Zool-02501 **Zoological Microtechniques** **2(1+1)**

General Laboratory Glassware and care, Laboratory safety measures. Elementary Microscopy and principals of optics: light microscopy, Magnification, Resolution, contrast. Types of microscopy: Phase contrast, Dark field and Electron microscopy. Collection and handling of material- (Anatomy, Chromosmal study and maycorrhiza) Assignment. Principles and methods involved in the killing, fixing and storage of materials, Chemicals used and their properties. Recopies and preparations of important fixatives, Dyes and related reagents and solutions. Assignment. Histochemistry, plant crystallography, localization of various chemicals in plants and animal tissues. Histological studies in plant and animal tissues by fixation, decalcification of animal tissues, dehydration. Impregnation and Embedding techniques, Section cutting staining and mounting procedures.

Practical

Preparation of anatomical and surface slides through hand and microtome sectioning. Study of morphology and reproductive structures of the types mentioned in theory. Handling of prepared slides for staining of plant material. Study of morphology and reproductive structures of the types mentioned in theory. Identification of various types mentioned from prepared slides and fresh collections. Handling of slides for animal tissues.

Recommended Books:

1. Latest additions of books will be recommended from stock available in the main library of the university

LAB SAFETY RULES

Lab safety rules:

Laboratory safety rules are a major aspect of every microbiology lab. Each student in microbiology laboratory must follow specific safety rules and procedures. Here are some safety rules:

i. Wear protective clothes.
- Gloves are essential.
- Lab coats are required.
- Safety glasses may be required to avoid splashes.

ii. Avoid touching objects(e.g; pencils, cell phones, door handles) while wearing gloves.

iii. Do not eat food or drink water in the lab. Do not use lab glassware as food or water containers.

iv. Pencils, labels, or any other materials should never be placed in your mouth.

v. Do not take any cultures out of the lab for any reason.

vi. Never touch, taste or smell a chemical unless instructed to do so.

vii. Always work in properly ventilated areas.

viii. Never use lab equipment that you are not approved or trained by your supervisor to operate.

ix. Do not pipette by mouth.

x. Wash hands after every lab.

xi. Long hair must be tied back or covered to minimize fire hazard or contamination of experiments.

xii. Caution must be taken when using gas burners. Be sure gas burners are turned off when finished.

xiii. Keep non essential books and clothing far away from your work area.

xiv. Dispose off waste products according to instructions.

Lab Safety Symbols

- They alert about the possible dangers in the lab.

General laboratory glassware:

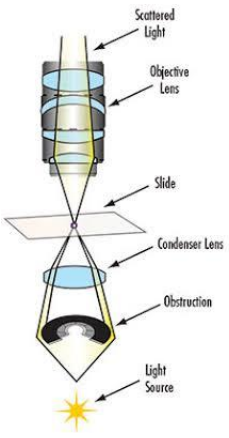
Laboratory glassware refers to a variety of equipment used in scientific work, and traditionally made of glass.

1. **Beakers:** beakers are simple cylindrical containers, in sizes from 10 ml to 4000 ml. one of the commonest glass equipment found in labs, they are used for holding solids and liquids ,and for pouring and decanting liquids.
2. **Test tubes:** test tubes are relatively smaller than beakers ,test tubes are cylindrical lab equipment used to store, mix and heat chemicals. They are useful in observational procedures.
3. **Watch glass:** this is a glass container which is used when the volume of liquid is small, but you need a higher surface area. It is good for crystallizing and evaporation and also can be used as a beaker.
4. **Funnel:** a funnel is a pipe with a wide(often conical) mouth and a narrow stem. It is used to channel liquid or fine grained substances into containers with a small opening.
5. **Volumetric flask**: a volumetric flask (measuring flask or graduated flask) is a piece of laboratory glassware, a type of laboratory flask, calibrated to contain a precise volume at a particular temperature.
6. **Spatula:** spatula are nothing but scientific spoon, which is used for taking sample in a small quantity.
7. **Volumetric pipette:** pipettes, also called pipets or chemical droppers, are small tubes of glass or plastic used to transfer a measureable amount of liquid from one container to another.
8. **Mortar-pestle:** these are basic apparatus available in every pharmaceutical laboratory especially in pharmacognosy departments, these are used to crush and make a pest of any given sample.
9. **Measuring cylinder:** a graduated cylinder also known as a measuring cylinder is one of the most common piece of laboratory glassware which is used to measure the volume of liquids.
10. **Condenser:** condenser are used to condense vapours to liquid and are most commonly used for distillation.
11. **Bunsen burner:** a Bunsen burner is a common piece of laboratory equipment which is used for heating and combustion.

DARK FIELD MICROSCOPY

➢ Introduction:

Dark field microscopy (also called ground field microscopy) describe microscopy which ideally used to illuminate unstained sample causing them to appear brightly lit against dark background. This type of microscope contain special condenser that scatter light and caused it to reflect off the specimen at an angle. Richard Zsigmonday in cooperation with Siedenkopf developed ultra-microscopy which is called dark field microscopy.



Living specimens may be observed more readily with dark field than bright field microscopy.

➢ Principle of dark field microscopy:

A dark field microscope is arranged so that the light source is bucked off causing light to scatter as it hits specimen.

- This is done for making objects bright against dark background.
- When light hits an object, rays are scattered in all directions. The design of dark field microscope is such that it remove the dispersed light, so that only the scattered beams hits the sample.
- Introduction of condenser ensures that these light rays will hit the specimen at different angles.
- The result is the "cone of light" where rays are diffracted, reflected, and refracted off the objects, allowing the individual to view a specimen in dark field.

➢ Uses of Dark field Microscope:

Following are the uses of dark field microscopy.

1. It is useful for the demonstration of very thin bacteria not visible under ordinary illumination since the reflection of the light makes them appear larger.
2. For the rapid demonstration of *Treponema pallidum* in clinical specimens.
3. It is used to study marine organisms such as algae, plankton as well as crystals and ceramics.
4. It is used to study mounted cells and tissues.
5. Ti is useful for examining external details than internal structures.

➢ Advantages of Dark field microscope:

1 .It is very simple yet effective technique.

2. It is well suited for uses involving live and unstained biological samples such as smear from a tissue culture or individual, water borne, single celled organisms.

3. The quality of images obtained from this technique is impressive.

4. This technique is entirely free from artifacts, due to nature of the process.

➢ Limitations of Dark Field microscope:

- The main limitation of dark field is the low light levels seen in the final image.
- The sample must be very strongly illuminated, which can cause damage to the sample.

ELECTRON MICROSCOPY

➢ Introduction:

It uses an electron beam to create an image of a sample. EM operates under vacuum which means the samples are placed in a vacuum system during analysis.

EM technique is for obtaining high resolution image of biological and non-biological specimens. It is used in biomedical research to investigate structure of tissue, cell, organelles etc.

➢ Working Principe of EM:

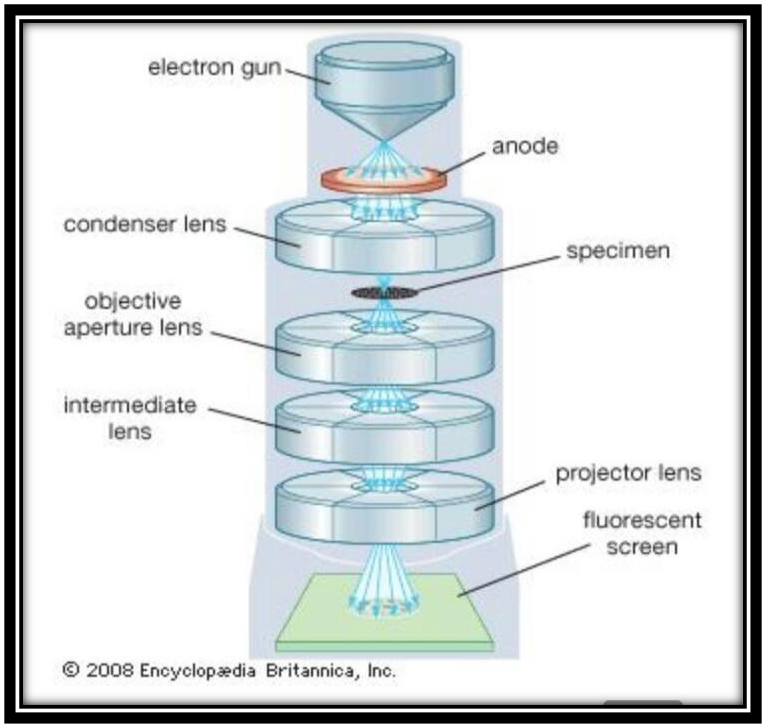
An EM uses an "electron beam" to produce the image of the object and magnification is obtained by "electromagnetic fields" in which light waves are used to produce the images and magnification is obtained by the system of optical lenses.

