Histological techniques (practical)

By

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Histological techniques

They are the methods by which histological sections are prepared for microscopic examination through series of processes

Tissue processing for paraffin method

The most common procedure.

Can be divided into the following steps:

1-Tissue sampling

2- Fixation

3- Dehydration & clearing

4-Impergnation & embedding in paraffin

5-Sectioning with a microtome

6- Mounting on microscope slides

7-Staining

Tissue processing for paraffin method

1-Obtaining the tissue: very small and fresh.

Tissue processing for paraffin method

2-Fixation:

treatment of tissues by **putting them in a fixative**(chemical or mixture of chemicals).

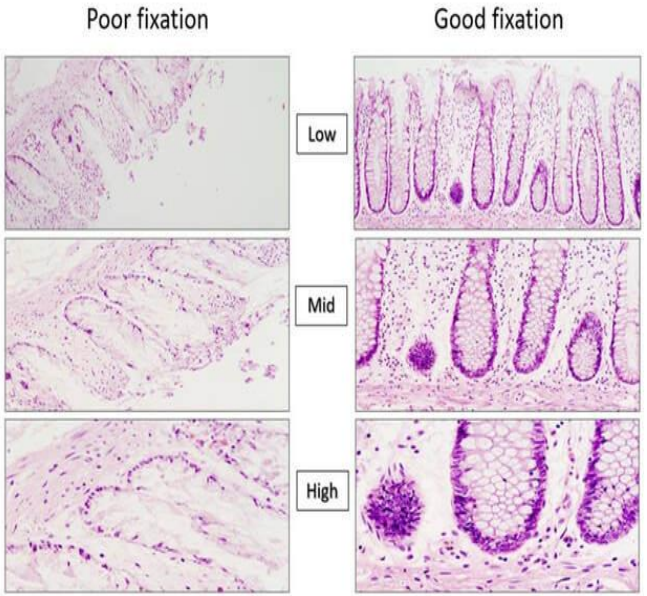
Aims:

- Harden the tissue to help in section cutting.
- Coagulates tissue proteins so preserve the cellular structure of the tissue close to its natural state & preserve the relations of tissue components.
- Prevent enzymatic digestions of cells by autolysis
- Increase affinity of tissue for stains.

Types of fixative:

- Simple fixatives: e.g. **formol saline** (formalin 10%) (***most common).***
- Combination of Simple fixatives: e.g. Bouins.

Duration: 24 hours



Tissue processing for paraffin method

3-Dehydration

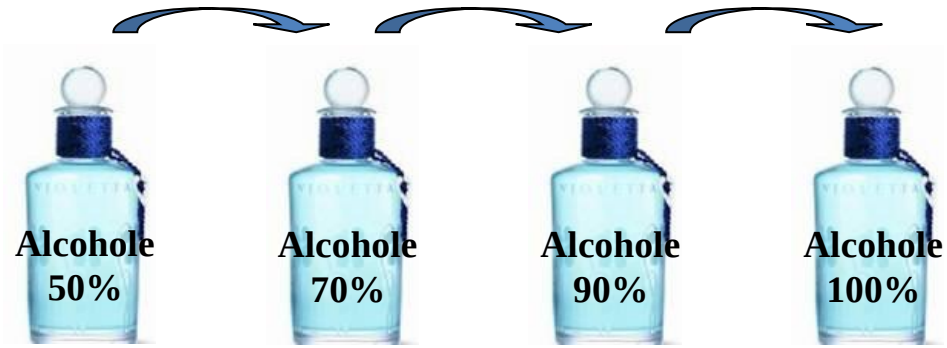
-Aim:

to remove excess water (why ????)

-Who?:

Putting the tissue pieces in **ascending grades of alcohol**(50%- 7o%- 90%) each for 30 m then **absolute alcohol** for 1 h. **Why???**

***Gradual dehydration*:** To prevent tissue shrinkage



Tissue processing for paraffin method

4- Clearing

Aim: to replace alcohol

By: **Xylol**

For: 2 h (until tissue is translucent)

Tissue processing for paraffin method

5- Impregnation

By: melted soft paraffin wax

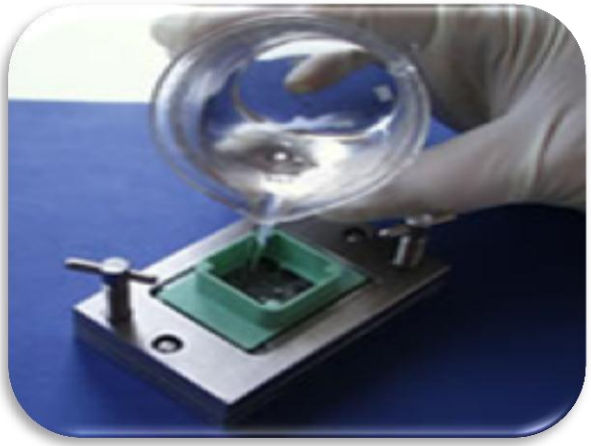
How: several change in oven

For : 2 h for each path

Aim:

