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EDO UNIVERSITY IYAMHO
Department of Biological Sciences
PBB 214 Biological Techniques

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Lectures: Tuesday & Thursday, 8am – 10am, NL2, 1pm – 3pm BioLab phone: (+234) 8135897349
Office hours: Wednesday, 12.30 to 1.30 PM (just before class), Office: Rm B3 Faculty of Law

Teaching Assistants: *None*

General overview of lecture: The course introduces some basic research techniques in biological sciences, These include Spectrophotometry, Chromatography, Manometry, Isotope methods, advanced microscopy (Scanning Electron Microscopy, Transmission Scanning Electron Microscopy, Fluorescence Microscopy); Micrometry, use of microtome; Permanent slides preparation, Plant tissue culture techniques, Sterilization & Culture techniques. Use of Counting chambers (eg hemocytometer, Sedgewick/Rafter cells and electronic particle counter, etc). Not only do they form the basic pedagogy in biological research, they are also the foundation of many branches of biological sciences, e.g. cell biology, molecular biology, plant transformation, plant physiology, etc. This course employs both theories and practical techniques in teaching and analyzing by using both formalism and examples.

Prerequisite: The students are expected to have a strong background in basic biological sciences, microscopy and research methodology. Some knowledge in biological researches, cell theory and plant systems will be helpful.

Learning outcomes: At the completion of this course, students are expected to:

i. better understand the basic research techniques in Biological sciences,
ii. gain experience with the use of spectrophotometry, chromatography and manometry,
iii. understand isotope methods and using advanced microscopy for the determination of cytotoxicity and genotoxicity studies,
iv. understand the importance and use of micrometry in specimen cutting, the relevance of permanent slide preparation,
v. understand extensively plant tissue culture techniques,
vi. know how to use the counting chambers (eg hemocytometer, Sedgewick/Rafter cells and electronic particle counter, etc)

Assignments: We expect to have 2 homework assignments and 1 seminar presentation throughout the course in addition to a Mid-Term Test and a Final Exam. Term papers are given at the beginning of the class and submission will be on the due date. Home works in the form of individual assignments, and group assignments are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams. There will also be 1

individual research project in this class. The goal of these project is to have the students experiment with the latest trend of researches in biological fields in Nigeria.

Grading: We will assign 10% of this class grade to home works, 10% for the research project, 10% for the mid-term test and 70% for the final exam. The Final exam is comprehensive.

Textbook: The recommended textbook for this class are as stated:

Title: *Transmission Electron Microscopy*

Authors: David B. Williams and C. Barry Carter

Publisher: Plenum Press, New York

ISBN: 0-306-45247-2

Year: 1996

Title: *Biological Techniques and Applications*

Edited by: Okhuoya J.A., F.I. Okungbowa and H.O. Shittu

Publisher: Uniben Press, Benin City, Nigeria

ISBN: 978-978-931-264-1

Year: 2012

Main Lecture: Below is a description of the contents. We may change the order to accommodate the materials you need for the projects.

Introduction to Biological techniques

Biology is a hands on science, and biology students are usually required to spend some time in the laboratory. The type of activities a student will perform vary depending on the exact field they are in. Plant geneticist, for example, sometimes spend time out in fields gathering plants, while molecular biologists may use complex equipment such as DNA sequencing machines. Even so, there are some basic techniques that all beginning lab students should understand.

Basic Research

Basic research is a scientific research aimed to improve scientific theories for improved understanding and prediction of natural or other phenomena. It can be referred to as pure or fundamental research and often driven by curiosity, basic research fuels applied science's innovations.

Manometry- Intro

The process of respiration involves the use of oxygen (O_2) by the cell or organism in the case of aerobic or no use of oxygen in the case of anaerobic respiration or fermentation. Thus, the measurement of O2 and CO2 is an important way to study respiration in the cell or whole organism.

Spectrophotometer – Intro

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength. Spectrophotometry is a measurement

of how much a chemical substance absorbs or transmits. Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc).

Chromatography - Intro

Chromatography was first employed in Russia by the Italian-born scientist Mikhail Tsvet in 1900. He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls. Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name. We have the planar chromatography, paper chromatography, thin layer chromatography, etc.

Microscope

In this course, users of laboratory microscopes, as well as those seeing the tool for the first time will learn about modern microscopes, a tool used in various health professions, research institutes, and Universities to magnify small objects that are difficult to see with the naked eye. The intension is to let you have a better understanding of the microscopes and their uses. There are various types of microscope, namely:

1. Compound Microscope

a. Complex compound microscope

Complex compound (CC) microscope with Condenser Chamber. (Photo from Bup Oyesiku Bryolab, 2011)