Types of Electron Microscope:

There are two types of electron microscope:

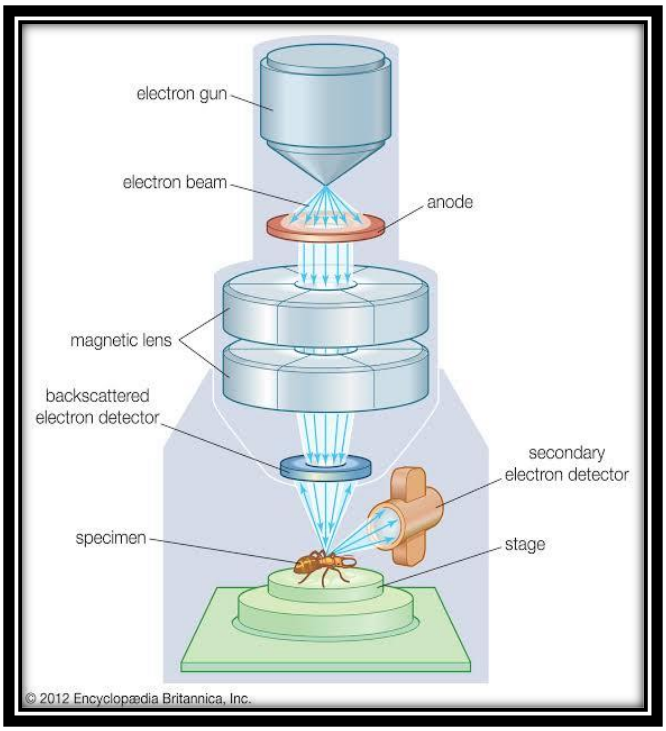
➢ The Transmission Electron Microscope:

The transmission electron microscope is used to view thon specimens through which electrons can pass generating a projection image. It is analogous in many ways to compound light microscope. It is used to image the interior of cells, the structure of protein molecules, the organization of molecules in viruses and cytoskeletal fragments, and the arrangement of protein molecules in cell membranes.



➢ The scanning Electron Microscope:

Scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen. Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope. It provide the detail images of surface cells and the whole organisms that are not possible with TEM. It is termed a scanning electron microscope because the image is formed by the scanning a focused electron beam onto the surface of specimen in a raster pattern.



➢ Uses of Electron Microscopes:

1. It is used to investigate the ultrastructure of wide range of biological and inorganic specimens including cells, large molecules, biopsy samples, metals, and crystals.
2. Electron microscopes are used for quality control and failure analysis.
3. Modern electron microscope produce electron micrographs using specialized digital cameras.
4. Science of microbiology owes its development to the electron microscope.

➢ Advantages of Electron Microscope:

- Very high magnification
- Incredibly high resolution
- Material rarely distorted by preparation
- It is possible to investigate a greater depth of field.
- Diverse applications

- **Disadvantages of Electron Microscope**:
  - The main disadvantage are cost, size, maintenance, researcher training and image artifacts resulting from specimen preparation.
  - This type of microscope is expensive piece of equipment, extremely sensitive to vibration and external magnetic fields.
- **Limitations of Electron Microscope:**
  - The live specimen cannot be observed.
  - As the penetration power is very low the object should be ultra-thin.
  - As the EM works in vacuum the specimen should be completely dry.
  - Expensive to build and maintain.
  - Requiring research training
  - Image artifacts resulting from specimen preparation.

FIXATIVES

A chemical substance used to preserve and stabilize biological material prior to microscopy or other examination.

Preparation of fixatives

1. Phosphate buffered formalin

Formulation

- 40% formaldehyde: 100 ml
- Distilled water: 900 ml
- Sodium dihydrogen phosphate monohydrate: 4 g
- Disodium hydrogen phosphate anhydrous 6.5 g
- The solution should have a pH of 6.8
- Fixation time: 12 – 24 hours

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2. Formal calcium

Formulation

- 40% formaldehyde: 100 ml
- Calcium chloride: 10 g
- Distilled water: 900 ml
- Fixation time: 12 – 24 hours

Recommended Applications

Recommended for the preservation of lipids especially phospholipids.

3. Formal saline

Formulation

- 40% formaldehyde: 100 ml
- Sodium chloride: 9 g
- Distilled water: 900 ml
- Fixation time: 12 – 24 hours

4. Zinc formalin (unbuffered)

Formulation

- Zinc sulphate: 1 g
- Deionised water: 900 ml
- Stir until dissolved then add –
- 40% formaldehyde: 100 ml
- Fixation time: 4 – 8 hours

5. Zenker's fixative

Formulation

- Distilled water: 950 ml
- Mercuric chloride: 50 g
- Potassium dichromate: 25 g
- Glacial acetic acid: 50 ml
- Fixation time: 4 – 24 hours

6. Helly's fixative

Formulation

- Distilled water: 1000 ml
- Potassium dichromate: 25 g
- Sodium sulphate: 10 g
- Mercuric chloride: 50 g
- Immediately before use add –
- 40% formaldehyde: 50 ml
- Fixation time: 4 – 24 hours

7. B-5 fixative

Formulation

Stock solution

- Mercuric chloride: 12 g
- Sodium acetate anhydrous: 2.5 g
- Distilled water: 200 ml

Working solution, prepare immediately before use

- B-5 stock solution: 20 ml
- 40% formaldehyde: 2 ml
- Fixation time: 4 – 8 hours

8. Bouin's solution

Formulation

- Picric acid saturated aqueous soln. (2.1%): 750 ml
- 40% formaldehyde: 250 ml
- Acetic acid glacial: 50 ml
- Fixation time: 4 – 18 hours

9. Hollande's

Formulation

- Copper acetate: 25 g
- Picric acid: 40 g
- 40% formaldehyde: 100 ml
- Acetic acid: 15 ml
- Distilled water: 1000 ml

Dissolve chemicals in distilled water without heat.

- Fixation time: 4 – 18 hours

10. Gendre's solution

Formulation

- 95% Ethanol saturated with picric acid: 800 ml
- 40% formaldehyde: 150 ml
- Acetic acid glacial: 50 ml
- Fixation time: 4 - 18 hours

REAGENTS

A substance added to a system to cause a chemical reaction, added to test if a reaction occurs.

Crystal violet

1. Dissolve 2.0 g certified crystal violet into 20.0 ml of 95% ethyl alcohol.
2. Dissolve 0.8 g ammonium oxalate into 80.0 ml distilled water.
3. Mix the two solutions together and allow them to stand overnight at room temperature (25°C).
4. Filter through coarse filter paper before use.
5. Store at room temperature (25°C).

Gram's iodine

- Grind 1.0 g iodine (crystalline) and 2.0 g potassium iodide in a mortar. Small additions of distilled water may be helpful in preparing the solution.
- Add to 300.0 ml distilled water.
- Store at room temperature (25°C) in a foil-covered bottle (to protect solution from light).

Preparation of Safranin

- Add 2.5 g certified safranin-O to 100.0 ml 95% ethyl alcohol.
- Add 10.0 ml safranin and ethyl alcohol solution made in step 1 to 90.0 ml distilled water.
- Store at room temperature (25°C).

DYES

Natural or synthetic substance used to add a colour to or change the colour of something.