-to replace xylol

- permeates the tissue and harden it from *inside*



Tissue processing for paraffin method

6- Embedding

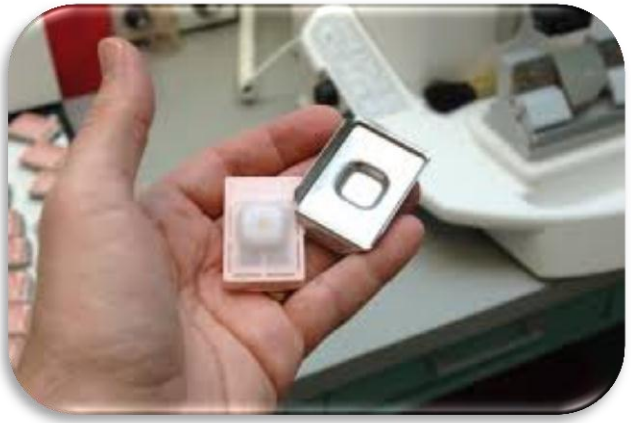
By: melted hard paraffin wax

Aim:

-to harden the tissue *from outside* forming paraffin **block**

- make thin section easy to cut

-preserve tissue for years

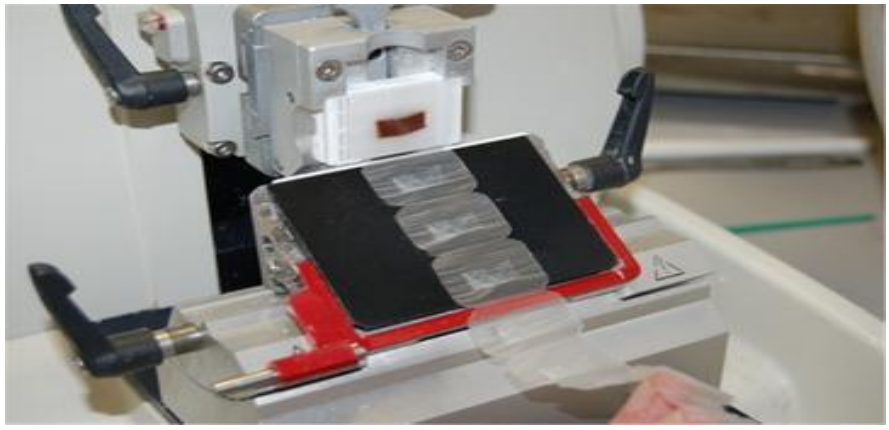


Tissue processing for paraffin method

7- section cutting

By: Rotatory microtome

Blocks are cut into thin sections (3-10micron).



