

WOLAITA SODO UNIVERSITY , SVM

VETERINARY HISTOLOGY I

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ECTS 5

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WOLAITA SOD ,

ETHIOPIA

Histological concepts

Definition

- The word “Histology” is derived from Greek words

Histos= tissue;

logos= the study of

- Therefore, histology is the study of tissues

Cont...

- Nowadays, the concept of histology is more than just the study of tissue
- It includes understanding of the structure & function of cells, tissues, organ and organ system.
- Microscopic Anatomy (histology) - the study of form & structure of an organism without naked eye.

Historical Background of Histology

A French scientist called Bichat (1771-1804) dissected animal body

- He observed grossly various layers and structures with different "weave or texture".
- He called the various structures- **tissue**
- After visual observation he applied various techniques
 - ✓ Putrefaction
 - ✓ Maceration
 - ✓ Cooking
 - ✓ Chemical disintegration

Cont...

- Based on effects of the above different techniques he classified tissues into several types.
- Though he was not correct, he laid a foundation for establishment of Histology.
- Antony van lee hook was the first person to show the application of microscope to the study of tissue.
- Based on the work of Lee van hook, scientists start to study tissues using microscope and histology is established as a separate discipline.

Elements of structural organization

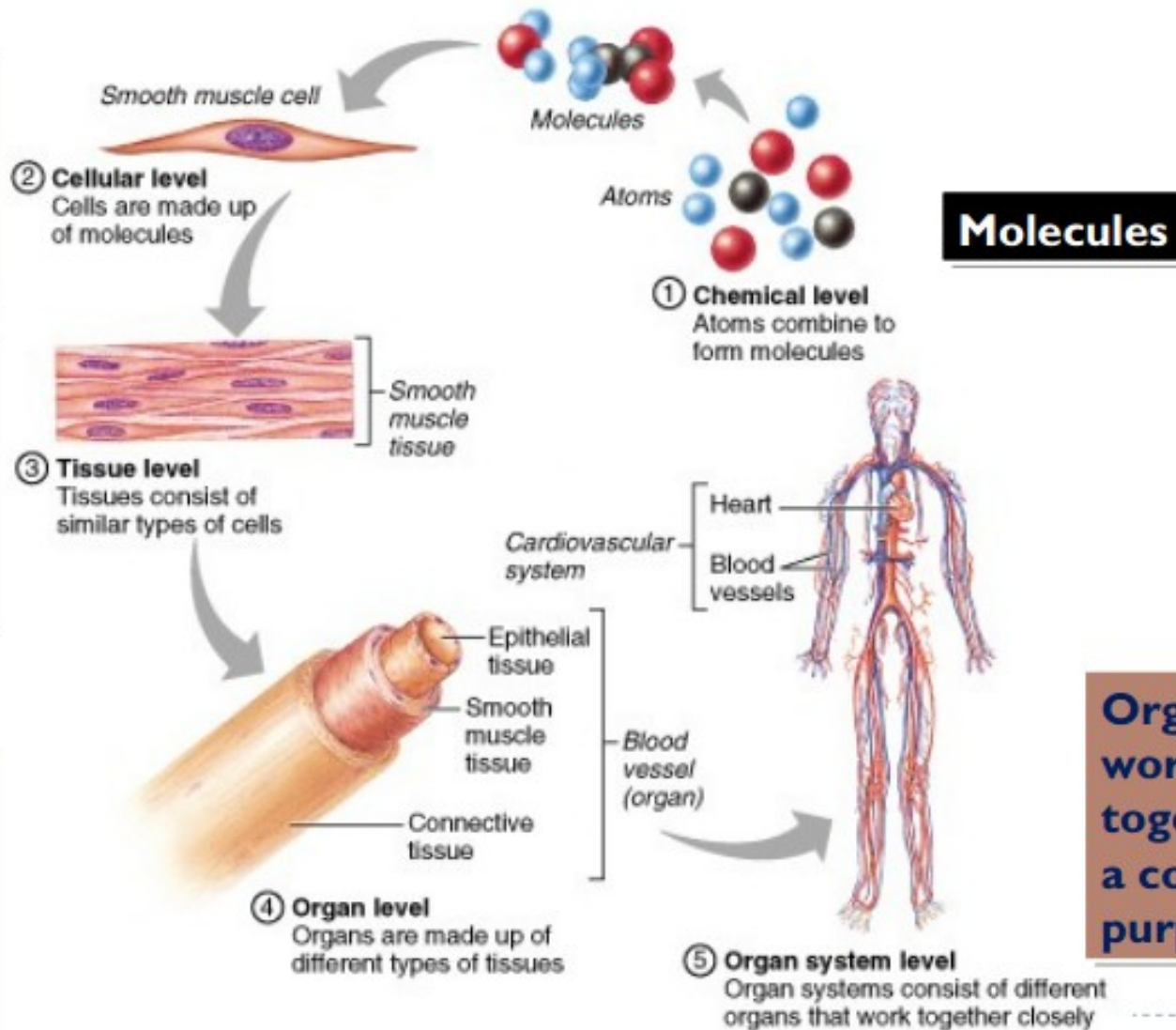
- Cells-the sum of organelles
 - Tissues-the sum of several cells
 - Organ-the sum of different tissues
 - Organism-the sum of organ components
- Tissue: a group of similar, identical, or dissimilar cells and their extra cellular products that perform specific function or spectrum of related function.
- **Generally**, the basic tissues then are the building blocks of complex organizational hierarchy- the organ

The Hierarchy of Structural Organization

Cells and their functional subunits

A group of cells performing a common function

A discrete structure made up of more than one tissue



Organs working together for a common purpose

Cont....

