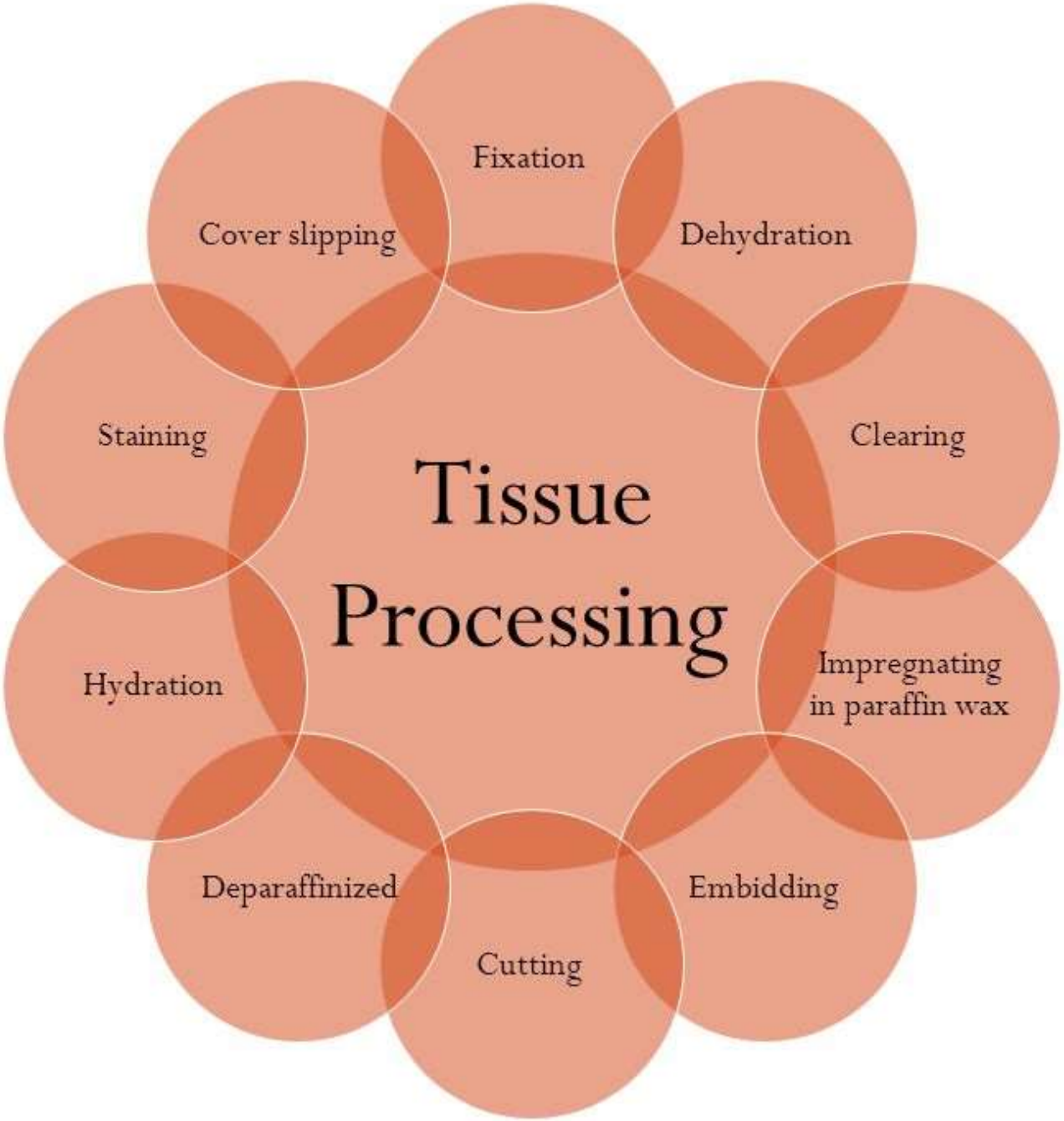

Good
Morning
Have a beautiful
day!

TISSUE PROCESSING


Tissue Processing
Fixation
Dehydration
Clearing
Impregnating in paraffin wax
Embidding
Cutting
Deparaffinized
Hydration
Staining
Cover slipping