Preparation of dyes:

1.janus green B

To prepare a 0.01% w/v dye, dissolve 10mg of dye in 2-3 ml of 100% ethanol and than make a final volume of 100 ml with distilled water

2. Eosin

To prepare a 0.5% w/v eosin solution , dissolve 0.5g of eosin Y in 100ml of distilled water

3. Gram's iodine

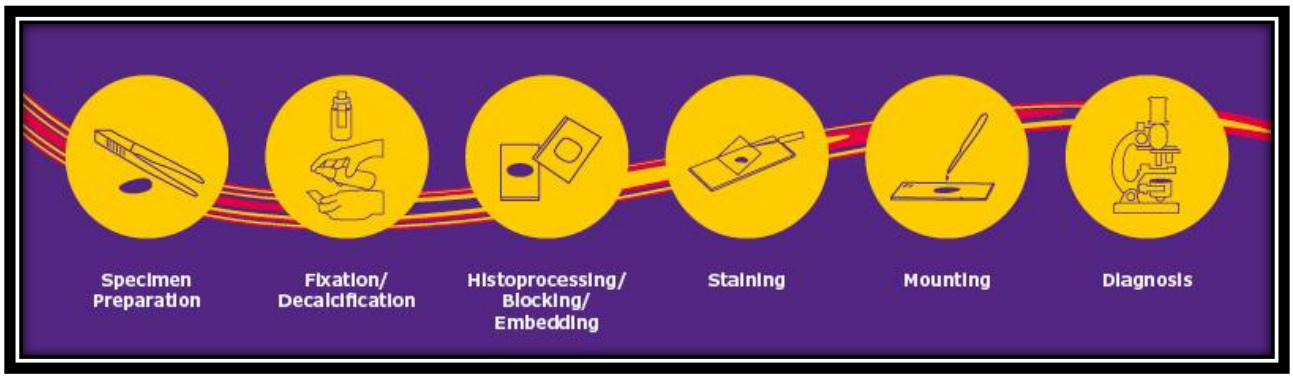
To prepare gram's iodine ,take 0.33g of iodine and 0.69 g of potassium iodide and dissolve it in distilled water to make a final volume of 100 ml

References

1. Eltoum I, Fredenburgh J, Myers RB, Grizzle WE. Introduction to the theory and practice of fixation of tissues. *J Histotechnol* 2001;24;173 -190.
2. Leong AS-Y. Fixation and fixatives. In Woods AE and Ellis RC eds. *Laboratory histopathology*. New York: Churchill Livingstone, 1994;4.1-1 - 4.1-26.
3. Hopwood D. Fixation and fixatives. In Bancroft J and Stevens A eds. *Theory and practice of histological techniques*. New York: Churchill Livingstone, 1996.
4. 111Carson FL. *Histotechnology*. 2nd ed. Chicago: ASCP Press, 2007.
5. Titford ME, Horenstein MG. Histomorphologic Assessment of Formalin Substitute Fixatives for Diagnostic Surgical Pathology. *Arch Pathol Lab Med* 2005;129;502-506.
6. Kothmaier H, Rohrer D, Stacher E, Quehenberger F, Becker K-F, Popper HH. Comparison of Formalin-free Tissue Fixatives: A Proteomic Study Testing Their Application for Routine Pathology and Research. *Arch Pathol Lab Med* 2011;135;744-752.

KILLING AND FIXATION

- Killing is the sudden stopping of all living process in all the cells of collected biological specimens.
- Whereas fixation is the preservation of all structural and cellular elements in a biological specimen in as near their original state as possible.
- Usually killing and fixing are done by a single fluid called fixative.



Principles of fixation

General principles of fixation are as:

- ✓ Amount of fixing fluid should be approximately 10 to 20 times more than the volume of tissue held in a container with a required fixation time.
- ✓ Temperature has an important effect i.e. increase in temperature can potentially increase the degeneration of specimen to be fixated.
- ✓ A lower temperature will retard fixation – reduce autolytic reaction.

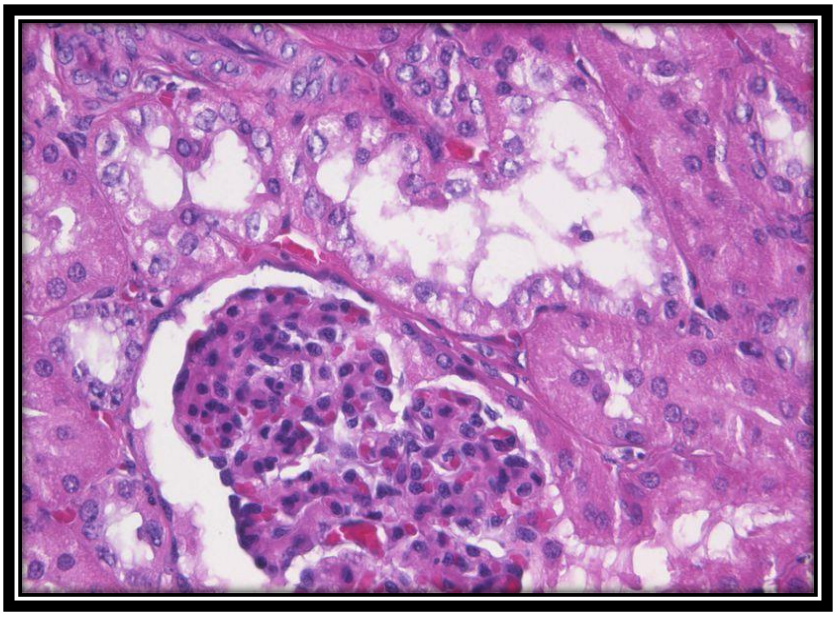
Methods of fixation

1. Before killing the animals from which the tissues are to be removed for fixation.

a) Obtain the necessary fixatives and containers.
b) Have on hand supply of physiological saline solution for rinsing blood and debris from the tissue and to prevent dry off tissue surfaces during subdivisions.
c) Have already prepared labels.

d) Have on hand the necessary instruments.
e) Everything should be arranged so no delay will occur once the animal has been killed. If a large number of different tissues are to be fixed from a single specimen than it is advisable for several people work together so the tissues are proceeded in shortest possible time.

2. Kill the animal.
3. As soon as the animal is dead it is desirable to drain as much blood from the body as possible. Open the blood vessels in the neck by incision or decapitation.
4. As quickly as possible remove the tissues to be fixed. Carefully subdivide them as necessary, rinse them in saline solution and place in the fixative with an appropriate label.
5. Record the accession numbers and necessary information in the catalogue.



process of fixation

Fixation and storage

1. **Bone:**

cut into short lengths and place into fixatives. It should be place into 70% alcohol from which it is transferred to an acid-alcohol solution.

- ✓ 2-4% hydrochloric acid in 70-80% alcohol gives satisfactory result.
- ✓ 1-5% Nitric acid in 70% alcohol may also be used.

2. **Cartilage:**

In young animals it is to be prepared in whole mounts in 10% formalin but in adults cut out blocks and fix as usual.

3. **Fat:**

Blocks of fat for frozen sections should be fixed in 10% formalin.

4. **Heart:**

Small hearts may be fixed intact, rinse with saline. Large hearts should be subdivided.

5. **Skin:**

Should be fixed flattened, or by pinning out on a cork.

6. **Lungs:**

Fix inflated by introducing fixative via bronchial tubes. Subdivide as necessary after initial hardening.

7. **Pancreas:**

Should be immediately fixed after the death of animal since breakdown is rapid.

- ✓ Fix in Helly's to retain cytoplasmic granules

storage and labeling

Chemicals used and their properties:

- Single chemicals are used for killing and fixing but combination of different chemicals are also used.
- These chemicals with some disadvantage should never be mixed.

  Like; Tissue hardening must be combined with tissue softening chemicals.

- Example: Ethyl alcohol, Formalin, Acetic acid, Picric acid crystals, Mercuric chloride etc

Properties:

Ethyl Alcohol:-

i. Boiling point is comparatively low (78.3°C)
ii. It is highly inflammable.
iii. It is a reducing agent.
iv. It dissolve fats and lipids of the cell
v. Miscible with water.