There are four basic tissues in animal

- ❑ **Epithelial tissue**-are found on surface of organ (tubular) organ, surface of skin, and internal surface of digestive system.
- ❑ **Connective tissue**-includes structures involved in providing support and connection
- ❑ **Muscular tissue**-are used for contraction and relaxation
- ❑ **Nervous tissue**- tissues involved in impulse conduction.

Relation of histology with other disciplines

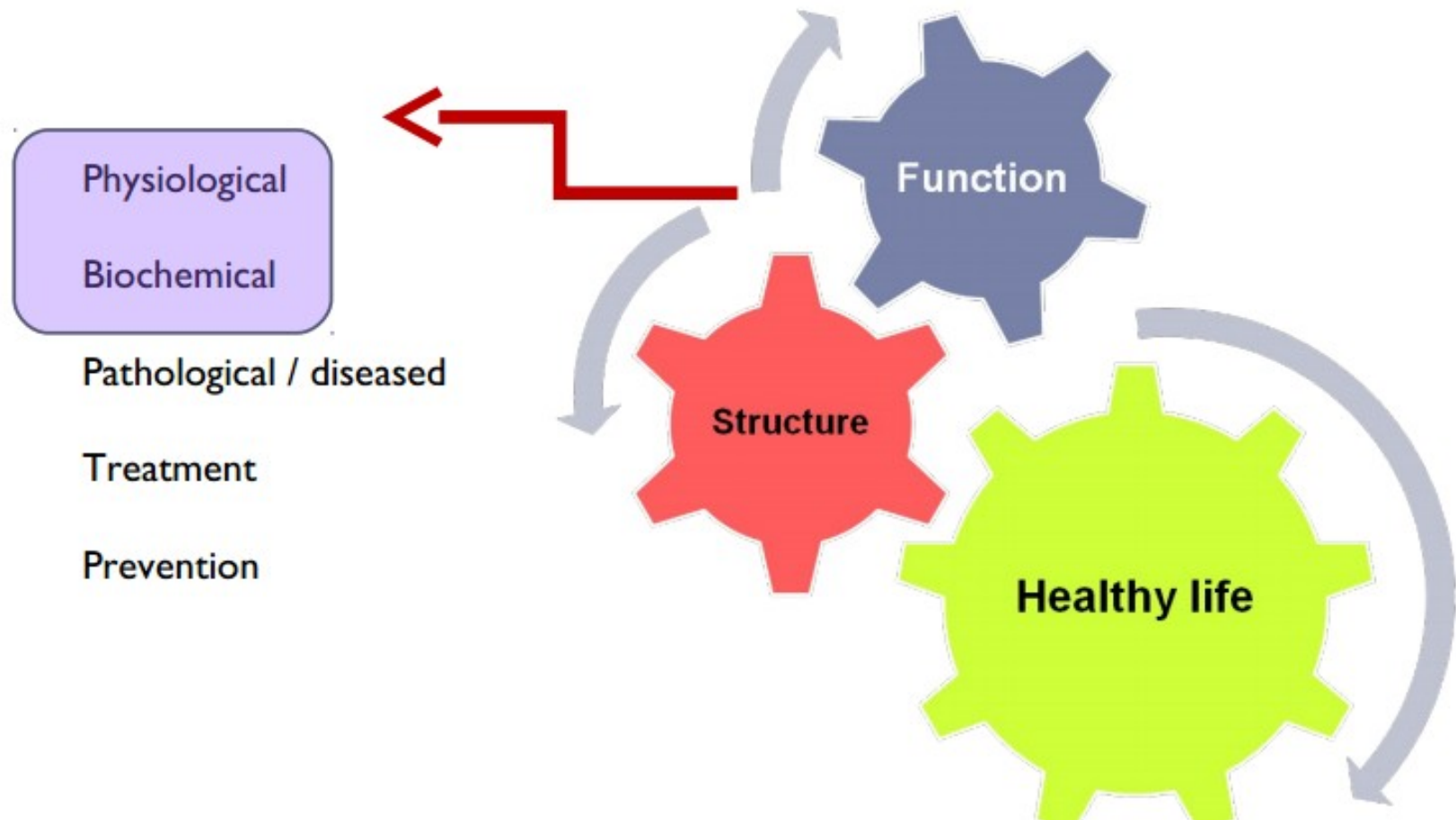
Histology has evolved into hybridization of

- **Gross anatomy**: study of all those structural features that are accessible for direct visual inspection, palpation and/or dissection whereas
- Studies structures which are not visible to the naked eye **microscopic anatomy**
- Histology is also related to **physiology** because one should be familiar with normal cells & tissues functions.
- It is also a prerequisite for **pathology** (study of changes in organs, tissues & cells due to disease).
- **Histopathology**; microscopic study of diseased tissues.