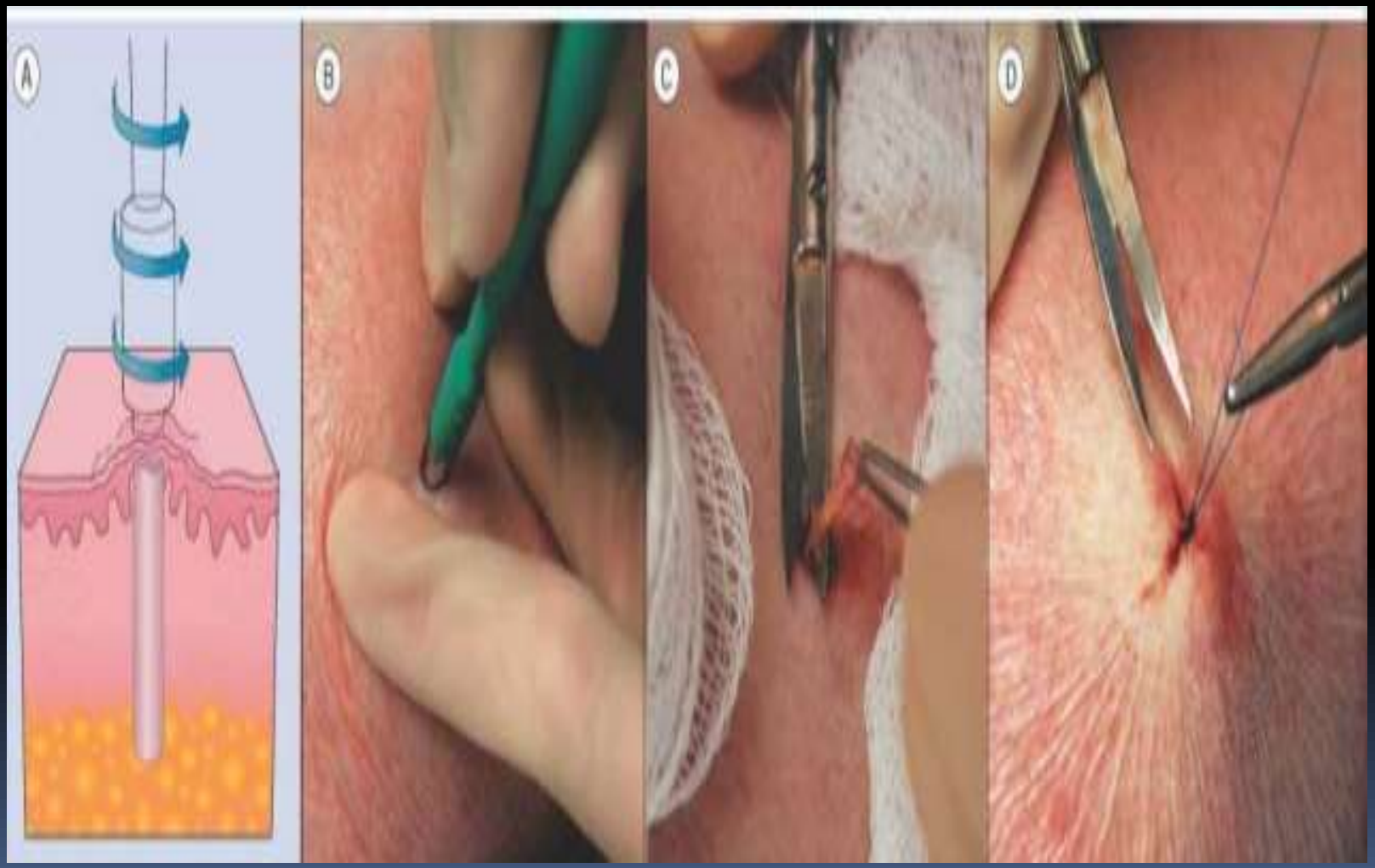
WHAT IS TISSUE PROCESSING ?

- Preparing a tissue to make it visible under the microscope is called tissue processing.
- There are various tissue processing methods available depending on the nature of the tissue.

What happens to the specimen?

- Specimens received in the lab (10% formalin)
- Grossed (appearance, measurements, noticeable, pathological changes etc) and kept for formalin fixation.
- Bits given from representative areas (not greater than 4 mm thick)
- Tissue processed…..
- Final outcome: stained slide for microscopic examination.

BIOPSY OF THE ORAL TISSUE

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Steps in tissue processing

Fixation(10% neutral buffered formalin)

Dehydration

(ascending grades of alcohol such as 50%, 70%,75%,80%,90%, 100%)

Clearing (xylene)

Impregnation

(paraffin wax/celloidin)

Embedding

(paraffin wax)

Sectioning

Staining

FIXATIVE

- Fixation is the procedure which preserves the tissue in a state as close to living tissues as possible by avoiding autolysis and putrefaction.

MECHANISM

- During this process, coagulation of proteins of occurs due to cross linking of the protein molecules, thus fixing the tissue.

Commonly used fixatives

- Formalin – 10% neutral buffered formalin in a volume of15 times more than that of the tissue is used as the fixative.

OTHERS:

- Picric acid
- Glutaraldehyde
- Alcohol
- Osmium tetraoxide.

- Depending on the size of the tissue, the fixation time may vary from 24Hours to 7 days.
- After fixation , the tissue is washed in running tap water for 10-15 minutes.

Dehydration

- This is done TO REMOVE WATER CONTENT FROM THE TISSUE

.

- To allow penetration of wax to make the tissue hard enough to be sectioned

- Once the water is removed the material used for dehydration occupies its place.

Dehydrating agent

- Tissue sent through grades of alcohol:

  50%,70%,75%,80%,90% and absolute alcohol are used.

- **TIME REQUIRED:**

  depends on the size of the tissue;

  generally, 30 minutes to 2 hours in each grades of alcohol may be required.