Formalin:-

i. Formalin is the trade name of 40% aqueous solution of formaldehyde.
ii. It is a reducing agent.
iii. Miscible with water.
iv. It is recommended for the fixation of nerve tissue.
v. It is a god cytoplasmic fixative, penetrates well and hardens the material.

Acetic Acid:-

i. It is also miscible with water.
ii. It precipitates the nucleoproteins.
iii. It destroys cytoplasmic structure such as Mitochondria, Golgi apparatus etc.
iv. It is usually used in combination with other fixers which have poor rate of penetration or do not fix nuclear material.

Picric Acid:-

i. Picric acid in combination with Acetic acid and Formalin gives excellent fixation for nuclear material.
ii. It is not favorable for cytoplasmic structure.
iii. Material can be stored in this solution without over hardening.
iv. Beat staining results are achieved if material is processed after fixation.

Mercuric Chloride;-

i. Has a tendency t destroy delicate structure such as cilia.
ii. It is used for the preservation of calcareous structure.
iii. It is highly poisonous so care should be taken.
iv. It penetrates and coagulates protein fairly well.

5th semester

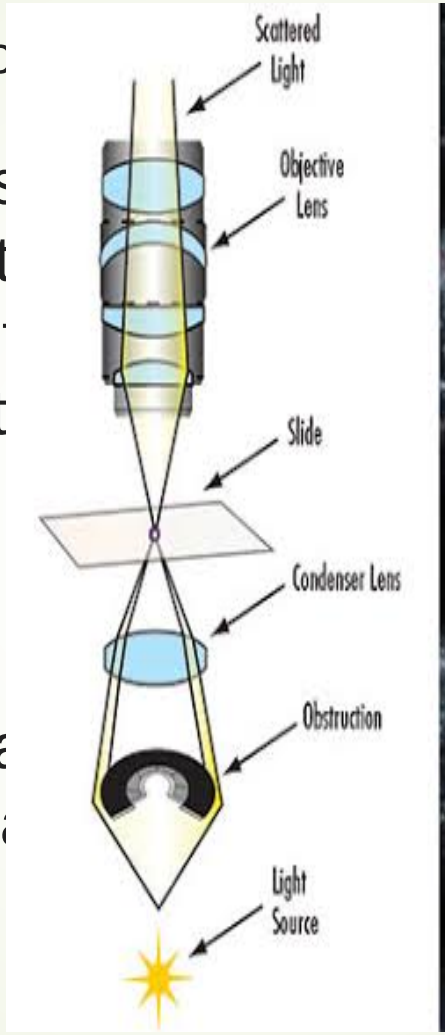
Zoological Microtecniques

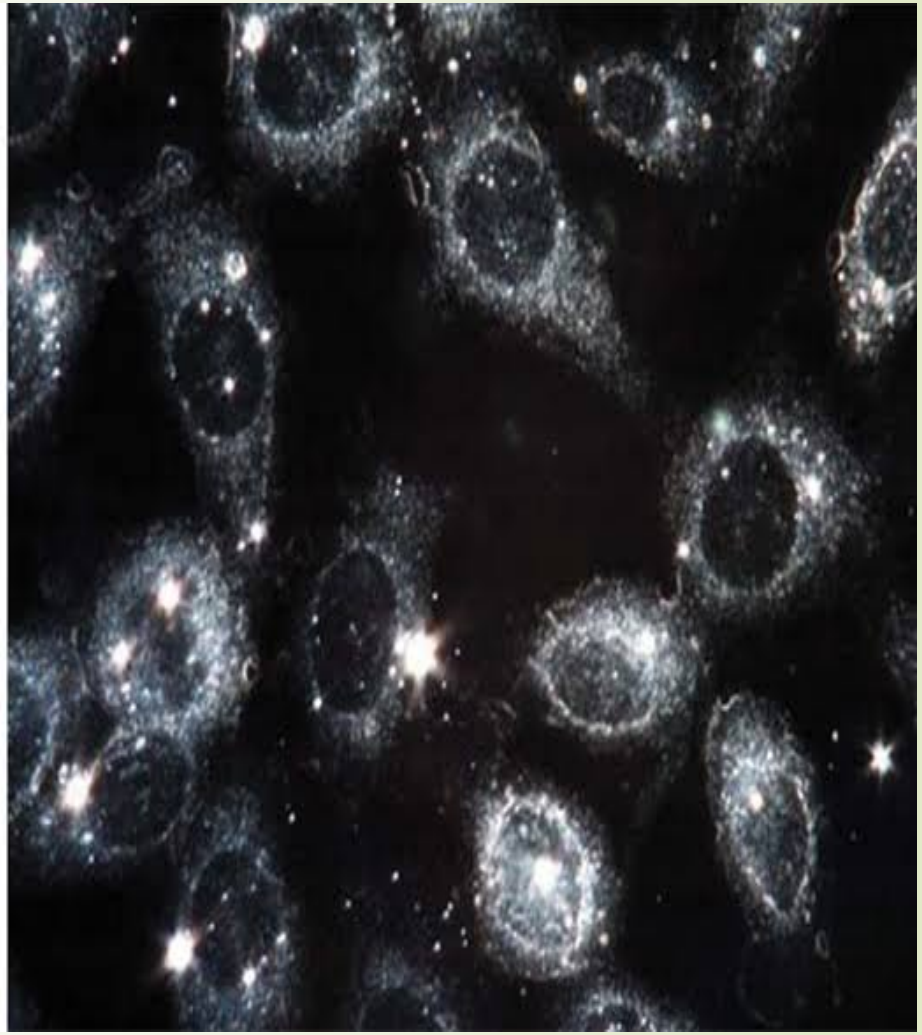
Types of Microscopy

1. Dark Field Microscopy
2. Electron Microscopy

Dark Field Microsco

- Microscopy us
  illuminate unst
  sample them
  appear bright
  against dark
  background.
- Have special
  condenser tha
  scatter light c
  to reflect





Principle of Dark Field Microscopy:

- Light hit the objet , rays are scattered in all directions
- A condenser is used , it ensures that the light rays will hit the specimen at different angles
- It resulted in “cone of light” where rays are reflected, refracted and diffracted
- It allow the individual to view the specimen in dark field

Dark field microscopy

Object appear bright against dark background

Object appear bright in dark field microscopy

Uses of Dark Field Microscopy:

it is used :

- For demonstration of very thin bacteria
- For rapid demonstration of *Treponema pallidum*
- To study marine organisms
- To study mounted cells and tissues
- For examining external details then internal

Advantages of Dark Field Microscopy:

- It is simple yet effective
- Quality of image obtained from this technique is impressive
- This is entirely free from artifacts

Limitations Of Dark Field Microscopy:

- Main limitation is the low light levels seen in the final image.
- Sample must be strongly illuminated, which can cause damage to sample.

Electron Microscopy

- It uses an electron beam to create an image of a sample. EM operates under vacuum which means the samples are placed in a vacuum system during analysis.
- EM technique is for obtaining high resolution image of biological and non-biological specimens. It is used in biomedical research to investigate structure of tissue, cell, organelles etc.

