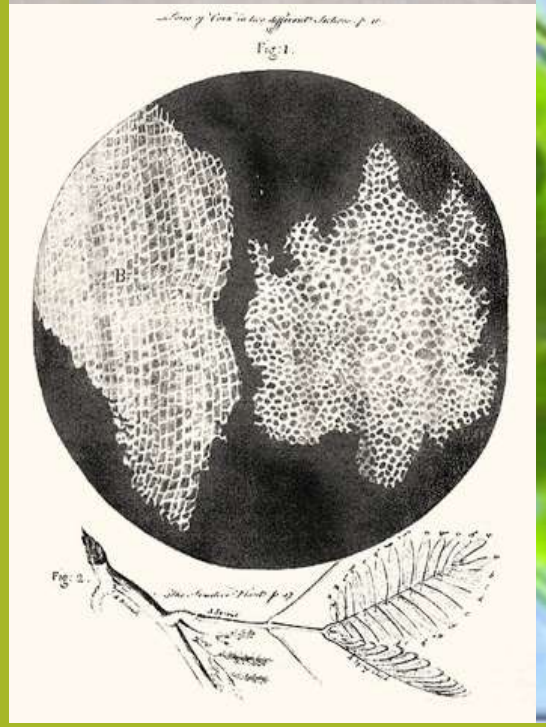
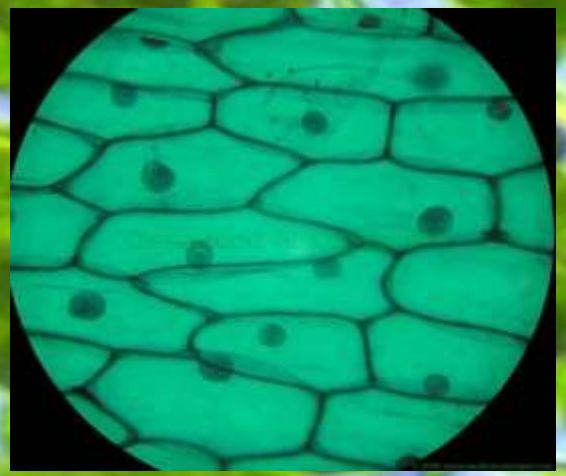


Plant Microtechnique

BOT213

التحضيرات المجهرية النباتية

213 نبت



Plant Microtechnique BOT213

Course Objectives: Managing the techniques of microscopic slides making, microscopic measurements and methods of identification of some organic compounds in plant cells.

Course Outcomes: After finishing this course, students should be able to:

- Make temporary microscopic slides, using different cutting techniques and permanent microscopic slides using paraffin method.

- Do microscopic measurements using image analyzing programs - detect the presence of different groups of organic compounds in plant material

Course Content:

Preparation of plant material for microscopic slides . Types of microscopic slides. Methods of sections. Types of microtomes and principles of their work. Methods of temporary and permanent microscopic slides. Temporary slides. Permanent slides – paraffin method. Special methods (maceration and squash methods). Microscopic measurements. Methods of microscopic measurement and data processing (standard and stereological method; measurements using Image Analyzing System and light microscope). Basic histological methods.

Total hours: Lectures, 1 hour: Practical, hours2 :

Methods of instruction: (Maximum points 100)

Final exam points = 40

Midexam I = 10 points

Midexam 2 =10 points

Practical = 30 points

Active participation in lectures = 2

Homework = 5

Quizzes = 3

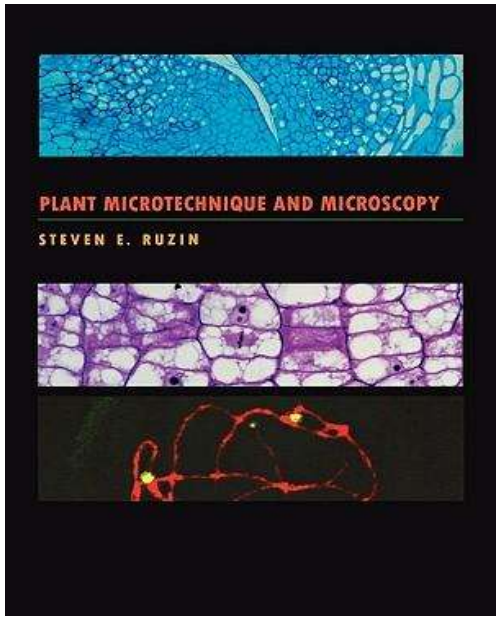
References:

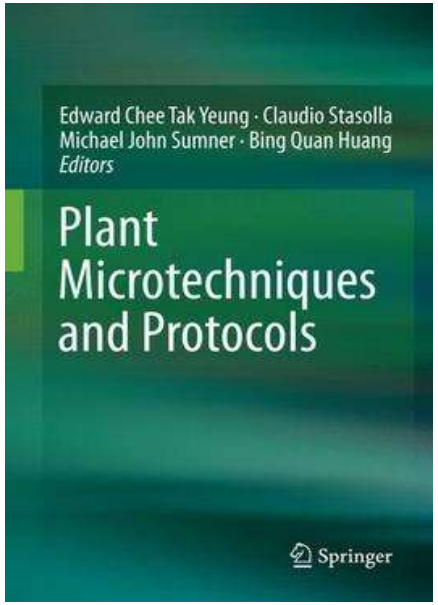
1. Jensen, W.A. (1962): Botanical Histochemistry. W.H. Freeman and Company, USA.

2. Ruzin, S. (1999): Plant Microtechnique and Microscopy. Oxford University Press Inc., Oxford.

3 Plant Microtechniques and Protocols Editors: Yeung, E.C.T., Stasolla, C., Sumner, M.J., Huang, B.Q. (Eds.) (2015)

4 - PLANTMICROTECHNIQUE BY DONALD ALEXANDER JOHANSEN 1 FIRST EDITION THIRDIMPRESSION McGRAW- HILL BOOK COMPANY, INC. NEWYORKANDLONDON 1940





Introduction

Microtechnique: Is an important experimental science that has led and continues to lead a great service for each branch of the life sciences: microbiology, genetics, embryology, morphology and science, also plays an important role in the development of medical studies of human anatomy. This includes knowledge of the preparations microscopic sample, whether plant or animal.

Sass (1958) was defined it as the science consists of three overlapping activities with each other: (1) Sample Preparation for microscopic study. (2) the proper use of the microscope and related devices help to explain the study samples. (3) codification of results and drawing which was replaced in modern imaging cameras , because of its major role in the transfer of the real image of the sample, so no interference from the researcher or the examiner in few modifications. And for the development and diversity of this science, it was divided into three sections:

Microtechnique: for the development and diversity of this science it was divided into three sections:

1. **Plant Microtechnique.**
2. **Animal Microtechnique .**
3. **Clinical Microtechnique.**

All share in most of the basics and methods of their preparations but may vary depending on their structures.

Plant Microtechnique is not limited to the preparation of the samples, but includes the selection of necessary equipment, reagents and materials, selection of appropriate dyes, and knowledge in ways that prepared and get good samples. **It depends on innovation and experimentation**.

Quiz:
Defined plant Microtechnique?

Answer: It is the science consists of three overlapping activities with each other:

(1) Sample Preparation for microscopic study.

(2) the proper use of the microscope and related devices help to explain the study samples.

(3) codification of results and drawing which was replaced in modern imaging cameras ,

because of its major role in the

Preparation of large plant Material

The Large plant Materials: either the Plant or part of it retains its dry or wet for reference when needed, either in Herbarium or museums and classrooms. The preparation divides into two types:

1- **Dry preparation**

2 - **Wet preparation**

Dry preparation:

The Plant, or a branch represents a complete plant, can be used for preparation, these samples or plant material must include **stem** and **leaves**, **flowers or fruits**, to identify the sample. In addition, the plant samples characterized by Morphological characters which must be concerned to remain for study. Since these samples will become dry and fragile, it must take care when used for the purposes of research and study.

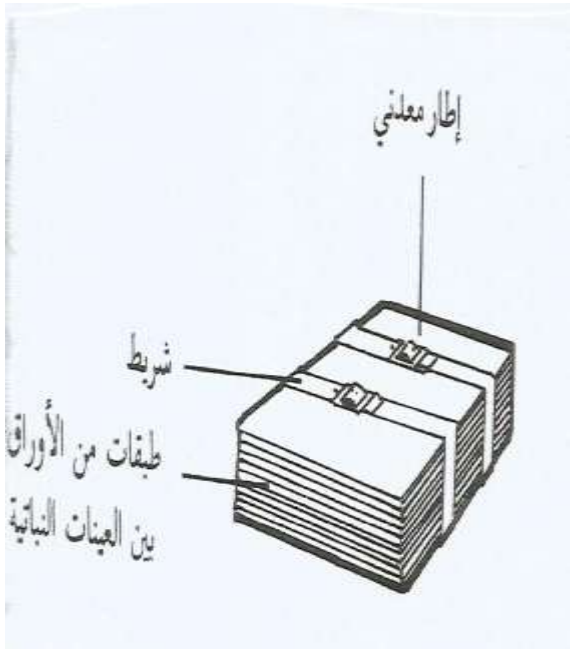
To prepare the samples in dry preparation the following method can use

Pressing Method:

Preparation of most plant material for display in a dry state, either as a whole plant or parts of it:

1 - by placing a plant or a part of it on a sheet of paper or newspapers leaves .

2- The plants are placed in a manner showing the external Morphology leaves ,flowers or fruits If they were overlapped to be spread.



3- Each plant material must be separated from other by few paper to prevent the plants from sticking to each others when removed.

4- Strand press used to process which may locally manufactured or bought from abroad which consists of two frames of metal or wood, either as a net or plate shapes.

5- then tie the two frames together including plant materials.

6- keep the plant materials to be dried in room temperature.

KSU No..... رقم العشبة

Bot. Name ..... الاسم العلمي

.....

Local Name..... الاسم المحلي

Family ..... الفصيلة

Place of Collection ..... الموطن

.....

