

Experiment No. : 01

Date : 15/7/21

Experiment Name : Microscope

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Microscope was Invented by Antony Van Leeuwenhoek in 1695. It is an instrument by which is Very small thing are made Visible by magnification.

A simple microscope be using one or more lenses the magnification is further increased and such system compound microscope.

Types of Microscopes:-

1. Simple microscope
2. Compound microscope
3. Electron microscope.

parts of a standard compound microscope

The parts can be conveniently grouped into 3 different categories according to the functional rules namely:

1. optical system
2. Illuminating system
3. mechanical adjustment and Supporting system.

parts of optical system:-

1. Eye-piece 5x, 10x, 100x
2. Draw Tube
3. Body Tube
4. Revolving nose piece
5. Objectives
6. Dry objectives, Low pressure - 40x
7. Immersion Objective, oil immersion - 100x.

parts of Illuminating System

1. Substage condensor
2. Iris diaphragm
3. mirror.

parts of Supporting & Adjustment System

1. Arm of the microscope
2. pillar of the microscope
3. Foot of the microscope
4. coarse of adjustment screw
5. fine adjustment screw
6. Adjustment screw for condensor
7. Stage for 1 spring clips & adjustment screw for moving the object.

methods of focusing:- Keep the desired object in the line with the optical axis, preferal start & the low power objectives looking in to the eye piece place the concave mirror in a particular angle so that the field of vision appear brightness and natural.

* Objective to be objective by looking from out edge than looking into the eye piece adjust taking in away from the objective until the image is clear than turn the high power objective until the image is clear Objective in the optical axis adjustment in the illuminating system is done for better illumination.

Calculation of power of microscope

1. Low power - $4\times$ power of objective $10\times - 40\times$
2. High power - $10\times$ power of objective $10\times 100\times$.

Care and use of the microscope

clean the microscope with clean soft cloth the objective and eye-piece must be cleaned with alone papers by 101 alcohol should be not used it dissolve the concept they bind the lens set up the microscope in convenient position facing the square of light plane the objectives on the stage adjust the mirror to be the illumination always to focus the illuminant always to focus the blue sky is artificial light is not available.

1. for unstained preparation

1. Lower the condenser
2. Use concave mirror
3. Adjust the iris diaphragm to on illumination of the microscope field.

2. for unstained preparation.

1. Lower the condenser
2. close the iris diaphragm
3. Use concave mirror
4. focus under the low power and then turn in high power.

3. for oil immersion estimation.

1. Rinse condenser completely
2. open the iris diaphragm
3. study the object then focus on oil immersion
4. After these remove the oil from objective.

Eye piece:- simple eye piece One \times on eye lenses alone end & field senses at the other and the inter the image of throwing into the focal length.

Demonstration eye piece:- In which esomature brist is incorporate which act as a pointer. It is used to point at particular cells in the field.

Double demonstration eye piece:- In this ordinary eye piece is attachment a tube at the end of which there is one directed through a prism that another observed from can be seen.

micrometer eye piece:- It is a small Circulum Glass disc on which the Graduations in microns is made kept with the eye length and lens & field lens the slide of the object can be measured directly using the scale.

Aim :- To process tissue blocks by dehydration, clearing and impregnation before embedding with paraffin wax.

