section with H&E.
3. Know examples for **special stains**.

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 Mode of Action

Chemical stain (Heamatoxylin and Eosin)

Physical stain
dissolves in tissue without any chemical reaction (Sudan III)

II- According to the reaction of stain:

Acidic (stains **basic** structures as cytoplasm) → **acidophilia** e.g: **Eosin** stain

Basic (stains **acidic** structures as nucleus) → **basophilia** e.g: **Heamatoxylin** stain

Neutral e.g: **Leishman** stain

III- Special stains

H&E (Routine histological stains)

1-Eosin (E):

red acidic dye (- ve charged)

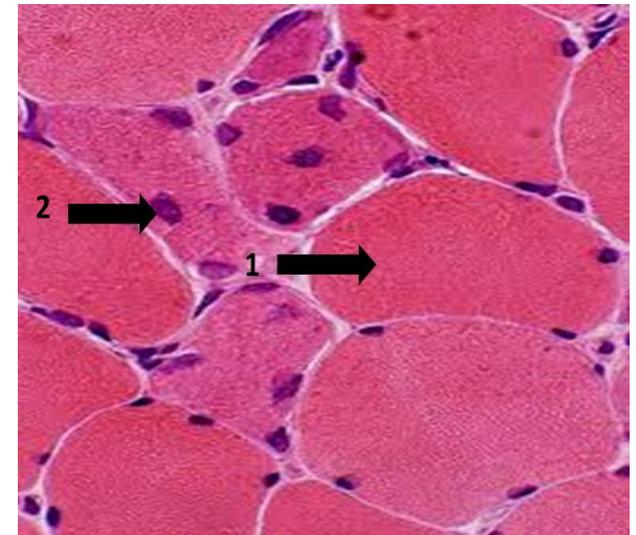
- Stains **basic** (cationic +ve) components of the cell with a **red color** e.g. some proteins of cytoplasm.



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).

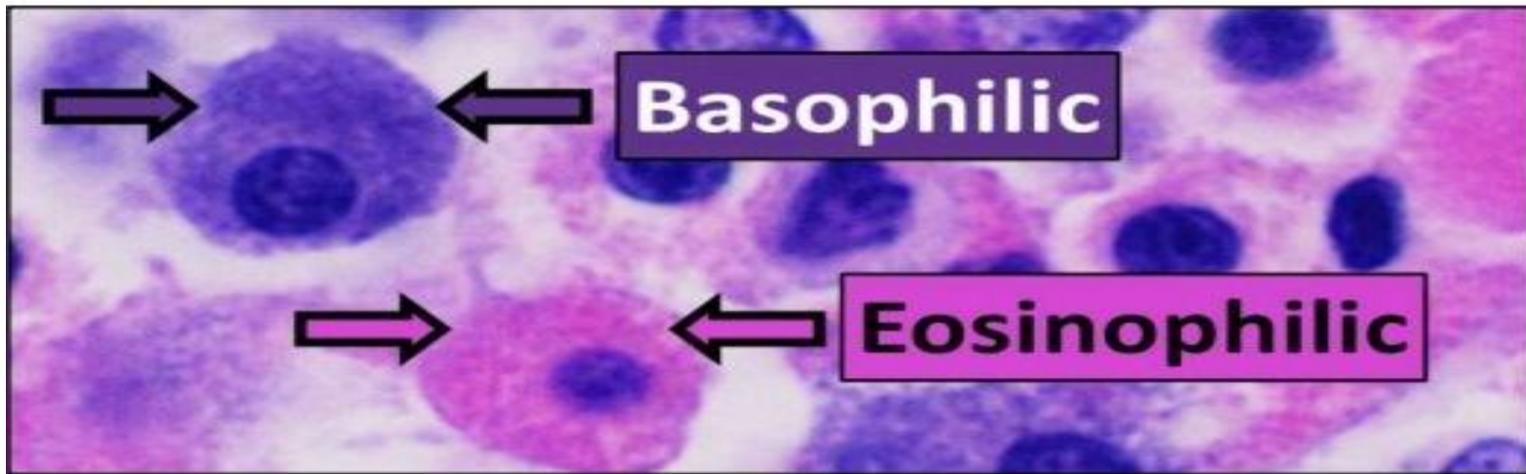


Stain ability of cell and tissue components:

Acidophilic (stains with acidic stains) e.g. acidophilic granules of Leucocytes.