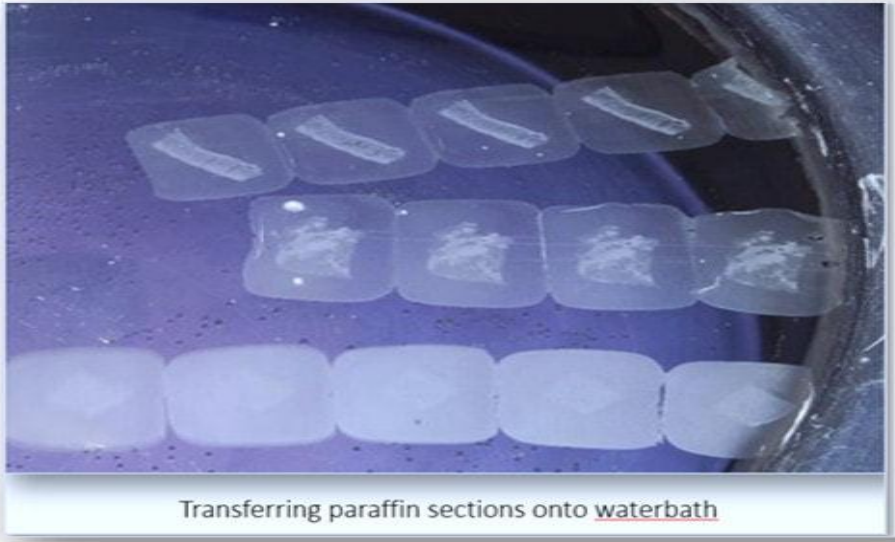
Tissue processing for paraffin method

8- Mounting sections

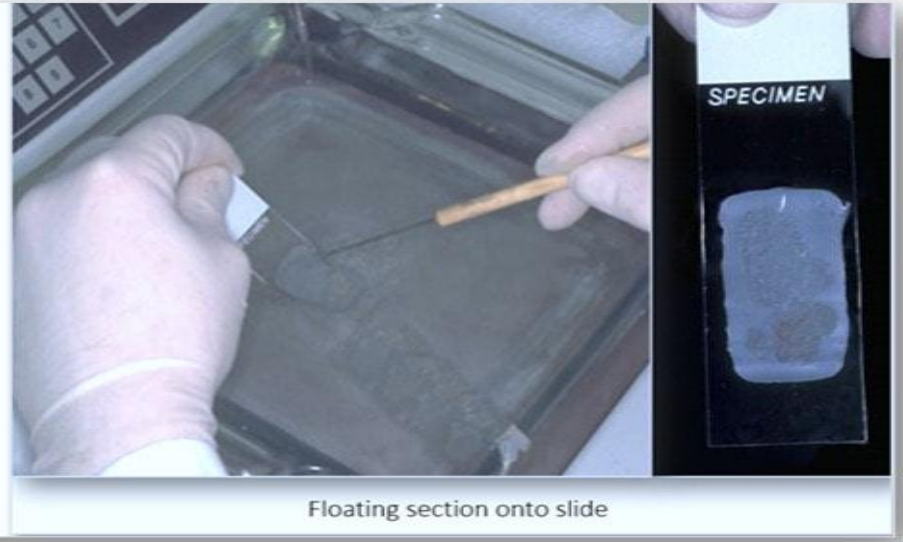
How???

-the sections are permanently attached to individual glass microscope slides.

-Slides are warmed on a hot plate, then dried in an incubator to be ready for staining .