Type of Soil ..... نوع التربة

Date of Collection ..... تاريخ الجمع

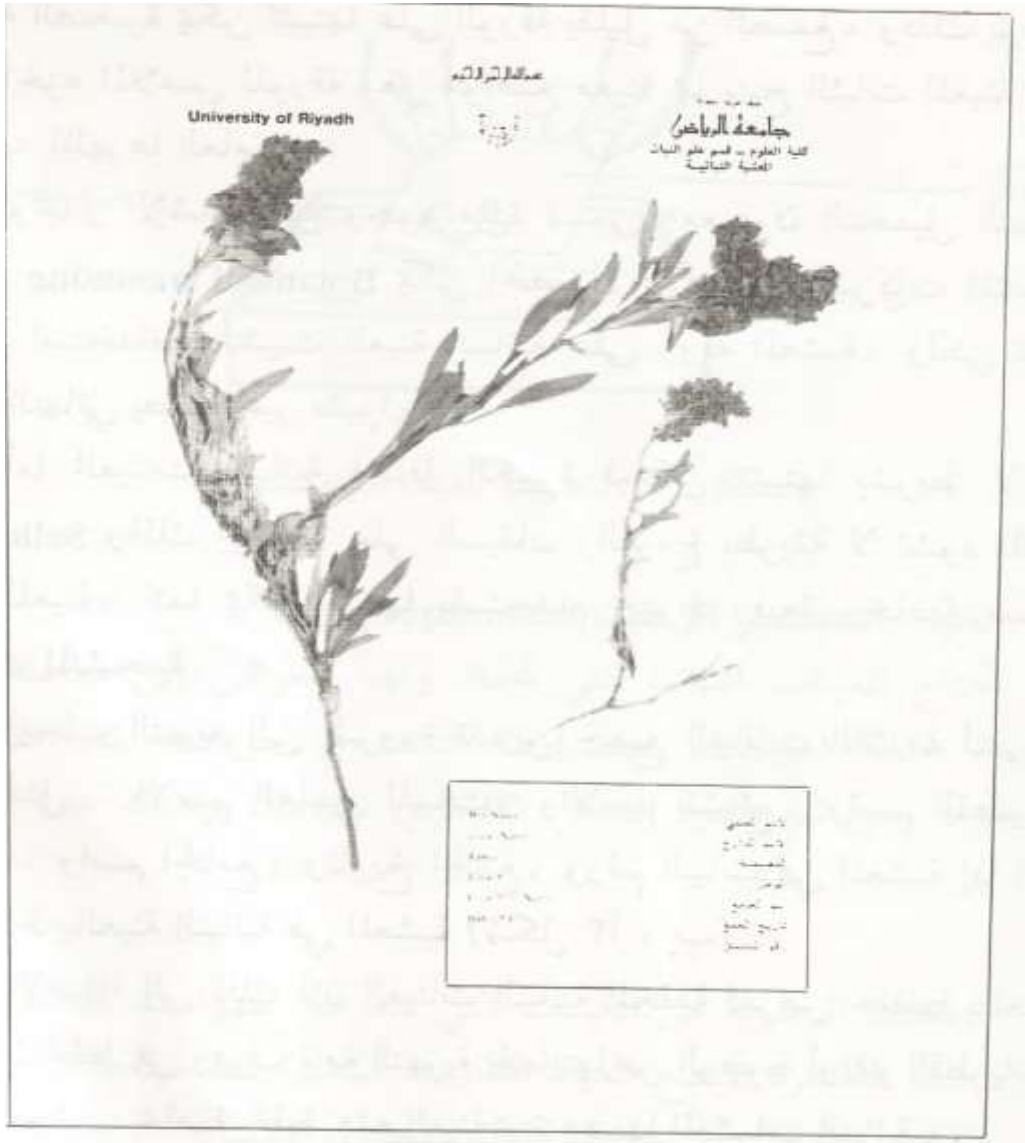
Collector ..... اسم الجامع

Notes ..... ملاحظات

.....

.....

.....



Wet preparation :

Plant samples need initial treatment with a fixative solution before it ready for display. When the plant removed or part of it, the tissues begin to decay or dry up, and then deform the morphology of the plant, and there are several reasons, including:

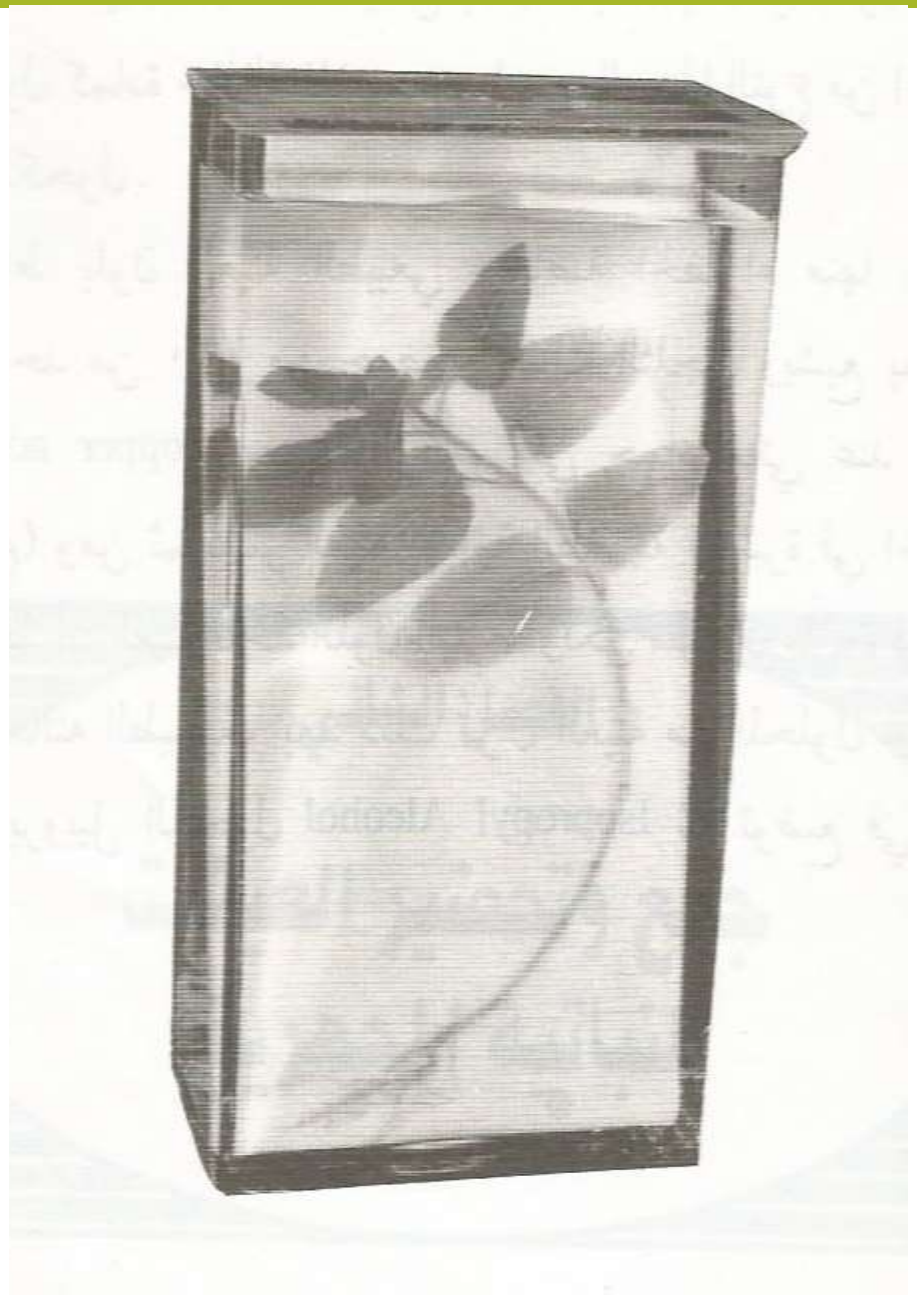
1. Loss of moisture from the samples that are exposed to the air.
2. Dead tissue exposure to a large number of micro-organisms, particularly bacteria and fungi.
3. The cells digest itself after the sample is separated from the plant, followed by the death of plant organ.

Sample preparation for display:

This is done by fixing plant sample to a plates of glass fits museum Jar or placed plant sample directly into the preservative Solution in museum Jar.

1 - Use a thin thread of nylon through certain holes in the board, and it must in this case be hidden behind the board and is not visible from any side in front of the display.

2. Use adhesive or adhesive tape do not dissolve in the solution



Quiz:

What are the Preparation types of large plant Material?

Answer:
1- Dry preparation
2 - Wet preparation

Retain the natural colour of the sample

To retain the natural colour of the Plant sample follow the steps:

1 - One liter of 20% glacial acetic acid solution

2 – Saturated the solution with Copper acetate in a large beaker and placed in a water bath at boiling point.

3 - then immersed fresh plant sample directly in the hot acid.

4 - notes that the sample take brown colour, but as soon as the green color back to its natural state.

5 – removed plant sample from the solution and washed in 50% Isopropyl Alcohol.

6 - then placed the sample in a preservative solution.

Quiz:

What are the solutions used to retain the natural colour of the wet Plant sample?

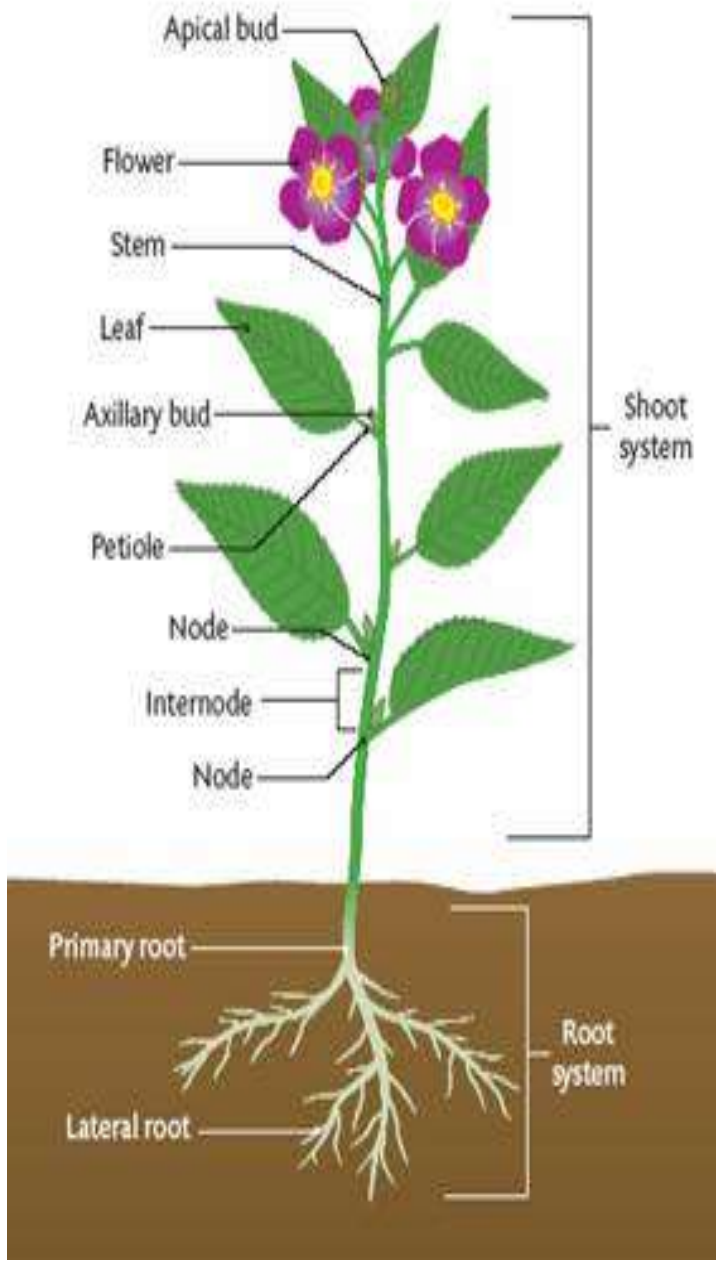
Answer:
1 - 20% glacial acetic acid solution
2 - Copper acetate

Collection of microscopic plant samples

Microscopic samples of plant material are small samples of the plant, which examined under a light microscope to illustrate the details of their structures which can not be seen and examined with the naked eye or manual lenses.