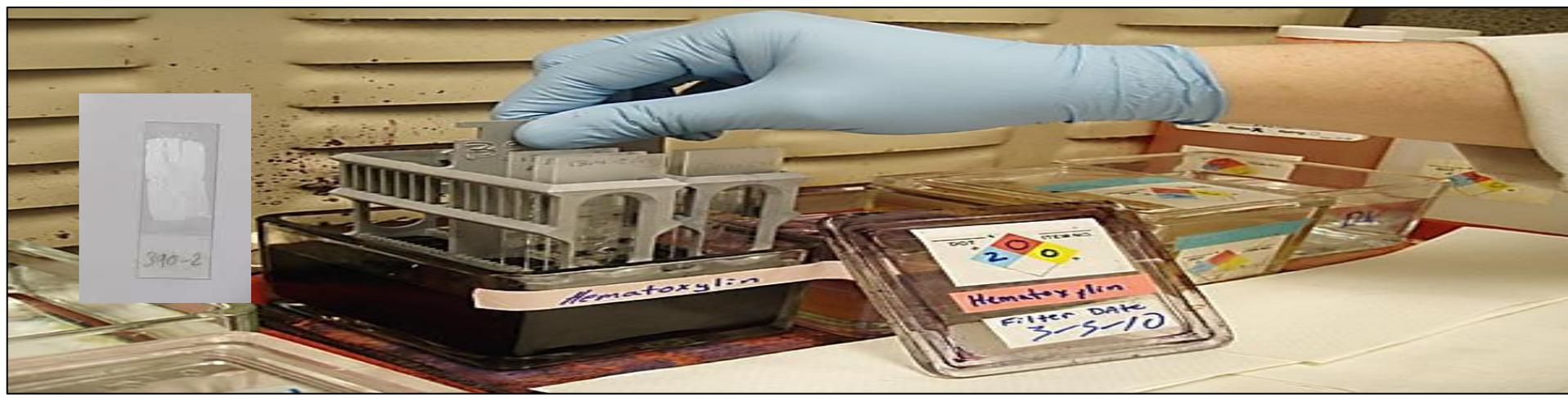
Basophilic (stains with basic stains) e.g. nucleus.