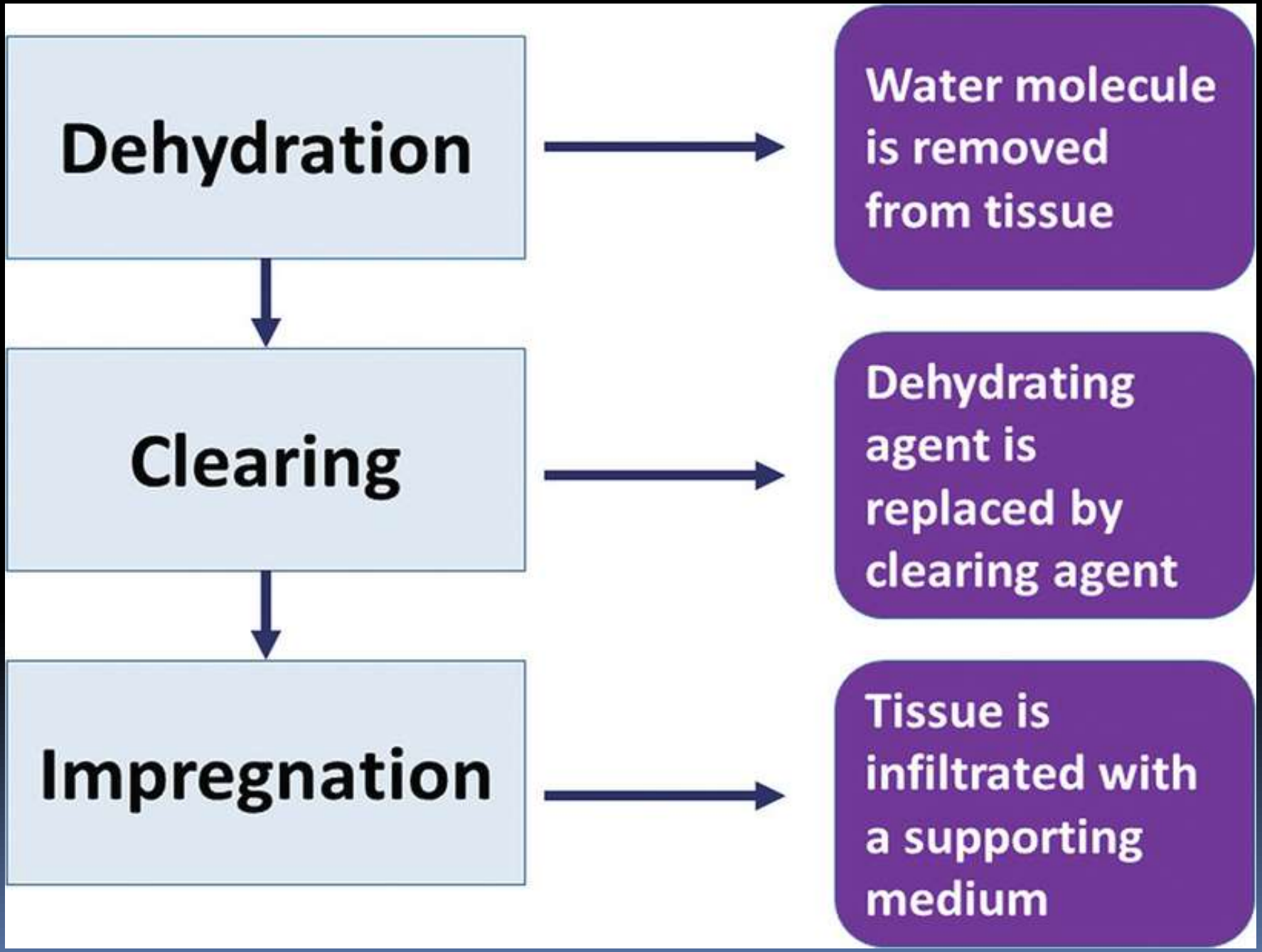
CLEARING

- In this the tissue is passed through clearing agent , which makes the tissue appears clear due the refractive index, which becomes close the glass during this step
- **XYLENE** is the most commonly used clearing agent.
- Xylene is changed twice during the procedure which lasts for **1-2 hours**


XYLENE - I
XYLENE - II
COCONUT OIL - I
COCONUT OIL - II

IMPREGNATION

- Empty spaces in the tissues and cells , after removal of the clearing agent , are taken by molten wax
- Hardens the tissue- helps in section cutting
- MELTING POINT of wax- 54 _ 62 degree C
- PARAFFIN WAX the routinely used material for impregnation


Dehydration
Water molecule is removed from tissue
Clearing
Dehydrating agent is replaced by clearing agent
Impregnation
Tissue is infiltrated with a supporting medium

TISSUE PROCESSOR

Embedding

- After the completion of impregnation ,the tissue is removed from the wax bath.

Embedding – with molten wax

wax blocks-

- metallic L(leuckahart's) blocks
- plastic moulds

Remove tissue from cassette

Fill mould with wax and orientate tissue

Embedding centre

- Wax reservoir
- Heated area for steel moulds
- Wax dispenser
- Separate hot and cold plates.

SECTIONING

- After the preparation of the wax block, it is fastened to a precision instrument called MICROTOME.(sections of 3-5micronmeter)

TYPES OF MICROTOMES:

1. Rotary- most common
2. sliding
3. Freezing
4. Rocking
5. Base -sledge

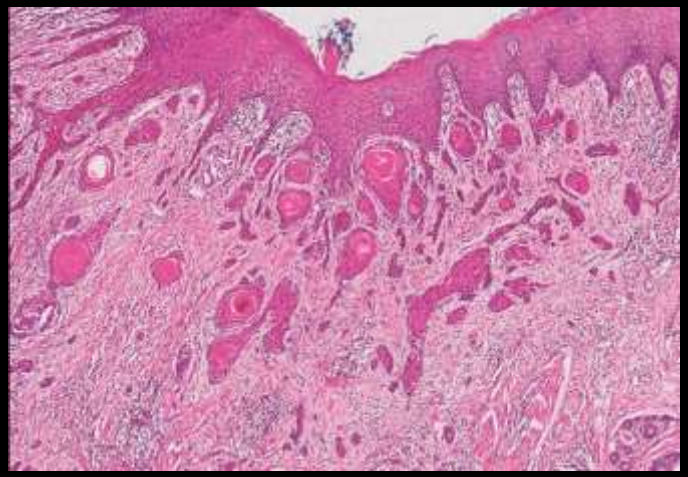

JUNG

HEMATOXYLIN & EOSIN STAINING PROCEDURE

Hematoxylin & Eosin Staining Procedure

DEPARAFFINISTATION AND REHYDRATION	XYLENE - I	5 MINS
	XYLENE – II	5 MINS
	90 % ALCOHOL	5 MINS
	70% ALCOHOL	5 MINS
	WATER WASH	10 MINS
NUCLEAR STAINING	HARRIS HEMATOXYLIN	8 MINS
	WATER WASH	2 MINS
DIFFERENTIATION	DIFF IN 1% ACID ALCOHOL	1 DIP
	WATER WASH	10 MINS
BLUING	1% LITHIUM CARBONATE	1 MIN
	WATER WASH	10 MINS
CYTOPLASMIC STAINING DEHYDRATION	1% EOSIN	1 MIN
	90% ALCOHOL	30SEC
	70% ALCOHOL	30 SEC
	XYLENE – I	5 MINS
	XYLENE – II	5 MINS
MOUNTING	DPX	

ROUTINE STAINING



- Haematoxylin- nuclear stain.
- Eosin- cytoplasmic stain
- Mounted in DPX/canada balsm
- End results;