The ultimate goals of Histology are:-

- To know the general & specific feature of tissues & organ
- To identify mammalian tissue quickly & accurately
- To understand how tissues are organized at all structural levels.
- To study tissue death and regeneration.
- To study various tissue reaction to injury or invading organism.
- To understand how cells and tissues are arranged in the normal organ system of the body, and
- To understand how these cells and tissues are specialized to perform the functions (structure-function relationship) most effectively.

- Learn the (microscopic) structure while correlating the structure with functions



- ❖ Examination of tissue at microscopic level is conducted mainly through **biopsy** & **autopsy**.
- ❖ Biopsy: histological examination of tissue taken from life organism.
 - Provides valuable information about the disease process while
- ❖ Autopsy: histological study of tissue after death of an organism.
 - It reveals tissue changes that have led the organism to death

METHODS OF HISTOLOGY

- ❖ The maximal information that is attainable with any particular technical approach requires
 - o A complete familiarity with the technique
 - o Understanding of the nature and limitation of the informational output.
 - o Comprehensive appreciation of its advantage and disadvantages.
- There are diversity of techniques that are available to examine living cells, and components cells
 - Cell culture
 - Vital and super vital staining
 - Paraffin technique

Cell culture

- Is the process of cultivating cells and tissues outside the body of an organism (invitro) in an artificial environment, w/c stimulates the invivo conditions such as temperature, nutrition and protection from microorganisms.
- It permits continual observation, manipulation and testing of explanted cells without any jeopardy to the donor organism.
- ❖ It is used to study:
 - ✓ Cell metabolism
 - ✓ Cellular differentiation
 - ✓ Cellular transformation
 - ✓ Cytogenesis
 - ✓ Cell to cell interaction
 - ✓ Host - parasite relation ship and other biological studies
 - ✓ Diagnostic virology, Vaccine development, & Vaccine production.
 - ✓ Culture media contains proper and complete media for that specific cell.

Vital and super vital staining techniques

- Used to understand the function of some cellular organelles and inclusions and extra cellular material.
- They are also useful to examine living cells because they have low toxicity.

Vital stain

- Inoculation of staining materials into living organism
- Lithium carmine and trypan blue are vital dyes and utilized to study phagocytosis