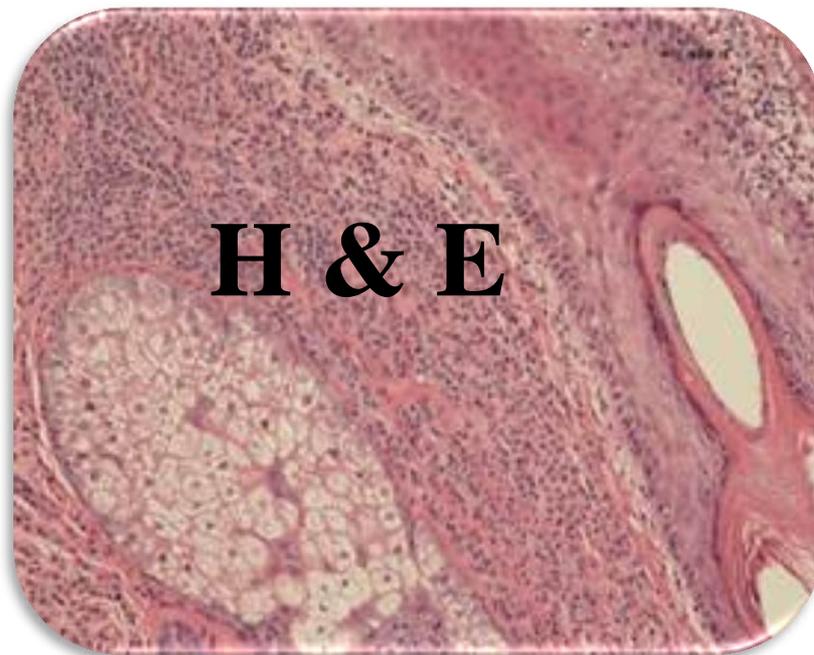
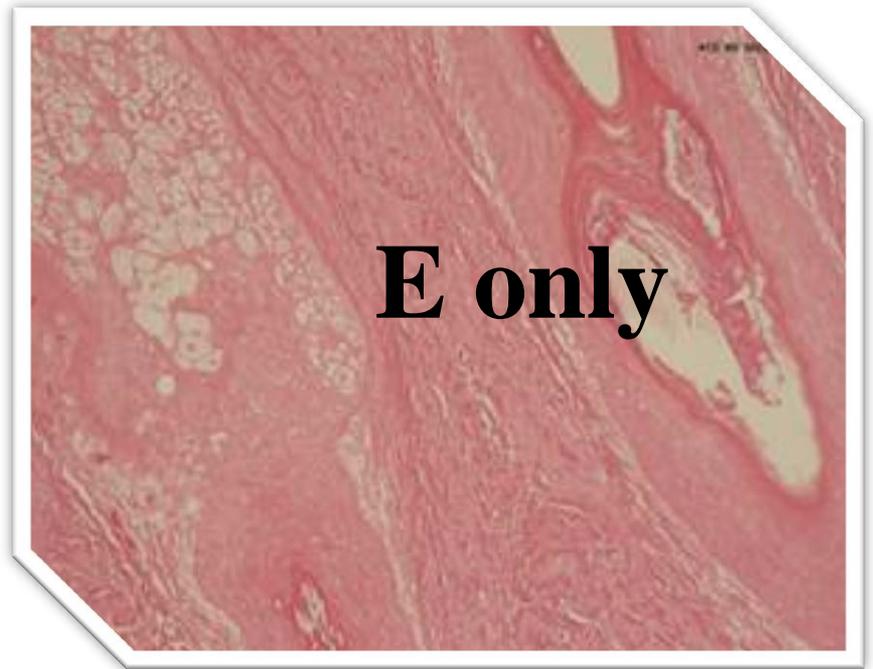
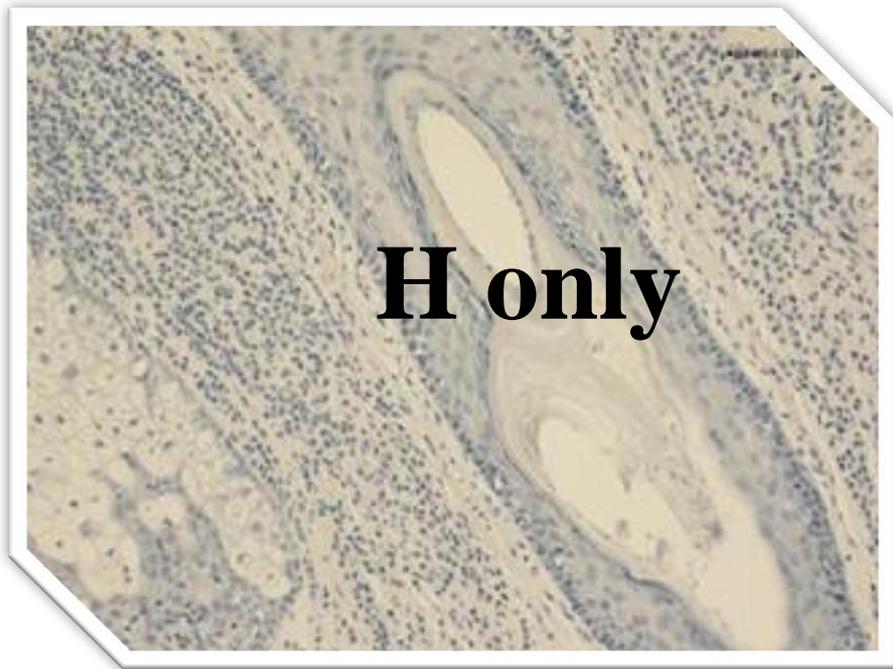
Amphoteric (stains with both acidic and basic stains) e.g. neutrophil granules of leucocytes.



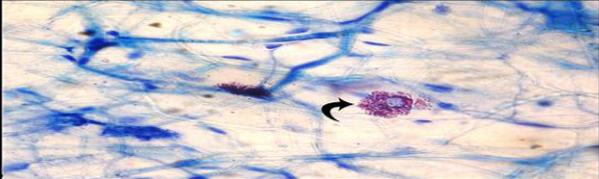
Staining of a paraffin section with

- 1-**Identify** the upper side by scratching the wax.
- 2-**Replace** paraffin by xylol.
- 3-**Replace** xylol by **absolute alcohol (100%)**.
- 4-**Replace** alcohol by **water** by put tinf the slide in descending grades of alcohol (%, 70%, 50%, distilled water).
- 5-**Stain** in **haematoxylin**
- 6-**Wash** in tap water
- 7-**Counter-stain** in **eosin**
- 8-**Dehydrate** in descending grades of alcohol.
- 9-**Clear** in xylol.
- 10-**Mount** in Canada balsam & cover with cover slip for permanent preservation.



