Doctoral School: **Biology Doctoral School**
Doctoral Program: Neuroscience and Human Biology

Subject code: **BIO/7/43**
Subject title: **Light microscopy techniques PR**
Teacher and Neptun code: **Dr. Schlett Katalin (KDC2T1)**
Credits: 4
Class hours: 2 hours/week, practical

Aims of the course
The course consists of 8 theoretical lectures, followed by a 2-day, blocked practical part to learn about light and fluorescence microscopes.
Contents of the course
1. General structure of the light microscope. Overview of major milestones in microscopy. Basic parts and structure of a light microscope. Comparison of Upright and Inverse Microscopes. Characteristics and selection aspects of lenses. The role of the condenser, correct setting.
2. Light microscopic contrast enhancing techniques. Principles and practical application of light microscopic contrast enhancement techniques: phase contrast, DIC, dark field, polarization and Hoffman modulation contrast microscopy.
3. Principles of fluorescence microscopy. Basic types of fluorescent dyes, molecules and crystals. Types of excitation light sources. Structure of the fluorescent filter cube. Types of filters and selection criteria. The phenomenon and treatment of spectral overlap and bleedthrough in fluorescence microscopy.
4. Optical slicing in fluorescence microscopy. Deconvolution and use of structured illumination. The concept and significance of the confocal principle, PSF. Laser scanning and spinning disc confocal microscopy. Two- and multiphoton microscopy.
5. Time-lapse and fluorescent living cell studies I. Use of fluorescent dyes and biosensors in neurobiology: Ca imaging, monitoring of changes in membrane potential, pH and ion concentrations.
6. Time-lapse and fluorescent live cell studies II. Use and application of fluorescent proteins in neurobiology. Phenomena of photoactivation, fotoswitching and photoconversion. FRAP, FLIM and PA techniques. Optogenetics.
7. Microscopic examination of protein-protein interactions. TIRF microscopy. The phenomenon of FRET and its measurement possibilities and limitations.
8. Super-resolution microscopy. Super-resolution microscopy: breaking the diffraction limit. 4Pi, STED, STORM and PALM microscopy presentation, application areas.
9. Center the light path, adjust the condenser properly. Lens types, maintenance - practical work. Köhler centering of the light microscope. Lens types and their cleaning. Maintenance and cleaning of the microscope.
10. Contrast enhancement techniques: phase contrast and DIC / Nomarski microscopy - practical work. Proper adjustment of light path and optical elements. Comparison of brightfield, darkfield, phase contrast, and DIC images of different preparations.
11. Epifluorescence microscope and its accessories - practical work. Replacing and cleaning the fluorescent filter cube. Centering excitation light. Multi-channel fluorescence recording. Bleed-through practice.
12. Live-cell fluorescence microscopy - practical work. Time-lapse imaging in cultures. Stabilizing the focal plane, ensuring proper physiological conditions. Wide-field microscopy.
13. Optical slicing in practice. Making confocal microscopic images. Determination of PSF. Multi-channel simultaneous and sequential scanning.
14. Spinning disc microscopy and FRAP in practice. Live cell studies by SD microscopy. Measurement of fluorescent signal return after fading in living cells.
Requirements
Written test covering the material of the theoretical and practical part.

Literature

online pdf and English-language tutorial sites