

FDITE02 Fluorescence spectroscopy in biophysics and biochemistry

(A. Sipos, T. Páli, P. Maróti)

Description:

In addition to presenting the physical foundations of fluorescence spectroscopy, the course discusses its role and benefits in biophysical and biochemical research and the study of molecular processes. In addition to presenting the theoretical foundations, it also covers the most important applications used in research practice.

Topics:

Basic concepts and types of luminescence: fluorescence, phosphorescence.

Characteristics of luminescent materials: absorption, emission, excitation spectra.

Quantum efficiency, lifetime, degree of polarization.

Spectra and their interpretation

Differences between absorption vs. excitation spectra.

Emission spectrum: $\Delta E/\Delta\lambda$ function, Stokes shift.

Mechanisms of fluorescence and phosphorescence.

Kasha rule, mirror symmetry.

Measurement setup (Spectrofluorimeter design, 90° detection, signal-to-noise ratio and the role of sensitive detectors)

Molecular factors (concept of chromophores and fluorophores, significance of excited state lifetime)

Photoselection and polarization (linearly polarized light, absorption and emission transition moments; phenomenon and significance of photoselection; degree of polarization as an indicator of molecular motion.

Applications of fluorescence (principle and advantages of fluorescent labeling; molecular and cell diagnostic applications). Fluorescent analytical methods (sensitivity and quantitative application of fluorescence analysis; calibration curve, linearity of dilute solutions)

Study of biological macromolecules (fluorescence of proteins and nucleic acids; intercalating dyes (ethidium bromide, propidium iodide); nucleic acid structure and dynamics studies, cell biology applications (membrane potential measurement, ion-specific fluorescent indicators, pH-sensitive dyes (BCECF).

Recommended literature:

Péter Maróti and Gábor Laczkó: Introduction to Biophysics, JATE Press 1993, 1995, 1998.

Sándor Damjanovich, Judit Fidy, János Szöllősi: Medical Biophysics, Medicina, Budapest, 2007

Ágoston Budó, Jenő Pócsa: Experimental Physics, Tankönyvkiadó, 2000