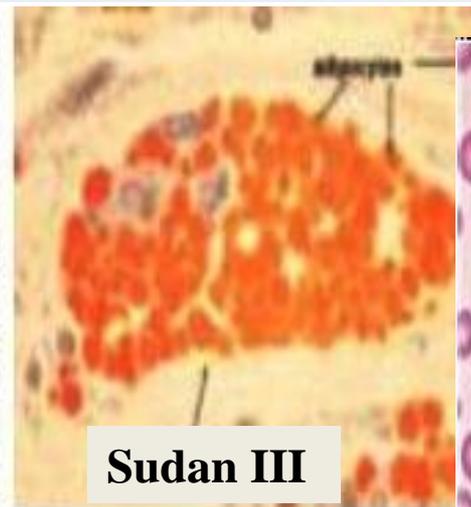
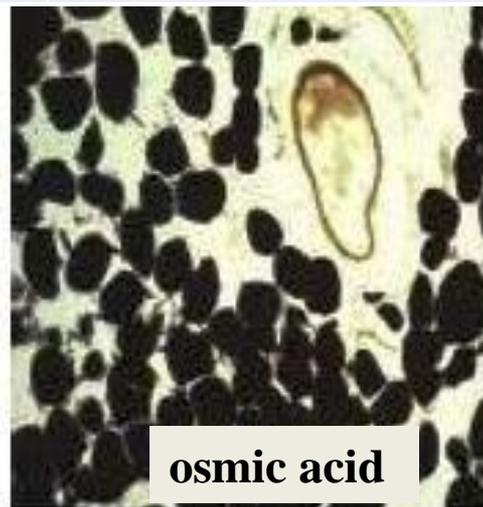
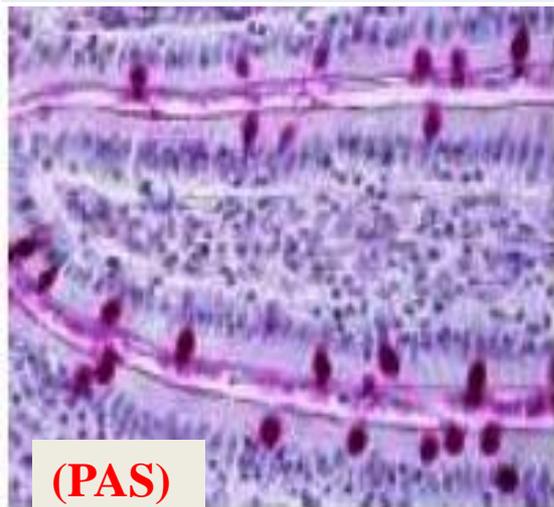


Special stains

	Explanation	Example
Vital stain	<ul style="list-style-type: none">-Staining living tissue inside the body by non toxic dyes-Done by injecting the dye into living animal prior to examine the tissue.	<u>Trypan blue stain</u> (Macrophages)  <p>Macrophage</p>
Supravital stain	Staining living tissues outside the body.	Brilliant <u>cresyl blue</u> staining reticulocytes
Metachromatic stain	Staining the tissues with a color different from the original color of stain	<u>Toluidine blue</u> for Mast cells 
Polychromatic stain	Staining the tissues with multiple colors in spite of using a single stain.	<u>Geimsa stain</u> for blood.

Some special stain for organic compound

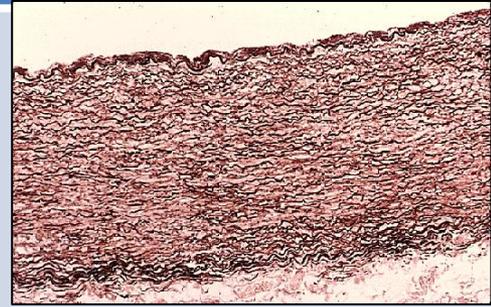
Carbohydrates	<ul style="list-style-type: none">- Periodic acid–Schiff (PAS) (carbohydrates & mucin/ magenta colour)
Lipids	<ul style="list-style-type: none">• Sudan III (orange)• osmic acid (black)
Blood	<ul style="list-style-type: none">- Giemsa stain



Some special stain for fibers

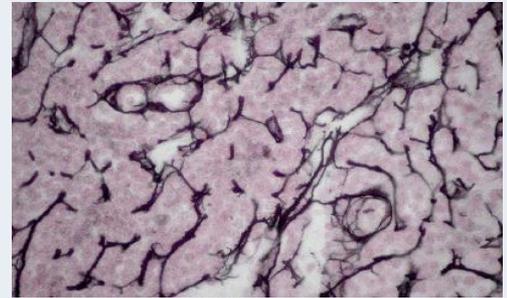
Elastic fibers

Orcein stain (brown)
(wall of aorta)