A- Flowering Plants

Plant body is different in this group (trees, shrubs, herbs), it may be a large body or small, so collected depends on the size of its body, if the plant is large in size sufficient part of it should be collected so that represents a complete plant, as the branch has a stem , leaves and flowers, but small plants can be fully collected:

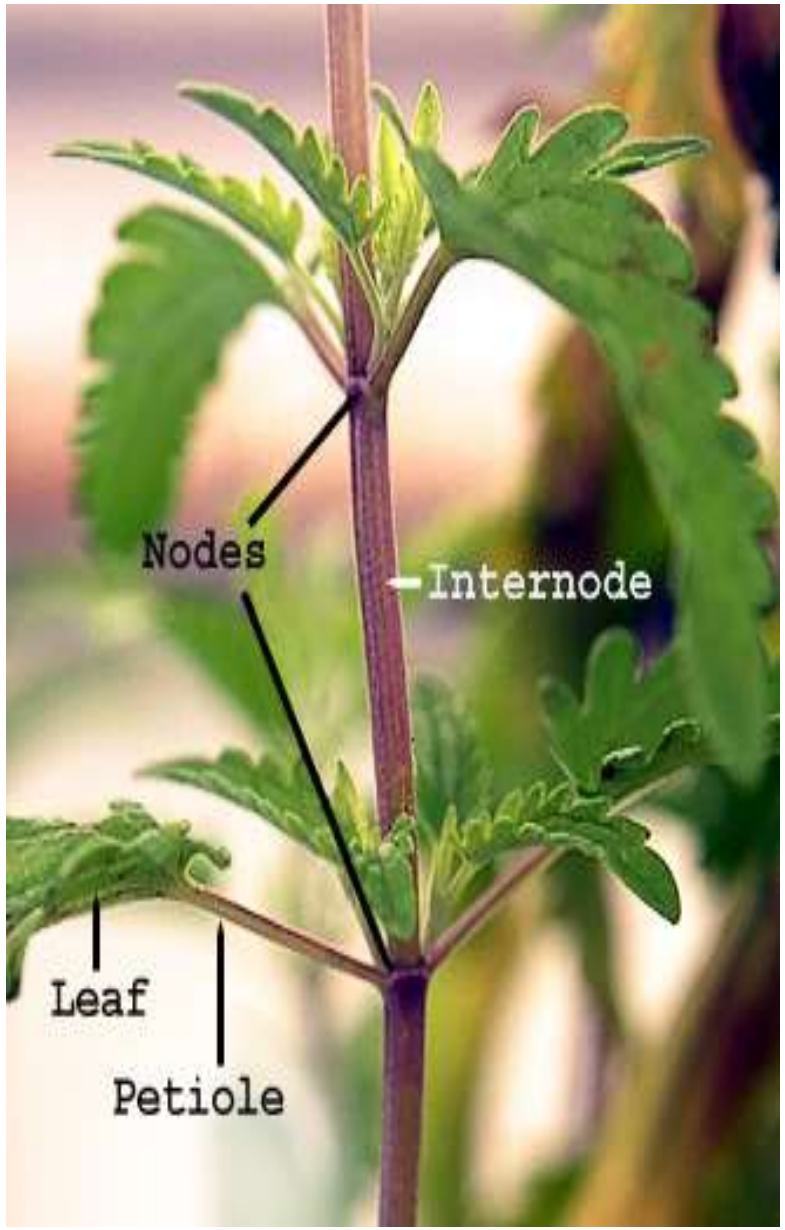


1- Leaves :

Cut a leaf or leaflet of the petiole with a sharp razor blade without squeezing or pulling to prevent tear off the tissues, or the separation of vascular bundles from the rest of the leaf tissues. Then placed it between two sheets wet paper and then placed in a closed container until it reaches the lab and then directly stored in a 70% solution of ethyl alcohol..

2 - Stems

Stem vary, herbaceous and woody or succulent, so its collection varies depending on its habitat, it can be kept fresh for several days by placing them in a container of water until it reaches the lab and then placed in the refrigerator. They can also cut into longest pieces and wrapped with moist paper with water and then put in a pot or box in a cool place without any pressure or bend or stored in the refrigerator for several weeks, Best of all of the above to keep these samples directly into the 70% solution of ethyl alcohol.



3 - Roots :

Root grows beneath the soil surface, and that is the root of all branches rubbing directly into the soil particles and removed directly from the soil causing significant damage, it affects the cortex tissue or remain under the surface of the soil, and to collect the roots without any harm to them must drill on the root and remove it with the amount of the surrounding soil and then wash it off with water cleans up the soil particles, may be necessary to use the brush to remove soil particles in some cases, then cut the roots into suitable pieces and wrap the pieces and store as the case of the stem, or directly putted into a suitable solution according to the type of study.

Salvadora persica " الأراك "

4 - Floral Parts or Organs:

Remove the entire flower of the plant and then wrap in wet paper and placed in a closed container in a cool place, e.g. Refrigerator, the large flowers can be placed in a cup with water until to be used. All types of flowers can preserved directly in the 70% solution of alcohol ethyl. Fruits may collected and stored in the same manner.

B- Liverworts & Mosses :

Remove groups of Plant material with abundant amount of soil and placed in a bowl and then wetted with water from time to time. In the laboratory remove soil, taking care not to damage Plant material. Choose the appropriate sample and place it in a fixing solution.

Moss

الحزازيات القائمة

Liverwort

الحزازيات المنبطحة

Hornwort

الحزازيات القرنية

Mosses

الزازيات القائمة

Liverworts

الحزازيات المنبطحة

C- Algae:

Often algae live in the water or the waterlogged ground, so it collected with the amount of water that grow in it, and keep in a cool place. Some filamentous algae decompose and rot if they remained in the lab for along, so holding adequate control of lighting and proper temperature in the lab for the species under study, If is best to fix algae directly into the killer solution and keep for certain types of study.

ج . الطحالب Algae:

غالباً ما تعيش الطحالب في الماء أو الأرض الغدقة، ولهذا فهي تجمع مع كمية من الماء الذي تنمو فيه، وتوضع في مكان بارد. إن بعض الطحالب الخيطية تتحلل وتتعفن إذا بقيت فترة طويلة في المعمل، لذا يجب عند الاحتفاظ بها في المعمل التحكم بالإضاءة الكافية ودرجة الحرارة المناسبة للأنواع تحت الدراسة، كما يفضل أن توضع الطحالب مباشرة في المحلول القاتل والمثبت عند حفظها لأنواع معينة من الدراسة.

D- Pathological materials

Particular care should be exercised to ensure that the condition of host tissue is not altered by handling the samples. When collecting these samples placed in a wet containers be careful not contaminated with bacteria or fungi or mixed with any other secondary organelles. Collect disease- free samples from the same plant to compare the infected samples, and must choose the best technology to preserving the the normal condition of the host before starting to interpret the results of **Pathological materials.**

Quiz:

Mention the types of the plant material to be prepared to examined under Light Microscope?

Answer
A- Flowering Plants(Root, stem . Leaves and Floral parts)
B- Liverworts & Mosses.
C- Algae.
D- Pathological materials

Types of sample preparation:

Sample preparation depends on the need of examination required , were divided into three types:

1- Temporary preparation

2 - Semitemporary preparation

3- Permanent preparation

Temporary preparation:

Sample usually mounted with water:

1-Place a drop of water on a clean glass slide.

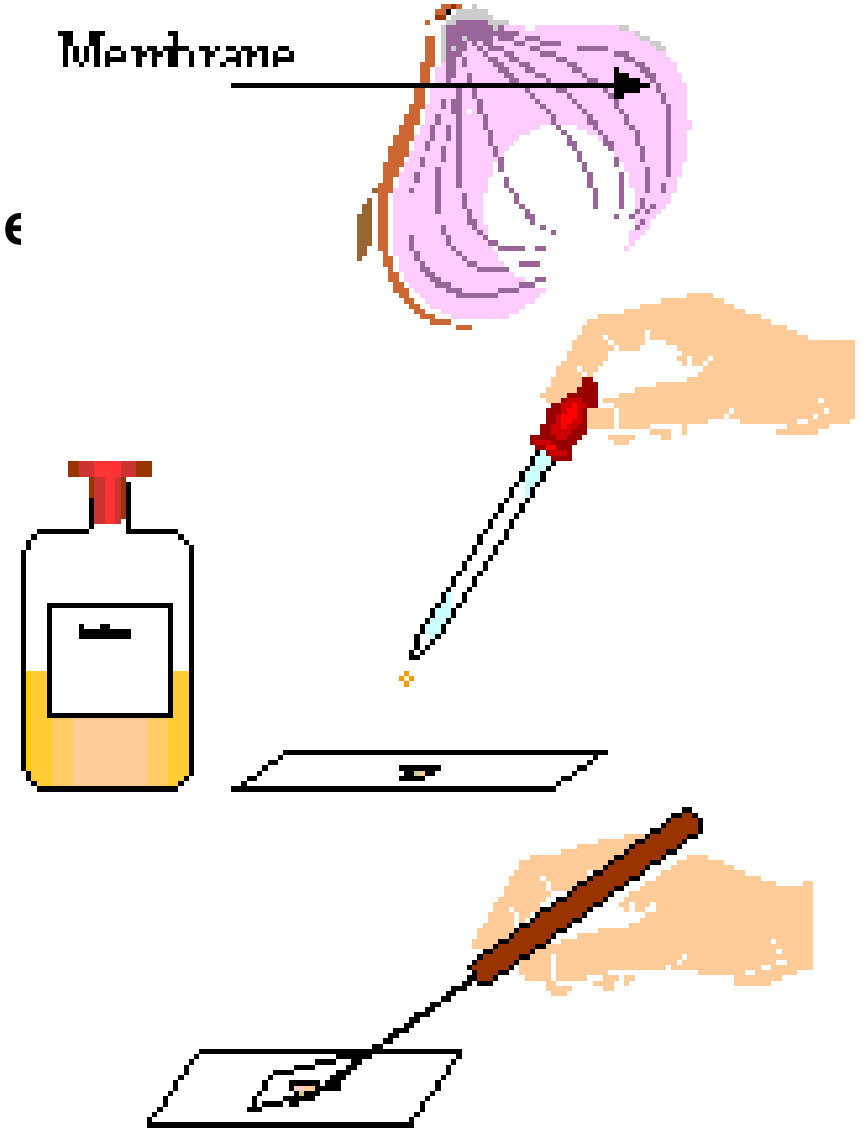
2- then place the sample and covered by the cover slide

3 – Immediately, examined under a light microscope.