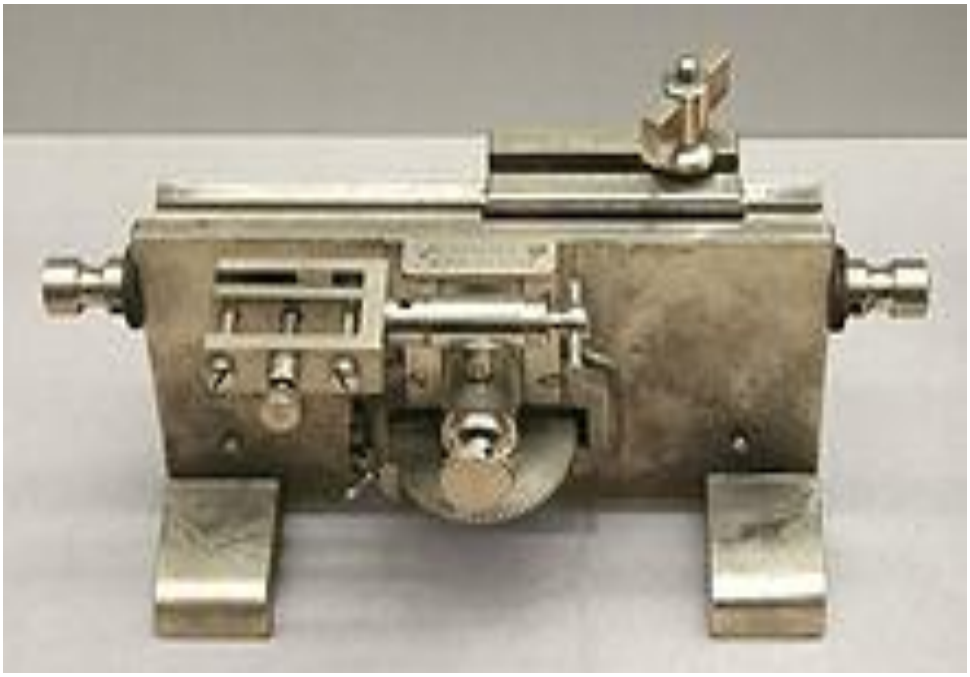
b. Simple compound microscope

Simple compound microscope without condenser lens beneath the stage. (Photo from Bup Oyesiku Bryolab, 2011)

2. Electron microscope
    a. Transmission electron microscope
    b. Scanning electron microscope

Microtome

A microtome (from the Greek mikros, meaning "small", and temnein, meaning "to cut") is a tool used to cut extremely thin slices of material, known as sections. Important in science, microtomes are used in microscopy, allowing for the preparation of samples for observation under transmitted light or electron radiation. They are used in a variety of fields for achieving various purposes. See diagram below.



A diagram of a microtome drawn by Cummings in 1770

Permanent Slide Preparation

We should bare in our minds that a good slide preparation is important in achieving a clear and understandable research about cellular life and for prolong reference. There are two main type of slide preparation namely; temporary and permanent. Temporary slides are for short term purposes and are usually discarded after use while permanent slides are for long term purposes and can be referenced to over the years.

Permanent Slide

If the slide is to be kept for long term reference e.g. for days or even years, it must be made as a permanent preparation. The techniques involved in permanent preparation are:

1. Fixation

For fresh tissues, the main aim of fixation is to kill tissues rapidly by precipitating proteins. This minimizes post-mortem changes. The reagent used is called a fixative; the most commonly used being 70% alcohol. Other common fixatives are Bouin fluid and formalin. Different fixatives are used depending on the nature of the tissue whether soft or hard. Tissues should be washed well after fixation, using the same solvent as the stain, in order to remove all traces of the fixative. If this is not done, tissues may not stain properly and some types of fixative may crystallize

out. However, it should be not that fixation is not necessary once the material is already preserved.

2. Staining

The object of staining is to accentuate the distinction between the different components of a tissue or organ.

3. Dehydration

The purpose of dehydration is to allow complete infiltration of tissues with a mountant (e.g Canada balsam). Unless all traces of water are removed, infiltration is incomplete, the tissues appear opaque and bacterial decay ultimately sets in. If carried out too rapidly, dehydration causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents. It should therefore be done gradually and sufficient time allowed for the complete extraction of water.

Dehydration is commonly effected by the passage of the stained specimen or slide through successively stronger solutions of ethanol (ethyl alcohol) ending with immersion in absolute alcohol 100% ethanol (2 changes).

Caution: Alcohol is a highly flammable material. Adequate ventilation and no naked flames are essential safely precautions.

Labelling of Slides

Slides act as a permanent record of tissues, organs and specimens. They may be of pathological origin, e.g. hospital patients, purchased material, prepared “in house”, etc. In all cases it is essential that the slide is properly indentified by adequate labelling. Labels should therefore carry the following information:

1. The name of the plant – If the whole organism is mounted then the slide can be marked Ac=Allium cepa or E=entire
2. The part of the plant used, e.g. leaf, root.
3. The type of preparation, e.g. smear; squash; TS = transverse section; VS = vertical section; LS = longitudinal section.

The following information is desirable but not essential;

4. Stains(s) used, e.g. H.E. = haematoxylin/eosin.
5. Dated
6. Signed.

It is common to use two labels, one on each end. Self-adhesive or gummed slide labels pre-printed with lines are available from lab suppliers – alternatively use ordinary self-adhesive or gummed labels. You will find it easier to write the label before you stick it on the slide. If the label is gummed (rather than self-adhesive) you must not lick it but use a wet sponge.

Tissue Culture

In biological research, tissue culture refers to a method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment, where they continue to function or grow. While fragments of a tissue are often used, it is important to note that entire organs are also used for tissue culture purposes. Here, such growth media as broth and agar are used to facilitate the process.

Tissue Culture by Linda Bartlett (Photographer) [Public domain or Public domain], via Wikimedia Commons0Save

Types of Tissue Culture

There are different types of tissue culture employed and are listed below, namely;

1. Seed culture
2. Embryo culture
3. Callus culture
4. Organ culture
5. Protoplast culture

Conclusion

In reality, there are numerous methods used for tissue culture given that there are different types of tissues that require specific conditions for the culture process yield desired results. Both plant and animal tissue can be used for tissue culture purposes for a wide range of purposes.