

# Histological techniques-2



By

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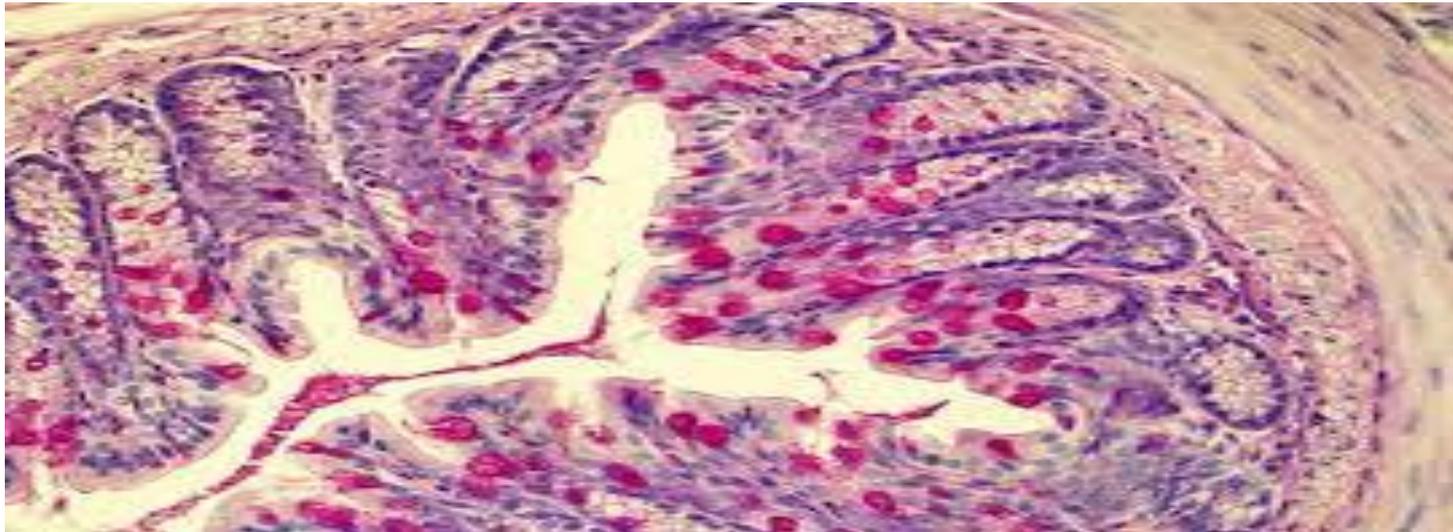
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# Learning outcomes

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)**

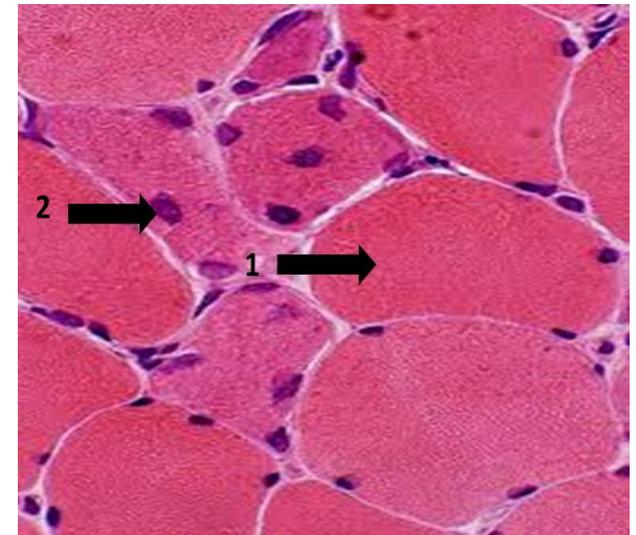
- Stains **basic** (cationic +ve) components of the cell with a **red color** e.g. some proteins of cytoplasm.