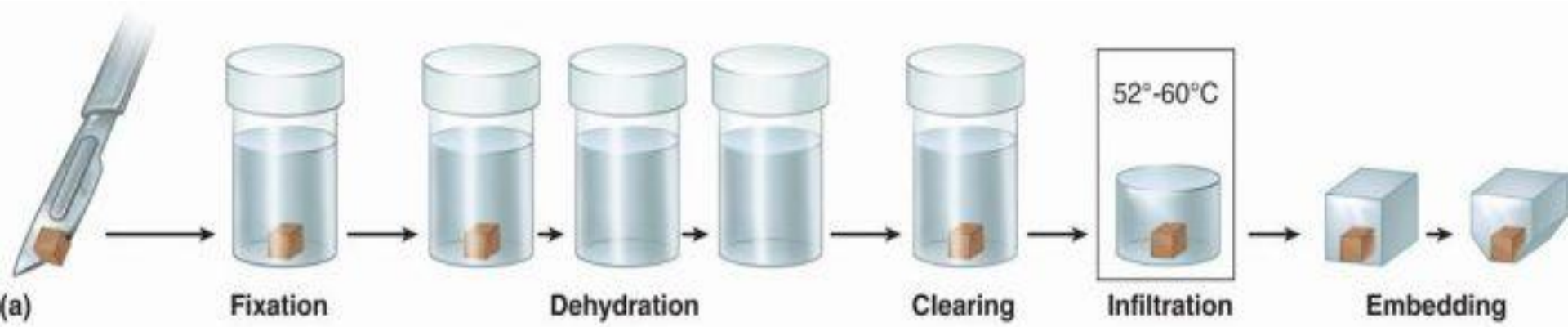


Transferring paraffin sections onto waterbath



Floating section onto slide

Tissue processing for paraffin method



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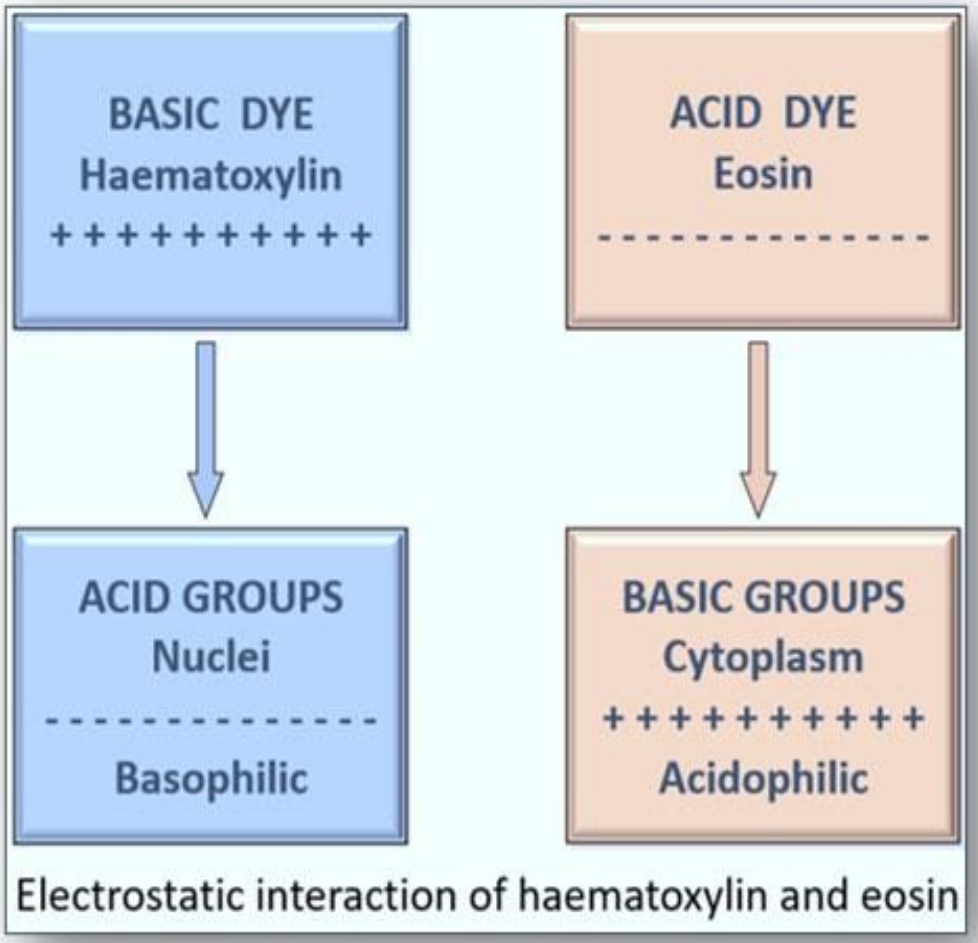
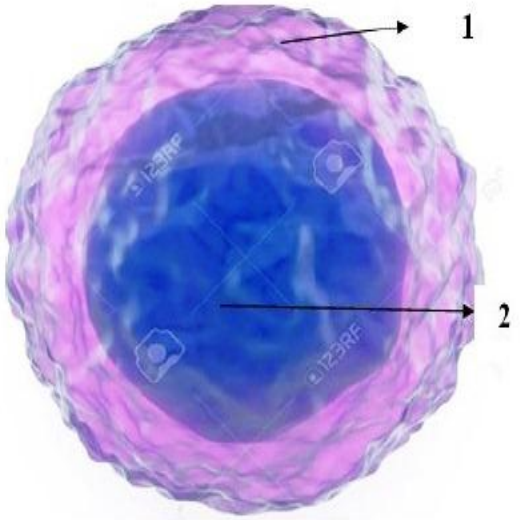
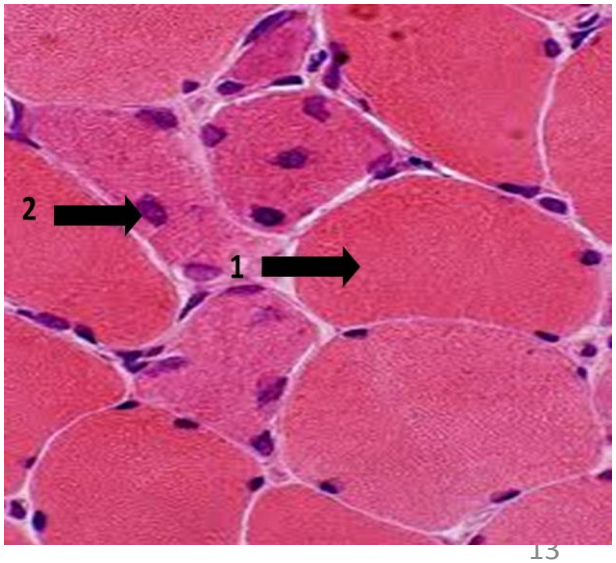
MLS

H&E (Routine histological stains)