Working Principle Of Electron microscope:

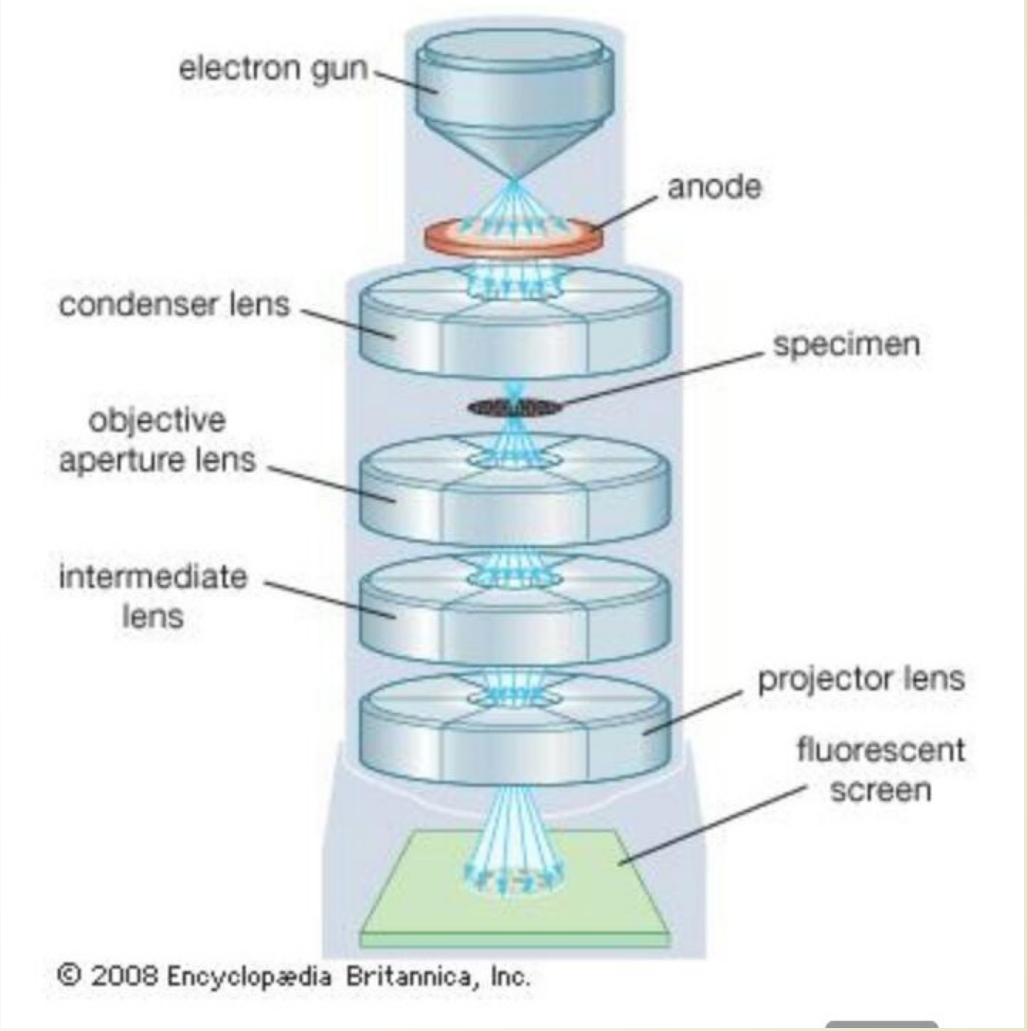
- An EM uses an “electron beam” to produce the image of the object and magnification is obtained by “electromagnetic fields” in which light waves are used to produce the images and magnification is obtained by the system of optical lenses.

Types of Electron Microscope:

- There are two types of electron microscope;
- Transmission electron microscope
- Scanning electron microscope

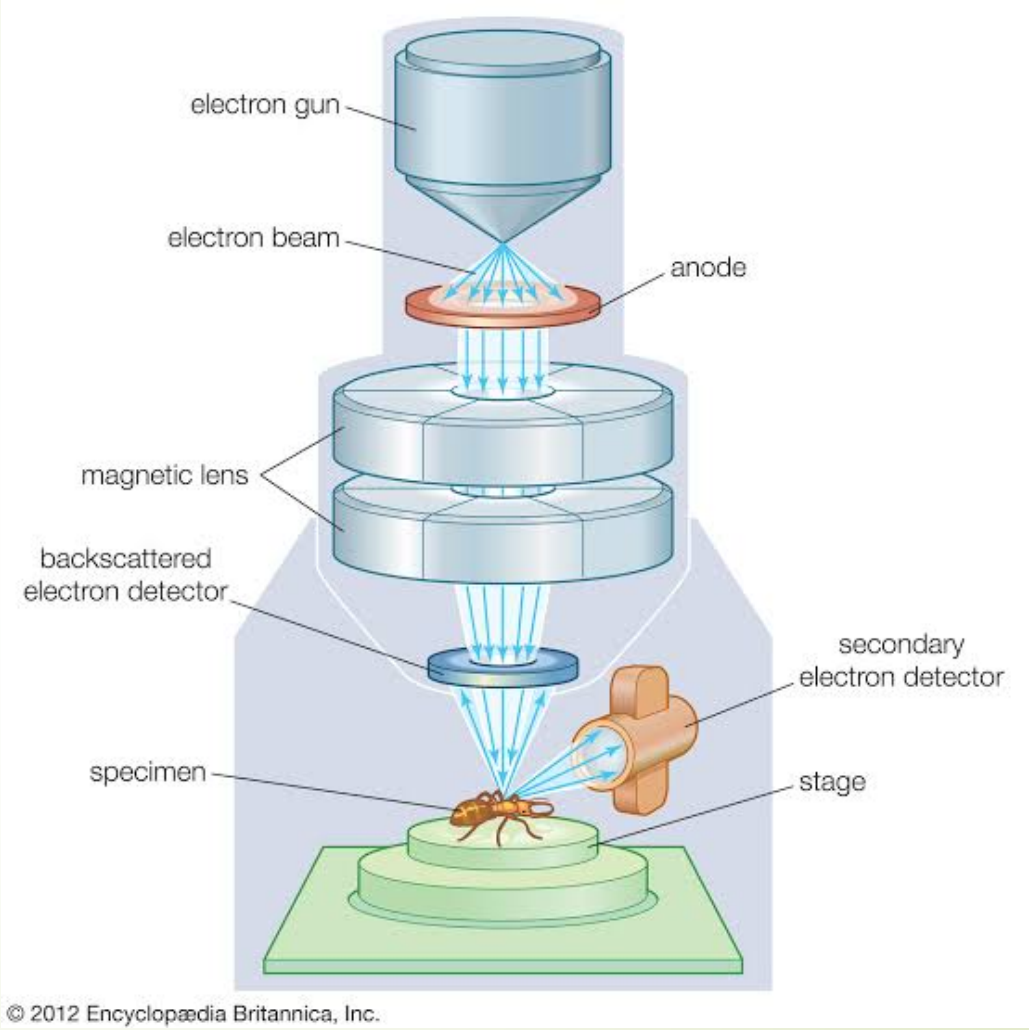
Transmission Electron Microscope:

- The transmission electron microscope is used to view thon specimens through which electrons can pass generating a projection image.
- It is used to image the interior of cells, the structure of protein molecules, the organization of molecules in viruses and cytoskeletal fragments, and the arrangement of protein molecules in cell membranes.



Scanning Electron Microscope:

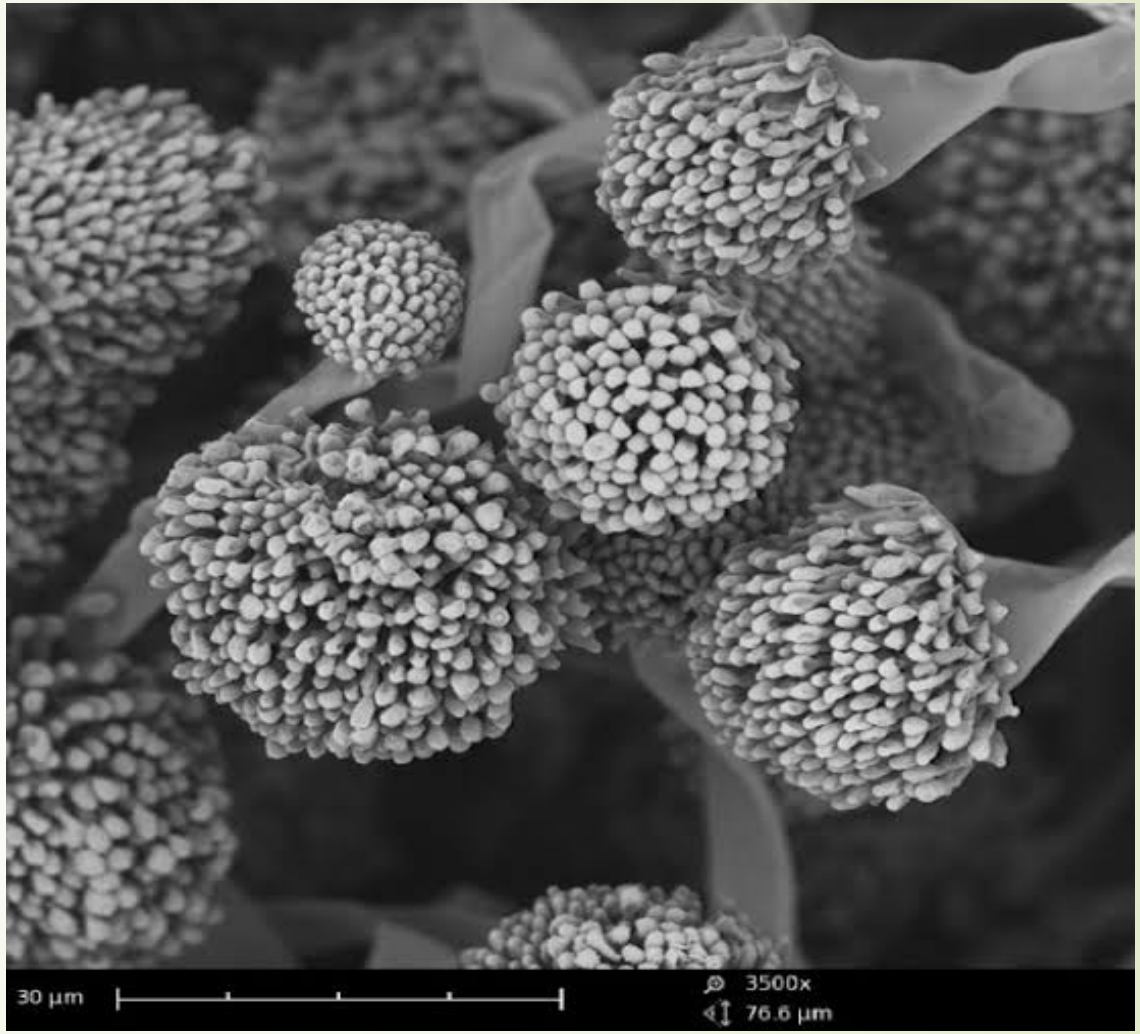
- It depends on the emission of secondary electrons from the surface of a specimen.
- a scanning electron microscope is the EM analog of a stereo light microscope.
- It provide the detail images of surface cells and the whole organisms that are not possible with TEM.
- It is termed a scanning electron microscope because the image is formed by the scanning a focused electron beam onto the surface of specimen in a raster pattern.



Uses of Electron Microscopes:

- It is used to investigate the ultrastructure of wide range of biological and inorganic specimens including cells, large molecules, biopsy samples, metals, and crystals.
- Electron microscopes are used for quality control and failure analysis.
- Modern electron microscope produce electron micrographs using specialized digital cameras.
- Science of microbiology owes its development to the electron microscope.

Object viewed by electron microscope



Advantages of Electron Microscope;

- Very high magnification
- Incredibly high resolution
- Material rarely distorted by preparation
- It is possible to investigate a greater depth of field.
- Diverse applications


diverse applications

Disadvantages of Electron Microscope:

- The main disadvantage are cost, size, maintenance, researcher training and image artifacts resulting from specimen preparation.
- This type of microscope is expensive piece of equipment, extremely sensitive to vibration and external magnetic fields.

Limitations of Electron Microscope:

- The live specimen cannot be observed.
- As the penetration power is very low the object should be ultra-thin.
- As the EM works in vacuum the specimen should be completely dry.
- Expensive to build and maintain.
- Requiring research training
- Image artifacts resulting from specimen preparation.

Principles and method involved in the killing and fixing and storage, chemicals and their properties

Killing and fixation

Killing:

- ✓ **sudden stopping of all living process in cells of biological specimen.**

Fixation:

- ✓ **Preservation of all structural and cellular elements in a biological specimen in as their original state.**

Principles of fixation

- **Fixing fluid should be approximately 10-20 times more than volume of tissues.**
- **Temperature has an important effect:**
  - ✓ **High temperature increase the degeneration of specimen tissues.**
  - ✓ **Low temperature will retard fixation.**

Methods of fixation

1)Before killing the animals from which the tissues are to be removed for fixation.

- **Obtain the necessary fixatives and containers.**
- **Have on hand supply of physiological saline solution for rinsing blood and debris from the tissue and to prevent dry off tissue surfaces during subdivisions.**
- **Have already prepared labels.**
- **Have on hand the necessary instruments.**

Methods of fixation

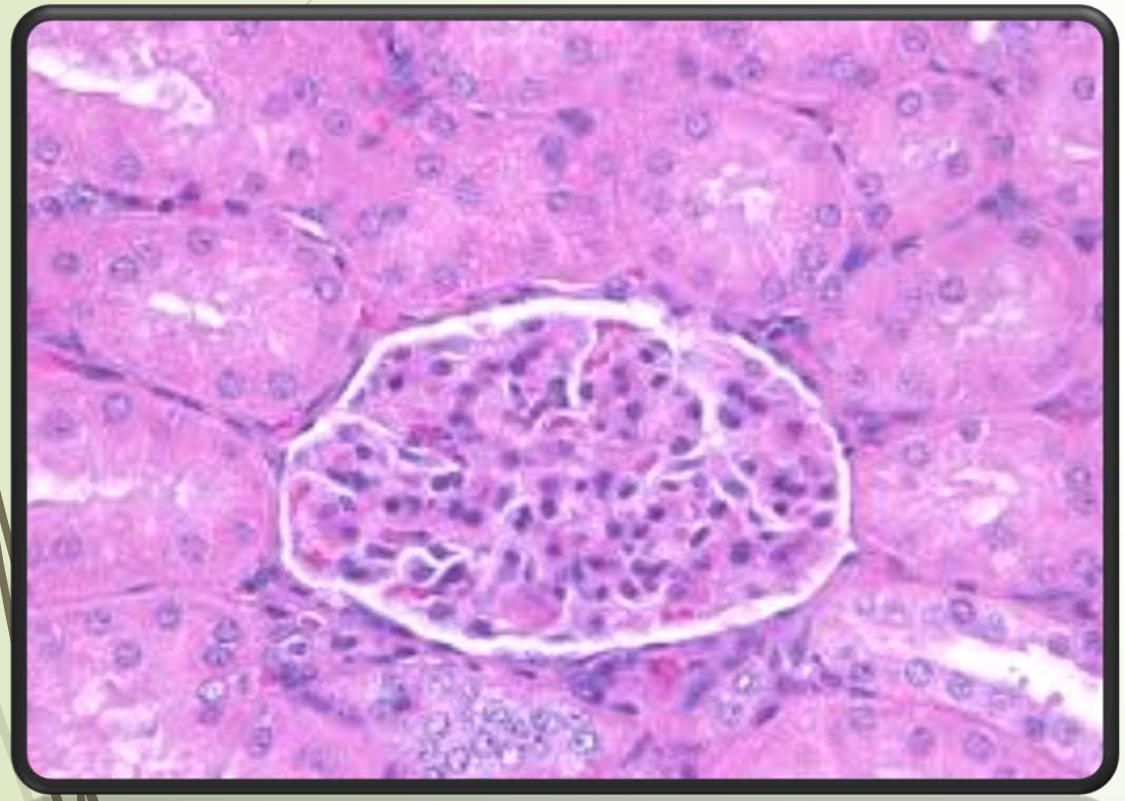
2) Kill the animal.

