Fénymikroszkópos technikák /Fluorescent microscopy

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The course consists of two parts:

1) The theoretical part consists of 11 lectures (see the topics given below), focusing on the basics of microscopy and on the biological applications of certain optical techniques. Emphasis is given on the practical considerations during experiments or during the interpretation of the data. The online tutoring is extensively used during the lectures, too. Students are strongly encouraged to ask about their own needs and considerations during the lectures. A written test is given on the last class (essay questions from different topics). The evaluation and the final mark of the course depends on the result of the written exam (as the course is considered as a practical, there is one chance to repeat the exam, on Dec 19).
2) Those who pass the exam with "excellent (5)" marks are eligible to participate in the practical training, held at the end of January / beginning of February (final date will be fixed with the potential participants). The training includes a 2-day-long hands-on experience with the cleaning and maintenance of microscopes, the use of wild-field, laser scanning and spinning disc confocal microscopes and a visit to the Imaging Centre of the Inst. of Experimental Medicine (MTA-KOKI) on a separate occasion. The practical training is not compulsory, so students eligible for participating can still skip the possibility without modifying their final mark.

Training materials:

- online tutorial sites (see the links given in the presentations)
- lecture presentations, including homepage links

Topics of the lectures:

1. Sept 9. Milestones in the development of microscopy techniques. Optical train in the microscope: advantages and drawbacks of the inverse and upright systems. Image formation in the microscope, numerical aperture and resolution. The point spread function. Type and choice of objectives.
2. Sept 16. The condensor and the correct Köhler illumination. Enhancing contrast in optical microscopy: phase contrast and DIC imaging. Practical usage and limitations of bright field microscopy.
3. Sept 23. Basic concepts in fluorescence microscopy. Main characteristics of fluorochromes and fluorescent proteins. Optical highlighter fluorescent proteins: photoactivation, photoconversion and photoswitching.
4. Sept 30. Optical elements of a fluorescent microscope: filters, fluorescent light sources. Choosing the right filters: crosstalk and bleed-through in fluorescent microscopy. Spectral imaging and linear unmixing.
5. Oct 7. Optical sectioning microscopy I. Deconvolution. Structured illumination microscopy (SIM).
6. Oct 14. Optical sectioning microscopy II. Point scanning and spinning disc confocal microscopy. Two- and multiphoton microscopy.

7. Oct 21. Light sheet microscopy. Fluorescent live cell imaging microscopy. Technical and practical considerations and limitations. Optimizing signal-to-noise ratio in live-cell imaging.
8. Nov 4. Use and importance of fluorescent biosensors (Ca imaging, pH and ion-selective measurements).
9. Nov 11. FRET microscopy: investigation of protein-protein interactions. FRAP, FLIM, FCS: use of optical highlighters in microscopy. Live cell applications and interpretation of the data.
10. Nov 18. Superresolution in fluorescent microscopy – the basic concepts. STED and PALM microscopy.
11. Nov 25. Laszlo Barna (Imaging Center, MTA-KOKI): STORM microscopy

Dec 9. (Monday) Written exam: essay questions based on the theoretical topics.

Dec 19. (Thursday) Repeated written exam