

Core II

Analytical Techniques in Plant Sciences

Course Objective

- To learn the principles and operations of microscopes of various complexity and their application in biological studies.
- To learn the techniques of centrifugation for separation of biological samples.
- To learn the methods of radioisotopes measurement in and their importance in study of biological materials and processes.
- To understand and the principles and applications of spectrophotometry and to understand the basic structural design of a standard instrument.
- To learn about various chromatographic techniques in separation of plant extracts.
- To acquaint the students with the advanced methods for characterization of biomolecules

Course Outcomes:

- Proper understanding of the microscopy and knowledge to analyze plant samples using electron microscopy and flow Cytometer.
- Separation of biomolecules and cell organelle and appropriate application of the knowledge of centrifugation for the same.
- Basic knowledge on the use of radioisotopes for analysis of biological samples.
- Extraction and qualitative and quantitative analysis of extracts as well as the assay mixtures using spectrophotometer.
- Skilful application of chromatographic techniques for separation of amino acids, pigments and biomolecules.
- Proper method for characterizing protein and nucleic acids and skill on handling electrophoresis equipment for preparation of gels.

Unit-I:

Learning Outcomes: Students will able to acquires knowledge about principles of microscopy and their types.

Imaging and related techniques: Principles of microscopy; Light microscopy; Fluorescence microscopy; Flow cytometry (FACS); Transmission and Scanning electron microscopy – sample preparation for electron microscopy, cryofixation, negative staining, shadow casting, freeze fracture, freeze etching.

Unit-II:

Learning Outcomes: Students will learn about the principles of centrifugation in biomolecule separation and importance of radiography in biological research

Cell fractionation: Centrifugation: Differential and density gradient centrifugation, Sucrose density gradient, CsCl₂ gradient, analytical centrifugation, ultracentrifugation. Radioisotopes: Use in biological research, auto-radiography, pulse chase experiment.

Unit-III:

Learning Outcomes: Students will learn about the components and working principle of different types of Spectrophotometer

Spectroscopy: Principles, Components and working mechanism of UV-Visible and Infra-Red spectroscopy, Fluorescence spectroscopy, Chlorophyll *a* fluorescence, Flame photometer, Bomb Calorimeter and Atomic Absorption Spectrophotometer.

Unit-IV:

Learning Outcomes: Students will learn about the separation methods for biomolecules using chromatography and electrophoresis instruments.

- **Chromatography:** Principle of chromatography, paper chromatography, column chromatography, TLC, HPLC, Ion-exchange chromatography, Molecular sieve chromatography, Affinity chromatography.
- **Characterization of proteins and nucleic acids:** Electrophoresis: AGE, PAGE, SDS-PAGE. Mass spectrometry; X-ray diffraction, X-ray crystallography.

Practicals:

1. Study of different microscopic techniques observation through simple and compound microscope
2. Study of PCR using demonstration.
3. To separate pigments by paper chromatography.
4. To separate phytochemicals by thin layer chromatography.
5. Qualitative analysis of total Carbohydrates, Proteins & Lipids.
6. Demonstration of SEM/ Electrophoresis/ Chromatography.
7. Measuring OD using spectroscopy.
8. Beer Lombard's law and its validation

Text Books:

- ✓ *Patil, C. S. (2017). Advanced Analytical Techniques, ABE Books, New Delhi.*
- ✓ *Pandey BP (2023). Botany for B.Sc. Students Semester I, NEP 2020; S. Chand publication, New Delhi*

Reference Books:

- ✓ *Aneja, K. R. (2014). Laboratory manual of microbiology and biotechnology, Medtech, New Delhi*
 - ✓ *Ausubel, F., Brent, R., Kingston, R. E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (1995). Short Protocols in Molecular Biology. John Wiley & Sons. 3rd edition.*
 - ✓ *Plummer, D.T. (1996). An Introduction to Practical Biochemistry. Tata McGraw-Hill Publishing Co. Ltd. New Delhi. 3rd edition.*
 - ✓ *Ruzin, S.E. (1999). Plant Micro technique and Microscopy, Oxford University Press, New York. U.S.A.*
- Pandey, B.P. (2023). Botany for B.Sc. Students Semester I: NEP 2020, S. Chand Publishing.*