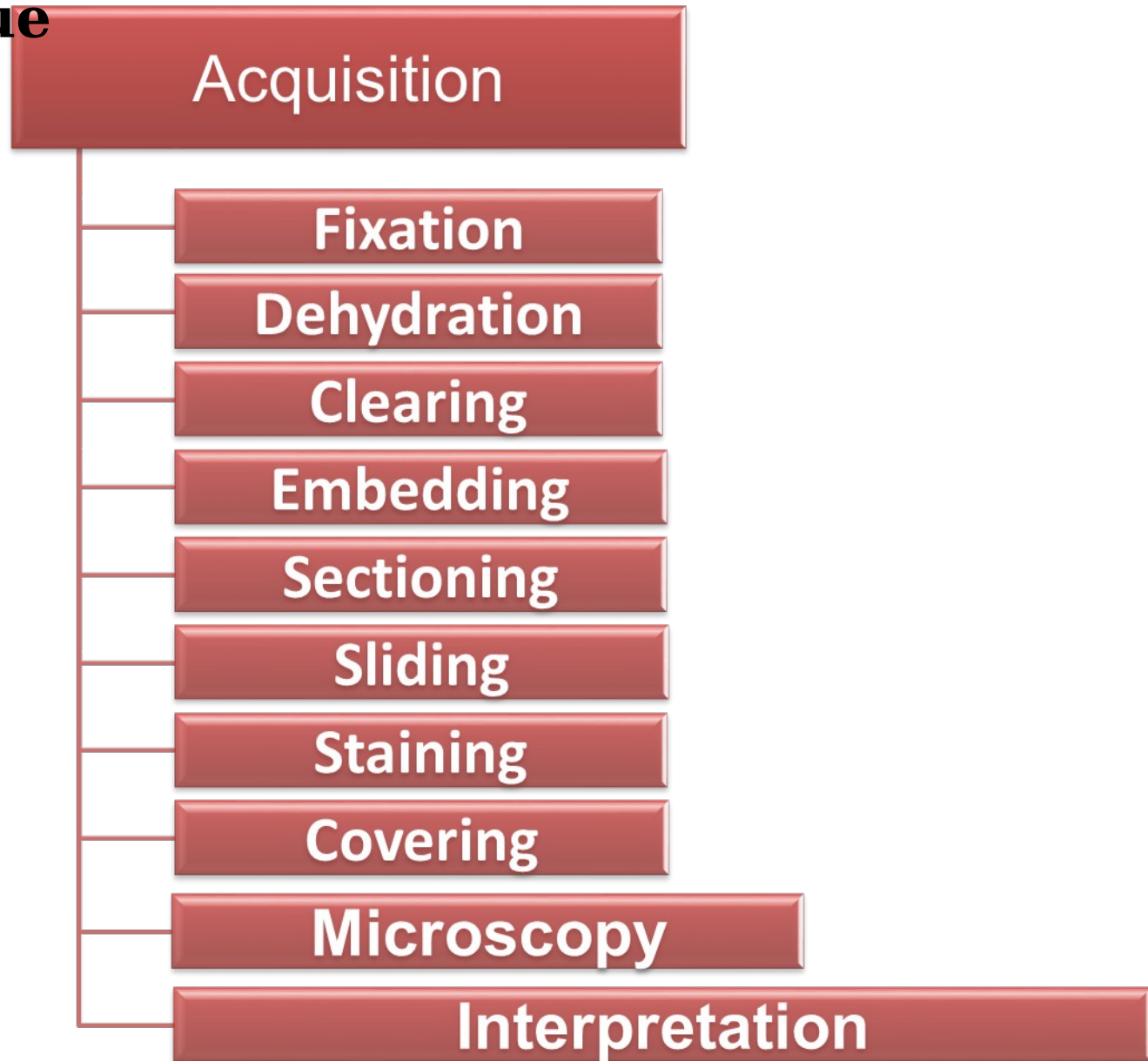
Supper vital staining

- Injection of staining materials into living cells extirpated from the organism.
- Janus green and neutral red are super vital stains used to study mitochondria and lysosomes

Paraffin technique

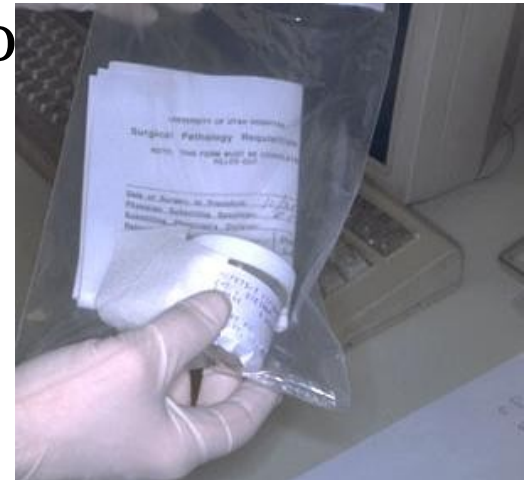
- Used for examination of dead cells and tissues.
- Living cells and tissues are difficult to examine because they are relatively transparent and thick.
- This problem is reduced, when thin sections of tissue are obtained, stained and examined.
- Tissues and cells for microscopic examinations are usually killed by careful fixation.

Steps in paraffin technique



Acquisition

- Clinical details
- Adequate specimen
- Identification of desired sample
- Removal of sample must be atraumatically.
- Excessive handling and poor extirpation techniques change morphology



Fixation

- This is achieved by exposing the tissue to chemical compounds, call fixatives.

Classification of Fixatives

❖ Tissue fixatives

- ✓ Buffered formalin, Buffered gluteraldehyde, Zenker's formal saline and Bowen's fluid

❖ Cytological fixatives

- ✓ Ethanol, Methanol and Ether

❖ Histochemical fixatives

- ✓ Formal saline, Cold acetone and Absolute alcohol

Factors Influencing Fixation

There are a number of factors that will affect the fixation process:

✓Temperature : 4°C.

✓Size: Ideal size: 5X5X3mm

Maximal size: 10X20X3mm.

✓Volume ratio: Tissue volume : Fixative volume at least 1:20.

✓Time

- Remove tissue from body to place the tissue to fixative.
- Duration Overnight (O/N) or 2days
- Time required to achieve fixation is variable and depends on : concentration of fixatives, diffusive property of fixatives and density of the tissues.
- Most formaldehyde achieves their fixation within 24hrs.

Cont...

The action of most fixatives is multiple

- To preserve the structure of tissue
- It is also used protect the tissue from the microorganisms
- Prevent post mortem autolysis by inactivating hydrolytic enzymes
- Facilitate sectioning of the tissues by hardening them
- Enhance staining by acting as a mordant
- Minimize the leaching of many constituents that results from subsequent processing.
- Stabilize structural components in near *in vivo* conditions as possible
- Protect the histologist through antiseptic properties of these substance

Dehydration

- Is the removal of extractable water from the tissue.
- Graduated strength of ethyl alcohol is routinely employed at series consisting of 70, 95 and 100% alcohol produce good result.
- Average time required is 20-30 min in each solution
- The sample is dehydrated as a preparatory stage to embedding.
- E.g. Ethanol, Dioxane, Buthanol and Is



Clearing

- Also called dealcoholation.
- The aim of this process is to replace alcohol by a solvent which is miscible with paraffin.
- Clearing of tissue is achieved by any of the following reagents: Xylene, toluene, benzene, Carbon tetrachloride and chloroform.

*Xylene is commonly used.

*Small piece of tissue are cleaned in 0.5 – 1 hour; *whereas larger (5cm or more thick) are cleaned in 2-4 hours.

Cont...

- Generally, dehydrating and embedding agents are not miscible in each other but clearing agents are miscible in dehydrating fluid and the embedding agents.
- Therefore, clearing agents replaces dehydrating agents & embedding agent replaces clearing substances

Embedding

- Before sectioning of tissue it must be embedded in a material which after hardening has a consistency that permits it to be cut into thin section.
- Paraffin wax is the most frequency used agent.
- During embedding process paraffin heated to 60 degree, and in cooling the paraffin with the tissue forms a firm tissue block.