NUCLEI	- BLUE
CYTOPLASM	
MUSCLE,COLLAGEN,	-PINK
KERATIN, COLLOID	
PROTEIN	-PINK
RBC's	-RED

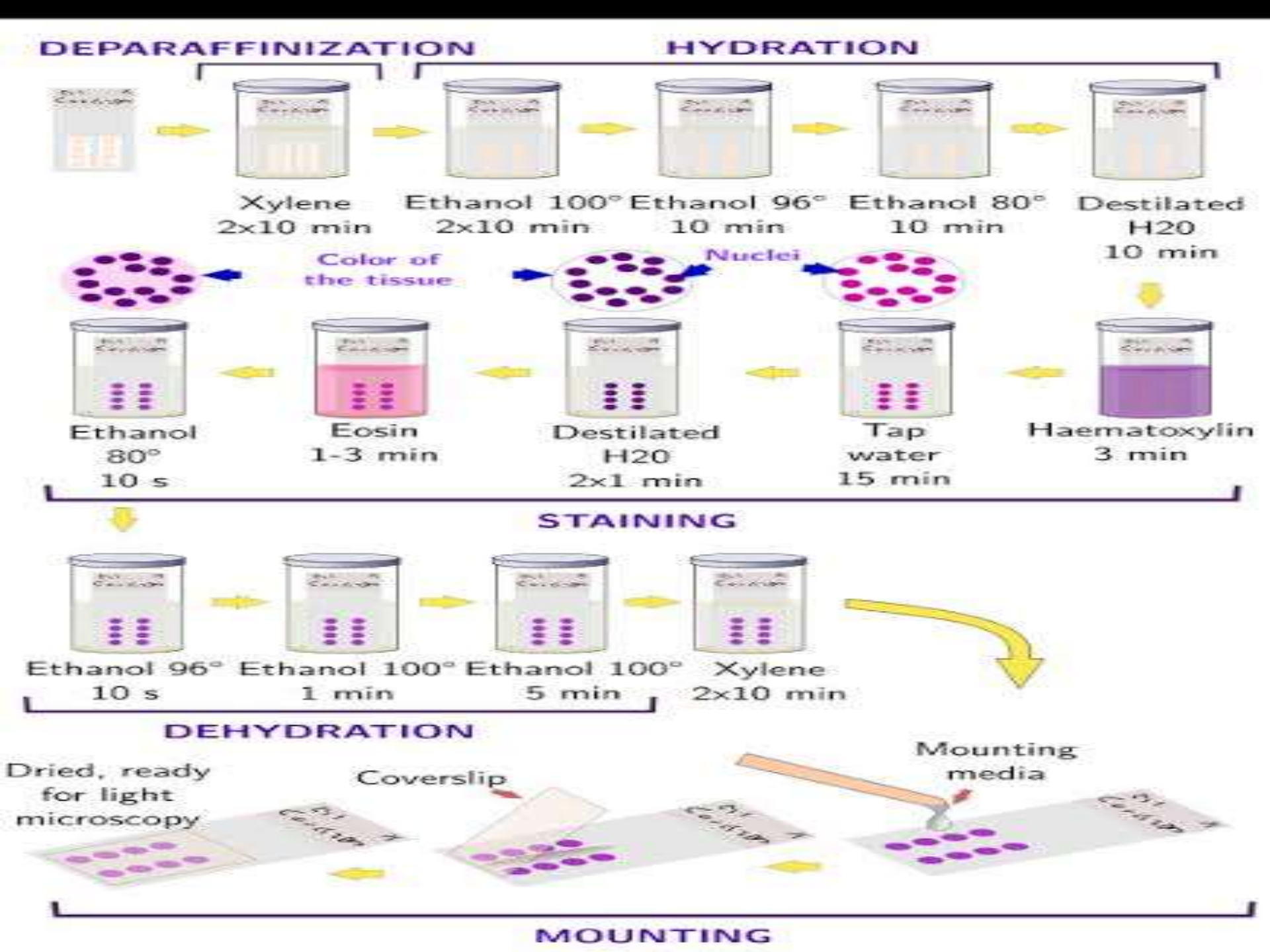
- **STAINING PROCEDURE** involves

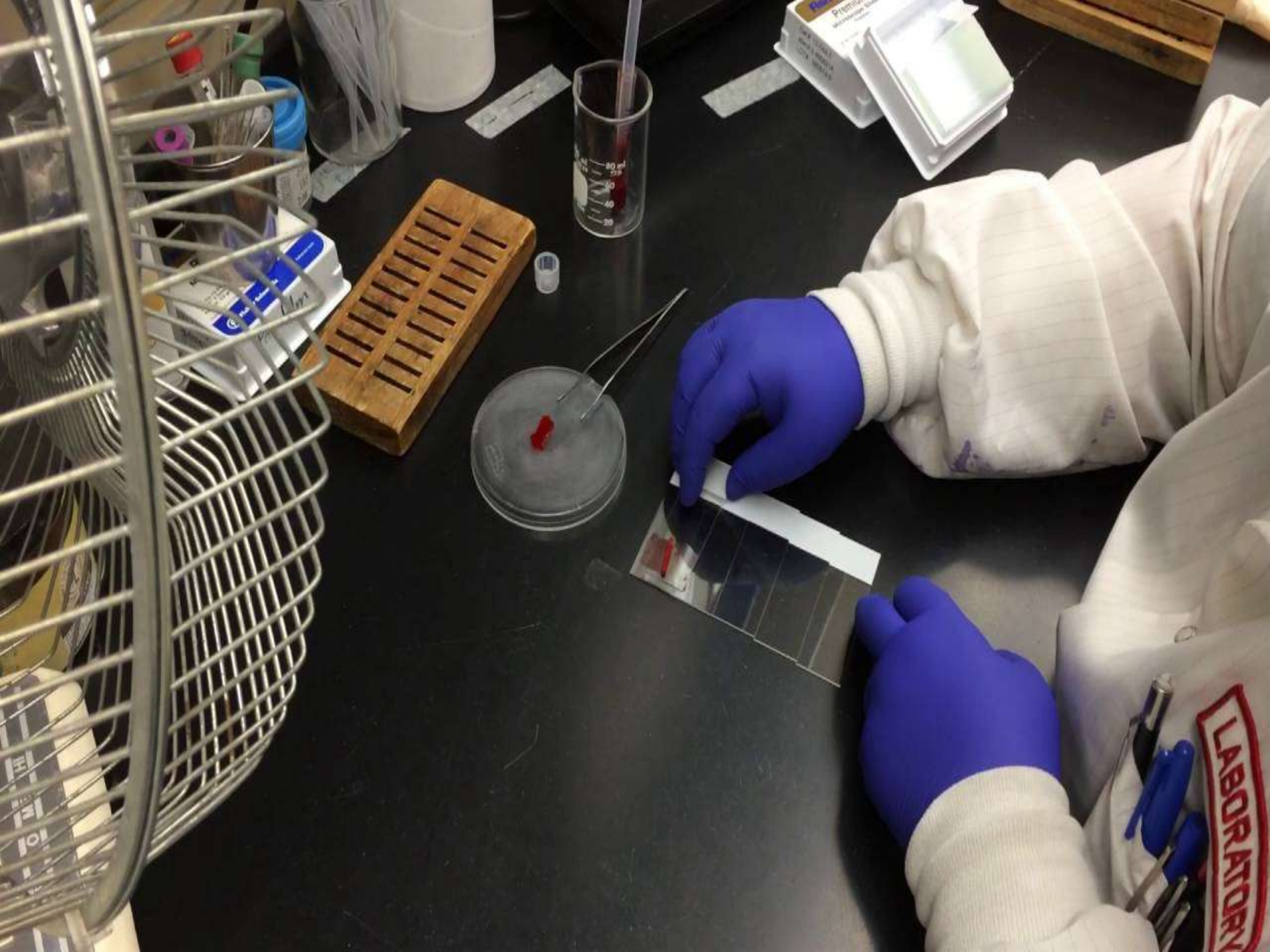
  -deparaFfinization(dewaxing)