Material required :-

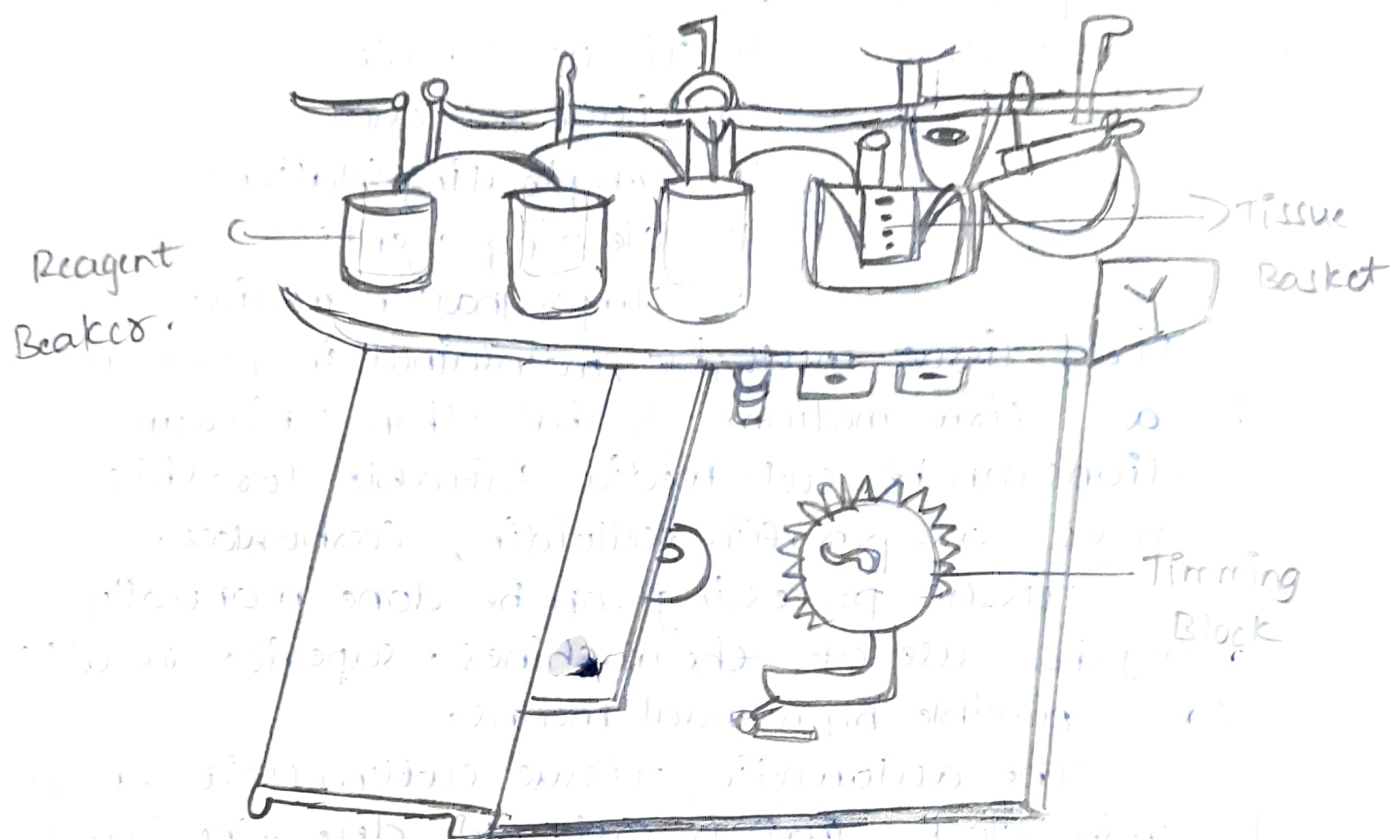
1. Tissue capsule
2. Tissue processor
3. Dehydrating Solutions
4. Clearing agents
5. Impregnation media.

Fixed Tissue must be maintained in position by a firm medium. so that thin, uniform sections can be cut. media suitable for this purposes are paraffin, celloidin, Carbowax.

Tissue processing can be done manually or by the use of the machines. Superior results are possible by manual means.

The automatic Tissue cutting unit consists of

1. Timing clock that is put to determine immersion periods of the tissue.
2. Reagent beakers of glass or plastic, which contain the reagents, required with covers.
3. Beaker platform for the precise alignment of beakers.
4. master shift carriage to automatically transfer tissue capsules from one fluid to another.
5. A displacer rotor, which provides constant rotation of the tissue basket during immersion of the fluids.