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

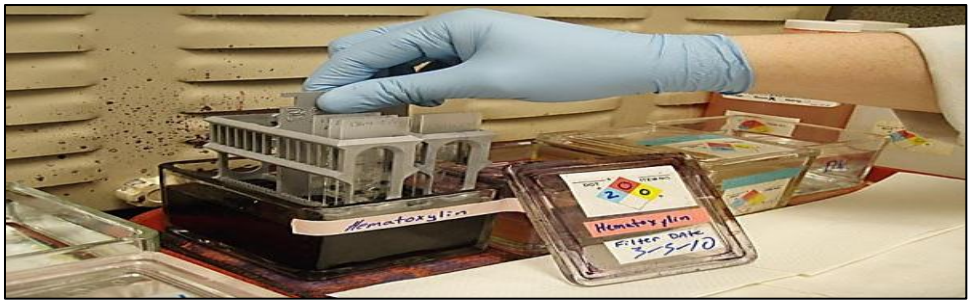
6-Wash in tap water

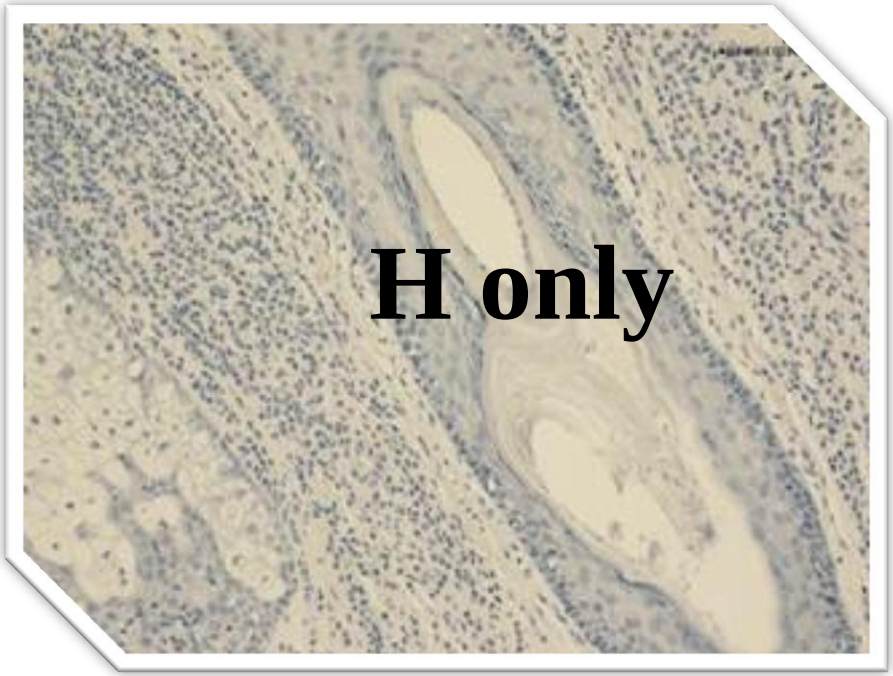
7-Counter-stain in eosin

8-Dehydrate in ascending grades of alcohol

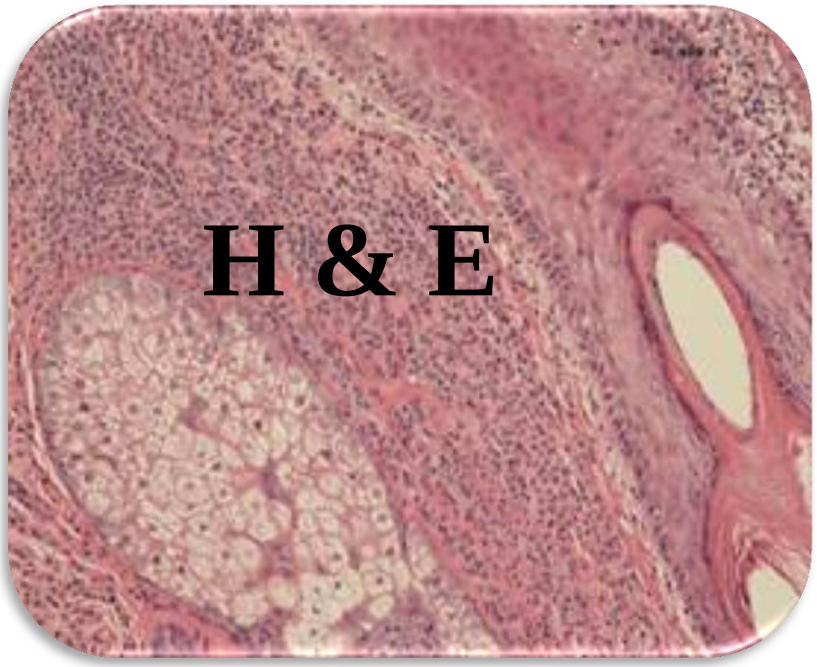
9-Clear in xylol

10-Mount in Canada balsam & cover with cover slip



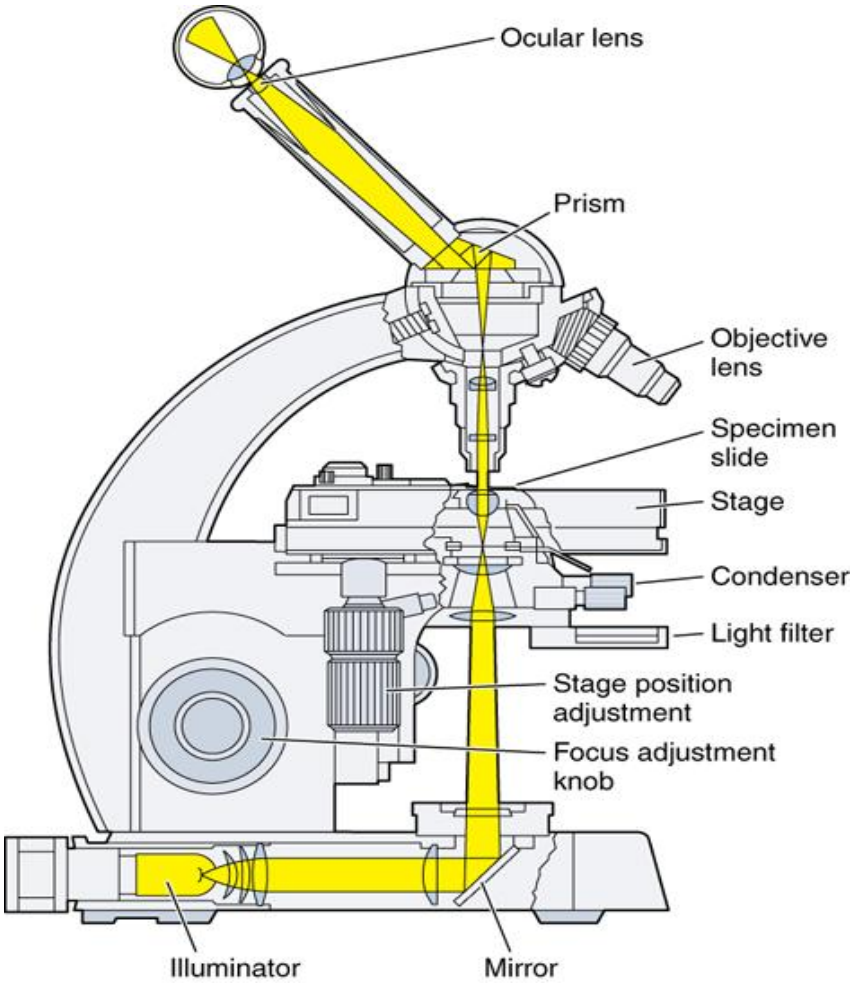

H only


E only

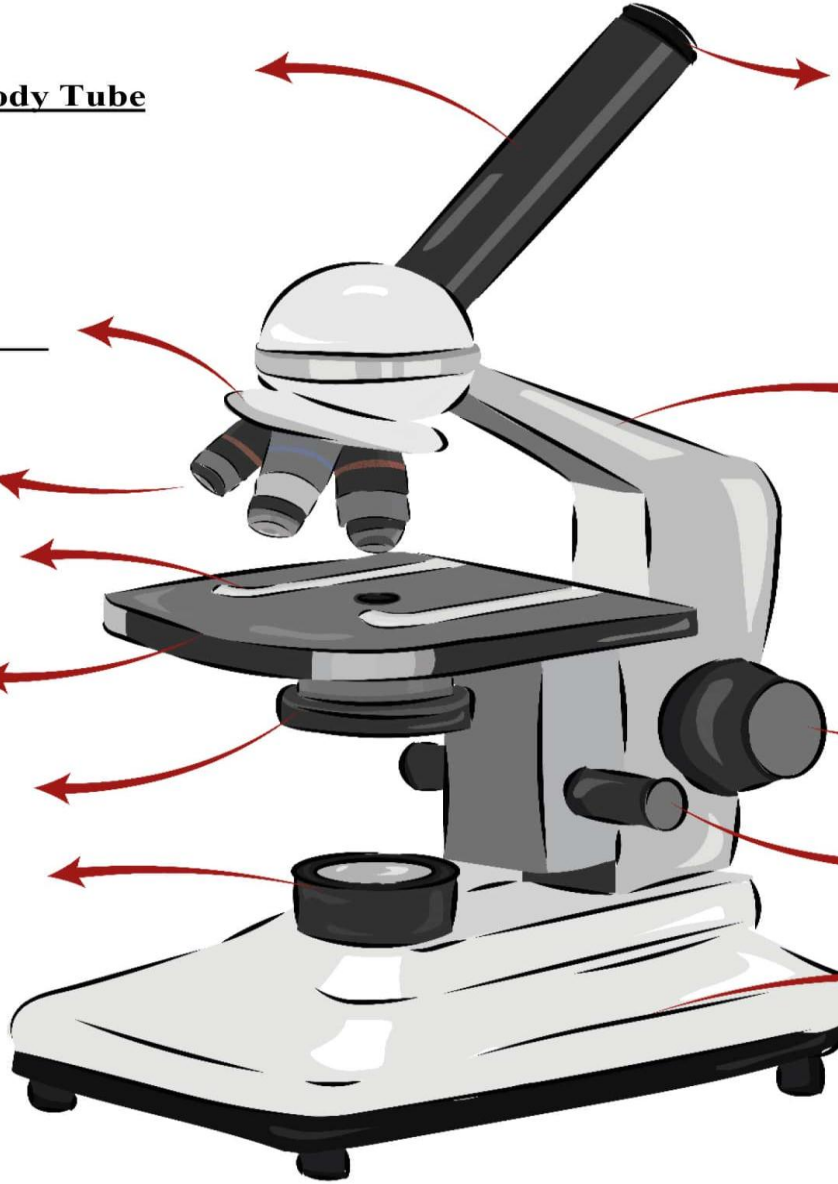

H & E

Light Microscope (LM)

- The widely used microscope
- LM uses visible light source + condenser lens (to send light through the object).



Parts of a Microscope Worksheet



1. _Eyepiece Tube or Body Tube
2. Nosepiece
3. Objective Lenses
4. Stage Clips
5. Stage
6. Diaphragm
7. Illuminator
8. _Eyepiece / Ocular Lens
9. Arm
10. _Coarse Focus
11. Fine Focus
12. Base

- The objective lenses (near the object)

- Groups of lenses fixed to metal wheel which turn round, and stop by a click.

