Master of Science (Botany)
M. Sc Botany Semester II

Course Code	PS02EBOT52	Title of the Course	Micro techniques
Total Credits of the Course	04	Hours per Week	04

Course Objectives:	1. To teach various light and electron microscopes. 2. To teach material processing for permanent slide preparation light and electron microscopes. 3. To teach various microtomes for wax embedded and resin embedded material. 4. To teach the techniques for enzyme localization

Course Content		
Unit	Description	Weightage* (%)
1.	Light microscopy Properties of lenses, Optical corrections, Properties and types of objectives, Oculars and Illumination. Light microscopes: Bright field, dark field, fluorescence, phase contrast, polarizing, differential interference contrast. Micrometry and photomicrography.	25
2.	Basic components of electron microscopes. Thermionic and field emission guns. Types of electron microscopes: TEM, SEM, STEM, ESEM and HVEM	25
3.	Maceration, squash and clearing techniques. Sample preparation for light microscopy. Classification of fixatives, formulas', (Plant and animal samples). Sample preparation for light microscopy: Fixation, dehydration and infiltration procedures. Embedding media for light microscopy. Stains and staining procedures- negative and positive staining procedures. Microtomes: Rotary, sliding, cryostat. Histochemical localization of metabolites for light microscopy: Starch, proteins, lipids, total carbohydrates, lignins, polyphenols, nucleic acid, histones, cutin, suberin and waxes. Localization of enzymes: Peroxidase, acid phosphatase and succinic dehydrogenase.	25
4.	Freeze etching and freeze fracturing. Sample preparation for Electron microscope: Fixatives, double fixation, dehydration and infiltration	25

	procedures, embedding media for electron microscopy. Fixation and embedding of particulate samples like bacteria, virus etc. ultra-microtome and freezing ultramicrotomesemi thin sectioning, ultrathin sectioning, grids, formavar coating, Staining for electron microscopy. Ultrastructural cytochemistry: Tannin, protein, cell wall polysaccharide, lignin and membrane. Enzymes: Peroxidase and phosphatase. Immunocytochemistry.	

Teaching-Learning Methodology	Topics of the course will be taught through interactive classes using appropriate tools and techniques. Students will be encouraged to explore different sources of data pertained to the course. Course materials will be provided from primary and secondary sources of information.

Evaluation Pattern		
Sr. No.	Details of the Evaluation	Weightage
1.	Internal Written / Practical Examination (As per CBCS R.6.8.3)	15%
2.	Internal Continuous Assessment in the form of Practical, Viva-voce, Quizzes, Seminars, Assignments, Attendance (As per CBCS R.6.8.3)	15%
3.	University Examination	70%

Course Outcomes: Having completed this course, students will be able to	
1.	Have thorough understanding of modern development in light and electron microscopy.
2.	Process plant/ animal samples for permanent slide preparation.
3.	Gain knowledge regarding various biological stains.
4.	Localize various enzymes in plant/animal tissue.
5.	Localize histochemical of proteins, lipids and nucleic acids.

Suggested References:	
Sr. No.	References
1	Marimuthu, R. (2019). *Microscopy and Microtechnique*. MJP Publisher.
2	O'Brien, T. P., & McCully, M. E. (1981). *The study of plant structure principles and selected methods* (No. 581.4 O2).
3	Johansen, D. A. (1940). *Plant microtechnique*. McGraw-Hill Book Company, Inc: London; 530p.
4	Berlyn, G. P. (1976). *Botanical microtechnique and cytochemistry*

On-line resources to be used if available as reference material
On-line Resources
Relevant review articles/research papers/handouts of latest development in the subject

LABORATORY EXERCISES

Practical

1. Demonstration and hands on training of brightfield, fluorescence, phase contrast, DIC and polarizing microscopes.

2. Slide preparation based on maceration, squash and whole mount.

3. Fixation and processing for wax embedding of plant/animal tissues.

4. Rotary microtome sectioning and staining.

5. Demonstration of cryocut and ultramicrotome.

6. Demonstration of plant/ animal tissue processing for electron microscope.