Automatic Tissue processing unit

Tissue processing involves the following stages:

Dehydration:- Some dehydrants used are ethanol, methylated spirit, methanol and isopropyl alcohol. Alcohol is most commonly used dehydrant usually starting with 80% continuing by upgrading the alcohol to absolute alcohol.

clearing:-

Use of clearing agent is necessary when the dehydrating agent, alcohol is not miscible with impregnation media. paraffin wax. As the dehydrant is removed, the tissues are cleared.

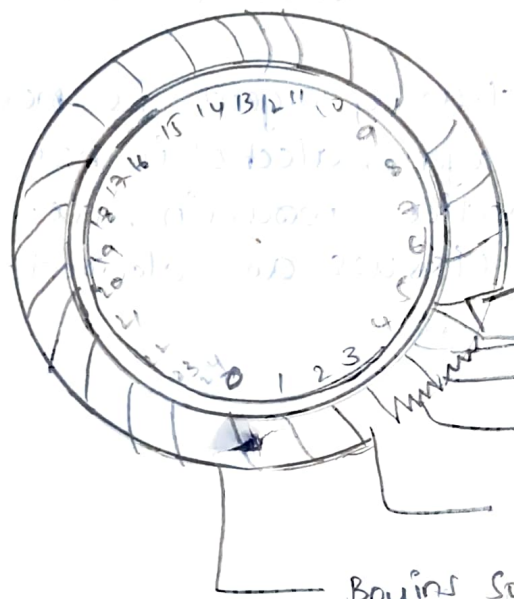
clearing agents are

- * Xylene
- * Toluene
- * Benzene
- * Chloroform
- * carbon tetrachloride.

Xylene, toluene, benzene all clear well, but are flammable and damaging on prolonged immersion of tissues. chloroform is clearing agent of most laboratories.

Impregnation:- paraffin wax is the most

common medium used. With paraffin, large number of tissue blocks may be processed in a comparatively short time and in addition sectioning and staining presents fewer difficulties than other media.



Timing disc for tooth specimens

Manual processing schedule (eg)

- | | |
|--------------------------|-------------------------|
| 1. 10% formalin | - over night |
| 2. 80% alcohol | - 9:00 to 11:00am |
| 3. 90% alcohol | - 11:00 to 1:00pm |
| 4. absolute alcohol | - 1:00 to 2:30pm |
| 5. Absolute alcohol | - 2:30 to 4:00pm |
| 6. Absolute alcohol | - over night |
| 7. Chloroform | - 9:00 to 11:00am |
| 8. Chloroform | - 11:00am to 1:00pm |
| 9. paraffin | - 1:00 to 3:00pm |
| 10. paraffin | - 3:00 to 4:00 pm |
| 11. Solidify | - 9:00 to 11:00am |
| 12. Warm and then Vacuum | - embed & cool quickly. |

Autotechnicon processing schedule (eg)

- | | |
|---------------------|------------|
| 1. 10% formalin | - 2 hours |
| 2. 70% alcohol | - 1 hour |
| 3. 90% alcohol | - 1 hour |
| 4. 95% alcohol | - 1 hour |
| 5. Absolute alcohol | - 1 hour |
| 6. Absolute alcohol | - 1 hour |
| 7. Absolute alcohol | - 1 hour |
| 8. Acetone | - 1 hour |
| 9. Acetone | - 1 hour |
| 10. chloroform | - 1 hour |
| 11. chloroform | - 1 hour |
| 12. paraffin | - 2 hours |
| 13. paraffin | - 2 hours. |

Replacement of processing Fluid and care of Machine.

- * Solutions on the tissue processors should be changed once a week. When an average of two baskets loads of tissue run each day.
- * The solution must be kept within one inch of the beaker on the processors.
- * Any odour of the clearing agent in the final Wax indicates that a change is required.
- * Any spillage of the fluid must be wiped away.
- * Accumulation of Wax must be removed from the beaker covers, using xylene.

Result

processed tissue blocks are ready for embedding.