Because of the water fast evaporation.

4- To prevent the evaporation of water place such as Petrolium jelly on the edges of the cover slide .

5- To keep a sample of a temporary preparation for longer replaces water with Glycerin which is less evaporation .



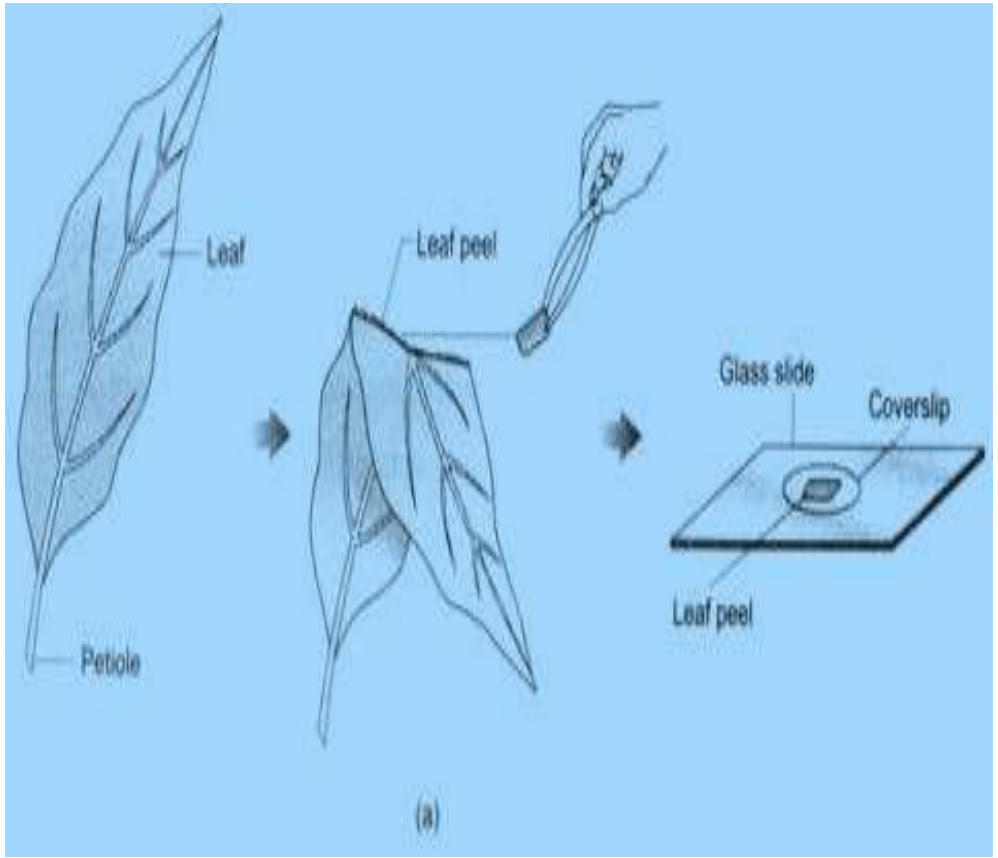
2 - Semi- temporary preparation:

Glycerin may be used as it is in temporary preparation , but also used Lactic acid and Phenol, particularly when preparing algae, fungi and ferns and thin sections and minute plant specimens, where the use of certain concentrations of fluids, including:

A- Lactophenol:

B - Phenol-glycerin

C - Glycerin – Jelly (glycerin gel)



3 - Permanent Preparation :

It means the preparation of plant sample, whether whole small plant or parts of large ones such as sections of individual organ . Large plant sample can not be mounted on a slide for examination under a microscope.

Permanent preparation of samples retain their shape and structures for several years and can be consulted when need arises, e.g. samples are used for practical lessons or research or samples kept for reference.

Note: Permanent preparation of plant samples and permanently dyed be better than samples prepared by temporary and semi- temporary preparation.

Quiz:

What are the types of sample preparation?

Answer:
1- Temporary preparation
2 - Semitemporary preparation
3- Permanent preparation:

Preparation Methods of microscopic samples and their applications

Preparation of microscopic samples of different plants depend on plant sizes and complexity of their structures. There are several methods, including:

1 - **Squash Method**
2 - **Smear Method**
3 - **Whole Mount**
4 - **Maceration Method**
5 - **Sectioning Method**

Quiz:

Mention the types of Preparation Methods of microscopic samples?

Answer:
1 - Squash Method
2 - Smear Method
3 - Whole Mount
4 - Maceration Method
5 - Sectioning Method

1- Squash Method

In some cases, a researcher needs to study the internal structures of botanical samples that are not shown by sections. **Squash Method** shows the internal structures of the samples and their relations.

This method used to study the spores that exist within the Sporangia, or study the phases of meiosis occurring within the **Pollen Mother Cells** in the anther to form pollens or to study Mitosis in stems and roots tips.

Squash Method summarized as follow:

1 - Place a sample on the center of a clean slide and covered by the cover slide.

2 - Press the cover slide to release the components

3 - and examine under a microscope.

4 - Pressure on the cover slide equal to avoid breaking it.

Mitosis:

Preparation of **Mitosis** division in order to study the different phases using a light microscope. Plant root tips are the best specimens to illustrate these phases. It was found that the roots of the plant *Allium cepa* (onions) are one of the best plant roots, especially when the study to be for who has no experience in the preparation of plant samples.

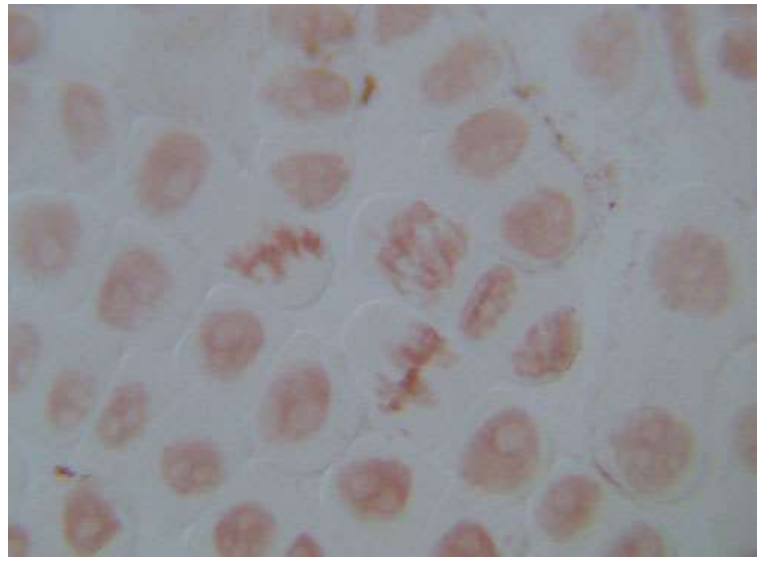
There are several Methods to prepare microscopic slides for phases **Mitosis** division, but our study here will be limited to the most important and most common, namely:

1- Feulgen Method

2- Acetocarmine Staining Method

3 - Colchicine Method

4 - The Karyotype



http://www.nuffieldfoundation.org/sites/default/files/PB_investigating-mitosis-in-allium-root-tip-squash-slide-b-zoomed-metaphase-500.jpg

1 - Feulgen Method:

1 - Feulgen Method was discovered by Feulgen in 1924, a technical study of chromosomes or DNA and summarize in the following steps:

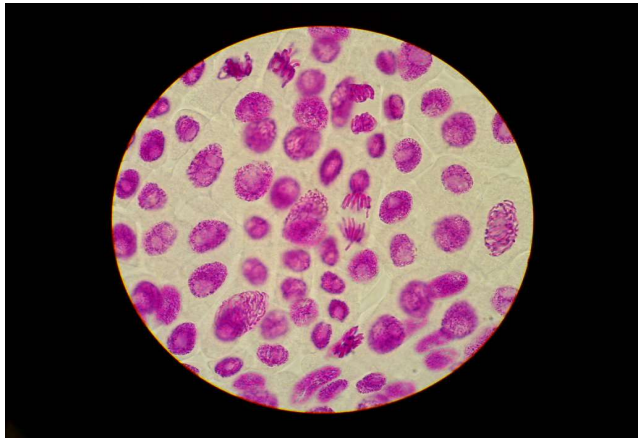
(A) Root Germination (onion roots)