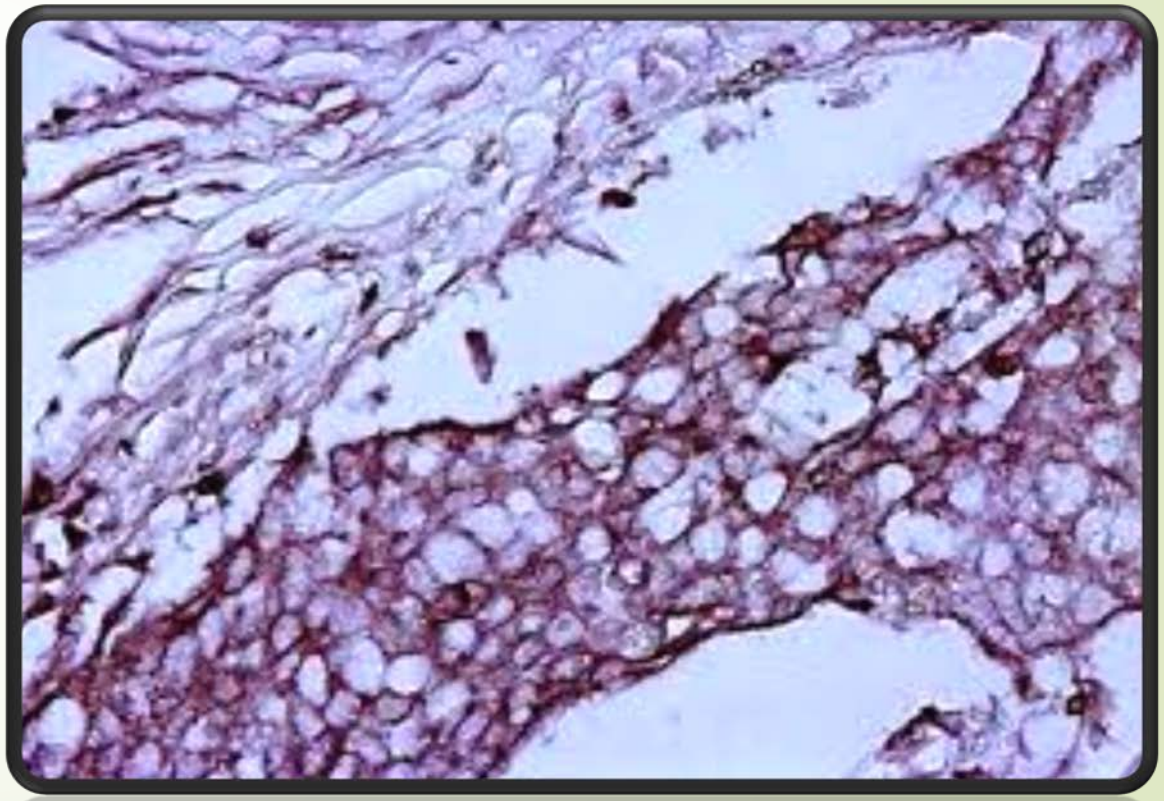
- **As soon as the animal is dead it is desirable to drain as much blood from the body as possible. Open the blood vessels in the neck by incision or decapitation.**

3) As quickly as possible remove the tissues to be fixed. Carefully subdivide them as necessary, rinse them in saline solution and place in the fixative with an appropriate label.

4) Record the accession numbers and necessary information in the catalogue.

Fixed tissues





Fixation and storage

1)Bone:

- cut into short lengths and place into fixatives. It should be place into 70% alcohol from which it is transferred to an acid-alcohol solution.
- 2-4% hydrochloric acid in 70-80% alcohol gives satisfactory result.
- 1-5% Nitric acid in 70% alcohol may also be used.

2)Cartilage:

- In young animals it is to be prepared in whole mounts in 10% formalin but in adults cut out blocks and fix as usual.

3)Fat:

- Blocks of fat for frozen sections should be fixed in 10% formalin.

Fixation and storage

4)Heart:

- **Small hearts may be fixed intact, rinse with saline. Large hearts should be subdivided.**

5)Skin:

- **Should be fixed flattened, or by pinning out on a cork.**

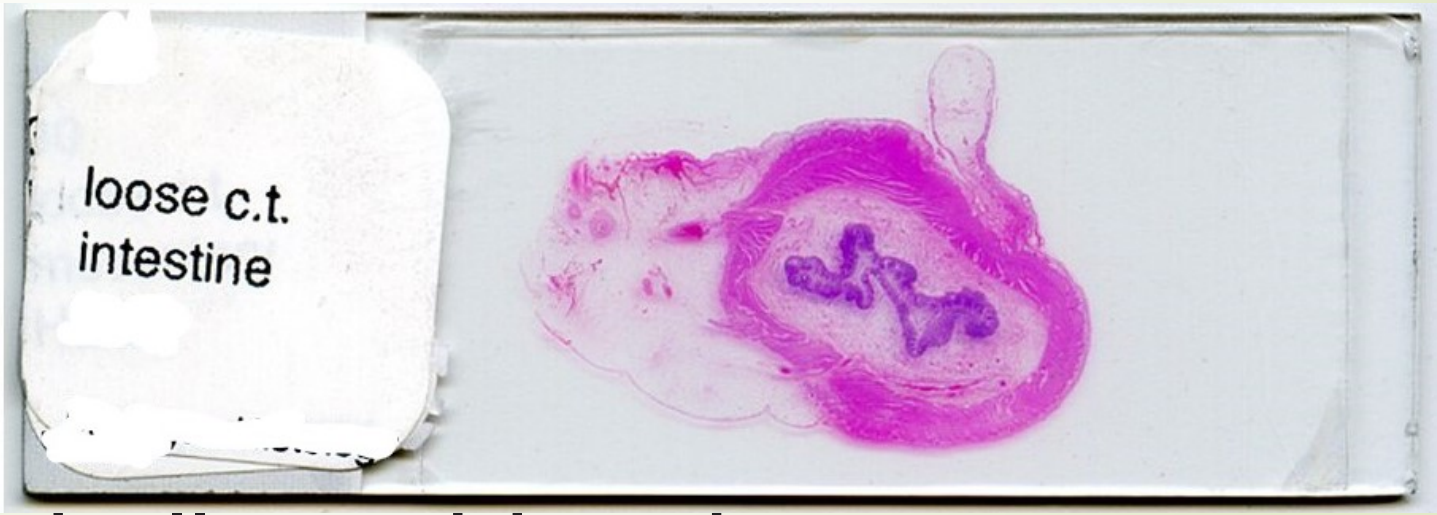
6)Lungs:

- **Fix inflated by introducing fixative via bronchial tubes. Subdivide as necessary after initial hardening.**

Fixation and storage

7)Pancreas:

- **Should be immediately fixed after the death of animal since breakdown is rapid.**
- **Fix in Helly's to retain cytoplasmic granules.**



Storage and labelling of fixed specimens

Chemicals used and their properties

- Mostly single chemicals are used for killing and fixing but combination of different chemicals are also used.

- **Examples:**
  - ethyl alcohol
  - formalin
  - acetic acid
  - picric acid
  - mercuric chloride

Chemicals and their properties

- **Ethyl alcohol:**

I. **Reducing agent.**

II. **Dissolve fats and lipids of the cell.**

III. **Miscible with water.**

- **Formalin:**

I. **Trade name of 40% aqueous solution of formaldehyde.**

II. **Recommended for the fixation of nerve tissues.**

III. **Good cytoplasmic fixative, penetrates well.**

Acetic acid:

I. Precipitates the nucleoproteins.
II. Destroys cytoplasmic structure such as Mitochondria.
III. Usually used in combination.

Picric acid:

I. In combination with acetic acid and formalin gives excellent fixation.
II. Not favorable for cytoplasmic structures.
III. Materials can be stored in this without over hardening.

Mercuric chloride:

- **Has tendency to destroy delicate structures like cilia.**
- **Used for the preservation of calcareous structures.**
- **Highly poisonous so care should be taken.**
- **Penetrates and coagulate proteins fairly well.**

Queries ????

