Module name	**Microscopic techniques**
Module code	B-BC.BE.223E
ISCED code	0511: Biology
Study cycle	*II*^{o}
Semester	summer
Responsible for this module	Main teacher Bożena Pawlikowska-Pawlęga, bozka1996@o2.pl, +48 815375991 Department of Functional Anatomy and Cytobiology Mgr Jurek Wydrych (1 meeting) Mgr Jarek Pawelec (1,5 meeting)
Language of instruction	English
Website	https://www.umcs.pl/en/biology-and-biotechnology,9741.htm.
Prerequisites	basic knowledge of English, the passed courses from cell biology, chemistry and biochemistry
ECTS	3
ECTS points hour equivalents	30 Contact hours with the lecturer in the form of classes 10 Student preparation for didactic classes 20 Student preparation for credit 15 Studying subject literature by a student **Total number of ECTS points for the module**
Learning outcomes verification methods	mid-term tests (W1-W5, U1-U4, K1-K2) attendance at exercises K-K01: attendance at lectures
Course full 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; Apoptotic and necrotic cells identification – staining with propidium iodide and Hoechst 33342. 15. Final assessment.
Bibliography	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
Learning outcomes	**KNOWLEDGE** K1. The student interprets the images visible on the preparations, i.e. he recognizes autophagy, apoptosis, normal and changed structure of tumor and tumor cells K2. The student has in-depth knowledge of physics and chemistry to the extent necessary to understand the theoretical foundations of the microscopic techniques used, including electron, confocal, light and fluorescence microscopy, i.e. he knows the phenomenon of fluorescence, phosphorescence, elastic and non-elastic scattering of electrons, diffraction, phase shift, chemical fixation in connection with other fields of science especially with physics and chemistry K3. Understands the principles of selection of individual microscopic methods and techniques used in biological sciences, i.e. for TEM ultrastructure research, for SEM surface topography, for life-long research - fluorescence and confocal microscopy K4. Knows the principles of safe and ergonomic work in the laboratory, especially during preparation procedures during fixation, trimming and work with normal and cancer cells from in vitro culture

	SKILLS S1. The graduate is able to select and use appropriate microscopic techniques for specific research purposes and modify standard procedures to achieve specific goals S2. The graduate is able to cooperate with other people as part of team work (trimming, preparation of preparations) in performing various tasks in the field of microscopic techniques, i.e. fluorescence, SEM and TEM and light microscopy **SOCIAL COMPETENCES** S1. The graduate is ready to lead the group and take responsibility for it and make decisions during the implementation of practical parts of the entire cycle of exercises S2. The graduate is ready to recognize the importance of knowledge in the field of microscopic techniques, i.e. in scientific, medical and pharmacological research (FLIM, TEM, EDX), related sciences, i.e. physics and chemistry in solving cognitive and practical problems
Practice	
Teaching methods	explanation, observation, practical preparation of samples and elements of the procedure for electron and confocal microscopy; lecture