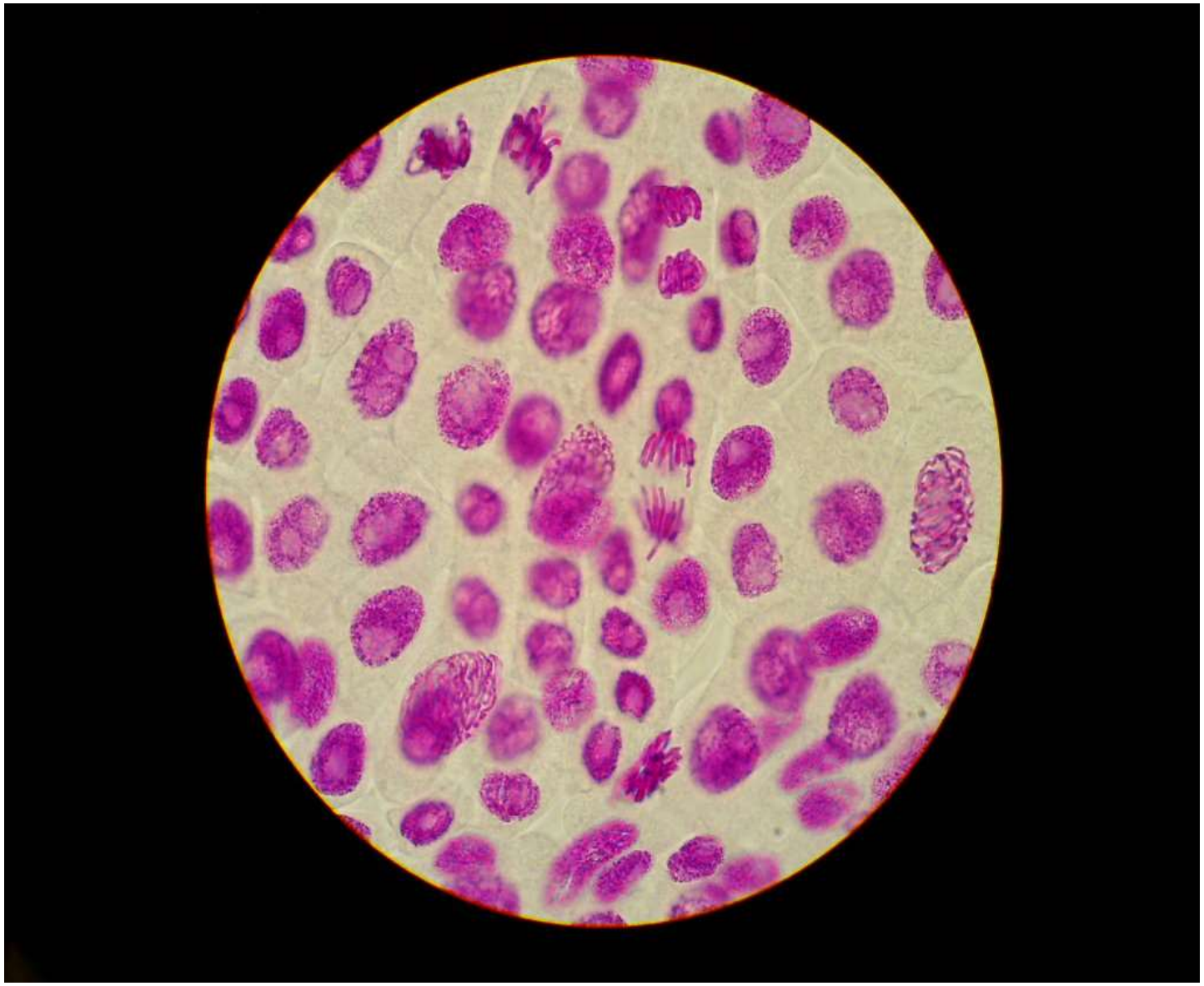
(B) Killing and Fixation: by Carnoy's fluid.

(C) Hydrolysis : the transfer of the roots to the test tube containing hydrochloric acid *N* HCl. Then placed in an oven or a water bath at 60 ° for 12 minutes.

(D) Staining: add Basic Fuchsin Stain

(E) Squashing: A clean glass slide is taken and then placed in the middle, a small drop of 45% acetic acid, a single stained root is transferred and dissected by forceps, squash it well and place cover slide and press on the cover so that the components of the root spread.



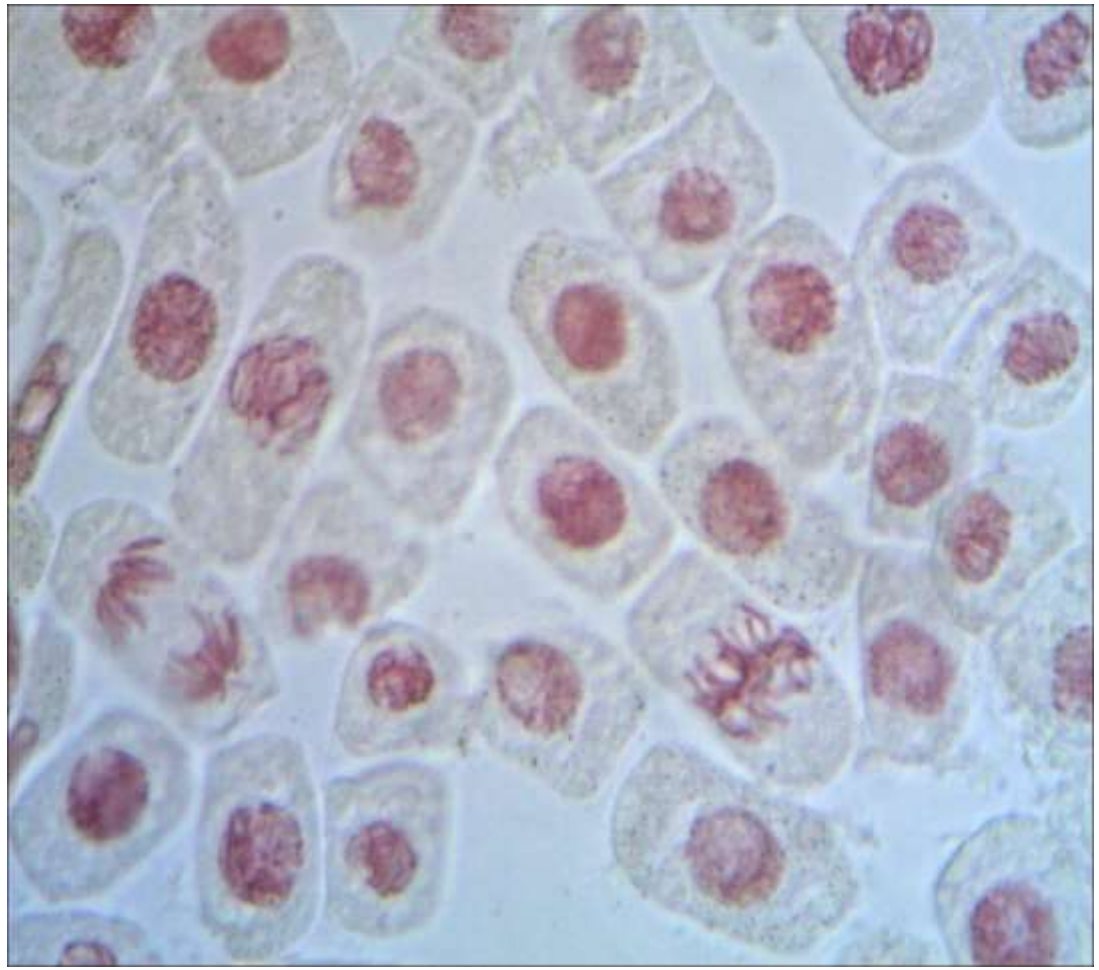


Phases of Mitosis division

2 - Acetocarmine Staining Method

The use of this technique by Belling (1926) for the study of chromosomes in each of Mitosis stages, he observed while working to use the Iron-Acetocarmine or Ferric acetate or iron resulting from the use of the rusty needle, was enough to stain chromosomes than other components of the cytoplasm. Belling also noted that heating the slide, including tissues reduces pigmentation of cytoplasm.

Orcien stain can be used to stain instead of Acetocarmine and Propionic acid instead of acetic acid, which gives good results with Acetocarmine.

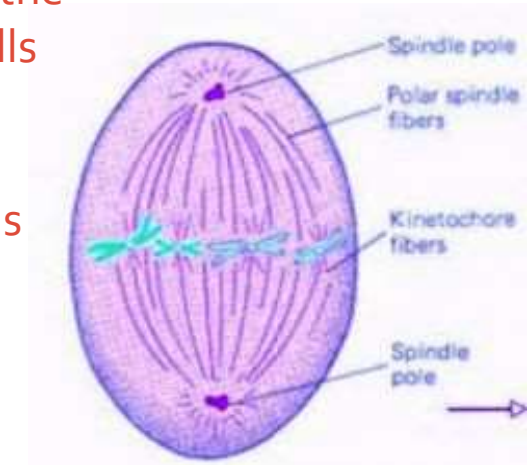


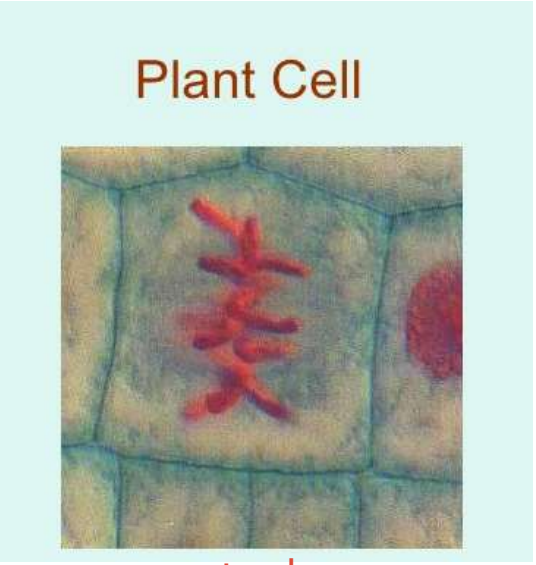
Light micrograph of onion (Allium ceba) root tip cells stained with acetocarmine to show nuclei and chromosomes. The field includes cells in interphase, prophase, metaphase, and late telophase

3 - Colchicine Method:

The purpose of the use of colchicine in the preparation of chromosomes is getting a lot of cells in the equatorial Metaphase as much as possible, because the chromosomes at this stage of the division has become the fully helical short and thick. Colchicine breaking the Spindle fibers in the Mitosis or prevent the chromosomes to clustered in the center of the cell during Metaphase, so this case is called Arrested Metaphase and hang the occurrence of Anaphase. chromosomes do not separate components to chromatids.

Chromosomes align at the metaphase plate. 2. Cells can be arrested by microtubule inhibitors (e.g., colchicine). 3. Cells can be isolated for karyotype ...