## 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).

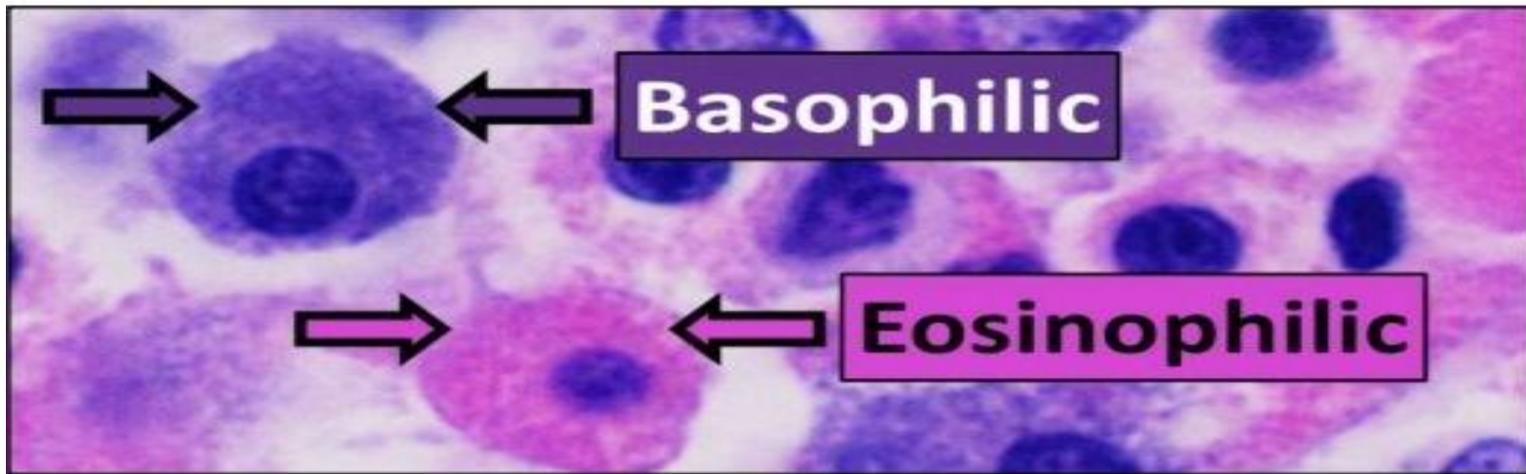


# Stain ability of cell and tissue components:

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

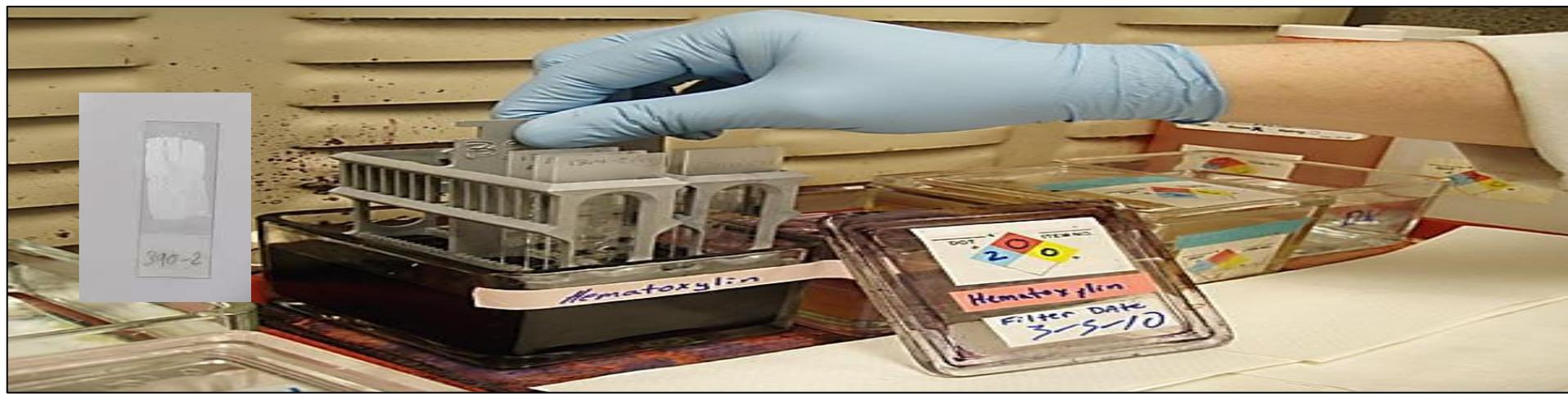
**Basophilic** (stains with basic stains) e.g. nucleus.