GROUP NO : 3

PRESENTED TO: DR. FAROOQ AHMED

PRESENTED BY:

MARYAM CH	10
BILAL SHABIR	11
SEHRISH AKHTAR	15
AYESHA FATIMA	17

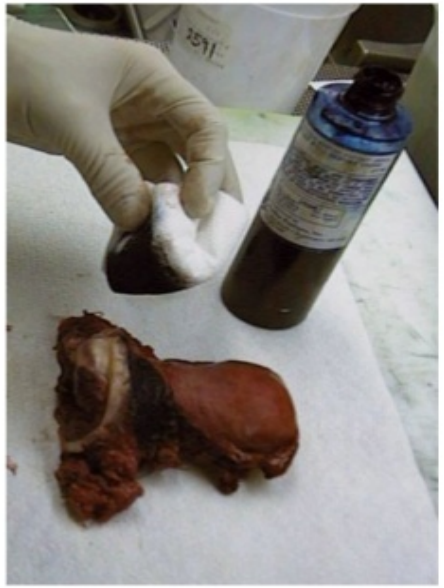
PREPARATION OF IMPORTANT FIXATIVES, REAGENT AND DYES

Fixatives:

A chemical substance used to preserve and stabilize biological material prior to microscopy or other examination.

AIM

- **Preservation** of cells & tissue constituents in a condition identical to that existing during life.
- To do this in a manner that will allow preparation of **thin, stained sections**.



PREPARTION OF FIXATIVES:

1. Phosphate buffered formalin:

FORMULATION

- ✓ 100 ml----------formaldehyde(40%)
- ✓ 900 ml----------distilled water
- ✓ 4g----------sodium dihydrogen phosphate monohydrate
- ✓ 6.5g----------disodium hydrogen phosphate anhydrous
- ✓ The solution should have pH 6.8
- ✓ 12- 24 Hours----------fixation time

2. FORMAL CALCIUM:

FORMULATION:

- ✓ 100ml---------formaldehyde (40%)
- ✓ 10g-----------calcium chloride
- ✓ 900ml----------Distilled water
- ✓ 12-24 Hours----------fixation time

Recommended Application:

Recommended for the preservation of lipids especially phospholipids.

3. FORMAL SALINE:

Formulation:

- ✓ 100ml---------formaldehyde(40%)
- ✓ 9g----------sodium chloride
- ✓ 900ml----------distilled water
- ✓ 12-24 Hours----------fixation time

4. ZINC FORMALIN (unbuffered)

Formulation:

- ✓ 1g---------zinc Sulphate
- ✓ 900ml---------- deionised water
- ✓ Stir until dissolved then add-
- ✓ 100ml----------formaldehyde(40%)
- ✓ 4-8 Hours----------fixation time

5. ZENKER'S FIXATIVES:

Formulation:

- ✓ 950ml----------distilled water
- ✓ 50g----------mercuric chloride
- ✓ 25g----------potassium dichromate
- ✓ 50ml----------glacial acetic acid
- ✓ 4-24 Hours----------fixation time

6. HELLY'S FIXATIVES:

Formulation:

- ✓ 1000ml----------distilled water
- ✓ 25g----------potassium dichromate
- ✓ 10g----------sodium sulphate
- ✓ 50g----------Mercuric chloride

Immediately before use add-

- ✓ 50ml----------formaldehyde(40%)
- ✓ 4-24 Hours----------fixation time

7. **B-5 FIXATIVE**

Formulation:

Stock solution

- ✓ 12g----------mercuric chloride
- ✓ 2.5g----------sodium acetate anhydrous
- ✓ 200ml----------distilled water

working solution, prepare immediately before use

- ✓ B-5 stock solution
- ✓ 2ml--------formaldehyde(40%)
- ✓ 4-8 Hours----------fixation time

8. BOUIN'S SOLUTION

Formulation:

- ✓ 750ml----------picric acid saturated aqueous soln(2.1%)
- ✓ 250ml----------formaldehyde(40%)
- ✓ 50ml----------acetic acid glacial
- ✓ 4-18 Hours----------fixation time

9. HOLLANDE'S

Formulation:

- ✓ 25g----------copper acetate
- ✓ 40g----------picric acid
- ✓ 100ml----------formaldehyde(40%)
- ✓ 15ml----------acetic acid
- ✓ 1000ml----------distilled water

Dissolved chemicals in distilled water without heat

- ✓ 4-18 Hours----------fixation time

10. GENDRE'S SOLUTION:

- **Formulation:**

- ✓ 800ml----------ethanol saturated with picric acid (95%)
- ✓ 150ml----------formaldehyde(40%)
- ✓ 50ml----------acetic acid glacial
- ✓ 4-18 Hours----------fixation time

REAGENTS:

A substance added to a system to cause a chemical reaction, or added to test if a reaction occours.

Preparation of reagents:

1. **Crystal violet:**

- ✓ Dissolve 2.0 g certified crystal violet into 20.0 ml of 95% ethyl alcohol.
- ✓ Dissolve 0.8 g ammonium oxalate into 80.0 ml distilled water.
- ✓ Mix the two solutions together and allow them to stand overnight at room temperature (25°C).
- ✓ Filter through coarse filter paper before use.
- ✓ Store at room temperature (25°C).

2.GRAM’S IODINE:

- ✓ Grind 1.0 g iodine (crystalline) and 2.0 g potassium iodide in a mortar. Small additions of distilled water may be helpful in preparing the solution.
- ✓ Add to 300.0 ml distilled water.
- ✓ Store at room temperature (25°C) in a foil-covered bottle (to protect solution from light).

3.Safranin:

- ✓ Add 2.5 g certified safranin-O to 100.0 ml 95% ethyl alcohol.
- ✓ Add 10.0 ml safranin and ethyl alcohol solution made in step 1 to 90.0 ml distilled water.
- ✓ Store at room temperature (25°C).

DYES:

Natural or synthetic substance used to add a colour to or change the colour of something.

Preparation of dyes:

1. JANUS GREEN B:

- ✓ To prepare a 0.01%(w/v)dye, dissolve 10mg of dye in 2-3 ml of 100% ethanol and than make a final volume of 100 ml with distilled water.

2.EOSIN:

- ✓ To prepare a 0.5%(w/v) eosin solution , dissolve 0.5g of eosin Y in 100ml of distilled water.

3.PYRONIN Y (ACETONE):

- ✓ To prepare 0.6% (w/v) pyronin Y , dissolve 0.6g of it , in 100 ml of acetone.

NAME: ----------ROLL No.----------

The Islamia University of Bahawalpur

Exam	Title Paper	Class/Subject	Time	Marks
Mid-term Spring 2020	**Zoological Microtechniques**	**BS/Zoology 5th Semester**	**6 Min**	**3**

Q # 1 (a). Mark each statement as True or False. Write true statement if false. (0.5x3=1.5 Marks)

1. Stereo microscopes use high power than compound microscopes do and have typical magnification level ranging from 100X to 1000X.

   ---

2. .

   ---

3. .

Q # 1 (b): Choose the right word for each statement. (0.5x3=1.5 Marks)

1. As the object is brought from a far point to the focus, ----------goes on increasing.

(A)	Resolution	(B)	Magnification	(C)	Distance	(D)	Resolving power

2. .

(A)		(B)		(C)		(D)	

3. Tick the statement/statements which is/are WRONG.

(A)	Water is added to acid to dilute the concentration.
(B)	Ether is flammable compound.
(C)	For electrical fires: Blue Circle With The Letter ‘C’ is used
(D)	The amount of radiation exposure decreases with distance

The Islamia University of Bahawalpur

Exam	Title Paper	Class/Subject	Time	Marks
Mid-term Spring 2020	**Zoological Microtechniques**	**BS/Zoology 5th Semester**	**6 Min**	**3**

Q. 2: Answer following short questions. **(0.5x4=2 Marks)**

1. What should be the first step after skin contact with potentially infectious material?
2. Which colour symbols are used to indicate i) Flammable ii) Instability iii) Health and iv) Special notice chemicals?
3. How our eyes distinguish different shades of colours?
4. What are the properties of condenser used in the dark-field microscope?

Q. 3: Write a note on Magnification, Resolution and Contrast. **1**