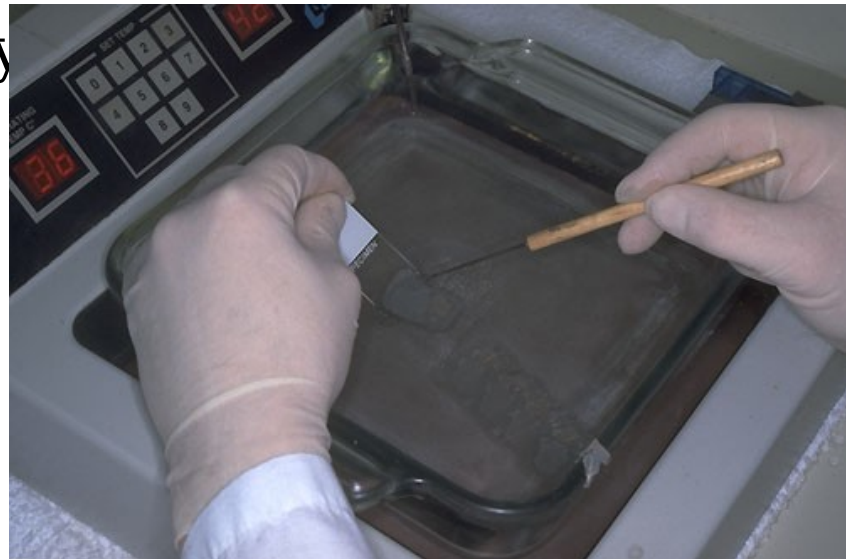
Sectioning

- Sectioning is carried out by the help of machine called *Microtome*.
- Usually 3-10 μm thick



Sliding

- ▶ Also called mounting of the slide.
- ▶ Clean microscopic glass slid are taken and the section which is floated in warm water is taken on the glass slid in such a way that no air bubble is trapped between them.
- ▶ The dish is always rounded edge



Staining

- Staining is a process by which we give colour to a section.
- Because the tissue of the body are colourless and it's difficult to study their details the staining techniques enhance natural contrast and permits distinction to be made between them.
- There are hundreds of stains available.
- Classification of Stains:
 - ✓ *Generally the stains are classified as:*
 - ✓ Acid stains
 - ✓ Basic stains
 - ✓ Neutral stains

Cont...

Acid Dyes:

- In an acid dye the basic component is coloured and the acid component is colourless.
- The colouring property of the dye is in the acidic radical the stain is called acidic dye and the stained structures called Acidophilic.
- Acid dyes stain basic components
*e.g. eosin stains cytoplasm red.

Basic Dyes:

- In a basic dye the acid component is coloured and the basic component is colourless.
- the colouring property of the dye is in the basic radical the stain is called basic dye and the stained structures called Basophilic
- Basic dyes stain acidic components
*e.g. basic fuchsin stains nucleus blue.

Cont...

Neutral Dyes:

- *When an acid dye is combined with a basic dye a neutral dye is formed.
- *As it contains both coloured radicals, it gives different colours to cytoplasm and nucleus simultaneously. This is the basis of Leishman stain.

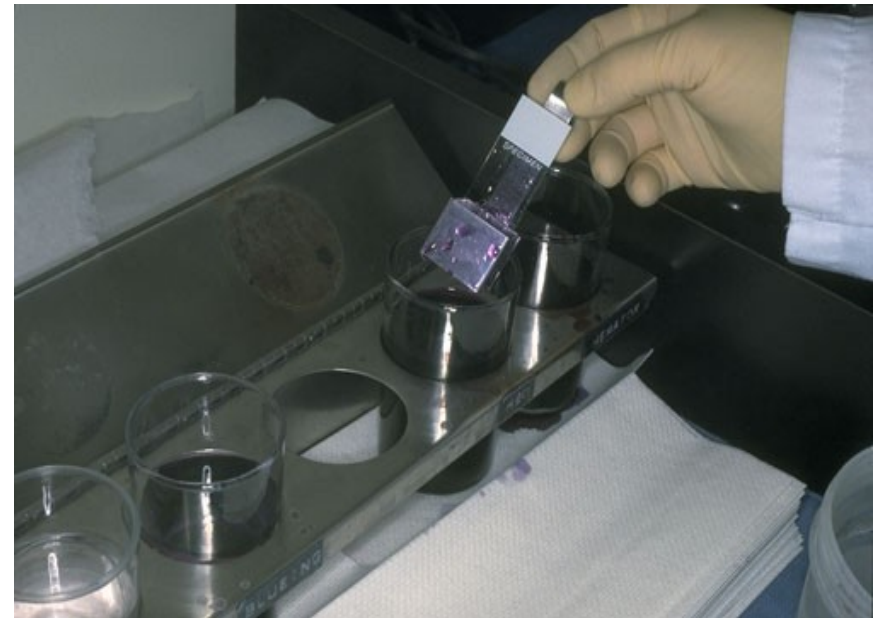
Staining can be done either manually or in an automatic stainer.