  -by placing in xylene for 30 min

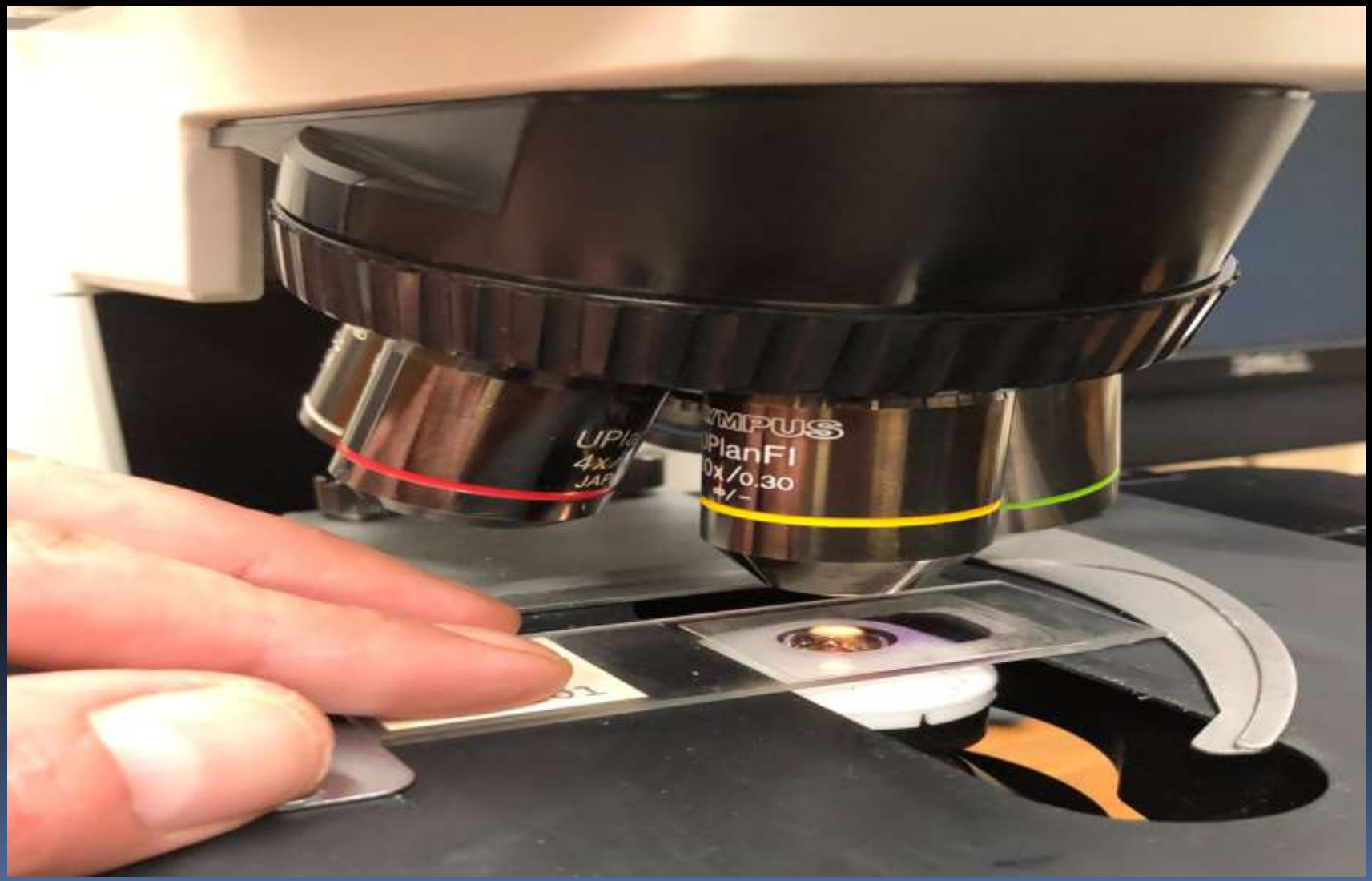
  - the slides are then placed in alcohol for 2 min and washed in tap water.