Reticular fibers

Silver stain



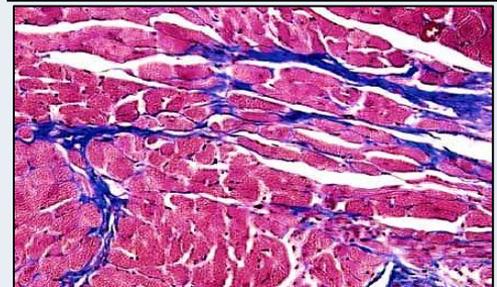
Neurofilaments

Silver stain



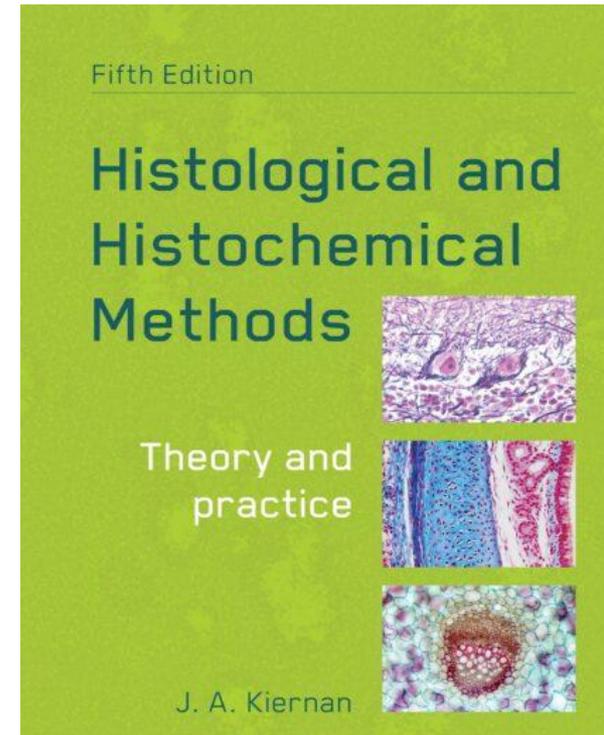
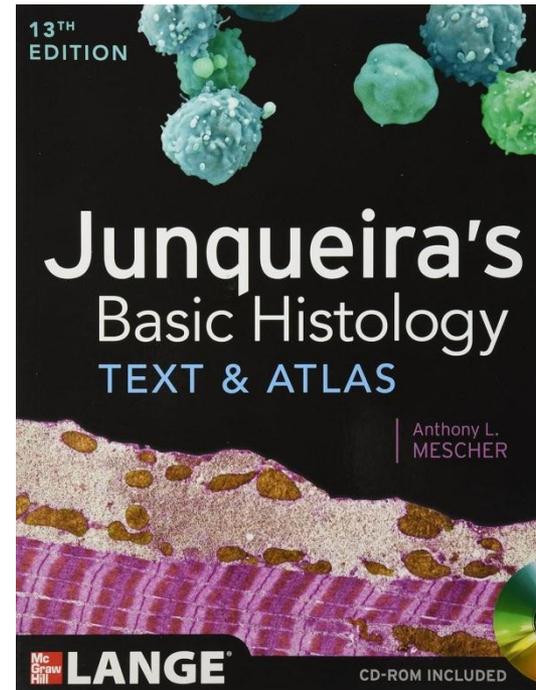
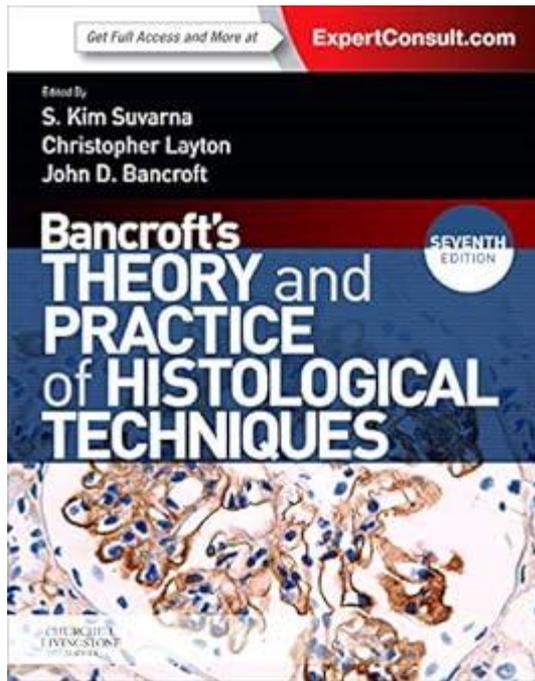
Collagen fibers

-Van Gieson
-Masson trichrome



References

Text books



Thank you