Automated



Manually



Hematoxylin and Eosin

- It is the most commonly used routine stain in histopathology
- Neutralization is mechanism of the H and E stain
- Hematoxylin the basic dye applied first.
- Hematoxylin imparts a bluish purple to acidic cellular components, chromatin, ergastoplasm and some secretory products.
- Eosin, the acidic dye ,imparts pink to red color to basic cellular components such as cytoplasm and numerous extra cellular products
- It stains nuclear structures are stained dark purple or blue and all cytoplasmic structures and intracellular substances are stained pink

Romanowsky stains

- Romanowsky stains are dye combinations that contain methylene blue and eosin.
- Common variants include Wright's stain, Jenner's stain, Leishman stain and Giemsa stain.
- Methylene blue oxidized readily to form azure dyes.
- Combination of methylene blue and azure dyes (azure A, B, & C) are called polychrome methylene blue.
- polychrome methylene blue has a broad staining range for acidic components.
- Eosin is the acidic counter-stain.

Cont...

- All are used to examine blood or bone marrow samples.
- They are preferred over H&E for inspection of blood cells .
- because different types of leukocytes (white blood cells) can be readily distinguished.
- All are also suited to examination of blood to detect blood-borne parasites.

Special stains

1. PAS (Periodic Acid Schiff) stain:

- It is used to mark carbohydrates (glycogen, glycoprotein, proteoglycans) in tissues (e.g., glycogen storage diseases).
- Involves two steps to achieve reactivity
 - The 1st reaction is oxidation of α -amino alcohols or 1,2 glycol groups to aldehyde groups by periodic acid.
 - These aldehydes are then subjected to Schiff reagent.
- Reaction of aldehydes with the reagent forms a complex that restores the magenta color.
- Hematoxylin is the usual counter-stain.

Cont...

2.Stains for micro-organism:

- ✓ Gram-stain:
- ✓ Ziehl_Neelsen stain: This stain detect acid fast bacilli.
- ✓ PAS stain: It is used for fungi, amoeba and Tricomonas.
- ✓ Modified Giemsa (2% Giemsa in water):For Helicobacter pylori.

3.Congo-red: It is used for identification of amyloid.

4.Sudan-Black: It is used for fat staining.

5.Masson's Trichrome: It is used for differentiation of connective tissue

Cont...

6. Histochemical stains

- used in histochemistry
- provides information about the presence and location of intracellular and extracellular molecules

Relies on the chemical reactivity, enzymatic activity associated with the cell component of interest.

- Is staining depending on enzymes.
- Ex. -Alkaline phosphatase
 - Acid phosphatase
 - Succinc dehydrogenase
 - Estrases

Cont...

7. Immunohistochemical stains

- o Staining depending on antibody antigen reaction .
- o Antibody against a particular macromolecule created and localised by reaction with the macromolecule
- o Visualized by labeling the antibody with a fluorescent dye or horseradish peroxidase.

Covering

- Applying a thin glass coverslips to protected



Microscopy and interpretation

- Microscopy is the study of microscopes.
- **Magnification:** ability to magnify objects
- ***Resolving power*** (R_p): is the capacity of a microscope to clearly differentiate between two near points.
- The display of closely spaced objects as separate images is the resolving power of a lens or lens system.
- Resolution is mathematically defined as

$$R = 0.61\lambda / NA.$$

- o Numerical aperture (NA)-measure of the ability of a microscope to collect diffracted light from fine details in the object

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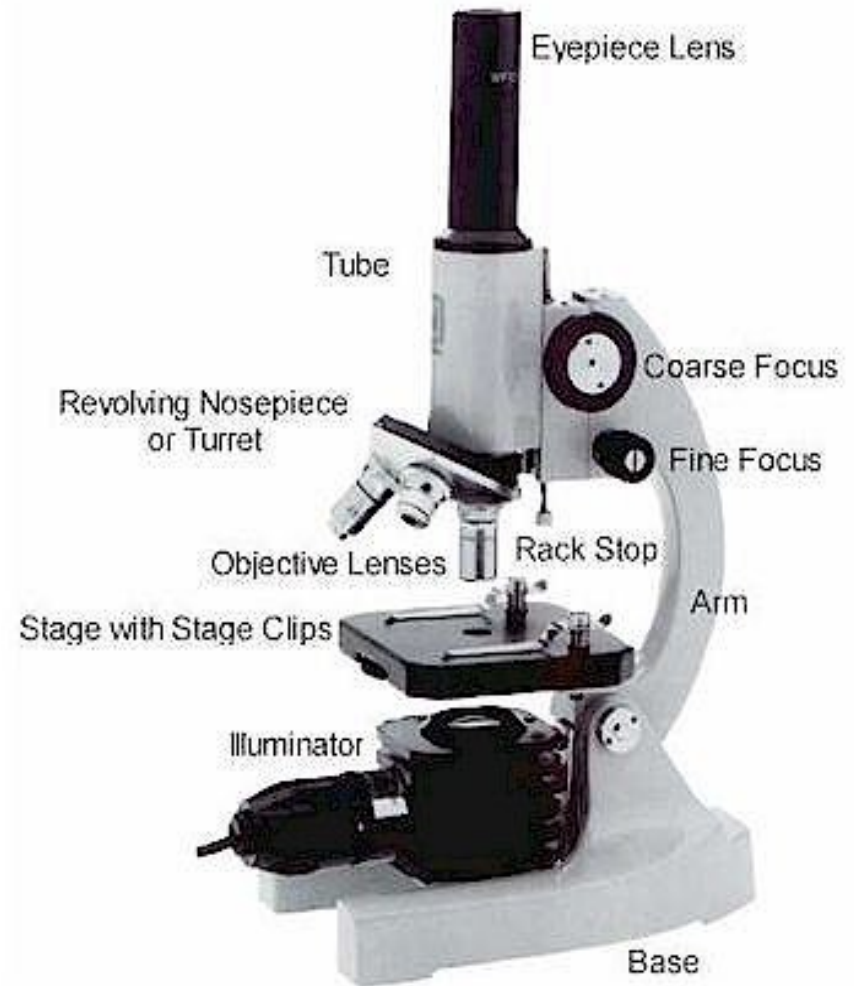
- The resolving power (R in $\text{\AA}^\circ$) is determined by the wavelength (λ in $\text{\AA}^\circ$) of the illuminating source and the numerical aperture (NA) of the objective lens.
- Usually the wavelength of light microscope is 5000 $\text{\AA}^\circ$ and NA in good oil immersion is 1.4.