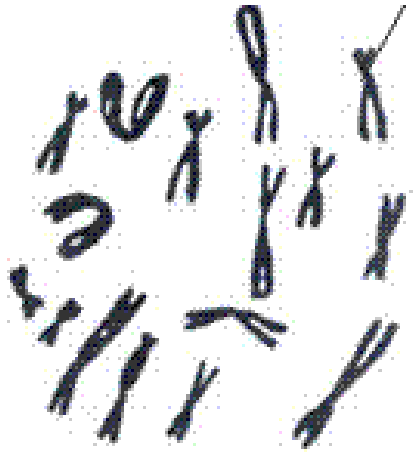




metaphase

4 - The Karyotype:

Cells of different types of plants contain chromosomes with the different numbers , shapes and sizes.. These chromosome characters can be seen when the cells of plants are in Mitosis, especially at Metaphase stage, chromosomes used to distinguish between different types of plants when studied in Cytogenetical methods. *Allium cepa* roots (onion) used as an example to show the karyotype (arrangement chromosomes), shapes sizes and the number of chromosomes.



The Karyotype of *Allium cepa*

Preparation of Meiosis Division:

Meiosis produces pollens in the anthers. sometimes pre-open before bud opening, each bud contains a number of anthers. Cut one of the good anthers and squash in Charmin over a glass slide and then examined to find any stage of **Meiosis**, if any. [1]

Meiosis in anthers represents easier **Meiosis** in living organisms can be clearly seen, because of the easy access to dividing cells, and the presence of many different stages to be examined.

Meiosis: consists of a series of phases :

1 - **Prophase 1**: chromosomes appear single in **Leptotene**, followed by convergence and the adhesion of identical chromosomes in **Zygotene**, then duplication in **Pachytene** followed by separation of the chromosomes to chromatids in **Diplotene** then the formation of the spindle in **Diakenesis.**

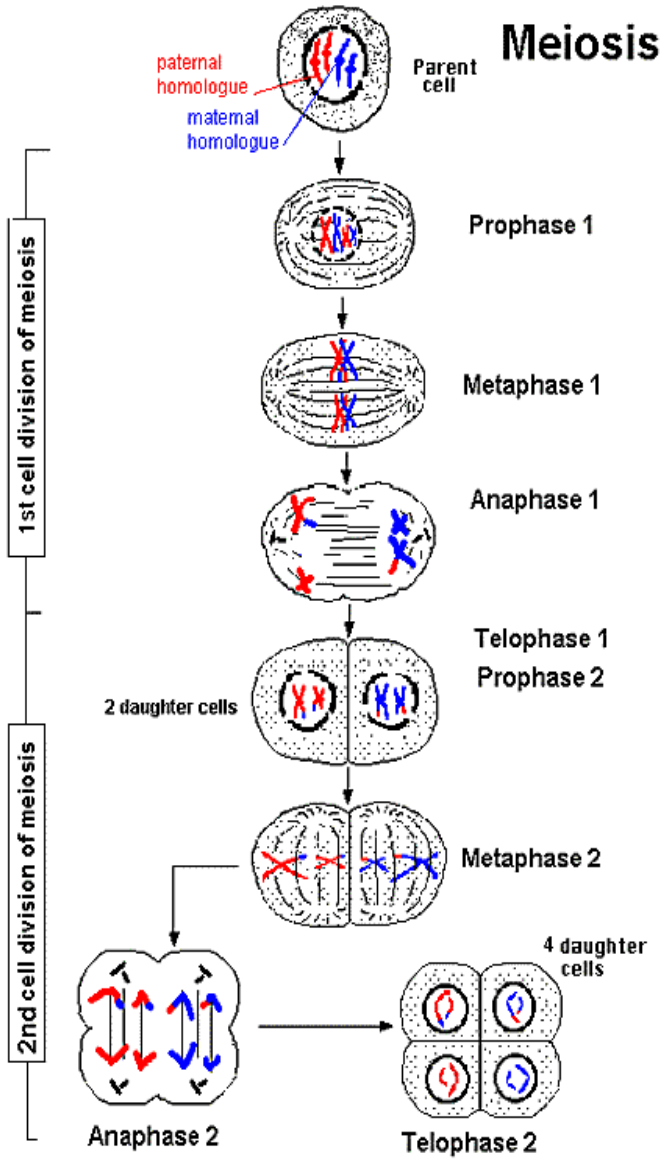
2 **Metaphase I**: Bilateral shrinking chromosomes in arranged in the center of spindle fibers.

3 - **Anaphase I :** the separation of identical chromosomes from each.

4 – Metaphase **II** : formation new regular bilateral chromosomes at the equator spindle.

5 - **Anaphase II**: The breakup of chromosomes to two groups.

6 - **Telophase II:** formation of four nuclei with a single chromosomal Haploid group. There are several methods to prepare phases of meiosis, including:



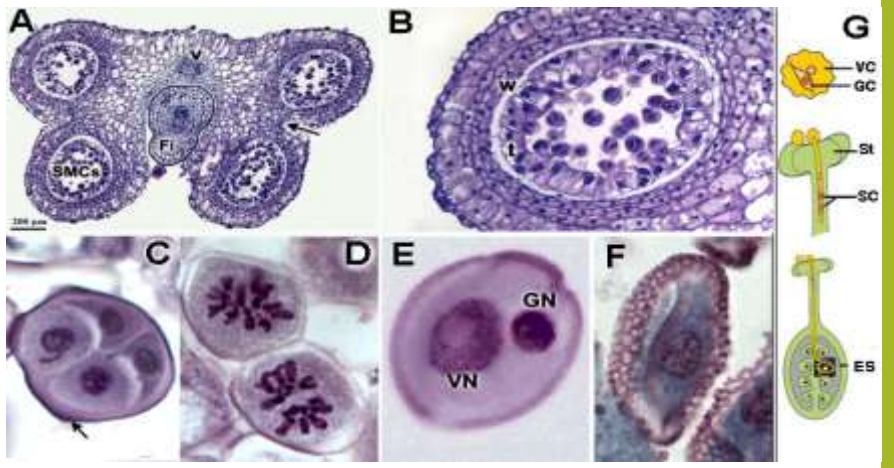
A – General Method

1 - Collect floral buds of plants to be studied.

2 - Squash in a drop of the selected stain, and then examine under a Light Microscope using a low-power (x 10) to see Anthers contain different stages of Meiosis,

3 - If desired phases clear. placed the cover slide. Gently heat with Bunsen Burner (avoid boiling dye), and then placed slide between two filter paper and press gently on the slide cover, and examine different stages of Meiosis, under a Microscope (High-power (x 40).

4 – Place Wax around the edges of the cover slide for temporary mounting.



B – Belling Method:

Belling method proposed by **Belling** in 1926 and it still used for the study of chromosomes during Meiosis in preparation of pollen grains using Iron-Acetocarmine dye (see practical).

D - Preparation of Pollen Grains :

There are three methods followed for the preparation of pollen grains depending on the quality of microscopic examination:

1 – Preparation of pollen grains, examined under a Light Microscope.

2 - Preparation of pollen grains, examined under a scanning Electron Microscope.

3 - Preparation of pollen grains, examined under the Transmission Electron Microscope.

1 - Preparation of pollen grains examine under a Light Microscope.

Preparation of pollen in one of two Methods:

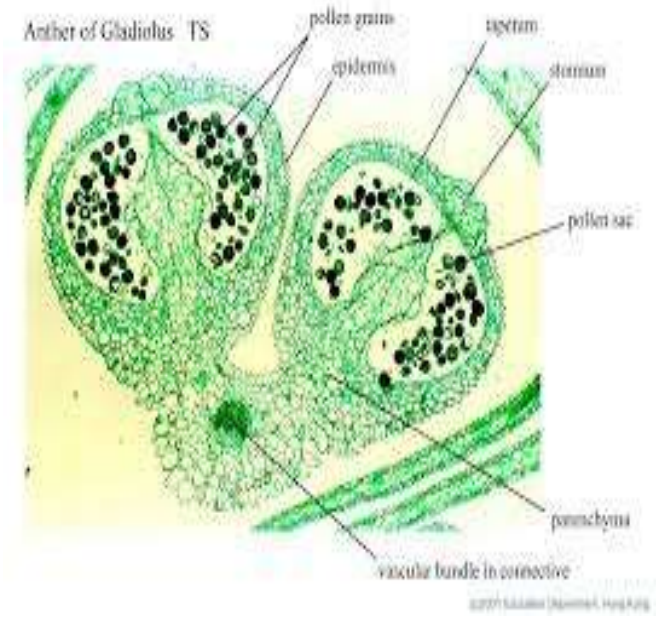
A - Erdtman Method (1971)

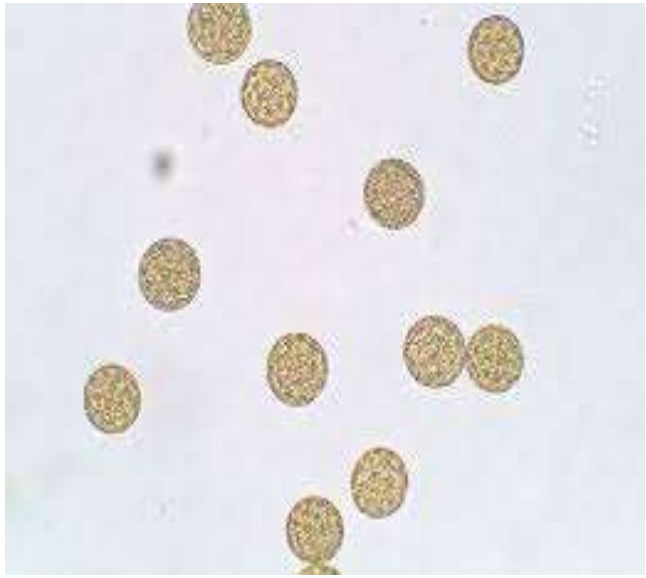
B - Punt Method 1962 and Hou, 1969).

A - Erdtman Method (1971)

Acetolysis mixture (Erdtman 1971)

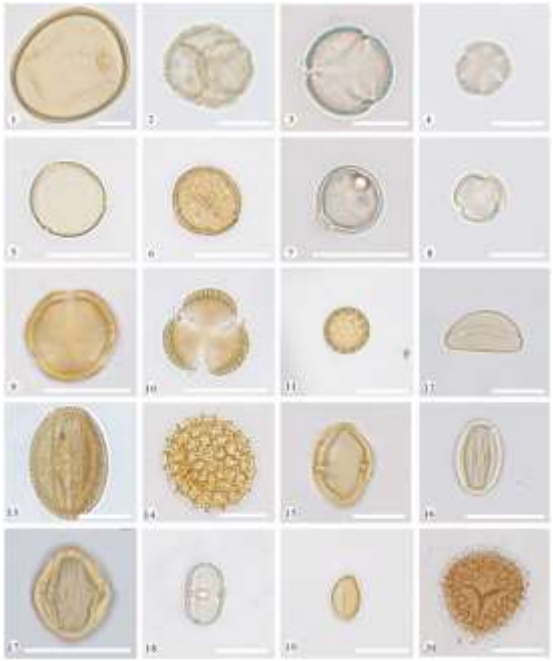
Add Acetolysis mixture to pollen when preparing (mixture consists of 9 parts of Acetic Anhydride + 1 part of Concentric Sulfuric Acid).