- Most microscopes have 3 objective lenses:
  - Low power (x10)
  - High power (x40)
  - Oil immersion (x100)

- ***Oil immersion lens*** looks at object through a drop of **oil.**

The image of this object is magnified by two sets of lenses:

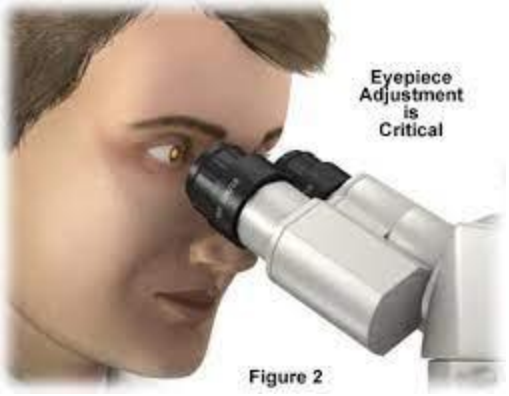
1. Ocular lens (10)
2. Objective lenses (5 ,10 , 40)

- **Total magnification power** = 1 x 2

  e.g.10 X40 =400

The Eye piece

- Close to your eye.
- At the top of the microscope.
- Easily comes out.
- Has two lenses, at the top and the bottom.
- Have different magnifying power (x5, x l0, x15).

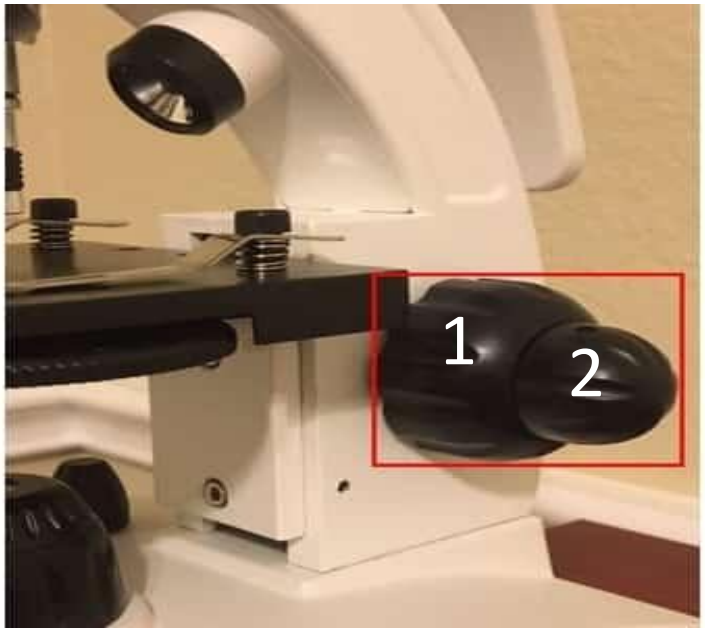


Adjustment

- The microscope moves by 2 kinds of knobs:

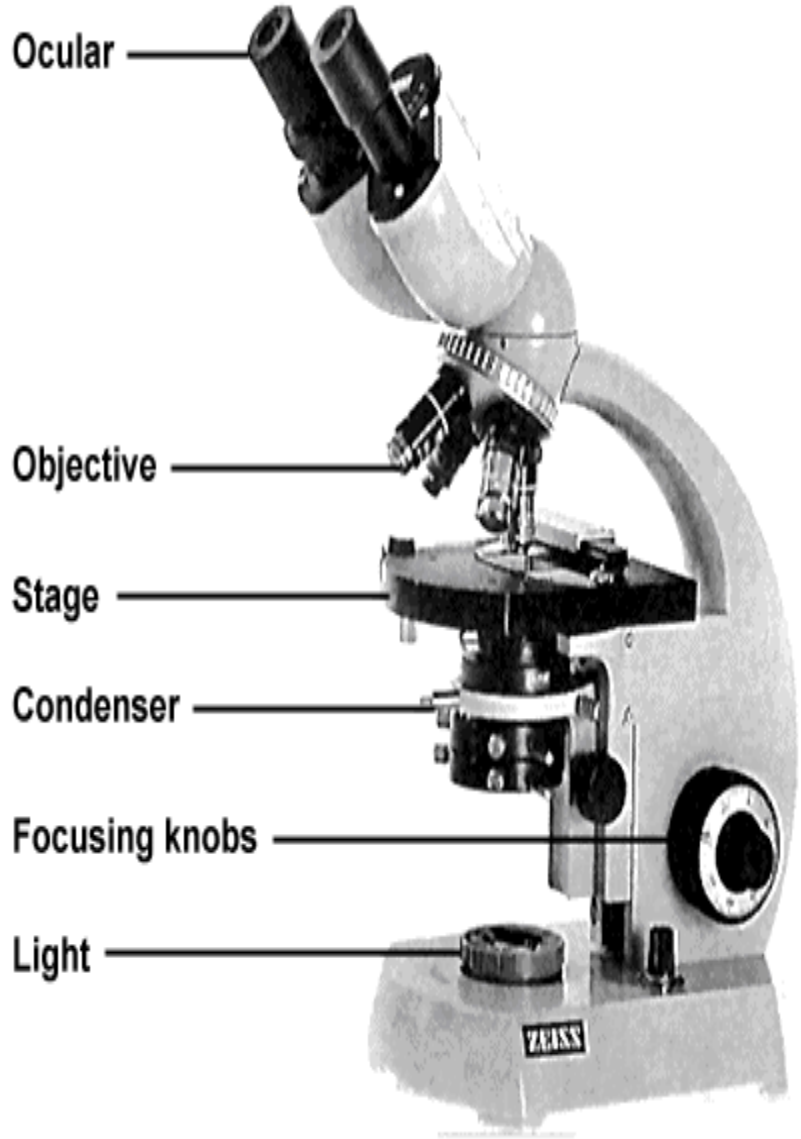
  1-Coarse adjustment: makes big movement so gets the object **near** the focus.