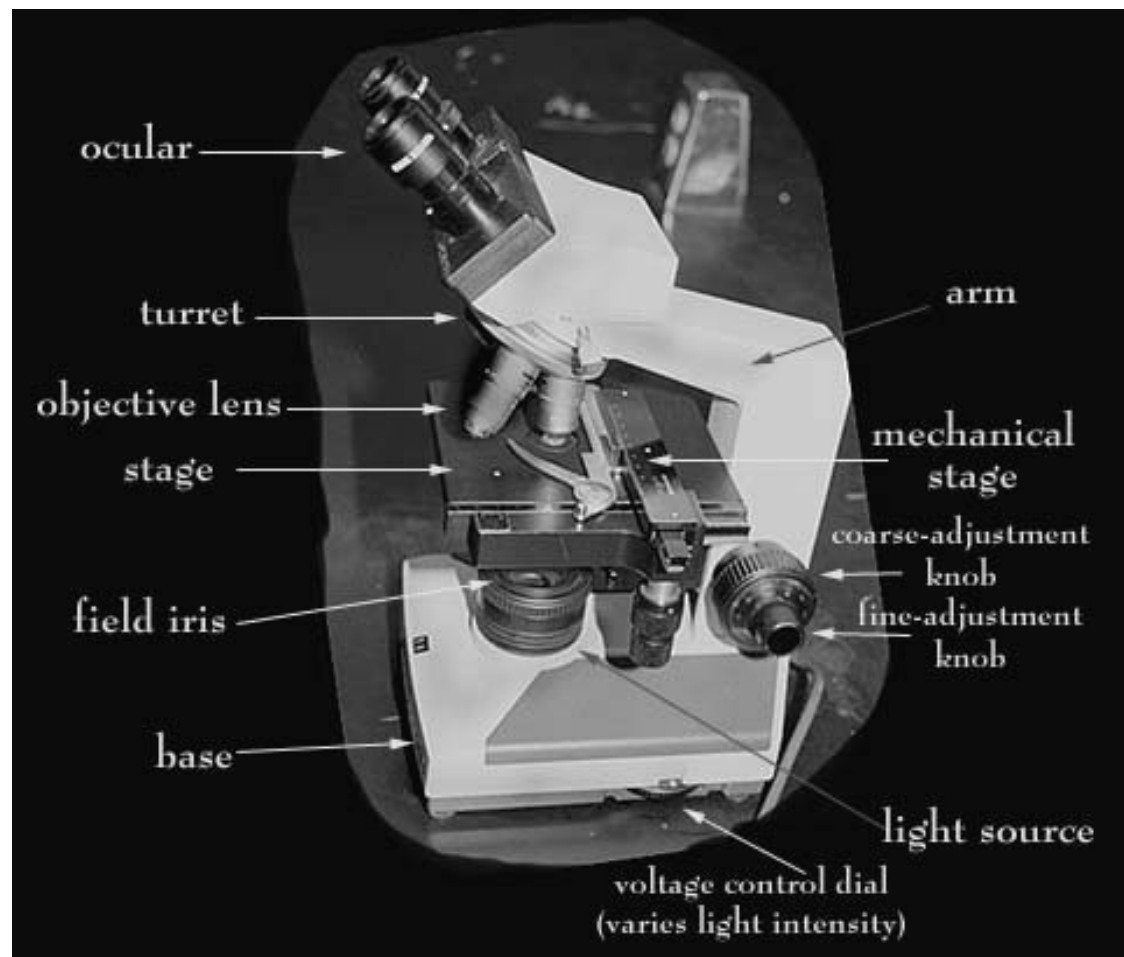
$$\text{RP} = \frac{0.61 \times 5000 \text{ \AA}^\circ}{1.4} = 2200 \text{ \AA}^\circ,$$

- But if the closeness is higher than resolution power one has to use another microscope.