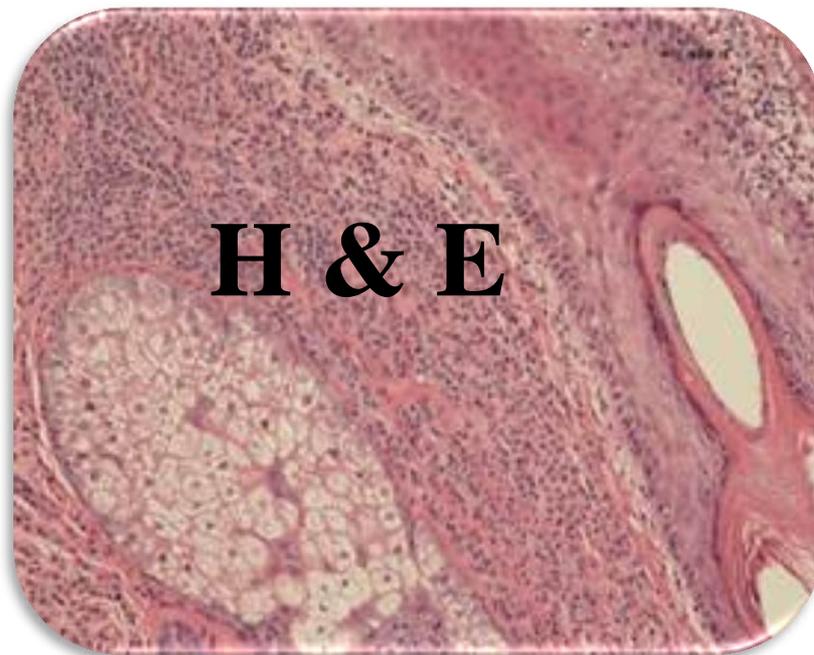
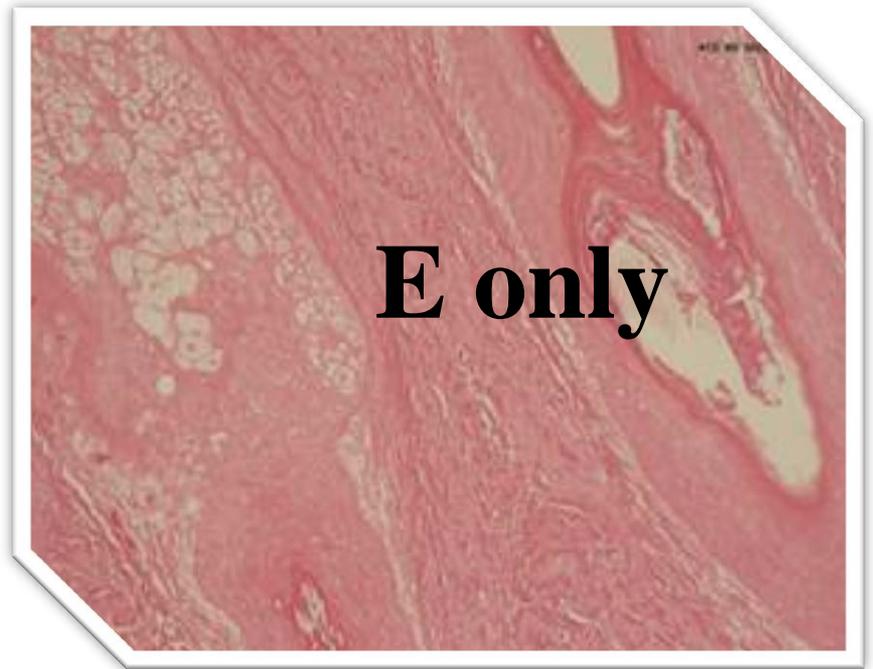
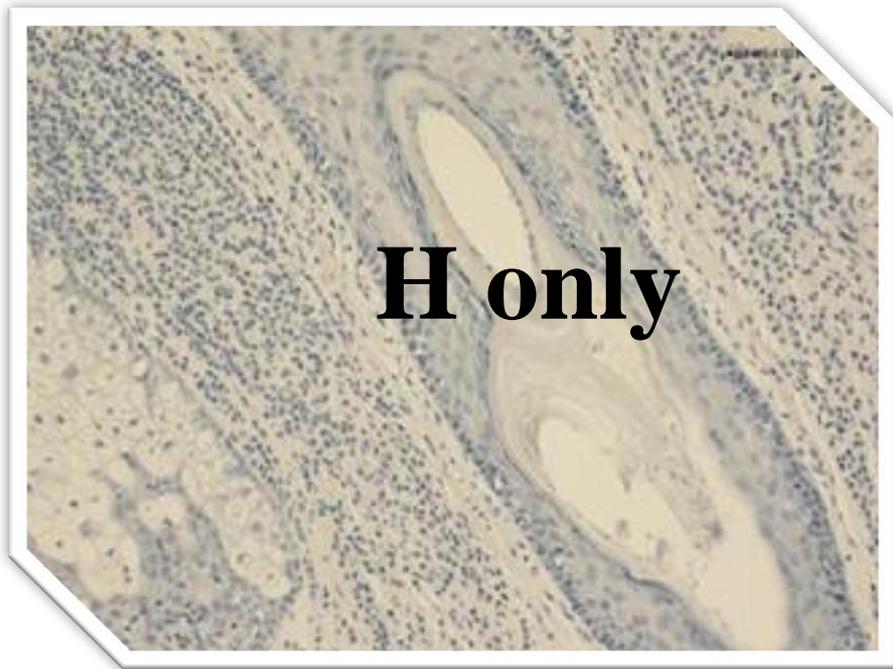
**Amphoteric** (stains with both acidic and basic stains) e.g. neutrophil granules of leucocytes.



