Microscopy and instrumental methods in biology

Program

L1. Light. Light absorption. Color. Spectrophotometers. Absorption-based methods. Dipole moment of molecules and absorption wavelength. (Shvadchak V).

S1 Light absorption and concentration (Shvadchak V)

L2. Fluorescence. Principles of fluorescence. Jablonsky diagram. Fluorescence quantum yield. Fluorophores. Tryptophan and other natural fluorophores. GFP. Brightness. Solvatochromism. (Shvadchak V).

S2 Real data processing: Protein-membrane binding followed by Trp fluorescence. Kd determination. Origin software for non-linear fitting.

L3. Advanced fluorescence methods.Protein labeling. Cys-, Lys- reactive dyes. Intercalating dyes. FRET and its application to study protein interactions. Polarized light. Fluorescence anisotropy. (Shvadchak V).

S3 Fluorescence anisotropy. FRET, stopped flow. (Shvadchak V)

L4. CD and IR. CD spectroscopy to determine protein structure. IR spectroscopy. (Shvadchak V).

L5. Methods to determine the size of molecules. Electrophoresis of proteins and oligonucleotides. DLS. FCS. FCCS. (Shvadchak V).

S4 CD, FCS, DLS, Electrophoresis. (Shvadchak V)

6. Chromatography HPLC principle, preparative and analytical applications. Types of columns. Ion exchange chromatography. Size-exclusion. (Shvadchak V).

S5 Ion exchange chromatography: construction of gradient. HPLC analysis. (Shvadchak V)

7. Mass-spectrometry. Mass-spectrometry. LC-MS. ESI, MALDI and other ionization methods. Types of mass detectors. Fragmentation. LC-MS in proteomics. (Shvadchak V).

S6 LC-MS (Shvadchak V)

8. Transmission and fluorescence microscopy. Transmission microscopy, phase contrast. Fluorescence microscopy. Principal schemes of microscopes. Lasers. Filters. Dichroic mirrors. Channels. Digital image collection. Image resolution, microns and pixels. Confocal microscopy. Z-slices. (Kovalchuk Yu.).

9. Applied fluorescence microscopy. Membrane trackers, staining of nuclei. ImageJ/Fiji. Colocalization. TIRF. (Kovalchuk Yu.).

S7 ImageJ and image processing (Shvadchak V)

10. Advanced fluorescence microscopy. FRET and detection of interactions in microscopy. Fluorescence lifetime and FLIM. Diffraction limit. Superresulution, STORM. PALM. Application to image actin fibrils. (Shvadchak V)

11. Fluorophores for microscopy. Fluorescent proteins. Small molecule dyes. Channel crosstalk and selection of fluorophores. Photodegradation during measurements. FRAP. Light intensity and damage to cells. Caged molecules and controllable photorelease. Photoswithchable molecules. (Shvadchak V)

S8 Fluorophores for microscopy. (Shvadchak V)

12. How to build or customize a microscope? (Khoroshyy P.)

13. Python for image processing (1) (Khoroshyy P.)

14. Python for image processing (2) (Khoroshyy P.)

S9 Python (Khoroshyy P.)

15. Electron microscopy. Principle. Resolution. Modes. Sample preparation. (Bondarenko N.)

16. CryoEM (Bondarenko N.)

S10 EM and CryoEM (Bondarenko N.)

17. Atomic Force Microscopy. Principle and scheme of microscopes. XY and Z resolution. Sample preparation. Scanning speed and sample damage. Application for protein unfolding. (Shvadchak V)

18. NMR and ESR. Spin. 13C and 15N protein labeling. NMR for protein structure analysis. Solid state NMR. ESR and free radicals. (Shvadchak V).

19. X-ray. Protein crystallization. SAXS. (Shvadchak V).

S11 How to select method to solve the problem?