TCIS Hyderabad

Course : Fluorescence Methods in Cellular Biophysics
Credits : 4
Coordinates : Monday 16.00 - 17.30 hrs. & Friday 09.30 - 11.00 hrs.
Contact Hours : 48 hrs.
Instructor/s : Aprotim Mazumder

Syllabus

Newton's laws of motion, Fluorescence microscopy is an essential tool of Cellular Biophsyics. Going beyond structure in fixed cells and tissues, advancements like Green Fluorescent Protein (GFP) technology have opened up a whole new vista of investigating protein dynamics in live cells. On the other hand, super-resolution techniques have pushed the bounds of structural resolution to well below the diffraction limit of light. So influential have been these methods, that both have won separate Nobel Prizes in Chemistry in a relatively short span of time after their development. This course will discuss both the classic and modern applications of fluorescence in Cellular Biophysics. Starting from the basics of fluorescence methods, we will go on to discuss methods for measuring dynamics like Fluorescence Anisotropy, Fluorescence Recovery After Photobleaching (FRAP), Fluorescence Correlation Spectroscopy (FCS), and also newly developed super-resolution methods that have been garnering a lot of interest. The following topics will be covered (approximately seven hours will be spent on each of the numbered points below on average):

- Basics of fluorescence - Jablonski diagrams, Stokes shifts, structures of fluorophores, quantum yields, fluorescence instrumentation - fluorescence microscopy and spectroscopy, light sources, filters, and detectors (PMTs, APDs and camera technologies - CCD, EMCCD, CMOS, sCMOS etc.)
- Forster Resonance Energy Transfer (FRET), Time-Correlated Single Photon Counting (TCSPC), fluorescence lifetime, quenching - theoretical ideas, technical details behind measurements and applications. Fluorescence polarization measurements - steady-state and time-resolved fluorescence anisotropy - theory, instrumentation and technical details.
- Widefield microscopy, effects of objective numerical aperture on resolution, diffraction limit of resolution of light microscopy, single molecule imaging of mRNA as an example, basics of flow cytometry.

- Confocal microscopy - point-scanning and spinning disk confocal microscopy; light sheet microscopy; Total Internal Reflection Fluorescence (TIRF) microscopy; considerations for temporal resolution; multiphoton microscopy.
- GFP technology and dynamics measurements in live cells - Single Particle Tracking (SPT), Fluorescence Correlation spectroscopy (FCS) - both APD and camera-based, Fluorescence Recovery After Photobleaching (FRAP), live cell mRNA dynamics measurements
- Super-resolution microscopy methods - stimulated emission depletion (STED), structured illumination microscopy (SIM), stochastic optical reconstruction microscopy (STORM), photo activated localization microscopy (PALM), point accumulation for imaging in nanoscale topography (PAINT) and others.

Text / References Books

- Principles of Fluorescence Spectroscopy - J R Lakowicz - Springer
- Fundamentals of Fluorescence Microscopy - P Mondal and A Diaspro - Springer

Other information

- **Grading:**
  - ❖ Assignments/examinations : 60 %
  - ❖ Presentations : 40%