  2-Fine adjustment: makes little movements to get the object **sharply** in focus.

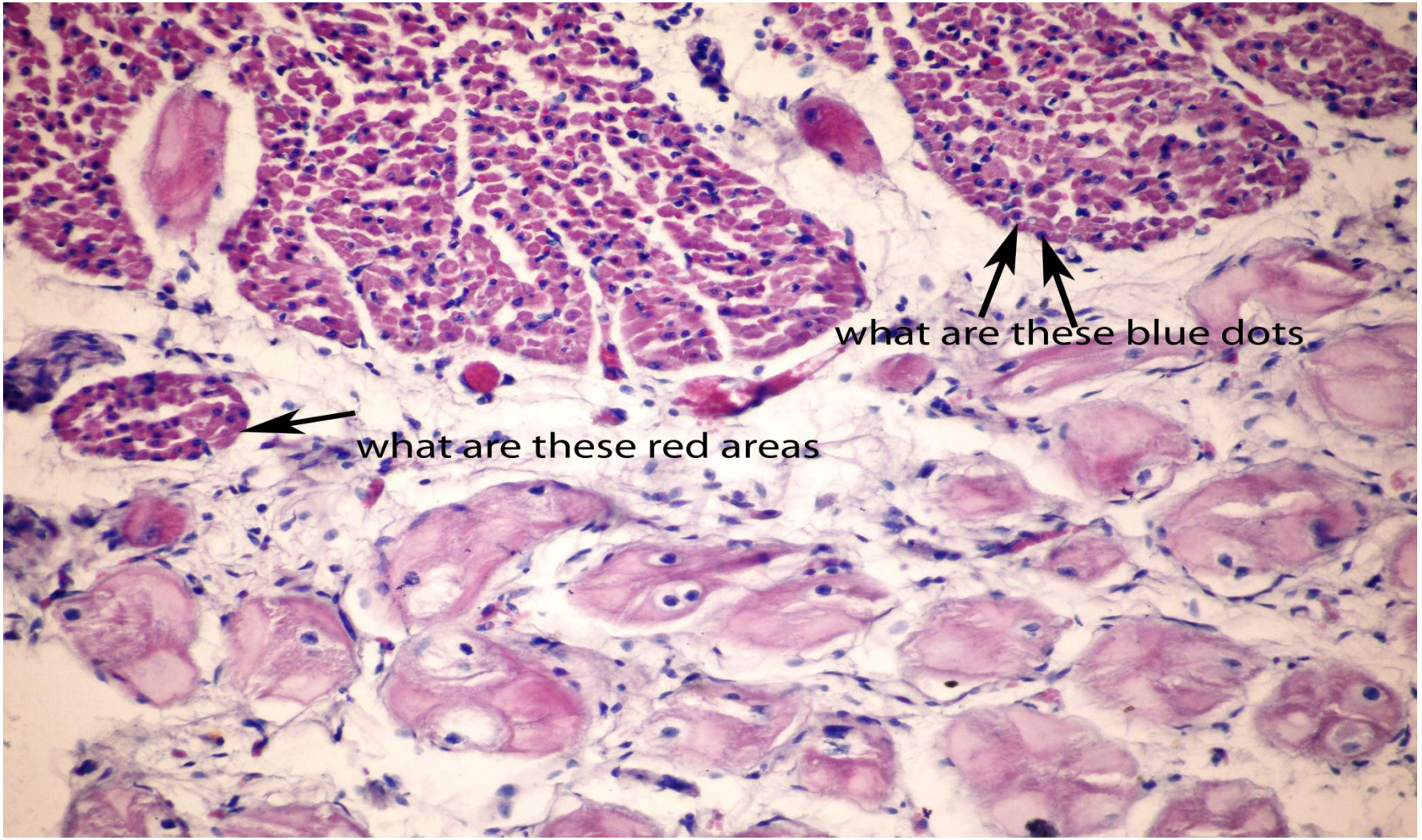


Parts of the microscope

1- Mirror.
2- Iris diaphragm.
3- Condenser.
4- Objective lenses.
5- Eye piece.
6- The tube.
7- Adjustment.
8- Mechanical stage.



Example of a section staine by (Hx & E)



Thank you