B - Punt Method (1962 and Hou, 1969).

1 - Add Methylated spirit solution (ethyl alcohol mixed with alcohol Mathele) to the pollen when preparing and then drop by drop to Acetolysis Mixture, which force mixture to move in the form of a loop to the edge of a glass watch, leaving the pollen in the middle. Add more Methylated spirit solution to pollen so not to dry out at this stage.

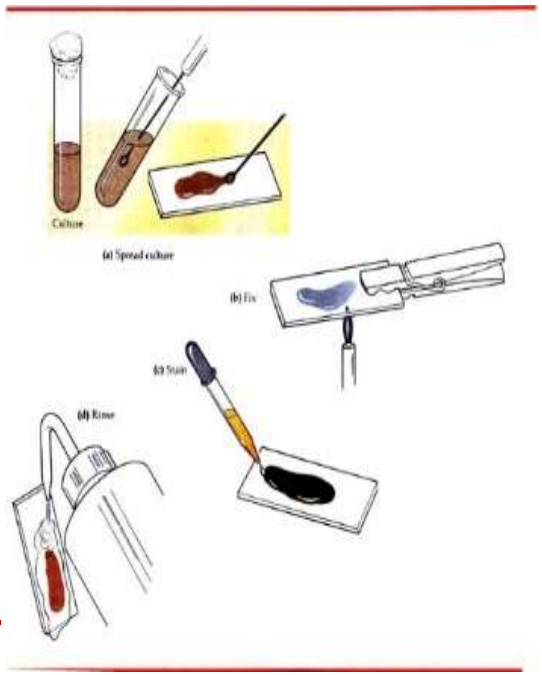


Photomicrographs of selected pollen grains recovered from the honey samples

2 - Smear method:

This preparation aims to spread the individual cells and cell organelles - suspended in a liquid and make them in a homogeneous single layer (membrane) on a glass slide in order to be killed and fixed at once without showing Artifacts. Generally all the cells or cell organelles which present stick on the slide and then no need for adhesives to fix them on the slide, because adhesives may be pigmented and give Artifacts.

- Dehydrate and stain the membrane (note that these last two stages are causing the loss of some cells or organelles of the membrane.



This method used for the preparation of plant specimens, their bodies consist of a single cell, like bacteria , some fungi and algae , isolated cells or cell organelles, e.g. Plastids and Mitochondria.

3 - Whole mount:

This method is used for minute plant specimens (small size) that do not need cutting or sectioning, e.g., filamentous algae, algal thallus , small leaves , parts of the Large leaf, stripped leaf and stem epidermis , hairs or pollen and parts of Flower.

Preparation of microscopic algae :

This method includes Isolation and Purification Microscopic algae, unicellular or filamentous, grow in fresh and sea water, soil and air. It should be noted that these methods can be used to prepare the other Blue green algae and Plankton. This method needs a lot of materials and the necessary tools and equipment.

Isolation microscopic algae:

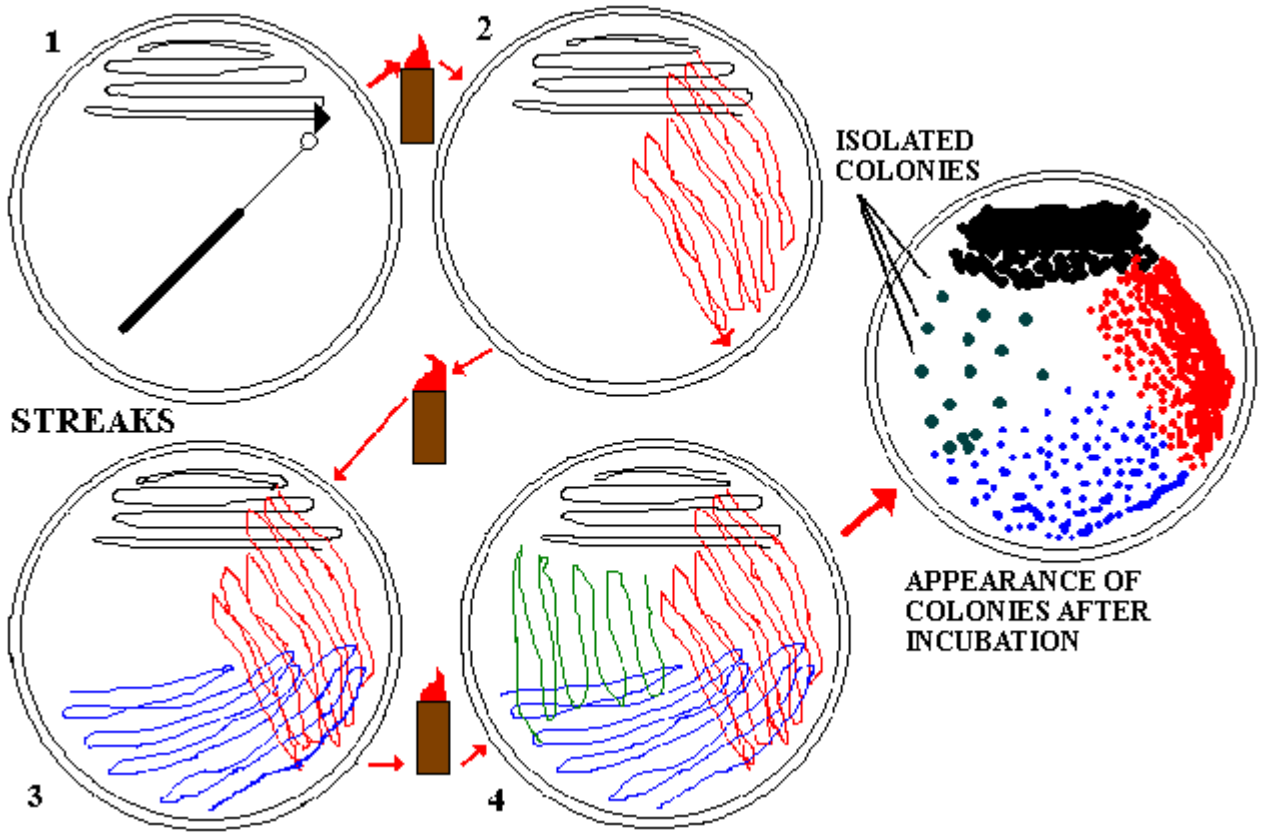
The term Algal Unit used for a single cell or colony or filamentous algae or thallus or reproductive parts. The isolation of Algal unit on suitable media for growth also be suitable to form a colony. Best methods: Capillary pipette and Streak plate.

(1)Sterile Pasteur type pipette

- Put Capillary pipette near the liquid above the algal unit, then dunk capillary tip and fluid and the algal unit will enter the Capillary pipette or pressing on the rubber, if necessary, to do so.

(2) Streak plating:

Algal units of less than 10 micrometers in diameter be easily isolated using the **Streak plate** to solid media in Petri dishes.



3) Isolation of Agar

Algal units Can be isolated from the rest of other contaminated organisms processed from the surface of agar or allowing them to isolate themselves away from other types of undesirable algae.

4)Isolation from atmosphere:

The isolation of algal units from atmospheric air an easy task, as the origin of the Brown, Larsion and Bold in 1964 suggested several simple methods to collect soil. and isolate the algae from the atmosphere. Luty and Hochaw (1967) described quoting after Osteen (1983), a successful method to isolate the algae from the atmosphere.

Isolation from atmosphere Method

a - Prepare agar medium petri dishes.

b - Exposed dishes to air in a vertical position directly to the atmosphere for 5-10 minutes from a car moving at 50-70 kilometers per hour.

c- Covered invert and incubate the exposed dishes for 2-6 weeks under suitable conditions for the growth of algae

d - follow the steps for streak plating.

Purification:

Often some physiological studies, Biochemistry on algae need pure culture. The isolation of algal units by **Capillary pipette** or **Streak plate** methods be used to liberated algal units from pollutants. **Centrifugation and Ultrasonic** vibrations can separate contaminated objects from algal units.

1 – Centrifugation:

Purification process conducted by repeat washing. transfer algal units through the liquid environment and centrifugation

2 - Ultrasonic :

This method is described by Brown and shows (1962). Used Ultrasonic water bath with a low-power 90 90 K cycles / second. Algal units will be separated from pollutants .

4 - Preparation of higher plant samples:

To prepare a complete samples of higher plants. small leaves or small parts of large leaves or flower parts or stripped leaf and stems epidermises, and other parts. Two methods include:

A - Clearing method:

This technique is suitable for the preparation of the whole samples. proposed by Shobe and Lersten (1967) which demonstrated extraordinary ability to prepare all of the fresh specimens or dry samples that taken from Herbarium.

Leaf fragments :

1 – Pl ace a fresh rectangular leaf fragment about 0.5 cm on a slide with its upper or lower surfaces against the slide.

2. Place a drop of chloral hydrate fluid and carefully balance a cover glass.

3. Fill in the space under the cover glass with clearing fluid and heat gently for few seconds, cool it and observe the specimen with low magnification of a bright-field microscope.

B - Stripped epidermis:

To study the characteristics of the Epidermal cells. There are several methods to prepare the plant samples, the simplest is performed stripping the epidermis layer of the leaf or stem of the plant, to study the types of epidermal cells, stomata , trichomes and Cuticle.

Stripped epidermis Method:

1- Take a fresh leaf of a plant.

