Course name: Microscopic techniques (USOS Code: B-BC.BE.223E)

ECTS: 3

No. of hours: 30 (0 lectures + 30 classes)

Course coordinator: dr hab. Bożena Pawlikowska-Pawlęga, prof. UMCS

Prerequisites:

Course description: 1. Construction of electron microscopes and their functioning. Characteristics of SEM and TEM parameters. 2. Visit in TEM ansd SEM labs. Microscopic observation of specimens from the selected cells, tissues, animal organs, viruses and bacteria. 3. Construction and operation of confocal microscope. Advanced Techniques dedicated for confocal microscopy: FRET (Fluorescence / Forster resonance energy transfer), FRAP (fluorescence recovery after photobleaching), FLIP (Fluorescence loss in photobleaching), FLIM (fluorescence lifetime imaging microscopy). 4. Visit in confocal microscopy lab of UMCS. Slides observation and saving images in confocal microscope. Convertion to three-dimensional image. 5. Test - electron microscopy: transmission, scanning and confocal microscopy. 6. Construction and operation of a light microscope (dark and bright field of view, phase contrast and fluorescence). 7. Preparation of samples for electron microscopy - the whole procedure. Collecting material, fixation, dehydration, saturation and embedding with resin, cutting on ultrathin sections, contrasting . Physical and chemical fixation. Carriers for fixatives and the criteria for their selection. 8. Trimming and grids preparation for electron microscopy. Manual trimming and trimming on ultramicrotome. 9. Cutting of biological samples. Cutting material into silver sections. Straightening of sections with chloroform. Collecting sections on copper grids. 10. Positive grid's contrasting. Positive contrasting - factors relevant for contrasting. Contrasting compounds: osmium tetroxide, uranyl acetate, lead salts, phosphotungstic acid, potassium permanganate. Negative contrasting. Contrasting compounds used in contrasting: uranyl acetate, Reynolds reagent :lead nitrate, sodium citrate. 11. Test- Construction and operation on light microscope (bright field, dark field, phase contrast and fluorescence; samples preparation for electron microscopy - all stages of the procedure. 12. Slides observation with application of light microscope. Staining of human cheek epithelium with methylene blue. HeLa cells from culture in vitro –semithin section stained with toluidine blue. Cerebellar cortex cells, hyaline cartilage of human trachea , squamous epithelial cells of the esophagus, ureter epithelium, seminiferous tubules of rat; 13. Preparation of sample for SEM from cells in vitro. Coating of sample with usage of coat sputter and recording images in electron microscope (SEM). 14. Slides observation in fluorescence microscopy. Identification of autophagic cells – acridine orange staining; Apototic and necrotic cells identification – staining with propidium iodide and Hoechst 33342. 15. Final assessment.

Recommended literature: 1. Alberts B, BrayD, Johnson A, lewis J, Raff M, Roberts K, Walter P. Podstawy biologii komórki. PWN 2005.; 2. Immunocytochemia. PWN 1999.; 3. Klyszejko-Stefanowicz L. Cytobiochemia. PWN 2002.; 4. Reid N. Ultramicrotomy [in:] Glanert AM. Practical methods in electron microscopy. Vol. 3,1975.; 5. J. Litwin, M. Gajda, WUJ 2011, Podstawy Technik Mikroskopowych.; 6. B. Wróbel, K. Zienkiewicz, D. Smoliński, J. Niedojadało, M. Świdziński, WUMK 2005, Podstawy Mikroskopii Elektronowej