Fluorescence – steady-state and time resolved fluorometers

In a nutshell: A well established and universal method to study molecular interactions, conformation, stability, and to perform various biochemical assays.

Services: Biophysics Facility offers fluorometers as open-access instruments. First-time users must complete a short training session before gaining access to the instrument reservation calendar.

Location: Building 50, room 3226

Description: Some substances reemit light after a finite delay subsequent to the absorption of the electromagnetic energy. This emission, called fluorescence, has longer wavelengths (lower energy of photons, or lower electromagnetic wave frequency) than the absorbed light. Both absorption and emission wavelengths are characteristics of the fluorophore—a functional group in the molecule responsible for its fluorescence—and are, in general, sensitive to the immediate environment. Fluorophores can be intrinsic, like the tryptophan residues in proteins, or they can be applied as extrinsic labels. The fluorometer permits the identification of the excitation and emission wavelengths and measures changes in fluorescence intensity, polarization, and lifetime. Fluorescence spectroscopy is very sensitive and allows quantitative measurements at very low concentrations, often at the nanomolar level. Several processes associated with fluorescence, like fluorescence quenching, resonance energy transfer (FRET) and fluorescence polarization, are used to study structural changes, dynamics and interactions of macromolecules.

Typical applications:

- Studies of macromolecular interactions and ligand binding by detecting changes in the fluorescence intensity, anisotropy, or in the extend of the energy transfer (FRET).
- Structural studies of proteins and nucleic acids (tertiary structure changes of proteins, bending of DNA, etc.) by measuring changes in the intrinsic fluorescence, quenching or FRET.
- Studies of conformational stability of macromolecules at varying temperature, pH or denaturant concentrations.
- Measurements of metal ions concentrations, pH values, and other parameters in living cells, using fluorescent indicators.

Basic instrument specifications:

PTI/Horiba QuantaMaster (spectral scans, fluorescence anisotropy, ratiometric fluorescence measurements, and tracking of slow kinetics):

- Geometry: double-detection in the T-format with Glan-Thompson polarizers
- Detectors: Peltier-cooled, extended range photomultipliers (R928, 185-900 nm)
- Temperature control: Peltier cell holder with temperature ramp capability

PTI EasyLife LS time-resolved fluorometer (boxcar-averager fluorescence lifetime system with LED excitation):

- Time resolution: 0.1 ns to 1 μs.
- PMT sensitivity range: 200 to 650 nm
- Excitation LEDs: 280 nm, 310 nm, 340 nm, 445 nm, 450 nm, 510 nm, 525 nm, 635 nm

Sample requirements and recommended buffers: Sample absorption should be measured ahead of time to calculate fluorophore concentration and to select the excitation wavelengths. Buffers should not have any background fluorescence – this is particularly important for measurements at low concentrations and with UV-excitation. Filter all buffers and centrifuge samples to eliminate light scattering. This is crucial for fluoresce anisotropy measurements where the polarized scattered excitation light will strongly affect the results.

Minimum sample amount: Both fluorometers accept standard 1 cm fluorescence cells and small-volume cells; the minimum sample volume for the micro cell is 120 μL.

Consumables: This instrument does not require consumables. Cells and filters are provided by the core.