2. Stretch and break it by applying pressure.

3. While breaking it, keep it stretched gently so that some peel projects out from the cut.

4. Remove this peel and put it in a Petri dish filled with water and add a few drops of safranin.

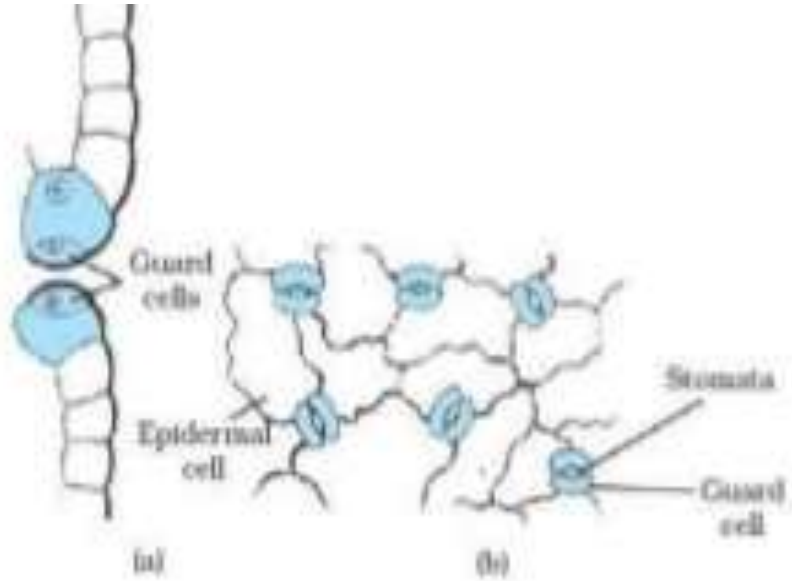
5. Wait for few minutes and then transfer it onto a slide.

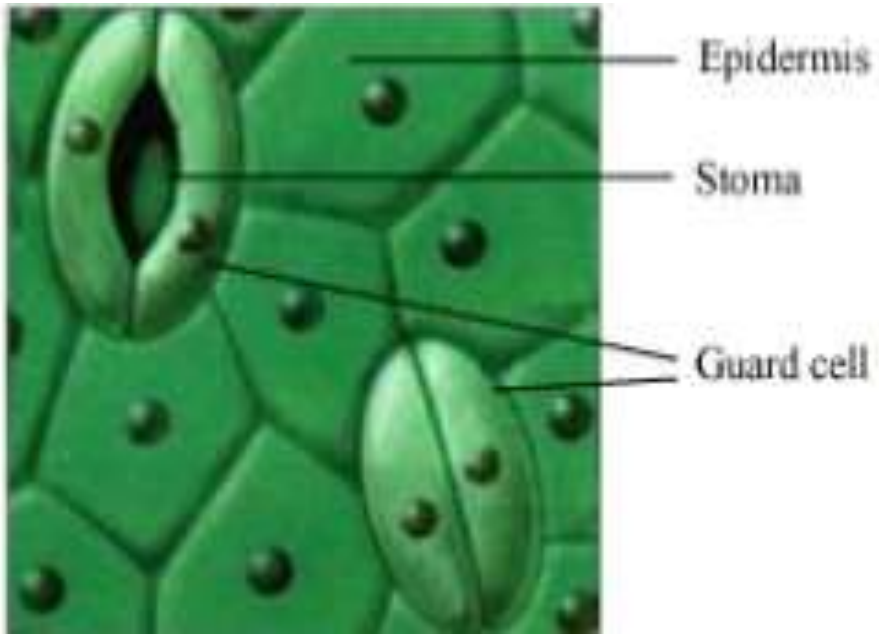
6 - Gently place a cover slip over it.

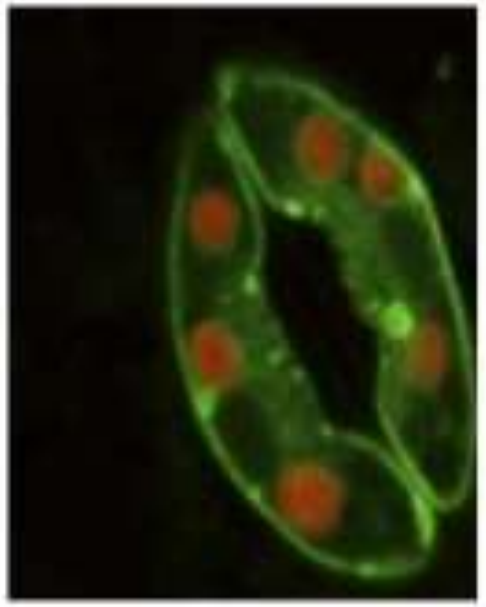
7 - and observe under microscope. When observed under Microscope. **Outermost layer of cells called EPIDERMIS (dermal tissue) is seen.**

Leaf Epidermis

Leaf Epidermis







Stripped epidermis of onion:

I - make wettable a perfectly clean slide (pass it through the flame of a spirit lamp, or a Bunsen burner, a couple of times on both sides).

2 - put a drop of water (or stain) in the center, and lay on it the sample, if possible with the face that was attached to the mesophyll downwards.

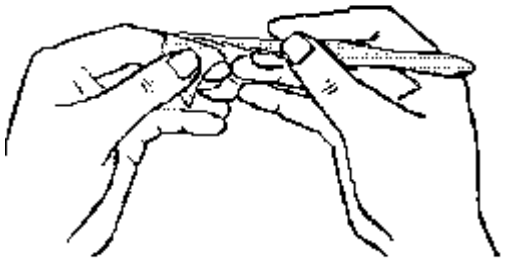
3 - Add a small drop of water (or stain) so that it does not dry out.

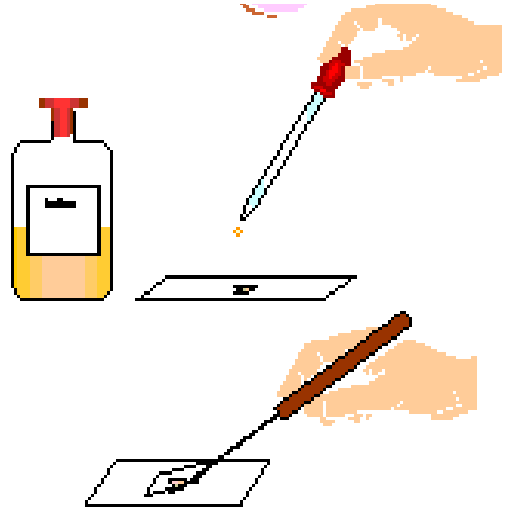
4 - Holding one bar with forceps, with a sharp scalpel cut with care next to the bar, freeing that end of the peel.

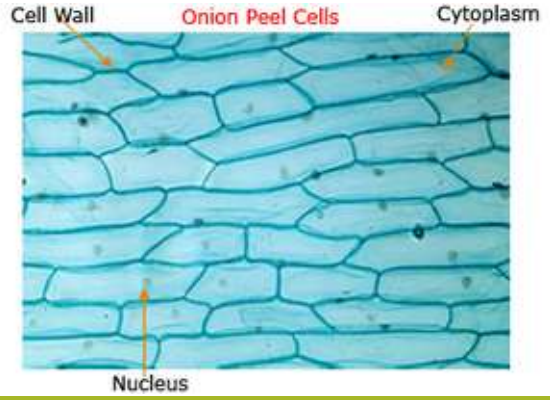
5 - Carefully apply the coverslip with common precautions and if it seems necessary, absorb a little liquid so that the weight of the coverslip flattens the epidermis.

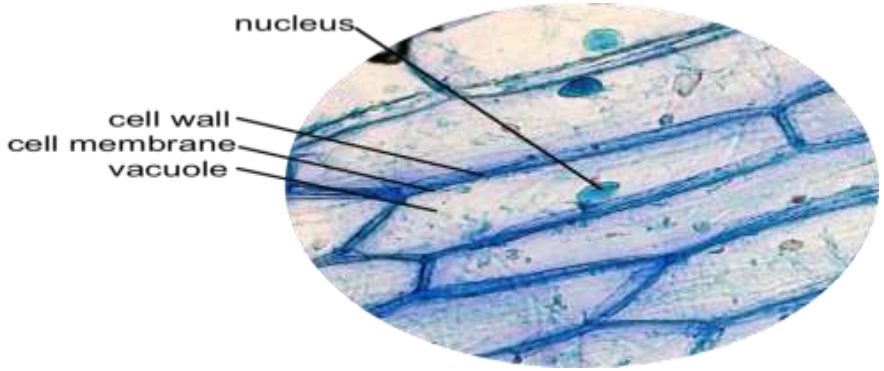
6 - The result is a peeling of a very good size of Onion Epidermis.

Detach epidermis from leaf









4 - Maceration Method:

When studying individual or isolated cells, the transverse or longitudinal sections of the samples are not enough to clarify those cells, so Maceration Method used. Various solutions are used to separate a tissue into its individual cells. These solutions dissolve or weaken the **middle lamella** so that the cells are easily shaken or teased apart.

1 - Boiling water:

This method is used to macerate samples of a few soft woody tissues and cells and its cell walls consist of Pictec and cellulosic substances or separating limited layers of tissue, for example, Parenchyma tissues

2 - 5% sodium or potassium hydroxide solution:

This method is used to separate individual cells, Parenchyma tissues or lignified elements within Parenchyma tissues or a group of a few lignified cells.

3 - 10% chromic acid solution and 10% nitric acid

This method is used to macerate some of the tissue that consists of a single layer of cells as Almgannh in his palace some seeds or group of cells Almgannh submerged in the textile Albornchimi.

4 -Schultze's Maceration Method.

Schultze used strong nitric acid and potassium chlorate:

1 - Put the material, which should be in very small pieces, into a test-tube.

2 - pour on just enough nitric acid to cover it, and then add a few crystals of potassium chlorate.

3 - Heat gently until bubbles are evolved, and let the reagent act until the material becomes white. Four or five minutes should be sufficient. The fumes are disagreeable and are very injurious to microscopes.

4 - Pour the contents of the tube into a dish of water. After the material is thoroughly washed in water, it may be teased with needles and mounted, or it may be put into a bottle of water and shaken until many of the cells become dissociated.

5 - Sectioning Method or Microtomy:

Preparation of large botanical specimens that can not be examined directly under Light Microscope must be cut into small pieces, involve to schedules appropriate time, and use of certain solvents, according to the nature of these samples. It is the first step to prepare a slide of the plant material for microscopic investigation. Fresh or preserved materials are cut into thin sections at suitable plane. It is essential to cut section thin enough to observe the details at the required level. Hand sectioning is carried out with sharp razor. Uniform section of given thickness can be obtained by special Machines called Microtome. Prior to microtome sectioning, material is processed which involves the following steps:

1 – fixation.
2 – dehydration.
3 – Clearing
4 – Sectioning
5 – embedding
6 – Staining
7 - Mounting

Quiz: What are the Sectioning Method or Microtomy steps?

Answer:

1 – fixation.
2 – dehydration.
3 – embedding
4 – Sectioning
5 – Staining
6 - Mounting