Different Parts of Microscope

- ▶ Eyepiece Lens
- ▶ Tube
- ▶ Arm
- ▶ Base
- ▶ Illuminator
- ▶ Stage
- ▶ Revolving Nosepiece
- ▶ Objective Lenses
- ▶ Condenser Lens
- ▶ Coarse & fine Focus
- ▶ Diaphragm





Cont...

- **Eyepiece Lens:** the lens at the top that you look through. They are usually 10X or 15X power.
- **Tube:** Connects the eyepiece to the objective lenses
- **Arm:** Supports the tube and connects it to the base
- **Base:** The bottom of the microscope, used for support.
- **Illuminator:** A steady light source (110 volts) used in place of a mirror
- **Stage:** The flat platform where you place your slides. Stage clips hold the slides in place.
- **Revolving Nosepiece or Turret:** This is the part that holds two or more objective lenses and can be rotated to easily change power.

Objective

Lenses

- A compound light microscope often contains four **objective lenses**:
 - scanning lens (4X),
 - low-power lens (10X),
 - high-power lens (40 X), and
 - oil-immersion lens (100 X).
- With an ocular lens that magnifies 10 times, the total magnifications possible will be 40 X with the scanning lens, 100 X with the low-power lens, 400 X with the high-power lens, and 1000 X with the oil-immersion lens.
- **Total magnification** = magnification achieved by objective lens X magnification achieved by ocular lens.

Cont...

- **Rack Stop** :This is an adjustment that determines how close the objective lens can get to the slide
- **Condenser Lens:**
 - to focus the light onto the specimen
- **Diaphragm or Iris:**
 - rotating disk under the stage.
 - used to vary the intensity and size of the cone of light that is projected upward into the slide

2. Ultraviolet microscope

- Utilizes an ultraviolet radiation source that emits radiation with wavelengths between 1000 $\text{\AA}$ and 3000 $\text{\AA}$.
- Resolution of this system is between 500 $\text{\AA}$ and 1500 $\text{\AA}$.
- Primary application is in microspectrophotometry as an adjunct to histochemical studies.

3. Fluorescence microscopy

- This is a form of visible light microscopy in which an ultraviolet emitter is used as the light source.
- It involves the use of fluorescent dyes that are either present in the specimen or applied on the specimen.
- These dyes have the ability to absorb UV-light and re-emit (reflect) it in the form of visible light.
- It is used in fluorescent antibody techniques

4. Dark field microscope

- This type of microscope is achieved by slightly modifying the bright-field microscope.
- The usual condenser is replaced by one that causes light to strike the image at oblique angle without any direct illumination reaching the objective lens.
- Dark field microscopes enable to examine living unstained specimen.

5. Phase-contrast microscope

- It is a modified conventional microscope that requires a special condenser (Zernike condenser) and a phase plate positioned behind objective lenses. .
- It is used to observe specimen or tissue sample having the same optical property to the medium .
- because it has the ability to alter the optical property of the specimen and the medium.
- Helpful in studying living and unstained specimens.
- Its single most advantage is the three-dimensional imaging of cells and tissues

6. Electron microscopy

- Sophisticated, advanced and latest.
- The source of illumination is electron.
- Electrons have a much smaller wavelength than light, so they can resolve much smaller structures.
- Images are received on a fluorescence screen or photographic plate.
- There are two types of electron microscope:
 - Scanning microscope
 - Transimission electron microscope

Cont...

Scanning microscope

- ✓ helps to study tissues from out side.
- ✓ It helps the display of three-dimensional images.

Transmission electron microscope

- ✓ Depending on the beams penetrates inside the tissue.
- ✓ The size, shape and arrangement of the particles which make up the specimen as well as their relationship to each other on the scale of atomic diameters.

7. Polarizing Microscope

- It can resolve structures of a cell less than $2000\text{\AA}$ apart.
- It is fitted with a material called polaroids, which make the light rays polarize in one direction.
- Enhance the visibility of the point of interest in detail.

Interpretati

- **on** Accurate indications of glycogens content are difficult are exposed routinely to water during processing.
- To study proteins and enzymes formalin must not be used
- Ability of microscope must be taken into consideration.
- make inferences toward largest structure.
- Color and morphology of specimen must be related with staining procedure.
- The tendency “to want to see more” by going directly to the highest magnification usually results in lack of orientation for the novice.
- Each increment in magnification should always be accompanied progressively or gradually from narrower field of vision.

Artifacts in histological section

- Structures or features in tissue that interfere with normal histological examination.
- These are not always present in normal tissue and can come from outside sources.
- Interfere with histology by changing the tissues appearance and hiding structures.

Cont...

Artifacts can be categorized into two.

- Pre-histology
- post histology

Pre-histology

- These are features and structures that have been introduced prior to the collection of the tissues.
- A common example of these include: ink from tattoos and freckles (melanin) in skin samples

Post-histology

- Artifacts can result from tissue processing. Processing lead to changes like shrinkage, color changes caused in a laboratory.
- the majority of post histology artifacts can be avoided or removed after being discovered.
- A common example is mercury pigment left behind after using Bouin's fixative to fix a section.

Cont...

- A number of artifacts that appear in stained slides may result from
 - ➔ Improper fixation
 - ➔ Type of fixative
 - ➔ Poor dehydration and paraffin infiltration
 - ➔ Improper reagents and poor microtome sectioning

Thank

you