  -staining in haematoxylin is done for 4-5min


DEPARAFFINIZATION
HYDRATION
Xylene
2x10 min
Ethanol 100°
2x10 min
Ethanol 96°
10 min
Ethanol 80°
10 min
Destilated
H20
10 min
Color of
the tissue
Nuclei
Ethanol
80°
10 s
Eosin
1-3 min
Destilated
H20
2x1 min
Tap
water
15 min
Haematoxylin
3 min
STAINING
Ethanol 96°
10 s
Ethanol 100°
1 min
Ethanol 100°
5 min
Xylene
2x10 min
DEHYDRATION
Dried, ready
for light
microscopy
Coverslip
Mounting
media
MOUNTING


LABORATORY

FINAL VISUALISATION

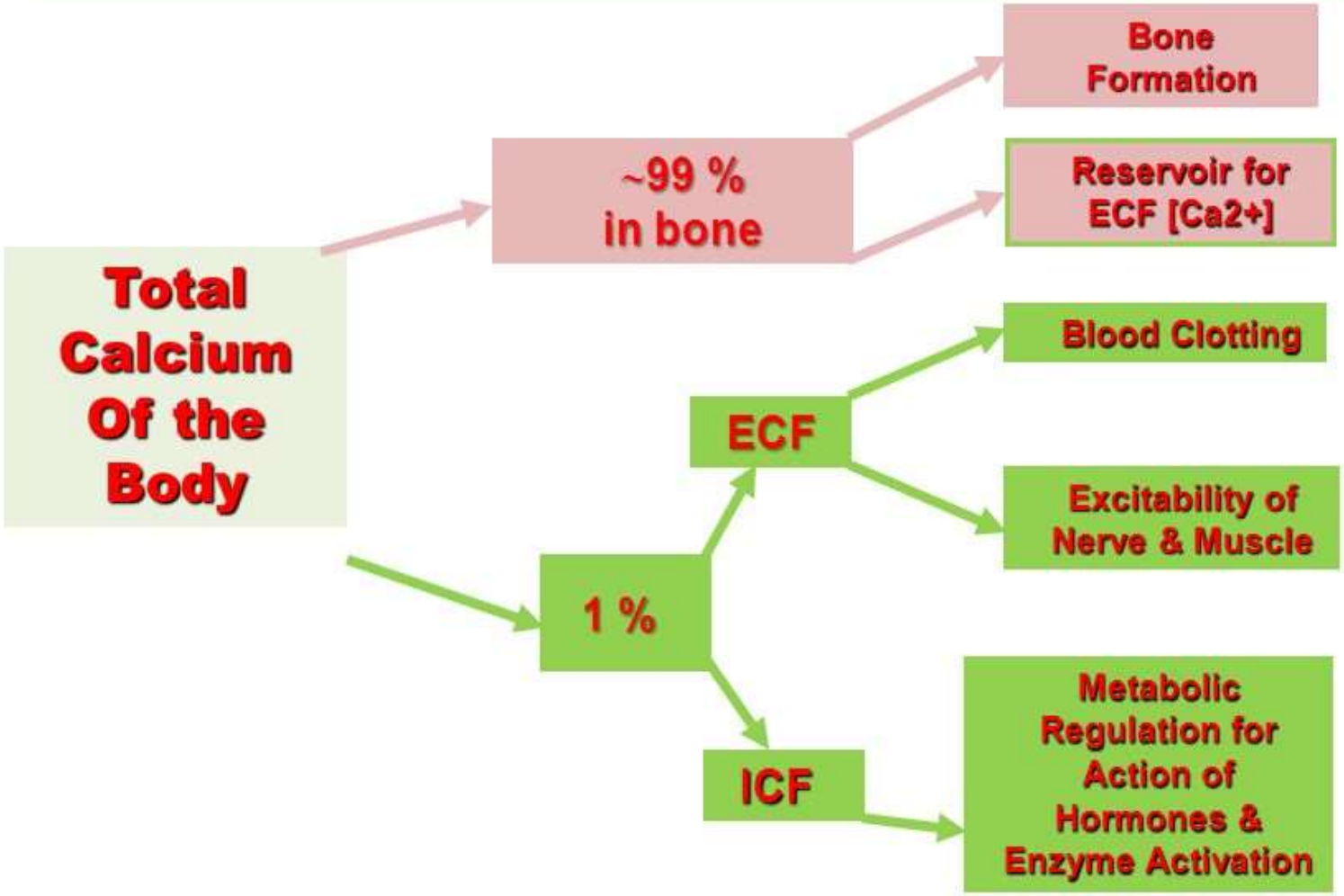


CALCIUM METABOLISM

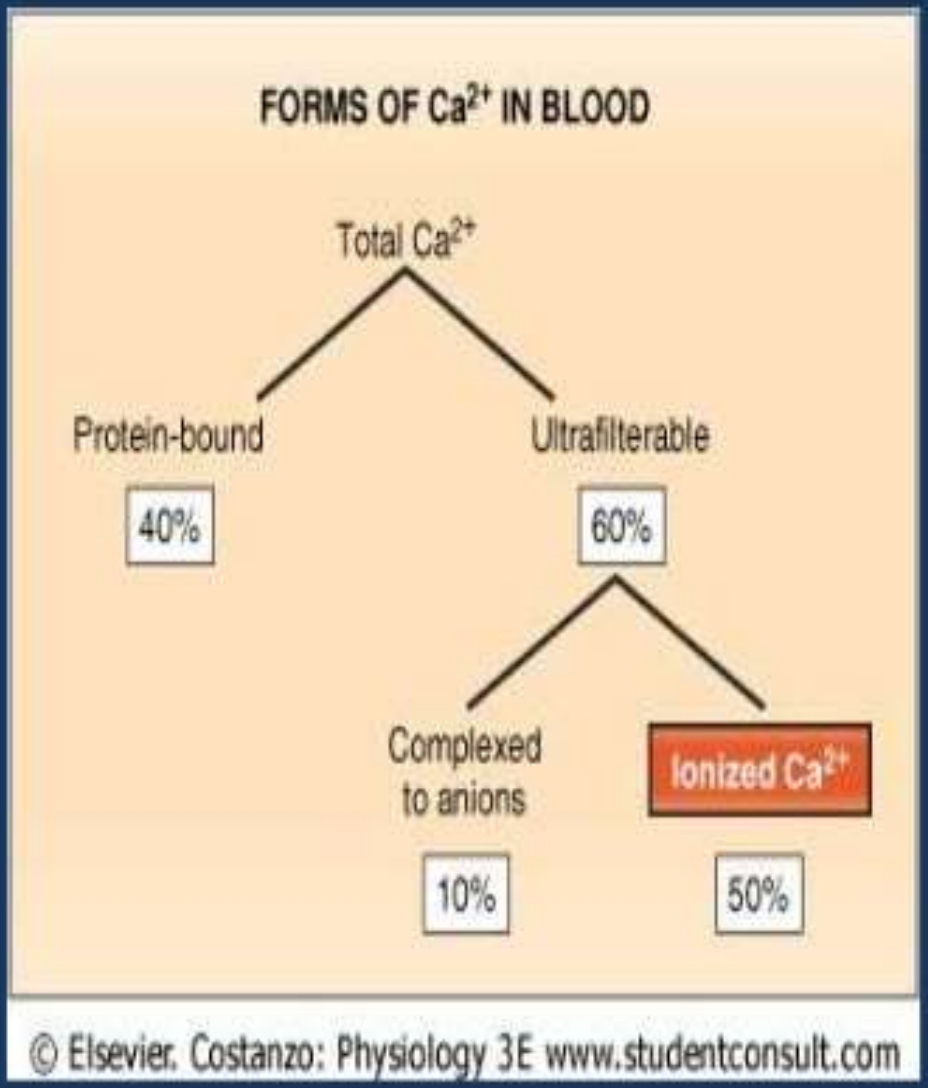
CALCIUM

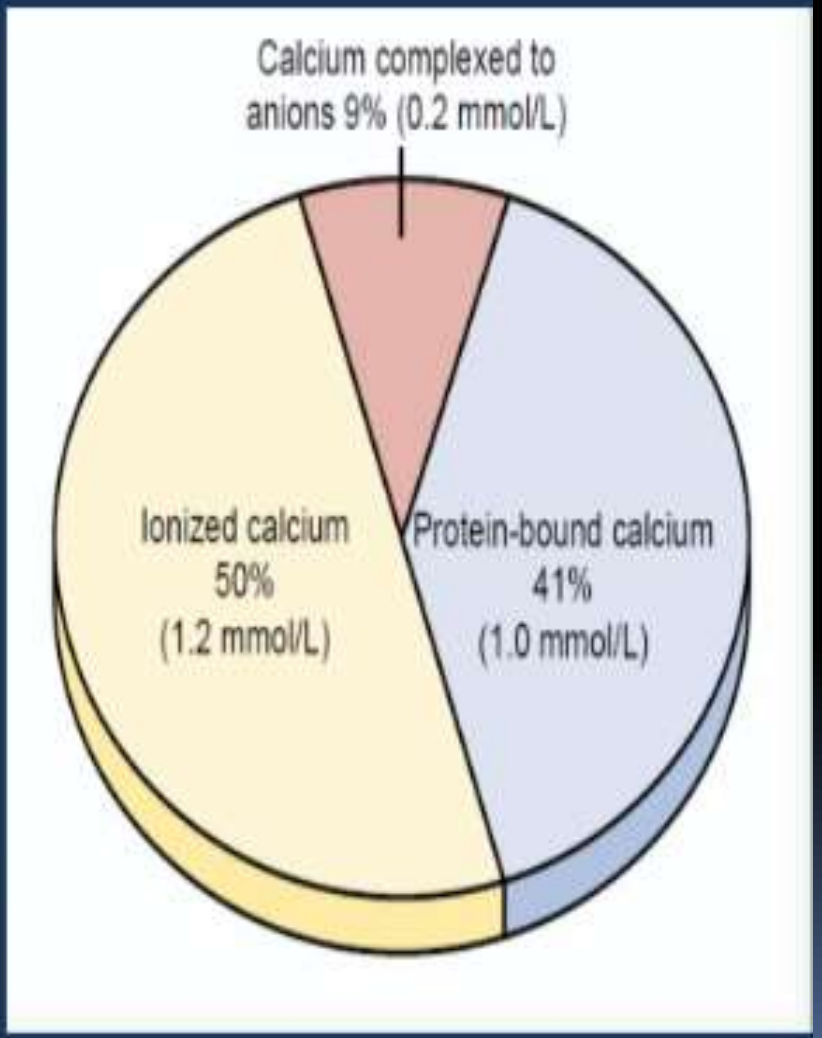
- The total body content of calcium in an adut is approximately 1.1 kg, of which around 98-99% is present in bone and teeth alone.

