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Correlated Imaging and Analysis of Single Cells: A Biophysical Perspective on Advanced Fluorescence Microscopy

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Fluorescence microscopy enables molecularly specific imaging in living cells using fluorescent labels that report the location and behaviour of selected targets. Widefield imaging is fast and simple, but it suffers from out-of-focus blur and background, especially in thicker samples.
In this report, these limitations are addressed by advanced fluorescence imaging techniques, such as confocal microscopy, which improves contrast through optical sectioning, and two-photon excitation, which confines excitation of fluorophores to the focal volume and supports deeper imaging in scattering tissue; both approaches are discussed in terms of their trade-offs in speed, signal, and phototoxicity. Super-resolution is discussed with a focus on stimulated emission depletion (STED), where a red-shifted, donut-shaped depletion beam suppresses fluorescence around the focal centre to achieve sub-diffraction resolution. Key practical limits in live cells include light dose, fluorophore photostability, labelling density and calibration. Multicolour fluorescence imaging is used to compare multiple targets within the same cell and to assess spatial overlap (co-localisation) between channels.
Dynamic imaging adds time-resolved information. FRAP/FLIP quantify mobility and exchange via recovery after photobleaching, while FRET and FLIM (including FLIM-FRET) probe nanometre-scale proximity and interaction dynamics. Overall, methods should be chosen according to the biological question and the required spatial–temporal scale, aiming to maximize information while minimizing perturbation of living cells.

Field of Research/Work

Beyond Physics

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