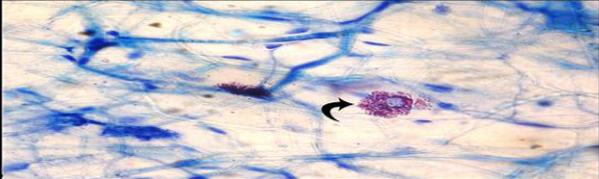
# Staining of a paraffin section with

- 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.



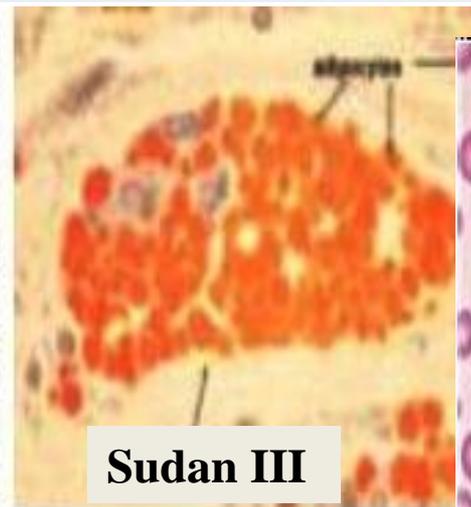
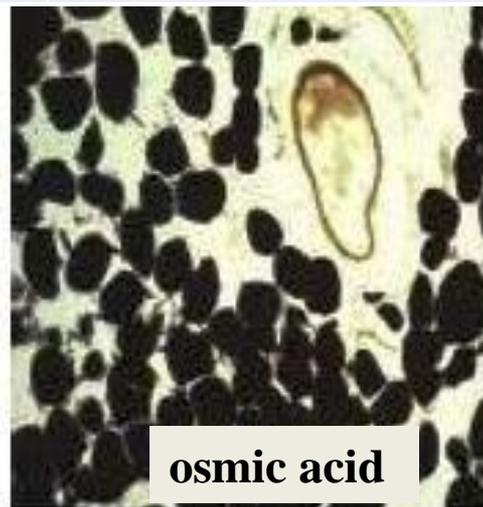
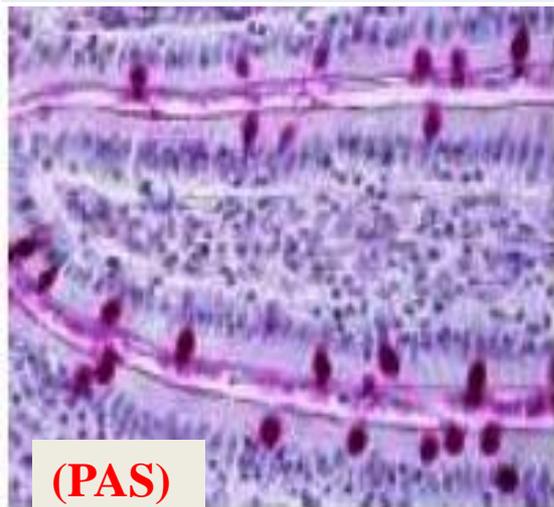


# Special stains

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

# Some special stain for organic compound

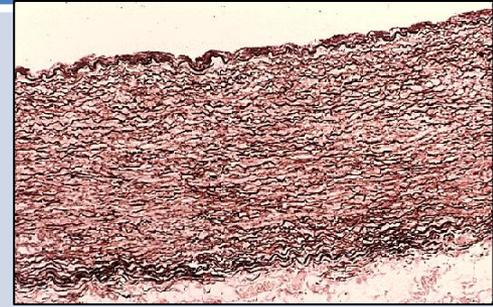
<b>Carbohydrates</b>	<ul style="list-style-type: none"><li>- <b>Periodic acid–Schiff (PAS)</b> (carbohydrates &amp; mucin/ magenta colour)</li></ul>
<b>Lipids</b>	<ul style="list-style-type: none"><li>• <b>Sudan III</b> (orange)</li><li>• <b>osmic acid</b> (black)</li></ul>
<b>Blood</b>	<ul style="list-style-type: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



# Some special stain for fibers

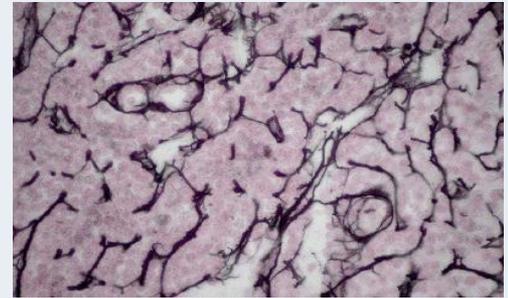
Elastic fibers

Orcein stain (brown)  
( wall of aorta)