Biological Functions of Calcium

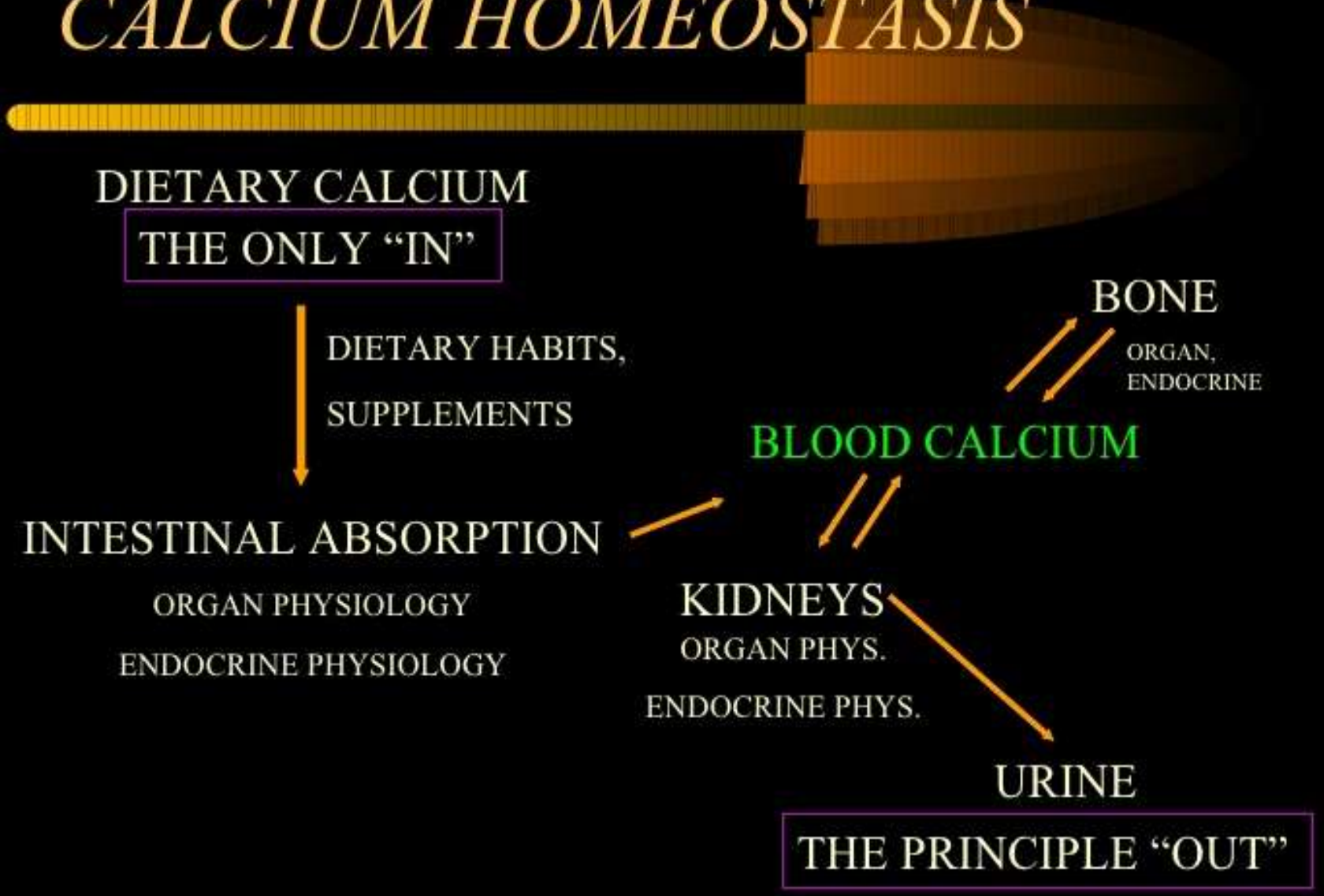


DISTRIBUTION OF CALCIUM

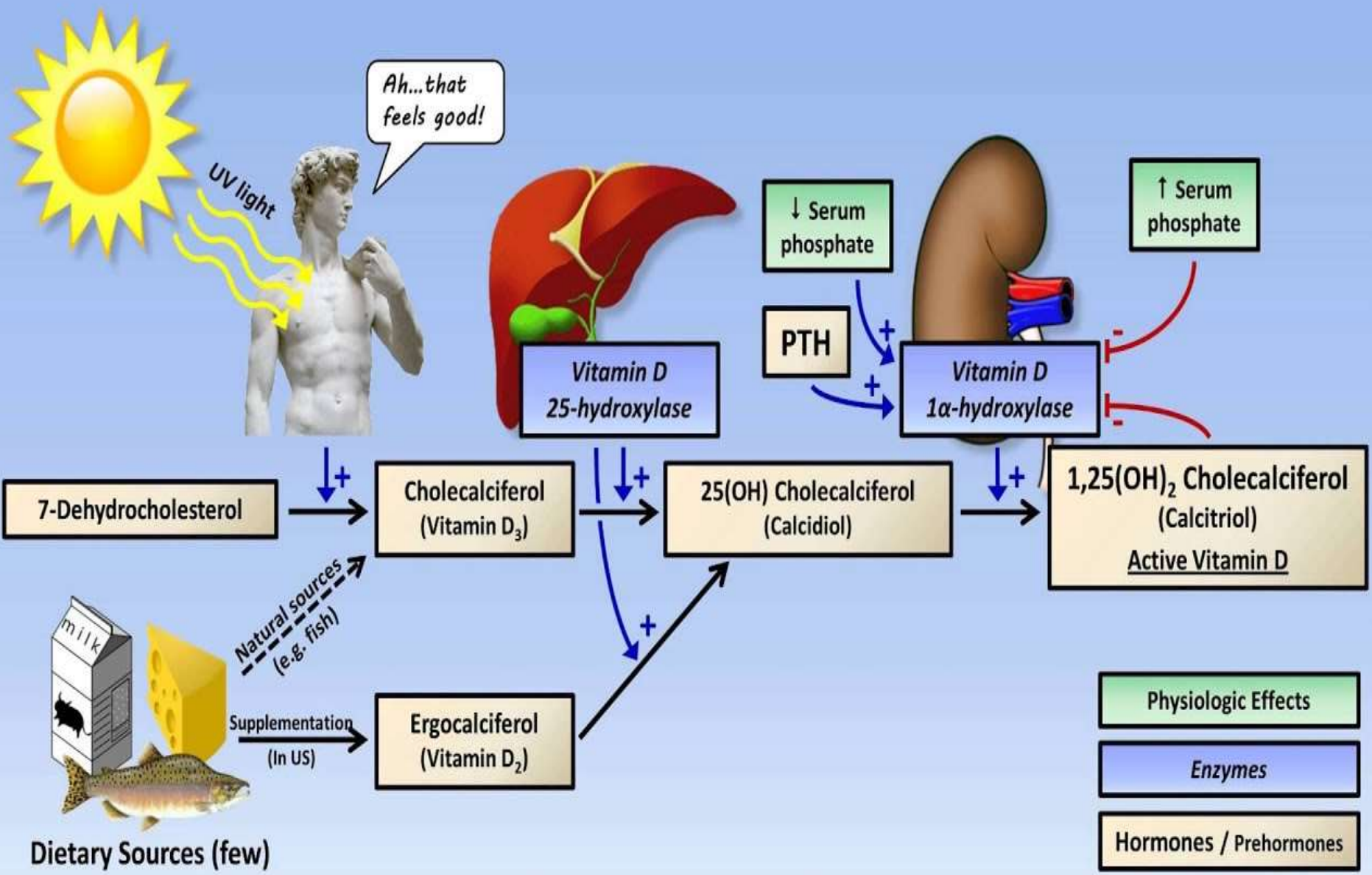




CALCIUM HOMEOSTASIS



Synthesis and Regulation of Calcitriol




THANK
YOU!