Reticular fibers

Silver stain



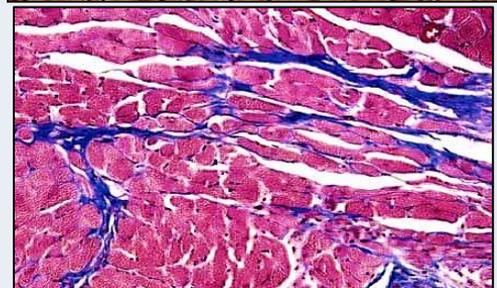
Neurofilaments

Silver stain



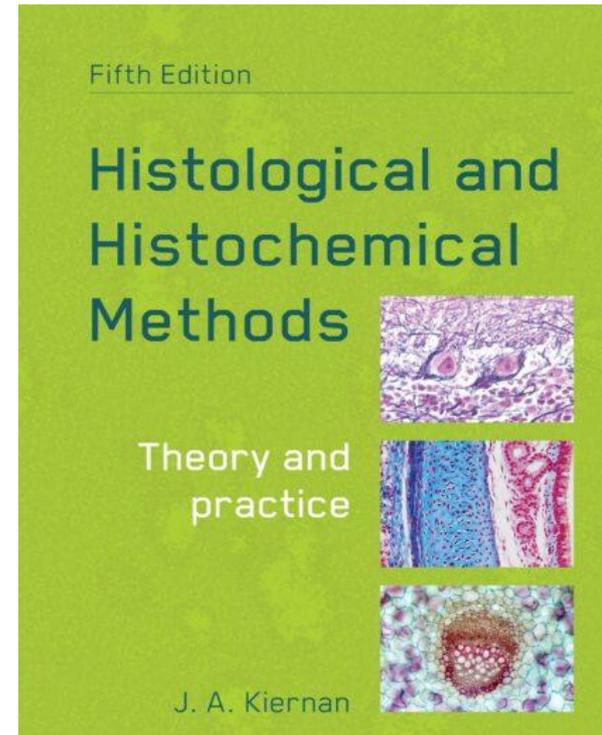
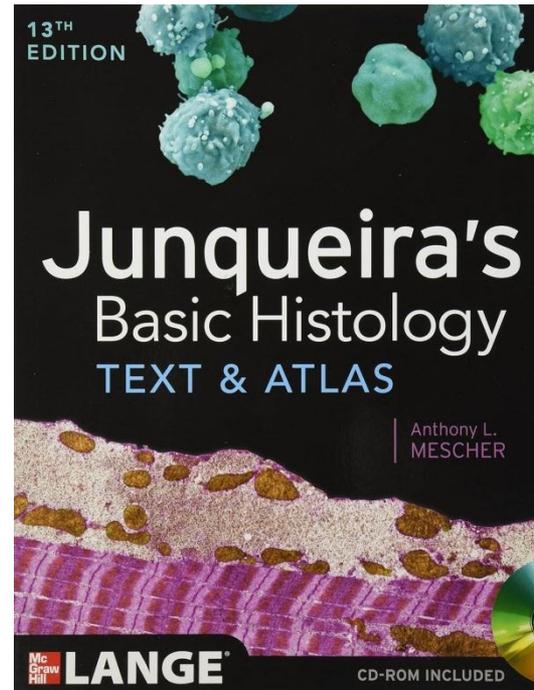
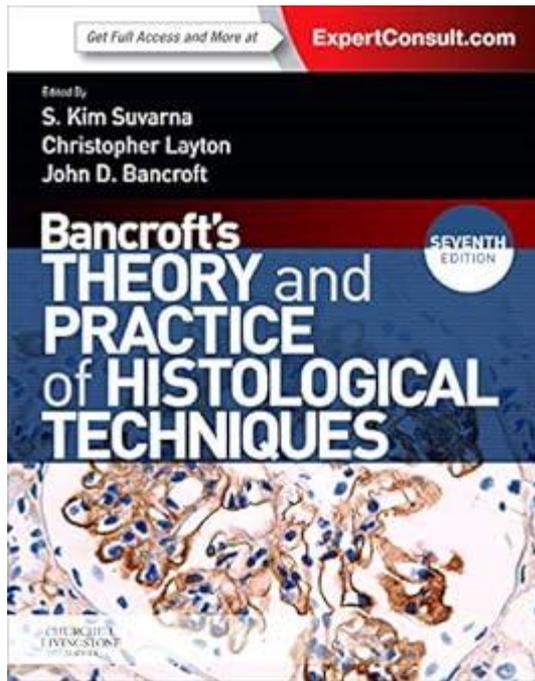
Collagen fibers

-Van Gieson  
-Masson trichrome



# References

## Text books



*Thank you*

